



HAL
open science

Multi-scale ^1H benchtop NMR analysis for milk analysis

Alper Soyler, Sevil Cikrikci, Cagri Cavdaroglu, Dylan Bouillaud, Jonathan Farjon,
Patrick Giraudeau, Mecit H.Oztop

► **To cite this version:**

Alper Soyler, Sevil Cikrikci, Cagri Cavdaroglu, Dylan Bouillaud, Jonathan Farjon, et al.. Multi-scale ^1H benchtop NMR analysis for milk analysis. *LWT - Food Science and Technology*, 2021, <10.1016/j.lwt.2020.110557>. <hal-03016342>

HAL Id: hal-03016342

<https://hal.science/hal-03016342v1>

Submitted on 20 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Journal Pre-proof

Multi-scale Benchtop ^1H NMR Spectroscopy for Milk Analysis

Alper Soyler, Sevil Cikrikci, Cagri Cavdaroglu, Dylan Bouillaud, Jonathan Farjon, Patrick Giraudeau, Mecit H. Oztop



PII: S0023-6438(20)31545-0

DOI: <https://doi.org/10.1016/j.lwt.2020.110557>

Reference: YFSTL 110557

To appear in: *LWT - Food Science and Technology*

Received Date: 15 July 2020

Revised Date: 30 September 2020

Accepted Date: 8 November 2020

Please cite this article as: Soyler, A., Cikrikci, S., Cavdaroglu, C., Bouillaud, D., Farjon, J., Giraudeau, P., Oztop, M.H., Multi-scale Benchtop ^1H NMR Spectroscopy for Milk Analysis, *LWT - Food Science and Technology*, <https://doi.org/10.1016/j.lwt.2020.110557>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Elsevier Ltd. All rights reserved.

Credit Author Statement

- The study is a part of the PhD dissertation of Dr. Alper Soyler
- Dr. Farjon and Mr. Bouillaud helped Dr. Soyler in the experiments.
- Dr. Cikrikci helped to arrange the data and Mr. Çavdaroğlu conducted and wrote the statistical analysis.
- Dr. Oztop and Dr. Giraudeau were the advisors of the study.

Journal Pre-proof

1 **Multi-scale Benchtop ^1H NMR Spectroscopy for Milk Analysis**

2

3 Alper Soyler¹, Sevil Cikrikci², Cagri Cavdaroglu³, Dylan Bouillaud⁴, Jonathan Farjon⁴, Patrick

4 Giraudeau^{4*}, Mecit H. Oztop^{1*}

5

6 ¹Department of Food Engineering, Middle East Technical University, Ankara, Turkey

7 ²Department of Food Engineering, Konya Food and Agriculture University, Konya, Turkey

8 ³Department of Food Engineering, Izmir Institute of Technology, Izmir, Turkey

9 ⁴Université de Nantes, CNRS, CEISAM UMR 6230, Nantes, France

10

Corresponding Authors* :

Dr. Mecit Oztop

Phone : + 90 312 210 5634

Fax : + 90 312 219 2767

E-mail : mecit@metu.edu.tr

Dr. Patrick Giraudeau

E-mail: patrick.giraudeau@univ-nantes.fr

Phone: + 33 251125709

Fax: + 33 251125712

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30 **ABSTRACT**

31 Benchtop NMR systems offers various advantages such as being easy to use, not requiring
32 constant maintenance and being available at affordable prices. In this study, multiple aspects
33 of benchtop NMR spectroscopy were explored to analyse milk in an industrial context, either
34 regarding the quality of production or regarding the differentiation of the final product. The
35 first part focuses on the production conditions of lactose hydrolysis in milk and quantitative
36 online NMR spectroscopy was adapted to follow lactose hydrolysis in milk in continuous flow
37 mode. The second part focuses on differentiating milk samples having different properties. 36
38 milk samples from France and Turkey were analysed and glycerol, fat and sugar contents were
39 measured from the NMR spectra. Combination of spectroscopic data with a proposed
40 Artificial Neural Network model enabled to classify milk of different origins and different
41 properties. This study shows that benchtop NMR spectroscopy is a versatile non-destructive
42 control method that can help controlling milk quality both during and after production.

43

44

45

46

47

48

49 **Keywords:** Benchtop NMR spectroscopy; milk; lactose hydrolysis; classification; flow NMR;
50 artificial neural network (ANN)

51

52

53

54 **INTRODUCTION**

55 Milk is a unique product including significant amounts of nutrients such as calcium, proteins
56 and vitamins (Dutra Rosolen, Gennari, Volpato, & Volken De Souza, 2015). It can be
57 considered as the 'magic liquid' due to its rich and various composition and confirmed
58 benefits on the newborns. In this context, accessible analytical tools are crucial to ensure the
59 quality of milk, both to optimize the production processes and after production to ensure its
60 characteristic properties.

61 The first focus of this paper is about the production stage, on the example of the potential
62 production of lactose-free milk. One of the milk constituents, lactose (*aka milk sugar*) is a
63 disaccharide composed of glucose and galactose that are linked by a β -1,4 glycosidic
64 bond (Dong & Zhong, 2019). However, lactose found in the milk or milk products cause
65 digestion problems in some part of the population. The absence of the digestive enzyme
66 lactase which is also known as 'lactose intolerance' is the reason of this problem. Significant
67 amount of world population suffers from lactose intolerance thus demand for lactose free
68 products is getting increasing attention (Churakova et al., 2019). Lactose free milk is produced
69 by the pre-digestion of the lactose in milk with the addition of the lactase enzyme. Controlling
70 the production of lactose-free milk during its production has a major industrial impact. There
71 exist many different techniques recognized by analytical standard agencies to determine the
72 lactose content of milk, such as polarimetry, mid-infrared detection, gravimetry, differential
73 pH, enzymatic methods detecting either the glucose or galactose moiety of lactose and HPLC
74 (Churakova et al., 2019). Each of these methods has its own advantages and disadvantages, but
75 to our knowledge none of them have the ability to monitor the hydrolysis process online in the
76 continuous mode. Therefore, developing new online tools for controlling this reaction is of
77 major importance for the milk industry.

78 On the other hand, the differentiation of milk products not only regarding the sugar type
79 (*lactose free or not*) but also according to the other parameters such as process, origin, fat

80 content, etc. requires considerable attention especially considering the regulatory
81 specifications and quality of the milk. In recent years, several studies have focused on
82 discriminating the products coming from different farming systems, animals or feeding
83 strategies, origin etc. (Bergamaschi, Cipolat-Gotet, Cecchinato, Schiavon, & Bittante, 2020).
84 Traditional methods like chromatographic techniques or sensory analyses might require highly
85 skilled operators and they could not be easily adapted to online monitoring. Moreover, they
86 could be time consuming and expensive. Easy, quick to use, minimum chemical requiring
87 methods have always been on the interest of manufacturers for ensuring the quality and
88 authenticity.

89 Nuclear magnetic resonance (NMR) spectroscopy, is a powerful, fingerprint tool for the
90 simultaneous identification and quantification of compounds in complex mixtures
91 (Monakhova, Kuballa, Leitz, Andlauer, & Lachenmeier, 2012). It is recognized as a valuable
92 tool for food analysis enabling not only the characterization of food matrices in terms of
93 authenticity and quality but also identification of counterfeits (Sobolev et al., 2019). Also,
94 studies of ^1H NMR-based metabolomics to get metabolite profiles during storage (Jansson et
95 al., 2014) or to analyze heritability of individual milk metabolites (Buitenhuis et al., 2013) in
96 animal based products have been explored (Bertram, 2018). However, most of the NMR
97 spectroscopy instruments work at high field (^1H frequencies >300 MHz) and requires a
98 massive dedicated place with specific installation and trained staff as well as a substantial
99 investment cost and expensive cryogenic fluids for maintenance (Bouillaud, Farjon,
100 Gonçalves, & Giraudeau, 2019). To overcome such limitations, benchtop systems capable of
101 conducting spectroscopy experiments have been developed (Perlo et al., 2005). These systems
102 have been successfully used to monitor authenticity in some kind of foods such as in coffee
103 (Defernez et al., 2017) and in meat (Jakes et al., 2015). Furthermore, thanks to their compact
104 character, NMR spectroscopy can easily be used for online monitoring under flow conditions
105 (Anderssen & McCarney, 2020; Bouillaud, Heredia, et al., 2019; Knox, Parkinson, Stone, &
106 Warren, 2019; Rönnols, Danieli, Freichels, & Aldaeus, 2020; Soyler, Bouillaud, Farjon,

107 Giraudeau, & Oztop, 2020), which makes them ideal candidates for the online control of food
108 production processes. For instance, benchtop NMR spectroscopy has been used to monitor
109 continuous processes such as enzymatic hydrolysis of marine by-products (Anderssen &
110 McCarney, 2020), sucrose hydrolysis (Soyler et al., 2020), *in vivo* monitoring of lipid
111 accumulation in microalgae (Bouillaud et al., 2020) and lignin analysis (Rönnols et al., 2020).
112 However it is important to note that implementing NMR spectroscopy in a flow system is
113 challenging and can introduce additional complexity relative to static samples (Knox et al.,
114 2019). The impact of flow rate on signal intensities should be investigated carefully and the
115 parameters should be set accordingly (Soyler et al., 2020).

116 To our knowledge, this will be 1st study where milk will be investigated by using a bench top
117 NMR spectroscopy system from different aspects. We have explored the versatility of
118 benchtop NMR on milk samples for two different purposes. In the first part of the study,
119 benchtop NMR spectroscopy was utilized in conditions mimicking the online control of
120 lactose hydrolysis in milk and lactose hydrolysis was monitored first in a model system, then
121 in a milk sample -with no need for deuterated solvents through the use of a tailored solvent
122 suppression pulse sequence (Gouilleux, Charrier, Akoka, & Giraudeau, 2017a). Kinetic
123 analysis of the reaction was also performed. In the second part, 36 milk samples from Turkey
124 and France; having different fat and sugar contents; production styles (conventional vs
125 organic) were analysed by NMR spectroscopy together with multivariate approaches to assess
126 the potential of benchtop NMR as a differentiation tool.

127 **MATERIALS AND METHODS**

128 **Materials**

129 Lactose in the form of *D-(+)-Lactose monohydrate* was purchased from Fluka BioChemika,
130 Switzerland. Lactase enzyme (Maxilact[®] LGi 5000) for lactose hydrolysis was supplied from
131 DSM, (Heerlen, The Netherlands). D-(+)-Glucose, D-(+)-Galactose, imidazole and 3-
132 (Trimethylsilyl) propionic-2,2,3,3-d₄ acid (TSP) were provided from Sigma-Aldrich Co. (St.

133 Louis, MO, USA). HPLC grade water from a water purification system (Nanopure Infinity,
134 Barnstead International, IA) was used for the preparation of the solutions. For the lactose
135 hydrolysis experiments in milk, a UHT whole milk (Lactel, France) bought from a grocery
136 store in France was used.

137 For the second part of the study, 36 different samples of milk (different cow milks: whole
138 milk, organic whole milk, low-fat milk, lactose-free milk and goat milk) were purchased from
139 local Turkish and French supermarkets. Information on the milk samples are given in Table 1.

140 **Sample Preparations**

141 For online monitoring analysis, hydrolysis reactions were performed both for a model system
142 and on a milk sample. As the model solution, a solution of glucose, galactose and lactose was
143 used. 5% (w/w) glucose, galactose and lactose solutions were prepared separately by
144 dissolving in non-deuterated HPLC grade water in a 50 mL tube. TSP was used as the internal
145 standard.

146 For milk differentiation experiments, 0.5% (w/w) imidazole was added to the 36 milk samples
147 (Table 1) as the internal reference for NMR measurements since TSP was masked with the
148 peaks of the fats present in the milk. Imidazole had ^1H chemical shifts of 7.26 ppm and 8.11
149 ppm. All the samples were stirred for 5 min for complete dissolution of imidazole in the milk.
150 Afterwards, the samples were transferred into 5 mm NMR tubes and stored in the dark and
151 refrigerated before analysis to prevent spoilage.

152 **NMR Spectroscopy**

153 NMR spectroscopy experiments were performed on a benchtop spectrometer operating at a 43
154 MHz ^1H frequency with a compact permanent magnet based on the Halbach design (Magritek
155 Spinsolve, Wellington, NZ) (E. Danieli, Perlo, Blümich, & Casanova, 2013; Ernesto Danieli,
156 Perlo, Blümich, & Casanova, 2010). The spectrometer was equipped with a gradient coil along
157 the B_0 axis in the transverse plane of the NMR tube that could produce a maximum field
158 gradient of $0.16 \text{ T}\cdot\text{m}^{-1}$ and also had an external lock system which allowed the use of non-

159 deuterated solvents. The experiments were performed at 29 °C since this was the temperature
160 at which the magnet stability was optimal. Since deuterated solvents were not used, an
161 experiment to suppress the water signal was used, based on a WET-180-NOESY (Gouilleux,
162 Charrier, Akoka, & Giraudeau, 2017b). This pulse sequence provides an optimal solvent
163 suppression for small molecules on a benchtop spectrometer, leading to a lower and narrower
164 water signal with a clean phase with a minimal impact on nearby peaks.

165 An inversion recovery sequence was used for measuring longitudinal relaxation times T_1 using
166 an inversion time range of 0.1-10,000 ms with 15 points.

167 The 1D ^1H spectra were obtained with 64 scans for a total experiment time of 6 min for model
168 lactose solution hydrolysis and 7 min for milk hydrolysis and '*milk differentiation*'
169 experiments. The 90° pulse angle was achieved by a pulse length of 6.7 μs at 0 dB. The FIDs
170 were recorded with 16 K points, a dwell time of 200 μs , and a repetition time of 6 s for model
171 lactose solution and 7 s for milk, corresponding to 5 times the longest T_1 in the sample to
172 ensure quantitative conditions. 1D data were processed with MestReNova. To align all the
173 spectra correctly, TSP was used to calibrate the chemical shift axis at 0 ppm for the hydrolysis
174 experiments. For milk spectra, the signal of imidazole at 7.26 ppm was used as the chemical
175 shift reference. All spectra were processed with a 0.2 Hz exponential apodization, an
176 automatic phase correction and an automatic baseline correction via a Whittaker smoother
177 algorithm. Manual phase correction and manual baseline correction were also performed in
178 addition to the automatic corrections. Relevant peak integrals were calculated by integration
179 with the MestReNova software. Deconvolution tools were also evaluated but yielded a slightly
180 lower performance than integration, probably due to the non-ideal line shapes.

181 ***Lactose hydrolysis in continuous flow system***

182 For on-line monitoring, the system included a glass flow cell with 5 mm outer diameter, a
183 peristaltic pump (Reglo Digital, Ismatec, Wertheim, Germany) and PEEK tubing. A heating
184 plate (RCT Basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) was added to the system

185 to control the temperature of the hydrolysis reaction.

186 NMR signal is very sensitive to the flow rates as the signal becomes broader and the resolution
187 decreases with increasing flow rates. The approach followed in a previous study(Soyler et al.,
188 2020) was used to determine the optimal flow rate of 0.5 mL/min.

189 *Differentiation milk samples*

190 Experiments were performed in 5 mm NMR tubes in static mode. For the quantitative analysis,
191 lactose, glycerol and fat contents were determined from the spectra as will be shown later.

192 Considering the factors given in Table 1 (country (TR/FR), milk type (Goat/Cow), production
193 style (Conventional/Organic), processing (UHT/Pasteurized), fat and sugar content), Analysis
194 of Variance (ANOVA), was conducted on the responses of glycerol; lactose and fat contents to
195 see if any of the responses showed differences with milk type. If significant difference was
196 detected, means were compared by the Tukey test ($p < 0.05$) using MINITAB (Version 19,
197 Coventry, U.K) software.

198 In addition, to obtain some grouping info for the given data set, an Artificial Neural Networks
199 (ANN) model was developed by using NMR spectra results. Artificial Neural Networks
200 (ANN) model is a nonlinear mathematical model with the capability of developing meaningful
201 relationships between input and output variables through a learning process (Zheng et al.,
202 2011). The function and organization of the human brain gave inspiration to its function and
203 structure (Bila et al., 1999). The mathematical model designed to classify milk samples with
204 respect to their fat and sugar contents, milk source, production and processing type was based
205 on nonlinear ANNs. These intelligent algorithms have been used to link chemical information
206 coming from 36 milk samples with milk type to achieve a potential tool for accurate
207 differentiation and quality control.

208 ANN model was implemented with the 'neural net package' (Version 1.44.2) in R (Stefan
209 Fritsch, Frauke Guenther, Marvin N. Wright, Marc Suling, & Sebastian M. Mueller, 2019).
210 For training (calibration), 2/3 of the data were used and 1/3 was separated to test the model

211 (external validation). In order to obtain the best score, ANN model was trained using different
212 number of neurons between 2 to 15 in the hidden layers. ANN model yielded possible
213 classifications of the samples and possibility ratios. R^2_{cal} was used to investigate the prediction
214 ability of the trained ANN model and R^2_{pred} for external validation. Error values were stated as
215 root mean square error of calibration (RMSEC) and root mean square error of prediction
216 (RMSEP). R^2 values should be close to 1 while error values should be low to obtain a robust
217 prediction model (Uncu & Ozen, 2019). Optimal number of learning steps was selected
218 according to the *minimum root means square error cross validation* (RMSECV) value for the
219 calibration dataset. A value for R^2 between 0.50 and 0.65 can be used for discrimination
220 between high and low concentrations. A value for R^2 between 0.66 and 0.81 shows
221 approximate quantitative predictions., R^2 between 0.82 and 0.90 are considered as good
222 prediction and above 0.91 are excellent (Saeys, Mouazen, & Ramon, 2005). Categorical
223 variables were used as binary data, while numeric data was used for normalization and scaling.

224 **RESULTS and DISCUSSION**

225 **I. Lactose hydrolysis in continuous flow system**

226 *1D solvent suppression enhanced NMR Spectra*

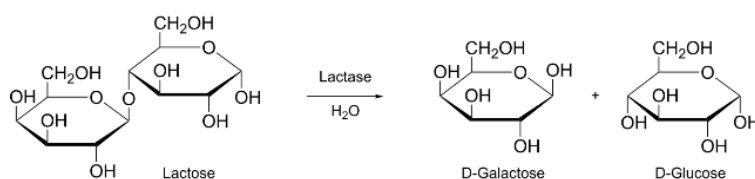
227 At first, 1D ^1H NMR experiments were carried out with a water signal suppression pulse
228 sequence on model solutions of isolated compounds to identify the peaks which could be used
229 to monitor the lactose hydrolysis reactions. Fig. 1 shows the stacked spectra of glucose,
230 galactose and lactose alone. Due to low magnetic field, the spectra of the sugars were highly
231 overlapped. The suppressed water peak was observed at 4.9 ppm. As can be seen in Fig. 1,
232 alpha anomeric proton peaks of glucose, galactose and lactose are overlapped at 5.3 ppm.
233 Although beta anomeric proton peaks of glucose and galactose are masked by the water peak,
234 the beta anomeric proton peak of lactose is seen as a distinctive peak at 4.4 ppm. Despite the
235 fact that it is difficult to integrate only alpha lactose site due to alpha glucose and galactose
236 signal overlap, it is still possible to monitor the lactose hydrolysis with the isolated beta
237 anomeric signal. Moreover, anomeric ratio between alpha and beta anomers is constant during

238 the monitoring therefore it is reliable to follow the beta anomeric site along the course of the
239 hydrolysis.

240

241 *Online Monitoring and Kinetic Modelling of Lactose Hydrolysis in the Model System*

242 Lactose when hydrolyzed yields glucose and galactose as seen below.



245 For model lactose solution hydrolysis, a 5% lactose solution was hydrolyzed with 20 μL
246 lactase enzyme. The NMR spectra were obtained at 6 min intervals for 240 min (Fig. 2a). The
247 consumption of lactose can be seen from β -lactose peaks (Fig 2b).

248 Concentrations of lactose for each time point were calculated using Equation 1. The number of
249 protons contributing to the signal was 9 for TSP, and 3 for imidazole.

250
$$C_x = \frac{A_x/N_x}{A_{REF}/N_{REF}} \cdot \frac{MW_x}{MW_{REF}} C_{REF} \quad (\text{Eq.1})$$

251 C , and MW were the concentrations in mg/g, the molecular weight. REF referred to the
252 internal reference. A and N , denoted the integral area in a fully relaxed ^1H NMR spectrum and
253 the number of hydrogens contributing to the signals. Hydrolysis experiments were repeated
254 three times and the change on the average concentrations of lactose from the experiments were
255 plotted as a function of time in Fig. 3.

256 Lactose data were fitted to Equation 2 ($R^2 > 0.99$) and the average rate constant was found as
257 $k = 1.66 \cdot 10^{-2} \text{ min}^{-1}$ at 29°C .

258
$$\frac{C - C_\infty}{C_0 - C_\infty} = e^{-kt} \quad (\text{Eq. 2})$$

259 *Lactose Hydrolysis in Milk*

260 Based on the good repeatability of the model lactose solution hydrolysis, milk was also
261 hydrolyzed with 20 μL of enzyme. Milk is a much more complex fluid than the model lactose
262 solution. The major constituents of milk are water, fat, proteins and lactose. Milk also contains
263 trace amounts of vitamins, minerals, organic acids, enzymes. Therefore, 1D ^1H NMR spectrum
264 of milk was quite different than the model lactose solution (Fig. 4a). The NMR spectra were
265 obtained at each 7 min for 280 min. As seen in Fig. 4b, the consumption of lactose was
266 followed using the peak of β -lactose. The average rate constant from the fractional conversion
267 model yield a value of $1.52 \times 10^{-2} \text{ min}^{-1}$ at 29°C which was consistent with the previous study of
268 Panesar (2007) where it was found as $1.56 \times 10^{-2} \text{ min}^{-1}$ at 30°C (Fig. 5).

269 Results of this part showed that lactose hydrolysis can be controlled online in a continuous
270 mode successfully by using benchtop NMR spectroscopy, thus the method has high potential
271 to be adapted for industrial processes. For lactose free milk production, it should be ensured
272 that residual lactose amounts are within the legal limits for that product category. This can
273 further be confirmed by other high-resolution spectroscopic techniques.

274 **II. Differentiation of Milk Samples by Benchtop ^1H -NMR Profiling and** 275 **Chemometrics**

276 In this part of the study, 36 milk samples of which the properties have been listed in Table 1
277 were analyzed. A sample spectrum of milk was given in Figure 4a. As seen in the spectrum,
278 there were broad fat peaks between 0.5 – 2.5 ppm. For the differentiation experiment of the
279 samples, integral area of *glycerol*, *lactose* and *fat* peak were used. The imidazole peak integral
280 area was used for the normalization of other peaks. The normalized integral areas are given in
281 Table 2. Lactose-free milks (LFM(T/F)) were found to have no lactose and half-fat milks
282 (HFM(T/F)) had nearly half fat content compared to whole milk samples.

283

284 Glycerol peak was observed next to β -lactose peak between 4.0 – 4.25 ppm. When the
285 glycerol contents were compared, Turkish and French milks were found to be significantly
286 different from each other; glycerol contents of French milks being higher ($p < 0.05$).

287 Two reasons could suggest for the presence of glycerol in the milk. Firstly, glycerol is used for
288 the treatment of ketosis which is a metabolic disease caused by the negative energy balance
289 during lactation (JOHNSON, 1954). And secondly, glycerol is used in the feed as an energy
290 source. Expansion of the biodiesel industry decreased the availability of corn for animal feed
291 and increased its prices. Therefore, glycerol which is also a by-product of the biodiesel
292 industry has started to be used as an energy source for cows (Carvalho, Schmelz-Roberts,
293 White, Doane, & Donkin, 2011). The energy concentration of glycerol (1.98-2.29 Mcal/kg) is
294 almost equal to corn starch (Schröder & Südekum, 1999). And inclusion of that in the diet was
295 eventually observed in the milk.

296 According to ANOVA results, only the fat and sugar status of the milk were found to be
297 significant for fat and lactose contents ($p < 0.05$). Production type, country and processing were
298 found to be insignificant on lactose and fat content ($p > 0.05$).

299 *Artificial Neural Network (ANN) Model*

300 As stated before, ANN was used as the multivariate approach for classification of the different
301 milk samples based on the outputs of the NMR spectra. The most commonly used quantitative
302 prediction multivariate technique is *Partial Least Square Regression (PLSR)*, which is mostly
303 used to analyse spectroscopic data, especially FTIR. However, ANN was found to show much
304 better differentiative prediction ability as compared to other classification models (Efenberger-
305 Szmechtyk, Nowak, & Kregiel, 2018). This is explained with several reasons. ANNs learn
306 relationship between the input variables and the output values through successive trainings and
307 the non-linear technique allows model to fit the data better. Analysis is noise insensitive which
308 provides accurate prediction in the presence of noisy data (Berrueta, Alonso-Salces, &
309 Heberger, 2007). There has been several studies on foods which show that ANN is superior to

310 PLSR in prediction (Panagou, Mohareb, Argyri, Bessant, & Nychas, 2011; Perai, Moghaddam,
311 Asadpour, Bahrapour, & Mansoori, 2010; Vasquez et al., 2018).

312 In this work, ANN model has been designed and optimized to discriminate milk samples
313 according to their glycerol, lactose and fat peak areas obtained from the 1D NMR spectra. The
314 mathematical tool employed six categorical parameters (country, fat content, sugar content,
315 milk source, production type and processing type) as variables to accurately classify the milk
316 samples.

317 RMSECV values of the model were used to determine ruggedness of the model. The
318 breakpoint in the RMSECV vs. iteration number (step) plot were identified as the number of
319 iteration (step) parameter for the analyses. Following such an approach prevented
320 overtraining of the model. Since the RMSECV values are usually an integral part of the ANN
321 analysis, they were not saved thus not reported in the model. Feedforward supervised method
322 was used for training ANN model with 5 neurons and 2 neurons in the two hidden layers.
323 Multilayer feedforward neural network diagrams are also given in the supplement. RMSEC
324 value was obtained as 0.017 and R^2_{cal} was 0.997. When external validation data was fitted to
325 model, RMSEP was observed as 0.128 and R^2_{pred} was 0.819 and these values indicated good
326 prediction. Each sample was correctly classified in the cross validation (24/24), while one of
327 the 12 samples was misclassified in the external validation set (Fig. 6). These results showed
328 that glycerol, fat and lactose contents, supplied with information such as production type,
329 processing method, milk source and analysed with ANN, could be used to locate the origin
330 (*geography*) of milk.

331 ANN was also used to differentiate organic milk samples from conventional milks samples,
332 and pasteurized milk samples from UHT processed milk samples. For classification of organic
333 and conventional samples, RMSEC value was obtained as 0.121 and R^2_{cal} was 0.893. When
334 external validation data was fitted to model, RMSEP was observed as 0.232 and R^2_{pred} was
335 0.419. Although R^2_{pred} was relatively low, 0.42 is adequate for discrimination between distinct
336 classes. Prediction ability of model according to samples' production type was 24 out of 24 for

337 calibration set and 9 out of 12 for validation set. In the validation set, two organic samples
338 were misclassified as conventional and one sample was misclassified as organic.

339 Same ANN parameters were used to classify pasteurized milk samples and UHT processed
340 milk samples. RMSEC for calibration set was 0.018 and RMSEP for prediction was 0.153. R^2
341 for calibration was obtained as 0.999 while R^2 for prediction was 0.851. Each sample in
342 calibration set and pasteurized samples in validation set were classified correctly while two of
343 UHT processed milk samples in validation set were misclassified. R^2 for prediction value
344 showed that model was good enough for prediction.

345 In summary, the ANN model was successfully used to predict origins of milk samples,
346 production type and process type. For all parameters evaluated in the study, the maximum
347 number of incorrect matches was 2 which was a good indicator for the strong classification
348 ability of the model. These results confirmed the existence of a relationship between the six
349 categorical parameters and three main components in the classification of milks, leading to
350 potential design of an objective evaluating method for classification of diverse milk samples.
351 The model supported potential use of benchtop NMR spectra for differentiating milk samples
352 and provided input for a more comprehensive authentication study.

353 CONCLUSION

354 Milk is a very valuable food for the human being. Ensuring its quality and authenticity is
355 significant. In this study, multiple aspects of benchtop NMR spectroscopy have been evaluated
356 to investigate its potential as a multi-scale control tool at different stages of milk production.

357 The first part of the study focused on the online monitoring of lactose hydrolysis in milk.
358 Enzymatic hydrolysis of lactose was successfully followed and a kinetic model was obtained
359 in agreement with previous studies. In the second part, milk samples that have different
360 properties were examined. Glycerol, fat, sugar content of the milks was obtained from the
361 NMR spectra and an Artificial Neural Network (ANN) model was developed to seek for
362 classifications based on the production, processing type, milk source and geographic origin.

363 The developed model supported the potential use of benchtop NMR spectra for differentiating

364 milk samples. Overall, it was shown that even for a complex fluid like milk, benchtop NMR
365 spectroscopy provides valuable information and the technique after a full analytical validation
366 that is out of the scope of this proof-of-concept study has the potential to be used in industry
367 for monitoring production, quality control and authenticity determination (*but on larger*
368 *sample set*).

369 **Funding:** Alper Soyler got funding from Short Term Scientific Mission (STSM) program
370 under COST Action CA15209 EURELAX – European Network on NMR Relaxometry
371 (http://www.cost.eu/COST_Actions/ca/CA15209) to conduct experiments at the University of
372 Nantes. Authors from Nantes acknowledge support from the Corsaire metabolomics core
373 facility (Biogenouest). J.F. acknowledges Sandrine Bouchet for a continuous essential
374 assistance. Middle East Technical University, Scientific Research Projects Coordination Unit
375 with grant # BAP-07-02-2017-004-077 is also acknowledged.

376 REFERENCES

- 377 Anderssen, K. E., & McCarney, E. R. (2020). Online monitoring of enzymatic hydrolysis of
378 marine by-products using benchtop nuclear magnetic resonance spectroscopy. *Food*
379 *Control*, 112(September 2019), 107053. <https://doi.org/10.1016/j.foodcont.2019.107053>
- 380 Bergamaschi, M., Cipolat-Gotet, C., Cecchinato, A., Schiavon, S., & Bittante, G. (2020).
381 Chemometric authentication of farming systems of origin of food (milk and ripened
382 cheese) using infrared spectra, fatty acid profiles, flavor fingerprints, and sensory
383 descriptions. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2019.125480>
- 384 Berrueta, L. A., Alonso-Salces, R. M., & Heberger, K. (2007). Supervised pattern recognition
385 in food analysis. *Journal of Chromatography A*, 1158(1–2), 196–214.
386 <https://doi.org/10.1016/j.chroma.2007.05.024>
- 387 Bertram, H. C. (2018). NMR-Based Metabolomics: Quality and Authenticity of Milk and
388 Meat. In *Modern Magnetic Resonance* (pp. 1–13). Springer.
- 389 Bila, S., Harkouss, Y., Ibrahim, M., Rousset, J., N’Goya, E., Baillargeat, D., ... Guillon, P.
390 (1999). Accurate wavelet neural-network-based model for electromagnetic optimization

- 391 of microwave circuits. *International Journal of RF and Microwave Computer-Aided*
392 *Engineering*. [https://doi.org/10.1002/\(SICI\)1099-047X\(199905\)9:3<297::AID-](https://doi.org/10.1002/(SICI)1099-047X(199905)9:3<297::AID-MMCE13>3.0.CO;2-W)
393 [MMCE13>3.0.CO;2-W](https://doi.org/10.1002/(SICI)1099-047X(199905)9:3<297::AID-MMCE13>3.0.CO;2-W)
- 394 Bouillaud, D., Drouin, D., Charrier, B., Jacquemmoz, C., Farjon, J., Giraudeau, P., &
395 Gonçalves, O. (2020). Using benchtop NMR spectroscopy as an online non-invasive in
396 vivo lipid sensor for microalgae cultivated in photobioreactors. *Process Biochemistry*,
397 *93*(January), 63–68. <https://doi.org/10.1016/j.procbio.2020.03.016>
- 398 Bouillaud, D., Farjon, J., Gonçalves, O., & Giraudeau, P. (2019). Benchtop NMR for the
399 monitoring of bioprocesses. *Magnetic Resonance in Chemistry*, *57*(10), 794–804.
400 <https://doi.org/10.1002/mrc.4821>
- 401 Bouillaud, D., Heredia, V., Castaing-Cordier, T., Drouin, D., Charrier, B., Gonçalves, O., ...
402 Giraudeau, P. (2019). Benchtop flow NMR spectroscopy as an online device for the in
403 vivo monitoring of lipid accumulation in microalgae. *Algal Research*, *43*(July), 101624.
404 <https://doi.org/10.1016/j.algal.2019.101624>
- 405 Buitenhuis, A. J., Sundekilde, U. K., Poulsen, N. A., Bertram, H. C., Larsen, L. B., &
406 Sørensen, P. (2013). Estimation of genetic parameters and detection of quantitative trait
407 loci for metabolites in Danish Holstein milk. *Journal of Dairy Science*.
408 <https://doi.org/10.3168/jds.2012-5914>
- 409 Carvalho, E. R., Schmelz-Roberts, N. S., White, H. M., Doane, P. H., & Donkin, S. S. (2011).
410 Replacing corn with glycerol in diets for transition dairy cows. *Journal of Dairy Science*.
411 <https://doi.org/10.3168/jds.2010-3581>
- 412 Churakova, E., Peri, K., Vis, J. S., Smith, D. W., Beam, J. M., Vijverberg, M. P., ... Winter, R.
413 T. (2019). Accurate analysis of residual lactose in low-lactose milk: Comparing a variety
414 of analytical techniques. *International Dairy Journal*, *96*, 126–131.
415 <https://doi.org/10.1016/j.idairyj.2019.02.020>
- 416 Danieli, E., Perlo, J., Blümich, B., & Casanova, F. (2013). Highly stable and finely tuned
417 magnetic fields generated by permanent magnet assemblies. *Physical Review Letters*.
418 <https://doi.org/10.1103/PhysRevLett.110.180801>

- 419 Danieli, Ernesto, Perlo, J., Blümich, B., & Casanova, F. (2010). Small magnets for portable
420 NMR spectrometers. *Angewandte Chemie - International Edition*.
421 <https://doi.org/10.1002/anie.201000221>
- 422 Defernez, M., Wren, E., Watson, A. D., Gunning, Y., Colquhoun, I. J., Le Gall, G., ...
423 Kemsley, E. K. (2017). Low-field ¹H NMR spectroscopy for distinguishing between
424 arabica and robusta ground roast coffees. *Food Chemistry*.
425 <https://doi.org/10.1016/j.foodchem.2016.08.028>
- 426 Dong, L., & Zhong, Q. (2019). Dispersible Biopolymer Particles Loaded with Lactase as a
427 Potential Delivery System to Control Lactose Hydrolysis in Milk. *Journal of*
428 *Agricultural and Food Chemistry*, 67(23), 6559–6568.
429 <https://doi.org/10.1021/acs.jafc.9b01546>
- 430 Dutra Rosolen, M., Gennari, A., Volpato, G., & Volken De Souza, C. F. (2015). Lactose
431 Hydrolysis in Milk and Dairy Whey Using Microbial β -Galactosidases. *Enzyme*
432 *Research*, 2015. <https://doi.org/10.1155/2015/806240>
- 433 Efenberger-Szmechtyk, M., Nowak, A., & Kregiel, D. (2018). Implementation of
434 chemometrics in quality evaluation of food and beverages. *Critical Reviews In Food*
435 *Science and Nutrition*, 58(10), 1747–1766.
436 <https://doi.org/10.1080/10408398.2016.1276883>
- 437 Gouilleux, B., Charrier, B., Akoka, S., & Giraudeau, P. (2017a). Gradient-based solvent
438 suppression methods on a benchtop spectrometer. *Magnetic Resonance in Chemistry*.
439 <https://doi.org/10.1002/mrc.4493>
- 440 Gouilleux, B., Charrier, B., Akoka, S., & Giraudeau, P. (2017b). Gradient-based solvent
441 suppression methods on a benchtop spectrometer. *Magnetic Resonance in Chemistry*.
442 <https://doi.org/10.1002/mrc.4493>
- 443 Jakes, W., Gerdova, A., Defernez, M., Watson, A. D., McCallum, C., Limer, E., ... Kemsley,
444 E. K. (2015). Authentication of beef versus horse meat using 60 MHz ¹H NMR
445 spectroscopy. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2014.11.110>
- 446 Jansson, T., Jensen, H. B., Sundekilde, U. K., Clausen, M. R., Eggers, N., Larsen, L. B., ...

- 447 Bertram, H. C. (2014). Chemical and proteolysis-derived changes during long-term
448 storage of lactose-hydrolyzed ultrahigh-temperature (UHT) milk. *Journal of Agricultural*
449 *and Food Chemistry*. <https://doi.org/10.1021/jf504104q>
- 450 Johnson, R. B. (1954). The treatment of ketosis with glycerol and propylene glycol. *The*
451 *Cornell Veterinarian*.
- 452 Knox, S. T., Parkinson, S., Stone, R., & Warren, N. J. (2019). Benchtop flow-NMR for rapid
453 online monitoring of RAFT and free radical polymerisation in batch and continuous
454 reactors. *Polymer Chemistry*, *10*(35), 4774–4778. <https://doi.org/10.1039/c9py00982e>
- 455 Monakhova, Y. B., Kuballa, T., Leitz, J., Andlauer, C., & Lachenmeier, D. W. (2012). NMR
456 spectroscopy as a screening tool to validate nutrition labeling of milk, lactose-free milk,
457 and milk substitutes based on soy and grains. *Dairy Science and Technology*, *92*(2), 109–
458 120. <https://doi.org/10.1007/s13594-011-0050-5>
- 459 Panagou, E. Z., Mohareb, F. R., Argyri, A. A., Bessant, C. M., & Nychas, G.-J. E. (2011). A
460 comparison of artificial neural networks and partial least squares modelling for the rapid
461 detection of the microbial spoilage of beef fillets based on Fourier transform infrared
462 spectral fingerprints. *FOOD MICROBIOLOGY*, *28*(4, SI), 782–790.
463 <https://doi.org/10.1016/j.fm.2010.05.014>
- 464 Perai, A. H., Moghaddam, H. N., Asadpour, S., Bahrampour, J., & Mansoori, G. (2010). A
465 comparison of artificial neural networks with other statistical approaches for the
466 prediction of true metabolizable energy of meat and bone meal. *Poultry Science*, *89*(7),
467 1562–1568. <https://doi.org/10.3382/ps.2010-00639>
- 468 Perlo, J., Demas, V., Casanova, F., Meriles, C. A., Reimer, J., Pines, A., & Blu, B. (2005).
469 High-Resolution NMR Spectroscopy with a Portable Single-Sided Sensor, (May).
470 <https://doi.org/10.1126/science.1108944>
- 471 Rönnols, J., Danieli, E., Freichels, H., & Aldaeus, F. (2020). Lignin analysis with benchtop
472 NMR spectroscopy. *Holzforschung*, *74*(2), 226–231. [https://doi.org/10.1515/hf-2018-](https://doi.org/10.1515/hf-2018-0282)
473 0282
- 474 Saeys, W., Mouazen, A. M., & Ramon, H. (2005). Potential for onsite and online analysis of

- 475 pig manure using visible and near infrared reflectance spectroscopy. *Biosystems*
476 *Engineering*, 91(4), 393–402. <https://doi.org/10.1016/j.biosystemseng.2005.05.001>
- 477 Schröder, A., & Stüdekum, K.-H. (1999). Glycerol as a by-product of biodiesel production in
478 diets for ruminants. *New Horizons for an Old Crop. Proc. 10th Int. Rapeseed Congr.*
- 479 Sobolev, A. P., Thomas, F., Donarski, J., Ingallina, C., Circi, S., Cesare Marincola, F., ...
480 Mannina, L. (2019). Use of NMR applications to tackle future food fraud issues. *Trends*
481 *in Food Science and Technology*, 91(March 2018), 347–353.
482 <https://doi.org/10.1016/j.tifs.2019.07.035>
- 483 Soyler, A., Bouillaud, D., Farjon, J., Giraudeau, P., & Oztop, M. H. (2020). Real-time
484 benchtop NMR spectroscopy for the online monitoring of sucrose hydrolysis. *LWT*.
485 <https://doi.org/10.1016/j.lwt.2019.108832>
- 486 Stefan Fritsch, Frauke Guenther, Marvin N. Wright, Marc Suling, & Sebastian M. Mueller.
487 (2019). Package “neuralnet” Training of Neural Networks, 1–15.
- 488 Uncu, O., & Ozen, B. (2019). A comparative study of mid-infrared, UV-Visible and
489 fluorescence spectroscopy in combination with chemometrics for the detection of
490 adulteration of fresh olive oils with old olive oils. *Food Control*, 105, 209–218.
491 <https://doi.org/10.1016/j.foodcont.2019.06.013>
- 492 Vasquez, N., Magan, C., Oblitas, J., Chuquizuta, T., Avila-George, H., & Castro, W. (2018).
493 Comparison between artificial neural network and partial least squares regression models
494 for hardness modeling during the ripening process of Swiss-type cheese using spectral
495 profiles. *Journal of Food Engineering*, 219, 8–15.
496 <https://doi.org/10.1016/j.jfoodeng.2017.09.008>
- 497 Zheng, H., Jiang, L., Lou, H., Hu, Y., Kong, X., & Lu, H. (2011). Application of Artificial
498 Neural Network (ANN) and Partial Least-Squares Regression (PLSR) to predict the
499 changes of anthocyanins, ascorbic acid, total phenols, flavonoids, and antioxidant
500 activity during storage of red bayberry juice based on fractal ana. *Journal of Agricultural*
501 *and Food Chemistry*, 59(2), 592–600. <https://doi.org/10.1021/jf1032476>
- 502

503

504

505

506

507

508

509

510

511

512

513

514 **Figure Captions**

515 **Figure 1.** Stacked spectra of glucose, galactose and lactose

516 **Figure 2. a)** Full superimposed spectra of lactose hydrolysis of model lactose solution **b)** β -
517 lactose region

518 **Figure 3.** Hydrolysis of lactose for the model system

519 **Figure 4.** 1D ^1H NMR spectrum of milk with the assignment for its main constituents (a)
520 Superimposed spectra of lactose hydrolysis in milk (b)

521 **Figure 5.** Hydrolysis of lactose in a real milk sample

522 **Figure 6** The confusion matrices for training dataset (left) and test dataset (right).

523

524

525

526

527

528

529

530

531

532

533

534

535

536 **TABLES**

537 *Table 1 Milk samples used in the study*

Sample Name	Country	Fat Content	Sugar Content	Milk Source	Production Style	Processing
WMF-1	France	Whole	Regular	Cow	Conventional	UHT
WMF-2	France	Whole	Regular	Cow	Conventional	UHT
WMF-3	France	Whole	Regular	Cow	Conventional	Pasteurized
WMF-4	France	Whole	Regular	Cow	Conventional	Pasteurized
OMF-5	France	Whole	Regular	Cow	Organic	UHT
OMF-6	France	Whole	Regular	Cow	Organic	Pasteurized
OMF-7	France	Whole	Regular	Cow	Organic	Pasteurized
HFMF-8	France	Half	Regular	Cow	Conventional	UHT
OHFMF-9	France	Half	Regular	Cow	Organic	Pasteurized
HFMF-10	France	Half	Regular	Cow	Conventional	Pasteurized
HFMF-11	France	Half	Regular	Cow	Conventional	Pasteurized
HFMF-12	France	Half	Regular	Cow	Conventional	UHT
OHFMF-13	France	Half	Regular	Cow	Organic	UHT
WMF-14	France	Whole	Regular	Cow	Conventional	UHT
OHFMF-15	France	Half	Regular	Cow	Organic	UHT
HFMF-16	France	Half	Regular	Cow	Conventional	UHT
OMF-17	France	Whole	Regular	Cow	Organic	Pasteurized
OHFMF-18	France	Half	Regular	Cow	Organic	UHT
GMF-19	France	Half	Regular	Goat	Conventional	UHT
GMF-20	France	Whole	Regular	Goat	Organic	UHT
GMF-21	France	Whole	Regular	Goat	Organic	UHT
GMF-22	France	Whole	Regular	Goat	Organic	UHT
WMT-23	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-24	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-25	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-26	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-27	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-28	Turkey	Whole	Regular	Cow	Conventional	UHT
OMT-29	Turkey	Whole	Regular	Cow	Organic	UHT
GMT-30	Turkey	Whole	Regular	Goat	Conventional	UHT
LFMF-31	France	Half	Lactose-Free	Cow	Conventional	UHT
LFMF-32	France	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-33	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-34	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-35	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-36	Turkey	Half	Lactose-Free	Cow	Conventional	UHT

538

539

540

541

542

543

544 *Table 2. Integral areas of glycerol, lactose and fat NMR peaks of analysed milks, normalized*545 *to the imidazole signal.*

Sample Name	Glycerol	Lactose	Fat	Sample Name	Glycerol	Lactose	Fat
WMF-1	1.126 ± 0.0003	18.023 ± 0.0108	40.585 ± 0.0209	GMF-19	0.723 ± 0.0006	20.1 ± 0.0301	27.899 ± 0.0089
WMF-2	1.204 ± 0.0037	21.068 ± 0.0582	49.632 ± 0.1252	GMF-20	0.749 ± 0.0004	17.719 ± 0.0146	47.585 ± 0.0178
WMF-3	0.845 ± 0.0005	18.416 ± 0.0047	44.177 ± 0.0169	GMF-21	0.841 ± 0.0017	20.372 ± 0.0191	46.935 ± 0.0752
WMF-4	1.022 ± 0.0019	21.37 ± 0.0097	49.785 ± 0.0029	GMF-22	1.006 ± 0.0012	18.727 ± 0.0215	52.168 ± 0.0439
OMF-5	1.006 ± 0.0001	18.631 ± 0.0031	47.615 ± 0.0068	WMT-23	0.863 ± 0.0006	19.08 ± 0.0208	44.018 ± 0.0429
OMF-6	1.043 ± 0.0003	20.927 ± 0.0033	55.941 ± 0.0076	WMT-24	1.007 ± 0.0024	19.483 ± 0.0295	48.226 ± 0.0474
OMF-7	0.576 ± 0.0010	19.352 ± 0.0090	48.897 ± 0.0210	WMT-25	0.279 ± 0.0020	22.412 ± 0.0668	45.022 ± 0.0609
HFMF-8	0.687 ± 0.0008	20.311 ± 0.0015	25.845 ± 0.0284	WMT-26	0.428 ± 0.0010	18.615 ± 0.0257	41.632 ± 0.0496
OHFMF-9	0.275 ± 0.0002	19.461 ± 0.0211	20.38 ± 0.0286	WMT-27	0.772 ± 0.0004	18.911 ± 0.0092	44.755 ± 0.0124
HFMF-10	0.472 ± 0.0004	20.384 ± 0.0047	25.257 ± 0.0235	WMT-28	0.23 ± 0.0006	19.651 ± 0.0275	44.747 ± 0.0299
HFMF-11	0.195 ± 0.0012	22.737 ± 0.0488	24.772 ± 0.0430	OMT-29	0.175 ± 0.0005	22.332 ± 0.0176	46.65 ± 0.0544
HFMF-12	0.55 ± 0.0004	22.638 ± 0.0188	25.964 ± 0.0748	GMT-30	0.163 ± 0.0012	19.955 ± 0.0248	44.893 ± 0.0698
OHFMF-13	0.7 ± 0.0015	23.062 ± 0.0313	28.13 ± 0.0998	LFMF-31	0.436 ± 0.0014	0	24.89 ± 0.0947
WMF-14	0.956 ± 0.0007	21.808 ± 0.0065	57.543 ± 0.0135	LFMF-32	0.123 ± 0.0004	0	24.288 ± 0.0186
OHFMF-15	0.522 ± 0.0009	18.763 ± 0.0165	22.294 ± 0.0364	LFMT-33	0.412 ± 0.0016	0	22.52 ± 0.0412
HFMF-16	0.502 ± 0.0052	18.866 ± 0.0429	22.594 ± 0.1573	LFMT-34	0.297 ± 0.0009	0	23.29 ± 0.01711
OMF-17	0.817 ± 0.0007	22.858 ± 0.0236	57.398 ± 0.0498	LFMT-35	0.373 ± 0.0006	0	20.793 ± 0.0204
OHFMF-18	0.482 ± 0.0005	20.006 ± 0.0202	26.536 ± 0.0403	LFMT-36	0.059 ± 0.0417	0	27.991 ± 0.0841

546

547

548

549

550

551

552

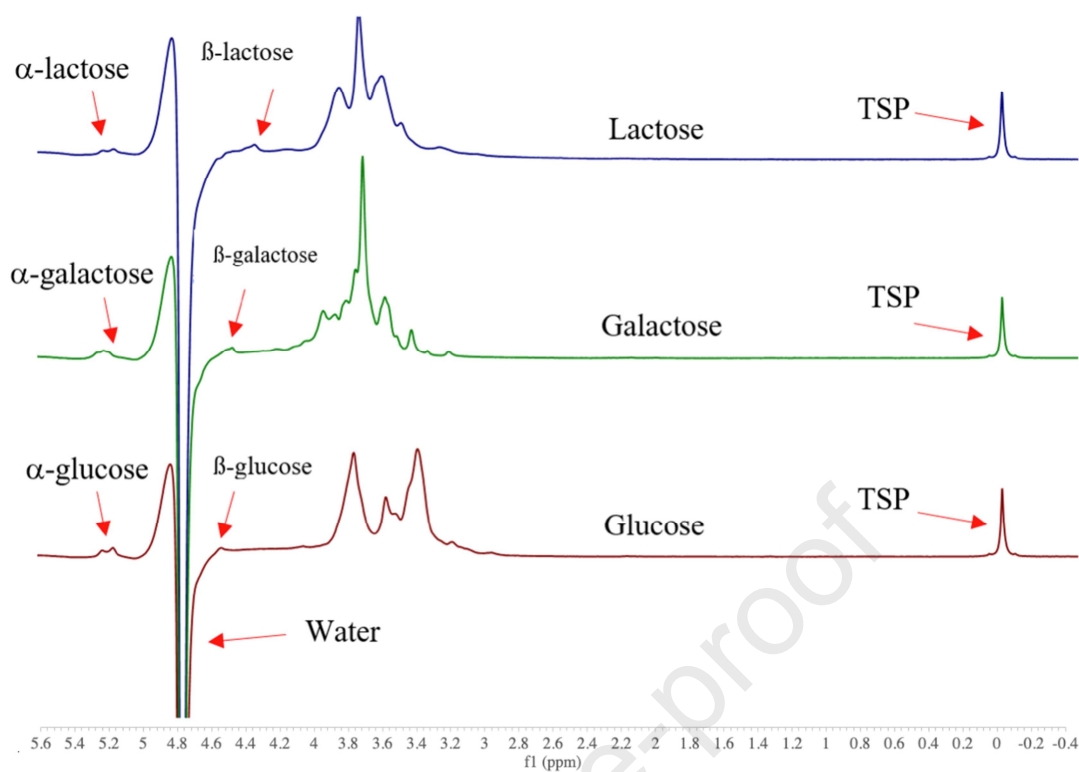
553

554

555

556

557



558

559

560 **Fig. 1**

561

562

563

564

565

566

567

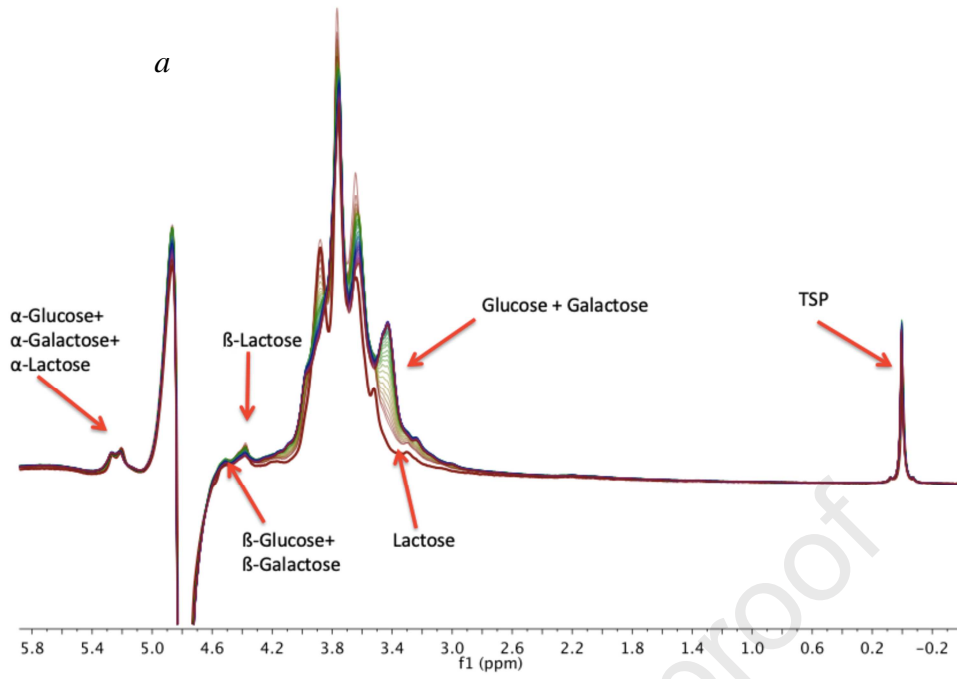
568

569

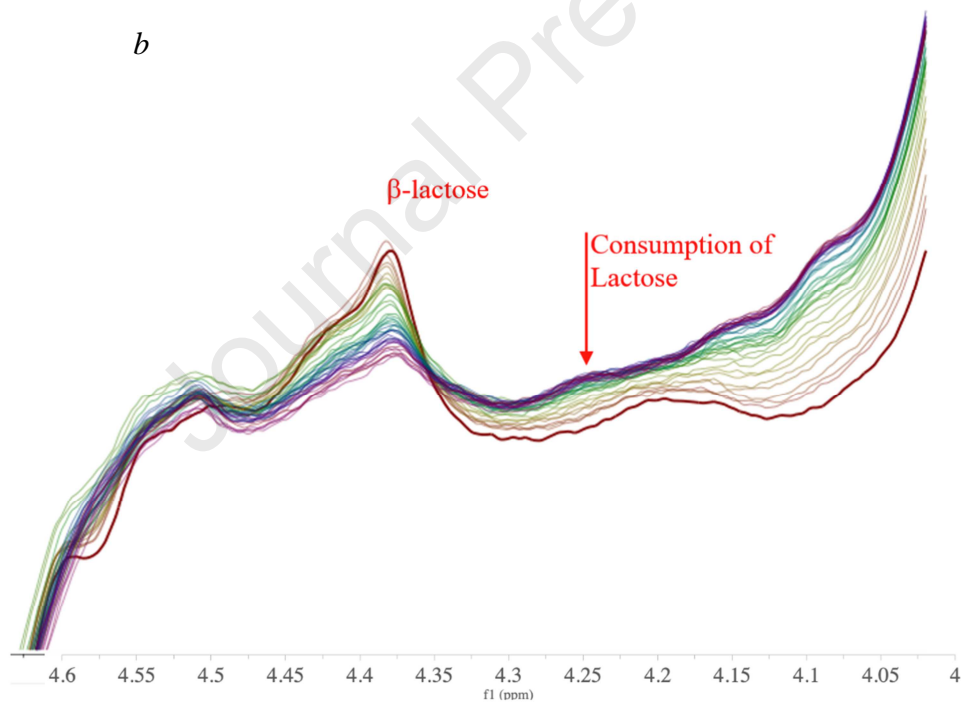
570

571

572



573

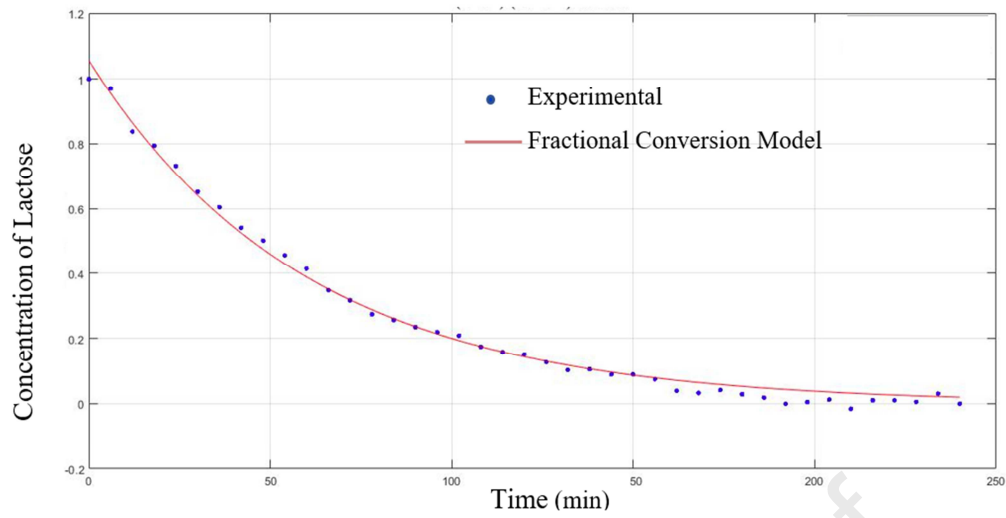


574

575 **Fig. 2**

576

577



578

579

580

581

582

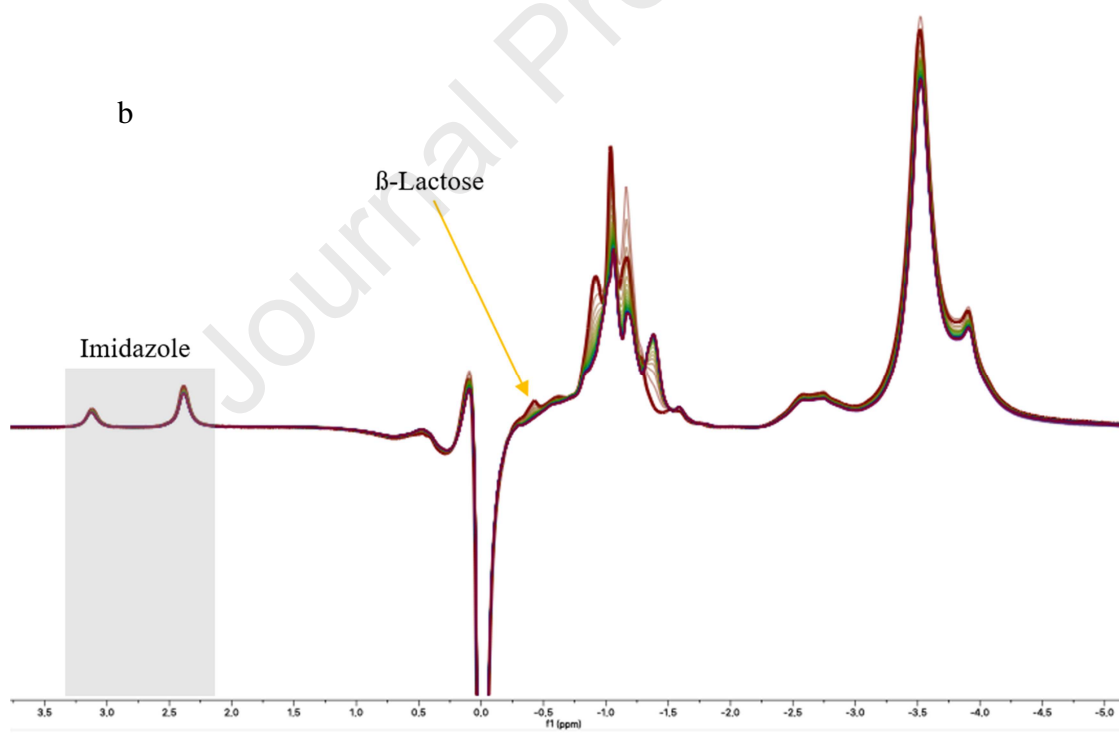
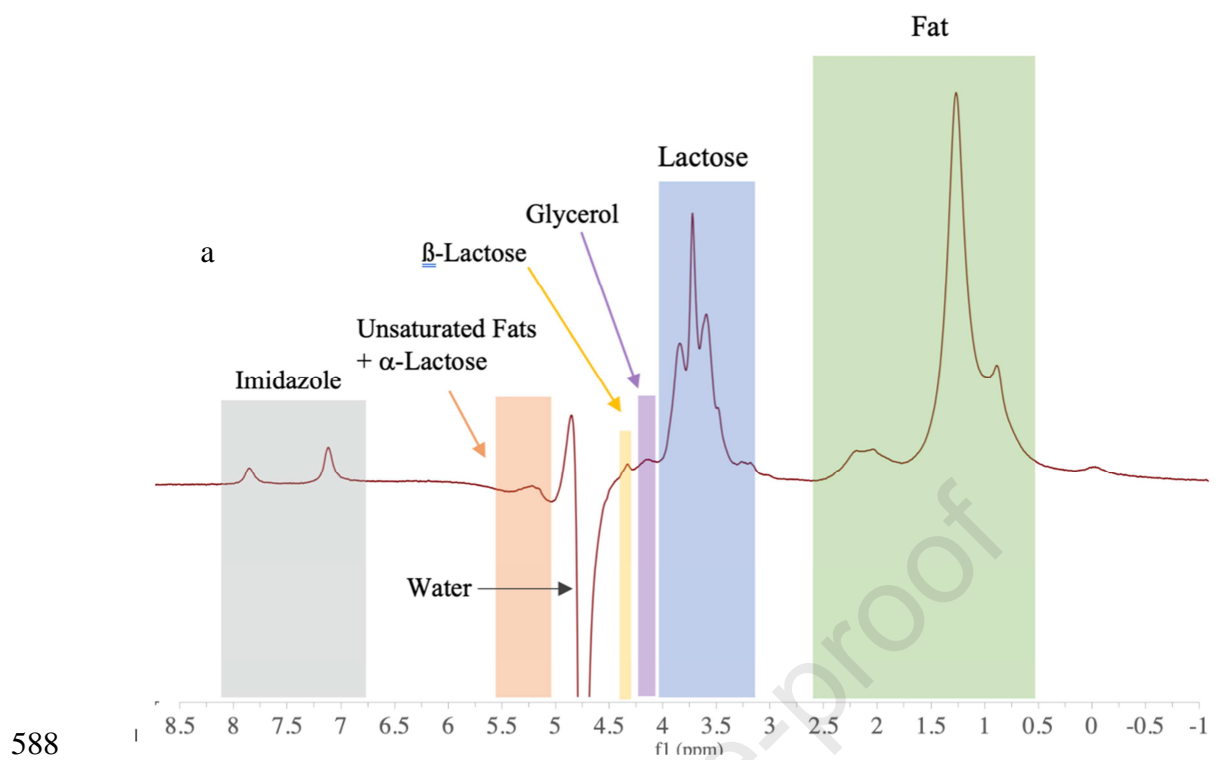
583

584 **Fig. 3**

585

586

587



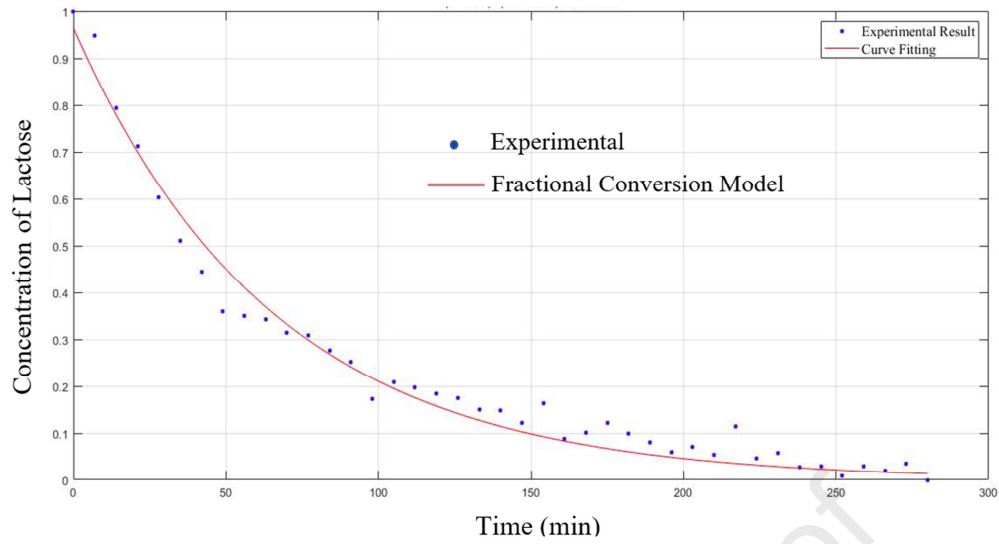
589

590

591

592

593 **Fig 4.**



594

595

596

597

598

599

600

601

602

603

604

605

606

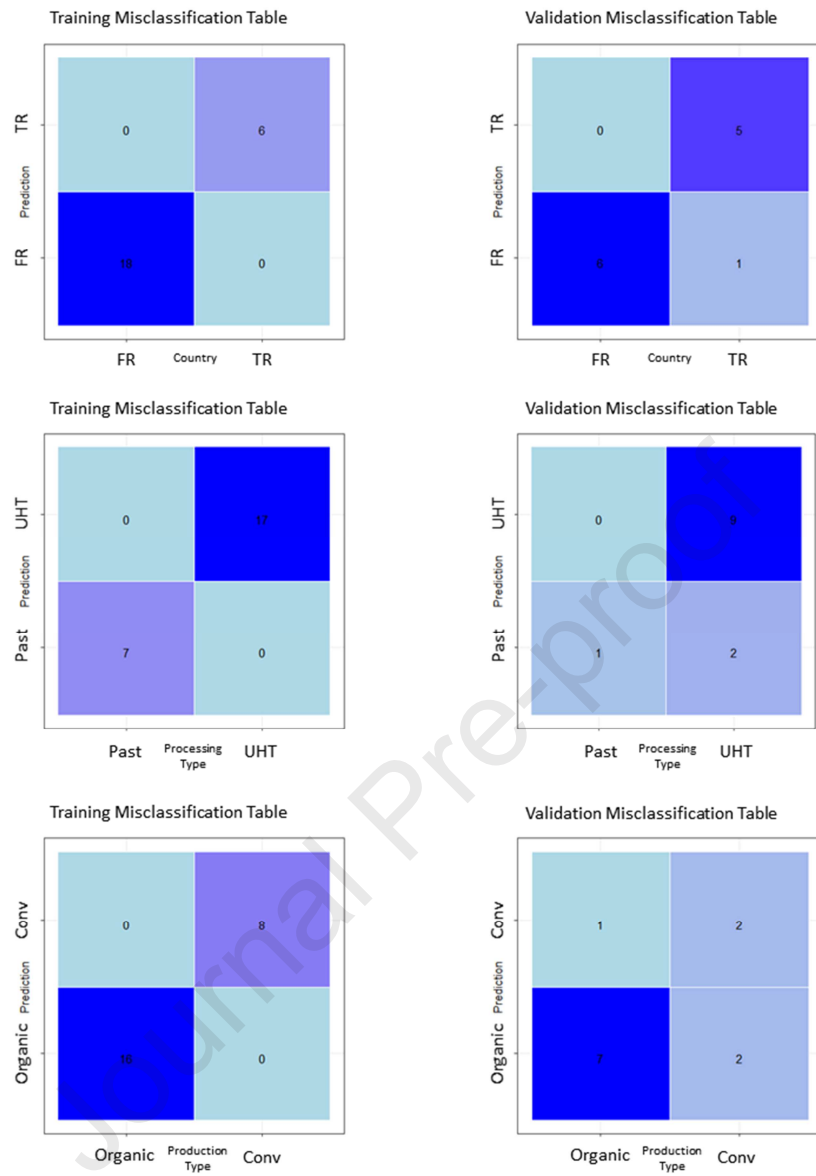
607

608

609

610

611 **Fig. 5**



612

613

614 **Fig. 6**

615

616

Sample Name	Country	Fat Content	Sugar Content	Milk Source	Production Style	Processing
WMF-1	France	Whole	Regular	Cow	Conventional	UHT
WMF-2	France	Whole	Regular	Cow	Conventional	UHT
WMF-3	France	Whole	Regular	Cow	Conventional	Pasteurized
WMF-4	France	Whole	Regular	Cow	Conventional	Pasteurized
OMF-5	France	Whole	Regular	Cow	Organic	UHT
OMF-6	France	Whole	Regular	Cow	Organic	Pasteurized
OMF-7	France	Whole	Regular	Cow	Organic	Pasteurized
HFMF-8	France	Half	Regular	Cow	Conventional	UHT
OHFMF-9	France	Half	Regular	Cow	Organic	Pasteurized
HFMF-10	France	Half	Regular	Cow	Conventional	Pasteurized
HFMF-11	France	Half	Regular	Cow	Conventional	Pasteurized
HFMF-12	France	Half	Regular	Cow	Conventional	UHT
OHFMF-13	France	Half	Regular	Cow	Organic	UHT
WMF-14	France	Whole	Regular	Cow	Conventional	UHT
OHFMF-15	France	Half	Regular	Cow	Organic	UHT
HFMF-16	France	Half	Regular	Cow	Conventional	UHT
OMF-17	France	Whole	Regular	Cow	Organic	Pasteurized
OHFMF-18	France	Half	Regular	Cow	Organic	UHT
GMF-19	France	Half	Regular	Goat	Conventional	UHT
GMF-20	France	Whole	Regular	Goat	Organic	UHT
GMF-21	France	Whole	Regular	Goat	Organic	UHT
GMF-22	France	Whole	Regular	Goat	Organic	UHT
WMT-23	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-24	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-25	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-26	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-27	Turkey	Whole	Regular	Cow	Conventional	UHT
WMT-28	Turkey	Whole	Regular	Cow	Conventional	UHT
OMT-29	Turkey	Whole	Regular	Cow	Organic	UHT
GMT-30	Turkey	Whole	Regular	Goat	Conventional	UHT
LFMF-31	France	Half	Lactose-Free	Cow	Conventional	UHT
LFMF-32	France	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-33	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-34	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-35	Turkey	Half	Lactose-Free	Cow	Conventional	UHT
LFMT-36	Turkey	Half	Lactose-Free	Cow	Conventional	UHT

Sample Name	Glycerol	Lactose	Fat	Sample Name	Glycerol	Lactose	Fat
WMF-1	1.126 ± 0.0003	18.023 ± 0.0108	40.585 ± 0.0209	GMF-19	0.723 ± 0.0006	20.1 ± 0.0301	27.899 ± 0.0089
WMF-2	1.204 ± 0.0037	21.068 ± 0.0582	49.632 ± 0.1252	GMF-20	0.749 ± 0.0004	17.719 ± 0.0146	47.585 ± 0.0178
WMF-3	0.845 ± 0.0005	18.416 ± 0.0047	44.177 ± 0.0169	GMF-21	0.841 ± 0.0017	20.372 ± 0.0191	46.935 ± 0.0752
WMF-4	1.022 ± 0.0019	21.37 ± 0.0097	49.785 ± 0.0029	GMF-22	1.006 ± 0.0012	18.727 ± 0.0215	52.168 ± 0.0439
OMF-5	1.006 ± 0.0001	18.631 ± 0.0031	47.615 ± 0.0068	WMT-23	0.863 ± 0.0006	19.08 ± 0.0208	44.018 ± 0.0429
OMF-6	1.043 ± 0.0003	20.927 ± 0.0033	55.941 ± 0.0076	WMT-24	1.007 ± 0.0024	19.483 ± 0.0295	48.226 ± 0.0474
OMF-7	0.576 ± 0.0010	19.352 ± 0.0090	48.897 ± 0.0210	WMT-25	0.279 ± 0.0020	22.412 ± 0.0668	45.022 ± 0.0609
HFMF-8	0.687 ± 0.0008	20.311 ± 0.0015	25.845 ± 0.0284	WMT-26	0.428 ± 0.0010	18.615 ± 0.0257	41.632 ± 0.0496
OHFMF-9	0.275 ± 0.0002	19.461 ± 0.0211	20.38 ± 0.0286	WMT-27	0.772 ± 0.0004	18.911 ± 0.0092	44.755 ± 0.0124
HFMF-10	0.472 ± 0.0004	20.384 ± 0.0047	25.257 ± 0.0235	WMT-28	0.23 ± 0.0006	19.651 ± 0.0275	44.747 ± 0.0299
HFMF-11	0.195 ± 0.0012	22.737 ± 0.0488	24.772 ± 0.0430	OMT-29	0.175 ± 0.0005	22.332 ± 0.0176	46.65 ± 0.0544
HFMF-12	0.55 ± 0.0004	22.638 ± 0.0188	25.964 ± 0.0748	GMT-30	0.163 ± 0.0012	19.955 ± 0.0248	44.893 ± 0.0698
OHFMF-13	0.7 ± 0.0015	23.062 ± 0.0313	28.13 ± 0.0998	LFMF-31	0.436 ± 0.0014	0	24.89 ± 0.0947
WMF-14	0.956 ± 0.0007	21.808 ± 0.0065	57.543 ± 0.0135	LFMF-32	0.123 ± 0.0004	0	24.288 ± 0.0186
OHFMF-15	0.522 ± 0.0009	18.763 ± 0.0165	22.294 ± 0.0364	LFMT-33	0.412 ± 0.0016	0	22.52 ± 0.0412
HFMF-16	0.502 ± 0.0052	18.866 ± 0.04292	22.594 ± 0.1573	LFMT-34	0.297 ± 0.0009	0	23.29 ± 0.01711
OMF-17	0.817 ± 0.0007	22.858 ± 0.0236	57.398 ± 0.0498	LFMT-35	0.373 ± 0.0006	0	20.793 ± 0.0204
OHFMF-18	0.482 ± 0.0005	20.006 ± 0.0202	26.536 ± 0.0403	LFMT-36	0.059 ± 0.0417	0	27.991 ± 0.0841

Highlights

- Multiple aspects of benchtop NMR spectroscopy were used to analyze milk.
- Benchtop system was adapted to follow lactose hydrolysis in milk in flow mode.
- Combination of spectroscopic and multivariate approaches enabled to classify milk.
- Benchtop NMR spectroscopy was shown as a method to control and ensure milk quality.

Journal Pre-proof

Declaration of Interest

All authors declare no conflict of interest.

Publication has been approved by all individual participants.

Journal Pre-proof