



REGULAR ARTICLE

PHYTOCHEMICAL SCREENING AND MINERAL CONTENTS OF ANNUAL PLANTS GROWING WILD IN THE SOUTHERN OF TUNISIA

Ahmed Akrou^t*, Hajer El Jani, Tarek Zammouri, Hédi Mighri, Mohamed Neffati

Laboratoire d'écologie pastorale, Institut des Régions Arides, Km 23, Route El Jorf, 4119 Médenine, Tunisia

SUMMARY

Eight annual species growing wild in the southern of Tunisia (*Diplo^taxis simplex*, *Chrysanthemum coronarium*, *Matthiola longipetela*, *Erodium glaucophyllum*, *Reseda alba*, *Diplo^taxis harra*, *Senecio gallicus* and *Papaver rhoeas*) were evaluated for their mineral contents and phytochemical screening. The mineral analysis showed that calcium and potassium were the most concentrated minerals (1.21-3.60% and 0.36-3.20% respectively) followed by sodium (0.12-1.38%), magnesium (0.16-0.41%) and phosphorus (0.05-28%). The preliminary phytochemical screening revealed the absence of anthraquinones in all studied plants whereas alkaloids were only present in *Papaver rhoeas*. *Senecio gallicus* and *Chrysanthemum coronarium* were the only species that contained essential oils. All species were found to contain saponins, flavonoids and tannins with the exception of *Erodium glaucophyllum* and *Papaver rhoeas*. These results indicate that some of these species may be used as fodder plants for livestock due to their high contents of minerals but the presence of some secondary metabolites may reduce their palatability. In the other hand, these secondary metabolites could be the origin of the medicinal properties of these species.

Keywords: Phytochemical screening, Tunisian annual plants, Mineral contents.

Ahmed Akrou^t et al. Phytochemical Screening and Mineral Contents of Annual Plants Growing Wild in the Southern of Tunisia. J Phytol 2 (2010) xxx-xxx

*Corresponding Author, Email: ahmed.akrou^t@ira.mrt.tn

1. Introduction

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of minerals contents of plants is useful to evaluate their nutritive potentialities as fodder for livestock and human. Therefore, the knowledge of the chemical constituents of plants would further

be valuable in discovering the actual value of folkloric remedies.

In Tunisia, there are 149 medicinal plants whose the majority are perennial and some of them are actually exploited for the production of essential oils (*Rosmarinus officinalis*, *Artemisia herba alba*, *Thymus capitatus*, *Myrtus communis*) [1]. Annual plants growing wild are generally consumed by livestock but not or little used in folk medicine in spite of their abundance especially during the rainy years.

The present study is a contribution to the determination of the chemical composition of

some annual plants growing wild in Tunisia: *Diplotaxis simplex*, *Chrysanthemum coronarium*, *Matthiola longipetala*, *Erodium glaucophyllum*, *Reseda alba*, *Diplotaxis harra*, *Senecio gallicus* and *Papaver rhoeas*. Some medicinal uses of these species are presented in Table 1 [2-4]. Several phytochemical and pharmacological studies were carried out on some of these species in other countries [5-19]. In Tunisia, no previous chemical and biological studies have been reported on these species except the two studies carried out by Hammami et al. who isolated and identified phenolic compounds, sterol glycosides and essential oil from the flowers of *Matthiola longipetala* [20,21].

The purpose of this work is the evaluation of nutritive and economic potentialities of

eight annual plants growing wild in the southern of Tunisia (*Diplotaxis simplex*, *Chrysanthemum coronarium*, *Matthiola longipetala*, *Erodium glaucophyllum*, *Reseda alba*, *Diplotaxis harra*, *Senecio gallicus* and *Papaver rhoeas*) by the determination of their macromineral contents and their phytochemical composition.

2. Materials and methods

Sample Preparation

The aerial parts of the studied plants (Table 1) were collected at the flowering stage (March-April) from El Fje region (33°30'N and 10°40'E) located at 22 Km from Médenine town in the southern of Tunisia.

Table 1: List of studied plants

Scientific name	Family	Voucher number	Vernacular name	Uses in folk medicine [2,4]
<i>Diplotaxis Simplex</i>	Brassicaceae	Ds01	"Jirjir"	Food for animals
<i>Matthiola Longipetala</i>	Brassicaceae	Ml01	"Chgara"	Anti-inflammatory
<i>Chrysanthemum coronarium</i>	Asteraceae	Cc01	"kahwana"	Stomachic, vermifuge and insecticide
<i>Erodium Glaucophyllum</i>	Geraniaceae	Eg01	"Toummir"	Skins tanning
<i>Reseda Alba</i>	Resedaceae	Ra01	"Dhil Khrouf"	Colic, diarrheas and poisonings
<i>Diplotaxis Harra</i>	Brassicaceae	Dh01	"El harra"	Food for animals
<i>Senecio Gallicus</i>	Asteraceae	Sg01	-	Uterine sedative, regulating venous circulation
<i>Papaver rhoeas</i>	Papaveraceae	Pr01	"Bouguaaroun"	disorders of sleep of the adult and the child, sedative effect against nervousness, anxiety and emotivity, effective, calming of cough and throat irritations

These samples were air-dried in shade at ambient temperature (20-22°C) until constant weight (about 20 days). The dried plant materials were then coarsely crushed with mortar, grounded in powder with a Molinex coffee mill and finally stored at 4°C in a tightly covered bottle until analysis. All the species were identified by Pr. Mohamed Neffati (Range Ecology Laboratory, Institut des Régions Arides, Médenine, Tunisia). Voucher

specimens have been deposited in the laboratory. Voucher numbers and medicinal uses of studied species are shown in Table 1.

Phytochemical Screening

Triplicate samples from each species were analysed for the presence of tannins, flavonoids, saponins, anthraquinones, alkaloids and essential oils using standard phytochemical procedures as following:

Tannins (ferric chloride solution), alkaloids (Bouchardat's, Mayers's and Dragendorff's reagents), flavonoids (Magnesium fragments and HCl), saponins (persistence of foam after shaking), anthraquinones (chloroform and ammonia solution) and essential oils (extraction with Clavenger apparatus) [22].

Determination of Mineral Contents

Mineral contents were determined using the AOAC methods adopted by Oshodi [23,24]: Triplicate samples from each species were dry ashed at 550 °C for four hours in a Heraeus furnace and then were prepared for mineral analysis by dissolving the ash with 20% HCl solution then completing the filtrate to 100 ml by deionised water. Sodium and potassium contents were analysed by a SHERWOOD 410 flame photometer. The concentration of calcium and magnesium were determined by a SHIMADZU AA6800 atomic absorption spectrophotometer. The percentage of Phosphorus was investigated using a modified vanado-molybdate method by a SHIMADZU UV-VIS Spectrophotometer.

3. Results and Discussion

Phytochemical Screening

The phytochemical screening revealed the presence of flavonoids, tannins and saponins in all studied plants except in *Erodium glaucophyllum* which was free of flavonoids and in *Papaver rhoeas* which did not contain saponins. Alkaloids were recorded only in *Papaver rhoeas* (Papaveraceae). Essential oils were present only in *Chrysanthemum coronarium* (Asteraceae) and *Senecio gallicus* (Asteraceae). Anthraquinones were absent in all studied plants. Some of these results are in agreement with those reported by several authors. Flavonoids and essential oils have been previously isolated and identified in *Chrysanthemum coronarium* and *Senecio gallicus* [7-9,13,14,16]. Different polyphenols and flavonoids have been separated and identified in *Diplotaxis simplex*, *Matthiola longipetala* and *Papaver rhoeas* [11,15,20,21]. The presence of

alkaloids in *Papaver rhoeas* has been also previously reported by Soulimani et al. [15]. The absence of essential oil in our *Matthiola longipetala* is in contradiction with the results reported by Hammami et al. [21] and this is can be attributed to the part and the method used for the extraction of this substance or to other parameters such as genetic factors, soil and weather conditions, origin and the stage of the sample the day of its harvest. However, the presence of tannins and saponins in our studied plants has not been previously reported.

The presence of these secondary metabolites in our studied plants may be at the origin of the therapeutic effects of these species and responsible for many pharmacological actions. Saponins have been known to have anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cells and cholesterol lowering activity [25]. The tannin-containing plant extracts are used as astringents, against diarrhoea, diuretics, against stomach and duodenal tumours, anti-inflammatory, antiseptic, antiviral, antibacterial, antioxidant, antitumour by inhibiting HIV replication [26]. Flavonoids were known to possess antioxidative, antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory properties [27]. Essential oils were known to exert antibacterial, anti-inflammatory, antiviral, antioxidant, insecticidal, antifungal and antimalarial activities. Alkaloids were known to be used as antiarrhythmic, anticholinergic, central nervous system stimulant, potent anticancer agent, topical anesthetic, adrenergic blocking agent, drug of abuse, relatively nonaddictive analgesic, antitussive, paralysis of motor nerves and anaesthesia, stimulants, orally active emetic, amoebicide, powerful narcotic analgesic, addictive drug of abuse, highly toxic, horticultural insecticide, peripheral stimulant of the parasympathetic system, antimalarial, antibacterial, sedative for motion sickness, violent tetanic poison, rat poison, antitumor

agent, nondepolarizing, muscle relaxant producing paralysis, adjuvant to anaesthesia and antineoplastic [29].

The biological activities of these secondary metabolites depend on their chemical

composition and their concentration in the studied plants. Further studies will be carried out to extract, identify and determine the biological activities of these substances.

Table 2: Mineral contents (g per 100 g dry matter) of studied plants

	Sodium	Potassium	Calcium	Magnesium	Phosphorus
<i>Diplotaxis Simplex</i>	0.47 ± 0.03	2.06 ± 0.11	1.69 ± 0.08	0.16 ± 0.02	0.24 ± 0.03
<i>Matthiola longipetela</i>	0.47 ± 0.04	1.87 ± 0.12	3.25 ± 0.20	0.23 ± 0.02	0.10 ± 0.01
<i>Chrysanthemum coronarium</i>	1.38 ± 0.06	1.80 ± 0.09	1.65 ± 0.11	0.22 ± 0.02	0.12 ± 0.02
<i>Erodium glaucophyllum</i>	0.12 ± 0.02	0.36 ± 0.08	1.26 ± 0.08	0.32 ± 0.03	0.05 ± 0.01
<i>Reseda alba</i>	0.42 ± 0.04	2.4 ± 0.26	1.21 ± 0.06	0.22 ± 0.02	0.25 ± 0.04
<i>Diplotaxis harra</i>	0.44 ± 0.05	1.93 ± 0.09	1.22 ± 0.08	0.18 ± 0.03	0.28 ± 0.03
<i>Senecio gallicus</i>	0.55 ± 0.05	1.03 ± 0.07	2.10 ± 0.24	0.16 ± 0.03	0.11 ± 0.01
<i>Papaver rhoeas</i>	0.66 ± 0.05	3.20 ± 0.26	3.60 ± 0.32	0.41 ± 0.04	0.19 ± 0.03

Mineral Contents

Results of minerals analysis (Table 2) showed that calcium and potassium were the most concentrated nutrients in all studied plants (1.21-3.60% and 0.36-3.20% respectively), followed by sodium (0.12-1.38%), magnesium (0.16-0.41%) and phosphorus (0.05-0.28%).

The calcium concentrations ranged from 1.21% to 3.60%, most species having contents between 1.21 and 1.69%. *Reseda alba* had the lowest Ca concentration and *Papaver rhoeas* the highest. *Papaver rhoeas*, *Matthiola longipetela* and *Senecio gallicus* can be considered as high calcium content plants (3.60, 3.25 and 2.10% respectively) whereas the others as medium [30]. The amount of calcium in all studied plants was above the required dietary level for feeding animals (0.18-0.33%) [31,32].

The potassium content varied between 0.36% in *Erodium glaucophyllum* and 3.20% in *Papaver rhoeas*. With the exception of *Erodium glaucophyllum* which recorded a very low amount of potassium (0.36%), *Senecio gallicus* which had a low content (1.03%) and *Papaver rhoeas* with a high percentage (3.20%), the remaining studied species contained medium percentage of this nutrient (1.82-2.40%) [30]

(Al-Jaloud et al., 1994). The majority of studied plants had potassium content to satisfy livestock requirements (0.18-0.25%) [31,32].

The amount of magnesium ranged between 0.16% in *Diplotaxis simplex* and 0.41% in *Papaver rhoeas*. All studied plants were found to contain almost the same percentage of this mineral (0.16-0.23%) except *Erodium glaucophyllum* and *Papaver rhoeas* that contained higher amounts (0.32 and 0.41% respectively). These species can be considered as plants with low to medium magnesium content but it seems to be enough to satisfy livestock requirements (0.08-0.25%) [30-32].

All studied species were found to contain almost the same amount of sodium (0.42-0.66%) except *Erodium glaucophyllum* with a lower content (0.12%) and *Chrysanthemum coronarium* with a higher content (1.38%). These species could be considered as plants with medium content of sodium (0.12-1.38%). The amount of sodium in all studied plants was above the required dietary level for feeding animals (0.18-0.33%) [30-32].

The phosphorus content was ranged from 0.05% in *Erodium glaucophyllum* to 0.28% in *Diplotaxis harra*. According to the classification

reported by Al-Jaloud et al. [30] for phosphorus content, *Diploaxis harra*, *Diploaxis simplex* and *Reseda alba* could be considered as plants with high content of this mineral (0.25-0.28%), *Papaver rhoeas* as plant with medium percentage (0.19%) and the other plants as plants with low amounts (0.05-0.12%). Among the studied species, only *Diploaxis harra*, *Diploaxis simplex*, *Reseda alba* and *Papaver rhoeas* had phosphorus content to satisfy livestock requirements (0.16-0.38%) [31,32].

The percentages of these minerals in our studied plants seem to be enough to satisfy livestock requirements with the exception of phosphorus which its amount in certain species is relatively low. The concentrations of these minerals were similar to those recorded in some medicinal and herbal species growing wild in arid and semi arid regions of the world [30,32-36], higher than those obtained in some medicinal plants growing wild in Egypt [31,37], some plants used as condiments in Turkey [38], some Mexican fruits and vegetables [39] and some cereals, fruits and vegetables from Finland [40].

It seems that plants growing wild in arid and semiarid regions contained sufficient amounts of macrominerals for optimal livestock and wildlife performance. These amounts were found to be higher than those in cereals, fruits and vegetables. Among these minerals, it appears that potassium is the most concentrated nutrient in the majority of fruits, vegetables, cereals and herbs and its content generally satisfy human and animals requirements.

Conclusions

The results of the present study have revealed the presence of flavonoids, tannins and saponins in the majority of the studied plants. Essential oil was present in only *Chrysanthemum coronarium* and *Senecio gallicus* whereas alkaloids were found in only *Papaver rhoeas*. The presence of these secondary metabolites in these plants might be the origin of some pharmaceutical properties. These plants could also serve as a good source for

food for animals due to their high mineral contents, particularly calcium and potassium, but the presence of certain secondary metabolites (tannins, alkaloids and essential oils) may limit the use of these species as fodder.

Further studies will be carried out to isolate, identify and evaluate the biological activities of these secondary metabolites and to determine the amount of other macronutrient (proteins, carbohydrates, fibres, etc.) and micronutrients (zinc, manganese, iron, nickel, selenium, etc.) in these species.

References

1. Chemli, R. 1997. Plantes médicinales et aromatiques de la flore de Tunisie. Cahiers Options Méditerranéennes, 23: 119-125.
2. Le Floch, H., E. 1983. Contribution à une étude ethnobotanique de la flore tunisienne. Publié par le ministère de l'enseignement supérieure et de la recherche scientifique, Tunis, Tunisie.
3. Boukef, M.K. 1986. Médecine traditionnelle et pharmacopée, les plantes dans la médecine traditionnelle en Tunisie. Edition ACCT, Tunisie.
4. Boukris, M., Chaieb, M. 1998. Flore succincte et illustrée des zones arides et sahariennes. Edition Or du Temps, Tunisie.
5. Gmelin, R., Kjaer, A. 1970. 2-Hydroxy-2-Methylpropyl glucosinolate in *Reseda alba*. Phytochemistry, 9: 599-600.
6. Harborne, J.B., Heywood, V.H., and Salah, N.A.M., 1970. Chemosystematics of the compositae: Flavonoid patterns in the Chrysanthemum complex of the tribe anthemideae. Phytochemistry, 9: 2011-2017.
7. Mansour, R.M.A., Saleh, N.A.M. 1980. Flavonoids of three local *Senecio gallicus* species. Phytochemistry, 20(5): 1180-1181.
8. El-Nasry, S., Abou-Dinia, A.H.A., Darwish, F.A., Abou-Karam, M.A., Greenz, M., Bohlmann, F. 1984. Sesquiterpene lactones from *Chrysanthemum coronarium*. Phytochemistry, 23 (12): 2953-2954.
9. Urones, J.G., De Pascual, J.T., Marcos, I.S.,

- Moro, R.F., Barcala, P.B., Guidrado J.S. 1987. Acetophenones and terpenoids from *Senecio gallicus* and *S. Adonidifolius*. *Phytochemistry*, 5: 1507-1510.
10. Urones, J.G., Barcala, P.B., Marcos, I.S., Moro, R.F., Esteben, M.L., Rodriguez, F. 1988. Pyrrolizidine alkaloids from *Senecio gallicus*. *Phytochemistry*, 4: 1113-1115.
11. Sanchez-Yelamo, M. D., Martinez-Labore, J.B. 1991. Chemataxonomic approach to *Diplotaxis muralis* (Cruciferae: Brassiceae) and related species. *Biochem. Syst. Ecol.*, 19 (16): 477-48.
12. Chuda, Y., Ono, H., Ohnishi-Kameyama, M., Nagata, T., Tsushida, T. 1996. Structural identification of two antioxidant quinic acid derivatives from Garland (*Chrysanthemum coronarium* L.). *J. Agri. Food Chem.*, 44: 2037-2039.
13. Alvarez-Castellanos, P.P., Bichop, C.D., Pascual-Villalobos, M.J. 2001. Antifungal activity of essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry*, 57: 99-102.
14. Alvarez-Castellanos, P.P., Pascual-Villalobos, M.J. 2003. Effect of fertilizer on yield and composition of flowerhead essential oil of *Chrysanthemum coronarium* (Asteraceae) cultivated in Spain. *Ind. Crop. Prod.*, 17: 77-81.
15. Soulimani, R., Younos, C., Jarmouni-Idrissi, S., Bousta, D., Khalouki, F., Laila, A. 2001. Behavioral and pharmaco-toxicological study of *Papaver rhoeas* L. in mice. *J. Ethnopharmacol.*, 74: 265-274.
16. Flamini, G., Cioni, P.L., Morelli I. 2003. Differences in the Fragrances of Pollen, Leaves, and Floral Parts of Garland (*Chrysanthemum coronarium*) and Composition of the Essential Oils from Flowerheads and Leaves. *J. Agr. Food Chem.*, 51: 2267-2271.
17. Gohar, A.A., Lahlob, M.F., and Niwa, M. 2003. Antibacterial polyphenol from *Erodium glaucophyllum*. *Z. Naturforsch. C. Biosci.*, 58(9,10): 670-674.
18. Kumar, A., Singh, S.P., and Bhakuni, R.S. 2005. Secondary metabolites of *chrysanthemum* genus and their biological activities. *Curr. Sci.*, 89(9): 1489-1501.
19. Lai, J.P., Lim, Y.H., Su, J., Shen, H.M., Ong, C.N. 2007. Identification and Characterization of major flavonoids and caffeoylquinic acids in three *Compositae* plants by LC/DAD-APCI/MS. *J. Chromat. B.*, 848: 215-225.
20. Hammami, S., Ciavatta, M. L., Ben Jannet, H., Cimino, G., Mighri, Z. 2006a. Three phenolic and a sterol glycosides identified for the first time in *Matthiola longipetala* growing in Tunisia. *Croat. Chem. Acta.*, 79(2): 215-218.
21. Hammami, S., Khoja, I., Ben Jannet, H., Ben Halima, M., and Mighri Z. 2006b. Flowers essential oil composition of Tunisian *Matthiola longipetala* and its bioactivity against *Tribolium confusum* insect. *J. Essent. Oil-Bear. Plants*, 10(2): 151-156.
22. Harborne, J.B. 1973. *Phytochemical methods*. Chapman and Hall Ltd., London.
23. AOAC (Association of Official Analytical Chemist). 1990. *Official methods of Analysis*. Association of Official Analytical Chemist, 15th Ed., AOAC Press, Gaithersburg, USA.
24. Oshodi, A.A. 1992. Proximate composition, nutritionally valuable minerals and functional properties of adenopus breviflorus benth seed flour and protein concentrate. *Food Chem.*, 45: 79-83.
25. Jimoh, F.O., Oladiji, A.T. 2005. Preliminary studies on *Piliostigma thonningii* seeds: Proximate analysis, mineral composition and phytochemical screening. *Afr. J. Biotechnol.*, 4(22): 1439-1442.
26. Khanbabaee, K., and Van Ree, T., 2001. Tannins: Classification and Definition. *Nat. Prod. Rep.*, 18: 641-649
27. Onyilagha, J. C., Grotewold, E. 2004. The biology and structural distribution of surface flavonoids. *Recent Res. Devel. Plant Sci.*, 2: 53-71.
28. Gershenzon, J., and Dudareva, N. 2007. The function of terpene natural products in the natural world. *Nat. Chem. Biol.*, 3: 408 - 414.

29. Kutchan, T. M. 1995. Alkaloid Biosynthesis: The Basis for Metabolic Engineering of Medicinal Plants. *Plant cell*, 7: 1059-1070.
30. Al-Jaloud, A.A., Chaudhary, S.A., Bashour, I.I., Qureshi, S., Al-Shanghitti, A. 1994. Nutrient evaluation of some arid range plants in Saudi Arabia. *J. Arid. Environ.*, 28: 299-311.
31. Shedid, M.G., Pulford, I.D., Hamed, A.I. 2006. Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert, Egypt. *J. Arid. Environ.*, 66: 210-217.
32. Ramirez, R.G., Gonzalez-Rodriguez, H., Ramirez-Orduna, R., Cerello-Soto, M.A., Juarez-Reyez, A.S. 2006. Seasonal trends of macro and micro minerals in 10 browse species that grow in northeastern Mexico. *Anim. Feed Sci. Tech.*, 128: 155-164.
33. Ekpa, O.D. 1996. Nutrient composition of three Nigerian medicinal plants. *Food Chem.*, 57(2): 229-232.
34. Ajasa, M.O., Bello, M.O., Ibrahim, A.O., Ogunwande, I.A., Olawore, N.O. 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chem.*, 85: 67-71.
35. Ramirez, R.G., Haenlein, G.F.W., Nunez-Gonzalez, M.A. 2001. Seasonal variation of macro and trace mineral contents in 14 browse species that grow in northeastern Mexico. *Small. Ruminant. Res.*, 39: 153-159.
36. Ramirez-Orduna, R., Ramirez, R.G., Gonzalez-Rodriguez, H., Haenlein, G.F.W. 2005. Mineral content of browse species from Baja California Sur, Mexico. *Small. Ruminant. Res.*, 57: 1-10.
37. El-Halawany, E.F., Mashaly, I.A., Omar, G. 2002. Economic potentialities of some plants growing naturally in the Nile Delta Region, Egypt. *Egyptian Journal of Desert Res.*, 52(1): 21-35.
38. Özcan, M. 2004. Mineral contents of some plants used as condiments in Turkey. *Food Chem.*, 84: 437-440.
39. Sanchez-Castillo, C.P., Dewey, P.J.S., Aguirre, A., Lara, J.J., Vaca, R., Leon de la Barra, P., Ortiz, M., Escamilla, I., James, W.P.T. 1998. The mineral content of Mexican fruits and vegetables. *J. Food. Comp. Anal.*, 11: 340-356.
40. Ekholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellström, J., Ovaskainen, M.L. 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *J. Food. Comp. Anal.*, 20: 487-495.