Utilization of front-face fluorescence spectroscopy for monitoring lipid oxidation during Lebanese Qishta aging

Mustapha Najib, Eliot Botosoa, Walid Hallab, Karim Hallab, Zaher Hallab, Monzer Hamze, Guillaume Delaplace, Romdhane Karoui, Nour-Eddine Chihib

Utilization of front-face fluorescence spectroscopy for monitoring lipid oxidation during Lebanese Qishta aging

Mustapha Najib[a,b,c], Eliot Patrick Botosoa[d], Walid Hallab[c], Karim Hallab[c], Zaher Hallab[c], Monzer Hamze[a], Guillaume Delaplace[b] ,Romdhane Karoui[d*],Nour-Eddine Chihib[b*]

[a]Health and Environment Microbiology Laboratory, Doctoral School of Sciences and Technology, Faculty of Public Health, Lebanese University, Tripoli, Lebanon

[b]UMET CNRS Laboratory, INRA*e*, UMR 8207-UMET-PIHM, Lille University, Villeneuve d'Ascq, France

[c]Hallab 1881 S.A.L., Tripoli, Lebanon

[d]Univ. Artois, INRA*e*, Ulco, Univ-Lille, Yncréa, Joint Research Unit 1154, ICV- Charles VIOLLETTE Institute, F-62300, Lens, France

*Corresponding authors:

Nour-Eddine CHIHIB ; Tel: +33 (0)3 59 63 22 46 ; E-mail : Nour-Eddine.chihib@univ-lille.fr

Romdhane Karoui ; Tel: +33 (0)3 21 24 81 03 ; E-mail : romdhane.karoui@univ-artois.fr

ABSTRACT

Front-face fluorescence spectroscopy technique coupled with chemometric tools was used for predicting the freshness state of a Lebanese dairy product called Qishta, stored up to 20 days. Acid, peroxide and thiobarbituric acid reactive substances (TBARS) values reached no more than 0.93 mg NaOH g^{-1} fat, 6.22 meq O_2 Kg^{-1} fat, and 0.0313 mg malonaldehyde (MA) kg^{-1} sample, respectively, throughout the investigated storage time. In parallel, fluorescence emission spectra of tryptophan and riboflavin, and fluorescence excitation spectra of vitamin A were recorded and showed the highest fluorescence intensity for the Qishta samples aged of 20 days and the lowest intensity for the fresh ones. The primary and secondary indicators of lipid oxidation showed that Qishta can be stored for 20 days without any alteration despite the increase in the TBARS after 16 days of storage. Principal component analysis (PCA) applied on riboflavin emission spectra allowed better discrimination between Qishta samples with a clear distinction of those aged 20 days while some overlapping was noticed between samples aged below 16 days. A high correlation (R^2 = 0.923) was observed between the peroxide value and the intensity of the riboflavin fluorescence recorded at 460 nm.

Keywords: Qishta, Fat Oxidation, Aging, Fluorescence spectroscopy, PCA

1. Introduction

Heat treatment is used as an efficient processing tool to increase the shelf life of food products such as dairy products. However, the heat treatment applied can generate undesirable effects such as fat oxidation and proteolysis (Ajmal et al., 2018; Vazquez-Landaverde, Torres & Qian, 2006). Nowadays, consumers are more conscientious and aim a category of fresh food free of artificial additives and being less processed.

Jensen, Ferris & Lammi-Keefe (1991) define the milk fat as an exceptional medium where different systems exist. Milk fat, which represents around 3 to 5 % in cow milk, is presented as small globules or droplets and dispersed in the aqueous phase of milk. Their diameters vary from 0.1 to 20 µm. Triglycerides are the principal components of milk fat. Moreover, di- and monoglycerides, fatty acids, sterols, carotenoids are present in milk giving it a yellow color (Jensen, 2002, Jensen, Ferris & Lammi-Keefe, 1991; Jensen et al., 1990).

In food products, the lipid may undergo autoxidation, photo-oxidation, thermal oxidation and enzymatic oxidation that differ in the type of free radical produced or oxygen species. Autoxidation is the most common oxidation process leading to oxidative deterioration, which can alter the texture and the flavor, generating some undesirable volatile products such as aldehydes, ketones, alcohols, esters, lactones, and hydrocarbons. The unsaturated aldehydes and ketones formed are mainly responsible for undesirable smells and taste in the dairy products known as rancidity (O'Brien & O'Connor, 2011).

Qishta is a Lebanese heated dairy product consumed as a dessert and used as a filler in some Oriental sweet production such as Knefeh, Mafrouke, etc. It can be defined as a lipo-protein product containing almost an equal amount of fat and protein (≈ 12%), pH quite similar

60 to milk (≈ 6.4), high a_w (≈ 0.98) and high moisture content (≈ 70 %). Due to these characteristics,
61 Qishta is considered as highly perishable items and has a shelf life of 5 days when stored at 4 °C
62 (Kassaify et al., 2010). Despite the wide consumption of Qishta in Lebanon and Middle East
63 countries, no bibliography was found investigating the effect of the fat oxidation on its shelf life.
64 Primary and secondary indicators of fat oxidation have been used as indicators of food
65 quality (Barriuso, Astiasarán & Ansorena, 2013; Botosoa, Chèné & Karoui, 2013; O'Brien &
66 O'Connor, 2011; Al-Rowaily, 2008). Al-Rowaily (2008) has studied the effect of heat treatment
67 on the chemical variations of lipids in some local dairy products using peroxide, *p*-anisidine,
68 TBARS, acid and TOTOX values. Even though the physico-chemical analyses had been proved
69 as efficient methods, they are considered as time-consuming and require the use of a lot of
70 chemical products.
71 Rapid screening techniques are mainly used nowadays to predict the fat oxidation level
72 instead of the laborious and destructive methods mentioned above. Front-face fluorescence
73 spectroscopy (FFFS) is considered today as a rapid, non-destructive, and relatively cheap
74 technique, for measuring quality parameters especially in the dairy field (Kamal & Karoui, 2017;
75 Botosoa, Chèné & Karoui, 2013; Karoui, Dufour & De Baerdemaeker, 2007; Karoui et al.,
76 2006a; 2006b; Miquel Becker et al., 2003). This technique was used to: i) predict the fat
77 oxidation of semi-hard cheeses during ripening (Karoui, Dufour & De Baerdemaeker, 2007); ii)
78 monitor the light-induced changes in plain yogurt and the effect of light on the oxidation of
79 cheese products during storage (Miquel Becker et al., 2003; Christensen, Povlsen & Sørensen,
80 2003; Mortensen et al., 2003; Wold, Jørgensen & Lundby, 2002). Finally, Karoui, Martin &
81 Dufour (2005) have utilized the FFFS technique to discriminate milk samples according to their
82 geographic origins. The high abundance of fluorophores in milk products such as vitamin A,

tryptophan, riboflavin and nicotinamide adenine dinucleotide (NADH), among several other fluorescent compounds, gave this technique an added value in the dairy field (Karoui et al., 2006b).

Our study aims to monitor fat oxidation of Qishta during its storage for up to 20 days at 4 °C using: i) primary and secondary indicators of oxidation; and ii) FFFS as a rapid and non-destructive technique to predict the chemical changes, especially lipid oxidation. To the best of our knowledge, this study is the first to aim in investigating the effect of storage on the lipid oxidation of Qishta.

2. Materials and Methods

2.1.Production of Qishta

According to Hallab 1881 company (Lebanon), the process of Qishta consists of heating whole milk in a large open shallow plate (diameter of 1 m and capacity of 12 L) from one side for 3 hours. Fifteen minutes after the beginning of heating, aggregates appear on top of the milk in the heated zone, where the temperature reaches 100 °C. These aggregates, defined as a mixture of protein and fat globule, are the main components of Qishta. Milk is added continuously in order to readjust its level in the plate due to the evaporation of water. The aggregates and/or the gel formed were gathered at the opposite side of the flame until a precise amount was obtained (usually between 2 and 3 Kg). Qishta was drained at 4 °C for one hour and then distributed in 14 plastic containers with a capacity of 150 g each, and stored at 4 °C during 0, 3, 6, 9, 16 and 20 day(s).

104 Milk was provided from Lactel (Lactel, France). It is an UHT whole milk having 3.6 % fat and
105 3.2 % protein. The plate (stainless-steel, 316 L) was provided from Nafco (Nafco, Lebanon) and
106 the burner was provided from AEM (AEM, Chelles, France) equipped with a pressure regulator.

107 **2.2.Physicochemical analysis**

108 Acetic acid glacial 100 %, dichloromethane, n-hexane, Celite® 545, sodium sulphate anhydrous,
109 ethanol denatured 95 % volume and propan-2-ol were purchased from VWR (France), while
110 Para-anisidine and 2-thiobarbituric acid reagent were provided from MERCK (Germany).
111 Roquette (France) and VWR (France) provided starch and chloroform respectively. Potassium
112 iodide 99 %, phenolphthalein solution (1 % ethanol) and pure sodium hydroxide (97 %) were
113 purchased from VWR (France) and LABOGROS (France). Finally, Isooctane (UV-IR-HPLC)
114 and sodium thiosulphate were provided from VWR (France) and Panreac Quimica (Spain),
115 respectively.

116

117 **2.3.Extraction of fat**

118 Fat extraction was held at the same day of the analyses and according to the Association
119 Françoise de Normalisation (AFNOR (1991). A volume of 400 mL of hexane/ethanol (3/1; v/v)
120 was added to 135 g of Qishta. The mixture was stirred at ambient temperature for 90 minutes
121 (10^3 rpm). The extracted fat was filtered by Whatman No. 1 filter paper. It was then eluted and
122 separated from the mixture by passing it through a 2 cm high column of Celite® and sodium
123 sulphate laid on the bottom of a Büchner filter. The filtrate was dried in a rotary evaporator
124 (Büchi, Rotavap R-3) at speed 4. Bath temperature was previously equilibrated at 40 °C. All the
125 physico-chemical analyses were performed in triplicate.

126

2.4.Measurement of acid value

Acid value of Qishta was determined according to AOCS (Cd 6d-63, 1997) method with a slight modification since only 2.5 g of the extracted fat were mixed with 15 mL of a solution of ethanol/dichloromethane (1/1; v/v) (Botosoa, Chèné & Karoui, 2013).

2.5.Measurement of peroxide values

Peroxide value of Qishta was analyzed according to AOCS (Cd 8-53, 1997) method with a slight modification, which corresponded to the fact that only 20 mL of a mixture of acetic acid / chloroform (3/2; v/v) and 15 mL of ultrapure water were necessary for 2.5 g of extracted fat. The titration was done using sodium thiosulfate ($Na_2S_4O_6$) (0.005 mol L^{-1}) (Botosoa, Chèné & Karoui, 2013).

2.6.Measurement of thiobarbituric acid

Thiobarbituric acid value of Qishta, during storage was determined using the direct method described by Pokorny and Dieffenbacher (1989) with a slight modification. 0.05 g of extracted fat was mixed with 5 mL of TBA solution (0.02 mol L^{-1}) and 5 ml of butanol and maintained in a heated bath (Büchi, France) equilibrated at 95 °C for 2 hours. The solution was cooled with tap water for 10 minutes before analyzing the absorbance values. TBA reacting with malonaldehyde (MA) forms a pink MA-TBA complex at 530–535 nm, which was measured using a spectrophotometer (UV 2600, Shimadzu, Noisiel, France).

2.7.Fluorescence spectroscopy measurements

149 Fluoromax-4 spectrofluorimeter (Jobin Yvon, Horiba, NJ, USA) was used in order to record the
150 fluorescence spectra. The incidence angle of the excitation radiation was set at 60° in order to
151 minimize the reflected light, the scattered radiation and the depolarization phenomena. The
152 spectrofluorimeter was supplied with a thermostated cell and Haake A25, AC 200 temperature
153 controller (Thermo-Scientific, France). A quartz cuvette filled with three grams of Qishta was
154 used for the analysis. Spectra of Qishta were recorded in duplicate. The sample was illuminated
155 by the photons of excitation (light beam: ~3 mm wide and ~0.3 mm high) at its center for 3 min,
156 limiting sample dehydration. Emission spectra of tryptophan ($305 < \lambda em < 450$ nm) and
157 riboflavin ($405 < \lambda em < 650$ nm) were recorded after excitation at 290 nm and 380 nm
158 respectively. Excitation spectra of vitamin A ($252 < \lambda ex < 390$ nm) were acquired with the
159 emission wavelength set at 410 nm. A rhodamine cell in the reference channel was used in order
160 to correct the recorded spectra.
161
162 **2.8.Mathematical analyses of data**
163 All spectra recorded were normalized by reducing the area under each spectrum to a value of 1 in
164 order to reduce the scattering effects (Karoui et al., 2007; 2008). Principal component analysis
165 (PCA) was applied on the normalized spectra to visualize the variations between Qishta samples
166 during storage. PCA is a descriptive, representative and exploratory method aiming to reduce the
167 variables into a lower number. It allows the visualization of correlations among the original
168 variables by finding a combination between them that describe the major trends in the data.
169 Variables are transformed into new orthogonal axes called principal components (PCs). In
170 addition, the eigenvectors are similar to spectra and are nominated spectral patterns. The
171 interpretation of the positive and negative peaks allows a better characterization of the emission

and excitation spectra. The correlation between the spectral patterns and the PCs allows the characterization of the potential wavelengths used to discriminate between spectra.

PCA and spectral patterns were performed using MATLAB version R 2013b and 2014a (The MathWorks Natick, MA, USA) and PLS Toolbox 7.9 and 8.0 (Eigenvector Research Inc., Wenatchee, WA, USA).

One-way analysis of variance (ANOVA) was conducted using the SPSS software for Windows (version 13.0, SPSS). A Duncan test was carried out to assess any significant differences between the means. The difference was considered statistically significant when $P \leq 0.05$.

3. Results and discussion

3.1. Evolution of the physico-chemical parameters during aging

3.1.1. Evolution of acid value during Qishta aging

Table 1 showed the variation of acid value during 20 days of Qishta storage at 4°C. Acid value reflects the quantity of acids resulting from the hydrolysis of triacylglycerols. In fact, the term free fatty acid refers to a fatty acid without glycerol (Mannion, Furey & Kilcawley, 2016). Acid values varied between 0.8 and 0.9 mg NaOH g^{-1} fat. The minimum value (0.8) was obtained for Qishta samples aged 20 days, while the highest one (0.93) was observed for those kept for 16 days at 4 °C. Fresh Qishta (0 day) exhibited an acid value of 0.89 mg NaOH g^{-1} fat, which is significantly higher compared with values usually reported for UHT milk. This significant increase ($P<0.05$) may be due to the impact of heat treatment (100 °C) during the production of Qishta related to lactose degradation and formations of acids such as formic acid. In addition, it was reported that half of this increase of acid value is due to the formation of organic acids from lactose; the remainder is due to the precipitation of calcium phosphate and dephosphorylation of casein (Fox et al., 2015).

196 The significant increase ($P<0.05$) of acid value observed between days 9 and 16 could be
197 ascribed to the development of microorganisms presenting lipolytic activity during storage at 4
198 °C. Thereby, psychrotrophic bacteria, yeasts and molds are usually present in refrigerated
199 products.
200
201 ***3.1.2. Evolution of primary oxidation products during Qishta aging***
202 Hydroperoxides formation, as a result of primary lipid oxidation, is responsible of undesired
203 reactions, giving rise to complexes of saturated and unsaturated ketones and aldehydes. These
204 molecules can negatively alter the flavor and the color of food products (Ramis-Ramos, 2003).
205 **Table 1** showed the variation of primary parameters of oxidation values. Concerning the
206 evolution of peroxide, the average value decreased from 6.22 to 3.47 meq O_2 kg^{-1} fat after 20
207 days of storage. Fatty products having a peroxide value higher than 20 meq O_2 kg^{-1} fat are
208 considered rancid and non-edible, while a value between 0 and 5 meq O_2 kg^{-1} fat corresponds to
209 a fresh high-quality product (O'Keefe & Pike, 2010). Fresh Qishta has a peroxide value of 6.22
210 meq O_2 kg^{-1} fat which reflects a medium level of oxidation. The sudden decrease between day 16
211 and 20 could be explained by the formation of the secondary products of oxidation. Rehman &
212 Salariya (2006) found an amount of 0.38 and 17.8 meq O_2 kg^{-1} fat for fresh and aged Khoa stored
213 for 10 days at 25 °C. Khoa is an Indian dairy product obtained by a similar process to that of
214 Qishta. Al-Rowaily (2008) noted an amount of 0.155 meq O_2 kg^{-1} fat for raw cow milk. The high
215 peroxide value obtained in fresh Qishta could be due to the use of UHT milk and the process
216 applied which consists of boiling milk for more than 3 hours.

Considering the primary indicators of fat oxidation, a decrease in both acid and peroxide values was observed after 20 days of storage; Qishta samples can be considered acceptable since the values are within the acceptable range.

3.1.3. Evolution of secondary products during Qishta aging

The oxidation level of Qishta was better represented by TBARS than by peroxide value since the noticeable increase in the TBARS of Qishta aged of 20 days was not reflected in peroxide value. Al-Rowaily (2008) reported a similar tendency while measuring the TBA value of microwave heated milk. TBARS measures mainly malonaldehydes (O'Keefe & Pike, 2010) but at those levels of oxidation TBARS could be more representative for oxidation status than peroxide value (Al-Rowaily, 2008). During 20 days of storage, the TBARS values increased from 0.0076 to 0.0313 (**Table 1**). Qishta exhibited the highest value of 0.0313 at 20 days of storage. The most important increase of TBARS (0.0175 to 0.0313) was observed between the 16^{th} and 20^{th} day of storage with a difference of 0,0138. Despite this increase, the values obtained reflected an acceptable quality of Qishta even at 20 days of storage at 4 °C. Indeed, these values, particularly TBARS of Qishta aged 16 days (0.0175), are significantly lower when compared to those obtained by Al-Rowaily, (2008), who reported TBARS value of 0.086 for yogurt and 0.021 for Labaneh produced by conventional method after 15 days of refrigerated storage. Ishak & Abdullah (2011) considered cakes as non-rancid if the TBARS value was less than 0.576 mg MA kg^{-1}. The TBARS values of raw cow milk and UHT milk were 0.014 and 0.027 mg MA kg^{-1} respectively (Al-Rowaily, 2008).

Based on these results (primary and secondary indicators of lipid oxidation), Qishta can be considered as acceptable and non-rancid and can be stored in the same conditions without

affecting the lipid oxidation. These results must be coupled with microbiological and sensory analysis in order to conclude on the shelf life of Qishta.

3.2.Evolution of fluorescence spectra during Qishta aging

The abundance of intrinsic fluorophores in dairy products, such as vitamin A, riboflavin, tryptophan, NADH and so on, has promoted the development of fluorescence spectroscopy methods. These techniques can provide, coupled with the chemometric tools, some chemical and physical properties of food products. FFFS is considered as a cheap, rapid and non-destructive method that could replace the physico-chemical analyses (Andersen & Mortensen, 2008; Karoui & De Baerdemaeker, 2007). The fluorescence spectra of vitamin A was reported to provide information related to: i) the interaction between proteins and lipids; and ii) the physical state of the triglycerides (Andersen & Mortensen, 2008); while the fluorescence spectra of riboflavin was linked with protein and fat oxidation (Karoui et al., 2006; Becker et al., 2003).

3.2.1. Fluorescence spectra of tryptophan acquired after excitation at 290 nm on Qishta samples during aging

In dairy products analysis, tryptophan emission spectra was considered as an indicator of the protein structure (Andersen and Mortensen, 2008). **Figure 1a** showed the normalized emission spectra acquired after excitation wavelength set at 290 nm. The emission spectra of all samples exhibited a maximum at around 375 nm. Except for the 20 days aged Qishta that had the highest fluorescence intensity and the largest width, all Qishta samples exhibited almost the same emission spectra. The observed shift from the maximum emission wavelength to the larger wavelength range can be explained by exposing more tryptophan residues to the aqueous phase

of aged Qishta samples, in agreement with previous findings of Karoui et al.(2008) who observed a red shift of tryptophan emission spectra acquired on aged egg albumen. Additionally, the exposed tryptophan residues in Qishta could be shielded from the aqueous phase by other protein molecules as a result of protein–protein interactions, the rate of which increases with protein unfolding. The high fluorescence intensity observed for Qishta aged 20 days could be due to protein aggregation that impact significantly the fluorescence intensity compared to the storage time-induced protein unfolding, which causes more tryptophan residues to become exposed to the aqueous phase of Qishta samples.

3.2.2. Fluorescence properties of vitamin A acquired after emission at 410 nm on Qishta samples during aging

Figure 1b showed the excitation fluorescence spectra recorded after emission wavelength set at 410 nm. These excitation spectra exhibited a maximum peak located at ~320 nm and two other minors located at 292 and 305 nm. These observations are in agreement with previous findings of Karoui and Dufour (2003) reporting that the maximum fluorescence intensity of vitamin A excitation spectra scanned on different varieties of soft cheese, after emission at 410 nm, was located at 322 and 305 nm. Again, as observed for tryptophan spectra, vitamin A spectra acquired on Qishta samples aged 20 days presented the highest fluorescence intensity. Indeed, Qishta samples aged 0 day had the lowest fluorescence intensity at 320 nm, while those kept up to 20 days had the highest one. It has been reported that the shape of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globules (Karoui et al., 2006a). The ratio of fluorescence intensity at 320nm/292 nm increase with the increase of

storage time which could be explained by the increase of the viscosity of triglycerides. This could be attributed to cristallization of triglyceride during storage, in agreement with previous findings (Andersen & Mortensen, 2008; Karoui et al., 2006a). Similar trend was observed during the ripening of semi-hard cheeses since changes in the fluorescence intensity ratios at 322 nm/295 nm were noted and ascribed to the crystallization of triglycerides between 1 day and 81 days of ripening (Dufour et al., 2000). Finally, the changes in the shapes of vitamin A spectra may also result from fluorescence transfer between tryptophan residues of proteins and vitamin A located in the fat globule membrane.

3.2.3. Fluorescence properties of riboflavin acquired after excitation at 380 nm on Qishta samples during aging

Considering the riboflavin fluorescence spectra (**Figure 1c**), the emission spectra exhibited 2 maxima located at 455 and 530 nm. Except for Qishta spectra acquired on day 0, the fluorescence intensity increased with the storage time. As observed for vitamin A and tryptophan spectra, the 20 days aged Qishta exhibited the highest fluorescence intensity.

An increase in the fluorescence intensity in the region located between 405 and 480 nm was noticed during the storage period of Qishta. This region was reported to reflect the oxidation resulted from the products formed by aldehydes and amino acids. In the same region, lumichrome, a photo breakdown product from riboflavin, exhibits fluorescence between 444–479 nm. In addition, β-carotene absorbs in the region located between 400–500 nm. β-carotene can also undergo photodegradation, which may influence the shape of riboflavin fluorescence spectra. The obtained results are in line with the findings of Karoui et al., (2007; 2006a) who observed an increase of the fluorescence intensity of spectra acquired after excitation set at 380

309 nm for both egg and cheese. Surprisingly, we noticed an increase of the fluorescence intensity at
310 530 nm, which is in discordance with the findings of Wold et al., (2002). An explanation could
311 arise from the transfer of energy that occurs between fluorescent compounds allowing an
312 increase of fluorescence intensity at 530 nm and/or from the presence of other fluorophores in
313 Qishta having maximum excitation at 530 nm.
314
315 ***3.3.Discrimination based on fluorescence spectra recorded on Qishta samples***
316 **3.3.1. Evaluation of the discriminant ability of fluorescence spectra of tryptophan**
317 **acquired after excitation wavelength set at 290 nm on Qishta samples during 20**
318 **days of storage.**
319
320 Most of the investigated spectra presented similar shapes, therefore it was of high importance to
321 find a mean in order to distinguish between the samples studied. Thus, PCA was used to extract
322 information from the data tables. This multidimensional statistical technique was applied to the
323 12 spectra collected on Qishta at different storage times (**Figure 2**). The map defined by PCs 1
324 and 2 (69.74 and 27.79 % of the total variance, respectively) of the PCA performed on spectra
325 acquired after excitation at 290 nm (corresponding to tryptophan) divided the samples into 2
326 groups. Group 1 consisted mostly of the samples aged from 0 to 16 days, while group 2
327 comprised the samples aged 20 days. A clear differentiation was shown between these 2 groups
328 since the former group is located mostly on the negative side of PC1, while the latter one is
329 positioned on the positive side. However, the distinction inside group 1 was not feasible since
330 Qishta samples were overlapped.
331

3.3.2. Evaluation of the discriminant ability of fluorescence spectra acquired on Qishta excitation during storage after emission wavelength at 410 nm on Qishta

Concerning PCA applied to the excitation spectra recorded after emission at 410 nm (excitation spectra of vitamin A), a better discrimination was obtained than that observed with the emission tryptophan spectra. In fact, the map defined by PCs 1 and 2 (93.42 % and 5.44 % of the total variance, respectively) showed some clear discrimination of Qishta samples according to their storage time (**Figure 3a**). Qishta samples aged 20 days were always distinguishable from all the other samples. Regarding PC1, all samples had negative score values except those aged 20 days and one sample aged 9 days.

The spectral pattern 2 showed an opposition between a negative peak located at 310 nm and a positive one at 375 nm (**Figure 3b**) indicating major changes at the molecular level between samples aged 0 and 3 days from the others. Karoui & Dufour (2003) have already obtained this spectral pattern while they were comparing the difference between the centers and the surfaces of ripened soft cheeses. They suggested that the shape of the spectral pattern of vitamin A reflects the variation occurred in the triglyceride molecules, as well as the interaction between proteins and fat globules during cheese ripening and storage. Botosoa et al., (2013) have used the spectral pattern of vitamin A in order to discriminate between cake samples during aging. According to the results obtained on the emission and excitation spectra of tryptophan and vitamin A respectively, it can be concluded that Qishta samples aged 20 days can be discriminated from other samples. The distinction between Qishta samples aged 0, 3, 6, 9 and 16 was not so clear due to the overlapping observed. The differences detected could be due to the interaction developed between protein and fat globule during the storage, aggravated by the high moisture

355 content which has been suggested to increase the molecular interactions in the food matrix
356 (Botosoa, Chèné & Karoui, 2013; Karoui and Dufour, 2003).
357
358 **3.3.3. Evaluation of the discriminant ability Fluorescence spectra of riboflavin**
359 **acquired after excitation at 380 nm on Qishta samples**
360 The map defined by PC 1 and 2 (97.13 % and 2.57 % of the total variance, respectively) showed
361 always a clear discrimination between samples aged 20 days and other samples (**Figure 4a**).
362 Concerning PC1, Qishta samples aged 0, 3 and 6 days had negative values while almost all the
363 other samples (age > 6 days) exhibited positive values. It can be concluded that PC1 divided the
364 samples according to their ages, and contrary to the previous spectra, the discrimination between
365 all Qishta samples was better with less overlapping. These results were in accordance with the
366 TBARS analysis since the map defined by PCs 1 and 2 divided the samples into 3 groups: the
367 first one consists of Qishta aged 0 day, the second one belongs to Qishta aged 3, 6, 9 and 16 days
368 and the last one contains Qishta aged 20 days. The ANOVA test held on the TBARS values has
369 also divided the Qishta samples into 3 groups significantly different and consisting of the same
370 Qishta samples.
371 The spectral pattern 2 (**Figure 4b**) showed a positive peak located around 460 nm suggesting the
372 formation of photo break down products from Qishta samples stored for 3, 6, 9 and 16 days.
373 The correlation between primary indicators of oxidation and high-intensity bands obtained at 460
374 nm of the spectra scanned after excitation set at 380 nm was investigated. A high correlation (R^2
375 = 0.923) was found between peroxide values and the fluorescence intensity. A negative
376 correlation was noticed between peroxide value and normalized fluorescence intensity at 460 nm

377 suggesting probably that this could be used as an indicator of the transformation of primary
378 products to secondary ones.
379
380 ## 4. Conclusion
381 From fat oxidation point of view, the physico-chemical results have shown that Qishta could be
382 stored for 20 days without any quality deterioration. TBARS value highly increased after 20 days
383 while peroxide value showed a slight decrease. Fluorescence spectra coupled with chemometric
384 tools were able to detect the variation that occurred during Qishta storage. In fact, PCA showed
385 clear discrimination between Qishta samples aged 20 days and all other samples. Tryptophan and
386 vitamin A showed an overlapping between samples aged from 0 to 16 days, while the
387 fluorescence spectra corresponding to riboflavin demonstrated its ability to determine the
388 freshness level of Qishta. A high correlation was observed between the fluorescence intensity at
389 460 nm and peroxide values. It could be concluded that riboflavin spectra could be used as an
390 effective tool for the evaluation of Qishta freshness.
391
392 **Acknowledgments**
393 Mr. Najib is grateful to Hallab 1881 Company Tripoli Lebanon for its financial grant of his
394 Ph.D.

References

AFNOR, (1991). Agricultural products and foodstuffs – Extraction of the crude fat with a view to its characterization. *In AFNOR (Ed.), (pp. 11). Paris*.

Ajmal, M., Nadeem, M., Imran, M. & Junaid, M. (2018). Lipid compositional changes and oxidation status of ultra-high temperature treated milk. *Lipids in Health and Disease*, *17*, 127.

Al-Rowaily, M. A. (2008). Effect of Heating Treatments, Processing Methods and Refrigerated Storage of Milk and Some Dairy Products on Lipids Oxidation. *Pakistan Journal of Nutrition*, 7, 118-125.Andersen, C. M. & Mortensen, G. (2008).

Fluorescence Spectroscopy: A Rapid Tool for Analyzing Dairy Products. *Journal of Agricultural and Food Chemistry*, *56*, 720–729.

AOCS (1997) Acid Value. Official Method Cd 3d-63. *In Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, Champaign, American Oil Chemists' Society Press.

AOCS (1997) Peroxide Value Acetic Acid - Chloroform Method. Official Method Cd 8-53. *In Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, Champaign, American Oil Chemists' Society Press.

Barriuso, B., Astiasarán, I. & Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: A challenging task. *European Food Research and Technology*, *236*, 1-15.

Becker, E., Christensen, J., Frederiksen, C. S. & Haugaard, V. K. (2003). Front-Face Fluorescence Spectroscopy and Chemometrics in Analysis of Yogurt: Rapid Analysis of Riboflavin. *Journal of Dairy Science*, *86*, 2508–2515.

418
419 Botosoa, E. P., Chèné, C. & Karoui, R. (2013). Use of front face fluorescence for monitoring
420 lipid oxidation during ageing of cakes. *Food Chemistry*, *141*, 1130–1139.

421 Christensen, J., Povlsen, V. T. & Sørensen, J. (2003). Application of fluorescence spectroscopy
422 and chemometrics in the evaluation of processed cheese during storage. *Journal of Dairy*
423 *Science*, *86* ,1101–1107.

424 Dufour, E., Mazerolles, G., Devaux, M.F., Duboz, G., Duployer, M.H., & Mouhous Riou, N.
425 (2000). Phase transition of triglycerides during semi-hard cheese ripening. *International Dairy*
426 *Journal, 10,* 81–93.
427 Dufour, E. & Riaublanc, A. (1997). Potentiality of spectroscopic methods for the characterisation
428 of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. *Le*
429 *Lait*, *77*, 657–670.

430 Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., & O'Mahony, J. A. (2015). Heat-induced
431 changes in milk. *In Dairy chemistry and biochemistry.* 345-375. Springer, Cham.
432
433 Ishak, I. & Abdullah, N. (2011). Evaluation of the antioxidant potential of some Malaysian
434 herbal aqueous extracts as compared with synthetic antioxidants and ascorbic acid in cakes.
435 *International Food Research Journal*, *18*, 583-587.

436 Jensen, R. G. (2002). The Composition of Bovine Milk Lipids: January 1995 to December 2000.
437 *Journal of Dairy Science*, *85*, 295–350.

438 Jensen, R. G., Ferris, A. M. & Lammi-Keefe, C. J. (1991). The composition of milk fat. *Journal*
439 *of Dairy Science*, *74*, 3228–3243.

440 Jensen, R. G., Ferris, A. M., Lammi-Keefe, C. J. & Henderson, R. A. (1990). Lipids of Bovine
441 and Human Milks: A Comparison1. *Journal of Dairy Science*, *73*, 223–240.

442 Kamal, M. & Karoui, R. (2017). Monitoring of mild heat treatment of camel milk by front-face
443 fluorescence spectroscopy. *LWT - Food Science and Technology*, *79*, 586–593.

444 Karoui, R. & De Baerdemaeker, J. (2007). A review of the analytical methods coupled with
445 chemometric tools for the determination of the quality and identity of dairy products. *Food*
446 *Chemistry*, *102*, 621–640.

447 Karoui, R., Dufour, E. & De Baerdemaeker, J. (2006a) Common components and specific
448 weights analysis: A tool for monitoring the molecular structure of semi-hard cheese throughout
449 ripening. *Analytica Chimica Acta*, *572*, 125-133.

450 Karoui, R. & Dufour, É. (2003). Dynamic testing rheology and fluorescence spectroscopy
451 investigations of surface to centre differences in ripened soft cheeses. *International Dairy*
452 *Journal*, *13*, 973–985.

453 Karoui, R., Dufour, É. & De Baerdemaeker, J. (2007). Front face fluorescence spectroscopy
454 coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout
455 ripening. *Food Chemistry*, *101*, 1305–1314.

456 Karoui, R., Nicolaï, B., de Baerdemaeker, J. (2008). Monitoring the egg freshness during
457 storage under modified atmosphere by fluorescence spectroscopy. Foo*d and Bioprocess*
458 *Technology, 1*, 346-356.
459

460 Karoui, R., Martin, B., & Dufour, É. (2005). Potentiality of front-face fluorescence spectroscopy
461 to determine the geographic origin of milks from the Haute-Loire department (France). *Lait*, *85*,
462 223–236.

463 Karoui, R., Mouazen, A., Dufour, É., Schoonheydt, R., & De Baerdemaeker, J. (2006b).
464 Utilisation of front-face fluorescence spectroscopy for the determination of some selected
465 chemical parameters in soft cheeses. *Lait*, *86*, 155–169.

466 Kassaify, Z. G., Najjar, M., Toufeili, I. & Malek, A. (2010). Microbiological and chemical
467 profile of Lebanese qishta [heat-coagulated milk]. *EMHJ - Eastern Mediterranean Health*
468 *Journal, 16,* 926-931.

469 Machado, G. S., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M. C. D., De Block, J. &
470 Heyndrickx, M. (2017). The biodiversity of the microbiota producing heat-resistant enzymes
471 responsible for spoilage in processed bovine milk and dairy products. *Frontiers in Microbiology*,
472 *8*, 302.
473
474 Mannion, D. T., Furey, A. & Kilcawley, K. N. (2016). Free fatty acids quantification in dairy
475 products. *International Journal of Dairy Technology*, *69*, 1–12.

476 Mortensen, G., Sørensen, J., Danielsen, B. & Stapelfeldt, H. (2003). Effect of specific
477 wavelengths on light-induced quality changes in Havarti cheese. *Journal of Dairy Research*, *70*,
478 413–421.

479 O'Brien, N. M. & O'Connor, T. P. (2002). Milk Lipids | Lipid Oxidation. *Encyclopedia of Dairy*
480 *Sciences*. 1600-1604.

Pokorny, J. & Dieffenbacher A. (1989). Determination of 2-thiobarbituric acid value : Direct method. *Pure and Applied Chemistry*, *61*, 1165-1170.

O'Keefe, S. F. & Pike, O. A. (2010). Fat Characterization. *Food Analysis*. 407-429.

Ramis-Ramos, G. (2003). ANTIOXIDANTS | Synthetic Antioxidants. *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. 265–275.

Rehman, Z. & Salariya, A. M. (2006). Effect of synthetic antioxidants on storage stability of Khoa – a semi-solid concentrated milk product. *Food Chemistry*, *96*, 122–125.

Tadesse, N., Reta, N. & Beyero, N. (2017). Level of Saturation and Anti-Oxidant Value of Heat and Spice Treated Animal Butter. *Food and Public Health*, *7*, 81–90.

Vazquez-Landaverde, P. A., Torres, J. A. & Qian, M. C. (2006). Effect of high-pressure-moderate-temperature processing on the volatile profile of milk. *Journal of Agricultural and Food Chemistry*, *54*, 9184–9192.

Wold, J. P., Jørgensen, K. & Lundby, F. (2002). Nondestructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *Journal of Dairy Science*, *85*, 1693–1704.

Table 1: Primary and secondary indicators of oxidation of Qishta stored at 4 °C for 20 days.

	Primary lipid products		Secondary lipid products
Aging time (days)	Acid value (mg NaOH g^{-1} fat)	Peroxide value (meq O_2 kg^{-1} fat)	TBARS (532 nm)
0	$0.89^{a,b}$	6.22^{a}	0.0076^{c}
3	0.86^{b}	6.06^{a}	0.0108^{b}
6	$0.84^{b,c}$	5.37^{b}	0.0110^{b}
9	0.86^{b}	5.56^{b}	0.0167^{b}
16	0.93^{a}	5.29^{b}	0.0175^{b}
20	0.80^{c}	3.47^{c}	0.0313^{a}

Means values within a column sharing a common alphabet do not differ significantly ($p < 0.05$); values presented are mean values for three samples ($n = 3$).

Figure 1: Normalized emission fluorescence spectra recorded after excitation wavelength set at (a) 290 nm, (b) 410 nm and (c) 380 nm on Qishta sample aged 0 (-), 3(– . -), 6(- . .), 9 (- - -), 16 (- . -) and 20 days (…).

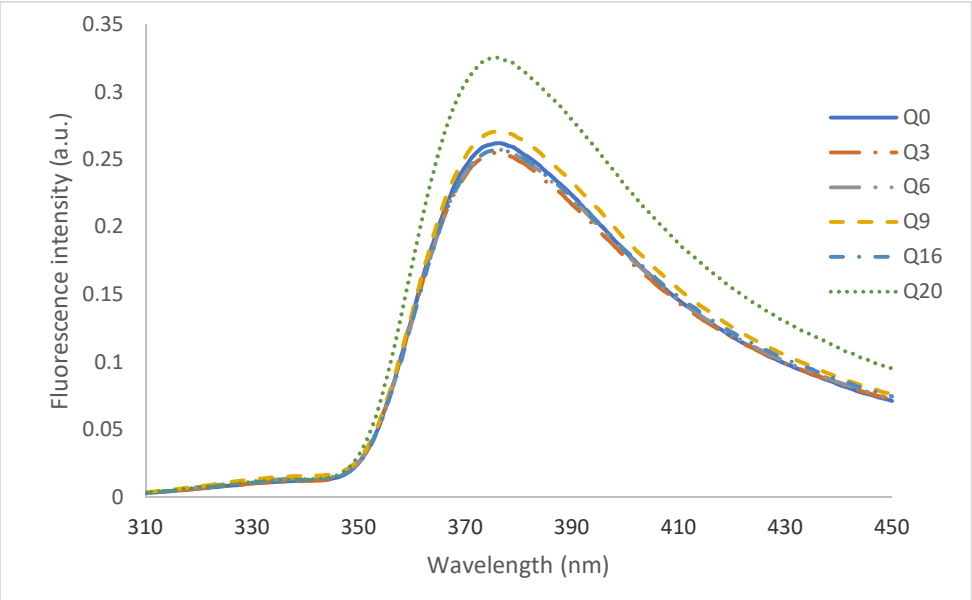
Figure 2: PCA similarity map defined by the principal components 1 and 2 after excitation wavelength set at 290 nm on Qishta samples during 20 days of storage.

Figure 3: (a) PCA similarity map defined by the principal components 1 and 2 after emission wavelength set at 410 nm on Qishta samples during 20 days of storage and (b) Spectral pattern corresponding to PC1 and PC2.

Figure 4: (a) PCA similarity map defined by the principal components 1 and 2 after excitation wavelength set at 380 nm on Qishta samples during 20 days of storage and (b) Spectral pattern corresponding to PC1 and PC2

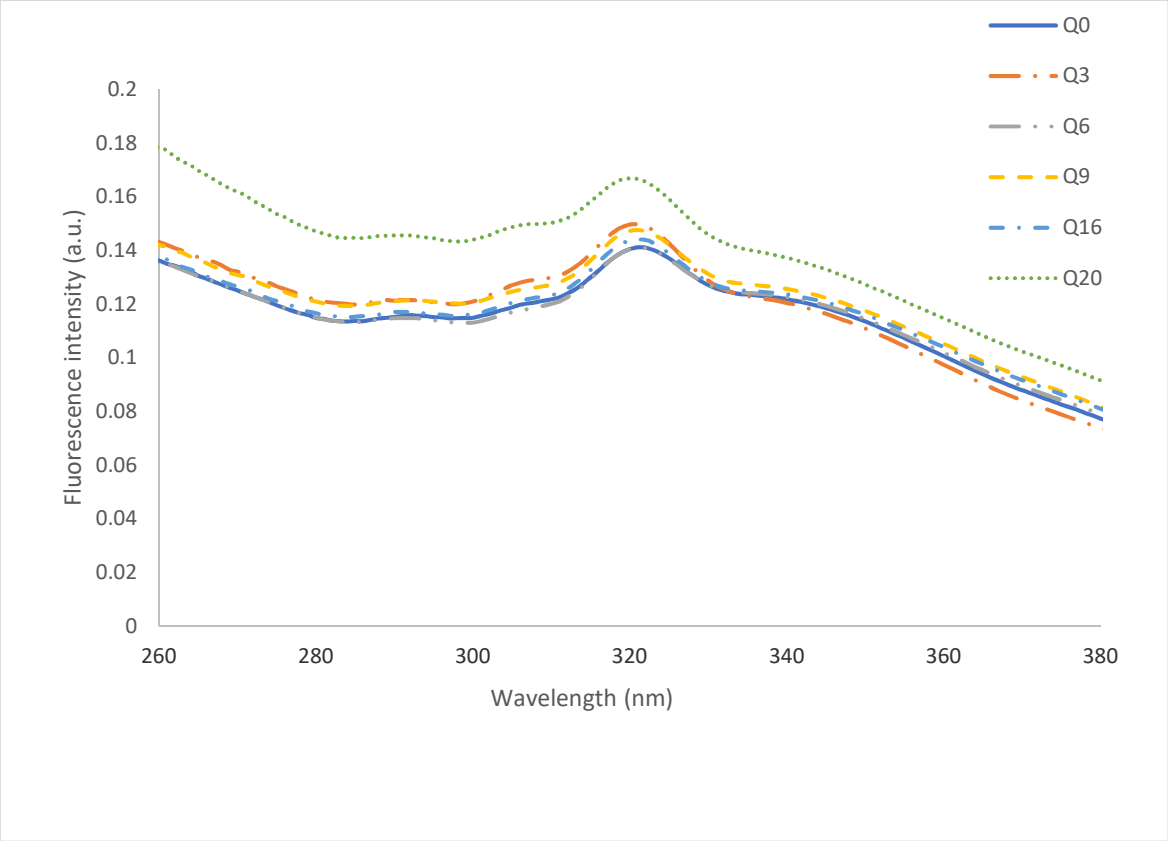
520 Figure 1a:

521



522

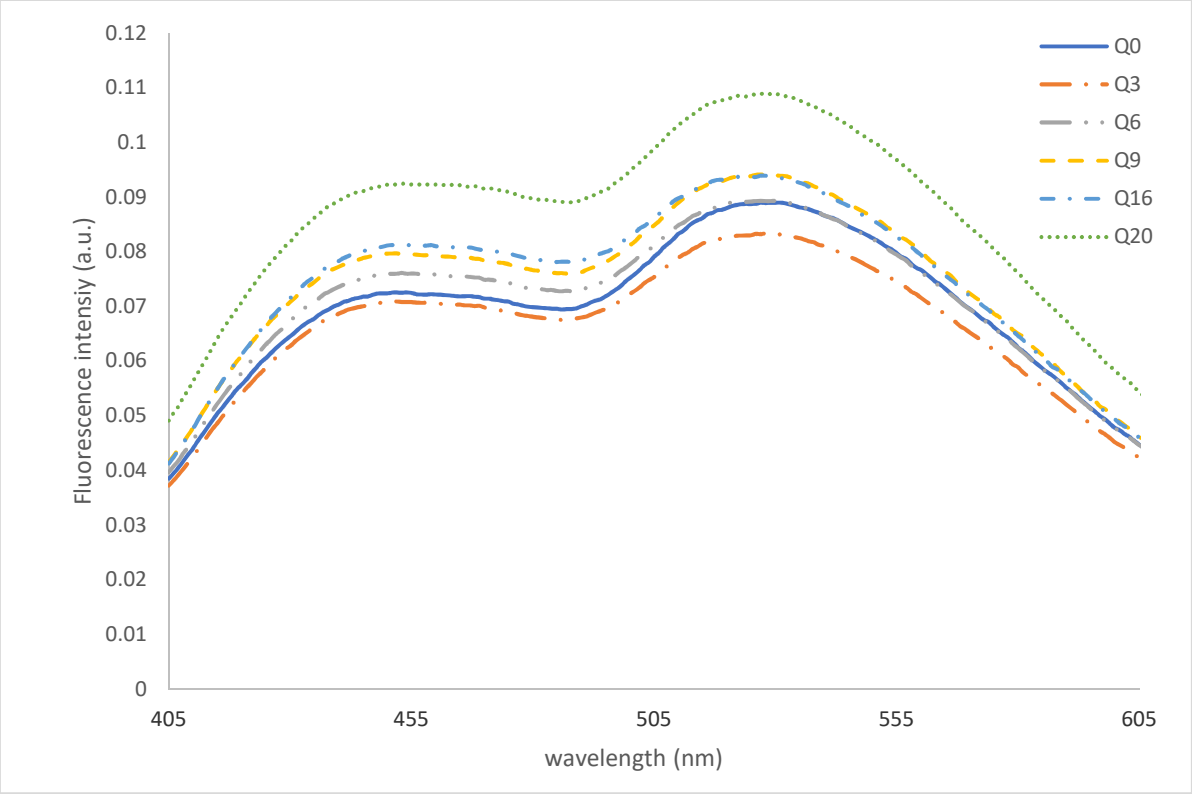
523 Figure 1b:



524

525

526 Figure 1c:

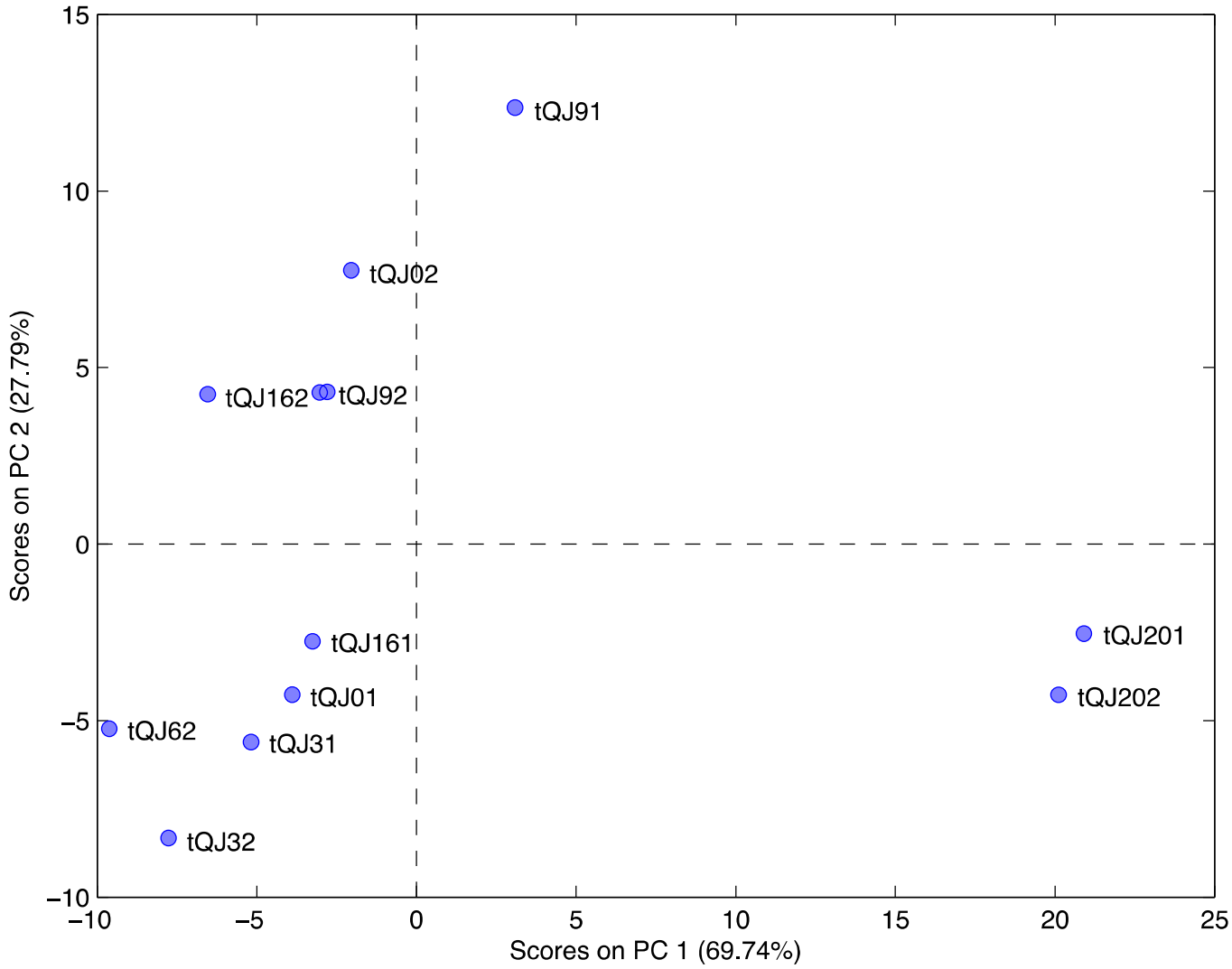


527

528

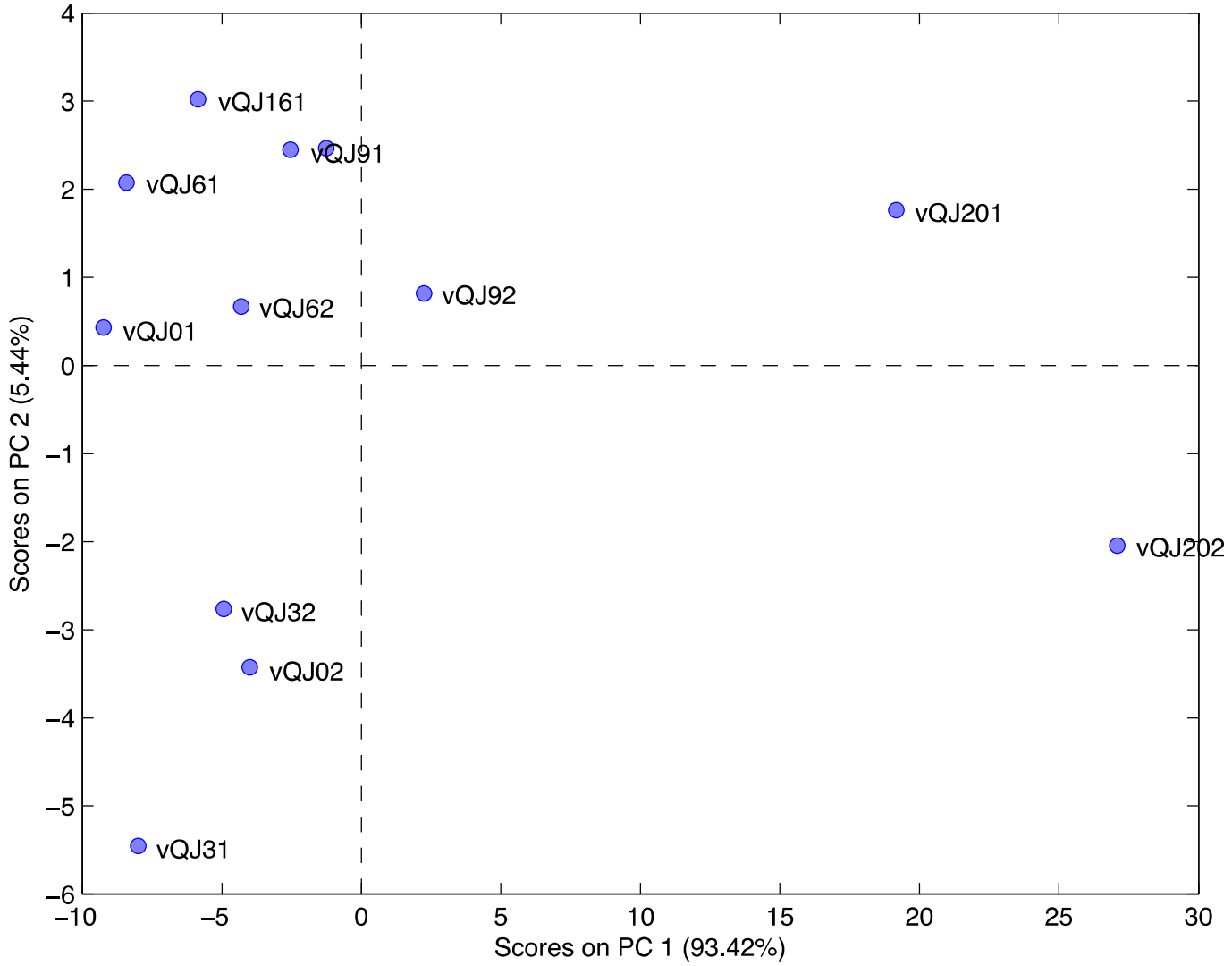
529

530 Figure 2:



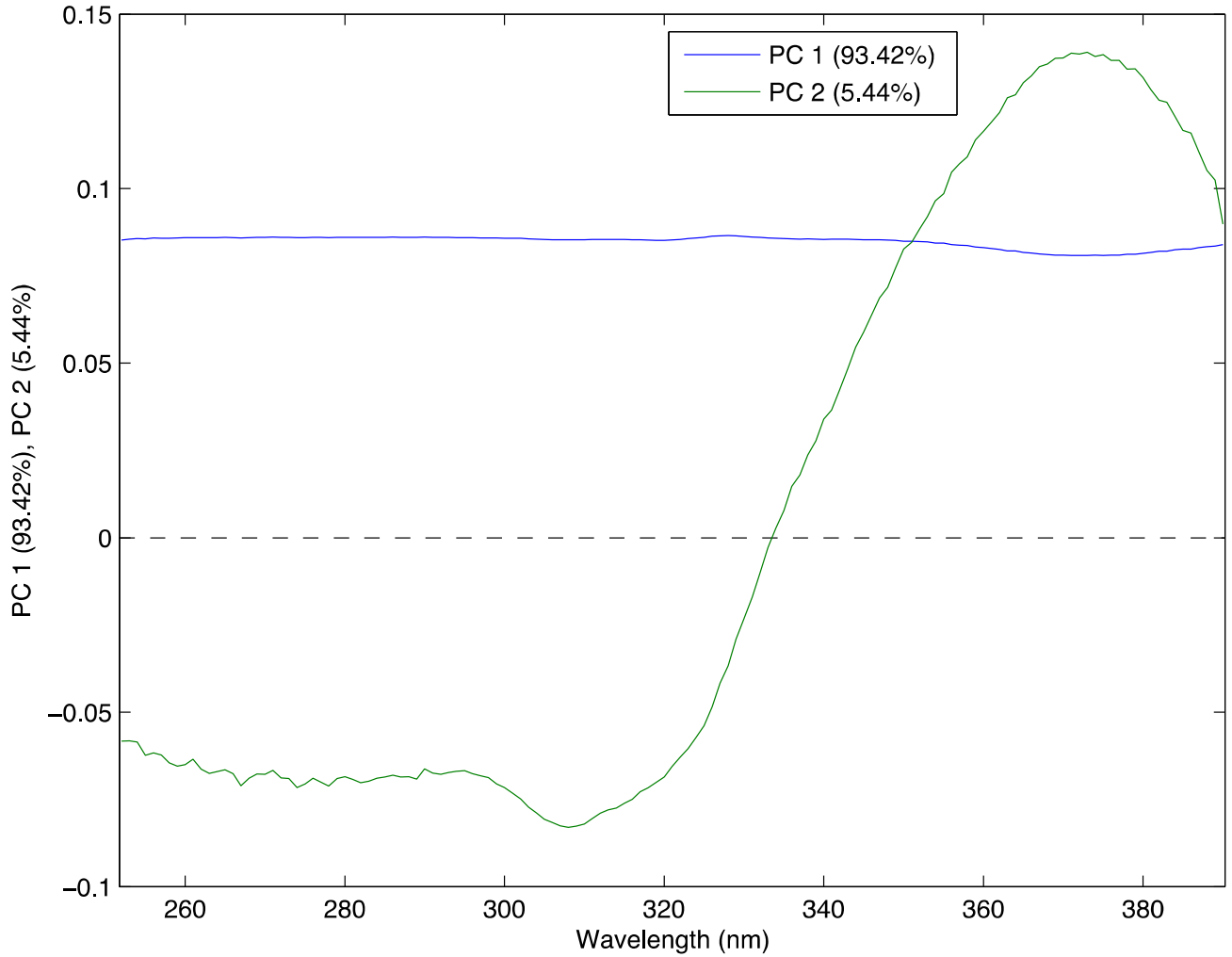
531

532 Figure 3a:



533

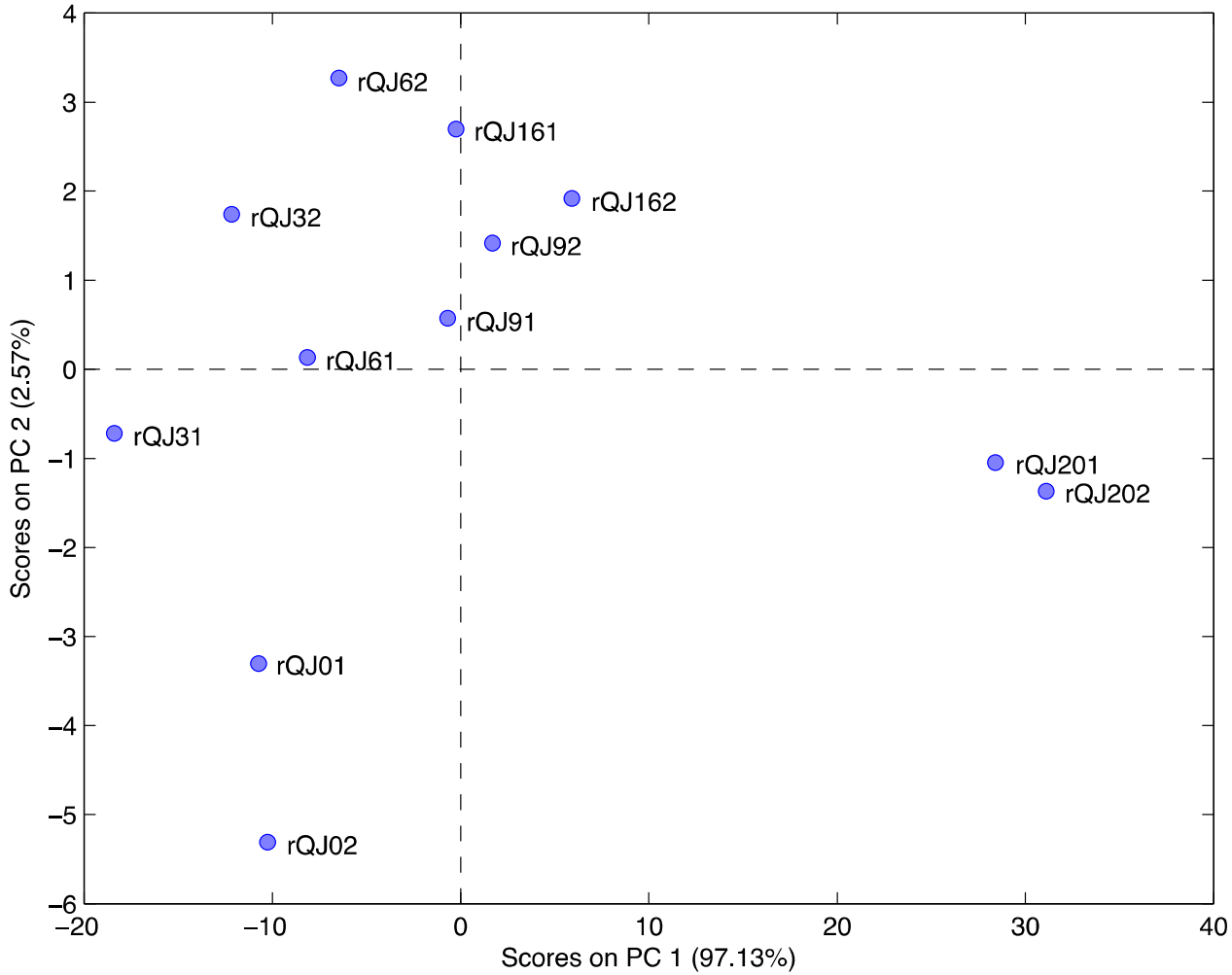
534 Figure 3b:



535

536

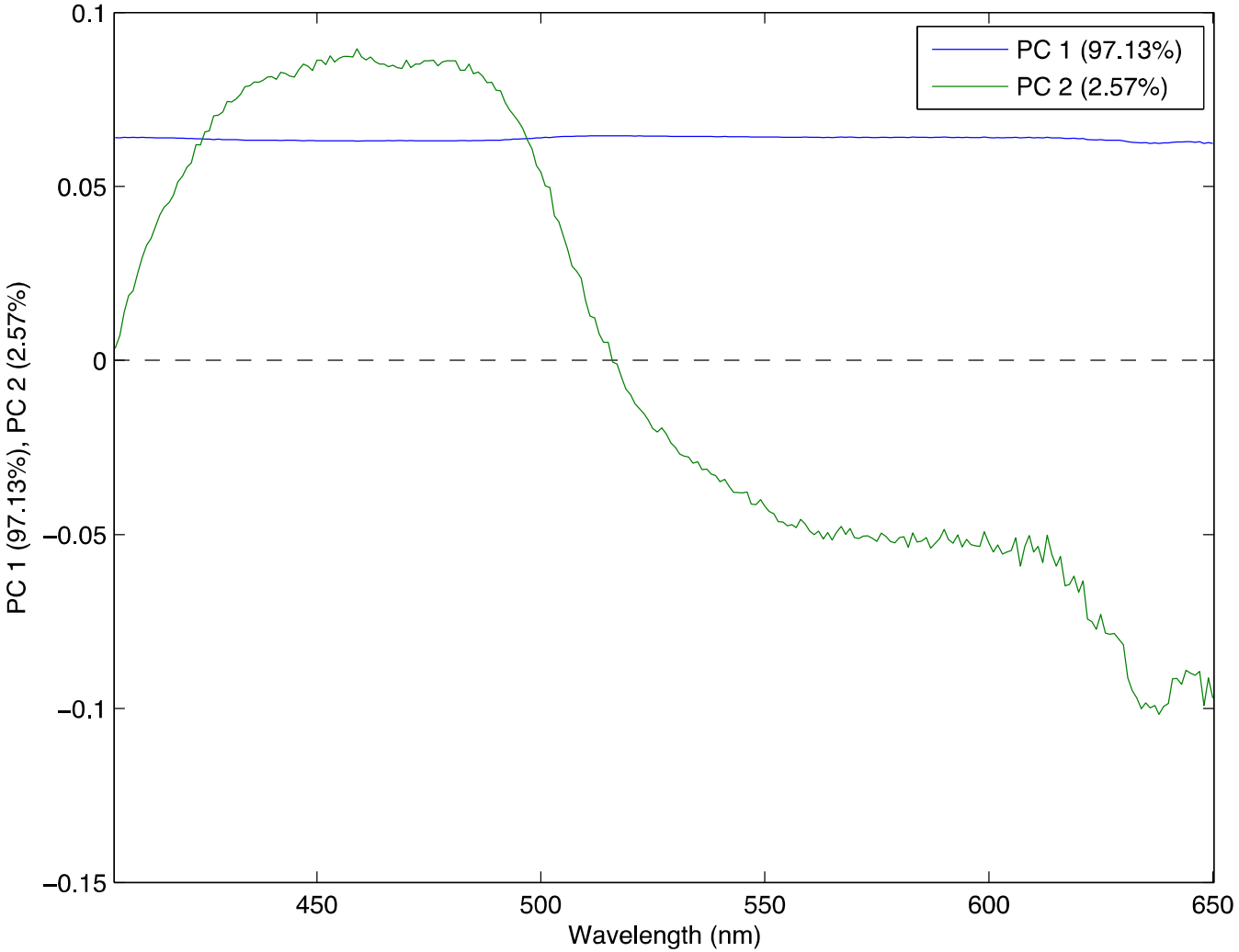
537 Figure 4a: Najib et al.



538

539

Figure 4b:


PC 1 (97.13%)
PC 2 (2.57%)
0.1
0.05
0
−0.05
−0.1
−0.15
PC 1 (97.13%), PC 2 (2.57%)
450
500
550
600
650
Wavelength (nm)