# A Multivariate Study of the Correlation Between Tocopherol Content and Fatty Acid Composition in Vegetable Oils

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**ABSTRACT:** The main biochemical function of the tocopherols is believed to be the protection of polyunsaturated fatty acids (PUFA) against peroxidation. A critical question that must be asked in reference to this is whether there is a biochemical link between the tocopherol levels and the degree of unsaturation in vegetable oils, the main source of dietary PUFA and vitamin E. We used a mathematical approach in an effort to highlight some facts that might help address this question. Literature data on the relative composition of fatty acids (16:0, 16:1, 18:0, 18:1, 18:2, and 18:3) and the contents of tocopherols (α-, β-, δ-, and γ-tocopherol) in 101 oil samples, including 14 different botanical species, were analyzed by principal-component analysis and linear regression. There was a negative correlation between α- and γ-tocopherols ($r = 0.633$, $P < 0.05$). Results also showed a positive correlation between linoleic acid (18:2) and α-tocopherol ($r = 0.549$, $P < 0.05$) and suggested a positive correlation between linolenic acid (18:3) and γ-tocopherol.
*JAOCS 74,* 375–380 (1997).

**KEY WORDS:** Biosynthesis, fatty acid, multivariate statistics, principal-component analysis, tocopherol, vegetable oils.

Tocopherols are essential for protection of polyunsaturated fatty acids (PUFA) in plants and animals against oxidative deterioration. They exert their antioxidant effect by numerous biochemical and biophysical mechanisms, including scavenging active oxygen species and free radicals, and through action as efficient chain terminators in lipid autoxidation reactions (1). Vegetable oils are the major sources of dietary PUFA and tocopherol. However, differences in fatty acid composition and tocopherol levels among vegetable oils should be described to provide basic information for research on the use and investigation of dietary fat.

Consumers and edible-oil producers are concerned about the low oxidative stability of linoleic acid (18:2) and the possible health hazards of saturated fatty acids from palm oil and of *trans* fatty acids that result from hydrogenation of other unsaturated oils. Modification of fatty acid composition to improve the oxidative stability of vegetable oils has been the focus of work on sunflower, soybean, and rapeseed (2–4). The existence of a correlation between tocopherols and fatty acids in vegetable oils has been suggested (5) but not well established. It would be of great interest to determine if tocopherol content and fatty acid composition are linked in at least some vegetable oils.

Multivariate data analysis is a suitable approach to find underlying structures in complicated biological systems. One of the most powerful and widely used methods is principal-component analysis (PCA), which reduces the number of variables to a limited number of principal components (PC) (6,7). Unlike measured variables, PC are orthogonal, and thereby describe independent variation structures in the data. The first PC always explains the greatest part, and the following PC successively explain smaller parts of the original variance. The presence of significant PC indicates structure in the data. Graphic overviews, ideally showing a large part of the variance in two dimensions, of the objects and variables are obtained by score and loading plots, respectively.

In this paper, we used PCA to study the interrelation between the tocopherols and the fatty acids in vegetable oils. The results from this analysis are discussed in relation to available chemical and biochemical knowledge.

## MATERIALS AND METHODS

Data used in this study were collected from previous references (8–18) with the prerequisite that analyses for both fatty acid profiles and tocopherol levels were performed on the same oil sample. The data set included 14 vegetable oils: cashew nut (*Anacardium occidentale,* Anacardiaceae), cottonseed (*Gossypium hirsutum,* Malvaceae), groundnut (*Arachis hypogea,* Leguminosae), linseed (*Linum usitatissimum,* Linaceae), maize (*Zea mays,* Graminae), olive (*Olea europea,* Oleaceae), nigella seed (*Nigella sativa,* Ranunculaceae), niger seed (*Guizotia abyssinica,* Compositae), palm (*Elaes* spp., Palmae), perilla seed (*Perilla frutescens,* Labiatae), rapeseed (*Brassica napus,* Cruciferae), sesame (*Sesamum* spp., Pedaliaceae), soybean (*Glycine max,* Leguminosae), and sunflower (*Helianthus annuus,* Compositae). These data were given codes to simplify processing and presentation. The data were retabulated so that values of zero were entered for all analytes listed as traces or as not detected. Values for α-, β-, and γ-tocotrienols in maize and palm oil were given in one reference and are shown in the footnote of Table 1.

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**TABLE 1**
**Mean Values for Fatty Acid Composition (%) and Tocopherol Levels (ppm) in 14 Vegetable Oils[a]**

| | | | Fatty acids (% of total fatty acids) | | | | | | Tocopherols (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oil type | Abbreviation | Number | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | α-T | β-T | γ-T | δ-T | References |
| Sunflower | SF | 55 | 5.2 | 0.1 | 3.7 | 33.7 | 56.5 | 0.0 | 671 | 23 | 4 | 0 | 8–10 |
| Groundnut | GN | 1 | 11.2 | 0.0 | 3.6 | 41.1 | 35.5 | 0.1 | 141 | 4 | 131 | 9 | 8 |
| Soybean | SB | 2 | 10.1 | 0.0 | 4.3 | 22.3 | 53.7 | 8.1 | 116 | 17 | 578 | 263 | 8,11 |
| Cottonseed | CT | 1 | 23.0 | 0.0 | 2.3 | 15.6 | 55.6 | 0.3 | 403 | 2 | 383 | 4 | 8 |
| Maize[b] | MZ | 2 | 11.6 | 0.0 | 2.5 | 38.7 | 44.7 | 1.4 | 222 | 1 | 570 | 23 | 8,11 |
| Olive | OL | 6 | 13.8 | 1.4 | 2.8 | 71.6 | 9.0 | 1.0 | 96 | 6 | 12 | — | 8,11,12 |
| Palm[c] | PL | 2 | 44.8 | 0.0 | 4.6 | 38.9 | 9.5 | 0.4 | 377 | 1 | 4 | — | 8,11 |
| Rapeseed | RS | 1 | 4.6 | 0.3 | 1.7 | 60.1 | 21.4 | 11.4 | 180 | — | 340 | — | 10 |
| Linseed | LS | 1 | 5.6 | 0.0 | 3.2 | 17.7 | 15.7 | 57.8 | — | — | 588 | 6 | 11 |
| Sesame | SE | 10 | 9.6 | 0.2 | 6.7 | 41.1 | 41.2 | 0.7 | 4 | — | 584 | 9 | 13,14 |
| Cashew nut | CN | 8 | 11.6 | 0.3 | 8.9 | 61.5 | 17.1 | 0.0 | 51 | — | 618 | 41 | 15 |
| Niger seed | NS | 6 | 8.8 | 0.0 | 6.8 | 7.5 | 76.7 | 0.0 | 727 | 8 | 31 | — | 16 |
| Nigella seed | ST | 1 | 11.4 | 0.0 | 2.9 | 21.9 | 60.8 | 0.0 | 40 | 50 | 250 | — | 17 |
| Perilla seed | PS | 5 | 6.4 | 0.0 | 1.6 | 13.8 | 15.5 | 62.6 | 10 | — | 526 | 31 | 18 |

[a]Mean values were obtained from literature values reported in the references listed.
[b]Maize oil also contained α-tocotrienol, 54 ppm; β-tocotrienol, 11 ppm; and γ-tocotrienol, 62 ppm.
[c]Palm oil also contained α-tocotrienol, 52 ppm; β-tocotrienol, 4 ppm; and γ-tocotrienol, 132 ppm.

PCA was performed with the software package SIRIUS (Patten Recognition System A/S, Bergen, Norway). Linear regression analysis was performed with the statistical analysis tools in Microsoft Excel, version 5.0 (Microsoft, Redmond, WA).

## RESULTS AND DISCUSSION

*PCA classification of the oils.* PCA initially was applied to the original literature data that described the relative composition of fatty acids [palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3)] and the contents of tocopherols (α-, β-, δ-, and γ-tocopherol) in a total of 101 oil samples from 14 different botanical species. PC are linear combinations of the original variables and are determined so that the first PC explains the largest part of the total variance. This means that correlated variables are explained by the same PC and less correlated variables by different PC. In the present analysis, the two first PC explained 37.0 and 18.7%, respectively, of the total variance in the 10 variables. The resulting score plot provides a conceptual overview of the samples by showing a total of 55.7% of the variance (Fig. 1). Samples with similar values for the variables explained by the PC appeared close together, and those with large positive or negative scores are extremes in some variables. Samples of the same oil type were found clustered in groups in the score plot, indicating that variations within groups were generally smaller than variations between groups. Thus, average values for each oil type could be used in the following evaluations. The use of mean values increased the accuracy of the analysis for oil types represented by many samples and gave all oil types equal weight in the data analysis.

*Pattern of variation for fatty acids and tocopherols.* Mean values obtained for each oil type are presented in Table 1, where the different oils showed significant differences in fatty acids and tocopherol composition. In a second PCA, two PC that explained 27.0 and 23.9% of the variance, respectively, were extracted from the mean values in Table 1. The scores obtained (Fig. 2A) reflect similarities and differences between the oil types. For example, perilla seed and linseed can be expected to have more characteristics in common than perilla seed and niger seed. The position of a sample in the score plot is, of course, determined by the values for the measured variables. The relationships between those variables and the PC

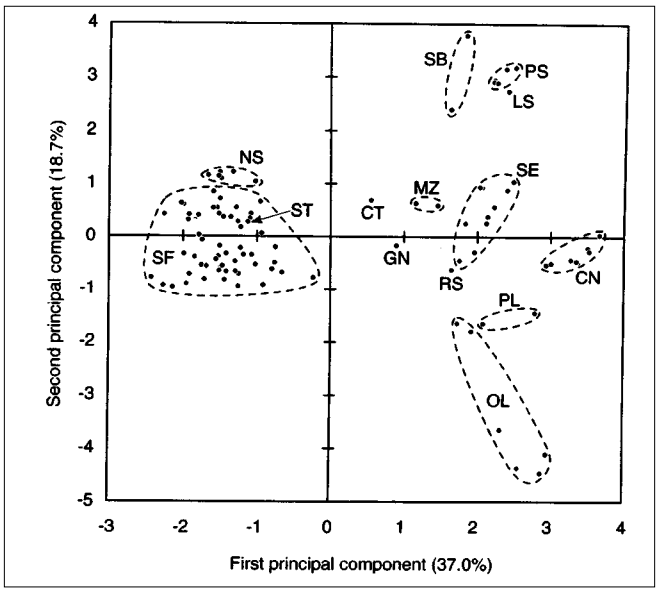
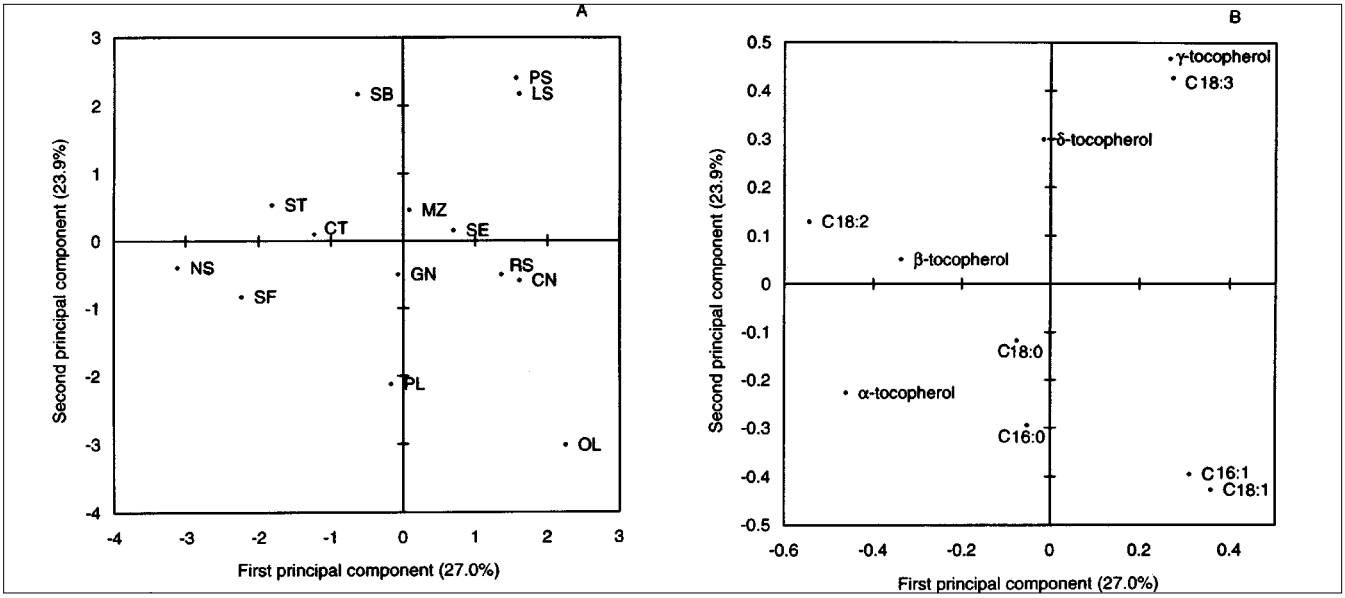



**FIG. 1.** Score plot of principal components 1 and 2 for 101 oil samples; NS, niger seed; ST, nigella seed; SF, sunflower; SB, soybean; PS, perilla seed; LS, linseed; MZ, maize; SE, sesame; CT, cottonseed; GN, groundnut; RS, rapeseed; PL, palm; CN, cashew nut; OL, olive.



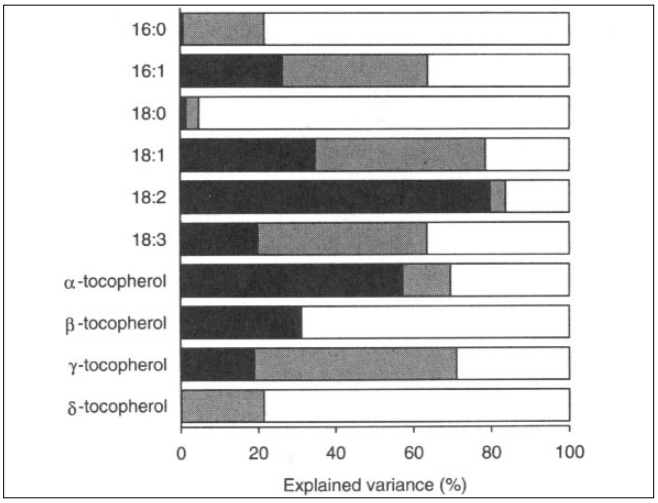




**FIG. 2.** Pattern of variation in fatty acid composition and tocopherol content for 14 different oil types: (A) Scores, (B) loadings, and (C) explained variance for the two first principal components (PC1, black bar; PC2, ruled bar; residual, white bar). See Figure 1 for abbreviations.

are defined as loadings. The pattern of covariation between the variables can be seen in the loading plot (Fig. 2B). Variables found in a similar direction and far from the origin are positively correlated, while those found at opposite sides of the plot are negatively correlated. For instance, the present loading plot indicated that γ-tocopherol was positively correlated to 18:3. However, the scores and the loadings only visualize the variance explained by the PC, and all variables are not equally explained (Fig. 2C). In this analysis, the saturated fatty acids (16:0 and 18:0) and β- and δ-tocopherol were substantially less explained than other variables and therefore had little influence on the model. The scores and loading plots can be interpreted together because objects with high scores for a specific PC also have high values for the variables with high loadings and low values for those with low loadings. Thus, the loading plot reveals that the similarity observed between perilla seed and linseed is due to their high contents of γ-tocopherol and 18:3. Olive oil is separated from the other oil types in the loading plot (Fig. 2B), and the possibility that this oil is an outlier is tested *vide infra*.

Olive oil is actually different from the other oils studied owing to its high level of monounsaturated fatty acids and relatively low total tocopherol level. Furthermore, it contains substantial amounts of chlorophyll and carotenoid pigments (19) as well as a number of phenolic compounds, e.g., tyrosol, hydroxy tyrosol, and caffeic acid (20). Plant phenolics are powerful inhibitors in lipid peroxidation *in vitro,* functioning both as chain-breaking and metal ion-chelating agents (21). Although the exact subcellular location of these phenolics is in doubt, plant secondary products are generally known to be located in the central vacuoles of the cells and not in the organelles that are subject to oxidation, such as the chloroplasts (22). Application of multivariate statistical techniques has shown that olive oil stability depends on both the tocopherol and phenolic compound contents (23).

The possibility that olive oil deviates from the other oils in

its tocopherol–fatty acid interrelation was tested by another PCA of the data in Table 1, with olive oil excluded. In this analysis (data not shown), the two first PC explained 30.0 and 22.7% of the variance, respectively, and the relative positions of the oil types in the score plot were retained. The loadings also showed a consistent pattern, and the influence by individual variables was similar to that for the previous model, although the tocopherols were less correlated to the second PC. The general trends illustrated by the previous PCA (Fig. 2) are thus valid and do not depend on the presence or absence of olive oil.

*Correlations between fatty acids and tocopherols*. Results showed that the pattern of variation for the fatty acids and tocopherols can be visualized by the loading plot obtained by PCA that includes all 14 different oils (Fig. 2B). This plot could serve as a basis for discussing the phenotypic linkages between the fatty acid composition and tocopherol contents of the oils studied. The plot showed that the saturated fatty acids (16:0 and 18:0) were not explained by the same PC as the tocopherols and were, thereby, likely to vary independent of tocopherols and other fatty acids. On the other hand, the two monounsaturated fatty acids (16:1 and 18:1) were positively correlated as confirmed by regression analysis ($r$ = 0.73, $P < 0.01$), and their position in the loading plot (Fig. 2B) indicates no correlation with other components. This plot also indicated a negative correlation between α- and γ-tocopherols ($r$ = 0.633, $P < 0.05$). α-Tocopherol and 18:2 were both highly explained by PC1 (Fig. 2C) and have both negative loading values for that PC. This indicates a positive correlation between them ($r$ = 0.549, $P < 0.05$). Also, 18:3 and γ-tocopherol were found close together in the upper right part of the loading plot (Fig. 2B), but the correlation between them was not statistically significant. This discrepancy between PCA and regression analysis is due to the fact that PCA selectively extracts the main covariance in the data while regression analysis uses all variance in each variable.

To confirm the findings from PCA and to obtain further insight into the correlations between tocopherols and fatty acids, 16:0, 18:1, 18:2, and 18:3 were plotted as a function of α- and γ-tocopherol contents (Fig. 3). The saturated fatty acids (e.g., 16:0), not explained by the PCA model (Fig. 2C), showed no obvious correlation with the tocopherols (Fig. 3A). The level of the monounsaturated fatty acid 18:1, found in a direction perpendicular to that representing the negative α-/γ-tocopherol correlation (Fig. 2B), also appeared to be independent of the tocopherol levels (Fig. 3B). Figure 3C

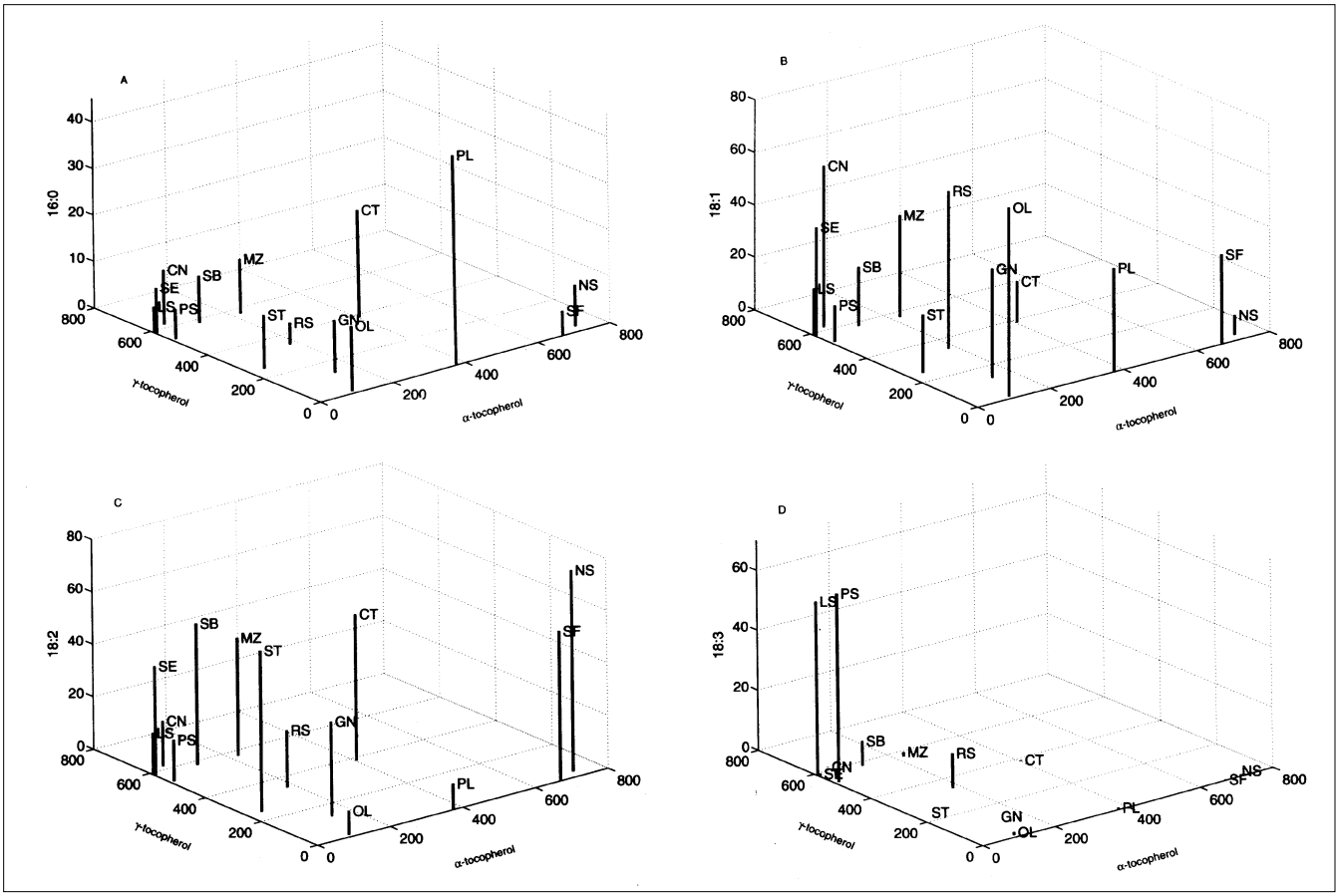



**FIG. 3.** Three-dimensional scatter plots of the correlation between α- and γ-tocopherols and (A) palmitic (16:0), (B) oleic (18:1), (C) linoleic (18:2), and (D) linolenic (18:3) acids for the 14 different oil types. See Figure 1 for abbreviations.

showed that the palm, soybean, nigella seed, and sesame oils deviate from the correlation between 18:2 and α-tocopherol found by both PCA and regression analysis. On the other hand, Figure 3D showed that the correlation between 18:3 and γ-tocopherol, previously indicated by PCA, was mainly due to the fact that linseed and perilla seed had much higher levels of 18:3 and, at the same time, contained high levels of γ-tocopherol compared with the other oil types.

So far in our discussion, we have tried to establish if there is any biochemical correlation between tocopherol contents and fatty acid percentages in 14 vegetable oils. Doing this, we realize that factors other than the degree of unsaturation, such as the nature of the lipids, lipid structure and the presence of anti- and/or prooxidants, can also influence the tocopherol levels. One such factor is the amount of chlorophyll and/or other photosensitizing pigments present in the oil. A typical example for such a case is olive oil. Although the total tocopherol level in olive oil is exceptionally low, α-tocopherol constitutes about 90% of that total, and its natural level varies from a few ppm up to 300 ppm (20). This may be due to the fact that 18:1, 18:2, and 18:3 oxidize at rate ratios of 1:1.7:2.3 during photosensitized oxidations (24) compared to rate ratios of 1:12:25 in normal autoxidation reactions (25). The fact that α-tocopherol is the most active tocopherol homologue in deactivating singlet oxygen (26) may explain why this form predominates in olive oil. Relevant to this observation is the fact that α-tocopherol is also known to be concentrated mainly in actively metabolizing tissues (e.g., cell chloroplasts), whereas the other tocopherols are more typical of dormant tissues and food reserves, such as in seed oils (27).

Regarding the correlation between fatty acids and tocopherols, both the tocopherol content and fatty acid composition in oil seeds are influenced by varietal differences, different stages of seed maturity at harvest as well as by the geographical and climatic conditions under which the oilseed is grown (28,29). In regard to the environmental influence, seeds from temperate regions are known to have a higher linoleic/oleic acid ratio (and thereby a higher degree of unsaturation) than those grown in warmer regions (30). Increases in temperature are also known to promote the biosynthesis of tocopherols (31), but whether the temperature has a selective effect on the relative amounts of the different tocopherols is not yet known.

The fact that a positive correlation between 18:3 and γ-tocopherol was observed in some of the vegetable oils analyzed in this study is interesting. It is generally believed that the main function of the tocopherols in biological systems is to act as antioxidant inhibitors of PUFA oxidation. However, whether α- or γ-tocopherol is the best antioxidant for vegetable oils is still a matter of debate (1). The effect from either of these tocopherols seems to depend not only on the degree of unsaturation of the oil but also on a multitude of chemical and physical factors that control oxidation. Chemically, α-tocopherol is more efficient than γ-tocopherol in scavenging free radicals, but it has the disadvantage of acting as a "prooxidant" under certain conditions. In plants, α-tocopherol is biosynthesized through intermediacy of β- or γ-tocopherol (32). The involvement of α-tocopherol in prooxidation (or co-oxidation) reactions may explain the finding that oils rich in 18:3 have low α-tocopherol levels.

An important question arising from the results obtained in this study is whether there is a genetic link between the fatty acid and tocopherol compositions of vegetable oils. This question is indeed difficult to answer because the data on the inheritance of tocopherol and fatty acid levels are limited. One study showed that the tocopherol composition in sunflower seeds is controlled by two nonallelic unlinked recessive genes, one controlling the α-/β-tocopherol ratio and the other controlling the α-/γ-tocopherol ratio (33,34), but similar reports are not available for the other oils. Another study on some soybean mutants showed that the genes that control 16:0 and 18:3 are inherited independently and that the levels of 18:1 and 18:2 are indirectly governed by these genes (35). It would be interesting to know whether the genes that determine the fatty acid composition of plant lipids are inherited dependently or independently of each other and whether they are linked to genes that control tocopherol levels.

By using statistical analyses of data from vegetable oils from 14 different plant species, we showed a positive correlation between linoleic acid (18:2) and α-tocopherol ($r = 0.549$, $P < 0.05$) and suggested a positive correlation between linolenic acid (18:3) and γ-tocopherol. Application of PCA or other multivariate techniques to data from different genotypes of the same species may provide additional information on whether tocopherol biosynthesis is genetically associated with PUFA biosynthesis within plant species.

## ACKNOWLEDGMENTS

We thank Dr. Sten Stymne (Department of Plant Breeding Research, SLU, Svalöv, Sweden) and Dr. Alexander Razdan from this department for kindly reading the manuscript and for useful suggestions.

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[Received July 11, 1996; accepted December 23, 1996]