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Rebuttal on 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

Sir: We thank Felsot and Rosen (*1*) for their interest in our work and thoughtful comments relating to the manuscript by Asami et al. (*2*). That paper describes the measurement of total phenolics (TPs) in marionberries, corn, and strawberries grown by organic, sustainable, and conventional agricultural practices. Additionally, the effects of three common food-processing methods (freezing, freeze-drying, and air-drying) on TPs were also investigated. Our results demonstrate a statistically significant trend of higher levels of TPs in samples taken from organic and sustainably grown produce as compared to samples grown by conventional agricultural practices. In all samples freeze-drying preserved higher levels of TP in comparison to air-drying.

The previous paper begins to address the question as to whether agricultural practices and certain plant stresses influence the level of phenolic compounds in food crops. In this instance we worked with growers and documented as well as possible the inputs that were used because there are no absolute definitions for “conventional” or “sustainable” farming and, although definitions exist for organically produced foods, conditions and inputs can vary greatly. At the other extreme, and equally important, is the evaluation of defined agricultural inputs in controlled settings so that direct comparisons of cause and effect can be made. We are presently designing additional investigations using matched organic and conventional plots at the University of California—Davis campus in order to evaluate relationships between agronomic inputs and the production of secondary plant metabolites. It is important to keep in mind that controlled studies will not reflect the dynamic grower environment, which changes with season, location, crop, and farm philosophy. Both types of investigation are important and relevant in the context of interpreting how agricultural practices affect food quality factors. The focus of the prior paper was on the existing grower environment.

We should clarify that in that paper we did not make the claim that there is a “nutritional benefit of certain foods grown by organic and/or sustainable methods in comparison to conventional methods” nor did we conclude that “organically grown produce benefits human health better than conventionally grown produce” as suggested by Felsot and Rosen. We do, however, state that, “given the increasing evidence indicating a role for plant phenolics in human health, effort needs to be directed in understanding relationships between cultural practices and phenolic levels in crops”. That being said, we emphasize that the hypothesis of this research is based upon a body of sound literature demonstrating that secondary plant metabolites are synthesized in response to a wide array of factors including soil quality, irrigation, weed population, insect, and pathogen pressures. Understanding how crop production practices and external pressures influence the synthesis of secondary plant metabolites is also important in the context of toxicity, because certain secondary plant metabolites may induce toxic effects.

We point out two additional studies demonstrating increased phenolic activity in produce grown by organic cultural practices as compared to conventional practices. In the first, Ren et al. demonstrate that organically grown spinach contains 120% higher antioxidant levels, whereas Welsh onion, Chinese cabbage, and qing-gen-cai contain 20−50% higher antioxidant levels as compared to their conventionally produced counterparts (*3*). That study demonstrates quercitin, caffeic acid, and baicalein levels that are 1.3−10.4 higher in juice obtained from these organic vegetables as compared to conventionally grown products. This study does not describe the cultivation practices used to characterize “conventional” agricultural practices other than to say that conventionally cultivated vegetables of the same varieties were purchased from an adjacent farm on the same day. However, samples were collected in triplicate from each site over three different dates and used as replicates, which strengthens the statistical analysis of this study. In the second study by Carbonaro et al., higher levels ($P < 0.01$) of TPs were found in peaches (*Prunus persica* L. cv. Regina bianca) and in two of three samples ($P < 0.05$) of pears (*Pyrus communis* L. cv. Williams) grown under organic cultivation conditions as compared to conventional cultivation conditions (*4*). A description of conventional cultivation conditions was not given in this study. To begin to interpret the factors responsible for these observations, information from farm records needs to be incorporated into these types of studies. Nonetheless, taken together these studies indicate a trend of higher levels of antioxidant activity in organically grown produce as compared to conventionally grown plants and warrant further investigation of this phenomenon.

We should clarify our interpretation of the literature that we used to build our hypothesis. Woese et al. evaluated results obtained from 150 studies of foods grown by organic or conventional cultivation methods published between 1926 and 1994 and concluded that many factors complicate comparisons of foods grown by organic or conventional cultivation methods (*5*). Those authors bring to light the fact that conclusive comparisons are very difficult to make because data lack details on the cultivation methods and do not provide information on the variety, age, and maturity of plants. Moreover, a variety of analytical methods were used to characterize quality factors. Nonetheless, Woese et al. concluded that despite the heterogeneity of the samples, some minor differences in quality between organic and conventionally produced foods could be identified. Secondary plant metabolites were not measured in the majority of these studies primarily because they compare data that span

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several decades, and the potential role of secondary plant metabolites in health was not recognized until recently. However, it is generally agreed that if quality differences are to be found between foods grown by organic and conventional systems, they will most likely involve differences in the levels of secondary metabolites and not necessarily macronutrients, vitamins, and minerals.

Felsot and Rosen believe that we misconstrued the conclusion of the paper by Brandt et al. (*6*) and that our misinterpretation of the literature has led to a false conclusion about the potential health benefits of organically produced foods. Felsot and Rosen claim that the statement by Brandt et al., "there is reason to believe that contents of many defense-related secondary metabolites are lower than optimal for human health, even for those where high levels are known to be harmful", is not referring specifically to comparisons of organic or conventional foods but to the fact that human intake of some secondary metabolites is inadequate. In contrast, we believed Brandt et al. were presenting an eloquent case for the need for investigating the influence agricultural practices have on levels of secondary metabolites in plants because their consumption has been linked to health. Brandt et al. intentionally construct their argument so that the reader will not lose sight of the fact that the most important issue regarding the consumption of optimal levels of secondary metabolites in human health is to increase the overall intake of fruits and vegetables in the diet. This critical message needs to be clearly conveyed to the public. Brandt et al. also point out that the content of secondary metabolites is probably higher in organic vegetables and fruits than in their conventional counterparts, and this leads to their statement "if defense-related secondary metabolites are the most important determinant of nutritional value of fruits and vegetables in the diet of developed countries, then vegetable and fruit products grown in organic agriculture would be expected to be more health-promoting than conventional ones". Correspondence with Brandt indicates that both interpretations of the manuscript are correct and not necessarily conflicting. We encourage interested investigators to read this thorough and thought-provoking paper.

Felsot and Rosen agree with our hypothesis of plant defense compound synthesis in response to pest control practices and suggest the use of a random block design in future studies. We strongly agree that a full random block design employing three replications and multiple-date sampling is the appropriate experimental design for future studies. A significant limitation of our study is a lack of this design. Nonetheless, the composite samples analyzed in our study represent a large (>2 kg) and adequately randomized sample and therefore do qualify for an ANOVA analysis. It is a reasonable strategy to employ a fractional factorial design in initial screening studies of potential stress factors (irrigation, pests, disease, soil, etc.) as resources are often limiting. To appropriately address the variability inherent in the dynamic grower environment, the experimental design should extend over multiple growing seasons. Because our data reflect only one growing season, variability in environmental conditions has not been appropriately been addressed. Felsot and Rosen claim that a major flaw in the experimental design was the inappropriate delineation of conventional and sustainable systems. We counter that this was not a flaw in our particular design, rather a lack of defined parameters in the agricultural systems growers classify as "conventional" or "sustainable". Felsot and Rosen correctly point out that there is no standard definition of "sustainable" or "conventional" cultural practices. However, conventional agriculture evolved in response to technological developments in mechanization/tillage, monoculture, synthetic fertilizer, irrigation, chemical pest and weed control, and genetics and breeding. Combinations of the use of these technologies are what distinguish conventional systems. Alternative systems are typically defined in terms of how they differ from conventional treatments. Felsot and Rosin state that the only way to actually differentiate between conventional and sustainable systems is to fully describe what management had occurred in the defined treatments. We agree. In fact, because we recognize the ambiguity inherent in the definitions of "conventional" or "sustainable" we provided all available data from farm records regarding agronomic inputs into tables within our original paper.

We also point out that the soil qualities among the three systems differed and likely played a key role in producing the effects we observed. In terms of these crops, the strawberries and corn were both grown in soil that had been in sustainable production for 10 years. The marionberries were grown in soil that had been in sustainable production for 2 years. By definition the certified organic soil had no pesticide treatments for 3 years prior to certification. Our original paper makes note of the potential for glyphosate drift on the strawberries as an attempt to be comprehensive in our documentation of inputs. Felsot and Rosen correctly conclude that if the glyphosate drift had been significant, the strawberries would most likely have died. As this did not occur we assume the exposure was minor.

In our text we incorrectly cited the use of Silohette in the sustainably grown corn. This was a spelling error. The material used was Silhouette. We agree with Felsot and Rosen that it is interesting that our results did not show increased production of ascorbic acid in herbicide-stressed plants, as previously noted by Brandt et al. Rather, our results indicate the opposite; that is, the corn crop grown organically had significantly higher levels of ascorbic acid (and polyphenolics) than the conventionally grown corn. However, the highest levels of ascorbic acid were obtained in the sustainably grown corn, which did receive herbicide treatment. The effect of herbicide stress obviously requires additional study.

We will address and clarify each of the major points brought up by Felsot and Rosen.

1. We would argue that the term "conventional" is still widely used to describe systems using synthetic fertilizers, chemical pest and weed control, and plants with modified genetics. The use of pesticides and fertilizers and the long-term sustainability associated with conventional farming are of public concern as reflected by increased interest in sustainable and organic farming and a growing consumer demand for organic foods. As for the results obtained by Robertson et al. (*7*), we agree with Felsot and Rosen that the conventionally managed system studied had the lowest net flux of global-warming gases. Our citation of this paper in the Introduction to our own paper was meant to point the reader to one particular study that compared conventional and organic systems.

2. The TP content was measured in these studies using a long accepted method that estimates the total phenol and polyphenol content in complex foods (Folin−Ciocalteu method; *8*). This method was employed to first determine if measurable differences exist between TPs in foods grown by various cultural practices under field conditions. Ideally one would want to quantitatively compare the profiles of specific secondary plant metabolites (e.g., quercetin, resveratrol, etc.) in response to exogenous environmental factors in order to assess the total antioxidant or toxicity potential of a food.

3. We agree that in future studies it will be important to match soil types, irrigation schemes, and plant age to the greatest extent

possible. As mentioned earlier, our study had limitations, yet is the first of what we hope to be many that address differences in the quality of products grown conventionally versus organically. We used real grower conditions and documented them as well as possible. Felsot and Rosen ask whether the age of a perennial planting changes its biochemical plantings. This is an excellent question, and one that we hope to address in the future.

4. Fruits and vegetables were brought in from each field to the processing plant in individual truckloads. Each truckload was assigned an identifying lot number. A 2−5 kg sample of fruit or corn was randomly selected from each lot and immediately individually quick frozen (IQF) in a blast tunnel run at −26 °F. Samples were then sent to Oregon Freeze-Dry, Inc., for freeze-drying or air-drying. The corn was removed from the cob. After processing at Oregon Freeze-Dry, samples were put into individual bags (one bag per sample) and shipped to the University of California at Davis on dry ice. When the samples were received, the contents of each bag were mixed and an ∼10−20 g sample was removed from each bag and homogenized. A 3 g (6 g for corn) aliquot of this homogenate was extracted as described in the text and analyzed in triplicate. This procedure was repeated on three independent samples of homogenates obtained from the original sample. The statistical analyses used to interpret the data presented in that manuscript were evaluated by the University of California−Davis Statistical Laboratory and stated by that laboratory to have been applied appropriately in a letter sent to the Editor of the *Journal of Agricultural and Food Chemistry*.

5. Felsot and Rosen bring up a very good point regarding possible variability in the content of sugar between organic and conventionally produced foods. We had not originally considered this point. Therefore, we used USDA literature values for sugar concentration to correct for the contribution of sugars in the TP assay. Future investigations relating these values should take into consideration the levels of soluble solids and measure °Brix values.

6. Levels of TP in sustainably grown and frozen strawberries were 19.1% higher than those in conventionally grown and frozen strawberries. However, this trend was not noticeable in comparisons of freeze-dried or air-dried fruits. We have no real explanation for this observation.

7. Felsot and Rosen claim that our results, with respect to fertilizer regimens, are incongruent with the hypothesis. As stated earlier, our hypothesis is that phenolic compounds are synthesized in response to a wide array of factors including soil quality, irrigation, weed population, insect, and pathogen pressures. Fertilizers are definitely one factor that may influence the production of secondary plant metabolites such as phenolics. In our study, the sustainable and conventional practices employed chemical fertilizers on marionberries and corn, whereas the organic system used chicken and/or cow manure. We conclude that soil nutrients may have influenced the production of phenolic compounds and again believe more investigation is needed to draw clear connections.

8. Felsot and Rosen are correct to point out that the conclusion obtained by Hakkinen and Törrönen, who compared three varieties of strawberries grown by organic and conventional cultivation techniques, was that organic cultivation had no consistent effect on levels of phenolic compounds in strawberries (*9*). Of the three varieties tested, only one demonstrated an elevated level of phenolic activity. This 12% increase in phenolic activity was the result of elevated levels of kaempferol and ellagic acid. Kaempferol is one of the antimicrobial compounds synthesized in response to pathogen attack. Our reference to this work was to offer a possible hypothesis (pathogenic pressure) for why we found higher levels of total phenolic activity in our samples obtained from organically and sustainably grown products on which no or low amounts of pesticides were used.

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