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Food Chemistry 97 (2006) 137–150
Food Chemistry
www.elsevier.com/locate/foodchem

An industrial approach in the search of natural antioxidants from vegetable and fruit wastes

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Received 21 October 2004; received in revised form 25 March 2005; accepted 31 March 2005

Abstract

Eleven fruit and vegetable byproducts and two minor crops were screened for industrial polyphenol exploitation potential by determination of their extraction yield, total phenolic content (TPC, Folin–Ciocalteu), and antioxidant activity (NTZ/hypoxanthine superoxide assay, ferric thiocyanate method). Extracts with the highest activity, economic justification and phenolic content were obtained from apple (TPC maximum 48.6 ± 0.9 mg Gallic acid equivalents g^{-1} dry extract), pear (60.7 ± 0.9 mg GAE g^{-1}), tomato (61.0 ± 3.0 mg GAE g^{-1}), golden rod (251.4 ± 7.0 mg GAE g^{-1}) and artichoke (514.2 ± 14.9 mg GAE g^{-1}). Apple, golden rod and artichoke byproducts were extracted at pilot plant scale and their antioxidant activity was confirmed by determination of their free radical scavenging activity (DPPH) and the inhibition of stimulated linoleic acid peroxidation (TBA and Rancimat® methods). The preservative effect of the three extracts (determination of the peroxide value in test crème formulations with 0.1–1.0% extract concentrations) was similar to the established antioxidants Oxynex® 0.1%, Controx® KS 0.15%, and butylated hydroxytoluene (BHT) 0.01%. This study demonstrates the possibility of recovering high amounts of phenolics with antioxidant properties from fruit and vegetable residuals not only for food but also cosmetic applications.

Keywords: Plant extracts; Agricultural wastes; Byproducts; Fruits; Vegetables; Antioxidant activity; Radical scavenging activity; Phenolic content

1. Introduction

Growing knowledge about the health promoting impact of antioxidants in everyday foods, combined with the assumption that a number of common synthetic preservatives may have hazardous effects (Krishnakumar & Gordon, 1996), has led to multiple investigations in the field of natural antioxidants. Until now, most attention has been paid to oral administration of natural radical scavengers as food supplements like green tea extract (Buetler, Renard, Offord, Schneider, & Ruegg, 2002) or as preserving food additives obtained from aromatic plants like rosemary and salvia extracts (Karpinska, Borowski, & Danowska-Oziewicz, 2000; Zupko et al., 2001). However, protection from hazardous reactive oxygen species is not only of nutritional relevance. Neither are oxidation reactions an exclusive concern of the food industry. Finally, the customer's awareness for

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doi:10.1016/j.foodchem.2005.03.033

"non-chemical" ingredients in health products has also to be faced by the cosmetic and pharmaceutical industry. These three sectors are drawn together and promoted products named functional food, food supplements, nutraceuticals or cosmeceuticals. Likewise practical and legal questions require interdisciplinary cooperation of academia and industry in this field (Schieber, Stintzing, & Carle, 2001). A recent increase in serious research on the commercial application of radical scavengers and flavonoids as beneficial anti-ageing and photoprotection ingredients in cosmetic products (Katiyar & Elmets, 2001; Lupo, 2001), and the demand for non-toxic antioxidants that are active in hydrophilic and lipophilic systems, led to the additional focus on the topical relevance of food derived extracts in the present study. As in food products two applications of phenolic antioxidants might be of interest: as a substitute for synthetic preservatives or as active ingredients, for example a skin-protecting additive in dermatology. Therefore, the three extracts developed here were also evaluated for their practical suitability in crème formulations.

Within the antioxidant literature the number of studied residual sources has been augmented considerably, which is caused by a value adding recycling interest of the agro- and food industry, but also increasing information on the specific location of active compounds and their modification during processing. However, only a few byproduct derived antioxidants have been developed successfully from the vast quantities of plant residues produced by the food processing industry in Europe, primarily grape seed and olive waste extracts (Alonso, Guillén, Barroso, Puertas, & García, 2002; Amro, Aburjai, & Al-Khalil, 2002). Follow-up crop candidates with a high annual production and already confirmed high antioxidant potential include apple (Du Pont, Bennett, Mellon, & Williamson, 2002; Paganga, Miller, & Rice-Evans, 1999), tomato (Fuhrman, Volkova, Rosenblat, & Aviram, 2000; Lavelli, Peri, & Rizzolo, 2000), and artichoke (Jiménez-Escrig, Dragsted, Daneshvar, Pulido, & Saura-Calixto, 2003; Zapolska-Downar et al., 2002). Recycling of the byproducts has been supported by the fact that polyphenols have been located specifically in the peels (Wolfe, Wu, & Liu, 2003), and that processing conditions are known to influence the phenolic content (Llorach, Espin, Tomás-Barberán, & Ferreres, 2002; Re, Bramley, & Rice-Evans, 2002; Rechner et al., 1999; Wang et al., 2003). Although the antioxidant potential of less important crops such as strawberry (Kähkönen, Hopia, & Heinonen, 2001), pear (Imeh & Khokhar, 2002), red beet (Kujala, Loponen, & Pihlaja, 2001), or broccoli (Kurilich, Jeffery, Juvik, Wallig, & Klei, 2002) is known, as yet little practical effort to utilize their byproducts for phenolic recovery has been reported. This might be caused by three limiting factors often overlooked in scientific studies: the effectiveness of recovery and extraction, the marketability of resulting extracts and the practical suitability for the food, cosmetic or pharmaceutical products.

Therefore, this study directly compares typical byproducts from apple, artichoke and tomato with eight other fruit and vegetable residues and two minor crops with interesting phenolic content but no reported detailed study of their antioxidant potential with regard to three aspects: (i) the general potential of plant residues as a source of antioxidants compared to already established ones; (ii) whether further investigation might be worthwhile in view of practical and economic limitations on production; (iii) first findings for applications in dermatology. In these three efforts, the institutes involved in the EC CRAFT project FAIR 98-9517 were joined by cosmetic and pharmaceutical companies, extraction companies, fruit juice producers and farmers from Germany and Spain. The practically-oriented screening was structured in four parts: (1) primary screening on economic aspects of the raw materials, the yield and the phenolic content of crude extracts (first screening); (2) two-step extraction of selected raw materials and determination of phenolic content and antioxidant activity by two assays (second screening); (3) pilot plant production of three selected extracts and characterization by five antioxidant assays; (4) suitability tests in crème formulations by sensory assessment and peroxide values (see also Fig. 1). The paper is completed by a discussion of methodological problems associated with an effective screening of natural phenolic sources.

2. Materials and methods

2.1. Raw materials and references

The materials investigated were (I) residues from juice production: red beet, apple, strawberry and pear; (II) waste from the canning industry: tomato, artichoke and asparagus; (III) remains after harvesting: chicory, endive, cucumber, and broccoli, and (IV) two cultivated medicinal herbs as references for primary plant material rich in polyphenols: golden rod herb (*Solidago virgaurea*) and woad herb (*Isatis tinctoria*) (Table 1). The fresh starting materials were dried either in lab scale between 25 and 60 °C or in pilot plant scale in accordance with the local equipment and production chain of the provider. The dry materials with a controlled loss-on-drying (vacuum oven at 105 °C for 2 h in accordance with European Pharmacopoeia, 1997, meth. 2.32.00) of less than 10% were crushed in a laboratory-size mill and passed through a sieve (No. 355 Eur. Ph., 2.1.4).

All the chemicals and reagents were of analytical grade. References used to determine antioxidant activity were butylated hydroxytoluene (BHT, Sigma, Germany) and Controx® KS (70–100% tocopherols, 10–20%

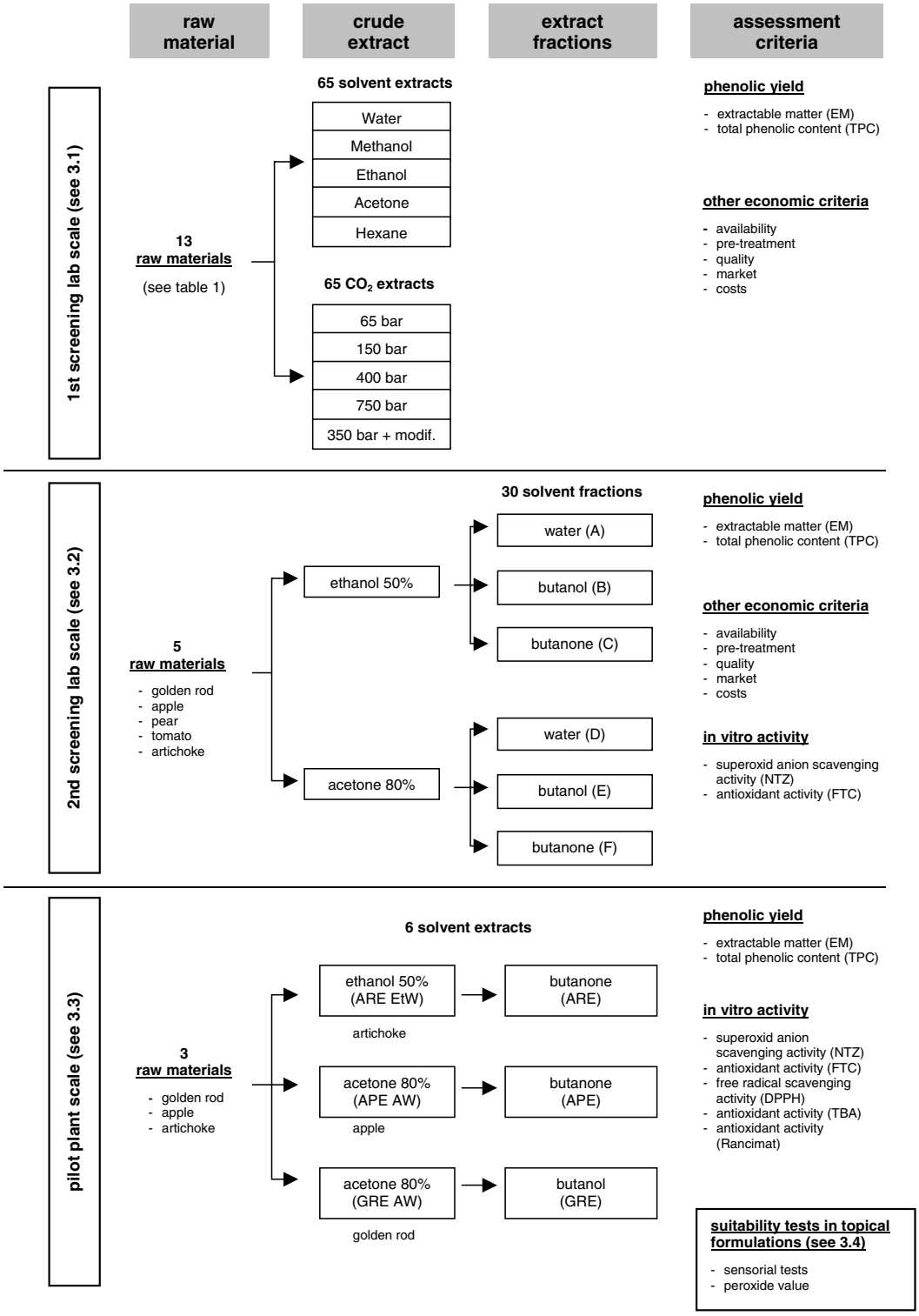

Fig. 1. Screening overview. Extraction, fractionation and assessment of the plant waste materials for suitability as antioxidants sources at the three main stages of this study.

hydrogenated palm glycerides citrate, Henkel, Germany), as well as the highly phenolic grape seed extract (GSE, Euromed, Mollet del Vallès, Spain), rosemary supercritical fluid extract (RSFE, "Stabiliton", RAPS, Kulmbach, Germany) and green tea extract (GTE, Flachsmann, Zürich, Switzerland).

Table 1
Source, provider and type of the raw materials investigated in this study

	Raw material	Type of residue	Provider
(I) Residues from juice production	Apple (*Malus* sp.)	Pressing residue	Beckers, Eisleben, Germany
	Strawberry (*Fragaria* sp.)	Pressing residue	Rauner, Tiefenbronn, Germany
	Pear (*Pyrus* sp.)	Pressing residue	Nufri, Lleida, Spain
	Red beet (*Beta vulgaris*)	Pressing residue	Eden-Waren GmbH, Hünfeld, Germany
(II) Waste from canning factory	Artichoke (*Cynara scolymus*)	Blanched outer bracts of the heads	Coop. Ntra. Sra. de Ocón Sociedad, Bernedo, Spain
	Asparagus (*Asparagus officinalis*)	Peels, stalks	Coop. Ntra. Sra. de Ocón Sociedad, Bernedo, Spain
	Tomato (*Lycopersicon lycopersicum*)	Peels	Coop. Ntra. Sra. de Ocón Sociedad, Bernedo, Spain
(III) Harvest remains	Broccoli (*Brassica oleracea* ssp. *oleracea* convar. *Botrytis* var. *italica*)	Stems, stalks	Coop. Agrícola El Progrés Garbi, Barcelona, Spain
	Cucumber (*Cucumis sativus*)	Stems, stalks	Coop. Agrícola El Progrés Garbi, Barcelona, Spain
	Endive (*Cichorium endivia*)	Stems, stalks	Coop. Agrícola El Progrés Garbi, Barcelona, Spain
	Chicory (*Cichorium intybus*)	Stems, stalks	Coop. Agrícola El Progrés Garbi, Barcelona, Spain
(IV) Minor crops	Golden rod (*Solidago virgaurea*)	Dried herb	Martin Bauer, Vestenbergsgreuth, Germany
	Woad (*Isatis tinctoria*)	Dried herb	Nuth-Chemie, Buchenau, Germany

2.2. Extract preparation

In the first screening, the raw materials were firstly extracted twice separately with water, methanol, ethanol, acetone and hexane, with a 10:1 solvent-raw material ratio, in closed vessels, by stirring at 25 °C for 4 h and being left to stand for another 4 h (using a soxhlet system for 6 h in the case of hexane). After filtration, the total solid content (TS) was determined in accordance with Eur. Ph. 1999, monography 765 ''Extracts'', and the maximum yield compared with the theoretically extractable matter (EM, according to British Pharmacopoeia, 1973) of the respective raw material. TS and EM measurements were made in duplicate, and repeated if deviation was higher than 5%.

The plant material selected in the primary screening was separately extracted with ethanol/water (50:50) and acetone/water (80:20) in a similar procedure to that described for the preliminary screening. These extracts were concentrated under vacuum and fractionated in triplicate in a liquid/liquid system of water/butanone and water/butanol. The solvents were chosen because they show a high sympathy for molecules containing hydroxyl groups, and also taking into account economic considerations imposed by the industrial context. The obtained fractions were filtered, concentrated under vacuum and dried in a vacuum oven at temperatures below 60 °C resulting in one aqueous fraction, one butanol fraction and one butanone fraction from each raw extract: fractions A–C from the ethanol raw extract and fractions D–F from the acetone one, as shown in Fig. 1.

For the scale up process, minor adaptations to the parameters were made for the pilot plant scale equipment of Euromed (Mollet del Vallès, Spain). For the CO_2 supercritical fluid extraction (RAPS Forschungszentrum Weihenstephan, Germany) the following range of conditions was chosen: 65 bar/60 °C/2 h, 150 bar/60 °C/2 h, 400 bar/60 °C/2 h, 750 bar/60 °C/2 h, and 350 bar/60 °C/2 h plus 30% (w/w) ethanol (50%) as modifier.

2.3. Determination of total phenolic content (TPC assay)

The total phenolic content (TPC) was determined in all the extracts and fractions following the Folin-Ciocalteu method (Singleton & Rossi, 1965). The reaction mixture was composed of 0.1 ml extract (1 or 10 mg ml^{-1}, depending on the activity), 7.9 ml distilled water, 0.5 ml of Folin–Ciocalteu's reagent, and 1.5 ml of a 20% sodium carbonate anhydrous solution (added 2 min after the Folin–Ciocalteu's reagent). After initial mixing the opaque flasks were allowed to stand for 2 h. The optical density of the blue-coloured samples was measured at 765 nm. The total phenolic content was determined as gallic acid equivalents (GAE) and values are expressed as mg of gallic acid/g of extract (in GAE).

2.4. Superoxide anion scavenging activity (NTZ assay)

The superoxide anion scavenging activity was measured with the neotetrazolium (NTZ) reagent in accordance with Masaki, Sakaki, Atsumi, and Sakurai (1995). To start the assay, 0.25 ml xanthine oxidase (1.2 U ml^{-1}) was added to a mixture containing 1.50 ml of 500 mmol l^{-1} Na–Pi buffer (pH 7.50 with 0.05 mmol l^{-1} EDTA) and 0.25 ml of 2.0 mmol l^{-1} hypoxanthine solution, 0.25 ml of 500 μmol l^{-1} neotetrazolium chloride solution and 0.25 ml sample. During

the first two screenings, sample concentrations of 10 or 20 $\mu g\,ml^{-1}$ were used. The optical density was measured at 560 nm. The inhibition was calculated in relation to the respective blank control at the reaction turning point after approximately 15 min.

2.5. Antioxidant activity in linoleic acid system with ferrothiocyanate reagent (FTC assay)

The antioxidant capacity was determined by the thiocyanate method (FTC) after Larrauri, Gosimni, Martín-Carrón, Rupérez, and Saura-Calixto (1996). A clear mixture of 0.5 ml extract or control sample ($2.5\,mg\,ml^{-1}$), 1.0 ml Na–Pi Buffer ($50\,mmol\,l^{-1}$, pH 7.0) and 0.5 ml of water or absolute ethanol was incubated for 1 h. Then 0.5 ml of ethanolic linoleic acid solution ($25\,mg\,ml^{-1}$) was added and shaken until saturated with oxygen. The brown test vials were put into an oven (40 °C) and after reacting for 96 h, an aliquot of 0.1 ml assay mixture was mixed with 9.7 ml ethanol (75%), 0.1 ml 1mmonium thiocyanate ($300\,g\,l^{-1}$) and $20\,mmol\,l^{-1}$ freshly prepared ferrous chloride solution (in 25% HCl). After 3 min the absorbance was measured at 500 nm. The inhibition of lipid peroxide oxidation was calculated against the blank sample and expressed in percent.

2.6. Free radical scavenging activity (DPPH assay)

The free radical scavenging activity using the 1.1-diphenyl-2-picryl-hydrazil (DPPH) reagent was determined after Brand-Williams, Cuvelier, and Berset (1995). To 0.75 ml of the extract sample at a concentration of 10 $\mu g\,ml^{-1}$ (in 50% methanol), 1.5 ml of freshly prepared methanolic DPPH solution (20 $\mu g\,ml^{-1}$) was added and stirred. The decolourizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control; in the case of coloured samples, an additional blind control contained the extract solution and pure methanol instead of DPPH. The solutions were freshly prepared and stored in darkness.

2.7. Inhibition of the $FeCl_2/H_2O_2$ stimulated linoleic acid peroxidation (TBA assay)

The pilot plant scale extracts were further characterized by determining the $FeCl_2/H_2O_2$-stimulated linoleic acid peroxidation by thiobarbituric acid (TBA) in accordance with Duh (1998). Extracts or synthetic antioxidants (0.2 ml) were added to a solution of 0.2 ml each of linoleic acid ($0.1\ mol\ l^{-1}$), $FeCl_2$–$4H_2O$ ($2.0\,mmol\,l^{-1}$), H_2O_2 ($2.0\,mmol\,l^{-1}$), and 5.0 ml phosphate buffer pH 7,4 ($0.2\,mmol\,l^{-1}$). After incubation for 24 h at 37 °C, 0.2 ml BHT (20 $\mu g\,ml^{-1}$), 1.0 ml TBA (1.0%) and 1.0 ml trichloroacetic acid (10%) were added to the mixture, which was heated at 100 °C for 30 min. After cooling, 5.0 ml chloroform was added and the mixture centrifuged at $1000 \times g$. The absorbance of the supernatant was measured spectrophotometrically at 532 nm. The inhibition was expressed as a percentage of BHT activity.

2.8. Rancimat® experiments

The inhibition of the oxidation in stressed linoleic acid was determined by colourimetric detection of secondary metabolites with the Rancimat® app. 743 (Metrohm, Herisau, Switzerland) with a slightly modified AOCS air oxidation method (AOM–AOCS Cd 12b-92) in accordance with Budincevic and Vrbaski (1995). In the standard method applied here generally pure linoleic acid is used as an oxygen-sensitive substrate. Depending on their specific solubility, 0.12 g of extract were pre-dissolved either in 9.88 g soybean oil, ethanol or Tween 80. 0.1 g of extract solution were added to 2.9 g of linoleic acid (Sigma L1626), previously treated with nitrogen flow for 5 min to displace oxygen. A permanent air-flow of 11 $cm^3\,min^{-1}$ oxidized the assay mixture heated to 60 °C. The retardation of induction time (due to the highly increased conductivity through volatile secondary oxidation products) was measured in triplicate and expressed as a percentage of the induction time of the negative control.

2.9. Suitability, stability and preservative effect in topical formulations

The extracts were evaluated for their general suitability in crème formulations through incorporation tests and sensory analysis (odour, colour, homogeneity, general aspect, handling) of standard formulations of water in oil (W/O) and oil in water (O/W) emulsion types ranging from 0.1% to 4.0% of oil. To determine the stability and antioxidant effect, the dry extracts selected were incorporated into a standard crème formulation type W/O with 5% standard evening primrose oil (EPO, Ropufa, Hoffmann La Roche, Switzerland) as an instable lipid component (0.1%, 0.5%, 1.0% and 3.0%) and an O/W-type formulation with 3% EPO (0.1%, 0.15%, 1.0% and 4.0%, partly pre-dissolved in propylene glycol). Before and after five weeks storage at 40 °C, the extracts were assessed by sensory analysis, and the peroxide value calculated as ionization potential in $meqO_2\ kg^{-1}$ (according to AOAS 965.33). Non-preserved crème served as the negative control and the standard preservation with 0.01% BHT, and the tocopherole derivatives 0.1% Oxynex® K liquid (Merck, Germany), or 0.15% Controx® KS (Henkel, Germany), respectively, as the positive control.

3. Results

3.1. Extraction yield and phenolic content of the raw extracts (first screening)

Taking into consideration the industrial requirements for extraction, both yields and economic parameters were primarily emphasized before detailed study of their antioxidant potential. The high moisture of the fresh byproducts investigated here (loss-on-drying of strawberry 65.2%, artichoke 89.8%, asparagus 92.7%, red beet 82.4%, tomato 71.2% and apple 80.0%) required a cost-intensive drying process with temperatures below 60 °C. As usual, the highest yield in one-step extraction was achieved by the polar solvents water and methanol (Table 2). Higher yields in the moderate polar area resulted for apple, strawberry, red beet, asparagus, tomato, cucumber, endives and chicory. Apple, strawberry and tomato wastes also yielded promising extractable matter with acetone and hexane.

In general terms, both the methanolic and ethanolic extracts exhibited higher phenolic contents than the other three conventional solvent extracts (i.e., apple, strawberry, cucumber, chicory, golden rod and woad). Red beet, artichoke, asparagus, tomato and broccoli seemed to deliver higher quantities of less polar phenolics. A multiple mixture of different phenolic classes in a wide range of polarities can be assumed for apple, red beet, artichoke, asparagus, tomato, and golden rod (Table 2). Evidently, lower extract:raw material ratios were

Table 2
Extraction yield (EY) and total phenolic content (TPC) of conventional solvent extracts from eleven byproducts and two minor crops (first screening)

Raw material	Water	Methanol	Ethanol	Acetone	Hexane
Extraction yield					
Residues from juice production					
Apple	33.7	30.2	26.0	6.7	3.7
Strawberry	7.8	17.1	14.2	9.9	9.9
Pear	11.4	4.3	2.2	1.4	1.0
Red beet	20.1	16.7	9.0	0.5	0.4
Waste from canning factory					
Artichoke	11.5	9.9	2.5	1.4	3.3
Asparagus	50.1	27.0	24.5	7.0	4.2
Tomato	39.9	11.6	10.6	15.0	11.9
Harvest remains					
Broccoli	21.8	6.5	2.1	1.2	2.1
Cucumber	25.1	19.6	28.0	6.4	1.0
Endive	36.1	9.0	3.5	1.6	2.2
Chicory	33.5	17.0	27.0	3.6	2.3
Minor crops					
Golden rod	15.0	11.0	5.3	3.4	2.2
Woad	24.4	12.8	4.9	2.4	3.0
Total phenolic content					
Residues from juice production					
Apple	46.00 ± 1.85	52.18 ± 4.80	41.56 ± 0.88	27.97 ± 0.76	31.93 ± 3.50
Strawberry	39.39 ± 4.60	59.77 ± 4.24	38.74 ± 2.40	34.55 ± 0.78	11.65 ± 0.75
Pear	12.90 ± 2.39	18.41 ± 2.12	12.09 ± 1.10	27.26 ± 2.65	13.38 ± 1.98
Red beet	91.74 ± 6.31	86.99 ± 7.02	121.95 ± 0.54	150.58 ± 6.51	124.15 ± 11.11
Waste from canning factory					
Artichoke	42.75 ± 12.17	95.65 ± 8.24	88.15 ± 4.99	102.33 ± 6.19	36.65 ± 5.87
Asparagus	89.40 ± 5.07	69.43 ± 7.06	60.14 ± 5.85	113.65 ± 17.73	29.33 ± 4.36
Tomato	12.15 ± 0.83	37.29 ± 2.08	42.00 ± 6.19	49.61 ± 9.52	30.24 ± 4.76
Harvest remains					
Broccoli	29.87 ± 1.58	25.58 ± 2.51	28.31 ± 1.69	36.18 ± 1.89	33.45 ± 2.32
Cucumber	18.41 ± 2.68	27.26 ± 1.80	16.96 ± 2.16	20.52 ± 2.59	26.71 ± 5.21
Endive	34.01 ± 6.79	17.18 ± 2.24	16.12 ± 2.48	23.66 ± 0.93	23.44 ± 2.46
Chicory	13.56 ± 1.81	25.51 ± 3.11	21.54 ± 3.58	14.16 ± 1.45	12.30 ± 1.80
Minor crops					
Golden rod	112.18 ± 3.25	181.68 ± 13.43	152.79 ± 9.47	91.10 ± 1.55	14.29 ± 0.21
Woad	65.72 ± 2.69	90.44 ± 11.55	76.95 ± 7.77	81.85 ± 6.46	29.82 ± 4.60

EY: values expressed as % of dry raw material; TPC: values expressed as mgGAE g^{-1} dry extract (means ± standard deviation of four measurements).

obtained by supercritical fluid extraction of five raw materials even in alcohol-modified conditions. The most effective extractions were, in decreasing order of yield: pear (10%) > strawberry (9%) > woad (4%) > apple (3.5%) > tomato (2%). The highest phenolic-containing supercritical fluid extracts (data not shown) were apple (450 bar/60 °C), pear (350 bar/60 °C), and strawberry (350 bar/60 °C + modifier), which gave TPC values of 69.2, 47.3 and 41.2 mg GAE g^{-1} dry extract, respectively.

To evaluate the procurement of raw material, economic characteristics such as availability (sufficient amount, season-independence), pre-treatment (low influence of treatment on active compounds, possible standardization according to *good agricultural practice* guidelines), quality (low water content, soil residues, homogeneity), market opportunities and costs (price, transport, additional pre-treatments required) were assessed. Thus even some byproducts with remarkable phenolic yield were regarded as too expensive or of little promise for the market, as for instance red beet, asparagus, or woad. After ranking of both the phenolic yield and practical issues, the following raw materials were selected for further investigation: apple, pear, artichoke, tomato, and golden rod.

3.2. Phenolic content and antioxidant activity of the selected extract fractions (second screening)

Purified and enriched extracts were obtained by two-step solvent extraction of selected starting raw materials (Fig. 1, Table 3). High phenolic content and acceptable yield resulted mainly from golden rod and artichoke byproducts (butanol and butanone fractions of 50%

Table 3
Extraction yield (EY), total phenolic content (TPC), superoxide anion scavenging activity (NTZ test) and antioxidant activity (FTC test) of fractions obtained from selected byproducts (second screening) (see Fig. 1 for fraction identification)

Raw material	Fraction	EY[a]	TPC[b]	NTZ[c]	FTC[c]
Apple	A	7.7	7.23 ± 0.62	14.81 ± 0.79	3.52 ± 0.90
	B	2.9	66.11 ± 0.36	20.22 ± 2.03	69.23 ± 0.12
	C	2.0	44.80 ± 0.34	18.59 ± 0.36	44.60 ± 0.56
	D	7.0	7.84 ± 0.11	24.61 ± 1.56	39.81 ± 0.85
	E	5.9	31.41 ± 0.22	27.54 ± 0.46	86.73 ± 0.86
	F	2.5	48.62 ± 0.93	25.80 ± 1.77	77.64 ± 0.71
Pear	A	1.1	19.04 ± 0.74	17.50 ± 1.50	79.04 ± 0.79
	B	2.6	57.68 ± 0.63	27.23 ± 2.52	88.60 ± 0.98
	C	2.9	53.33 ± 0.78	23.29 ± 0.65	n.d.
	D	0.1	14.13 ± 0.82	20.31 ± 1.87	84.78 ± 0.96
	E	1.0	60.67 ± 0.86	32.04 ± 2.16	77.09 ± 0.19
	F	1.5	48.49 ± 0.76	28.52 ± 1.47	77.63 ± 0.81
Artichoke	A	10.4	65.81 ± 2.43	4.03 ± 4.87	88.72 ± 2.04
	B	5.3	495.04 ± 14.05	26.00 ± 1.38	85.58 ± 1.89
	C	2.7	514.18 ± 14.92	8.76 ± 0.62	86.04 ± 1.64
	D	3.4	162.40 ± 4.97	8.04 ± 1.10	88.31 ± 2.21
	E	4.2	365.03 ± 9.12	20.45 ± 2.42	84.20 ± 1.97
	F	3.3	363.22 ± 10.17	15.81 ± 0.69	87.49 ± 2.12
Tomato	A	8.8	27.62 ± 1.98	28.76 ± 1.30	85.04 ± 1.87
	B	1.6	61.04 ± 3.02	32.81 ± 1.35	82.78 ± 1.49
	C	0.7	20.83 ± 0.69	28.34 ± 0.89	85.30 ± 2.06
	D	3.9	36.60 ± 2.24	32.88 ± 1.14	76.04 ± 0.95
	E	3.7	26.65 ± 1.76	34.50 ± 0.80	63.44 ± 0.78
	F	1.3	16.91 ± 0.82	33.86 ± 1.22	80.09 ± 2.08
Golden rod	A	6.7	58.36 ± 1.84	13.38 ± 0.70	84.10 ± 1.91
	B	5.8	203.34 ± 5.95	32.34 ± 2.20	86.13 ± 2.22
	C	3.7	251.40 ± 7.03	26.50 ± 1.84	86.41 ± 2.18
	D	1.7	72.51 ± 2.55	11.41 ± 1.55	84.04 ± 1.93
	E	6.2	217.69 ± 6.22	14.33 ± 1.85	84.38 ± 2.07
	F	3.3	200.43 ± 5.87	13.65 ± 1.76	88.52 ± 3.02
Reference extract	GSE		831.33 ± 19.26	37.91 ± 2.98	81.83 ± 1.49
	RSFE		147.10 ± 3.67	4.29 ± 0.06	83.90 ± 1.27

n.d., not detected; GSE, grape seed extract; RSFE, rosemary supercritical fluid extract.
[a] Values expressed as % of dry raw material.
[b] Values expressed as mgGAE g^{-1} dry extract (mean of three replicates ± standard deviation).
[c] Values expressed as percentage of inhibition at 10 $\mu g ml^{-1}$ extract (mean of three replicates ± standard deviation).

ethanol or 80% acetone raw extracts). The apple residue (toasted directly by the producer in large industrial scale at 400 °C) showed a phenolic content lower than that obtained during the first screening, when dried at 40 °C.

There was no general correlation between phenolic content (TPC), superoxide scavenging activity (NTZ) and inhibition of linoleic acid peroxidation (FTC). Similar phenolic classes can be assumed by comparing the TPC of butanol (B and E) and butanone (C and F) fractions of each raw material. Partly differing radical scavenging activity may be caused by lower availability of these extracts in the assay or by interactions with other constituents (e.g., artichoke). The butanol extract of golden rod (B) and pear (E), just as all the water, butanol and butanone extracts from tomato, reached superoxide scavenging activity nearly as high as the grape seed extract (GSE) (Table 3). At a 10 $\mu g ml^{-1}$ extract concentration in the FTC assay, almost all the selected extracts inhibited the oxidation process. The artichoke and golden rod extracts, and some fractions from tomato (A, C), apple (E) and pear (B, D) surpassed the effect of GSE and RSFE (Table 3).

Though all raw materials would justify the further investigation, only apple, artichoke and golden rod were selected for the scale up here. The decision was influenced by economic factors important for plant extract, cosmetics and pharmaceutical industries, as for instance the low extractable matter of pear, or the possible low customer acceptance of tomato in cosmetics.

3.3. Phenolic content and antioxidant activity of the pilot plant scale extracts (scale up)

Three starting raw materials were extracted on a pilot plant scale and the extracts compared in detail to common antioxidant references using the same methodology as used before and by three additional test systems (Table 4). Despite the lower total phenolic content than the reference plant extracts, both the golden rod (GRE) and artichoke (ARE) extracts showed a high radical scavenging activity in hydrophilic test systems and in the Rancimat® method. In contrast, the apple extract (APE) exhibited low phenolic content and radical scavenging activity, but high efficiency in the FTC and TBA assays.

Purification of the extracts did not lead automatically to a further concentration of phenolics and increased activity in all assays. A partial removal of active substances is suggested by the fraction results from the DPPH (APE), FTC (ARE, GRE) and TBA (ARE) assays (Table 4). The Rancimat® results exhibited a wide variance and depended strongly on the chosen pre-solution of the extracts (Table 5). In this test, BHT was clearly superior to any of the pilot plant obtained extracts, followed by Controx® KS, and by the artichoke, green tea and golden rod extracts, when they were predissolved in ethanol (Table 4). Surprisingly, low results were obtained with apple, grape seed extract and rosemary supercritical fluid extracts.

3.4. Suitability and antioxidant effect in dermatological formulations

The two cosmetic companies involved in this project evaluated first the general handling and incorporation properties of the extracts in standard crème formulations. In two formulations, water in oil emulsion (W/O) and oil in water emulsion (O/W), the golden rod extract (GRE) was easily mixable and dispersible up to concentrations of 3%. In contrast, both the apple (APE) and artichoke (ARE) extracts were hard to homogenize. In O/W formulations the miscibility and

Table 4
Extraction yield (EY), total phenolic content (TPC), radical scavenging activity (NTZ and DPPH tests), antioxidant activity (FTC and TBA tests) and oxidative stability (Rancimat® test) of pilot plant scale extracts (see Fig. 1 for extract identification)

Extract	EY[a]	TPC[b]	NTZ[c]	DPPH[c]	FTC[c]	TBA[c]	Rancimat
APE AW	30.4	31.60 ± 0.71	10.82 ± 0.83	77.63 ± 0.42	71.52 ± 1.44	76.72 ± 1.95	n.a.
APE	5.1	51.52 ± 0.83	27.41 ± 1.51	27.41 ± 0.55	75.20 ± 1.49	82.53 ± 1.88	48.40 ± 5.25
ARE EtW	18.2	145.37 ± 3.84	10.51 ± 1.26	41.82 ± 1.04	76.44 ± 1.98	91.33 ± 2.03	n.a.
ARE	2.1	193.24 ± 4.17	38.88 ± 1.68	87.03 ± 2.15	68.04 ± 1.56	64.11 ± 1.66	228.19 ± 31.97
GRE AW	14.3	220.40 ± 6.68	27.90 ± 2.50	91.77 ± 2.64	77.43 ± 2.31	12.55 ± 0.27	n.a.
GRE	3.5	207.71 ± 5.09	30.64 ± 1.34	91.55 ± 3.03	67.08 ± 1.85	67.86 ± 1.39	129.13 ± 20.41
Reference standard/extract							
BHT	–	–	55.50 ± 5.72	75.14 ± 1.29	92.14 ± 1.26	100.02 ± 1.89	609.13 ± 41.65
Controx® KS	–	71.03 ± 0.41	26.86 ± 0.53	45.30 ± 0178	86.96 ± 0.82	88.53 ± 1.76	249.40 ± 14.56
GSE	–	831.33 ± 19.26	63.80 ± 3.80	90.03 ± 1.63	81.83 ± 1.49	91.30 ± 2.21	0.82 ± 0.15
GTE	–	357.18 ± 10.11	57.41 ± 1.77	65.81 ± 0.74	71.91 ± 0.97	n.a.	228.01 ± 21.57
RSFE	–	147.10 ± 3.67	4.45 ± 0.57	22.88 ± 1.15	83.90 ± 1.27	81.84 ± 2.06	36.29 ± 4.31

n.a., not available; GSE, grape seed extract; GTE, green tea extract; RSFE, rosemary supercritical fluid extract.
[a] Values expressed as % of dry raw material.
[b] Values expressed as mgGAE g^{-1} dry extract (mean of three replicates ± standard deviation).
[c] Values expressed as % of inhibition at 10 (DPPH, FTC and TBA) and 20 (NTZ) $\mu g ml^{-1}$ extract (mean of three replicates ± standard deviation).

Table 5
Inhibition of lipid oxidation by the three pilot plant extracts in the Rancimat® test under different assay mixtures and conditions: I, pre-dissolution in ethanol, linoleic acid, 60 °C; II, pre-dissolution in 0.1% Tween 80, linoleic acid, 60 °C; III, direct dispersing in linoleic acid only, 60 °C; IV, evening primrose oil, 100 °C (see Fig. 1 for extract identification)

Extract	Treatment			
	I	II	III	IV
APE	48.40 ± 5.25	22.89 ± 5.42	28.55 ± 6.75	−5.60 ± 1.70
ARE	228.19 ± 31.97	207.90 ± 19.45	17.91 ± 2.96	−3.04 ± 0.82
GRE	129.13 ± 20.41	26.93 ± 5.96	14.33 ± 1.50	−7.61 ± 1.41
Reference standard/extract				
BHT	609.13 ± 41.65	–	–	–
Controx®	249.40 ± 14.56	–	–	–
GSE	0.82 ± 0.15	24.41 ± 4.39	–	–
GTE	228.01 ± 21.57	121.33 ± 11.56	–	59.20 ± 4.01
RSFE	36.29 ± 4.31	–	64.34 ± 3.72	–

Values represent the prolongation of the inhibition time, and are expressed as % (mean of three replicates ± standard deviation); GSE, grape seed extract; GTE, green tea extract; RSFE, rosemary supercritical fluid extract.

homogeneity of ARE could be improved by pre-dissolution in propylene glycol. With regard to odour and colour, concentrations of 0.1% and 0.5% of APE would be acceptable from the cosmetic point of view. GRE and ARE formulations with concentrations higher than 0.1% were apparently yellow-brown or greenish-coloured and with a characteristically fruity or spicy smell.

After five weeks storage at room temperature or 40 °C, the galenic stability and rancidity of the prototypes were analysed by the cosmetic companies using organoleptic criteria. In the sensitive emulsions selected, an upper limit of 0.5% can be given for both the W/O and the O/W formulations. Higher concentrations caused emulsion instabilities probably due to the high phenolic content. This can be considered as a reverse side effect of high phenolic antioxidants.

The onset of rancidity in the crèmes was evidenced by the peroxide value (PV). W/O formulations of all byproduct extracts had a peroxide rate lower than the negative control, 0.01% BHT and 0.1% Oxynex® samples (Fig. 2). In O/W emulsions, 0.1% APE, 1.0% GRE, and the two concentrations of ARE surpassed both the Controx® reference and the control, whereas 0.1% GRE and 1% APE had no relevant preservative effect (Fig. 3). In a complementary analysis of crème formulations stored at room temperature for five months,

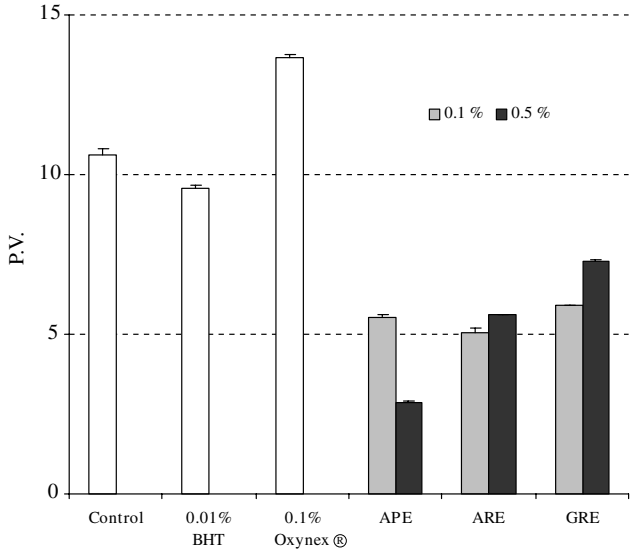

Fig. 2. Peroxide value of evening primrose oil (5%) containing W/O lotions (five weeks storage at 40 °C) supplemented with two concentrations of the three pilot plant extracts: apple (APE), artichoke (ARE) and Golden Rod (GRE) vs. BHT and Oxynex® in common concentrations and the unstabilized control. Values are the mean of three replicates + standard deviation.

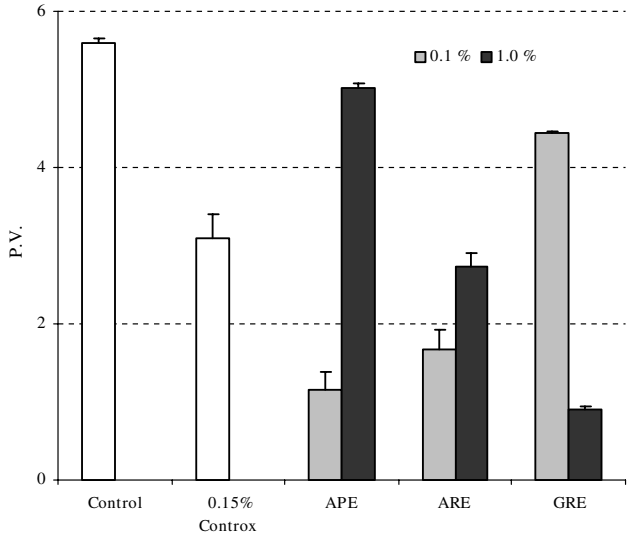

Fig. 3. Peroxide value of evening primrose oil (3%) containing O/W lotions (five weeks storage at 40 °C) supplemented with two concentrations of the three pilot plant extracts: apple (APE), artichoke (ARE) and Golden Rod (GRE) vs. Controx® in common concentrations and the unstabilized control. Values are the mean of three replicates + standard deviation.

no major differences were observed between the crèmes preserved by BHT and those preserved by 0.5% of plant extract in W/O formulations (PV values ranged between 3.42 and 6.28). In O/W formulations the antioxidant effect for GRE and ARE 1% (PV < 0.3 both), but not for APE (PV 2.75), was confirmed (PV control 2.69).

3.5. Considerations on the screening methodology

The heterogeneous results of this study demonstrate the usefulness of a wide range of methods to simulate physiological conditions rather than just one test system to characterize the antioxidant potential of plant extracts. Besides the general division between lipophilic and hydrophilic compounds, different matrices, assay conditions and interactions between extract components must be considered (Aruoma, 2003; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002). Nevertheless, it often remains difficult to differentiate between quantitative (real availability of the antioxidant) and qualitative reasons (multiple interactions of extract compounds and the test system) even within in vitro methods. However, feasible test systems with non-biological matrices can reduce the cost of analysis for screenings with high numbers of samples and prevent misleading results, as has been described, for instance, for reducing interactions of apple polyphenols with cell culture media in anti-proliferation assays (Lapidot, Walker, & Kanner, 2002). Thus, the general ranking of high sample numbers by few methods and generalized concentrations can be considered as no more than a useful first step in the further investigation of promising raw materials. This is demonstrated below in the discussion of two popular assays (NTZ and Rancimat®) used in this study.

The superoxide anion radical is regarded not as one of the most aggressive radicals, but as an initiator of radical chain reactions that generate other more reactive radicals, and thus gives strong evidence for the antioxidative potential of a substance (Nishikimi, Rao, & Yagi, 1972). The neotetrazolium/hypoxanthine assay (NTZ) showed some disadvantages in the evaluation of plant extracts, mainly caused by the sensitive hypoxanthine (HX)/xanthinoxidase (XOD) enzyme system and its limitation to aqueous conditions (Tsai, Chang, Chiou, & Liu, 2003). Therefore, only water or ethanol extracts could be used. In these solvents insoluble parts of e.g., acetone extracts could not be characterized completely. For a number of antioxidants and flavonoids, intrinsic potential and direct reductive influences on biological matrices can cause false positive results, as has been described for the MTT tetrazolium assay in tests of natural compounds (Bruggisser, von Daeniken, Jundt, Schaffner, & Tullberg-Reinert, 2002). For the NTZ assay using the HX/XOD system, direct influences on the enzyme or the NTZ reaction should be investigated as proposed by Soares, Dini, Cunha, and Almeida (1997). To exclude confounding effects of the three high phenolic extracts investigated here, first an additional analysis was performed with xanthine instead of hypoxanthine (detection at 295 nm without NTZ), and second, the NTZ reduction detected without XOD in the assay mixture.

The low resistance of the enzymatic system to light, temperature changes and other substances dictated a high level of standardization. Even deviations between levels of activity in different batches of standardized XOD products were noticed. Therefore, non-enzymatic test systems would be recommended for the primary screening of materials. In crude extracts, results may be influenced by compounds with certain pro-oxidant activity. Such effects were evident in this study by: (a) the addition of chlorophyll (10 $\mu g\,ml^{-1}$, CAS No. 1404-65-1, Roth, Karlsruhe, Germany) to the highly active grape seed extract; (b) the comparison of rosemary extract with chlorophyll free rosemary extract (both supercritical fluid extracts supplied by RAPS, Germany) (Fig. 4). The fact that the activity of crude extracts detected before fractionation was lower in some cases despite higher phenolic content could be explained in part by that effect.

The Rancimat® measurement of fat and oil oxidation stability was used by some authors to evaluate substances that retard oxidation processes (Budincevic & Vrbaski, 1995). Despite its practical advantages, the solubility or dispersion of more polar antioxidants in the assay matrix is of decisive influence on what activity is determined. The standard method applied here generally uses pure linoleic acid as a lipid component to avoid any falsification by natural antioxidants in oils. The activity of RSFE changed from 64.3%, when directly dispersed in linoleic acid to 36.3%, when pre-dissolved in ethanol, and that of GSE from 0.8% to 24.4% when it was pre-

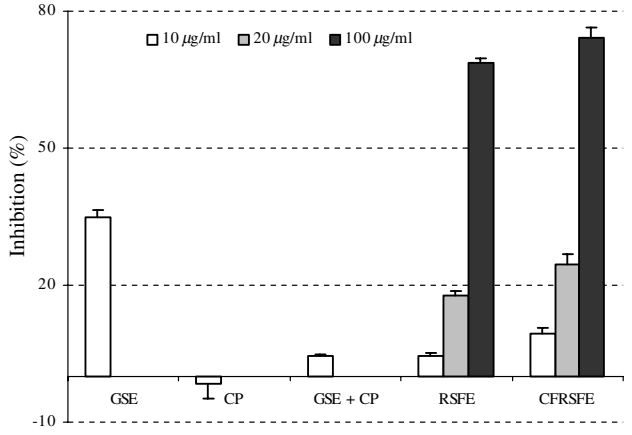

Fig. 4. Influence of chlorophyll (CP) on the superoxide anion scavenging activity detection of grape seed extract (GSE) and rosemary supercritical fluid extract (RSFE) (see Section 3.5 for treatment details; CFRSFE, chlorophyll free rosemary supercritical fluid extract). Values are the mean of three replicates + standard deviation.

dissolved in Tween 80 instead of ethanol (Table 5). To estimate the influence of temperature, the extracts were also measured in EPO at 100 °C. Whereas the GTE had a lower but still detectable antioxidant effect at this temperature, the three extracts investigated no longer showed any activity at all.

4. Discussion

4.1. Industrial exploitation potential of fruit and vegetable byproducts

The results confirmed that agroindustrial and agricultural wastes contain high amounts of phenolics and suggest the antioxidant recycling of the wastes of artichoke, apple and tomato; furthermore strawberry, asparagus or red beet. If the phenolic content of waste-derived raw extracts is compared to that of the original fruits and vegetables, an advantage of byproduct use for further enrichment of phenolics is also apparent. For example, it was possible to obtain a pilot plant artichoke extract (from the blanched outer bracts only) with 145.4 mgGAE g^{-1} dry extract (= 2.64% TPC in dry raw material) and later to enrich it to 193.2 mgGAE g^{-1}. According to Wagenbreth and Eich (2000), HPLC analysis revealed that whole heads contained 1.4% caffeic acid derivatives and 0.06% flavonoids calculated as cynaroside. These findings confirm the results of Llorach et al. (2002), who detected a caffeic acid derivate content in artichoke heads after blanching (103–243 mg g^{-1} dry extract) higher than in unprocessed material (99–154 mg g^{-1}). Further detailed investigation of phenolic substances in the artichoke byproduct extract (ARE) has led to the identification of 45 phenolic compounds (Sánchez-Rabaneda et al., 2003).

High antioxidant effects of apple extracts with relatively low phenolic content (12.1 mgGAE g^{-1}) have already been described by Kähkönen et al. (1999). The TPC of apple juice with 1.02 mgGAE ml^{-1} (Netzel et al., 1999) is considerably exceeded by fresh apple peel (5.0-5.9 mgGAE g^{-1} according to Wolfe & Liu, 2003). Our acetone/water extract obtained at pilot plant scale contained 31.6 mgGAE g^{-1} dry extract (=9.6 mgGAE g^{-1} in the dried and 1.9 mgGAE g^{-1} in the fresh pressing residue). The utility of recycling apple residue from pressing is supported by the results of van der Sluis, Dekker, Skrede, and Jongen (2002), who reported that most of the antioxidants in fresh apples were retained in the solid matter rather than being transferred into the juice during pressing. The detailed study of this apple waste extract (APE) has been recently performed by Sánchez Rabaneda et al. (2004), trying to establish the connection between its antioxidant activity and chemical composition as a basis for further industrial applications.

Golden rod, which originally was run in this screening as a phenolic-rich plant reference, has already been investigated for its diuretic, antiphlogistic, spasmolytic, antimycotic and anti-tumor activity (Schilcher, 1999). However, no detailed study on the antioxidant activity of golden rod has been concluded yet. Our data demonstrate for the first time the high radical scavenging activity of its extracts.

4.2. Byproduct exploitation limits

As multiple polyphenolic sources are known, investigation efforts in the early stage should be influenced by economic factors such as purchasing expenditure, transport, drying costs, required changes in the production chain, and measurements for standardization and extraction costs; but also the image and marketing aspects for application in cosmetics and as nutriceuticals. This study revealed three typical problems with obtaining high-quality standardized phenolic extracts from byproducts.

(1) The heterogeneity of the batches and missing specification of cultivars, provenance, storage time and conditions of processed fruits and vegetables often lead to a low degree of standardization. The local variation in the production chain demands tailored adaptations and solutions. In this study, for example, the apple juice producer recycles the dried storable residue by selling it for pectin production. However, in this case the common practice of applying enzymes (pectinase) and higher temperatures to significantly increase juice yields are not permitted. The here investigated alternative apple residue recycling could be of commercial interest due to higher pressing efficiency and indirect lower drying costs. Such substantial change in the production chain would require a guaranteed demand for high amounts of byproduct to be processed for phenol extraction. Thus, the final raw material price would be a function of the market size for an antioxidant apple extract like the one developed here.
(2) High costs arose for fast but soft drying conditions of what is generally extremely wet material (e.g., artichoke byproduct dry:fresh mass ratio around 1:9) to prevent the deterioration of polyphenols through heat and enzymes. Drying should be realized immediately and as close to the production location as possible. Existing industrial production lines facilitate procurement, reduce transport costs and avoid long-term storage of voluminous wet material. However, one example for overstressed industrial drying is given in this study, where the TPC value of the apple residue was 15.74 mgGAE g^{-1}dry matter in the first screening (40 °C), and

3.10 mgGAE g^{-1} dry matter in the second one (dried up to 400 °C for a few minutes at industrial scale) (data not shown). Similarly, wet artichoke waste batches showed lower TPC and antioxidant activity after longer storage and transport without cooling.

(3) The more expensive supercritical fluid extraction can be assumed to be economically less feasible due to the low yield of high active phenolic fractions. It would only be justified if some extracts exceeded the content of antioxidants considerably, or for the specific enrichment of unique high-value constituents, as for example, it was suggested for lycopene enrichment of tomatoes (Rozzi, Singh, Vierling, & Watkins, 2002). The one-step concentration of phenolic constituents obtained by the conventional solvent extraction is remarkably high. However, further purification is recommended for certain substances with inconvenient physico-chemical extract properties, such as carbohydrates. Possible technical modifications according to the specific demands of end producers (colour, dryness, miscibility, etc.) depend on the acceptable price for standardized extracts.

4.3. Suitability of phenolic rich plant extracts as antioxidants in foods and topical formulations

This investigation revealed the high exploitation potential of plant residues and encourages future, detailed studies of processing conditions and economic parameters. The direct comparison of data from byproducts to each other and to established antioxidants, the proven activity in a variety of assays and the differences according the extract polarity should facilitate decisions of the food producing industry for polyphenolic recycling. The economically based exclusion of some active extracts at an early stage of screening might be different when other companies, than in this study, pursue other product sectors and efficiency limits differ. In general food derived extracts exhibit certain advantages in contrast to (semi)synthetic antioxidants or isolated natural compounds in terms of consumer acceptance and legal needs for the market access. Despite efforts for harmonized safety requirements on the European level, extracts from food plants are often still recognized as safe in national or international law both in food as in cosmetics (“INCI list”) or underlie at least a simplified proof of safety.

The general advantageous name recognition of food plant derived extracts can be restricted by less favourable associations linked with some vegetables when used in cosmetics (e.g., potato, broccoli), but is not a problem in food supplements. Topical formulations are limited by colour, odour and physico-chemical properties that may cause instability or skin irritation. In addition, concentrated natural extracts can be restricted by an adstringent or unpleasant taste when added to a food product. Our findings show that concentrations higher than 0.1% are not recommended for general use in cosmetic formulations. For the specific extract utilization as an active ingredient, concentrations of up to 2% should be possible in appropriate matrices depending on the sensitivity of the formulations in question. The maintenance of the antioxidant activity can be assumed but this should be confirmed in further investigations involving longer stability tests and higher temperatures during the production. The partial substitution of synthetic or expensive established plant antioxidants could be successful, when upper concentration limits are defined with regard to galenic stability and skin irritation, and lower concentration limits with regard to the preservative effect.

Combinations of various low concentrated antioxidant extracts tailored to the organoleptic quality needs of individual products might be a future strategy for the cosmetic as also for the food industry (Beddows, Jagait, & Kelly, 2001). The synergistic action of a variety of antioxidants could be an appropriate way to diminish detrimental interactions sometimes associated with products. Following the strategy of lower total antioxidant concentrations and higher consumer acceptance an extended pool of non-toxic natural antioxidants would be valuable – even if the price of the extracts is still higher than that of ascorbic acid derivates, modified tocopherol products or BHT.

Furthermore the extracts could be suitable as functional ingredients in the food industry (Milo-Ohr, 2004). For example, a concentrate of apple juice may protect the brain against oxidative damage and improve cognitive performance (Rogers, Milhalik, Ortiz, & Shea, 2003), or broccoli may help the body fight prostate cancer (Milo-Ohr, 2004). More nutritional studies, however, are needed to measure the necessary level of extract to observe the beneficial effect. They should not only consider the influence of different conditions, including temperature, moisture, oxygen and added ingredients on these bioactive compounds (Bell, 2001), but specifically byproducts and byproduct derived extracts, as apparently many desired constituents are at higher concentrations in the residuals. (Schieber et al., 2001).

Acknowledgements

The financial support from the European Commission and the collaboration and technical support by all partners involved in the CRAFT project FAIR CT-98-9517, especially Nuth-Chemie (Buchenau, Germany), Euromed S.A. (Mollet del Vallès, Spain)., RAPS-Forschungszentrum (Weihenstephan, Germany), Kuhs

GmbH & Co. KG (Langenfeld, Germany), Laboratorios Rubio S.A. (Barcelona, Spain), and Becker GmbH & Co. Eislebener Fruchtsaft oHG (Eisleben, Germany) is gratefully acknowledged.

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