Available online at www.sciencedirect.com
ScienceDirect
Innovative Food Science and Emerging Technologies 9 (2008) 311–319
Innovative Food Science & Emerging Technologies
www.elsevier.com/locate/ifset

In vitro evaluation of olive- and grape-based natural extracts as potential preservatives for food

Ana Teresa Serra [a], Ana A. Matias [a], Ana V.M. Nunes [a], M.C. Leitão [b], Dulce Brito [c], R. Bronze [d], Sandra Silva [d], Antonio Pires [d], M.T. Crespo [e], M.V. San Romão [b], C.M. Duarte [a,*]

[a] *Nutraceuticals and Delivery Laboratory, IBET/ITQB-UNL, Oeiras, Portugal*
[b] *Physiology of Environmentally Conditioned Microbiota Laboratory, IBET/ITQB-UNL, Oeiras, Portugal, Estação Vitivinicola Nacional, INIAP, Portugal*
[c] *Food Microbial Technology, ITQB-UNL, Oeiras, Portugal, Estação Agronómica Nacional/INIAP, Portugal*
[d] *Analytical Chemistry Laboratory, IBET/ITQB, Portugal*
[e] *Microbiology of Man-made Environments Laboratory, IBET/ITQB-UNL, Oeiras, Portugal*

Received 30 January 2007; accepted 30 July 2007

Abstract

The use of natural antimicrobial compounds, especially extracted from plants, as food preservatives is nowadays widely used, since plant matrices possesses antimicrobial natural products to protect themselves from microbial infection and deterioration. Plant phenolics are currently of growing interest due to their likely human health benefit properties.

In the present study, the antimicrobial activities of two waste-derived extracts—from olive oil and wine production—, both rich in polyphenols, and three standard well recognized antioxidants (quercetin, hydroxytyrosol and oleuropein) were investigated against five microbial species (*Escherichia coli*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans*). The tests were carried out using a microplate photometer assay. The results obtained suggest that the natural extracts may have important applications in the future as natural antimicrobial agents for food industry as well as for medical use. The natural extracts showed more antimicrobial activity than shown by the selected antioxidants alone against all microorganisms.

Keywords: Natural extracts; Olive; Grape; Hydroxytyrosol; Quercetin; Oleuropein; Antimicrobial activity

Industrial relevance: This manuscript is a step forward in the development of effective natural preservatives for food. It is focused on the antimicrobial activity against five microbial species (*Escherichia coli*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans*) of two waste-derived extracts—from olive oil and wine production— and three standard well recognized antioxidants (quercetin, hydroxytyrosol and oleuropein).

1. Introduction

Food-borne illness resulting from the consumption of foods contaminated with pathogenic bacteria and yeasts have been of great concern for public health. Currently there is a worldwide effort to minimize the use of chemical preservatives due to consumer preferences towards more natural and healthier products. Consequently, we are assisting to an increasing scientific interest in the searching for natural antimicrobial compounds as biopreservatives. Antimicrobial effects of various plant extracts against certain pathogens have been studied by a number of researchers (Rauha et al., 2000; Ahmad & Beg, 2001; Ebi, 2001; Puupponen-Pimia et al., 2001; Fukai, Marumo, Kaitou, Kanda, Terada, & Nomura, 2002; Erasto, Bojase-Moleta and Majinda, 2004; Tepe, Deferera, Sokmen, Polissiou, & Sokmen, 2004). For instance, residues obtained during olive oil production, as well as their extracts, have been investigated regarding antibacterial activity against *Bacillus megaterium*) (Rodriguez, Perez, Ramos-Cormenzana, & Martinez, 1988). Also, grape

* Corresponding author. Fax: +351 21 4421161.
E-mail address: cduarte@itqb.unl.pt (C.M. Duarte).

1466-8564/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ifset.2007.07.011

seeds and bagasse extracts have been studied for their antimicrobial activity and preservative properties (Jayaprakasha, Selvi, & Sakariah, 2003; Baydar, Ozkan, & Sagdiç, 2004). Recently Rhodes, Mitchell, Wilson and Melton (2006) reported antilisterial activity of a grape juice and skin and seed extract of a *Vitis vinifera* variety.

Polyphenols of plant origin have been reported to have a variety of biological effects, including antioxidant, anticarcinogenic, antiinflammatory and antimicrobial activities. Specifically some phenolic compounds such as resveratrol, hydroxytyrosol, oleuropein, quercetin and a number of phenolic acids have been reported to inhibit various pathogenic microorganisms (Aziz, Farag, Mousa, & Abo-Zaid, 1998; Bisignano, et al, 1998; Papdopoulou, Soulti, & Roussis, 2005).

Olive oil and wine production wastes constitute a cheap source of phenolic compounds and are rich in such components. Hydroxytyrosol has been referred as a potent chemo-preventive agent (D'Angello et al., 2005), and is considered as a highly potent antioxidant. Activity of hydroxytyrosol was firstly attributed, to its ability to prevent the oxidation of the low density lipoprotein (LDL) (Visioli, Bellomo, Montedoro, & Galli, 1995) and the aggregation of blood platelet (Petroni, et al., 1995).

Quercetin is a flavonol that occurs widely in plants and is significantly present in grapes and red wine. Recently, quercetin has been shown to confer many beneficial effects in human and animals models due to its strong antioxidant activity (Miller, 1996). For example, quercetin prevents LDL oxidation (Howard, et al., 2002), cardiovascular diseases (Hertog, Feskens, & Hollman, 1993), diabetes (Vessal, Hemmati, & Vasei, 2003), and cancer (Ginter, 1995). Moreover, quercetin has demonstrated antimicrobial activities against human pathogens (Aziz et al., 1998).

This paper presents results concerning the antimicrobial activities against several food-borne pathogenic microorganisms, of two natural extracts obtained with integrated clean technologies from residues of winery and olive oil industries. The olive extract (OE) was isolated from olive oil semi-solid residues, obtained from a modern two-phase processing technique for olive oil production. The grape extract (GE), was isolated from residues of white wine production, especially grape skins and seeds. Due to their phenolic content, the above mentioned natural extracts have demonstrated a positive bioactivity, such as high antioxidant capacity.

Five different microbial species were used (*Escherichia coli*, *Salmonella poona*, *Bacillus cereus*, *Saccharomyces cerevisiae* and *Candida albicans*). For comparison, the antimicrobial action of three standard antioxidants (quercetin, hydroxytyrosol and oleuropein) was also investigated.

Of the species used, *B. cereus* is one of the most common gram-positive bacteria often associated with two kinds of food-borne

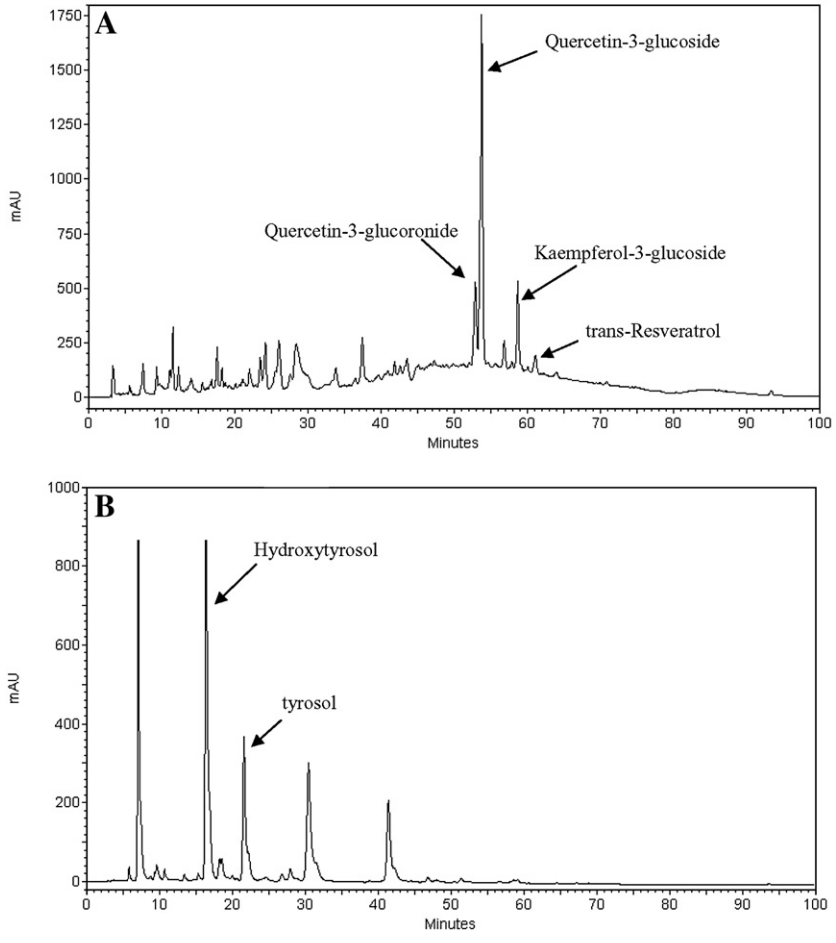

Fig. 1. HPLC profiles at 280 nm of (A) natural grape extract and (B) natural olive extract.

illness, a diarrhoeal and an emetic syndrome (Valero and Giner, 2006). Gram-negative bacteria are represented by *E. coli* and *S. poona*. *E. coli* belongs to the normal flora of humans, but there is an enterohaemorrhagic strain that has caused serious cases of food poisoning (Rahua et al., 2000). Infection may produce mild diarrhoea, or a severe fatal illness. *Salmonella* is reported as the most frequent cause of food-borne outbreaks of gastroenteritis in the world. Diet contamination with *S. cerevisiae* and *C. albicans* are commonly observed and they are causative agents of opportunistic mycoses and mouth infections, respectively (Rahua et al., 2000).

Quercetin and hydroxytyrosol were chosen as standards because they are the major phenolic compounds identified in GE and OE, respectively, and have been reported as having antimicrobial action against various pathogens (Aziz et al., 1998 and Bisignano et al., 1999). Oleuropein (the bitter molecule present in large amounts in olives) was also studied as this compound can produce hydroxytyrosol by acidic or enzymatic hydrolyses.

2. Materials and methods

2.1. Natural extracts

2.1.1. Preparation of natural extracts

Both natural extracts were recovered from the residues of olive oil and white wine production, using clean technologies, namely extraction with biocompatible solvents followed by a membrane-based process.

Grape aqueous extract was prepared from grape residues (grape skins and seeds) of Arinto variety (Bucelas, Portugal) at room temperature. The liquid obtained was centrifuged at 9000 rpm at 20 °C during 15 min and concentrated by rotary evaporator. The extract was filtered before storage at −20 °C.

The olive extract was obtained from olive oil semi-solid residues (Moura, Portugal) through a patented process (Duarte, et al., 2006).

2.1.2. Analysis of phenolics

The total concentrations of phenolic compounds present in the natural extracts were determined according to the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). Briefly, the appropriate diluted solutions of extracts were oxidized with a Folin–Ciocalteu reagent (Panreac, Spain) and the reaction was neutralized with sodium carbonate. The absorbance of the samples was measured at 765 nm on a Spectrophotometer (Genesys™ 10UV) after 30 min at 40 °C. Gallic acid (Fluka, Germany) was used as standard, and the result was expressed as means of three replicates (mg of gallic acid equiv/L of extract — mg GAE/L).

The phenolic composition of natural extracts was analysed by HPLC, coupled with a UV detector (Bravo, Silva, Coelho, Boas, & Bronze, 2006). Quercetin and hydroxytyrosol were quantified at 280 nm. Mass spectroscopy was used to confirm the identification of quercetin glycosides (Silva, et al., 2005).

2.2. Antioxidants

Hydroxytyrosol and Oleuropein were purchased from Extrasynthèse (Genay, France). Quercetin dihydrate was obtained from Sigma–Aldrich (Madrid, Spain).

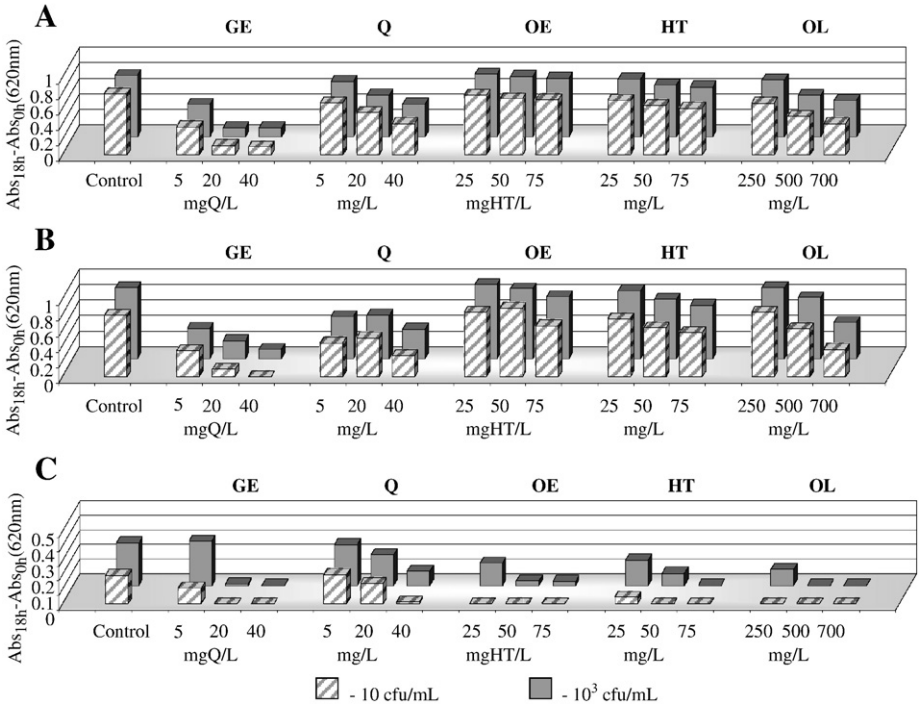

Fig. 2. Antibacterial effect of natural extracts (GE and OE) and standard antioxidants (Q, HT and OL) on (A) *E. coli*, (B) *S. poona* and (C) *B. cereus*. Growth of the three bacteria strains on Nut medium at 18 h with or without (control) NE or standard antioxidants. The results are the difference between the 18 h and the 0 h absorbance.

2.3. Antimicrobial activity tests

2.3.1. Microbial strains

All microorganisms, bacterial strains (*E. coli* C7085L, *S. poona* C6009L and *B. cereus* C1220) and yeasts (*S. cerevisiae* C8201L and *C. albicans* C1503L) used for the assay were recovered from culti-loops purchased from Oxoid (Hampshire, England).

2.3.2. Culture media and inoculum

Bacterial strains were maintained in Mueller–Hinton (MH) (Biokar Diagnostic, France) and Nutrient (Nut) broth. Nutrient broth was prepared with Bacto Tryptone (BD, Sparks, USA), Meat Extract dry (Merck KGaA, Darmstadt, Germany) and Sodium Chloride (Panreac, Química SA, Barcelona, Espanha). Yeasts were grown on Potato Dextrose broth (PDB) (Merck KGaA, Darmstadt, Germany) and on a commercially available apple juice.

Each microorganism was suspended in 5 mL broth and incubated overnight at 37 °C for Gram(–) bacteria, 30 °C for *B. cereus* and 25 °C for yeasts.

Cultures growth was followed by the turbidity measurement at 620 nm and adjusted to obtain an inoculum of 10^8 cfu/mL.

2.3.3. Antimicrobial assay

A microplate photometer method (Devienne and Roddi, 2002) was used to determine the antimicrobial activity of the natural extracts and standard antioxidants. Briefly, the wells of 96-well plate were filled with 100 μL of growing culture (10 and 10^3 cfu/mL) and added with 100 μL of various concentrations of natural extract or standard antioxidant. Controls were prepared adding 100 μL of culture medium instead of extract. The assay was performed in MH and Nut broth for bacteria and in PDB and apple juice for yeasts.

Plates were incubated at specific conditions according to the microbe under assay (37 °C for Gram(–) bacteria, 30 °C for *B. cereus* and 25 °C for yeasts). At different times (18, 24, 48 and 72 h) plates were agitated and the absorbance was read at 620 nm.

The results presented correspond to at least three repetitions.

The mean values and the standard deviation were calculated from the data obtained and the maximum experimental error associated was <5%.

3. Results and discussion

3.1. Phenolic content

The natural extracts obtained from olive and grape residues presented a high content of total phenolics — GE: 3400 mg GAE/L; OE: 400 mg GAE/L.

Chromatograms presented in Fig. 1 were obtained for grape and olive natural extracts using UV–VIS detection. Quercetin glycosides, kaempferol and resveratrol were identified in the grape extract (Fig. 1A). Quercetin content in the GE was found to be 100 mg/L and hydroxytyrosol was the major compound present in OE (Fig. 1B), with a concentration of 280 mg/L.

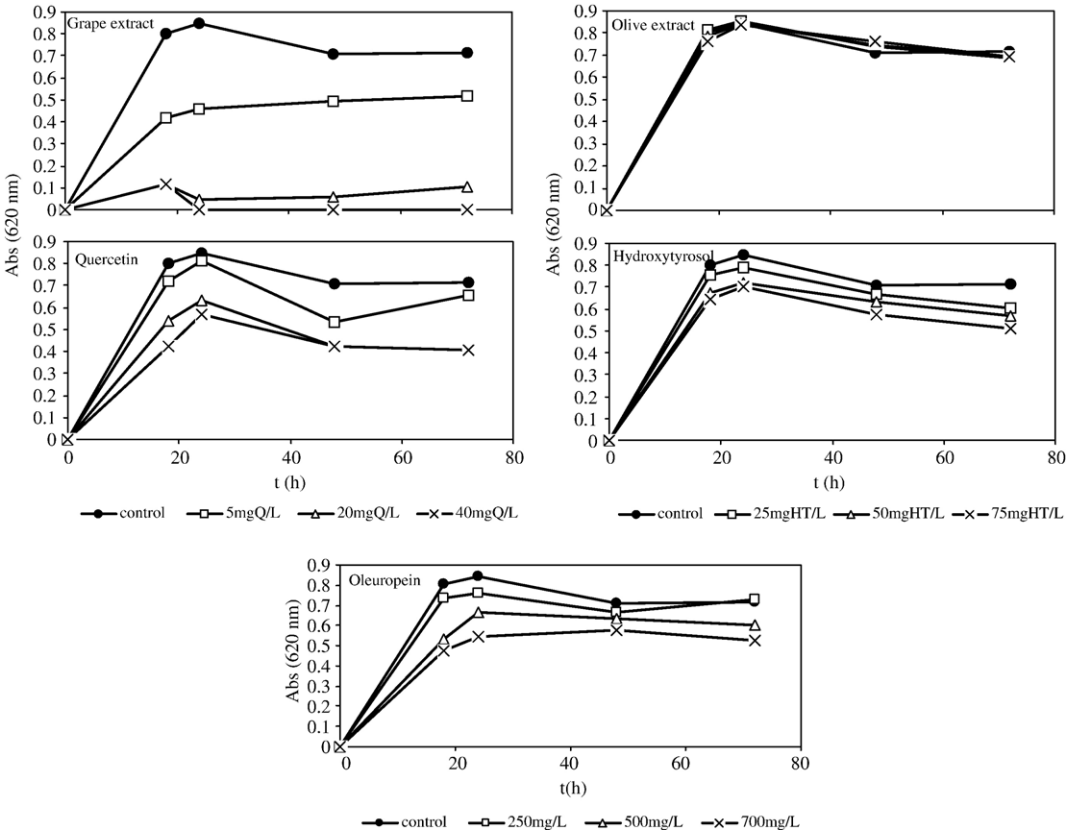

Fig. 3. Effect of natural extracts and antioxidants on the growth kinetics of *E. coli*. Bacteria strain was grown in nutrient culture medium with an inoculum of 10^3 cfu/mL. The results are the difference between the 18, 24, 48 and 72 h with the 0 h absorbance.

3.2. Effect of natural extracts and standard antioxidants in bacterial growth

Antimicrobial activities of the two natural extracts (OE and GE) and the standard antioxidants against *E. coli*, *S. poona* and *B. cereus* are presented in Fig. 2. Results obtained show that, within the compounds assayed, GE was the most effective antibacterial agent. This natural extract inhibited drastically the growth of the three bacteria strains in a dose dependent-manner, and the inhibitory effect was more efficient in Gram(+) strain *B. cereus* (Fig. 2C) than Gram-negative *E. coli* (Fig. 2A) and *S. poona* (Fig. 2B). These results were in agreement to those obtained by Jayaprakasha et al. (2003). They reported that acetone:water:acetic acid and methanol:water:acetic acid extracts from grape seeds inhibited more easily Gram(+) bacteria such as *S. aureus*, *B. cereus* and *B. subtilis* than Gram(−) ones such as *E. coli*.

In addition, GE exhibited higher antimicrobial activity than pure quercetin for the same concentration. For example, in the case of *B. cereus*, GE with a quercetin content of 20 mg Q/L, totally inhibited bacteria growth in both different inoculums (Fig. 2C), whereas the same concentration of synthetic quercetin still allowed some bacterial growth. This result suggests there might be a positive synergetic effect within the constituents of GE that reinforce the response and/or the natural extract contains other components in the matrices with antimicrobial activity.

Some authors attributed the extent of the inhibitory effects of the extracts to their total phenolic concentration and composition. For example, Baydar et al. (2004) found that grape seed extract exhibited a significant antibacterial activity in contrast to grape bagasse extracts; and those results coincided with higher polyphenolic content existent in the first extract. Our grape extract was obtained from residues of white wine production, containing grape seeds and skins. In terms of total phenolic compounds, GE inhibited bacterial growth at concentrations of 680 mg GAE/L and 1360 mg GAE/L for Gram(+) and Gram(−) bacteria, respectively. Comparing these results with those previously reported by Jayaprakasha et al. (2003), also using grape seeds extracts, their extracts seem to be more effective antibacterial agents than GE. In their study a minimal inhibitory concentration of 340−390 mg GAE/L and 475−575 mg GAE/L was obtained for Gram(+) and Gram(−) bacteria, respectively. These differences might be attributed to a different extraction.

Olive extract, synthetic HT and OL showed lower antimicrobial activity against *E. coli* and *S. poona* than against *B. cereus* (Fig. 2), suggesting that Gram(−) bacteria were more resistant to olive phenolics than Gram(+) strains. This observation has been reported for olive oil waste waters by Moreno, Quevedo-Sarmeinto, and Ramos-Cormenzana (1989), and can be attributed to differences in the structure of bacteria cell wall. The less complex structure of the cell wall in the Gram(+) bacteria makes it more permeable to the antimicrobial compounds.

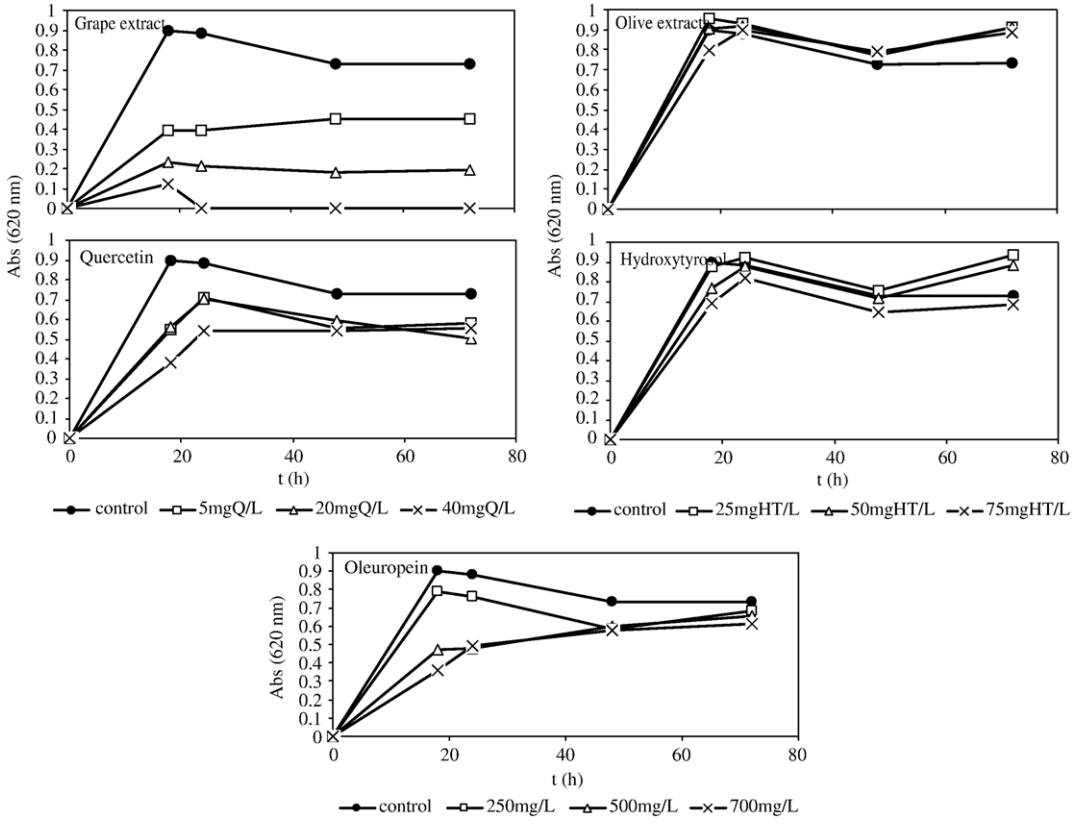

Fig. 4. Effect of natural extracts and antioxidants on the growth kinetics of *S. poona*. Bacteria strain was grown in nutrient culture medium with an inoculum of 10^3 cfu/mL. The results are the difference between the 18, 24, 48 and 72 h with the 0 h absorbance.

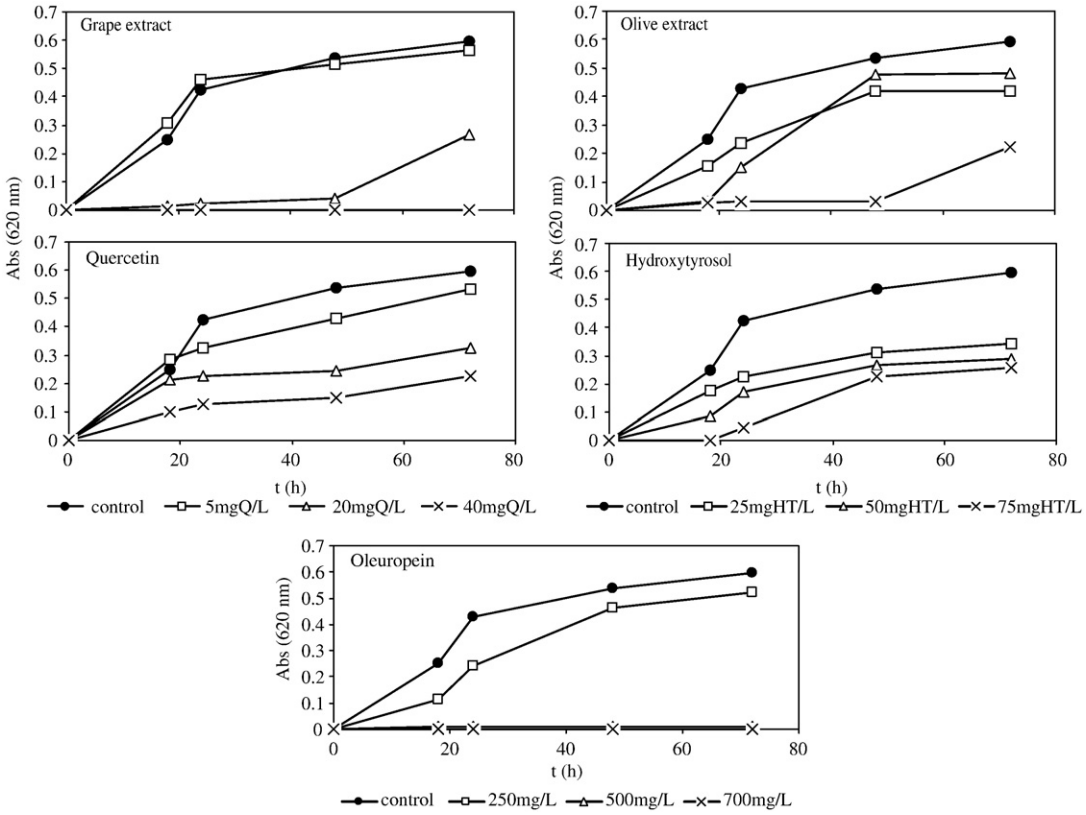

Fig. 5. Effect of natural extracts and antioxidants on the growth kinetics of *B. cereus*. Bacteria strain was grown in nutrient culture medium with an inoculum of 10^3 cfu/mL. The results are the difference between the 18, 24, 48 and 72 h with the 0 h absorbance.

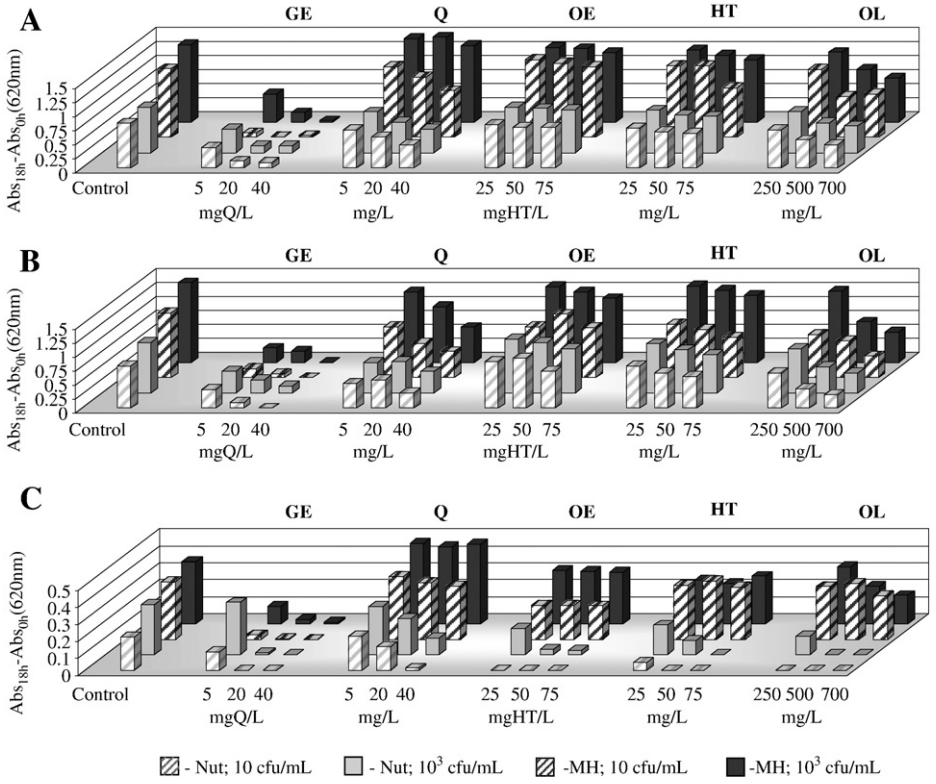

Fig. 6. Antimicrobial activity of natural extracts (GE and OE) and standard antioxidants (Q, HT and OL) on the growth of (A) *E. coli*, (B) *S. poona* and (C) *B. cereus* at 18 h in Muller–Hinton (MH) and Nutrient broth (Nut). The results are the difference between the 18 h and the 0 h absorbance.

3.3. Effect of the natural extracts and antioxidants on the growth kinetics of bacteria

The effect of natural extracts and standard antioxidants on the growth kinetics of the three bacteria strains (inoculum of 10^3 cfu/mL) was evaluated during 72 h (Figs. 3, 4 and 5).

3.3.1. Gram(−) strains

Figs. 3 and 4 show the results for the Gram(−) strains. For *E. coli* and *S. poona*, respectively, GE was the most powerful antimicrobial agent inhibiting the bacteria growth during 72 h in a dose dependent-manner. Total inhibition was only obtained with 40mg Q/L during the 3 days. With 20 and 5 mg Q/L, the same natural extract also exhibited antibacterial activity reducing the bacteria density during that time. Indeed, for these two concentrations the bacterial growth stopped after 18–24 h as in the control experiment. Olive extract and standard HT did not show antibacterial activity during the 3 days. Growth kinetics curves for *E. coli* and *S. poona* obtained with OE and HT were equivalent to the control. Antimicrobial activity during 24 h was obtained with oleuropein at concentrations of 500 and 700 mg/L.

3.3.2. Gram(+) strain

Concerning to *B. cereus*, a more significant antimicrobial activity was obtained for both natural extracts (Fig. 5). Herein GE achieved the best result where a concentration of 40 mgQ/L totally inhibited *B. cereus* growth during a 72 h period. Also, 20 mgQ/L of GE strongly reduced bacterial growth during 48 h. After that, *B. cereus* proceeded to replicate in a normal fashion approach. The same concentrations of synthetic quercetin did not show such remarkable effect in bacterial growth.

The natural extract OE reduced bacteria growth in a weaker manner; a strong effect was observed at concentrations equivalent to 75 mg/L of HT. After 48 h of exposure, the bacteria recovered its growth ability. The same concentration of hydroxytyrosol tested alone did not have such effect. Above 500 mg/L, oleuropein alone showed totally inhibition of *B. cereus* growth.

3.4. Effect of culture medium in antibacterial activity of natural extracts and antioxidants

In order to access if the culture medium is an important factor for the antimicrobial activity of natural extracts, the assay was performed in two different media — MH and Nut broth — for the three bacteria strains. The results are presented in Fig. 6.

Considering results of the control experiments (bacteria growing in MH and Nut broth without natural extracts and synthetic antioxidants), dependence of the composition of culture medium in bacteria growth is clearly evident. All microorganisms exhibited a fast growth on the most nutritious medium–MH.

Figs. 6A and B show that the culture medium did not affect the antimicrobial behaviour of NE and standard antioxidants on the growth of Gram(−) strains. For *E. coli* and *S. poona*, the highest concentrations of GE studied strongly inhibited bacteria growth in both culture media while OE did not show antimicrobial activity, under the same conditions.

For the Gram(+) strain studied (Fig. 6C), the media showed to interfere with the OE and standards antioxidants antimicrobial activity. In the more nutritious medium, the antibacterial effect was null. In contrast, GE showed strong antimicrobial action in both culture media.

3.5. Effect of natural extracts and antioxidants on yeast growth

Antimicrobial activity against two yeasts strains of *S. cerevisiae* and *C. albicans*, was also studied. For this assay, both yeasts were grown during 18 h in PDB medium with

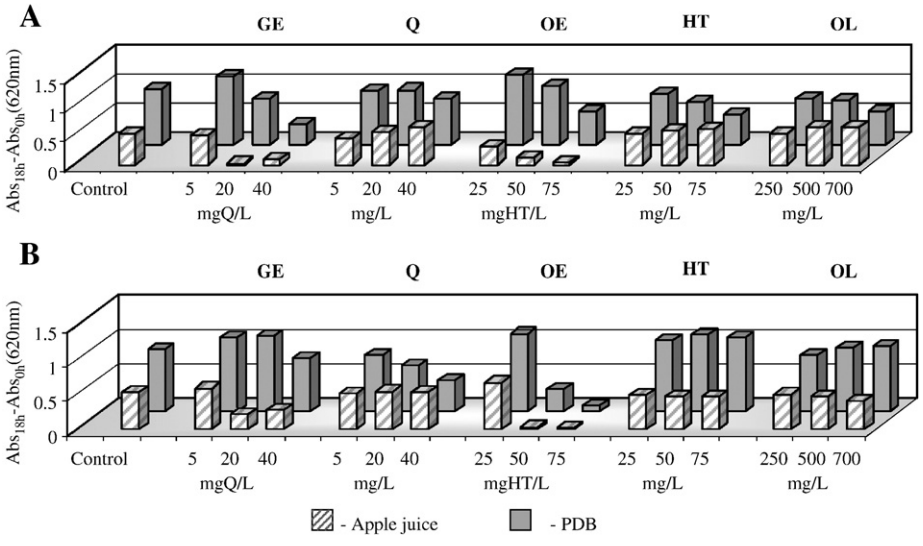

Fig. 7. Antiyeast activity of natural extracts (GE and OE) and standard antioxidants (Q, HT and OL) *C. albicans* and (B) *S .cerevisiae* growth at 18 h in potato dextrose broth (PDB) and apple juice. The yeast inoculum's is about 10^6 cfu/mL. The results are the difference between the 18 h and the 0 h absorbance.

natural extracts and synthetic antioxidants taken individually. Control experiments were done only in culture medium. The results are present in Fig. 7.

The results revealed that natural extracts were the most powerful anti-yeast agents. GE and OE strongly inhibited *S. cerevisiae* and *C. albicans* growth in a dose dependent-fashion. The same concentration range of quercetin and hydroxytyrosol did not show the similar effect. This result suggests that it is likely to exist a positive synergetic effect within the constituents of natural extracts that reinforce the response. In case of oleuropein, the three concentrations studied did not inhibit yeast growth.

Taking into account the data presented in this work and considering that yeasts are one of the most contaminant microorganisms responsible for fruit juice deterioration, we considered interesting to analyze the anti-yeast effect of these natural extracts and antioxidants on apple juice. The assay was performed using the same methodology described previously but using apple juice as culture medium. Results are also shown in Fig. 7. Control experiments showed that yeast growth is lower in apple juice than in culture medium. This fact may be attributed to the difference between composition and pH of the two media (pH 7 for PDB and pH 4 for apple juice). Antimicrobial activity of NE in juice was also verified and found to be stronger than in PDB.

4. Conclusions

Antimicrobial activities of a grape extract (GE), an olive extract (OE), both rich in polyphenols, and three standard well recognized antioxidants (quercetin, hydroxytyrosol and oleuropein) were investigated against five microbial species (*E. coli*, *S. poona*, *B. cereus*, *S. cerevisiae* and *C. albicans*).

The natural extracts showed an evident antimicrobial effect against yeast and bacteria in a dose dependent-manner. GE, the natural extract from wine production wastes, was the most effective antimicrobial agent for all food-borne bacteria studied. OE is more specific for Gram(+) bacteria.

The action of the synthetic antioxidants alone was much less effective than NE, suggesting i) a positive synergetic effect within the constituents of GE, that reinforce the response and/or, ii) the presence of other components in the matrices with antimicrobial activity.

From the results presented above, we can assume that the natural extracts GE and OE are promising natural preservatives, with application in food industry.

Acknowledgements

Teresa Serra and Ana Nunes acknowledge the financial support received from FCT (Fundação para a Ciência e Tecnologia), through the grants SFRH/BD/19238/2004 and SFRH/BGCT/15295/2004, respectively.

Ana Matias is grateful to FCT — MCES, Portugal, and Sovena — Comércio e Industria de Produtos Alimentares, S.A. for financial support, through the grant SFRH/BDE/15535/2005.

References

Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, *74*, 113−123.

Aziz, N. H., Farag, S. E., Mousa, L. A. A., & Abo-Zaid, M. A. (1998). Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios*, *93*, 43−54.

Baydar, N. G., Ozkan, G., & Sagdiç, O. (2004). Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control*, *15*, 335−339.

Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccella, N., & Saija, A. (1999). On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *Journal of Pharmacy and Pharmacology*, *51*(8), 971−974.

Bravo, M. N., Silva, S., Coelho, A. V., Boas, L. V., & Bronze, M. R. (2006). Analysis of phenolic compounds in Muscatel wines produced in Portugal. *Analytica Chimica Acta*, *563*, 84−92.

D'Angelo, S., Ingrosso, D., Migliardi, V., Sorrentino, A., Donnarumma, G., Baroni, A., Masella, L., Tufano, M. A., Zappia, M., & Galletti, P. (2005). Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biology and Medicine*, *38*, 908−919.

Devienne, K. F., & Roddi, M. S. R. (2002). Screening for antimicrobial activity of natural products using a microplate photometer. *Brazilian Journal of Microbiology*, *33*, 166−168.

Duarte, C. M., Nunes, A. M., Matias, A. A., Santos, J. L., Goulão-Crespo, J. P., & Nunes da Ponte, M. N. (2006). *Method of Obtaining a Natural Hydroxytyrosol-rich Concentrate from Olive Tree Residues and Subproducts using Clean Technologies, PCT/IB2006/052552.*

Ebi, G. C. (2001). Antimicrobial activities of *Alchornea cordifolia*. *Fitoterapia*, *72*, 69−72.

Erasto, P., Bojase-Moleta, G., & Majinda, R. R. (2004). Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*. *Phytochemistry*, *65*, 875−880.

Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., & Nomura, T. (2002). Antimicrobial activity of licorice flavonoids against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*, *73*, 536−539.

Ginter, E. (1995). The role of antioxidants in the prevention of tumors. *Bratislavské lekárske listy*, *96*, 195−209.

Hertog, M. G., Feskens, E. J., & Hollman, P. C. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, *342*, 1007−1011.

Howard, A., Chopra, M., Thurnham, D. I., Strain, J. J., Fuhrman, B., & Aviram, M. (2002). Red wine consumption and inhibition of LDL oxidation: What are the important components. *Medical Hypotheses*, *59*, 101−104.

Jayaprakasha, G. K., Selvi, T., & Sakariah, K. K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International*, *36*, 117−172.

Miller, A. L. (1996). Antioxidant flavonoids: Structure, function and clinical usage. *Alternative Medicine Review*, *1*(2), 103−111.

Moreno, E., Quevedo-Sarmiento, J., & Ramos-Cormenzana, A. (1989). Antibacterial activity of wastewaters from olive oil mills. In P. N. Cheremisoff (Ed.), *Encyclopedia of Environmental Control Technology* Houston, TX: Gulf Publishing.

Papdopoulou, C., Soulti, K., & Roussis, I. G. (2005). Potential antimicrobial activity of red and white wine extracts against strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Food Technology and Biotechnology*, *43*(1), 41−46.

Petroni, A., Blasevich, M., Salami, M., Papini, N., Montedoro, G. F., & Galli, C. (1995). Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thrombosis Research*, *78*, 151−160.

Puupponen-Pimia, R., Nohynek, L., Meier, C., Kahkonen, M. M., Heinonen, A., & Hopia, Oksman-Caldentey, K. M. (2001). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology*, *90*, 494−507.

Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H., & Vuorela, P. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology*, *56*, 3−12.

Rhodes, P. L., Mitchell, J. W., Wilson, M. W., & Melton, L. D. (2006). Antilisterial activity of grape juice and grape extracts derived from *Vitis vinifera* variety Ribier. *International Journal of Food Microbiology*, *107*, 281−286.

Rodrigues, M. M., Perez, J., Ramos-Cormenzana, A., & Martinez, J. (1988). Effect of extracts obtained from olive oil mill waste-waters on *Bacillus-megaterium* ATCC-33085. *Journal of Applied Bacteriology*, *64*(3), 219−225.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144−158.

Silva, S., Matias, A., Nunes, A., Duarte, C., Coelho, A. V., & Bronze, M. R. (2005). Identification of flavonol glycosides in winemaking by-products by HPLC with different detectors and hyphenated with mass spectrometry. *Ciência e Técnica Vitivinicola*, *20*(1), 17−33.

Tepe, B., Daferera, D., Sokmen, M., Polissiou, M., & Sokmen, A. (2004). In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii*. *Journal of Agricultural and Food Chemistry*, *52*, 1132−1137.

Valero, M., & Giner, M. J. (2006). Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. *International Journal of Food Microbiology*, *106*, 90−94.

Vessal, M., Hemmati, M., & Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. *Comparative Biochemistry and Physiology Part C*, *135*, 357−364.

Visioli, F., Bellomo, G., Montedoro, G. F., & Galli, C. (1995). Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis*, *117*(1), 25−32.