We thought that the differences noted in the cloud-o-grams and cloud points of the materials containing the same average moles of ethylene oxide might lie in the distribution of moles around the average. Therefore, samples were made by combining samples of different average moles to contain an average of 11.5 ±0.2 moles of ethylene oxide and were processed through this procedure. Figure 5 is a graphic representation of the distribution of the moles of ethylene oxide of these combinations based on the Poisson Distribution of each component. Figure 6 illustrates the cloud-o-grams and cloud points obtained from these combinations.

As illustrated, we have a preponderance of low molecular weight material (8 moles) but enough of the higher weights to average out at the desired level. However, the cloud points obtained are not what one expects of 11.5 mole material, but more like that of a 9 or 10 mole material. Although the average moles are nearly equal, the cloud-o-grams reflect the differences in the distribution around the average.

Additional confirmation of the effect of the mole distribution around the average was obtained by plotting the data of Mayhew and Hyatt (3) on arithmetical probability paper.

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Solidification of Unsaturated/Saturated Fatty Acid Mixtures and Its Relationship to Chilling Sensitivity in Plants

JAMES M. LYONS and CRAIG M. ASMUNDSON
Department of Vegetable Crops, University of California, Riverside

Abstract

Freezing points of mixtures of palmitic and linoleic, or palmitic and linolenic acids, the predominant fatty acids in plants, decrease slowly as the unsaturated fatty acid is increased to 60 mole %. Beyond this per cent the freezing point is depressed quite markedly by each addition of unsaturated fatty acid. Linoleic and linolenic acids have similar effects on the freezing points of the mixtures until about 82 mole % unsaturated fatty acid. Differences of less than 5% in the amount of unsaturated fatty acid have a marked effect on the freezing point of mixtures at the approximate composition of fatty acid in plant membrane lipids.

Introduction

LIPIDS MAY PLAY an important role in chilling injury of plants (injury which occurs in some plant species at temperatures below about 10C but above freezing) (4,6,7); however, it has not been possible to relate lipid composition to the basic cause of chilling injury until concepts were reported relating the role of lipids in membrane systems at a subcellular level (2). Recent work (6) has demonstrated a relationship between fatty acid composition of mitochondrial membranes and sensitivity toward chilling of species from which the mitochondria were derived. In general, species which were sensitive to chilling temperatures possessed mitochondria with a higher amount of saturated fatty acids in their mitochondrial membranes than chilling-resistant species. In the work of Wheaton (12), fatty acid compositions of phospholipids extracted from root tissues of a number of plant species were surveyed and a tendency toward the sensitive species having the greater amount of saturation existed. Correlation of sensitivity to chilling and fatty acid composition was not precise, and it appeared that taxonomic relationships were equally important. A relationship of the amount of unsaturation of fatty acids in mitochondrial membranes to cold sensitivity was more clearly demonstrated in animal studies where it has been shown that mitochondria derived from warm-blooded animals possessed more saturated fatty acids than those derived from cold-blooded animals (8,9). These studies indicated that the mitochondria from warm-blooded animals with the more saturated fatty acids could not maintain their flexibility at lower temperatures, implying a disruption of metabolism and energy supply, while those from cold-blooded animals with more unsaturated fatty acids could maintain their flexibility at lower temperatures.

In evaluating the physical characteristics of fatty acids in relationship to their influence on membrane properties, it is known that chain length, number of double bonds, and position of the double bonds, all play a role in determining the physical properties of the lipid. The predominant fatty acids in nongreen plant tissues are palmitic (28%), linoleic (40%), and linolenic (21%), with lesser amounts of stearic (3%) and oleic (5%), and others in trace amounts (12). Assessment of the role of these fatty acids in relation to the physical properties of membrane lipid raises the question as to whether the difference between two double bonds in linoleic and three double bonds in linolenic can significantly alter the freezing point or solidification of the membrane lipid. Since the double bond index (the summation of weight % of each acid in a mixture multiplied by the number of double bonds it contains per molecule, divided by 100) has been used to classify the degree of unsaturation in a lipid, it is important to evaluate this point critically, because a small change in the distribution of either linoleic or linolenic can markedly alter the double bond index. A freezing point curve for mixtures of palmitic (the saturated fatty acid of major importance in plant material) and oleic is available in the literature (10). It seemed of importance to prepare diagrams for palmitic and linoleic, or palmitic and linolenic so that the role of these mixtures may be adequately considered.

Materials and Methods

Fatty Acids

Purified samples of palmitic, linoleic, and linolenic acids were obtained from Applied Sciences Laboratories, State College, Pennsylvania. These fatty acids were gas chromatographically homogeneous and 99+ % pure. Mixtures were made by placing 10 mg of one fatty acid in a sample tube and adding small increments of the second fatty acid until the desired mixture was obtained. Liquid fatty acids were weighed and transferred in a tared 50 μl syringe. To insure complete mixing, the total sample in the tube was dissolved in redistilled reagent anhydrous ether, and the ether removed under a stream of prepurifid nitrogen in a water bath maintained at a temperature slightly above the melting point of the mixture.

Freezing Point Determination

An apparatus was assembled to determine the freezing point with a thermocouple consisting of a platinum vs. a 13% rhodiumplatinum double junction, with the cold junction maintained at the melting point of water. Conditions were selected such that 0C registered on the baseline of a Brown 1 mv full scale recorder, with a switch included to make it possible to change the polarity for measurement of potentials below 0C. The cooling curves of suitably melting pure compounds were determined to calibrate the apparatus. The freezing point of mixtures could be determined with this method to a sensitivity of 0.1C. In order to control the cooling rate, the sample and probe were placed in small vacuum Dewar tubes. Repeated runs of the freezing point curves were then made in order to determine a reliable freezing point.

Results and Discussion

The freezing points of these mixtures decrease slowly as the mole % of unsaturated fatty acid is increased, until around 60 mole % is reached. As the amount of unsaturated fatty acid is increased beyond 60 mole %, the freezing point is depressed quite markedly by each increment of unsaturated fatty acid. By about 82 mole %, the freezing point is depressed below 0C with essentially no difference observed up to this point whether linoleic or linolenic is used as the unsaturated acid. As the amount of unsaturated acid is increased beyond the eutectic point, the linoleic mixture does not have as low a freezing point as the linolenic mixture, but otherwise the curves are similar. The curve indicating the freezing points of palmitic and oleic reported by Smith (10) is shown in Figure 1 for comparative purposes.

Since the difference in the number of double bonds between linoleic or linolenic acids does not influence the freezing point of these mixtures in a temperature range applicable to plant physiology, representation of the amount of unsaturation in these lipids by the double bond index is not meaningful and gives too much weight to the linolenic content. Rather, the unsaturated acids (of which linoleic and linolenic are reported to contribute over 90% in plant membranes and phospholipids) should be considered equally regardless of the number of double bonds and a more accurate measure is that provided by a ratio of the mole % of all unsaturated to saturated fatty acids. In contrast, the double bond index can be used with more reliability when considering animal fatty acids since their composition is more complex (8,9).

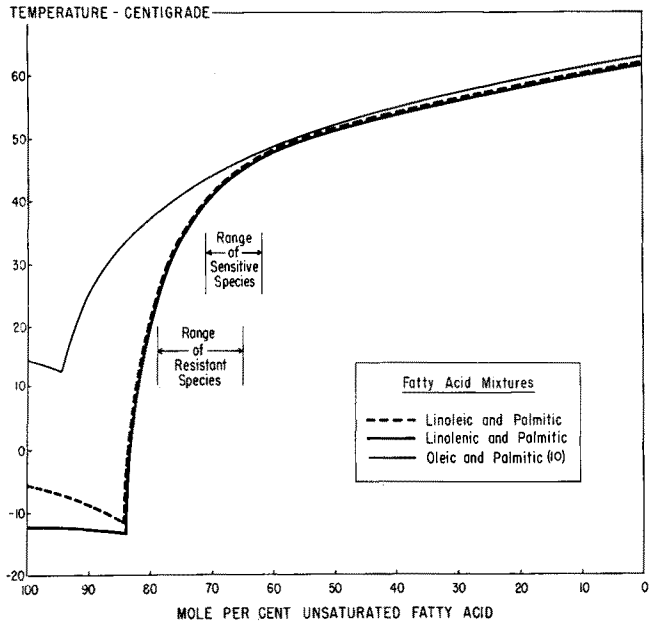

FIG. 1. Phase diagrams showing the freezing point of mixtures of palmitic and linoleic and palmitic and linolenic fatty acids.

The data of Wheaton (12) presenting the fatty acid composition of root phospholipids from 9 plant species sensitive to chilling temperatures and 11 species resistant to chilling temperatures, and of Lyons et al. (6) of the fatty acid composition of mitochondrial membranes derived from roots, shoots, fruits, and buds of 4 sensitive and 3 resistant species are calculated as a ratio of the mole % total unsaturated to saturated fatty acids and shown in Figure 2. Correlation of the ratio of mole % unsaturated/saturated fatty acids with chilling sensitivity is not precise; however, there is an obvious distribution which indicates the sensitive species tend to have more saturated fatty acids than the resistant species.

These data were also calculated as mole % unsaturated fatty acid and the distribution of this percentage for both sensitive and resistant species is

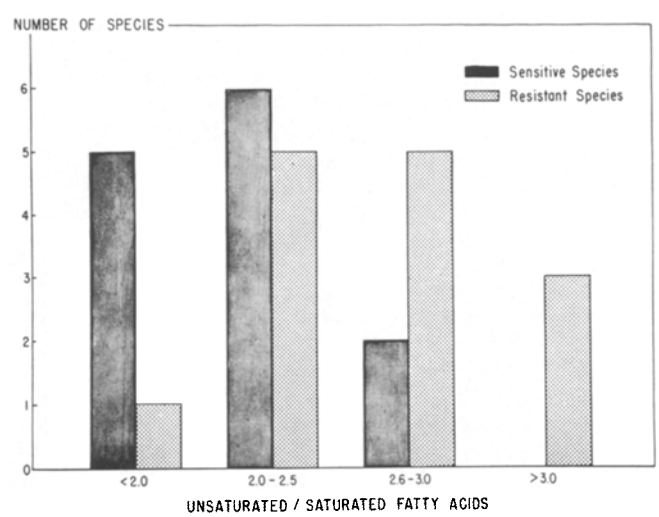

FIG. 2. Bar graphs indicating the distribution of the ratio of the mole % of unsaturated/saturated fatty acids from the phospholipids of 13 plant species sensitive and 14 resistant to chilling injury. Data from Wheaton (12) and Lyons et al. (6).

indicated in Figure 1. The overlap between the sensitive and resistant species is readily apparent but it is also of interest to observe that the distribution between the saturated and unsaturated fatty acids of these species occur at the critical composition where a small increment of added unsaturated fatty acid has a marked effect on the freezing point of that mixture. Freezing points were not determined for 3 or 4 component mixtures to evaluate the influence of oleic acid on mixtures of palmitic, linoleic, and linolenic. Oleic acid would certainly raise the freezing point of a mixture in proportion to the amount present, however, it occurs in relatively small amounts in the membrane and phospholipid fractions.

Several hypotheses have been presented to explain the mechanism of injury to chilling-sensitive plants including disorganization of protein molecules, disruption of enzymatic activity, accumulation of toxins, altered membrane permeability, and physical changes in lipids (1,4). None of these hypotheses has been proven or disproven, but with increased knowledge on composition and function of subcellular membranes additional support is placed on the hypotheses relating to physical changes in lipids. Luzzati and Husson (5) indicated that the physical condition of a lipoprotein complex such as a cell membrane is on the borderline of a phase transition from a liquid-crystalline structure to a coagel. When one of the parameters is altered (such as temperature), the hydrocarbon chains crystallize, thus blocking some physiological activity of the lipid. Similarly, Byrne and Chapman (3) reported that when temperature is increased from a low temperature, the hydrocarbon moiety of a phospholipid begins to twist and flex until finally the melting of the chain occurs. In his discussion on the thermostability of proteins, Ushakov (11) points out that cellular proteins are complexed with other protoplasmic substances, such as lipids, and thermostability of these proteins is a function of the properties of the complexing substances as well as their own properties.

The phase diagrams presented here indicate that differences of less than 5 mole % in the amount of unsaturated fatty acid in a fatty acid mixture have a marked effect on the solidifaction of these mixtures. This effect could be of significance in determining the sensitivity to chilling of a given plant species.

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Gas-Solid Chromatography of Hydrocarbons on Activated Alumina[1]

G. R. LIST, R. L. HOFFMANN and C. D. EVANS
Northern Regional Research Laboratory,[2] Peoria, Illinois

Abstract

Volatile hydrocarbons representative of those in autoxidizing fats were analyzed on a single column of activated alumina by gas-solid adsorption chromatography (GSC). Mixtures of C_1 to C_8 paraffins and α-olefins were readily separated from one another, and from several branched hydrocarbons in less than 40 min. Semilog plots of carbon number versus log retention time for these individual mixtures indicate that good separations may be expected when all components are present simultaneously. Alumina is a unique chromatographic substrate for these separations. Since no liquid phase is employed, wide temperature ranges may be applied, column bleeding is eliminated and the system becomes ideal for temperature programming even on single column instruments. This system of GSC offers a convenient and direct method for hydrocarbon analysis since more polar materials such as aldehydes, ketones and esters are irreversibly adsorbed on alumina. It shows promise not only for the analysis of volatiles in the flavor evaluation of edible oils, but also as an aid in solving many other food and biological problems.

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[2]A laboratory of the No. Utiliz. Res. and Dev. Div., ARS, USDA.

Introduction

ALIPHATIC HYDROCARBONS are becoming important factors in everwidening areas of biological and medical research. The need for their detection and identification is no longer limited to the field of synthetic chemicals. Pertinent areas of investigation are smog and air pollution studies (7), ripening and aging of fruit (4,5), fat autoxidation (8), enzyme and microbiological studies (17) and food storage and stability (6). In all these investigations trace amounts of hydrocarbons present must be rapidly identified and their concentrations determined. Other areas of application would be synthetic and structure investigations where high-temperature hydrogenation is used to obtain complete reduction and splitting of the compound to yield hydrocarbon fragments that are charactertistic of the original compound (1–3).

Alumina has been previously employed in gas chromatography as a support for liquid phase. McKenna and Idleman (16) have reported complete resolution of C_1 to C_4 paraffins and olefins on alumina coated with propylene carbonate. Early work by Greene et al. (9–10) showed that alumina, silica gel or a mixture of the two was effective for gas-solid adsorption chromatography (GSC) of atmospheric gases and light hydrocarbons. They reported good separation of homologues up to butadiene by temperature programming from +5 to 170C with an analysis time of approximately 1 hr. Investigations