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Changes in nutraceutical composition of lemon juices according to different industrial extraction systems

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Abstract

Flavonoids and ascorbic acid have gained especial relevance due to their functional properties. Their level in processed foodstuffs depend not only on the raw material composition but also on the processing system employed. The contents of these two nutraceuticals in juice are determined in two lemon varieties. The highest ascorbic acid contents are in Fino lemon juice, irrespective of the system of extraction used. However, a clear technological effect of the extraction system has been found on the levels of the different types of flavonoids. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Nowadays, it is widely accepted that the beneficial health effects of fruits and vegetables, in the prevention of heart disease and certain types of cancer, are due to their bioactive components (Benavente-García, Castillo, Marín, Ortuño, & Del Río, 1997; Block, 1992; Block, Patterson, & Subar, 1992). Some reports suggest that ascorbic acid (vitamin C) and flavonoids may inhibit these diseases due to their antioxidant potential (Hertog, Feskeens, Hollman, Katan, & Kromhout, 1993; Salah, Miller, Paganga, Tijburg, Bolwell, & Rice Evans, 1995).

The influence of ascorbic acid in processes such as the absorption of iron, aminoacid metabolism, hormones and cell oxidoreduction processes, is well-established (Buettner, 1993). This has led to the suggestion that vitamin C, as a natural antioxidant, may inhibit the development of major clinical conditions and certain cancers (Diplock, 1991). Flavonoids are also attracting more and more attention due to their properties as antioxidants, anticarcinogenic agents, antiinflammatories and because of their lipid antiperoxidation effects (Elangovan, Sekar, & Govindasamy, 1994; Godeberge, 1994; Jean & Bodinier, 1994; Marín, Frutos, Pérez-Alvarez, Martinez-Sánchez, & Del Río, in press; Meyer, 1994; Rice Evans, Miller, & Paganda, 1997).

Citrus fruits are important sources of ascorbic acid and flavonoids (Benavente-García, Castillo, & Del Río, 1993; Castillo, Benavente-García, & Del Río, 1993; Del Río, Arcas, Benavente-García, Sabater, & Ortuño, 1998; Ortuño, Arcas, Benavente-García, & Del Río, 1999) and are frequently used in industry for the extraction of flavanones (Baer, Borrego, Benavente-García, Castillo, & Del Río, 1990; Del Río, Fuster, Sabater, Porras, García Lidón, & Ortuño, 1997). During the industrial process, the fruits, which are used to obtain concentrates, juices and nectars, undergo different extraction processes which may alter the contents of these compounds in the end-product. Degradations may also occur, especially of ascorbic acid, as a consequence of heat-treatment and storage (Kabasakalis, Siopidou, & Moshatou, 2000). In this respect, there are few reports available on the contributions of flavonoids and vitamin C to the antioxidant activity in citrus juices. Those that have been published are scarce and do not quantify the contributions of the different types of flavonoids to the TAA (Total Antioxidant Activity), nor the effect of the process on possible interactions between the two types of compound. In the main they are confined to widely consumed juices, such as orange, grapefruit, apple or pineapple (Gardner, White, McPhail, &

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Duthie, 2000; Leuzzi, Caristi, Panzera, & Licandro, 2000).

Our objective in this work was to study the contents of flavonoids and vitamin C, as well as their effects on the antioxidant potential in juices from two varieties of lemon juice (Fino and Verna), obtained by manual extraction and by means of two different industrial systems. The study underlines the different compositions of these bioactive compounds undergoing the different processes and thus their different nutritional quality.

2. Material and methods

2.1. Vegetable samples

Lemon fruits (*Citrus limon*) were used from main-flowering season floration harvests and were of the Fino (clon 49) and Verna (clon 50) varieties, rootstock on *Citrus macrophylla*, collected from orchards situated in Murcia (Spain).

2.2. Extraction systems of the juice

The extraction systems of the juice from the lemons were the following:

Extraction System 1 (ES-1): non-industrial system. The juice was obtained by squeezing the lemons and filtering through a nylon sieve

Extraction System 2 (ES-2): "in line" extraction system from Food Machinery Corporation (FMC, USA). Extraction was done by squeezing after incisions had been made in the polar areas of the fruit. The pulp and juice were separated by means of a prefinisher tube with a pore size of 0.062 mm.

Extraction System 3 (ES-3): "Indelicato" System from Fratelli Indelicato (Italy). The extraction was performed in two stages. The first consisted of an extraction using two rollers turning in opposite directions [Polycitrus (ES-3.1)]. The second stage consisted of a pressing of the fruits from ES-3.1, using a screw press [Polypress (ES-3.2)]. The juices obtained by ES-3 were mixed in variable proportions to obtain the final juice. In this case the proportion was 60:40 (ES-3.1:ES-3.2).

2.3. Methods of analysis

The determinations of the acidity, expressed in g of citric acid/100 mL, were performed using method No. 3 of the IFJU (International Fruit Juice Union).

The content of soluble solids, expressed in °Brix, was obtained by refractometer, in an ATAGO 1T table refractor meter (Atago Co. Ltd., Japan).

The content of essential oils, expressed in ppm, was obtained by means of method No. 30021 of the AOAC.

The content of ascorbic acid, expressed in mg/l, was obtained by means of method No. 43056 of the AOAC.

For the quantification of flavonoids, an ODS-C18 (250×4.6 mm i.d.) analytical column was used with an average particle size of 5 μm, using water: acetonitrile: methanol: acetic acid (15:2:2:1) as the eluent with a flow rate of 1 ml/min at 30 °C. The absorbance change was monitored at 280 and 350 nm with a Hewlett Packard mod. HP 1100 UV°Vis diode array detector (Castillo, Benavente-García, & Del Río, 1994).

For the identification of flavonoids, the procedure described by Castillo et al. (1994) was used. All compounds isolated were identified by their melting points (Gallenkamp, England), mass spectrum (EIMS) (Hewlett-Packard Co., USA) and the 1H NMR (200 MHz) and 13C NMR (50 MHz) (Brucker, Germany) in hexadeutero-DMSO.

The TAA (total antioxidant activity) in the juices was measured spectrophotometrically using the Miller, Rice-Evans, Davies, Gopinathan, and Milner method (1993), based on the relative capacity of different substances to stabilize the radical cation of ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), compared to a standard antioxidant (Trolox) by means of a response–dosage curve. The contribution to the TAA, of the different components, was obtained by measuring this in dissolutions prepared from the analytical data obtained.

3. Results and discussion

3.1. Differences in the acidity, soluble solids, essential oils and ascorbic acid between the juices from lemons (Fino and Verna varieties)

The results obtained reflect a greater acidity in the juice obtained by the non-industrial process (ES-1) in both the Fino variety (7.70 g/100 mℓ) and Verna (5.53 g/100 mℓ) (Table 1). The juice from the Fino variety presents higher levels of acidity than Verna, irrespective of the extraction system used. Thus, values obtained were higher by about 39, 27 and 21%, respectively, for ES-1, ES-2 and ES-3 in juice from Fino lemons than to that from Verna (Table 1).

Higher soluble solids levels were observed in juice from Fino lemons than from Verna, irrespective of the system of extraction employed. Thus, values obtained were higher by about 40, 37 and 12%, respectively, for ES-1, ES-2 and ES-3 in juice from Fino lemons than that from Vernas (Table 1).

The contents of essential oils in the juices obtained using ES-1 and ES-2, in both varieties, were very similar. The differences for both were less than 5% (Table 1). However, when ES-3 was used to obtain the juice, the contents of essential oils were found to be 4.71 and 2.03

Table 1
Composition of the lemon juices from the Fino and Verna varieties obtained by means of different extraction systems

	Acidity (g/100 mℓ)	°Brix	Essential oils (ppm)	Ascorbic Ac (mg/ℓ)	Flavonoids (mg/ℓ)
Fino					
ES-1	7.7±0.9	9.0±0.5	21.0±1.2	532±20.2	220±10.2
ES-2	6.4±0.7	9.2±0.6	21.5±2.3	573±40.6	580±30.7
ES-3	5.8±0.3	9.0±0.4	4706±823	457±37.2	583±40.5
ES-3.1	6.8±0.5	8.5±0.3	840±30.1	252±31.2	173±20.9
ES-3.2	4.5±0.3	9.5±0.2	10520±1654	763±22.6	1205±332
Verna					
ES-1	5.53±0.5	6.5±0.6	20.0±1.7	261±18.8	371±20.4
ES-2	5.05±0.6	6.7±0.9	20.5±1.9	310±25.2	686±27.1
ES-3	4.83±0.4	7.5±0.9	2033±324	361±27.4	854±42.5
ES-3.1	5.33±0.7	7.5±0.7	241±12.3	294±32.7	253±38.3
ES-3.2	4.09±0.5	7.9±0.4	4721±216	462±15.0	1751±121

The values are expressed as an average±ES ($n=3$).

ppm for Fino and Verna varieties, respectively.This gives a 130% higher content in Fino than in Verna (Table 1).

As for the acidity and soluble solids, the levels of ascorbic acid in Fino lemon juice, irrespective of the system of extraction used, were higher than those found in Verna juice. Values obtained were higher by about 104, 85 and 27%, respectively, for ES-1, ES-2 and ES-3 in juice from Fino lemons than that from Vernas (Table 1).

The effect of the system of extraction on the parameters studied is practically irrelevant as regards acidity, soluble solids and ascorbic acid, although it is interesting to note the high amount of ascorbic acid produced when the juice was obtained using ES-3. On the hand, greater differences are seen in the amounts of essential oils, depending on the system of extraction used. The greatest effect is observed in the content of essential oils produced by ES-3. In this case, the contents of essential oils were 224 and 101 times higher in Fino and Verna, respectively, than that which would be obtained by means of a system of extraction (ES-1) similar to a domestic one. On the other hand, the juice obtained using ES-2 had a content of essential oils similar to the juice obtained manually (ES-1), irrespective of the variety studied (Table 1). The huge amounts of essential oils observed in those juices obtained by ES-3 is explained by the nature of the system used. During the first step (ES-3.1), the fruit was subjected to pressure between two rollers turning in opposite directions and in which not only juice was obtained but also the essential oils present in the rind. Thus, levels of essential oils in ES-3.1 of approximately 840 and 240 ppm were obtained, in Fino and Verna respectively. Next, the fruits were pressed in a screw-press (ES-3.2) yeilding contents of essential oils, in the juice of this second squeezing about 500 and 236 times greater, in the juice from Fino and Verna lemons, respectively, than that obtained in the juice extracted manually (ES-1). On the other hand, in ES-2 the essential oils in the rind were eliminated by the washing process, quantified by an internal circuit of the extractor. They did not come into contact with the pulp which was subjected to extraction in the interior of the prefinisher tube, which accords with the low levels of essential oils found in the juice, in both Verna and Fino, obtained by ES-2. These great differences give the juices obtained by ES-3 their distinct organoleptic characteristics

Table 2
Levels of predominant flavonoids (mg/ℓ) in lemon juice of the Fino and Verna varieties, obtained by different extraction systems

	Eriocitrin	Hesperidin	Diosmin	Luteolin-7-*O*-rutinóside
Fino				
ES-1	81±5	104±15	10±2	22±9
ES-2	203±20	240±30	41±3	65±7
ES-3	205±13	253±28	51±8	64±4
Verna				
ES-1	111±12	224±20	13±3	15±2
ES-2	193±22	395±31	41±12	42±15
ES-3	391±22	410±40	28±9	28±5

The results are expressed as an average value±ES ($n=3$).

The greatest technological improvement of the extraction systems on the content of ascorbic acid was observed in the juices obtained by the ES- 3.2 method. In this case, the contents of ascorbic acid were 43 and 77% higher, in Fino and Verna, respectively, than by using ES-1 (Table 1). This great difference, disappears after mixing the juices obtained by ES-3.1 and ES-3.2 (Table 1).

3.2. Levels of flavonoids in the juice of lemon (Fino and Verna varieties)

Flavonoids appeared to be higher in Verna lemon juice, than Fino, regardless of the system of extraction used. Thus, for ES-1, ES- 2 and ES-3, values approximately 68, 17 and 45% greater, respectively,

Table 3
Total antioxidant activity (TAA) and percentage contribution to TAA of the different components of the juice

	TAA (mg/ℓ)	Flavonoids (% TAA)	Ascorbate (% TAA)	Unidentified antioxidants
Fino				
ES-1	808±20	9.89±3	65.9±10	24.2±7
ES-2	1101±24	19.0±4	52.0±15	29.1±11
ES-3	997±33	23.4±5	49.8±12	26.8±9
Verna				
ES-1	781±19	16.1±6	33.3±11	50.6±17
ES-2	1.049±22	21.8±9	29.6±12	48.6±16
ES-3	1135±25	21.8±7	29.6±10	49.6±15

TAA is expressed as antioxidant capacity equivalent to a solution of ascorbic acid (mg/l). The values are expressed as an average±ES ($n=3$).

were obtained in the juice from Vernas than that from Fino (Table 1). However, an effect is observed, regarding the total content, dependent on the system of extraction. Thus, for both varieties, the flavonoid content was lower in juices obtained by the manual method (ES-1) than when the industrial processes (ES-2 and ES-3) were used. In both cases, the amounts of flavonoids in industrial juices (ES-2 and ES-3) were at least double that of the juice obtained manually (ES-1) (Table 1).

In the juices from both varieties of lemon, the presence of eriocitrin and hesperidin flavonones was noted, as well as luteolin-7-*O*-rutinoside and diosmin flavones. These results agree with those obtained by Horowitz and Gentili (1977) for this species of citrus. Eriocitrin, luteolin-7-*O*-rutinoside, hesperidin and diosmin constitute approximately 94%, (for juices from Fino and Verna lemons extracted by ES-2), to approximately 98% (in the remaining lemon juices), of the total flavonic content present in the juices of the varieties studied herein (Table 2). In the juices of both varieties, regardless of the extraction process used, the flavanones (hesperidin and eriocitrin) predominate over the flavones (luteolin-7-*O*-rutinoside and diosmin).

There are clear differences regarding the relationship between flavanones and flavones. Thus, the flavonones, eriocitrin and hesperidin, are found in greater proprtion in the juice from the Verna lemon than Fino, regardless of the system of extraction used (Table 2). On the other hand, the luteolin-7-*O*-rutinoside and diosmin flavones are found in greater proportions in the juices of Fino lemons than in those of Verna. In this way, the total content is greater in Fino lemon juice than in Verna, despite the fact that the total content in flavonoids is greater in the jucie of Verna lemons. Also, it is interesting to note that, while the luteolin-7-*O*-rutinoside:-diosmin ratio is very close to 1 in the juices from Verna lemons, regardless of the system of extraction used, in Fino lemons the content of luteolin-7-*O*-rutinoside is always greater than that of diosmin, regardless of the method of extraction used (Table 2). These data may be important from a nutritional point of view, since several of these, for example the flavone diosmin, have a tested pharmacological effect on certain illnesses of the circulatory system (Jean & Bodinier, 1994) and as protectors against carcinogenic processes started by benzanthracenes, which appear when foodstuffs are being cooked (Ciolino, Wang, & Yeh, 1998).

A clear technological effect on the composition of flavonoids can also be seen, depending on the extraction system used. Thus, while the percentage of flavones, is approximately 15% in Fino lemon juice obtained by the ES-1 system, this figure reaches percentages of about 18% in the juice obtained by using ES-2 and 20% in juice obtained with the ES-3 system (Table 2), which would imply a possible greater contribution of albedo and flavedo in these systems of extraction (ES-2 and ES-3) compared to the manual method. Also, it was observed that, in Fino lemon juice obtained using ES-2 and ES-3, the luteolin-7-*O*-rutinoside:diosmin ratio was lower than ES-1. While this ratio is equal to 2.2 for juice obtained using ES-1, it is 1.58 for that from ES-2 and 1.25 for that from ES-3, thus increasing the functionality of the industrial juices (ES-2 and ES-3) compared to those obtained manually, at least with respect to the content in diosmin. Likewise, these results agree with the distribution of these compounds in the different tissues of the fruit (Marín & Del Río, 2001) and a greater supply of flavedo and albedo in industrial extractions than in manual extractions.

3.3. Antioxidant activity in lemon juice (Fino and Verna varieties)

Lemon juices from the Fino and Verna varieties, obtained by the ES-1 system, showed similar antioxidant activities (Table 3). The Fino lemon juice showed a total antioxidant activity equivalent to a disolution of 808 mg of ascorbic acid/ℓ and that of Verna of 781 mg of ascorbic acid/ℓ.

However, there are important differences regarding the contributions which the different bioactive components make to the TAA. Thus, while in Fino lemon juice, ascorbic acid contributes approximately 66% of the total antioxidant activity, in Verna juice it contributed approximately 33% (Table 3). These results agree with the greater content of ascorbic acid in Fino lemon juice than in Verna lemons (Table 1). The flavonoids contribute approximately 10–16% of TAA in the juice from Fino and Verna lemons. The percentage of TAA which corresponds to unidentified antioxidants is approximately a quarter of the total from non-industrial Fino lemon juice and about half in Verna lemons, where it is the most important fraction from this point of view. Due to the fact that, when the tests were being performed, no synergic effect was observed, in the proprtions used, the percentage of TAA which corresponds to unidentified compounds might be explained by the presence of carotenoids and essential oils with antioxidant activity. The ES-1 juice from Fino lemons is characterised by a large contribution of ascorbic acid to the TAA while that of Verna lemons by the contribution of unidentified antioxidants and flavonoids which, together, amount to approximately 66% of TAA.

When the technological effect is compared to the extraction effect on TAA it is observed that, in the ES-1 juices (manual extraction), the contribution of ascorbic acid to TAA is greater, regardless of the variety, than that produced in ES-2 and ES-3 juices. Since the levels of ascorbic acid are similar in the juices obtained by the three extraction systems (Table 1) this variation must be due to the increase in the contents of flavonoids observed in ES-2 and ES-3 juices, regardless of the variety (Table 1). This also explains the net increase of TAA in ES-2 and ES-3 juices compared to ES-1 juices, where this is over 30% higher in Verna lemon juice (Table 3).

In both varieties of lemon (Fino and Verna), and for systems ES-2 and ES-3, the contribution of flavonoids to the TAA is greater in juices obtained using ES-3 than ES-2. This contribution would amount to approximately 23 and 29% in Fino and Verna, respectively, for ES-3 juices compared to a contribution to TAA of approximately 19 and 22% in Fino and Verna, respectively, for ES-2 juices (Table 3). However, regarding the contribution of ascorbic acid to TAA, the opposite occurs. In this case the greatest contribution is produced in ES-2 juices, regardless of variety (Table 3).

If losses in bioactive components are not taken into account (during pasturisation and possibly during processes of concentration) the juices obtained by ES-2 and ES-3 have higher contents of bioactive compounds. Similar or higher contents of ascorbic acid have been found than in ES-1 juices and are clearly greater with respect to flavonoids. Between these two systems, and due to the low content in essential oils, the ES-2 system seems to resemble more the composition, and (probably) the organoleptic characteristics of the juice obtained manually.

Cossins, Lee and Packer (1998) proposed a model of a cell antioxidant chain in which both the ascorbic acid and flavonoids constitute a fundamental part. On the other hand, Fino lemon juice presents values of TAA similar to those of Verna (Table 3). However, the contributions of flavonoids and ascorbic acid to TAA are clearly different. Thus, in the juices from Fino lemons these compunds contribute approximately 60–75% to TAA while, in Verna lemon juice they constitute 50%. In the light of these results it is possible to state that Fino lemons supply a potential antioxidant (nutraceutical composition) more suited to the cell oxidant chain model than Verna lemons.

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