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Thermal stability of antioxidants obtained from wood and industrial wastes

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Abstract

The thermal stability of two synthetic food antioxidants (BHA and BHT) and three biomass-derived fractions with antioxidant activity (ethyl acetate soluble-fraction from *Eucalyptus globulus* acid hydrolysates, ethyl acetate soluble-fraction of autohydrolysis liquors from red grape pomace after fermentation and distillation and washing water of the same feedstock) were assessed. In the case of BHA and BHT, the non-volatile fraction and the antioxidant activity were measured at 100, 150 or 200 °C in assays lasting up to 120 min. In the case of biomass-derived fractions, the percentage of recovered phenolics in solid phase was also determined. The susceptibility of synthetic antioxidants towards volatilisation was higher than those of biomass-derived fractions, which also showed a remarkable ability to retain antioxidant activity after heating.

Keywords: Antioxidants; Ethyl acetate extraction; *Eucalyptus* wood; Hydrolysates; Red grape pomace; Thermal stability

1. Introduction

The major compounds recovered in the liquid phase from the mild acid processing of lignocellulosics are monomeric and oligomeric hemicellulosic sugars, sugar dehydration products, organic acids, extractives and phenolic compounds arising from the depolymerization of the lignin fraction (Klinke, Schmidt, & Thomsen, 1998; Tran & Chambers, 1985). Lignin degradation products are simple phenols, mainly derived from guaiacyl, syringyl or *p*-hydroxyphenyl groups, depending on the origin of the raw material. Phenolic and cinnamic acids, aldehydes, alcohols and ketones have been identified in hydrolysates from mild acid hydrolysis of hardwoods or agricultural residues (Ando, Arai, Kiyoto, & Hanai, 1986; Jönsson, Palmqvist, Nilvebrant, & Hahn-Hägerdal, 1998; Larsson et al., 1999; Tran & Chambers, 1985; Tran & Chambers, 1986). These compounds are prejudicial to a further fermentative processing of hydrolysates because they can inhibit growth and metabolism of microorganisms. In order to facilitate the benefit of hydrolysates, this type of compound can be selectively removed by solvent extraction (Clark & Mackie, 1984; Parajó, Domínguez, & Domínguez, 1998), leading to crude fractions with antioxidant activity. Higher radical-scavenging capacity than BHT, and of the same order as BHA, has been reported for ethyl acetate extracts of liquid phases from the hydrolytic processing of lignocellulosic materials (Cruz, Domínguez, Domínguez, & Parajó, 2001; Garrote, Cruz, Domínguez, & Parajó, 2003; González, Cruz, Domínguez, & Parajó, 2004).

Exposure to high temperatures can result in decomposition and/or evaporation of a given food antioxidant. Studies to assess the susceptibility of the considered antioxidant to thermal decomposition in air and the identification of degradation products have been reported (Hamama & Nawar, 1991), whereas assays involving exposure to high

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temperatures in oil are useful for predicting the behaviour during frying (Sanhueza, Nieto, & Valenzuela, 2000).

The aim of this work was to assess the thermal stability of the three crude fractions obtained from biomass: ethyl acetate soluble-fraction from *Eucalyptus globulus* acid hydrolysates, ethyl acetate soluble-fraction from autohydrolysis liquors of red grape pomace after fermentation and distillation and washing water of red grape pomace after fermentation and distillation. For this purpose, the effects caused by heating (volatilisation and modification of phenolic content and antioxidant activity) on the crude fractions have been evaluated. The results are compared to data corresponding to two commercial food antioxidants, BHA and BHT.

2. Materials and methods

2.1. Antioxidant extracts

Ground samples of *Eucalyptus* wood were hydrolysed with 5% H_2SO_4 for 60 min at 130 °C, using a liquid:solid ratio (LSR) of 8:1 g/g (González et al., 2004). The ethyl acetate-soluble solids from hydrolysates of *E. globulus* wood were freeze-dried and the extract (EWH) was used for further studies. Red grape pomace, after fermentation and distillation (an industrial waste obtained from "Cooperativa Vitivinícola del Ribeiro", Ourense, Spain), was subjected to an autohydrolysis reaction in aqueous media by autoclaving (at 130 °C for 90 min) a suspension containing 7.5 g water/g solid (Cruz, Domínguez, & Parajó, 2004) and the liquid phase was extracted with ethyl acetate. The freeze-dried, ethyl acetate soluble-fraction (here denoted RGPH) was used in further experimentation. Alternatively, the red grape pomace after fermentation and distillation was washed with water at 60 °C for 1 h using 25 g water/g pomace (Cruz et al., 2004), and the washing liquors were freeze dried to isolate a fraction (here denoted RGPWL) to be used in further experiments.

2.2. Non-volatile fraction

Samples (0.01 g each) of the synthetic antioxidants BHA (Analema), BHT (Analema), EWH, RGPH or RGPWL were individually placed in 2 ml test tubes and heated in air at the desired temperature (in the range 100–200 °C) for 2 h. After the desired heating time, tubes were periodically removed, cooled to room temperature, weighed and assayed for antioxidant activity and (in the case of EWH, RGPH or RGPWL samples) for total phenolics. All the assays were run in triplicate.

2.3. Recovery of phenolic compounds

Total phenols in original or heated EWH, RGPH and RGPWL were determined by absorbance readings, at 760 nm, of the complex formed with the Folin–Denis reagent. A standard curve with gallic acid (Sigma Chem. Co.) was used to express the concentrations of phenolics as gallic acid equivalents. In order to facilitate the interpretation of results, the phenolic content of samples exposed to heating is expressed as a percentage with respect to the results obtained for the samples not subjected to thermal treatment.

2.4. DPPH radical-scavenging activity

Two millilitre of a 6×10^{-5} M methanolic solution of DPPH (α,α-diphenyl-β-picrylhydrazyl) were added to 50 μl of a methanolic solution of the antioxidant considered (BHA, BHT, EWH, RGPH and RGPWL). Separate sets of experiments were carried out for each of them, using concentrations leading to inhibition percentages (IP) of the DPPH radical (calculated as the percentage of reduction in absorbance at 515 nm between 0 and 16 min) above and below than 50, and the concentrations leading to IP = 50 were calculated by interpolation. The concentrations leading to 50% inhibition were 0.23 g BHA/l, 2.78 g BHT/l, 0.50 g EWH/l, 0.25 g RPGH/l and 1.37 g RGPWL/l. Methanolic solutions of BHT, EWH, RGPH and RGPWL, previously subjected to heating in air, were prepared at the above concentrations and assayed for antioxidant activity.

3. Results and discussion

As a first approach to measuring the thermal stability of antioxidants, the weight of solid remaining after exposition to air at the desired temperature and the antioxidant activity were determined for BHA, BHT, EWH, RGPH and RGPWL. The results from the gravimetric analysis allowed the determination of the "non-volatilised fraction" (NVF), which was expressed as a percentage with respect to the initial weight. The antioxidant activity of non-volatilised solids was expressed as inhibition percentage (IP) and referred to the mass of solids after thermal treatment.

Figs. 1 and 2 show the dependence of NVF and IP on processing time for BHA and BHT at the temperatures selected in this study. The data of Figs. 1a and 2a show that both compounds were stable when exposed for 1 h at 100 °C (NVF near 100%), whereas 90% NVF was determined after 2 h by heating at the same temperature. High temperatures resulted in marked volatilisation: for example, NVF near zero was determined in the experiment carried out at 200 °C for 120 min. Figs. 1b and 2b show that temperature also was very influential on antioxidant activity: the antioxidant activity of BHA decreased by more than 1/3 after 120 min heating at 150 °C, whereas it was deactivated by 80% after 120 min heating at 200 °C. Comparatively, after heating at 100 °C for 2 h, BHT retained 74% of its initial DPPH radical-scavenging capacity, but it was completely inactive after 120 min heating at 150 °C or after 75 min heating at 200 °C.

The decrease in antioxidant activity of the non-volatilised fraction observed for BHA and BHT can be caused