Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 89 (2005) 57–61
Food Chemistry
www.elsevier.com/locate/foodchem

Mexican lime peel: Comparative study on contents of dietary fibre and associated antioxidant activity

J. Ubando-Rivera, A. Navarro-Ocaña, M.A. Valdivia-López *

Departamento de Alimentos y Biotecnología, Facultad de Química "E" – UNAM, Cd. Universitaria, 04510 México, D.F., Mexico

Received 15 January 2004; accepted 28 January 2004

Abstract

High dietary fibre (DF) powders from Persian and Mexican lime peels were prepared and their dietary fibre composition and antioxidant capacities determined. The total dietary fibre (TDF) contents of both varieties were high; 70.4% and 66.7%, respectively. Both lime peel varieties had an appropriate ratio of soluble/insoluble fractions. The water-holding capacities (WHC) of DF concentrates are high (6.96–12.8 g/g). The WHC was related to the soluble dietary fibre (SDF) which was higher in the DF concentrate of Mexican lime. As part of this analysis, the antioxidant activity (AA) of total extractable polyphenols (TEP) was studied, using three methods: azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical-scavenging activity, α,α-diphenyl–picrylhydrazyl (DPPH) and β-carotene-linoleic acid antioxidant assay. DF concentrates of Persian lime peel had greater polyphenol contents than those of Mexican lime peel. The polyphenols associated with the DF in both lime peel varieties showed a good AA. From a nutritional standpoint, DF lime concentrates may be suitable as food additives.

Keywords: Dietary fibre; Antioxidant capacity; Mexican lime; Persian lime; Polyphenols

1. Introduction

Lime is the second most important citrus fruit, in both fresh consumption and industrial uses. It is one of the main crops in Mexico and it is the fifth largest in harvested area worldwide. The two main varieties in the Mexican production of this fruit are the Persian lime (*Citrus latifolia*) and the Mexican lime (*Citrus aurantifolia*).

DF has shown beneficial physiological effects on the human body. The nutritional value of fruit DF concentrates is considerable, due to the presence of significant amounts of bioactive compounds, such as flavonoids and carotenoids. The high fibre content, the soluble/insoluble DF ratio, the water-holding capacity (WHC), the low energy value and phytic acid content also play important roles in its nutritional quality (Saura-Calixto, 1998).

Noteworthy are the natural sources of DF that combine antioxidant properties with the physiological effects of the fibre itself. The progress in the development of nutraceutical products from citrus peels underlines the importance of secondary metabolites such as flavones.

Flavonoids in citrus are found as compounds, namely methoxylated flavones and glycoside flavones. These are characteristic of citrus and because of specific patterns in each variety, they constitute good indicators of authenticity of commercial juices (Bocco, Cuvelier, Richard, & Berset, 1998). The outstanding features of these compounds are in the antioxidant properties that are useful for obtaining natural ingredients that can replace synthetic antioxidants.

Free radicals attack the saturated fatty acids in the biomembrane. They cause lipid peroxidation, permeation decrease and protein membrane damage, resulting in cellular inactivation. DNA is also subject to mutations which lead to cancer. An important correlation of cancer prevention, antimutation, and antioxidant properties exists (Yen & Hsieh, 1998). Antioxidants act as breakers of chain-reactions caused by free radicals.

* Corresponding author. Fax: +52-5622-5312.
E-mail address: mavald@servidor.unam.mx (M.A. Valdivia-López).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.076

The purpose of this study is to characterize the DF concentrates of lime peel obtained from the main Mexican varieties and to investigate the antioxidant activity as a potential functional ingredient in food products.

2. Materials and methods

2.1. Materials

The Mexican variety of lime (*C. aurantifolia*) was obtained in Apatzingan, Michoacan Mexico. The Persian lime (*C. latifolia*) came from the Central de Abastos Ciudad de México (Commodities Distribution Center at Mexico City). To prepare the DF concentrates, the juice was extracted and the remaining pulp was ground and washed with 96% ethanol, dried in a forced convection cabinet drier (Apex, model SSE70) at 60 °C, air flow 5 m/s, until moisture content was less than 8% and ground (Flour Grinding Mill, type SC, Chus Boeki Goshi Kaisha) a second time to a particle size of less than 0.5 mm.

2.2. Analysis of dietary fibre

Total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined as described elsewhere (Mañas, Bravo, & Saura-Calixto, 1994); a modification of the AOAC method was used (Prosky, Asp, Schweizer, Devries, & Furda, 1988) in which dialysis was used instead of ethanolic precipitation to avoid SDF loss.

2.3. Water-holding capacity and swelling determination

The WHC and swelling of the DF lime concentrates were determined according to the method described elsewhere (Robertson et al., 2000).

2.4. Determination of total extractable polyphenols

DF lime concentrate samples (500 mg) were sequentially extracted with 40 ml of methanol:water (50:50, v/v) and 40 ml acetone:water (70:30, v/v) for 1 h, in each case. Once centrifuged at 2500*g* for 15 min, the combined supernatants from the two previous extractions were concentrated in a vacuum rotatory evaporator at 50 °C, freeze-dried and dispersed in absolute ethanol. A spectrophotometric test was used on the total extractable polyphenols (TEP), following the Folin–Ciocalteau method, with gallic acid as standard (Larrauri, Rupérez, & Saura-Calixto, 1997).

2.5. Determination of antioxidant activity

The activity of antioxidants in foods and biological systems depends on a multitude of factors; a reliable antioxidant protocol demands measuring more than one property relevant to either foods or biological systems. The antioxidant capacity of polyphenolic extracts from Mexican and Persian lime peels was determined through three complementary assay procedures.

2.5.1. Scavenging effect on DPPH radical

The capacity of extracts to scavenge the DPPH radical was estimated according to the procedure described by others (Siddhuraju, Hohan, & Beckera, 2000). A volume of 2 ml of 3.6×10^{-5} M methanolic solution of DPPH (Sigma) was added to 50 μl of a methanolic solution (200 $mg\,l^{-1}$) of the antioxidant. The decrease in absorbance at 515 nm was continuously recorded in a spectrophotometer (Perkin–Elmer Model C618-0432) for 16 min. The scavenging effect, expressed as the decrease of absorbance at 515 nm, was plotted against time and the percentage of DPPH radical-scavenging ability (SA) of the sample was calculated from the absorbance value at the end of 16 min as follows:

$$\mathrm{SA} = \left(\frac{A_0 - A_{16}}{A_0}\right) 100,$$

where A_0 = absorbance at 0 min; A_{16} = absorbance at 16 min.

2.5.2. Scavenging effect on ABTS radical

The method measures the capacity of different components to scavenge the ABTS radical cation ($ABTS^{+}$) (Arnao, Cano, & Acosta, 2001). The hydrophilic antioxidant activity was measured in a reaction mixture that contained 0.5 ml of H_2O_2 15 μM, 0.5 ml of ABTS 2 mM, and 0.5 ml of horseradish peroxidase (1 mg/ml in 50 mM Na-phosphate buffer (pH 7.5); donor was hydrogenperoxideoxidoreductase; EC 1.11.1.7, 50,000 U, P-8250, Sigma). The reaction was monitored at 730 nm, until a stable absorbance was reached. Then, 0.5 ml of the extract (50 mg/kg) was added to the reaction medium and the decrease in absorbance was recorded during a 12 min period (Arnao et al., 2001). Scavenging ability was calculated as follows:

$$\mathrm{SA} = \left(\frac{A_0 - A_{12}}{A_0}\right) 100,$$

where A_0 = absorbance at 0 min; A_{16} = absorbance at 12 min.

2.5.3. Determination of antioxidant activity by the β-carotene bleaching method

Twenty milligrammes of linoleic acid and 200 mg of Tween 80 were transferred into a flask, and 1 ml of a solution of β-carotene (0.2 mg/ml) in chloroform was added. Chloroform was removed at 40 °C under vacuum. Then, 50 ml of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. To an aliquot of 5 ml of this

emulsion, 0.2 ml of an antioxidant solution was added, and the absorbance was immediately measured at 470 nm. The tubes were placed in a water bath at 50 °C and the absorbance was measured (Matthaüs, 2002). The antioxidant activity (AA) of the extracts was calculated from the absorbance value at the end of 2 h as follows:

$$AA = \left[\frac{A_{E2} - A_{C2}}{A_{C0} - A_{E2}}\right] 100,$$

where A_{E2} = absorbance of extract at 2 h; A_{C2} = absorbance of control at 2 h; A_{C0} = absorbance of control at 0 h.

2.6. Statistics

Data obtained were analyzed using the ANOVA technique. Significance of differences between samples to a 5% level was evaluated using Duncan's multiple range test.

3. Results and discussion

3.1. Dietary fibre content and functional properties

Soluble, insoluble and total dietary fibre contents of Mexican lime peels concentrates are shown in Table 1. Fibre must have a balanced composition of soluble and insoluble fractions in order to retain all its properties. Such is the case for all the Mexican products derived from the Mexican and Persian varieties of lime peel. These products contain 27–31% of SDF from total dietary fibre while wheat bran has only 6.46% (Grigelmo & Martín, 1999).

Insoluble dietary fibre was the predominant fraction in all the samples. The main components of IDF are neutral sugars (NS) which make up to 25–31% of total dietary fibre. The content of Klason lignin found (7.45–7.67%) is similar to the values of other citrus fruits, as in the case of orange peel with 11.6% (Mañas et al., 1994). Lignin is related to the hypocholesterolemic effect associated with fibre consumption due to its capacity to absorb bile acids. The ratio of soluble to insoluble fractions in DF must be within the range of 1.0–2.3 to be able to exert the physiological effect associated with both fractions in dietary fibre (Grigelmo, Martín, & Martín, 1999). According to what was previously mentioned, both the Mexican and the Persian varieties of lime contain the best SDF–IDF ratio (2.2–2.7). Lime peel is a good source of dietary fibre (DF) that contains a balanced proportion of soluble and insoluble fractions (Larrauri, Rodríguez, Fernández, & Borroto, 1994).These results indicate that the dietary fibre in these varieties may confer benefits from a nutritional and health standpoint.

3.2. Water-holding capacity and swelling

Dietary fibre from Mexican limes is outstanding, due to its ability to retain more than 10 times its weight of water (Table 2) as a result of a greater content of SDF. WHC is high in both cases when compared with fibre in other fruits such as pineapple peel (3.5), apple (6.3) and pear (6.8) (Grigelmo et al., 1999). The high WHC opens the possibility of using DF lime concentrate as a functional ingredient for reducing calories, avoiding syneresis and modifying the viscosity of formulated foods. The swelling values obtained (10.2–14.2 ml/g DF) are similar to those reported in DF of citrus fruits (Robertson et al., 2000).

3.3. Total extractable polyphenols and antioxidant activity

The contents of total phenolic compounds in crude extracts obtained from each lime are presented in Fig. 1; the results are reported as milligrammes of gallic acid per grammes of DF.

The TEP values in DF concentrates of Mexican and Persian lime were 10.55 and 19.90 mg/g, respectively. The highest content of TEP was found in the DF concentrate of Persian lime (Fig 1). It is likely that the main polyphenol components of fibre extracts are hesperidin, fe-

Table 2
WHC and swelling of lime DF concentrates

Sample	WHC (grammes of water per gramme of DF)	Swelling (ml/g of DF)
Mexican	12.84 ± 0.54^{a}	13.64 ± 0.52^{a}
Persian	6.96 ± 0.65^{b}	11.34 ± 0.34^{b}

Values in a column with different letters are significantly different at $p \leqslant 0.05$.

Table 1
Dietary fibre composition of powdered lime peel (dry weight base, g/100 g)

Sample	Soluble dietary fibre		Insoluble dietary fibre			Total dietary fibre
	UA[A]	NS[B]	UA	NS	KL[C]	
Mexican	14.3 ± 3.89^{a}	7.59 ± 1.29^{a}	13.1 ± 1.50^{a}	27.9 ± 2.91^{a}	7.67 ± 0.47^{a}	70.4^{a}
Persian	13.2 ± 3.95^{a}	7.06 ± 1.68^{a}	12.2 ± 0.91^{a}	26.8 ± 1.25^{a}	7.45 ± 0.92^{a}	66.7^{b}

Values in a column with different letters are significantly different at $p \leqslant 0.05$.
[A] Uronic acids.
[B] Neutral sugars.
[C] Klason lignin.

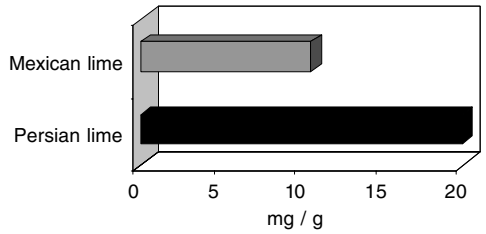

Fig. 1. TEP in DF concentrates of lime in milligrammes of gallic acid per gramme of DF.

rulic acid, ellagic acid, kaempferol, quercetin and caffeic acid (Larrauri, Rupérez, Bravo, & Saura-Calixto, 1996).

3.4. Antioxidant activity in extracts from Mexican and Persian limes

Three in vitro antioxidant assays were examined as standard ways to assess the potential AA of extracts from Mexican and Persian lime peels.

3.5. DPPH radical-scavenging activity

The radical-scavenging activity, using a DPPH generated radical, was tested with the polyphenolic extract from Mexican and Persian lime peels and with Trolox. It can be observed in the kinetics (Fig. 2) that the radical-scavenging activity was faster in the Persian lime peel extract than in the Mexican lime extract. Trolox showed the fastest radical-scavenging activity and the greatest percentage of inhibiting activity. A rapid absorbance decrease indicates a large antioxidant activity in terms of the ability of the compound to act as a hydrogen donor (Siddhuraju et al., 2000). In this test, taking place in an organic system, the TEP concentrate of lime peel extracts has a low hydrogen-donating ability.

3.6. ABTS radical-scavenging activity

Due to the reaction medium, the ABTS test estimates the hydrophilic antioxidant activity of the extracts. The effects on ABTS of the extract of DF concentrates of Mexican and Persian lime, are shown in Fig 3. There was no significant difference between the two varieties. However, the Persian lime extract seems to have a faster radical-scavenging activity than the Mexican lime extract, but essentially the same as that of Trolox.

3.7. Determination of antioxidant activity with the β-carotene bleaching method

The oxidative destruction of β-carotene by the products of linoleic acid degradation is measured by the decrease in absorbance at 470 nm. Table 3 shows the percentages of antioxidant activity of lime peel extracts on β-carotene.

In this test, the antioxidants must act in an emulsion of linoleic acid and β-carotene. In contrast with the triacylglycerols, linoleic acid forms micelles in aqueous systems. These micelles have colloidal properties that strongly affect both the behaviour of oxidation initiators and antioxidants (Frankel & Meyer, 2000).

The lime extracts show high antioxidant activity, largely preventing the bleaching of β-carotene which indicates a good capacity for reduction of the radicals generated by the oxidation of linoleic acid.

Both extracts showed a high scavenging capacity by the ABTS test (for hydrophilic antioxidants). The lipidic systems formed by the micelles in the aqueous media, such as linoleic acid, greatly enhance the hydrophilic and polar antioxidant activity. The results of both tests could be due to a higher concentration of polar polyphenols in the lime peel extracts. In contrast, the lime peels showed lower AA with the DPPH test (for hydrophobic antioxidant).

The relationship between the total phenolic content and the radical-scavenging activity, as determined by all of the assays tested here, was very close in the Persian lime peel. These data agree with other works (Holasova et al., 2002; Gorinstein et al., 2003).

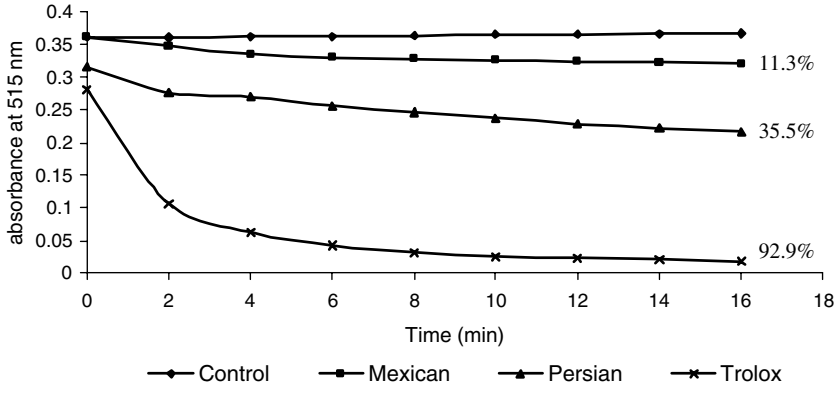

Fig. 2. Kinetics of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging capacity of DF concentrate of lime extracts and Trolox. End values at $t = 16$ min indicate the radical-scavenging percentage.

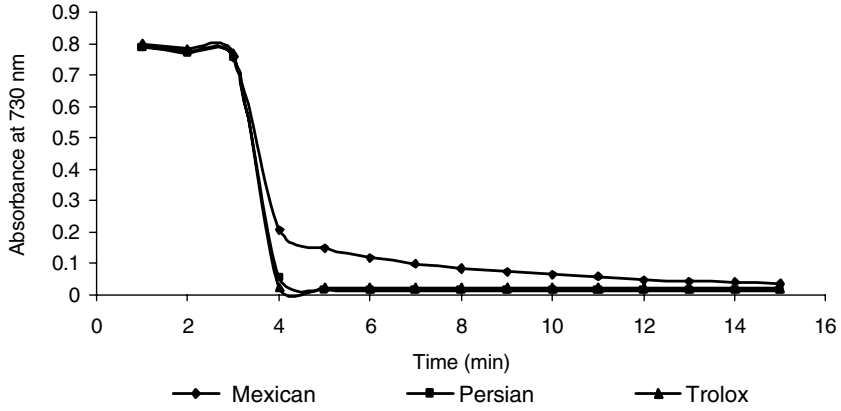

Fig. 3. Hydrophilic antioxidant activity estimated using the ABTS/HRP discoloration method.

Table 3
Antioxidant activity determined by β-carotene bleaching

Sample	%AA
Mexican	88.4 ± 0.32^{a}
Persian	89.1 ± 0.86^{b}
TROLOX	89.5 ± 0.56^{b}

Values in a column with different letters are significantly different at $p \leqslant 0.05$.

Acknowledgements

The authors are grateful to the Fundación Produce Michoacán, A.C. for their financial support.

References

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry, 73*, 239–244.

Bocco, A., Cuvelier, A., Richard, H., & Berset, C. (1998). Antioxidant activity and phenolic composition of citrus peel and seed extracts. *Journal of Agricultural and Food Chemistry, 46*, 2123–2129.

Frankel, E. N., & Meyer, A. S. (2000). Review: The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Gorinstein, S., Martín-Belloso, O., Katrich, E., Lojek, A., Ciz, M., Gligelmo-Miguel, N., Haruenkit, R., Park, Y., Jung, S., & Trakhtenberg, S. (2003). Comparison of the contents of the main biochemical compounds and the antioxidant activity of some spanish olive as determined by four different radical scavenging tests. *Journal of Nutritional Biochemistry., 14*, 154–159.

Grigelmo, N., & Martín, O. (1999). Characterization of dietary fibre from orange juice extraction. *Food Research International, 31*(5), 355–361.

Grigelmo, N., Martín, O., & Martín, M. (1999). Comparison of dietary fibre from by-products of processing fruits and greens and from cereal. *Lebensmittel-Wissenschaft und -Technologie, 32*, 503–508.

Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, J., & Vavreinova, S. (2002). Buckwheat the source of antioxidant activity in functional foods. *Food Research International., 35*, 207–211.

Larrauri, J. A., Rodríguez, J. L., Fernández, M., & Borroto, B. (1994). NOTA. Fibra dietética obtenida a partir de hollejos cítricos y cáscaras de piñas. *Revista Española de Ciencia y Tecnología de los Alimentos, 34*, 102–107.

Larrauri, J. A., Rupérez, P., Bravo, L., & Saura-Calixto, F. (1996). High dietary fibre powders from orange and lime peels: Associated polyphenols and antioxidant capacity. *Food Research International, 29*(8), 757–762.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Pineapple shell as a source of dietary fibre with associated polyphenols. *Journal of Agricultural and Food Chemistry, 45*, 4028–4031.

Mañas, E., Bravo, L., & Saura-Calixto, F. (1994). Sources of error in dietary fibre analysis. *Food Chemistry, 50*, 331–342.

Matthaüs, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural and Food Chemistry, 50*(12), 3444–3452.

Prosky, L., Asp, N. G., Schweizer, T. F., Devries, J. W., & Furda, I. (1988). Determination of insoluble, soluble and total dietary fibre in foods and food products: Laboratory study. *Journal of the Association of Official Analytical Chemists, 75*(2), 360–367.

Robertson, J. A., Monredon, F. D., Dysseler, P., Guillon, F., Amdó, R., & Thibault, J. F. (2000). Hydration properties of dietary fibre and resistant starch: A European Collaborative Study. *Lebensmittel-Wissenschaft und-Technologie, 33*, 73–79.

Saura-Calixto, F. (1998). Antioxidant dietary fibre product: A new concept and a potential food ingredient. *Journal of Agricultural and Food Chemistry., 46*, 4303–4306.

Siddhuraju, A., Hohan, B., & Beckera, K. (2000). S. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of rude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry, 79*, 61–67.

Yen, G. C., & Hsieh, C. L. (1998). Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) towards various lipid peroxidation models in vitro. *Journal of Agricultural and Food Chemistry, 46*, 3952–3957.