Advances in Dairy Research
ISSN: 2329-888X

Calvano and Zambonin, Adv Dairy Res 2013, 1:1
DOI: 10.4172/2329-888X.1000101

Research Article Open Access

MALDI-Q-TOF-MS Ionization and Fragmentation of Phospholipids and Neutral Lipids of Dairy Interest Using Variable Doping Salts

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Abstract

This paper reports the potential of matrix assisted laser desorption ionization quadrupole-time-of-flight (MALDI-Q-TOF) mass spectrometry (MS) for the analysis of lipids of dairy interest, i.e., glycerophospholipids (Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol), sphingolipids (Sphingomyelin) and glycerolipids (Triacylglycerols, Diacylglycerols), respectively. The matrix used was 2,5-dihydroxybenzoic acid (DHB), together with cationizing agents such as sodium and lithium salt that enhanced fragmentation of most lipid classes. The alkaline adducts obtained helped to define in a fast way the polar head groups of different glycerophospholipid classes and provided information about the constituent fatty acid residues. Moreover, information about the position (sn-1, and -2) of the fatty acid residue on the glycerol backbone was obtained. Besides, lithium ion adducts appeared to be the most informative for the structural characterization of glycerophospholipids, glycerolipids and sphingolipids. The present approach proved then to be a convenient, fast and informative way for lipid analysis.

Keywords: Glycerophospholipids; Sphingolipids; Glycerolipids; MALDI-Q-TOF-MS; Fragmentation; Alkaline adducts

Introduction

Lipids in food matrices can include different chemical moieties that are organized into structural classes (polar headgroup), subclasses, and individual molecular species. Glycerophospholipids (GPLs), sphingolipids (SLs) and glycerolipids (GLs) are important food constituents that significantly affect its quality even if present as minor components [1]. They are also among the most chemically unstable food components since they readily undergo, during processing or storage, free radical chain reactions with a consequent food deterioration and oxidative fragments production [2]. Besides, food fat content is extremely variable, both quantitatively and qualitatively; as an example the milk lipid content depends on lactation stage, season, breed, genotype and feeding [3]. Thus, lipid composition could reflect the difference among species, being very informative in the assessment of milk authenticity [4,5]. Therefore, for quality control purposes, analytical methods for their determination in food matrices could represent an important breakthrough.

Traditionally, lipids have been separated using different chromatographic techniques such as gas chromatography, supercritical fluid chromatography, thin layer chromatography [6,7], and liquid chromatography (LC) [8-10] often coupled with UV detection. However, the use of UV spectroscopy leads to considerable differences in the detectability of lipids containing fatty acids with different degree of saturation and different UV absorbance [11]. Moreover, these techniques are tedious, time-consuming, relatively insensitive, and they deliver poor structural information.

For these reasons, mass spectrometry coupled to either gas or liquid chromatography, with its characteristic high sensitivity and specificity, has become increasingly popular for determining these compounds. Lipid analysis was primary performed by using a prefractionation step such as thin layer chromatography or reverse phase-HPLC [12], hydroxylation and derivatization of compounds followed by GC-MS analysis. Even though these compounds could be clearly resolved by GC, drawbacks regarding compound volatilization or thermal degradation could be observed. Moreover, information on the fatty acid prime location is lost [13]. More recently, MS soft ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ ionization (MALDI) have been applied to food lipids characterization. Initially, ESI-MS has been widely used to characterize lipid mixtures prepared from different sources [14-16], in contrast to MALDI-MS that has been limited by the presence of matrix clusters interfering with analyte signals or complexity in reaching efficient ionization/desorption of matrix-hydrophobic lipid analyte clusters. However, the introduction of matrixes such as 2,5-dihydroxybenzoic acid [17], 9-aminoacridine [18], lumazine [19] or ionic liquids [20,21] has permitted the growth of MALDI for high-throughput lipid analysis. Besides, MALDI offers a number of advantages compared to other techniques [22-24]. In fact, the introduction of samples into the instrument is simple, the ionization of lipids occurs also in the presence of contaminating substances, small amounts of material are needed to produce signals without the require for derivatization, the sample preparation can be easily performed, and an excellent sensitivity in absolute terms can be achieved [25]. Furthermore, lipid samples and the common used matrices are readily soluble in organic solvents leading to a homogeneous crystallization of the analyte/matrix mixture on the MALDI target [17]. This results in reproducible and well resolved MALDI mass spectra. A further important MALDI progress for lipid analysis has been the possibility to obtain detailed structural information using fragmentation techniques such as post-source decay (PSD) [26,27] or tandem quadrupole-TOF analyzers (Q-TOF) [28]. In the first case, PSD produces fragment ions with relatively poor resolution while Q-ToF provides excellent MS/MS data, making the structural elucidation quite easy and improving both sensitivity and quality of spectra.

In this work, MALDI-Q-TOF was exploited for the analysis of

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Received February 07, 2013; **Accepted** March 05, 2013; **Published** March 07, 2013

Citation: Calvano CD, Zambonin CG (2013) MALDI-Q-TOF-MS Ionization and Fragmentation of Phospholipids and Neutral Lipids of Dairy Interest Using Variable Doping Salts. Adv Dairy Res 1: 101. doi:10.4172/2329-888X.1000101

various lipid classes such as glycerophospholipids (Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol), sphingolipids (Sphingomyelin) and glycerolipids (Triacylglycerols, Diacylglycerols). The goal of this research was to enhance the abundances of structurally informative ions and to illustrate the fragmentation pathways occurring in MALDI-Q-TOF. Thus, lithium and sodium ion salts have been used to increase ionization efficiencies and facilitate lipids structural analysis.

Experimental Methods

Chemicals

Chloroform, methanol, trifluoroacetic acid (TFA), lithium and sodium chloride were obtained from Sigma (Sigma Aldrich, St. Louis, MO, USA). Lipids and phospholipids standard were obtained from Sigma and stored following the relevant guidelines. 2,5-dihydroxybenzoic acid (DHB) was from Fluka (St. Louis, MO). Water was obtained from a Milli-Q system (Millipore, Bedford, MA). All chemicals and reagents were of the highest grade commercially available.

Lipids and phospholipids standard mixture

The following target analytes standards were purchased: Glycerophospholipids: D-α-phosphatidylcoline-dipalmitoyl (DPPC, 733.56 Da), D-α-phosphatidylcoline-distearoyl (DSPC, 789.62 Da), L-α-phosphatidylethanolamine-oleyl-palmitoyl (POPE, 717.53 Da), L-α-phosphatidylinositol-palmitoyl-linoleoyl ammonium salt (PI, 850.54 Da).

Sphingolipids: Sphingomyelin nervonic (SM, 812.68 Da).

Glycerolipids: 1,3-dipalmitin (PP, 569.51 Da) and tristearin (SSS, 891.84 Da).

Different salts and organic solvents at different concentrations were tested. In the optimized procedure, standard solutions were prepared in chloroform: methanol (1:1, v/v) at a concentration of 1 mg/ml for each target analyte. The solution was mixed (1:1, v/v) with DHB (40 mg/ml in 0.1% TFA in MeOH) and 1 µL of the resulting mixture was deposited on the MALDI target plate and analyzed.

In order to promote the generation of certain types of ion adducts, solutions of alkali salts (sodium or lithium chloride) were mixed with DHB matrix solution to get final salt concentrations between 10 and 50 mM ("salt doping technique").

Mass spectrometry

Positive ion MALDI Q-TOF mass spectra were acquired with a Micromass Q-TOF Ultima MALDI mass spectrometer (Waters, MA). The instrument was operated in V mode with a mass resolution of 10,000. Laser pulses were generated by a nitrogen laser (337 nm) with laser energy of 350 µJ per pulse. Mass spectra were acquired and processed by Masslynx 3.5 software (Micromass Ltd., Manchester, United Kingdom). For MS experiments, a total of 40-60 scans were averaged per mass spectrum in the m/z range 500-1200, the background was subtracted, and the spectrum was smoothed using a mass window appropriate for the significant peak widths. A tryptic digest of lactoglobulin was used as internal mass standard. For MS/MS experiments, the opportune acquisition window was selected for each ion using collision ion energy around 30 eV. No signal smoothing and baseline subtraction were applied.

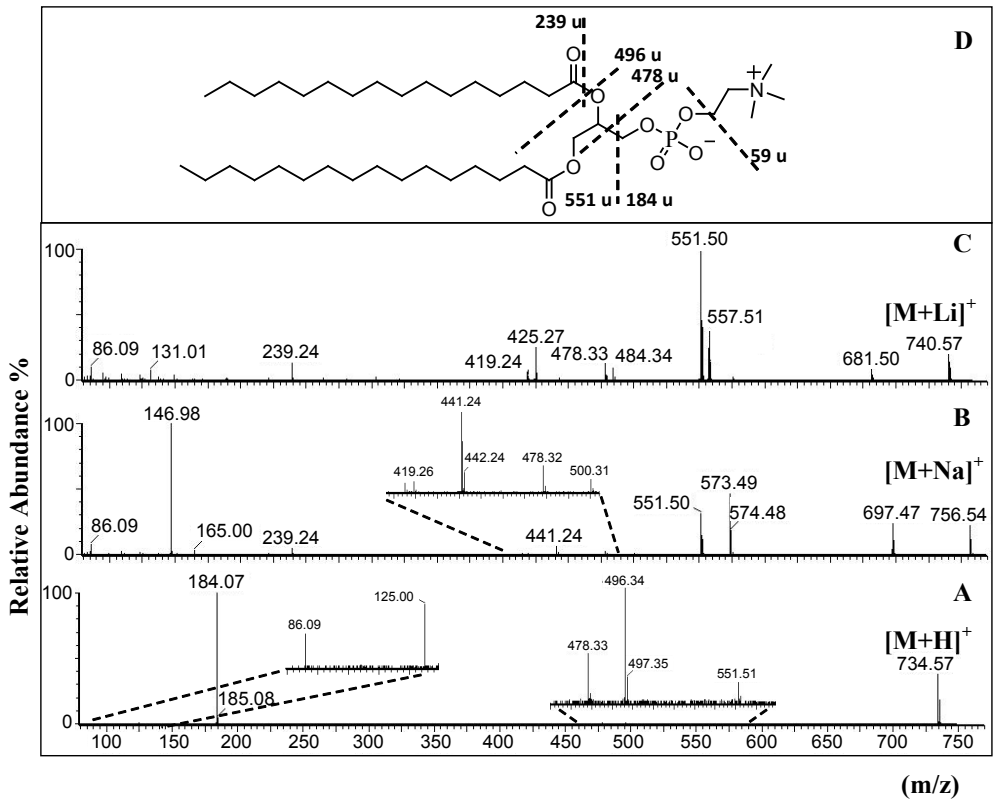

Figure 1: MALDI-Q-TOF spectra of DPPC (16:0/16:0) obtained inducing the fragmentation of (a) $[M+H]^+$ (m/z 734.57), (b) $[M+Na]^+$ (m/z 756.54), and (c) $[M+Li]^+$ (m/z 740.57), (d) Scheme of the important fragments observed in figure 1A.

Results and Discussion

The main aim of the work was to show the utility of MALDI-Q-TOF analyses to identify the head group of many lipid classes and to generate fragments useful for fatty acid composition identification. Moreover, a comparison of spectra obtained with or without alkali cations attachment is provided showing the results and some fragmentation behavior for selected lipid standards.

Phosphatidylcholine

MS/MS experiments were performed by inducing fragmentation on protonated, sodiated and lithiated precursor ions derived from DPPC and DSPC, respectively (Figures 1 and 2). The spectra (Figures 1a and 2a) arising from both the protonated analytes $[M+H]^+$ (m/z 734.57 and 790.62) show one significant fragment ion at m/z 184.07 corresponding to cholinephosphate [29-31], and the less intense m/z ions 125.00 (cyclophosphane) and 86.09 ("dehydrocholine"). These ions are diagnostic for the choline headgroup and could be considered a "fingerprint" of the PC class; however, this is the only information obtainable from these spectra since no characteristic fragment ions derived from the loss of the fatty acid residues were observed, as confirmed by other published data [29-31]. However, increasing collision energy and laser fluence, three more ions appeared in the range m/z 450-550 in both spectra. In particular, in the case of DPPC (see inset in figure 1a) the m/z ions 551.51, 496.34 and 478.33 showed up, corresponding to $[M-183+H]^+$ (loss of cholinephosphate), $[M-C_{16}H_{30}O+H]^+$ (loss of ketene palmitic acid), and $[M-C_{16}H_{32}O_2+H]^+$ (loss of palmitic acid). Similarly, in the case of DSPC (Figure 2a) the m/z ions 607.55, 524.37 and 506.35, corresponding to $[M-183+H]^+$ (loss of cholinephosphate), $[M-C_{18}H_{34}O+H]^+$ (loss of ketene stearic acid), and $[M-C_{18}H_{36}O_2+H]^+$ (loss of stearic acid), were detected.

Contrary to previously reported data obtained performing post source decay experiments starting from sodiated PC precursors ions [32], that showed a poor fragmentation, a significant number of fragment ions, including those relevant to the loss of fatty acid substituents (Figures 1b and 2b), were obtained by MALDI-Q-TOF. In particular, the fragmentation induced on the sodium adduct $[M+Na]^+$ of DPPC (m/z 756.54) lead to (Figure 1b) the m/z ions 697.47, resulting from the neutral loss of trimethylamine (59u), 573.49, resulting from the neutral loss of cholinephosphate (183u), 551.50, corresponding to the loss of sodiated cholinephosphate (205 u), 146.98, corresponding to sodiated cyclophosphane and 86.09, corresponding to "dehydrocholine". At lower abundance, the m/z ions 500.31, corresponding to $[M-C_{16}H_{32}O_2+Na]^+$, and 478.32, corresponding to $[M-C_{16}H_{32}O_2+H]^+$, both relevant to the loss of palmitic acid substituent, were also observed. Furthermore, the m/z ions 441.24 corresponding to $[M-59-C_{16}H_{32}O_2+Na]^+$, and 419.26, corresponding to $[M-59-C_{16}H_{32}O_2+H]^+$, resulted from the neutral loss of trimethylamine and palmitic acid, while the m/z ion 239.24 was attributed to palmitic acilium ion. The same fragmentation pattern was originated by the sodiated ion $[M+Na]^+$ of DSPC (Figure 2b).

Figures 1c and 2c report the MS/MS spectra relevant to the fragmentation mainly originated by the $[M+Li]^+$ adducts of DPPC (m/z 740.57) and DSPC (796.64), respectively. In the case of DPPC (Figure 1c), the most abundant m/z ions 551.50 and 557.51 correspond to the neutral loss of cholinephosphate (183 u) from the $[M+H]^+$ and $[M+Li]^+$ adducts, respectively. The loss of trimethylamine from $[M+Li]^+$ generates a small peak at m/z 681.50. Furthermore, fragment ions resulting from the loss of the acyl groups could be detected at higher intensities in the m/z range 400-500. In particular, the m/z ion 425.27, corresponding to $[M-59-C_{16}H_{32}O_2+Li]^+$ originates

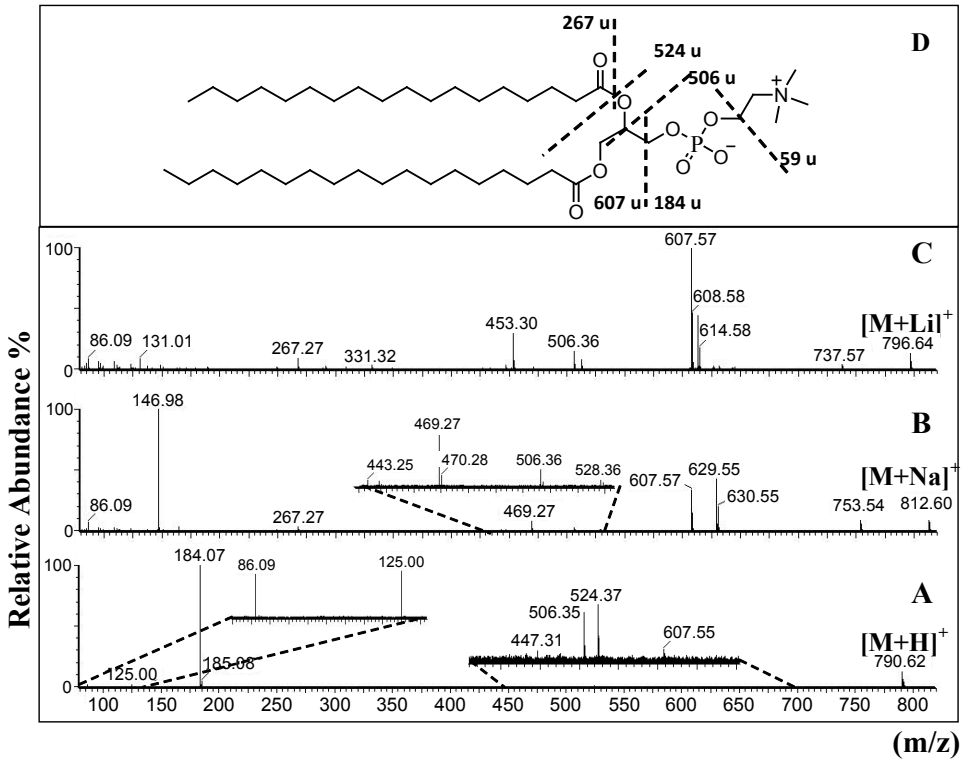

Figure 2: MALDI-Q-TOF spectra of DSPC (18:0/18:0) obtained inducing the fragmentation of (a) $[M+H]^+$ (m/z 790.62), (b) $[M+Na]^+$ (m/z 812.60), (c) $[M+Li]^+$ (m/z 796.64), (d) Scheme of the important fragments observed in figure 2A.

from a combined loss of palmitic acid and trimethylamine; the corresponding non lithiated m/z ion 419.26 is also present. Further fragments are observable at m/z 484.34 and 478.33 corresponding to $[M-C_{16}H_{32}O_2+Li]^+$ and $[M-C_{16}H_{32}O_2+H]^+$, respectively, and once again, at m/z 239.24, corresponding to palmitic acylium ion. Finally, in the low mass range, peaks at m/z 131.01 and m/z 86.09, corresponding to lithiated cyclophosphane and "dehydrocholine", respectively, were obtained. The same fragmentation behavior was observed for the lithiated ion $[M+Li]^+$ of DSPC (Figure 2c).

Phosphatidylethanolamine

Figures 3a and 3b report the MALDI-Q-TOF spectra of $[M+Na]^+$ and $[M-H+2Li]^+$ ions derived from POPE. Even without salt addition, the $[M+Na]^+$ adduct, i.e., the m/z ion 740.57, was the most abundant quasi-molecular ion while the $[M+H]^+$ adduct was not observable. The spectrum (Figure 3a) relevant to the fragmentation of the precursor ion $[M+Na]^+$ shows five major fragment ions at m/z 697.47, 599.50, 577.50, 164.01, and 120.97 which are characteristic for the PE class. In particular, the m/z ion 697.47 arises from the loss of ethanolamine (43 u), while the highly abundant m/z ions 599.50 and 577.50 arise from the loss of the neutral polar head ethanolaminephosphate (141 u) and sodiated ethanolaminephosphate (163 u), respectively. The signal at m/z 164.01 corresponds to the sodiated polar head, while the peak at m/z 120.97 corresponds to the loss of ethanolamine (43 u) from the sodiated polar head. In addition, it is possible to see in the range 400-500 m/z the fragmentation pathway generated by the loss of *sn*-1 and *sn*-2 acyl chains, as ketene (R=C=O) or free fatty acid (RCOOH), combined with the loss of 43u. The product ion formed by the loss of R_1COOH and ethanolamine ($[M+Na-R_1COOH-43]^+$, m/z 441.24) shows higher relative abundance compared to the fragment formed by the loss of R_2COOH and ethanolamine ($[M+Na-R_2COOH-43]^+$, m/z 415.22). In the same way, the product ion arising from the loss of R_1COOH ($[M+Na-R_1COOH]^+$, m/z 484.34) shows higher relative abundance than the product ion formed by loss of R_2COOH ($[M+Na-R_2COOH]^+$, m/z 458.23). These two ion couples allow the identification of both fatty acyl residues of the PEs, as well as their specific location in the glycerol backbone. Thus, these diagnostic ions can be potentially used for differentiation of isomeric PEs. A further peak at m/z 239.24 was attributed to the acylium ion R_1CO^+, confirming the preferential loss of the *sn*-1 acyl chain. It is worth noting that using MALDI-Q-TOF, it was possible to obtain detailed structural information, comparable to those obtainable by ESI [33]; on the contrary, PSD [32] allowed only the attribution of polar head.

The precursor ion $[M-H+2Li]^+$ (m/z 730.55) provides an even more complex spectrum (Figure 3b). Again, very intense fragments due to headgroup losses could be observed. The m/z ions 687.47 and m/z 601.54 correspond to the loss of ethanolamine (43u) and the neutral loss of lithiated ethanolaminephosphate (129u), respectively. Furthermore, a fragmentation pattern similar to the $[M+Na]^+$ adduct was observed for the loss of the fatty acids residues. The $[M-H+2Li-(RCOOLi)-43]^+$ and $[M-H+2Li-(RCOOH)-43]^+$ ions show a higher signal for the loss from the *sn*-1 (m/z 425.27 and 431.28, respectively) than from the *sn*-2 position (m/z 399.26 and 405.31, respectively). The low abundant m/z ion 474.31 corresponds to the $[M-H+2Li-(RCOOH)]^+$ ion due to the loss of palmitic acid. The two low abundant m/z ions 339.30 and 313.28 were attributed to $[M-H+2Li-129-(RCOOLi)]^+$, corresponding to the release of lithiated carboxylates of the fatty acids (RCOOLi) from the *sn*-1 (m/z 339) and *sn*-2 position (m/z 313) of the m/z ion 601.54; these ions are present together with their corresponding lithiated at m/z 345.30 and 319.28 arising from the release of the carboxylates fatty acids $[M-H+2Li-129-(RCOOH)]^+$ from the *sn*-1 (m/z 345) and *sn*-2 position

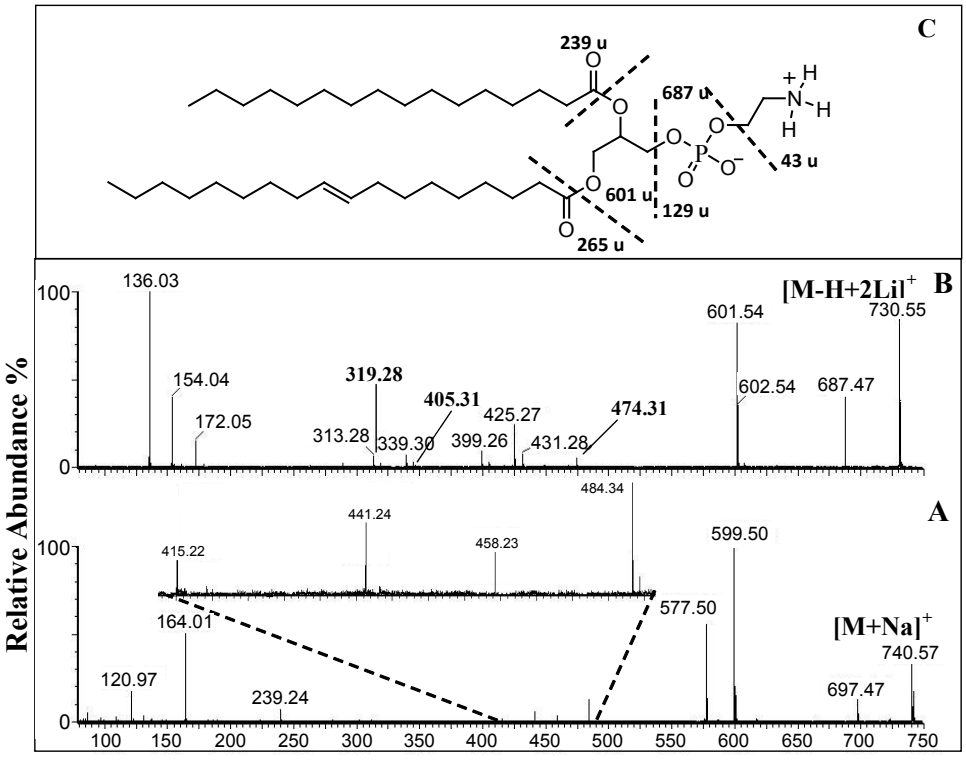

Figure 3: MALDI-Q-TOF spectra of POPE (16:0/18:1) obtained inducing the fragmentation of (a) $[M+Na]^+$ (m/z 740.57), and (b) $[M+2Li-H]^+$ (m/z 730.55) and (c) Scheme of the important fragments observed in figure 3B.

(m/z 319) [34] of the m/z ion 601.54. This peculiar fragmentation pathway seems to occur only in the case of dilithiated molecules. In the low mass range, the fragment ions corresponding to dilithiated amidophosphane (m/z 136.03), ethanolaminephosphate (m/z 154.04) and hydrated ethanolaminephosphate (m/z 172.05) were also present.

Phosphatidylinositol

As observed in the case of phosphatidylethanolamine, the $[M+H]^+$ adduct was never observable in the spectra. Figures 4a-4c report the MALDI-Q-ToF spectra of $[M-H+2Li]^+$ (m/z 847.55), $[M+Na]^+$ (m/z 857.50) and $[M-H+2Na]^+$ (m/z 879.49) adducts, respectively, derived from PI. The spectrum of dilithiated PI (Figure 4a) shows a simple fragmentation pattern. The low signal at m/z 685.49 was due to the neutral loss of inositol (162 u). The peaks at m/z 599.53 and 605.55 could be trilithiated and tetralithiated ion adducts correlated to the neutral loss of inositol 1-phosphate (260u) from the quasi-molecular ion. The ions detected at m/z 429.26 and 405.26 correspond to $[M-H+2Li-162-(RCOOH)]^+$ indicating a probable combined loss of inositol and palmitic (256 u) or linoleic acid (280 u), respectively. The signal intensity ratio of almost 3:1 between the two peaks accounts for the localization of linoleic acid at the *sn*-2 position [35]. Two more m/z ions, 273.06 and 255.04, were attributed to dilithiated inositol 1-phosphate and dilithiated inositol 1-phosphate after elimination of a water molecule. The MALDI-Q-TOF spectrum of the $[M+Na]^+$ adduct, shown in figure 4b, resulted to be very simple. The low peaks at m/z 597.48 and 575.50 arise from the neutral loss of inositol 1-phosphate (260u) and of the corresponding sodium adduct (282u), respectively, while the signal at m/z 283.02 is attributable to sodiated inositol 1-phosphate ion. On the contrary, the fragmentation of the $[M-H+2Na]^+$ adduct (m/z 879.49), reported in figure 4c, shows a definitely more informative pattern. The m/z ion 717.45 originates from the neutral loss of inositol (162u), while in the m/z range 400-700 fragment ions reflecting the loss of acyl groups are observable. The m/z ions 601.28 and 577.28 are attributable to $[M+Na-(RCOOH)]^+$ ions, corresponding to the neutral losses of palmitic (256 u) and linoleic acid (280 u), respectively. The peaks at m/z 599.28 and 623.25 can be ascribed to $[M-H+2Na-(RCOOH)]^+$ ions, corresponding to the neutral losses of palmitic (256 u) and linoleic acid (280 u), respectively. In addition, $[M-H+2Na-(RCOOH)-162]^+$ ions indicating a combined loss of acyl groups and inositol were detected at m/z 461.21 and 437.21, respectively. Smaller signals corresponding to the monosodiated ions $[M+Na-(RCOOH)-162]^+$ were detected at m/z 439.23 and 415.24, respectively. The m/z ions observed at m/z 305.01 and 286.99 can be attributed to disodiated inositol 1-phosphate and disodiated inositol 1-phosphate after elimination of a water molecule.

J Adv Dairy Res, an open access journal
ISSN:2329-888X

Sphingomyelin

MS/MS experiments were performed inducing the fragmentation of protonated, sodiated and lithiated precursor ions derived from SM (Figures 5a-5c). The spectrum (Figure 5a) relevant to the fragmentation of the precursor ion $[M+H]^+$ (m/z 813.69) was found to be not peculiar for SM, since it is completely identical to that of the PC class, with the only exception for a fragment ion at m/z 184.07, corresponding to cholinephosphate [36]. The sodium and lithium ions adducts (m/z 835.67 and 819.69, respectively) exhibited a more informative fragmentation pattern. The adduct $[M+Na]^+$ yields (Figure 5b) fragments at m/z 776.59, corresponding to the neutral loss of trimethylamine ($[M+Na]^+$-N(CH_3)$_3$), at m/z 652.61 corresponding to

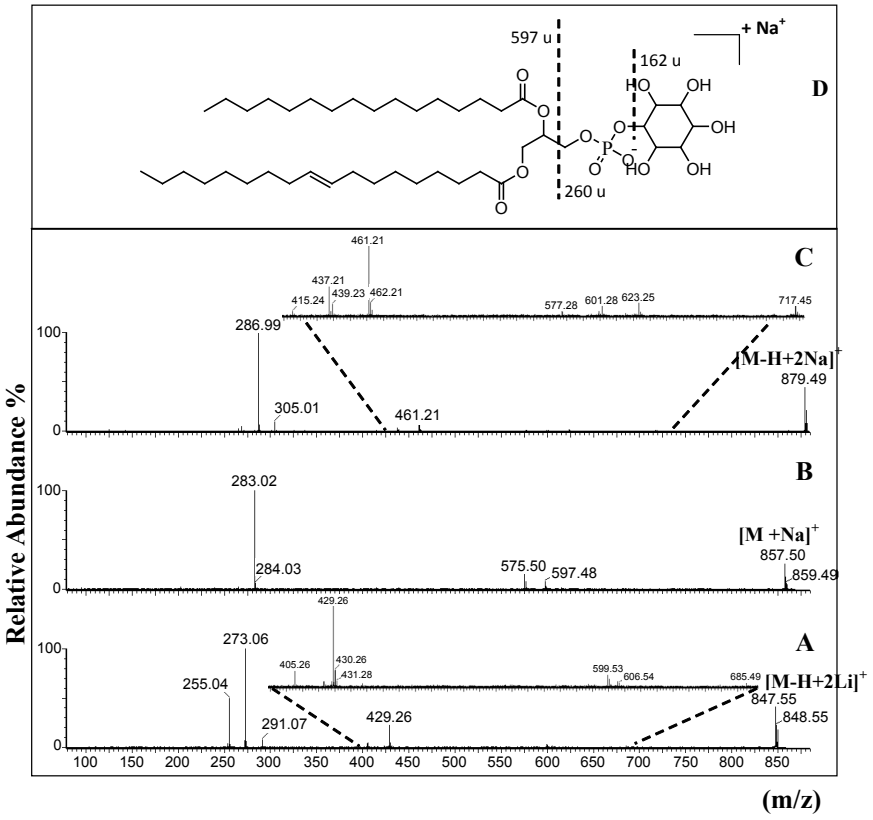

Figure 4: MALDI-Q-TOF spectra of PI obtained inducing the fragmentation of (a) $[M+2Li-H]^+$ (m/z 847.55), (b) $[M+Na]^+$ (m/z 857.50), (c) $[M+2Na-H]^+$ (m/z 879.49) and (d) Scheme of the important fragments observed in figure 4B.

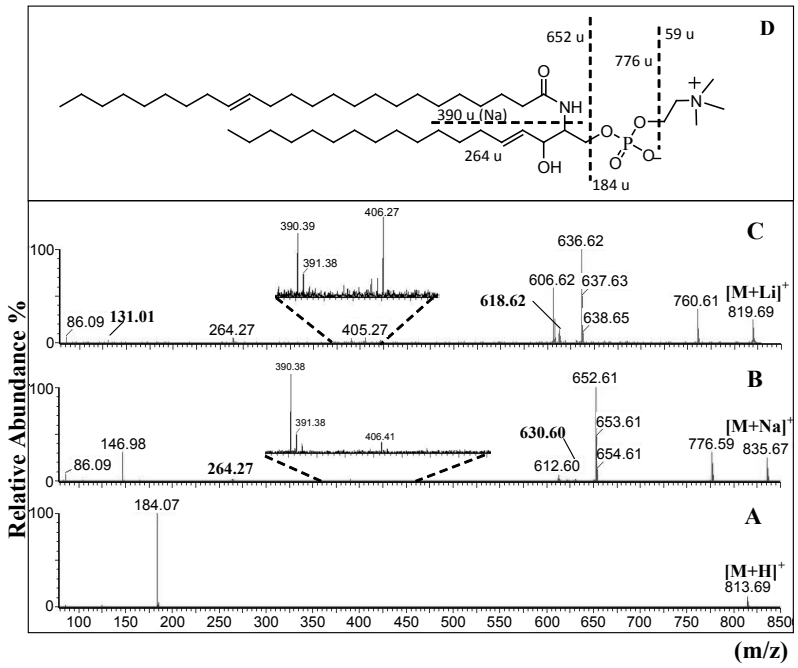

Figure 5: MALDI-Q-TOF spectra of SM (18:1/24:1) obtained inducing the fragmentation of (a) $[M+H]^+$ (m/z 813.69), (b) $[M+Na]^+$ (m/z 835.67), and (c) $[M+Li]^+$ (m/z 819.69) and (d) Scheme of the important fragments observed in figure 5B.

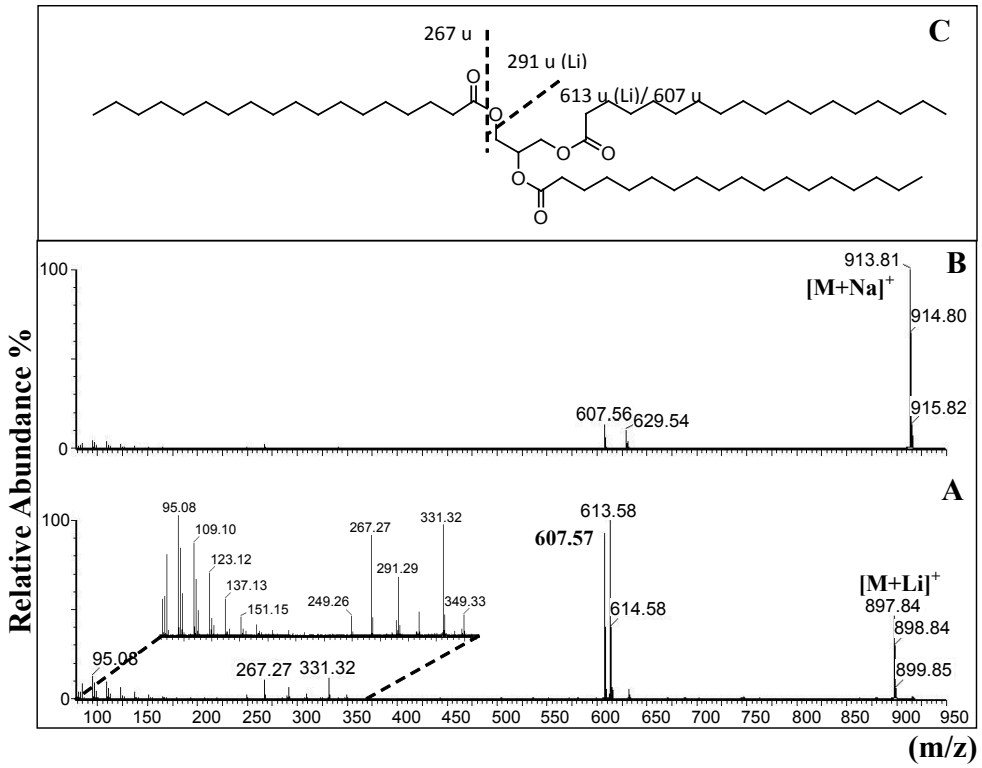

Figure 6: MALDI-Q-TOF spectra of SSS (18:0/18:0/18:0) obtained inducing the fragmentation of (a) $[M+Li]^+$ (m/z 897.84), (b) $[M+Na]^+$ (m/z 913.81) and (c) Scheme of the important fragments observed in figure 6A.

the ion ($[M+Na]^+$-183), arising from the neutral loss of cyclophosphane and at m/z 630.60, corresponding to the ion ($[M+H]^+$-183), arising from the loss of sodium cyclophosphanate. Further elimination of H_2O from the m/z ion 630.60 via charge-remote fragmentation yields a diagnostic ion ($[M+H]^+$-183-H_2O) at m/z 612.60. In addition, the spectrum contains two characteristic ions [37] at m/z 264.27 and

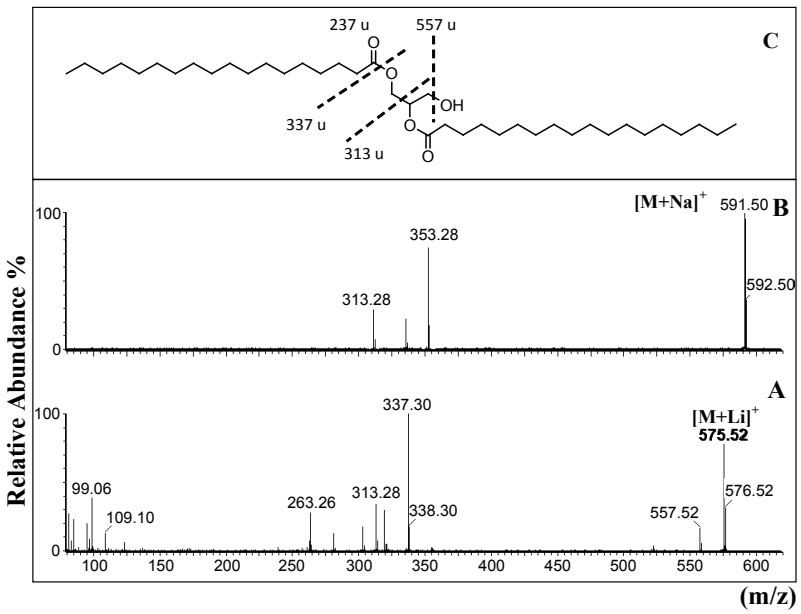

Figure 7: MALDI-Q-TOF spectra of PP (16:0/16:0) obtained inducing the fragmentation of (a) $[M+Li]^+$ (m/z 575.52), (b) $[M+Na]^+$ (m/z 591.50) and (c) Scheme of the important fragments observed in figure 7A.

390.38, corresponding to a 18:1 chain and a 24:1 chain, respectively, that allow the characterization of SM structure in d18:1/24:1. In the low mass range, signals from sodiated cyclophosphane (m/z 146.98) and dehydrocholine (m/z 86.09) were also observed.

Similar results (Figure 5c) were obtained by inducing the fragmentation of the lithium ion adducts. Distinctive fragments arising from the neutral loss of trimethylamine (at m/z 760.61), and the neutral loss of cyclophosphane (at m/z 636.62) were observed in the spectrum. Further elimination of CH_2O and H_2O from the m/z ion 636.62 via charge-remote fragmentation yields an abundant peak ($[M+Li]^+$-183-CH_2O) at m/z 606.62 and a low abundant peak ($[M+Li]^+$-183-H_2O) at m/z 618.62. These ions could be used as diagnostic for SM, since this fragmentation behavior was not showed by PC. As mentioned above, a further confirmation of the identity of the fatty acid constituents is the presence in the spectrum of the low intensity ions at m/z 390.39 and m/z 264.27. Finally, the m/z ions 131.01 and 86.09, representing lithiated cyclophosphane and CH_2=CH-N$(CH_3)_3^+$, respectively, were observed in the low mass range [36]. As apparent, the MALDI-Q-TOF permits the fast structural characterization of sphingomyelin, with results comparable to ESI-MS/MS.

Tri- and diacylglycerols

The fragmentation (Figure 6a) of the precursor ion $[M+Li]^+$ (m/z 897.84) relevant to SSS originates abundant fragment ions that reflect the identities and positions of the analyte fatty acid substituents. The m/z ions 613.58 and 607.57 arise from the neutral losses of stearic acid (284 u) and correspond to the lithium and proton ion adducts of the fragment, respectively. The same behavior was observed in the case of the fragmentation (Figure 6b) of the precursor ion $[M+Na]^+$ (m/z 913.81), where the losses of the fatty acid substituent produced the sodium (m/z 629.54) and proton ion (m/z 607.56) adducts of the fragments. Unfortunately, no more useful information was provided in this spectrum. In fact, as already reported for ESI experiments, lithium ion adducts of GL yielded more informative MS/MS spectra using low collision energy [38,39] than sodium adducts [40,41]. In fact, more fragment ions that reflect the mass of each fatty acid substituent are observed in the mass region m/z 240-350. In particular, the m/z ions 331.32, 291.29 and 267.27 correspond to $[M+Li-(RCO_2H)-(RCH=CHCO_2H)]^+$, $[RCO_2H+Li]^+$ and to the acylium ($RnCO^+$) ion derived from 18:0 fatty acid, respectively; a further loss of water from the m/z ion 267.27 lead to the [$RnCO^+$-18] ion at m/z 249.26. Other fragments were observed in the m/z range 30-200: these include a series of alkyl ions at m/z 81, 95, 109, 123, 137 arising from the decomposition of $[M+Li-(RCO_2Li)]^+$ ions, similar to those observed under electron impact conditions from alkyl chains, due to charge driven fragmentation mechanisms [42,43]. As far as the analysis of alkali adducts of PP is concerned (Figures 7a and 7b), the same results discussed above were obtained.

Conclusions

MALDI-Q-TOF proved to be a powerful technique to obtain structural information on various lipids of dairy significance, allowing a fast characterization of the different classes inducing the fragmentation of sodium and lithium ion adducts. In fact, very informative fragment ions related to the polar head groups and reflecting the fatty acid constitution of the molecules (acyl group composition) were easily observed. The fragmentation was strongly dependent on the type of precursor ions and the number of attached alkali ions. In particular, lithium ion adducts provided the most important structural elucidation.

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