Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 100 (2007) 1059–1064
Food Chemistry
www.elsevier.com/locate/foodchem

Thermal stability of antioxidants obtained from wood and industrial wastes

J.M. Cruz [a,b,*], E. Conde [a], H. Domínguez [a], J.C. Parajó [a]

[a] Departamento de Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, 32004 Ourense, Spain
[b] Área de Nutrición y Bromatología, Departamento de Química Analítica Nutrición y Bromatología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela – La Coruña, Spain

Received 30 May 2005; received in revised form 3 November 2005; accepted 3 November 2005

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

* Corresponding author. Fax: +34 981 594 912.
E-mail address: jmcruz@usc.es (J.M. Cruz).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.11.012

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

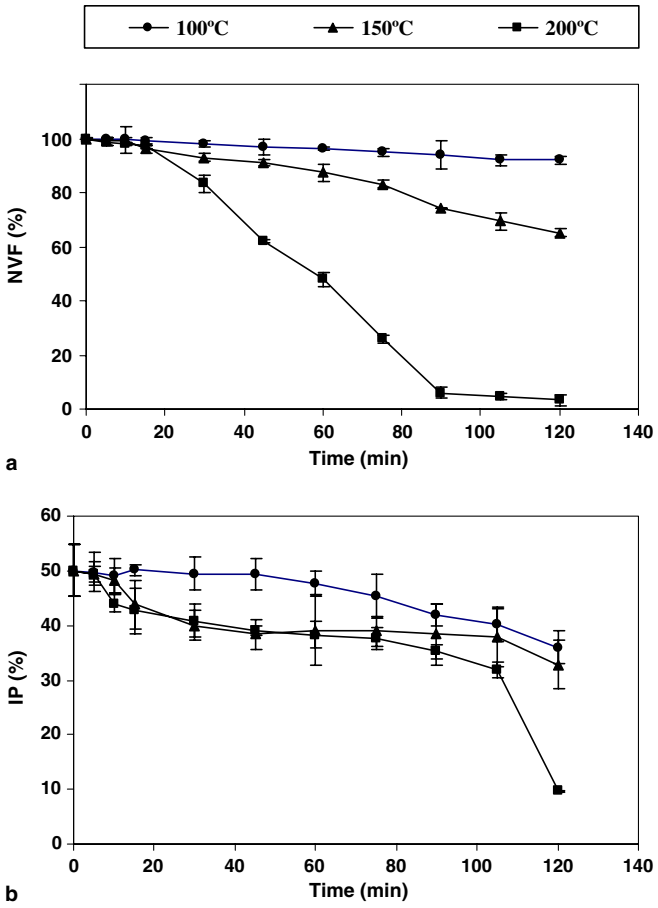

Fig. 1. Results determined for the synthetic antioxidant BHA upon air exposure at 100, 150 or 200 °C: time-dependence of: (a) non-volatile fraction of solids (NVF) and (b) inhibition percentage of the DPPH radical (IP).

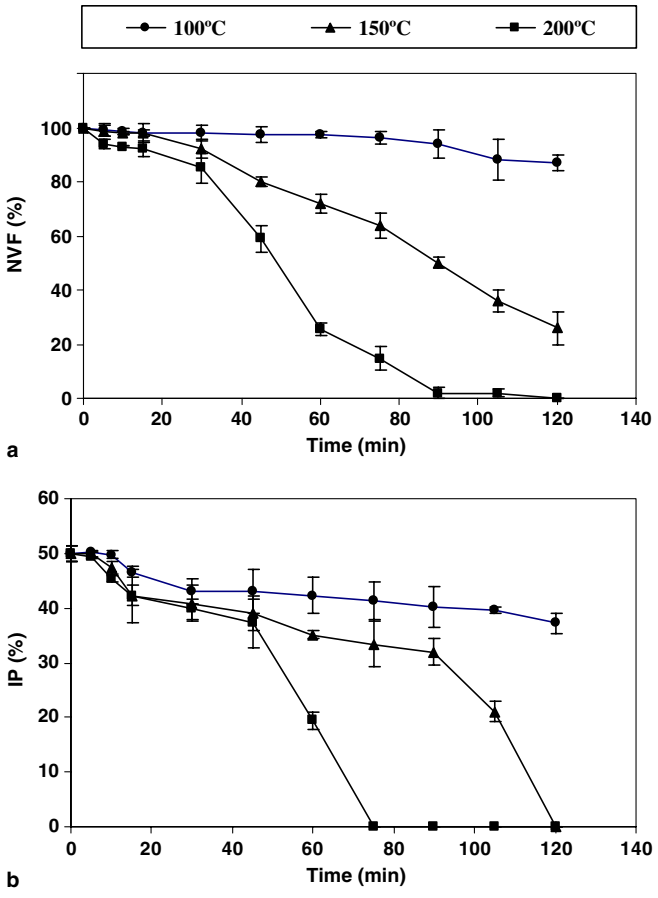

Fig. 2. Results determined for the synthetic antioxidant BHT upon air exposure at 100, 150 or 200 °C: time-dependence of: (a) non-volatile fraction of solids (NVF) and (b) inhibition percentage of the DPPH radical (IP).

by the weight loss of the solid (volatilisation) and/or chemical alteration, leading to products in the solid phase with lower antioxidant activity than the original fractions. As the IP have been determined for the same concentrations of solids (before or after heating), the results obtained for BHA and BHT, at all the temperatures considered in this work, show that the decomposition products remaining in solid phase have lower specific antioxidant activities than the initial product. Hamama and Nawar (1991) detected 2,3′-di-*tert*-butyl, 2′-hydroxy, 4,5′-dimethoxydiphenyl ether, 2,2′-dihydroxy-5,5′-dimethoxy-3,3′-di-*tert*-butylbiphenyl and free radical species as the thermal decomposition products of BHA and up to four free-radical resonant species as degradation products from BHT.

Fig. 3 shows the time-dependence of NVF, percentage of recovered phenolics (%RP) and IP for experiments carried out with EWH samples at the selected temperatures. The same information is given in Fig. 4 for RGPH and in Fig. 5 for RGPWL. A comparative evaluation of the results determined for NVF showed that EWH, RGPH and RGPWL had higher thermal stabilities than had BHA or BHT: in the case of the biomass-derived fractions, NVF in the range 70–80% were determined, even after prolonged heating at 200 °C, conditions under which the synthetic antioxidants were almost completely volatilised. Thermal processing affected the phenolic content of EWH, which was reduced by 20–40% after prolonged heating at 100–200 °C. Phenolics in RGPH were more stable than those in EWH, as they were not significantly volatilised at 100–150 °C and showed high %RP (72%) under the severest conditions assayed (120 min heating at 200 °C). In experiments at 100 °C, the phenolic content of RGPWL was not significantly affected by the heating time within the experimental range considered in this study, but higher temperatures resulted in decreased phenolic contents; for example, 26.5% and 70% reductions in phenolic content were observed after 120 min heating at 150 and 200 °C, respectively. Temperature had a comparatively lower influence on the phenolic content and antioxidant activity of RGPH samples, as can be inferred from the experimental profiles shown in Fig. 4.

The antioxidant activity of EWH, RGPH and RGPWL was fairly constant with time at the lowest temperature assayed (100 °C), but presented different behaviour at 150 and 200 °C, particularly with prolonged heating times; the IP of EWH and RPGWL decreased during the

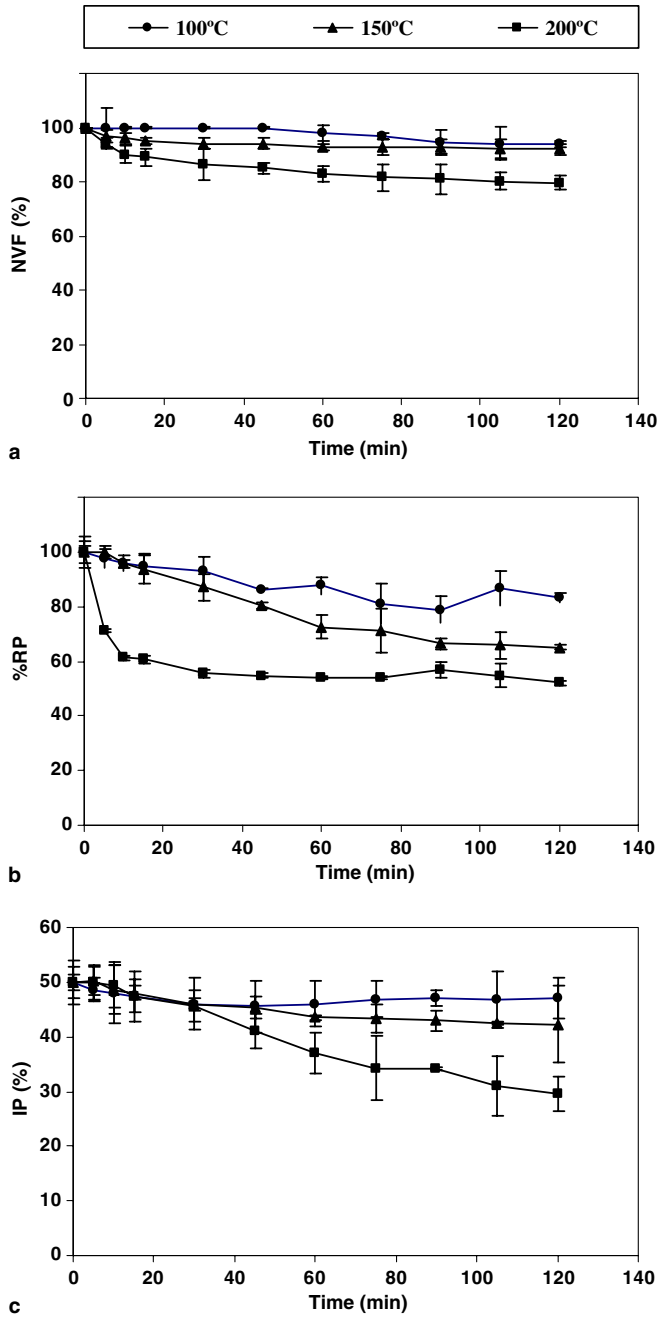

Fig. 3. Results determined for EWH samples (ethyl acetate soluble-fraction from *Eucalyptus* wood hydrolysates) upon air exposure at 100, 150 or 200 °C: time-dependence of: (a) non-volatile fraction of solids (NVF); (b) Percentage of recovered phenolics in solid phase (%RP) and (c) inhibition percentage of the DPPH radical (IP).

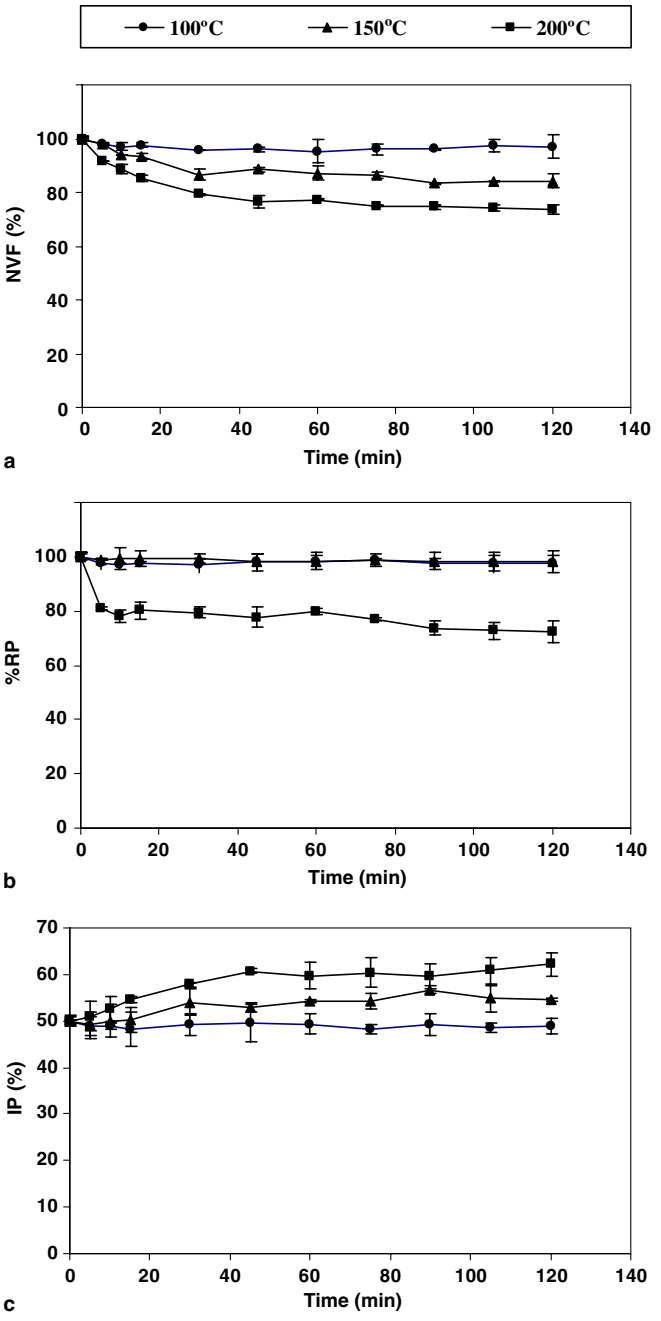

Fig. 4. Results determined for RGPH samples (ethyl acetate soluble-fraction from autohydrolysis liquors of red grape pomace after fermentation and distillation) upon air exposure at 100, 150 or 200 °C: time-dependence of: (a) non-volatile fraction of solids (NVF); (b) Percentage of recovered phenolics in solid phase (%RP) and (c) inhibition percentage of the DPPH radical (IP).

treatments, whereas the same variable increased for RGPH, indicating that the products from thermal degradation were more active antioxidants than were the parent extracts. In related experiments, Guillot, Malnoee, and Stadler (1996) observed that the decomposition products of caffeic acid were more potent antioxidants than was the original product. In the case of RPGWL, there was a remarkable similarity between the experimental profiles of %RP and IP (see Fig. 5b and c), suggesting that the loss of antioxidant activity during heating could be directly ascribed to the decomposition of phenols.

4. Conclusions

In the present work, the superior thermal stability of some active extracts from residual sources over synthetic ones was confirmed. The ethyl acetate solubles from acid

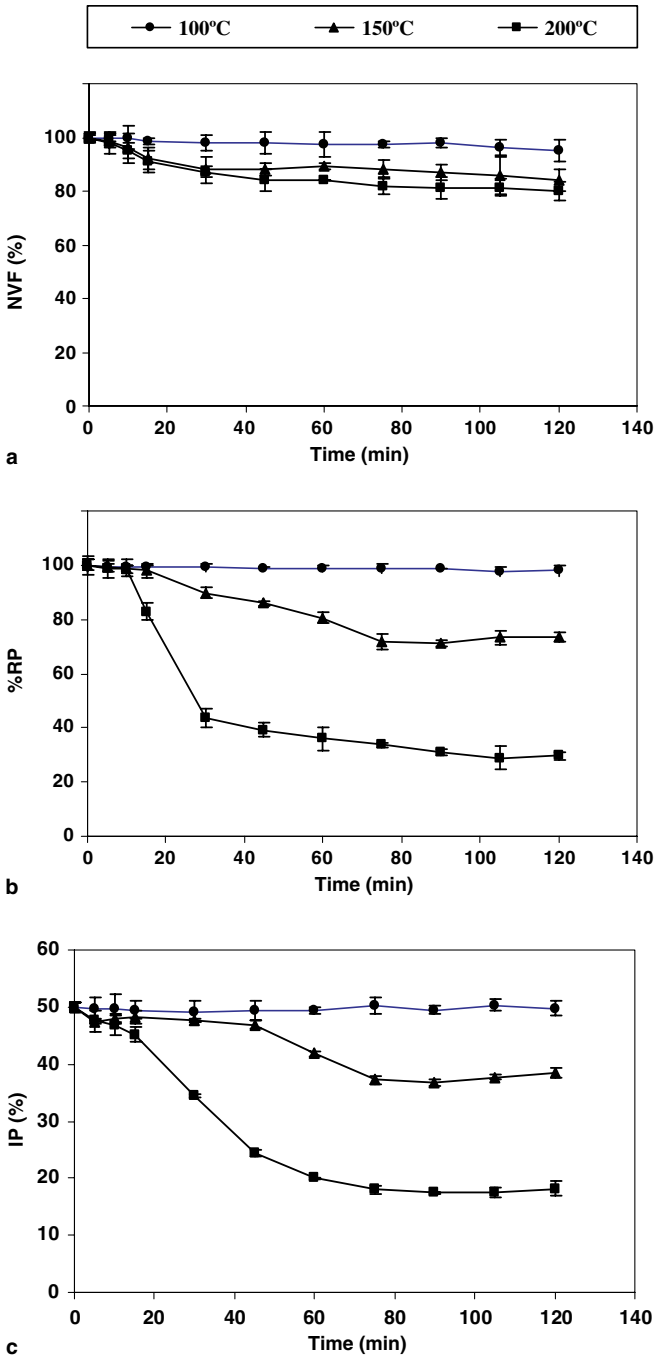

Fig. 5. Results determined for RGPWL samples (washing liquors from red grape pomace after fermentation and distillation) upon air exposure at 100, 150 or 200 °C: time-dependence of: (a) non-volatile fraction of solids (NVF); (b) Percentage of recovered phenolics in solid phase (%RP) and (c) inhibition percentage of the DPPH radical (IP).

hydrolysates of *E. globulus* wood, from autohydrolysis and from washing of distilled red grape pomace showed higher thermal stability than BHA or BHT. The non-volatile fractions, from natural extracts, in the range 70–80%, were determined even after prolonged heating at 200 °C, conditions under which the synthetic antioxidants were almost completely volatilised. In addition, the natural-derived antioxidants showed greater ability than synthetics to retain antioxidant activity after heating.

Acknowledgements

Authors are grateful to CICYT (PPQ2000-0688-C05-02), Xunta Galicia (PGIDT, 01PXI38302PN) and to the "Ramón y Cajal" Programme financed by the Ministry of Education of Spain for the financial support of this work.

References

Ando, S., Arai, I., Kiyoto, K., & Hanai, S. (1986). Identification of aromatic monomers in steam-exploded poplar and their influences on ethanol fermentation by *Saccharomyces cerevisiae*. *Journal of Fermentation Technology, 64*, 567–570.

Clark, T. A., & Mackie, K. L. (1984). Fermentation inhibitors in wood hydrolysates derived from the softwood *Pinus radiata*. *Journal of Chemical Technology and Biotechnology, 34B*, 101–110.

Cruz, J. M., Domínguez, H., & Parajó, J. C. (2004). Assessment of the production of antioxidants from winemaking waste solids. *Journal of Agricultural and Food Chemistry, 52*, 5612–5620.

Cruz, J. M., Domínguez, J. M., Domínguez, H., & Parajó, J. C. (2001). Antioxidant and antimicrobial effects of extracts from hydrolysates of lignocellulosic materials. *Journal of Agricultural and Food Chemistry, 49*, 2459–2464.

Garrote, G., Cruz, J. M., Domínguez, H., & Parajó, J. C. (2003). Valorisation of waste fractions from autohydrolysis of selected lignocellulosic materials. *Journal of Chemical Technology and Biotechnology, 78*, 392–398.

González, J., Cruz, J. M., Domínguez, H., & Parajó, J. C. (2004). Effect of the operational conditions for the production of antioxidants from Eucalyptus wood by acid hydrolysis. *Food Chemistry, 84*, 243–251.

Guillot, F. L., Malnoee, A., & Stadler, R. H. (1996). Antioxidant properties of novel tetraoxygenated phenylindan isomers formed during thermal decomposition of caffeic acid. *Journal of Agricultural and Food Chemistry, 44*, 2503–2510.

Hamama, A. A., & Nawar, W. W. (1991). Thermal decomposition of some phenolic antioxidants. *Journal of Agricultural and Food Chemistry, 39*, 1063–1069.

Jönsson, I. J., Palmqvist, E., Nilvebrant, N. O., & Hahn-Hägerdal, B. (1998). Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*. *Applied Microbiology and Biotechnology, 49*, 691–697.

Klinke, H. B., Schmidt, A. S., & Thomsen, A. B. (1998). Identification of degradation products from wheat straw in relation to pretreatment conditions. In H. Kopetz, T. Weber, W. Palz, P. Chartier, & G. L. Ferrero (Eds.), *Proceedings of the biomass for energy and industry* (pp. 484–487). London, UK: James & James (Science Publishers) Ltd.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tenborg, C., Stenberg, K., Zacchi, G., et al. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme and Microbial Technology, 24*, 151–159.

Parajó, J. C., Domínguez, H., & Domínguez, J. M. (1998). Biotechnological production of xylitol. Part 3: operation in culture media made from lignocellulose hydrolyzates. *Bioresource Technology, 66*, 25–50.

Sanhueza, J., Nieto, S., & Valenzuela, A. (2000). Thermal stability of some commercial synthetic antioxidants. *Journal of the American Oil Chemists' Society, 77*, 933–936.

Tran, A. V., & Chambers, R. P. (1985). Red oak wood derived inhibitors in the ethanol fermentation of xylose by *Pichia stipitis* CBS 5776. *Biotechnology Letters, 7*, 841–846.

Tran, A. V., & Chambers, R. P. (1986). Ethanol fermentation of red oak acid prehydrolysate by the yeast *Pichia stipitis* CBS 5776. *Enzyme Microbiology & Technology, 8*, 439–445.