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

Sir: Lombardi-Boccia et al. (*1*) compared nutrient concentrations in yellow plums grown conventionally and organically. Unfortunately, apparently all of their statistical comparisons were calculated using an inflated value for *n*, the number of statistical observations or replications. As a result, most differences that were said to be statistically significant (values of *p* from <0.001 to <0.05) are not significant ($p > 0.05$).

The authors carried out their study for three harvest years. They measured nutrient contents as a "triplicate analysis for each year." In Tables 1−3 they reported their analytical results, described as follows: "values are the $M \pm$ SD of triplicates for each harvest year" (Table 1) and "values are the "$M \pm$ SD of three harvest years" (Tables 2 and 3). The 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. However, by collapsing their analytical data into yearly means (verified by correspondence), the authors also reduce their total *n* from the nine analytical observations to three more-reliable "yearly mean" observations. Thus, in the authors' *t*-tests used to make statistical comparisons between the *M* values for conventional and organic samples grown on tilled soil (Tables 1 and 2), the *n* associated with the SDs should be 3. However, the authors mistakenly proceeded as though they had 9 independent observations for each cultivation. I verified that the authors used $n = 9$ in their *t*-test calculations by recalculating *p* values using both $n = 3$ and $n = 9$ and by correspondence. All of the authors' (unreported) *t* values calculated with $n = 9$ were too large by a factor of $\sqrt{3} = 1.73$, and thus the published *p* values are too small, usually by substantial amounts. Also, four *p* values proved to be too small, even for $n = 9$ (e.g., for Zn and Mg, Table 1, conventional vs tilled organic).

The authors also reported values of $M \pm$ SD for two alternate forms of organic cultivation. They used analysis of variance (ANOVA) to test for differences among the *M* values of the three organic crops (Tables 1 and 3). Apparently the ANOVA and subsequent tests also used $n = 9$ for each cultivation group. Unlike the *t*-tests, the ANOVA results cannot be recalculated without access to unpublished data. Furthermore, the authors do not report what statistical method they used, after the ANOVA tests, to make pairwise comparisons among the three organic cultivations. However, any valid pairwise comparison among three values necessarily will control for the inflation of type-I error rates and result in fewer significant values than a pairwise comparison by *t*-test. As I will illustrate, in most cases even "overly optimistic" *t*-test comparisons using the appropriate $n = 3$ are sufficient to disprove the authors' reported statistically significant comparisons among the three organic samples.

The authors' Table 1 (corrected in ref *2*) shows $M \pm$ SD for 28 minerals, vitamins, and other nutrients. For these values, only a few statistical comparisons were reported, all in narrative form. The following corrections are needed on pages 91 (end) and 92:

1. "... the organically grown plums (tilled soil) were richest ($p < 0.001$) in K, Mg, and Zn compared to the conventional cultivation; Na and Cu were higher ($p < 0.05$) in the conventional cultivation ...." Actually, $p > 0.05$ for all five comparisons.

2. "... [for total fiber] significant differences ($p < 0.05$) were evidenced between the other two organically grown plums." Actual *p* values must exceed 0.16 (from a *t*-test of the most reliable difference, organic tilled vs organic trifolium).

3. "... [for total sugar] significant differences ($p < 0.05$) were observed among the three organic cultivations ...." All *p* values must exceed 0.10 (from a *t*-test of the most reliable difference, organic tilled vs organic meadow).

4. "β-carotene concentration in organically grown plums (tilled soil) was higher than that found in conventionally grown plums ...." Table 1 shows the opposite result, for which $p = 0.002$.

The noted superiority of conventional plums (on tilled soil) for α-tocopherol and vitamin K remains significant ($p = 0.04$ and 0.02, respectively). The superiority of organic-meadow cultivation for α-tocopherol is probably reliable, but the true *p* must be greater than the *t*-test value of 0.0004. For γ-tocopherol, the *t*-test value of $p = 0.011$ leaves some doubt whether the true *p* is <0.05.

The authors' Table 2 compares polyphenols in conventional and organic plums grown on tilled soil, based on *t*-tests. It includes 10 significant *p* values in two categories, $p < 0.001$ (6 cases) and $p < 0.05$ (4 cases). After correction for $n = 3$, five significant *p* values remain: $p < 0.001$ for quercetin and $p < 0.05$ for protocatechuic, ferulic, chlorogenic, and kaempferol. Corresponding changes apply to the discussion (p 92).

Table 3 compares the same polyphenols among the three organic cultivations. On the basis of ANOVA and unspecified pairwise tests, 24 of 30 possible comparisons were reported to have $p < 0.001$ (no other *p* levels are reported). However, pairwise *t*-tests are consistent with $p < 0.001$ for at most three comparisons (caffeic, trifolium vs meadow and tilled vs meadow; neo-chlorogenic, trifolium vs meadow).

Finally, it seems appropriate to note that when a large number of independent statistical comparisons are made, as in this study, a few are expected to appear significant by chance alone (1 in 20 at $p = 0.05$).

Further correspondence failed to resolve these statistical issues.

LITERATURE CITED

(1) Lombardi-Boccia, M.; Lucarini, M.; Lanzi, S.; Aguzzi, A.; Cappelloni, M. Nutrients and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic productions: a comparative study. *J. Agric. Food Chem.* **2004**, *52*, 90−94.

(2) Correction. *J. Agric. Food Chem.* **2006**, *54*, 3764.

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