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Simultaneous determination of carbohydrates and simmondsins in jojoba seed meal (*Simmondsia chinensis*) by gas chromatography

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Abstract

Separate methods for the analyses of soluble carbohydrates in different plants and simmondsins in jojoba seed meal are described. A reliable gas chromatographic procedure for the simultaneous quantification of D-pinitol, *myo*-inositol, sucrose, 5-α-D-galactopyranosyl-D-pinitol, 2-α-D-galactopyranosyl-D-pinitol, simmondsin, 4-demethylsimmondsin, 5-demethylsimmondsin and 4,5-didemethylsimmondsin as trimethylsilyl derivatives in jojoba seed meal has been developed. The study of different extraction mixtures allowed for the quantitative recovery of the 9 analytes by a mixture of methanol–water (80:20, v/v) in the concentration range between 0.1 and 4%. Comparison of the separation parameters on three different capillary stationary phases with MS detection allowed for the choice of the optimal gas chromatographic conditions for baseline separation of the analytes.

Keywords: *Simmondsia chinensis*; Food analysis; Jojoba seed meal; Carbohydrates; Simmondsins

1. Introduction

The seeds of the jojoba plant (*Simmondsia chinensis*), an evergreen shrub native to the Sonoran desert, deliver a liquid wax commonly referred as jojoba oil. The oil is of great economical value with many applications especially in the cosmetic industry. The seed flour, remaining after winning the oil, is composed of about 30% protein, dietary fiber and carbohydrates and could possibly serve as an animal feed supplement. However, when used as an animal feed ingredient, the meal causes food intake reduction and growth retardation. The inhibitory effect on food intake is mainly due to the presence of simmondsin [1–3] and renders the meal unsuitable as a livestock feed. The meal contains about 5% simmondsin together with minor quantities of the simmondsin analogues 4-demethylsimmondsin, 5-demethylsimmondsin and 4,5-didemethylsimmondsin [4–6]. Different authors describe methods for the inactivation or elimination of simmondsins from

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jojoba meal. Detoxification procedures by chemical methods [7,8], microbiological methods [9–13] and solvent extraction methods [14–16] are described. Different methods can, however, result in a rather important loss of nutritious soluble carbohydrates present in jojoba meal [17]. For the evaluation of jojoba meal treatment procedures an accurate and quick method for the simultaneous quantitative determination of carbohydrates and simmondsins is necessary. Separate methods for the determination of simmondsins in jojoba seed meal [12,18–20] or carbohydrates in plant material [21,22] are described; however, no method for the simultaneous quantitative determination of both soluble carbohydrates and simmondsins in jojoba meal is yet available.

2. Experimental

2.1. Apparatus

2.1.1. Gas chromatography

A Chrompack 9000 gas chromatograph equipped with a flame ionisation detector was used for the analysis. Because of the complex mixture of soluble carbohydrates and simmondsins in the jojoba meal extracts, separations were studied by means of three different capillary columns. The separations were performed by fused-silica capillary columns with chemically bonded phases (Chrompack, Middelburg, The Netherlands) of, respectively, 100% dimethylpolysiloxane gum or CP-Sil 5 (25 m×0.25 mm diameter, 0.25-μm film), (14%) cyanopropylphenyl (86%) dimethylpolysiloxane gum or CP-Sil 19 (25 m×0.25 mm diameter and 0.25-μm film) and a (50%) phenyl (50%) dimethyl polysiloxane gum or CP-Sil 24 (40 m, 0.25 mm diameter, 0.2-μm film). Samples of 1 μl were injected by means of a split/splitless injector. To protect the column a special insert glass liner was used. Injector and detector temperatures were set at 300 °C. Detector gases were 45 ml/min of hydrogen and 300 ml/min of air. Helium was used as carrier gas at 25 cm/s (set at 60 °C). An identical temperature program was used for the three mentioned columns. Injections were made at 180 °C; this temperature was kept at 180° for 2 min and programmed to 280 °C at 10°/min and kept at 280 °C for 20 min. Data acquisition and processing were done with a Merck-Hitachi 2500 Chromato-Integrator.

2.1.2. Gas chromatography–mass spectrometry

Mass spectra were obtained with a Hewlett-Packard 890 Series II gas chromatograph equipped with a Hewlett-Packard 5971A mass-selective detector and with an electron impact ion source (electron energy 70 eV), a quadrupole mass filter and an electron multiplier detector allowing to register ions up to m/z 650. The already mentioned columns were also used for the GC–MS analysis; the columns were coupled with the ion source without interface. Extracts were introduced by splitless injection at 70 °C; the injector temperature was kept at 70 °C for 2 min and programmed to 100 °C at 35 °C/min and from 100 to 280 °C at 10 °C/min.

2.1.3. Extraction and concentration

A rotary mixer (Labin, Belgium) was used for the extraction of jojoba meal by means of extraction tubes. Extracts were concentrated on a SC110 Speed-Vac concentrator (Savant Instrument, Hicksville, NY, USA).

2.2. Reagents

Jojoba seed meal was obtained from EMEC Agro Industries (Antwerp, Belgium). Simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucopyranoside], 4-demethylsimmondsin [2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-D-glucopyranoside], 5-demethylsimmondsin [2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-D-glucopyranoside] and 4,5-didemethylsimmondsin [2-(cyanomethylene)-3,4,5-trihydroxy-cyclohexyl β-D-glucopyranoside], used as references (simmondsins) were isolated from jojoba meal [23–25].

The carbohydrates D-pinitol, sucrose, 5-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 5-α-D-galactopyranosyl-D-pinitol and 2-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 2-α-D-galactopyranosyl-D-pinitol, used as references, were also isolated from jojoba meal [17].

Phenyl β-glucopyranoside (internal standard), *myo*-inositol and methanol of capillary GC grade were obtained from Aldrich (Bornem, Belgium). Tri-Sil was obtained from Pierce (Antwerp, Belgium).

Blank jojoba seed meal was prepared from defatted jojoba meal by Soxhlet extraction with methanol during 48 h.

2.3. Standard preparation

Primary stock solutions of references of 4 mg/ml were prepared. The different products are quantitatively transferred in separate 10.0-ml flasks and dissolved in methanol. Stock solutions were kept in the refrigerator at 4 °C. Aliquots of the different standard stock solutions were combined in a 20-ml volumetric flask and diluted with methanol or concentrated to obtain calibration standards of 0.1; 0.25; 0.5; 1.0; 2.0 and 4.0 mg/ml.

An internal standard solution of 1 mg/ml in methanol was prepared and used for constructing the calibration graphs and the quantitative determinations.

2.4. Extraction of jojoba meal

Procedures for the extraction of carbohydrates from plant material have been described [21,22]. They include extraction with water with various concentrations of alcohols. Simmondsins can be quantitatively extracted from the meal with 95% methanol [19]. For that reason we examined methanol and methanol with increasing amounts of water as extraction solvent for the simultaneous determination of five soluble carbohydrates and four simmondsins, present in jojoba seed meal.

Measured samples (~100 mg) of jojoba meal were sonicated for 5 min in extraction tubes with 20 ml of extraction solvent of, respectively, 100% methanol and mixtures of methanol–water with the following compositions: 90:10, 80:20, 70:30, 60:40 and 50:50 (v/v). After sonicating for 5 min, the tubes were rotated on a rotatory mixer for 60 min and subsequently centrifuged at 2000 rpm for 5 min. Aliquots of 1 ml from the organic layers were brought in glass vials together with 1 ml of internal standard solution (1 mg/ml). The extracts were evaporated by means of the SpeedVac concentrator at 45 °C and the residues dissolved in 1.0 ml of Tri-Sil. The silylated mixtures were subsequently analysed by GC and GC–MS by in injecting 1-μl aliquots using the conditions described in Section 2.1.

2.5. Silylation procedure

For the optimalization of the silylation procedure 1 ml of Tri-Sil was added to a residue of 0.5 mg quantities of D-pinitol, *myo*-inositol, sucrose, simmondsin, 5-α-D-galactopyranosyl-D-pinitol and 2-α-D-galactopyranosyl-D-pinitol, simmondsin, 4- and 5-demethylsimmondsin, didemethylsimmondsin, phenyl β-glucopyranoside along with 0.5 mg of C_{18} as an internal standard. Aliquots of about 1 μl were injected after 15, 30, 60, 120 and 240 min reaction at room temperature and after 15, 30 and 60 min at 50 °C.

2.6. Calibration

Linearity was verified for simmondsin, 5-demethylsimmondsin, didemethylsimmondsin, D-pinitol, *myo*-inositol, sucrose and 5-α-D-galactopyranosyl-D-pinitol. For this reason 0.1, 0.25, 0.5, 1, 2 and 4 mg of the mentioned compounds were added to blank jojoba meal samples (100 mg) and extracted by means of 20 ml of a methanol–water mixture. The calibration graphs were constructed with the extraction mixture, which was shown previously to deliver the highest yields for the extraction of the different components. The extraction solvent was separated from the meal by centrifugation and 1-ml aliquots of the supernatant liquids were transferred to reaction vials containing 1 mg of internal standard. After evaporation of the solvent by means of SpeedVac concentrator at 45 °C, the samples were silylated according to the optimised silylation procedure. Separate calibration curves were constructed for seven different analytes by plotting the peak area ratios of standards to internal standard against weights of the individual substances. The calibration graph for 5-demethylsimmondsin was applied for the determination of 4-demethylsimmondsin and the

calibration graph for 5-α-D-galactopyranosyl-D-pinitol was also applied for the determination of the 2-α-D-galactopyranosyl-D-pinitol isomer.

2.7. Recovery

The recoveries of D-pinitol, *myo*-inositol, sucrose, 5-α-D-galactopyranosyl-D-pinitol, simmondsin, 5-demethylsimmondsin and 4,5-didemethylsimmondsin from jojoba seed meal were measured by spiking blank jojoba meal with 20 mg g^{-1} and extracting the samples with an extraction mixture consisting of 80% of methanol and 20% of water. The spiked samples were analysed in the same way as already described. The obtained ratios between standards and internal standard for the spiked samples were compared to the same ratios obtained by adding the internal standard to the described standards without extraction.

$$\text{Recovery (\%)} = [(\text{measured mg/g})/(\text{added mg/g})] \times 100$$

3. Results and discussion

3.1. Silylation

Silylation was complete for the individual analytes and the internal standard after 60 min reaction at room temperature or after 15 min reaction at 50 °C, as could easily be seen from the ratios between peak areas of standards and internal standard to the peak area of C_{18}.

3.2. Selection of the analytical column

The purpose of this work was the optimization of the separation of the nine analytes and the internal standard by capillary gas chromatography. The retention times of the soluble carbohydrates and simmondsins obtained with the three different columns are represented in Table 1. From this table can be seen that the CP-Sil 5 column resulted in incomplete separation of several of the mentioned compounds: 5-demethylsimmondsin is not separated from 5-α-D-galactopyranosyl-D-pinitol, 4,5-didemethylsimmondsin is not separated from 2-α-D-galactopyranosyl-D-pinitol and 4-demethylsimmondsin is incompletely separated from 4,5-didemethylsimmondsin. The CP-Sil 19 column shows no separation between the different simmondsin; simmondsin, 4- and 5-demethylsimmondsin and 4,5-didemethylsimmondsin elute as one single peak. The soluble carbohydrates, on the other hand, are very good separated by the present stationary phase and do not interfere with the simmondsins. If the separation of the different simmondsins is not important, adaptation of the temperature program allows the quantitation of soluble carbohydrates and the sum of simmondsins in jojoba meal by this column in less than 15 min. Baseline separation of the carbohydrates (D-pinitol, *myo*-inositol, sucrose, 5-α-D-galactopyranosyl-D-pinitol and 2-α-D-galactopyranosyl-D-pinitol) and the simmondsins (simmondsin, 4- and 5-demethylsim-

Table 1
Retention times from jojoba constituents and internal standard as trimethylsilyl derivatives on three capillary columns

Compound name	Compound no.	Retention time (min)		
		CP-Sil 5	CP-Sil 19	CP-Sil 24
D-Pinitol	1	6.90	7.00	14.07
myo-Inositol	2	9.61	9.88	17.61
Phenyl β-glucopyranoside	I.S.	11.44	15.03	23.05
Sucrose	3	16.10	19.19	24.58
Simmondsin	9	18.16	24.47	33.40
4-Demethylsimmondsin	8	18.88	24.47	30.96
5-Demethylsimmondsin	7	19.85	24.47	31.33
4,4-Didemethylsimmondsin	6	19.90	24.47	29.33
5-α-D-Galactopyranosyl-D-pinitol	4	18.88	21.34	26.62
2-α-D-Galactopyranosyl-D-pinitol	5	19.90	23.00	28.40

mondsin and 4,5-didemethylsimmondsin) is only obtained by the CP-Sil 24 column without the presence of interfering peaks. For this reason the CP-Sil 24 column was retained for the simultaneous determination of the five soluble carbohydrates and four simmondsins present in jojoba meal. If the separation of 4- and 5-demethylsimmondsin is not essential, the gas chromatographic procedure for the simultaneous determination of the soluble carbohydrates and simmondsins in jojoba seed meal extracts can be reduced to 15 min. Fig. 1 shows a gas chromatogram of the trimethylsilyl derivatives of a jojoba meal extract obtained with the mentioned column.

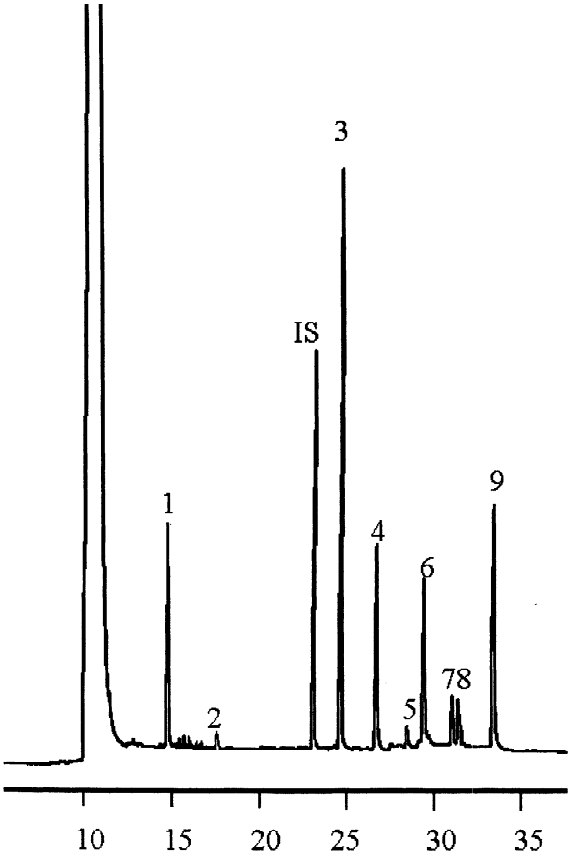

Fig. 1. Gas chromatogram of the trimethylsilyl derivatives of a jojoba meal extract. The numbers of the peaks correspond to the numbers represented in Table 1.

3.3. Identification of the individual carbohydrates and simmondsins

The assignment of the different peaks was done by comparison of retention times and mass spectra of different silylated derivatives, present in the extracts, with the corresponding data obtained from reference compounds previously isolated from jojoba meal.

The most important fragment ions from the different silylated carbohydrates and simmondsins, identified in jojoba meal extracts are shown in Table 2. Molecular ions could not be registered because of the range of the apparatus allowing to register ions up to m/z 650.

3.4. Recovery

The influence of the composition of the extraction solvent on the extraction yields is represented in Table 3. As can be seen from this table the simmondsins show the highest extraction yields for mixtures containing at least 10% of water. The recoveries for sucrose and galactosides were quantitative with extraction mixtures containing at least 20% of water; the recoveries for D-pinitol and *myo*-inositol were quantitative for mixtures containing at least 10% water. For the mentioned reasons a mixture of methanol–water (80:20, v/v) was used for the simultaneous quantitative extraction of simmondsins and carbohydrates from jojoba seed meal.

3.5. Precision, linearity and reproducibility

The precision of the method was assessed using six replicate injections of mixtures from 1.0 mg/ml solutions of nine analytes with the internal standard. The relative standard deviation (RSD) of the proportion between the analytes and the internal standard was between 0.2 and 0.4%.

The calibration curves obtained by replicate analysis ($n=3$) of series of analyte concentrations corresponding to 0.1, 0.25, 0.5, 1.0, 2.0 and 4.0% in jojoba seed meal, with the extraction mixture of methanol–water (80:20, v/v), were subjected to linear regression analysis. The obtained calibration graphs showed ranges of linearity ($r^2>0.995$) for concentrations between 0.1 and 4%.

Table 2
Most important fragment ions as m/z with relative abundance (%) from the trimethylsilyl derivatives of the soluble carbohydrates and simmondsins present in jojoba meal extracts

D-Pinitol
73 (100); 129 (13); 133 (11); 147 (47); 191 (26); 207 (12); 217 (26); 265 (8); 305 (37); 318 (39); 367 (3); 393 (3); 433 (4); 507 (1)
Myo-inositol
73 (100); 147 (50); 191 (22); 204 (12); 217 (36); 265 (7); 305 (25); 318 (14); 367 (3); 433 (3); 507 (1)
Sucrose
73 (98); 103 (27); 117 (7); 129 (20); 147 (35); 217 (59); 243 (10); 271 (15); 319 (7); 361 (100); 362 (30); 437 (16); 451 (8).
5-α-D-Galactopyranosyl-D-pinitol
73 (31);147 (14); 180 (4); 191 (8); 204 (100); 205 (10); 217 (12); 305 (5); 361 (4); 375 (2).
2-α-D-Galactopyranosyl-D-pinitol.
73 (33);147 (11); 180 (2); 191 (7); 204 (100); 205 (10); 217 (15); 305 (4); 361 (6); 375 (2)
Simmondsin
73 (89); 103 (13); 133 (10); 147 (27); 180 (10); 204 (100); 217(22); 236 (10); 268 (8); 357 (4); 361 (4); 386 (10)
5-Demethylsimmondsin
73 (63); 103 (20); 133 (12); 147 (23); 180 (12); 204 (100); 217 (21); 236 (6); 326 (5); 361 (7); 415 (5); 444 (9)
4-Demethylsimmondsin
73 (82); 103 (18); 133 (10); 147 (25); 180(9); 204 (100); 217 (19); 236 (3); 326 (3); 361 (7); 415 (5); 444 (7)
4,5-Didemethylsimmondsin
73 (68); 103 (21); 133 (8); 147 (45); 180 (12); 204 (100); 217 (29); 294 (8); 331 (3); 361 (9); 473 (6); 502 (4)

Table 3
Percentage of recoveries (±RSD) of soluble carbohydrates and simmondsins from jojoba seed meal with methanol–water mixtures. The results are from triplicate analyses of jojoba seed meal spiked with 20 mg/g

Compound	Recovery, % (mean±RSD, %) for CH_3OH–water mixtures (v/v)			
	50:50, 60:40, 70:30	80:20	90:10	100% CH_3OH
D-Pinitol	101±2	99±2	97±2	90±3
myo-Inositol	99±2	100±2	100±2	101±3
Sucrose	101±2	101±2	97±2	95±2
Simmondsin	101±2	100±2	100±1	94±2
5-Demethylsimmondsin	102±2	100±2	99±2	99±2
Didemethylsimmondsin	101±2	99±2	101±2	99±2
5-α-D-Galactopyranosyl-D-pinitol	99±2	101±2	97±2	94±2

Table 4
Concentrations of soluble carbohydrates and simmondsins (±RSD) in extracts from two different jojoba meal samples

Compound	Concentration (% in jojoba seed meal)	
D-Pinitol	1.41±0.02	1.20±0.02
myo-Inositol	0.91±0.02	0.12±0.02
Sucrose	3.16±0.04	4.82±0.05
5-α-D-Galactopyranosyl-D-pinitol	0.88±0.02	0.98±0.02
2-α-D-Galactopyranosyl-D-pinitol	0.16±0.02	0.13±0.02
Simmondsin	4.62±0.05	5.02±0.05
5-Demethylsimmondsin	0.55±0.01	0.48±0.01
4-Demethylsimmondsin	0.37±0.01	0.53±0.01
4,5-Didemethyl-simmondsin	1.95±0.03	2.51±0.03

To evaluate the reproducibility, jojoba seed meal samples were extracted three times on 3 different days and injected three times. The obtained data were subjected to analysis of variance. Intraday repeatability was good with a mean standard deviation (RSD)$<$3.5% for the calculated concentrations and below 0.1% for the retention times. Mean RSD values for inter-day variations were also $<$3.5 and $<$0.1% for the retention times.

3.6. Sample composition

The method was applied to determine concentrations of the soluble carbohydrates and simmondsins in two different samples of jojoba seed meal. The obtained results are represented in Table 4.

4. Conclusion

Capillary gas chromatography with a phenyl(50%) dimethylpolysiloxane phase has been validated for the simultaneous determination of D-pinitol, *myo*-inositol, sucrose, 5-α-D-galactopyranosyl-D-pinitol, 2-α-D-galactopyranosyl-D-pinitol, simmondsin, 4-demethylsimmondsin, 5-demethylsimmondsin and 4,5-didemethylsimmondsin as trimethylsilyl derivatives in jojoba seed meal in the concentration range between 0.1 and 4%. The described method shows good selectivity, repeatability, sensitivity and linearity.

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References

[1] N. Booth, C. Elliger, A. Waiss, Life Sci. 15 (1974) 1115.
[2] M. Cokelaere, J. Buyse, P. Daenens, E. Decuypere, R. Kühn, M. Van Boven, J. Agric. Food Chem. 41 (1993) 1444.
[3] M. Cokelaere, H.D. Dangreau, S. Arnouts, R. Kühn, E. Decuypere, J. Agric. Food Chem. 40 (1992) 1839.
[4] C. Elliger, A. Waiss, R. Lundin, Phytochem. Rep. 13 (1974) 2319.
[5] M. Van Boven, P. Daenens, M. Cokelaere, G. Janssen, J. Agric. Food Chem. 42 (1994) 2684.
[6] M. Van Boven, R. Busson, M. Cokelaere, G. Flo, E. Decuypere, Ind. Crops Prod. 6 (1997) 147.
[7] C. Elliger, A. Waiss, A. Booth, US Pat. 3 919 432 (1974).
[8] A. Verbiscar, T. Baningan, W. Weber, B. Reid, J. Trei, E. Nelson, R. Raffauf, D. Kosersky, J. Agric. Food Chem. 28 (1980) 571.
[9] G. Sodoni, M. Canella, A. Patricelli, US Pat. 4 148 928 (1979).
[10] A. Cotgageorge, B. Weber, B. Reid, R. Price, in: D. Yermanos (Ed.), Proceedings of the Third International Conference on Jojoba and Its Uses, Riverside, California, 1978, p. 171.
[11] T. Abbott, L. Nakamura, T. Nelsen, H. Gasdorf, G. Bennett, R. Kleiman, Appl. Microbiol. Biotechnol. 34 (1990) 270.
[12] A. Verbiscar, T. Baningan, J. Agric. Food Chem. 26 (1978) 1456.
[13] A. Verbiscar, T. Baningan, W. Weber, B. Reid, J. Spencer-Swingle, J. Trei, E. Nelson, J. Agric. Food Chem. 29 (1981) 296.
[14] S. Erhan, T. Abbott, H. Nabetani, H. Purcell, Ind. Crops Prod. 6 (1997) 147.
[15] R. Holser, T. Abbott, Ind. Crops Prod. 10 (1999) 41.
[16] T. Abbott, R. Holser, B. Plattner, R. Plattner, H. Purcell, Ind. Crops Prod. 10 (1999) 65.
[17] M. Van Boven, T. Leyssen, R. Holser, M. Cokelaere, G. Flo, E. Decuypere, J. Agric. Food Chem. 49 (2001) 4278.
[18] T. Abbott, R. Peterson, L. Nakamura, T. Nelsen, M. Bagby, in: A.R. Baldwin (Ed.), Proceedings of the 7th Conference on Jojoba and its Uses, AOCS, Champaign, IL, 1988, p. 440.
[19] M. Van Boven, P. Daenens, J. Tytgat, M. Cokelaere, J. Agric. Food Chem. 44 (1996) 2239.
[20] M. Van Boven, R. Holser, M. Cokelaere, G. Flo, E. Decuypere, J. Agric. Food Chem. 48 (2000) 4083.

[21] K. Bach Knudsen, B. Li, J. Agric. Food Chem. 39 (1991) 689.
[22] H. Nygaard Johansen, V. Glitso, K. Bach Knudsen, J. Agric. Food Chem. 44 (1996) 1470.
[23] M. Van Boven, N. Blaton, M. Cokelaere, P. Daenens, J. Agric. Food Chem. 41 (1993) 1605.
[24] M. Van Boven, P. Daenens, M. Cokelaere, G. Janssen, J. Agric. Food Chem. 43 (1994) 1193.
[25] M. Van Boven, R. Busson, M. Cokelaere, G. Flo, E. Decuypere, Ind. Crops Prod. 12 (2000) 203.