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Rebuttal to Comment on Nutrients and Antioxidant Molecules in Yellow Plums (*Prunus domestica* L.) from Conventional and Organic Productions: A Comparative Study

Sir: The scientific purpose of our study was to monitor changes in the synthesis of bioactive molecules in organically grown plums versus conventionally grown plums in relation to agronomic practices. To reach this goal we planned a long-term trial (three harvest years) on experimental fields ad hoc cultivated; this allowed us to have both organic and conventional fruits harvested in the same pedo-climatic conditions. On these bases we monitored the plums during three consecutive years to "capture" a behavior, if any.

Dr. Davis raises questions about the statistical analysis performed in our work. Essentially, Dr. Davis maintains that we obtained *p* values that are too low because we used an incorrect sample size ("using an inflated value for *n*"). Davis interprets that we analyzed the annual means of the measurements, rather than individual measurements, and thus we overstated the sample size. Davis's critique is also related to the quality of analytical data ("triplicate analyses (instrumental replications) within each year served to assess instrumental variability and, when averaged within each year, improved the accuracy of measured concentrations for each year").

It is plain that the "sampling" is the crux of the comment, so a better description of it should help to clarify any doubt: Our working plan was to analyze plums grown in four orchards, one conventional orchard (tilled soil) and three organic orchards that utilize three different types of soil management (tilled soil, trifolium, and natural meadow). A sample of about 10 kg of plums for each cultivation type was delivered to the laboratory from the experimental fields (plums were picked randomly from trees by agronomists of the Experimental Institute of Fruitculture).

The primary objective in "sampling" is to collect food samples that are representative and to ensure that changes in composition do not take place between collection and analysis. Generally, to analyze food it is usual to constitute a pool. Therefore, equal amounts of noninjured plums (taken from the 10 kg of plums delivered to the laboratory) for each type of cultivation were pooled; we prepared a total of three pool/subsamples (see **Figure 1**) per cultivation type and than we froze them.

Each of the frozen pools (three per cultivation type) was utilized to analyze all of the nutrients and bioactive molecules included in the study. In each year we made at least three independent analyses (three pool, three analyses) per molecule per cultivation type. Therefore, we obtained for each molecule, in a given harvested year, three data points from three different homogenized masses of plum.

This means that after 3 years we had for each of the four cultivation types at least nine measurements for molecules (9 numbers across 3 harvest years) consisting of 3 replicates for 1999, 3 replicates for 2000, and 3 replicates for 2001. We had 9 independent observations per molecule, and we made statistical

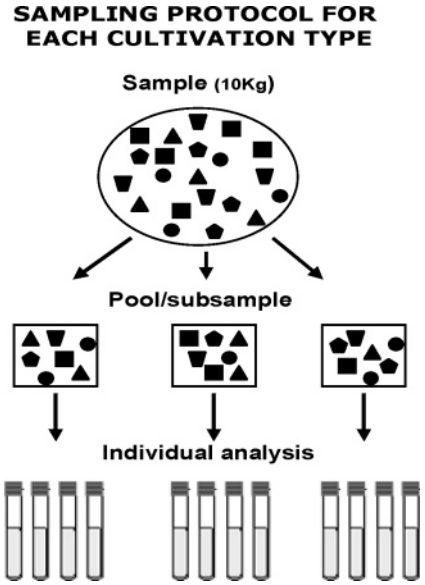

Figure 1. Different stages in "sampling" and analysis utilized in the study for each cultivation type.

analyses only at the end of the study (we never made "yearly means" as Dr. Davis supposes). Therefore, we think that the observation reported by Dr. Davis in his comment "in their *t* test they mistakenly proceed as though they had nine independent observations for each cultivation group" is wrong and that our statistical comparisons were calculated using a correct value for *n*. We had nine independent observations, and we did not have any reason to group our results into yearly means because we were not interested in evidencing seasonal effects.

We would like to make it clear again that our triplicate analyses did not derive from the same homogenized mass of plums but from different pools/subsamples of plums prepared per each cultivation type.

Furthermore, triplicate analyses made from different pools/subsamples of fruit do not assess "instrumental variability". To avoid further misunderstanding, we would like to explain it more fully: when we analyzed, for example, total flavonols, we took one sample from pool A, one sample from pool B, and one sample from pool C; this procedure does not assess instrumental variability but, from our point of view, fruit variability.

Concerning analytical data, quality is determined, in part, by the validity of analytical methods (Mangels, A. R.; Holden, J. M.; Beecher, G. R.; Forman, M. L.; Lanza, E. Carotenoid content of fruit and vegetables: an evaluation of analytical data. *J. Am. Diet. Assoc.* **1993**, *93*, 284−296). As reference for the production of analytical data we follow the guideline developed by Greenfield and Southgate (Greenfield, H.; Southgate, D. A.

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T. *Food Composition Data: Production, Management and Use*; Elsevier Applied Science: London, U.K., 2003).

The *instrumental variability* is assessed (e.g., in HPLC analysis) by the study of variability between injections from the *same vial* (e.g., we do this evaluation when we perform the system suitability test for the HPLC instrument). In contrast, the *precision of a method* of analysis is assessed by carrying out replicate analysis on the same sample (in our case *pool/subsample*; see **Figure 1**), which must be homogeneous and stable. The U.S. FDA's description of the *accuracy of a method* is "the degree of closeness of the determined value to the nominal or known *true value* under prescribed conditions. This is sometimes termed *trueness"* (FDA. *Bioanalytical Method Validation*; Washington, DC, May 2001, guidelines). The concept of a "true value" is, of course, hypothetical because the true value for a nutrient in a food is not known. The accuracy of a method is usually determined by the analysis of standard reference materials (SRMs) or certified reference materials (CRMs), as we briefly reported in our work under Minerals and Trace Elements. Unfortunately, the range of nutrients for which SRMs or CRMs are available is limited; therefore, the recovery studies of standards added to the foods are useful to estimate the accuracy (however, recovery studies do not provide unequivocal proof of the accuracy of a method because they assume that the added nutrient may be extracted with the same efficiency as the intrinsic nutrient (Wolf, W. R. Trace element analysis in food. In *Clinical, Biochemical and Nutritional Aspects of Trace Elements*; Prasad, A., Ed.; Alan R. Liss: New York, 1982; pp 427−446).

Concerning the ANOVA tests, we used ANOVA one-way to compare the three organic cultivations; the F values found were significant with $p \leq 0.001$. Furthermore, a Duncan's multiple-comparison test ($p = 0.05$) was applied; this post hoc test has been designed to counter the problems that arise with inflecting type I error when all possible pairs of means are compared.

The mistake highlighted on page 92, lines 67−68 (the substitution of conventionally with organically), was an error in the proof; the respective numbers presented in Table 1 for β-carotene content are correct.

Probably footnotes were not well written and can generate misunderstanding. Therefore, if footnotes are judged to be misleading, we suggest to change them in the following way: "Each value is $M \pm$ SD of nine measurements consisting of three replicates for each year from three different homogeneous pool/subsamples".

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