

 Open access • Journal Article • DOI:10.1021/JF405527T

## Innovative Nondestructive Measurements of Water Activity and the Content of Salts in Low-Salt Hake Minces — [Source link](#)

Kirsti Greiff, Kirsti Greiff, Ana Fuentes, Ida Grong Aursand ...+4 more authors

**Institutions:** Norwegian University of Science and Technology, SINTEF, Polytechnic University of Valencia

**Published on:** 11 Mar 2014 - Journal of Agricultural and Food Chemistry (American Chemical Society)

**Topics:** Low sodium, Ion selective electrode, Ion chromatography and Sodium

Related papers:

- [Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments](#)
- [Study of water dynamics in the soaking, steaming, and solid-state fermentation of glutinous rice by LF-NMR: a novel monitoring approach.](#)
- [Water properties and salt uptake in Atlantic salmon fillets as affected by ante-mortem stress, rigor mortis, and brine salting: A low-field <sup>1</sup>H NMR and <sup>1</sup>H/<sup>23</sup>Na MRI study](#)
- [Origin of multiexponential T\(2\) relaxation in muscle myowater.](#)
- [Temperature Invariance of NaCl Solubility in Water: Inferences from Salt–Water Cluster Behavior of NaCl, KCl, and NH<sub>4</sub>Cl](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/innovative-nondestructive-measurements-of-water-activity-and-y6d0q18dww>

Document downloaded from:

<http://hdl.handle.net/10251/66102>

This paper must be cited as:

Greiff, K.; Fuentes López, A.; Aursand, IG.; Erikson, U.; Masot Peris, R.; Alcañiz Fillol, M.; Barat Baviera, JM. (2014). Innovative non-destructive measurements of salt in low-salt hake minces. *Journal of Agricultural and Food Chemistry*. 62(12):2496-2505.  
doi:10.1021/jf405527t



The final publication is available at

<https://dx.doi.org/10.1021/jf405527t>

Copyright American Chemical Society

Additional Information

1 **Innovative Non-Destructive Measurements of Water Activity and the**  
2 **Content of Salts in Low-Salt Hake Minces**

3

4 Kirsti Greiff<sup>a,b</sup>, Ana Fuentes<sup>c</sup>, Ida G. Aursand<sup>a</sup>, Ulf Erikson<sup>a</sup>, Rafael Masot<sup>d</sup>, Miguel Alcañiz<sup>d</sup>,  
5 and Jose M. Barat<sup>c</sup>

6

7 <sup>a</sup> SINTEF Fisheries and Aquaculture, Brattørkaia 17C, N-7465 Trondheim, Norway

8 <sup>b</sup> Department of Biotechnology, Norwegian University of Science and Technology, Sem  
9 Sælandsvei 6/8, N-7491, Trondheim, Norway

10 <sup>c</sup>Departamente de Tecnología de Alimentos (DTA), Universitat Politècnica de València, Cno  
11 de Vera s/n 46022 València, Spain

12 <sup>d</sup>Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Centro Mixto  
13 Universitat Politècnica de València, Cno de Vera s/n 46022 València, Spain

14

15 Running title header: Sodium reduction, potassium chloride, spectroscopic impedance,  
16 sodium measurement, low field NMR

17

18 \*corresponding author:

19 Kirsti Greiff, SINTEF Fisheries and Aquaculture, Brattørkaia 17C, 7465 Trondheim, Norway

20 Tel: +4747903891, Fax: +47 93 27 07 01, E-mail: kirsti.greiff@sintef.no

21

22 **Abstract**

23 Impedance spectroscopy (IS), Low-field proton Nuclear Magnetic Resonance (LF <sup>1</sup>H NMR),  
24 chloride titration, ion chromatography and ion selective electrode were used to investigate  
25 physicochemical parameters and measure sodium and potassium contents in low salt brines and  
26 fish. Salt solutions (0-3 w/w,%) and model products of minced hake with added NaCl (0.5–3.0  
27 w/w,%), or a mixture of NaCl and KCl (50/50 w/w,%) were analyzed. Good correlation was  
28 observed between the sodium content determined by ion selective electrode method and ion  
29 chromatography ( $R^2=0.97$ ). In both salt solutions and fish minces, the impedance spectroscopy  
30 measurements could detect the difference in salt contents in mince with salt contents down to  
31 0.5%. The NMR transversal relaxation time  $T_2$  measurements clearly distinguished samples  
32 with 0, 0.5% and 1.0 - 3.0% salt, based on the principal component analysis (PCA). Therefore,  
33 LF <sup>1</sup>H NMR seems to be a suitable technique for studies of low-salt products.

34 **Key words:** Impedance spectroscopy, low sodium, low salt, low-field NMR,  $T_2$  relaxation,  
35 hake

36

## 37 INTRODUCTION

38 A high consumption of sodium has been directly associated with a greater likelihood of  
39 increased blood pressure, which in turn has been directly related to the development of  
40 cardiovascular and renal diseases. <sup>1</sup> For these reasons, national and international bodies have  
41 set targets for a reduction in sodium consumption. <sup>2</sup> Salt is commonly employed in fish  
42 processing, because it helps increase shelf-life, reduce water activity ( $a_w$ ) and it has an  
43 important effect on water holding capacity, fat binding, color, flavor, and texture. <sup>3-5</sup> Fish is  
44 used as a main ingredient in numerous seafood products, such as fish sausages, surimi and  
45 surimi-based products, like fish puddings.

46 The development of low-sodium fish products without affecting product quality and  
47 safety is of interest, especially considering the otherwise good nutritional characteristics of fish.  
48 The partial substitution of NaCl by KCl has shown to be one of the best alternatives for reducing  
49 sodium content. <sup>6-8</sup> Indeed, both salts have similar properties and the health effects of increased  
50 potassium intake are continuously evaluated by the international health authorities. <sup>9-11</sup>  
51 Replacement of NaCl by to high concentrations of KCl may have a negative influence on the  
52 flavor intensity and produce bitter tastes. <sup>12</sup>

53 Parameters such as  $a_w$  and salt content have important implications for product shelf-  
54 life and consumer safety. In this regard, the development of rapid, accurate, and non-destructive  
55 methods for monitoring, these parameters, independently of the sodium replacement, is of  
56 industrial interest as well as to determine the sodium content accurately in food. The increasing  
57 use of salt- replacers such as potassium chloride <sup>8</sup> makes it necessary to find new rapid  
58 techniques for determining the sodium content directly, since measuring the chloride content  
59 no longer represents the sodium content in the food.

60 Analytical methods for the determination of salt include flame atomic absorption  
61 spectrophotometry (FAAS), inductively coupled plasma/MS (ICP/MS), ionic chromatography,

62 and sodium selective electrodes <sup>13</sup>. Other methods, such as Volhart method (AOAC method  
63 971.27) <sup>14</sup> and potentiometric titration <sup>15</sup> measure the chloride contents and the sodium content  
64 is then calculated stoichiometrically.

65 To meet the objective of developing fast, non-destructive methods to monitor product  
66 quality as affected by sodium reduction, electronic sensors based on impedance spectroscopy  
67 (IS) may be an option. The relationship between sodium chloride content and impedance  
68 measurements has already been demonstrated. <sup>16-19</sup> In the IS technique, an electrical sinusoidal  
69 stimulus is applied to the electrodes to measure the impedance of the sample at different  
70 frequencies. The module and phase of the impedance can vary significantly according to the  
71 charges present (free ions), types of microstructure and electrolytes, as well as texture,  
72 geometry, and the electrodes used. <sup>18</sup> However, this technique has not yet been applied to food  
73 products in which sodium has been replaced by other cations.

74 The effect of salting can also be determined indirectly, for example by using low-field  
75 (LF) <sup>1</sup>H NMR to monitor changes in proton relaxation behavior as a result of salt addition. In  
76 foods, the NMR proton signals basically originate from small molecules like water and fat.  
77 Changes in tissue microstructure due to salting will affect proton exchange with the surrounding  
78 environment. For example, tissue swelling after the addition of salt leads to a more open  
79 microstructure causing higher water mobility. Several studies have been carried out where LF  
80 NMR has been used to monitor changes during fish salting processes. <sup>20-26</sup> However, since none  
81 of these studies have dealt with low-salt tissues, it would be of interest to explore the method  
82 further as a potential tool for low-salt applications.

83 The objectives of the present research are to (1) evaluate the application of impedance  
84 spectroscopy to monitor physicochemical parameters in salted fish products with, and without  
85 sodium replacement, (2) establish a fast and consistent method to measure sodium and

86 potassium contents in fish products, and (3) assess the feasibility of employing LF NMR in  
87 low-salt tissues.

88

## 89 **MATERIALS AND METHODS**

### 90 **Chemicals**

91 Ammonium Chloride ( $\text{NH}_4\text{Cl}$ ), Ammonium Hydroxide ( $\text{NH}_4\text{OH}$ ), Ammonium  
92 Hydrogen Fluoride ( $\text{NH}_4\text{FHF} < 1\%$ ,  $\text{LD}_{50}$  mg/kg not found), Chloroform ( $\text{CHCl}_3$ ), Ethanol  
93 ( $\text{C}_2\text{H}_5\text{OH}$ ), Sulfuric acid ( $\text{H}_2\text{SO}_4$ ), Potassium sulfate ( $\text{K}_2\text{SO}_4$ ), Copper Sulfate ( $\text{CuSO}_4$ ),  
94 Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) and Sodium hydroxide ( $\text{NaOH}$ ) (Scharlau, S.A. or Thermo Fisher  
95 Scientific, USA). All the chemicals were of analytical-reagent grades.

### 96 **Experimental protocol**

97 Experiments using the impedance system were carried out in two phases. In the first phase, the  
98 system`s capability to distinguish between different types and quantities of salts was evaluated.  
99 The second phase evaluated the impedance system for discriminating between fish samples  
100 salted with different salt mixtures and quantities of salt.

### 101 **Phase I: Salt solutions**

102 Different brines were prepared by using  $\text{NaCl}$ ,  $\text{KCl}$ , and a mixture of  $\text{NaCl/KCl}$  (50/50, w/w,  
103 %) at different contents. The total salt contents assessed were 0.0, 0.1, 0.5, 1.0 1.5, 2.0 2.5 and  
104 3.0% (g salt/100 g distilled water).  $\text{NaCl}$  and  $\text{KCl}$  reagents (analytical-reagent grade) were  
105 obtained from Panreac Química S.A.U. (Barcelona, Spain). The brines were prepared the day  
106 before analysis to ensure that all of the salt was completely dissolved. The parameters measured  
107 in brines were  $a_w$ , pH, conductivity and sodium and chloride contents. Sodium content in brines  
108 was determined by a Na-selective electrode. All measurements were done in triplicate.  
109 Impedance spectroscopy measurements were also carried out on the same brine solutions.

110 **Phase II: fish minces**

111 Fresh hake (*Merluccius paradoxus/capensis*) were used as raw material. The fish were caught  
112 in June 13. 2012 by trawling of the coast of South Africa (FAO fishing area 47, Atlantic  
113 Southeast) and were obtained June 19<sup>th</sup> 2012 from a local supermarket in Valencia (Spain). The  
114 fish specimens were placed in styrofoam boxes with ice and transported immediately to the  
115 laboratory. Upon arrival to the laboratory, two fish were headed and gutted. Then, the fish were  
116 filleted, skinned, and the flesh was chopped with a standard food processor at low speed  
117 (Minirobot D81, Moulinex, Group SEB Iberica, Barcelona, Spain). Samples were prepared by  
118 mixing the fish mince (fish mince and salt, 200 g total) with an exact amount of salt before  
119 homogenising for 1 min. in the food processor. The amount of salt added to the fish mince had  
120 been pre-weighed to achieve an exact salt content (NaCl or NaCl/KCl) in the final sample 0.0,  
121 0.5, 1.0, 2.0 and 3.0 % salt (g NaCl or NaCl/KCl/100 g salted fish mince). The homogenized  
122 fish minces were divided into five plastic containers (40 g. in each). Three of the plastic  
123 containers were used for the physicochemical analyses and impedance spectroscopy, whereas  
124 the remaining two containers were used for LF-NMR measurements. According to the results  
125 obtained by Sánchez-Alonso,<sup>27</sup> the mince composition does not suffer significant alterations  
126 during the frozen storage period. The samples were stored at -18°C and thawed to 4°C during  
127 18 h before analysis. Moisture, lipid, protein, ash,  $a_w$ , pH, chloride, sodium and potassium  
128 contents, were determined in the same subsamples as were subjected to impedance  
129 spectroscopy. The minces assigned for LF NMR analysis were kept frozen for 86 days before  
130 the measurements were carried out.

131 **ANALYTICAL METHODS**

132 **Physicochemical analyses**

133 Moisture, lipid, protein and ash contents were assayed by AOAC Methods 950.46, 991.36,  
134 928.08, and 920.153, respectively,<sup>28</sup> whereas pH and conductivity of brines were determined



135 by using a multimeter MM 40 (Crison Instruments, S.A., Barcelona, Spain). The pH  
136 measurements of fish were carried out using a digital pH-meter micropH 2001 (Crison  
137 Instruments) with a puncture electrode (Crison 5231). Water activity was assessed in brines and  
138 fish minces with a fast water activity-meter (GBX FAsT/lab, Romans sur Isère Cedex, France).

139 The chloride and sodium contents in brines were measured directly in the solutions,  
140 using a Chloride Analyzer (Sherwood mod. 926, Cambridge, UK) and a Dual Star™ pH/ISE  
141 Meter ( Thermo Fisher Scientific, Waltham, MA, USA) with a Na-selective electrode (Ross®  
142 Sodium Ion Selective Electrode, Thermo Fisher Scientific, USA), respectively. Chloride,  
143 sodium (by two different analytical methods) and potassium contents of fish minces were  
144 measured in an extract of the sample. For preparing the extract, 1.5 g of the mince was  
145 homogenised in ultra-pure water using an Ultra-turrax T-25 (IKA, Labortechnik, Staufen,  
146 Germany) at 9000 rpm for 1 min. Then, samples were warmed up to 90 °C for 30 min, cooled  
147 down to room temperature, transferred to a volumetric flask and deluted up to 200 mL with  
148 ultra-pure water. Finally, samples were filtered through a cellulose filter paper (Whatman n° 1,  
149 Whatman International Ltd., Maidstone, UK). For chloride and sodium determinations, an  
150 aliquot of the extract was measured at room temperature by using the Chloride Analyzer and  
151 the Na-selective electrode as described above. The Na-selective electrode method was a  
152 modification of the Kivikari method.<sup>29</sup> In this study the direct calibration method was used,  
153 contrary to the method of Kivikari, where the known addition method was used. A calibration  
154 curve was made by using three standards of analytical-grade NaCl from Panreac Química  
155 S.A.U. (Barcelona, Spain). Sodium ion strength adjustor (Sodium ionic strength adjustor,  
156 Thermo Fisher Scientific, USA) was added to all solutions to ensure that samples and standards  
157 had similar ionic strength. Sodium and potassium contents of the samples were determined by  
158 ion chromatography (Compact IC 761, Metrohm® Ltd., Herisau, Switzerland) by using an ion  
159 exchange column (Metrosep C2, 250/4.0, Metrohm® Ltd., Herisau, Switzerland). The

160 separation was monitored by using a regulated (20°C) conductivity detector and the IC Net 2.3  
161 (Methrom® Ltd.) software was used for data collection and processing. Prior to analysis,  
162 samples were filtered through 0.45 µm nylon syringe filters. The isocratic elution was carried  
163 out using a solution of tartaric acid (4.0 mM)/dipicolinic acid (0.75 mM) at a flow rate of 1  
164 mL/min. Samples were injected using a 20 µl loop injector. The content of each cation was  
165 determined by interpolation in the corresponding calibration curve. The calibration was  
166 established using a triplicate set of standard solutions of Na<sup>+</sup> (Fluka, Buchs, Switzerland) and  
167 K<sup>+</sup> (Sigma-Aldrich, St. Louis, MO, USA).

### 168 **Impedance spectroscopy**

169 The impedance spectroscopy measurement system was developed by the Instituto de  
170 Reconocimiento Molecular y Desarrollo Tecnológico (IDM) at the Universidad Politécnica de  
171 València (UPV).<sup>18</sup> It consists of a software application that runs on a PC, electronic equipment  
172 and an electrode (for more information look in the supplementary information).

173 Using the software application the user chooses the frequencies and the amplitudes of  
174 the sinusoidal voltage signals. For each of the frequencies the electronic equipment generates  
175 the corresponding sinusoidal voltage waveform to the electrode. The current (I) and voltage (V)  
176 signals at the electrode are then sampled and the collected data are sent to the PC where a  
177 Discrete Fourier transform analysis (DFT) is performed to determine their amplitude and phase.  
178 The module  $|Z|$  and the phase ( $\phi$ ) of the *impedance* are then calculated using Eq. 1, where  $v(t)$   
179 is the voltage signal,  $i(t)$  the current signal,  $f$  the frequency of the signals, and  $\Delta t$  is the time  
180 interval between the zero crossing of the voltage and current signals (**Figure 1**).

$$181 \quad Z = |Z|e^{j\phi} \quad \begin{cases} |Z| = \frac{|v(t)|}{|i(t)|} & \text{Module} \\ \phi = 2\pi f\Delta t & \text{Phase} \end{cases} \quad (1)$$

182 The electronic equipment includes a digital processing block based on two CPLD's and three  
183 Random-access memories (RAM), one digital-to-analog converter, two analog-to-digital  
184 converters and some analog signal adaption circuits.<sup>18</sup>

185 The sensor employed in this study is a double electrode designed at IDM-UPV. The  
186 sensor consists of two steel needles 1.5 cm long and 1 mm in diameter, separated by a distance  
187 of 1 cm in a non-conductive frame. This design keeps the separation between both needles  
188 constant during measurements.

189 The impedance measurements were taken by inserting the sensors into the middle of the  
190 plastic containers (n=3) containing the solutions or the fish minces. Ten parallel measurements  
191 were performed in each plastic container. The penetration depth of the electrodes was constant  
192 in all the analyses (1.5 cm). All measurements were carried out at room temperature.

193 Preliminary Impedance Spectroscopy measurements showed that information given by  
194 low frequencies was not relevant for this study. Therefore all the measurements were carried  
195 out in the range of [10 kHz-1 MHz]. Seventeen frequencies were chosen in this range, thus a  
196 set of 34 values (17 module values and 17 phase values) were obtained for each sample.

### 197 **Low field <sup>1</sup>H- NMR**

198 LF <sup>1</sup>H NMR measurements were made on all fish minces. After thawing, approximately 2 grams  
199 samples were taken from each subsample of fish mince (n=2), and placed in NMR tubes  
200 (diameter 10 mm). There were analyzed three parallels from each subsample with fish mince  
201 in the LF <sup>1</sup>H NMR measurements. The tubes were immediately placed in ice and kept there  
202 until the NMR measurements were carried out. The measurements were performed using a  
203 Bruker minispec mq 20 (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field  
204 strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz. The instrument  
205 was equipped with a 10 mm temperature-variable probe. A built-in heating element was  
206 connected to the temperature control unit (BVT3000, Bruker Optik GmbH). The temperature

207 in the probe was regulated to 4°C by blowing compressed air through the sample holder.  
208 Transversal (T<sub>2</sub>) relaxation was measured using the Carr-Purcell-Meiboom-Gill pulse sequence  
209 (CPMG).<sup>30, 31</sup> The T<sub>2</sub> measurements were performed with a time delay between the 90° and  
210 180° pulses (τ) of 150 μs. Data from 4000 echoes were acquired from 16 scan repetitions. The  
211 repetition time between two succeeding scans was set to 3 s. All even echoes were sampled.  
212 The NMR transverse relaxation data were analyzed using two different calculation methods.  
213 (1) Biexponential analysis of T<sub>2</sub> relaxation data was performed by fitting of the following  
214 equation to the experimental CPMG curves, similar to that reported by Erikson et al.<sup>21</sup> and  
215 Lambelet et al.<sup>32</sup> :

$$216 \quad S_i = A_1 e^{-t/T_{21}} + A_2 e^{-t/T_{22}} \quad (\text{Eq. 2})$$

217 where T<sub>21</sub> and T<sub>22</sub> are the relaxation time components, and A<sub>21</sub> and A<sub>22</sub> are the corresponding  
218 amplitudes, 4000 data points were used, and the calculations were made using MatLab (The  
219 Mathworks Inc., Natick, MA). Since the absolute relaxation amplitudes are proportional to the  
220 amount of water and fat in the sample, the relative amplitudes within samples were used. The  
221 T<sub>21</sub> populations are calculated as: A<sub>21</sub>/(A<sub>21</sub> + A<sub>22</sub>).

222 For the biexponential fitting, the populations sum up to 100%. Three parallel samples  
223 from each fish mince (n=2) were averaged. (2) Multivariate data analysis was performed for all  
224 raw relaxation (CPMG) curves. These curves were normalized by setting the first sampled echo  
225 to a value of 100, and thereafter scaling the rest of the echo-train. The first 600 data points were  
226 used for the principal component analysis (PCA).

### 227 **Statistical analyses**

228 Statistical treatment of the data was performed using the Statgraphics Centurion (Statpoint  
229 Technologies, Inc., Warrenton, VA, USA). A multifactor analysis of variance (ANOVA) was  
230 conducted for each evaluated parameter to test whether there were significant differences  
231 between the samples. These analyses were performed for the salt solutions and fish mince

232 samples (phases I and II); in both cases, the physicochemical parameters were considered as  
233 dependent variables in these analyses. The type of cations and salt content, as well as its  
234 interaction were the factors. The Tukey test (least significant difference) was used to test for  
235 differences between averages at the 5% significance level.

236 In order to evaluate the measurement techniques used in this paper, different multivariate  
237 analyses<sup>33</sup> were carried out using the software SOLO PLS\_Toolbox (Eigenvector Research,  
238 Inc., Wenatchee, WA).

239 Principal Component Analysis (PCA) was used to discriminate the salt content level for  
240 NaCl, KCl and mixtures. Typically, in PCA projects a multi-dimensional data set onto a new  
241 coordinate base formed by the orthogonal directions with data maximum variance. The  
242 eigenvectors of the data matrix are called principal components and they are uncorrelated  
243 between them. The principal components (PCs) are ordered so that PC1 displays the greatest  
244 amount of variance, followed by the next greatest PC2 and so forth. The main features of PCA  
245 are the coordinates of the data in the new base (scores plot) and the contribution to each  
246 component of the sensors (loads plot).

247 To create predictive models of physicochemical parameters, Partial Least Square (PLS)  
248 regressions were applied to both impedance spectroscopy and NMR measurements. The main  
249 objective of PLS is to predict one or more parameters (dependent variables Y) from a set of  
250 measured data (independent variables X). First, the set of independent variables is projected  
251 onto a new coordinate space by maximizing the covariance between Y and X. The axes of this  
252 new space are called latent variables (LV's). The important information that correlates Y and  
253 X is contained in the first LV's. Then a prediction model is built by applying a multiple  
254 regression to a reduced number of the LV's. PLS prediction models for  $a_w$ , Na, K, NaCl, and  
255 solute contents (g/100g) as well as solutes content in the water phase (g solutes/100g liquid  
256 phase) were created using a set of experimental data (calibration set). First, cross validation was

257 used to select the number of LV's. The model was then validated with a new set of experimental  
258 data (validation set).

259 In the case of impedance measurements PCA's and PLS regressions were performed  
260 using impedance module and phase values obtained for the 17 frequencies in the range from  
261 [10 kHz to 1 MHz]. In the case of NMR measurements, the relaxation times for each defined  
262 frequency were used.

263

## 264 **RESULTS AND DISCUSSION**

### 265 **Phase I: Salt solutions**

#### 266 **Physicochemical parameters**

267 The results of the physicochemical analyses carried out for the salt solutions are shown in **Table**  
268 **1**. As expected, the  $a_w$  of brines decreased with increasing brine content regardless of type of  
269 salt and the conductivity increased as salt content increased. Conductivity correlates with the  
270 total dissolved solids independently of the solute composition. In water, ions pass the electricity  
271 from one to another, therefore, the more  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  the solution contain the more  
272 electricity is carried and the higher the conductivity. This explained the fact that the  
273 conductivity was affected by the amount of salt but not by the sodium replacement. The initial  
274 conductivity of distilled water employed for preparing the salt solutions was  $0.025 \pm 0.003$   
275 mS/cm. The value increased with increasing salt content, from 2 to 60 mS/cm for the lowest  
276 and the highest salt content, respectively. **Table 1** also shows the resulting contents of  $\text{Na}^+$  and  
277  $\text{Cl}^-$  after different salt additions to distilled water. In the solution prepared from KCl only,  
278 sodium was present in the range of 0.3 to 2.2 mg/L. The observed differences in chloride content  
279 depending on the type of salt are due to the different atomic mass of sodium and potassium (23  
280 and 39 atomic mass units, respectively) owing to the fact that the salts were added equally by  
281 weight.

282           **Impedance spectroscopy**

283   Module and phase impedance spectra of KCl solutions are shown in **Figure 2a** and **2b**,  
284   respectively. Differences in both module and phase of impedance were observed to depend on  
285   salt content.

286           The module of the impedance decreased as the salt content increased, and the values  
287   were much higher for the lowest content (0.1% KCl) than for the other contents. Similar  
288   differences between salt contents were observed for NaCl and the mixture of NaCl:KCl (data  
289   not shown). These results are in agreement with those observed for the conductivity parameters  
290   for the brine (**Table 1**). The results are in accordance with previous studies on impedance  
291   spectroscopy.<sup>16-18</sup> The correlation can be explained by the conductance of an aqueous solution  
292   as a function of the ion content of the samples, and in fact impedance measurements are related  
293   with the ions capability of movement under the influence of an electrical field in this aqueous  
294   solution. In the present study, the behavior observed for NaCl solutions was similar to what was  
295   observed for KCl and NaCl:KCl solutions, which would indicate that impedance values were  
296   highly correlated with solute content. These results were confirmed by ANOVAs carried out  
297   for each impedance value (module and phase of impedance for each frequency), which  
298   established significant differences for solute content ( $p < 0.001$ ) but not for the type of salt  
299   ( $p > 0.05$ ) (ANOVA data not shown).

300           A PCA was performed with the data obtained in the impedance measurements (**Figure**  
301   **3**). The statistical analysis was able to reduce the initial variables (34 variables, 17 values of  
302   module and 17 values of phase of impedance) into a set of values of linearly uncorrelated  
303   variables called principal components (PCs), being the number of principal components less  
304   than or equal to the number of original variables. Most of the variation in the sample was  
305   explained by PC 1 (68.75%) and PC 2 (27.82%). According to the results obtained, the  
306   impedance spectroscopy method could distinguish between salt contents; however, it was

307 difficult to establish a correct classification of solutions according to the type of salt (**Figure**  
308 **3**).

## 309 **Phase II: Fish mince**

### 310 **Physicochemical analyses**

311 The composition of the frozen/thawed raw material was determined. Moisture, protein, lipid  
312 and ash contents for unsalted hake were  $80.2 \pm 0.1$  (**Table 2**),  $15.6 \pm 1.3$ ,  $0.5 \pm 0.2$  and  $1.20 \pm$   
313  $0.02$  g/100 g, respectively. These results are similar to those reported in other studies carried  
314 out with the same fish species.<sup>27, 34</sup>

315 The results of the physicochemical analyses for salted hake mince are summarized in  
316 **Table 2**. As expected, adding salt to the mince led to a reduction in moisture, from about 80.2  
317 % (mince without additions ) to 78.3 % and 77.8 % , for minces containing sodium- and  
318 potassium chloride (Na:K) and minces containing sodium chloride (Na), respectively. Due to  
319 the increase in mineral contents (up to 3.0g/100g mince) the  $a_w$  decreased from 0.992 (mince  
320 without additions) to 0.974 (Na:K) and 0.969 (Na). The moisture and  $a_w$  were significantly  
321 lower in minces containing 3.0% salt compared to minces containing less. Both the type of salt  
322 and their contents had a significant effect on the  $a_w$ , compared to the brines where the  $a_w$   
323 correlates only with the contents of salt. As expected, slightly higher water activities were found  
324 in the NaCl:KCl minces than in minces containing NaCl. Water activity decreases with  
325 increasing number of colligative units dissolved per volume. As  $K^+$  is a larger ion than  $Na^+$ ,  
326 replacing NaCl with an equal amount by weight of KCl will lead to a lower number of dissolved  
327 ions (colligative units) per volume and thus an increase in  $a_w$  of the product.

328 The pH of the unsalted mince (pH 6.97) was reduced after preparing the mince with  
329 different salts (Na) and (Na:K) and content (**Table 2**). The pH values of the raw material  
330 employed in this study is in accordance with the results obtained in other studies<sup>34, 35</sup> for fresh  
331 hake. A decrease in pH was observed when salt was added to our minces, a little more



332 pronounced in case of Na than with most Na:K mixtures. Similar results have been observed in  
333 a study by Leroi & Joffraud,<sup>36</sup> indicating that pH decreases in fish flesh by the addition of salt  
334 due to the increase of the ionic strength of the solution inside the cells. Another explanation  
335 might be that an increased amount of chloride ions would open the myosin filament and the  
336 more dissociable acidic groups would be water-accessible.<sup>37</sup> Samples containing Na exhibited  
337 lower pH than the corresponding Na:K samples: pH 6.76 vs 6.81, respectively. Similar results  
338 with fish products subjected to partial sodium replacement have also been observed.<sup>38</sup>

339 The measured contents of sodium, potassium and chloride are shown in **Table 2**. The  
340 sodium (0.05-0.06 g/100 g) and potassium contents (0.35 g/100 g) of fresh fish mince (**Table**  
341 **2**) agree with those reported in another study<sup>39</sup> for deboned hake. The chloride content in mince  
342 without additions was 0.21 g/100 g. When only NaCl was added to the minces, the potassium  
343 levels remained almost constant at 0.30 – 0.40 g/100g, resembling the level in mince without  
344 additions.

345 **Table 2** shows a comparison between sodium contents in the different minces as  
346 determined by the ion selective electrode and by ion chromatography. Good correlation was  
347 observed between the sodium content determined by the ion selective electrode method and ion  
348 chromatography, which was confirmed by a simple regression carried out on the data obtained  
349 by both methodologies ( $y=1.066x+7.961$ ,  $R^2=0.967$ ).

### 350 **Impedance Spectroscopy measurements**

351 Impedance spectroscopy was used to detect changes in the fish mince adding different salt  
352 content and type of salt. A PCA was performed on the impedance spectroscopy measurements  
353 in fish mince samples with different type of salts.

354 The discrimination between the different salt contents observed in the PCA plot for fish  
355 minces was better than the one obtained for salt solutions (**Figure 4**). The percentage of  
356 variance explained by the first principal component in **Figure 4** is 90.17% while in **Figure 3**

357 PC1 only explains 68.75% of the total variance. This means that the correlation between  
358 impedance spectroscopy data and salt content is stronger in fish samples than in solutions. A  
359 possible explanation for this behavior is the salting-in effects on muscle proteins.<sup>37</sup> At salt  
360 contents lower than 0.5 M, the swelling of myofibrils starts and reaching a maximum at 0.8–1  
361 M.<sup>37</sup> This usually causes a decrease myofibril volume, because the myofibril tends to dissolve.  
362 However, in our study, the highest content in the minces corresponded to 0.65M and 0.55M for  
363 the minces with 3.0% NaCl and NaCl:KCl, respectively. The conformational changes, together  
364 with the increase in the conductivity, could be responsible for the different behavior in the IS  
365 observed among our hake minces and in the solutions. At some contents, the method also  
366 distinguished between types of cations ( $\text{Na}^+$  or  $\text{Na}^+/\text{K}^+$ ) in the fish mince, a behavior that can  
367 be explained by the different effects of sodium (kosmotrope, water-structure maker) and  
368 potassium (chaotrope, water-structure breaker) in actin and myosin.<sup>40</sup> Further work is needed  
369 to reveal significant differences between cations in the minces.

### 370 **LF NMR**

371 A LF-NMR  $T_2$  relaxation method was used to study the relaxation behaviors in the mince when  
372 different types of salt were added to the mince in different amounts. The two transversal  
373 relaxation times with corresponding populations obtained from fitting of NMR data, are shown  
374 in **Table 3**. In fish muscle, typically two or three relaxation components are reported Erikson  
375 et al. and references therein<sup>41</sup>. The two major ones have relaxation times in the range of 40-60  
376 ms ( $T_{21}$ ) and 150-400 ms ( $T_{22}$ ), similar to those of the present research. The mean  $T_{21}$  and  $T_{22}$   
377 relaxation times for the unsalted hake mince were 54 and 219 ms, respectively. The  
378 interpretation of such data have been controversial, but it is now becoming more accepted that  
379 the observed changes in relaxation behavior are due primarily to chemical and diffusive proton  
380 exchange between water molecules and biopolymers (e.g. proteins).<sup>42, 43</sup> A number of studies

381 have nevertheless shown that these processes are linked to the morphology of the sample that  
382 in turn can be affected by, example.g., processing, such as salting and mincing.<sup>23, 25, 41</sup>

383 After addition of 0.5% NaCl or NaCl/KCl to the mince, the proton relaxation times,  
384 found by bioexponential fitting, increased to 59-61 ms in case of T<sub>21</sub> whereas the T<sub>22</sub> value  
385 remained largely unchanged. Addition of more salt led to an increase in both T<sub>21</sub> and T<sub>22</sub>  
386 relaxation times, with mean values of 67-71 ms and 286-496 ms, respectively.

387 By comparison, when frozen/thawed Atlantic salmon fillets were salted to 2.7% NaCl  
388 in the head part of the fillet, T<sub>21</sub> increased from 47 ms (unsalted) to 48 ms (salted), whereas the  
389 tail part of the fillet had 2.9 % NaCl and T<sub>21</sub> increased from 47 ms (unsalted) to 50 ms (salted),  
390 respectively. No significant changes were observed in T<sub>22</sub> (140-150 ms (head part) and 140-169  
391 ms (tail part)).<sup>21</sup> Similar values were obtained when fillets of the same species were salted in a  
392 15 % NaCl brine.<sup>20</sup> Thus, it seems that the magnitude of change in T<sub>21</sub> can be similar in highly  
393 concentrated brines (whole, lean fillets) as in our lean hake mince. Notably, the mincing of cod  
394 fillets does not alter the magnitude of the T<sub>21</sub> values<sup>44</sup>. A stronger effect of salting of cod was,  
395 however, reported<sup>24</sup> where T<sub>21</sub> values increased from 51 ms (raw material) to 86 to 94 ms after  
396 presalting by different methods (12 % salt).

397 A PCA score plot of the relaxation time curves is presented in **Figure 5**. Most of the  
398 variation in the sample was explained by PC 1 (76.60%) and PC 2 (20.54%) and it separates  
399 between minces with 0, 0.5% and 1.0-3.0% salt. Otherwise, the relaxation data did not reveal  
400 any clear trends, that is, between the magnitudes of the relaxation times at increasing salt  
401 contents above 0.5 %. The increase in relaxation times when 0.5 % salt was added, reflecting  
402 higher water proton mobility, suggests that a more open mince microstructure was formed. This  
403 was possibly caused by the binding of chloride ions to myosin filaments which would induce  
404 electrostatic repulsive forces causing an increase of filament spacing.<sup>37</sup>

405 In contrast to the increase in  $T_{21}$  as a result of the addition of 0.5 % salt, the  
406 corresponding population ( $T_{21}$  pop) did not change accordingly. The  $T_{21}$  pop values remained  
407 similar to those in the mince without additions (85-87 %). With further addition of salt, the  
408 values increased to 96-99 %, regardless of type and amount of salt (1.0, 2.0 or 3.0 % salt) with a  
409 corresponding decrease in  $T_{22}$  pop. The latter population with high mobility decreased to 1-4  
410 %. The changes in  $T_2$  populations reflect a shift of the proton populations, increasing the amount  
411 of protons with higher mobility and decreasing the amount of protons with lower mobility. This  
412 may be explained by the changes in muscle structure due to the salting-in effect previously  
413 discussed.

414 Based on PCA analyses of the NMR  $T_2$  relaxation data, a clear separation between  
415 samples with 0, 0.5 and 1.0 - 3.0% of salt was obtained. However, the LF NMR method was  
416 unable to distinguish between minces with different types of cations. To sum up, the fact that  
417 the most pronounced changes in relaxation behavior occurred at low contents of salt (0 – 0.5%)  
418 that LF  $^1\text{H}$  NMR can be a suitable tool for indirectly studies of structural changes in low-salt  
419 systems. <sup>41</sup>

#### 420 **Partial Least Square (PLS) results**

421 In order to create predictive models of physicochemical parameters PLS regression were  
422 applied to both impedance spectroscopy and LF NMR measurements. **Table 4** shows the values  
423 of the determination coefficient ( $R^2$ ), the root-mean-square error of prediction (RMSEP) and  
424 the number of latent variables corresponding to the prediction models built for  $a_w$ ,  $\text{Na}^+$   
425 (mg/100g),  $\text{K}^+$  (mg/100g), NaCl (g/100g), gram salts (g/100g) and gram solutes/100g liquid  
426 phase using impedance spectroscopy data. Models for  $a_w$ , gram salts (g/100g) and gram  
427 solutes/100g liquid phase show very good behavior with  $R^2$  values close to, or higher than 0.9.  
428 However, the results obtained for  $\text{Na}^+$  (mg/100g),  $\text{K}^+$  (mg/100g) and NaCl (g/100g)  
429 demonstrate that the proposed technique is not able to discriminate between the different types

430 of salt. As shown in **Figure 3**, in the PCA plots most of the total variance corresponds to PC1.  
431 In the module and phase plots a similar discrimination between the different salt content levels  
432 could be obtained considering the module and phase values for all the frequencies or  
433 considering just the module value for one frequency (for example 1MHz). Based on this idea,  
434 new PLS models were built for  $a_w$  and gram salts (g/100g) using only one latent variable. The  
435  $R^2$  values for these new models are similar to those obtained using the number of latent variables  
436 established by cross-validation. This opens the the possibility to limit impedance measurements  
437 to the module at one single frequency so that the measurement process would be greatly  
438 shortened and the prediction could be made using a simple regression. There were no significant  
439 correlations between the LF NMR measurements and the physicochemical results. In  
440 conclusion, the PLS models of impedance spectroscopy measurements showed good  
441 correlations with  $R^2$  values close to or higher than 0.9 for  $a_w$ , solute content and solute content  
442 in the liquid phase. However, the results obtained for  $\text{Na}^+$  (mg/100g),  $\text{K}^+$  (mg/100g) and NaCl  
443 (g/100g) demonstrate that the proposed technique is not able to discriminate between the  
444 different types of salt.

445

446 In conclusion, good correlations were observed between the sodium content determined  
447 by ion selective electrode method and ionic chromatography, which was confirmed by a simple  
448 regression, carried out using the data obtained by both methodologies. In both salt solutions and  
449 fish minces, the impedance spectroscopy measurements could separate between different salt  
450 contents down to 0.5%. However, the results obtained for cation determinations demonstrate  
451 that the proposed technique is not able to discriminate between the different types of salt.  
452 Furthermore, impedance spectroscopy measurements showed good correlations for  $a_w$ , solute  
453 content and solute content in the liquid phase. The NMR transversal relaxation time  $T_2$ , clearly  
454 distinguishes samples with 0, 0.5% and 1.0 - 3.0% salt, based on the principal component

455 analysis (PCA). We conclude that LF <sup>1</sup>H NMR can be a suitable technique for studies of low-  
456 salt products. However, the LF NMR method was unable to distinguish between minces with  
457 different types of cations.

458

## 459 **ACKNOWLEDGEMENTS**

460 The authors would like to thank the co-workers at UPV Isabel Fernández-Segovia, Arantxa  
461 Rizo and Lupis Hernandez and Marte Schei at SINTEF Fisheries and Aquaculture for their  
462 support and valuable participation in discussions regarding planning of the experiments,  
463 production of fish mince, and guidance related to the use of the different measuring techniques.  
464

465 **SUPPORTING INFORMATION**

466 Supporting Information Available: System Block Diagram. This material is available free of  
467 charge via the Internet at <http://pubs.acs.org>.

468

469 **REFERENCES**

- 470 1. He, F. J.; MacGregor, G. A., Effect of modest salt reduction on blood pressure: a meta-  
 471 analysis of randomized trials. Implications for public health. *J Hum Hypertens* **2002**, *16*, 761-  
 472 70.
- 473 2. WHO *Reducing salt intake in populations. Report of a WHO Forum and Technical*  
 474 *Meeting*; World Health Organization, Paris: 2006.
- 475 3. Martínez-Alvarez, O.; Gómez-Guillén, M. C., Effect of brine salting at different pHs on  
 476 the functional properties of cod muscle proteins after subsequent dry salting. *Food Chem.* **2006**,  
 477 *94*, 123-129.
- 478 4. Thorarinsdottir, K. A.; Arason, S.; Bogason, S. G.; Kristbergsson, K., The effects of  
 479 various salt concentrations during brine curing of cod (*Gadus morhua*). *Int. J. Food Sci.*  
 480 *Technol.* **2004**, *39*, 79-89.
- 481 5. Fuentes, A.; Fernandez-Segovia, I.; Barat, J. M.; Serra, J. A., Physicochemical  
 482 characterization of some smoked and marinated fish products. *J. Food Process. Preserv.* **2010**,  
 483 *34*, 83-103.
- 484 6. Aliño, M.; Fuentes, A.; Fernández-Segovia, I.; Barat, J. M., Development of a low-  
 485 sodium ready-to-eat desalted cod. *J. Food Eng.* **2011**, *107*, 304-310.
- 486 7. Fuentes, A.; Fernández-Segovia, I.; Barat, J. M.; Serra, J. A., Influence of sodium  
 487 replacement and packaging on quality and shelf life of smoked sea bass (*Dicentrarchus labrax*  
 488 L.). *LWT- Food Sci. Technol.* **2011**, *44*, 917-923.
- 489 8. Toldrá, F.; Barat, J. M., Strategies for salt reduction in foods. *Recent Pat. Food Nutr.*  
 490 *Agric.* **2012**, *4*, 19-25.
- 491 9. Geleijnse, J. M.; Witterman, J. C. M.; Stijnen, T.; Kloos, M. W.; Hofman, A.; Grobbee,  
 492 D. E., Sodium and potassium intake and risk of cardiovascular events and all-causes mortality:  
 493 The Rotterdam study. *Eur. J. Epidemiol.* **2007**, *22*, 763-777.
- 494 10. FSAI, Salt and health: review of the scientific evidence and recommendations for public  
 495 policy in Ireland. In 2005.
- 496 11. EFSA, Opinion of the Scientific Panel of Dietetic Products, Nutrition and Allergies on  
 497 request from the Commission related to the Tolerable Upper Intake Level of Potassium. *The*  
 498 *EFSA Journal* **2005**, *193*, 1-19.
- 499 12. Desmond, E., Reducing salt: A challenge for the meat industry. *Meat Sci.* **2006**, *74*, 188-  
 500 196.
- 501 13. Ehling, S.; Tefera, S.; Earl, R.; Cole, S., Comparison of Analytical Methods to  
 502 Determine Sodium Content of Low-Sodium Foods. *J. AOAC Int.* **2010**, *93*, 628-637.
- 503 14. AOAC, Official Methods of Analysis of the Association of Official Analytical  
 504 Chemists. In 15th ed.; AOAC: Arlington, Virginia, 1990.
- 505 15. Pérez-Olmos, R.; Herrero, R.; Lima, J. L. F. C.; Montenegro, M. C. B. S. M., Sequential  
 506 potentiometric determination of chloride and nitrate in meat products. *Food Chem.* **1997**, *59*,  
 507 305-311.
- 508 16. Chanet, M.; Rivière, C.; Eynard, P., Electric impedance spectrometry for the control of  
 509 manufacturing process of comminuted meat products. *J. Food Eng.* **1999**, *42*, 153-159.
- 510 17. Guerrero, L.; Gobantes, I.; Oliver, M. À.; Arnau, J.; Dolors Guàrdia, M.; Elvira, J.; Riu,  
 511 P.; Grèbol, N. s.; Monfort, J. M., Green hams electrical impedance spectroscopy (EIS) measures  
 512 and pastiness prediction of dry cured hams. *Meat Sci.* **2004**, *66*, 289-294.



- 513 18. Masot, R.; Alcañiz, M.; Fuentes, A.; Schmidt, F. C.; Barat, J. M.; Gil, L.; Baigts, D.;  
514 Martínez-Mañez, R.; Soto, J., Design of a low-cost non-destructive system for punctual  
515 measurements of salt levels in food products using impedance spectroscopy. *Sensor Actuat. A-*  
516 *Phys* **2010**, *158*, 217-223.
- 517 19. Rizo, A.; Fuentes, A.; Fernández-Segovia, I.; Masot, R.; Alcañiz, M.; Barat, J. M.,  
518 Development of a new salmon salting–smoking method and process monitoring by impedance  
519 spectroscopy. *LWT- Food Sci. Technol.* **2013**, *51*, 218-224.
- 520 20. Aursand, I. G.; Gallart-Jornet, L.; Erikson, U.; Axelson, D. E.; Rustad, T., Water  
521 Distribution in Brine Salted Cod (*Gadus morhua*) and Salmon (*Salmo salar*): A Low-Field <sup>1</sup>H  
522 NMR Study. *J. Agric. Food. Chem.* **2008**, *56*, 6252-6260.
- 523 21. Aursand, I. G.; Veliyulin, E.; Böcker, U.; Ofstad, R.; Rustad, T.; Erikson, U., Water and  
524 Salt Distribution in Atlantic Salmon (*Salmo salar*) Studied by Low-Field <sup>1</sup>H NMR, <sup>1</sup>H and  
525 <sup>23</sup>Na MRI and Light Microscopy: Effects of Raw Material Quality and Brine Salting. *J. Agric.*  
526 *Food. Chem.* **2008**, *57*, 46-54.
- 527 22. Erikson, U.; Veliyulin, E.; Singstad, T. E.; Aursand, M., Salting and desalting of fresh  
528 and frozen-thawed cod (*Gadus morhua*) fillets: A comparative study using <sup>23</sup>Na NMR, <sup>23</sup>Na  
529 MRI, low-field <sup>1</sup>H NMR, and physicochemical analytical methods. *J. Food Sci.* **2004**, *69*.
- 530 23. Aursand, I. G.; Erikson, U.; Veliyulin, E., Water properties and salt uptake in Atlantic  
531 salmon fillets as affected by ante-mortem stress, rigor mortis, and brine salting: A low-field <sup>1</sup>H  
532 NMR and <sup>1</sup>H/<sup>23</sup>Na MRI study. *Food Chem.* **2010**, *120*, 482-489.
- 533 24. Gudjónsdóttir, M.; Arason, S.; Rustad, T., The effects of pre-salting methods on water  
534 distribution and protein denaturation of dry salted and rehydrated cod – A low-field NMR study.  
535 *J. Food Eng.* **2011**, *104*, 23-29.
- 536 25. Gudjonsdottir, M.; Gunnlaugsson, V. N.; Finnbogadottir, G. A.; Sveinsdottir, K.;  
537 Magnusson, H.; Arason, S.; Rustad, T., Process Control of Lightly Salted Wild and Farmed  
538 Atlantic Cod (*Gadus morhua*) by Brine Injection, Brining, and Freezing-A Low Field NMR  
539 Study. *J. Food Sci.* **2010**, *75*, E527-E536.
- 540 26. Gudjonsdottir, M.; Lauzon, H. L.; Magnusson, H.; Sveinsdottir, K.; Arason, S.;  
541 Martinsdottir, E.; Rustad, T., Low field Nuclear Magnetic Resonance on the effect of salt and  
542 modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage. *Food Res.*  
543 *Int.* **2011**, *44*, 241-249.
- 544 27. Sánchez-Alonso, I.; Martinez, I.; Sánchez-Valencia, J.; Careche, M., Estimation of  
545 freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) by low field  
546 NMR. *Food Chem.* **2012**, *135*, 1626-1634.
- 547 28. AOAC, Official Methods of Analysis of the Association of Official Analytical  
548 Chemists. In 16th ed.; AOAC: Arlington, Virginia, 1997.
- 549 29. Kivikari, R. *Analysis of sodium in meat products using an Na-selective electrode. (In*  
550 *Finnish). Proc. of Meat Day Seminar 1996, Nr, 536; 1996; pp 64-66.*
- 551 30. Carr, H. Y.; Puncell, E. M., Effects of diffusion on free precession in nuclear magnetic  
552 resonance experiments. *Am. J. Physiology* **1954**, *94*, 630-638.
- 553 31. Meiboom, S.; Gill, D., Modified spin-echo method for measuring nuclear times. *Rev.*  
554 *Sci. Instrum* **1958**, *29*, 688-691.
- 555 32. Lambelet, P.; Renevey, F.; Kaabi, C.; Raemy, A., Low-Field Nuclear Magnetic  
556 Resonance Relaxation Study of Stored or Processed Cod. *J. Agric. Food. Chem.* **1995**, *43*, 1462-  
557 1466.
- 558 33. Martens, H.; Martens, M., *Multivariate Analysis of Quality. An introduction.* John Wiley  
559 & Sons Inc.: 2001.

- 560 34. Orban, E.; Nevigato, T.; Di Lena, G.; Masci, M.; Casini, I.; Caproni, R.; Rampacci, M.,  
561 Total volatile basic nitrogen and trimethylamine nitrogen levels during ice storage of European  
562 hake (*Merluccius merluccius*): A seasonal and size differentiation. *Food Chem.* **2011**, *128*, 679-  
563 682.
- 564 35. Pastoriza, L.; Sampedro, G.; Herrera, J. J.; Cabo, M. L., Influence of sodium chloride  
565 and modified atmosphere packaging on microbiological, chemical and sensorial properties in  
566 ice storage of slices of hake (*Merluccius merluccius*). *Food Chem.* **1998**, *61*, 23-28.
- 567 36. Leroi, F.; Joffraud, J. J., Salt and smoke simultaneously affect chemical and sensory  
568 quality of cold-smoked salmon during 5 °C storage predicted using factorial design. *J. Food*  
569 *Prot.* **2000**, *63*, 1222-1227.
- 570 37. Offer, G.; Trinick, J., On the Mechanism of Water Holding in Meat: The Swelling and  
571 Shrinking of Myofibrils. *Meat Sci.* **1983**, *8*, 245-281.
- 572 38. Fuentes, A.; Fernández-Segovia, I.; Serra, J. A.; Barat, J. M., Effect of partial sodium  
573 replacement on physicochemical parameters of smoked sea bass during storage. *Food Sci.*  
574 *Technol. Int.* **2012**, *18*, 207-217.
- 575 39. Martínez-Valverde, I.; Jesús Periago, M.; Santaella, M.; Ros, G., The content and  
576 nutritional significance of minerals on fish flesh in the presence and absence of bone. *Food*  
577 *Chem.* **2000**, *71*, 503-509.
- 578 40. Puolanne, E.; Halonen, M., Theoretical aspects of water-holding in meat. *Meat Sci.*  
579 **2010**, *86*, 151-165.
- 580 41. Erikson, U.; Standal, I. B.; Aursand, I. G.; Veliyulin, E.; Aursand, M., Use of NMR in  
581 fish processing optimization: a review of recent progress. *Magn. Reson. Chem.* **2012**, *50*, 471-  
582 480.
- 583 42. Halle, B., Molecular theory of field-dependent proton spin-lattice relaxation in tissue.  
584 *Magnet. Reson. Med.* **2006**, *56*, 60-72.
- 585 43. Belton, P., Spectroscopic Approaches to the Understanding of Water in Foods. *Food*  
586 *Rev. Int.* **2011**, *27*, 170-191.
- 587 44. Andersen, C. M.; Jørgensen, B. M., On the Relation Between Water Pools and Water  
588 Holding Capacity in Cod Muscle. *J. Aquat. Food Prod. Technol.* **2004**, *13*, 13-23  
589
- 590 This research was conducted when Kirsti Greiff visited Universidad Politécnica de Valencia  
591 (UPV) as a part of the project Low salt products, Project No. 185063/O10, supported by the  
592 Research Council of Norway.

## Figure captions

**Figure 1.** Scheme of impedance measurement and registered signals. ( Module  $|Z|$ , phase ( $\phi$ ) ,  $v(t)$  is the voltage signal,  $i(t)$  the current signal,  $f$  the frequency of the signals, and  $\Delta t$  is the time interval between the zero crossing of the voltage and current signals)

**Figure 2.** Mean values of modulus (a) and phase (b) of impedance spectra for the KCl solutions with different salt contents (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g salt/100 g distilled water, respectively)

**Figure 3.** PCA score plot of data obtained from the impedance spectroscopy measurements in solutions with different types of salt (NaCl, KCl and NaCl/KCl (NaK) , 50/50 w/w%) and contents 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g salt/ 100 g distilled water, respectively)

**Figure 4.** PCA score plot of data obtained from the impedance spectroscopy measurements in hake minces with different salt contents (NaCl (Na) or NaCl/KCl (Na:K) 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 g/100 g salted mince, respectively)

**Figure 5.** PCA score plot of LF  $^1\text{H}$  NMR  $T_2$  relaxation data obtained from fish mince with salt content (NaCl (Na) or NaCl/KCl (Na:K) 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 g/100 g salted mince, respectively)

**Table 1**

Physicochemical parameters of brine solutions prepared with different salts (S: KCl (K), NaCl:KCl (Na:K) and NaCl (Na)) and contents (C: g salt/100 g brine). Mean values  $\pm$  SD (n=3). ANOVA F-ratio for each of the 2 factors (S and C) and its interaction in the physicochemical parameters.

S	C	$a_w$	Conductivity (mS/cm)	Na (g/L)	Cl (g/L)
K	0.1	0.999 $\pm$ 0.000 <sup>aA</sup>	2.10 $\pm$ 0.11 <sup>A</sup>	(0.34 $\pm$ 0.02)*10 <sup>-3aA</sup>	1.03 $\pm$ 0.06 <sup>aA</sup>
	0.5	0.993 $\pm$ 0.001 <sup>aB</sup>	10.27 $\pm$ 1.15 <sup>B</sup>	(0.76 $\pm$ 0.00)*10 <sup>-3aB</sup>	3.50 $\pm$ 0.14 <sup>aB</sup>
	1.0	0.996 $\pm$ 0.003 <sup>aB</sup>	19.31 $\pm$ 1.24 <sup>C</sup>	(0.89 $\pm$ 0.01)*10 <sup>-3aC</sup>	5.78 $\pm$ 0.13 <sup>aC</sup>
	1.5	0.990 $\pm$ 0.002 <sup>aC</sup>	31.20 $\pm$ 0.28 <sup>D</sup>	(1.26 $\pm$ 0.02)*10 <sup>-3aD</sup>	8.30 $\pm$ 0.26 <sup>aD</sup>
	2.0	0.985 $\pm$ 0.001 <sup>aC</sup>	41.67 $\pm$ 1.21 <sup>E</sup>	(1.37 $\pm$ 0.02)*10 <sup>-3aE</sup>	10.54 $\pm$ 0.09 <sup>aE</sup>
	2.5	0.980 $\pm$ 0.002 <sup>aCD</sup>	51.23 $\pm$ 3.61 <sup>F</sup>	(1.69 $\pm$ 0.04)*10 <sup>-3aF</sup>	12.64 $\pm$ 0.13 <sup>aF</sup>
	3.0	0.984 $\pm$ 0.002 <sup>aD</sup>	63.30 $\pm$ 5.30 <sup>G</sup>	(2.18 $\pm$ 0.16)*10 <sup>-3aG</sup>	14.68 $\pm$ 0.33 <sup>aG</sup>
K: Na	0.1	0.995 $\pm$ 0.002 <sup>bA</sup>	2.07 $\pm$ 0.04 <sup>A</sup>	0.26 $\pm$ 0.00 <sup>bA</sup>	1.08 $\pm$ 0.08 <sup>bA</sup>
	0.5	0.989 $\pm$ 0.002 <sup>bB</sup>	9.90 $\pm$ 0.42 <sup>B</sup>	0.77 $\pm$ 0.00 <sup>bB</sup>	3.74 $\pm$ 0.21 <sup>bB</sup>
	1.0	0.991 $\pm$ 0.001 <sup>bB</sup>	19.33 $\pm$ 1.26 <sup>C</sup>	1.67 $\pm$ 0.02 <sup>bC</sup>	6.44 $\pm$ 0.17 <sup>bC</sup>
	1.5	0.985 $\pm$ 0.004 <sup>bC</sup>	30.17 $\pm$ 3.06 <sup>D</sup>	2.60 $\pm$ 0.01 <sup>bD</sup>	9.12 $\pm$ 0.19 <sup>bD</sup>
	2.0	0.985 $\pm$ 0.001 <sup>bC</sup>	38.27 $\pm$ 0.81 <sup>E</sup>	3.45 $\pm$ 0.01 <sup>bE</sup>	11.86 $\pm$ 0.2 <sup>bE</sup>
	2.5	0.980 $\pm$ 0.002 <sup>bCD</sup>	50.63 $\pm$ 3.10 <sup>F</sup>	4.23 $\pm$ 0.07 <sup>bF</sup>	14.72 $\pm$ 0.3 <sup>bF</sup>
	3.0	0.981 $\pm$ 0.001 <sup>bD</sup>	63.10 $\pm$ 4.27 <sup>G</sup>	5.17 $\pm$ 0.02 <sup>bG</sup>	16.52 $\pm$ 0.15 <sup>bG</sup>
Na	0.1	0.998 $\pm$ 0.002 <sup>bA</sup>	2.57 $\pm$ 0.68 <sup>A</sup>	0.39 $\pm$ 0.00 <sup>cA</sup>	1.05 $\pm$ 0.08 <sup>cA</sup>
	0.5	0.989 $\pm$ 0.001 <sup>bB</sup>	10.44 $\pm$ 1.50 <sup>B</sup>	1.60 $\pm$ 0.01 <sup>cB</sup>	3.63 $\pm$ 0.21 <sup>cB</sup>
	1.0	0.984 $\pm$ 0.004 <sup>bB</sup>	19.37 $\pm$ 0.97 <sup>C</sup>	3.56 $\pm$ 0.02 <sup>cC</sup>	6.72 $\pm$ 0.28 <sup>cC</sup>
	1.5	0.980 $\pm$ 0.002 <sup>bC</sup>	28.37 $\pm$ 1.51 <sup>D</sup>	5.53 $\pm$ 0.03 <sup>cD</sup>	10.01 $\pm$ 0.19 <sup>cD</sup>
	2.0	0.981 $\pm$ 0.002 <sup>bC</sup>	39.87 $\pm$ 2.00 <sup>E</sup>	7.65 $\pm$ 0.00 <sup>cE</sup>	12.64 $\pm$ 0.05 <sup>cE</sup>
	2.5	0.983 $\pm$ 0.001 <sup>bCD</sup>	50.80 $\pm$ 3.10 <sup>F</sup>	9.45 $\pm$ 0.19 <sup>cF</sup>	16.20 $\pm$ 0.24 <sup>cF</sup>
	3.0	0.984 $\pm$ 0.002 <sup>bCD</sup>	59.10 $\pm$ 3.05 <sup>G</sup>	11.07 $\pm$ 0.06 <sup>cG</sup>	18.78 $\pm$ 0.26 <sup>cG</sup>
F- ratio					
S		22.45 <sup>***</sup>	0.61 <sup>ns</sup>	69815.08 <sup>***</sup>	600.85 <sup>***</sup>
C		80.43 <sup>***</sup>	589.51 <sup>***</sup>	14371.42 <sup>***</sup>	10525.71 <sup>***</sup>
S x C		7.90 <sup>***</sup>	0.29 <sup>ns</sup>	5104.16 <sup>***</sup>	69.82 <sup>***</sup>

p-values : \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; ns: non significant

Different lower-case letters indicate significant differences ( $p < 0.05$ ) for factor S (salt composition).

Different capital letters indicate significant differences ( $p < 0.05$ ) for factor C (salt content).

**Table 2**

Physicochemical parameters of fish mince prepared with different salts (S: NaCl (Na) and NaCl:KCl (Na:K) and contents (C: g salt/100 g fish mince). Mean values  $\pm$  SD (n=3).

S	C	Moisture (%)	$a_w$	pH	Chloride (g/100g)	Sodium (g/100g) (ISE)	Sodium(g/100g)(IC)	Potassium (g/100/g)	Ionic strength (mol/ kg solvent)
Fish mince	0	80.15 $\pm$ 0.08	0.992 $\pm$ 0.004	6.97 $\pm$ 0.13	0.21 $\pm$ 0.01	0.05 $\pm$ 0.00	0.06 $\pm$ 0.01	0.35 $\pm$ 0.00	0.11 $\pm$ 0.00
Na:K	0.5	80.37 $\pm$ 0.24 <sup>aA</sup>	0.987 $\pm$ 0.004 <sup>aA</sup>	6.81 $\pm$ 0.01 <sup>aA</sup>	0.43 $\pm$ 0.01 <sup>aA</sup>	0.12 $\pm$ 0.01 <sup>aA</sup>	0.12 $\pm$ 0.04 <sup>aA</sup>	0.48 $\pm$ 0.09 <sup>aA</sup>	0.18 $\pm$ 0.03 <sup>aA</sup>
Na:K	1.0	79.13 $\pm$ 0.41 <sup>aA</sup>	0.984 $\pm$ 0.003 <sup>aAB</sup>	6.71 $\pm$ 0.04 <sup>aA</sup>	0.69 $\pm$ 0.00 <sup>aB</sup>	0.19 $\pm$ 0.00 <sup>aB</sup>	0.19 $\pm$ 0.05 <sup>aA</sup>	0.56 $\pm$ 0.12 <sup>aA</sup>	0.26 $\pm$ 0.04 <sup>aB</sup>
Na:K	2.0	79.57 $\pm$ 0.09 <sup>aA</sup>	0.980 $\pm$ 0.005 <sup>aB</sup>	6.83 $\pm$ 0.02 <sup>aA</sup>	1.29 $\pm$ 0.06 <sup>aC</sup>	0.43 $\pm$ 0.04 <sup>aC</sup>	0.42 $\pm$ 0.03 <sup>aB</sup>	0.79 $\pm$ 0.11 <sup>bB</sup>	0.47 $\pm$ 0.03 <sup>aC</sup>
Na:K	3.0	78.32 $\pm$ 0.41 <sup>aB</sup>	0.974 $\pm$ 0.001 <sup>aC</sup>	6.69 $\pm$ 0.02 <sup>bB</sup>	1.70 $\pm$ 0.03 <sup>aD</sup>	0.57 $\pm$ 0.02 <sup>aD</sup>	0.68 $\pm$ 0.08 <sup>aC</sup>	1.17 $\pm$ 0.14 <sup>cC</sup>	0.69 $\pm$ 0.05 <sup>aD</sup>
Na	0.5	79.09 $\pm$ 0.94 <sup>aA</sup>	0.985 $\pm$ 0.002 <sup>bA</sup>	6.76 $\pm$ 0.05 <sup>bA</sup>	0.50 $\pm$ 0.01 <sup>bA</sup>	0.19 $\pm$ 0.00 <sup>bA</sup>	0.26 $\pm$ 0.04 <sup>bA</sup>	0.40 $\pm$ 0.004 <sup>bA</sup>	0.22 $\pm$ 0.01 <sup>bA</sup>
Na	1.0	80.47 $\pm$ 0.88 <sup>aA</sup>	0.982 $\pm$ 0.001 <sup>bAB</sup>	6.78 $\pm$ 0.03 <sup>bA</sup>	0.82 $\pm$ 0.06 <sup>bB</sup>	0.35 $\pm$ 0.01 <sup>bB</sup>	0.38 $\pm$ 0.07 <sup>bA</sup>	0.30 $\pm$ 0.06 <sup>bA</sup>	0.30 $\pm$ 0.03 <sup>bB</sup>
Na	2.0	78.67 $\pm$ 0.14 <sup>aA</sup>	0.978 $\pm$ 0.002 <sup>bB</sup>	6.67 $\pm$ 0.04 <sup>bA</sup>	1.34 $\pm$ 0.11 <sup>bC</sup>	0.76 $\pm$ 0.06 <sup>bC</sup>	0.78 $\pm$ 0.02 <sup>bB</sup>	0.40 $\pm$ 0.04 <sup>bB</sup>	0.52 $\pm$ 0.03 <sup>bC</sup>
Na	3.0	77.77 $\pm$ 0.06 <sup>aB</sup>	0.969 $\pm$ 0.001 <sup>bC</sup>	6.67 $\pm$ 0.04 <sup>bB</sup>	1.90 $\pm$ 0.03 <sup>bD</sup>	1.01 $\pm$ 0.00 <sup>bD</sup>	1.09 $\pm$ 0.11 <sup>bC</sup>	0.39 $\pm$ 0.04 <sup>bC</sup>	0.71 $\pm$ 0.03 <sup>bD</sup>
F-ratio									
S		2.79 <sup>ns</sup>	6.82 <sup>***</sup>	6.42 <sup>***</sup>	28.40 <sup>***</sup>	527.67 <sup>***</sup>	116.86 <sup>***</sup>	112.43 <sup>***</sup>	8.26 <sup>***</sup>
C		15.07 <sup>***</sup>	31.63 <sup>***</sup>	10.26 <sup>***</sup>	819.47 <sup>***</sup>	720.83 <sup>***</sup>	153.16 <sup>***</sup>	21.40 <sup>***</sup>	300.80 <sup>***</sup>
S x C		7.80 <sup>***</sup>	0.67 <sup>ns</sup>	12.58 <sup>***</sup>	2.71 <sup>ns</sup>	60.63 <sup>***</sup>	6.65 <sup>***</sup>	17.82 <sup>***</sup>	0.17 <sup>ns</sup>

Different lower-case letters indicate significant differences (p<0.05) for factor S (salt composition). Different capital letters indicate significant differences (p<0.05) for factor C (salt content).

**Table 3**

Biexponential fitting of LF  $^1\text{H}$  NMR  $T_2$  relaxation data obtained in fish mince and fish mince prepared with different salts (S: NaCl and NaCl:KCl) and concentrations (C: g salt/100g fish mince). Mean values  $\pm$  SD (n =2)

S	C (%)	$T_{21}$ (ms)	$T_{22}$ (ms)	$T_{21}$ pop (%)	$T_{22}$ pop (%)
Fish mince	0	$54 \pm 1$	$219 \pm 7$	$86 \pm 3$	$14 \pm 3$
NaCl/KCl	0.5	$61 \pm 1^{\text{aA}}$	$226 \pm 10^{\text{aA}}$	$87 \pm 2^{\text{aA}}$	$13 \pm 2^{\text{aD}}$
NaCl/ KCl	1.0	$67 \pm 1^{\text{aB}}$	$286 \pm 46^{\text{aB}}$	$96 \pm 1^{\text{aBD}}$	$4 \pm 1^{\text{aAC}}$
NaCl/ KCl	2.0	$71 \pm 1^{\text{aD}}$	$496 \pm 42^{\text{aD}}$	$99 \pm 0^{\text{aBC}}$	$1 \pm 0^{\text{aBC}}$
NaCl/ KCl	3.0	$68 \pm 2^{\text{aBC}}$	$342 \pm 15^{\text{aC}}$	$98 \pm 0^{\text{aCD}}$	$2 \pm 0^{\text{aAB}}$
NaCl	0.5	$59 \pm 1^{\text{bA}}$	$215 \pm 10^{\text{aA}}$	$85 \pm 1^{\text{bA}}$	$15 \pm 1^{\text{bD}}$
NaCl	1.0	$69 \pm 1^{\text{bC}}$	$366 \pm 48^{\text{bB}}$	$98 \pm 1^{\text{bB}}$	$2 \pm 1^{\text{bC}}$
NaCl	2.0	$67 \pm 0^{\text{bB}}$	$427 \pm 34^{\text{bD}}$	$99 \pm 0^{\text{aCD}}$	$1 \pm 0^{\text{aAB}}$
NaCl	3.0	$68 \pm 0^{\text{aBCD}}$	$423 \pm 34^{\text{bC}}$	$99 \pm 0^{\text{aD}}$	$1 \pm 0^{\text{aA}}$
F - ratio					
S		9.20**	4.99*	0.30 <sup>ns</sup>	0.30 <sup>ns</sup>
C		214.59***	132.32***	609.87***	609.87***
S x C		22.49***	17.43***	12.14***	12.14***

p-values : \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; ns: non significant

Different lower-case letters indicate significant differences ( $p < 0.05$ ) for factor S (salt composition).  
Different capital letters indicate significant differences ( $p < 0.05$ ) for factor C (salt concentration).

**Table 4**

Parameters of the PLS models of physicochemical parameters from the impedance measurements.

	<b>LV</b>	<b>R<sup>2</sup></b>	<b>RMSEP</b>
<b>a<sub>w</sub></b>	5	0.886	0.003
	1	0.812	0.003
<b>Na (mg/100g)</b>	4	0.480	276.154
<b>K (mg/100g)</b>	4	0.094	269.090
<b>NaCl (g/100g)</b>	4	0.425	0.730
<b>KCl (g/100g)</b>	3	0.393	0.628
<b>Solute content (g/100g)</b>	5	0.950	0.210
	1	0.953	0.203
<b>Solute content in liquid phase (g/100g)</b>	4	0.946	0.286

LV: number of Latent Variables; R<sup>2</sup>: coefficient of determination; RMSEP: Root Mean Square Error of Prediction

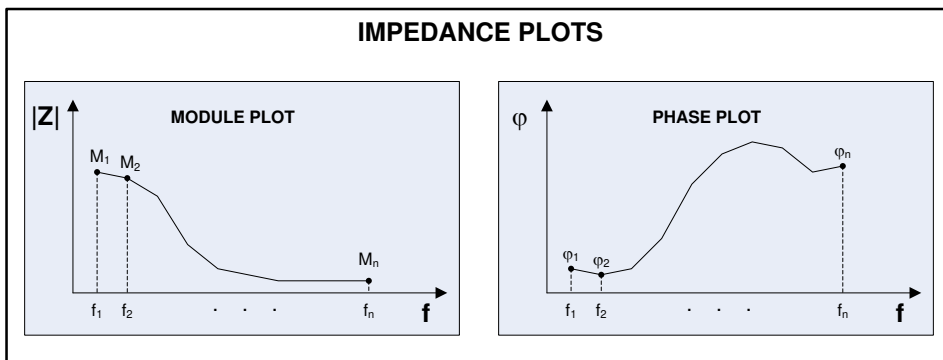
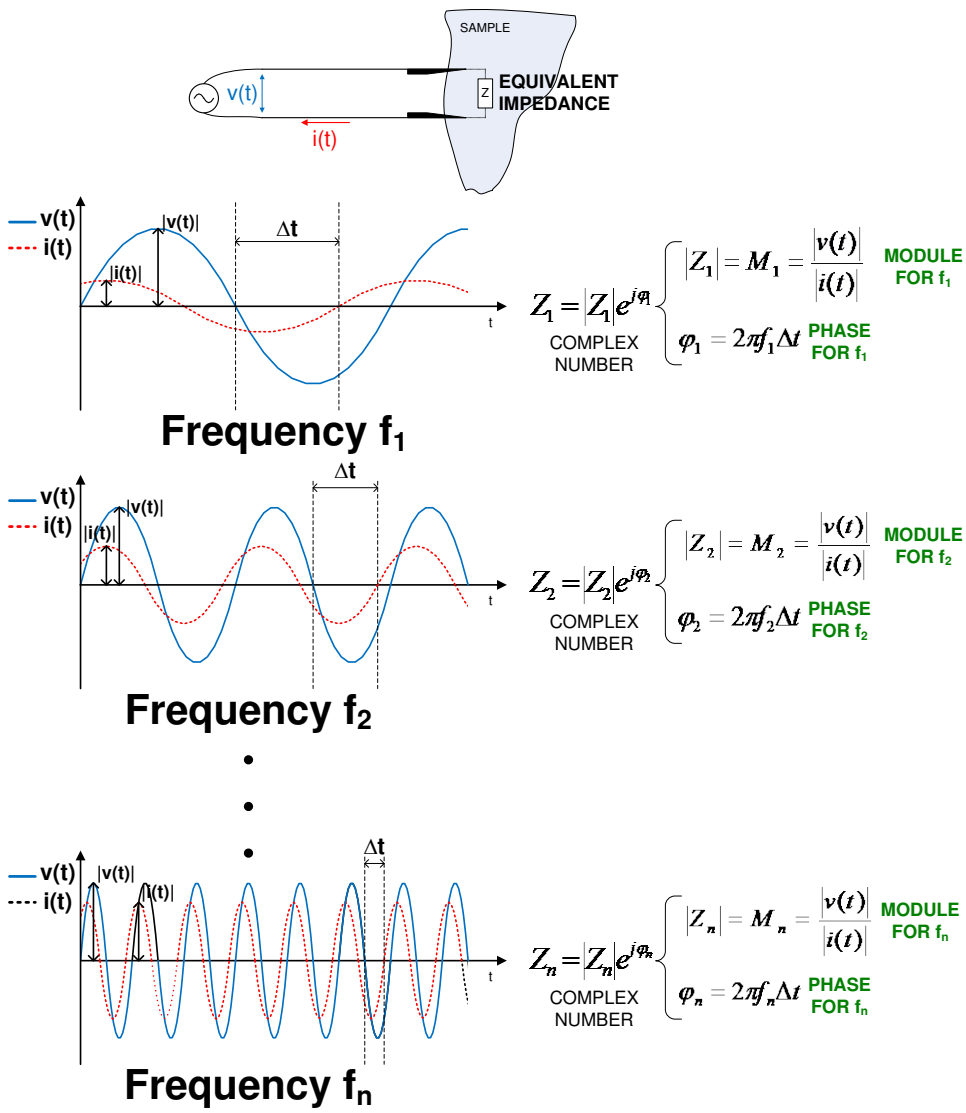
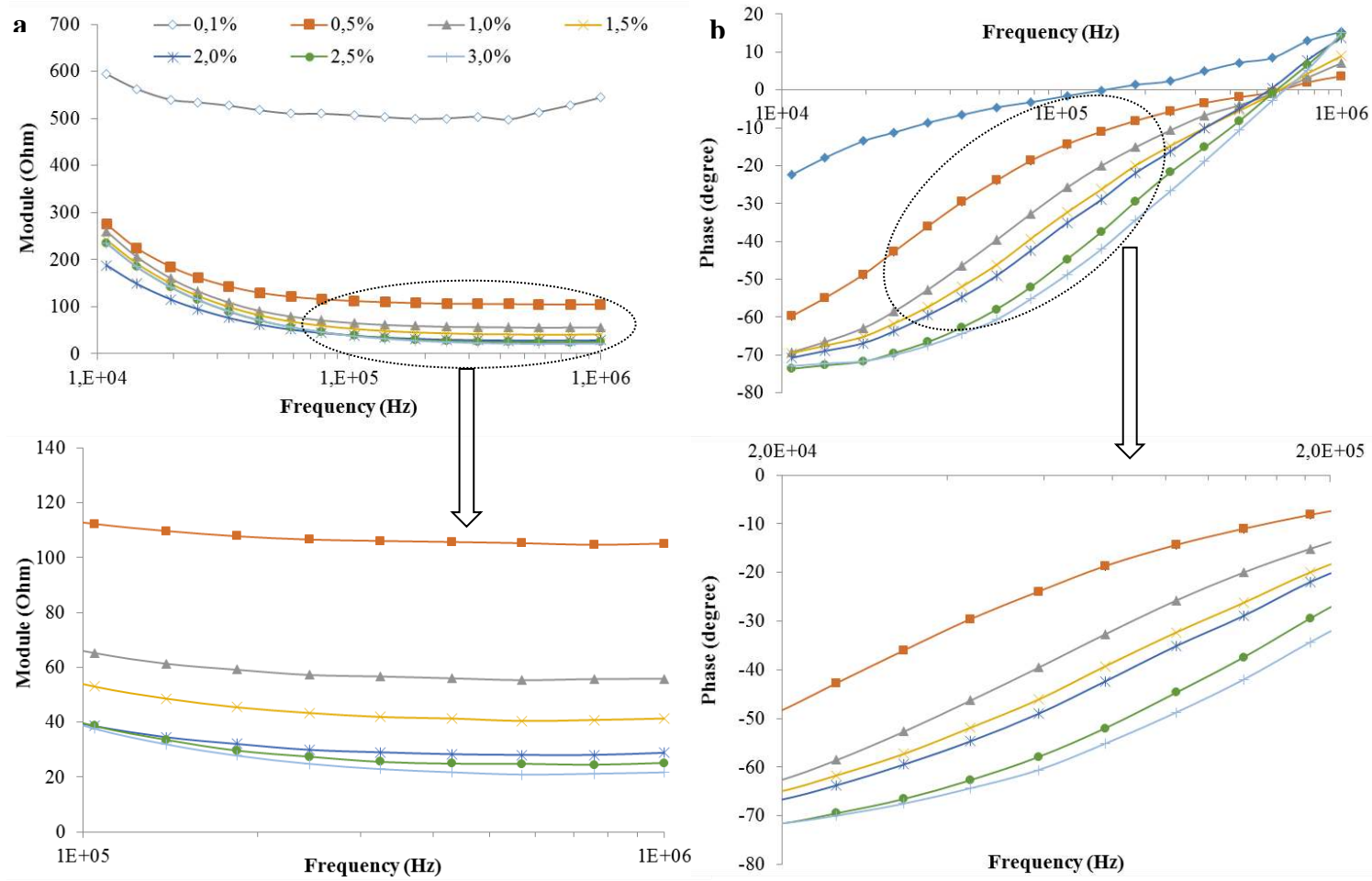
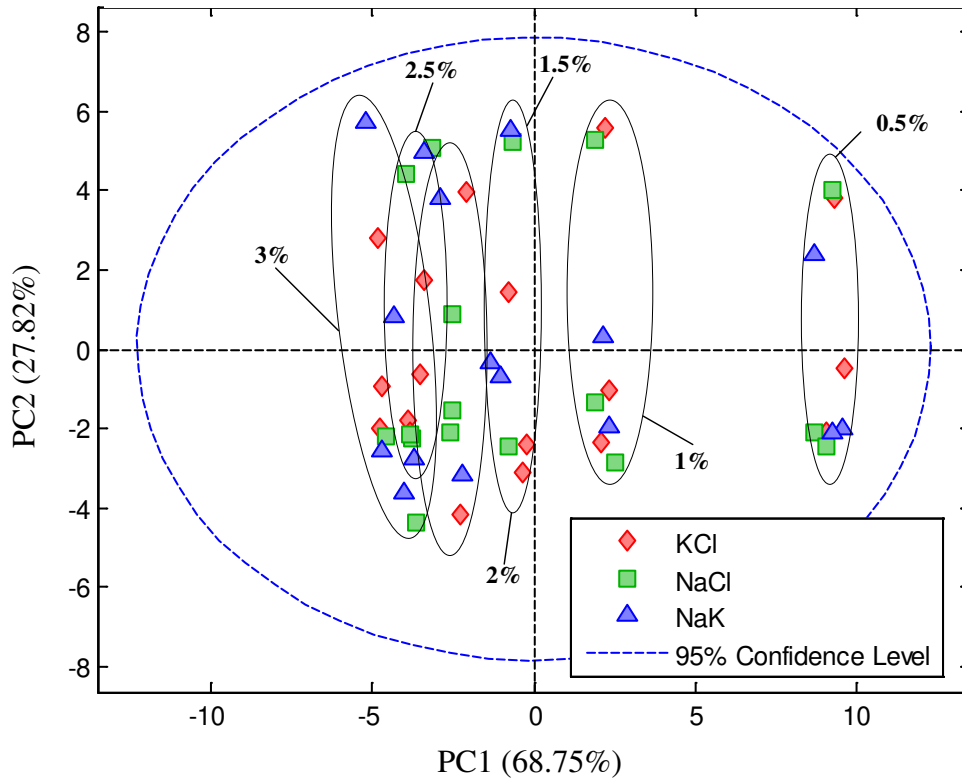


Figure 1





**Figure 2**



**Figure 3**

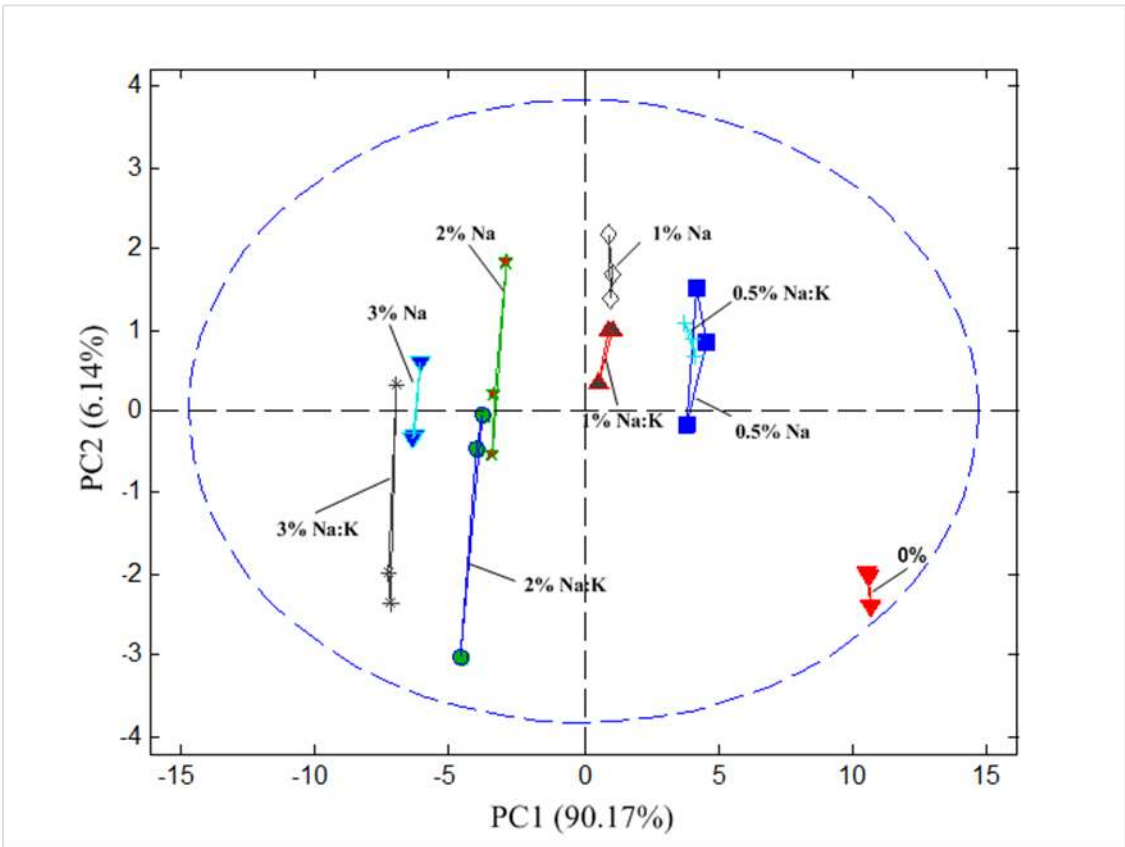


Figure 4

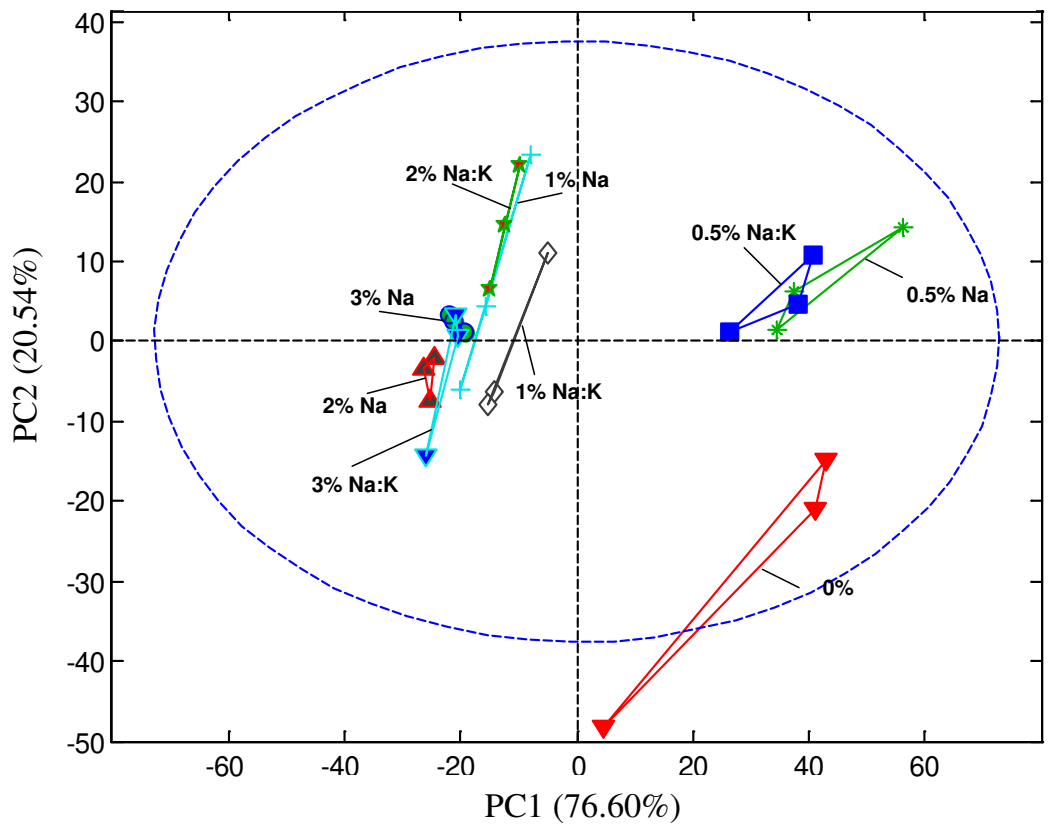


Figure 5

# TOC graphic

