Assessment of Selected Antioxidants in Tomato Pomace Subsequent to Treatment with the Edible Oyster Mushroom, *Pleurotus ostreatus*, under Solid-State Fermentation

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Tomato pomace, delignified by the edible oyster mushroom, *Pleurotus ostreatus*, could be used as a poultry feed ingredient to provide α-tocopherol for retardation of lipid oxidation in postmortem meat if the antioxidant were retained in pomace after fungal fermentation. Experiments were conducted to investigate changes in the content of α-tocopherol, lycopene, and β-carotene in tomato pomace after sterilization and treatment with *P. ostreatus* from 0 to 104 days. α-Tocopherol (39.26 to 31.15 μg/g) and lycopene (17.42 to 11.19 μg/g) significantly decreased during sterilization while β-carotene (42.56 to 35.44 μg/g) did not. The content of carotenoids decreased by day 26 as compared to 0 day for the control and for treated samples. α-Tocopherol decreased during fungal fermentation at day 26 as compared to 0 day for the control and increased during the same period for treated samples. By 104 days, only α-tocopherol in control pomace was present in a significant amount. The α-tocopherol content of mushroom fruit grown in pomace (74.10 μg/g) and in wheat straw (51.36 μg/g) was not significantly different. Tomato pomace could be used as a substrate to successfully grow edible mushrooms; however, the initial level of selected antioxidants and their reduction during fungal fermentation must be considered when delignified pomace is utilized for selected nutrient content in animal feed or products for human consumption.

KEYWORDS: Antioxidant; β-carotene; fermentation; lycopene; *Pleurotus ostreatus*; α-tocopherol; tomato pomace

INTRODUCTION

The world, the United States, and California, in particular, produce great amounts of fruits, vegetables, and nuts; through direct consumption and subsequent food processing, a considerable mass of agricultural byproducts is accumulated. Fadel (*1*) studied the status of selected plant byproducts worldwide. The selected byproducts represent almost 2 trillion Mcal of energy. The study also indicated that the world's total byproducts almost equal 1 billion metric tons of dry matter. These enormous quantities will continue to increase, particularly in developed and some fast-changing developing countries. Bioconversion of accumulated byproducts, as animal feed, may become more important as the world's natural resources become limited and the population continues to expand (*2*).

Analysis of 10 years (1996–2005) of data from the California Agricultural Statistics Service (CASS) indicates that California's annual mean production of 10.1 million tons of processing tomatoes represents more than 90% of the United States' total production (*3, 4*). This generates up to 4.0 million tons of tomato

waste or pomace (*5*). Tomato pomace consists of peels, cores, culls, trimmings, seeds, liquor, and unprocessed green tomatoes picked by harvest machinery. The nutritional value of the dried pomace consists of 94.6% total dry matter, 22.9% protein, up to 30.2% fiber, and 23.4% nitrogen-free extract (*6*). The total tomato dregs are an excellent source of nutrients such as α-tocopherol, lycopene, and β-carotene.

α-Tocopherol has been added to poultry feed as a potential antioxidant to retard lipid oxidation in processed meat. King and Zeidler (*7*) suggested that α-tocopherol from tomato pomace could be used in broiler diets to retard lipid oxidation during subsequent long-term frozen storage or heating of meat. However, high fiber in diets caused by adding pomace adversely affected feed conversion and cost, thereby reducing its usefulness as a feed ingredient. Knoblich et al. (*6*) examined tomato peel and seed byproducts to determine if their carotenoids could be transferred to yolk. While detectable amounts of lycopene were transferred to yolk, these investigators suggested that poor digestibility of high fiber content in tomato pomace would be a major obstacle in feeding it to poultry. Thus, ways to maximize the potential utilization of pomace as an ingredient in feed while maintaining useful components such as antioxidants are needed.

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One possibility is to treat the pomace with the edible oyster mushroom, white rot fungi (*Pleurotus ostreatus*), under solid state fermentation (SSF) to transform the fibrous ingredients into digestible nutritional components while retaining antioxidants. *P. ostreatus* easily colonizes a variety of substrates; almost any fibrous agricultural waste can be a proper substrate (*8–11*).

Substrates for *P. ostreatus* are generally sterilized to eliminate competing bacteria and other microorganisms. Several investigators have determined the effect of heating on levels of antioxidants after sterilization and processing of tomatoes and their products (*12–16*). Chun et al. (*17*) noted that the α-tocopherol content of unpeeled raw tomatoes grown in the United States was 0.53 + 0.22 mg/100 g, while that reported for whole canned tomatoes was 0.79 + 0.16 mg/100 g. Seybold et al. (*18*) made tomato juice from canned tomatoes (origin not stated), soup from Spanish tomatoes, and sauces from both Dutch and Spanish tomatoes. The juice was sterilized (121 °C for 2 min) and pasteurized (80 °C for 20 min). The soup and sauces were heated on hot plates (medium heat) for various lengths of time (0–50 and 210 min). Homogenization, sterilization, and pasteurization significantly reduced the α-tocopherol content on a wet and dry weight basis for juice. However, Seybold et al. (*18*) noted that the antioxidant was very stable against thermal degradation in a model reaction (180 °C for 60 min) or after short-term heating (15–50 min) of tomato sauces or soup.

Seybold et al. (*18*) analyzed tomato sauces for lycopene and β-carotene content after heating on a hot plate to simulate household conditions. Sauce 1 (Dutch tomatoes) was heated for 0–50 min, and sauce 2 (Spanish) was heated for 0–210 min. (*E*)-Lycopene and (*E*)-β-carotene levels in tomato sauces significantly increased after heating for 0–50 min when measured on a wet weight basis. (*E*)-Lycopene and (*E*)-β-carotene in soup and in sauce 1 significantly decreased on a dry weight basis, while the levels of (*E*)-lycopene significantly increased on a dry weight basis in sauce 2 after heating for 30 min.

Heating seemed to slightly increase the β-carotene content of tomatoes in some comparisons. Red, ripe, canned, whole, regular pack tomatoes (240 g) contained 6480 μg of β-carotene, while the same quantity of red, ripe, raw tomatoes contained 6175 μg of this carotenoid (*19*). Abushita et al. (*20*) reported an increase in the lycopene content (dry weight basis) after processing, while Takeoka et al. (*21*) reported the opposite for a wet weight basis. Also, other results have also shown a decrease in β-carotene and isomerization after processing (*20, 22, 23*).

Because delignified tomato pomace in broiler and laying hen diets is a possible source of α-tocopherol in processed meat and lycopene in egg yolks, respectively, the fate of these antioxidants and others in tomato pomace during the process of delignification by fungal fermentation should be evaluated. Thus, the purpose of the work reported here was to investigate the effect of fermentation by *P. ostreatus* on the content of α-tocopherol, lycopene, and β-carotene in tomato pomace.

MATERIALS AND METHODS

Substrate Preparation and Sterilization. Tomato pomace was prepared as a substrate following procedures of Stamets (*8*) and Davis (*11*). Tomato pomace (70% moisture) was obtained from the Processed Foods Research Unit (Western Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, Albany, CA). For experiment I, 95 g of pomace was placed in flasks and covered with cotton plugs overlaid with aluminum foil. The pomace in a predetermined number of flasks was sterilized in an autoclave (Steris model SV-120 Sterilizer) at 121 °C and 28 pounds per square inch pressure

for 1 h. For experiment II, 2 kg of pomace was placed in autoclavable plastic bags and sterilized. All control flasks, sterilized flasks, and sterilized bags were cooled for 24 h at 24–26 °C.

Tomato Pomace Treatment with *P. ostreatus* under SSF. After they were cooled, flasks and bags were inoculated with 5% (on a wet weight basis) of sterile sawdust spawn of the white rot fungi, *P. ostreatus* (Fungi Perfecti LLC, Olympia, WA), under a laminar flow hood (Nuaire model 201-430, Plymouth, MN). For experiment I, sterilized pomace in flasks was treated and stored in the same manner for 26 days of incubation. For experiment II, the bags were treated and stored for 49 days. After full colonization and formation of the aggregated mass of mycelia, the bags were transferred to a fully controlled environmental growth chamber (Conviron model E15). The conditions of the chamber—18 °C, ventilation, relative humidity of 98%, and 4 h of light per day using four 60 W incandescent light bulbs—initiated the oyster mushroom fruiting stage. Samples of mushrooms grown on wheat straw were obtained from a local farmers' market. The spent (the residue of tomato pomace substrate after mushroom cultivation) was sampled at 104 days after mushroom harvesting. Harvested mushrooms and the spent were stored at −80 °C until analyzed.

Analyses of Antioxidants. α-Tocopherol and carotenoid (lycopene and β-carotene) assays were performed at Analysis, Research, Consulting Laboratories (San Francisco, CA), using high-performance liquid chromatography (HPLC)-UV method 992.03 (*24*) and Craft et al. (*25*), respectively. The method is briefly described as follows. Samples were saponified with KOH and ascorbic acid. The mixture was extracted with a solvent containing hexane:acetone:ethanol:toluene, in a 10:7:6:7 ratio plus 200 mg/L BHT. The sodium sulfite-dried extract was evaporated by vacuum and transferred to a solvent composed of acetonitrile/methanol/ethylacetate for HPLC analysis. HPLC reverse phase separation was followed by monitoring at dual wavelengths of 294 and 450 nm. Tocopherol isomers (DLα-, β-, and Δ-tocopherols) and lutein, zeaxanthin, β-carotene, lycopene, and β-cryptoxanthin were used as standards to determine retention times of the compounds of interest. All standards were obtained from Sigma Chemical Co. except for β-cryptoxanthin from Indofine Chemicals.

Experimental Designs. In experiment I, samples were analyzed before and after sterilization as a 2 (treatments) × 3 (triplicate samples) × 3 (analyses of selected antioxidants) × 2 (replication) experimental design. For determining the effect on antioxidants during fungal fermentation in experiment 1, samples were analyzed as a 2 (*P. ostreatus* treatment and control) × 3 (sampling times at 0, 17, and 26 days) × 3 (triplicate samples) × 3 (analyses of selected antioxidants) × 2 (replication) experimental design. For experiment II, samples were analyzed as a 2 (type of samples) × 3 (triplicate sample) × 3 (analyses of selected antioxidants) × 2 (replication) experimental design.

Statistical Analysis. All data were analyzed by analysis of variance (*26*). The statistical model for analysis of variables (α-tocopherol, lycopene, and β-carotene) before and after sterilization in treatment 1 was $y_{ijk} = \mu + \text{treatment}_i + \text{sample}_j + \text{treatment} \times \text{sample}_{ij} + e_{ijk}$. Data for the effect of fungal fermentation over time in treatment 1 were analyzed as $y_{ijkl} = \mu + \text{treatment}_i + \text{rep}_j + \text{day}_l + \text{treatment} \times \text{rep}_{ij} + e_{ijk}$. Generally, only trace amounts of selected antioxidants were detected at 104 days; therefore, the data were not included in the analyses. The Tukey–Kramer test was used to separate means in treatment I, and the Welch two-sample *t* test was used to separate means in treatment II.

RESULTS AND DISCUSSION

Retention of Antioxidants during Sterilization and Fungal Fermentation. Tomato pomace substrate was sterilized to eliminate microbial contamination. The retention of α-tocopherol, lycopene, and β-carotene after sterilization was 79, 64, and 83%, respectively (**Table 1**).

Variability in the content of α-tocopherol after the heating of tomatoes has been reported (*19, 20*). Our results (wet weight basis) agreed with those of Seybold et al. (*18*) for tomato juice after processing. These investigators reported that homogeniza-

Table 1. Retention of Selected Antioxidants (μg/g) in Tomato Pomace after Sterilization[a]

samples	means[b]		
	α-tocopherol	lycopene	β-carotene
unsterilized	39.26 A	17.42 A	42.56 A
sterile	31.15 B	11.19 B	35.44 A
p values	0.0044	0.0059	0.0537

[a] Sterilized by autoclaving at 121 °C and 28 PSI for 1 h. [b] Means (six samples) within columns with the same letter are not significantly different at the stated *p* value.

tion and sterilization of tomato juice significantly reduced the α-tocopherol content measured on a wet or dry weight basis.

The retention of lycopene was the lowest among the three antioxidants. As discussed above in the Introduction, Seybold et al. (*18*) noted that lycopene level changed after heating depending on the cultivar. Somewhat contrary to the findings of Seybold et al. (*18*), Dewanto et al. (*27*) observed an increase in bioaccessible lycopene due to thermal processing. The *trans*-lycopene content increased from 2.01 ± 0.04 for raw tomatoes to 3.11 ± 0.04, 5.45 ± 0.02, and 5.32 ± 0.05 mg of *trans*-lycopene/g after heating at 88 °C for 2, 15, and 30 min, respectively. These investigators reported an enhancement in nutritional value of tomatoes due to elevated bioaccessible lycopene content and total antioxidant activity and suggested that the nutritional value of fresh produce may not always be higher than that of processed fruits and vegetables. Graziani et al. (*28*) observed that longer heating (>2 h in oil at 100 °C) decreased the lycopene content due to the release of the carotenoid from its binding site. Our findings agreed with those of Graziani et al. (*28*). Apparently, in our study, heating of tomato pomace containing 70% moisture for 1 h under pressure also released lycopene and caused significant degradation.

After sterilization, β-carotene was reduced by about 17%. Jajyalakshmi et al. (*29*) collected 46 green leafy vegetables from the forests of India and found losses of from 2 to 25% β-carotene after processing in various ways. Takeoka et al. (*21*) also found a decrease in this carotenoid on a wet weight basis after the processing of tomatoes. Our results were not supportive of those of Takeoka et al. (*21*) and were similar to those of Seybold et al. (*18*) who observed no significant changes for this carotenoid in tomato sauce from tomatoes of Spain. It is not known if cultivars of tomatoes grown in California are more resistant to losses of β-carotene during heating.

The remaining content of antioxidants after sterilization was sufficient to continue investigating fungal bioconversion of the pomace. α-Tocopherol significantly decreased (52%) in the control by day 26; there was no further decrease by day 104 (**Table 2**). These findings supported often cited reports on the stability of α-tocopherol (*17, 18*). The almost 2-fold quantity of α-tocopherol in treated samples at day 17 as compared to day 0 may have been caused by low quantities of heat generated (*18*) during the fermentation process and/or release of the vitamin by *P. ostreatus*. The antioxidant (88%) was retained from day 17 to 26 in treated samples but was no longer present by day 104 (data not shown).

Table 2 shows a statistically significant decrease ($>40\%$) for lycopene and β-carotene in the control from 0 to 26 days. Quantities of both carotenoids remaining in the spent (104 days) for the control were less than 0.3 μg/g (data not shown). Lycopene and β-carotene in treated samples greatly decreased over time with only about 8 and 5% retention at 26 days, respectively. Caroteinoids were negligible (trace amounts, data not shown) in the spent from treated samples at 104

Table 2. Effect of *P. ostreatus* Fungal Fermentation on Selected Antioxidants in Tomato Pomace

pomace samples (day)	means[a]		
	α-tocopherol (μg/g)	lycopene (μg/g)	β-carotene (IU/100 g)
0 control	31.15 B	11.19 A	35.44 A
17 treated[b]	60.26 A	2.04 C	4.20 D
17 control	18.56 C	5.53 B	24.91 B
26 treated[c]	52.87 A	0.89 C	1.66 D
26 control	14.87 C	6.32 B	21.09 C

[a] Means (six samples) within columns with one or more of the same letters are not significantly different ($p \leq 0.05$). [b] Partially colonized with *P. ostreatus* mycelia. [c] Fully colonized with *P. ostreatus* mycelia.

days. Overall, these results revealed the unstable nature of carotenoids following sterilization and storage at 25 ± 1 °C. Heat produced during fungal fermentation in the anaerobic environment of the flasks and UV light most likely contributed to destruction of antioxidants along with that caused by *P. ostreatus* at 25 ± 1 °C.

Content of Antioxidants in Edible Oyster Mushrooms. Only trace amount of carotenoids were detected in edible oyster mushrooms grown in tomato pomace and wheat straw. α-Tocopherol in mushroom fruit (74.10 μg/g) from pomace was numerically higher, although not statistically different ($p \leq 0.05$), than that for mushrooms (51.36 μg/g) from wheat straw. Possibly, α-tocopherol was released from treated pomace during the fermentation process and stored in the mushroom fruit.

ABBREVIATIONS USED

CASS, California Agricultural Statistics Service; SSF, solid state fermentation; PSI, pound per square inch pressure; AOAC, Association of Official Analytical Chemists.

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