Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/fpsl

Anti-yeast activity of natural compounds: *In vitro* and *in vivo* tests

Daniela Gammariello, Amalia Conte, Annalisa Lucera, Marianna Mastromatteo, Matteo Alessandro Del Nobile *

Department of Agricultural Science, Food and Environment, University of Foggia, Via Napoli, 25-71121 Foggia, Italy

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
9 October 2013
Accepted 11 October 2013
Available online 13 November 2013

Keywords:
Natural compounds
Anti-yeast activity
Juice
Quality
Shelf life

ABSTRACT

The anti-yeast activity of some natural compounds (trans-2-hexanal, cinnamon oil, eugenol and thymol) was addressed by *in vitro* and *in vivo* tests. First, their efficacy was tested against some yeasts. By a sensory evaluation the thymol was discarded and the other three natural preservatives were tested in grape juice inoculated with a cocktail of selected yeasts. The most effective compound was trans-2-hexanal. A Central Composite Design (CCD) was also carried out to highlight possible synergistic effects between trans-2-hexanal, cinnamon oil and eugenol. Results showed that compounds were all effective against yeasts and their combination further reduced the active agent concentration. To finally assess the activity of the six most effective combinations of the CCD, a fresh grape juice added with compounds and stored at two storage temperatures, was monitored during time. Results confirmed the effectiveness of the three natural compounds in providing an immediate and significant protection of juice to yeast proliferation.

1. Introduction

Essential oils are volatile, natural compounds characterized by a strong odour, formed by aromatic plants as secondary metabolites (Burt, 2004). These substances are known for their antiseptic and medical properties and their fragrance and are gaining success in food research as natural compounds with a prominent activity against bacteria (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Chang, Chen, & Chang, 2001; Joshi, Verma, & Mathela, 2010), yeasts (Tserennadmid et al., 2011) and moulds (Lanciotti et al., 2004). Essential oils are claimed to possess a broad spectrum of active properties due to the high content in phenolic derivatives (eugenol, citral, pinene, thymol, cinnamon, carvacrol) and minor components. Cinnamon oil is one of the essential oils commonly used in food industry for its special aroma (Cava, Nowak, Taboada, & Marin-Iniesta, 2007). Cinnamonum is a genus in the family *Lauraceae*, many species of which are used for spices. Its main inhibitory component is cinnamaldehyde (Chang et al., 2001) and its effectiveness against moulds and yeasts has been reported by Lopez-Malo, Alzamora, and Palou (2002). Eugenol, the major phenolic component of clove essential oil, is widely used in medical and dental practice due to its potent fungicidal, bactericidal, anaesthetic, antioxidant and anti-inflammatory properties (Guenette, Beaudry, Marier, & Vachon, 2006; Konning, Agyare, & Enninson, 2004; Vázquez, Fente, Franco, Vázquez, & Cepeda, 2001). Trans-2-hexanal is the most important aroma compound of fruit and vegetables tested for preservation of tea-based beverage, sliced apples packed under modified atmosphere and grapes (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000). Thymol, a major essential oil component of thyme, has been tested for antibacterial effects against a wide range of microorganisms and oral bacteria (Corbo et al., 2009; Dorman & Deans, 2000). The activity of essential oils, plant extracts or pure compounds has been investigated more abundantly

* *Corresponding author*. Tel.: +39 881 589 242.
E-mail address: matteo.delnobile@unifg.it (M.A. Del Nobile).
2214-2894/$ – see front matter
http://dx.doi.org/10.1016/j.fpsl.2013.10.003

against bacteria (Konning et al., 2004; Periago, Delgado, Fernández, & Palop, 2004; Sacchetti et al., 2005; Shan, Cai, Brooks, & Corke, 2007), than yeasts (Pinto et al., 2006; Tampieri et al., 2005; Tserennadmid et al., 2011; Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, & Pérez-Álvarez, 2008), even if yeasts are broadly distributed in nature and are able to spoil many foods such as wine, cheese, vinegar, beverages, juices, fruits, salads, sugar and meat.

Candida, *Pichia*, *Rhodotorula*, *Torulopsis*, *Saccharomyces*, *Zygosaccharomyces*, *Hansenula* and *Trichosporon* are some important examples of food spoiling yeasts (Deak & Beuchatl, 1987). Their contamination can cause changes in odour, colour, taste and texture. Pasteurization and aseptic packaging are generally efficient methods to prevent yeast proliferation in food but these systems cannot be always applied. In these last cases chemically synthesized compounds are taken into account even though consumers demand for diffusion of natural preservatives in food industry (Corbo et al., 2009; Mastromatteo, Barbuzzi, Conte, & Del Nobile, 2009). Therefore, due to the lack of knowledge dealing with the effects of natural agents from plants on common spoiling yeasts in real foods, the aim of this study was to determine the efficacy of trans-2-hexanal, cinnamon oil, eugenol and thymol. A preliminary trial was carried out by *in vitro* test and then the anti-yeast activity was tested on grape juice, thus determining the proper active combination of compounds.

2. Materials and methods

2.1. Yeasts

Rhodotorula mucilaginosa, *Cryptococcus laurentii* and *Candida famata* yeasts used for the experiments were isolated from table grape. Yeast strains were isolated by plating the diluted samples on Sabouraud agar (SAB, Oxoid, Italy) incubated at 25 °C for 48 h. The spread plate technique was used. The identification was carried out on five colonies isolated from the last positive dilution by API20C Aux system (BioMérieux, Marcy l'Etoil, France) and confirmed by VITEK automatic system. Isolates were maintained on SAB agar slants at 4 °C.

2.2. Antibacterial assay by agar diffusion method

The test yeasts were screened for susceptibility to the different natural agents using the agar diffusion method (Costa, Lucera, Conte, Contò, & Del Nobile, 2013). Stock solutions (from 500 to 10,000 mg/L) of eugenol (Sigma–Aldrich, Italy), cinnamon oil (Sigma–Aldrich, Italy) and thymol (Sigma–Aldrich, Italy) were prepared in water–ethanol (1:1) while trans-2-hexanal (Sigma–Aldrich, Italy) was dissolved in distilled water. Working cultures were prepared by inoculating a loop of each strain into 10 mL of Sabouraud broth and incubating for 2 days at 25 °C. Serial dilutions in sterile saline solution were prepared to obtain approximately a final concentration of 10^6 CFU/mL. These cultures were placed on Sabouraud agar to test the anti-yeast activity of natural compounds. Effectiveness of active compounds was determined by inhibition zone assay inoculating, separately, the substrates with 1 mL of each cell suspension. For each natural compound, 0.1 mL of active solutions was poured into wells (9 mm diameter) previously cut with a sterilized cork borer into SAB. The plates were incubated at 25 °C for 48 h. Positive controls were prepared using the same solvent employed to dissolve the compounds. The anti-yeast activity was evaluated by measuring the zone of inhibition in mm. The experiments were repeated in triplicate and results were expressed as average values.

2.3. Sensory evaluation

To assess the sensory properties of grape juice added with natural compounds of plants origin, a sensory evaluation was carried out. The various prepared active solutions were diluted in grape juice at concentration levels recorded from previous *in vitro* tests. All active solutions were prepared 24 h before the panel test and held at 10 °C in an odour-free environment. The sensory evaluation was carried out by 7 trained judges, members of the Food Packaging Laboratory of the University of Foggia (Gammariello, Di Giulio, Conte, & Del Nobile, 2008). Our panellists had at least several years of experience in evaluation of food products prior to this study. The panellists were retrained for this study in two sessions held over two days (1 session/day, 2 h/session). Retraining samples included fresh and pasteurized grape juice. After retraining, experienced graders were able to evaluate product odour. Panel tests were conducted in individual booths where panellists were asked to score the odour of grape juice samples by using a 5-point scale and were also invited to highlight defects or off-odours.

2.4. Application of active agents to grape juice

Concentrate pasteurized and fresh white grape juice (65°Brix) were kindly provided from a local winemaker of southern Italy. The pasteurized juice was diluted with sterile water up to 16°Brix. Then, duplicate samples were packaged in polypropylene tubs after inoculating a yeast cocktail (1 mL/100 mL) consisting of equal proportions of *R. mucilaginosa*, *C. laurentii* and *C. famata* (approximately 10^3 CFU/mL each), and each natural compound. Inoculated grape juice without any additions was used as control. The antimicrobial compounds were singly dissolved into product in order to reach final concentrations from 50 to 10,000 mg/L. The samples were stored at 4 °C for 72 h. Microbiological analyses were carried out after 1, 24 and 72 h, plating the appropriate decimal serial dilutions onto SAB agar. The incubation was performed at 25 °C for 48 h. On the inoculated grape juice, the combined effects of the three antifungal compounds were also studied. The concentration values varied according to a three-factor, three-level Central Composite Design (CCD) (Box, Hunter, & Hunter, 1978). The 16 variable combinations used are listed in Table 1; each combination was repeated twice. The samples were stored at 4 °C for 72 h and then analyzed as reported above. Finally, the six combinations of agents that exerted the best anti-yeast effects after 24 h of contact (yeasts' concentration below the detection limit) were tested in fresh grape juice stored at 4 and 9 °C.

2.5. Statistical analysis

All the experimental data were subjected to analysis of variance (ANOVA). Sources of variation were the concentrations of active