Fourier Transform Near-Infrared Spectroscopy Application for Sea Salt Quality Evaluation

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ABSTRACT: Near-infrared (NIR) spectroscopy in diffuse reflectance mode was explored with the objective of discriminating sea salts according to their quality type (traditional salt vs "flower of salt") and geographical origin (Atlantic vs Mediterranean). Sea salts were also analyzed in terms of Ca^{2+}, Mg^{2+}, K^+, alkalinity, and sulfate concentrations to support spectroscopic results. High concentrations of Mg^{2+} and K^+ characterized Atlantic samples, while a high Ca^{2+} content was observed in traditional sea salts. A partial least-squares discriminant analysis model considering the 8500—7500 cm^{-1} region permitted the discrimination of salts by quality types. The regions 4650—4350 and 5900—5500 cm^{-1} allowed salts classification according to their geographical origin. It was possible to classify correctly 85.3 and 94.8% of the analyzed samples according to the salt type and to the geographical origin, respectively. These results demonstrated that NIR spectroscopy is a suitable and very efficient tool for sea salt quality evaluation.

KEYWORDS: Sea salt, FT-near-infrared spectroscopy, PLS-DA, quality control, geographical origin, calcium, potassium, magnesium concentration, authenticity

■ INTRODUCTION

The production of traditional sea salt is seen as a sustainable activity with important benefits for the ecosystem and for the economy of the regions where this type of activity is carried out.[1] Most of the sea salt marketed in Portugal comes from local producers located in regions bordered by the Atlantic sea. The major producers of sea salt are from the Algarve region (south of Portugal), where they have an old and large experience in sea salt exploration; other producers of sea salt come from the Aveiro region, which has a minor sea salt activity; and finally, the rest of the marketed salt in Portugal comes from Spain, which has large sea pans explorations in regions bordered by the Mediterranean Sea.

Sea salt is a broad term that refers to unrefined salt deriving directly from the sea. The extraction of sea salt is an ancient and hand-labor activity that requires the correct management of brine flows coming from the sea, which travels through well-defined sea pans areas until NaCl crystallizes. The salt pans have specific characteristics in terms of dimension (area) to promote the formation of NaCl crystals and its extraction. These pans are called reservoir, evaporation, and crystallization areas. In the reservoir area, the water from the sea is received, and most of the organic material (i.e., algae and other subproducts) is precipitated. Then, the seawater passes to the evaporation area where the water content is reduced until a relative density of 20 °Be (degrees Baume). Finally, in the crystallization area, a salinity gradient is established, and along this process, salts with low solubility like calcium carbonate ($CaCO_3$) precipitate.[2] Sodium chloride is obtained at a relative density between 26 and 28.5 °Be; between this range (25—26 °Be), other important salts like

magnesium chloride ($MgCl_2$) and magnesium sulfate ($MgSO_4$) are also precipitated. During traditional sea salt production, no mechanical devices are used, and once the salt is harvested, no washing and artificial drying are allowed; then, the other salts associated with NaCl are also maintained.

Sea salt is a seasonal product. Depending on the weather conditions, its production starts in June and ends in August. Salt production counts basically on high light intensity and temperatures and low relative humidity, all conditions found during summer months. In days of exceptional weather and in association with a moderate breeze, a thin layer of salt crystals is formed; this is known as "flower of salt". This type of salt is considered a superior class of salt that has specific physicochemical characteristics (i.e., long and soft crystals with bright white color), which makes it a premium product with prices that can reach 10 times more the prices of the traditional sea salt. For the mentioned reasons, "flower of salt" is considered today a gourmet product with distinctive physicochemical characteristics.

Recent studies about sea salt have demonstrated that sea salts have unique sensorial characteristics.[3,4] Near-infrared (NIR) spectroscopy techniques have been widely used in studies of authenticity and geographic traceability of food products.[5,6] The advantages of NIR measurements have been well documented in several works with different food matrices; some advantages are multiparametric and in situ determinations[7,8] with a minimum sample preparation.[9] NIR methodology has been used for the

determination of sodium chloride content in food products[10,11] and for the determination of seawater salinity.[12] However, as far as we know, there is no precedent about the use of Fourier transform (FT) NIR for the discrimination between sea salts on the basis of their geographical origin and quality type ("flower of salt" vs traditional sea salt). The main purpose of the work presented here is to ascertain whether this technique, in combination with multivariate analysis, has the capacity to discriminate sea salts with different geographical origins and quality type characteristics.

■ MATERIALS AND METHODS

Sea Salt Samples. A total of 61 sea salt samples were analyzed; some were commercial samples (n = 37) bought at different gourmet and local stores in Europe (France, Spain, and Portugal). The rest of the samples (n = 24), noncommercial samples, came from particular collections. Samples had different origins being from Atlantic and Mediterranean sea salt explorations.

Reagents and Solutions. All chemicals were of analytical grade. Single-element stock standard solutions of 1000 mg L^{-1} of Ca^{2+}, Mg^{2+}, and K$^+$ (Merck, Germany) were used to prepare the working standard solutions for calcium ranging from 0 to 3 mg L^{-1}, for magnesium ranging from 0.2 to 0.8 mg L^{-1}, and for potassium ranging from 1 to 6 mg L^{-1}. A lanthanum(III) solution with a concentration of 100000 ppm was used for the Ca^{2+} and Mg^{2+} determinations. Deionized water was used throughout.

Alkalinity determination was based on a reaction with acetic acid and the corresponding color change of bromocresol green (BG). The preparation procedures and solution concentrations are described by Mesquita and Rangel.[13] A 1 g L^{-1} hydrogen carbonate stock solution obtained by dilution of 0.164 g of potassium hydrogen carbonate in 100 mL of water was used to prepare alkalinity working standards in the range of 8−60 mg L^{-1}. A stock solution of BG 0.05% was obtained by dissolving 50 mg of the solid in 10 mL of ethanol and diluted to 100 mL of water. Acetic acid (0.8 mol L^{-1}) was prepared (from proper dilution from the concentrated acetic acid d = 1.05; 100%), and 0.125 mL of this solution was mixed with 11 mL of BG and diluted to 50 mL.

The solutions for sulfate determination were prepared according to Morais et al.[14] A standard sulfate stock solution (1000 mg L^{-1}) was obtained by dissolution of K$_2$SO$_4$ previously dried and was used to prepare several working standard solutions in the range 66−440 mg L^{-1}. A 0.10% (m/v) polyvinyl alcohol (PVA) solution was prepared by suspending 0.5 g of solid in boiling water under continuous stirring. A 10% (m/v) barium chloride solution was obtained by dissolution of the solid (BaCl$_2$·2H$_2$O) in the 0.10% PVA solution. A buffer solution containing 40 g of EDTA, 7 g of ammonium chloride, and 57 mL of concentrated ammonia was obtained by dissolving the different chemical in 600 mL of deionized water and diluting to 1 L. A solution of 0.07 M HNO$_3$ was prepared from proper dilution from the concentrated nitric acid (d = 1.39; 65%).

Humidity Determination. Humidity was evaluated by the AOAC protocol.[15] An exactly weighed quantity of salt (approximately 5 g) was dried in the oven at 103 °C for 12 h. The dried salt was transferred to a desiccator until the samples reached a constant room temperature, and then, it was weighed. The weight loss expressed as a percentage was taken as a percentage of moisture.

Granulometry Determination. Sea salt samples were classified by experts in sea salt production into coarse (crystal size > 3 mm), fine (crystal size between 1 and 3 mm), and extra-fine (crystal size < 1 mm). The flower of salt has different crystal configurations so these samples were classified just as "flower of salt".[1]

Determination of Calcium, Magnesium, Potassium, Alkalinity, and Sulfate in Sea Salts. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) contents were determined using an atomic absorption spectrometer, model 969 with an air-acetylene flame (Thermo Scientific, United States). Potassium (K$^+$) determination was carried out using a flame photometer model PFP7 with an air-propane flame (Jenway, United Kingdom). The alkalinity expressed as HCO$_3^-$ and sulfate was expressed as SO$_4^{2-}$ were determined according to sequential injection analysis (SIA) protocols developed in our research group.[13,14] The solutions were propelled with a Gilson Minipuls 3 peristaltic pump with PVC pumping tube connected to the central channel of an eight-port electrically actuated selection valve (Valco VICI 51652-E8). All tubing connecting the different components of the sequential injection system was made of PTFE from Omnifit (0.8 mm i.d.). A Unicam 5625 UV/vis spectrophotometer with a Hellma 178.711-QS flow cell (10 mm light path, 80 μL inner volume) was used as a detection system. The signal was recorded using Kipp & Zonen BD111 strip chart recorder. The wavelength was set at 611 nm for determining the alkalinity and at 420 nm for determining the sulfates content. Sample solutions were prepared by dissolving appropriate amounts of sea salt and "flower of salt" in deionized water. These solutions were diluted to fit in the linear range of the previously established calibration curves. Quantification of the analytes was based on the interpolation of the recorded signal of the sample solutions on the calibration curves. Results were obtained as the means of triplicate determinations and were expressed as mg of the ions per 100 g of salt.

NIR Spectroscopy. NIR spectra were recorded in a Fourier transform near-infrared analyzer (FTLA2000, ABB Bomem, Québec, Canada) equipped with an indium−gallium−arsenide (InGaAs) detector. The analyzer was equipped with diffuse reflectance sampling accessory (ABB ACC101), which provides the means for performing NIR analysis in glass containers. The instrument was controlled by the GRAMS/AI (ThermoGalactic, MA) software. Spectra were acquired with a 2 cm^{-1} resolution with an average of 64 scans over a wavenumber range between 10000 and 4000 cm^{-1}. The measurements were performed by placing a glass flask containing the samples in the illuminated area (6 mm diameter) of the diffuse reflectance accessory. The background was measured at the beginning of each analysis by placing a flask containing a reference substance (Teflon) in the measurement area. Spectra were acquired under controlled conditions of temperature (19 °C) and relative humidity (39%). Spectra were measured in triplicate and averaged, yielding a data matrix with dimensions 61 × 6223.

Data Analysis. Metal ions data were analyzed by principal components analysis (PCA),[16] and NIR spectra were analyzed by partial least-squares discriminant analysis (PLS-DA).[17] The former was used to extract common patterns from sea salts samples, while the latter was used to develop calibration models for discrimination purposes. PCA was applied to model ions data using autoscaling as a preprocessing method. PLS-DA was adopted for spectra modeling processed using a Savitzky−Golay filter (15 filter size, third order polynomial, and second order derivative) followed by standard normal variate (SNV) and mean centering. These preprocessing methods were found to produce the best results among the other methods tested including the application of multiplicative scatter correction (MSC) and the extended multiplicative scatter correction (EMSC) methods. The strategy for PLS-DA models validation was previously described by Preisner et al.[18] This strategy is based on a series of calibrations/tests through the random division of the original data set calibration (∼70% samples) and test (∼30% samples). For each random division, a correct balance between the different classes (e.g., "flower of salt" and traditional sea salt) in calibration and test sets is ensured. Several repetitions of this procedure are required to ensure statistical significance of prediction errors. It was found for this data set that 200 repetitions were enough to ensure statistical validity of the results (P < 0.01). For each procedure repetition, the optimal number of latent variables (LVs) to use was estimated by cross-validation using only the calibration set.[16] Validation results are expressed as confusion

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Table 1. Principal Characteristics and Composition Values of the Studied Sea Salt and "Flower of Salt" Samples

salt code	type of salt[b]	denomination	class	humidity (%)	concn (mg per 100 g of salt)[a]				
					alkalinity[c]	sulfates	Mg^{2+}	Ca^{2+}	K^+
F2	flower of salt	Atlantic	noncommercial	3.68	49.5 ± 0.3	1396.4 ± 6.8	824.0 ± 3.2	113.2 ± 7.1	250.3 ± 17.0
F3	flower of salt	Atlantic	noncommercial	ND	39.4 ± 0.3	582.1 ± 13.6	448.1 ± 1.6	93.7 ± 4.5	114.7 ± 3.6
F4	flower of salt	Atlantic	noncommercial	ND	42.9 ± 0.6	1779.3 ± 68.1	507.1 ± 3.2	114.2 ± 0.0	295.1 ± 1.5
F14	flower of salt	Atlantic	noncommercial	9.36	65.0 ± 0.3	691.9 ± 7.7	678.9 ± 10.7	72.9 ± 3.7	196.4 ± 4.3
F15	flower of salt	Atlantic	noncommercial	6.69	56.3 ± 0.2	678.1 ± 8.6	598.7 ± 3.8	62.8 ± 2.8	171.9 ± 9.5
F16	flower of salt	Atlantic	noncommercial	9.12	68.3 ± 1.0	78.9 ± 0.7	1141.2 ± 3.1	67.8 ± 1.5	292.3 ± 3.0
F17	flower of salt	Atlantic	noncommercial	6.51	55.2 ± 0.8	285.4 ± 3.2	475.6 ± 3.1	78.7 ± 0.0	128.6 ± 1.4
F18	flower of salt	Atlantic	noncommercial	4.48	50.4 ± 0.3	204.2 ± 3.0	402.9 ± 4.0	9.5 ± 0.3	274.2 ± 8.4
F23	flower of salt	Atlantic	noncommercial	8.03	60.8 ± 0.4	478.1 ± 0.7	339.6 ± 6.4	45.4 ± 1.3	98.9 ± 3.6
F1	flower of salt	Atlantic	commercial	2.47	38.7 ± 0.2	1033.5 ± 22.7	407.9 ± 2.8	51.4 ± 1.1	156.4 ± 1.5
F6	flower of salt	Atlantic	commercial	3.66	48.8 ± 1.1	1538.3 ± 29.2	676.3 ± 8.5	88.0 ± 4.5	289.4 ± 9.7
F7	flower of salt	Atlantic	commercial	4.74	49.0 ± 0.8	1011.8 ± 12.6	791.8 ± 1.6	182.2 ± 0.0	315.3 ± 9.5
F8	flower of salt	Atlantic	commercial	4.84	45.6 ± 0.3	1252.2 ± 14.5	567.6 ± 1.6	39.0 ± 2.2	260.9 ± 2.6
F9	flower of salt	Atlantic	commercial	4.62	53.3 ± 1.1	1168.3 ± 39.2	545.9 ± 2.8	80.3 ± 4.5	371.3 ± 19.1
F11	flower of salt	Atlantic	commercial	2.75	39.1 ± 0.3	632.3 ± 43.7	521.9 ± 2.8	94.4 ± 1.1	241.8 ± 4.5
F12	flower of salt	Atlantic	commercial	3.43	39.6 ± 0.5	1571.6 ± 21.4	498.9 ± 0.0	64.0 ± 3.9	154.1 ± 0.6
F20	flower of salt	Atlantic	commercial	4.40	45.1 ± 0.3	218.2 ± 2.5	629.9 ± 3.0	10.4 ± 0.5	158.4 ± 8.3
F21	flower of salt	Atlantic	commercial	6.35	48.0 ± 0.3	671.2 ± 2.4	411.3 ± 2.3	139.6 ± 4.1	107.1 ± 5.5
F26	flower of salt	Atlantic	commercial	6.90	72.2 ± 0.6	63.3 ± 0.3	70.7 ± 1.4	87.4 ± 6.6	6.0 ± 0.3
F10	flower of salt	Mediterranean	commercial	0.87	46.2 ± 0.0	1271.1 ± 29.0	169.6 ± 3.2	433.3 ± 0.0	75.5 ± 5.0
F13	flower of salt	Mediterranean	commercial	9.48	68.9 ± 1.2	600.8 ± 6.8	919.5 ± 7.1	77.8 ± 0.5	275.3 ± 6.5
F19	flower of salt	Mediterranean	commercial	0.94	46.9 ± 0.9	309.7 ± 2.3	9.58 ± 0.0	227.9 ± 0.0	75.3 ± 4.6
F22	flower of salt	Mediterranean	commercial	1.08	34.1 ± 0.1	210.7 ± 8.2	159.7 ± 4.2	173.6 ± 8.4	71.4 ± 2.7
F24	flower of salt	Mediterranean	commercial	0.94	33.1 ± 0.6	305.5 ± 9.4	296.6 ± 5.8	72.2 ± 4.0	73.8 ± 2.2
F25	flower of salt	Mediterranean	commercial	4.15	55.2 ± 0.4	239.3 ± 2.3	384.7 ± 1.9	101.7 ± 0.0	128.4 ± 5.1
F27	flower of salt	Mediterranean	commercial	2.20	55.5 ± 0.7	732.8 ± 5.6	349.3 ± 3.0	108.3 ± 0.0	141.1 ± 3.3
F28	flower of salt	Mediterranean	commercial	2.15	45.5 ± 0.3	665.1 ± 2.4	262.4 ± 1.2	173.3 ± 0.0	101.1 ± 2.4
A11	salt (fine)	Atlantic	noncommercial	4.84	51.7 ± 0.0	1644.0 ± 17.0	873.6 ± 2.8	202.1 ± 4.1	258.0 ± 6.7
A12	salt (fine)	Atlantic	noncommercial	5.67	51.6 ± 0.5	1448.2 ± 33.4	1135.5 ± 3.2	221.0 ± 4.1	224.0 ± 4.8
A13	salt (fine)	Atlantic	noncommercial	1.67	37.1 ± 0.5	1317.6 ± 26.4	281.9 ± 3.2	296.3 ± 0.0	84.0 ± 0.9
A14	salt (fine)	Atlantic	noncommercial	2.50	41.0 ± 0.7	870.3 ± 11.6	493.5 ± 3.2	225.6 ± 4.1	134.5 ± 4.6
A15	salt (fine)	Atlantic	noncommercial	5.20	37.0 ± 0.1	1113.7 ± 40.7	618.6 ± 5.8	245.9 ± 4.1	181.5 ± 2.9
A27	salt (fine)	Atlantic	noncommercial	2.43	37.1 ± 0.5	1154.0 ± 24.6	535.9 ± 3.2	209.5 ± 4.4	124.7 ± 1.9
A30	salt (fine)	Atlantic	noncommercial	8.09	71.5 ± 1.4	70.4 ± 3.8	968.6 ± 7.5	69.7 ± 7.2	259.2 ± 9.8
A31	salt (fine)	Atlantic	noncommercial	4.24	47.1 ± 0.06	569.8 ± 5.5	324.4 ± 3.1	96.0 ± 4.3	87.4 ± 3.4
A32	salt (fine)	Atlantic	noncommercial	7.28	172.6 ± 1.6	389.3 ± 6.2	608.2 ± 11.5	147.7 ± 8.4	173.3 ± 11.2
A33	salt (fine)	Atlantic	noncommercial	8.14	62.3 ± 0.6	401.7 ± 5.8	727.8 ± 6.3	79.4 ± 2.9	224.4 ± 4.1
A34	salt (fine)	Atlantic	noncommercial	6.74	65.7 ± 0.3	469.8 ± 15.0	804.2 ± 7.1	140.9 ± 2.1	215.2 ± 11.5
A35	salt (fine)	Atlantic	noncommercial	6.34	54.8 ± 0.2	289.4 ± 7.6	473.8 ± 2.3	108.8 ± 9.9	158.0 ± 7.7
A36	salt (fine)	Atlantic	noncommercial	7.04	54.6 ± 0.2	318.2 ± 6.6	277.8 ± 2.3	89.5 ± 2.1	163.8 ± 4.1
A37	salt (fine)	Atlantic	noncommercial	6.78	41.2 ± 0.5	318.2 ± 5.8	411.4 ± 1.1	141.7 ± 9.1	83.5 ± 2.5
A38	salt (fine)	Atlantic	noncommercial	6.21	32.0 ± 0.7	2.9 ± 0.0	29.0 ± 1.4	66.8 ± 0.0	15.7 ± 1.2
A51	salt (fine)	Atlantic	noncommercial	6.14	50.5 ± 0.8	365.6 ± 12.4	47.7 ± 0.3	107.8 ± 6.4	125.8 ± 2.1
A17	salt (coarse)	Atlantic	commercial	2.78	37.8 ± 0.3	744.1 ± 16.8	341.9 ± 0.0	252.0 ± 9.0	124.3 ± 2.5
A18	salt (coarse)	Atlantic	commercial	2.63	34.2 ± 0.2	1320.2 ± 34.9	624.8 ± 3.2	182.2 ± 9.0	125.4 ± 1.4
A19	salt (coarse)	Atlantic	commercial	4.13	44.2 ± 2.8	1243.6 ± 33.4	584.3 ± 1.6	205.2 ± 2.3	310.5 ± 2.2
A20	salt (coarse)	Atlantic	commercial	3.76	40.3 ± 0.2	730.5 ± 29.8	309.0 ± 0.0	167.2 ± 7.7	115.2 ± 1.9
A24	salt (coarse)	Atlantic	commercial	0.19	24.3 ± 0.1	957.4 ± 14.8	44.2 ± 1.6	354.6 ± 7.1	12.4 ± 0.4
A40	salt (coarse)	Atlantic	commercial	0.41	29.0 ± 0.6	637.8 ± 5.4	114.8 ± 0.0	154.2 ± 0.0	44.9 ± 1.6
A42	salt (fine)	Atlantic	commercial	0.31	43.2 ± 1.0	136.5 ± 1.7	170.4 ± 7.4	57.5 ± 1.2	60.5 ± 2.0
A43	salt (fine)	Atlantic	commercial	2.63	38.4 ± 0.3	918.6 ± 16.2	684.7 ± 7.6	151.0 ± 6.3	119.3 ± 3.1
A41	salt (extra-fine)	Atlantic	commercial	0.63	49.6 ± 0.2	350.4 ± 8.4	238.1 ± 3.4	29.9 ± 1.5	73.3 ± 4.5

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Table 1. Continued

salt code	type of salt[b]	denomination	class	humidity (%)	concn (mg per 100 g of salt)[a]				
					alkalinity[c]	sulfates	Mg^{2+}	Ca^{2+}	K^+
A44	salt (extra-fine)	Atlantic	commercial	0.21	62.6 ± 1.2	165.5 ± 9.0	102.3 ± 0.9	128.9 ± 9.3	29.0 ± 1.0
A45	salt (extra-fine)	Atlantic	commercial	1.49	46.0 ± 0.5	278.3 ± 2.3	414.6 ± 2.3	112.5 ± 0.0	100.7 ± 3.0
A16	salt (coarse)	Mediterranean	commercial	0.09	37.1 ± 0.0	250.0 ± 12.6	20.9 ± 0.0	261.7 ± 0.0	20.7 ± 0.9
A50	salt (coarse)	Mediterranean	commercial	1.96	35.3 ± 0.0	189.7 ± 1.0	109.7 ± 5.2	181.0 ± 2.5	49.6 ± 1.4
A21	salt (fine)	Mediterranean	commercial	0.88	27.4 ± 0.6	1032.8 ± 14.3	7.2 ± 0.0	206.3 ± 4.1	28.3 ± 0.7
A39	salt (fine)	Mediterranean	commercial	0.28	26.6 ± 0.4	213.1 ± 6.0	39.5 ± 1.4	89.5 ± 1.2	15.9 ± 1.2
A49	salt (fine)	Mediterranean	commercial	1.34	39.3 ± 0.1	117.3 ± 5.4	44.6 ± 1.4	61.7 ± 0.9	21.6 ± 1.8
A47	salt (extra-fine)	Mediterranean	commercial	0.22	33.1 ± 0.2	11.2 ± 1.3	76.8 ± 2.0	128.6 ± 3.2	19.6 ± 0.8
A48	salt (extra-fine)	Mediterranean	commercial	1.71	45.2 ± 0.7	117.8 ± 1.8	59.2 ± 1.1	70.8 ± 0.0	16.6 ± 0.3

[a] Average value ($n = 3$) $\pm$ SD. [b] Sea salt granulometry is in parentheses. [c] Expressed as mg HCO_3^- L^{-1}.

matrices, obtained by averaging the correct classification rate for each predicted sample considering only the test sets. Confusion matrices compare the attribute (type of salt or geographical origin) of each sample with the corresponding NIR-based prediction. The results were expressed as percentages. The objective of these matrices is not only to estimate the number of correctly predicted samples but also to diagnose which samples are being incorrectly predicted, identifying the most similar salts in terms of NIR spectra. All chemometrics methods and spectra data processing were performed using Matlab version 6.5 (Mathworks, Natick, MA).

■ RESULTS AND DISCUSSION

Humidity and Granulometry in Sea Salts. Samples were analyzed in a randomized arrangement, and a specific code was given to each one (Table 1). Most of the analyzed samples had Atlantic origins; in this group, 19 samples correspond to "flower of salt", and 27 correspond to traditional sea salts; in the other group of the Mediterranean samples, there are eight samples of "flower of salt" and seven samples of traditional sea salts. In total, there are 25 noncommercial samples.

In relation to the granulometry, 23% of sea salt samples were classified coarse, 62% of the samples were classified fine, and 15% were classified extra-fine (Table 1). According to the guideline[19] that defines the rules for the production and commercialization for the traditional sea salt, the maximum percentages of humidity allowed for commercial sea salt and "flower of salt" are 6 and 8%, respectively. Most of the sea salt samples were below the maximum recommended value of humidity (6%); an exception was observed for some noncommercial sea samples (Table 1). For the "flower of salt" group, just three samples were above the maximum level of humidity recommended, which were two noncommercial samples and just one (F13) a commercial one. Taking just the commercial samples into consideration, it was possible to observe that the "flower of salt" samples presented higher levels of relative humidity in comparison with sea salt samples.

Calcium, Magnesium, Potassium, Alkalinity, and Sulfate Contents in Sea Salts. The results of the concentrations of Ca^{2+}, Mg^{2+}, K^+, alkalinity, and sulfate for the 61 analyzed sea salt samples are presented in Table 1. Triplicates for each ion concentration were averaged yielding a data matrix with dimensions 61×3. In relation with the alkalinity expressed as HCO_3^-, the sample A32, a noncommercial salt sample from the Atlantic region, presented a higher alkalinity content in relation to the

other samples (Table 1). The sulfate results showed that the Atlantic samples presented in average (729.6 ± 493.7 mg/100 g of salt) higher sulfate (SO_4^{2-}) concentration than the Mediterranean ones (424.5 ± 358.1 mg/100 g of salt) ($P = 0.019$). The magnesium results shows that the mean concentration value of Mg^{2+} for Atlantic salts (495.2 ± 268.8 mg/100 g of salt) was significantly higher than the mean concentration value observed for Mediterranean salts (194.0 ± 237.2 mg/100 g of salt) ($P < 0.0001$). A similar tendency was observed for the K^+ concentration for which the mean value (163.3 ± 90.4 mg/100 g of salt) for Atlantic samples was significantly higher than the mean value observed for the Mediterranean salts (74.3 ± 69.0 mg/100 g of salt) ($P < 0.0001$).

By comparison of the samples of the traditional sea salt vs the "flower of salt", it is readily apparent that the traditional sea salt samples had higher concentrations of Ca^{2+} than the samples of "flower of salt". The mean value of calcium for traditional salts (160.5 ± 84.5 mg/100 g of salt) was significantly higher than the mean value of Ca^{2+} observed for "flower of salt" samples (107.0 ± 84.5 mg/100 g of salt) ($P = 0.011$). However, a specific sample of "flower of salt" coded as F10 presented an unexpected high concentration of Ca^{2+} (Table 1). The high amount of Ca^{2+} found in sea salt samples is normal as certain forms of calcium, like calcium sulfate and/or calcium carbonate, can be present in salt brines during NaCl crystallization.[2] The concentrations of calcium and sulfate of Mediterranean samples are correlated ($r = 0.671$), indicating that some sources of Ca^{2+} and SO_4^{2-} are effectively present in Mediterranean samples.

Principal component analysis (PCA) was employed for further analysis of the ions results. A PCA model was performed after autoscaling the data matrix. Figure 1 represents the two main principal components coded according to salt type (Figure 1a) and geographical origin (Figure 1b). Loadings were superimposed to interpret the variation in the scores; the scores representations are the averaged triplicates of each ion concentration. The representation of the scores according to the salt type (Figure 1a) shows that Ca^{2+} ion has an important contribution in the separation of traditional sea salts from the "flower of salt" samples, since traditional salts show a shift toward the direction of the Ca^{2+} ion (upper left side of the Figure 1a). These results also permitted us to confirm that some samples of "flower of salt" presented an unexpected high Ca^{2+} concentration; confirming the presence of F10 in the group of sea salts and other samples were also shifted in the same direction, and they were identified as F19, F22, and F28 (Figure 1a). It is important
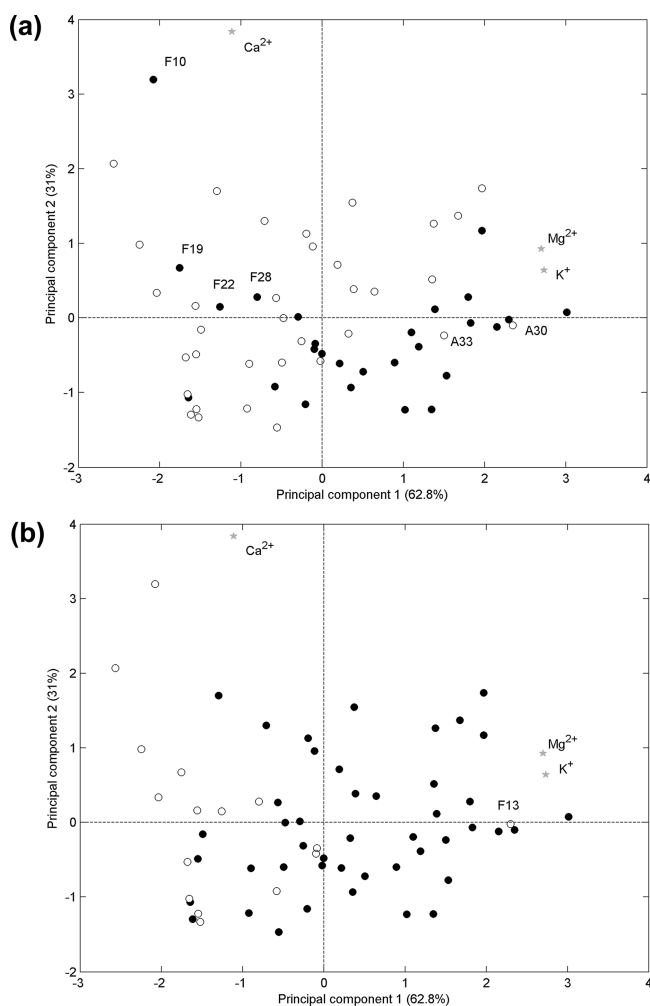
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(a)

(b)

Figure 1. Representation of the scores resulting from a PCA model applied to the three ions concentrations, depicted according to the type of salt (a) (black dot, "flower of salt"; white dot, traditional sea salt) and geographical origin (b) (black dot, Atlantic; white dot, Mediterranean). The represented concentrations are the mean of triplicate measurements. The loadings corresponding to the three metal ions Ca^{2+}, Mg^{2+}, and K^+ were superimposed for scores distribution interpretation (gray star).

to notice that all of the mentioned "flower of salt" samples were commercial samples from Mediterranean origin (Table 1). It was also readily apparent that two traditional salts (A30 and A33), noncommercial samples, were located within the group of the "flower of salt" (Figure 1a).

In relation to the Mg^{2+} and K^+ concentrations, the loadings show that Mg^{2+} is highly correlated with K^+ ($r = 0.811$) (Figure 1b). These ions allowed a distinction according to the geographical origin (Figure 1b). The Atlantic salt samples (mostly located on the lower right side of Figure 1b) have higher concentrations of these ions when compared to Mediterranean samples (mostly located on the left side of Figure 1b). An exception was detected, namely, the sample F13, a commercial Mediterranean sample, which is located within the Atlantic salts group.

NIR Spectroscopy Analysis. Figure 2 represents the normalized averaged NIR spectra for Atlantic and Mediterranean salts. The NIR spectra of the analyzed samples show two broad spectral
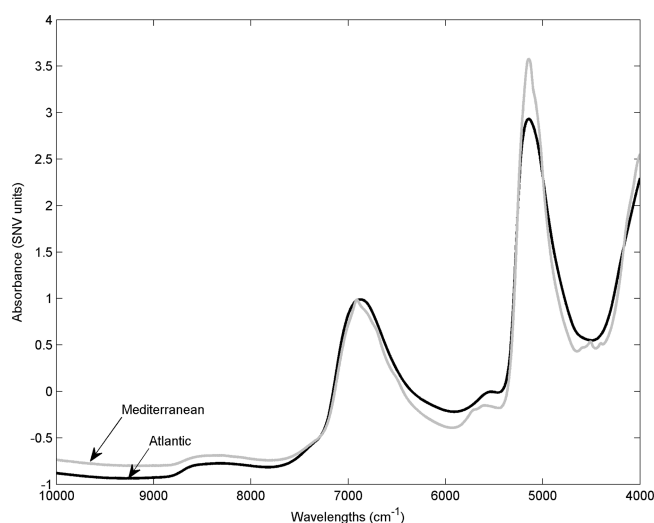

Figure 2. Average NIR spectra of Atlantic (black line) and Mediterranean (gray line) sea salt samples.

bands centered around 6910 and 5145 cm^{-1} (Figure 2), which arise from the presence of water in different coordination states, respectively, assigned to the O−H stretching overtone and O−H combination.[20] It is evident that on average Atlantic salts and Mediterranean salts presented differences in various spectral regions. Those differences are clearly observed in the regions 7000−6500, 4650−4350, and 5900−5500 cm^{-1}. Moreover, it is possible to see that the shape of the overlapped band at 5300−5000 cm^{-1} is different for the Mediterranean samples. In addition, major bands attributed to OH vibrations (around 6900 and 5150 cm^{-1}) are distinctive, too; these features are ascribed to a different humidity content of the samples (Table 1). Finally, a distinct pattern is also observed toward 10000 cm^{-1}, indicating a difference in terms of granulometry for Mediterranean samples when compared with the Atlantic ones. Scientific studies demonstrated the ability of NIR spectroscopy in the 10000−7500 cm^{-1} region to estimate the granulometry (particle size distribution).[21]

In an attempt to understand the most important bands found in sea salt spectrum, a comparison was made using as references the spectra of pure compounds, that is, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), and other salts. A representation of the spectra of NaCl, $MgCl_2$, $CaSO_4 \cdot 2H_2O$, two Mediterranean (A21 and F10), and two Atlantic (A19 and F8) salts is shown in Figure 3a. The calcium sulfate characteristic bands at 6912, 6706, 6500, 5715, 5640, 5145, 5080, 4508, and 4410 cm^{-1} were all easily visible in the Mediterranean salts (sea salt and "flower of salt"), therefore, indicating the presence of this compound. The Atlantic salts analyzed do not have these calcium sulfate distinctive bands. The overlaid spectra of pure NaCl and $MgCl_2$, displaying broad bands, were not especially useful to differentiate the salt samples. Because of the interference of the humidity content in the samples (Table 1), a similar analysis was followed using the same samples after drying conditioning. A representation of the NIR spectrum of those samples and the pure compounds after drying is shown in Figure 3b. These results confirm the conclusions taken before. In this situation, the sharp band centered at 5124 cm^{-1} characteristic of anhydrous $CaSO_4$ and the broad band at 5613 cm^{-1} are easily visible in the Mediterranean salts

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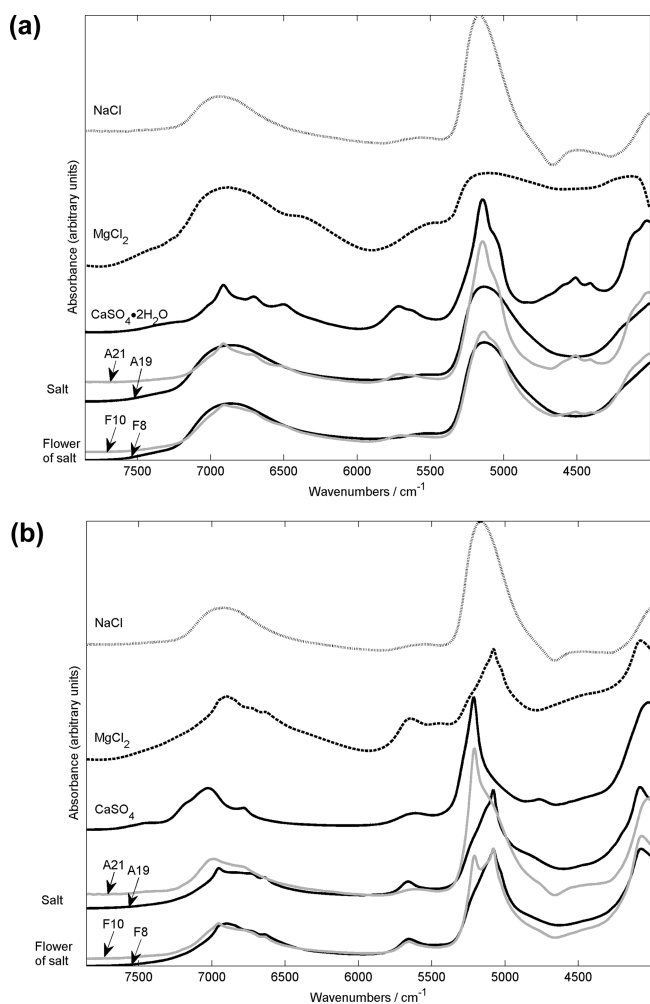
Figure 3. NIR spectra of selected sea salt samples and pure compounds (a) (black line, "flower of salt" F8; gray line, "flower of salt" F10; black line, traditional salt A19; and gray line, traditional salt A21) and NIR spectra of selected sea salt samples and pure compounds after drying for 12 h at 105 °C (b) (black line, "flower of salt" F8; gray line, "flower of salt" F10; black line, traditional salt A19; and gray line, traditional salt A21).

Figure 4. PLSDA model scores considering the NIR region $8500-7500$ cm^{-1} marked according to the type of salt (a) (black dot, "flower of salt"; white dot, traditional sea salt) and PLSDA model scores considering the NIR regions $4650-4350$ and $5900-5500$ cm^{-1} marked according to the geographical origin (b) (black dot, Atlantic; white dot, Mediterranean).

and absent in the Atlantic ones. Additionally, it is clear that the bands arising at 5647, 5080, and 4076 cm^{-1} characteristic of anhydrous MgCl$_2$ are all observed in the selected Atlantic salts.

Sea salt samples (identified in Table 1) were analyzed by NIR spectroscopy. Spectra were tested in terms of their capacity to discriminate by salt type and geographical origin. Because of the variable intensity of the OH$-$ (water) vibration bands at $5300-4800$ and $7200-6300$ cm^{-1}, which have a rather large variation mainly because of the differences on the natural drying conditions of the sea salts, these regions were excluded from the analysis. The region $8500-7500$ cm^{-1} was found to be the most important in terms of discriminating by salt type. This region was mainly affected by the particle size, which influences the light scattering phenomena. The light scattering phenomena are very important in the NIR spectrum, and the effect depends essentially on the crystal (or particle) size distribution. As crystal mean sizes increase, the spectral baseline shifts up, and the absorbance increases as the wavenumber decreases.

Figure 4a shows the LVs 1 and 2 obtained from PLS-DA model calibrated with the 61 salts according to the salt type
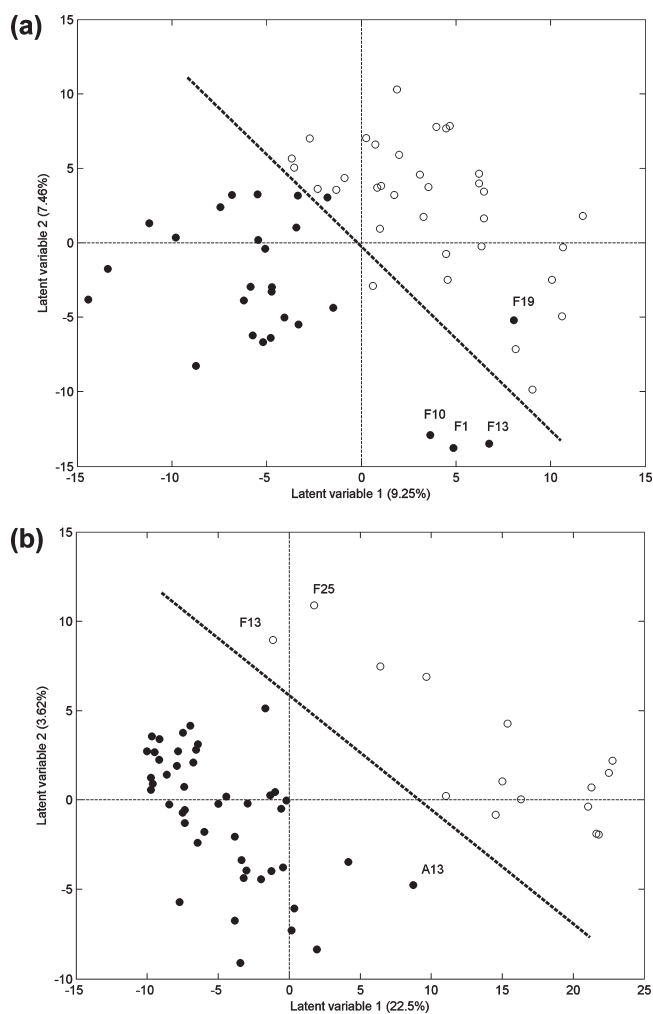
(three LVs). The represented scores were obtained using the cross-validation "leave-one-out" method[16] (also for Figure 4b). Therefore, each sample score was obtained by projecting the corresponding spectrum on a model calibrated with the remaining samples. It is known, however, that the "leave-one-out" method can produce overoptimistic results in some conditions. This is why a more robust procedure for testing the model performance was adopted (see Table 2). Figure 4a shows a good separation between traditional sea salt and "flower of salt" samples. Both types of salts form homogeneous clusters although some samples are more distant from the cluster center. Sample F19 was found to be inside the cluster formed by traditional sea salts. A group consisting of "flower of salt" samples F1, F10, and F13 were also located aside from the main cluster formed by "flower of salt" samples. The validation procedure applied to this model yielded the results expressed in Table 2 (top). The model is able to correctly assign in average, 85.3% of the 61 samples according to the salt type. The 14.7% error is divided in 4.9 (traditional sea salt samples predicted as "flower of salt") and 9.8% ("flower of salt" samples predicted as traditional sea salts).

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Table 2. Confusion Matrices for PLSDA Models Discriminating According to the Type of Salt (Top) and According to the Geographical Origin (Bottom) (Values Are in %)

NIR method	reference method		
	type of salt 8500−7500 cm^{-1} (3 LV)		
	"flower of salt"	traditional sea salt	sum
"flower of salt"	35.2	4.9	40.1
traditional sea salt	9.8	50.1	59.9
sum	45.0	55.0	100.0
	geographical origin 4650−4350 cm^{-1}; 5900−5500 cm^{-1} (4 LV)		
	Atlantic	Mediterranean	sum
Atlantic	73.7	5.2	78.9
Mediterranean	0.0	21.1	21.1
sum	73.7	26.3	100.0

The samples that are systematically predicted in a different class are F1, F10, F13, F19, A40, and A16. In Figure 4a, it is possible to observe that F1, F10, F13, and F19 are on the edge of the separation boundary between the two types of salts. As was previously observed, the samples F10 and F19 were the samples of 'flower of salt', which presented an unforeseen high Ca^{2+} concentration. The high calcium concentration in those commercial Mediterranean samples, which are from the same producer, might come from CaSO$_4$, which could crystallize together with NaCl, by the addition of calcium carbonate (CaCO$_3$) during the process of purification of sea salt or by addition as anticaking agent.[22]

As was previously referred, it is relatively easy to discriminate between Mediterranean and Atlantic salts just by visual inspection of the NIR spectra (Figure 2). To confirm this observation, a second PLS-DA model was calibrated for the geographical origin using the spectral regions 4650−4350 and 5900−5500 cm^{-1} (four LVs). The obtained LVs demonstrate that the discrimination according to the geographical origin is clear; therefore, the discrimination is possible (Figure 4b). The validation procedure applied to this model yielded the results expressed in Table 2 (bottom). The model was able to correctly assign on average 94.8% of the 61 samples according to the geographical origin. On average, none of the Atlantic salt samples is predicted as Mediterranean. On the other hand, 5.2% of the Mediterranean samples are being assigned as Atlantic. Considering the 200 different performed tests, the samples that were shown to be more often incorrectly classified were F13, F25, and A48, all being commercial samples. Taking into consideration the previous results, it is possible to say that the sample F13, a "flower of salt" labeled as Mediterranean sample, could be an Atlantic salt.

The presented results have demonstrated that this NIR technique is a valuable tool that can be used in a straightforward way for the discrimination of sea salts according their geographical origin (Atlantic vs Mediterranean) and according to their quality type (traditional sea salt vs "flower of salt"). The NIR spectra provide information about O−H vibrations resulting from the interaction between water and different salts, with NaCl, MgCl$_2$, and CaSO$_4$ being identified in these samples.

This new application opens the possibility of using NIR as a simple tool for routine quality control uses in the sea salt production and commercialization areas; this methodology can be used for the identification of counterfeiting in traditional sea salts, especially those labeled as "flower of salt" or traditional salts, which does not comply with production specifications (i.e., refine process and/or addition of anticaking agents), and also for denomination origin purposes.

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