ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Smartphone-Based Spectrophotometer for Facile and Fast Determination of Lipid Peroxidation in Local Fried Food

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Citation:
Khawasung, T.; Utaipan, T.; Treewanjutha, W. Smartphone-based spectrophotometer for facile and fast determination of lipid peroxidation in local fried food. *ASEAN J. Sci. Tech. Report.* **2024**, *27*(2), 79-89. https://doi.org/10.55164/ajstr.v27i2.252346

Article history:
Received: January 10, 2024
Revised: February 27, 2024
Accepted: February 28, 2024
Available online: February 29, 2024

Abstract: During lipid peroxidation in foods, deterioration rancidity occurred, and a toxic by-product also accumulated. The well-known marker of lipid peroxidation in food is malondialdehyde (MDA), suspected to be carcinogenic and mutagenic in humans. MDA level is determined using thiobarbituric acid (TBA) assay. The pink color of the MDA-TBA2 complex after the reaction can be measured spectrophotometrically at 530-540 nm. Several analytical methods, including smartphone-based methods, have been used to determine the MDA-TBA2 complex, such as UV-Vis spectrophotometry and HPLC-DAD. Therefore, this research aimed to determine lipid peroxidation in fried food using a simple smartphone-based spectrophotometer. The device was established using a paper box, LED lamps, and a test tube. Various concentrations of MDA were reacted with TBA reagent and then submitted to the device. RGB intensity data were converted to absorbance values and used to construct linear regression. Results showed that the G value from the smartphone-based spectrophotometer provided consistent results with R^2 of 0.9869, including 0.93 and 95.17% precision and accuracy, respectively. Then, the developed device was finally used to determine the MDA in local fried food samples. The concentration of MDA in fried foods was successfully determined with high precision (0.96) and accuracy (88.33 %) compared to the traditional UV-Vis spectrophotometric method. Thus, this study provides a practical guideline for developing quick and easy accessibility, portability, and low-cost spectrophotometer for lipid peroxidation assessment in fried food and other future food matrices.

Keywords: Smartphone; Malondialdehyde; Thiobarbituric acid reactive substances; Fried food

1. Introduction

Frying is a fast, simple, and cost-effective cooking method used for producing and preparing food, both on industrial and domestic scales [1]. Fried food has unique sensory characteristics, which consumers highly appreciate. These characteristics include a pleasant flavor, golden-brown color, and crispy texture. During the frying process, the oil used is subjected to high temperatures, which accelerate the formation of lipid oxidation [2]. Mild oxidation of fried food continuously occurs without promoting noticeable sensory changes during processing and storage. The longer fried food is

exposed to high temperatures and atmospheric air, the higher lipid peroxidation and oxidative products are generated [3]. Among the various oxidation products, aldehydes are mostly significant, affecting food flavor and nutritional quality. They are also the main cause of food rancidity during preparation and storage [4-5]. One widely known lipid peroxidation product is malondialdehyde (MDA) [6]. MDA is a final product of lipid peroxidation and is considered a universal lipid oxidation marker of oxidative stress. It had been regarded as a cytotoxic, neurotoxic, mutagenic, and possibly carcinogenic in humans [7]. The thiobarbituric acid (TBA) assay is an empirical method frequently used to measure oxidation in food products. The assay has been utilized due to its simple and quick derivatization of MDA before the detection process [8]. Since free MDA is very low in concentration, thus harsh conditions of acidity and temperature are needed to treat proteins and break down peroxides. One molecule of malonaldehyde reacts with two TBA molecules, forming a pink-colored MDA-TBA2 complex that absorbs light at 530-540 nm and can be quantified as shown in Fig. 1. However, the reaction is not specific because the other molecules in the sample metric such as ketones, acids, esters, sugars, amino acids, oxidized proteins, and vitamins, can also react with TBA, hence known as TBA-reactive substances or TBARS [9-10]. Meanwhile, the intensity of a pink-colored complex corresponds to the level of lipid peroxidation in the sample.

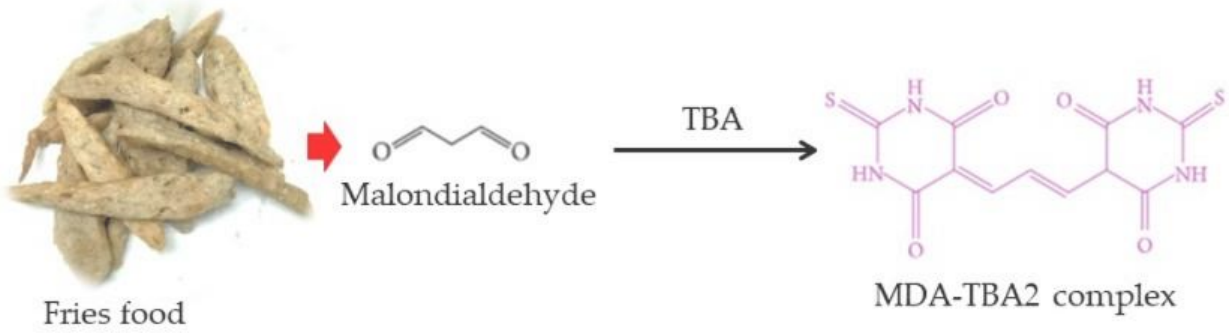

Figure 1. Reaction mechanism of malondialdehyde (MDA) with thiobarbituric acid (TBA) in the presence of acid and heat to produce MDA-TBA2 colored product [10].

Several analytical methods have been used to determine TBARS, such as spectrophotometrically [11], spectrofluorometrically [12] for screening analysis, and chromatographic, such as HPLC-DAD which is suggested for more specific performance [13], but the longer duration and cost of the analysis will also increase. Spectrophotometry, therefore, is a preferable method for its simplicity, high sample throughput, and low cost. Recently, the use of smartphones has rapidly increased in terms of accessibility for every single person. The capabilities of smartphone technology are more efficient in terms of computational power connectivity, including camera and imaging capability. Smartphone imaging has been increasingly applied across various scientific fields and several applications [14-15]. The RGB (Red–Green–Blue) color system has frequently been used as an alternative for quantitative determinations in analytical chemistry due to its real-time, convenient, and inexpensive [16]. Mostly, smartphone colorimetric data will be converted to analyze concentrations using a high-resolution built-in camera smartphone [17-18].

In this work, the developed smartphone-based spectrophotometer, in combination with Red-Green-Blue (RGB) analysis, was used to trace MDA in the local fried food after a derivatization step with TBA. An in-house smartphone-based spectrophotometer was constructed for field analysis of MDA in fried food samples. The influence of the dimension of the component effective in developing smartphone-based spectrophotometer sensitivity was evaluated and optimized. The reliability of the developed device for MDA in fried food evaluation was compared with the standard UV-Vis spectrophotometry method.

2. Materials and Methods

2.1 Constructing a smartphone-based spectrophotometer

Inside the developed device, there is the sample cell (small test tube, 16 mm x 10 mm, Pyrex, USA.) with base, background panel, and LED board accommodated in a paper box which has a small channel for taking pictures of the solution in a sample cell from outside as shown in Fig. 2.

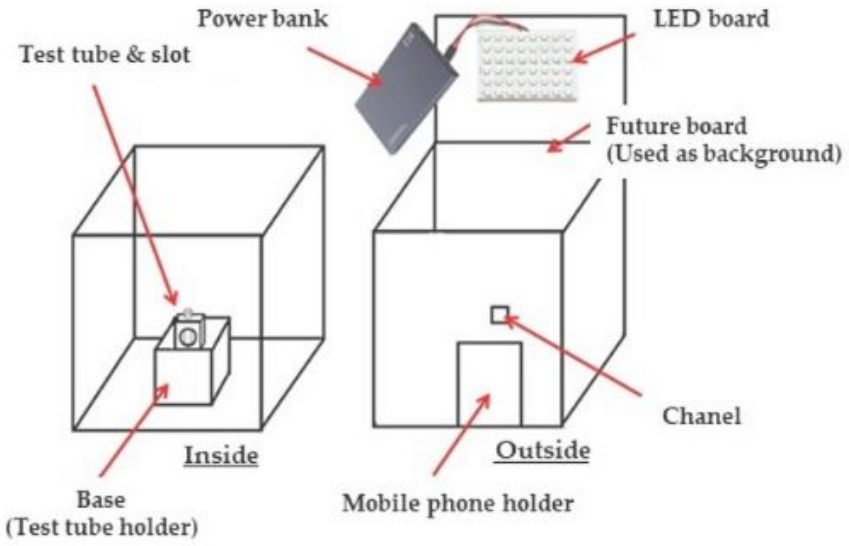

Figure 2. The design of the smartphone-based spectrophotometer to measure RGB value using a flat-palette application.

The dimensions of the device are listed in Table 1. The best distance and position of the sample cell, background, and LED panel were optimized by varying the distance between the camera and sample cell and the distance between the sample cell and the background. The best parameters have been chosen for device construction and further experiments. The digital picture of the MDA-TBA2 solution was captured by a smartphone camera (flat-palette application, Samsung Galaxy S10 Plus, screen is 6.4 inches, 103.8 cm^2 (88.9% screen-to-body ratio), with a resolution of 1440 x 3040 pixels, 19:9 ratio (522 ppi density), Korea). The image was then decomposed into three color components: red, green, and blue (R, G, and B).

Table 1. Apparatus design of the smartphone-based spectrophotometer.

List	Details
Box	Size 21 cm x 30 cm x 23 cm
White LED light bulbs	Size 5 mm x 18 bulbs
LED board	Size 4.5 cm x 17.5 cm
Test tubes	Size 16 mm x 10 mm
Slot	Size 2.5 cm x 6 cm x 6 cm
Base	Size 5 cm x 10.6 cm x 10 cm
Chanel	Size 1 cm x 2 cm
Phone holder	Size 10 cm x 13 cm
Camera Chanel	Size 1 cm x 2 cm
Feature board	Size 21 cm x 23 cm
Power bank	Capacity 11000 mAh

2.2 Optimization of components of the developed device

The pink solution of the MDA-TBA2 complex was prepared from the derivatization of 100 μM MDA standard and TBA solution, which was then measured at 532 nm. The red, green, and blue (RGB) colors were detected using a flat-palette application on the smartphone-based spectrophotometer. To achieve the best results, the distance between the camera and the sample cell was varied from 1 to 6 cm. While the LED board was fixed at 3 cm above the sample cell, the background was fixed at 15 cm behind the sample cell. Three different colors (white, blue, and green) of backgrounds were used to find the most appropriate color. The photos were converted to RGB values using the "flat-palette application." Flat-palette is a color manager app that combines useful color scheme ideas and functions, including RGB slider adjustment, center point color extraction, and image color scheme creation. Therefore, the captured image will provide the RGB value, a color commonly seen on a smartphone screen. The RGB obtained values were then converted to absorbance using equation (1) [17].

$$A = -\log\left(\frac{I}{I0}\right) \quad (1)$$

When

I = R, G, or B value of sample solution
I_0 = R, G, or B value of blank solution

The condition that provided the absorbance comparable to that obtained from a standard UV-Vis spectrophotometer was chosen for further studies.

2.3 Standard curve of MDA-TBA2 complex

The standard solution of TBA was prepared by using 0.375% of TBA dissolved in 15% trichloro acetic acid (TCA). The solution of TBA was prepared freshly before experimenting. BHT stock solution was prepared using 1 g of BHT dissolved in 100 mL of 95% ethanol. Standard solution of MDA (100 mmol/L) was prepared by diluting 99% MDA in 25 mL of distilled water. Then, MDA with concentrations of 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 μmol/L were prepared. The derivatization of TBA and MDA standard in various concentrations was done by mixing 3.5 mL of 0.375% TBA in 15% TCA and 0.5 mL of MDA standard solutions. The solution was then heated in a water bath at 95°C for 10 min [19]. After cooling, the pink solution was measured at 532 nm using a UV-Vis spectrophotometer. The red, green, and blue (RGB) colors were also measured using pictures from the developed smartphone-based spectrophotometer. All experiments were performed in triplicate.

2.4 Extraction of MDA in fried food samples

Two grams of fried food powder (S1 = fried fish cracker crisps, S2= fried fish cracker sticks, S3= French fries) were taken into a 15 mL test tube, and 5 mL of the glacial acetic acid (50% v/v) was added, followed by 2 mL of BHT (0.01%) for preventing further oxidation [10]. The samples were shaken for one hour and filtered. The filtrate was centrifuged when required.

2.5 Determination of MDA in fried food samples

0.5 mL of sample solution was mixed with 3.5 mL of thiobarbituric acid (TBA), then heated in a water bath at 95°C for 10 min. After the mixture had cooled down at room temperature, the absorbance at 532 nm was measured using the UV-Vis spectrophotometer and developed device. MDA content obtained from the device was compared to MDA content obtained from the UV-Vis spectrophotometer. The limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and the relative error of the methods were validated. The analysis of all samples was performed in triplicate. The data was expressed as standard deviation (SD).

2.6 Data analysis

Triplicate measurement was done for each standard and sample. All values were displayed as mean ±SD. LOD, LOQ, precision, accuracy, and error were calculated as compared to the absorbance from the UV-Vis spectrophotometer.

3. Results and Discussion

3.1 The optimization and construction of a smartphone-based device.

The optimization of the device with a smartphone camera was performed to find the best distance between the camera and the sample cell, the sample cell to the background, and the color of the background for TBARS measurement. Several conditions were tested for the analysis of the MDA-TBA2 complex by changing the distance between the camera and the sample cell. Three different backgrounds, white, green, and blue, were also investigated. R G B values after conversion to absorbance using a different camera and sample cell distances and different backgrounds (white, green, and blue) are shown in Table 2. The most efficient distance of each part was selected by considering the similarity of absorbance of the tested MDA-TBA2 complex solution when measured by the UV-Vis spectrophotometer at 532 nm (Abs. = 0.200). The results from Table 2 revealed that the reverse of the green value (G value) gave a positive absorbance value in all backgrounds used and was proportional to the concentration of MDA. However, the closest value to the UV-Vis spectrophotometer was most achieved on a blue background. However, the distance between the camera and sample cell that gives similar absorbance from the UV-Vis spectrophotometer was 3 and 4 cm, with the absorbance value of 0.165 and 0.245, respectively. The results were confirmed additionally, and the values of MDA-TBA2 levels were similar to the previous experiments, whereby 3 cm between the camera and sample cell was chosen. Therefore, the optimum dimensions of the smartphone-based spectrophotometer were 3 cm, 15 cm, and 3 cm for the distance from the camera to the sample cell and from the sample cell to the background and LED panel positions (in the above position), respectively. The absorbance, which was calculated using equation (1) and G value from a flat-palette application on a smartphone-based spectrophotometer, was found to exhibit a correlation with the value obtained from the UV-Vis spectrophotometer because both red chromogen and blue background are absorbing the green light (G value) according to "The light theory of color" [20]. Then, the G value and blue background were used as the analytical signal for further study. The optimum distance of the developed device is shown in Table 3.

3.2 Reaction of standard MDA and the TBA reagent for the standard curve

In this study, the reaction mixture was heated at 95 °C for 10 minutes due to the indicated experimental data that these conditions yield maximal product [10]. In reaction with TBA (TBA assay), MDA forms an intensely pink-colored complex that is easily assessed spectrophotometrically; therefore, this method is frequently used to detect lipid peroxidation in several samples. The pink color of the solution indicated the absorption of the green color wavelength; thus, the red color wavelength was transmitted and then observed. Beer's Law mentioned that the higher the concentration, the lower the light intensity that passes through a chemical solution [21].

The absorbance obtained from the UV-Vis and smartphone-based spectrophotometer increased correspondingly to the MDA concentration, as shown in Table 4. However, the absorbance from the developed device was slightly lower than that obtained from the UV-Vis spectrophotometer. The calibration curve of five points in triplicate (n=3) was established in the concentration range of 6.25 to 100 μmol/L. Linear regression shows a correlation coefficient (R^2) of 0.9984 with the equation of $y = 0.0144x$ and 0.9865 with an equation of $y = 0.0073x$-0.0532 for a commercial system and smartphone-based spectrophotometer, respectively, as shown in Fig. 3A and 3B. Meanwhile, the linear regression slope revealed a sensitivity of the method, in which the UV-Vis spectrophotometer provides higher sensitivity (slope = 0.0144) compared to the smartphone-based device (slope = 0.0073). The performance of the commercial UV-Vis spectrophotometer in terms of limit of detection and quantification was 3.352 μmol/L and 10.157 μmol/L, respectively, which revealed notably better results. Each run using the smartphone-based spectrophotometer took almost 1 min, compared to less than 5 s for the commercial spectrophotometer. However, this research aimed to provide a handheld device available to everyone to determine the MDA content onsite. Thus, the above performance was acceptable in relation to the robustness, ease of operation, and ultra-low cost of the smartphone-based spectrophotometer. Thus, measuring the concentration of MDA or TBARS in fried food samples can be applicable.

Table 2. The absorbance of MDA-TBA2 complex from R, G, and B values conversion using three different color backgrounds.

Distant between camera and sample cell (cm)	Absorbance (Abs.) value								
	White background			Green background			Blue background		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
1	-0.008 ± 0.001	0.054 ± 0.002	-0.032 ± 0.001	-0.075 ± 0.004	0.060 ± 0.005	-0.015 ± 0.002	-0.102 ± 0.008	0.126 ± 0.000	0.008 ± 0.005
2	0.015 ± 0.002	0.082 ± 0.002	-0.008 ± 0.000	0.104 ± 0.050	0.114 ± 0.021	0.060 ± 0.020	-0.159 ± 0.027	0.139 ± 0.010	0.025 ± 0.003
3	**-0.014 ± 0.003**	***0.039 ± 0.002***	**-0.036 ± 0.003**	**-1.652 ± 0.052**	***0.095 ± 0.041***	**0.079 ± 0.040**	**-0.031 ± 0.013**	***0.165 ± 0.013***	**0.017 ± 0.002**
4	**-0.015 ± 0.003**	***0.035 ± 0.004***	**-0.038 ± 0.004**	**-0.134 ± 0.284**	***0.095 ± 0.025***	**0.049 ± 0.027**	**-0.028 ± 0.014**	***0.245 ± 0.005***	**0.037 ± 0.004**
5	0.010 ± 0.006	0.113 ± 0.017	-0.050 ± 0.001	-0.815 ± 0.027	0.054 ± 0.006	0.000 ± 0.027	-0.195 ± 0.029	0.103 ± 0.016	0.024 ± 0.008
6	-0.016 ± 0.006	0.078 ± 0.009	-0.022 ± 0.003	-0.435 ± 0.088	0.021 ± 0.015	-0.004 ± 0.013	-0.598 ± 0.032	0.136 ± 0.025	0.002 ± 0.010

Data are expressed as mean±SD from triplicate samples.

Table 3. The optimum dimension of the smartphone-based spectrophotometer.

Details	Optimum
Distance between camera to sample cell	3 cm
Distance between sample cell to background	15 cm
Distance of LED panel (from top)	3 cm
R, G, B color	Green
Color of background	Blue

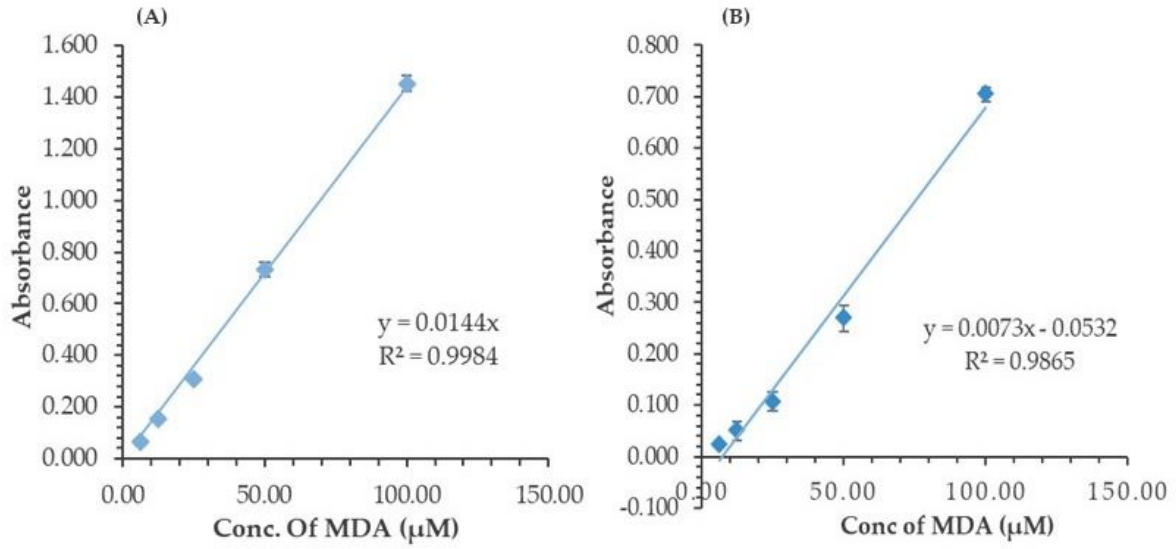

Figure 3. (A) MDA standard curve obtained from the UV-Vis spectrophotometer, (B) MDA standard curve obtained from the smartphone-based spectrophotometer.

Table 4. Absorbances were obtained from the UV-Vis spectrophotometer and the smartphone-based spectrophotometer.

concentration of MDA (μmol/L)	Absorbance from UV-Vis spectrophotometer	Absorbance from Smartphone-based spectrophotometer.
6.25	0.063 ± 0.013	0.024 ± 0.005
12.50	0.154 ± 0.008	0.050 ± 0.018
25.00	0.308 ± 0.011	0.107 ± 0.018
50.00	0.735 ± 0.028	0.269 ± 0.026
100.00	1.454 ± 0.029	0.704 ± 0.014

Data are expressed as mean±SD from triplicate samples**.**

The limit of detection (LOD) and quantification (LOQ) were evaluated from the slope and residual standard deviations of the standard curve. The LOD and LOQ can be calculated as described in the following equation: LOD = 3.3σ/S and LOQ = 10σ/S, respectively, where σ is the standard deviation of the response, and S is the slope of the calibration curve [22]. Results showed that the LOD and LOQ of the UV-Vis spectrophotometer were 3.352 μmol/L and 10.157 μmol/L while the LOD and LOQ developed spectrophotometer were 11.490 μmol/L and 34.819 μmol/L. The obtained results are in good agreement with previous studies in the determination of TBARS in fried fast food using a simple spectrophotometric method, which showed that the LOD was 1.758 μM, while LOQ was 5.859 μM [23]. The higher LOD and LOQ of the developed spectrophotometer should be remarked on compared to the commercial system. However, the LOD of MDA by the device achieved 11.490 μmol/L, which is quite below or lower than the recommended values, whereby the acute toxicity of MDA in the diet is considered not high. Still, the lethal dose of LD_{50} in rats accounts for 632 mg/ kg body weight [24]. The analytical performance was also validated for the smartphone-based spectrophotometric method, including accuracy, precision, and error. Three concentrations of MDA (20, 40, and 60 μmol/L) were used for this purpose. Accuracy was reported as the recovery percentage, while the precision and error represented the repeatability of the tested method. Instrumental precision was determined by the replicate (n=9) analysis of standard compounds. The accuracy, precision, and error of the developed device were 95.17%, 0.933, and 4.83 %, respectively, as shown in Table 5. Poor color intensity may decrease accuracy when the MDA is used at a lower concentration than 20 μmol/L. The recoveries ranged from 87 to 95%.

The results exhibited evidence and demonstrated the performance of the device for the analysis of different MDA amounts in different fried food samples.

Table 5. Precision, accuracy, and error of the developed smartphone-based spectrophotometer.

MDA (μM)	Precision	% Accuracy	% Error
20	0.879	87.102	12.898
40	0.926	94.118	5.882
60	0.933	95.169	4.831

3.3 Determination of MDA in fried food samples

To evaluate the performance of the smartphone-based spectrophotometer with real sample matrices, the method was finally applied to analyze MDA in fried food samples found in the local markets. MDA concentrations were determined parallelly between the smartphone-based method and the traditional UV-Vis spectrophotometer. Three samples of street foods were selected since they were popular among consumers and widely consumed. These include fried fish cracker crisps, fried fish cracker sticks, and French fries. The results of this experiment and the short description of these foods are given in Table 6. As determined by spectrophotometry, MDA concentration in fried fish cracker crisps, fried fish cracker sticks, and French fries were 55.84, 53.21, and 58.89 μmol/L, respectively. Consequently, MDA concentration in the same samples using the developed device was 63.21, 41.118, and 142.84 μmol/L. Zeb and Ullah (2016) revealed that fried fast foods possessed an amount of TBARS in the range of 0.372 ± 0.03 to 2.911±0.13 μmol/g when 50% acetic acid was used as an extraction solvent [10]. Pandey et al. [25] also reported that deep fried Shami kebab possessed the values of TBARS 0.38 to 0.68 mg/kg. The difference may be due to the difference in the laboratory and food preparation, TBARS in sample or frying medium. These results indicate that the methods used in this study, UV-Vis spectrophotometry and the developed device, give similar tendency results to the other works. The precision, accuracy, and error of the device were calculated. The best reliability was found in fried fish cracker crisps (S1), as shown by low error percentage, high precision, and high accuracy percentage. This result was consistent with the color of the reactant. The reactant solution of S1 was pale pink, indicating the expression of TBARS since oxygen can result in oxidative deterioration of the fried fish cracker. Meanwhile, MDA in S3 detected by the device was positively faulty compared to the UV-Vis spectrophotometry due to the yellow color of the reactant, which was derived from the food dye. Thus, the yellow solution's absorption or visible light transmission possibly interfered with the detection system. The precision and accuracy of the device are correlated to the color of the reactant solution. The pink solution shows high precision and accuracy with a lower error percentage. The errors in MDA detection using the device may be due to the color of the solution. The device could determine MDA in fried fish cracker crisps extract (S1) precisely compared to the spectrophotometer result. Fried fish cracker crisps are generally consumed instantly after preparation. Hence, the high amounts of lipids and the packaging process can result in high oxidative deterioration of the fried fish cracker. Thus, this result indicated that the developed device was suitable for evaluating MDA in fried food samples used.

Table 6. The concentration of MDA in fried food samples using a UV-Vis spectrophotometer and a smartphone-based device.

Sample	Description	The concentration of MDA from UV-Vis spectrophotometer (μmol/L)	Concentration of MDA from developed device (μmol/L)	Precision	% Accuracy	% Error
S1= fried fish cracker crisps	Fried wheat flour mixed with fish meat and seasoning (Thin piece) 	55.842 ± 2.22	63.216 ±8.30	0.96	88.334	11.666
S2= fried fish cracker sticks	Fried wheat flour mixed with fish meat and seasoning (Thick piece) 	53.214 ± 4.82	41.118 ±36.12	0.86	78.181	21.819
S3=French fries	Fried potato chips 	58.899 ± 3.23	142.843 ±22.32	0.93	56.760	43.240

Data are expressed as mean±SD from triplicate samples

4. Conclusions

The smartphone-based spectrophotometer was constructed and applied to determine MDA concentration in fried food samples compared to the standard UV-Vis spectrophotometric method. The developed method was simpler, lens-free, portable, cost-effective, and yielded high sample throughput. No power supply is needed for the infield analysis. This device can be handmade, constructed in minutes, and used to determine MDA in fried food samples ranging from 6.25 to 100 μmol/L precisely and accurately. Compared to a commercial instrument, the readout of this device revealed similar analytical features; nevertheless, the limitations are its lower sensitivity and higher LOD. This research would accommodate TBARS evaluation in other food matrices for quality control in the food industries or regular food inspection systems in the future.

5. Acknowledgements

This research was funded by the Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus. High appreciation is also rendered to the staff in the chemistry division. PSU, Pattani.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, Weeraya Treewanjutha and Tanyarath Utaipan; methodology, Weeraya Treewanjutha and Trin khawsung; software, Tanyarath Utaipan; validation, Weeraya Treewanjutha and Tanyarath Utaipan; formal analysis, Tanyarath Utaipan; investigation,Weeraya Treewanjutha and Tanyarath Utaipan; resources, Weeraya Treewanjutha, Tanyarath Utaipan and Trin khawsung; data curation, Tanyarath Utaipan; writing—original draft preparation, Weeraya Treewanjutha and Tanyarath Utaipan; writing—review and editing, Weeraya Treewanjutha and Tanyarath Utaipan; visualization, Weeraya Treewanjutha; supervision, Weeraya Treewanjutha; project administration, Faculty of Science and Technology,PSU; funding acquisition, Faculty of Science and Technology, PSU. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: This research was funded by funded by the Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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4. Conclusions

There is a contamination of *E. coli* O45 in meats at high levels. Even though O45 strains in this study are not in the EHEC/STEC group, some contain virulence factors and belong to phylogenetic group D, indicating the potential to cause illnesses. Moreover, they are resistant to numerous antimicrobial agents, and almost half of them show a multi-drug resistant phenotype and the possibility of gaining a *stx*$_2$-phage that makes them more dangerous in the future. These data are important from a public health standpoint.

5. Acknowledgements

We thank the Department of Microbiology, Division of Biological Science, Faculty of Science, Prince of Songkla University for providing the essential facilities.

Author Contributions: Conceptualization, P.S.; methodology, P.S.; software, P.R.; validation, P.S., P.R. and A.S.; formal analysis, P.S. and A.S.; investigation, A.S.; writing—original draft preparation, A.S.; writing—review and editing, P.S. and P.R.; supervision, P.S.; project administration, P.S.; funding acquisition, P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported in part by Prince of Songkla University (grant no. SCI600588S).

Conflicts of Interest: The authors declare no conflict of interest.

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