

1 **TITLE:** Detection of adulteration in edible oil using FT-IR spectroscopy and machine learning
2
3

4 **ABSTRACT**

5 **Aims:** To detect the adulterant in edible oil rapidly

6 **Study Design:** Authenticity and adulteration detection in edible oils are the increasing
7 challenges for researchers, consumers, industries and regulatory agencies. Traditional
8 approaches may not be the most effective option to combat against adulteration in edible oils as
9 that's are complex, laborious, expensive, require a high degree of technical knowledge when
10 interpreting data and produce hazardous chemical. Consequently, a cost effective, rapid and
11 reliable method is required.

12 **Place and Duration of the Study:** The experiment was conducted jointly in the laboratory of
13 the Department of Food Technology and Rural Industries, Bangladesh Agricultural University,
14 Mymensingh and the Institute of Food Science and Technology, BCSIR, Dhaka.

15 **Method:** In this study, Fourier Transform Infrared spectroscopy coupled with multivariate
16 analysis was used for adulteration detection in sunflower and rice bran oil. Sunflower oil was
17 adulterated with soybean oil in the range of 10-50% (v/v) and rice bran oil was adulterated with
18 palm oil in the range of 4-40% (v/v) at approximately 10% and 5% increments respectively.
19 FTIR spectra were recorded in the wavenumber range of 4000-650cm⁻¹.

20 **Results:** FTIR spectra data in the whole spectral range and reduced spectral range were used
21 to develop a partial least square regression (PLSR) model to predict the level of adulteration in
22 sunflower and palm oils. Good prediction model was obtained for all PLSR models with a
23 coefficient of determination (R²) of >= 0.985 and root mean square errors of calibration
24 (RMSEC) in the range of 0-1.7325%.

25 **Conclusion:** The result suggested that FTIR spectroscopy associated with multivariate analysis
26 has the great potential for a rapid and non-destructive detection of adulteration in edible oils
27 laborious conventional analytical techniques.
28

29 **Keywords:** FT-IR, Spectroscopy, Adulteration, Edible oil, Authenticity
30
31

32 **1. INTRODUCTION**

33 History across the world reminds that our food is under continuous threat from adulteration.
34 It has existed if food has been made and sold. Since prehistoric times humans have altered the state of
35 food to extend its longevity or improve its taste. However, the eighteenth and nineteenth centuries saw an
36 explosion of food adulteration when foodstuffs were often adulterated with inedible and even
37 poisonous/toxic substances, as all stakeholders' (i.e. farmers, suppliers, and grocers) tried to maximize
38 their profits [1] and now a global issue addressed by several researchers, involving economic, quality,
39 safety, ethical and socio-religious issues [2]. Major food adulteration events appear to regularly occur, for
40 instance adulteration of spices with Sudan Red dye in 2003, milk powder with melamine in 2008, dioxins
41 in pork in 2008, milk with detergent, fat, and even urea in 2012 and processed beef products with
42 horsemeat in 2013 [3-5]. In some cases, there might be no issue related to safety (i.e. horsemeat
43 scandal), however, such adulteration is always a concern with individuals allergic to species, or those with
44 religious taboos or ethical aversions [5, 6]. Adulteration varies widely among the thousands of food
45 products, range from tragic, as in the toxic oil syndrome or simply toxic syndrome disaster in Spain in
46 1981 where thousands were hospitalized and an approximately 600 people died due to rapeseed oil
47 intended for industrial use being sold as olive oil [7, 8] to authentication of the species [9, 10], variety [11,
48 12], purity [13-15] and geographical origin of a product [6, 16-18]. Although the extent and dangers of
49 food adulteration have received huge public attention, the prevalence of fraud is not easy to assess [19].
50 Basically, detecting adulteration is difficult without resorting to highly sophisticated analytical techniques
51 because the adulterant components are usually very similar to the authentic product [2, 20]. On the other
52 side, fraudsters are always one step ahead of the regulatory agencies and their techniques are
increasingly becoming more and more sophisticated with time. Once a specific test has been developed

53 by the scientists to identify an adulterant, fraudsters can become aware of this and then add or remove
54 that component from the adulterated foodstuff [3]. Many methods are available for the detection of
55 qualitative and/ or quantitative adulteration in food. Currently the methods often used for food
56 authentication and detection of fraud include polymerase chain reaction (PCR), chromatography,
57 electrophoretic separation of proteins, immunological procedure and DNA based techniques, and
58 enzymatic assays [19], all of which are well documented. However, these techniques are invasive, time
59 consuming, laborious, demand highly skilled personnel, and thus they are not suitable for online
60 application and routine analysis. Consequently, a cost effective, efficient, rapid, and reliable method is
61 required. There is a great interest in developing non-destructive optical technologies that have the
62 capability of monitoring in real-time assessment.

63 Among optical sensing technologies, FT-IR spectroscopy is more sensitive and perhaps more suited to
64 detect and quantify the presence of adulterants within complex food matrices. The high spectral signal-to-
65 noise ratio obtained from FT-IR analysis allows the detection of constituents present in very low
66 concentrations as well as subtle compositional differences between and among multi constituent
67 specimens. Basically, the IR spectrum is formed because of the absorption of electromagnetic radiation at
68 characteristic bands/wavenumbers that correlate to the vibration of specific sets of chemical
69 bonds/functional groups from within a molecule. Different functional groups absorb characteristic
70 frequencies of IR radiation. Analysis of these absorption characteristics reveals details about the
71 molecular structure of the sample. However, this is complex task due to the overlap of frequencies
72 characteristic and also because of overtone and combinations bands [8]. There are several alternatives
73 for quantifying a compound/functional group in a multicomponent mixture based on IR spectra, ranging
74 from the univariate calibration method, which correlates the intensity (i.e. absorbance) of an isolated,
75 intense band in the spectrum to the concentration of the compound/functional group, through the peak
76 fitting, which enables quantification of the compound/functional group from the absorption by scaling fixed
77 peak shapes to the spectrum, or fitting parameterized line shapes (such as Gaussian) to particular
78 regions of the spectrum, up to the more sophisticated multivariate approach in which nearly entire spectra
79 are utilized by carrying out correlation between spectral data and the concentrations or other measurable
80 properties obtained from the ordinary laboratory measurements. Univariate analysis or peak fittings can
81 become complex when absorbance peaks overlap [21, 22] or if there are multiple absorption peaks for
82 the same functional group. On the other side, multivariate calibration approach is especially useful when
83 data are highly collinear [23] as in the case for FT-IR spectra. Several chemometric
84 algorithms/multivariate analyses are available to appropriately extract meaningful information in an
85 efficient way from the spectra. By applying chemometric tools, one can perform reliable quantitative
86 analysis of a multicomponent system even in the case of very complex spectra. In its ample applications,
87 FT-IR spectroscopic technique in tandem with multivariate analysis has proved its potential for detecting
88 adulteration and authenticity in edible oils.

89 Edible oils are routinely used as cooking oils, salad oils, shortenings, spreads and ingredients in several
90 food products formulation and a large variety of edible oils treated and marketed in Bangladesh. Some
91 edible oils are expensive compared to others as tempting to adulterate with the lower price edible oils.
92 Adulteration of high value edible oils can occur either by mislabeling of less expensive oil or by adding
93 less expensive oils to increase the volume and therefore profits. Different physical parameters such as
94 refractive index, viscosity, melting point, saponification and iodine value can be measured to detect
95 adulteration in edible oils. However, these parameters are not anymore practical to detect adulteration as
96 these properties are easy to manage in adulterated oils to mask the adulteration. On the other side, it is
97 also possible to use both major (triacylglycerols) and minor components (sterols, carotenoids,
98 tocopherols, chlorophylls etc.) as detection tool. Among different edible oils, some have particular
99 component at a known level which is absent in other one. Therefore, the presence and amounts of this
100 particular component can be considered as a detection tool [9]. Many analytical techniques can be used
101 to detect adulteration in edible oils. Most of these techniques are based upon the chromatographic
102 methods, which rely on the determination of fatty acids [24]. However, these methods are time
103 consuming, complex, laborious, expensive and destructive, require a skilled operator and produce
104 hazardous chemical waste. In this study, an attempt has been made to develop a rapid and accurate
105 analytical protocol based on FT-IR spectroscopy for the determination of adulteration (palm oil in rice bran
106 oil and soybean oil in sunflower oil) in edible oils and apply machine learning technique such as PLSR to
107 develop calibration model for detecting adulteration in edible oils.

108

109 2. MATERIALS AND METHODS

110

111 2.1 MATERIALS

112 Four different edible oils such as sunflower oil, soybean oil, rice bran oil and palm oil were purchased
113 from the local super market.

114

115 2.2 PREPARATION OF ADULTERATED SAMPLES

116 The first study was carried out for detection of palm oil in rice bran oil (palm oil is cheaper than rice bran
117 oil) and the second study was carried out for detection of soybean oil in sunflower oil (sunflower oil is
118 expensive compared to soybean oil). For the first study, rice bran oil was adulterated with palm oil in the
119 range of 5-40% (v/v) at approximately 5% increments. For the second study, sunflower oil was
120 adulterated with soybean oil in the range of 10-50% (v/v) at approximately 10% increments. A total of 8
121 samples were prepared for palm oil adulteration in rice bran oil and only 5 samples were prepared for
122 soybean oil adulteration in sunflower oil. Additionally, a spectrum of each pure oil was also extracted to
123 compare the IR spectra of different oils.

124

125 2.3 FTIR SPECTRA ACQUISITION

126 Fourier transform infrared spectroscopy (FT-IR) was performed using a Perkin Elmer Universal ATR
127 spectrophotometer (UATR-FT-IR, USA) equipped with a Zn Se crystal for the FT-IR spectroscopy.
128 Transmittance was measured as the function of the wave number between 4000 and 650 cm^{-1} with their
129 solution of 4 cm^{-1} and the number of scans equal to 12.

130

131 2.4 SPECTRA PRE-PROCESSING

132 In spectral instruments, sample physical properties and discrepancies in instrument response can cause
133 undesired effects such as light scattering and random noise. These effects can induce spectral variations
134 that are not associated with the studied responses and affect the reliability of multivariate calibration
135 models. These effects can be eliminated from the spectral data by applying some mathematical
136 treatments. However, there is still no single recipe available to select the best pre-treatment technique
137 that needs to be applied. In this study, various pre-processing techniques such as first derivative, second
138 derivative, multiplicative scatter correction (MSC), and standard normal variate (SNV) were separately
139 applied to the spectral data prior to the development of multivariate model.

140

141 2.5 ANALYSIS OF SPECTRAL DATA

142 Partial least squares regression (PLSR) was developed to correlate the FT-IR spectra of different oil
143 samples and their corresponding level of adulteration. It was developed to calibrate the FT-IR spectra of
144 laboratory standards to their corresponding moles of functional group. A detailed description of the PLS
145 can be found in our previous studies [25-27]. In recent years, PLSR has become the *de facto* standard in
146 multivariate spectral analysis in different fields [28-31]. PLSR is emerging as the most robust and reliable
147 chemometric method for constructing multivariate models when the measured variables are many and
148 highly collinear (correlated) as in the case of FTIR spectra [32]. PLS regression is used to find the
149 fundamental relations between the predictors (X) and the responses (y), thus reducing the original
150 predictors to a new variable set called latent variables (LVs), which have the best predictive power. These
151 LVs are statistically independent i.e. uncorrelated and ideally carry all relevant information to be
152 correlated with reference values (i.e., true measured values) leading to more stable predictions [23, 33].
153 Generally, the PLS regression builds a linear model by decomposing both X (n, m) and y ($n, 1$) and
154 constructs the following relations [34]:

$$155 X = TP^T + E \quad (1)$$

$$156 y = Tq^T + f \quad (2)$$

157 where, P (m, k) is the matrix of X-loadings, T(n, k) is the matrix of X-scores, q ($k, 1$) is the loading vector
158 of y, E (n, m) and f ($n, 1$) are error terms which are not explained by the model, and k is the number of
159 LVs used in the PLS model.

160 The X-scores are predictors of y and model X, i.e., both y and X are assumed to be, at least partly,
161 modeled by the same LVs. The scores can be computed by a linear combination of the variables in X with
162 the weights \hat{W} (m, k) as $T=X\hat{W}$. These weights are computed so that each of them maximizes the
163 covariance between responses and the corresponding scores.

164 The regression coefficients b ($m, 1$) of y on X can then be calculated through the weights W^* as $b=W^*q^T$,
165 where $W^* = W(P^T W)^{-1}$.

166 Finally, the PLS latent variable model can be re-organized as a simple prediction equation similarly as for
167 multiple linear regression:

$$168 \hat{y} = Xb + f \quad (3)$$

169 To avoid either over- or under-fit problem of the model, cross validation using leave-one-out method was
170 used during the calibration step to select the optimum number of LVs for PLSR model. It was determined
171 at the minimum value of the root mean square error of cross-validation (RMSECV). To estimate the actual
172 predictive capability of the calibration model, the performance of the developed model was validated
173 using an independent test set with samples not included in the original calibration samples.

174

175 2.5 EVALUATION OF THE CALIBRATION MODELS

176 The predictive capabilities of the calibration model were evaluated by examining the coefficient of
177 determination (R^2), and the root mean square errors (RMSE). The R^2 and RMSE are defined as follows:

$$178 R^2 = 1 - \frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{\sum_{i=1}^N (\hat{y}_i - \bar{y}_i)^2} \quad (4)$$

$$179 RMSEC \text{ or } RMSECV = \sqrt{\frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{N}} \quad (5)$$

180 where \hat{y}_i = predicted value of the i^{th} sample, y_i = measured value of the i^{th} sample, N = number of
181 samples, N_c = number of samples in the calibration set and N_p = number of samples in the validation
182 (testing) set.

183 Generally, the accuracy of multivariate calibration model is considered as excellent when the R^2 is 0.90 or
184 higher [35, 36]. However, it is always expected to obtain R^2 as close as 1 with errors as close as 0.

185

186 2.6 SOFTWARE

187 All spectral transformations were carried out in Unscrambler (CAMO AS, Trondheim, Norway). The PLSR
188 analysis was carried out in MATLAB.

189

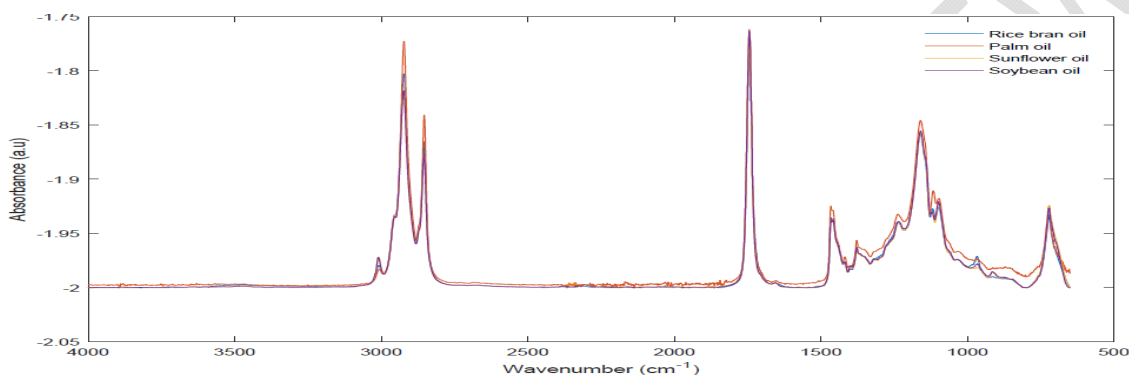
190

191 3. RESULTS AND DISCUSSION

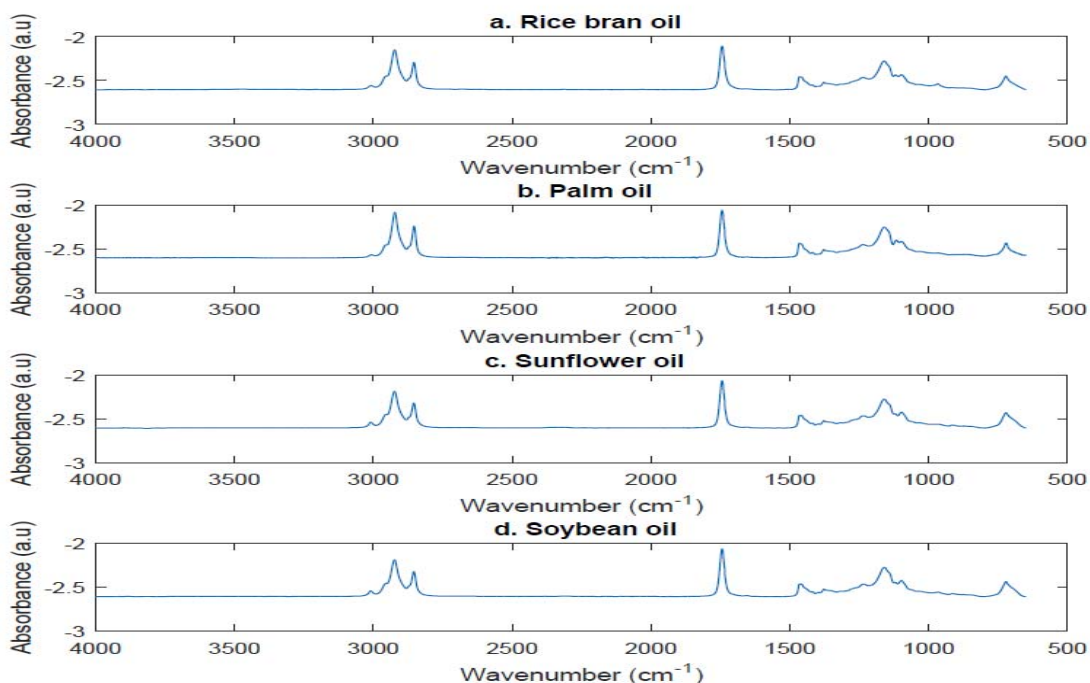
192 3.1 FT-IR SPECTRA OF THE DIFFERENT PURE AND ADULTERATED EDIBLE OILS

193 FT-IR spectra of four different pure oils (rice bran, palm, sunflower and soybean) in the spectral range of
194 4000-650 cm^{-1} are shown in Fig 1 and individual spectra shown in Fig 2. The spectra of the tested
195 samples of different oils showed similar trends throughout the whole spectral range. Despite the similarity,
196 the studied original spectra were different in absorbance values. Although, the difference in the spectral
197 profile is not clear by naked eye in the whole spectral range, it is clearly seen if the spectra are zoomed at
198 some selected spectral range Fig 3. In general, objects present similar spectral patterns indicate their
199 similarity in chemical composition. However, different concentrations of the major chemical compositions
200 in the tested object make the difference in absorbance values. It is seen that the differences between
201 different oils is small and the spectra in the FTIR region have well-resolved bands that can be assigned to
202 different functional groups present in the oils. Basically, the IR spectrum is formed as a consequence of
203 the absorption of electromagnetic radiation at characteristics bands/wavenumbers that correlate to the
204 vibration of specific sets of chemical bonds/functional groups from within a molecule. Different functional
205 groups absorb characteristic frequencies of IR radiation. Analysis of these absorption characteristics
206 reveals details about the molecular structure of the sample [37]. Chemically, fats and oils are glycerol
207 esterified with fatty acids. Some of the fats and oils might have quite similar composition; consequently, it
208 is often difficult to detect adulteration of fats and oils physically [13, 38]. However, because of its

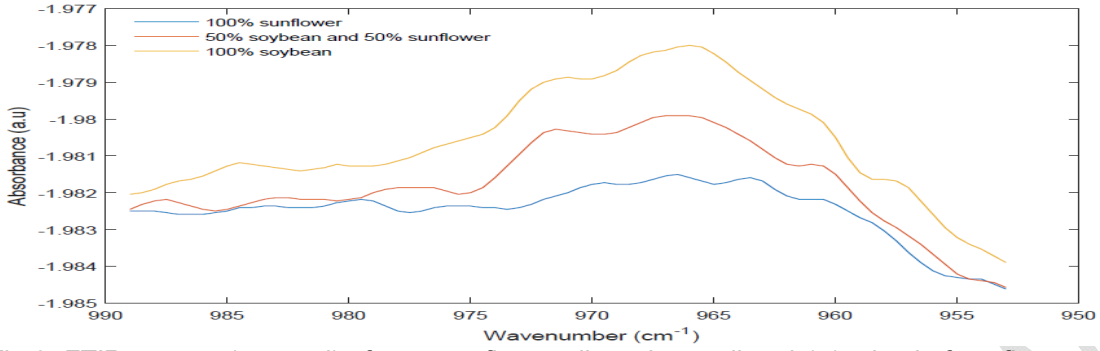
209 capability as a fingerprint technique, IR spectroscopy allows one to differentiate authentic oils and those
 210 adulterated with others by observing the spectra changes due to the adulteration [7, 39].
 211 The assignment of functional groups, shown in Fig 1, are dominated by some peaks at 3013, 2924, 2855,
 212 1745, 1650, 1462, 1416, 1376, 1242, 1160, 1114, 1099, 1033, 968 and 723 cm^{-1} . The observed
 213 absorption bands were consistent with reported peak assignments based on published literature [40].
 214 Absorbance between 3008 and 2852 cm^{-1} are due to bands arising from CH_2 stretching vibrations,
 215 asymmetric and symmetric, respectively. The major peak at 1745 cm^{-1} arises from $\text{C}=\text{O}$ stretching
 216 vibrations of aldehydes and ketons. The stretching $\text{C}=\text{C}$ was observed at 1650 cm^{-1} . The bands at 1462,
 217 1416 and 1376 cm^{-1} arise from CH_2 and CH_3 scissoring vibration of ethers, while those at 1242, 1160,
 218 1114 1099 and 1033 cm^{-1} are associated with the $\text{C}=\text{O}$ stretching vibration. The $\text{C}=\text{C}$ component in oil
 219 samples was observed at 968 cm^{-1} . A small peak at 723 cm^{-1} corresponds to CH_2 rocking mode. These
 220 observations are in agreement with the results of other studies performed with oils [41, 42], which have a
 221 composition and spectra very similar to the ones obtained in this work.
 222



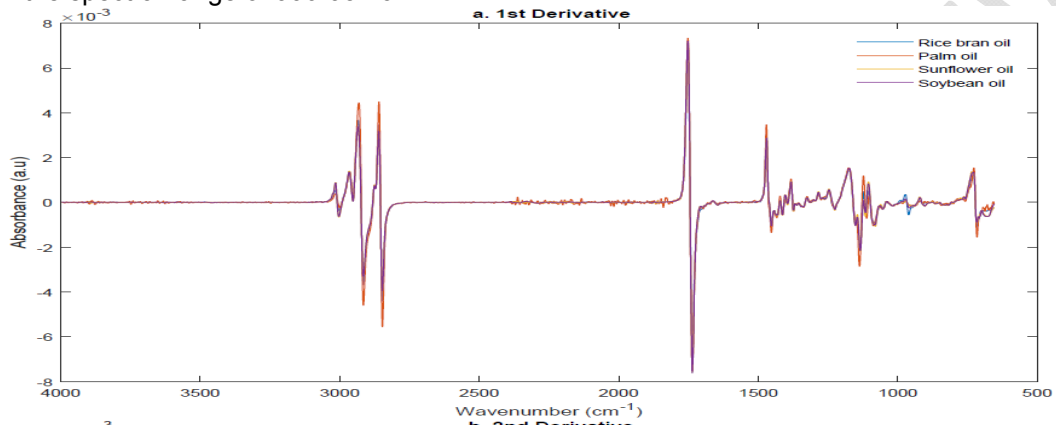
223
 224 Fig1. FTIR spectra of rice bran, palm, sunflower, and soybean oils in the spectral range of 4000-650 cm^{-1} .



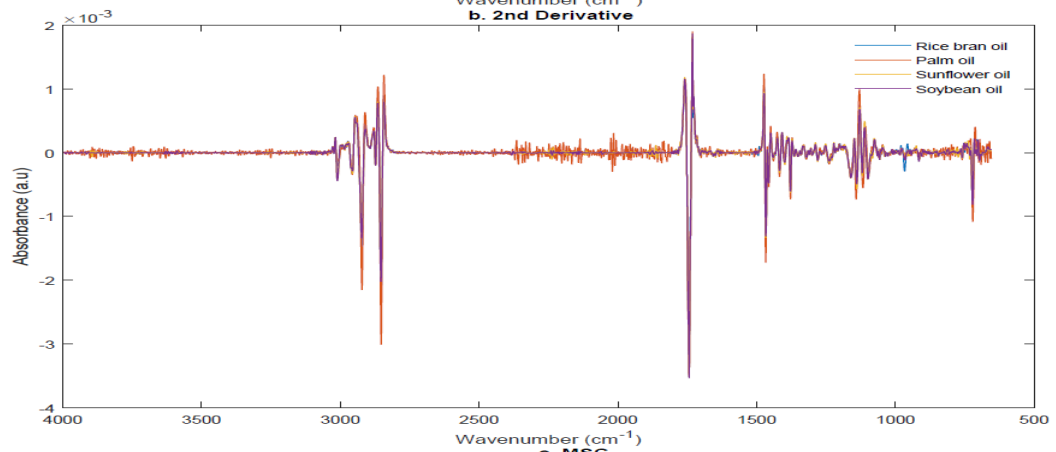
225
 226 Fig 2: Spectra of rice bran, palm, soybean and sunflower oil in the spectral range of 4000-650 cm^{-1} .
 227



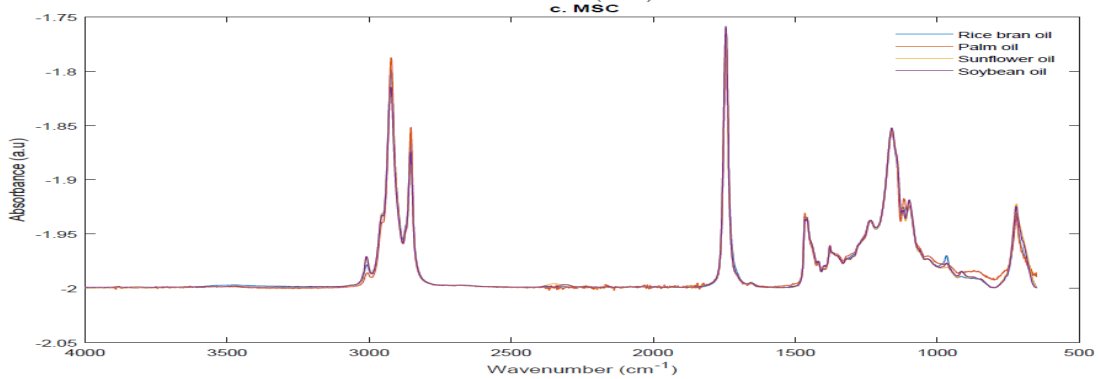
228
229 Fig 3: FTIR spectra (zoomed) of pure sunflower oil, soybean oil and 1:1 mixed of sunflower and soybean
230 in the spectral range of 990-954 cm⁻¹.



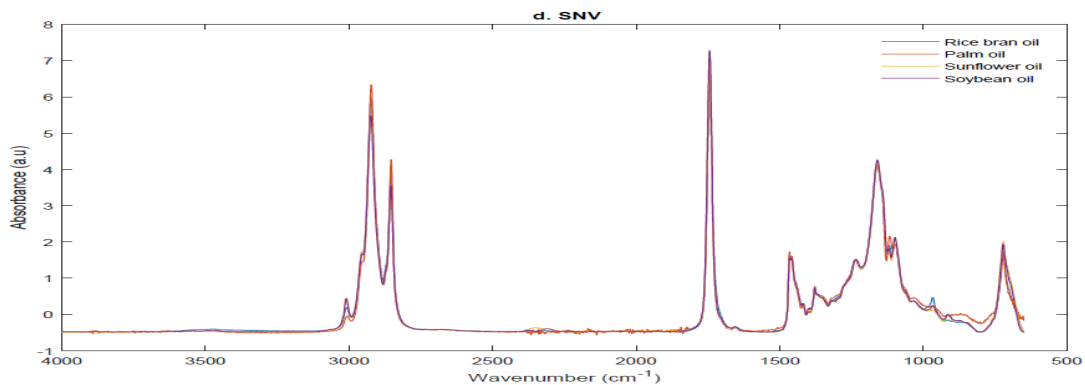
231



232



233

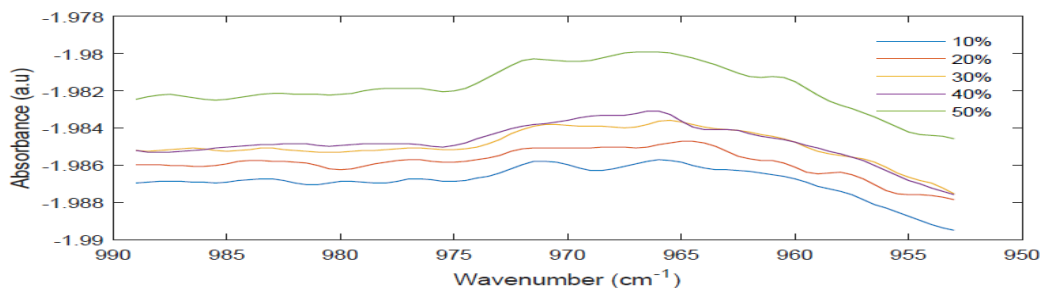
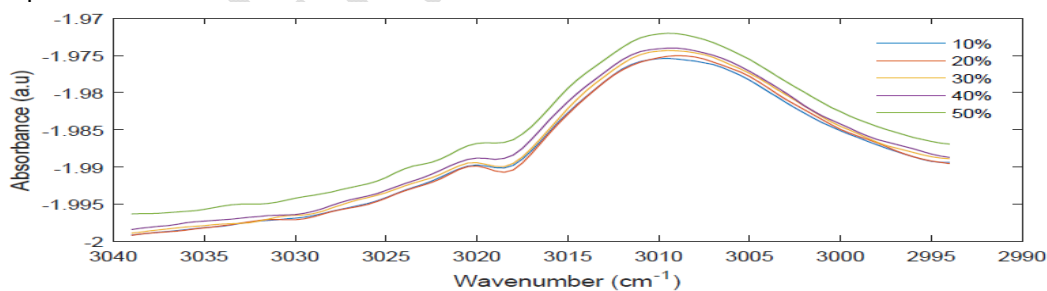


234
235 Figure 4: Spectral features with various pre-treatment procedures in the spectral range of 4000-650 cm^{-1} :
236 (a) first derivative, (b) second derivative, (c) MSC and (d) SNV

237 To correct the scatter effect, different spectral pre-treatment techniques such as derivatives (both first and
238 second derivative) MSC and SNV were applied and the resulting spectra are shown in Fig 4. It is
239 apparent that all the pre-treatments effectively suppressed the scatter effect. SNV and MSC worked
240 similarly in data preprocessing and provided equivalent results as shown in Fig 4 (c, d) and this agreed
241 well with some previous investigations [43, 44]. As expected, several new absorption spectral bands are
242 apparent in the derivative spectra as illustrated in Fig 4 (a,b); those were difficult to understand in the
243 original reflectance spectra as shown in Fig 1.

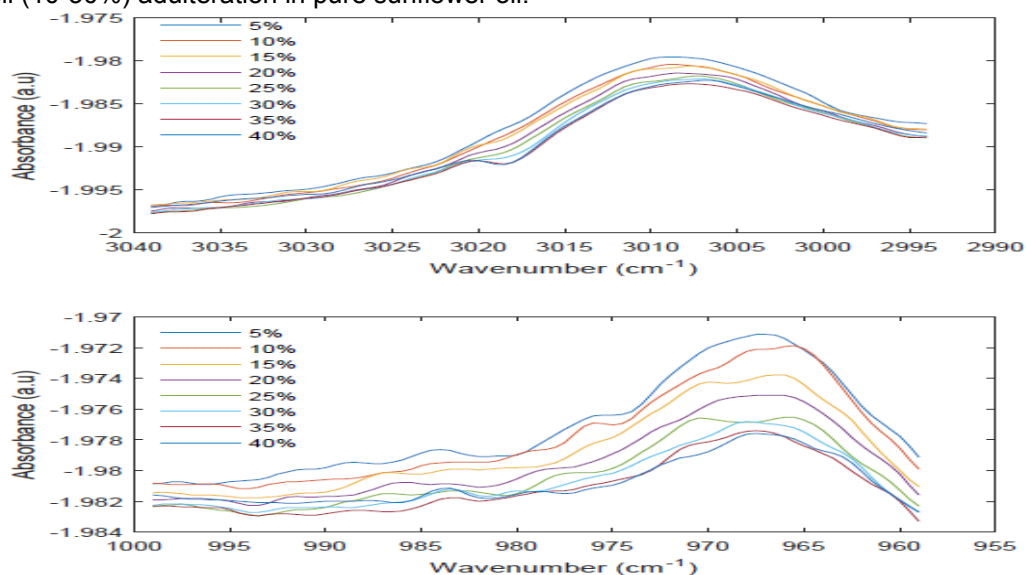
244
245 **3.2 SELECTION OF SPECTRAL RANGE**

246 It is not always possible to distinguish infrared spectra of adulterated samples from pure samples with
247 visual inspection. Therefore, it is necessary to analyze the data by multivariate methods to develop
248 predictive models and to achieve an accurate study due to the fact that some regions could be statistically
249 different. Similar changes in the absorbance on some regions are proportional to the degree of
250 adulteration. In this study, the most sensitive region from whole spectra was selected for multivariate
251 analysis based on visual inspection [40]. The region 3040-2995 cm^{-1} and 1000-960 cm^{-1} were selected for
252 detecting palm oil adulteration in rice bran oil, while the regions 3040-2995 cm^{-1} and 990-954 cm^{-1} were
253 selected for detecting soybean oil adulteration in sunflower oil. The FTIR spectra depicted Fig 5 and 6
254 revealed the differences in absorbance in these range due to the change in concentration of functional
255 groups for the addition of adulterants (i.e. palm oil in rice bran and soybean oil in sunflower) in different
256 level. These spectral ranges, in addition of whole spectral range, were used to develop multivariate
257 calibration for detecting adulteration in edible oil. Therefore, a total of six calibration models was
258 developed for adulteration detection in rice bran oil and sunflower oil.



259

260 Fig 5: FTIR spectra in the spectral range of 3040-2995 cm^{-1} (top) and 990-954 cm^{-1} (bottom) for soybean
 261 oil (10-50%) adulteration in pure sunflower oil.



262 Fig 6: FT-IR spectra in the spectral range of 3040-2995 cm^{-1} (top) and 1000-960 cm^{-1} (bottom) for palm oil
 263 adulteration (5-40%) in pure rice bran oil.
 264
 265

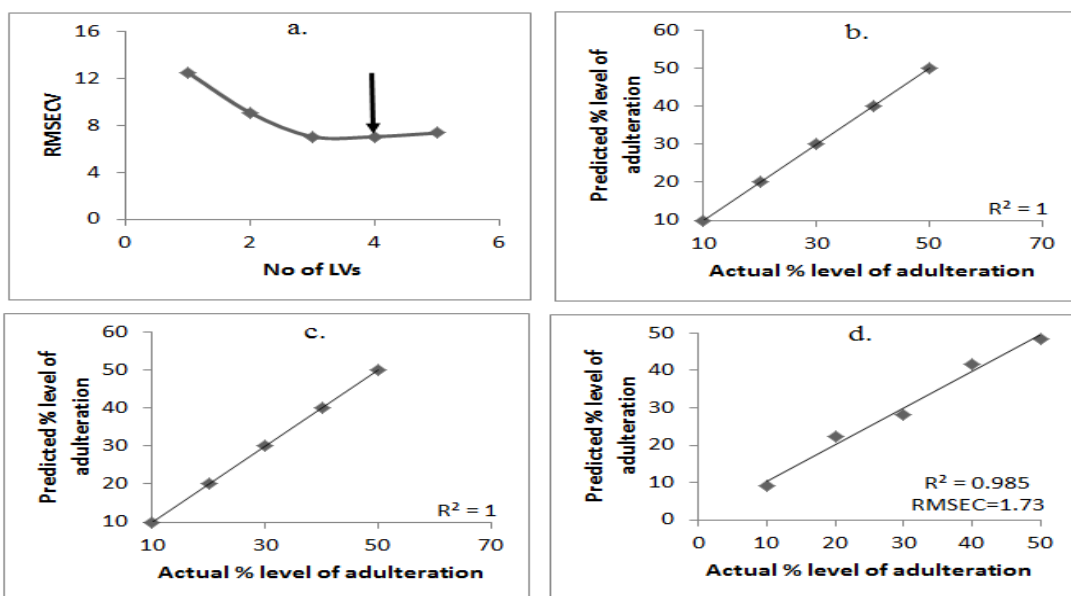
266 3.3 DEVELOPMENT OF CALIBRATION MODEL BASED ON FT-IR SPECTRA

267 Application of IR spectroscopy combined with chemometric methods is a relatively new approach to
 268 determine authenticity and adulteration detection in edible oil industry. Use of chemometric technique as
 269 an analytical procedure is fast and very cheap, not very accurate but enough accurate for many actual
 270 problems. In this study, a chemometric algorithm called partial least squares regression (PLSR) was
 271 used. Three different PLSR models were developed using full spectra of 4000-650 cm^{-1} and reduced
 272 spectra of 3040-2995 cm^{-1} and 1000-960 cm^{-1} for palm oil adulteration in rice bran oil. On the other hand,
 273 another three models were developed in the spectral range of 4000-650 cm^{-1} , 3040-2995 cm^{-1} and 990-
 274 954 cm^{-1} for soybean oil adulteration detection in sunflower oil. The selection of the optimum number of
 275 LVs is the key step in building a robust PLSR model to obtain efficient and reliable prediction [30].
 276 Selecting many or few LVs may lead to over- or under-fitting of the model. There are numerous ways to
 277 select optimum number of LVs. In this study, the optimum number of LVs was selected at the minimum
 278 value of RMSECV (Fig 7a and Fig 8a). The calibration statistics of different PLSR models are
 279 summarized in Table 1. To visualize the PLSR calibration models, the actual percent level of adulteration
 280 and its predicted percent level of adulteration obtained from the PLSR models are plotted and displayed
 281 in Fig 7 (b, c, d) and Fig 8 (b, c, d). The PLSR models developed using the raw spectra were very
 282 accurate with $R_c^2 > 0.985$ for all three spectral ranges for both adulterants. The results found in this study
 283 were similar to those mentioned by [40, 45] for predicting adulteration in edible oils using FT-IR
 284 spectroscopy. Quiñones-Islas et al. [40] reported R^2 of > 0.99 using PLSR for predicting sunflower, canola
 285 and soybean adulteration in avocado oil [15, 45], whereas [45] obtained R^2 of 0.999 for predicting
 286 sunflower and corn oil in extra virgin coconut oil.

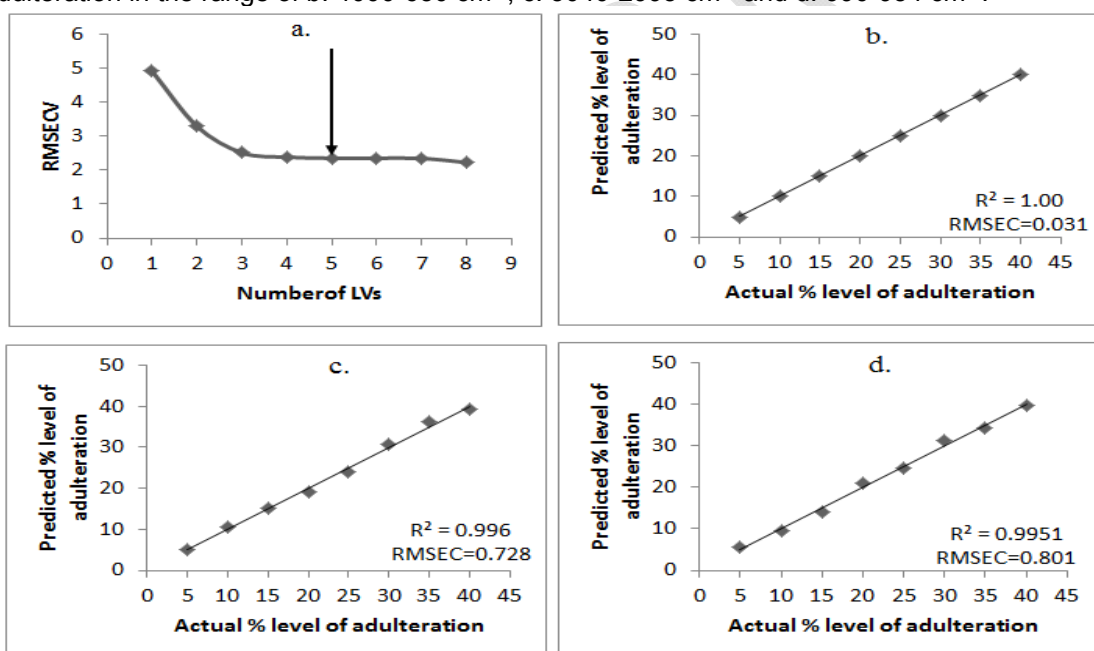
287
 288 **Table1:** Performance of PLS model for detecting soybean oil and palm adulteration in sunflower oil and
 289 rice bran oil, respectively.
 290

Adulterants	Spectral range (cm^{-1})	LVs	RMSEC	R^2
Soybean	4000-650	4	0.000	1.000
	3040-2995	4	0.000	1.000
	990-954	2	1.732	0.985
Palm oil	4000-650	5	0.031	1.000
	3040-2995	2	0.728	0.996
	1000-960	2	0.801	0.995

291



292
293 Fig. 7: Prediction of soybean oil adulteration in sunflower oil using PLSR models. a. An example of
294 determination of LVs at the minimum value of RMSECV. a. Measured vs. PLSR prediction of % level of
295 adulteration in the range of b. 4000-650 cm^{-1} , c. 3040-2995 cm^{-1} and d. 990-954 cm^{-1} .



296
297 Fig. 8: Prediction of palm oil adulteration in rice bran oil using PLSR models. a. An example of
298 determination of LVs at the minimum value of RMSECV. Measured vs. PLSR prediction of % level of
299 adulteration in the range of b. 4000-650 cm^{-1} , c. 3040-2995 cm^{-1} and d. 1000-960 cm^{-1} .

300
301
302
303
304
305
306

307
308

Table 2. Measured and PLSR predicted values of palm oil adulteration in rice bran oil.

% Measured	% Predicted (4000-650 cm ⁻¹)	% Predicted (3040-2995 cm ⁻¹)	% Predicted (1000-960 cm ⁻¹)
5.00	4.98	4.99	5.53
10.00	10.00	10.67	9.45
15.00	15.00	15.01	14.09
20.00	20.06	19.16	21.01
25.00	24.97	24.12	24.75
30.00	30.00	30.64	31.36
35.00	34.95	36.12	34.17
40.00	40.02	39.20	39.63

309

Table 3. Measured and PLSR predicted values of soybean oil adulteration in sunflower oil.

% Measured	% Predicted (4000-650 cm ⁻¹)	% Predicted (3040-2995 cm ⁻¹)	% Predicted (990-954 cm ⁻¹)
10.00	10.00	10.00	9.02
20.00	20.00	20.00	22.36
30.00	30.00	30.00	28.25
40.00	40.00	40.00	41.81
50.00	50.00	50.00	48.55

310
311

4. CONCLUSION

312 The samples were collected from the local market and the preparation was done in the laboratory. This
313 study mainly concerned with the development of optical sensing technique based on FTIR spectroscopy
314 for adulteration detection in edible oils. Two types of oils such as palm oil and soybean oil were selected
315 as adulterants. Rice bran oil was adulterated with palm oil at 5% increments upto 40% (v/v), while
316 sunflower oil was adulterated with soybean oil at 10% increments upto 50% (v/v). FTIR spectra in the
317 wave number interval of 4000–650 cm⁻¹ were then collected at room temperature using a Perkin Elmer
318 Universal ATR spectrophotometer (UATR-FT-IR, USA). Various pre-processing techniques such as first
319 derivation, second derivative, multiplicative scatter correction (MSC), and standard normal variate (SNV)
320 were separately applied to the spectral data prior to the development of multivariate model. Partial least
321 squares regression (PLSR) was developed to correlate the FT-IR spectra of different oil samples and their
322 corresponding level of adulteration. Cross validation using leave-one-out method was used during the
323 calibration step to select the optimum number of LVs for PLSR model. It was determined at the minimum
324 value of the root mean square error of cross-validation (RMSECV). Six different PLSR models were
325 developed based on whole spectral range as well as selected spectral range. For all models, the level of
326 adulteration in pure oil was predicted with determination coefficients (R²) of > 0.985. This study revealed
327 that FT-IR spectroscopy coupled with PLSR can be successfully utilized as a rapid screening technique to
328 detect and quantify the level of adulteration in edible oil.
329 Adulteration of food products involves the replacement of high cost ingredients with inferior quality
330

331 substitutes. Expensive edible oil is sometimes adulterated with cheaper oil as a means of illegally
332 increasing profit. Therefore, the challenge is to develop a cost effective and efficient analytical method to
333 detect adulteration and confirm authenticity. Using FTIR spectroscopy coupled with multivariate analysis
334 could be used as an alternative analytical tool to detect adulteration in sunflower oil and rice bran oil.
335 Therefore, the laborious and time-consuming conventional analytical techniques could be replaced by
336 spectral data to provide a rapid and nondestructive testing technique. More results compare with other
337 analytical techniques need to be addressing to validate the models.
338

339 **COMPETING INTEREST**

340 Authors have declared that no competing interests exist.
341

342 **REFERENCES**

- 343 1. Filby FA. (1976). A history of food adulteration and analysis. Allen & Unwin, London.
- 344 2. Alamprese, C., Casale, M., Sinelli, N., Lanteri, S., & Casiraghi, E. (2013). Detection of minced beef
345 adulteration with turkey meat by UV-vis, NIR and MIR spectroscopy. *LWT - Food Science and*
346 *Technology*, 53(1), 225-232. doi:10.1016/j.lwt.2013.01.027.
- 347 3. Ellis, D. I., Brewster, V. L., Dunn, W. B., Allwood, J. W., Golovanov, A. P., & Goodacre, R. (2012).
348 Fingerprinting food: current technologies for the detection of food adulteration and contamination.
349 *Chem Soc Rev*, 41(17), 5706-5727. doi:10.1039/c2cs35138b.
- 350 4. Kamruzzaman, M., Makino, Y., Oshita, S., & Liu, S. (2015). Assessment of Visible Near-Infrared
351 Hyperspectral Imaging as a Tool for Detection of Horsemeat Adulteration in Minced Beef. *Food*
352 *and Bioprocess Technology*, 8(5), 1054-1062. doi:10.1007/s11947-015-1470-7.
- 353 5. Zhao, M., Downey, G., & O'Donnell, C. P. (2014). Detection of adulteration in fresh and frozen
354 beefburger products by beef offal using mid-infrared ATR spectroscopy and multivariate data
355 analysis. *Meat Sci*, 96(2 Pt A), 1003-1011. doi:10.1016/j.meatsci.2013.10.015.
- 356 6. Urickova, V., & Sadecka, J. (2015). Determination of geographical origin of alcoholic beverages using
357 ultraviolet, visible and infrared spectroscopy: A review. *Spectrochim Acta A Mol Biomol*
358 *Spectrosc*, 148, 131-137. doi:10.1016/j.saa.2015.03.111.
- 359 7. Beaten V, Fernández Pierna J A, Dardenne P, Meurens M, García-González D L and Aparicio-Ruiz R
360 (2005). Detection of the presence of hazelnut oil in olive oil by FT-Raman and FT-MIR
361 spectroscopy. *Journal of Agriculture and Food Chemistry*, 53, 6201-6206.
- 362 8. García-González, D. L., Aparicio-Ruiz, R., & Aparicio, R. (2008). Virgin olive oil - Chemical implications
363 on quality and health. *European Journal of Lipid Science and Technology*, 110(7), 602-607.
364 doi:10.1002/ejlt.200700262.
- 365 9. Espiñeira, M., Vieites, J. M., & Santaclara, F. J. (2010). Species authentication of octopus, cuttlefish,
366 bobtail and bottle squids (families Octopodidae, Sepiidae and Sepiolidae) by FINS methodology
367 in seafoods. *Food Chemistry*, 121(2), 527-532. doi:10.1016/j.foodchem.2009.12.042.
- 368 10. Kamruzzaman, M., Barbin, D., ElMasry, G., Sun, D.-W., & Allen, P. (2012a). Potential of hyperspectral
369 imaging and pattern recognition for categorization and authentication of red meat. *Innovative*
370 *Food Science and Emerging Technologies*, 16, 316-325.
371 doi:http://dx.doi.org/10.1016/j.ifset.2012.07.007.
- 372 11. Manley M, De Bruyn N and Downey G (2003). Classification of three-year old, unblended South
373 African brandy with near-infrared spectroscopy. *NIR News*, 14, 8-9.
- 374 12. Ruoff K, Luginbuhl W and Kunzli R (2006). Authentication of the botanical and geographical origin
375 of honey by mid-infrared spectroscopy. *Journal of Food Agricultural Chemistry*, 54, 6873-
376 6880.
- 377 13. Li, B., Wang, H., Zhao, Q., Ouyang, J., & Wu, Y. (2015). Rapid detection of authenticity and
378 adulteration of walnut oil by FTIR and fluorescence spectroscopy: a comparative study. *Food*
379 *Chem*, 181, 25-30. doi:10.1016/j.foodchem.2015.02.079.
- 380 14. Mamani-Linares, L. W., Gallo, C., & Alomar, D. (2012). Identification of cattle, llama and horse meat
381 by near infrared reflectance or transfectance spectroscopy. *Meat Sci*, 90(2), 378-385.
382 doi:10.1016/j.meatsci.2011.08.002.
- 383 15. Sun, X., Zhang, L., Li, P., Xu, B., Ma, F., Zhang, Q., & Zhang, W. (2015). Fatty acid profiles based
384 adulteration detection for flaxseed oil by gas chromatography mass spectrometry. *LWT - Food*
385 *Science and Technology*, 63(1), 430-436. doi:10.1016/j.lwt.2015.02.023.

- 386 16. Mohammed Kamruzzaman, Y. M., Seiichi Oshita. (2014). An appraisal of hyperspectral imaging for
387 non-invasive authentication of geographical origin of beef and pork *Proceedings International*
388 *Conference of Agricultural Engineering, Zurich*.
- 389 17. Longobardi F, Casiello G and Cortese M (2015). Discrimination of geographical origin of lentils
390 (*Lens culinaris Medik.*) using isotope ratio mass spectroscopy combined with chemometrics.
391 *Food Chemistry*, 05, 020.
- 392 18. Picque D, Cattenoz T and Corrieu G (2005). Discrimination of red wines according to their
393 geographical origin and vintage year by the use of mid-infrared spectroscopy. *Science*
394 *Aliments*, 25, 207- 220.
- 395 19. Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Sci*, 86(3), 577-587.
396 doi:10.1016/j.meatsci.2010.06.001.
- 397 20. Rasmus Bro, Van Den Berg F., Anette Thybo, Charlotte M. Anderseny, Bo M. Jørgensenx and Henrik
398 Andersen. (2002). Multivariate data analysis as a tool in advanced quality monitoring in the food
399 production chain. *Trends in Food Science & Technology*, 13, 235-244.
- 400 21. Russell, L. M., Bahadur, R., Hawkins, L. N., Allan, J., Baumgardner, D., Quinn, P. K., & Bates, T. S.
401 (2009). Organic aerosol characterization by complementary measurements of chemical bonds
402 and molecular fragments. *Atmospheric Environment*, 43(38), 6100-6105.
- 403 22. Takahama, S., Johnson, A., & Russell, L. M. (2013). Quantification of carboxylic and carbonyl
404 functional groups in organic aerosol infrared absorbance spectra. *Aerosol Science and*
405 *Technology*, 47(3), 310-325.
- 406 23. Svante Wold, M. S. a. L. E. (2001). PLS-regression: a basic tool of chemometrics *Chemometrics and*
407 *Intelligent Laboratory Systems*, 58, 109-130.
- 408 24. Safwan M. Obeidat, M. S. K. a. W. M. O. (2009). Classification of Edible Oils and Uncovering
409 Adulteration of Virgin Olive Oil Using FTIR with the Aid of Chemometrics *Australian Journal of*
410 *Basic and Applied Sciences*, 3(3), 2048-2053.
- 411 25. Kamruzzaman, M., Makino, Y., & Oshita, S. (2016). Rapid and non-destructive detection of chicken
412 adulteration in minced beef using visible near-infrared hyperspectral imaging and machine
413 learning. *Journal of Food Engineering*, 170, 8-15. doi:10.1016/j.jfoodeng.2015.08.023.
- 414 26. Maggio, R. M., Cerretani, L., Chiavaro, E., Kaufman, T. S., & Bendini, A. (2010). A novel chemometric
415 strategy for the estimation of extra virgin olive oil adulteration with edible oils. *Food Control*, 21(6),
416 890-895. doi:10.1016/j.foodcont.2009.12.006.
- 417 27. Oussama, A., Elabadi, F., Platikanov, S., Kzaiber, F., & Tauler, R. (2012). Detection of Olive Oil
418 Adulteration Using FT-IR Spectroscopy and PLS with Variable Importance of Projection (VIP)
419 Scores. *Journal of the American Oil Chemists' Society*, 89(10), 1807-1812. doi:10.1007/s11746-
420 012-2091-1.
- 421 28. Abdel-Rahman, E. M., Mutanga, O., Adam, E., & Ismail, R. (2014). Detecting Sirex noctilio grey-
422 attacked and lightning-struck pine trees using airborne hyperspectral data, random forest and
423 support vector machines classifiers. *ISPRS Journal of Photogrammetry and Remote Sensing*, 88,
424 48-59. doi:10.1016/j.isprsjprs.2013.11.013.
- 425 29. Kamruzzaman, M., ElMasry, G., Sun, D. W., & Allen, P. (2012). Prediction of some quality attributes
426 of lamb meat using near-infrared hyperspectral imaging and multivariate analysis. *Anal Chim*
427 *Acta*, 714, 57-67. doi:10.1016/j.aca.2011.11.037.
- 428 30. Perez-Rodriguez, M., Horak-Terra, I., Rodriguez-Lado, L., & Martinez Cortizas, A. (2016). Modelling
429 mercury accumulation in minerogenic peat combining FTIR-ATR spectroscopy and partial least
430 squares (PLS). *Spectrochim Acta A Mol Biomol Spectrosc*, 168, 65-72.
431 doi:10.1016/j.saa.2016.05.052.
- 432 31. Ruthenburg, T. C., Perlín, P. C., Liu, V., McDade, C. E., & Dillner, A. M. (2014). Determination of
433 organic matter and organic matter to organic carbon ratios by infrared spectroscopy with
434 application to selected sites in the IMPROVE network. *Atmospheric Environment*, 86, 47-57.
435 doi:10.1016/j.atmosenv.2013.12.034.
- 436 32. Gurdeniz, G., & Ozen, B. (2009). Detection of adulteration of extra-virgin olive oil by chemometric
437 analysis of mid-infrared spectral data. *Food Chemistry*, 116(2), 519-525.
438 doi:10.1016/j.foodchem.2009.02.068.
- 439 33. Hirri A, Gammouh M, Gorfti A, Kzaiber F, Bassbasi M, Souhassou S, Balouki A and Oussama A
440 (2015). The use of Fourier transform mid infrared (FT-MIR) spectroscopy for detection and

- 441 estimation of extra virgin olive oil adulteration with old olive oil, *Sky Journal of Food Science*,
442 4, 060-066.
- 443 34. Kowalski, P. G. B. R. (1986). Partial Least-Squares Regression: A Tutorial. *Analytica Chimica Acta*,
444 186, 1-17.
- 445 35. Rinnan, Å., Berg, F. v. d., & Engelsen, S. B. (2009). Review of the most common pre-processing
446 techniques for near-infrared spectra. *TrAC Trends in Analytical Chemistry*, 28(10), 1201-1222.
447 doi:10.1016/j.trac.2009.07.007.
- 448 36. Urbano Cuadrado, M., Luque de Castro, M. D., Perez Juan, P. M., & Gomez-Nieto, M. A. (2005).
449 Comparison and joint use of near infrared spectroscopy and Fourier transform mid infrared
450 spectroscopy for the determination of wine parameters. *Talanta*, 66(1), 218-224.
451 doi:10.1016/j.talanta.2004.11.011.
- 452 37. Zhang, Q., Liu, C., Sun, Z., Hu, X., Shen, Q., & Wu, J. (2012). Authentication of edible vegetable oils
453 adulterated with used frying oil by Fourier Transform Infrared Spectroscopy. *Food Chem*, 132(3),
454 1607-1613. doi:10.1016/j.foodchem.2011.11.129.
- 455 38. Christy AA, K. S., Du Y and Ozaki K. (2004). The detection and quantification of adulteration in olive
456 oil by near-infrared spectroscopy and chemometrics. *Analytical Sciences*, 20, 935-940.
- 457 39. Yap, K. Y.-L., Chan, S. Y., & Lim, C. S. (2007). Infrared-based protocol for the identification and
458 categorization of ginseng and its products. *Food Research International*, 40(5), 643-652.
459 doi:10.1016/j.foodres.2006.11.009.
- 460 40. Quiñones-Islas, N., Meza-Márquez, O. G., Osorio-Revilla, G., & Gallardo-Velazquez, T. (2013).
461 Detection of adulterants in avocado oil by Mid-FTIR spectroscopy and multivariate analysis. *Food*
462 *Research International*, 51(1), 148-154. doi:10.1016/j.foodres.2012.11.037.
- 463 41. Mauer, B. F. O. A. L. J. (2002). Detection of Hazelnut Oil Adulteration Using FT-IR Spectroscopy.
464 *Agricultural and Food Chemistry*, 50, 3898-3901. doi:doi:10.1021/jf0201834.
- 465 42. Tay A, Singh RK, Krishnan SS and Gore JP (2002). Authentication of olive oil adulterated with
466 vegetable oils using Fourier transform infrared spectroscopy. *LWT-Food Science and*
467 *Technology*.35, 99-103.
- 468 43. Elzey, B., Pollard, D., & Fakayode, S. O. (2016). Determination of adulterated neem and flaxseed oil
469 compositions by FTIR spectroscopy and multivariate regression analysis. *Food Control*, 68, 303-
470 309. doi:10.1016/j.foodcont.2016.04.008.
- 471 44. Rohman, A., & Man, Y. B. C. (2011). The use of Fourier transform mid infrared (FT-MIR)
472 spectroscopy for detection and quantification of adulteration in virgin coconut oil. *Food Chemistry*,
473 129(2), 583-588. doi:doi:10.1016/j.foodchem.2011.04.070.
- 474 45. Rohman, A., & Man, Y. C. (2010). Fourier transform infrared (FTIR) spectroscopy for analysis of extra
475 virgin olive oil adulterated with palm oil. *Food Research International*, 43(3), 886-892.
476 doi:doi:10.1016/j.foodres.2009.12.006.
- 477
478
479