Rapid detection of adulteration of sesame oil with peanut oil and other edible oils

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Abstract

Sesame oil has culinary, medicinal, and industrial uses. It is valued for its distinct aroma, flavour, and health benefits. Due to its high demand and the higher price, the merchants intentionally adulterate low-priced, low-quality edible oil with sesame oil for economic gains without considering consumer health. The present study focused on developing rapid, cost-effective, and straightforward methods for the detection of adulteration of peanut oil with a dual emphasis on facilitating on-site detection as well as enabling laboratory in-house sample analysis. Pure sesame and peanut oil samples were prepared at the laboratory by the expeller method. Using UV-VIS Spectrophotometric analyses, two approaches were established in the first stage of the investigation. For the in-laboratory sample analysis, the absorption spectra of adulterated samples in the UV range were obtained and compared with those of pure sesame and peanut oil. A significant variation in both the highest absorbance level and the corresponding wavelength was observed once the adulteration level reached 50%, enabling detection within the adulteration range of 50% to 100%. To detect adulteration levels between 0% and 50%, the absorbance of the chromogenic complex resulting from the reaction between sesame oil and ethanolic furfural solution was utilized in developing a spectrophotometric analysis. Absorbances recorded at 520 nm for each adulteration exhibited a strong correlation with the sesame oil percentage allowing for detection within the adulteration range spanning 0% to 50%. The latter part of the study focused on establishing a simple chromogenic test for on-site sample analysis to detect total adulteration level. Hence two chromogenic tests were developed using acidic permanganate and acidic dichromate solutions by availing the higher antioxidant activity of sesame oil.

Keywords: Sesame Oil, Adulteration, UV-VIS Spectrophotometry, Chromogenic Reaction, Rapid Test

Introduction

Sesame oil is an edible oil that has been heavily used in the culinary section and most Asian cuisines and also in the Middle East, Africa, Europe, America, Canada, and the Asia Pacific region as a traditional cooking oil for centuries.[1] Consumption of sesame lignans or sesame oil has been shown to lower blood pressure in several types of hypertensive animals and humans.[2] Also, sesame oil has been known to have anti-inflammatory properties, which makes it effective in reducing atherosclerosis and the risk of cardiovascular disease.[3] Sesame oil is rich in various bioactive compounds including phytosterols, tocopherols, and lignans such as sesamin, sesamolin, and sesaminol, which are known to play an important role in the antioxidant activity of the oil.[4] The cosmetic value of sesame oil comes with its high abundance of linoleic acid. Linoleic acid is the most frequently used fatty acid in cosmetic products as it moisturizes the skin, aids in the healing process of dermatoses and sunburns, and is used to treat common chronic skin diseases.[5]

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In the market/industries, the price for pure sesame oil is much higher than other common vegetable oils, which strongly motivates unscrupulous producers to blend pure sesame oil with common, low-cost edible oils and sell it for the same price as genuine sesame oil.[6] These fraudulent practices of adulteration of sesame oil by substandard edible oils cause the violation of consumer rights.[7] Therefore, authentication of sesame oil and detection of its adulterants in both qualitative and quantitative manner are important. At present, many sophisticated methods utilizing high-end instruments have been developed to analyze adulteration and purity such as liquid chromatography (LC), gas chromatography (GC), mass spectrophotometry (MS), and nuclear magnetic resonance spectroscopy (NMR)[8]. However, there are several difficulties encountered when using these instruments due to their complexity, such as the use of high-cost solvents/chemicals, strenuous sample preparation[9], the requirement of knowledgeable analyst on instrumentation, their time-consuming nature, and so on.[10] Hence, the emphasis was placed on developing simple, rapid, more cost-effective, and straightforward procedures to distinguish pure sesame oil from adulterated samples. UV-VIS spectrophotometry is a versatile and precise method[11] that has been utilized in various aspects of food chemistry-related analyses due to the instrumentation accessibility, economy, efficiency, simplicity of procedures, and accuracy of the methods.[12] For simple detection of adulteration of sesame oil with peanut oil, pure sesame oil and pure peanut oil samples were acquired from the genuine oil expeller after monitoring the process. Upon scrutinizing the spectra of the adulterated samples alongside the two uncontaminated samples of pure sesame and peanut oil in the UV range using a dual-beam spectrophotometer, findings revealed a notable variation in both the peak absorbance intensity and corresponding wavelength, beginning at the 40% adulteration level compared to the spectrums exhibited by the pure samples. Hence an unknown adulteration percentage of 40% above could be identified just by comparing it with a pre-established compilation of spectrums of intentionally adulterated samples.

To determine the adulteration percentage between 0% and 50%, an alternate method was tested using a microplate reader. A calibration curve for the instrument can be used to determine the concentration from recorded absorbance for each sample.[13] Sesamolin and Sesamol lignans that are present in sesame oil result crimson-red colour complex upon treatment with conc. HCl and alcoholic furfural solution.[14] The wavelength at maximum absorbance of this coloured complex was reported at 520 nm in a previous study.[15] This chromogenic reaction can be used for the detection of actual sesame oil content in an adulterated sample. However, to plot a calibration curve, either high-purity commercially available standards[16] or extractions from methods like GC and HPLC are required.[17] These commercial standards are not readily available and highly expensive.[15] As a result, this study aimed to design a method to determine adulteration that was not based on commercial standards. Hence the methodology devised in this section diverges from the conventional practice of employing a calibration curve reliant on commercial standards to ascertain the concentration of the unknown analyte. Instead, it has been modified to operate independently of these standards.

In the latter part of the study, two simple chromogenic tests were developed to rapidly detect adulteration in sesame oil by treating the oil with oxidizing agents. Sesame oil has significant antioxidant action, owing to its high concentration of lignans, particularly sesamol.[18] These chemicals have significant free radical scavenging activities, which serve to reduce oxidative stress and prevent cellular damage[19] and are also capable of undergoing redox reactions in the presence of a strong oxidizing agent.[20] The monitored gradual alteration in colour, stemming from the interaction between potent oxidizing agents and the antioxidative constituents within sesame oil, occurred at a precise moment during the reaction. This observation facilitated the quantification of adulteration levels present within the sample. Peanut

oil was employed as the primary adulterant across all segments of the study.

Materials and Methods

Sample preparation

Pure sesame and peanut oil samples were prepared and collected in the laboratory using the expeller method. For this process, high-quality white sesame seeds (*Sesamum indicum*) and locally sourced bold-type peanuts (*Arachis hypogaea*) were selected. The seeds and nuts were first cleaned and dried to remove any impurities and moisture and were then fed into an expeller press, where they were mechanically pressed at a controlled temperature to extract the oil. The extracted oil was collected and filtered to remove solid particles, resulting in pure sesame and peanut oil samples. These samples served as genuine references throughout the study.

To investigate the rapid detection of adulteration, adulterated samples were synthetically prepared by mixing pure sesame oil with pure peanut oil in varying volume percentages. Each adulterated sample was thoroughly mixed to ensure homogeneity, creating a range of samples with different adulteration levels of peanut oil. These samples were then subjected to various analytical methods to evaluate the efficacy of the detection techniques employed in this study.

Wavelength Scans of adulterated samples

Synthetically adulterated sesame oil samples were prepared using peanut oil with adulteration percentages varying from 5% to 90% volume vice. A portion of the 1 mL oil sample was dissolved in 4 mL hexane and a wavelength scan was performed in the UV range using SHIMADZU UV-2600. Figure 2 illustrates how the maximum absorbance and its corresponding wavelength vary as the level of adulteration increases.

Quantification of modified Villavecchia Test

Synthetically adulterated oil samples were prepared using peanut oil as the adulterant with adulteration percentages of 0%, 10%, 20%, 30%, 40%, 50%, and 100%. A portion of 1 mL oil was dissolved in 2 mL hexane. From this solution, an aliquot of 50 µL was taken and further diluted by adding another 2 mL of hexane. To this, 1 mL conc. HCl and 200 µL volume of 2% Furfural solution in ethanol were added and quickly swirled. Right after 15 seconds, the absorbance of the bottom layer was measured using a Spectra-Max Spectrophotometer (microplate reader) at 520 nm.[15] Absorbance vs adulteration percentage was plotted in a graph using MS-Excel (2010). The linear correlation between the absorbance of the coloured complex and the percentage variation in adulteration is portrayed in Figure 3.

Chromogenic Test with potassium permanganate

Sesame oil samples adulterated with peanut oil with adulteration percentages varying from 5% to 90% were prepared. A portion of 1 mL oil was dissolved in 2 mL hexane and 1 mL of conc. HCl was added. After mixing for a few seconds, 500 µL of 0.5 M $KMnO_4$ solution was added. The test tube was swirled for 10 seconds occasionally with 10 seconds interval for 9 minutes. The colour was observed at the 9-minute mark. Figure 4 illustrates the colour changes associated with different degrees of adulteration.

Chromogenic Test with potassium dichromate

Sesame oil samples adulterated with peanut oil with adulteration percentages varying from 5% to 90% were prepared. A portion of 200 µL oil was dissolved in 2 mL hexane and 1 mL of conc. HCl was added. After mixing for a few seconds, 400 µL of 0.25 M $K_2Cr_2O_7$ solution was added. The test tube was swirled for 10 seconds occasionally with 10 seconds interval for 9 minutes. The colour was observed at the 9-minute mark. Figure 5 illustrates the colour changes associated with different degrees of adulteration.

All the quantitative data were collected in triplicate to ensure accuracy and reliability. The results were statistically analyzed using one-way ANOVA, conducted by Minitab® 18 software, to determine the significance of differences between the values.

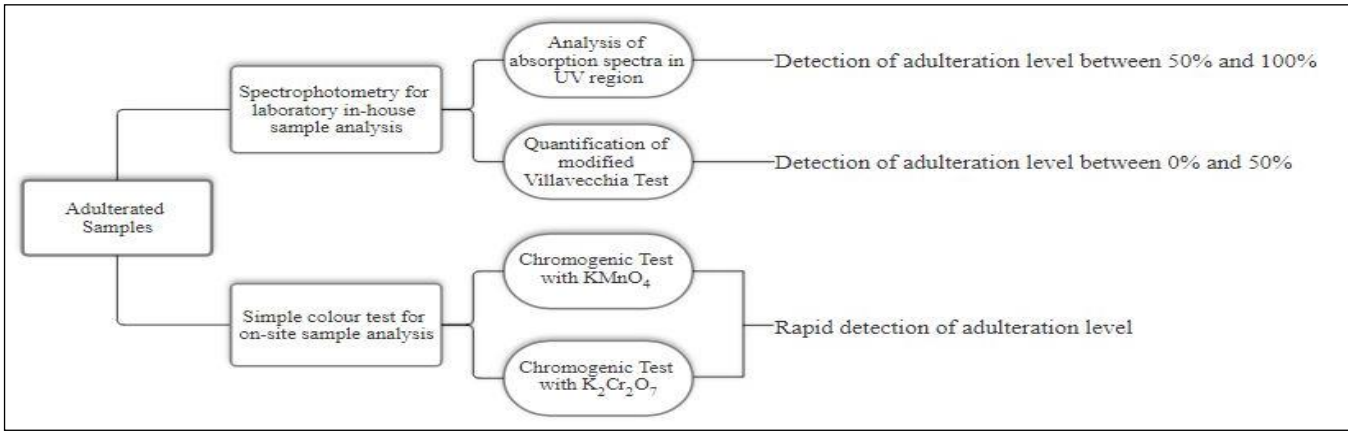

Fig.1: Concise illustration of the methodology followed

Results

Wavelength scans of adulterated samples

Following the scanning of adulterated and pure samples, their absorption spectra recorded in the UV range were obtained. Detailed analysis was conducted on each spectrum to determine characteristics such as the maximum absorbance and the corresponding wavelength. One-way ANOVA was conducted for both maximum absorbance and wavelengths separately considering adulteration percentage as the independent variable (Figure 2 and Table 1).

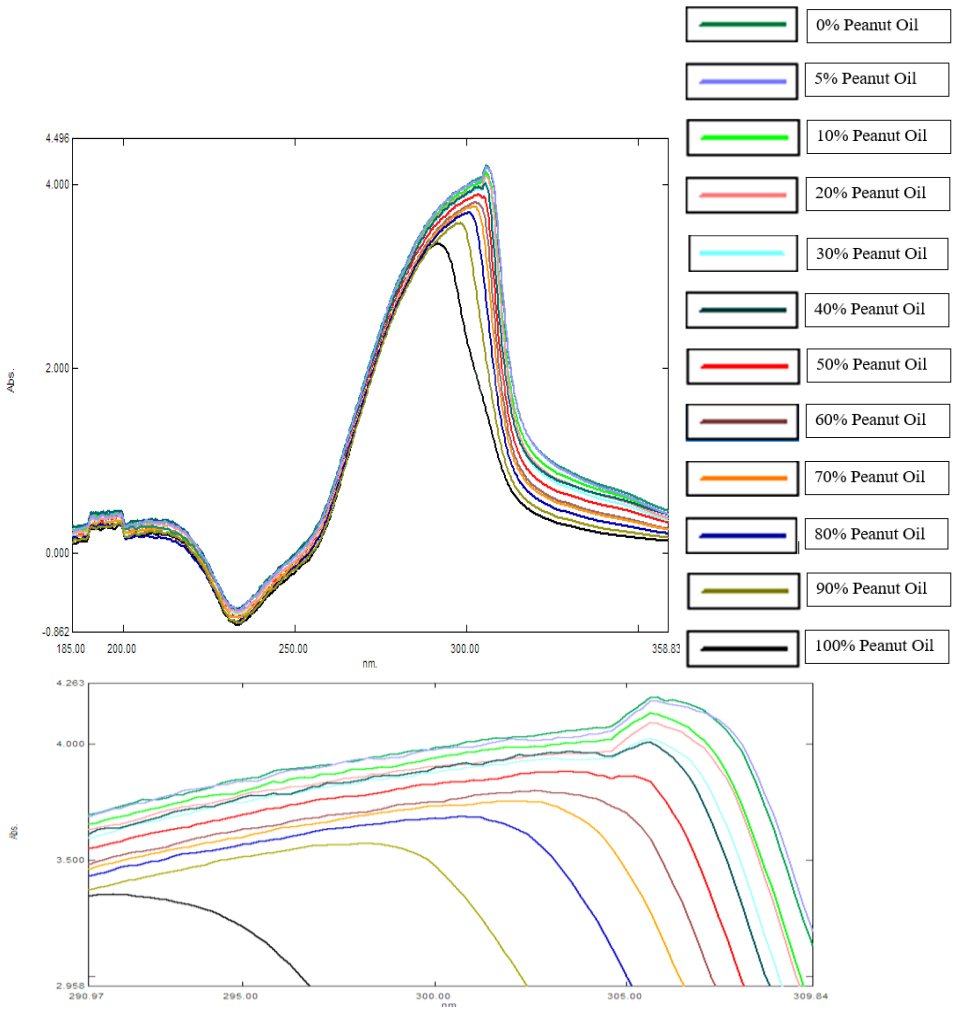

Fig. 2: Variation in wavelength and maximum absorbance with the increment of adulteration percentage

Table 1: **Maximum absorbance and corresponding wavelength recorded in UV range for each adulteration**

Adulteration %	Maximum Absorbance	Wavelength at maximum Absorbance
0 (pure sesame oil)	4.2030 ± 0.08^{A}	305.6 nm ± 0.00^{A}
5	4.1870 ± 0.10^{A}	305.6 nm ± 0.00^{A}
10	4.1360 ± 0.08^{A}	305.6 nm ± 0.00^{A}
20	4.0940 ± 0.12^{A}	305.6 nm ± 0.00^{A}
30	4.0260 ± 0.06^{A}	305.6 nm ± 0.00^{A}
40	4.0090 ± 0.05^{A}	305.6 nm ± 0.11^{A}
50	3.8830 ± 0.05^{B}	303.6 nm ± 0.11^{B}
60	3.8010 ± 0.05^{C}	302.6 nm ± 0.41^{C}
70	3.7550 ± 0.06^{D}	301.8 nm ± 0.30^{D}
80	3.6890 ± 0.04^{D}	300.8 nm ± 0.23^{E}
90	3.5730 ± 0.05^{D}	298.2 nm ± 0.52^{F}
100 (pure peanut oil)	3.3530 ± 0.04^{E}	290.2 nm ± 0.00^{G}

Note: The superscript letters (A, B, C, D, E) assigned for maximum absorbance values indicate statistically significant differences (p<0.005) between the analyzed samples (n=3). The superscript letters (A, B, C, D, E, F, G) assigned for wavelength at maximum absorbance values indicate statistically significant differences (p<0.005) between the analyzed samples (n=3).

Quantification of modified Villavecchia Test

Absorbances recorded at 520 nm for adulteration levels ranging from 0% to 50% are shown in Table 2. Based on these values a graph was plotted to analyze the linear relationship between the oil content and absorbance (Figure 3).

Table 2: Absorbance recorded for each adulteration percentage

Peanut Oil %	Sesame Oil %	Absorbance at 520 nm
0	100	0.7376 ± 0.0201^{A}
10	90	0.6466 ± 0.0550^{B}
20	80	0.5576 ± 0.0404^{C}
30	70	0.3956 ± 0.0136^{D}
40	60	0.3386 ± 0.0116^{D}
50	50	0.2056 ± 0.0185^{E}

Note: The superscript letters (A, B, C, D, E) assigned for maximum absorbances at 520 nm indicate statistically significant differences (p<0.005) between the analyzed samples (n=3).

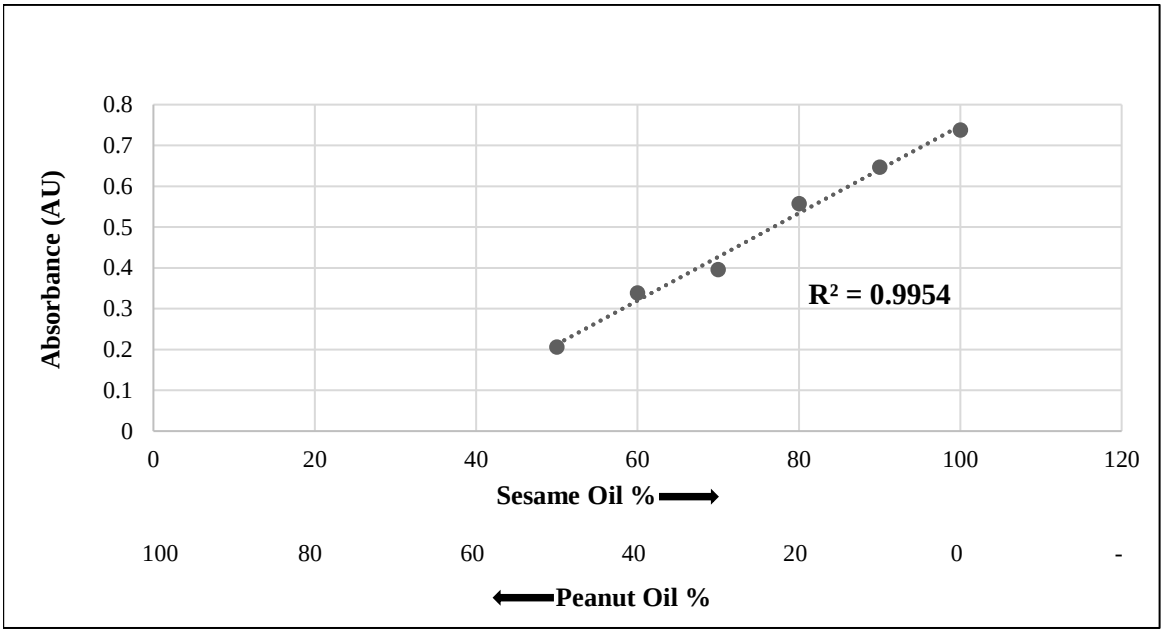

Fig. 3: Plot of the linear relationship between absorbance and sesame oil percentage

Chromogenic reaction with $KMnO_4$ solution

The observed results of the chromogenic reaction with $KMnO_4$ solution shows in Figure 4. The colour change of each adulteration is given based on sesame oil percentage.

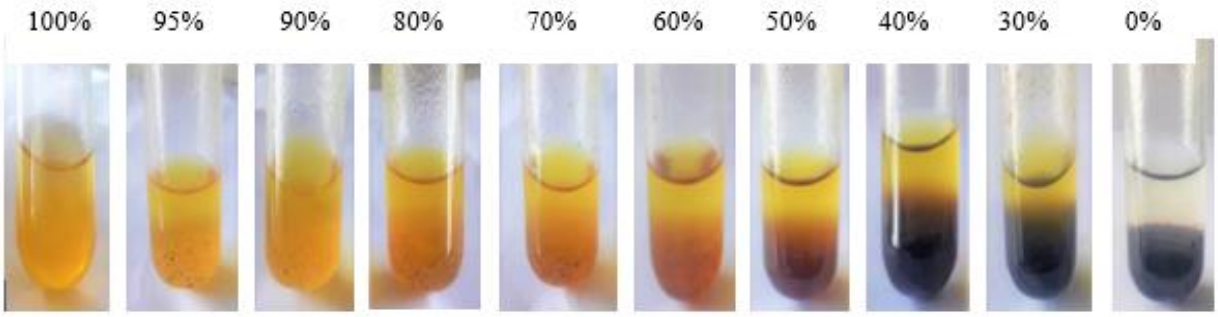

Fig.4: Colour observed for each percentage with $KMnO_4$ solution

Chromogenic Reaction with $K_2Cr_2O_7$ Solution

The observed results of the chromogenic reaction with $K_2Cr_2O_7$ shows in Figure 5**.** The colour change of each adulteration is given based on sesame oil percentage.

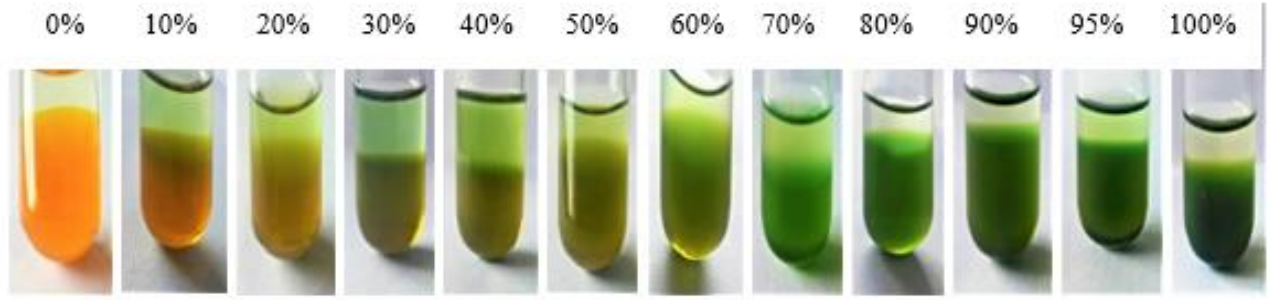

Fig. 5: Colour observed for each percentage with $K_2Cr_2O_7$ solution

Discussion

Analysis of absorption spectra

In the present study, it was attempted to develop simple, straightforward, and cost-effective methods to analyze the adulteration of sesame oil using peanut oil as the main adulterant in both a qualitative and quantitative manner. In the initial phase of the study, absorption spectra (wavelength scans) of adulterated samples in the UV range were analyzed and compared along with the pure sesame oil and peanut oil without any derivatizations to maintain the clarity of the analysis. Sesame oil reported its maximum absorbance of (4.2030±0.08) at 305.6 nm and that of pure peanut oil was (3.3530±0.04) at 290.2 nm. All the other adulterations exhibited their maximum absorbances and respective wavelengths between the two values recorded for pure oils. Both absorbances and respective wavelengths for adulterated samples were shown in decreasing order with the increment of adulteration percentage, between the respective values for pure sesame and pure peanut oil. Absorption peak between 230-300 nm indicates the presence of di-unsaturated fatty acids and tri-unsaturated fatty acids.[21] Both oils are primarily composed of unsaturated fats, but sesame oil contains a higher proportion of unsaturated fats compared to peanut oil.[22] Sesame oil is rich in both monounsaturated and polyunsaturated fatty acids, while peanut oil contains more monounsaturated fats than polyunsaturated fats.[22] This clarifies the absorbance variation occurs around 300 nm. Figure 2 depicts the propagation of the maximum absorbance and wavelength of each sample. Even though for the lowest adulteration, there is an apparent change in absorbance from the pure value, a high standard deviation was recorded. Statistical analysis also indicates no significant difference. The condition remained consistent up to the 40% adulteration. The split between the respective peak and the adjacent peak began to widen at a level of 40% adulteration and the wavelength at maximum absorbance of each adulterated sample started to decrease as well. To identify the adulteration solely through comparing wavelength scans in an edible oil sample as demonstrated in this section of the study, the sample must exhibit distinctive peaks and corresponding absorbances for each adulterant present apart from the main analyte of interest. The greater the discrepancies between the peaks and absorbances detected in each sample of pure sesame oil and pure peanut oil, the more precise the outcomes are likely to be. In this scenario, sesame oil and peanut oil exhibited their major peaks and corresponding wavelengths in close proximity, with no additional peaks besides their primary ones. The spectrum obtained from the adulterated samples comprised an overlap of the major peaks of both pure oils and up to 40% adulteration, values were recorded with a significant standard deviation. Statistical analysis also depicts that there is no significant difference in both maximum absorbances and their corresponding wavelengths recorded for 0% to 40% adulteration levels. Therefore, only an adulteration above 40% could be discerned employing this approach. Another constraint arises when significant levels of other adulterants, such as cheaper oils like palm oil and coconut oil, are present. In such instances, the resulting peak may deviate from its typical shape, posing challenges in accurately identifying adulteration caused by peanut oil. In such scenarios, alternative methods like GC-MS or HPLC must be employed. Thus, it is imperative to ascertain the presence of major adulterants, aside from peanut oil, by scrutinizing the obtained spectra before proceeding with the analysis.

Quantification of modified Villavecchia Test

In the lack of a sesamol standard, an attempt was made to develop a spectrophotometric analytical method in the quantification of the modified Villavecchia test[15]. The absorbance given by the coloured complex between sesamol lignans and ethanolic furfural solution of each adulterated sample was measured at 520 nm and these absorbances showed a strong correlation (R^2=0.9954) with the sesame oil percentage. (Shown in Figure 3) Samples were prepared in a way that the highest and lowest absorbance falls into the linear

range of the Beer-Lambert law.[23] A drawback observed in this part of the study was peanut oil also gave a faint pink colour with furfural. Furfural is capable of reacting with phenolic compounds and hence producing a chromogenic product.[15] Resveratrol is a phenolic compound found naturally in peanuts. During the oil extraction and processing a small amount of resveratrol can get passed into the oil which gives a faint positive colour.[24] If the primary adulterant is any oil other than peanut oil, it's crucial to recognize that necessary modifications to the methodology are necessary to accurately determine the amount of sesame oil present in the sample. The authors attempted to utilize the standard approach of UV-VIS spectrophotometry by establishing a calibration curve to determine the actual sesame oil content in a sample by using pure sesame oil as the standard. The dilution processes were carried out using hexane and it was observed that there was a volume contraction in the oil samples dissolved in hexane complicating its accurate inclusion into the current investigation.

Chromogenic Tests

During the final stretch of the study, two new chromogenic tests were developed using $KMnO_4$ and $K_2Cr_2O_7$ solutions for the rapid identification of actual sesame oil content in an unknown sample. (Shown in Figure 4 and Figure 5 respectively) In the first test, 0.5 M $KMnO_4$ solution was used. In the absence of sesame oil, the dark brown colour imparted by unreacted permanganate was readily observable. As the sesame oil percentage increased, the dark brown colour gradually decreased and for the pure sesame oil, a clear golden yellow colour was evident. There were no distinguishable differences observed between 30%, 20%, and 10% sesame oil percentages. In the second test, 0.25 M $K_2Cr_2O_7$ solution was used. For the 100% adulteration which was pure peanut oil, dichromate colour could be seen resulting from unreacted dichromate. As the sesame oil percentage increased, the orange colour of the dichromate gradually decreased and the green colour imparted by Cr(III) resulting from the reduction of dichromate gradually appeared. For pure sesame oil, an intense green colour was observed. There was no apparent change in colour within samples containing 30%, 40%, 50%, and 60% sesame oil. The perception of colour can be elucidated through the antioxidant characteristics of oils. Sesame oil is rich in antioxidants[25] which are capable of undergoing oxidation upon reacting with strong oxidizing agents.[20] It was observed that the initial colour observed for peanut oil started to change after one day in both chromogenic tests. The FRAP value, which quantifies the antioxidant capacity of a substance, is higher for sesame oil.[26] It is approximately eight times higher than that of peanut oil.[27] This indicates the higher feasibility of sesame oil reacting with strong oxidizing agents like $KMnO_4$ and $K_2Cr_2O_7$ than peanut oil. Both of these tests did not yield a positive result with other edible oils such as cottonseed oil, palm oil, coconut oil, cow ghee, and vegetable ghee within observation time, therefore can be used to determine sesame oil content in samples blended with the above-mentioned oils.

Conclusion

The comparison of absorption spectra of adulterated sesame oil samples with peanut oil obtained in the UV range can be used to detect 40%, 50%, 60%, 70%, 80%, and 90% adulteration within a range with the minimum being 40% after meticulously analyzing both maximum absorbances and their corresponding wavelengths. It is needed to aid more sophisticated methods and high-end instruments to detect the exact adulteration level. From the quantification of the Villavecchia test, sesame oil content between 50% and 100% can be determined, hence the total adulteration percentage between 0% and 50% can be assessed. Both chromogenic tests developed in this study showed a positive result in determining the sesame oil content in an edible oil sample if the adulterating oil mixed was one out of Cottonseed oil, Palm oil, Coconut oil, Cow ghee, and Vegetable ghee. An initial aim of the study was to devise a chromogenic test tailored to exclusively detect the presence of peanut oil in any unknown

sample which could not achieved with the present study. Therefore, the authors suggest the development of a simple chromogenic test specifically designed for the detection of peanut oil in sesame oil which is not yet recorded.

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