LIPID OXIDATION OF MILK FAT IN FREEZE-DRIED CELLULOSE MODEL SYSTEMS OF VARYING WATER ACTIVITIES

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ABSTRACT

LIPID OXIDATION OF MILK FAT IN
FREEZE-DRIED CELLULOSE MODEL SYSTEMS
OF VARYING WATER ACTIVITIES

By

Sang Choul Han

The organoleptic deterioration of milk fat components in dehydrated food systems has been a serious problem from the standpoint of consumer acceptance.

The effects of several different water activities on autoxidation of milk fat in a freeze-dried microcrystalline cellulose model system were investigated using a peroxide test, a 2-thiobarbituric acid test and flavor panel evaluation. The results of autoxidation of milk fat were compared with the calculated BET monolayer value. The correlation of the 2-thiobarbituric acid test to the other tests are discussed.

Three different water activities, a_w 0.12, a_w 0.47 and a_w 0.75 of freeze-dried milk fat - microcrystalline cellulose model systems were prepared for these studies.

The calculated BET monolayer value of the model system was a_w 0.23 and 0.37% of moisture content, dry basis.

Sang Choul Han

In comparison of results of TBA to peroxide value and sensory evaluation during 2 months storage periods at 20 $\pm$ 0.5°C, the TBA test showed a poor correlation with the peroxide test and the sensory evaluation; the peroxide test proved to be the most applicable test for the autoxidation studies of the freeze-dried model systems for long term storage stability.

The effects of varying water activities on the autoxidation studies demonstrated that the model system a_w 0.47 (above BET monolayer value) demonstrated a protective effect, while the model system, a_w 0.12 and a_w 0.75 (below BET monolayer value and far above BET value) showed a prooxidant effects.

Possible reasons are suggested for the poor correlation of the TBA test results with those of the peroxide test and the sensory evaluation.

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INTRODUCTION

The milk of animals was used for human food before the dawn of history. Milk has long been referred to as one of the most nearly perfect foods. In the last half of this century the dairy industry has made remarkable progress in the technology of fluid and dehydrated foods. In recent years, research related to dry by-products has intensified, partially as a result of declining per-capita consumption of fluid milk in developed nations.

The milk lipids are recognized as a major factor in determining economic value as well as consumer acceptability of most dairy products. Many dehydrated dairy products have been produced: nonfat dry milk, dry cream, dry whole milk, ice cream mix, dehydrated butter, dehydrated cheese and coffee whiteners. These products offer great promise since their versatility, novelty and convenience of use have inherent consumer appeal. However, their mediocre storage stability has been a major obstacle to wider acceptance.

Initially, in the preparation of dehydrated foods, the objective was to produce a product as dry as possible. But for most food, it was found that for optimum stability a small amount of water must be left in the products.

The presence of a small amount of water content appears to be an extremely important factor in preventing or inhibiting fat oxidation.

Much research on lipid oxidation in various freeze-dried model systems has been done recently. Most of these studies have been based upon the method of oxygen uptake which uses rather purified unsaturated fatty acid in metal catalyzed conditions. However, the method of oxygen uptake provides good kinetic results in oxidation studies of lipids, but in complex food systems such as dairy products which contain large amounts of a low peroxide fatty acid constituent, it is not very applicable for the practical routine examinations for a long term storage stability. This is because the errors caused by the empirical oxygen uptake method are often greater than those detectable by chemical or sensory evaluation of oxidation of milk fat.

For practical purposes, the peroxide test, the 2-thiobarbituric acid test and sensory evaluation have been widely used in studies of organoleptic deterioration of high moisture content food systems. But in the case of dehydrated food systems which contained low peroxide fatty acid, the correlation of the TBA test to other tests such as the peroxide test and sensory evaluation have not been fully investigated.

The purpose of this research was to determine the effects of water activity on milk fat oxidation in a model system and also to find the correlation of TBA to other chemical and sensory tests.

LITERATURE REVIEW

Water Activity and Lipid Oxidation

One of the major problems in research on dehydrated food is the prevention of deleterious change in the products during storage. Removing water from a food by freeze dehydration results in a product that has a porous, sponge-like matrix which permits ready access of oxygen to the components of the food, thereby facilitating oxidation changes (Maloney et al., 1966).

Historically, in the preparation of dehydrated food, the objective was to produce a product as dry as possible. But for most foods (the exception being those high in sugar), it was found that a small amount of water must be left in the product. Without a residue of water, good stability generally cannot be attained. Moreover, water activity appears to be an extremely important factor in preventing or inhibiting lipid oxidation (Rockland, 1957; Salwin, 1963).

Definition of Water Activity

The availability of water for spore germination, microbial growth and chemical deterioration of food is closely

related to its relative vapor pressure (Scott, 1957), commonly designated as water activity. Water activity (a_w) is defined as the ratio of the vapor pressure (P) of water in the food to the vapor pressure of water (P_s) at the same temperature (Brockman, 1970). It can also be approximated as the mole fraction of water; that is, the moles of water divided by the sum of the moles of water and the moles of solute.

$$a_w = \frac{P}{P_s} = \frac{Nw}{Nw + Ns} = \frac{RH}{100}$$

where

P = water vapor pressure exerted by the food

P_s= vapor pressure of pure water at temperature T

T = equilibrium temperature of systems

Nw= moles of water

Ns= sum of mole fraction of all soluble constituents.

Moisture Adsorption Isotherm

Moisture adsorption isotherms have been used to calculate the Brunauer, Emmet and Teller (BET) monomolecular layer value, and they have been used to predict the optimum moisture content of numerous food (Rockland, 1969; Salwin, 1963).

The adsorption isotherm of a food is best described as a plot of the amount of water adsorbed as a function of the relative humidity or activity of the vapor space surrounding

the material all at a constant temperature. Many investigators (Taylor, 1961; Stitt, 1958; Rockland, 1960) have described the procedures for obtaining water vapor isotherms for food.

One general method is that the dehydrated food material is placed in a vacuum desiccator containing a specific saturated salt solution known to provide a definite equilibrium relative humidity necessary for determination of the isotherms.

The other method involves measurement of the vapor pressure of water in equilibrium with a food at given moisture content and temperature, using a sensitive manometric system or electric-hygrometer.

Theoretical Description of Isotherms

According to the theory of Brunauer et al. (1938), water is bound (strongly adsorbed) in a monomolecular layer in the region of the first slope of a moisture adsorption isotherm, up to point A from Figure 1. Above point A, in the linear section, bi- or multimolecular adsorption occurs. From about B on, water is condensed in capillaries with increasing water activity (Acker, 1969).

The Storage Stability of Food Products and Water Activity

The effect of water and water activity on lipid oxidation in foods has been studied in model systems by several

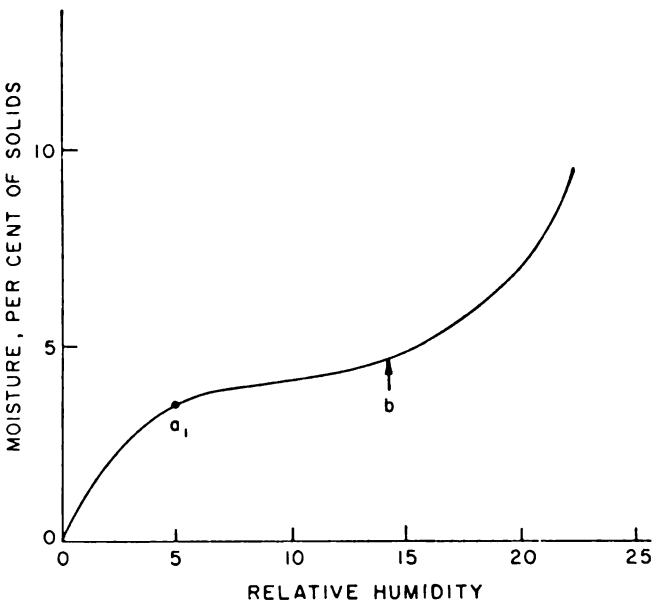


Figure 1. Moisture isotherm curve of food products.

investigators (Heidelbaugh et al., 1970; Labuza et al., 1966; Chou et al., 1973; Heidelbaugh et al., 1971). In general it was found that at very low water activities, lipid oxidation was relatively rapid. Addition of water up to a critical level reduced the rate of lipid oxidation. Salwin (1959, 1963) conjectured that the monolayer moisture acts as a film, protecting the food from attack by oxygen. Exceptions to this concept appear to be mainly food of high carbohydrate content.

Maloney and Karel (1966) reported that water had an inhibitory effect on the oxidation of a freeze-dried model system consisting of micro-crystalline cellulose and methyl linoleate, varying with water activity up to 0.5, and then at intermediate moisture levels, presumably a_w 0.5 to a_w 0.75, the lipid oxidation of model systems becomes accelerate (Labuza et al., 1969, 1970; Heidelbaugh et al., 1971). A series of related studies on the intermediate moisture range were carried out in an attempt to determine the rate of lipid oxidation. Labuza and Chou (1973) observed that in a non-swellable cellulose system at low metal content, the rate of oxidation increased as the a_w increased into the range of moisture that describes intermediate moisture foods. Labuza et al. (1970), concluded that with high water activity, where the water is not bound tightly, the moisture content of the system has a strong effect on control of

oxidation rate. At an extremely high trace metal content, 500 ppm, the oxidation rate pattern was completely reversed with the rate of oxidation being faster at the lower water content and lower water activity.

Hypotheses Explaining the Protective Effect of Water

Several hypotheses have been suggested to explain the protective effect of water in retarding lipid oxidation. The most important are:

1. That water has a protective effect due to retardation of oxygen diffusion (Halton and Fisher, 1937).
2. That water lowers the effectiveness of metal catalysts such as copper and iron (Uri, 1956).
3. That water is attached to sites on the surface thereby excluding oxygen from these sites (Salwin, 1959).
4. That water promotes non-enzymatic browning and browning can result in the formation of antioxidant compounds (Lea, 1958).
5. That water forms hydrogen bonds with hydroperoxide and retards hydroperoxide decomposition (Maloney, 1966).

Oxidation Mechanism of Milk Lipids

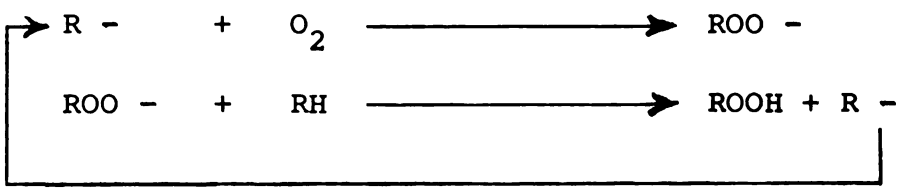
Theoretical Aspects of Autoxidations

The autoxidative deterioration of lipid containing foods has been a serious problem in the food industry because it leads to a decrease in organoleptical quality and nutritional value. A good understanding of the principal mechanism will be of value in examining the studies of the correlation between the peroxide test and the 2-thiobarbituric acid test in the autoxidized milk fat.

Badings (1960) and Holman et al. (1962), have presented a good review of the theoretical aspects of autoxidation processes in lipids.

The free radical chain reaction is a well-established explanation for the majority of autoxidation mechanisms. Peroxides are usually the primary products and the major general reactions are:

1. Initiation: formation of free radicals
2. Propagation: the free radical chain reactions



3. Termination: formation of non-reactive products with R-, ROO-, RO-, etc.

The rate of autoxidation is dependent on the energy required for the rupture of the α-methylene bond. The intermediately formed α-methylene radical is stabilized by resonance.

$$- \overset{*}{C}H - CH = CH - \longleftrightarrow - CH = CH - \overset{*}{C}H -$$

The two structures contributing to the resonance hybrid give rise to the formation of isomeric hydroperoxides. The reaction can be accelerated by pro-oxidant factors such as metal, free radicals, UV light, elevated temperature and moisture (which presumably increases mobility of pro-oxidants). On the other hand, autoxidation is inhibited by compounds which react with free radicals to form non-radical products.

The autoxidation pattern is often complicated by numerous free radical reactions such as:

a. Formation of epoxides
b. Polymerization
c. Formation of poly-peroxides
d. Formation of cyclic peroxides
e. Formation of secondary autoxidation products by dismutation of the hydroperoxides.

Dismutation of Hydroperoxides and Off-flavor Production

The hydroperoxides formed in the autoxidation of unsaturated fatty acid are usually not stable and will

decompose. The formation of these dismutation products is a serious problem in food products which contain lipids because of the formation of very objectionable off-flavor products such as short carbon chain aldehydes, ketones, alcohols and other volatile carbonyl compounds.

The Composition of Milk Lipid and Its Properties

Milk fat consists chiefly of triglycerides of fatty acid. The composition of cow's milk is presented in Table 1. The unsaturated acids are responsible for the

Table 1. The Composition of Milk Lipid*

Class of lipid	Range of occurrence %
Triglycerides of fatty acid	97-98
Di-glycerides of fatty acid	0.25-0.48
Mono-glycerides	0.016-0.038
Phospholipids	0.2-1.0

*Source: Byron H. Webb and Arnold H. Johnson, 1965.

development of oxidized flavor in the milk fat because of the lability of the carbons alpha to the unsaturated bonds.

The fatty acid composition of cow's milk fat is presented in Table 2.

As Tables 2 and 3 indicate, the major unsaturated fatty acid components are carbon chain 18 and the number of unsaturated double bonds are principally monoene and diene (oleic and linoleic acid).

Autoxidation of Saturated Fatty Acid of Milk Fat

From a practical standpoint, the breakdown of saturated fatty acids through oxidation can be ignored. Brodnitz (1968) observed that at 100°C the rate of saturated fat oxidation was about 100 times slower than for unsaturated linoleate.

Autoxidation of Oleic Acid

Oleic acid, which is a major unsaturated fatty acid of milk fat, has two alpha-methylene groups and these are the point of attack in the free radical chain reaction. Farmer et al. (1943); Ross et al. (1949); and Privett et al. (1953) have proved that four hydroperoxides are formed in oleic acid oxidation. Badings (1960) quoting from Horikx and Schogot (1959) observed that saturated aldehydes with a chain length of C_5 to C_{10} and the 2-enals with a chain length of C_6 to C_{11} are formed in the initial phase of the autoxidation of the fatty acid.

Table 2. The Fatty Acid Composition of Milk Fat

Fatty acid	Number of carbon atoms	Percentage of total fat
Butyric	4	3.7
Caproic	6	2.0
Caprylic	8	1.3
Capric	10	2.7
Lauric	12	4.0
Myristic	14	7.9
Palmitic	16	23.8
Stearic	18	10.7
Arachidonic*	20	0.5*
Oleic*	18	38.5*
Linoleic*	18	4.7*
Unsaturated*	20 to 22	0.4*

*Major unsaturated fatty acid.

Source: Hilditch and Thompson, 1936.

Table 3. Comparison of Unsaturated Fatty Acid in Mole Percent of Milk Fat (Total Unsaturated Fatty Acid Components)

Length of carbon chain	Jack and Henderson 1945	Hilditch and Longenecker 1938	Smith and Dastur 1938	Hilditch and Paul 1940
Iodine value	31.9	37.5	36.6	46.9
C_{10}	0.5	0.4	0.3	0.2
C_{12}	0.3	0.9	0.3	0.2
C_{14}	1.3	1.7	1.0	0.9
C_{16}	3.6*	3.7*	3.0*	2.8*
C_{18}	16.4*	24.8*	30.5*	31.4*
C_{20}	1.4	0.2	0.6	0.5
C 18:2	1.7*	2.9*	1.0*	4.9*

*Major unsaturated fatty acid components.

Source: Henderson, 1970.

Oxidative Deterioration of Dairy Products

It is generally accepted that in the oxidation of the unsaturated fatty acid of milk, triglycerides and phospholipids are always involved (Greenbank, 1953; Lea, 1953). El-Negoumy et al. (1962, 1968), and Lea (1957) also concluded that off-flavor production is closely connected with phospholipid deterioration in butter, since phospholipid-free butter fat, on oxidation, gives rise only to "oily" and ultimately, "painty" flavors.

Swanson and Sommer (1940) isolated the crude phospholipid fraction from milk which had developed a copper-induced oxidized flavor and showed that it had decreased in iodine value by a large amount, while the triglyceride fat had hardly changed. The copper induced "carboard-like" flavor of whole milk is largely due to a series of C_4 to C_{11} 2-unsaturated and 2:4 di-unsaturated carbonyl compounds, particularly the C_7 to C_9 aldehydes present at concentrations as low as 1 part in 10^9 of milk. These compounds are typical products of the autoxidation of a poly-unsaturated fatty acid (Lea, 1953).

Chemical Tests for Lipid Oxidation and Correlation to Sensory Evaluation

2-Thiobarbituric Acid Test for Measurement of Oxidative Rancidity

Since Kohn and Liversedge (1944) reported that a red color resulted when tissue had been incubated aerobically

with 2-thiobarbituric acid (TBA), the production of malonaldehyde has been widely used as a measurement of the oxidation of unsaturated fatty acid in food (dairy products: Patton et al., 1951; Biggs et al., 1953; Sidwell et al., 1954; Jennings et al., 1955; King, 1962; fish: Sinnhuber, 1958; pork: Younathan et al., 1960; and bakery products: Caldwell, 1955).

The Principal TBA Reactants

The mechanism and chemical structure of TBA-reactive materials are not very well understood even at the present time, though numerous investigators have postulated possible TBA-reactive materials. Bernheim and Bernheim and Wilber (1947); for example, suggested that TBA pigment from oxidized lipids involves the reaction of a three carbon compound with the reagent. Patton and Kurtz (1951) observed that malonaldehyde, a three carbon compound, gives the characteristic absorbance at 532 nm in the test. Sinnhuber et al. (1958) indicated that the principal TBA-reactive material is malonaldehyde and Patton and Kurtz (1955) reported that freshly prepared or unsaturated aldehyde did not give the TBA test, but when they were autoxidized or heated with Cu^{++} ions a positive reaction resulted.

Sinnhuber et al. (1958) demonstrated that crystalline TBA derivatives could be isolated from rancid salmon oil,

sulfadiazine and malonaldehyde. The molecular configuration of the pigment is believed to be a condensation product of two molecules of TBA with one molecule of malonaldehyde. Holman et al. (1962) observed that an α,β-unsaturated peroxide radical is a precursor of malonaldehyde and has a relation to the TBA color production. Finally, Marcuse and Johansson (1971) reported that TBA color production is quantitatively related to the amount of dismutation products of hydroperoxide such as conjugated triene, tetraene, pentaene and hexaene.

TBA Reactive Material and Water Extraction

The true chemical structure and the origin of the total TBA-reactive materials are still unknown. However, Patton and Kurtz (1955) reported that the TBA-reactive material from milk fat was water soluble and of low molecular weight.

Tarladgis et al. (1964) indicated that free malonaldehyde is produced as a result of the oxidative breakdown of the unsaturated fatty acid and can be extracted with water. King (1962) demonstrated a sensitive TBA test of an intact milk sample and suggested that the TBA reaction should not be carried out in the presence of the oxidized or oxidizing lipids since further oxidation may occur during the reaction with TBA. The pigments derived probably do not entirely reflect the organoleptic property of the intact sample.

The Correlation Between TBA-Reactive Materials and Rancid Odor of Food Products

The correlation between TBA values and rancid odor of food products has not been fully investigated. Dunkley (1951) observed, however, that the color production in the 2-thiobarbituric acid test and the organoleptic flavor score of a whole milk sample having a oxidized flavor are closely correlated. Lillard and Day (1961) examined the relationship between the off-flavor of milk fat oxidized in diffused daylight to different levels and several criteria of chemical change, including peroxide value and TBA value. They reported that TBA and peroxide test correlate well with oxidized flavor in this particular system. King (1962) also reported that the TBA test closely reflects the organoleptic conditions of the intact samples in the studies of oxidized flavor in the model systems containing fat globule material of cow's milk and ascorbic acid. Holman et al. (1954) also demonstrated that the TBA color production and peroxide value have an essentially linear relation below a peroxide value of 1000 in oxidized poly-unsaturated fatty acids which contain conjugated diene, triene, tetraene, hexaene and pentaene.

However, other investigators have reported a poor correlation between TBA test and off-flavor production. For example, Holland (1971) observed that in freeze-dried meat

the TBA values were low and could not be correlated with the appearance of off-odor or the absorption of oxygen. Tarladgis et al. (1960) and Corliss et al. (1963) also observed that the TBA value reached a peak at an early stage of oxidation and later fell.

The relationship between TBA reactive materials and rancid odor in dehydrated food systems needs further investigation with respect to the possible decomposition products of lipid oxidation.

EXPERIMENTAL

Separation of Milk Fat

Three different lots of fresh, raw, winter creams were obtained from the Michigan State University dairy plant, pasteurized at 77°C for 10 minutes, cooled, and held at refrigerator temperature for subsequent churning. The milk fat was separated by conventional churning in a 5 liter Erlenmeyer flask at 12°C-16°C.

After the cream was churned, the butter was recovered and carefully melted in a 50°C water bath. The melted milk fat was then washed with 50°C distilled water until a fairly clean aqueous solution was obtained. The washed milk fat was then dried under vacuum and filtered through filter paper in a 50°C incubator.

Preparation of Freeze-dried Model Systems

The freeze-dried model system, which contained microcrystalline cellulose and milk fat, was prepared as follows.

Components of Model Systems:

1. One part milk fat and six parts of microcrystalline cellulose, dry basis W/W.

2. The melted milk fat was homogenized with 10 parts of distilled water with 2,500 psi at 45°C by using a laboratory homogenizer. The small portion of unhomogenized milk fat was separated with a separatory funnel, re-homogenized and combined with the major portion.
3. The homogenized milk fat was mixed with 6 parts of micro-crystalline cellulose.
4. The thoroughly mixed model system was freeze-dried at 100 microns absolute pressure.

Adjusting Water Activity by Moisture Equilibration

The sifted freeze-dried model system was mixed uniformly in a nitrogen inflated vinyl bag. An accurately weighed amount of the freeze-dried system was humidified in vacuum desiccators which contained a specific saturated salt solution for desired water activity (see Table 4).

Determination of Water Activity

An apparatus capable of measuring a_w by either the Makower-Meyers (1943) or the Taylor (1961) methods was used to determine the water activity of the freeze-dried model systems (see Figure 2). In the present study a

Table 4. Relative Humidities of Saturated Salt Solutions at Given Temperature

Saturated salt solution	Relative humidity, %	Temperature °C, and reference
Lithium chloride (LiCl)	11	20 (1)
	12	25 (4)
	12	22.5 (1)
Potassium thiocyanate (KSCN)	47	25 (3)
Sodium chloride (NaCl)	75.8	20 (4)
	75	25 (1,2,4)
	75.1	22.5 (3)

References:
1. Weston et al., 1954
2. Rockland, 1957
3. Handbook of Chemistry and Physics, 55th ed.
4. Wexler and Hasegawa, 1954

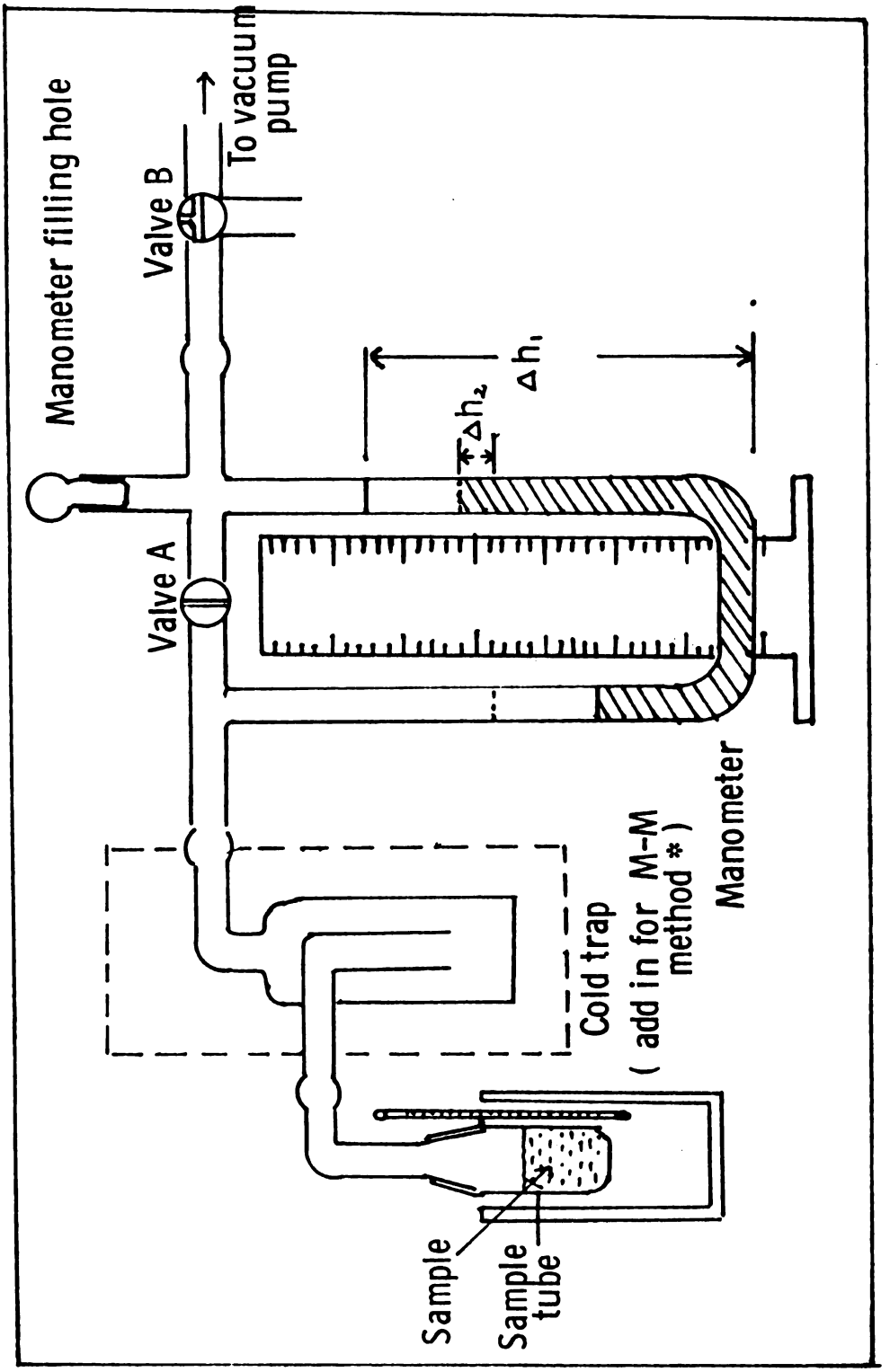


Figure 2. Simplified Makower-Meyer/Taylor apparatus.

modified Taylor method was used. The sample tube was filled quickly with 3 g. of freeze-dried sample and then stoppered with a greased ground-glass cap. After the sample tube was frozen in a mixture of dry ice and acetone for 30 minutes, the tube was quickly installed in the Taylor apparatus and held an additional 10 minutes in the dry ice-acetone mixture.

The initial reading of the difference of oil "B", manometric fluid, levels between the right and left legs was made in the following ways:

1. The valve A was set so that the vacuum pump would exhaust air from both legs of the manometer for five minutes after the pump had approached maximum vacuum as indicated by pump sound.

2. The valve A was then set to pump the air only from the left leg of the manometer. The initial pressure difference in the system was read after reaching constant oil levels.

The freezing solution was then replaced by water at ambient temperature and the equilibrated food sample was maintained at a given temperature under the constant vacuum pressure so that a maximum pressure change could be read. The total pressure change by the equilibrium moisture content of food sample was calculated by subtracting initial pressure difference of both legs from the total pressure difference after reaching equilibrium moisture content at given temperature.

Possible errors which might be caused by trapped air in the sample were minimized by refreezing the sample tube 10 minutes and subtracting the difference of the pressure change caused by the refreezing from the total pressure difference by the food sample.

Calculation of Water Activity

In Taylor's apparatus the water activity can be calculated as follows:

$$a_w = \frac{P}{P_s} = \frac{\Delta h_1 - \Delta h_2}{P_{(\text{oil B})}}$$

where

$P_{(\text{oil B})}$ = vapor pressure of pure water at temperature T., in terms of mm of oil B.

Δh_1 = pressure difference of manometer at equilibrium relative humidity after thawing with water at ambient temperature (at T.)

Δh_2 = pressure difference of manometer after re-freezing the sample.

Chemical Test for Lipid Oxidation

Peroxide Test

Peroxide values were determined by a minor modification of the method used by Stine et al. (1954)--fat extraction, which was modified by the butanol-salicylate

de-emulsification method of Pont (1955), proceeded in the following manner:

Ten g. of the freeze-dried model system was reconstituted with 25 ml. of distilled water and 15 ml. of the de-emulsification reagent were added. The mixture was then transferred quantitatively to a 9 g. 50% cream test bottle. After the reagent was thoroughly mixed, the bottle was placed in a 70°C water bath for 10 minutes and then centrifuged for 3 minutes in a heated Babcock centrifuge. Distilled water at 70°C was added to bring the fat into the neck of the Babcock bottle and the bottle was again centrifuged for 3 minutes. The peroxide determination of a weighed amount of milk fat was accomplished by the method described (Stine et al., 1954).

2-Thiobarbituric Acid Test

The 2-thiobarbituric acid test of King (1962) was modified to accommodate a freeze-dried model system based on micro-crystalline cellulose and milk fat by using water extraction of TBA-reactants, increasing reaction time with the TBA reagent and the chromatographic separation of pink TBA pigment.

Five g. of the freeze-dried model system was reconstituted with 20 ml of distilled water and shaken vigorously for 10 seconds in a glass stoppered 50 ml Erlenmeyer flask

and tempered for 10 minutes at 45°C. One ml of trichloroacetic acid solution (1 g. TCA/1 ml) was added. The solution was tempered for 5 minutes at the same temperature and was shaken vigorously.

The sample was then filtered through No. 42 Whatman ashless filter paper. Four ml of filtrate and 2 ml of TBA reagent (0.05 M) were reacted in the 50 ml screw capped glass tube for exactly 35 minutes at 100°C. The interference of yellow pigment caused by high heat treatment was eliminated by passing the reactants through a chromatographic column.

Chromatographic Separation of Pink TBA Pigment

The TBA reacted solution was poured into a chromatographic column and forced through under 5 psi nitrogen pressure. The preparation of chromatographic column with cellulose (Whatman standard grade) used the procedure of Caldwell et al., 1955.

Each column was washed with 2 ml of the washed reaction mixture from the test tube, then with two 2-ml portions of distilled water. A 10 ml volumetric flask was placed under each column, and absorbed red pigment eluted with aqueous pyridine (water:pyridine, 5:1), exactly 10 ml being collected.

Optical density was read at 532 nm against distilled water using a Beckman Model DU-2 spectrophotometer.

RESULTS AND DISCUSSION

Lack of sensitivity of chemical methods in analyzing lipid oxidation of low-peroxide fat has been an obstacle to the study of autoxidation of purified milk fat. In recent years most studies of lipid oxidation in dehydrated food systems have been based upon the various methods of oxygen uptake methods. But for practical purposes, other methods have been required. There is also scant information regarding the effects of water activity on off-flavor development; correlating of the 2-thiobarbituric acid test with other tests such as peroxide test and sensory evaluation. The purpose of this investigation was to determine the effects of water activity on milk fat oxidation and the correlation of two chemical tests, the TBA test and the colorimetric peroxide test to sensory evaluation of freeze-dried milk fat model systems.

Preparation of Model Systems

Three different lots of fresh, raw winter creams were pasteurized at 77°C for 10 minutes to inactivate enzymes and effectively inhibit microbial growth, which might alter the result of autoxidation studies.

The churned milk fat was melted carefully and washed with distilled water at 50°C to prevent possible coagulation of protein residue.

For the further separation of water soluble components, the washed milk fat was concentrated under vacuum at 50°C, and the clear lipid layer was decanted. Any insoluble matter was filtered off at the same temperature.

In the preparation of freeze-dried milk fat model systems utilizing micro-crystalline cellulose (6 parts) as the carrier, the melted milk fat (1 part) was homogenized in distilled water (10 parts) to attain homogeneity.

The freeze-dried systems were sifted with a stainless steel screen and stored at -30°C under nitrogen.

Moisture Equilibration of the Milk Fat Model Systems

Table 6 and Figures 3 and 4, respectively, present the moisture change and moisture equilibration curves during the humidification periods at 20 $\pm$ 0.5°C under reduced pressure. The equilibrium moisture data were obtained by exposing the freeze-dried milk fat model systems to the required equilibrium moisture content (RH) at 20 $\pm$ 0.5°C in vacuum desiccators. The equilibration temperatures and vacuum were selected in order to obtain optimum determination conditions of water activities by the Taylor method

(manometric) and to prevent further lipid oxidation during humidification periods. The saturated salt solutions which were used in adjusting water activity are presented in Table 5.

Table 5. Saturated Salt Solutions and Equilibrium Moisture Content

Saturated salt solution	Relative humidity by Taylor method *	RH by reference at 25°C **
LiCl	12.1	12.0
KSCN	46.8	47.0
NaCl	74.7	75.8

*Measurement water activity at 20.5°C.
**Data from Rockland, 1957; Wexler and Hasegawa, 1954; in Strolle et al., 1965.

The moisture content of the freeze-dried model systems during moisture equilibration was periodically determined on duplicate samples by the method described in A.O.A.C. (1965). At the final stage of the moisture equilibration curve, the water activities of the model systems were systematically determined until constant values were obtained.

Table 6. Change of Moisture Content of the Model System During Attainment of Equilibrium Moisture

Run 1			
	% moisture in sample, wet basis*		
Time (hrs)	over satd LiCl	over satd KSCN	over satd NaCl
24	0.95	3.45	5.97
48	1.78	4.65	7.15
72	2.26	5.30	7.51
144	2.50 (a_w 0.12)	5.40 (a_w 0.447)	7.70 (a_w 0.685)
168	2.51 (a_w 0.12)	5.45 (a_w 0.462)	7.75 (a_w 0.745)
192	2.50 (a_w 0.12)	5.45 (a_w 0.465)	7.75 (a_w 0.745)
Runs 2 and 3			
Time (days)			
3	1.82	3.05	5.84
6	2.27	4.32	6.78
9	2.35	5.1	7.30
13	2.45	5.15	7.55 (a_w 0.67)
20	2.50 (a_w 0.12)	5.40 (a_w 0.447)	7.75 (a_w 0.742)
24	2.50 (a_w 0.12)	5.40 (a_w 0.467)	7.74 (a_w 0.746)

*Average of duplicate determination.

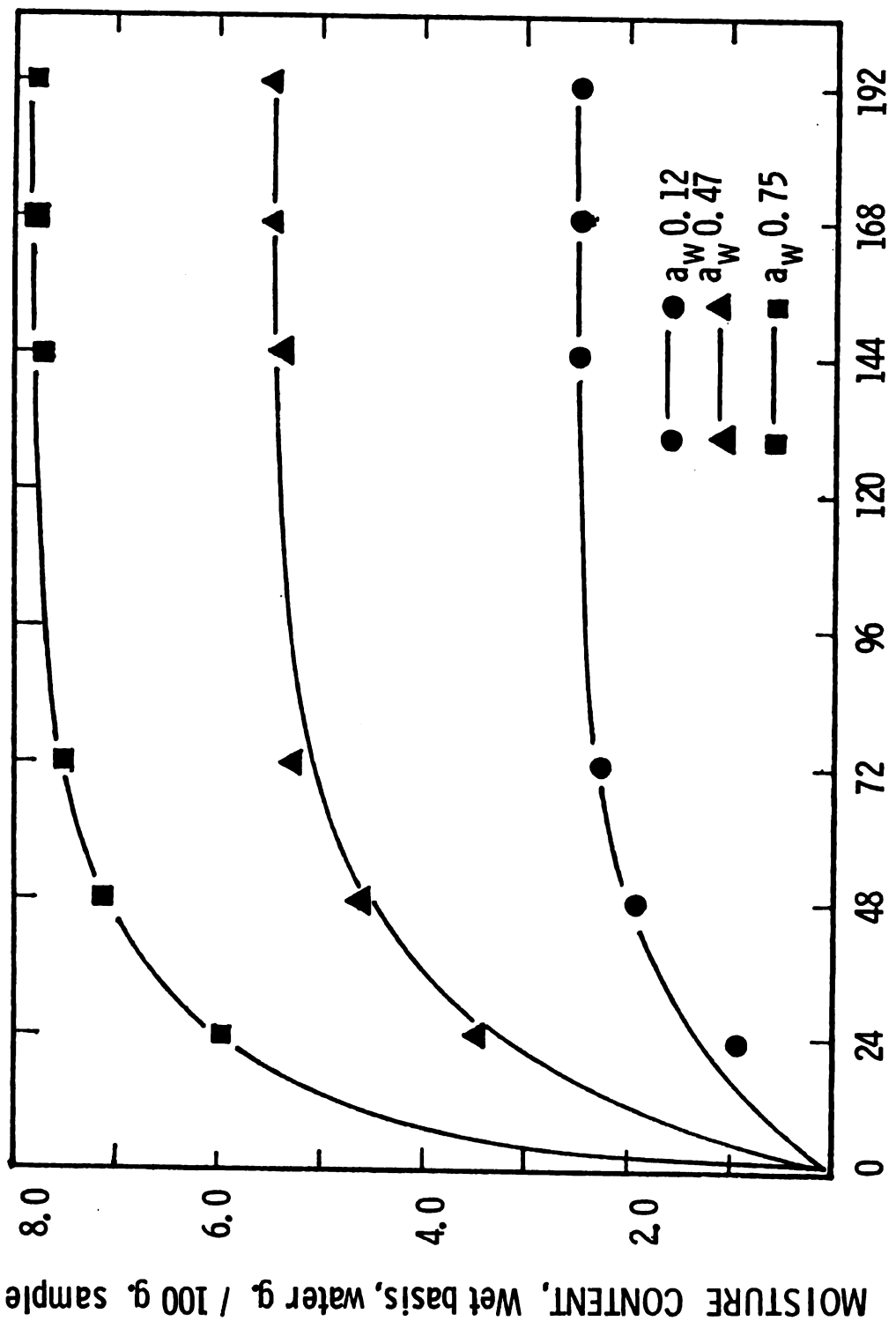


Figure 3. Moisture equilibrium curve of a milk fat-cellulose model system at 20 ± 0.7°C (Run 1).

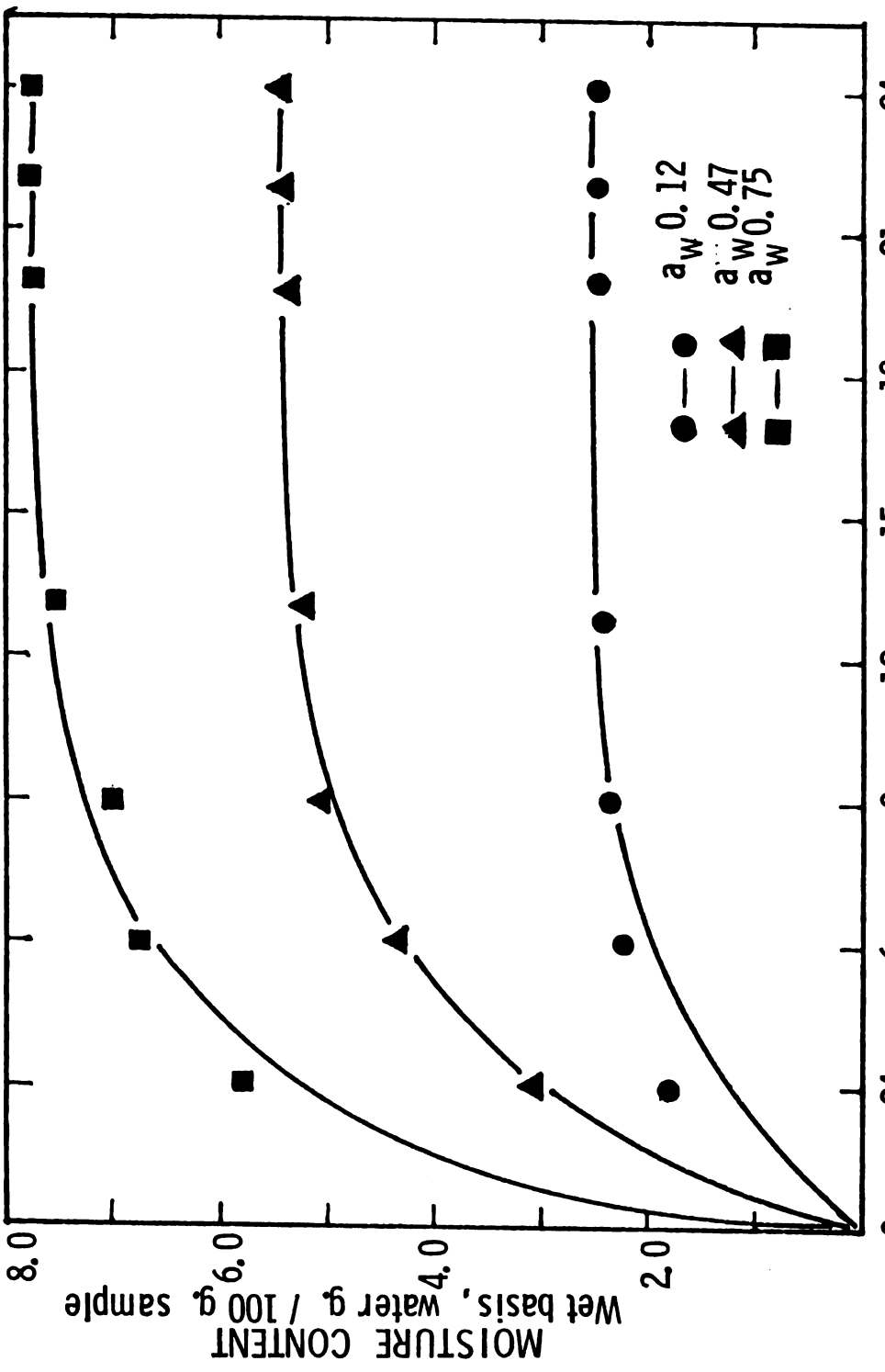


Figure 4. Moisture equilibrium curve of a milk fat-cellulose model system at 20 ± 0.7°C (Runs 2 and 3).

Adsorption Moisture Isotherm Curve of the Model Systems

The relation between water activity and moisture content of the freeze-dried model systems can be precisely described by the moisture adsorption isotherm depicted in Table 7 and Figure 5.

Table 7. Change in Water Activity of the Freeze-dried Model System at Varying Moisture Contents

Moisture content, dry basis %	Water activity at 20°C
2.51	0.12
3.53	0.208
4.23	0.28
5.60	0.465
6.95	0.618
8.43	0.745

Figure 5 shows the typical sigmoid curve of a general moisture adsorption isotherm. Labuza (1968) and Acker (1969) have described theoretically the moisture isotherm curve in three different regions depending on the physical state of the water present in food matrix.

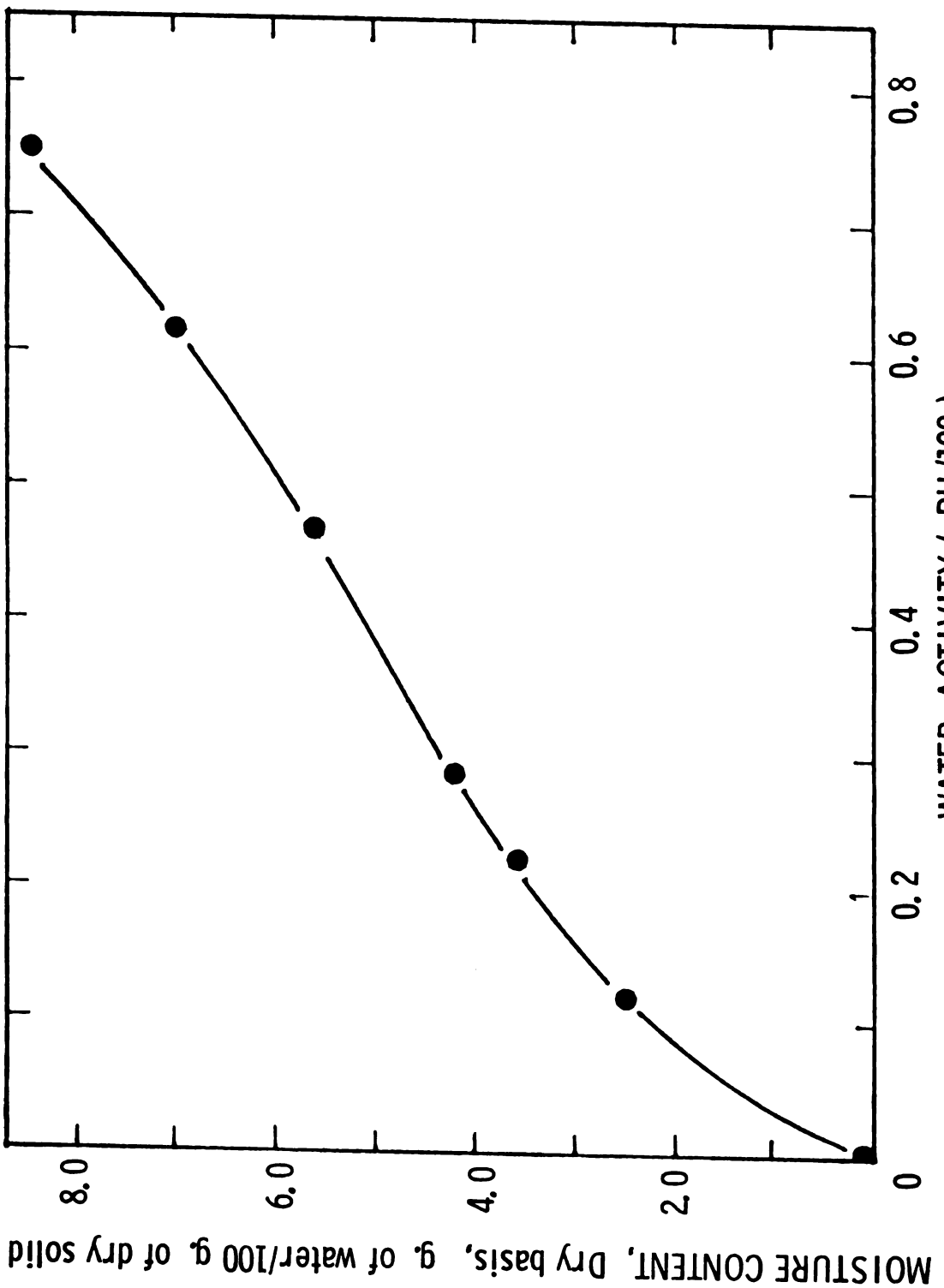


Figure 5. Moisture adsorption isotherm of the freeze-dried model system at 20 ± 0.2°C.

The selection of water activities for the lipid oxidation studies of milk fat in freeze-dried model systems was based on an arbitrary figure representing each of these three regions: a_w 0.12, a_w 0.47 and a_w 0.75.

Several theories and hypotheses have been offered to explain the moisture adsorption isotherms by Langmuir (1918), Brunauer et al. (1938), Henderson (1952) and Rockland (1957). The BET isotherm by Brunauer et al. (1938) has been most widely used. The limitation of the BET theory is confined to its ability to explain the observed facts in the range of water activity between 0.1 to 0.4. The BET monolayer value has frequently been used to predict the optimum moisture content of a food in relation to shelf life.

The BET monomolecular layer value of the milk fat model systems was calculated from the moisture adsorption isotherm data by the following procedure:

BET equation:

$$\frac{a}{(1 - a)\, m} = \frac{1}{m_1 C} + \frac{(C - 1)\, a}{m_1 C}$$

where

m: Grams of water per 100 g. of dry matter corresponding to a relative vapor pressure

C: Constant related to the heat of adsorption

$$C = \exp\ (Qs/RT)$$

where

Qs = the site of interaction energy of adsorption of water molecules to the solid surface at temperature T

R = universal gas constant

T = absolute temperature

m_1: Grams of water equivalent to monolayer absorbed on 100 g. of dried solid.

The data a(100)/(1 - a)m and relative humidity are presented in Table 8 and Figure 6.

Table 8. The Value for BET Monomolecular Layer Plot

Water activity (a)	Moisture content Dry basis (m)	$\frac{a(100)}{(1 - a)m}$
0.12	2.51	5.43
0.208	3.53	7.44
0.28	4.23	9.19
0.465	5.60	15.52
0.618	6.95	23.20
0.745	8.43	34.65

From the straight line and intercept of the line, the monolayer value was calculated. The BET monolayer value was water activity of 0.23 and at the moisture content of 0.37 g. of water per 100 g. dry solid.

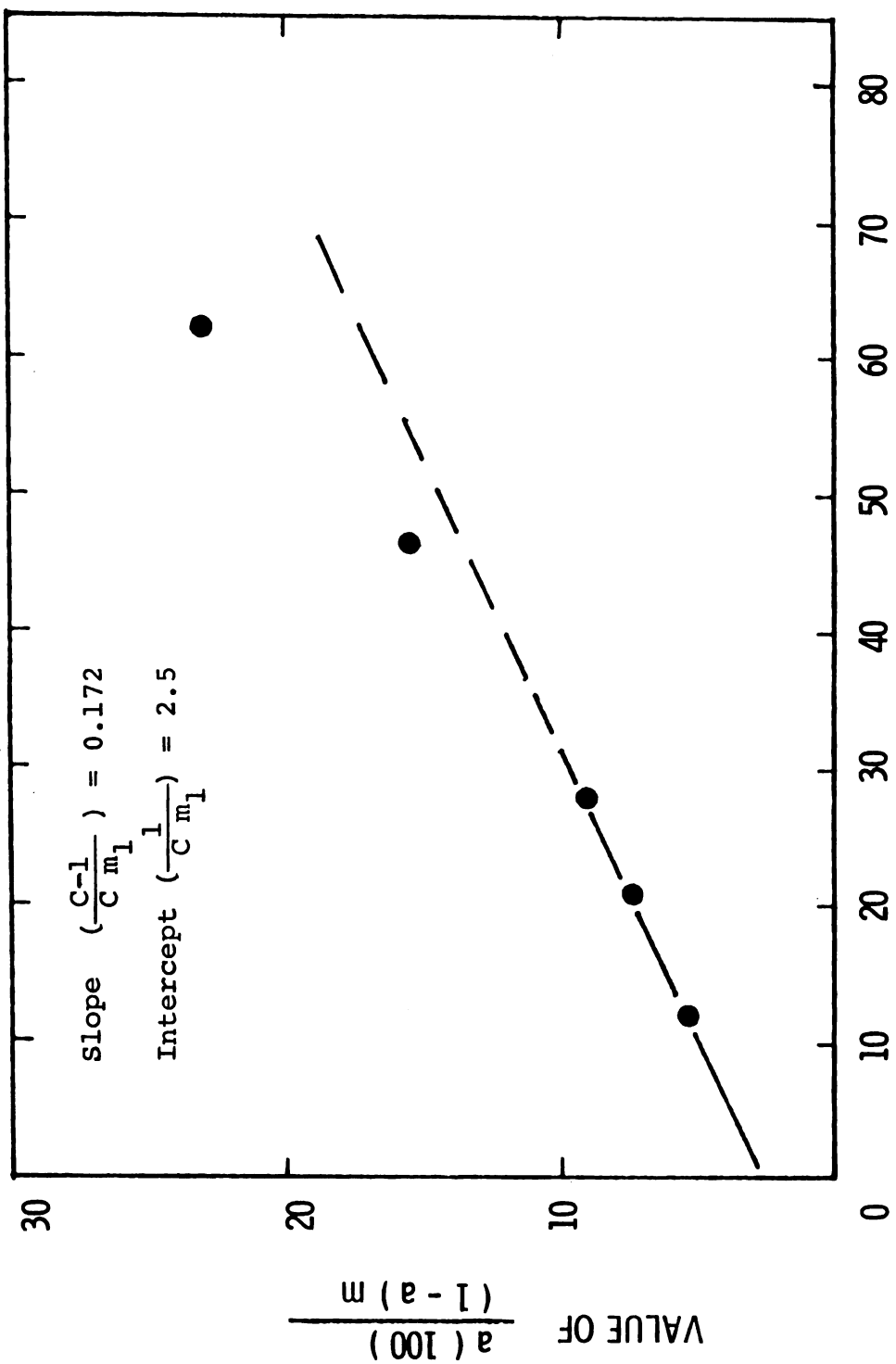


Figure 6. The BET monolayer plot of the freeze-dried model system at 20 ± 0.2°C.

The Change in Moisture Content of the Milk Fat Model Systems During Autoxidation Periods

The model systems, which were adjusted to water activity figures a_w 0.12, a_w 0.47 and a_w 0.75 were placed in containers with air tight covers in 10 g. portions and stored over the saturated salt solutions in desiccators to provide the same relative humidity conditions as the sample and also to prevent additional moisture change.

During two months of storage, the changes of moisture content were monitored by the A.O.A.C. method (1965) at the initial stage, after 1 month, after 2 months by the random selection of 3 samples of each water activity through duplicate determination. The average moisture change of the model systems during the storage periods was negligible.

Sensory Evaluation

Sensory evaluation of the freeze-dried model systems was performed periodically during two months of storage at 20 $\pm$ 0.5°C. The scoring system, adapted from a similar method used by Dunkley (1951), is as follows:

a. Excellent (no detectable oxidized flavor)..... 40 points
b. Good (detectable oxidized flavor... 37-40 points
c. Fair (slightly oxidized flavor..... 34-37 points
d. Poor (distinctly oxidized flavor... 25-34 points

The selection of panel members are based upon their ability to differentiate three standard samples: fresh, a two month old sample at room temperature, a mixture of 50% fresh, and a 50% two month old sample.

In the preparation of sample, 5 g of duplicate portions of the freeze-dried sample in glass bottles with air-tight plastic lids were distributed to the panel members in random order during mid-morning or mid-afternoon. The three criteria based on different degrees of oxidized sample were used to obtain consistent results.

The sensory evaluation of tasting was not applicable in the freeze-dried model systems by confusing of characteristic cellulose flavor against the mild oxidized flavor.

However, in sensory evaluation, some difficulties and confusing of flavor judgments were encountered at the initial stage of the autoxidation by a small amount of chemical change responsible for oxidized flavor defects. The total pattern of off-flavor production at two month storage periods was able to differentiate among the model systems, a_w 0.12, a_w 0.47 and a_w 0.75.

The best storage stability was obtained at a water activity of 0.47, while the samples stored a_w 0.12 showed slightly increased off-flavor production and those held at a_w 0.75 produced a strongly detectable off-flavor after a two month storage period.

The off-flavor production shows some correlation with the peroxide test, but not with TBA test. The results of off-flavor evaluation in freeze-dried model systems contained various amounts of moisture content is presented in Table 9 and Figure 7 (see the following four pages).

Effects of Varying Water Activities on Autoxidation of Milk Fat in Freeze-dried Model Systems

Lipid oxidation of milk fat in freeze-dried model systems based on micro-crystalline cellulose was determined by the peroxide test, TBA test and off-flavor evaluation during a two month storage period. The major difficulties encountered in this investigation included the resistant nature of purified milk fat to autoxidation and the narrow range of chemical change which occurred during storage. This may be due to the composition of the milk fat which is high in oleic and low in polyunsaturated fatty acids.

A good example of this resistant nature over a longer period was reported by Sidwell et al. (1954). His data for the storage stability of butter fat are presented in Table 10 (see page 47).

The peroxide test, TBA test and sensory evaluation were used to monitor the degree of lipid oxidation in this study; the most applicable chemical test was the peroxide test. The results of the TBA test showed a poor correlation to both the peroxide test and the sensory evaluation. In the

Table 9. Flavor Scores of Freeze-dried Model Systems

Number of Run	Storage days	Average Score* a_w 0.12	a_w 0.47	a_w 0.75
Run 1	0	38.2	38.7	37.3
	13	38.3	38.2	34.7
	20	37.4	36.7	34.8
	30	36.0	36.9	32.8
	43	35.1	36.4	31.7
Run 2	0	38.0	37.8	38.3
	14	36.8	37.3	36.1
	30	36.5	37.1	32.0
	48	35.2	36.5	31.2
Run 3	0	37.5	37.4	37.7
	8	37.4	36.9	35.7
	21	36.5	37.3	34.0
	35	35.5	36.7	31.6
	46	35.0	36.4	31.3

*The average score of duplicate evaluation from the results of 6 panel members.

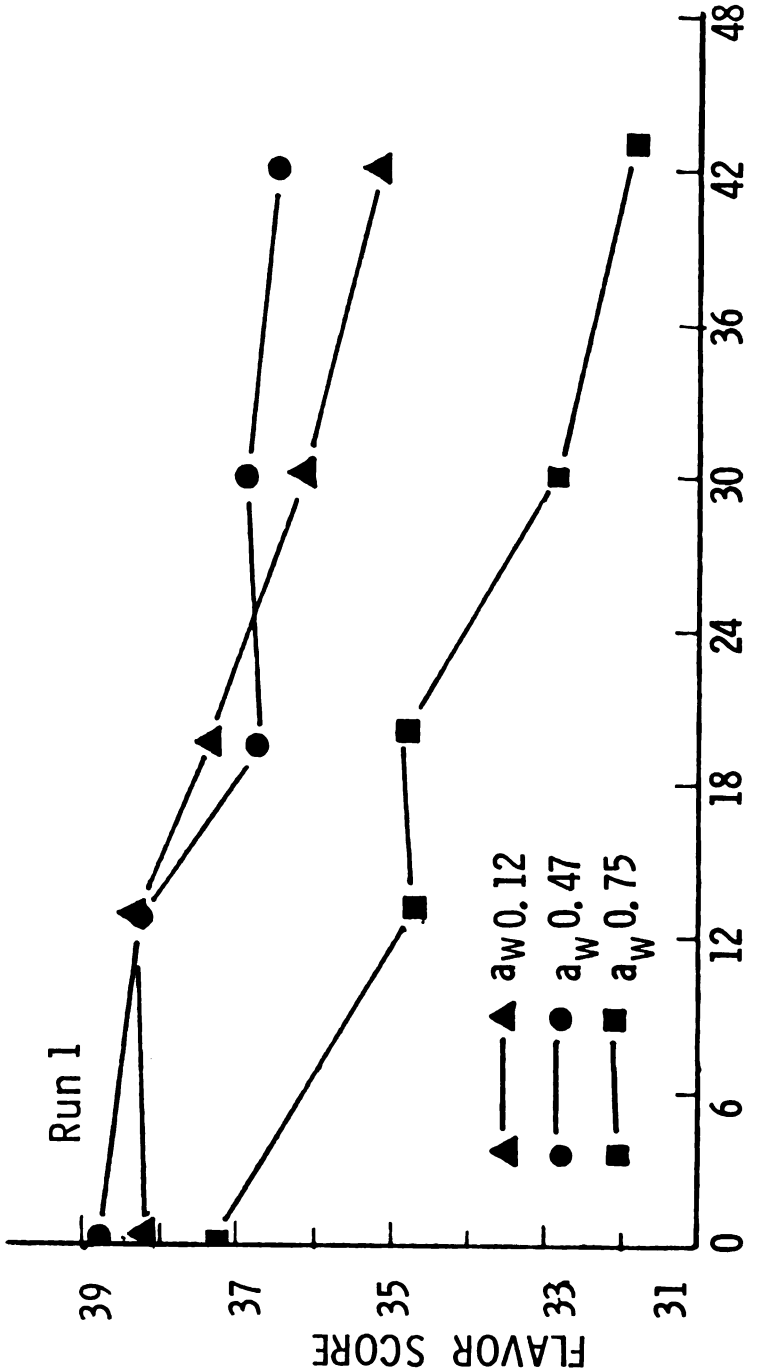


Figure 7-1. Sensory evaluation of freeze-dried model systems during storage at 20°C and a_w of 0.12, 0.47 and 0.75.

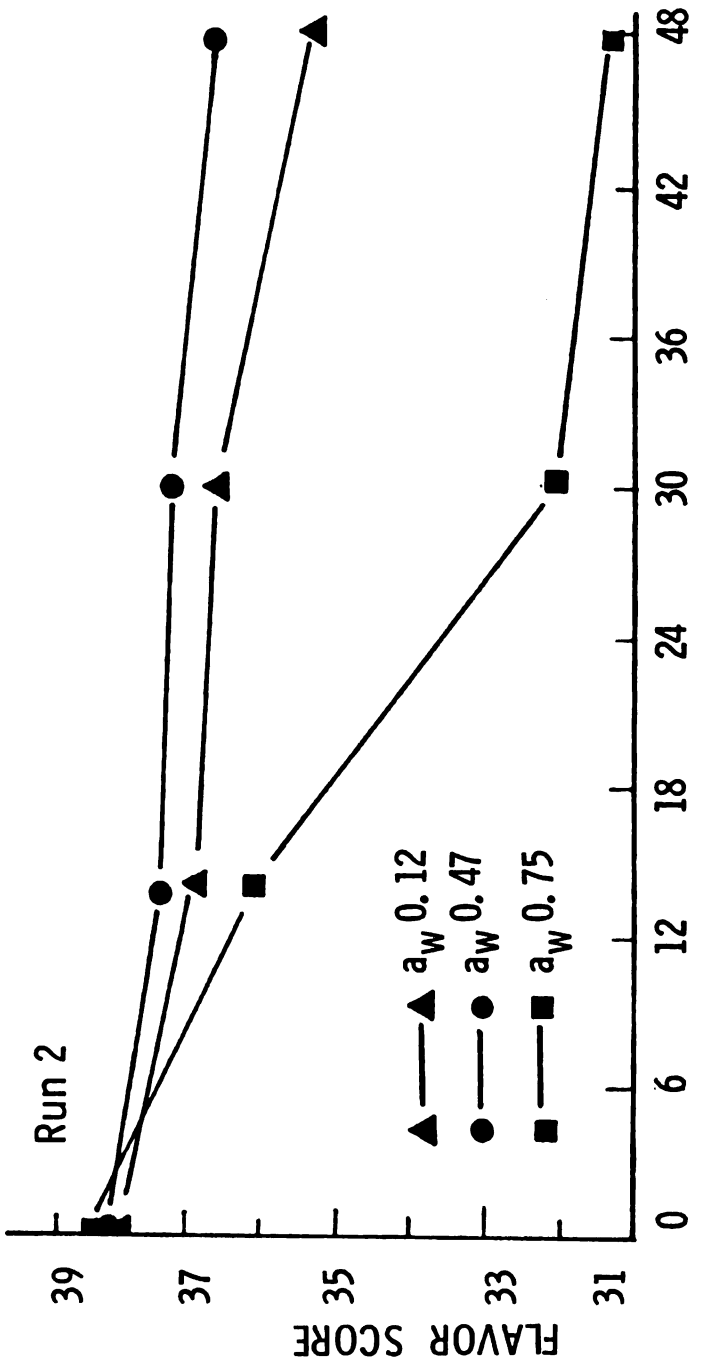


Figure 7-2. Sensory evaluation of freeze-dried model systems during storage at 20 ± °C and a_w of 0.12, 0.47 and 0.75.

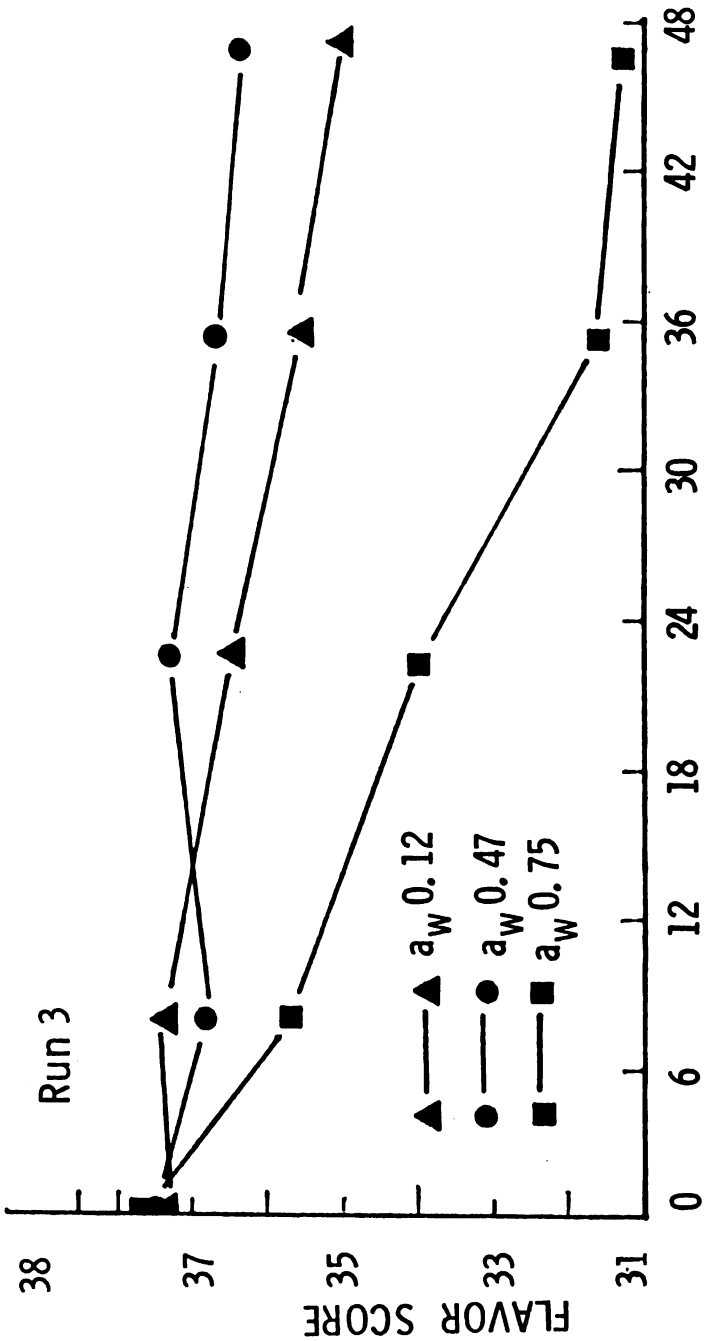


Figure 7-3. Sensory evaluation of freeze-dried model systems during storage at 20°C and a_w of 0.12, 0.47 and 0.75.

Table 10. TBA Values and Peroxide Values of Butter Fat Stored for Approximately 2 Years at -20, 0, 72 and 100 °F

Storage temperature of sample °F	TBA Value	Peroxide Value
-20	0.29	2.00
0	0.30	1.33
72	0.51	4.32
100	0.87	3.33

Source: Sidwell et al. (1954).

case of sensory evaluation, there were some difficulties at the early stage of milk fat oxidation because of low levels of compounds contributing off-flavor, and some confused judgment among panel members as to off-flavor production.

The results of peroxide value for samples in Runs 1-3 are presented in Table 11 and Figures 8, 9 and 10 respectively. The results show that lipid oxidation in the freeze-dried model systems was affected by different levels of water activity. In comparison to the sample of water activity 0.47, the rate of autoxidation increased slightly at a_w 0.12 and notably at a_w 0.75.

The decrease of lipid oxidation at a_w 0.47 compared to a_w 0.12 may be explained by the hypotheses of Labuza et al. (1966), Karel et al. (1967), and Maloney et al. (1966).

Table 11. Peroxide Value of Milk Fat Model System Runs 1, 2 and 3 During Autoxidation Periods

Number of Run	Storage days	Water Activity a_w 0.12	a_w 0.47	a_w 0.75
Run 1	0	0.62	0.61	0.64
	3	0.65	0.61	0.76
	5	0.70	0.64	0.83
	13	0.81	0.68	0.98
	21	0.92	0.81	0.13
	24	--	--	1.60
	32	1.39	1.16	2.05
Run 2	0	0.60	0.62	0.63
	5	0.62	0.60	0.67
	8	0.61	0.60	0.68
	13	0.66	0.64	0.75
	22	0.69	0.63	0.77
	28	0.75	0.68	0.83
	36	0.82	0.74	0.97
	51	0.97	0.87	1.14
Run 3	0	0.65	0.61	0.61
	8	0.68	0.61	0.73
	10	0.70	0.62	0.63
	18	0.72	0.65	0.80
	30	0.85	0.73	0.91
	46	0.97	0.82	1.05

Note: The peroxide values represent the average of duplicate data.

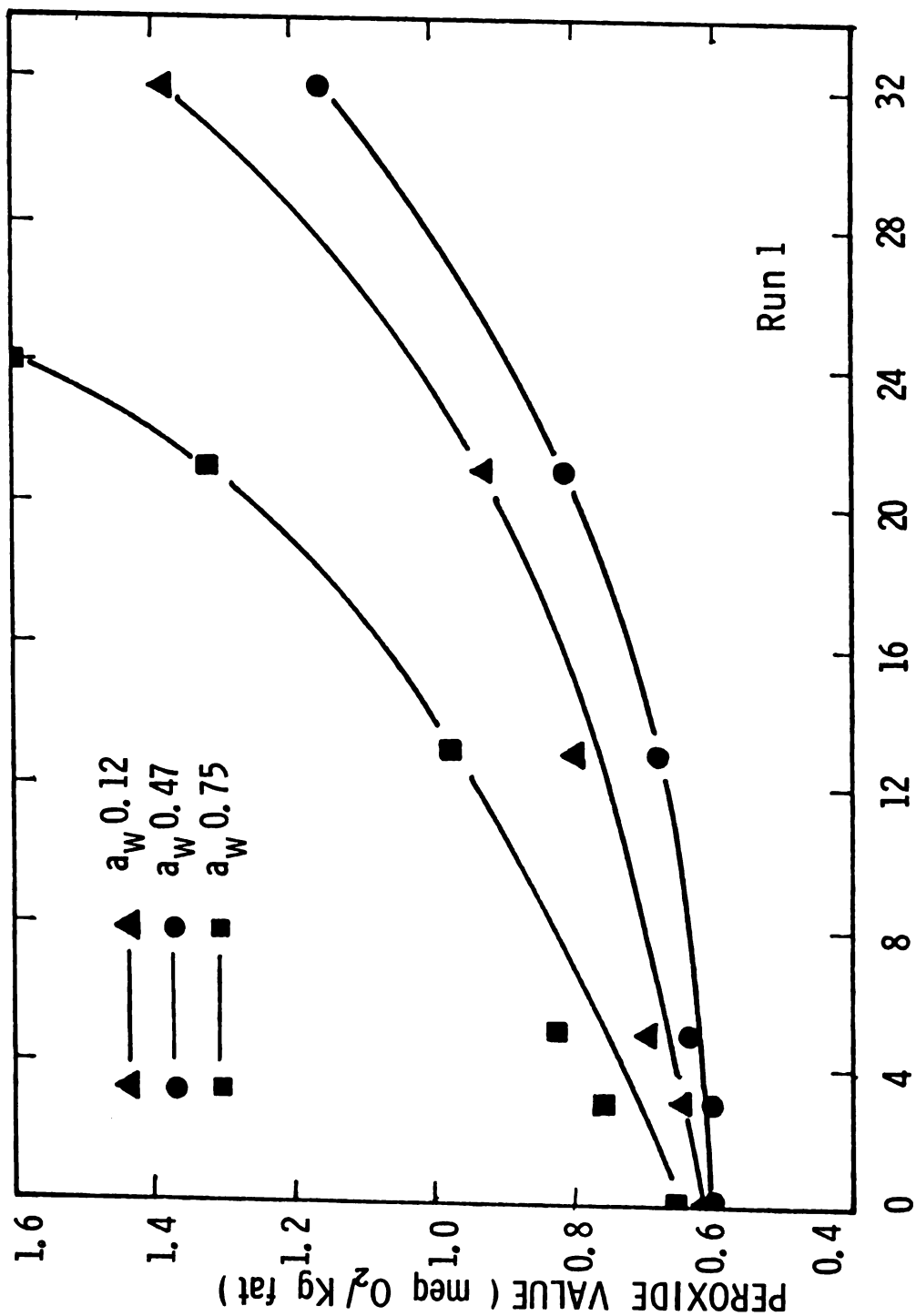


Figure 8. Lipid oxidation in freeze-dried model systems in varying water activities at 20 ± 0.5°C (Run 1).

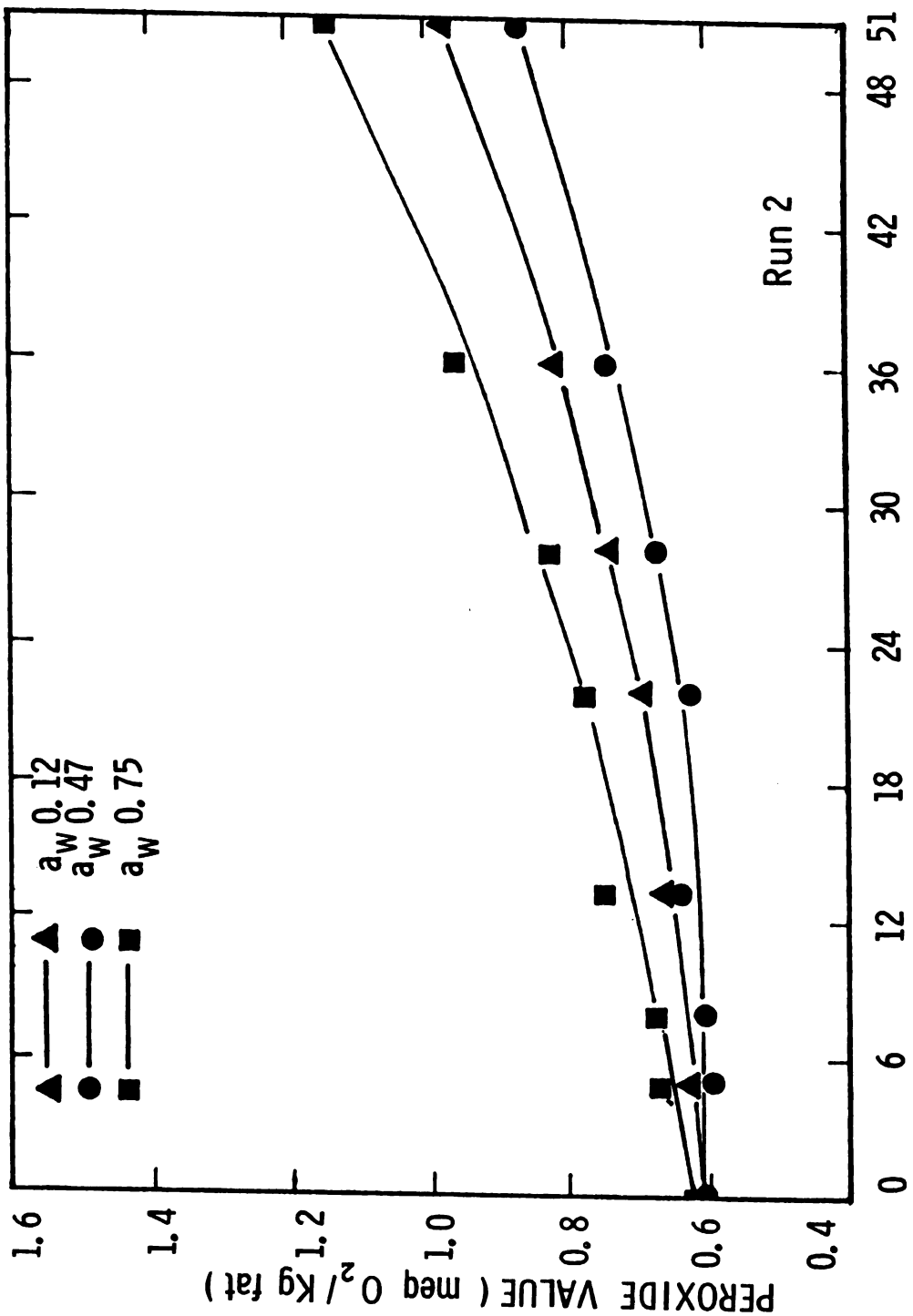


Figure 9. Lipid oxidation in freeze-dried model systems in varying water activities at 20 ± 0.5°C (Run 2).

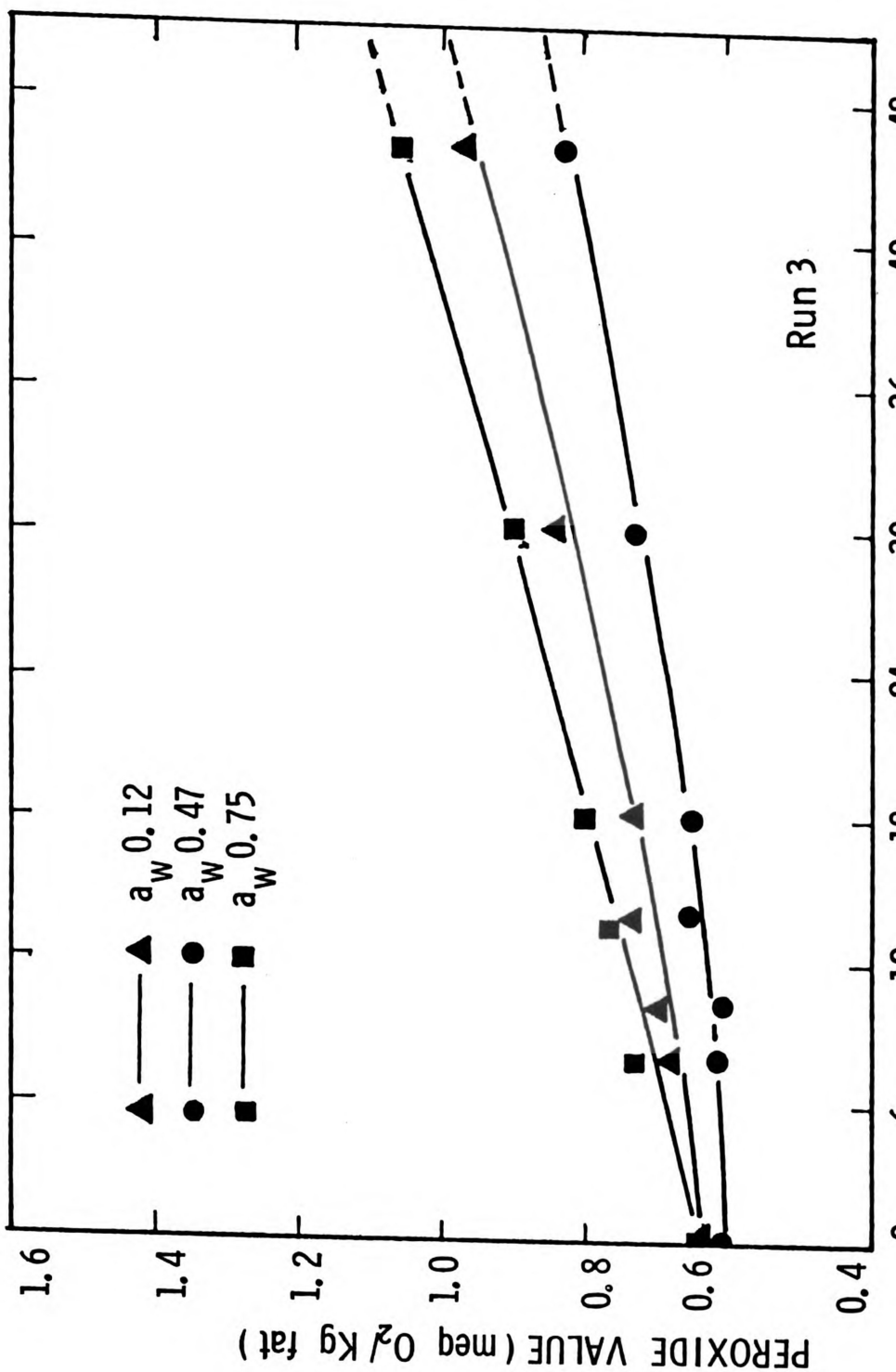


Figure 10. Lipid oxidation in freeze-dried model systems in varying water activities at 20 ± 0.5°C (Run 3).

They indicated that the formation of hydrogen bonding of hydroperoxide with water alters the decomposition rate of hydrogen peroxide.

Another explanation by Chung and Pfost (1967) can describe part of this phenomenon. The physical adsorption causes intermolecular forces between the molecules of water vapor and the surface of the absorbant of food materials. As a consequence, as the moisture content increases up to a certain level, the water will increase the reaction rate with the polar site of food components such as -OH, -COOH, $-CONH_2$, -NH-, etc. and thereby the water will exclude oxygen adsorption from these sites. Still another possible explanation could be the inactivation of metal catalysts by hydration.

At the highest water activity of 0.75, a pro-oxidant effect has been observed. This effect may be explained by the hypotheses of Labuza et al. (1970, 1972), and Heidelbaugh et al. (1971) which suggest that the effect is due to increasing the mobility of reactants and catalysts which were not inactivated and possibly to the swelling of the porous matrix which allows for more contact with atmospheric oxygen.

The Effect of Water Activity on the Rate of Milk Fat Oxidation

A plot of oxidation rates of milk fat in model systems vs. water activity is presented in Table 12 and Figure 11.

The oxidation rates of milk fats were obtained by drawing the least square line of the peroxide values at the early stage of oxidation. However, the oxidation rates of Runs 1, 2 and 3 for varying water activities were different, due possibly to the compositional difference of milk fat. The data in Figure 11 demonstrate clearly the optimum moisture content for varying water activities.

In the comparison of autoxidation rates of the freeze-dried milk fat model systems with various water activities of a_w 0.12, a_w 0.47 and a_w 0.75, the water activitiey which was a little above a BET monomolecular layer value demonstrated an identically lower oxidation rate at the initial stage of the oxidation, while a little below a BET monomolecular value or far above a BET monolayer value showed a much faster oxidation rate. The data of autoxidation rate of the three different freeze-dried model systems are presented in Table 12.

Table 12. The Effects of Water Activity on Autoxidation Rate of Milk Fat in Freeze-dried Cellulose Model Systems at 20 $\pm$ 0.5°C (MEQ O_2/KG FAT per day)

Number of model systems	a_w 0.12	a_w 0.47	a_w 0.75
Run 1	0.0125	0.00625	0.025
Run 2	0.0041	0.0013	0.071
Run 3	0.0070	0.0027	0.0092

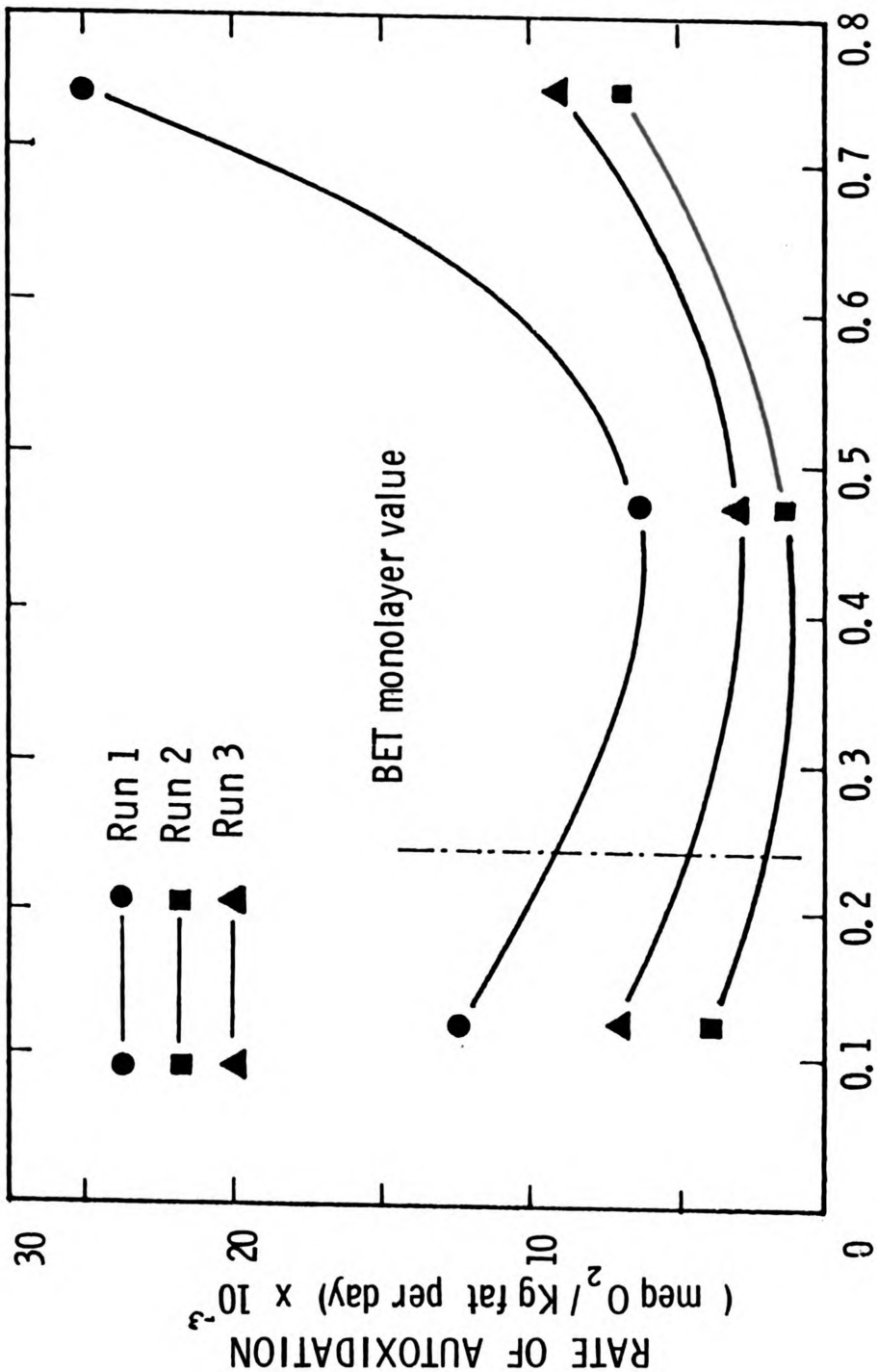


Figure 11. The effects of various water activities on milk fat oxidation in the model systems at 20 ± 0.5°C.

2-Thiobarbituric Acid Test in Freeze-dried Milk Fat Oxidation

In spite of abundant literature, the usefulness of 2-thiobarbituric acid test for the determination of the rancidity of dairy products is still debatable with respect to suitable conditions for the reaction, the appropriate area of its application and the significance of the results as relatable to sensory properties.

The TBA test is based on a color reaction between a TBA reagent and TBA-reactive materials which are produced during oxidation of a poly-unsaturated fatty acid.

Many investigators (Biggs, 1953; Dunkley and Jennings 1951; Keeney et al., 1959; King, 1962; Patton et al., 1951, 1955; Sidwell et al., 1955) have reported that at the early stage of autoxidation, dairy products show a much lower production of TBA color than do other food products, which contain more poly-unsaturated fatty acid. In the preliminary investigation a considerable effort was made to increase the TBA color production with washed milk fat by some modification of published TBA methods. But the results were unsatisfactory with the exception of King's method (1962). The principal reason for lower color production of absorbance at 532 nm may be the influence of the composition of fatty acid and the kind of dismutation products of autoxidized milk fat.

Effects of Fatty Acid Component of Purified Milk Fat on TBA Test

The comparatative data for the fatty acid composition of milk fat were presented in the Literature Review in Table 2. Inspection of these data reveals that the principal unsaturated fatty acids are oleic acid (ca 30%), linoleic acid (3-5%) and a trace amount of unsaturated acid C_{20-22} in carbon chain. In the case of churned and washed milk fat, in which most of the phospholipids are removed, the amount of polyunsaturated fatty acid will be much smaller.

In the autoxidation studies it was demonstrated that the purified milk fat has a much slower oxidation rate than that of unpurified milk fat. This may be an example of the effect of phospholipid on the oxidation rate. In fact, Lea (1957), King (1962) and El-Negoumy et al. (1965) have shown that oxidation of milk fat and TBA color production are remarkably influenced by the amount of phospholipid in the milk fat globule membrane.

Dunkley and Jennings (1951) observed that the amount of red color correlated with the intensity of oxidized flavor but the formation of yellow compound (Patton et al., 1951) was variable and did not show a consistent relationship to red color production.

In the studies of the relationship of purified milk fat to TBA color production, some modification of King's

TBA method was desired; such as, increasing reaction time with the TBA reagent and the chromatographic separation of interfering yellow pigments. Interfering yellow pigment with adsorption maxima at 450-460 nm was observed in oxidized milk fat. Keeney et al. (1959) reported that the early browning intermediate of non fat dried milk could be converted to TBA reactive materials such as 5-hydroxymethylfurfural (HMF) and partial HMF by heating non fat dried milk in acid systems.

Reinhard et al. (1971) noted that several aldehydes which are commonly found in autoxidized milk fat may produce a yellow color with the reaction of TBA reagent at a lower reaction temperature. But in this particular milk fat-cellulose model system, which has no protein constituents, the possible major yellow interfering pigment may result from dismutation products of hydroperoxides of milk fat such as short chain carbon compounds--aldehydes and ketones. The data from the TBA test and the autoxidation curve of freeze-dried model systems are presented in Table 13 and Figure 12 respectively.

In the investigation of the correlation between the TBA test and the peroxide test, the results showed some correlation at the early stage of the induction period of autoxidation with respect to the lipid oxidation rate, even though the individual TBA values were not reflected

Table 13. TBA Color Production at 532 nm of Autoxidized Milk Fat in Freeze-dried Cellulose Model Systems at Various Ranges of Water Activity

Time (days)	Water Activity a_w 0.12	a_w 0.47	a_w 0.75
Run 2			
0	0.061	0.080	0.039
2	0.064	0.082	0.043
5	0.074	0.075	0.051
8	0.083	0.071	0.060
12	0.049	0.075	0.067
15	0.049	0.037	0.040
20	0.038	0.045	0.053
27	0.059	0.055	0.056
Run 3			
0	0.058	0.075	0.045
4	0.079	0.074	0.057
6	0.088	0.076	0.063
9	0.072	0.082	0.066
13	0.058	0.065	0.050
22	0.035	0.043	0.043
28	0.043	0.052	0.046

Note: The data are based on the average of duplicate results of TBA test.

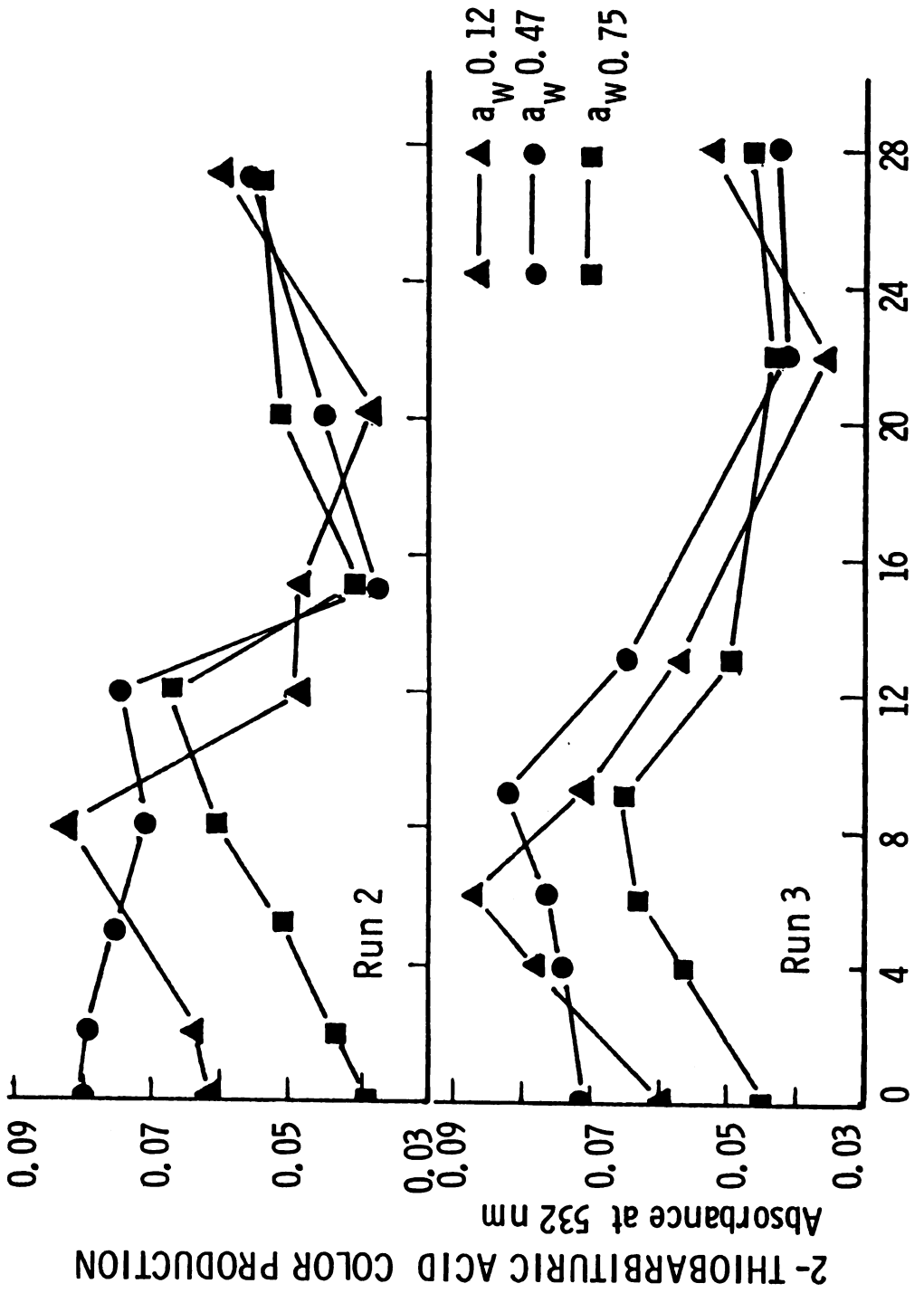


Figure 12. TBA color production of oxidized milk fat in cellulose model systems at various water activities.

proportionally to the peroxide value. However, at the end of the induction period of the autoxidation the TBA values began to decline and showed a poor correlation with the peroxide values as well as sensory evaluation, while the peroxide value and sensory evaluation were very closely correlated throughout the whole oxidation period.

The Theoretical Reasons for Poor Correlation Between the TBA and Peroxide Test or Sensory Evaluation

TBA Color Production and Decomposed Products of the Oxidized Fat

Kohn and Liversedge (1944) reported that the color production of TBA test is due to a three carbon compound containing aldehyde or ketone groups derived from the oxidation of fatty acid. Patton and Kurtz (1951) suggested that malonaldehyde was the compound responsible for the red color maximum at 532 nm and that this compound was present in oxidized milk fat.

However, Holman et al. (1962) reported that the TBA color production increases as poly-unsaturated fatty acid content is increased, even at the same peroxide value. Oleic acid, and linoleic acid give no TBA color at the early stage of oxidation. These researchers suggested that the TBA color production is very closely related to the content of β,γ-unsaturated radicals. The example of autoxidation studies (Holman et al., 1962) of methylene interrupted

polyene is presented in Table 14 and Figure 13.

Table 14. Number and Type of Isomeric Peroxide Radicals Formed in Early Stage of Autoxidation of Methylene Interrupted Polyene*

Polyene	Isomeric peroxide radicals	Unsaturated peroxide radicals	Praction of β,γ-unsaturated radicals
Diene	2	0	---
Triene	4	2	0.5
Tetraene	6	4	0.67
Pentaene	8	6	0.75
Hexaene	10	8	0.80

*Source: Holman et al., 1962.

Similar results were obtained by Marcuse et al. (1971) in the studies of TBA color production with different aldehydes and varying reaction times and temperatures. They observed that the TBA color production, absorbance at 530 nm, is closely related to the conjugated diene, but not with alkanal and alkenal which are most commonly found in oxidized lipids from monoene acids. Their data are presented in Table 15.

As a consequence, the TBA test and off-flavor evaluation will be much less correlated as the decreasing

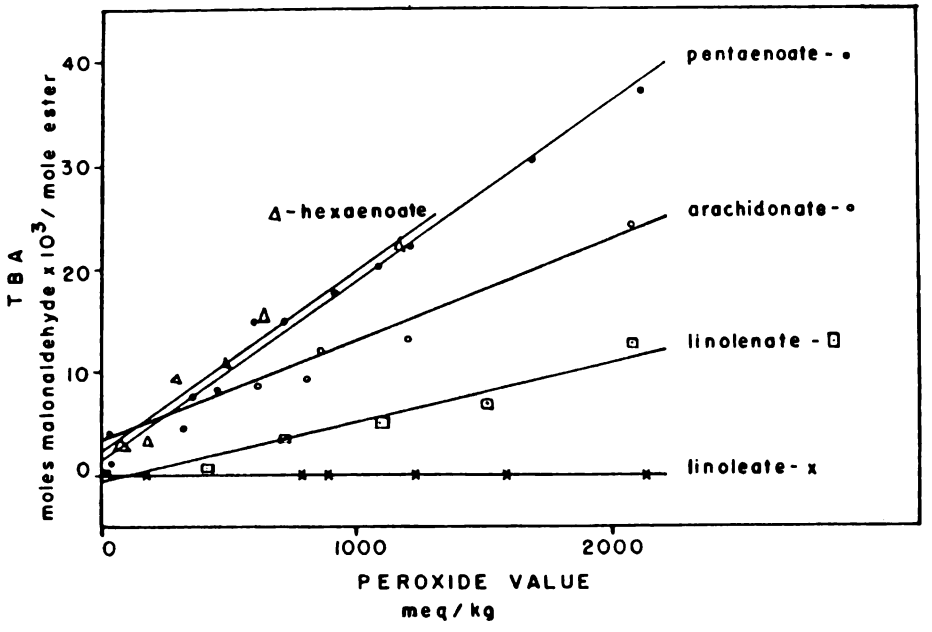


Figure 13. Relationship between peroxide value and TBA value in the oxidation of poly-unsaturated acids. (Source: Holman et al., 1962)

Table 15. Absorbance of Products of TBA Reaction with Different Types of Aldehydes per Mole of Aldehyde

Aldehyde	450 nm, 2 hr, 50C			450 nm, 2 h5, 70C			530 nm, 1 hr, 95C		
	MV	SD	n	MV	SD	n	MV	SD	n
Alkanals									
Pentanal	6.9	1.0	(3)	1.1	0.1	(3)	0.0	0.0	(3)
Hexanal	4.2	0.8	(3)	0.7	0.1	(3)	0.0	0.0	(3)
Heptanal	5.5	1.0	(3)	0.7	0.0	(3)	0.0	0.0	(3)
Decanal	8.3	2.8	(3)	2.7	0.6	(3)	0.0	0.0	(3)
Alkenals									
2-Hexenal	9.8	1.6	(6)	0.7	0.0	(6)	0.1	0.0	(6)
2-Heptenal	11.3	1.0	(6)	0.7	0.2	(7)	0.2	0.0	(6)
2-Octenal	5.1	0.9	(5)	0.9	0.1	(5)	0.2	0.0	(5)
2-Nonenal	8.2	1.2	(3)	1.0	0.1	(3)	0.1	0.0	(3)
2-Decenal	9.5	2.1	(3)	1.0	0.2	(3)			
Alkadienals									
2,4-Hexadienal	2.0	0.3	(4)	0.6	0.1	(4)	2.1	0.8	(4)
2,4-Nonadienal	5.4	0.4	(2)	1.0	0.0	(2)	2.6	0.4	(2)
2,4-Decadienal	3.2	0.6	(3)	0.8	0.2	(3)	2.5	0.4	(2)
2,4-Undecadienal	5.0	0.6	(3)	0.6	0.1	(3)	1.9	0.1	(3)
2,4-Dodecadienal	3.5	0.7	(4)	0.5	0.1	(4)	1.1	0.2	(4)

*Mean values (MV) ± standard deviation (SD) and number of determinations (n).

Source: Marcuse et al. (1971).

poly-unsaturated fatty acid compositions in food systems such as purified milk fat because the ratio of the fraction of β,γ-unsaturated, polyene conjugated components derived from autoxidized milk fat to the total off-flavor component will be much less than that of higher unsaturated fatty acid.

The Effects of Chemical Reactivity of Unsaturated Fatty Acid in Milk Fat and TBA Reagent

Kenstone et al. (1955) observed that oxidized linolenate (C 18:3) produced 60 to 100 times as much TBA color as oxidized linoleate (C 18:2); whereas, oleate (C 18:1) produced no color when all were measured at the same level of autoxidation indicated by peroxide value.

From the standpoint of autoxidation and chemical reactivity of poly-unsaturated fatty acid with TBA reagent, it may be true that the early stage of milk fat oxidation is predominantely governed by the trace amount of poly-unsaturated fatty acid components.

The reasons for the higher oxidation rates at the initial period of the TBA test in this investigation may be due to the oxidative behavior of trace poly-unsaturated fatty acids.

The Effects of Physical Structure of Freeze-dried Model Systems on the Accumulation of TBA Reactive Materials

In the comparison of the TBA color reactive materials of the milk fat oxidation (Runs 2 and 3, see Figure 14 on the following page), the possible total accumulation of TBA color reactants during the autoxidation period can be represented as a total integrated area of the autoxidation curves in the plots of TBA absorbance at 532 nm vs. time of storage period. The integrated areas at the early stages of oxidation increased as did the order a_w 0.75, a_w 0.12 and a_w 0.47, which represent an inversion of the oxidative pattern of the oxidative curves. From these results, it can be postulated that the TBA color reactants could be further oxidized to produce a less TBA reactive, volatile, very unstable and water soluble short carbon chain compounds probably saturated aldehyde, ketone, alcohols in freeze-dried model systems.

In the nature of porous and sponge like matrix of dehydrated food systems, the holding capacity of the water soluble, volatile, and unstable TBA reactive materials will be affected in the different ways from high moisture content food or liquid food systems. This could be a part of the explanation as to why many researchers have failed to demonstrate a correlation of the TBA test to other chemical and

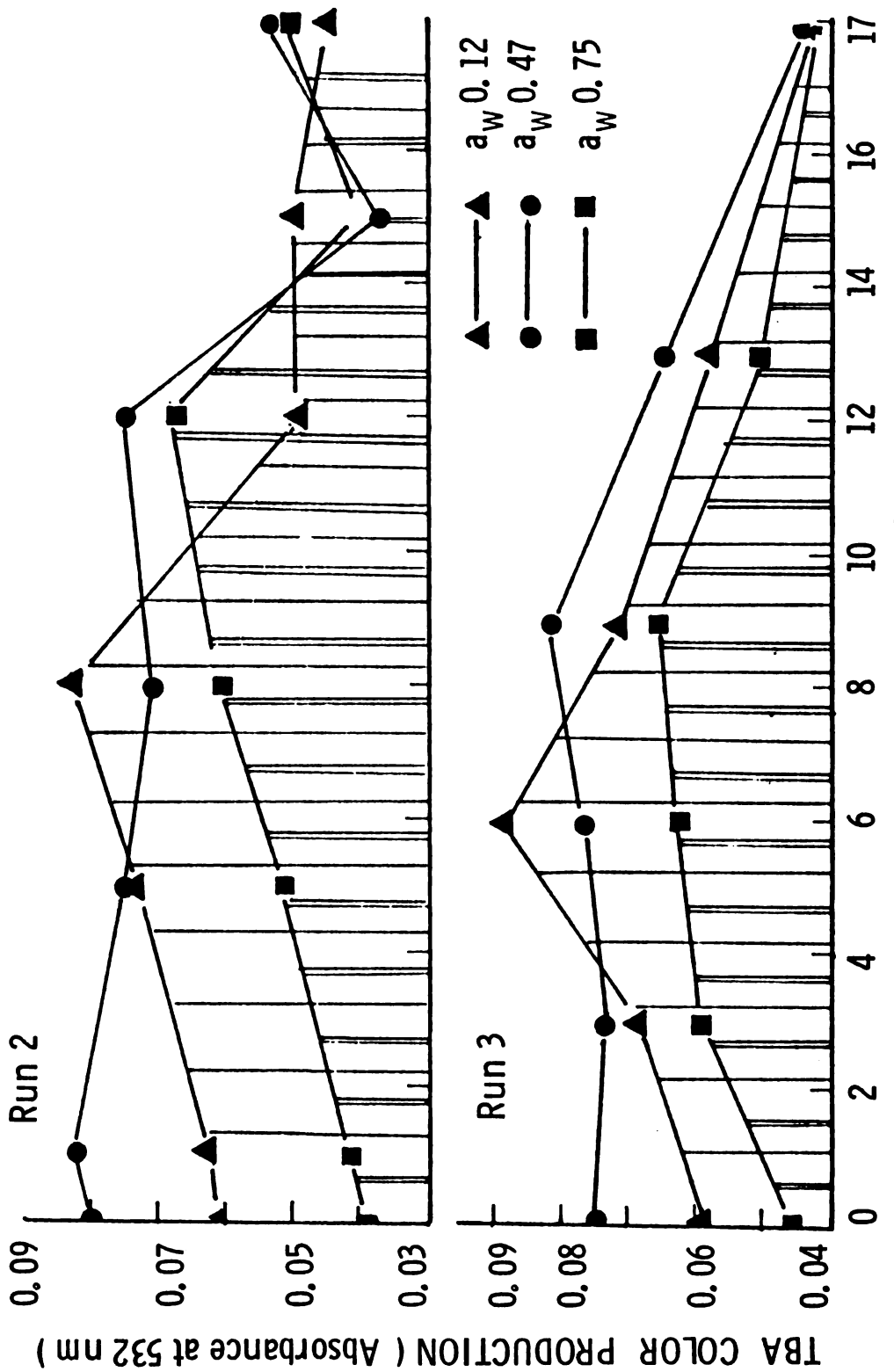


Figure 14. The accumulation of TBA reactive materials and effects of water activity in freeze-dried milk fat model systems.

sensory evaluation of oxidized lipid in dehydrated systems (Tarladgis, 1960; Holland, 1971; Corliss, 1963) as well as this investigation.

SUMMARY AND CONCLUSION

The organoleptic deterioration of milk fat components in dehydrated food systems has been a serious problem from the standpoint of consumer acceptance.

In the investigation of autoxidation, three different lots of milk fats were separated from 40% cream by a conventional churning method. The fat was melted, washed and concentrated under vacuum.

Three different water activities, a_w 0.12, a_w 0.47 and a_w 0.75 of freeze-dried fat-cellulose model systems were prepared by exposing the samples to specific relative humidity conditions. The effect of water activity on autoxidation of milk fat and the correlation of the 2-thiobarbituric acid test to other analytical methods of autoxidized lipid determination were examined by comparing the results of the peroxide test and off-flavor evaluation.

The conclusions of the milk fat oxidation studies for varying water activities are summarized as follows:

1. The moisture adsorption isotherm curve demonstrated a typical sigmoid pattern. The BET monolayer value was calculated by this moisture isotherm curve. The BET monolayer value of the milk fat model

systems based on micro-crystalline cellulose was a_w 0.23 and 0.37% of moisture content by dry base.

2. Autoxidation of milk fat in model systems was monitored and compared to the peroxide test, TBA test and off-flavor evaluation. The most applicable test for the autoxidation studies of the milk fat model systems was the peroxide test.

3. Autoxidation of washed milk fat in freeze-dried model systems showed a very slow oxidation rate in comparison to un-washed milk fat containing membrane lipids. This may be due to the effects of different amounts of phospholipid constituents.

4. The effects of varying water activities on the autoxidation of milk fat show protective (in a_w 0.47) and pro-oxidant (at a_w 0.12 and a_w 0.75) effects.

In the comparison of the oxidation rates for the milk fat in model systems with various water activities, at initial stage the water activity a little above the BET monolayer value was demonstrated to be most stable to lipid oxidation, while the water activity below the BET monolayer value (a_w 0.12) and well above the BET monolayer value (a_w 0.75) showed a rapid increase in autoxidation rate.

5. Although at the early stage of autoxidation of the milk fat model systems, there were difficulties in off-flavor evaluation and some variation in flavor judgments, the total off-flavor evaluation during a two-month period showed a correlation to the peroxide test but not to the TBA test.

6. The possible reasons are suggested for the poor correlation of the TBA test to the peroxide test and sensory evaluations:

   a. The off-flavor components of the autoxidized milk fat are, for the most part, constituents of low TBA reactivity, and the proportional increase of TBA color production is only found at the initial stage of autoxidation. As a consequence, the TBA test cannot represent the total off-flavor production and cannot be correlated with the peroxide test for a long storage period.

   b. The ratio of TBA color reactant to the total off-flavor components which are derived from the decomposition of hydroperoxide of autoxidized milk fat can be affected by the amount of poly-unsaturated fatty acid composition.

   c. The holding capacity of water soluble, volatile unstable TBA color reactant is very low in dehydrated food systems compared with high moisture

content food. The amount of accumulation of TBA reactant in freeze-dried model systems was affected by varying water activities and oxidation rates. The model systems with a_w 0.47 showed a higher accumulation of TBA color reactants, while the a_w 0.75 showed a lower rate of accumulation. The TBA color reactant seems to be oxidized further to produce more unstable and less TBA color reactive materials.

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