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Removal of Grapefruit Juice Furanocoumarins by Four Edible Fungi

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Furanocoumarins (FCs), a class of phenolic compounds, are known to inhibit the human cytochrome P450 3A4 (CYP 3A4) activity responsible for the metabolism of certain medications. This inhibition increases the oral bioavailability of these medications, leading to potential toxic effects. These interactions, overriding the potential health benefits of grapefruit consumption, have adversely affected the grapefruit industry, and have led to a need to remove the FCs from grapefruit juice (GFJ). Previously, we showed that autoclaved *Aspergillus niger* adsorbs FCs in GFJ, and that the fungus-treated GFJ showed a reduced inhibition of CYP 3A4. However, *A. niger* is not an edible fungus, thus prompting us to investigate edible fungi. In this study, autoclaved edible ascomycetes (*Morchella esculenta* and *Monascus purpureus*) and basidomycetes (*Pleurotus sapidus*, and *Agaricus bisporus*) were mixed with GFJ, and the levels of two of the major furanocoumarins 6′,7′-dihydroxybergamottin (DHB) and bergamottin (BM) were compared in the treated GFJ and in the control, untreated GFJ. These FCs were removed by the heat-killed fungi, suggesting that production of FC-removed GFJ may be achieved.

Furanocoumarins (FCs), a class of phenolic compounds, are produced in certain plants, such as grapefruit, bergamot, limes, parsnips, and celery (Murray et al., 1982; Tatum and Berry, 1979). In grapefruit juice (GFJ), two of the major FCs include 6′,7′-dihydroxybergamottin (DHB) and bergamottin (BM), while a large number of other minor-occurring FCs occur differentially distributed within specific tissues of the fruit (De Castro et al., 2006; Manthey et al., 2006; Tatum and Berry, 1979). The FCs in GFJ have been shown to inhibit a class of cytochrome P450 (CYP) enzymes, including CYPs 3A4, 2B6, 3A5, 2D6, and 2C9, responsible for the metabolism of a number of medications in humans (Girennavar et al., 2007; Guo et al., 2000; Lin et al., 2005; Wangensteen et al., 2003). These interactions have adversely affected the grapefruit component of the citrus industry for years, even though grapefruit possesses numerous health-beneficial phytochemicals that potentially act against cancer and cardiovascular diseases (Kiani and Imam, 2007; Mertens-Talcott et al., 2006).

Recently, in order to reduce the occurrence of the drug interactions associated with GFJ consumption, there have been attempts to remove FCs from GFJ using chemical, physical, and microbiological methods (Myung et al., 2008a; Paine et al. 2006; Uesawa and Mohri 2006a,b). The method developed by Paine et al. (2006) used a series of chemical extractions and reconstitutions of compounds in GFJ to produce a FC-free GFJ, while methods studied by Uesawa and Mohri (2006a,b) used UV irradiation and heat. Meanwhile, we investigated the use of autoclaved ascomycete *Aspergillus niger* to adsorb and remove FCs in GFJ (Myung et al., 2008a). In this present study, we examined whether edible fungi including two ascomycetes, *Morchella esculenta* and *Monascus purpureus*, and two basidomycetes, *Pleurotus sapidus* and *Agaricus bisporus*, can remove DHB and BM from GFJ.

Materials and Methods

FUNGAL ORGANISMS AND CULTURES. A culture of *Morchella esculenta* Dill. ex Pers. (#FP-140146) was obtained from the USDA Forest Service, Center for Forest Mycology Research in Madison, WI. *Monascus purpureus* Tiegh. was obtained from the ARS Culture Collection (NRRL # 1596) at the National Center for Agricultural Utilization Research. *Pleurotus sapidus* (Fr.) P. Krumm. nom. cons. was obtained from the American Type Culture Collection (#24987). *Agaricus bisporus* L. culture was prepared from an isolation made from the trama tissue of the pileus from a fresh basidiocarp.

The cultures of *M. esculenta* and *M. purpureus* were placed on potato dextrose agar (PDA) (BD/Difco Sparks, MD), and these cultures were maintained to provide initial inoculum by placing a few small sections of the culture into 100-mL flasks of YM broth (10.0 g glucose, 5.0 g peptone, 3.0 g yeast extract, 3.0 g malt extract in 1 L water) at 100 rpm, 23 °C. Mycelial pellets from these cultures were broken apart and aliquots of the resulting suspension were poured into several 2-L flasks containing 1 L of YM broth. The flasks were shaken on a large orbital shaker at 140 rpm, 20 °C for 2 weeks. *P. sapidus* and *A. bisporus* were maintained on YM agar containing thiamine (100 $\mu L \cdot L^{-1}$) and inoculated on YM broth as described above with the addition of thiamine.

The fully grown fungal tissues were autoclaved for 20 min at 121 °C to kill the fungus. After cooling, the material was macerated in a 1-L Waring stainless steel blender cup for 2 min. The resulting fungal material was vacuum-filtered from the broth and used as adsorbent.

INTERACTION OF GRAPEFRUIT JUICE WITH FUNGAL MATERIALS.

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The GFJ samples were prepared from fresh grapefruit by juicing white Marsh grapefruit (*Citrus paradisi*), which was a gift from Dr. Mark Ritenour (University of Florida, Indian River Research and Educational Center). The pH of the GFJ samples was further adjusted to 5.0. Portions of the autoclaved and macerated fungal material (2, 4, and 6 g), as described above, were added to 50-mL GFJ samples and mixed for 4 h at 250 rpm, 25 °C. After the interaction, the samples were vacuum-filtered through a Whatman No.1 filter, and the resulting filtrates and the fungal materials on the filters were collected.

Analysis of furanocoumarins in grapefruit juice. Both filtrates and fungal materials were extracted three times with 100-mL portions of ethyl acetate. The extracts were dried using a rotary evaporator. The resulting pellets were dissolved in 10 mL acetone, and 40-μL aliquots were subjected to HPLC analysis, as previously described (Myung et al., 2008b). The FCs obtained from control (no fungi added) and treated (various amounts of edible fungi added) GFJ samples were identified by comparing their elution times, UV absorbance at 320 nm, and MS data to authentic FC standards, and analyzed as previously described (Myung et al., 2008b). Concentrations (means ± standard deviations) of DHB and BM in GFJ samples produced from fresh grapefruit were 5.32 ± 0.46 and 3.34 ± 0.37 ppm, respectively.

Statistical procedures. All experiments in this study were conducted with three replicates. Student *t*-test was used to compare the differences in changes between controls and treatments if applicable. Two-tailed *P* values were calculated to report significant differences in the mean values.

Results and Discussion

To determine whether edible fungi can bind and remove FCs in GFJ, *M. esculenta, M. purpureus, P. sapidus,* and *A. bisporus* were mixed with GFJ for 4 h, and the two major FCs, DHB and BM in the GFJ were analyzed. Indeed, FCs in GFJ were removed by these edible fungi (Table 1). *M. esculenta* and *M. purpureus* (2.0 g) removed 11.4% and 24.8% of DHB, and 31.7% and 70.9% of BM, respectively, and the removal of both FCs was proportionally increased with the amount of fungus added. The removal of DHB and BM from GFJ was also shown with two other fungi, *P. sapidus* and *A. bisporus*, where approximately 50% and 95% of DHB and BM, respectively, was removed from GFJ by 6 g of these fungi. The maximum binding of DHB and BM to 6 g of these fungal hyphae did not significantly differ among fungi ($P>0.05$), suggesting that the binding may be a general interaction between FCs and fungal tissues, regardless of the fungal type.

In summary, the FCs in GFJ were removed by an edible fungus, *M. esculenta*. The removal of DHB and BM was also observed from other edible fungi, *M. purpureus*, *P. sapidus*, and *A. bisporus*, suggesting that the binding of FCs to the fungal hyphae is a passive interaction and these fungi contain components responsible for the binding. The removal of FCs in GFJ was also observed with dry *M. esculenta*, making it possible to use the dry material for the removal of FCs in GFJ and characterize constituents binding to the FCs.

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Table 1. Removal of two major furanocoumarins (FCs), 6′, 7′-dihydroxybergamottin (DHB) and bergamottin (BM), in grapefruit juice (GFJ) by autoclaved edible fungi, *Morchella esculenta, Monascus purpureus*, *Pleurotus sapidus*, and *Agaricus bisporus*. GFJ (50 mL) was mixed with three different amounts (2, 4, and 6 g, wet weight) of autoclaved fungi and DHB and BM in GFJ were analyzed. Data represent means ± standard deviations of triplicates.

Fungal material used (g)	% removal of FCs in GFJ by fungi											
	M. esculenta			*M. purpureus*			*P. sapidus*			*A. bisporus*		
	2	4	6	2	4	6	2	4	6	2	4	6
DHB	11.4±3.1	38.6±3.8	41.0±13.3	24.8±8.9	39.1±9.1	58.9±4.1	15.1±1.6	48.6±1.8	55.1±1.6	15.2±2.6	25.2±8.2	44.6±4.3
BM	31.7±1.2	47.0±8.9	89.4±11.3	70.9±9.0	93.6±2.0	97.2±0.6	46.3±2.8	82.2±5.3	96.2±2.2	26.8±7.8	44.6±6.4	75.7±4.9