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Molecular weight distribution of the triacylglycerols of berry seed oils analysed by negative-ion chemical ionization mass spectrometry

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Abstract Seed oils of 22 northern wild berry species collected in Finland were studied by ammonia negative-ion chemical ionization mass spectrometry using a direct exposure probe for sample introduction. The berries belonging to 13 genera (*Vaccinium*, *Oxycoccus*, *Arctostaphylos*, *Empetrum*, *Hippophaë*, *Chamaepericlymenum*, *Sambucus*, *Rosa*, *Fragaria*, *Rubus*, *Sorbus*, *Prunus* and *Ribes*) represented the most important Scandinavian edible berry species. The mass spectrometric analyses provided information about the different molecular weight species of triacylglycerols and their proportions in the seed oils studied. Triacylglycerols with 54 acyl carbons were the most abundant components in all berry oils (72–97 mol%). The proportions of triacylglycerols with 52 and 56 acyl carbons were only 2–23 mol% and 0–6 mol%, respectively. The results were in good agreement with the fatty acid compositions of the berry seed oils reported elsewhere. The species-specific molecular weight patterns varied from the random fatty acid distribution most clearly in the seed oils of *P. padus* ($P = 0.03$) and *Rubus chamaemorus* ($P = 0.02$). Negative-ion chemical ionization mass spectrometry is a useful tool for the screening of triacylglycerol profiles, as shown in the present study. Measurement of the oil or fat authenticity or of the mixing ratios of different oils is one potential application of the fast analytical technique described.

Key words Beery seed oils · Triacylglycerols · Ammonia negative-ion chemical ionization mass spectrometry

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Introduction

Palmitic, stearic, oleic, linoleic and α-linolenic acids dominate the triacylglycerols of plants, as a consequence of the nature of the fatty acid synthesis and modification reactions, as reviewed by Gurr [1] and Murphy [2, 3], for example. In addition, some oil seeds synthesize unusual, often species- or genus-specific, fatty acids, which occur almost exclusively in the triacylglycerol fraction. The molecular association of fatty acids in triacylglycerols is primarily dependent on the relative amounts of different fatty acids available in the cytoplasmic membrane fraction, presumably the endoplasmic reticulum of the oleogenic plant tissues during triacylglycerol synthesis. The non-random distribution of fatty acid moieties between the *sn*-1, *sn*-2 and *sn*-3 positions of the glycerol backbone is affected by the specificity of the synthesizing enzymes.

In addition to the fatty acid composition, information concerning the molecular structures of triacylglycerols is important from the nutritional and technological points of view. Different chromatographic methods, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and thin-layer chromatography (TLC), have been applied to the analytical separation of triacylglycerols. These methods are fairly effective for the separation of complex, natural mixtures of triacylglycerols; however, the identification of the compounds is often a problem. Mass spectrometric methods provide both structural and quantitative information about triacylglycerols. Chemical ionization (CI) mass spectrometry is the technique mostly used for the analyses of triacylglycerols, due to the formation of abundant molecular or quasi-molecular ions [4–8]. Recently, the formation of abundant $[M–H]^-$ ions of triacylglycerols by ammonia negative-ion CI mass spectrometry (NICI-MS) has been reported [9, 10]. This is a fast and sensitive

method for the determination of the molecular weight distribution of triacylglycerols and it has been applied to the analysis of, for example, seed oils [9, 11–13], human milk [14, 15] and bovine milk [16, 17] fat.

Only a few studies of the triacylglycerols of berry seed oils have been reported. The triacylglycerol composition of *Ribes nigrum* seed oil has been determined by reversed-phase HPLC [7, 18, 19] and by capillary GC and desorption mass spectrometry in positive-ion chemical ionization mode [7]. SFC has been applied to the characterization of triacylglycerols of *Rubus chamaemorus*, *Hippophaë rhamnoides*, *Ribes nigrum* and *Ri. alpinum* seed oils [11, 12].

According to the fatty acid composition, it is possible to calculate the theoretical random distribution of triacylglycerols, in which each stereochemical position of a triacylglycerol molecule has an equal probability. This study was aimed at determining the molecular weight distribution of triacylglycerols of various edible northern berry seed oils by mass spectrometry using ammonia negative-ion chemical ionization. The mass spectrometric results were compared with the theoretical random triacylglycerol profiles to indicate the possible selectivity of the molecular association of fatty acids during the biosynthesis of plant triacylglycerols.

Materials and methods

Seed material. The northern edible berry species investigated were: *Vaccinium vitis-idaea*, *V. myrtillus*, *V. uliginosum*, *Oxycoccus quadripetalus*, *Arctostaphylos uva-ursi*, *A. alpina*, *Empetrum nigrum*, *E. hermaphroditum*, *Hippophaë rhamnoides*, *Chamaepericlymenum suecicum*, *Sambucus racemosa*, *Rosa dumalis*, *Fragaria vesca*, *Rubus idaeus*, *R. saxatilis*, *R. arcticus*, *R. chamaemorus*, *Sorbus aucuparia*, *Prunus padus*, *Ribes nigrum*, *Ri. spicatum* and *Ri. alpinum*. The berry samples were collected in the years 1992 and 1993 in Finland and processed further as described elsewhere [20].

Samples preparation. The lipids were extracted with a mixture of chloroform and methanol and the fatty acids analysed as methyl esters by GC as reported elsewhere [20]. The triacylglycerols were purified by elution (hexane:diethylether, 4:1 v:v) from a short Florisil column and dissolved in hexane (approximately 1 mg/ml) for mass spectrometric analyses.

Mass spectrometric determinations. Triacylglycerols were chemically ionized with ammonia ($\geq$ 99.998%, Prax Air, Oevel, Belgium) in negative-ion mode according to the method introduced by Kallio and Currie [9] and by Kallio and Rua [15]. All experiments were performed with a Finnigan MAT TSQ-700 triple quadrupole mass spectrometer (Finnigan Mat, San Jose, Calif., USA) equipped with a combined EI/CI ion source. The pressure of ammonia was approximately 1.13 kPa (8500 mtorr) and the ion source temperature 200°C. The electron energy was 70 eV and the filament current 400 μA. An aliquot of 0.5 μl of purified triacylglycerols dissolved in hexane was applied to the rhenium wire of the direct exposure probe. After the evaporation of the solvent, the probe was introduced into the ion source, and the heating of the rhenium wire with current (4 mA/s) was started in order to vaporize the sample. Negatively charged ions with m/z values from 700 to 1100 were scanned using a scan time of 0.5 s. Each sample was analysed four times. The spectra were averaged and displayed.

Theoretical triacylglycerol profiles. The random distribution of triacylglycerols was calculated according to the fatty acid composition, the stereochemical distribution of the fatty acids in a triacylglycerol molecule being ignored. The proportions of triacylglycerols with the same number of acyl carbons (ACN; acyl carbon number) and double bonds (DB) were combined. The 30 most abundant ACN:DB species, comprising 97.4–100.0 mol% of the total calculated triacylglycerols, were taken into account in the comparisons of results. Each of the ACN:DB species left out of the calculations had a relative proportion of less than 0.1 mol%.

Statistical analysis. The differences in the triacylglycerol profiles determined by mass spectrometry and theoretical calculations were tested by the Pearson Chi-square statistical test. For the statistical analyses, the proportions of triacylglycerols with the same ACN were combined and each ACN group rather than the individual molecular weight species was compared.

Results and discussion

Molecular weight distribution of the triacylglycerols analysed by ammonia NICI-MS

CI with ammonia resulted in the formation of deprotonated triacylglycerol ions ($[M–H]^-$). There were no other ions interfering at this molecular weight region of the mass spectra. According to the $[M–H]^-$ ions, the molecular weight distribution of triacylglycerols was determined. The m/z values of the $[M–H]^-$ ions define the combined number of acyl carbons and double bonds in the acyl chains of triacylglycerols, whereas the abundances of the $[M–H]^-$ ions define the proportions of different molecular weight species. No detailed information about the fatty acid constituents or their combinations in triacylglycerols was obtained with this method. Seed oils typically consist of triacylglycerols with 50 to 56 acyl carbon atoms, therefore, the amount of ^{13}C has to be taken into account while calculating the proportions of triacylglycerols.

The molecular weight species of triacylglycerols having 54 acyl carbon atoms were most abundant in all the oils studied, comprising 72–97 mol% of the total triacylglycerols (Fig. 1–6). In addition, the seed oils contained 2–23 mol% triacylglycerols with 52 acyl carbons and 0–6 mol% molecules with 56 acyl carbons. These results are in good accordance with the fatty acid compositions reported elsewhere [20]. The proportion of C18 fatty acids was more than 91 mol% in all the oils studied, which explains well the high proportion of triacylglycerols with 54 acyl carbons. The combined number of DB in the major triacylglycerol species was from four to nine. Saturated triacylglycerols were found in *O. quadripetalus* (0.3 mol%) and *H. rhamnoides* (0.5 mol%) only, which is supported by the low proportion of saturated fatty acids in the berry seed oils.

In the following text, the triacylglycerol distributions within each genus or family are compared. In the genus *Ribes* the triacylglycerol profiles of different berry species resembled each other closely (Fig. 1). The

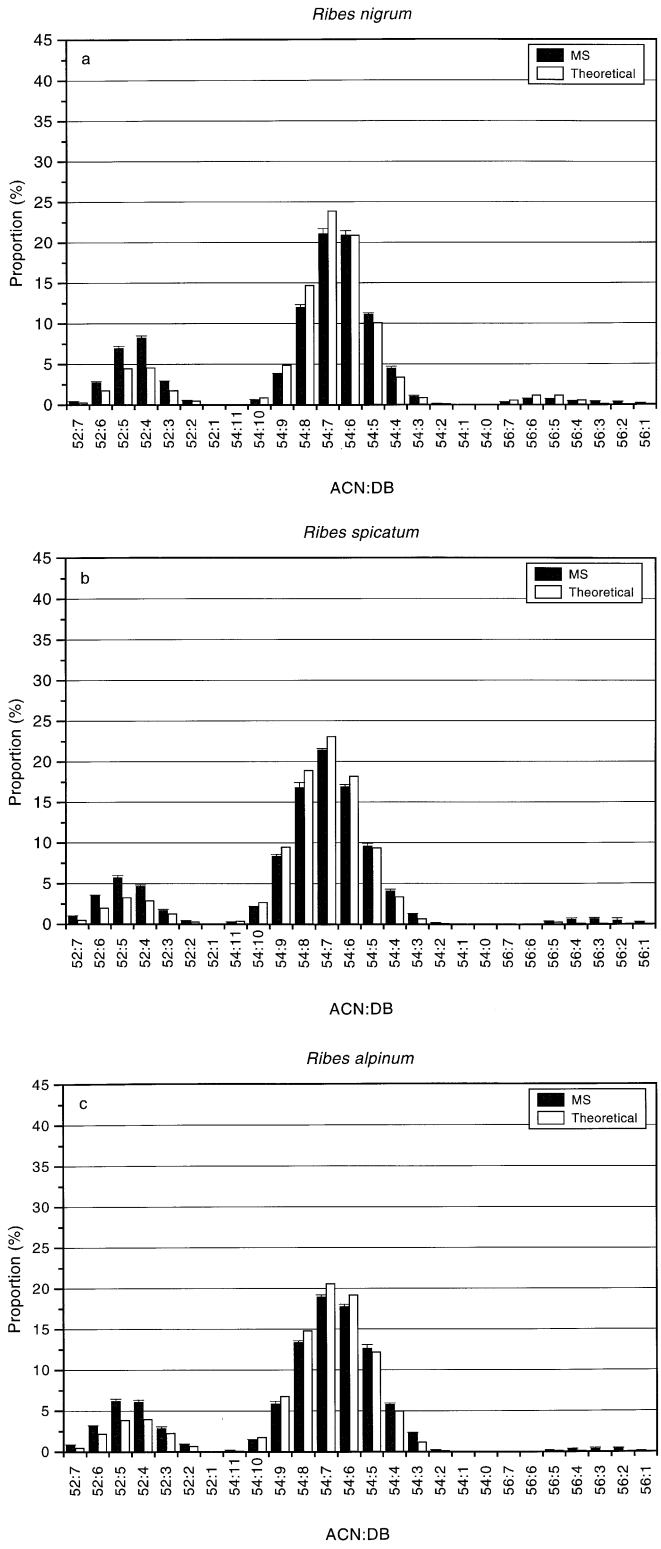

Fig. 1 Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Ribes nigrum*, **b** *Ri. spicatum* and **c** *Ri. alpinum*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

proportions of triacylglycerols with ACN 52, 54 and 56 were 17–22 mol%, 75–81 mol% and 2–3 mol%, respectively. The greatest differences between the *Ribes* species were determined within the ACN 54 triacylglycerols with 8–11 DB. The combined proportion of the triacylglycerols 54:8, 54:9, 54:10 and 54:11 in the *Ri. spicatum* seed oil was over 27 mol% of the total triacylglycerols, whereas the corresponding values of *Ri. nigrum* and *Ri. alpinum* were 16 mol% and 21 mol%, respectively. The fatty acid compositions of the seed oils of *Ribes* berries explain the differences. The seed oil of *Ri. spicatum* contained a total of 35 mol% fatty acids with three DB, i.e. α-linolenic [18:3($n-3$)] and γ-linolenic acids [18:3($n-6$)], thus exceeding the corresponding values of *Ri. nigrum* and *Ri. alpinum* by approximately 6 and 4% units, respectively [20]. Whether this is due to genetic properties or to the northern growth site (70°N Lat) of *Ri. spicatum* is worthy of further investigation. A typical feature of the triacylglycerol profiles of the genus *Ribes* berry seed oils is a relatively high proportion of ACN 52 triacylglycerols.

The triacylglycerol profiles within the pairs *R. saxatilis*/*R. arcticus* and *R. idaeus*/*R. chamaemorus* in the genus *Rubus* were highly similar (Fig. 2). The relative amounts of ACN 52, 54 and 56 triacylglycerols in the oils of *R. saxatilis* and *R. arcticus* were 4 mol%, 95–96 mol% and 0.2–1.6 mol%, respectively. The corresponding values of *R. idaeus* and *R. chamaemorus* were 7–8 mol%, 87–91 mol% and 2–6 mol%. The most abundant triacylglycerol species in *R. saxatilis* and *R. arcticus* was 54:6 with the proportions of 44 mol% and 39 mol%, respectively. The seed oils of *R. idaeus* and *R. chamaemorus* contained nearly equal amounts, 23–28 mol%, of the two most abundant molecular weight species of triacylglycerols, i.e. 54:7 and 54:6. The combined proportion of ACN 56 triacylglycerols in *R. chamaemorus* oil (6 mol%) was much higher than in all the other *Rubus* oils (0.2–1.9 mol%), which accords well with the fatty acid compositions reported elsewhere [20]. The amount of long-chain ($\geq$ C20) fatty acids was clearly higher in *R. chamaemorus* seed oil than in the oils of the other *Rubus* berries.

The triacylglycerol profiles of *S. aucuparia* (Fig. 3c) and *P. padus* (Fig. 3d) differed remarkably from those of the other berry species in the family Rosaceae (species in Figs. 2 and 3). The proportions of less unsaturated triacylglycerols were higher than in the other Rosaceae species, in particular *P. padus* contained triacylglycerol species 54:5 (33 mol%) and 54:4 (36 mol%) in abundance. *P. padus* was exceptional also because of the total absence of ACN 56 triacylglycerols in the profile. The triacylglycerol distributions of *R. dumalis* and *F. vesca* (Fig. 3a, b) resembled those of *R. idaeus* and *R. chamaemorus* (Fig. 2a, d). The results agree well with the fatty acid compositions of the oils of Rosaceae berries [20]. In contrast to the other Rosaceae species, the seed oils of *S. aucuparia* and *P. padus* did not contain significant amounts of α-linolenic acid,

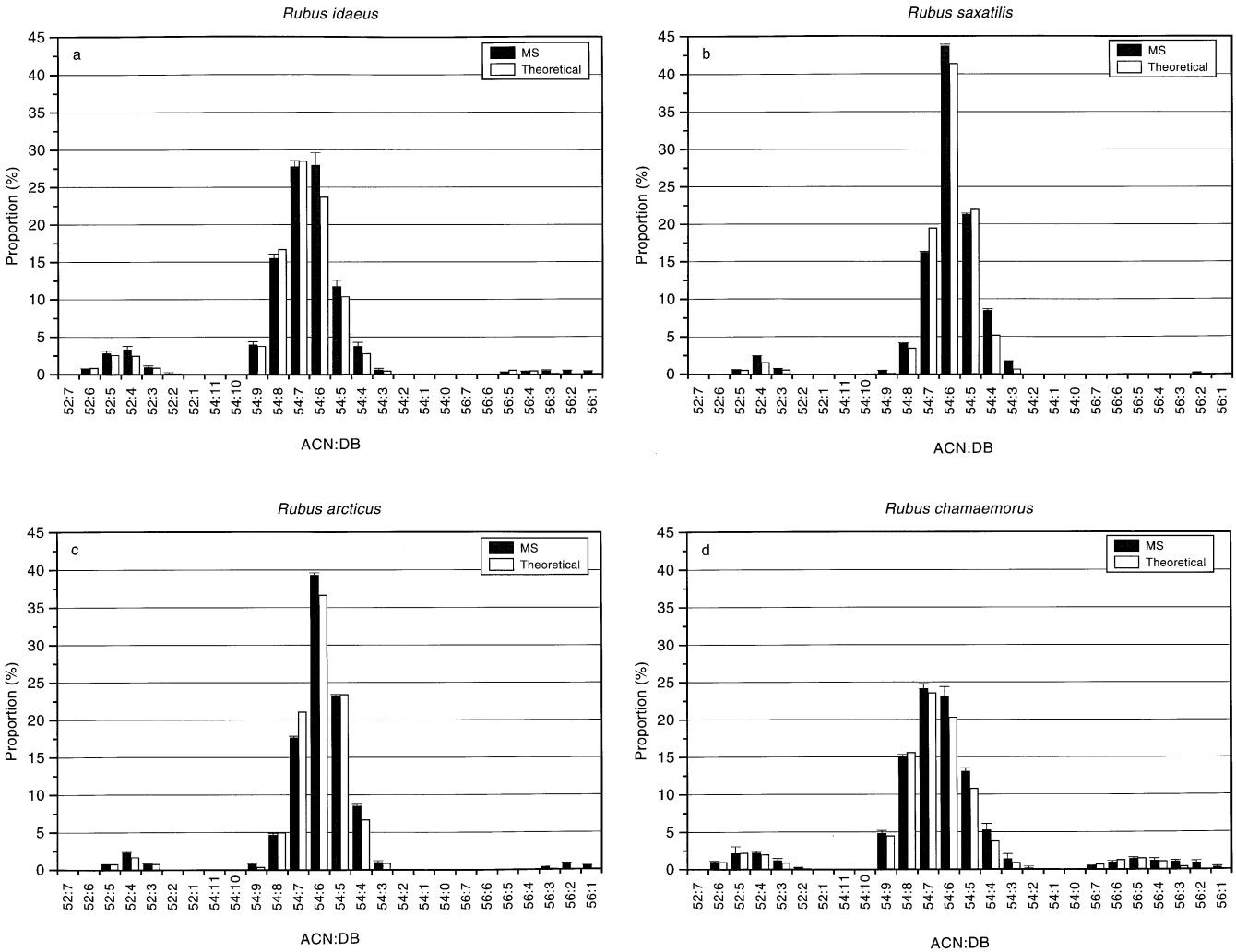

Fig. 2 Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Rubus idaeus*, **b** *R. saxatilis*, **c** *R. arcticus* and **d** *R. chamaemorus*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

which explains the low proportion of the ACN 54 triacylglycerols with more than six DB. Also, the proportion of fatty acids with 20 or more acyl carbons in *P. padus* was less than 0.5 mol%.

Generally, the triacylglycerol profiles of *Vaccinium* and *Oxycoccus* species were relatively similar (ACN 52, 3–16 mol%; ACN 54, 82–96 mol%; ACN 56, 1–2 mol%) (Fig. 4). The triacylglycerol compositions of *V. myrtillus* and *O. quadripetalus* were almost identical, which was expected according to the very similar fatty acid compositions of these species [20]. The abundance of 54:9, 54:8 and 54:7 species, making a total of 66 mol%, in the triacylglycerol profile of *V. vitis-idaea* results from the high amount of α-linolenic acid (50 mol%) in the seed oil [20]. *V. vitis-idaea* and *V. uliginosum* contained only about 1 mol% palmitic acid (16:0), which explains the low amount (3 mol%) of ACN 52 triacylglycerols.

Although the berry species of genera *Arctostaphylos* and *Empetrum* belong to different families (Ericaceae and Empetraceae, respectively), the triacylglycerol profiles (ACN 52, 2–8 mol%; ACN 54, 91–97 mol%; ACN 56, 1–2 mol%) of the seeds oils of these berries were highly similar (Fig. 5). Only *A. alpina* differed to some extent by containing much less ACN 52 triacylglycerols (2 mol%) compared with the other *Arctostaphylos* and *Empetrum* species (7–8 mol%).

C. suecicum (Fig. 6b) has a very special, narrow profile of triacylglycerols, which is quite similar to the profile of *S. aucuparia* (Fig. 3c). This is due to the very low proportion of triunsaturated fatty acids in the seed oils of *C. suecicum* (1.4 mol%) and *S. aucuparia* (0.8 mol%) [20]. *H. rhamnoides* contained the highest proportion of ACN 50 triacylglycerols (1.6 mol%) in the seed oil of all the berry species studied (Fig. 6a). In addition, *H. rhamnoides* was the only species that

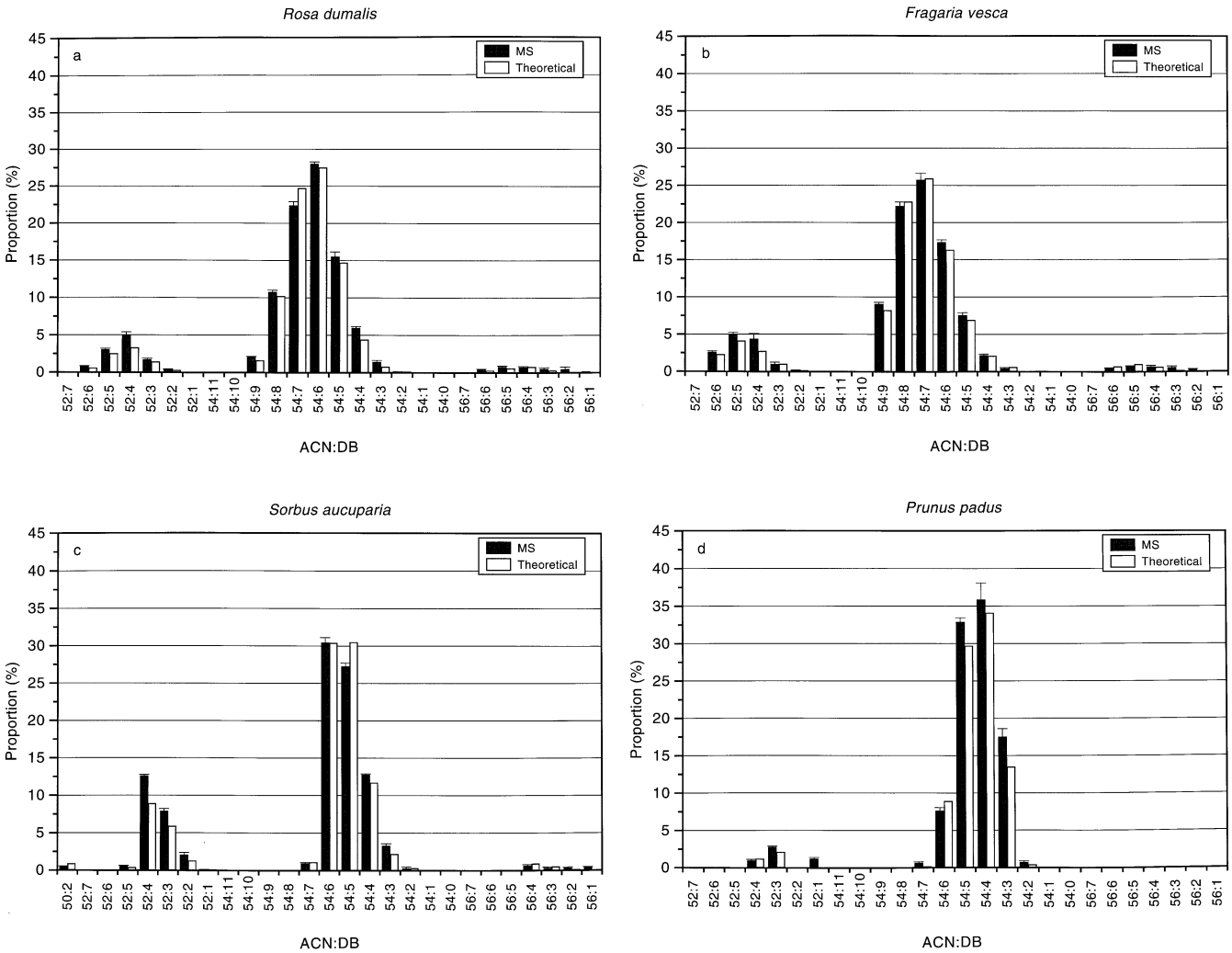

Fig. 3 Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Rosa dumalis*, **b** *Fragaria vesca*, **c** *Sorbus aucuparia* and **d** *Prunus padus*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

contained detectable amounts of triacylglycerols with 51 acyl carbon atoms.

Comparison of the triacylglycerol composition and the random distribution model of fatty acids in triacylglycerols

The Pearson Chi-square test found statistically significant differences between the mass spectrometric and theoretical triacylglycerol profiles of *P. padus* ($P = 0.03$) and *R. chamaemorus* ($P = 0.02$) only (Figs. 3d and 2d). The combined proportion of triacylglycerols with more than 56 or less than 52 acyl carbon atoms was approximately 9% in the theoretical profiles of the berries, whereas triacylglycerols outside the ACN range of 52–56 were not detected in the mass spectrometric determinations. Although triacylglycerols outside the ACN range of 52–56 were also found in the theoretical profiles of the other berry species, the differences between the mass spectrometric and the calculated profiles were not significant. Thus, certain fatty acid combinations are impossible, or at least very rare, in the natural seed oils of plants bearing evidence of the enzyme specificity during the oil biosynthesis.

Visual examination revealed that the triacylglycerol profiles of most berry seed oils differed by the greater abundance of ACN 52 triacylglycerols in the analysed profile than in that calculated (Figs. 1–6). It was also notable that the ACN 50 triacylglycerols were almost invariably absent from the analysed profiles, whereas a 0.1–1.4 mol% proportion of the ACN 50 triacylglycerols was achieved by theoretical calculation. This is a natural result of the rare combination of two palmitic acids in one triacylglycerol molecule of seed oils. The differences between the analysed and

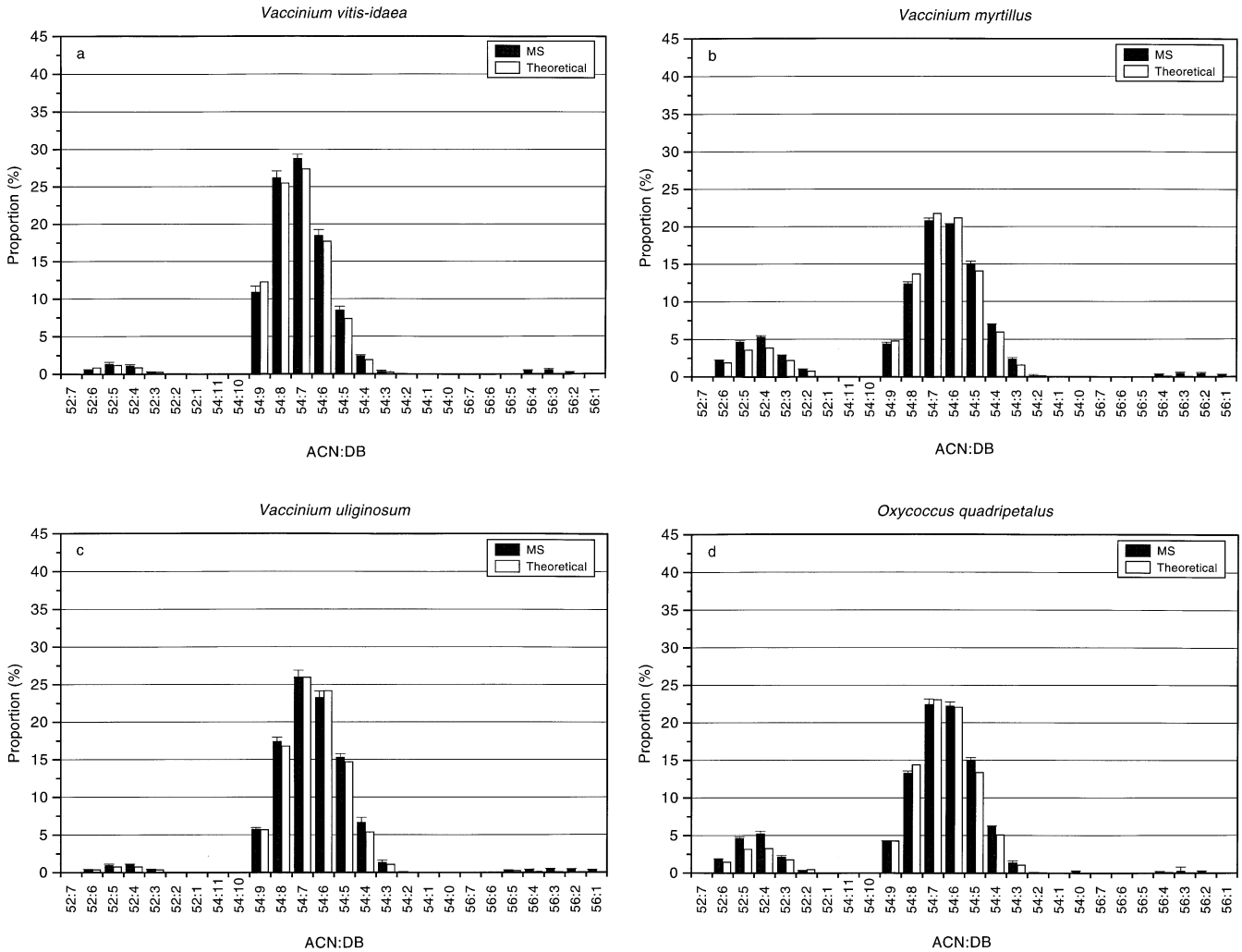

Fig. 4. Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Vaccinium vitis-idaea*, **b** *V. myrtillus*, **c** *V. uliginosum* and **d** *Oxycoccus quadripetalus*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

calculated theoretical triacylglycerol profiles may be explained partially by the molecular weight discrimination of triacylglycerols under the mass spectrometric conditions used [9, 14, 16, 17]. The analytical conditions can, however, be optimized to avoid discrimination according to the molecular weights of triacylglycerols [21]. In the *Ribes* berries the combined proportion of triacylglycerols with 52 acyl carbons was 48–65% higher than that obtained by random calculation (Fig. 1). The triacylglycerol compositions concerning the ACN 52 triacylglycerols were more alike in the other berry species: the analysed relative amount of ACN 52 triacylglycerols was, on average, 23% (0–49%) higher than that calculated (Figs. 2–6).

Differences in the distribution of triacylglycerols with ACN 54 were variable. The trend was clear in *Ribes*: the combined proportion of the ACN 54 triacylglycerols determined by mass spectrometry was 3–5% lower than that calculated. In *P. padus*, *R. chamaemorus*, *R. idaeus* and *A. alpina* the analysed proportion of ACN 54 triacylglycerols was higher. Other berries showed an almost equal proportion of ACN 54 triacylglycerols determined either by mass spectrometry or calculated according to the fatty acid composition. The combined proportion of analysed ACN 56 triacylglycerols in all berry species except *Ri. nigrum*, *F. vesca*, *S. aucuparia*, *R. saxatilis*, *A. uva-ursi* and *S. racemosa* was remarkably higher than that calculated.

A general phenomenon was observed when the individual molecular weight species within the ACN 54 cluster were examined; the proportions of the less unsaturated triacylglycerols, from 54:2 to 54:5, analysed by mass spectrometry were higher than those determined by theoretical calculations. In the case of more unsaturated triacylglycerols, the calculated

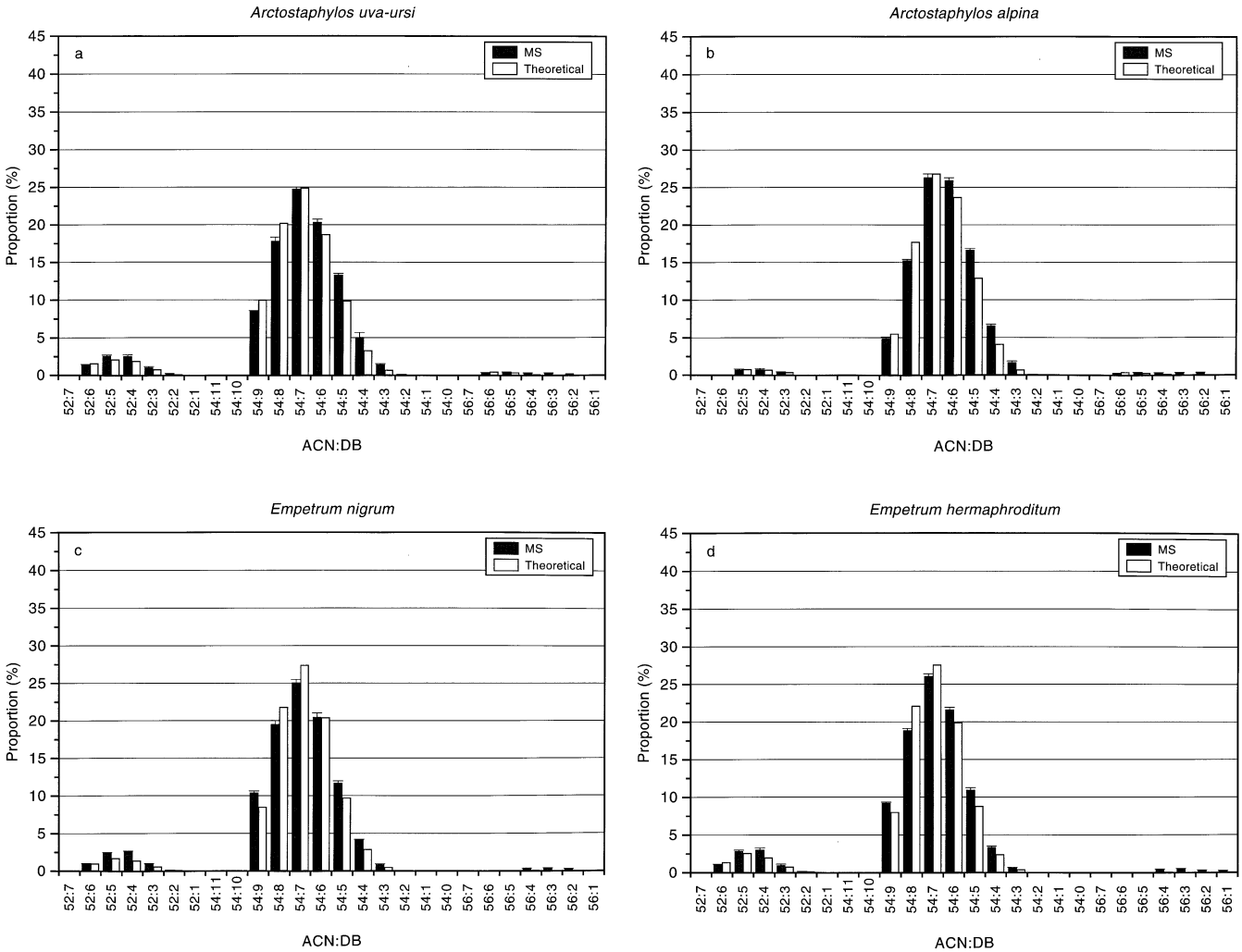

Fig. 5 Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Arctostaphylos uva-ursi*, **b** *A. alpina* **c**, *Empetrum nigrum* and **d** *E. hermaphroditum*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

proportions were higher. However, the abundance of the most unsaturated triacylglycerol, usually 54:9, is, in most berry species, nearly equal in both profiles or somewhat higher in the profile determined by mass spectrometry.

The combined proportions of ACN 52 and ACN 54 triacylglycerols of *Ri. nigrum* seed oil analysed by mass spectrometry in this study were 7–10% units higher and 10–12% units lower, respectively, than those determined by Rezanka and Mareš [7] and Perrin et al. [19]. The triacylglycerols with ACN 56 were detected only in the present study.

In addition to the natural variation of the seed oil composition, the different determination methods could explain the differences between the studies. The discrimination of triacylglycerols is often a problem in both chromatographic and mass spectrometric methods. In this study the mass spectrometric determinations of all berry seed oils were performed under constant analytical conditions with the same tuning of the instrument; therefore, the possible discrimination was of the same order of magnitude in all cases. The difference between the mass spectrometric and theoretical triacylglycerol profiles varied specifically with the species or the genus, and the most significant differences of the ACN 52 species were found in the *Ribes* berries (refer to Fig. 1). The similarity of the triacylglycerol profiles of *R. chamaemorus* seed oil determined by SFC coupled with a flame ionization detector and by ammonia NICI-MS also gives evidence for a very little or negligible discrimination [11]. Rezanka and Mareš [7] and Perrin et al. [19] characterized only about ten different ACN:DB species of *R. nigrum* seed oil, whereas in our study a total of 22 ACN:DB species were identified and quantified, which clearly demonstrates the resolving power and

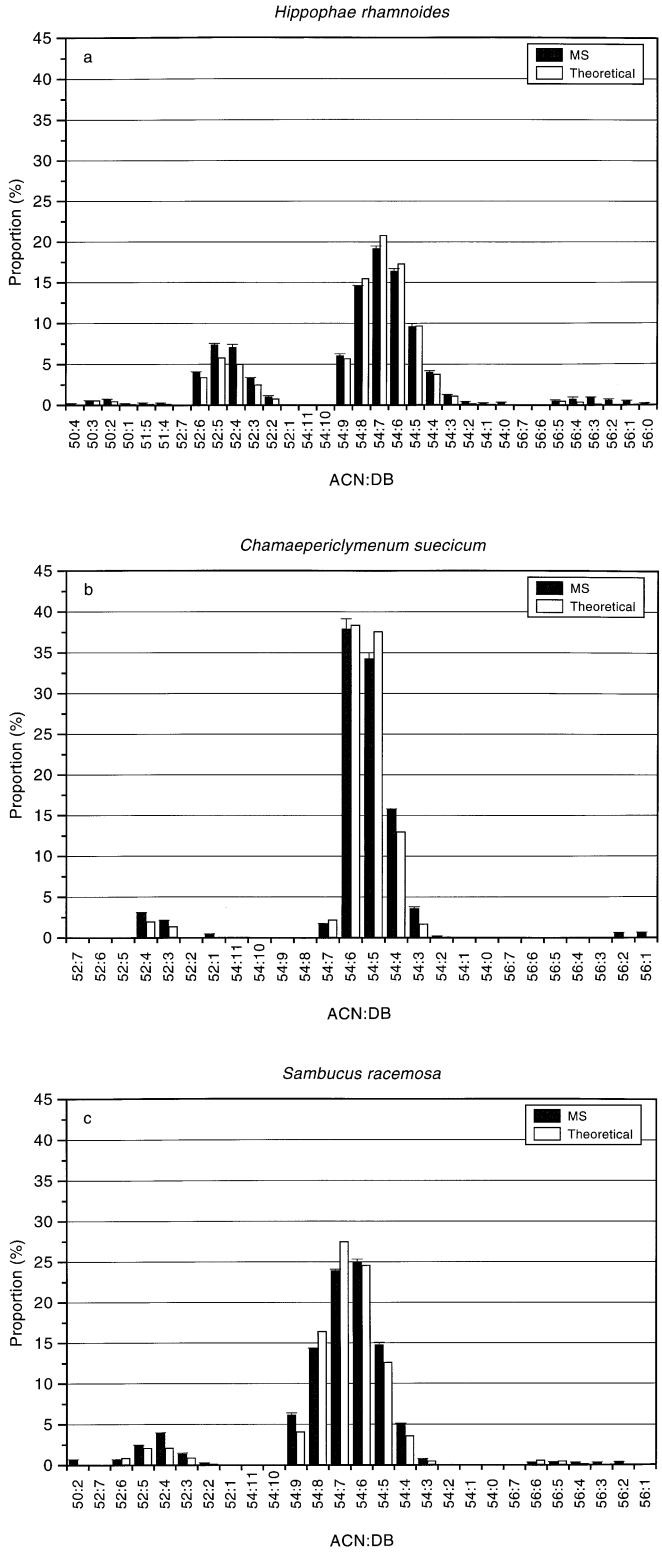

Fig. 6 Distribution of triacylglycerols according to the acyl carbon numbers (*ACN*) and number of double bonds (*DB*) in the seed oils of **a** *Hippophaë rhamnoides*, **b** *Chamaepericlymenum suecicum* and **c** *Sambucus racemosa*. The profiles were determined by ammonia negative-ion chemical ionization mass spectrometry (*MS*) and calculated according to the fatty acid composition of the oils (*Theoretical*). In MS profiles the relative standard deviations of the proportions of triacylglycerols in four analyses are also presented

sensitivity of the ammonia NICI-MS technique in the analysis of triacylglycerol mixtures. Perrin et al. [19] also noticed that the composition analysed by reversed-phase HPLC differs somewhat from the random distribution of triacylglycerols, which they believe to be a consequence of selectivity in oil biosynthesis.

In conclusion, this study shows the usefulness of NICI-MS for the screening of triacylglycerol profiles of various seeds oils. The method is fast and sensitive and allows therefore a large number of samples to be analysed in a relatively short time. This method would be a useful analytical tool for plant breeding experiments.

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