

## Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices

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Secondary phenolic metabolites play an important role in plant defense mechanisms, and increasing evidence indicates that many are important in human health. To date, few studies have investigated the impact of various agricultural practices on levels of secondary plant metabolites. To address this issue, the total phenolic (TP) content of marionberries, strawberries, and corn grown by sustainable, organic, or conventional cultural practices were measured. Additionally, the effects of three common postharvest processing treatments (freezing, freeze-drying, and air-drying) on the TP content of these agricultural products were also investigated. Statistically higher levels of TPs were consistently found in organically and sustainably grown foods as compared to those produced by conventional agricultural practices. In all samples, freeze-drying preserved higher levels of TPs in comparison with air-drying.

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**KEYWORDS:** Phenolics; ascorbic acid; sustainable agriculture; organic agriculture; conventional agriculture; strawberry; corn; marionberry

### INTRODUCTION

A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (1–4). Of special interest are plant-based phenolic metabolites due to their potent antioxidant activity and wide range of pharmacologic properties including anticancer, antioxidant, and platelet aggregation inhibition activity (5–9). Phenolic metabolites are common constituents of fruits and vegetables that function in the defense against insect and animal herbivory (10). The synthesis of phenolics correlates to insect pressure, UV light exposure, and microorganism pressures, which suggests they have a protective role in preventing insect predation, photo-oxidation, and bacterial or fungal infections (11, 12). There is a growing concern that the levels of some phenolics may be lower than optimal for human health in foods grown using conventional agricultural practices (13, 14). This concern arises because conventional agricultural practices utilize levels of pesticides and fertilizers that can result in a disruption of the natural production of phenolic metabolites in the plant (15).

Factors such as resource availability, soil quality, climate, and insect and animal herbivory pressures are known to affect levels of nutrients in plants. For example, increased soil nitrogen

generally produces an increase in protein content (16), whereas low phosphorus soil levels result in increased sugar content (17). However, there is very little information on the impact various cultural practices have on the production of secondary phenolic metabolites in plants (13). Given that increasing evidence indicates a role for plant phenolics in human health, efforts need to be directed in understanding relationships between cultural practices and phenolics levels in crops.

Conventional, organic, and sustainable agriculture are the primary cultural practices used in the production of foods in the United States. The goal of each of these practices differs greatly with respect to crop yield, land and pesticide use, and environmental impact. Conventional agricultural practices utilize high-yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term sustainability associated with these practices (18). Organic farming must adhere to National Organic Standards set by the U.S. Department of Agriculture. Organic crops cannot be genetically engineered, irradiated, or fertilized with sewage sludge. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years prior to harvest. Disease-resistant cultivars are often used, and plant nutrients are supplied through crop rotation, cover crops, and animal manure. Sustainable agriculture is based upon the principle that agricultural practices

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**Table 1.** Agricultural Conditions, Soil Type, Irrigation Source, and Chemical Applications

crop	agricultural practice	soil type	crop age, years	previous crop	irrigation	chemical application
marionberry	conventional		21–22		creek	
	organic		4		creek, well	
	sustainable		2		well	
strawberry	conventional	clay, loam	2		well	glyphosate drift from neighboring field
	sustainable	clay, loam	1		well	none
corn	conventional	sandy, Ritzville loam		wheat	well	Partner at 3 lb/acre (pre-emergence) atrazine at 1 pt/acre (pre-emergence)
	organic sustainable	sandy, clay, loam clay, loam		green beans winter squash	creek, well creek, well	none glyphosate at 1.5 pt/acre (preplant) Silhouette at 2 pt/acre (pre-emergence) Frontier at 25 oz/acre (pre-emergence)

**Table 2.** Fertilizer Usage Records

crop	agricultural practice	fertilizer	description	rate	timing
marionberry	conventional	standard commercial chemical fertilizers	NA <sup>a</sup>		
	organic	cow/chicken manure		40–60 yd <sup>3</sup> /acre	postemergence
	sustainable	ammonium nitrate boron Solution 32	32% N	20 lb total 1 lb/acre 8.74 gal/acre	postemergence postemergence postemergence
strawberry	conventional	none			
	sustainable	none			
corn	conventional	standard commercial chemical fertilizers	NA		
	organic	chicken manure		14–18 yd <sup>3</sup> /acre	pre-emergence
	sustainable	Solution 32 planting blend	32% N <sup>b</sup> 9% N, 27% P, 1.7% K, 2.3% S	17.5 gal/acre 25.5 gal/acre	preplant planting

<sup>a</sup> NA, information not available. <sup>b</sup> N, nitrogen; P, phosphorus; K, potassium; S, sulfur.

must meet the needs of consumers without compromising the ability of future generations to meet their own needs. Sustainable farming systems are designed to promote both environmental health and the social and economic equity of a region. Sustainable agriculture practices are hard to define because conditions can vary greatly depending on the crop, environment, and issues important to a region.

There is abundant anecdotal evidence indicating that, on average, organic foods likely contain higher levels of phenolic metabolites than conventionally produced fruit and vegetables; however, few studies directly address this issue (14). Differences between the content of phenolic metabolites in organically and conventionally produced fruits and vegetables allows for the possibility that organically grown produce may benefit human health better than corresponding conventionally grown produce. The problem with this supposition is that there are very few studies available to resolve this question (13). Existing studies have compared the nutritional quality of organically and conventionally grown plants in terms of macronutrients, vitamins, and minerals, and the results of over 150 of these studies were recently reviewed (14). These data demonstrate inconsistent differences in the nutritional quality of conventionally and organically produced vegetables with the exception of nitrate and ascorbic acid (AA) in vegetables. These results are difficult to interpret because cultivar selection and growing conditions varied widely and different methods of sampling and analysis were used in the investigations. Additionally, these studies did not address levels of secondary plant metabolites in conventionally and organically grown foods, although it is the levels of secondary plant metabolites that are expected to differ the most between these two practices.

In the present study, total phenolic (TP) levels, as measured by the Folin–Ciocalteu method, and AA were determined in marionberries and corn grown by conventional, sustainable, and

certified organic agricultural practices and in strawberries grown by conventional and sustainable agricultural practices. Fruit and vegetable selection was based upon the availability of similarly matched and controlled fields that were harvested at the same time. The impacts of three common postharvest processing methods, controlled freeze-drying, air-drying, and flash freezing, on the levels of TPs and AA were also compared. To our knowledge we show for the first time a correlation between the applied agricultural system and levels of TPs in marionberries, strawberries, and corn and demonstrate the impact of postharvest drying processes on TPs.

## MATERIALS AND METHODS

**Chemicals.** Folin–Ciocalteu phenol reagent, gallic acid monohydrate, and theobromine were purchased from Sigma (St. Louis, MO). HPLC grade acetone, glacial acetic acid, L-ascorbic acid, and metaphosphoric acid were obtained from Fisher Scientific (Tustin, CA). Sodium carbonate was obtained from EM Science (Gibbstown, NJ). Reagent grade, bacteria-free water was generated by a Barnstead E-pure four-module deionization system.

**Agricultural Conditions and Soil Characteristics.** All commodities used in these studies were grown under controlled conditions and supplied by Stahlbush Island Farms, Inc. (Corvallis, OR). Sample selection was based upon matched crop availability from this farm. The strawberry variety was Northwest Totem, blackberries were of the marionberry variety, and the corn variety was Supersweet Golden Jubilee. Records were kept on agricultural conditions, soil type, irrigation source, and chemical applications and are defined in **Table 1**. Fertilizer usage, rates of application, and times of application are defined in **Table 2**. Commodities grown by various conditions were harvested at the same time to ensure the same degree of ripeness for TP comparisons.

**Sample Freezing.** Fresh samples were individually quick frozen (IQF) in a blast tunnel run at  $-26^{\circ}\text{F}$ . Ambient temperature samples were frozen to  $-5^{\circ}\text{F}$  in 10 min. Frozen samples were analyzed within 3 months of freezing.

**Freeze-Drying Method.** A vertical freezer with 17.5 ft<sup>2</sup> of shelf space, designed and built by Oregon Freeze-Dry, Inc. (Albany, OR), was utilized for freeze-drying samples. Samples were placed in a single layer on solid anodized aluminum trays and dried in three (corn) or four (marionberries and strawberries) stages. At each stage, a progressively lower temperature of ethylene glycol was used as the heating medium in the dryer shelves. The condenser temperature was approximately -45 °C. Freeze-drying marionberries and strawberries to 0.1–0.2% moisture required 22 h, whereas corn required ~20–24 h to reach a moisture content of 0.7–1.1%.

**Air-Drying Method.** Samples were dried in an air-dryer designed and built by Oregon Freeze-Dry, Inc., which had a capacity of 22 ft<sup>2</sup> of shelf space. Air-drying was carried out using horizontal air flow over products, which were placed in a single layer on anodized aluminum trays. Drying occurred in two stages, with an initial relatively short period of time at 76.7 °C followed by a longer drying period at 48.9 °C. Drying time for marionberries and strawberries was 88 h to reach moisture contents of 2.2–2.5% in marionberries and 3.9–5.4% in strawberries. Corn required 20–25 h to dry to a moisture content of 4.0–5.2%.

**Extraction of Phenolics.** Samples were homogenized for 1 min at maximum speed in a Waring blender. A 3 g aliquot (6 g was used for corn) of the homogenized sample was then transferred to polypropylene tubes and extracted with 40 mL of a mixture containing acetone, water, and acetic acid (70:29.5:0.5 v/v). Samples were vortexed and allowed to stand for 1 h at room temperature to allow for complete solvent extraction. Extracts were centrifuged at 1640g for 15 min at 20 °C. The supernatant plus a subsequent 15 mL water rinse was filtered through a Whatman no. 1 filter (Whatman Inc., Clifton, NJ), after which the filtrate was concentrated using a rotary evaporator under partial vacuum at 40 °C. Samples were concentrated to 25 mL and were brought up to a total volume of 30 mL with Nanopure water. Extractions were repeated on three independent samples of the initial homogenate to give triplicate readings.

**Measurement of Total Phenolics.** TP concentrations were measured using the Folin–Ciocalteu assay (19). Briefly, 5 mL of Nanopure water, 0.5–1.0 mL of sample, and 1.0 mL of Folin–Ciocalteu reagent were added to a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5–8 min at room temperature. Next, 10 mL of a 7% sodium carbonate solution was added, followed by the addition of Nanopure water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2 h. Sample aliquots were filtered through a Whatman 0.45 µm poly(tetrafluoroethylene) filter prior to the determination of total phenols concentration using a Beckman DU 7400 spectrophotometer monitoring 750 nm. TP content was standardized against gallic acid and expressed as milligrams per liter of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5–5.0 mg/L GAE ( $R^2 = 0.9990$ ), giving an absorbance range of 0.050–0.555 AU.

**HPLC Analysis of Ascorbic Acid.** Samples were homogenized for 1 min at maximum speed in a Waring blender. The homogenate (1–5 g) was added to 20 mL of 4.5% metaphosphoric acid and vortexed. Extracts were centrifuged at 1640g for 15 min at 20 °C. The supernatant was filtered through a Whatman no. 1 filter and diluted to 25 mL with the 4.5% metaphosphoric acid. AA concentrations were measured according to established methods with minor modifications (20). Analysis was performed using a Waters 515 HPLC pump equipped with a Waters 486 tunable absorbance detector (Waters, Milford, MA). Reverse-phase separation was attained using an Agilent (Palo Alto, CA) Zorbax 5 µm Eclipse XDB-C18 (4.6 × 250 mm). The mobile phase was Nanopure water brought to pH 2.2 with sulfuric acid. The flow rate was 0.5 mL/min, and the detection wavelength was 245 nm. Sample aliquots were filtered through a 0.45 µm poly(tetrafluoroethylene) filter prior to injection. All samples were run in triplicate. The linearity range was determined from 0.005 to 0.04 µg/µL with a 20 µL injection volume ( $R^2 = 0.9997$ ), yielding an absorbance range of 0.055–0.500 AU.

**Ascorbic Acid Correction.** Because AA contributes to the response of the Folin–Ciocalteu assay, a correction factor for AA was determined. This was determined by taking known amounts of AA, corresponding to the lowest, midrange, and highest concentrations found

in samples, through the extraction procedure and measuring absorbance in the Folin–Ciocalteu assay. The AA contribution was consistent in a weight-to-weight ratio of 0.640:1.00, gallic acid versus AA. The correction factor of 0.640 was applied to the AA concentrations measured through HPLC analysis. The reduced AA values thus represented the impact of AA upon the concentrations of TPs in terms of milligrams of gallic acid per 100 g of fresh weight and were deducted from the spectrophotometrically determined TP values.

**Correction for Sugar Content.** Sugars may interfere with the TP analysis if the sugar level is high (21). This effect can be compensated for by applying a standard correction (21, 23). On the basis of the invert sugar contents of the samples (24), the correction factors for marionberry, strawberry, and corn were applied to data as 0.92, 0.94, and 0.98, respectively.

**Statistical Analysis.** Statistical analysis was performed with SAS software (SAS Institute, Cary, NC). Separate models were created for each crop using two-factor analysis of variance (ANOVA) with interaction, and differences between sample means were analyzed by Tukey's method of multiple comparison at  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

TP and AA concentrations were measured in marionberries and corn grown by conventional, sustainable, and certified organic agricultural practices and in strawberries grown by conventional and sustainable practices. The impact of freeze-drying and air-drying on TP content was also evaluated and compared to the TP content in fresh samples that were frozen and stored at -12 °C. In a previous study of peaches, we demonstrated that freezing and storing at -12 °C for up to 6 months produced no significant decrease in TPs as compared to fresh samples (Asami et al., *J. Sci. Food Chem.*, in press). Therefore, levels of TPs in air-dried and freeze-dried samples were compared to those found in frozen samples because the fresh samples were not available for analysis.

Concentrations of AA were measured separately by HPLC because AA produces an oxidative–reduction reaction that contributes to the absorbance measurement in the Folin–Ciocalteu assay. Reported TP values were adjusted for the contributory effect of AA in the analysis of TP activity. Levels of AA in organically grown and sustainably grown samples were consistently higher than the levels for the conventionally grown crops (Table 3). A statistically significant decrease in AA content was also observed in freeze-dried and air-dried samples as compared to frozen samples. Air-dried samples contained the lowest levels of AA. Measurements of AA content for all frozen crops were consistent with other published values (25, 26). Comparative measures for air-dried and freeze-dried crops are not available.

Sustainably grown and frozen strawberries contained higher levels (20.3%) of AA as compared to conventionally grown strawberries. The average AA levels in sustainably grown and freeze-dried or air-dried fruit were significantly lower ( $P < 0.05$ ) at 44.2 and 16.2% of levels in frozen strawberries. The only marionberry samples found to contain detectable levels of AA were the sustainably grown and frozen berries, which had 2.9 mg of AA/100 g of fresh weight. The AA concentrations of organic and sustainably grown and frozen corn were 52.4 and 66.7% higher, respectively, than conventionally grown and frozen corn. AA was not detectable in air-dried or freeze-dried corn.

The average TP content of conventionally grown and frozen marionberries, strawberries, and corn were 412, 241, and 24.7 mg/100 g of fresh weight, respectively (Figure 1). On a dry weight basis, average levels of TPs reported here for dried strawberries (2200 mg/100 g of dry weight) are consistent with previously reported values of 1600–2410 mg/100 g of dry weight

**Table 3.** Ascorbic Acid Concentrations (Milligrams per 100 g of Fresh Weight)

crop	agricultural practice	sample preparation	ascorbic acid <sup>a</sup>
marionberry	conventional	frozen	nd
		freeze-dried	nd
		air-dried	nd
	organic	frozen	nd
		freeze-dried	nd
		air-dried	nd
	sustainable	frozen	2.9
		freeze-dried	nd
		air-dried	nd
strawberry	conventional	frozen	27.1a
		freeze-dried	9.8b
		air-dried	3.6c
	sustainable	frozen	32.6d
		freeze-dried	14.4e
		air-dried	5.3f
corn	conventional	frozen	2.1a
		freeze-dried	nd
		air-dried	nd
	organic	frozen	3.2b
		freeze-dried	nd
		air-dried	nd
sustainable	frozen	3.5c	
	freeze-dried	nd	
	air-dried	nd	

<sup>a</sup> Values are means of triplicate analyses for each treatment; nd, not detected. Values for each crop with different letters were significantly different at  $\alpha = 0.05$  based on Tukey's method of multiple comparison. Detection limit is  $0.27 \mu\text{g/mL}$  ( $3 \times \text{SD}$  of blank).

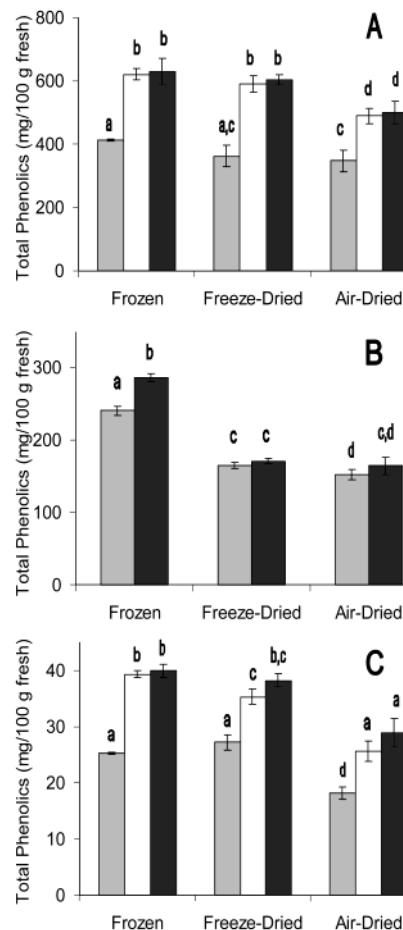
(27). In contrast, the levels of TPs we report for frozen strawberries and marionberries are 2 (28) and 6 times higher (29) than previously reported values. Comparative values for corn were not available.

TP levels were significantly higher ( $P < 0.05$ ) in frozen marionberries grown using organic (620 mg/100 g of fresh weight) and sustainable (630 mg/100 g) agricultural methods as compared with conventionally grown and frozen marionberries (412 mg/100 g). The TP levels in air-dried marionberries were 15.6–21.1% lower than those found in frozen fruit (**Figure 1a**). Differences in TP levels between freeze-dried and frozen marionberries were not significant.

TP levels in sustainably grown and frozen strawberries (**Figure 1b**) were 19.1% greater than those in conventionally grown and frozen strawberries. The levels of TPs in freeze-dried and air-dried samples were 31.5–42.6% lower than that of the frozen strawberries. There was no statistical difference ( $P < 0.05$ ) in TP levels between air-dried and freeze-dried samples.

The levels of TPs in frozen, freeze-dried, and air-dried corn grown by sustainable methods were 58.5, 40.7, and 58.4% higher than levels measured in conventionally grown samples, respectively. On average, the TP content of air-dried corn was 30.1% lower than that of frozen corn. There was no significant difference between frozen and freeze-dried corn samples grown by conventional methods.

The highest levels of TPs were consistently found in the extractions of frozen samples, followed by freeze-dried and then air-dried. In general, air-drying at temperatures  $>60^\circ\text{C}$  is regarded as unfavorable due to the possibility of inducing oxidative condensation or decomposition of thermolabile compounds, such as (+)-catechin. Conversely, freeze-drying may lead to higher extraction efficiency of TPs because freeze-drying



**Figure 1.** Total phenolics (mg/100 g of fresh weight) corrected for ascorbic acid in frozen, freeze-dried, and air-dried (A) marionberries, (B) strawberries, and (C) corn grown by conventional (gray bars), organic (white bars), or sustainable (black bars) agricultural practices. Values for each crop with different letters were significantly different at  $\alpha = 0.05$  based on Tukey's method of multiple comparison. Results are presented as means  $\pm$  SD for triplicate analyses. Maximum relative SD is 9.2%.

can lead to the development of ice crystals within the plant matrix. Ice crystals can result in a greater rupturing of plant cell structure, which may allow for better solvent access and extraction (30). With air-drying there is little or no cell rupture and there is the added effect of heat, which can cause losses in phenolics and ascorbic acid.

Flavonols such as quercetin and kaempferol are antimicrobial compounds synthesized by plants in response to pathogen attack (31). Because organically and sustainably grown products were produced by cultural methods utilizing no or very little pesticides, pathogenic pressures may explain the higher TP levels found in the organically and sustainably grown samples. Enhanced levels of TPs in strawberries cultivated by organic methods as compared to conventional methods were recently reported by Törrönen et al., who determined that the increases were a result of elevated amounts of ellagic acid and kaempferol (32).

These results demonstrate a statistically relevant trend of higher levels of TPs in organically and sustainably produced crops. More interestingly, our results indicate that TPs were highest in the crops grown by sustainable agricultural methods as compared to organic methods. This may reflect balance between adequate nutrition, as all sustainable crops were treated with synthetic fertilizers, and the requisite pathogenic pressures



that lead to the synthesis of TPs. We feel that these results warrant further studies investigating links between specific agricultural practices and levels of TPs in important food crops.

#### ABBREVIATIONS USED

TP, total phenolic; AA, ascorbic acid; GAE, gallic acid equivalent; HPLC, high-performance liquid chromatography.

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