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Ceramides as Possible Nutraceutical Compounds: Characterization of the Ceramides of the Moro Blood Orange (*Citrus sinensis*)

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ABSTRACT: Ceramides are presented as nutraceutical compounds for protection of colon carcinoma and as important cosmetic preparation components, increasing absorption through the skin. Therefore, the ceramide (Cer) content of Moro blood oranges was determined by mass spectrometry. A total of 114 Cer species were identified: ∼160 mg in the peels and ∼140 mg in the pulp per kilogram of oranges, expressed as "milligram equivalents of d18:1,17:0 Cer". The predominant ceramides contained 4-hydroxy-8-sphingenine (t18:1$^{\Delta8}$) and 4-hydroxysphinganine (t18:0) as long-chain bases (LCBs) and fatty acids (FAs) with different structures. In the pulp, t18:1$^{\Delta8}$- and t18:0-containing Cer species comprised 50.5 and 33.5% of the total, respectively, 11.5 and 3.5% non-hydroxylated FAs, respectively, 32.0 and 21.0% α-hydroxylated FAs, respectively, and 7.0 and 9.0% α,β-hydroxylated FAs, respectively. In the peels, t18:1$^{\Delta8}$- and t18:0-containing species comprised 49.5 and 34.5% of the total, respectively, 16.0 and 1.5% non-hydroxylated FAs, respectively, 31.5 and 29.0% α-hydroxylated FAs, respectively, and 2.0 and 4.0% α,β-hydroxylated FAs, respectively.

KEYWORDS: *Ceramide, orange, citrus, peel, pulp, cosmetic, skin*

■ INTRODUCTION

Ceramide (Cer) (compounds **1** and **3** in Figure 1), a double-chain lipid composed of a long-chain base (LCB) (compounds **2** and **4** in Figure 1) and a fatty acid (FA),[1] is a highly hydrophobic structure. The Cer family is a very heterogeneous group of species that differ in both their LCB and FA structures.[2−4] Cer is the lipid moiety of sphingolipids, such as sphingomyelin and glycolipids, which are amphiphilic components of cell membranes. Cer is a minor component of all cell membranes and is abundant in skin.[5−7] In mammalian cells, Cer is produced from sphingomyelin and glycosphingolipids by plasma membrane sphingomyelinase and glycosphingolipid hydrolases,[8] respectively, and may act as an apoptotic compound under certain conditions.[9] Sphingolipids in mammalian organisms contain mainly sphingosine (*trans*-4-sphingenine, d18:1$^{\Delta4}$) and sphinganine (d18:0) as LCB. They are also present in plants,[4,10−12] and they contain mainly 4-hydroxysphinganine (t18:0, phytosphingosine) and 4-hydroxy-8-sphingenine (t18:1$_{\Delta8}$). The prefixes "d" and "t" designate di- and trihydroxy sphingoid backbones, respectively (Figure 1). Sphingolipids are present in high quantity in the human diet in the form of meat, milk, cheese, soybeans, and soybean derivatives.[3] In the human intestine, sphingolipids are digested by specific enzymes to yield Cer. There is evidence that high levels of Cer, derived from a diet rich in sphingolipids, reduce the risk of colon carcinoma.[13,14]

Cer in human skin has a very complex composition and functions as a highly hydrophobic barrier that protects the body from the absorption of water.[15] Some of the Cer species present in cell membranes contain complex and very long FA chains that reach the opposite layer, allowing interdigitation between the outer and inner membrane leaflets. Cer species containing C26−C36 FAs are produced by the activity of Cer synthase CerS3, one of the six types of Cer synthases.[15] Interestingly, some Cer species in skin belong to the phyto-Cer series (compound **3** in Figure 1), derived from phyto-LCB (compound **4** in Figure 1).

A recent study demonstrates that some Cer orally administered to rats is distributed gradually in the dermis after gastrointestinal absorption, followed by transfer from the dermis to the epidermis.[16] Because of the above properties, Cer has great potential application as a nutraceutical compound for the prevention of colonic tumors[17] and enhancing the skin barrier function.[18] There is consequently a demand for large amounts of Cer and commercially available sources from which Cer can be extracted.

In the literature, studies on the characterization of ceramides in different plant species are reported, for instance, in potatoes and sweet potatoes,[11] in scarlet runner beans and kidney beans,[10] nuts and seeds,[19] etc.[20] Citrus fruits have been reported to contain Cer species having structures very similar to that of human skin Cer.[21] The present study is the first in a series of detailed studies of the Cer composition of orange species, in this case the Moro blood orange, *Citrus sinensis*, which has been cultivated for many centuries on the Italian island of Sicily. Moro blood oranges are covered by the Indicazione Geografica Protetta (IGP) trademark and have been shown to contain
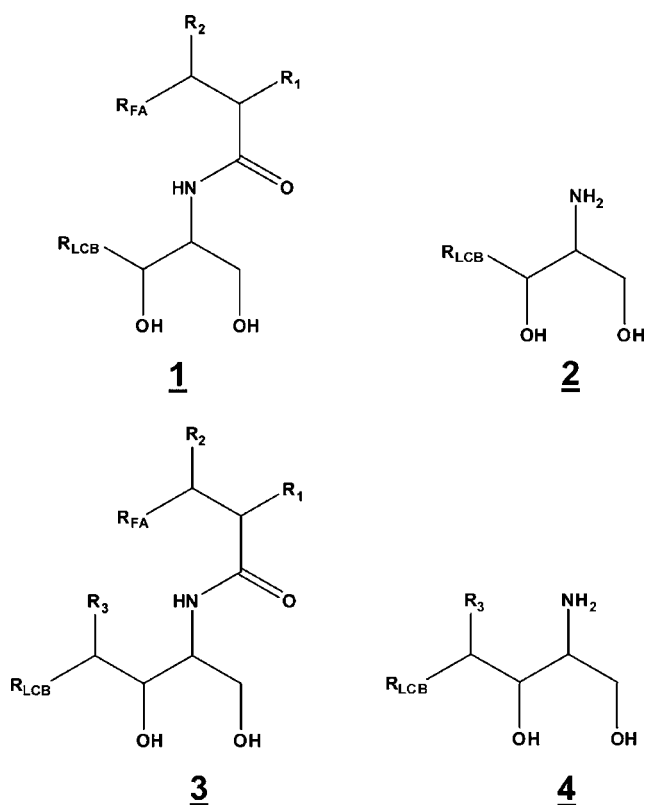
Figure 1. Basic structures of Cer and LCB. An abbreviated nomenclature was used, in agreement with other authors,[28,29,40] with slight modifications: R_{FA} and R_{LCB} = alkyl or alkenyl chains, FA- or LCB-related, respectively; R_1 = H or OH; R_2 = H or OH; species denoted with R_3 = H have a dihydroxy base; and species denoted with R_3 = OH have a trihydroxy base (4-phyto series).

Figure 2. Scheme for the extraction and fractionation of Cer species.

several health-promoting compounds.[22] Several commercial products are obtained from these oranges: juice (from the pulp), liqueurs (prepared using aromatic oils extracted from the peel), and antioxidant compounds (separated from the oils).

Recently, liquid chromatography tandem mass spectrometry (LC−MS/MS) has been shown to be a useful method for the structural characterization of ceramides.[23−29] By high-performance liquid chromatography−electrospray ionization−mass spectrometry (HPLC−ESI−MS), we found that several species of Cer are components of both the peels and pulp of *C. sinensis*.

■ MATERIALS AND METHODS

The chemicals used were the purest commercially available; the common solvents were distilled before use; and water was doubly distilled in a glass apparatus. The reagents were all obtained from Sigma Chemical Co. (St. Louis, MO). Silica gel 60 for column chromatography (0.040−0.063 mm, 230−400 mesh, ASTM) and silica gel thin-layer chromatography (TLC) plates were from Merck (Darmstadt, Germany). C17-Cer used as an internal standard was prepared by the acylation of sphingosine, which was prepared from cerebroside.[30]

Moro blood oranges were bought at the fruit market of Catania, Sicily, Italy. The outer skin was removed with a knife and discarded. The oranges were squeezed to give a turbid suspension, and the peels were stored together with thin peels of cloves (Sample **1** in Figure 2). The suspension was centrifuged at 1000g to obtain pelleted orange pulp (Sample **2** in Figure 2). Sample **1** and **2** were lyophilized and extracted using organic solvents. A total of 5 g of each sample was extracted 3 times with 100 mL each of the following solvents in series: (1) petroleum ether, (2) chloroform, and (3) chloroform/methanol (2:1 by vol). After drying, fractions 2 and 3 were applied on a silica gel column (2 × 22 cm)
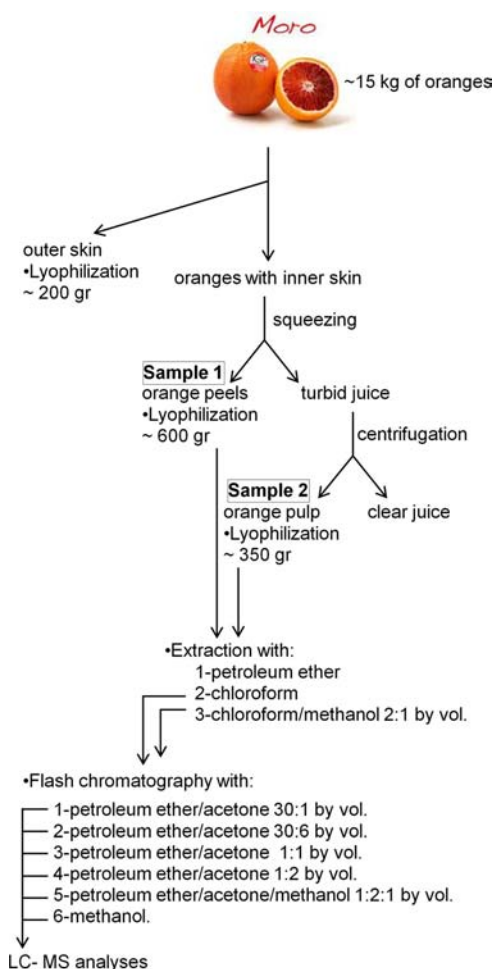
and subjected to flash chromatography using 100 mL each of the following solvents in series: (1) petroleum ether/acetone (30:1 by vol), (2) petroleum ether/acetone (30:6 by vol), (3) petroleum ether/acetone (1:1 by vol), (4) petroleum ether/acetone (1:2 by vol), (5) petroleum ether/acetone/methanol (1:2:1 by vol), and (6) methanol. Fractions 1−6 were subjected to analysis by HPLC−MS. All of the extracts were subjected to TLC using several solvent systems, and Cer was detected using Pancaldi spray reagent $[(NH_4)_6MoO_4, Ce(SO_4)_2, H_2SO_4, and H_2O]$.

MS analyses of ceramides[23,31] were performed using a Thermo-Quest Finnigan LCQ Deca ion-trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electrospray ionization (ESI) ion source, an Xcalibur data system, and a TSP P4000 quaternary pump. Separations were obtained on a LiChrospher 100 RP8 column (5 μm, 250 × 4 mm; Merck). Elution was carried out at a flow rate of 0.5 mL/min, with gradient formed by the solvent system A, consisting of methanol/water (90:10 by vol), and solvent system B, consisting of methanol, both containing 5 mM ammonium acetate. The gradient elution program was as follows: 5 min with solvent A, 5 min with a linear gradient from 100% solvent A to 100% solvent B, 15 min with 100% solvent B, and 5 min with a linear gradient from 100% solvent B to 100% methanol. Methanol was also used to wash the column for 10 min, followed by an equilibration procedure with solvent A for 15 min. The optimal conditions for MS analyses, achieved with an internal standard but also with different molecular species of Cer without substantial differences, were as follows: sheath gas flow, 50 arbitrary units; spray voltage, 4 kV; capillary voltage, −47 V; capillary temperature, 260 °C; and fragmentation voltage (used for collision-induced dissociation), 40−60%. For all experiments, the source ion optics were adjusted to achieve desolvation of ions while

Table 1. Cer Species of Moro Blood Oranges (*C. sinensis*)[a]

LCB	FA C_n:1	FA C_n:0	α-OH FA C_n:2	α-OH FA C_n:1	α-OH FA C_n:0	α,β-OH FA C_n:1	α,β-OH FA C_n:0
	FA		α-OH FA			α,β-OH FA	
Cer Species of the Pulp of *C. sinensis*						
$d18:1^{\Delta4/8}$					cis C_{23}–C_{27} tr	cis C_{23}–C_{26} tr	
$d18:0$				cis C_{23}–C_{27} tr	cis C_{22}–C_{27} tr	cis C_{23}–C_{26} tr	cis C_{22}–C_{28} tr
$t18:2^{\Delta4,8}$ (6-OH)[b]	$^{X}C_{21}$–C_{27} 3.0%			$^{X}C_{21}$–C_{27} 2.0%			
			cis C_{23}–C_{25} tr	cis C_{21}–C_{27} 1.5%			
$t18:1^{\Delta8}$ (4-OH)		cis C_{21}–C_{28} 11.5%			cis C_{21}–C_{28} 32.0%		cis C_{21}–C_{27} 7.0%
$t18:1^{\Delta4/8}$ (6-OH)[b]	$^{X}C_{21}$–C_{26} 1.0%		$^{X}C_{21}$–C_{27} tr	$^{X}C_{20}$–C_{27} 4.0%			
				cis C_{20}–C_{27} 2.0%			
$t18:0$ (4-OH)	$^{X}C_{22}$–C_{26} tr			$^{X}C_{22}$–C_{26} 1.5%		$^{X}C_{22}$–C_{26} 0.5%	
	cis C_{22}–C_{26} tr	cis C_{22}–C_{27} 3.5%			cis C_{22}–C_{28} 21.0%		cis C_{22}–C_{28} 9.0%
$t18:0$ (6-OH)[b]			$^{X}C_{20}$–C_{27} tr	$^{X}C_{20}$–C_{26} tr			
				cis C_{20}–C_{26} 0.5%			
Cer Species of the Peels of *C. sinensis*						
$d18:1^{\Delta4/8}$		cis C_{20}–C_{24} 9.0%					cis C_{22}–C_{26} tr
$d18:0$		cis C_{20}–C_{24} 7.0%				cis C_{23}–C_{26} tr	
$t18:1^{\Delta8}$ (4-OH)		cis C_{21}–C_{26} 16.0%			cis C_{22}–C_{26} 31.5%	cis C_{22}–C_{24} tr	cis C_{21}–C_{27} 2.0%
$t18:0$ (4-OH)	cis C_{24}–C_{26} tr	cis C_{21}–C_{27} 1.5%		cis C_{22}–C_{26} tr	cis C_{22}–C_{28} 29.0%	cis C_{24}–C_{26} tr	cis C_{22}–C_{28} 4.0%

[a]An abbreviated nomenclature for sphingoid bases was used according to Merrill et al.,[23] with slight modifications: $\Delta4,8$ was for the position of two double bonds in 4 and 8, and $\Delta4/8$ was for the position of the single double bond in 4 or 8. Results are expressed in "term of equivalents" of the internal standard, even if the MS response of ceramides is usually very dependent upon the structure. The table gives only a percentage of distribution and not real quantitative data. X = unknown Cer species. [b](6-OH) = supposed 6-phyto series but not characterized.

minimizing fragmentation. Mass spectra were acquired over a m/z 200−1000 range.

The uncommon $d18:1,17:0$ Cer[31] was used as an internal standard. A 50 μM stock solution of internal standard in 5 mM ammonium acetate in methanol was prepared quantitatively and stored at −20 °C. Serial dilutions were prepared from the stock solution and used for calibration curves. An internal standard was added to samples just before LC−MS analyses.

■ RESULTS AND DISCUSSION

Fresh oranges were subjected to fractionation by the scheme shown in Figure 2. A series of solvents for Cer extraction and Cer enrichment by column chromatography was determined in preliminary experiments.

Analyses were performed using 15 kg of fresh oranges. The outer skin was removed to prevent analytical interference from the aromatic oil. The oranges were squeezed, and the juice was centrifuged to separate the pulp. After lyophilization, 600 g of peels and 350 g of pulp were subjected to extraction and fractionation. Cer is the trivial name of the structure shown in Compound **1** and **3** in Figure 1 and corresponds chemically to several molecular species that differ in both the LCB and FA structures. Ceramides play an important role in establishment and maintenance of the water-retaining properties of fruit peels and human skin. Not surprisingly, these Cer species comprise a very heterogeneous group of structures differing in their polarity.

To simplify the MS analyses, we fractionated the Cer mixture with a series of chromatographic solvents at increasing polarity (Figure 2). Solvents 1 and 2 gave very small quantities of Cer, whereas solvents 3−6 gave Cer at increasing polarity. These last fractions contained about 300 mg of ceramides in total per kilogram of oranges (∼160 mg in the peels and ∼140 mg in the pulp). This quantity was obtained by the weight of the fractions subjected to LC/MS analyses, in terms of equivalents of the internal standard $d18:1,17:0$ Cer. Although this is not the more correct method to have exhaustive quantitative data, in the present study, we want to show the recovery amount of ceramides per kilogram of oranges and only qualitative distribution of Cer molecular species.

On the basis of the molecular mass, we detected a total of 114 Cer species containing non-hydroxylated, monohydroxylated, and dihydroxylated FAs. The FAs comprised saturated, mono-unsaturated, and diunsaturated chains ranging from 20 to 28 carbons. A minor quantity of unknown Cer species, with the same m/z of cis double-bond FA-containing Cer but different retention times, was found (Table 1). Cer species with LCB of the "d" series were present in trace quantity in pulp but comprised approximately 16% of total Cer in peels. In both pulp and peels, species with $t18:0$ and $t18:1^{\Delta8}$ LCB (belonging to the phyto-Cer series) were the major Cer components, comprising 33.5 and 50.5% of the total in pulp and 34.5 and 49.5% of the total in peels, respectively. In pulp, Cer species with phyto-LCB
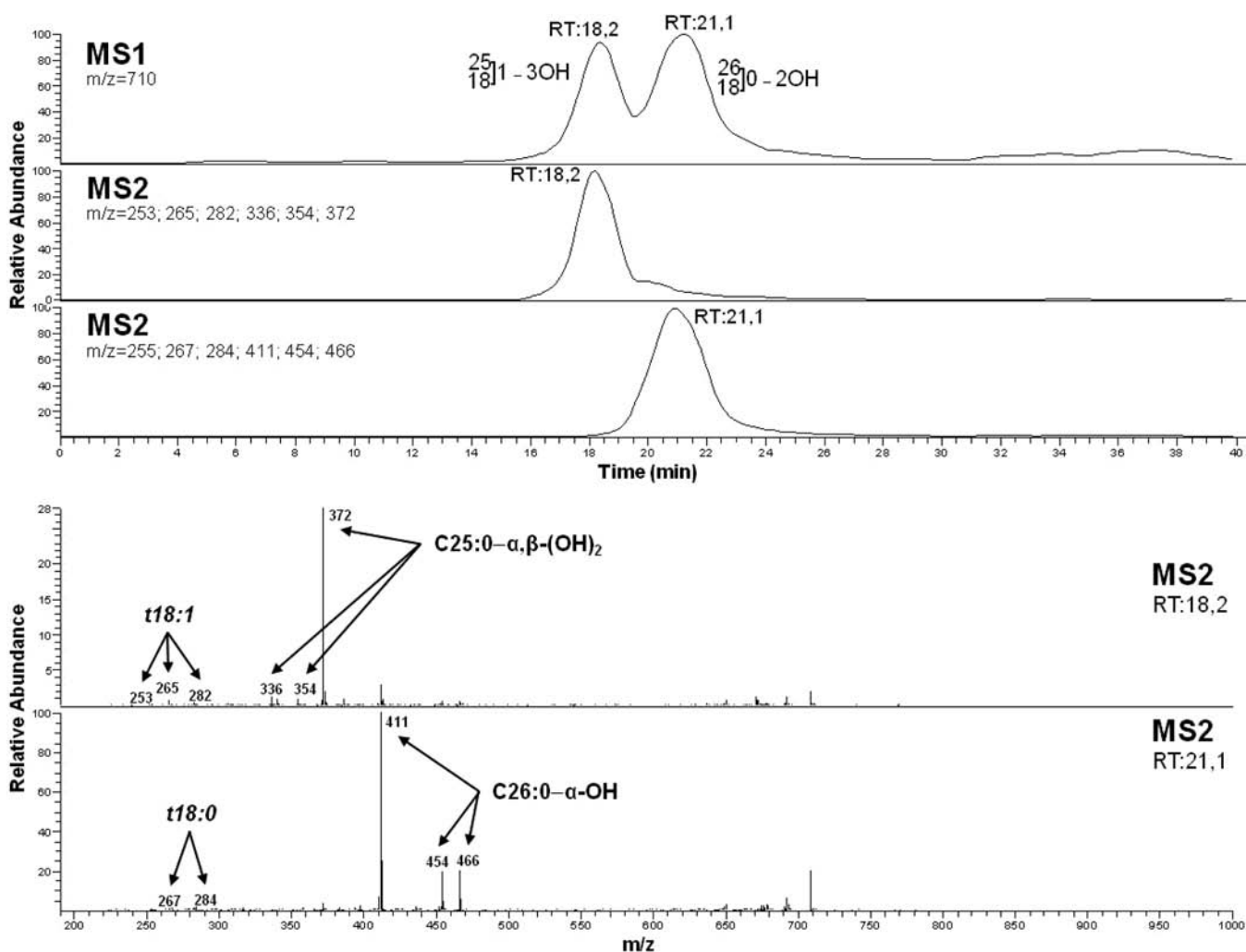
Figure 3. (Upper panel) HPLC/MS1 extracted ion chromatogram at m/z 710 corresponding to the $[M − H]^-$ ion and two HPLC/MS2 chromatograms corresponding to a series of ions allowing for characterization of $t18:1^{\Delta 8}/C25:0−\alpha,\beta$-$(OH)_2$ and $t18:0/C26:0−\alpha$-OH Cer species. (Lower panel) MS2 spectra associated with the chromatograms in the upper panel.

having OH in position 4 were predominant, but some species having OH in a different position were also detectable. The results suggest the compounds could contain phyto-LCB having OH in position 6 because of their chromatographic and mass spectrometric behavior, slightly different from the correspondent 4-phyto species (data not shown). However, further analyses are necessary to confirm our assumptions. Existence of 6-phyto ceramides in human skin have already been reported in the literature.[5,6,32−34] The Cer composition of orange pulp and peels is summarized in Table 1.

For complex sphingolipids (i.e., ceramides), an internal standard with uncommon FAs can be used.[28] Our quantitative data were referred to the synthetic internal standard of Cer containing $d18:1$ sphingosine and 17:0 FA. A linear correlation between a series of solutions of this compound and the ion peak intensities was obtained by multiple reaction monitoring (MRM) analysis. We had a correlation coefficient of 0.998 and a slope of 69.64 for the internal standard in a range from 1 to 100 pmol.[31] Obviously, it is desirable to have separate internal standard compounds with and without double bonds in the sphingoid base backbone (or with a hydroxy-sphingoid base backbone if this category is being analyzed), but for a general survey, its properties were reasonably similar to those of the ceramides of Moro blood orange. Therefore, quantization was

achieved by direct correlation of the peak areas generated by the internal standard with those of the found species.

The mass spectrometer was programmed to monitor specific, individually optimized precursor and product ions in specific time frames. The signal generated by each ion transition uniquely identified molecular species by retention time, MS1 and MS2 spectra. In several cases, the same precursor ion was associated with two peaks having different HPLC retention times. Under our HPLC conditions, molecular species of ceramides did not separate completely, but a complete baseline separation is not necessary because of the specificity of the mass spectrometric detection. In fact, a minimum separation should be achieved with respect to the reliable applicability of the method (MRM detection)[28] to complex mixtures. Still, we were able to obtain definitive information on the Cer structures from the MS analyses. As an example, Figure 3 illustrates the characterization of two Cer species that were separated by HPLC (retention times of 18.2 and 21.1 min) and displayed the same $[M − H]^-$ ion at m/z 710. MS2 suggests that the two species could be $t18:1^{\Delta 8}/C25:0−\alpha,\beta$-$(OH)_2$, whose existence had already been reported in a previous paper,[10] and $t18:0/C26:0−\alpha$-OH. Figures 4 and 5 show the fragmentation patterns for the two species as deduced by the ions present in the mass spectra. The species containing α-hydroxylated FAs gave MS2 fragment ions at m/z 411, 454,
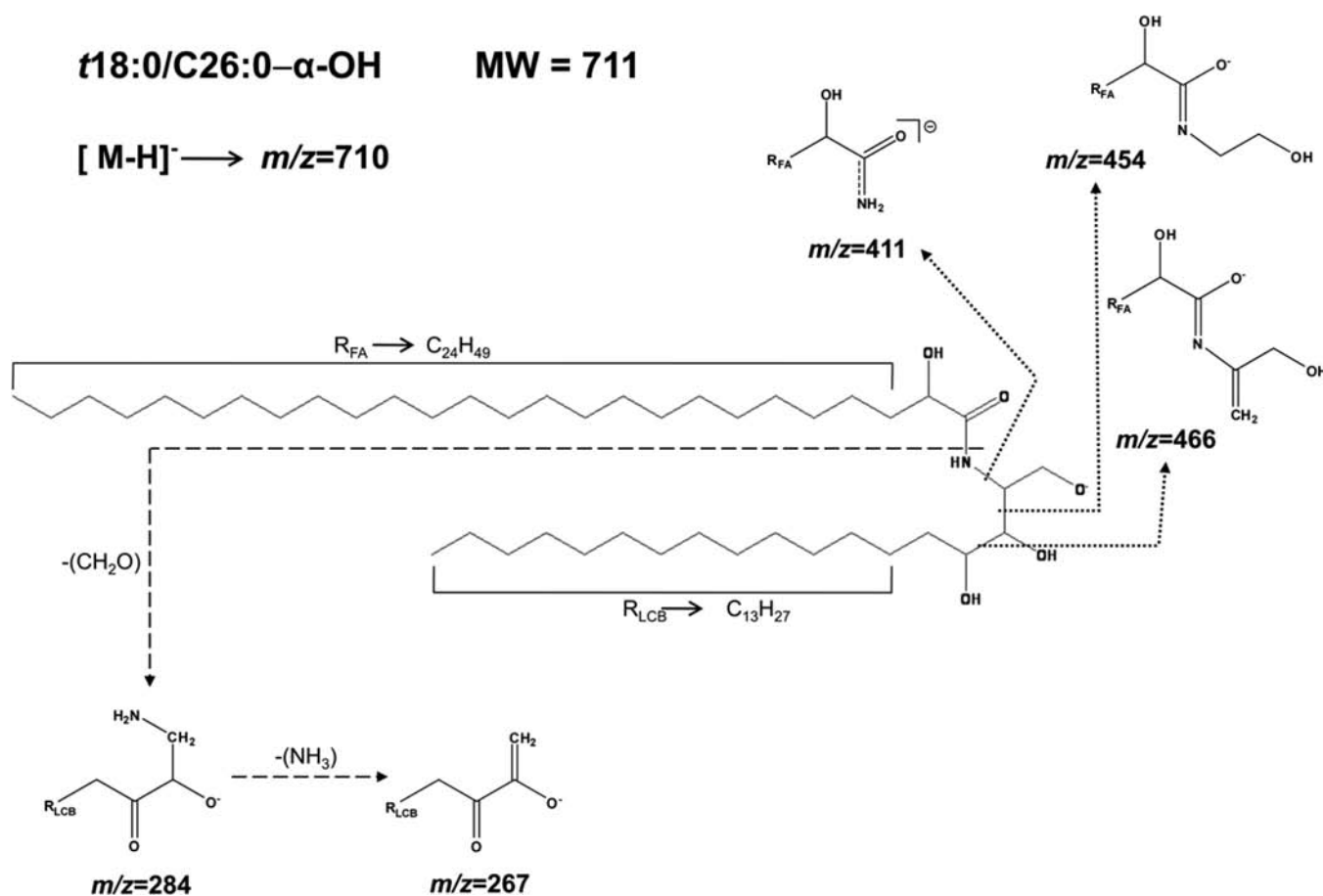
Figure 4. Proposed MS fragmentation scheme for $t18:0/C26:0-\alpha$-OH Cer species. The figure shows only one possible resonance structure of each fragment ion.

and 466 that corresponded to the total acyl chain associated with portions of the LCB. In agreement with previous results,[29,32,35] these ions made possible the identification of the FA structure. In contrast, the ions at m/z 284 and 267 allowed us to characterize the phyto-LCB structure, as previously reported.[29,35]

Dihydroxy-FA-containing Cer fragmentation was different from that of other Cer species; mass spectra of mono- or non-hydroxy-FA-containing ceramides showed FA- and LCB-related ions. Indeed, in this case, we could find only LCB-related ions also containing a portion of the FA chain. MS2 fragmentation of Cer containing α,β-$(OH)_2$ FA probably occurs between the two FA hydroxyl groups, and the sequence of fragment ions at m/z 372 → 354 → 336 therefore allowed us to identify the FA structure based on the differences from the precursor ions. The fragments at m/z 282, 265, and 253 provided structural information on the length and degree of unsaturation of LCB. Tables 2–4 show the "fingerprints" of the major Cer species of *C. sinensis*. All precursor and product ions reported in Tables 2–4 have been identified.

Cer is an ubiquitous component of mammalian cells and plays many biochemical and physiological roles. Lysosomal catabolism of very complex sphingolipids produces Cer and LCB; most of the LCB is recycled. Cer is also produced by catabolism mediated by sphingomyelinase and glycosphingolipid hydrolases at the plasma membrane surface, where it forms large platforms that help modulate the cell signaling that leads to apoptosis. Cer is also a natural component of all of the membranes of mammalian cells and is particularly abundant in the skin. Human skin

contains a heterogeneous group of Cer species characterized by very long acyl chains that are able to interdigitate across the membrane bilayer and that contain hydroxyl groups. Cer molecules play an important role in establishment and maintenance of the water-retaining properties of skin. Because of its unique properties, Cer has potential application as a nutraceutical molecule for the prevention of colonic tumors and is widely used in the cosmetic industry for the preparation of skin creams and hair shampoos.

According to this above, Cer attracted the interest of many scientists and opened a large discussion on its effective role as a natural and nutraceutical compound. Therefore, there is a large amount of literature for or against Cer and its roles.[10,17,36−39]

Recent studies have shown that citrus fruits contain Cer species that are structurally similar to those found in human skin. These fruits are therefore useful sources for the extraction and purification of various useful Cer species.

Oranges have been cultivated from many centuries in southern Italy. One of the major crop species is the Moro blood orange, *C. sinensis*. Much of the production of this species is used for the preparation of canned juice, leaving a great quantity of "waste" peels and pulp.

The present study showed that Cer is a major component of both peels and pulp from *C. sinensis* and comprises a very heterogeneous mixture of species that belong mostly to the phyto series and contain very long and hydroxylated FAs. Interestingly, many of them have structures also found in human skin Cer.
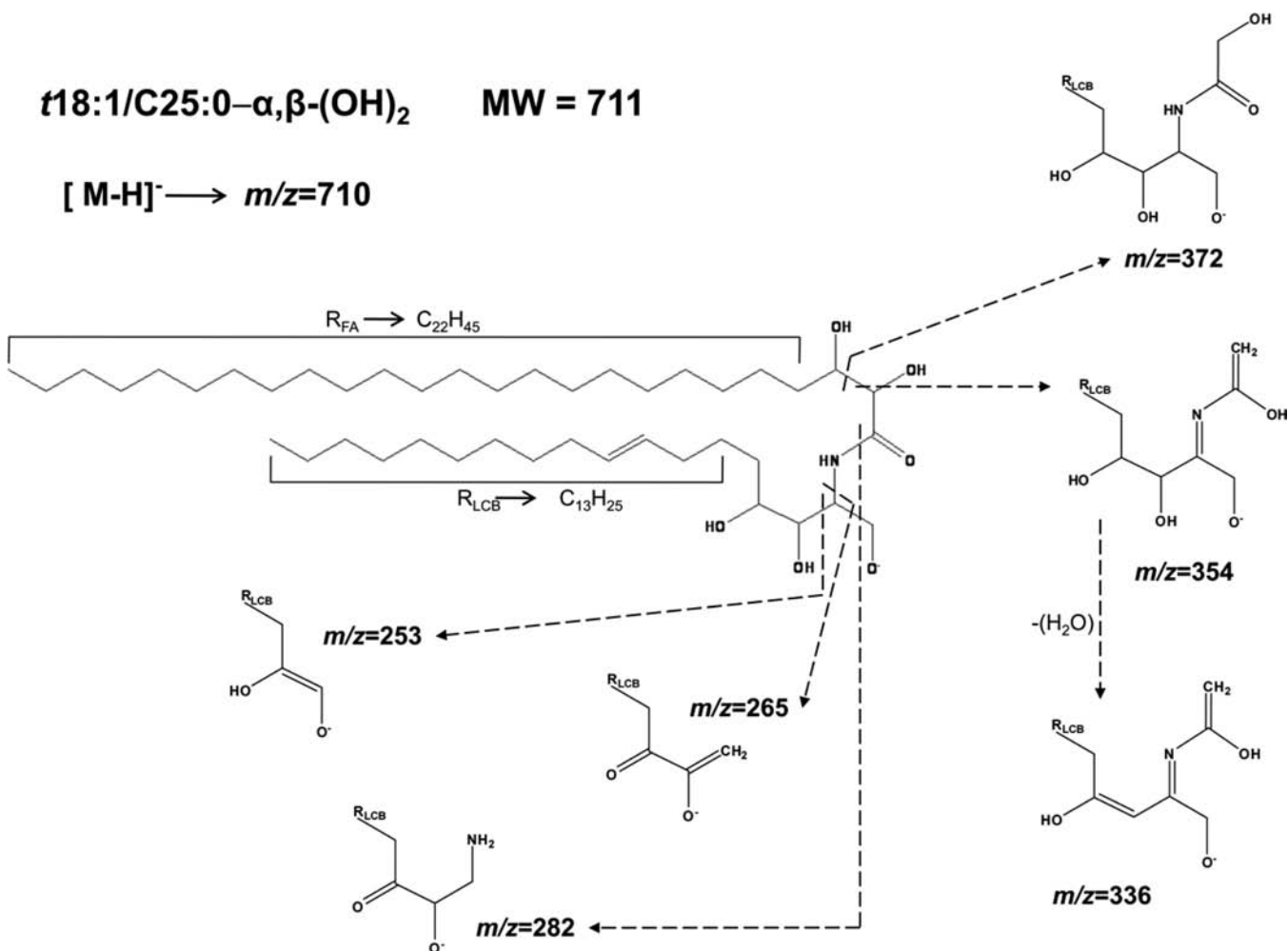
Figure 5. Proposed MS fragmentation scheme for $t18:1/C25:0-\alpha,\beta\text{-}(OH)_2$ Cer species. The figure shows only one possible resonance structure of each fragment ion.

Table 2. Summary of Precursor/Product Ion m/z Values for MRM Detection of Ceramides Containing 4-Phyto-LCBs and Non-hydroxy-FAs of Different Lengths

| structure of phyto-Cer | precursor and common ions of phyto-Cer | | | | | FA-related ions | | LCB-related ions | | |
	$[M + \text{acetate}]^-$	$[M - H]^-$	$[M - H]^- - (H_2O)$	$[M - H]^- - (2H_2O)$	$[M - H]^- - (CH_2O) - (H_2O)$	non-hydroxy		1	2	3
t18:1/C21:0	682	622	604	586	574	368	380	253	265	282
t18:0/C21:0	684	624	606	588	576	368	380	255	267	284
t18:1/C22:0	696	636	618	600	588	382	394	253	265	282
t18:0/C22:1	696	636	618	600	588	380	392	255	267	284
t18:0/C22:0	698	638	620	602	590	382	394	255	267	284
t18:1/C23:0	710	650	632	614	602	396	408	253	265	282
t18:0/C23:1	710	650	632	614	602	394	406	255	267	284
t18:0/C23:0	712	652	634	616	604	396	408	255	267	284
t18:1/C24:0	724	664	646	628	616	410	422	253	265	282
t18:0/C24:1	724	664	646	628	616	408	420	255	267	284
t18:0/C24:0	726	666	648	630	618	410	422	255	267	284
t18:1/C25:0	738	678	660	642	630	424	436	253	265	282
t18:0/C25:1	738	678	660	642	630	422	434	255	267	284
t18:0/C25:0	740	680	662	644	632	424	436	255	267	284
t18:1/C26:0	752	692	674	656	644	438	450	253	265	282
t18:0/C26:1	752	692	674	656	644	436	448	255	267	284
t18:0/C26:0	754	694	676	658	646	438	450	255	267	284
t18:1/C27:0	766	706	688	670	658	452	464	253	265	282
t18:0/C27:0	768	708	690	672	660	452	464	255	267	284
t18:1/C28:0	780	720	702	684	672	466	478	253	265	282

Table 3. Summary of Precursor/Product Ion *m/z* Values for MRM Detection of Ceramides Containing 4-Phyto-LCBs and *α*-Hydroxy-FAs of Different Lengths

structure of phyto-Cer	precursor and common ions of phyto-Cer					FA-related ions			LCB-related ions		
	[M + acetate]⁻	[M − H]⁻	[M − H]⁻ − (H₂O)	[M − H]⁻ − (2H₂O)	[M − H]⁻ − (CH₂O) − (H₂O)	*α*-hydroxy			1	2	3
t18:1/C21:0	698	638	620	602	590	341	384	396	253	265	282
t18:1/C22:0	712	652	634	616	604	355	398	410	253	265	282
t18:0/C22:1	712	652	634	616	604	353	396	408	255	267	284
t18:0/C22:0	714	654	636	618	606	355	398	410	255	267	284
t18:1/C23:0	726	666	648	630	618	369	412	424	253	265	282
t18:0/C23:1	726	666	648	630	618	367	410	422	255	267	284
t18:0/C23:0	728	668	650	632	620	369	412	424	255	267	284
t18:1/C24:0	740	680	662	644	632	383	426	438	253	265	282
t18:0/C24:1	740	680	662	644	632	381	424	436	255	267	284
t18:0/C24:0	742	682	664	646	634	383	426	438	255	267	284
t18:1/C25:0	754	694	676	658	646	397	440	452	253	265	282
t18:0/C25:1	754	694	676	658	646	395	438	450	255	267	284
t18:0/C25:0	756	696	678	660	648	397	440	452	255	267	284
t18:1/C26:0	768	708	690	672	660	411	454	466	253	265	282
t18:0/C26:1	768	708	690	672	660	409	452	464	255	267	284
t18:0/C26:0	770	710	692	674	662	411	454	466	255	267	284
t18:1/C27:0	782	722	704	686	674	425	468	480	253	265	282
t18:0/C27:0	784	724	706	688	676	425	468	480	255	267	284
t18:1/C28:0	796	736	718	700	688	439	482	494	253	265	282
t18:0/C28:0	798	738	720	702	690	439	482	494	255	267	284

Table 4. Summary of Precursor/Product Ion *m/z* Values for MRM Detection of Ceramides Containing 4-Phyto-LCBs and *α,β*-Dihydroxy-FAs of Different Lengths

structure of phyto-Cer	precursor ion of phyto-CER	LCB-related ions					
	[M − H]⁻	in phyto-Cer with *α,β*-dihydroxy-FA					
t18:1/C21:0	654	336	354	372	253	265	282
t18:1/C22:1	666	336	354	372	253	265	282
t18:1/C22:0	668	336	354	372	253	265	282
t18:0/C22:0	670	338	356	374	255	267	284
t18:1/C23:1	680	336	354	372	253	265	282
t18:1/C23:0	682	336	354	372	253	265	282
t18:0/C23:0	684	338	356	374	255	267	284
t18:1/C24:1	694	336	354	372	253	265	282
t18:1/C24:0	696	336	354	372	253	265	282
t18:0/C24:1	696	338	356	374	255	267	284
t18:0/C24:0	698	338	356	374	255	267	284
t18:1/C25:0	710	336	354	372	253	265	282
t18:0/C25:1	710	338	356	374	255	267	284
t18:0/C25:0	712	338	356	374	255	267	284
t18:1/C26:0	724	336	354	372	253	265	282
t18:0/C26:1	724	338	356	374	255	267	284
t18:0/C26:0	726	338	356	374	255	267	284
t18:1/C27:0	738	336	354	372	253	265	282
t18:0/C27:0	740	338	356	374	255	267	284
t18:0/C28:0	754	338	356	374	255	267	284

The structural characterization of Cer species requires advanced methodologies, particularly when dealing with a large, heterogeneous group. MS analysis in combination with simple HPLC separation allows for the identification of a very large group of species. We applied this procedure to analyze the Cer composition of the peels and pulp of *C. sinensis*. Remarkably, these oranges contained 114 Cer species that differed in both LCB and FA structures. The major species, accounting for 80−90% of the 114 found, were those belonging to the t18:0 and t18:1$^{\Delta 8}$ series. To the best of our knowledge, this is the first report on the Cer composition of oranges.

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Notes

The authors declare no competing financial interest.

■ **ABBREVIATIONS USED**

Cer, ceramide; FA, fatty acid; LCB, long-chain base; MS, mass spectrometry

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