# The Determination of Small Proportions of Linolenic Acid in Vegetable Oils by Alkaline Isomerization

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Brice and Swain [1] have devised a method for the accurate determination of small proportions of polyenoic fatty acids in vegetable oils based on the ultraviolet spectra of the oils before and after alkaline isomerization. The method, which includes corrections for background absorption, may be used on a variety of fatty acid mixtures containing conjugated and unconjugated dienoic and trienoic acids, but requires about 200 mg fatty acids for each analysis. The microisomerization method of Herb and Riemenschneider [2] has made it possible to use as little as 1 – 10 mg fatty acids. The present paper concerns the determination of small proportions of trienoic acid by the isomerization method of Herb and Riemenschneider [2] and the calculation method of Brice and Swain [1].

*Reagents and methods* are described by Nørby [3]. *Symbols:* $k_{262}$, $k_{268}$, and $k_{274}$ are observed specific extinction coefficients $(E^{1\text{g/l}}_{1\text{cm}})$ before isomerization, the subscripts denoting the wave lengths. $k'_{262}$, $k'_{268}$, and $k'_{274}$ are the same coefficients after isomerization. $k_3$ is the specific extinction coefficient before isomerization at 268 mμ (maximum for triene conjugation) after correction for bakground absorption according to eqn. II of Brice and Swain [1]. $k'_3$ is the same coefficient after isomerization.

*Calculation of $k'_3$.* Two equations can be used for the calculation of $k'_3$:

$$k'_3 = k'_{268} - k_{268} \tag{I}$$

$$k'_3 = 4.1\,[k'_{268} - \tfrac{1}{2}(k'_{274} + k'_{262})] - k_3 \tag{II}$$

As $k'_3$ is the specific extinction coefficient of the sample after isomerization due to triene conjugation produced by the isomerization, eqn. I is used when the background absorption is not changed during isomerization. In this case $k'_{268} - k_{268}$ is the increase due to triene conjugation.

If, however, isomerization increases the background absorption, $k'_{268} - k_{268}$ will be higher than $k'_3$, and eqn. I can not be used. Brice and Swain [1] have shown that if the increased background absorption is linear in the region 262 – 274 mμ, eqn. II will give the correct value for $k'_3$ ($k_3$ is zero if no conjugated material is present before isomerization). The correction factor 4.1 ($= F_3$) was derived from experiments on isomerized pure linolenic acid as:

$$F_3 = 4.1 = \frac{k'_{268}}{k'_{268} - \frac{1}{2}(k'_{274} + k'_{262})} \tag{III}$$

From the paper of Brice and Swain [1] it is seen that $F_3$ is the total height of the triene peak at 268 mμ divided by the height of the peak taken with a straight line between the minima at 262 and 274 as a base line. For a given sample the denominator will not change if a linear background absorption is added and hence II will give the correct $k'_3$ in such cases.

When $k'_3$ is known, % triene is $1.105 \cdot k'_3$ (Herb and Riemenschneider [2]).

*Results. Determination of correction factor $F_3$ for the method of Herb and Riemenschneider* [2]. Different amounts of two samples of linolenic acid (Hormel Foundation) and soybean oil (containing 51.8 % diene and 7.67 % triene) were isomerized and $k'_{262}$, $k'_{268}$, and $k'_{274}$ determined. At the same time $k_{262}$, $k_{268}$, and $k_{274}$ were determined on unisomerized samples. As all samples analyzed showed some absorption before isomerization, the following expression, which is a modification of that of Brice and Swain [1] (III), was used for the calculation of $F_3$:

$$F_3 = \frac{k'_{268} - k_{268}}{k'_{268} - k_{268} - \frac{1}{2}[(k'_{274} - k_{274}) + (k'_{262} - k_{262})]} \tag{IV}$$

In IV $k' - k$ is the increase in specific extinction coefficient due to isomerization of pure linolenic acid.

The value 4.12 found for $F_3$ (Table 1) agrees well with 4.1 found by Brice and Swain [1] for their method. $F_3$ did not vary with sample size in the range indicated in Table 1.

*Determination of small amounts of triene.* In the first experiment (Table 2) the specific extinction coefficients before and after isomerization were determined for five samples of linoleic acid (Hormel Foundation)

*Table 1.* Determination of correction factor $F_3$ for the method of Herb and Riemenschneider.

| Sample | Sample weight mg | Number of determinations | $F_3$ | SD |
|---|---|---|---|---|
| Linolenic acid I | 2.3 – 3.5 | 9 | 4.124 | 0.012 |
| " " II | 0.08–12.2 | 22 | 4.112 | 0.013 |
| Soybean oil | 1.8 –40 | 19 | 4.123 | 0.018 |

*Table 2.* Determination of small amounts of triene calculated according to equation II and I.

|  | Sample weight | | % triene | | |
|---|---|---|---|---|---|
| Sample | mg | % diene | eqn. II | eqn. I | $F_3$ |
| Linoleic acid concentrate | 1.16 | 99.2 | 1.18 | 2.19 | 7.47 |
| " " " | 1.86 | 99.4 | 1.09 | 1.85 | 6.82 |
| " " " | 2.61 | 99.2 | 1.07 | 1.21 | 4.68 |
| " " " | 4.39 | 97.2 | 1.20 | 1.44 | 4.91 |
| " " " | 9.04 | 98.1 | 1.20 | 1.48 | 5.06 |
| Sunflower seed oil | 4.5 | 58.2 | 0.13 | 0.27 | 8.36 |
| " " " | 4.7 | 58.9 | 0.11 | 0.27 | 8.00 |
| " " " | 5.6 | 60.0 | 0.09 | 0.37 | 13.60 |
| " " " | 9.4 | 59.2 | 0.10 | 0.27 | 11.04 |
| " " " | 17.8 | 56.5 | 0.10 | 0.25 | 10.81 |

*Table 3.* Determination of triene in mixtures of linoleic or linolenic acid and soybean oil after calculation of $k'_3$ by eqn. I or II (see text).

|  | Sample weight | Calculated composition | | % triene found | | Recovery % | | |
|---|---|---|---|---|---|---|---|---|
| Sample | mg | % diene | % triene | eqn. II | eqn. I | eqn. II | eqn. I | $F_3^{\text{sample}}$ |
| Soybeen oil | 4.3 | 88.5 | 2.59 | 2.55 | 3.03 | 98.5 | 117.0 | 4.94 |
| + linoleic | 3.0 | 82.4 | 3.44 | 3.40 | 3.91 | 98.8 | 113.7 | 4.78 |
| acid | 2.6 | 77.1 | 4.14 | 4.17 | 4.49 | 100.7 | 108.5 | 4.46 |
| Soybean oil | 8.9 | 47.5 | 15.2 | 15.6 | 15.3 | 102.6 | 100.7 | 4.05 |
| + linolenic | 7.6 | 39.7 | 28.9 | 28.6 | 29.0 | 99.6 | 100.3 | 4.15 |
| acid | 5.5 | 24.5 | 55.6 | 54.6 | 55.6 | 98.2 | 100.0 | 4.19 |

*Table 4.* $k'_{233}$ $k'_{268}$ and $F_3^{\text{sample}}$ for all experiments mentioned in the present paper.

| Sample | $k'_{233}$ | $k'_{268}$ | $k'_{233}/k'_{268}$ | $F_3^{\text{sample}}$ |
|---|---|---|---|---|
| Sunflower seed oil | ca. 54 | 0.27–0.39 | 142–199 | 5.82–13.6 |
| Linoleic acid | ca. 90 | 1.4 –1.8 | 50–64 | 4.7 –11.3 |
| Soybean oil + linoleic acid | 72–82 | 2.8 –4.2 | 17–29 | 4.4 – 4.9 |
| Soybean oil | 51 | 7 | 7.3 | 4.12 |
| Soybean oil + linolenic acid | 49–52 | 14–63 | 0.8–3.7 | 4.18 |
| Linolenic acid | 46 | 92 | 0.5 | 4.11 |

and sunflower seed oil. The content of triene was determined after calculation of $k'_3$ by eqn. I or eqn. II and $F_3^{\text{sample}}$ was calculated by eqn. IV. It is seen that $F_3^{\text{sample}}$ is consistently higher than 4.12 indicating that a background absorption has developed during isomerization. In accordance with this, calculation of $k'_3$ by eqn. I gives higher values than by eqn. II.

In order to determine whether the use of eqn. II, when $F_3^{\text{sample}}$ is higher than 4.12, will give the *correct* value for % triene, a number of recovery experiments were carried out. Known mixtures of soybean oil and linoleic or linolenic acid were isomerized and % triene determined after calculation of $k'_3$ by eqn. I or II. $F_3^{\text{sample}}$ was calculated by eqn. IV. The results in Table 3 confirm, that when $F_3^{\text{sample}}$ is higher than 4.12, as seen in the isomerized mixtures of soybean oil and linoleic acid, calculation of $k'_3$ by eqn. II gives the correct value for % triene.

In conclusion it can be said that the value of $F_3^{\text{sample}}$ will indicate which of the eqns. I or II will give the correct value for $k'_3$ and thereby the correct value for the triene content of the sample. If $F_3^{\text{sample}}$ is higher than 4.12 eqn. II should be used, in all other cases eqn. I will give the correct $k'_3$.

In an attempt to standardize the spectrophotometric determination of polyenoic fatty acids and to simplify the calculations involved, Brice *et al.*[4] have recommended the use of eqn. II only when $k'_{268}$ is lower than 1. In Table 4 the values for $k'_{233}$ $k'_{268}$, $k'_{233}/k'_{268}$ and $F_3^{\text{sample}}$ found in the experiments described in this paper are collected. It is clearly seen that in several experiments, in which $k'_{268}$ is greater than 1, eqn. II must be used since $F_3^{\text{sample}}$ is higher than 4.12. From Table 4 it furthermore appears that if the ratio $k'_{233}/k'_{268}$ is higher than 7, $F_3^{\text{sample}}$ is higher than 4.12. Inspection of spectral curves for isomerized samples of this type clearly shows that the diene peak, with maximum at 233 mμ, contributes significantly to the absorption in the region 262—274 mμ. As this "tail absorption" is approximately linear, eqn. II will give the true $k'_3$ for these samples.

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2. Herb, S. F. and Riemenschneider, R. W. *Anal. Chem.* **25** (1953) 953.
3. Nørby, J. G. *Acta Chem. Scand. To be published.*
4. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L. and Riemenschneider, R. W. *J. Am. Oil. Chemists' Soc.* **29** (1952) 279.

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# Studies on Rennin

## IV. Chromatographic Fractionation of Rennin

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In previous papers of this series rennin was considered a rather homogeneous protein. Only one peak was observed on moving boundary electrophoresis at pH 5.8, and in a solubility test the enzyme behaved nearly as a single-component system (Foltmann [1,2]).

On the other hand, Ernstrom[3] reported that although rennin only showed one peak on electrophoresis in buffers of ionic strength 0.1—0.2, it would split up in 3—4 peaks on moving boundary electrophoresis in buffers of ionic strength 0.033 and pH 6.8. In a personal communication to the author, Ernstrom added that on zone-electrophoresis in buffers of ionic strength 0.033 he had observed a slight displacement of enzymatic activity as compared with optical density; however, no separation into discrete peaks was achieved by such zone-electrophoresis.

Jirgensons *et al.*[4] have reported chromatography of a commercial crystalline rennin on diethylaminoethylcellulose. They found that the rennin could be fractionated into one inactive and two active fractions. However, these experiments were carried out at pH 6.7, which is somewhat above the stability range of the enzyme (Foltmann [1]).

The present communication deals with fractionation at a more appropriate pH of rennin by chromatography on diethylaminoethylcellulose (DEAE-cellulose) as described by Peterson and Sober[5]. The ion-exchange cellulose used was a commercial