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Formation of 2-acetoxyethanol in cocoa beans and cocoa powder during treatment with ethylene oxide

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In 1965, *Wesley* et al. (1) reported the presence of toxic ethylene chlorohydrin in foodstuffs which had been fumigated with ethylene oxide for sterilization purposes. Since that time, several publications were issued and the following residues were identified: ethylene bromohydrin by *Scudamore* and *Heuser* (3) in foods already fumigated with methylbromide and N-hydroxyethylisoleucine by *G. Schlögel* (4).

In our laboratory, raw materials are routinely examined for residues of ethylene chlorohydrin. This work has recently been extended to the search for other reaction products of ethylene oxide with normal food constituents.

During an investigation concerning the chemical changes provoked in cocoa powder during ethylene oxide fumigation, we observed a considerable amount

of a reaction product that was present in the volatile fraction of this commodity. The same phenomenon was observed when whole cocoa beans were subjected to a small scale ethylene oxide treatment in our laboratory. The formation of this compound was accompanied by a decrease in the volatile acid content of the cocoa products. Knowing that acetic acid is one of the principal volatiles present in these commodities, we postulated that ethylene oxide reacted with this acid to form 2-acetoxyethanol, also known as ethylene glycol monoacetate (EGMA).

Identity of this compound was confirmed by coincidence of retention times on different gas chromatographic columns with an authentic sample of the compound, by reduction of the amount of acetic acid with simultaneous formation of EGMA in a steam distillate of untreated cocoa powder upon addition of ethylene oxide, and by its rapid decomposition on treatment with alkali.

We learned from model studies with pure chemicals that equimolecular weights of ethylene oxide and acetic acid yielded EGMA at room temperature. It was also obtained by treatment of ethylene glycol with acetic acid under the same conditions. However, in this case the reaction proceeded with time to form ethylene glycol diacetate.

Ethylene oxide was also found capable of withdrawing acetic acid from aqueous solutions of acetates. A few drops of ethylene oxide added to a 1 per cent aqueous solution of magnesium acetate readily yielded EGMA, causing magnesium hydroxide to precipitate. This reaction can be explained by the pseudo-basic activity of ethylene oxide. It is, therefore, not excluded that the formation of EGMA could also occur in neutral or slightly alkaline cocoa powders. This possibility should be investigated.

Ethylene glycol monoacetate is a rather simple compound, but there is little information available about its chemical, physical and toxicological properties. Most manuals do not mention it, but the Merck index (1960) lists the following properties: colourless liquid, miscible with water and ethanol, density 1,108, boiling point 182 ° C, LD₅₀ in mice 1,45 g/kg. The very high acute lethal dose indicates that EGMA residues may be considered harmless or at least tolerable.

It should be pointed out that the amount of EGMA formed during ethylene oxide treatment of cocoa powder far exceeds that of ethylene chlorohydrin. Even excessively fumigated cocoa powders seldom contain more than 50 ppm of the latter compound and this low amount disappears quite rapidly during storage. On the other hand, EGMA levels found in fumigated cocoa powders from different origin ranged from 200—700 ppm and these quantities do not seem to decrease with time. In fact, EGMA was readily detected in samples which had been stored at room temperature for several years.

The presence of EGMA in cocoa beans or cocoa powder can be considered as an infallible sign that these commodities have been subjected to ethylene oxide treatment.

The determination of EGMA can be carried out by cold extraction of 5 g of the finely divided sample with 25 ml of acetonitrile-water 5 : 1, v/v according to the procedure for residues of fumigants as described by *Heuser and Scudamore*

(2). Gas chromatographic analysis of the extract can be performed on a 5 ft \times $\frac{1}{8}$ inch column with a packing of Chromosorb W, impregnated with 10% Carbowax 1540. At a column temperature of 110 °C with a carrier gas flow of 30 ml nitrogen per minute, EGMA is sharply separated from the cocoa aroma compounds which are present as co-extractives. When using a flame ionisation detector the minimum detectable amount is approximately 50 nanograms. The retention time of EGMA is 4,5 times that of ethylene chlorohydrin. The latter compound is eluted in approximately three minutes.

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Neuere Methoden der Analytik von Tocopherolen (Eine Literaturrecherche)

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1. Einleitung

Das ständige Anwachsen der Literatur über das Vitamin E zeugt von seiner zunehmenden Bedeutung, sei es als Vitamin selbst oder als Antioxydans in der Ernährung von Mensch und Tier. Die vorliegende Literaturzusammenstellung ist keineswegs vollständig, obschon nur über die Analytik berichtet werden soll und Arbeiten vor 1966 nicht berücksichtigt wurden. Gegenstand einer weiteren Arbeit wird es sein, eine konzentrierte Uebersicht über die Rolle des Vitamins E in der Tierernährung zu geben.

2. Zur Chemie des Vitamins E

In der Natur wurden bis heute (1971) 8 verschiedene Tocopherole gefunden, die in der Folge der Kürze wegen mit den Vulgärausdrücken bezeichnet werden. Sie leiten sich ab entweder vom Tocol = 2-Methyl-2-phythyl-chroman-6-ol: