LOW VS HIGH FIELD ^{1}H NMR SPECTROSCOPY FOR THE DETECTION OF ADULTERATION OF COLD PRESSED RAPESEED OIL WITH REFINED OILS.

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21 **ABSTRACT**

22 Cold pressed rapeseed oil (CPRO) is a relatively recent development in rapeseed processing,
23 which produces a quality product with a high market value. High field NMR (400 MHz) is a
24 well-established tool in food analysis, while low-field NMR (60 MHz) is much less studied.
25 This study aims to establish the effectiveness of both techniques in identifying binary
26 adulteration in CPRO. Three adulteration scenarios were investigated; a). CPRO and refined
27 rapeseed oil (RRO), b). CPRO and refined sunflower oil (RSO), c) CPRO and RRO or RSO.
28 A range of classification techniques were trialled as well as partial least squares regression to
29 gauge predictive quantification performance. The 400 MHz NMR achieved classification rates
30 of 100% in the scenarios with a single adulterant, and 93% in the multiple adulterant scenario.
31 The 60 MHz NMR produced lower but still encouraging classification rates (RSO 92%; RRO
32 85%; both RRO and RSO 87%).

33

34 **Keywords:** NMR, authentication, cold pressed rapeseed oil, chemometrics, fraud

35

36 **Abbreviations:** CPRO – Cold pressed rapeseed oil; RRO – Refined rapeseed oil; RSO –
37 Refined Sunflower oil; TPR – True positive rate; FNR – False negative rate

1. INTRODUCTION

Cold pressed rapeseed oil (CPRO) is a relatively new culinary oil in the UK and Ireland, as well as other parts of the world, that is locally cultivated, harvested and pressed. The seeds from the oilseed rape crop are mechanically crushed at a low temperature and the oil is collected. The oil is filtered and bottled, which concludes the processing (McDowell, Elliott, Koidis, 2017a). The final product has desirable organoleptic attributes and it is marketed towards the top end of the retail market. High quality edible oils such as this are particularly susceptible to adulteration, due to the ease with which low value oils can be mixed in to increase profit (McDowell, Elliott, Koidis, 2017a, b). One research paper has recently investigated cold pressed edible oil authentication with FT-IR spectroscopy (Ozulku, Yildirim, Toker, Karasu, & Durak, 2017), however cold pressed rapeseed oil was not covered. The utilisation of fraud monitoring systems have been established as an important component in reducing fraud vulnerability in food supply chains (van Ruth, Luning, Silvis, Yang, & Huisman, 2018). As cold pressed rapeseed oil continues to grow in popularity (SFD, 2013), it is important that techniques are developed which can ensure the sector maintains its high standards of quality.

Refined rapeseed oil (RRO) – sometimes simply branded as “vegetable oil” in retail – is a low cost oil which has been used extensively in the food industry (Przybylski, Mag, Eskin, & McDonald, 2005). The seeds from the oilseed rape plant undergo a much more complex processing pathway than the cold pressed sequence (Ghazani, García-Llatas, & Marangoni, 2014). More specifically, the seeds are mechanically crushed and solvent extracted to ensure maximum levels of oil are recovered. This oil then undergoes a range of intensive processing techniques that include degumming, neutralisation, bleaching and deodorisation which removes phospholipids, chlorophyll, free fatty acids and odour compounds associated with colour, flavour and odour defects. The resulting oil is low value and used exclusively for high temperature cooking. Refined rapeseed oil is, in terms of fatty acid composition, very similar

63 to CPRO, most of the variation between these two oils comes from the unsaponifiable fraction
64 which remains in the oils after either cold pressing or industrial extraction (Ghazani et al.,
65 2014). It is because of its similarities to CPRO and its low cost that RRO has been suggested
66 as a potential adulterant in this study. A similar adulteration scenario became a problem for the
67 extra-virgin olive oil industry when high value extra-virgin olive oil was being adulterated with
68 lower value refined olive oil (Yang & Irudayaraj, 2001). This has led to the utilisation of
69 spectroscopic techniques which can detect fraud in a quick non-destructive manner (Georgouli,
70 Rincon, & Koidis, 2017; Jiménez-Carvelo, Osorio, Koidis, González-Casado, & Cuadros-
71 Rodríguez, 2017).

72 Another potential candidate for adulterating CPRO is refined sunflower oil (RSO) due to its
73 lower price and widespread availability. It is a popular culinary oil used across the world and
74 would therefore be easily accessible in large quantities. Refined sunflower oil is produced from
75 sunflower seeds by undergoing similar processing to that described for RRO. Being produced
76 from a different seed, it differs much more in fatty acid composition from CPRO than RRO. It
77 would therefore be expected that RSO should be easier to detect in CPRO using analytical
78 techniques although no studies exist in the literature for these two cases of adulteration.

79 Oil speciation is a challenge which has been tackled by both chromatographic (Aparicio and
80 Aparicio-Ruíz 2000) and spectroscopic (Che Man et al. 2011; Koidis and Osorio-Argüello
81 2013) techniques. Raman and FT-IR are the most dominant types of vibrational spectroscopy
82 currently used (Osorio, Haughey, Elliott, & Koidis, 2014). Proton NMR is less well utilised in
83 this area mainly due to the size and cost of its operation compared to other spectroscopic
84 techniques. There have however been recent interesting developments in low field ^{1}H NMR
85 systems, which are much smaller (lower footprint), less resource-heavy and less expensive than
86 the high-field counterparts.

87 In the literature, high field ^{1}H NMR spectroscopy has been shown to be an effective tool for
88 extra virgin olive oil quality assessment and authentication (Dais & Hatzakis, 2013). This type
89 of spectroscopy is powerful enough to produce peaks which yield much more comprehensive
90 structural information than other spectroscopic techniques. This information can be quantitated
91 to show the ratios of unsaturated fatty acids/mono-unsaturated fatty acids/poly-unsaturated
92 fatty acids, or even identify the molar percentage of individual fatty acids (Siciliano et al.,
93 2013). Low field ^{1}H NMR produces spectra which do not have as sharp peaks as in high field
94 ^{1}H NMR, and with more overlap between signals; nevertheless, certain regions can still be
95 associated with specific fatty acid moieties and minor compounds. This ^{1}H NMR signal can be
96 utilised when coupled with chemometrics for qualitative analysis of food and drinks (Jakes et
97 al., 2015). With regards to edible oil authentication, 60 MHz ^{1}H NMR has been shown to be
98 able to detect hazelnut oil adulteration in olive oil down to a limit of 11.2% w/w (Parker et al.,
99 2014) and peaks can be used to estimate the amount of *Robusta* coffee in *Arabica* coffee with
100 an approximate detection limit of 10-20% w/w (Defernez et al., 2017). It has also been recently
101 show that 2D NMR can be utilised to further improve the performance of low field NMR
102 (Gouilleux, Marchand, Charrier, Remaud, & Giraudeau, 2018).

103 The aim of this study therefore was to evaluate the effectiveness of 60 MHz and 400 MHz
104 proton NMR in identifying refined rapeseed oil and refined sunflower oil as adulterants in cold
105 pressed rapeseed oil.

106

107 2. MATERIALS AND METHODS

108 2.1. Oil Samples and Reagents

109 The CPROs in this study were donated by reputable producers from the UK, Ireland and
110 France. The RROs and RSOs were donated by international food companies with certificates
111 of authenticity. All the RROs and the RSOs were fully refined i.e. no partially refined oils were

112 included in this study. The total number of different oil brands used in this study was 21 CPRO,
113 16 RRO and 15 RSO. Analytical grade chloroform and deuterated chloroform (Sigma Aldrich,
114 Dorset, UK) were the solvents used in this study. All oil samples were analysed for acidity and
115 peroxide value using standard methods to confirm their identity and to identify if there were
116 any outliers.

117 **2.2. Preparation of oil admixtures**

118 Forty-five binary mixtures of either CPRO and RRO, or CPRO and RSO (both 2-98% CPRO),
119 were prepared by weighing (±0.001 g) the desired ratio of oils in a 20 mL vial. The mixtures
120 (90 in total) were then vortexed and stored at -20°C in 30mL amber glass vials and defrosted
121 at room temperature when required.

122 **2.3. 60 MHz ^{1}H NMR acquisition**

123 All samples (pure and admixtures) were diluted with analytical standard chloroform, as this
124 was shown to produce a more defined spectrum (narrower line width) than pure oil after initial
125 tests. An aliquot of 300 µl of oil was pipetted directly into an Aldrich ColorSpec NMR tube,
126 followed by 700 µl of chloroform. The tube was inverted several times until the oil was
127 completely dissolved in the chloroform. Spectra were recorded on an Oxford Instruments 60
128 MHz benchtop NMR (Pulsar, Oxford Instruments, Oxford, UK). Instrument optimisation was
129 performed with 12% TMS (tetramethylsilane) in chloroform before samples were analysed.
130 The linewidth was maintained at an acceptable level by daily checking and shimming as and
131 when necessary. The acquisition parameters were 16 scans and 10 sec recycle delay. All sample
132 tubes were analysed three times and averaged. Each full spectrum consisted of 5,933 data
133 points. The data was processed in MNOVA (phase-correction) and saved as text file before
134 being further manipulated and analysed in Matlab.

135

136 **2.4. 400 Hz ^{1}H NMR acquisition**

137 All oil samples were diluted in deuterated chloroform in the ratio 60 µl of oil: 500 µl deuterated
138 chloroform in an Aldrich ColorSpec NMR tube. The samples were inserted into a Bruker
139 Ultrashield 400 Plus ^{1}H NMR (Bruker, Rheinstetten, Germany). Acquisition of the NMR data
140 was done using the zg30 pulse program with a pulse time of 11 µsec and a recovery delay
141 of 1 sec. Acquisition used a 30° excitation pulse. The number of scans was set at 16 and each
142 full spectrum consisted of 65,536 data points. Acquisition time was set to 4.089 sec and the
143 dwell time was set to 62.400 µsec. Transmitter frequency used in these experiments was 400.13
144 MHz. Samples were measured downfield from TMS (0 ppm). Acquisition was obtained at
145 a temperature of 298 K.

146

147 **2.5. Statistical analysis**

148 Three different oil adulteration scenarios were explored: 1. CPRO adulterated with RSO; 2.
149 CPRO adulterated with RRO; 3. CPRO adulterated with either RRO or RSO (Table 1). It
150 should be noted that the CPROs used in scenario 1 were different oils/brands than scenario 2,
151 the aim of this was to increase the diversity of oils in the adulteration test. Scenario 3 used
152 CPRO which were present in both scenario 1 and 2.

153 Firstly, we looked to carry out a classification analysis of these scenarios. Samples relevant to
154 each scenario were selected and their spectra were allocated to either the calibration or the
155 validation set (60% and 40% of the total dataset respectively), on the condition that the oils
156 (pure and admixtures) featured in the calibration set were not used again in the validation set.
157 The mixtures of binary oils used in the calibration sets ranged from 4 – 97% CPRO, and in the
158 validation sets from 2 – 93 % CPRO. Secondly, we looked a quantification analysis, partial
159 least squares regression (PLS-R) was used to quantify the level of adulteration in the first two

160 scenarios. For this, all spectra listed in the first two scenarios (RSO as an adulterant and RRO
161 as an adulterant) were used.
162 The spectra were examined in MNOVA and saved as text files, before being read into
163 MATLAB. All chemometric data analysis was performed with in-house MATLAB routines
164 (Mathworks Inc. MA, USA). For classification analysis, four different chemometric techniques
165 were used to illustrate the most effective: Partial Least Squares – Discriminant Analysis (PLS-
166 DA) (Ballabio & Consonni, 2013), Soft Independent Modelling by Class Analogy (SIMCA)
167 (Brereton, 1992), Linear Discriminant Analysis - K-Nearest Neighbour (LDA-KNN), and
168 Linear Discriminant Analysis - Support Vector Machine (LDA-SVM) (Xanthopoulos,
169 Pardalos, & Trafalis, 2013). Classification results were expressed as the rate of true positives
170 (TPR %) and the rate of false negatives (FNR %) that occurred in the validation dataset. The
171 TPR was calculated by number of true positives/ (number of true positives + number of false
172 positives). The FNR was calculated by number of false negative/ (number of false negative +
173 number of true negatives). Quantification analysis was carried out with partial least squares
174 regression (PLS-R) analysis. Three spectral regions were extracted (0.52-3ppm, 3.9-4.56ppm,
175 and 4.94-5.8ppm) and each region was baseline-corrected using a polynomial function. The
176 three regions were bolted together (with 3217 data points in total) and the data normalised on
177 the glyceride peak (3.9-4.56ppm). Leave-one-out cross-validation was used at the calibration
178 stage, and the optimal model applied to the validation data. The results show the predicted
179 versus actual composition values, root mean square error (RMSE) and the R^2 value of the
180 regression, obtained from the cross-validation calibration and validation sets. Limit of detection
181 was calculated as 2 times the RMSEP (Downey & Kelly, 2004).
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183
184

3. RESULTS AND DISCUSSION

3.1. Spectral characteristics in different oils and instrumental operating frequencies

The spectra produced when edible oils are analysed with NMR are representations of the resonances of ^{1}H nuclei within the oil. Hydrogen nuclei are present in both the glycerol and the fatty acid sections of triacylglycerols (TAGs). Edible oil tends to be around 95-98% TAGs and 2-5% minor compounds (Cert, Moreda, & Pérez-Camino, 2000), therefore the peaks produced are directly related to TAG composition (Table 2). Minor compounds found in edible oil like phytosterols and tocopherols can be detected with ^{1}H NMR, although are better represented when extracted from oil to avoid spectral suppression from the more dominant TAG peaks (Alonso-Salces et al., 2010). The spectra of the three types of pure oil used in this study can be seen in Fig. 1. As expected the structure of the peaks in the 60 MHz spectra is less detailed than that of the 400 MHz spectra. The 400 MHz NMR spectrum (Fig. 1A) shows a signal with low noise and clearly identifiable regions associated with specific fatty acid groups. Cold pressed rapeseed oil and refined rapeseed oil have very similar fatty acid compositions (Wroniak, Krygier, & Kaczmarczyk, 2008) which would explain the close similarities between the two spectra (Fig. 1). Sunflower oil does however differ from rapeseed oil in its fatty acid composition (Kostadinovic Velickovska & Mitrev, 2013), and this can be seen when the 400 MHz spectra of CPRO and RSO are compared.

On the other hand, the spectra produced by the 60 MHz ^{1}H NMR contains less specific detail compared with the 400 MHz spectra (Fig. 1). The reduction in field-strength means that the peaks' structure corresponding to specific nuclei interactions (J-coupling) is not immediately visible. Instead a broader spectrum is produced, with many overlapped peaks and a few isolated ones (glyceryl CH_2 at 4.2 ppm and *bis*-allylic CH_2 at 2.8 ppm). The 60 MHz spectra of the three types of pure oils used in this study can be seen in Fig. 1B. There appears to be little visible variation in the three spectra apart from the peak at 1.3 ppm which has a different shape

210 in RSO compared to rapeseed oils. Subtle differences can also be seen in the *bis*-allylic CH_2
211 peak at 2.8 ppm, as well as the peak at 1 ppm. It is unclear as to the exact fatty acids responsible
212 for this variation, although it is likely to be at least partly due to the difference in unsaturated
213 fatty acid composition between RSO and CPRO (Supplementary data).

214 To highlight the extent of spectral variation within oil admixtures, multiple spectra (ranging
215 from 3-97% CPRO) were superimposed (Fig. 2 and 3). The superimposed NMR spectra of
216 mixtures of RRO and CPRO (Fig. 2) show there is no discernible variation in the 400 MHz
217 spectra. This illustrates the close similarities between CPRO and RRO. There is some variation
218 within the 60 MHz superimposed spectra (Fig. 2A); however, this is not systematic and is likely
219 to be due to instrumental variation rather than to chemical composition. It should be
220 remembered that the 60 MHz NMR operates at room temperature using rare earth magnets,
221 therefore the operating parameters cannot be as tightly controlled as the larger, cryogen-cooled
222 400 MHz instrument (Parker et al., 2014). This initial exploration would suggest that RRO
223 could be difficult to detect in CPRO, by both types of NMR spectroscopy. When mixtures of
224 sunflower oil (RSO) and cold pressed rapeseed oil (CPRO) in 400 MHz and 60 MHz were
225 superimposed, there is clear variation in many of the peaks both at 60 and 400 MHz (Fig. 3).
226 Refined sunflower oil has a much more dissimilar fatty acid composition than CPRO and
227 should therefore be easier to be detected as an adulterant, due to the difference in omega-3 to
228 omega-6 ratios (Supplementary data).

229 **3.2. Classification with Multivariate Analysis (PLS-DA, SIMCA, and LDA)**

230 Classification analysis was carried out on spectra produced from both 60 MHz and 400 MHz
231 instruments. The results were expressed in terms of the true positive rate (TPR) and the false
232 negative rate (FNR), which together portray the sensitivity and specificity of each chemometric
233 model (Table 3). In general, for a good model, the aim is for FNR to be kept as lower as possible
234 (<5%) whereas at the same time the TPR remains as high as possible. In the dataset containing

235 CPRO, RSO and its mixtures (modelling scenario 1), the 60 MHz NMR instrument had the
236 best classification outcome of TPR 92% and FNR 4% when using the LDA-KNN classifier.
237 The 400 MHz instrument provided data that when coupled with LDA-SVM or PLS-DA could
238 achieve 100% TPR and an FNR of 0%. The high classification rate for 400 MHz NMR is
239 explained by the clear spectral variation associated with oil type deriving from the richer
240 molecular structural information it provides. This becomes evident when comparing the
241 spectral data (Fig. 4B) to the PLS-R regression coefficient vector. This vector directly relates
242 the spectra to the compositional information. There are many regions where the magnitude of
243 the regression coefficients is large, and these correspond to regions of the spectra where CPRO
244 and RSO differ most. These include regions 2.70-2.90 ppm where *bis*-allyic peaks associated
245 with linolenic (C18:3) and linoleic (C18:2) fatty acids are present. Rapeseed oil has higher
246 levels of linolenic fatty acids while sunflower oil has higher levels of linoleic fatty acids
247 (Supplementary data). This is reflected in the regression coefficients, where there is a negative
248 peak corresponding to linoleic acid at very slightly lower chemical shifts than a positive peak
249 corresponding to linolenic acid (Guillén & Ruiz, 2001). The region 2.00-2.10 ppm is also
250 associated with linoleic, linolenic, as well as oleic fatty acids, but this time in relation to the
251 allyl nuclei response. There is a negative coefficient peak attributed to the linoleic region and
252 a positive peak associated with the linolenic and oleic region. The region between 0.95-0.99
253 ppm corresponds to terminal methyl protons which are only present in CPRO. The absence of
254 any peaks in this region from RSO confirms these are the terminal methyl protons from the
255 omega 3 - linolenic fatty acids in CPRO. The regression coefficient vector corresponds to this
256 by showing a large positive peak with no negative values associated with RSO. The region
257 between 0.85-0.92 ppm corresponds to the terminal methyl protons of saturated,
258 monounsaturated and polyunsaturated fatty acids. It is unclear exactly which fatty acids are
259 responsible for the coefficient values in this region, although it is undoubtedly an important

260 and potentially discriminant region of the spectra. Differences in saturated fatty acid content
261 are evident in 1.23-1.39 ppm which corresponds to methylene protons on saturated carbon
262 chains. The coefficient peaks come from the difference in abundance of C16:0, C18:0 and
263 C20:0 between the two oils. The region between 5.20-5.45 ppm is associated with unsaturated
264 fatty acids and protons found on the middle branch of glycerol in triglycerides. There are
265 therefore multiple fatty acids which produce peaks in this region and the subsequent loading
266 vectors could therefore not be attributed to one fatty acid.

267 In the 60 MHz NMR spectra which had a less defined shape than the 400 MHz ones, it is seen
268 that the regions which have the greatest regression coefficient magnitude differ (Fig. 4A). The
269 region with the largest coefficient was 1.20-1.40 ppm, which corresponds to saturated fatty
270 acids. The terminal methyl protons in the region of 0.94 -1.00 ppm exhibited large coefficient
271 values for 400 MHz spectra, but were less defined in the 60 MHz and consequently did not
272 have such influential coefficients. The region 2.70-2.90 ppm corresponds to *bis*-allylic protons
273 associated with linolenic and linoleic fatty acids, although the effect of there being different
274 relative amounts of these fatty acids in rapeseed and sunflower oils is less directly interpretable
275 than in the high-field, due to increased peak overlap. It is also worth noting that for the 400
276 MHz data, coefficient values associated with the spectral baseline were essentially zero
277 throughout, whereas there is clearly some (unwanted) variance in the 60 MHz baseline that is
278 entering the regression coefficients. This is a consequence of the greater comparative stability
279 of the 400 MHz instrumentation.

280 Although the spectra produced by the low-field are less clear than the high-field NMR with
281 regards to peak definition, the 60 MHz instrument still produced competitive classification
282 results which could be used to screen for detecting RSO in CPRO presence. This is a positive
283 outcome, in view of the comparative ease-of-use and significantly lower cost of the
284 spectrometer. With regards to the 400 MHz NMR, its sensitivity and faultless classification

285 rate with RSO means it could be used as a screening tool or as a confirmatory procedure. As
286 implied earlier, the reason such high classification rates were achieved was that the highly-
287 detailed spectra exhibited many differences associated with variation in fatty acid composition
288 between the two oils.

289 The second adulteration scenario (RRO, CPRO and their mixtures) was predicted to be more
290 difficult due to the similarities between the two oils in terms of chemical composition and
291 corresponding spectral fingerprints (Fig. 1). The 60 MHz instrument achieved a top
292 classification rate of 85% TPR and 7% FNR. It is less clear which regions of the 60 MHz
293 spectra are important for CPRO and RRO classification. It can be seen in Fig. 5A, that the
294 spectral regions from 0.8-2.4 ppm have the largest effect on loading intensity. This region
295 corresponds to many fatty acids and it is therefore not possible to specifically identify the cause
296 of the differentiation between the two oils.

297 The 400 MHz instrument was again able to produce faultless classification regarding refined
298 rapeseed oil as an adulterant. The PLS-R regression coefficients go some way towards
299 explaining how compositional differences between the two oils enabled successful
300 classification with 400 MHz spectra. The coefficient region amongst the largest magnitudes is
301 0.95-1.0 ppm, which corresponds to linolenic acid (Fig. 5B). The superimposed spectra of the
302 two oils show that RRO appears to exhibit slightly higher levels of this fatty acid which would
303 explain the importance of this region. A region which seems more important when RRO is an
304 adulterant rather than RSO, is 1.53-1.69 ppm which corresponds to the six methylene protons
305 in the *beta* position from the carbonyl carbon. The other regions of the spectra all appear to
306 have an influence on the regression coefficients, which is most likely due to subtle differences
307 in fatty acid composition. It is well known that CPRO has a greater abundance of pigments
308 than RRO (Wroniak et al., 2008). However, in the NMR spectra we would not expect to see
309 peaks specifically assigned to pigments or other minor compounds.

310 A larger adulteration scenario (no.3) including both RRO and RSO was also tested with both
311 60 MHz and 400 MHz instruments. Unlike the previous three-class scenarios, no technique
312 could achieve 100% correct classification rate with a five-class scenario. The 60 MHz NMR
313 could produce classification results of 87% TPR and 3% FNR while the 400 MHz NMR
314 produced 93% TPR and 2% FNR. This would suggest that both types of NMR could be used
315 as screening tools for cold pressed rapeseed oil authentication. With regards to the choice of
316 classification model, either LDA-KNN or LDA-SVM were the best performing classifiers
317 depending on the NMR field-strength and dataset (Table 3).

318 **3.3. Quantification with multivariate analysis (PLS-R)**

319 Partial least squares regression (PLS-R) is a multivariate regression technique, closely related
320 to PLS-DA, but where the dependent variable is continuous (e.g. composition data) rather than
321 discrete (i.e. classification group). PLS-R analyses were carried out for the modelling scenarios
322 as detailed in Table 4. The amount of CPRO in each of the validation set samples is plotted
323 against the amount of CPRO predicted by each model in Fig. 6. The R^2 value indicates the
324 effectiveness of the regressions, and as seen in Table 4, when RSO is the adulterant the models
325 perform to a higher standard. Both the 400 MHz and 60 MHz gave high R^2 values and low
326 standard deviation of residuals. The 400 MHz NMR was shown to be more sensitive than 60
327 MHz NMR, as it is able to detect RSO adulteration down to 8% compared to 12.2%. The PLS
328 model for CPRO/RRO mixtures was much less accurate (Fig. 6), showing that RRO is a more
329 difficult to quantify adulterant. Neither the 400 MHz NMR nor the 60 MHz NMR could
330 achieve acceptable levels of detection, with limits of 72% and 74% respectively.

331 These results show that both 400 MHz and 60 MHz NMR can detect and quantify RSO in
332 CPRO down to low levels with 95% certainty. 60 MHz NMR may not be suitable however, if
333 adulteration is taking place below levels of 12% RSO. It is unlikely that adulteration would be
334 economically viable for potential fraudsters at levels below 8% RSO, therefore the 400 MHz

NMR would be suitable for quantification of RSO in CPRO in real world situations. In contrast, the limits of detection of RRO in CPRO were unacceptably high as RRO in CPRO would likely be below 75% RRO. This indicates that both the 400 MHz NMR and the 60 MHz are inadequate tools for quantifying the amount of RRO in CPRO with the experimental conditions explored in this study.

4. CONCLUSION

As expected the 400 MHz NMR produced superior classification and quantification results when compared with the 60 MHz NMR. In spite of this the 60 MHz NMR could still be considered competitive, especially when size and instrument cost are factored. When assessing the two NMR instruments for the potential to authenticate cold pressed rapeseed oil, the 60 MHz NMR produced classification results which were only slightly less sensitive than the more powerful 400 MHz NMR. The 400 MHz NMR was able to produce almost faultless classification results. Quantification analysis showed that 400 MHz NMR was better able to estimate the percentage of adulteration than the 60 MHz NMR.

The inherent strengths of the techniques investigated in this paper are their speed, non-destructive nature and high throughput. Their disadvantages are difficulty in quantifying adulteration and targeted analysis. Both 400 MHz and 60 MHz NMR have shown potential to act as rapid screening techniques to classify potentially adulterated cold pressed rapeseed oils.

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364

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