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Colorimetric film sensor for monitoring rancidity reaction of intermediate-moisture (Cake)

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ABSTRACT

KEY WORDS:

Colorimetric films, Rancidity, Glucose oxidase, Alpha amylase, Hydrogen peroxide, peroxide value (PV)

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A colorimetric film sensor based on wheat gliadin and natural dyes (anthocyanin, chlorophyll, and beta-carotene) was developed for real-time monitoring of cake rancidity. The addition of 0.9% glucose oxidase (270 U/0.09 g), 0.9% alpha-amylase (117 U/0.09 g), and 5% natural pigments significantly enhanced the sensor's sensitivity to rancidity. The enzyme-substrate mixture significantly impacted the intensity of color changes in the films. Examination of the active film's color attributes revealed that chlorophyll changes from green to yellow, beta-carotene from yellow to pale yellow, and anthocyanin from red to brown with increasing storage duration and oxidant concentration. These color changes can be used to detect the rancidity reaction of cake fat. Absorbance measurements at 494, 450, and 666 nm, respectively, showed a considerable decrease in the intensity of anthocyanin, beta-carotene, and chlorophyll films due to the rancidity reaction in cake samples stored for 3 weeks at room temperature (30-35°C) and 25-30% relative humidity. Furthermore, all treated samples exhibited a decrease in color pigment intensity compared to the control within 15 minutes of exposure to 1 ppm hydrogen peroxide, the lowest concentration at which the films are susceptible to oxidation. Additionally, after 4 weeks of storage under the same conditions, cake samples developed a rancid odor, and the peroxide value of the cake fat increased significantly, from 1.3 to 8 mEqO₂/kg, approaching the maximum permissible limit set by Codex regulations. This increase in peroxide value and rancidity also led to a complete change in the color of the film sensor

غشاء لوني مستشعر لرصد تفاعل التزنخ في (الكيك) متوسط الرطوبة

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علوم الأغذية ومراقبة الجودة ، كلية علوم الهندسة الزراعية ، جامعة السليمانية ، العراق

الخلاصة

تم إنشاء أغشية لونية مستشعرة للتزنخ على أساس مكون من جليادين الحنطة مع الأصباغ الطبيعية (بما في ذلك الأنثوسيانين والكلوروفيل وبيتا كاروتين) وأنزيمات الكلوكون أوكسيديز وألفا أميليز / النشا / لاكتشاف تفاعل التزنخ في الكيك و لمراقبة الوقت الفعلي لحدوثه ووصفه في هذه الدراسة. إضافة إنزيم الجلوكون أوكسيديز 0.9% (نشاط 270 وحدة/0.09 غم) ، 0.9% (نشاط 117 وحدة/0.09 غم) من ألفا أميليز و 5% من الأصباغ الطبيعية المستخلصة وبنسبة 1:0.1 من غليادين / غليسرول ونشا المكونة لتلك الأغشية أدت إلى زيادة حساسية تلك الأغشية لتفاعل التزنخ . أدت إضافة الخليط المكون من الإنزيمات-المادة الأساس إلى تحسين شدة تغير الأغشية الملونة. بالإضافة إلى ذلك ، عندما تم فحص سمات الأغشية الملونة النشطة ، حيث تم اكتشاف أن لون غشاء الكلوروفيل يتغير (من الأخضر إلى الأصفر) في حين أن غشاء بيتا كاروتين من (أصفر إلى أصفر شاحب) وغشاء الأنثوسيانين (تم تغييره من أحمر إلى بني) مع زيادة مدة التخزين ونسبة الأكسدة ، والتي يمكن استخدامها للكشف عن تفاعل التزنخ لدهن الكيك. وكما أظهر الامتصاص عند 494 و 450 و 666 نانومتر ، على التوالي ، أن شدة الأنثوسيانين والبيتا كاروتين والأغشية الملونة بالكلوروفيل قد انخفضت بشكل كبير نتيجة تفاعل التزنخ في عينات الكيك بعد 3 أسابيع من التخزين في درجة حرارة الغرفة 30-35 درجة مئوية و 25-30% من الرطوبة النسبية. بالإضافة إلى ذلك ، بعد 15 دقيقة من التعرض لبيروكسيد الهيدروجين المضاف (1 جزء في المليون) ، وهو أقل تركيز تكون فيه الأغشية عرضة للأكسدة ، أظهرت جميع العينات انخفاضاً في كثافة شدة اللون مقارنة إلى عينات السيطرة. علاوة على ذلك ، بعد 4 أسابيع من التخزين ، لوحظت عينات الكيك تظهر منها رائحة الزناخة بشكل واضح في نفس الظروف المحيطة ، وزادت قيمة بيروكسيد دهون الكيك بشكل كبير ، من 1.3 إلى 8 ملي مكافئ / 2 / كغم كانت قريبة تقريباً من الحد الأقصى المسموح به في لوائح الدستور الغذائي وبالتالي لوحظ تغيير لون الأغشية المستشعرة للتزنخ بالكامل نتيجة لزيادة الرقم البيروكسيدي.

الكلمات المفتاحية: الأغشية الملونة ، التزنخ ، كلوكون أوكسيديز ، ألفا أميليز ، بيروكسيد الهيدروجين ، قيمة البيروكسيد

INTRODUCTION

Legal requirements for food traceability are increasing, especially in the European Union. This establishes a chain of accountability along the entire food supply chain. In order to check the freshness of food products in real time, the food industry, retailers, consumer advocates, and food safety regulators are all interested in creating precise, economical, rapid, reliable, non-invasive, and non-destructive methods or equipment. Development of intelligent packaging in the form of a food deterioration indicator to monitor freshness state is an alternate concept to address this demand (Sonneveld, 2000). Cake belongs to the category of intermediate-moisture foods (IMF), which are those with moisture contents between 15 and 50 percent and water activity values between 0.60 and 0.85. (Jay *et al*, 2008). Sugar is added to a product as Thai dessert to keep it fresh. Osmotolerant yeasts are chiefly responsible for the spoiling of intermediate-moisture desserts. "Golden drop" (Hong yod in Thai) is a common intermediate-moisture Thai dessert that is derived from a Portuguese egg-

based treat (Yu, 2003). Golden drop is also considered a high-end dessert. Golden drop manufacture is currently classified as a small and medium enterprise (SME) in Thailand. As a result, when it relates to the distribution of golden drop on the market, shelf life is a significant concern. Due to their often-high water activity, most Thai desserts are easily deteriorated by microorganisms. The customer is dissatisfied with these negative changes in color, flavor, odor, and texture over the duration of distribution and storage (in't Veld, 1996).

Cake is one of the bakery products that undergo deterioration during its shelf life because it includes fat ingredients, commonly in significant amounts, exposing it susceptible to lipid oxidation and chemical changes (rancidity). Lipid oxidation is considered to be the primary cause of deterioration for these products (Caruso *et al*, 2017). Lipid oxidation via a three-stage free radical chain reaction: initiation, propagation, and termination. In the initiation stage, an initiator used unsaturated fatty acids to form free radicals. A free radical creates a proxy radical when it interacts with oxygen. In the propagation step, the proxy radical selectively removes hydrogen from nearby lipid molecules, producing hydro peroxides as the major byproduct of oxidation along with additional free radicals (Yin *et al*, 2011, Kanner *et al*, 1987). Lipid free radicals are converted to non-radicals at the termination stage. Alkenes, aldehydes, ketones, and other substances that alter the flavor and odor of food are secondary lipid oxidation products (Woo and Lindsay, 1983). The majority of methods for detecting rancidity reactions exist, but they take time in laboratories and are difficult to comprehend. Rancidity is the flavor and odor that results from lipid alterations. The most common cause of oil deterioration is rancidity, which happens as a result of lipid oxidation. This technology has the potential to be used in a variety of products. In order to monitor the rancidity reaction of oxygen-sensitive items, a new rancidity indication for detecting hexanal and acetic acid formation from oxidation and hydrolysis is intended to be a freshness colorimetric indicator (Belitz *et al*, 2008).

Protein-based films have been deemed appropriate packaging materials because they have a high ability to limit oxygen penetration (Swain *et al*, 2012). Gliadin is a protein, and gluten makes up roughly 80% of the protein in wheat grains (Domenek *et al*, 2004). Gliadin dissolves in alcohol, while glutenin is only soluble in dilute acids and may with reducing sugars. These two compounds can be refined by washing their bonded starch since they are insoluble in water. The oxidation conditions that can be employed to oxidize foods for intelligent packaging change the film's color properties. Glycerol could be used as a gelatin plasticizer to produce flexible and uniform films (Fu *et al*, 2009). Aldehyde indicator pad for chemical and industrial applications to detect aldehyde quickly. It was discovered that when exposed to glutaraldehyde, this indicator pad changed color from yellow to red. When adding glutaraldehyde, the color of the aldehyde indicator changed as the pH was reduced (Kulchan *et al*, 2016).

The aims of this study were to monitoring and detecting rancidity reaction of moisture cake spoilage kept at room temperature ambient conditions. Furthermore, to emphasize the safety of these products, on the one hand, by employing natural safe materials rather than artificial materials, and on the other hand, by using alternative approach rather than chemical classic procedures for rancidity detection. Finally, to make customer aware of how to detect rancidity by using a new and quick method involving visual colorimetric films by naked eye.

Firstly, Glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide (Tao *et al*, 2009). When film exposed to volatile substances such as hexanal, acetic acid, and H_2O_2 are influence on intensity color caused by the response of the starch-glucose oxidase –alpha amylase and pigments complex is proportionate to the glucose concentration and changes color the combination, and this mixture can be successfully used for monitoring rancidity reaction. The above standardized complicated mixture was sealed on all sides in 2×2 cm low density polyethylene (LDPE) bags.

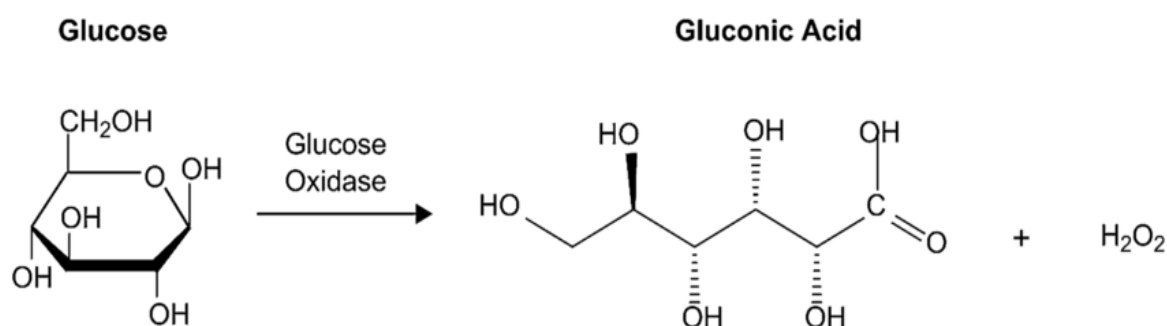


Fig.1 Chemical equation showing GOx catalyzing glucose reaction

MATERIAL AND METHODS

Materials

Roselle (*Hibiscus sabdariffa* var. *sabdariffa*) dehydrated calyces, chard, carrot, and gliadin were extracted from commercially available wheat gluten (ALTUNSA company, Turkey) and imported (cake with milk cream) were obtained from the local market in Sulaimani city, Iraq. Other components, such as soluble starch, Glycerol, ethanol absolute 99 % (C_2H_6O) (Scharlau, Spain), petroleum ether and glacial acetic acid (Chem-Lab, Belgium), glucose oxidase 0.9% (activity 270U/0.09g) and alpha-amylase 0.9% (117U/0.09g) enzymes were purchased from AVOCHEM Ltd. Chemical Co. (Cheshire, Sk116PJ.U.K.). Utilizing potassium iodide (Scharlau, Spain), sodium thiosulphate (Merck, Germany), chloroform (Alfa CHEKA, India), Hydrogen peroxide 30% (Merk, Germany) and measuring relative humidity and temperature by Temperature /Hygrometer (Mebus Sender/Aussensender 40424/China). Oven Ufp500/Memmert, Germany) was used in the drying process. Soxhlet fat extraction apparatus (BUCHI Laborate 1000ml, Switzerland). Heating mantle 250W (Lab Tech, China). Scanning pigments were applied by using UV-Vis spectrophotometry (Jenway -7205- the UK by Cole - Parmer Ltd).

Anthocyanins Extraction

The calyces were manually separated, washed with distilled water, and drained. After that, the calyces were dried in the oven dryer (Ufp500/Memmert) at 45° C. The dried calyces were packed in double Polythene (PE) pouches, kept in a black PE bag, and stored at -18°C (deep fridge) until use. Before analysis, the calyces were milled in a mixer blender (Mulino) based on the approach in (Gartaula and Karki, 2010) with various variations. The aqueous extraction of the powdered using citric acid monohydrate and sodium bicarbonate are used respectively. The Roselle powder was extracted in a water bath at a temperature of (70.2 °C) for (20, 40, and 60min). After the completion of heat treatment, the conical flasks were removed, warmed to room temperature (32°C), and immediately filtered, with a solid-liquid ratio of 1:10 in the initial set of tests. In the second set of tests, the extraction was carried out for 20 minutes at 70.2 °C with a pH 7 solvent. The solid-to-liquid ratio likewise changed, going from 1:50 to 1:100 to 1:150. The aqueous extraction was performed by the procedure outlined in (Gartaula and Karki, 2010). To extract all of the anthocyanins, roselle powder was steeped in distilled water and mashed in a mortar and pestle. The process was repeated up until the residue was colorless. In a conical flask, 50 ml made up the volume. The extract was then filtered in preparation for additional analysis.

Anthocyanin Content

A- Using a UV-V spectrophotometer, the anthocyanin content of the extract powder was determined absorbance with using differentials specified in Method (Tsai *et al*, 2002) (Jenway -7205-UK by Cole - Parmer Ltd.). 60 mL of distilled water were used to dissolve the anthocyanin extract powder (2.4g), and 1 mL of anthocyanin was then diluted with 1 mL of the solution. At 400 and 700 nm, the sample's absorbance was determined. The amount of anthocyanins was specified as mg/ml. Calculation: Total anthocyanins (mg/ml) = (Total OD/ml)/ the total anthocyanin was expressed on a % dry basis. Total OD/ 60 ml = (OD* volume made up*60/ ml juice taken).

Beta carotene extraction

Conditions of extraction: By the procedure described in Slovak Technical Standard 56 0053 (Fikselová *et al*, 2008), slices of roots were used (width 2 mm, length 1 cm). At various temperatures (20-25°C), the extraction yield of carotenes was examined using ethanol (96%) and 40 g of chopped carrot samples were added to 240 ml of 96 % ethanol and 10 ml of distilled water and placed inside a beaker covered in aluminum foil. Carrot slices were taken out of a water bath at 60 degrees Celsius and shaken every 10 minutes. After each hour of extraction, 10 ml of the sample was removed and combined with petroleum ether (10 ml).

Determination of carotenes

Measurement of carotenes Using a spectrophotometer (Jenway -7205- the UK by Cole - Parmer Ltd.), the absorbency was measured at a wavelength of 450 nm to estimate the amount of -carotene contained in the petroleum-ether extract. The carotenoid concentration represented as β -carotene (mg/100 ml) was estimated using the following response parameters and response factors:

$$\beta\text{-carotene} = A \times d \times V / E1\% \text{ } 1\text{cm} \times w$$

Where: A – absorbency d – dilution E1% – coefficient of absorbency (2592 for petroleum-ether) 1cm w – weight of the sample (g) V – volume (ml)

$\text{Carotene (mg/100g)} = \text{Absorbance} \times \text{total volume} \times \text{Dilution} / \text{wt. of sample} \times \text{volume of cell (cuvette)} \times 1000 = \text{the result} \times 100$

Chlorophyll Extraction

Chard leaves were obtained from a local grocery store in Sulaimani city, Iraq. Simple mechanical-thermal extraction utilizing water as the solvent was used to produce the leaf extract. Filtering helped to separate the extract. 50 grams of leaves and 100 ml of solvent made up the 1:2 mass ratios of the leaves and solvent used in the study, heated the mixture for 2 minutes, and then separated chlorophyll extracted and dried it in a dark place at room temperature overnight.

Chlorophyll extracted pigment 100mg/ml dissolved in 1ml of ethanol 70% solution. An aluminum foil-wrapped conical flask was used to extract the chlorophyll to shield it from light and maintain the stability of the reaction. Although chlorophyll is stable, when it is removed from plant tissue, it is rapidly degraded by heat, light, oxygen, acid, and enzymes (Humphrey, 2004).

Total chlorophyll content (chlorophyll a and b) (mg/l) was analyzed using UV-Vis Spectrophotometer with the following equation (Humphrey, 2004).

$$\text{Total chlorophyll content} = 20, 31A_{645} + 8, 05A_{663} \quad (\text{Jinheng et al, 2009})$$

With A_{645} = the absorbance at 645 nm wavelength and A_{663} = the absorbance at 663 nm wavelength

Preparation of the colorimetric films

Each film was created by pouring 2 ml of the film-forming solution (gliadin-glycerol) (0.2 per cent) into a spotless, smooth, and 10 mm diameter plastic petri dish. A certain quantity of anthocyanins extracts powder was then added based on the estimated anthocyanins content (5 per cent solution). Pigments were added (5%) of beta-carotene and chlorophyll concentrations, respectively. In addition, the same concentration of enzymes and substrate (starch) (0.9%) were added to both compositions. These concentrations were included because they represent the best and lowest concentrations for the effect of enzyme activity on color intensity of pigments. Then films were dried at 25-30°C and 33-35 %RH for 24 hours according to the procedure with modifications as mentioned in method. Also, relative humidity and optimal temperature were chosen as the storage parameters since they are equivalent to the environment for storing cakes.

Gliadin extraction

According to the technique (Reddy and Yang, 2010), some ethanol concentration changes .99.16 ml of 96 per cent ethanol was added to 119 g of wet gluten to extract gliadin using 60% aqueous ethanol (final concentration of ethanol in combination) centrifuged ethanol/water mixture that has been swirled overnight at room temperature at 9000 rpm for 15 minutes at 25 degrees Celsius the supernatant was collected, which included the gliadin-rich fraction after extraction gluten from wheat flour .Ethanol was evaporated from the supernatant to obtain the dissolved gliadin. Film-forming gliadin solution the precipitate, which was agitated for 1 hour at 25°C.

Imported Cake formulation

Cacao cake 84%, wheat flour(gluten), sugar, egg, glucose syrup, emulsifier mono and diglycerides of vegetable fatty acids, Poly-glycerol esters of vegetable fatty acids, sorbitol, salt, sodium bicarbonate, Sodium acid pyrophosphate (citric acid), xanthan gum, cream filling with milk flavor 15%, palm oil, preservative materials(potassium sorbate), Anti-oxidant (tocopherol), E322 chocolate flavors.

Cake's fat extraction

To extract the oil using Soxhlet system according to the AOAC official Method no.920.39C using petroleum ether as a solvent (AOAC, 2016). Weighed three samples for each cake sample, 40 g in overall, in order to extract fat using the Soxhlet method.

Peroxide value

The peroxide value was measured according to AOAC method No.965.33 (AOAC, 2016). The reaction between a saturated potassium iodide and oil sample is the basis of the method, and the iodine formed is titrated against sodium thiosulfate with starch as an endpoint indicator. It was expressed as milli equivalent of peroxide oxygen per Kg sample (mEqO_2/kg).

$$PV (\text{mEqO}_2/\text{kg oil}) = \frac{[(S-B) * \text{Normality}] \times 1000}{\text{Weight of sample (kg)}}$$

Sample wt. (g)

Where S=titration of test sample (mL), B=titration of blank (mL)

Colorimetric film sensor development with H₂O₂

H₂O₂ is significant as a defense mechanism, oxidative stress marker, and aging agent and is the intermediate molecule formed in reactions involving numerous oxidases such as glucose oxidase, alcohol oxidase, cholesterol oxidase, lactate oxidase, and glutamate oxidase and it is measured utilizing a variety of techniques, including electrochemical, optical including spectrophotometry, chemiluminescence, and fluorescence (Giorgio *et al*, 2007; Sies, 2017 and Aziz *et al*, 2019). When hydrogen peroxide was added (1ppm) to vials containing colorimetric -dye-based indicator solution. Three different types of film sensors were tested with H₂O₂ (oxidant agent), including anthocyanin, chlorophyll, and beta-carotene, and the film sensors were changed after 15 minutes from addition time at room temperature.

Statistical Analysis

The complete randomized design (CRD) was used with three replicates. XLSTAT, 2016 statistical program was used to analyze mean of each data point by Duncan test at the level of 5%.

RESULT AND DISCUSSION

Case study of cake which is oxygen-sensitive. The peroxide value is a quality index that is utilized early in the lipid oxidation process (Nopwinyuwong *et al*, 2010). Due to utility of indicator colorimetric films, high moisture and changing peroxide value have an effect on indicator color changes. The color changes of sensor films labels in sealed bags held at room temperature (20-25°C) and relative humidity 25-27% were shown in this experiment. When exposed to volatile substances such as hexanal, acetic acid, and H₂O₂, the chlorophyll colorimetric indicator labels gradually changed from brilliant green to yellow, anthocyanin film red color to brown, and beta carotene film bright yellow to pale yellow, respectively. Increasing the concentration of PV throughout storage time was linked to changing the color of the indicator labels and that compatible with Augspole and Rakcejeva (2013).

To examine the effect of H₂O₂, optimum concentration added 1ppm was has impact on film sensor sensitivity after 15 minutes from addition time and this result nearly similar to outcome of the study that includes using chemiresistive H₂O₂ sensors operate within a millimolar concentration change, with one exception that operated lower to the Nano molar range (Hnaïen *et al*, 2010). The bar chart showed there is a significant changing while addition 1ppm of peroxide hydrogen on (beta carotene, glucose oxidase, alpha amylase and starch). There were significant differences between the treatments for all kinds of sensors. The result recorded that the absorbance decreased from 0.509 to 0.208 at 450nm of beta carotene pigment and that compatible with the result in Augspole and Rakcejeva (2013).

Figure 3 demonstrated that significant differences are available among the entire film sensors (anthocyanin, chlorophyll and beta-carotene) to present 1 ppm hydrogen peroxide in their

environments. It reduced the colors of pigments and lowered the absorbance values. Chlorophyll was highly stable to reduce (0.05 value) than anthocyanin and beta-carotenes with change in 0.3 value. In contrast, anthocyanin had highest value in reduction by hydrogen peroxide which reduces its level to half with 0.333 value lost (0.7 to 0.367). These results are compatible with results in Augspole and Rakcejeva (2013). The oxidation of phenols, also have deleterious effects on anthocyanins (Nopwinyuwong *et al*, 2010).

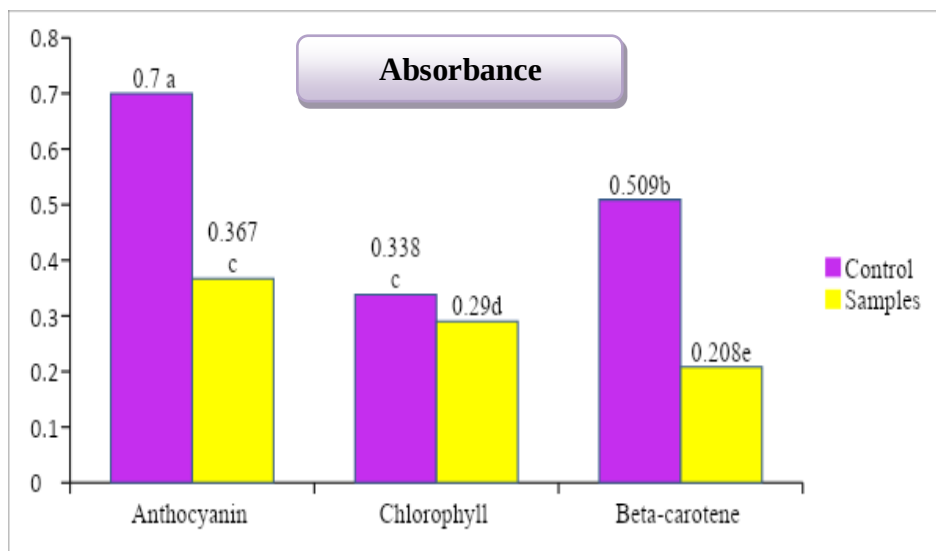


Fig. 2. Effect of H₂O₂ (1 ppm) on pigments sensors

H₂O₂ decomposition and dissociation products were found to be responsible for the oxidation and subsequent degradation of phenolic compounds (Yao *et al*, 2006).

Additionally, oxidizing reagent H₂O₂ had an impact noticeable color change of anthocyanin mixture from red to brown, and its concentration was reduced by approximately a half when compared to the control sample. Moreover, that green color of chlorophyll mixture was affected by H₂O₂ and was altered to yellow color and the absorbance obtained at 666 nm was declined in the control sample from 0.338 to 0.290 in the mixture sample and that related to the bleaching of chlorophyll caused by the addition of hydrogen peroxide and these results are almost similar to those observed in Chen *et al* (2006).

Peroxide value is the parameter which used as an indicator for primary oxidation and rancidity of the oils (Poiana, 2012). Figure 5 showed the peroxide values of the fat extracted from the cake, which were 1.6, 1.5 and 1.3 mEqO₂/ kg, respectively, at zero time, within the permitted level. There were significant differences between the peroxide values of cakes among the month for all samples. The changes of peroxide value were close among the samples and sharply increased four times. In contrast to PV, the absorbance decreased approximately to half value in chlorophyll and beta-

carotenes. Similar to PV, significant differences were found to absorbances. Anthocyanin presented to largest change in color in comparison with another colorimetric films (anthocyanin and beta-carotene). Value of absorbance of anthocyanin after four weeks decreased closely to one third of control absorbance. According to the Codex Alimentary (210-1999) (Idun- Acquah *et al*, 2016), peroxide value of oils should be less than 10 mEqO₂/ kg. As a result, it may be stated that the initial peroxide value in this study, which was stored at 25-30°C and exposed to natural light, complied with Codex standards. Whereas, after storage cake with film sensors for 4 weeks with 30-35 °C and 25-30% of RH the results revealed that the rancidity reaction and hydrogen peroxide have a substantial impact on the intensity of film colors compared to the control. Decreasing color film sensor intensity in relation to increasing PV levels in cake's fat sample was 8 mEqO₂/ kg approximately 6 times higher than initial levels and that close to allowable maximum level according to that findings are somewhat similar to those in Almeida (2018) and Ghanbari *et al* (2018).



Fig.3 colorimetric film sensor with cake samples

As shown in figure4, pigments response films were raised by increasing concentration of PV values after storage for 4 weeks and rancidity reaction. There is a significant decreasing in absorbance 0.217, 0.282, 0.145 for anthocyanin, chlorophyll and beta-carotene films sensors respectively compared to controls at zero time. The rancidity of the product caused the colorimetric film that had been applied to the sample to change colors due to lipid oxidation. As a result of the two parameters increased PV values of cake's fat and the rancidity reaction, the color of the films has changed and this result similar to findings in the studies (Kulchan *et al*, 2016 and Dirpan *et al*, 2018) Lipase causes

rancidity in hydrolysis when catalyzed (Onilude *et al*, 2010). However, based on these results, moisture content significantly affects lipid oxidation as shown in PV values.

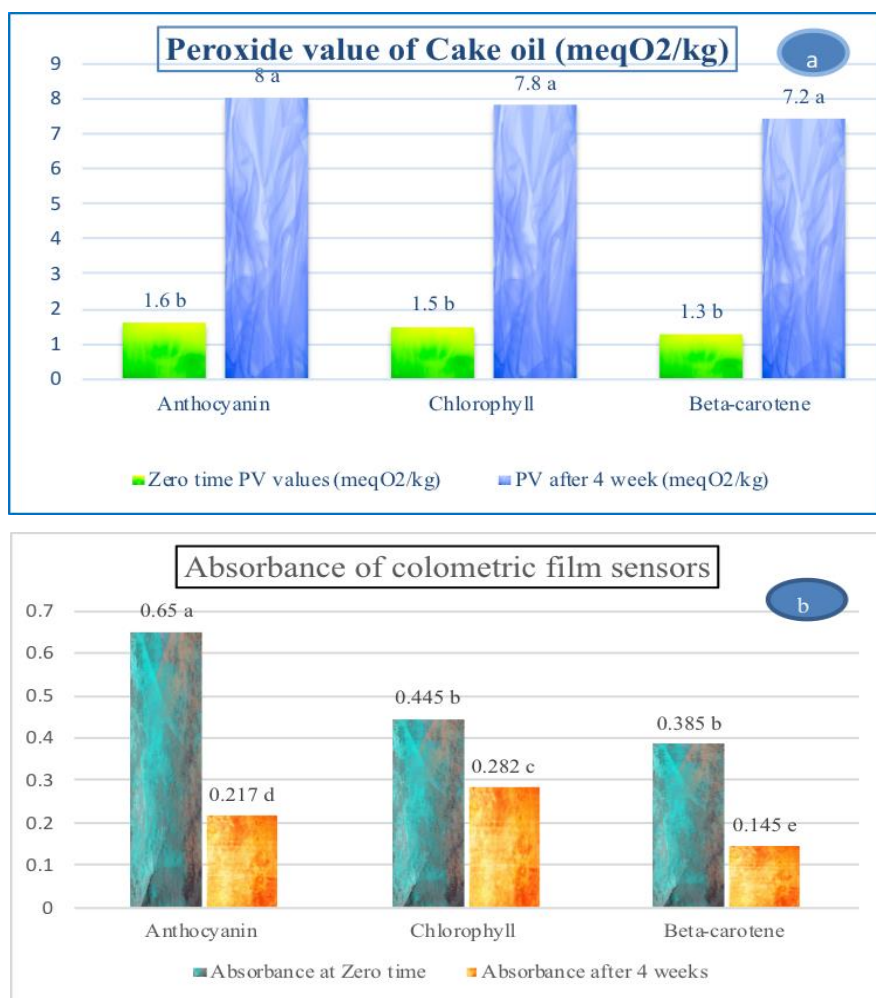


Fig. 4. Impact Peroxide value of Cake's fat (a) on absorbance film sensor (b) at zero time and after storage 4 weeks

CONCLUSION

The results of this research showed that a colorimetric approach is capable of sensitively detecting spoilage metabolites (rancidity) in an intermediate-moisture product as cake. The improved guarantee of the safety of food products is certainly of the utmost importance to customers. This colorimetric film sensor food deterioration indicator offers visual monitoring of the best-before date as well as the dynamic freshness and rancidity reaction, allowing the food item to have a usable shelf life. In addition to expanding future studies may focus on applying the idea of a colorimetric film sensor-based food spoilage indicator to additional food products, such as processed foods, baked products, desserts, and fresh-cut fruits and veggies.

REFERENCES

- Alimentarius, C. (1999). Codex standard for named vegetable oils. *Codex stan*, 210, 1-13. Cited by Idun-Acquah, N., Obeng, G. Y., & Mensah, E. (2016). Repetitive use of vegetable cooking oil and effects on physico-chemical properties—case of frying with redfish (*Lutjanus fulgens*). *Sci Technology*, 6(1), 8-14.
- Almeida, D. T. D., Viana, T. V., Costa, M. M., Silva, C. D. S., & Feitosa, S. (2018). Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). *Food Science and Technology*, 39, 211-217.
- AOAC (Association of Official Agricultural Chemists) (2016). In George W. Latimer, Gaithersburg (Eds). Official methods of analysis of AOAC International (20th ed.). Gaithersburg, Md. USA: AOAC International.
- Augspole, I., & Rakcejeva, T. (2013). Effect of hydrogen peroxide on the quality parameters of shredded carrots. In *Proceedings of Annual 19th International Scientific Conference Research for Rural Development* (pp. 91-97).
- Aziz, A., Asif, M., Ashraf, G., Azeem, M., Majeed, I., Ajmal, M., ... & Liu, H. (2019). Advancements in electrochemical sensing of hydrogen peroxide, glucose and dopamine by using 2D nanoarchitectures of layered double hydroxides or metal dichalcogenides. A review. *Microchimica Acta*, 186(10), 1-16.
- Belitz, H. D., Grosch, W., & Schieberle, P. (2008). *Food chemistry*. Springer Science & Business Media.
- Caruso, M. C., Galgano, F., Colangelo, M. A., Condelli, N., Scarpa, T., Tolve, R., & Favati, F. (2017). Evaluation of the oxidative stability of bakery products by OXITEST method and sensory analysis. *European Food Research and Technology*, 243(7), 1183-1191.
- Chen, X. B., Zhao, X. H., Zhu, Y., Gong, Y. D., Li, L. B., Zhang, J. P., & Kuang, T. Y. (2006). Hydrogen peroxide-induced chlorophyll a bleaching in the cytochrome b 6 f complex: a simple and effective assay for stability of the complex in detergent solutions. *Photosynthesis research*, 90(3), 205-
- Dirpan, A., Latief, R., Syarifuddin, A., Rahman, A. N. F., Putra, R. P., & Hidayat, S. H. (2018, May). The use of colour indicator as a smart packaging system for evaluating mangoes Arummanis (*Mangifera indica* L. var. *Arummanisa*) freshness. In *IOP Conference Series: Earth and Environmental Science* (Vol. 157, No. 1, p. 012031). IOP Publishing.
- Domenek, S., Feuilloley, P., Gratraud, J., Morel, M. H., & Guilbert, S. (2004). Biodegradability of wheat gluten-based bioplastics. *Chemosphere*, 54(4), 551-559.
- Fikselová, M., Šilhár, S., Mareček, J., & Frančáková, H. (2008). Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. *Czech Journal of Food Sciences*, 26(4), 268-274.
- Fu, X., Xu, S., & Wang, Z. (2009). Kinetics of lipid oxidation and off-odor formation in silver carp mince: The effect of lipoxygenase and hemoglobin. *Food Research International*, 42(1), 85-90.
- Gartaula, C., & Karki, D. B. (2010). Optimization of Extraction of Anthocyanins from Roselle (*Hibiscus sabdariffa* var. *sabdariffa*) in Aqueous Medium. *Journal of Food Science and Technology Nepal*, 6, 69-72.

- Ghanbari, R., Mousazadeh, M., Moussavi, S. P., & Rostami, R. (2018). Determining Hydrogen Peroxide Index of Cooking Oil: Fast Food Shops in Northern Iran. *Journal of Biochemistry Technology* (2): 128-130
- Giorgio, M., Trinei, M., Migliaccio, E., & Pelicci, P. G. (2007). Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nature reviews Molecular cell biology*, 8(9), 722-728.
- Hnaïen, M., Lagarde, F., & Jaffrezic-Renault, N. (2010). A rapid and sensitive alcohol oxidase/catalase conductometric biosensor for alcohol determination. *Talanta*, 81(1-2), 222-227.
- Humphrey, A. M. (2004). Chlorophyll as a color and functional ingredient. *Journal of food science*, 69(5), C422-C425.
- In't Veld, J. H. H. (1996). Microbial and biochemical spoilage of foods: an overview. *International journal of food microbiology*, 33(1), 1-18.
- Jay, J. M., Loessner, M. J., & Golden, D. A. (2008). *Modern food microbiology*. Springer Science & Business Media.
- Jinheng, Z., Chao, H., & Zhiheng, L. (2009). Absorption spectrum estimating rice chlorophyll concentration: preliminary investigations. *Journal of Plant Breeding and Crop Science*, 1(5), 223-229.
- Kanner, J., German, J. B., Kinsella, J. E., & Hultin, H. O. (1987). Initiation of lipid peroxidation in biological systems. *Critical Reviews in Food Science & Nutrition*, 25(4), 317-364.
- Kulchan, R., Boonsupthip, W., Jinkarn, T., & Suppakul, P. (2016). Developing a novel colorimetric indicator for monitoring rancidity reaction and estimating the accelerated shelf life of oxygen-sensitive dairy products. *International Food Research Journal*, 23(3), 1092.
- Nopwinyuwong, A., Trevanich, S., & Suppakul, P. (2010). Development of a novel colorimetric indicator label for monitoring freshness of intermediate-moisture dessert spoilage. *Talanta*, 81(3), 1126-1132.
- Onilude, A. A., Igbinadolor, R. O., & Wakil, S. M. (2010). Effect of varying relative humidity on the rancidity of cashew (*Anacardium occidentale L.*) kernel oil by lipolytic organisms. *African Journal of Biotechnology*, 9(31), 4890-4896.
- Poiana, M. A. (2012). Enhancing oxidative stability of sunflower oil during convective and microwave heating using grape seed extract. *International Journal of Molecular Sciences*, 13(7), 9240-9259.
- Reddy, N., & Yang, Y. (2010). Developing water stable gliadin films without using crosslinking agents. *Journal of Polymers and the Environment*, 18(3), 277-283.
- Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox biology*, 11, 613-619.
- Sonneveld, K. (2000). What drives (food) packaging innovation. *Packaging Technology and Science: An International Journal*, 13(1), 29-35.
- Swain, S. K., Priyadarshini, P. P., & Patra, S. K. (2012). Soy protein/clay bionanocomposites as ideal packaging materials. *Polymer-Plastics Technology and Engineering*, 51(12), 1282-1287.

- Tao, Z., Raffel, R. A., Souid, A. K., & Goodisman, J. (2009). Kinetic studies on enzyme-catalyzed reactions: oxidation of glucose, decomposition of hydrogen peroxide and their combination. *Biophysical journal*, 96(7), 2977-2988.
- Tsai, P. J, McIntosh, J, Pearce, P, Camden, B, Jordan, B, R. (2002). Anthocyanin and Antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. *Food Research International*, 35(4), 351-356.
- Woo, A. H., & Lindsay, R. C. (1983). Statistical correlation of quantitative flavor intensity assessments and individual free fatty acid measurements for routine detection and prediction of hydrolytic rancidity off-flavors in butter. *Journal of Food Science*, 48(6), 1761-1766.
- Yao, R. S., Sun, M., Wang, C. L., & Deng, S. S. (2006). Degradation of phenolic compounds with hydrogen peroxide catalyzed by enzyme from *Serratia marcescens* AB 90027. *Water research*, 40(16), 3091-3098.
- Yin, H., Xu, L., & Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. *Chemical reviews*, 111(10), 5944-5972.
- Yu, S. M. (2003). Thai Egg-Based Sweets: The Legend of Thao Thong Keap-Ma.