Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *83* (1) 31–38 (2018)
JSCS–5056
UDC 582.736+581.19:547.972:
547.918:615.279–188(65)
Short communication

SHORT COMMUNICATION

Chemical composition and antioxidant activity of *Astragalus monspessulanus* L. growing in semiarid areas of Algeria

SAWSEN BOUREZZANE[1], HAMADA HABA[1], CHRISTOPHE LONG[2] and MOHAMMED BENKHALED[1]*

[1]*Laboratory of Chemistry and Environmental Chemistry (L.C.C.E), Department of Chemistry, Faculty of Sciences, Batna-1 University, Algeria and* [2]*USR 3388 CNRS-Pierre Fabre, 3 Avenue Hubert Curien BP 13562, 31035 Toulouse, France*

(Received 12 December 2016, revised 19 June, accepted 3 July 2017)

Abstract: This paper reports a phytochemical study of the aerial parts of *Astragalus monspessulanus* L. growing in Algeria. It deals with the isolation and structure elucidation of 13 known compounds. From the *n*-butanol extract, seven flavonoids **1**–**7**, two saponins and one lignan were isolated. In addition, two phytosterols and one triterpenoid were isolated from the ethyl acetate extract. The structures of all the isolated products were determined by using 1D and 2D-NMR techniques, measurement of optical rotation, mass spectrometry ESI-MS and by comparison with literature data. Furthermore, the antioxidant activity of the *n*-butanol extract of the aerial parts of *Astragalus monspessulanus* L. was investigated using the DPPH radical scavenging and ferrous ion chelating assays. The *n*-butanol extract showed low (EC_{50} = 2.09±0.434 mg mL^{-1}) to moderate (IC_{50} = 63.60±0.01 μg mL^{-1}) antioxidant activities depending on the test method.

Keywords: Fabaceae; *Astragalus monspessulanus* L.; flavonoids; lignan; saponins; NMR; antioxidant activity.

INTRODUCTION

Astragalus is the largest genus in the Fabaceae family comprising over 3000 species of flowering plants.[1] Many of these species are famed in traditional medicine as anti-perspirants, diuretics as well as tonics and for the treatment of nephritis, diabetes, leukemia and uterine cancer. Previous phytochemical investigations on *Astragalus* genus revealed the presence of saponins, polysaccharides, phenolics and alkaloids.[2–5]

In continuation of on-going studies on the constituents of *Astragalus* species,[6–8] a phytochemical study on *Astragalus monspessulanus* L. was realized.

* Corresponding author. E-mail: mbenkhaled@yahoo.fr
https://doi.org/10.2298/JSC161212085B

This plant is very common in semi-arid land and the Tell Atlas Mountains in Algeria.[9] Very recently, a phytochemical investigation on *A. monspessulanus* L., growing in Bulgaria reported the isolation of flavoalkaloids and flavonoids, with hepatoprotective and antioxidant activities.[10] A previous biochemical study performed on this plant revealed that a mixture of non-identified saponins exhibited a cytotoxic effect on HepG2 cell line, at the highest concentration of 4 mg mL^{-1} after 48 and 72 h.[11]

The aim of the present study was to examine the chemical composition of *A. monspessulanus* species (aerial parts) and to evaluate its antioxidant activity.

In the current work, 13 compounds were isolated from the ethyl acetate and *n*-butanol extracts of *A. monspessulanus* L. (Fig. 1). Thus, this study focused on the phytochemistry of this species, structure determination of the isolated compounds and the antioxidant activity of the *n*-BuOH extract using DPPH radical scavenging and ferrous ion chelating assays.

EXPERIMENTAL

General

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance II spectrometer (Bruker, France) equipped with a cryoprobe (500 MHz for ^{1}H and 125 MHz for ^{13}C) in MeOD or DMSO-d_6 solution and a Bruker Avance spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) in CD_3OD. 1D and 2D NMR experiments (COSY, HSQC and HMBC) were used to assign the individual ^{1}H and ^{13}C resonances. ESI-MS spectra were obtained on a Bruker ion trap Esquire LC. Column chromatography (CC) was performed using silica gel Merck Kieselgel 60 (70–230 mesh), Merck Lobar Lichroprep RP-18 (40 μm×63 μm), Polyamide SC6 and Sephadex LH-20. Column fractions were monitored by TLC; individual TLC plates were performed on silica gel plates Kieselgel 60 F_{254S} (Merck, Darmstadt, Germany) or RP-18 plates and visualized by spraying with 50 % sulfuric acid reagent or vanillin sulfuric acid then heating. A pre-packed C_{18} reversed-phase column (XTerra RP-18, 19×250, 30×100 and 4.6×100 mm, 0.45 μm) was used for preparative HPLC with binary gradient elution (solvent A: H_2O and solvent B: MeCN) at 25 °C, a flow rate of 25 mL min^{-1} and the chromatogram was monitored at 250 and 280 nm. Absorption measurements were performed on a Sedico VIS7220G UV–Vis spectrophotometer (Sedico, Cyprus).

Plant material

The aerial parts of *Astragalus monspessulanus* L. were collected in June 2012 near Batna-Aures (Algeria), and identified by Prof. Bachir Oudjehih of the Agronomic Institute of the University of Batna-1, where a voucher specimen was deposited (No. 745/ LCCE).

Extraction and isolation

Powdered air-dried material of *A. monspessulanus* (1000 g) was extracted three times with EtOH/H_2O (70/30, 10 L) each for 3 days at room temperature. After filtration and evaporation, the crude extract suspended in H_2O was partitioned successively with petroleum ether, ethyl acetate and *n*-butanol to give three different polar parts. Evaporation to dryness yielded the following extracts: petroleum ether (8.3 g), ethyl acetate (12.1 g), and *n*-butanol (15.33 g).

The *n*-butanol extract (7 g) was submitted to vacuum liquid chromatography (VLC) on RP-18, using a gradient of H_2O/MeOH (80:20 to 0:100) as eluent to obtain 15 fractions (Fr_1–Fr_{15}). Further chromatographic separation and purification using CC, TLC, HPLC and precipitation on fractions Fr_1 (5.53 g) and Fr_4 (60 mg) allowed the isolation of 10 compounds (**1**–**10**).

The ethyl acetate extract (7 g) was subjected to RP-18 vacuum liquid chromatography (VLC) using a gradient system of H_2O/MeOH (80:20 to 0:100) to afford 9 fractions (Fr_1–Fr_9). Fractions Fr_9 (232 mg), Fr_6 (695 mg) and Fr_7 (780 mg) were chromatographed on successive silica gel CC followed by precipitation to provide 3 compounds (**11**–**13**).

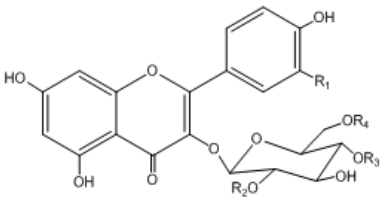
Details of the isolation and purification procedures are given in the Supplementary material to this paper, along with the analytical and spectral data of the compounds.

RESULTS AND DISCUSSION

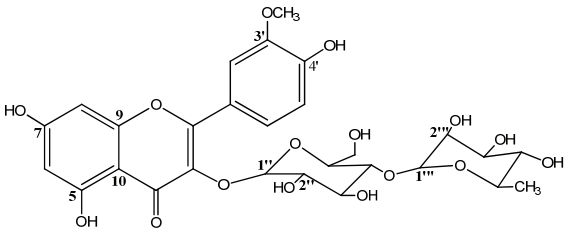
In this study, 13 known compounds were isolated, including seven flavonoids, identified as kaempferol 3-*O*-rutinoside (nicotiflorin, **1**),[12] isorhamnetin 3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (calendoside III, **2**),[13] quercetin 3-*O*-rutinoside (rutin, **3**),[12] kaempferol 3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (**4**),[14] quercetin 3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**5**),[15] 3′,5′-di-*C*-β-D-glucopyranosylphloretin (**6**) and hovetrichoside C (**8**),[16,17] one lignan, *i.e.*, isolariciresinol 9′-*O*-β-D-glucopyranoside (**7**),[18] two saponins, *i.e.*, soyasaponin I (**9**) and dehydrosoyasaponin I (**10**),[19,20] two sterols, *i.e.*, β-sitosterol (**11**) and β-sitosterol 3-*O*-glucoside (**12**),[21] and one triterpenoid, *i.e.*, lupeol (**13**).[22] Their structures were elucidated by extensive spectroscopic methods including 1D (^{1}H and ^{13}C) and 2D NMR (COSY, HSQC and HMBC) experiments as well as ESI-MS analysis (see Supplementary material) and by comparison with those reported in literature (Fig. 1).

Although the presence of flavonoids as dominant compounds was observed in the same species from the flora of Bulgaria,[10] only quercetin 3-*O*-rutinoside (rutin, **3**) is common. This compound was previously reported in the *Astragalus* genus, such as in *A. corniculatus*.[23] The present study reports for the first time the isolation and identification of the triterpenic saponins **9** and **10** in *A. monspessulanus* L. Triterpenic saponins are the major constituents of the *Astragalus* genus, besides flavonoids and polysaccharides.[24] Soyasaponin I (**9**) was previously isolated from many *Astragalus* species.[7,19,25] However, this is only the second report of the occurrence of dehydrosoyasaponin I (**10**) in this genus.[7]

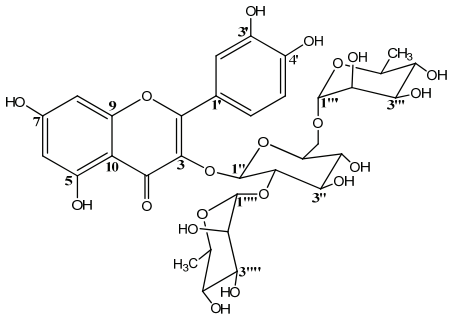
According to the literature data, this is also the first occurrence of the compounds **2**–**8** in the genus *Astragalus*. Calendoside III (**2**) was identified in *Calendula officinalis* L. (Asteraceae).[13] Compound **4** was reported in *Oxandra sessiliflora* (Annonaceae) and *Acacia pennata* Willd (Mimosaceae).[14,26] Compound **5**, called manghaslin, was previously isolated from *Glycine max* (Fabaceae).[27] It was first found in *Cerbera manghas* (Apocynaceae).[28] Compound **6** is present in the Fabaceae family, such as in *Cyclopia subternata*, and could contribute to the antioxidant activity of this species.[29]



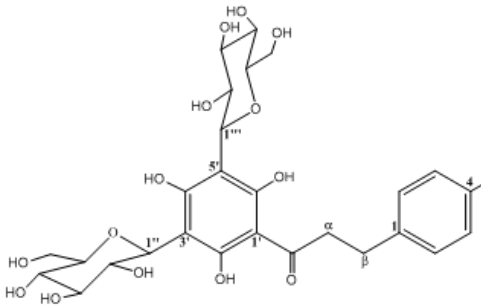
1 R_1=R_2=R_3=H, R_4=Rha
3 R_1=OH, R_2=R_3=H, R_4=Rha
4 R_1=R_2=R_4=H, R_3=Rha



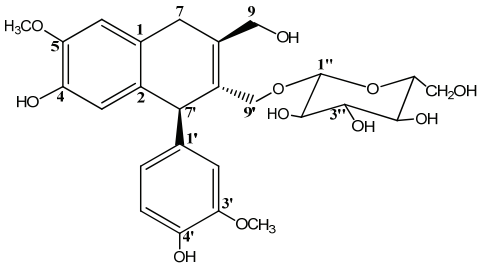
2



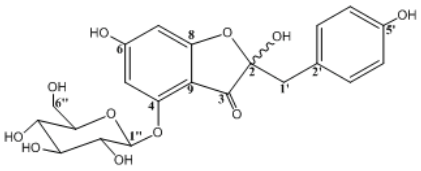
5



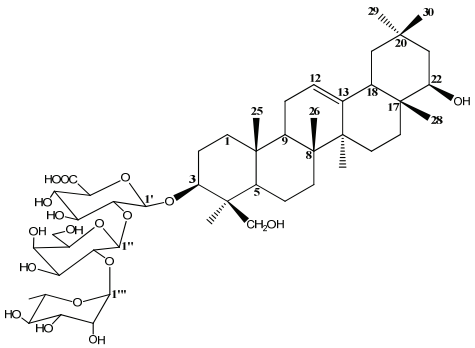
6



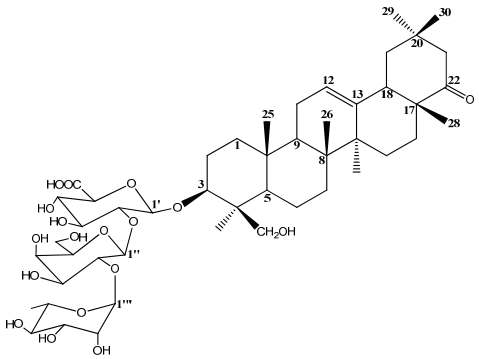
7



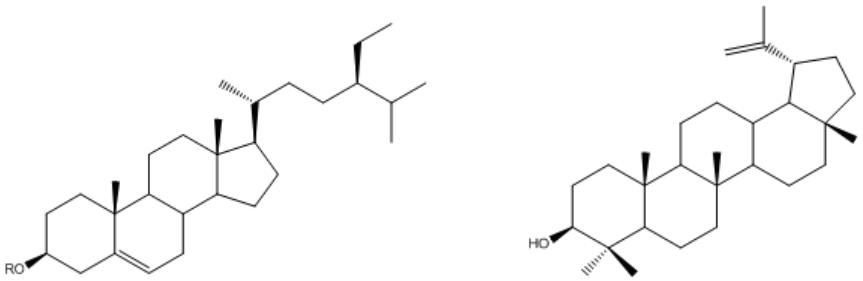
8



9



10



11 R=H, **12** R= Glu **13**

Fig. 1. Chemical structures of compounds **1**–**13** isolated from *Astragalus monspessulanus* L.

Compound **7** was isolated from *Pelargonium reniforme* (Geraniaceae) and *Pedicularis densispica* (Scrophulariaceae).[18,30] Hovetrichoside C (**8**) was previously obtained from *Hovenia trichocarea* (Rhamnaceae) and *Punica granatum* (Punicaceae).[17,31]

Nicotiflorin (**1**) is common in the *Astragalus* genus. It has been detected in several *Astragalus* species, such as *A. verrucosus* Moris,[3] *A. icmadophilus* Hand.-Mazz.[32] and *A. cruciatus* Link.[6]

Antioxidant activity

In this study, the antioxidant activity of the *n*-butanol extract of *A. monspessulanus* was assessed *in vitro* using two different methods, *i.e.*, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferrous ion chelating activities. The crude extract possessed a moderate scavenging effect (IC_{50} = 63.60±0.01 μg mL^{-1}, Supplementary material, Fig. S-4) in a dose-dependent manner compared to ascorbic acid as a standard (IC_{50} = 3.15 μg mL^{-1}). This antioxidant activity may be due to the presence of phenolic compounds, such as 3′-5′-di-*C*-*β*-D-glucopyranosylphloretin (**6**), which is known for its interesting antioxidant activities.[29]

The results of the ferrous ion chelating activity (Supplementary material, Fig. S-6) revealed that the *n*-butanol extract of *A. monspessulanus* possesses a low metal chelating activity (EC_{50} = 2.09±0.44 mg mL^{-1}) compared to EDTA as a standard (EC_{50} = 0.529±0.031 μg mL^{-1}). This low activity could be explained by the nature of chemical compounds in the extract. Many studies reported that the metal chelating ability of phenolic compounds is linked to their unique phenolic structure and the number and location of hydroxyl groups.[33,34]

CONCLUSIONS

In conclusion, 13 known compounds were isolated from the ethyl acetate and *n*-BuOH extracts of the aerial parts of *Astragalus monspessulanus*. According to literature data, the isolated compounds **2**–**8** are identified for the first time in *Astragalus* genus, and compounds **1**, **9** and **10** in the species. Our report con-

firms that saponins and flavonoids are the major constituents of the *Astragalus* genus. Interestingly, this investigation highlights a significant difference between flavonoids reported in the present study and those isolated from *A. monspessulanus* of Bulgarian origin, except compound **3** (rutin) which is common. It is worth noting that it is the first report of saponins and lignans in *A. monspessulanus*. Indeed, the accumulation of **9** (soyasaponin I) is a generic trait of the genus *Astragalus*. This compound is used as a chemotaxonomic marker for this genus and the Fabaceae family.[35]

Data from the presented results revealed that the *n*-butanol extract of *A. monspessulanus* acts as an antioxidant agent. Thus, the antioxidant capacity is correlated to the phenolic composition of the corresponding extract, including flavonoids and lignan.[36] In fact, the flavonoid class is the most important plant antioxidant.[37]

SUPPLEMENTARY MATERIAL

Details of the isolation and purification procedures, along with the analytical and spectral data of the compounds, are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. We are thankful to the Algerian Minister of Higher Education and Scientific Research for providing a research grant. We are also grateful to USR 3388 CNRS-Pierre Fabre, Toulouse, France for providing research facilities and technical support.

ИЗВОД

ХЕМИЈСКИ САСТАВ И АНТИОКСИДАТИВНА АКТИВНОСТ ЕКСТРАКАТА ИЗОЛОВАНИХ ИЗ *Astragalus monspessulanus* L. КОЈИ РАСТУ У ПОЛУСУВИМ ОБЛАСТИМА АЛЖИРА

SAWSEN BOUREZZANE[1], HAMADA HABA[1], CHRISTOPHE LONG[2] и MOHAMMED BENKHALED[1]

[1]Laboratory of Chemistry and Environmental Chemistry (L.C.C.E), Department of Chemistry, Faculty of Sciences, Batna-1 University, Algeria и [2]USR 3388 CNRS-Pierre Fabre, 3 Avenue Hubert Curien BP 13562, 31035 Toulouse, France

У раду је приказано фитохемијско испитивање надземних делова биљке *Astragalus monspessulanus* L. која расте у Алжиру. Описано је изоловање и утврђивање структуре 13 познатих једињења. Из *n*-бутанолног екстракта изоловано је седам флавоноида (**1**–**7**), два сапонина и један лигнан. Такође, два фитостерола и један тритерпенод изоловани су из етил-ацетатног екстракта. Структуре свих изолованих једињења одређене су применом 1D- и 2D-NMR спектроскопије, мерењем оптичке ротације, ESI-MS масеном спектрометријом и поређењем са подацима из литературе. Осим тога, испитана је антиоксидативна активност *n*-бутанолног екстракта надземних делова *A. monspessulanus* L. Применом DPPH хватача радикала и гвожђе(II) теста, испитивани *n*-бутанолни екстракт показује ниску (EC_{50} = 2,09±0.44 mg mL^{-1}) до умерену (IC_{50} = 63,60±0,01 μg mL^{-1}) антиоксидативну активност у зависности од примењеног модела за тестирање.

(Примљено 12. децембра 2016, ревидирано 19. јуна, прихваћено 3. јула 2017)

REFERENCES

1. V. H. Heyword, *Flowering plants of the world*, Oxford University Press, London, 1978

2. M. I. Choudhary, S. Jan, A. Abbaskhan, S. G. Mucharraf, S. A. Samreen Sattar, *J. Nat. Prod*. **71** (2008) 1557
3. L. Pistelli, I. Giachi, E. Lepori, A. Bertoli, *Pharm. Biol*. **41** (2003) 568
4. J. Echeverria, S. M. Espinoza, H. M. Niemeyer, *Nat. Prod. Res*. **31** (2017) 89
5. K. Guo, X. He, D. Lu, Y. Zhang, X. Li, Z. Yan, B. Qin, *Nat. Prod. Res.* **31** (2017) 314
6. W. Benchadi, H. Haba, C. Lavaud, D. Harakat, M. Benkhaled, *Rec. Nat. Prod*. **7** (2013) 105
7. L. Maamria, H. Haba, C. Lavaud, D. Harakat, M. Benkhaled, *J. Serb. Chem. Soc.* **80** (2015) 137
8. L. Maamria, C. Long, H. Haba, C. Lavaud, M. Benkhaled, *Phytochem. Lett., B* **11** (2015) 286
9. P. Quezel, S. Santa, *Nouvelle Flore de l'Algérie et des régions désertiques méridionales*. Vol. 1–2, CNRS, Paris, 1963
10. I. Krasteva, V. Braktov, F. Bucar, O. Kunert, M. Kollhoser, M. Kondeva-Burdina, I. Ionkova, *J. Nat. Prod.* **78** (2015) 2565
11. V. Bratkov, M. Kondeva-Burdina, R. Simeonova, V. Tzankova, I. Krasteva, *Eur. J. Med. Plants* **4** (2014) 522
12. K. Kazuma, N. Noda, M. Suzuki, *Phytochemistry* **62** (2003) 229
13. D. N. Olennikov, N. I. Kashchenko, *Chem. Nat. Compd*. **50** (2014) 633
14. E. A. De Sousa, A. A. C. A. Da Silva, A. J. Cavalheiro, J. H. G. Lago, M. H. Chaves, *J. Braz. Chem. Soc*. **25** (2014) 704
15. B. Schwarz, T. Hofmann, *J. Agric. Food Chem*. **55** (2007) 1394
16. K. Ogawa, A. Kawasaki, M. Omura, T. Yoshida, Y. Ikoma, M. Yano, *Phytochemistry* **57** (2001) 737
17. K. Yoshikawa, K. Eiko, N. Mimura, Y. Kondo, S. Arihara. *J. Nat. Prod*. **61** (1998) 786
18. K. P. Latté, M. Kaloga, M. Schäfer, H. Kolodziej, *Phytochemistry* **69** (2008) 820
19. A. M. El-Hawiet, S. M. Toaima, A. M. Asaad, M. M. Radwan, N. A. El-Sebakhy, *Braz. J. Pharmacogn.* **20** (2010) 860
20. H. Miyao, Y. Sakai, T. Takeshita, J. Kinjo, T. Nohara, *Chem. Pharm. Bull.* **44** (1996) 1222
21. D. N. Olennikov, L. M. Tankhaeva, V. V. Partilkhaev, A. V. Rokhin, *Braz. J. Pharmacogn.* **22** (2012) 490
22. J. Anaya, M. C. Caballero, M. Grande, J. J. Navarro, I. Tapia, J. F. Almeida, *Phytochemistry* **28** (1989) 2206
23. I. Krasteva, S. Nikolov, *Quim. Nova* **31** (2008) 59
24. L. J. Rios, G. P. Waterman, *Phytother. Res.* **11** (1997) 411
25. S. Yahara, M. Kihjyouma, H. Kohoda, *Phytochemistry* **53** (1990) 469
26. A. B. Dongmo, T. Milyamoto, K. Yoshikawa, S. Arihara, M. A. Lacaille-Dubois, *Planta Med*. **73** (2007) 1202
27. B. R. Buttery, R. I. Buzell, *Can. J. Bot.* **53** (1975) 219
28. A. Sakushima, S. Hisada, Y. Ogihara, S. Nushiba, *Chem. Pharm. Bull*. **28** (1980) 1223
29. A. Kokotkiewicz, M. Luczkiewicz, P. Sowinski, D. Glod, K. Gorynski, A. Bucinski, *Food Chem.* **133** (2012) 1373
30. H. B. Chu, G. Z. Zeng, M. J. Zhu, W. J. He, Y. M. Zhang, N. H. Tan, *Z. Naturforsch., B: J. Chem. Sci.* **66** (2011) 641
31. T. Yuan, C. Wan, H. Ma, N. P. Seeram, *Planta Med.* **79** (2013) 1674
32. I. Horo, E. Bedir, A. Perrone, F. Özgökçe, S. Piacente, Ö. Alankuş-Çalişkan, *Phytochemistry* **71** (2010) 956

33. I. F. Cheng, K. Breen, *Biomaterials* **13** (2000) 7
34. J. Santoso, Y. Yoshie-Stark, T. Suzuki, *Fish. Sci. (Tokyo, Jpn.)* **70** (2004) 183
35. W. Oleszek, A. Stochmal, *Phytochemistry* **61** (2002) 165
36. L. Bravo, *Nutr. Rev.* **56** (1998) 317
37. J. V. Higdon, B. Frei, *Crit. Rev. Food Sci.* **43** (2003) 89.