Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 1021–1030
Food Chemistry
www.elsevier.com/locate/foodchem

A new integrated membrane process for the production of concentrated blood orange juice: Effect on bioactive compounds and antioxidant activity

Gianni Galaverna [a,*], Gianluca Di Silvestro [c], Alfredo Cassano [b], Stefano Sforza [a], Arnaldo Dossena [a], Enrico Drioli [b], Rosangela Marchelli [a]

[a] *Dipartimento di Chimica Organica e Industriale, Università di Parma, V.le G. P. Usberti 17/A, I-43100 Parma, Italy*
[b] *ITM-CNR, Istituto per la Tecnologia delle Membrane, c/o University of Calabria, Via P. Bucci, 17/C, I-87030 Rende (CS), Italy*
[c] *Centro Ricerche Parmalat, Via San Vitale, 22, I-43038 Castellaro di Sala Baganza (PR), Italy*

Received 1 February 2007; received in revised form 11 May 2007; accepted 10 July 2007

Abstract

The production of high quality concentrated blood orange juice according to a new integrated membrane process, alternative to thermal evaporation, was evaluated in terms of preservation of the total antioxidant activity and of the bioactive antioxidant components of the juice (ascorbic acid, anthocyanins, hydroxycinnamic acids, flavanones). The process was based on the initial clarification of freshly squeezed juice by ultrafiltration (UF); the clarified juice was successively concentrated by two consecutive processes: first reverse osmosis (RO), used as a pre-concentration technique (up to 25–30 °Bx), then osmotic distillation (OD), up to a final concentration of about 60 °Bx. During the concentration process of the liquid fractions, a slight decrease of total antioxidant activity (TAA) was observed (−15%), which was due to the partial degradation of ascorbic acid (ca. −15%) and anthocyanins (ca. −20%). Nevertheless, this degradation was lower than that observed with thermally concentrated juice: TAA, −26%; ascorbic acid, −30%, anthocyanins, −36%. The possibility to operate at room temperature allowed reduction in thermal damage and energy consumption. On the basis of these results, the integrated membrane process may be proposed as a valuable alternative to obtain high quality concentrated juice, as the final product still showed a very high antioxidant activity and a very high amount of natural bioactive components, showing a brilliant red colour and a pleasant aroma, characteristics that were significantly lost during traditional thermal evaporation.

Keywords: Total antioxidant activity; Orange juice; Ultrafiltration; Reverse osmosis; Osmotic distillation; Polyphenols

1. Introduction

Orange juice is probably the best known and most widespread fruit juice all over the world, particularly appreciated for its fresh flavour and considered of high beneficial value for its high content in vitamin C and natural antioxidants, such as flavonoids and phenylpropanoids (Gardner, White, McPhail, & Duthie, 2000; Miller & Rice-Evans, 1997; Rapisarda et al., 1999). Indeed, according to recent epidemiological studies, high consumption of orange juice is associated with a reduced risk of free radical related oxidative damages and diseases such as different types of cancer, cardiovascular or neurological diseases (Ames, 1998; Baynes & Thorpe, 1999; Bazzano et al., 2002; Finkel & Holbrook, 2000; Franke, Pra, Erdtmann, Henriques, & da Silva, 2005; Kaur & Kapoor, 2001; Sauvaget et al., 2003; Vinson et al., 2002). Blood orange juice is a typical Italian product characterized by the presence of higher amounts of these health promoting substances, in comparison with blond orange juices. In particular, it is very rich in ascorbic acid and hydroxycinnamic acids and characterized by the presence of anthocyanins,

* Corresponding author. Tel.: +39 0521 906196; fax: +39 0521 905472.
E-mail address: gianni.galaverna@unipr.it (G. Galaverna).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.018

which are responsible of the bright red colour (Proteggente, Saija, De Pasquale, & Rice-Evans, 2003; Riso et al., 2005). Three types of this product are mainly present on the European market: fresh juices, obtained by simple squeezing and mild pasteurization (fresh squeezed), not from concentrate juices (NFC) obtained by freezing after squeezing and juices reconstituted from concentrate (RFC). A large part of the market is based on the latter products, as the concentration process (up to 60 °Bx final concentration of dissolved solids) allows to reduce storage volumes (thus reducing transport and storage costs) and to facilitate preservation. Nevertheless, when concentration is carried out by traditional multistep vacuum evaporation, a severe loss of the volatile organic flavour/fragrance components occurs as well as a partial degradation of ascorbic acid and natural antioxidants, accompanied by a certain discolouration and a consequent qualitative decline (Arena, Fallico, & Maccarone, 2001; Maccarone, Campisi, Cataldi Lupo, Fallico, & Nicolosi Asmundo, 1996). These effects are mainly attributable to heat transfer to the juice during evaporation. In order to overcome some of these problems and to better preserve the properties of the fresh fruits, several new "mild" technological processes have been proposed in the last years for juice production (Drioli & Romano, 2001; Jiao, Cassano, & Drioli, 2004). Cryoconcentration (Jariel et al., 1996) preserves juice quality, but the achievable concentration is lower (about 50 °Bx) than that obtained by evaporation (60–65 °Bx) and with a significant energy consumption. An alternative approach is based on membrane processes: thus, juice clarification, stabilisation, depectinization and concentration are typical steps where membrane processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have been successfully utilised and are today very efficient systems to preserve the nutritional and organoleptic properties of the fresh product (absence of cooked flavour) owing to the possibility of operating at room temperature with low energy consumption (Alves & Coelhoso, 2006; Fukumoto, Delaquis, & Girard, 1998; Hernandez, Chen, Shaw, Carter, & Barros, 1992; Paulson, Wilson, & Spatz, 1985; Rektor, Vatai, & Bekassy-Molnar, 2006; Silva, Jardine, & Matta, 1998; Singh & Eipeson, 2000; Todisco, Tallarico, & Drioli, 1998). More recently, osmotic distillation (OD) has been proposed as an attractive process allowing very high concentrations (up to 65 °Bx) to be reached under atmospheric pressure and at room temperature, thus avoiding thermal and mechanical damage; the process is based on a water vapour transfer across the pores of a hydrophobic microporous membrane induced by the difference in water activity between the feed (juice) and a hypertonic salt solution (concentrated brine) as stripping phase (Deblay, 1995; Jiao et al., 2004; Lefebrve, 1988). In the last years, the potential for concentrating fruit juice by these membrane processes has been investigated and applied to different fruit juices (orange, apple, kiwi fruit, passion fruit, etc.): several papers report benefits in terms of higher product quality (flavour, colour, nutrients) and lower energy consumption in comparison with traditional thermal evaporation (Barbe, Bartley, Jacobs, & Johnson, 1998; Hogan, Canning, Peterson, Johnson, & Michaels, 1998; Shaw et al., 2001; Vaillant et al., 2001). Full exploitation of the potential of these techniques may be achieved by the integration of the different processes (UF, RO, OD): indeed, separating the suspended solids and pectins from juices by MF or UF decreases viscosity and increases flux of RO and OD, maximizing yield and minimizing nutrient and flavour losses (Alvarez et al., 2000; Bailey, Barbe, Hogan, Johnson, & Sheng, 2000; Cassano, Jiao, & Drioli, 2004). Recently, we investigated the technical feasibility of an integrated membrane process (Cassano et al., 2003) based on (i) initial separation of the liquid and the pulp fractions of freshly squeezed juice by ultrafiltration; (ii) concentration of the liquid fractions by two consecutive processes: first reverse osmosis, then osmotic distillation or, alternatively, only the latter. The product obtained showed very high preservation of colour and flavour and of the total antioxidant activity (Cassano et al., 2003), measured by the ABTS assay (Re et al., 1999).

The aim of the present work was to study the behaviour of the different bioactive compounds (ascorbic acid, anthocyanins, hydroxycinnamic acids, flavanones), in order to understand the effect of the different processes and to evaluate their efficiency in preserving the natural antioxidant components and to maintain a high, total antioxidant activity of the juice. The results obtained with the new technologies were compared with those obtained concentrating the same juice by the traditional thermal technology.

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were obtained from Sigma (Milan, Italy); sodium hydrogen and dihydrogen phosphate were from Carlo Erba (Milan, Italy); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was from Aldrich (Milan, Italy); narirutin, cyanidin-3-glucoside were from Extrasynthese (Genay, France); hesperidin and naringin were from Roth (Karlsruhe, Germany); *p*-coumaric acid, caffeic acid, ascorbic acid were from Fluka Chemika–Biochemika (Milan, Italy); ferulic acid and sinapic acid were from Ega Chemie (Steinheim, Germany); all solvents were from Carlo Erba (Milan, Italy) and were of analytical grade; bi-distilled water was produced in our laboratory by using an Alpha-Q system (Millipore, Marlborough, MA, USA). Calcium chloride dihydrate 4.1–4.5 M (60–66% w/w) solution (Carlo Erba, Milan, Italy) was recirculated in the tube side of the OD plant as stripping solution.

2.2. Orange juice samples

Blood orange juice (Tarocco variety) were produced from fruits cultivated in Sicily and were supplied by Parma-

lat S.p.A. (Parma, Italy): TSS concentration of the raw juice was about 12.0–12.6°Bx with a pH = 3.5. Traditionally, concentrated orange juice was produced by a multiple effect thermally accelerated short time evaporator (TASTE) evaporator at a final concentration of 56.3°Bx by Parmalat S.p.A. Samples of fresh, clarified and concentrated orange juice were collected and stored refrigerated at −20 °C until analyses.

2.3. UF unit and procedures

UF was performed by using a laboratory pilot plant supplied by Verind SpA (Rodano, Milan, Italy) equipped with a Koch tubular membrane module (type Series-Cor HFM-251, PVDF, nominal molecular weight cut-off 15 kDa, surface membrane area 0.23 m^2, pore diameter 59 Å, pressure operating range 0.8–5.5 bar, temperature operating range 0–55 °C, pH operating range 2–11). Experiments were carried out in the batch concentration mode to concentrate the juice up to a recovery factor of 85%. The membrane module was rinsed with tap water for 30 min after the treatment of the juice; then it was submitted to a cleaning procedure with the alkaline detergent Ultrasil 10 (Henkel Chemicals Ltd., Dusseldorf, Germany) at a concentration of 0.2% w/w% and at a temperature of 40 °C for 60 min. A final rinse of the system with tap water for at least 20 min was carried out.

2.4. RO unit and procedures

The permeates coming from the UF treatment were submitted to a preliminary concentration by RO using a laboratory unit supplied by Matrix Desalination, Inc. (Florida, USA). The equipment consisted of a 12 l feed tank, a cooling coil working with tap water, a high pressure feed pump, a stainless steel housing, a permeate flowmeter and a pressure control system. The plant was equipped with an Hydranautics spiral-wound membrane module (type SWC2-2521, composite polyamide, salt rejection minimum 99.0%, nominal membrane area 1.12 m^2, pressure operating range 1–69 bar, temperature operating range 0–45 °C, pH operating range 3–10). All the experiments were performed according to the batch concentration mode. The membrane module was rinsed with tap water for 30 min after the treatment of the juice; then it was submitted to a cleaning procedure using NaOH (Carlo Erba, Milan, Italy) solution at 0.01% (w/w). The solution was circulated for 60 min at a temperature of 40 °C and at a transmembrane pressure of 5 bar. A final rinse of the system with tap water for at least 20 min was carried out.

2.5. OD units and procedure

The retentates coming from the reverse osmosis unit were submitted to OD experiments using a laboratory plant equipped with a Hoechst-Celanese Liqui-Cell membrane contactor (Liqui-Cel® Extra-Flow 2.5 × 8 in., effective surface area 1.4 m^2, effective area/volume 29.3 cm^2/cm^3, fibre potting material polyethylene, max. transmembrane differential pressure 4.08 bar, temperature operating range 1–40 °C) containing microporous polypropylene hollow-fibres of Celgard membrane (Hoechst–Celanese Corporation, Wiesbaden, Germany). The clarified juice was pumped through the shell side of the membrane module; calcium chloride dihydrate at 60% (w/w) by Carlo Erba (Milan, Italy), used as stripping solution, was pumped through the fibre lumens (tube side). Both solutions were recirculated back to their reservoirs after passing through the contactor. Circulation of both brine and juice was counter-current. Inlet and outlet pressures for both tube side and shell side streams were registered by pressure gauges in order to control the pressure differentials between the two sides of the membrane. OD system was generally operated with a slightly higher pressure on the shell side of the module than the lumen side in order to avoid the leakage of the brine strip into the product. The clarified juice was recirculated in the shell side of the OD membrane module with an average flow rate of 28.7 l/h. The stripping solution was recirculated in the tube side with an average flow rate of 30.3 l/h. The temperature of both, juice and brine, was 25 ± 1 °C whereas the average transmembrane pressure (TMP) was 0.28 bar. After each trial, the pilot plant was cleaned first by rinsing the tube side and shell side with de-ionised water. Then, a NaOH solution at 2% (w/w) was circulated for 1 h at 40 °C. After a short rinsing with de-ionised water a citric acid solution at 2% (w/w) was circulated for 1 h at 40 °C. Finally, the circuit was rinsed with de-ionised water.

2.6. Physicochemical assays

TSS measurements were carried out using hand refractometers (Atago Co., Ltd., Tokyo, Japan) with scale range of 0–32, 28–62 and 58–90 °Bx. pH was measured by a Mod. 691 pH meter (Metrohm Italiana, Origgio, VA, Italy).

2.7. Determination of the total antioxidant activity (TAA)

The total antioxidant activity was determined by an improved version of the ABTS assay in which the radical cation is generated by reaction with potassium persulfate before the addition of the antioxidant (decolourization assay) (Re et al., 1999). This method gives a measure of the antioxidant activity of pure substances and of mixtures by monitoring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. Spectrophotometric measurements were performed with a Lambda Bio 20 model spectrophotometer (Perkin–Elmer, Norwalk, USA) equipped with a peltier system PTP 6 (Perkin–Elmer) for temperature control. Quartz cuvettes were from Hellman (Müllheim, Germany). Mixing was performed by a vortex model SA6 (Stuart Scientific, Redhill, England). TAA of liquid fractions was determined after adjusting concentration of the concentrated samples to 12.6 °Bx by

addition of bi-distilled water. ABTS was dissolved in water at 2 mM concentration: $ABTS^{\cdot+}$ was produced by reacting 10 ml of ABTS stock solution with 100 μl of 70 mM potassium persulfate solution ($ABTS:K_2S_2O_8 = 1:0.35$ molar ratio) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Work solution was prepared diluting 1 ml of the $ABTS^{\cdot+}$ solution to 25 ml with PBS buffer (5 mM Na_2HPO_4, 5 mM NaH_2PO_4, NaCl 9 g/l, pH = 6.8) to a final UV absorbance of 0.70 ± 0.02 at 734 nm (eventually, adjusting with small drops of the two solution). After addition of 10 μl of sample (juice or pulp extract) to 10 ml of ABTS work solution, the absorbance at 734 nm was recorded every min for a total of 6 min. The value at 5 min was used to calculate the results reported as total antioxidant activity, expressed in terms of mM trolox equivalent. Each determination was performed in triplicate. Results are expressed as mean ± SD of three samples.

2.8. Determination of ascorbic acid

Ascorbic acid was determined by HPLC, according to Sánchez-Mata, Camara-Hurtado, Diez-Marques, and Torjia-Isasa (2000). The liquid fractions were directly injected (after filtration on 0.45 μm HPLC filters), whereas the concentrated ones were previously rediluted to the concentration of the fresh juice (12.6 °Bx). Juices with pulps (fresh juice, thermal concentrate, UF retentate) were mixed with an equal volume (20 ml) of an extracting solution (4.5% metaphosphoric acid), homogenized, then centrifuged at 6000 rpm for 15 min, in order to remove the pulp fraction (ALC 4237 R centrifuge, Chemifarm, Parma, Italy), adjusted to 50 ml and finally filtered and injected. HPLC-analyses were performed with a Waters model 2690 separation module (Waters, Milford, MA, USA) equipped with a Waters model 2487 dual-band UV–Vis detector ($\lambda = 254$ nm) and a C_{18} Spherisorb RP-column (3 μm, 250 × 2.1 mm ID) (Waters), thermostated at 30 °C. The solvent system used was a 0.2 M phosphate buffer pH = 3.0 (isocratic). Flow rate was set at 0.3 ml/min. Analyses were performed in duplicate (20 μl injection).

2.9. HPLC–MS analyses: identification of antioxidant compounds

HPLC–MS analyses were performed with a Waters model 2690 separation module linked to a Photo Diode Array detector (PDA model 996) and a Micromass ZMD mass spectrometer (Micromass, Manchester, UK), equipped with an electrospray source and a single quadrupole mass analyser. Analyses were performed with a C_{18} RP-column (5 μm, 250 × 4.6 mm ID, 300 Å) (Jupiter Phenomenex). The elution conditions were as follows: flow rate, 1 ml/min; temperature, 30 °C. The solvent system used was a gradient of solvent A (water with 0.2% v/v formic acid) and solvent B (water:acetonitrile = 60:40 v/v with 0.2% formic acid). The following gradient was applied: 0–30 min linear from 100% A to 100% B, 30–32 min linear to 100% A, 32–45 min isocratic 100% A for reconditioning the column. 95% of the eluate was discharged by a T-tube before entering the electrospray source. Data were acquired by the software Masslynx 3.4. PDA chromatogram (200–600 nm scan range) and mass chromatogram (positive ion mode, capillary voltage 3500 V, cone voltage 40 V, desolvation flow (N_2) = 4631 l/h, nebulizer flow (N_2) 981 l/h, scan range m/z = 100–700 Da, scan time 4.1 s) were compared in order to identify the different peaks according to UV and mass spectra.

2.10. Determination of flavanones

Flavanones were determined by HPLC (same system as above), according to Justesen, Knuthsen, and Leth (1998), using a Phenomenex C18 column (250 × 4.6 mm, 5 μm) and UV detector (280 nm) by direct injection of the juice for the liquid fractions and of the extract for the pulp fractions. Pulp fractions (5 g) were extracted with $MeOH/H_2O$ = 80:20 (1% HCOOH) (20 ml × 2). Solvent was evaporated under vacuum and the residue redissolved in 1 ml $MeOH/H_2O$ = 80:20 (1% HCOOH) for HPLC analysis. The elution conditions were as follows: flow rate, 1 ml/min; temperature, 30 °C. The solvent system used was a gradient of solvent A (water with 0.2% v/v formic acid) and solvent B (water:acetonitrile = 60:40 v/v with 0.2% formic acid). The following gradient was applied: 0–30 min linear from 100% A to 100% B, 30–32 min linear to 100% A, 32–45 min isocratic 100% A for reconditioning the column.

2.11. Determination of hydroxycinnamic acids

Hydroxycinnamic acids were determined after alkaline hydrolysis of the bound form (mainly esters of glucose) and extraction with ethyl acetate, according to the literature procedure (Rapisarda, Crollo, Fallico, Tomaselli, & Maccarone, 1998). To 10 ml of clear juice, after addition of internal standard *o*-coumaric acid (ca. 30 ppm), 20 ml of 1 N NaOH were added. Complete hydrolysis of the bound forms of hydroxycinnamic acids occurred in 4 h, at room temperature and in the dark. The solution was then acidified to pH = 2 with 1 N HCl and free hydroxycinnamic acids extracted with ethyl acetate (3 × 20 ml). After evaporation of the solvent under vacuum, the residue was dissolved in $THF:H_2O$ = 80:20 v/v and analysed by HPLC. In the case of the pulp fractions, hydrolysis was performed after extraction with a $MeOH/H_2O$ = 80:20 mixture (acidified with 0.2% HCOOH, 2 × 20 ml). Hydroxycinnamic acids were analysed on a C_{18} Spherisorb RP-column (3 μm, 250 × 2.1 mm ID) (Waters) and UV detector (330 nm). The elution conditions were as follows: flow rate, 0.2 ml/min; temperature, 30 °C. The solvent system used was a gradient of solvent A (water with 2% v/v acetic acid) and solvent B (water:tetrahydrofuran = 20:80 v/v). The following gradient was applied: 0–21 min isocratic 84% A and 16% B, 21–33 min linear to 40% B, 33–40 min isocratic 40% B, 40–41 min linear to 16% B, 41–50 min 16% B for reconditioning the column.

2.12. Determination of anthocyanins

Anthocyanins were determined by HPLC according to the literature procedure (Maccarone, Rapisarda, Fanella, Arena, & Mondello, 1998; Mondello, Cotroneo, Errante, Dugo, & Dugo, 2000). Typically, 5 ml of clear juice was eluted through Waters Sep-Pack C_{18} cartridges (500 mg), previously conditioned with 10 ml ethanol and 10 ml bi-distilled water. After washing with water, anthocyanins adsorbed on the column were eluted with 2 ml HCl/MeOH (1%). In the case of orange pulps, 5 g were extracted with MeOH/H_2O = 80:20 mixture (acidified with 1% HCOOH, 2 × 20 ml), the combined extracts concentrated under vacuo and applied to the Sep-Pack cartridge as above. HPLC analyses were performed on a C_{18} Spherisorb RP-column (3 μm, 250 × 2.1 mm ID) (Waters) with UV detector (510 nm). The elution conditions were as follows: flow rate, 0.3 ml/min; temperature, 30 °C. The solvent system used was a gradient of solvent A (water with 2% v/v formic acid) and solvent B (water:formic acid:acetonitrile = 40:10:50 v/v). The following gradient was applied: 0–20 min linear from 15% to 35% B, 20–22 min linear to 100% B, 22–27 min isocratic 100% B, 27–30 min linear to 15% B, 30–35 min isocratic 15% B for reconditioning the column.

2.13. Statistical analysis

Results were given as mean ± SD of three independent determinations. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered to be significant at $P < 0.05$. All statistical analyses were performed with SPSS 13.0 (SPSS, Inc., Chicago, IL).

3. Results and discussion

3.1. The blood orange juice

The orange juice under investigation was obtained by industrial squeezing of Sicilian oranges (mostly, Tarocco variety) and was preserved refrigerated (−20 °C) until processing and analyses. The freshly squeezed blood orange juice was characterized by a pH = 3.5 and by a TSS concentration of 12.0 °Bx. The juice showed a very high total antioxidant activity (TAA), 8.65 ± 0.07 mM trolox: this value is larger than that usually found for blond orange juice and also somewhat higher or similar to the value obtained for freshly squeezed blood oranges by Arena et al. (2001) (TAA = 5.08–5.18 mM) or by Rapisarda et al. (1999) (TAA = 3.76–7.05). The high values are related to the high content of antioxidant components of these fruit varieties, mainly ascorbic acid and polyphenols.

The polyphenolic profile of fresh blood orange juice is very rich: several known polyphenolic components were present, mainly flavanones (hesperidin and narirutin) (Justesen et al., 1998), hydroxycinnamic acids derivatives (ferulic, *p*-coumaric, sinapic and caffeic acids) (Rapisarda et al., 1998) and anthocyanins (mainly, cyanidin-3-glucoside and cyanidin-3-glucoside-6″-malonyl) (Maccarone et al., 1998; Mondello et al., 2000).

These compounds can be easily identified by using the HPLC–DAD–MS system by comparing the mass spectra, the UV spectra and, when possible, by spiking with original standards: identified components are reported in Table 1.

HPLC–MS with electrospray ionisation allows a very easy identification of the different compounds. Indeed, most compounds are detected as protonated pseudo-molecular ions $[M + H]^+$. Other molecular ion species are adducts with sodium. Particularly important for the structural information is the partial fragmentation occurring as a consequence of collisionally induced dissociation (CID) in the electrospray source. In the case of anthocyanins and flavonoids, mostly present as *O*-glycosides, fragmentation mainly occurs at the glycosidic bond: thus $[M + H]^+$ is often accompanied by the aglycone pseudo-molecular ion $[A + H]^+$. Moreover, when disaccharides are linked to the aglycone, also the fragment obtained by loss of one sugar molecule is obtained. In the case of hydroxycinnamates, the most abundant ion is the acylium ion, obtained by loss of OH^- from the carboxylic group. Hydroxycinnamic acids (ferulic and *p*-coumaric, the most abundant) are present mainly as the sugar (glucose) ester, as confirmed also by analysis after alkaline hydrolysis. The most abundant flavanones are hesperidin and narirutin, but also a small

Table 1
HPLC–DAD–MS analysis of blood orange juice (M = molecular ion, A = aglycone)

Compounds	MS fragments	UV spectra (λ_{max}, nm)
Ascorbic acid	177 ($M + H^+$), 141, 113	243
Feruloyl glucose	379 ($M + Na^+$), 195 ($A + H^+$), 177 (A-OH), 145 (A-OH–OCH_3)	323
p-Coumaroyl glucose	349 ($M + Na^+$), 165 ($A + H^+$), 147 (A-OH)	310
Tannin	595 ($M + H^+$)	280
Narirutin	581 ($M + H^+$), 273 ($A + H^+$)	280
Hesperidin	611 ($M + H^+$), 303 ($A + H^+$)	280
Isosakuranetin-7-rutinoside	595 ($M + H^+$), 287 ($A + H^+$)	280
Rutin	449 ($M + H^+$), 303 ($A + H^+$)	284, 324
Cyanidin-3-glucoside	449 (M^+), 287 (A^+)	280, 520
Cyanidin-3-glucoside-6″-malonyl	535 (M^+), 287 (A^+)	280, 520

amount of isosakuranetin-7-rutinoside was identified in these juices. The latter is typically found in the peel, so that in this case it could be an impurity due to industrial squeezing. Quercetin is the most abundant flavonol, mainly present as rutine (quercetin-7-rutinoside). The two main anthocyanins, cyanidin-3-glucoside and cyanidin-3-glucoside-6″-malonyl, are easily identified also by examining the chromatogram at 510 nm. As the whole juice chromatogram was very complicated, quantitative analyses of hydroxycinnamates and anthocyanins were performed by HPLC, after extraction and purification, according to the literature procedures (Maccarone et al., 1998; Rapisarda et al., 1998).

3.2. The integrated process

The different steps of the integrated membrane process here applied to the concentration of orange juice have been optimized in terms of technical parameters (temperature, transmembrane pressure, feed flow rate, membrane fouling and cleaning procedures) and were described in detail in a previous paper (Cassano et al., 2003). The process was based on the initial separation of liquid and pulp fractions of freshly squeezed blood orange juice by ultrafiltration (UF). The clarified juice was successively concentrated by two consecutive processes: first reverse osmosis (RO), as a pre-concentration step up to 25–30 °Bx, then osmotic distillation (OD), to obtain a final concentration of ca. 60 °Bx. Alternatively, concentration could be performed also only by the latter method (Fig. 1).

Ultrafiltration (UF) membranes retain microorganisms and large molecules as lipids, proteins and colloids, while small solutes such as vitamins, salts, sugars, are allowed to flow through the membrane with water. Thus, the possibility of microbial contamination in the permeate stream is minimised, avoiding thermal treatments and, consequently, loss of volatile aroma compounds. Moreover, the ultrafiltration step allowed to obtain clarified juice more suitable to the following membrane based concentration steps: indeed, UF completely removed the suspended solid and the resulting clarified juice had lower viscosity and negligible turbidity. This step is a fundamental pre-requisite in order to apply high flow rate and maximize yield during the subsequent RO or OD treatment (Bailey et al., 2000; Cassano et al., 2004).

During the reverse osmosis process, water is efficiently removed from the juice: nevertheless, since the osmotic pressure of the juice increases rapidly with the increase of sugar concentration (100 and 200 bar for concentrations of 42 and 60 °Bx, respectively), this process was used only as a pre-concentration technique to reach a final concentration of 21.4 °Bx (Singh & Eipeson, 2000; Vaillant et al., 2001). The concentration of the juice was continued by osmotic distillation, a new membrane process also called "isothermal membrane distillation" which can be used to selectively remove water from aqueous solutions under atmospheric pressure and at room temperature (Hogan et al., 1998; Shaw et al., 2001).

OD involves the use of a microporous hydrophobic membrane to separate two circulating aqueous solutions at different solute concentrations: a dilute solution and a hypertonic salt solution. The difference in solute concentrations and, consequently, in water activity between the solutions, generates at the vapour–liquid interface a vapour pressure difference which induces a vapour transfer from the dilute solution towards the stripping solution.

Clarified orange juice was concentrated to a final value of ca. 61 °Bx in both operating modes (UF–RO–OD or UF–OD).

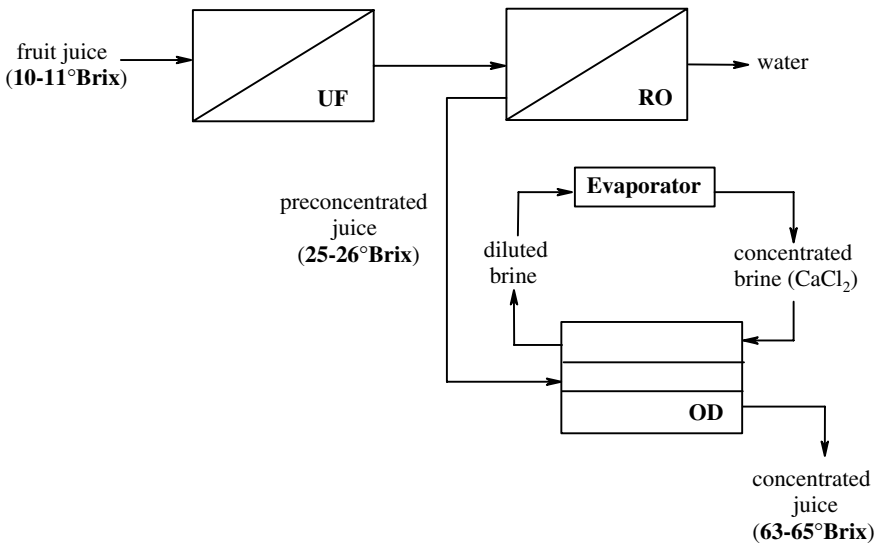

Fig. 1. General scheme of the integrated membrane process: UF, ultrafiltration unit; RO, reverse osmosis unit; OD, osmotic distillation unit.

3.3. Effect of ultrafiltration and concentration processes on the total antioxidant activity (TAA) and on the bioactive components of the juice

In order to evaluate the effect of the new proposed processes, we measured the TAA in several samples of the initial fresh juice, of the ultrafiltrated (UF) permeate and retentate fractions, of the concentrate obtained by reverse osmosis (RO) and of the concentrate produced by osmotic distillation (OD) at different degrees of concentration.

The antioxidant activity was measured after rediluting the concentrated juices to the same °Bx of the fresh juice (12.0–12.6 °Bx), in order to allow a direct comparison between the different juices. Two different configurations were evaluated: UF–RO–OD, in which reverse osmosis was used as a pre-concentration treatment, and UF–OD, in which the reverse osmosis treatment was omitted. The results obtained with the two configurations are reported in Table 2 (the final concentration in °Bx achieved by the various treatments is indicated).

During the ultrafiltration process TAA was maintained (UFP, 8.21 ± 0.12 mM trolox). A decrease was observed with the reverse osmosis step (RO, 7.47 ± 0.24 mM trolox), probably on account of the high pressure (50 bar) experienced by the juice during the treatment. After this step, the subsequent concentration treatment by osmotic distillation did not induce other significant changes in TAA: the highly concentrated sample at 60.6 °Bx still showed a high value of TAA (7.33 ± 0.20 mM trolox), higher than that obtained by the traditional thermal evaporation (6.40 ± 0.24 mM trolox). A TAA percentage reduction of 15% was measured for the concentrate, whereas a 26% reduction was observed for the traditionally evaporated juice.

Recently, an integrated membrane process based upon UF–OD (thus bypassing the RO step) was successfully applied to the concentration of kiwi fruit juice with high preservation of antioxidant activity (Cassano et al., 2004).

In the case of orange juice, the results obtained with the configuration UF–OD showed that only a slight decrease of TAA was observed (ca. 13%), confirming the particular mildness of the treatment (Table 2). Nevertheless, the final TAA value obtained by UF–OD was not significantly different from that obtained by UF–RO–OD.

In order to better understand these findings, HPLC quantitative analysis of several antioxidant components was performed on samples before and after each technological treatment.

If we consider the entire scheme of the new process (Tables 3 and 4), we can see that for ascorbic acid a slight decrease was observed in particular during ultrafiltration (about 15%).

The subsequent concentration steps by reverse osmosis and osmotic distillation induced only a very small decrease and the final amount of ascorbic acid was very high (ca. 600 ppm).

Anthocyanins were also slightly affected by the process, decreasing in particular during the reverse osmosis step: at the end of the process a reduction of about 23% was measured.

On the contrary, no significant variations were observed for hydroxycinnamic acids and for flavanones, which

Table 2
TAA variation (mM trolox) observed for the different process sequence in comparison with traditional thermal evaporation

Sample	TAA		
	UF–RO–OD	UF–OD	Thermal evaporation
Fresh juice (12.6 °Bx)	8.65 ± 0.07a	8.61 ± 0.15a	8.63 ± 0.11a
UFP (12.4 °Bx)	8.21 ± 0.12a	8.48 ± 0.17a	–
UFR (13.5 °Bx)	8.29 ± 0.11a	8.52 ± 0.09a	–
RO retentate (21.4 °Bx)	7.47 ± 0.24b	–	–
OD retentate (60.6 °Bx)	7.33 ± 0.22b	7.66 ± 0.20b	–
TC (63.0 °Bx)			6.40 ± 0.24c

Values are means ± SD, $n = 6$. Mean values within a column with different letters are significantly different at $P < 0.05$. UFP, ultrafiltration permeate; UFR, ultrafiltration retentate; RO, reverse osmosis; OD, osmotic distillation; TC, thermal concentration.

Table 3
Variation of ascorbic acid and anthocyanins during ultrafiltration and concentration processes (data in mg/l)

Sample (°Bx)	Ascorbic acid	Cyanidin-3-glucoside	Cyanidin-3-glucoside-6″-malonyl	Total anthocyanins
FJ (12.6)	701.0 ± 8.3a	22.7 ± 2.2a	24.8 ± 2.4a	56.3 ± 3.2a
UFP (12.4)	636.5 ± 7.1b	22.2 ± 2.3a	24.1 ± 2.3a	55.0 ± 3.5a
UFR (13.5)	624.9 ± 9.5b	22.1 ± 1.1ab	24.8 ± 1.9a	55.3 ± 2.8a
RO (21.4)	610.6 ± 8.1b	17.9 ± 2.5b	19.5 ± 1.9b	44.7 ± 3.2b
OD (60.6)	594.2 ± 7.9c	17.2 ± 1.9b	18.8 ± 1.8b	43.3 ± 2.2b
TC (56.3)	486.3 ± 9.4d			35.8 ± 1.9c

Values are means ± SD, $n = 6$. Mean values within a column with different letters are significantly different at $P < 0.05$. UFP, ultrafiltration permeate; UFR, ultrafiltration retentate; RO, reverse osmosis; OD, osmotic distillation; TC, thermal concentration.

Table 4
Variation of hydroxycinnamic acids and flavanones during ultrafiltration and concentration processes (data in mg/l)

Sample (°Bx)	Sinapic acid	Caffeic acid	Ferulic acid	*p*-Coumaric acid	Narirutin	Hesperidin
FJ (12.6)	6.6 ± 0.5a	6.8 ± 0.6a	51.3 ± 1.1a	33.5 ± 0.8a	50.7 ± 2.1a	45.1 ± 2.2a
UFP (12.4)	6.6 ± 0.4a	6.8 ± 0.5a	51.7 ± 1.3a	34.9 ± 0.9a	50.8 ± 1.9a	45.5 ± 1.7a
UFR (13.5)	6.1 ± 0.3a	7.0 ± 0.4a	53.9 ± 0.9a	33.9 ± 0.7a	49.9 ± 3.3a	42.1 ± 2.8a
RO (21.4)	6.0 ± 0.5a	7.4 ± 0.6a	51.1 ± 1.2a	34.3 ± 1.1a	50.2 ± 1.8a	46.6 ± 2.0a
OD (60.6)	5.6 ± 0.4a	7.6 ± 0.5a	51.0 ± 1.1a	33.5 ± 0.9a	48.7 ± 2.3a	45.3 ± 2.5a
TC (56.3)	3.7 ± 0.4b	14.6 ± 0.5b	11.8 ± 0.6b	13.9 ± 0.7b	38.2 ± 1.6b	35.2 ± 2.2b

Values are means ± SD, $n = 6$. Mean values within a column with different letters are significantly different at $P < 0.05$. UFP, ultrafiltration permeate; UFR, ultrafiltration retentate; RO, reverse osmosis; OD, osmotic distillation; TC, thermal concentration.

appeared to be very stable under these particular conditions.

To better understand the effect of the different treatments, we omitted the reverse osmosis concentration step and performed concentration only via osmotic distillation.

Results of this experiment are reported in Tables 5 and 6.

As before, hydroxycinnamic acids and flavanones were practically unaffected, whereas ascorbic acid and anthocyanins slightly decreased (13.5% and 23%, respectively): for ascorbic acid the highest decrease was observed during UF, whereas anthocyanins were more affected by the concentration steps.

In both configurations, TAA variations were particularly due to variations in ascorbic acid content, the latter being the most important antioxidant component of the juice.

In conclusion, the final values obtained both for TAA and for antioxidant compounds by UF–RO–OD and by UF–OD are very similar so that, owing to the longer time necessary to perform concentration only via membrane distillation, the pre-concentration step via reverse osmosis should be maintained, as it allows to save time and increase efficiency without affecting the quality of the final product in a significant way.

On the basis of the experimental data obtained here, this new methodology appears to combine high efficiency and mildness.

A larger decrease of the amount of the antioxidant components was observed in the case of the traditional thermally evaporated juice: both anthocyanins and hydroxycinnamates (particularly, ferulic and *p*-coumaric acid) underwent a reduction of 36% and 55%, respectively, ascorbic acid of about 30% and flavanones of ca. 23%. The data obtained for the thermal concentration process are worse than those obtained by other authors (Arena et al., 2001) investigating the effect of industrial concentration processes, probably on account of the different performances of the TASTE evaporators. Nevertheless, in agreement with their findings, the product showed an evident colour change, which turned to red brick: this effect is linked to degradation of anthocyanins probably accompanied by a different distribution of carotenoids in the serum and to the formation of Maillard reaction products as suggested by the same authors (Arena et al., 2001). Indeed, in most cases Allura Red AC (E129) is added to a large part

Table 5
Variation of ascorbic acid and anthocyanins during ultrafiltration and osmotic distillation processes (data in mg/l)

Sample (°Bx)	Ascorbic acid	Cyanidin-3-glucoside	Cyanidin-3-glucoside-6″-malonyl	Total anthocyanins
FJ (12.0)	701.0 ± 8.7a	22.9 ± 2.2a	26.5 ± 2.5a	60.4 ± 3.1a
UFP (11.2)	642.2 ± 7.3b	21.0 ± 1.9a	23.8 ± 2.3b	54.7 ± 2.8b
UFR (13.5)	640.4 ± 9.1b	22.7 ± 2.3a	26.8 ± 2.1a	60.6 ± 2.9a
OD (61.0)	605.1 ± 7.5c	18.2 ± 1.8b	20.4 ± 1.9b	47.2 ± 2.6c

Values are means ± SD, $n = 6$. Mean values within a column with different letters are significantly different at $P < 0.05$. UFP, ultrafiltration permeate; UFR, ultrafiltration retentate; OD, osmotic distillation.

Table 6
Variation of hydroxycinnamic acids and flavanones during ultrafiltration and osmotic distillation processes (data in mg/l)

Sample (°Bx)	Sinapic acid	Caffeic acid	Ferulic acid	*p*-Coumaric acid	Narirutin	Hesperidin
FJ (12.0)	6.6 ± 0.6a	7.1 ± 0.6a	51.9 ± 2.1a	30.7 ± 0.8a	46.7 ± 2.1a	32.4 ± 2.3a
UFP (11.2)	6.4 ± 0.4a	6.7 ± 0.5a	51.4 ± 1.9a	29.7 ± 0.9a	46.8 ± 1.7a	33.7 ± 1.5a
UFR (13.5)	6.8 ± 0.5a	7.2 ± 0.5a	52.4 ± 1.8a	31.1 ± 0.8a	47.6 ± 2.5a	34.4 ± 2.6a
OD (61.0)	6.5 ± 0.5a	7.1 ± 0.6a	51.8 ± 2.0a	30.9 ± 0.9a	47.3 ± 1.7a	34.5 ± 1.5a

Values are means ± SD, $n = 6$. Mean values within a column with different letters are significantly different at $P < 0.05$. UFP, ultrafiltration permeate; UFR, ultrafiltration retentate; OD, osmotic distillation.

of blood orange juice present on the market to maintain a red vivid colour.

On the contrary, the bright red colour is perfectly preserved in the juice with the proposed process, despite the partial degradation of anthocyanins. Moreover, the juice concentrated in the traditional evaporation has also a typical "cooked flavour" always perceived by the panellists when a thermal treatment is applied to the juice.

4. Conclusions

The new membrane-based integrated process for the concentration of fruit juice is very efficient in preserving the total antioxidant activity (TAA) of the final product even at high concentration (60 °Bx). Among the different antioxidant components a slight decrease is observed only for ascorbic acid (ca. −15%) and anthocyanins (ca. −23%), whereas flavanones and hydroxycinnamic acids are very stable. During the proposed process, the decrease is similar in both configuration (UF–RO–OD or UF–OD) and lower than that observed with the traditional thermal treatment. Moreover, the concentrated juice retains its bright red colour and its pleasant aroma, which is, on the contrary, completely lost during thermal concentration.

On the basis of these results an integrated membrane process for the production of high quality concentrated orange juice may be envisaged, taking also into account that in the final process all the technological steps will be performed sequentially: in our case the different treatments were performed on different pilot plants, with freezing and defreezing steps to preserve the juice, so that even better results could be obtained on a fully integrated pilot plant.

Acknowledgements

This work was financially supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR, Rome, Italy) and by Parmalat, S.p.A (Parma, Italy).

References

Alvarez, S., Riera, F. A., Alvarez, R., Coca, J., Cuperus, F. P., Bouwer, S., et al. (2000). A new integrated membrane process for producing clarified apple juice and apple juice aroma concentrate. *Journal of Food Engineering, 46*, 109–125.

Alves, V. D., & Coelhoso, I. M. (2006). Orange juice concentration by osmotic evaporation and membrane distillation: A comparative study. *Journal of Food Engineering, 74*, 125–133.

Ames, B. N. (1998). Micronutrients prevent cancer and delay aging. *Toxicology Letters, 102–103*, 5–18.

Arena, E., Fallico, B., & Maccarone, E. (2001). Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. *Food Chemistry, 74*, 423–427.

Bailey, A. F. G., Barbe, A. M., Hogan, P. A., Johnson, R. A., & Sheng, J. (2000). The effect of ultrafiltration on the subsequent concentration of grape juice by osmotic distillation. *Journal of Membrane Science, 164*, 195–204.

Barbe, A. M., Bartley, J. P., Jacobs, A. L., & Johnson, R. A. (1998). Retention of volatile organic flavour/fragrance components in the concentration of liquid foods by osmotic distillation. *Journal of Membrane Science, 145*, 67–75.

Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complication: A new perspective of an old paradigm. *Diabetes, 48*, 1–9.

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., Vupputuri, S., Myers, L., et al. (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults: The first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *American Journal of Clinical Nutrition, 76*, 93–99.

Cassano, A., Drioli, E., Galaverna, G., Marchelli, R., Di Silvestro, G., & Cagnasso, P. (2003). Clarification and concentration of citrus and carrot juices by integrated membrane processes. *Journal of Food Engineering, 57*, 153–163.

Cassano, A., Jiao, B., & Drioli, E. (2004). Production of concentrated kiwifruit juice by integrated membrane processes. *Food Research International, 37*(2), 139–148.

Deblay, P. (1995). Process for at least partial dehydration of an aqueous composition and devices for implementing the process. US Patent 5,382,365, 26 January.

Drioli, E., & Romano, M. (2001). Progress and new perspectives on integrated membrane operations for sustainable growth. *Industrial and Engineering Chemistry Research, 40*, 1277–1300.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*, 239–246.

Franke, S. I. R., Pra, D., Erdtmann, B., Henriques, J. A. P., & da Silva, J. (2005). Influence of orange juice over the genotoxicity induced alkylating agents: An in vivo analysis. *Mutagenesis, 20*, 279–283.

Fukumoto, L. R., Delaquis, P., & Girard, B. (1998). Microfiltration and ultrafiltration ceramic membranes for apple juice clarification. *Journal of Food Science, 63*(5), 845–850.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry, 68*, 471–474.

Hernandez, E., Chen, C. S., Shaw, P. E., Carter, R. D., & Barros, S. (1992). Ultrafiltration of orange juice: Effect on soluble solids, suspended solids, and aroma. *Journal of Agriculture and Food Chemistry, 40*, 986–988.

Hogan, P. A., Canning, R. P., Peterson, P. A., Johnson, R. A., & Michaels, A. S. (1998). A new option: Osmotic distillation. *Chemistry and Engineering Progress*(July), 49–61.

Jariel, O., Reynes, M., Courel, M., Durand, N., Dornier, M., & Deblay, P. (1996). Comparison of some fruit juice concentration techniques. *Fruits, 51*, 437–450.

Jiao, B., Cassano, A., & Drioli, E. (2004). Recent advances on membrane processes for the concentration of fruit juices: A review. *Journal of Food Engineering, 63*, 303–324.

Justesen, U., Knuthsen, P., & Leth, T. (1998). Quantitative analysis of flavonols, flavones and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *Journal of Chromatography A, 799*, 101–110.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables – The millennium's health. *International Journal of Food Science and Technology, 36*, 727–735.

Lefebrve, M. S. M. (1988). Method of performing osmotic distillation. US Patent 4,781,837, 1 November.

Maccarone, E., Campisi, S., Cataldi Lupo, M. C., Fallico, B., & Nicolosi Asmundo, C. (1996). Thermal treatments effects on the red orange juice constituents. *Industria Bevande, 25*, 335–341.

Maccarone, E., Rapisarda, P., Fanella, F., Arena, E., & Mondello, L. (1998). Cyanidin-3(6″-malonyl)-β-glucoside. One of the major anthocyanins in blood orange juice. *Italian Journal of Food Science, 10*, 367–372.

Miller, N. J., & Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry, 60*, 331–337.

Mondello, L., Cotroneo, A., Errante, G., Dugo, G., & Dugo, P. (2000). Determination of anthocyanins in blood orange juices by HPLC analysis. *Journal of Pharmaceutical and Biomedical Analyses, 23*, 191–195.

Paulson, D. J., Wilson, R., & Spatz, D. D. (1985). Reverse osmosis ultrafiltration. In *ACS symposium series* (Vol. 281, pp. 325–344).

Proteggente, A. R., Saija, A., De Pasquale, A., & Rice-Evans, C. A. (2003). The compositional characterisation and antioxidant activity of fresh juices from Sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. *Free Radical Research, 37*, 681–687.

Rapisarda, P., Crollo, G., Fallico, B., Tomaselli, F., & Maccarone, E. (1998). Hydroxycinnamic acids as markers of Italian blood orange juices. *Journal of Agriculture and Food Chemistry, 46*, 464–470.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A., & Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agriculture and Food Chemistry, 47*, 4718–4723.

Rektor, A., Vatai, G., & Bekassy-Molnar, E. (2006). Multi-step membrane processes for the concentration of grape juice. *Desalination, 191*, 446–453.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radicals in Biology and Medicine, 26*, 1231–1237.

Riso, P., Visioli, F., Gardana, C., Grande, S., Brusamolino, A., Galvano, F., et al. (2005). Effects of blood orange intake on antioxidant bioavailability and on different markers related to oxidative stress. *Journal of Agriculture and Food Chemistry, 53*, 941–947.

Sánchez-Mata, M. C., Camara-Hurtado, M., Diez-Marques, C., & Torjia-Isasa, M. E. (2000). Comparison of high-performance liquid chromatography and spectrofluorimetry for vitamin C analysis of green beans (*Phaseolus vulgaris* L.). *European Food Research and Technology, 210*, 220–225.

Sauvaget, C., Nagano, J., Hayashi, M., Spencer, E., Shimizu, Y., & Allen, N. (2003). Vegetables and fruit intake and cancer mortality in the Hiroshima/Nagasaki Life Span Study. *British Journal of Cancer, 88*, 689–694.

Shaw, P. E., Lebrun, M., Dornier, M., Ducamp, M. N., Courel, M., & Reynes, M. (2001). Evaluation of concentrated orange and passion-fruit juices prepared by osmotic evaporation. *Lebensmittel-Wissenschaft und-Technologie, 34*, 60–65.

Silva, F. T., Jardine, J. G., & Matta, V. M. (1998). Concentration of orange (*Citrus sinensis*) juice by reverse osmosis. *Ciencia e Tecnologia des Alimentos, 18*, 99–104.

Singh, N. G. I., & Eipeson, W. E. (2000). Concentration of clarified orange juice by reverse osmosis. *Journal of Food Science and Technology, 37*, 363–367.

Todisco, S., Tallarico, P., & Drioli, E. (1998). Modelling and analysis of the effects of ultrafiltration on the quality of freshly squeezed orange juice. *Italian Food and Beverage Technology, 12*, 3–8.

Vaillant, F., Jeanton, E., Dornier, M., O'Brien, G. M., Reynes, M., & Decloux, M. (2001). Concentration of passion fruit juice on an industrial pilot scale using osmotic evaporation. *Journal of Food Engineering, 47*, 195–202.

Vinson, J. A., Liang, X. Q., Proch, J., Hontz, B. A., Dancel, J., & Sandone, N. (2002). Polyphenol antioxidants in citrus juices: In vitro and in vivo studies relevant to hearth disease. *Flavonoids in Cell Function – Advances in Experimental Medicine and Biology, 505*, 113–122.