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Plant essential oils and components on growth of spoilage yeasts in microbiological media and a model salad dressing

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ABSTRACT

Essential oils (EOs) and EO components have been widely tested for their antimicrobial properties against microorganisms. However, less is known about the inhibitory properties of essential oil components against microorganisms in foods and, more specifically, spoilage yeasts in a food matrix. Clove bud, cinnamon bark, and thyme oils, and the EO components, *trans*-cinnamaldehyde, cinnamic acid, eugenol, carvacrol, and thymol first were evaluated for their antimicrobial activity against yeasts in microbiological media. The yeasts tested included *Torulaspora delbrueckii, Candida krusei, Schizosaccharomyces pombe,* and *Zygosaccharomyces bailii*. The most efficacious EO components in media were applied to a model mayonnaise-based salad dressing as a food model to determine the effect on spoilage yeast growth. *Trans*-cinnamaldehyde and cinnamon bark oil were the most effective against yeasts in microbiological media among the compounds tested with minimum inhibitory concentrations (MICs) of 50 mg/L. Thymol and carvacrol had the next most inhibitory activity against yeasts with MICs of 200 mg/L. In a model salad dressing at pH 4.2, *trans*-cinnamaldehyde at 500 mg/L was most effective among the EO components inhibiting *S. pombe* and *Z. bailii* during 4- and 5-day storage, respectively, at 22 °C.

1. Introduction

Yeasts are capable of grow in foods, especially those with high concentrations of sugar and organic acids or with lower water activity and pH, such as ketchup, beer, wine, salad dressing, fruit juices and soft drinks (Fabian & Wethington, 1950; Fleet, 1992; Loureiro & Malfeito-Ferreira, 2003; Tokuoka, 1993; Tornai-Lehoczki, Peter, & Dlauchy, 2003). Contamination by spoilage yeasts frequently leads to a decreased food product shelf-life due to gas production and sometimes undesirable off odors or flavors.

Salad dressings are among the many foods which are subject to contamination and spoilage by yeasts (Fabian & Wethington, 1950; Kurtzman, Rogers, & Hesseltine, 1971). *Zygosaccharomyces bailii* has been isolated from spoiled salad dressing and mayonnaise (Couto, Hartog, Veld, Hofstra, & vanderVossen, 1996) and can grow in products with a pH of 3.6 and water activity of 0.89 (Meyer, Grant, Luedecke, & Leung, 1989). Typically the high acidity (pH < 4.5) of salad dressings is relied on to prevent the growth of spoilage and pathogenic bacteria (Smittle, 2000). However, high acid environments do not inhibit many strains of yeast with some species surviving pH as low as 3.0 (Deak, 2007).

Although heat treatment is one of the most effective methods for microbial control in foods, it may cause undesirable changes to sensitive food products. Another microbial control measure for foods is the incorporation of organic acid preservatives, such as benzoic or sorbic acid. Activity of these preservatives is dependent on a range of factors, most importantly pH, but also water activity, storage temperature and composition of the food. They are most effective at a low pH making them useful in high acid products, such as salad dressings. However, some yeasts possess genetic or acquired resistance mechanisms to weak organic acids, including the ability to degrade them or to pump out dissociated anions (Dawidowicz & Rado, 2010; Mollapour & Piper, 2001; Piper, Calderon, Hatzixanthis, & Mollapour, 2001). Moreover, exposure to sublethal concentrations of organic acids may lead to subsequent resistance development (Piper et al., 2001).

With an increase in consumer demand for “preservative-free”

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and "natural" food products, interest has increased in the food industry for natural and/or naturally-derived antimicrobials to maintain microbiological control in foods. Plant extracts and plant essential oils (EOs), which have generally recognized as safe (GRAS) status in the US, have been repeatedly shown to exhibit antimicrobial activity against both foodborne pathogenic and spoilage microorganisms (Burt, 2004; Hyldgaard, Mygind, & Meyer, 2012; Kalemba & Kunicka, 2003; Peter & Nirmal Babu, 2012). Thus, there is potential for EOs and EO components to be used as natural antimicrobial alternatives to control spoilage yeasts in foods. Among plant EOs, thyme, clove and cinnamon oils have been shown to be active against many pathogenic and spoilage bacteria using broth dilution and agar diffusion assays (Davidson, Critzer, & Taylor, 2013; Kalemba & Kunicka, 2003; Zaika, 1988). Their activity is mainly attributed to their major components, including thymol, eugenol, carvacrol and *trans*-cinnamaldehyde (Bullerman, Lieu, & Seier, 1977; Kim, Marshall, & Wei, 1995; Lambert, Skandamis, Coote, & Nychas, 2001). Previous studies have shown that EOs and EO components, including eugenol, carvacrol, *trans*-cinnamaldehyde, cinnamon bark oil, citral, and clove bud oil were inhibitory against some yeast strains, including *Saccharomyces cerevisiae*, *Debaryomyces hansenii,* and *Z. bailii*, *in vitro* (Bang, Lee, Park, & Rhee, 2000; Bennis, Chami, Chami, Bouchikhi, & Remmal, 2004; Chami, Chami, Bennis, Bouchikhi, & Remmal, 2005; Curtis, Shetty, Cassagnol, & Peleg, 1996; Rivera-Carriles, Argaiz, Palou, & Lopez-Malo, 2005). However, while there have been a few studies on the efficacy of EOs (sage, juniper, lemon, lemon grass, marjoram, mentha) and their components (thymol, carvacrol) against spoilage yeasts in fruit juices and wine (Chavan & Tupe, 2014; Tserennadmid et al., 2011; Tyagi, Gottardi, Malik, & Guerzoni, 2013, 2014), studies in foods are limited. The objectives of this research were to determine the minimum inhibitory concentrations (MICs) of EOs of cinnamon bark (*Cinnamomum zeylanicum*), clove bud (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) and their components *in vitro* against four spoilage yeasts known to commonly contaminate and cause spoilage in high acid foods, and to further evaluate the application potential of those EO components *in situ* using a salad dressing as a food model.

2. Materials and methods

2.1. Microbial strains and preparation

Z. bailii, Candida krusei, and *Schizosaccharomyces pombe* isolated from spoiled commercial ketchup samples and *Torulaspora delbrueckii* obtained from the University of Tennessee culture collection were used in this study. All yeast strains were cultured in a shaking incubator at 32 °C. *T. delbrueckii,* and *C. krusei* were cultured in Yeast Extract Peptone Dextrose broth (YPD, Difco Becton Dickinson, Sparks, MD) and *S. pombe* was cultured in Yeast Extract Glucose broth (YEG) containing 30 g of glucose (Difco) and 5 g of yeast extract (Difco) per liter for 24 h at 32 °C. *Z. bailii* was cultured in YPD broth for 48 h at 32 °C. Cultures were transferred 3 times prior to use. For antimicrobial MIC determination, cultures were serially diluted in 0.1% peptone solution (Difco) to ca. 4 log CFU/mL. For salad dressing inoculation, *S. pombe* and *Z. bailii* cultures were concentrated and washed by centrifugation and re-suspended in 0.1% peptone solution to achieve an initial count of ca. 9.4 log CFU/ml.

2.2. Antimicrobial preparation

The plant EOs examined in this study include cinnamon bark (≥98% purity), clove bud (≥98% purity) and thyme (≥98% purity) oils (Sigma–Aldrich, St. Louis, MO), and the EO components include carvacrol (≥98% purity; PubChem CID:10364), cinnamic acid (≥98% purity; PubChem CID: 444539), *trans*-cinnamaldehyde (≥98% purity; PubChem CID: 637511), eugenol (≥98% purity; PubChem CID: 3314), and thymol (≥98% purity; PubChem CID: 6989) (Sigma–Aldrich). Stock solutions of EOs and EO components were prepared in 95% ethanol.

2.3. Determination of in vitro antimicrobial activity of EOs and EO components

MICs of test compounds were determined using a modified agar dilution assay (Davidson & Parish, 1989). EOs and EO components at desired concentrations were aseptically added into YEG or YPD agar in sterile bottles. The mixtures were then poured into petri plates and allowed to solidify. Each EO agar plate was spot inoculated (3 spots, 10 μL/spot) with 10^4 CFU/mL of yeast. Inocula were allowed to absorb; then, plates were incubated at 32 °C for at least 7 d. A positive control (yeast inoculated on YEG or YEPD plate without EO), ethanol control (yeast inoculated on YEG or YEPD plates with ethanol at the maximum concentration used in the treatments) and negative controls (un-inoculated plates containing EO and EO components) were included. MIC was determined as the lowest concentration of EO or EO component that completely inhibited visible growth of yeasts after 7-day incubation. Experiments were conducted in triplicate with duplicate sampling.

2.4. Salad dressing model

A model salad dressing was prepared based on one described by Yang, Luedecke, Swanson, and Davidson (2003). Sterile deionized (DI) water (281 g), 160 g of corn syrup, 76 g of distilled white vinegar, 46 g of cornstarch, 22 g of sucrose, and 15 g of salt were combined, heated until a starch paste was formed, and allowed to cool to room temperature. Then, 54 g of egg yolk, 312 g of soybean oil, 30 g of sterile DI water and 76 g of white vinegar were combined in a sterile mixer to form a mayonnaise base. Cooled starch paste was then whipped in with the mayonnaise base to form salad dressing with a final volume of 1.2 L. In preliminary experiments, it was found that the yeast did not grow during the incubation time with the natural pH (pH = 3.3) of the salad dressing. Thus, the pH of the model salad dressing was adjusted from 3.3 ± 0.2 to pH of 4.2 ± 0.2 using 5 N NaOH which did allow for yeast growth in 48–72 h.

2.5. Determination of antimicrobial effect of EO components in salad dressing

Approximately 8 log CFU of washed *S. pombe* and *Z. bailii* cells in 40 μL were added to 39 g of salad dressing and thoroughly mixed for 60 s. Carvacrol, eugenol and *trans*-cinnamaldehyde were applied to 960 μL salad dressing to achieve 10 times the MIC (determined in Section 2.2) in the final salad dressing. After addition of the EO components, the inoculated salad dressing was mixed vigorously by hand shaking for 30 s. Sorbic (0.03 and 0.1% w/v) and benzoic (0.05 and 0.1% w/v) acids (Sigma–Aldrich) were used as reference antimicrobials. Inoculated salad dressing without EO components, inoculated salad dressing with 2.3% ethanol, and un-inoculated salad dressing with EO components were included as controls. Samples were incubated at 22 °C for up to 96 h. Yeast were enumerated at 0, 6, 12, 24, 48 and 72 h, with an additional sampling time at 96 h for *Z. bailii* by plating on YEG agar, followed by incubation at 32 °C. All experiments were conducted in triplicate with duplicate sampling.