# AGRICULTURAL AND FOOD CHEMISTRY

# CORRESPONDENCE/REBUTTAL

### **Editor's Comment**

The Editor appreciates the considerable thought and effort put forth by commenters Felsot and Rosen, responders Mitchell and Barrett, and the reviewers of the accompanying comment and rebuttal. The area of science dealing with the effects, if any, of environment, production, and cultivation practices on the composition and quality of foods is an important one. The *Journal of Agricultural and Food Chemistry* will welcome future manuscripts dealing with topics in this general area that fall within the scope of the Journal. Such issues as the delineation of the conditions of production, experimental design and replication, sampling, and data anaylsis should be addressed along the lines indicated in the accompanying comment and rebuttal. Results from analyses for total content of a class or classes of compounds, such as phenolics and antioxidants, should be accompanied by analytical data for individual components of those classes.

The accompanying comment and rebuttal should be consulted and put to use when one is planning, conducting, and reporting studies of this type to be submitted to the *Journal of Agricultural and Food Chemistry* for publication consideration.

James N. Seiber Editor in Chief

JF030737I

## Comment 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:* An American Chemical Society press release alerted us to volume 51 (issue 5) of *Journal of Agricultural and Food Chemistry* and the paper titled "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" (*1*), authored by D. K. Asami, Y.-J. Hong, D. M. Barrett, and A. E. Mitchell. We read this article with critical interest because it seemed to provide evidence of nutritional benefits of certain foods grown by "organic" and/or "sustainable" methods in comparison to "conventional" methods. Although such a finding would have many positive benefits for the marketing of certified organic produce, our analysis of the paper and references used to support its suppositions have uncovered significant technical and conceptual flaws.

In the Introduction, Asami et al. cite literature to assert that secondary plant metabolites have human health promoting properties. They then iterate that synthesis of the antioxidant phenolics correlates with a host of factors that include insect and pathogen infestations. The phenolics are supposed to represent responses of plants in defense against exogenous stresses. We agree that such a hypothesis is more or less supported by the published literature. From this point, Asami et al. attempt to make the case that conventionally produced food has phenolic levels that are too low to be optimal for human health, putatively because of the use of crop protection agents

#### Correspondence

that reduce pest pressure and thus "result in a disruption of the natural production of phenolic metabolites in the plant". Asami et al. then argue that anecdotal evidence suggests organic foods likely contain higher levels of phenolic metabolites than conventionally produced foods.

Although not explicitly stated, we infer the hypothesis of Asami et al. to be that phenolic metabolites in foods grown by certified organic methods are sufficiently higher than in foods grown by conventional methods to warrant the conclusion that organically grown produce benefits human health better than conventionally grown produce. We base our inference on the following statements in the Introduction (p 1238): "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."

Our inference was further premised on the content of the remainder of the Introduction, which expounded on methodological issues. Thus, Asami et al. seem to have taken up the challenge of testing the stated supposition by comparing ascorbic acid and total phenolics (TP) content in marionberries, strawberries, and corn grown under different management practices on a single farm in Oregon.

Our first criticism of this paper stems from a misinterpretation of the literature that Asami et al. use to build their hypothesis. Specifically, the authors cite papers by Woese et al. (2) and Brandt et al. (3) to conclude that phenolics may be lower than optimal for human health in foods grown using conventional agricultural practices. Nowhere in either of those papers did the authors conclude that conventionally grown foods had phenolic levels that were less than optimal for human health. In truth, Woese et al. (2) did not focus their review on secondary metabolites at all. In the one paragraph devoted to secondary metabolites (namely, betaine, mustard oils, lycopene), they concluded no reliable differences could be established for vegetables produced under different agronomic practices. Furthermore, an examination of fertility parameters and rearing performance in animals fed conventionally produced or organically produced food led to the conclusion that results were contradictory.

Asami et al. also misconstrued the conclusions of the paper by Brandt et al. (3). In their abstract, the latter authors make the statement that "contents of many defence-related secondary metabolites in the diet are lower than optimal for human health". However, a careful reading of the contents of the Brandt et al. (3) paper reveals that the aforementioned statement was not referring specifically to organic or conventional foods but to the fact that human intake of foods rich in secondary metabolites was inadequate. Indeed, Ames et al. (4) have reviewed the biochemical benefits of antioxidants and lamented that only 9% of Americans, and fewer in most other countries, are eating the recommended amounts of fruits and vegetables. In short, we believe that the misinterpretations of the literature have led to a false conclusion about potential health benefits of organically produced foods in comparison to conventionally produced foods.

Nevertheless, to determine whether differences in synthesis of plant-defense-initiated compounds occur as a result of the reduction in exogenous pest control practices is a worthy hypothesis to test. Ideally, such an experiment would deploy a random block design in which the experimental units are replicated and sampling is truly random. The raw data emanating from such a design would be amenable to parametric statistical analysis. However, such a design may be impossible for testing organic production practices in direct comparison to conventional practices because of the need to meet organic certification standards. However, such a design can be deployed effectively on nearby blocks of land using replicated sampling and nonparametric statistics. The ability to make definitive conclusions from such an experiment would depend on how well matched the soil types and water management practices were. Woese et al. (2) reviewed some of the pitfalls of doing comparative tests.

Unfortunately, Asami et al. have not instituted any of the logically recommended designs in their experiment, or if they did, they did not communicate them in the published paper. Indeed, given the information on management practices that were presented in Tables 1 and 2, we do not understand how they were able to arrive at the conclusion that total phenolics and ascorbic acid were higher in commodities grown under organic and sustainable systems than under conventional systems.

A major flaw in experimental design was the inappropriate delineation of conventional and sustainable systems. A scientifically fundamental definition of a "sustainable" system does not exist. In reality, so-called conventional systems use practices also employed by organic systems and vice versa. No-tillage and cover crops are not exclusive domains of sustainable or organic systems today. Similarly, pest control practices used by organic growers are also used by so-called conventional growers (for example, both types of growers use sulfur fungicide and codling moth pheromone dispensers for mating disruption; both use pest-resistant cultivars). In essence, the terms conventional and sustainable are clichés. The only way to differentiate them would be to fully describe in detail what management had occurred in the defined treatments, but the description of systems by Asami et al. was insufficient to delineate between the socalled conventional and sustainable systems.

Examination of pesticide use provides one example of the failure to provide sufficient information that could explain the results. There were no pesticide applications of any kind to the marionberries in any of the agricultural treatments. For strawberries, no differentiation in pesticide use from "sustainable" practices was noted other than an unspecified incidence of glyphosate drift. No further details are offered about this drift event, but one can logically conclude that if it had been severe enough, then it would have caused physiological distress and perhaps death of the plants. Ironically, herbicide stress is one of the factors cited by Brandt et al. (*3*) that supposedly increases

ascorbic acid content. Older literature has shown increases in flavonoids (5) and carotene (6) in crops exposed to herbicides.

In corn, both the sustainable and conventional systems used herbicides at pre-emergence, although the types of herbicides were from different chemical classes. A third material (Silohette) was also used in the sustainable corn treatment, but we were unable to determine the active ingredient of this product, even after we searched this term in three different databases (*Herbicide Handbook*; *Farm Chemicals Handbook*; Washington State University's Pesticide Information Center On-Line, http:// picol.cahe.wsu.edu/labels/). Curiously, we did find a listing for the formulation Silhouette that has a registration in the state of Oregon. It is a formulation of glyphosate.

Asami et al. have not presented any evidence from their own work or from the literature that weeds can induce the same type of defensive responses thought to be induced by pathogens or insects. Furthermore, we noted that no insecticides or fungicides were listed as treatments in the conventional practices. Yet, insecticides and fungicides are the type of chemicals that would relieve the stresses known to induce plant defense mechanisms. Therefore, if herbicides are used in both the conventional and sustainable corn systems (but not the strawberries or marionberries) and no insecticide or fungicide was used in any of the systems, then why are the sustainable and conventional treatments considered different if one of the working hypotheses is that pesticides reduce the plant's need for synthesis of secondary metabolites?

The authors seemed to have assumed that stresses from pests would be greater in organic and sustainable systems than in conventional systems but provided no literature references to support the assumption. As a matter of fact, research in grape vineyards has shown that organic practices significantly reduce Phylloxera damage and associated pathogen infection without the use of pesticides (7). Indeed, control of these pests in the organic system was superior to control in the conventional system. Thus, grapes grown organically would not be expected to be under greater stress than those grown conventionally. Growers of certified organic fresh produce would not be able to market their product if it did not have an appearance of quality. We were under the impression that the organic production goal was to manage the so-called exogenous stresses by ecological means rather than by pesticides. In short, Asami et al. presented no evidence to prove that organically produced commodities suffer more stress that would lead to defensive production of phenolics in greater amounts than in conventionally grown crops.

In addition to the fundamental flaw of a lack of differentiation in basic practices used in the conventional and sustainable production systems, there are other more specific problems that lead us to question the validity of the paper's conclusions.

1. In the paper's Introduction, Asami et al. cite Robertson et al. (8) in support of the concerns over the negative biological and environmental consequences of "conventional" agriculture and therefore its incompatibility with long-term sustainability. Considering that the term "conventional" is losing its meaning and modern agricultural production has greatly evolved over

the past 30 years, the level of concern is highly debatable. We do not argue whether different perspectives exist. However, we find disingenuous the support of a position by citing a paper [i.e., Robertson et al. (8)] that makes no claim or statement about the negative attributes of conventional agriculture and by implication the benefits of organic agriculture. Robertson et al. (8) studied the global-warming potential of various agricultural management techniques and compared several types of gaseous emissions from these systems to natural ecosystems. Robertson et al. (8) concluded that the agricultural management system giving the lowest net flux of global-warming gases was a conventionally managed (i.e., herbicides and mineralized fertilizers were used) corn-soybean-wheat rotation using no-tillage soil management. Organic and low-input legume cover crop systems had higher global-warming potential and less carbon sequestration potential, possibly because tillage was used to prepare the soil for planting and to control weeds. We are very curious about how the results in the Robertson et al. (8) paper were translated by Asami et al. to negative biological and environmental consequences of "conventional" agriculture.

2. The analysis of total antioxidant activity is somewhat misleading when conclusions are being made about the health benefits of TP because certain antioxidants are more effective than others, and we do not know what an effective dose is (in other words, no matter how the plants are grown, they may have enough antioxidant to do the job). Ironically, a number of compounds with antioxidant activity also test positive in rodent carcinogenicity assays (9)—probably for the same reason that many pesticides do: the artifact of high-dose testing. Thus, the results obtained by the authors could instead be used by critics of organic/sustainable agriculture to aver that organic/sustainable practices cause the production of more cancer-causing chemicals.

3. The crops for the differently defined agronomic practice treatments seem to have been grown on different soils and irrigated differently. Soil type (as well as location) can make a difference in biochemical parameters. The conventional marionberry crop was 21-22 years old compared to the organic (4 years) and sustainable (2 years) crops. Is it possible that the age of a perennial planting changes biochemical parameters?

4. Materials and Methods omits any details on sampling methodology. What kind of experimental design was used? How were the crops spatially oriented? How many replicate samples were taken from the field and then analyzed? In the absence of these data, one can conclude only that one sample of fruit or vegetable grown by each of the methods was collected as a composite sample of everything harvested in the field. We deduced that each sample was divided into three subsamples and either frozen, freeze-dried, or air-dried. The authors stated, "Extractions were repeated on three independent samples of the initial homogenate to give triplicate readings." We therefore further deduced that the preparation treatment subsamples were then analyzed in triplicate from a single extraction homogenate. Because a single homogenate was analyzed to generate the socalled replicates and no information was given about repeated sampling in the field, the sampling design indicates that Asami

#### Correspondence

et al. have used pseudo-replication and thus do not meet the requirements for conducting an ANOVA statistical analysis using farming practices as a class variable. To meet the requirements for conducting a parametric test, the replicates of the class variables must be independent of one another and the error term must be random (10). We do not believe that subsampling a single homogenate of a composite plant sample meets this requirement.

5. The authors acknowledge that sugars interfere with total phenol analysis, but instead of measuring sugar content in each sample in order to apply the appropriate corrections (as they did to correct phenolics content for ascorbate interferences), they rely on USDA literature values for the sugar concentrations. How can we be sure that growing food organically does not have some, as yet unexplained, effect on sugar content?

6. The "significant" increase in total phenolics for sustainably grown strawberries appears to be limited to frozen strawberries. The authors do not explain why they found no differences in TP between conventional and sustainable strawberries that were freeze- or air-dried.

7. One of the agronomic factors that could influence levels of secondary metabolites is soil fertility, as pointed out in ref 3. When we examine the fertilizer regimens, we find results that are incongruent with the hypothesis that the different agronomic practices could have affected phenolic levels. For example, neither the conventional nor sustainable strawberries received fertilizer. Thus, any differences in TP cannot be explained by fertilization treatment. For the marionberries and corn, the conventional treatment was an unspecified "standard commercial chemical fertilizer". This is a curious designation for a chemistry journal; it can only be assumed that the authors meant a mineralized nitrogen fertilizer, but absolutely no descriptive information was given. Ironically, ammonium nitrate was used on sustainable marionberries (how is this different from mineralized N?). The sustainable corn used Solution 32 and a planting blend (no commercial names were given). Solution 32 seems to be UAN 32% solution. It is a nitrogen fertilizer solution composed of urea and ammonium nitrate. Considering that both conventional and sustainable marionberries/corn received a form of mineralized N, fertilizer treatment cannot explain TP differences for those crops either. However, if N application rate as well as availability had been noted, then it might have been possible to know if soil fertility was a factor in physiological differences among the treatments.

8. To back up their hypothesis, the authors refer to a paper by Hakkinen and Torronen (11), "who determined that the increases (of TP in organically grown strawberries) were a result of elevated amounts of elliagic acid and kaempferol" (antimicrobial compounds synthesized by plants in response to pathogen attack). However, the results in ref 11 showed that of the three identical strawberry varieties grown under conventional and organic conditions, one set had increases in the two metabolites, whereas two sets did not. Hakkinen and Torronen (11) concluded that "compared to conventional cultivation techniques, organic cultivation had no consistent effect on the levels of phenolic compounds in strawberries". In summary, it seems to us that the conclusions of Asami et al. do not follow logically from the experimental design and details as presented in the paper. The hypothesis that organic foods promote health better than conventional foods seems to be based on a misreading of the literature cited, and the data presented in Asami et al. do not support the validity of such a hypothesis.

#### LITERATURE CITED

- (1) Asami, D. K.; Hong, Y.-J.; Barrett, D. M.; Mitchell, A. E. Comparison of the total phenolic and ascorbic acid content of freeeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J. Agric. Food Chem. 2003, 51, 1237–1241.
- (2) Woese, K.; Lange, D.; Boess, C.; Bogl, K. W. A comparison of organically and conventionally grown foods—results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281– 293.
- (3) Brandt, K.; Molgaard, J. P. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? J. Sci. Food Agric. 2001, 81, 924–931.
- (4) Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915–7922.
- (5) Rubin, B.; Penner, D.; Saettler, A. W. Induction of isoflavonoid production in *Phaseolus vulgaris* L. leaves by ozone, sulfur dioxide and herbicide stress. *Environ. Toxicol. Chem.* **1983**, 2, 295–306.
- (6) Sweeney, J. P.; Marsh, A. C. Effects of selected herbicides on provitamin A content of vegetables. J. Agric. Food Chem. 1971, 19, 854–856.
- (7) Lotter, D. W. Reduced root damage in organically managed *Phylloxera*-infested vineyards in California. *Proceedings of the* 6th International Congress on Organic Viticulture; 2000; pp 183–191, http://www.soel.de/inhalte/publikationen/s\_77.pdf.
- (8) Robertson, G. P.; Paul, E. A.; Harwood, R. R. Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. *Science* 2000, 289, 1922– 1925.
- (9) Ames, B. N.; Profet, M.; Gold, L. S. Dietary pesticides (99.99% all natural). Proc. Natl. Acad. Sci. U.S.A. 1990, 87, 7777–7781.
- (10) Hurlbert, S. H. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **1984**, *No.* 54, 187–211.
- (11) Hakkinen, S.; Torronen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Res. Int.* 2000, *33*, 517–524.

Received for review April 6, 2003.

### Allan S. Felsot<sup>†</sup>

Food and Environmental Quality Laboratory, Washington State University, 2710 University Drive, Richland, Washington 99352

#### Joseph D. Rosen<sup>§</sup>

Professor of Food Science, Cook College, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901

#### JF0305332

 $<sup>^\</sup>dagger$  Telephone (509) 372-7365; fax (509) 372-7460; e-mail afelsot@ tricity.wsu.edu.

<sup>&</sup>lt;sup>§</sup> Telephone (732) 932-9611, ext. 229; e-mail jrosen@ aesop.rutgers.edu.

### 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

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 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 convention-ally 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 im-

mediately 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  $\sim 10-20$  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.

#### LITERATURE CITED

- (1) Felsot, A. S.; Rosen, J. D. Comment on comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using conventional, organic, and sustainable agricultural practices. *J. Agric. Food Chem.* 2003, 51, 147–150.
- (2) Asami, D. K.; Hong, Y.-J.; Barrett, D. M.; Mitchell, A. E. Comparison of the total phenolic and ascorbic acid contents of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J. Agric. Food Chem. 2003, 51, 1237–1241.
- (3) Ren, H.; Hideaki, E.; Hayashi, T. Antioxidative and antimutagenic activities and polyphenol content of pesticide-free and organically cultivated green vegetables using water-soluble chitosan as a soil modifier and leaf spray. J. Sci. Food Agric. 2001, 81, 1426–1432.
- (4) Carbonaro, M.; Mattera, M. Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams). *Food Chem.* **2001**, 72, 419–424.
- (5) 5. Woese, K.; Lange, D.; Boess, C.; Bogl, K. W. A comparison of organically and conventionally grown foods—Results of a review of the relevant literature. J. Sci. Food Agric. 1997, 74, 281–293.
- (6) Brandt, K.; Møgaard, J. P. Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? J. Sci. Food Agric. 2001, 81, 924–931.
- (7) Robertson, G. P.; Paul, E. A.; Harwood, R. R. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. *Science* 2000, 289, 1922– 1925.
- (8) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. In *Methods in Enzymol*ogy, Oxidant and Antioxidants (Part A); Packer, L., Ed.; Academic Press: San Diego, CA, 1999; Vol. 299, pp 152–178.
- (9) Haikkinen, S.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Res. Int.* 2000, *33*, 517–524.

Received for review July 18, 2003.

Alyson E. Mitchell<sup>†</sup> and Diane M. Barrett

Department of Food Science and Technology, University of California—Davis, One Shields Avenue, Davis, California 95616

JF030515K

 $<sup>^\</sup>dagger$  Telephone (530) 752-7926; fax (530) 752-4759; e-mail ae<br/>mitchell@ucdavis.edu.