1938

# A critical study of the precision and value of halogen addition reactions applied to dairy research

Delbert F. Breazeale
*Iowa State College*

## Recommended Citation

Breazeale, Delbert F., "A critical study of the precision and value of halogen addition reactions applied to dairy research " (1938). *Retrospective Theses and Dissertations*. 13448.
https://lib.dr.iastate.edu/rtd/13448

# A CRITICAL STUDY OF THE PRECISION AND VALUE OF HALOGEN ADDITION REACTIONS APPLIED TO DAIRY RESEARCH

By

167

Delbert F. Breazeale

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subjects
Dairy Industry--Chemistry

Approved:

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Signature was redacted for privacy.

Heads of Major Departments

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1938

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

1126-56

ACKNOWLEDGEMENTS

The writer wishes to express his appreciation to Dr. Emerson W. Bird for designing and constructing the special apparatus employed, for suggestions during the progress of the investigation and for assistance in the preparation of this manuscript. The writer is also indebted to Dr. M. P. Baker for photographing the apparatus and preparing the pictures presented.

## TABLE OF CONTENTS

# A CRITICAL STUDY OF THE PRECISION AND VALUE OF HALOGEN ADDITION REACTIONS APPLIED TO DAIRY RESEARCH

## INTRODUCTION

### Introduction

In 1932 a project was initiated in this laboratory to study the distribution of free acids between the aqueous and fat phases in butter. It seemed desirable to determine not only the total amounts of acids present, but also some of the properties of these acids. The characteristics of the fat soluble acids that were considered of particular interest were the average molecular weight and the relative degree of unsaturation.

A suitable extraction procedure was developed for separating and recovering the free acids contained in filtered butter fat. Preliminary experiments indicated that the amounts of these acids which would be available for determining the degree of unsaturation would range usually from 20 to 50 mg. and would probably never exceed 110 mg. Therefore a micro or semi-micro method would have to be employed.

The degree of unsaturation of fats and fatty acid mixtures is determined, generally, by the use of the iodine number (percentage of iodine, or other halogens calculated as iodine, taken up by the sample). Furthermore, the relative amounts of different unsaturated acids present in a mixture have been calculated, following iodine number determinations by a combination of appropriate methods.

## Statement of the problem

The Association of Official Agricultural Chemists designate both the Wijs and Hanus methods as "Official". The Hanus reagent can be prepared more conveniently and is considerably more stable than the Wijs reagent; it has, therefore, been employed as a routine method in this laboratory for determining the iodine number of fats. It was assumed that this method, with some modifications, would be satisfactory with the fatty acids extracted from butter fat. The amount of Hanus reagent and the concentration of the sodium thiosulfate were each reduced to one-fifth of their recommended values because approximately one-fifth of the usual weight of sample was available. The results obtained with this method were unsatisfactory. Replicate determinations frequently showed considerable variation and the values were found to depend somewhat upon the weight of sample.

A brief search through the literature indicated that much research had been done on iodine numbers. However, it appeared that none of the methods, without some changes, could be applied to the present problem. At that time no semi-micro method had been described and only three micro methods. The literature was found to contain controversial statements with regard to the reaction obtained with some of these methods. Some investigators claimed that substitution as well as addition occurred while others refuted these statements. Very few iodine number studies included butter fat or the mixed acids obtained from butter fat and these materials were not used in any of the more fundamental investigations.

The problem, at its beginning, did not appear to be simple and it was decided, therefore, to study (on a semi-micro scale) several of the more important iodine number methods described in the literature with especial reference to the following points:

1. The relationship between the iodine number of butter fat and the mixed insoluble acids obtained from it.
2. The reproducibility of results.
3. The effect of experimental conditions.
4. The determination of the optimum conditions for the best method.
5. The relative amounts of addition and substitution obtained.

It was hoped that this study would permit the evaluation of the iodine number as a tool for certain dairy research.

## REVIEW OF LITERATURE

In 1928 Kolthoff and Furman (53) reviewed that part of the literature on iodine number determinations which was of particular interest from the standpoint of quantitative analysis and made the following comment: "A fundamental physico-chemical investigation of halogen addition (velocity of reaction under varying conditions and disturbing influences) is greatly to be desired. The present very extensive literature has a purely empirical character and does not yet enable us to draw generally valid conclusions." Since 1928 the literature on iodine numbers has increased steadily and although few studies have been reported on reaction velocities, considerable valuable information has been published.

Types of reagents. Hübl (33) found that iodine reacted very slowly with fats at room temperature and at higher temperatures the reaction was not smooth. Margosches and Hinner (58) observed that when fats in carbon tetrachloride solution were treated with aqueous iodine-potassium iodide solution the iodine numbers obtained varied inversely with the ratio of potassium iodide to iodine.

Hübl (33) obtained what he considered a satisfactory reaction of fat in chloroform solution with 0.2N alcoholic iodine containing one mole of mercuric chloride per mole of iodine. He assumed that the addition product was the iodochloro-derivative of the unsaturated fatty acid in the fat. Wijs (90) showed that the active agent in Hübl's reagent is iodine monochloride and is formed by the following reaction:

$$HgCl_2 + 2I_2 \rightleftharpoons HgI_2 + 2ICl$$

He also stated that HgICl may be formed as an intermediate product. Wijs (91) proposed a new method for the preparation of iodine monochloride solution. He dissolved 13 gm. of iodine in a liter of acetic acid, then bubbled chlorine through the solution until there was a definite color change. Marshall (63) prepared iodine monochloride in carbon tetrachloride solution in a similar manner. A somewhat different method was used by Hunter and Hyde (35); they allowed 0.05 mole of toluene p-sulfondichloramide to react with 0.10 mole of potassium iodide in one liter of glacial acetic acid.

Hanus (26) introduced iodine monobromide as a halogenating agent for fats. This was prepared by finely triturating 20 gm. of iodine to which were added dropwise 13 gm. of bromine. The reaction mixture was kept cool and was stirred continuously. Twenty grams of the compound were dissolved in one liter of glacial acetic acid. This solution was more stable than were those prepared by Hübl or Wijs. Hunt (34) simplified the method of preparing iodine monobromide by dissolving iodine in glacial acetic acid and adding enough bromine to double the titration value. Ralls (69) prepared

0.1N iodine monobromide in carbon tetrachloride by the same method.

Margosches, Hinner and Friedmann (60, 61) published a rapid iodine number method in which hypoiodous acid was the active agent. A 0.2N iodine solution in 96 per cent alcohol is added to the fat sample which is dissolved in absolute alcohol at room temperature or in 96 per cent alcohol at 50°C. The contents of the flask are well mixed, emulsified with water, allowed to react 3 to 5 minutes and the excess iodine is titrated with sodium thiosulfate. Margosches and Hinner (59) used 0.1N alcoholic iodine solution containing 1/60 equivalent of iodic acid. This solution was not stable.

Kaufmann and Grosse-Oetringhaus (48, 49) found that as little as two per cent iodine greatly stabilized solutions of thiocyanogen. They studied the reactions of solutions containing equivalent amounts of thiocyanogen and iodine and found the following equilibrium reaction:

$$I_2 + (SCN)_2 \rightleftarrows 2I(SCN)$$

Bromine has been used by many investigators as a halogenating agent for fats. Rosenmund and Kuhnhenn (74) employed a 0.1N solution of pyridine sulfate dibromide in glacial acetic acid. Kaufmann (39) and Kaufmann and Hansen-Schmidt (50) used 0.1N bromine in methyl alcohol saturated with sodium bromide. Volmar and Samdahl (85) recommended that fats be brominated in ether solution at 0°C. by adding bromine dropwise until a yellow color persists. Hehner (28) added bromine directly to fats dissolved in chloroform; the excess was removed by heating on a water bath. Bromine vapor has been employed by the following investigators: Becker (9), Toms (82,83), Böeseken and Polls (13), Wollschitt (93) and Rossman (71,72). Bülmann (10) studied the action of bromine water on unsaturated compounds.

Kaufmann (40) found that thiocyanogen could be used to determine simple ethylenic linkages. A catalytic hydrogenation method, the results of which are calculated in terms of iodine number, was described by Kaufmann and Baltes (46). Maleic anhydride has been used to determine conjugated double bonds by Kaufmann, Baltes and Büter (47), Ellis and Jones (22) and Kaufmann (42).

Fat solvents. André (1) found that the reactivity of iodine towards fats was somewhat greater in chloroform than in carbon bisulfide, carbon tetrachloride or acetic acid. Bankston and Vilbrandt (6) observed that higher and more consistent results were obtained when chloroform and carbon tetrachloride were used as fat solvents than when ether, alcohol or benzene were used.

The relative reactivities of some fat solvents with bromine have been reported by Kaufmann (41) and Kaufmann and

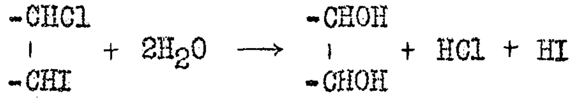
Hansen-Schmidt (50). Named in order of increasing loss in titer they are: carbon tetrachloride, acetic acid, carbon bisulfide, chloroform, methyl alcohol and ethyl alcohol.

Acid formation in reactions with Hübl's reagent. Hübl's reagent has been criticized for its lack of stability or loss in titer by many investigators including Fahrion (23), Hehner (28), Waller (87), Auguent (4), Wijs (89,90) and Schmidt-Nielsen and Owe (76). Fahrion observed that the stability of the reagent could be increased greatly by storing the iodine and mercuric chloride solutions separately and recommended that they be added separately to the fat. Although the French official method specified that the solutions be held separately, Auguent found that the alcoholic iodine solution gradually increased in acidity. As much as 10 gm. per liter of hydriodic acid were found in old solutions. The reaction is given as:

$$C_2H_5OH + I_2 \longrightarrow C_2H_4O + HI$$

Traces of acetic acid were found. Waller found that Hübl's reagent could be almost completely stabilized by the addition of 5 per cent of concentrated hydrochloric acid.

Ingle (37) believed that the addition of hydrochloric acid to Hübl's reagent caused the formation of the following compound: $ICl \cdot HCl$, and that because of the affinity of ICl for HCl its reactivity towards ethylenic compounds was diminished. Ingle (36) proposed the following mechanism, for the formation of acid during the reaction of Hubl's reagent on ethylenic linkages:



$$\begin{matrix}-CHCl\\ |\\ -CHI\end{matrix} + 2H_2O \longrightarrow \begin{matrix}-CHOH\\ |\\ -CHOH\end{matrix} + HCl + HI$$

Wijs (89) stated that false values were obtained when the acid formed in the Hübl reaction was calculated as iodine and subtracted from the total. He also observed that the constitution of the compound greatly influenced the amount of acid formed. The acids calculated as percentages of the total halogen consumed were linseed oil 11.3, peanut oil 12.0, oleomargarine 10.8, coconut fat 10.7, allyl alcohol 22.3 and cholesterol 50.8.

Schmidt-Nielsen and Owe (76) emphasized the fact that the change in titer of the blank determinations during the Hübl reaction caused serious errors. They recommended that the reaction period be at least 12 hours and preferably 24 hours. A formula was presented for the calculation of iodine numbers which was designed to correct for the change in titer of the blanks. The formula is:

$$J = \frac{127f}{100i}\left[(b_o - a) - \left(\frac{a}{b_t}\right)^2 (b_o - b_t)\right]$$

When $J$ = iodine number, $b_o$ = titration of blank at beginning of reaction, $b_t$ = titration of blank at end of reaction, $a$ =

titration of sample, f* = normality factor and i = sample weight.

Substitution reaction. Marshall (63) described a sensitive test for substitution when neutral solvents are employed. After the completion of the usual titration, potassium iodate was added and the titration then continued to the disappearance of the starch iodide color. No substitution was encountered when moisture was excluded from the halogenation reaction. Iodine monochloride was employed in carbon tetrachloride. The reaction which Marshall believed occurred in the presence of moisture was:

$$3ICl + 3H_2O \longrightarrow HIO_3 + 3HCl + 2HI$$

If substitution occurred in the absence of water the halogen acid formed was determined as follows:

$$6HCl + KIO_3 + 5KI \longrightarrow 6KCl + 3H_2O + 3I_2$$

and the liberated iodine was titrated with standard thiosulfate.

Hunt (34) prepared iodine monochloride and iodine monobromide in a commercial grade of carbon tetrachloride and with these solutions and Marshall's method he determined the amounts of acids formed with several oils. These values calculated in terms of iodine number ranged from 0.45 to 1.16

---

*If (f) is the normality of the sodium thiosulfate, the value (100) should be changed to (10).

units. He believed that the actual halogen added could be obtained by subtracting the halogen equivalent to twice the amount of halogen acid titrated from the total consumed. He also called attention to the fact that the end-points with this method were not as sharp as when acetic acid was used as the halogen solvent in the Wijs and Hanus methods.

Schmidt-Nielsen and Owe (76) criticized the Wijs method because increasing values were obtained with increasing periods of reaction. They assumed that substitution played an important part in the reaction. Wijs (92) refuted this statement with the argument that their curves did not have enough points to determine the course of reaction.

Werner (88) using iodine monochloride in carbon tetrachloride solution determined the amounts of acid formed in the reaction with some derivatives of cholesterol. With dihydrocholesterol the halogen consumed per mole varied from 0.27 at 1 hour to 1.25 at 24 hours and remained constant up to 144 hours. The halogen acid values were erratic and varied from 8 to 47 per cent of the total halogen consumed.

Kaufmann and Lutenberg (52) titrated the acids formed when 0.1N bromine in carbon tetrachloride reacted with China wood oil and $\beta$-elaeostearin. These values were low and there seemed to be little tendency for them to increase with increasing reaction period. The iodine numbers of $\beta$-elaeostearin were fairly constant from three to six hours, indicating reaction with two of the three double bonds. The values were slightly higher at 22 hours. They believed that substitution did not occur.

Rossmann (72) found that halogen acid was produced in his "titrimetric" bromine vapor method. However, when this acid was subtracted from the total halogen consumed, the values were much lower than the theoretical values of pure unsaturated compounds. Theoretical values were obtained when this acid was not considered. He assumed that the acid did not result from hydrogen substitution but probably from a reaction of the addition product with water and potassium iodide.

After determining the acids formed in a large number of compounds when treated with 0.1N iodine monobromide in carbon tetrachloride, Ralls (69) made the following statement: "Study of the results given shows that we cannot put forth the described method as the 'universal' in which all reactions, save addition, are completely prevented in all cases. Nor do we feel that the custom of subtracting twice the halogen acid from the total halogen and calling the result the halogen of addition is fully justified--because substitution is not the only side reaction producing halogen acid."

<u>Effect of experimental conditions on the reaction.</u> Hanus (26) stated that the values obtained with his method checked

Hübl values when he used about 80 per cent excess reagent for substances having iodine numbers less than 120 and 100 per cent excess for substances having higher values. A fairly complete study was presented by Schmidt-Nielsen and Owe (76) on the effect of variations in excess reagent and variable periods of reaction. Several fats and oils were used and the methods of the following authors were compared: Hübl, Wijs, Hanus, Waller and Winkler. Hanus and Wijs values were especially dependent upon excess reagent and duration of reaction. The Waller method gave much lower values which lacked reproducibility. The Winkler method was very sensitive to light, but if the reactions were performed in a dark room with red light the results were satisfactory. The chief advantage of the method is that the halogenating solution (potassium bromate) is stable. The Hübl method, with the results calculated according to the formula which Schmidt-Nielsen and Owe developed, was found to give nearly constant values under the conditions studied.

Jamieson (38) determined the iodine number of a few oils by the Hanus method using 1/2 and 3/4 hour reaction periods and concluded that there was no advantage in using the latter. Hawley (27) found that iodine values obtained by the Rosenmund and Kuhnhenn method were not appreciably altered by variations in either the excess of reagent or in the duration of the reaction. Dam (17) studied the effect of variable reaction periods and excesses of reagent on cholesterol with the methods of Hübl, Waller, Wijs, Hanus, Winkler and Rosenmund and Kuhnhenn. The last two methods gave by far the most nearly constant values and also most nearly approached the theoretical values.

Margosches, Friedmann and Tschörner (57) observed that iodine numbers determined by the method of Margosches, Hinner and Friedmann (61) increased with increasing periods of reaction and that the halogen acid increased also. The magnitude of these increases varied with different oils. The difference between the iodine number obtained at 5 minutes and at 24 hours was termed the "periodine number". Margosches, Ludwig, Scheinost and Tschörner (62) used this method to determine the relative amounts of different oils in mixtures.

Ralls (68) stated that iodine number-time curves of cholesterol with Hanus solution diluted 1 : 1 with acetic acid showed no flat portion up to 90 minutes. The values ranged from 137 to 175 per cent of the theoretical when chloroform was used as the solvent and from 112 to 117 per cent when carbon tetrachloride was used. Ho, Wan and Wen (31) studied the reaction of the Wijs reagent on tung oil. The iodine number was found to be increasing slowly at the end of a reaction period of 12 days. If the temperature were raised

10°C., approximately the same iodine number was obtained in half the reaction period. A straight line was obtained when iodine numbers were plotted against the logarithm of excess reagent (cg. $I_2$ per gm. fat).

Equilibrium constants. Van der Steur (77,78,79) allowed the reaction of 0.1N iodine in carbon tetrachloride with fats and fatty acids to proceed until an equilibrium had been reached and then calculated the equilibrium constants as follows:

$$K = \frac{\text{C iodine addition compound}}{(\text{C double bonds})(\text{C iodine})}$$

The units in which these concentration terms (C) are expressed were not defined. The Hübl method was used to determine the concentration of double bonds. The values of K varied when concentrations of reactants were varied with oils, which contained glycerides of different unsaturated acids. These values were constant for pure acids. At 0°C. (in carbon tetrachloride solution) K for oleic acid was 94.7 while that of its isomer elaidic acid was only 5.0. At 19.5°C. these values were 26.3 and 2.0 respectively. In benzene solution the corresponding values were 9.2 to 9.8 and 0.56 to 0.72. Thus these acids take up only about one-third as much iodine in benzene as in carbon tetrachloride.

Dehalogenation. Michael (64) dissolved the dibromides of ethylene, propylene and iso-butylene in ether and refluxed them with zinc for 4.5 hours. The percentages of bromine liberated were 45.4, 66.8 and 91.8 respectively. Van Duin (20,21) studied the velocity of this reaction:

$$RCHBr \cdot CHBrR' + 2MeI \longrightarrow RCH = CHR' + I_2 + 2MeBr$$

The rates of reaction were found to be somewhat greater in 0.5N potassium iodide than in 1 normal which indicates that iodide ions are more important than undissociated molecules. The rates of these reactions were influenced very markedly by different substituent groups. The reaction velocities could be increased by the addition of sodium, potassium or calcium ions.

Böeseken and Gelber (12) published a method for the determination of iodine numbers which was based upon the fact that iodides remove halogen from the addition product. At the end of the reaction calomel or finely divided silver was shaken with the reaction mixture to remove the free halogen. The filtrate from this mixture was treated with alcoholic sodium iodide and the halogen which was removed from the addition product was titrated with sodium thiosulfate. This method is especially useful in the determination of the iodine number of compounds containing a phenyl or carboxyl group adjacent to the double bond. Heat and light were found to increase the rate at which halogens were removed from the addition product.

Velocity of addition reaction. Herz and Mylius determined the velocity of the addition of bromine to cinnamic acid (29) and the addition of iodine to allyl alcohol (30). The velocity constants (k) were calculated according to the bimolecular reaction formula:

$$k = \frac{2.303}{t(a-b)} \cdot \log \frac{b(a-x)}{a(b-x)}$$

Where a = concentrations of unsaturated compound, b = concentration of halogen and t = period of reaction. They found that the rate of reaction was three to four times greater in carbon tetrachloride than in chloroform solution. These reactions were performed in sealed tubes. Bauer and Moser (8) determined the velocity constants of the reaction of bromine on stilbene and methyl stilbene. They found that the velocities were greater in chloroform than in carbon tetrachloride solution. Glass stoppered bottles were used for these reactions which may explain the discrepancy between their results and those of Herz and Mylius.

Sudborough and Thomas (80,81) found that bromine added to crotonic acid nearly 100 times more rapidly in daylight than in the dark. They studied the effect of substituent groups on the velocity of the reaction and concluded that (a)

$\alpha,\beta$-unsaturated acids combine less readily with bromine than their isomers in which the double bond is farther away from the carboxyl group, (b) introduction of a methyl group to the olefinic carbon atom makes addition of bromine easier and (c) when the acid contains a conjugated system of double bonds, one of which is in the $\alpha,\beta$ position to the carboxyl group, addition takes place more readily than when the acid contains only the $\alpha,\beta$ double bond. Bauer (7) observed that bromine added readily to acrylic and crotonic acids but failed to add to tribromoacrylic acid or to dibromocrotonic acid.

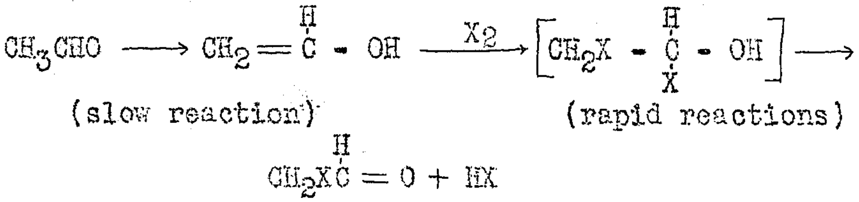
Böeseken and Blumberger (11) determined reaction velocities of iodine and unsaturated compounds in chloroform solution. They found that when old solutions of iodine in chloroform were used the velocity constants diminished with increasing periods of reaction and that this did not occur when fresh solutions were used or when benzene was used as the solvent. Their explanation is that iodine monochloride, which has a much greater activity than iodine, is formed slowly according to the following reaction:

$$CHCl_3 + I_2 \longrightarrow CHCl_2I + ICl$$

Dawson, Burton and Ark (18) showed that in neutral, aqueous solution aldehydes are oxidized by halogens as in the following example:

$$CH_3CHO + Br_2 + H_2O \longrightarrow CH_3COOH + 2HBr$$

The reaction velocity was about 800 times greater with bromine than with iodine. In normal solutions of hydrochloric, hydrobromic or sulfuric acids the reaction velocity was independent of the kind of halogen or its concentration. Under these conditions substitution occurred according to these reactions:



$$CH_3CHO \longrightarrow CH_2=\overset{H}{C}-OH \xrightarrow{X_2} \left[CH_2X-\overset{H}{\underset{X}{C}}-OH\right] \longrightarrow$$

(slow reaction) (rapid reactions)

$$CH_2X\overset{H}{C}=O + HX$$

As they ascended the aldehyde series, the rate of reaction increased. Dawson and Wheatley (19) observed that the velocity of the reaction between ketones and iodine depended upon the rate at which the ketones enolized, and that this change was greatly influenced by the groups attached to the carbonyl carbon. For example, when the velocity of the reaction with acetone was considered as unity, the relative values for phenyl ethyl ketone, acetophenone and benzophenone were 0.82, 0.37 and 0.0 respectively.

Caldwell and Piontkowski (15) obtained nearly theoretical values with the Hanus and thiocyanogen (Kaufmann's) methods on oleic and erucic acids. With ricinoleic acid the thiocyanogen values were about theoretical but the Hanus values were too high. With both methods low values were obtained with furoic acid, and maleic acid did not react. Kaufmann and Baltes (44) believed that the carbonyl group in licanic acid caused serious errors with the usual iodine number methods. Thiocyanogen gave nearly theoretical values for one double bond since this reagent does not substitute hydrogen. Rossmann (73) observed that with the bromine vapor method on compounds containing hydroxyl groups close to the double bond, as ricinoleic acid, slow reaction occurred up to double the theoretical value. Similar reaction was found with oxidized drying oils. Böeseken and Polls (13) indicate that a trace of moisture will cause esterification in the reaction of ricinoleic acid and bromine vapor. The reactions are:

$$H_2O + Br_2 \longrightarrow HBr + HOBr \qquad \text{and}$$

$$-\overset{|}{\underset{|}{C}}-OH + HBr \longrightarrow -\overset{|}{\underset{|}{C}}-Br + H_2O$$

Silver analysis of the brominated product showed 44.6 per cent silver bromide against 46.0 per cent calculated for tribromostearic acid. Böeseken and Polls claimed that correct values could be obtained if the compound were first acetylated. Yoshiyuka and Ishikawa (96) obtained 12-hydroxystearic acid from hydrogenated castor oil and determined iodine numbers by the Wijs and Rosenmund and Kuhnhenn methods on this compound. At 25°C. the Wijs values varied from 2.8 to 9.8 at 0.5 to 4.0

hour reaction periods, while those of the Rosenmund and Kuhnhenn method varied from 14.7 to 30.0 at 0.5 to 2.0 hour periods. Both methods gave somewhat higher values at 30°C.

No evidence has been found in the literature to indicate whether or not the iodine number of butter fat is affected by the presence of hydroxyl or carbonyl groups. Hafner, Swinney and West (25) reported that butter fat contained hydroxy compounds. Expressed as the mg. of acetyl bound per gm. of fat the value given for a sample of butter fat was 2.7 while that for castor oil was 125.0. The acids obtained from the butter fat had a lower acetyl value than did the fat, although this increased with the acids at a more rapid rate during storage than it did with the fat.

It is well known that the ethylenic bonds in different fatty acids show different reactivities towards halogens. With $\beta$-elaeostearic acid, which contains a conjugated system of three double bonds, Kaufmann (41) and Kaufmann and Lutenberg (52) reported that (a) thiocyanogen values correspond to one double bond, (b) values obtained with 0.1N bromine in methyl alcohol saturated with sodium bromide, correspond to two double bonds, and (c) 0.1N bromine in carbon tetrachloride gives values corresponding to two double bonds when the reaction is carried out in the dark, but all three bonds will become saturated in the presence of ultra violet light. Böeseken and Gelber (12) also pointed out that conjugated double bonds reacted much more slowly than non-conjugated bonds.

The addition product. A few investigators have analyzed the addition products obtained with some of the iodine number methods. Mahle (56) found that the addition product from oleic acid and Hübl's reagent contained nearly equivalent amounts of iodine and chlorine. Ingle's (36) results show that stilbene iodochloride was formed in the reaction of stilbene with the Wijs reagent. Holde and Gorgas (32) obtained the following addition products:

oleic acid + HOI (Margosches method) → iodohydroxystearic acid
erucic acid + HOI " " → iodohydroxybehenic acid
erucic acid + IBr (Hanus method) → iodobromobehenic acid
linoleic acid + IBr " " → diiododibromostearic acid

Werner's (88) analyses showed that the amount of chlorine was somewhat greater than the amount of iodine in the product from oleic acid and Hübl's reagent.

Relative amounts of different unsaturated glycerides in fats. Attempts have been made to calculate the amounts of different unsaturated glycerides in fats from the data secured by determining iodine numbers, thiocyanogen numbers, diene numbers and hydrogen numbers. Kaufmann (40) found that thiocyanogen reacted with only one of the two double bonds in linoleic acid. Since the usual iodine number methods react with both double bonds the percentages of oleic and linoleic acids in mixtures can be calculated. Arup (2) used the Wijs iodine number and the Kaufmann thiocyanogen number on several samples of butter secured from widely separated geographical areas. The calculated linoleic acid values ranged from 3.3 to 4.3 per cent. Kaufmann and Baltes (43,45) presented many equations by means of which different combinations of unsaturated acids could be calculated when present in mixtures. Bolton and Williams (14) obtained good agreement between the calculated values of elaeostearin in China wood oil and the values obtained by a determination of polymerizable glycerides.

Comparisons of iodine number methods. Many investigators have compared two or more methods--generally against some official method, such as Hübl's or Wijs'. This type of research was severely criticized by Volmar and Wagner (86) because the so-called official methods do not give correct values with many substances. Rossmann (73) stated that all volumetric iodine number methods are relative and their values depend upon temperature, light, concentration of reagent, duration of reaction and the presence of conjugated bonds. Since these statements can not be denied, it seems sufficient to give the following list of authors who have presented some work on comparisons of methods: Hunt (34), Tolman and Munson (84), Ingle (37), MacLean and Thomas (55), Schmidt-Nielsen and Owe (76), Dam (17), Yasuda (95), Ralls (68), Werner (88), Godbole, Ketkar, Sharma and Kamath (24), Pelikan and Mikusch (67), Netto (65), Yamaguchi, Matsumura and Takagi (94) and Yoshiyuka and Ishikawa (96).

Micro and semi-micro iodine number methods. Micro adaptations of the Rosenmund and Kuhnhenn method have been described by Page, Pesternack and Burt (66) and by Yasuda (95). Babkine (5) used a semi-micro Hübl method. Margosches reagent was employed by Ruziczka (75) in a micro method. The Hanus reagent was used by Chargaff (16). Ralls (69) employed 0.1N iodine monobromide in carbon tetrachloride solution for micro work. The bromine vapor method of Becker (9) has been used with small sample weights by Böeseken and Polls (13), Rossmann (73) and Wollschitt (93). Kaufmann and Hartweg (51) described a semi-micro method in which 0.1N bromine in methyl alcohol saturated with sodium bromide was employed.

# EXPERIMENTAL

## General methods

Apparatus. The reaction flasks used for the semi-micro iodine determinations were of conventional design, had a capacity of 125 ml., ground-glass stoppers and a rim around the neck for liquid seal. Special pipettes were employed for measuring the halogenating solutions and aliquots of fat or fatty acid solution. The pipette used to measure the samples was calibrated to deliver 2 ml. at 25°C., while the one used for halogenating reagents delivered 5 ml. at the same temperature. The stopcocks which were sealed onto these pipettes aided materially in the measurement of volatile solutions. Reading errors were minimized by using capillary tubing for the portion of the pipettes which contained the calibration marks. The 2 ml. pipette is shown in plate 1.

In the experiments in which only one halogenating solution was used, the siphon filling pipette (plate 1) was employed. Except for the siphon into the reagent bottle, this pipette was similar in construction to those previously mentioned.

Ordinary 50 ml. burettes graduated to 0.1 ml. were used for the titrations with standard $Na_2S_2O_3$. A 10 ml. automatic filling burette graduated to 0.02 ml. was used in some phases

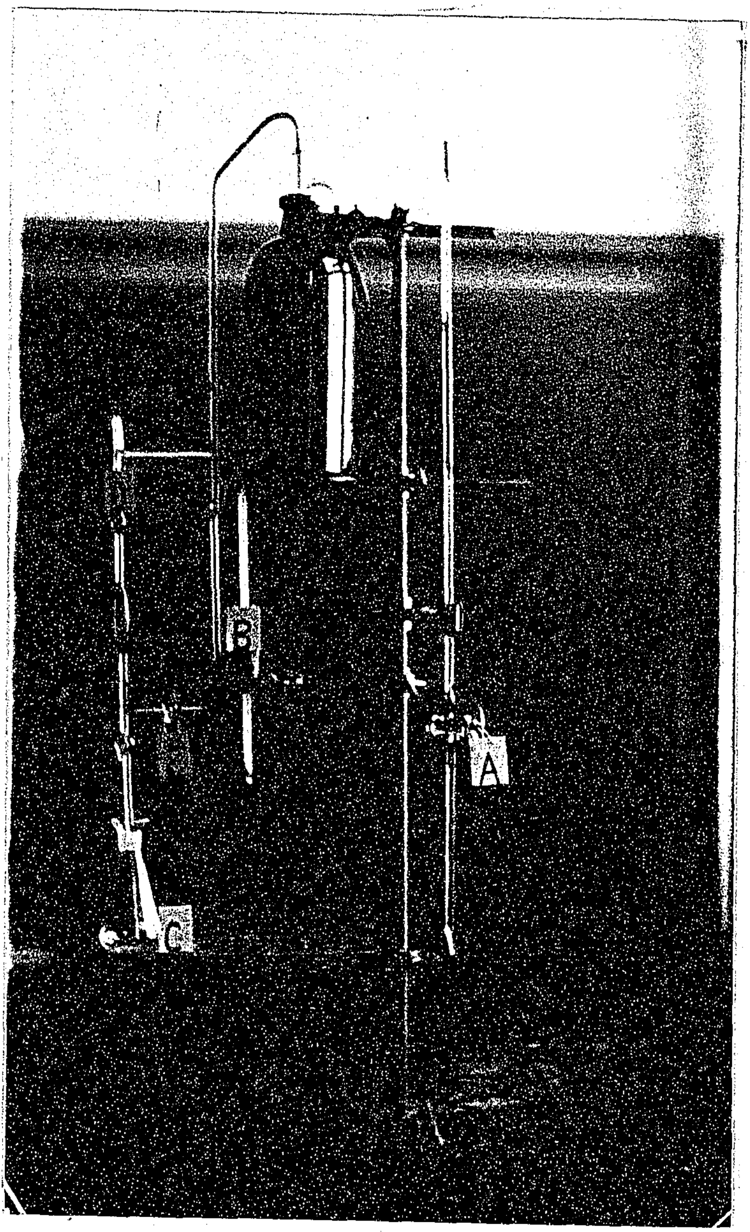


Plate 1. Pipettes used for samples (A) and halogenating solutions (D).

of the work. Calibrated (Bureau of Standards) pipettes and volumetric flasks were used in the preparation and standardization of $KIO_3$ and $Na_2S_2O_3$ solutions.

In the early part of the investigation a chainomatic balance was the only one available. The sensitivity of this balance was such that 0.36 mg. displaced the zero point 1 scale space. Therefore, it should have been possible to obtain sample weights which were correct to 0.1 mg. Because of mechanical imperfections of the balance (which developed while this study was in progress), greater variations in weights occurred at times. However, these errors were probably very small for the majority of the data which were obtained with this balance, because fairly large samples of substances were weighed into volumetric flasks, made to volume with the appropriate solvent and 2 ml. aliquots pipetted into the iodine flasks. A more sensitive balance was used for the last part of the work. The sensitivity of this balance was such that 0.18 mg. displaced the zero point 1 scale space.

A constant temperature of 25°C. ± 0.4° was used for all of the reactions. A thermostatically controlled chamber from which light was excluded was employed.

Preparation of butter fat and insoluble butter acids. The butter fat was obtained by melting butter at 50° to 60°C. until the fat had separated from the serum. The fat was then filtered at about the same temperature.

The insoluble butter acids were obtained as follows: Ten grams of the filtered fat were refluxed with 75 ml. 1N alcoholic KOH for 30 min.; 200 ml. $H_2O$ were added and the heating was continued until nearly all of the alcohol was removed. The solution was acidified with dilute $H_2SO_4$, boiled until the acids separated as an oily layer and cooled until the acids formed a solid cake. These acids were washed with water, were boiled in water for several minutes and were cooled and washed. This process was repeated until very little odor of butyric acid remained. The acids were dissolved in petroleum ether (b. p. 30° to 40°C.), dried overnight with anhydrous $Na_2SO_4$ and filtered. The petroleum ether was distilled off and the last traces were removed under 20 inches vacuum at 90° to 100°C. The samples were stored at 0° to 5°C. (in the dark until needed).

Preparation and standardization of reagents. Different concentrations of sodium thiosulfate have been employed in this investigation depending on the amount and concentration of halogenating reagent required for the different types of experiments. The concentration of sodium thiosulfate was regulated so that between 40 and 50 ml. were required to titrate the total halogen, i.e. blanks, of each reagent. The three principal concentrations of sodium thiosulfate employed were 0.11N, 0.022N and 0.01N.

It is well known that sodium thiosulfate solutions are not completely stable and that rapid changes in titer may occur unless these solutions are carefully prepared and stored. This is especially true of 0.01N solutions. Solutions prepared according to the following procedure have maintained nearly constant titer for several months. The flasks and bottles were cleaned with chromic acid cleaning solution. The water used was redistilled from an all-glass still to eliminate copper contamination and then boiled and cooled to expel dissolved oxygen and to destroy bacteria. The required amount of reagent grade of sodium thiosulfate pentahydrate was dissolved and diluted to the correct volume. The addition of 0.02 per cent of sodium carbonate as recommended by Kolthoff and Furman (53) was employed to prevent oxidation of the thiosulfate. Obviously, sodium carbonate can not be added when the acids formed in the reaction are to be determined iodometrically. Approximately 1 ml. of toluene per liter of solution was added to inhibit the growth of bacteria. As a further precaution, the air which entered the solution bottle was washed through alkaline pyrogallol solution.

Solutions of potassium dichromate, purified iodine in potassium iodide and potassium iodate have been used as primary standards to determine the concentration of sodium thiosulfate. The most satisfactory reagent, especially for 0.01N solutions, is potassium iodate. The methods of Kolthoff and Sandell (54) were followed for the standardization of solutions of sodium thiosulfate against potassium iodate and against iodine in potassium iodide. The normalities of a sodium thiosulfate solution calculated from the results of these two methods were 0.11130 and 0.11129 respectively. The potassium iodate and sodium thiosulfate solutions were each diluted 1 : 10 and when the same method of standardization was applied, the normality found for the diluted thiosulfate was 0.011123.

Starch indicator solutions were prepared by adding slowly a thin starch paste containing 5 gm. starch to one liter of boiling water and boiling the mixture for 2 to 3 min. The starch solution was transferred to 150 ml. bottles and sterilized in an autoclave for 25 min. at 15 lbs. pressure. Such solutions maintained their sensitivity for many months.

In some of the experiments a solution of iodine in potassium iodide was used to back titrate a slight excess of sodium thiosulfate. Since iodine has an appreciable vapor pressure, such solutions rapidly drop in titer unless special precautions are taken. A ratio of $I_2$ : KI of 1 : 4 was found to be satisfactory. The solution was used with an automatic filling burette in a closed system to prevent volatilization. The air which was admitted into the system was washed through a solution having the same concentration of potassium iodide as the solution used for titration but from 2 to 4 times as much iodine.

The reagents used for the Wijs and Hanus methods were prepared according to the directions given by the Association of Official Agricultural Chemists (3). Since Schmidt-Nielsen and Owe (76) have studied the Hübl method exhaustively, their methods were followed for the preparation and use of this reagent. The methyl alcohol-Hübl reagent was prepared in the same manner except that absolute methyl alcohol was used instead of 95 per cent ethyl alcohol. The directions given by Kaufmann and Hansen-Schmidt (50) and Rosenmund and Kuhnhenn (74) were followed for the preparation of the reagents used for their methods.

Procedure. Although the details of the methods varied somewhat with the different types of experiments, some general procedures were followed throughout the investigation. In the experiments in which a series of iodine numbers were determined on a sample, one sample was weighed into a volumetric flask, dissolved in the solvent under consideration, and after it had reached the same temperature as was maintained in the constant temperature chamber it was diluted to volume. This solution was transferred to a larger glass-stoppered flask, thoroughly mixed and 2 ml. aliquots were pipetted into the iodine flasks. The halogenating solution (5 ml.) was added to the sample, the flask was quickly stoppered, the contents were mixed by gently rotating for a few seconds and the flask was placed in the constant temperature chamber. At the end of the reaction period the required amount of potassium iodide was added quickly to the solution and was thoroughly mixed by rotating for approximately 20 sec. The stopper was rinsed into the flask with the dilution water. The solution was titrated with standard sodium thiosulfate as rapidly as possible. When the end point was nearly reached, 4-5 ml. of 0.5 per cent starch solution were added, the flask was stoppered and shaken violently, the stopper was rinsed into the flask and the titration was continued to the disappearance of the blue iodine-starch color. In order to increase the sensitivity of the end points a slight excess of sodium thiosulfate was added in some of the experiments and this excess was titrated with a more dilute solution of iodine in potassium iodide.

Some investigators have indicated that they believed some abnormal results might have been caused by the presence of traces of heavy metals. For this reason the following procedure was used to clean the reaction flasks: The reaction mixture was rinsed out with hot tap water, the flasks were immersed in sulfuric acid at 100° to 150°C. to which a little nitric acid was added at frequent intervals. After at least 30 min. in the acid bath the flasks were rinsed several times, in the order named, with tap water, distilled water and finally with redistilled water from the all-glass still. The flasks were dried in a hot air oven before they were used.

## Results

Preliminary results. Eighteen samples of butter fat were analyzed in duplicate by the Hanus method and by a semi-micro adaptation of this method. The average iodine number obtained for the regular method was 32.00 while the average for the semi-micro method was 33.16. The sample weights were approximately 0.4 gm. for the regular method while with the semi-micro method the sample weights ranged from 20 to slightly more than 100 mg. Differences among the light and heavy samples of the same fat were not great enough to account for difference between the averages. The quantities of reagents used with the Hanus methods were: Regular - 25 ml. halogenating reagent, 10 ml. of 15 per cent potassium iodide, 100 ml. distilled water (added immediately after the potassium iodide) and 0.11N sodium thiosulfate; for the semi-micro method 5 ml. halogenating reagent, 2 ml. potassium iodide, 25 ml. distilled water (added as above) and 0.022N sodium thiosulfate.

In an attempt to determine the reason for the discrepancy between the two methods, some of the experimental conditions were varied with the semi-micro method. In table 1 data are presented which show that increasing amounts of potassium iodide had little or no effect on the iodine numbers.

Table 1

Effect of the Amount of Potassium Iodide on the Iodine Number of Butter Fat. Semi-micro Method.

| ml. 15% KI | Iodine number | Average |
|---|---|---|
| 2 | 33.75<br>33.73 | 33.74 |
| 3 | 33.54<br>33.84 | 33.69 |
| 5 | 33.72<br>33.89 | 33.81 |

Apparently the amount of water which is used to dilute the reaction mixtures after the addition of potassium iodide is not important when the titrations are performed immediately. The data in table 2 do not show any general trends with increasing amounts of water.

Table 2

Effect of the Amount of Dilution on the Iodine Number of Butter Fat. Semi-micro Hanus Method.

| ml. $H_2O$ | Iodine number | Average |
|---|---|---|
| 0 | 33.93<br>33.84 | 33.88 |
| 20 | 33.91<br>33.43 | 33.67 |
| 30 | 34.05<br>34.09 | 34.07 |
| 40 | 33.85<br>33.93 | 33.89 |

In some experiments it is much more convenient to add the potassium iodide solution and dilution water to a series of reactions before making the titrations than to complete a titration before adding potassium iodide and water to the next flask. In order to determine whether or not potassium iodide would remove halogen from the fat addition product, the data in table 3 were secured. When the reaction mixtures were diluted immediately after the potassium iodide was added, the changes in iodine numbers were relatively small if the samples were titrated within two hours. The iodine numbers decreased appreciably when the dilution water was not added until the time of the titration.

The work of Schmidt-Nielsen and Owe (76) indicated that when their special calculation was employed, the Hübl method yielded results that varied less with excess reagent and

### Table 3

Effect of the Length of the Reaction Period of Potassium Iodide on the Iodine Number of Butter Fat. Semi-micro Hanus Method.

| Reaction period of KI in hours | 25 ml. $H_2O$ added immediately after the addition of KI | | 25 ml. $H_2O$ added at the end of the reaction period | |
|---|---|---|---|---|
| | Iodine number | Average | Iodine number | Average |
| 0.0 | 34.28<br>33.81 | 34.04 | 34.04<br>33.67 | 33.86 |
| 0.5 | 34.41<br>33.98 | 34.19 | 32.01<br>32.49 | 32.25 |
| 2.0 | 33.87<br>33.77 | 33.82 | 31.86<br>31.93 | 31.90 |

variable reaction periods than any other iodine number method which they studied. It seemed desirable, therefore, to include the Hübl method in the present study.

A semi-micro Hübl method was designed in which from 10 to 100 mg. samples, 5 ml. Hübl reagents and a 24 hour reaction period were employed. The reaction was arrested with 3 ml. of 15 per cent potassium iodide solution, 25 ml. of water were added and the titrations were performed with standard sodium thiosulfate solution. Blanks on the reagents were titrated at the beginning and end of the reaction period. The iodine numbers were calculated either (A) by subtracting the titration of the sample from the average of the beginning and end blanks or (B) by the Schmidt-Nielsen and Owe formula (page 13). Data are presented in table 4 which were secured with this method

Table 4

Effect of Variations in Sample Weights on the Iodine Number of Insoluble Butter Acids. Semi-micro Hanus (30 min. reaction) and Semi-micro Hübl (24 hr. reaction)

| Semi-micro Hanus | | Semi-micro Hübl | | |
|---|---|---|---|---|
| Sample weight | Iodine number | Sample weight | Iodine number A | Iodine number B |
| 0.0159 | 41.56 | 0.0149 | 48.70 | 40.17 |
| 0.0507 | 41.13 | 0.0492 | 42.59 | 41.73 |
| 0.1060 | 39.98 | 0.1023 | 41.41 | 41.84 |
| 0.1795 | 37.95 | 0.1622 | 41.15 | 41.82 |

Each value in table 4 is an average of triplicate determinations.

A. Average of beginning and final blanks used in calculations.

B. Schmidt-Nielsen and Owe formula used to calculate iodine numbers.

and with the semi-micro Hanus method. Sample weights were allowed to vary over a wide range in this trial. The iodine numbers were most nearly constant when calculated by the Schmidt-Nielsen and Owe formula, although even in this case the maximum variation was 1.67 iodine units. The maximum variation with the Hanus method was 3.61 units; the variations in sample weights with this method were slightly greater than were those with the Hübl method.

The results of the above experiment indicated that the semi-micro Hübl method deserved further study. The rate at which acid increases in Hübl solutions is much greater when the alcoholic solutions of iodine and mercuric chloride are mixed together than when they are stored separately. In the work herein reported the two solutions were mixed together 24 hours before they were used because the titer drops much more rapidly during the first few hours after mixing than it does later. It was known from a study of the literature that old Hübl solutions, even when stored separately, contain a considerable amount of acid--as much as 10 gm. of HI per liter. In order to determine the effect which this acid would have on iodine numbers, comparisons were made on a sample of insoluble butter acids using an old solution, a freshly prepared solution and the freshly prepared solution to which hydriodic acid had been added in an amount approximating that contained in the old solution. The results of this experiment, which are presented in table 5, show that the highest iodine numbers were obtained with the acidified solution and the lowest with the freshly prepared, unacidified solution. The differences between the two methods of calculating the results were least with the old solution and greatest with fresh solution.

Table 5

Effect of the Age of Hübl Reagents and the Addition of Hydriodic Acid to a Freshly Prepared Solution on the Iodine Number of Insoluble Butter Acids.
(Reaction Period 24 hours.)

| | Fresh solutions | Fresh solution + 6.6 gm. HI per liter | Reagents about 6 mo. old |
|---|---|---|---|
| I no. A | 38.50 | 38.82 | 38.71 |
| I no. B | 37.976 | 38.60 | 38.55 |

Each value in table 5 is an average of triplicate determinations.

A. Average of beginning and final blanks used in calculations.

B. Schmidt-Nielsen and Owe formula used to calculate iodine numbers.

An attempt was made to determine whether or not a general relationship existed between the iodine number and the amount of hydriodic acid contained in Hübl reagents. The data presented in table 6 show that (a) the iodine numbers of butter fat and insoluble butter acids increase with increasing additions of acid, (b) additions of acid were not high enough to obtain a maximum iodine number, although twice the concentration of acid was used that has been reported present in old Hübl solutions and (c) the differences between the two methods of calculating iodine numbers become less as the hydriodic acid concentration is increased.

It seemed reasonable that Hübl reagents might be more satisfactory if a non-reactive solvent could be employed.

Table 6

Effect of the Concentration of Hydriodic Acid Added to Freshly Prepared Hübl Solutions on the Iodine Number of Butter Fat and Insoluble Butter Acids.
(Reaction Period 24 hours.)

| Grams HI per liter | | 0.0 | 2.5 | 5.0 | 10.0 | 20.0 |
|---|---|---|---|---|---|---|
| Butter fat | I no. A | 32.44 | 32.88 | 33.05 | 33.15 | 33.50 |
| | I no. B | 31.90 | 32.52 | 32.79 | 32.98 | 33.32 |
| Insoluble butter acids | I no. A | 38.16 | 38.42 | 38.47 | 38.64 | 38.77 |
| | I no. B | 37.66 | 38.15 | 38.29 | 38.53 | 38.68 |

Each value in table 6 is the average of triplicate determinations.

A. Average of beginning and final blanks used in calculations.

B. Schmidt-Nielsen and Owe formula used to calculate iodine numbers.

Then a mathematical formula would not be necessary to correct for the change in titer of the blanks from the beginning to the end of the reaction. In the majority of organic solvents which do not react appreciably with iodine, mercuric chloride has very limited solubility. The alcohols were the only solvents which appeared to be worth consideration in this connection.

In order to determine, in a general way, the relative reactivity of different alcohols with iodine, the following experiment was designed: An approximately 0.1N solution of iodine was prepared in carbon tetrachloride; 10 ml. of this solution were pipetted into 100 ml. volumetric flasks and were made to volume with the alcohols under consideration. These solutions were transferred to bottles the glass stoppers of which were well lubricated with graphite. At frequent time intervals 5 ml. of these solutions were transferred to iodine flasks and 2 ml. of 15 per cent potassium iodide followed immediately by 25 ml. of water were added. Approximately 0.01N sodium thiosulfate was used to determine the concentration of iodine. The initial and final (at the end of the experiment) concentrations of iodine are given in table 7.

Following the iodine titrations the amounts of acids formed in the above reactions were titrated with the sodium thiosulfate solution after the addition of 5 ml. of 3 per cent potassium iodate. The amounts of acids titrated were much too low to account for the loss of iodine. In the case of methyl alcohol there was no acid formed and it was assumed, therefore, that the loss of iodine resulted largely from volatilization.

A few trials were run in which methyl alcohol was used instead of ethyl alcohol as the solvent for Hübl reagents. The methyl alcohol employed was a C. P. grade and labeled "absolute". It was further purified by refluxing with 80 mesh magnesium metal and mercuric chloride for two hours and was then distilled.

Table 7

Reaction of Iodine with Alcohols

| Alcohol | Concentration of $I_2$ in milliequiv. per liter | | |
|---|---|---|---|
| | initial | after 35 days | loss |
| (Control)<br>Carbon tetrachloride | 10.507 | 10.443 | 0.064 |
| Methyl | 10.507 | 9.730 | 0.777 |
| Ethyl | 10.507 | 10.257 | 0.250 |
| $\beta$-chloroethyl | 10.507 | 9.021 | 1.486 |
| n-propyl | 10.507 | 3.133 | 7.374 |
| iso-propyl | 10.507 | 7.136 | 3.371 |
| n-butyl | 10.507 | 6.127 | 4.380 |
| iso-butyl | 10.507 | 4.036 | 6.471 |
| sec-butyl | 10.507 | 4.486 | 6.021 |
| ter-butyl | 10.507 | 9.424 | 1.083 |

The drop in titer of the blanks over a 24-hour reaction period was very much less when methyl alcohol was used rather than ethyl alcohol as the solvent for Hübl reagents. A comparison of the iodine numbers obtained with the two solutions on butter fat and insoluble butter acids is given in table 8. The methyl alcohol reagent gave higher values than did the ethyl alcohol reagent.

Table 8

A Comparison of the Iodine Numbers of Butter Fat and Insoluble Butter Acids When Methyl and Ethyl Alcohols Were Used As Solvents for the Hübl Reagents

| Solvent | Iodine number | |
|---|---|---|
| | butter fat | insoluble butter acids |
| Methyl alcohol | 33.76 | 39.34 |
| Ethyl alcohol A | 33.02 | 38.29 |
| Ethyl alcohol B | 31.93 | 37.14 |

Each value in table 8 is an average of triplicate determinations.

A. Average of beginning and final blanks used in calculations.

B. Schmidt-Nielsen and Owe formula used to calculate iodine numbers.

On several occasions, when Hübl reagents had reacted with butter fat for a few days, there was an odor of low molecular weight fatty acids in the reaction mixture. It appeared, therefore, that hydrolysis of fat was taking place. It was not known whether or not the amount of this hydrolysis was sufficient to account for the gradual increase in iodine numbers with increasing periods of reaction, assuming that the liberated glycerol radicals would react with the halogenating reagent.

The following methods were used in attempts to determine the relative amounts of weak acids (fatty acids) and strong acids (HI or HCl) left in the reaction mixtures after the excess halogen had been titrated with sodium thiosulfate; (a) Electrometric titrations, (b) conductometric titrations and (c) double indicator titrations. All of these methods failed to show definitely the presence of a weak acid. The conductometric titration method probably would have been satisfactory if a high concentration of salts (NaI, KI and $Na_2S_4O_6$) had not been present. The proportion of the total current which was carried by the acid and base ions was very small and for this reason, the method did not detect small amounts of weak acids.

The sensitivity of the double indicator method was tested on mixtures of 0.01N hydriodic and 0.01N butyric acids. Ten ml. of these mixtures were pipetted into 125 ml. Erlenmeyer flasks, 7 ml. of 15 per cent potassium iodide and 85 ml. of water were added and the solutions were titrated with 0.1N sodium hydroxide from a semi-micro burette. Methyl orange and phenolphthalein were used as indicators. The end points were matched against color standards containing these indicators in a buffered solution. The results of the titrations of known mixtures of acids, which are presented in table 9, indicate that the method should detect less than 10 per cent of a weak acid or mixture of weak acids in an unknown solution having approximately the same salt concentration.

Table 9

Titrations of 10 ml. of Mixtures of 0.01N Hydriodic and 0.01N *n*-Butyric Acids With 0.1N Sodium Hydroxide

| % HI | 100 | 95 | 90 | 80 | 66 2/3 |
|---|---|---|---|---|---|
| % $C_3H_7COOH$ | 0 | 5 | 10 | 20 | 33 1/3 |
| ml. NaOH (phenolphthalein) | 1.15 | 1.15 | 1.13 | 1.11 | 1.10 |
| ml. NaOH (methyl orange) | 0.85 | 0.82 | 0.73 | 0.62 | 0.53 |
| Difference | 0.30 | 0.33 | 0.40 | 0.49 | 0.57 |
| ml. NaOH ≎ $C_3H_7OOH$ | 0.00 | 0.03 | 0.10 | 0.19 | 0.27 |
| % $C_3H_7COOH$ found | 0.0 | 1.7 | 10.4 | 17.1 | 24.5 |
| ml. NaOH ≎ HI | 1.15 | 1.12 | 1.03 | 0.92 | 0.83 |
| % HI found | 100.0 | 98.3 | 89.6 | 82.9 | 75.5 |

Each titration value in table 9 is the average of triplicate determinations.

The method just described was applied to the solutions obtained in iodine number determinations of butter fat with Hübl reagents in ethyl alcohol. The results are recorded in table 9a. There is no tendency for the difference between the two titration end points to increase with increasing reaction period which indicates (at least in this case) that hydrolysis of the fat is not a function of time.

It was planned to plot the titration differences (table 9) against percentage composition of the known mixture of acids

Table 9a

Titrations of the Acids Produced in the Hübl
Iodine Number Determination of Butter Fat.
(Sample Weight, 0.0506 gm.)

| Reaction period (hours) | 1 | 11 | 23 | 47 | 73 | 95 | 120 |
|---|---|---|---|---|---|---|---|
| ml. 0.1N NaOH (phenolphthalein) | 0.94 | 1.07 | 1.17 | 1.36 | 1.56 | 1.66 | 1.81 |
| ml. 0.1N NaOH (methyl orange) | 0.74 | 0.82 | 0.92 | 1.10 | 1.30 | 1.43 | 1.58 |
| Difference | 0.20 | 0.25 | 0.25 | 0.26 | 0.26 | 0.23 | 0.23 |

and to use this curve to determine, roughly, the percentage of weak acids in the unknown solutions. When the data in table 9a were obtained, the difference values approximated those for 100 per cent hydriodic acid in table 9 and indicated that no quantity of organic acid large enough to be detected by this method was formed.

The variation in the iodine numbers, determined by several methods, with change in length of reaction period. The preliminary studies just presented indicated that neither the Hanus nor the Hübl reagents would yield results which were entirely satisfactory. It seemed advisable, therefore, to compare different halogenating reagents under the same conditions. For this reason all the halogenating agents, described by other investigators, which, as nearly as could be judged from the literature, would be expected to be of value in this study were employed. Sample weights and reaction temperature were practically constant; the only intended variable in these experiments was the length of the reaction period. Titrations were made at frequent intervals during the first 12 hours of the reactions, when the rate of change in iodine numbers was expected to be greatest, and as a general rule, at daily intervals after the first 24 hours. In each case the reactions were continued for one week. The data are presented graphically in figures 1, 2 and 3.

Iodine numbers of a single sample of butter fat are recorded in figure 1; those for the insoluble acids prepared from the same fat are in figure 2. The values with the Kaufmann and Hübl methods on butter fat are nearly constant from about 30 hours to 168 hours. The Kaufmann reagent gave values with the acids that gradually increased throughout the reaction period. With the original Hübl method the curve on acids rises during the first 75 hours and then remains nearly constant to the end of the experiment. No other curve presented shows constant values during any part of the reaction period with either fat or acids.

The data presented in figure 3 are sections of the graphs of figure 1 from 0 to 12 hours and are plotted on a much larger scale. The Rosenmund and Kuhnhenn curve is nearly flat from 2 to 4 hours while the Kaufmann curve shows

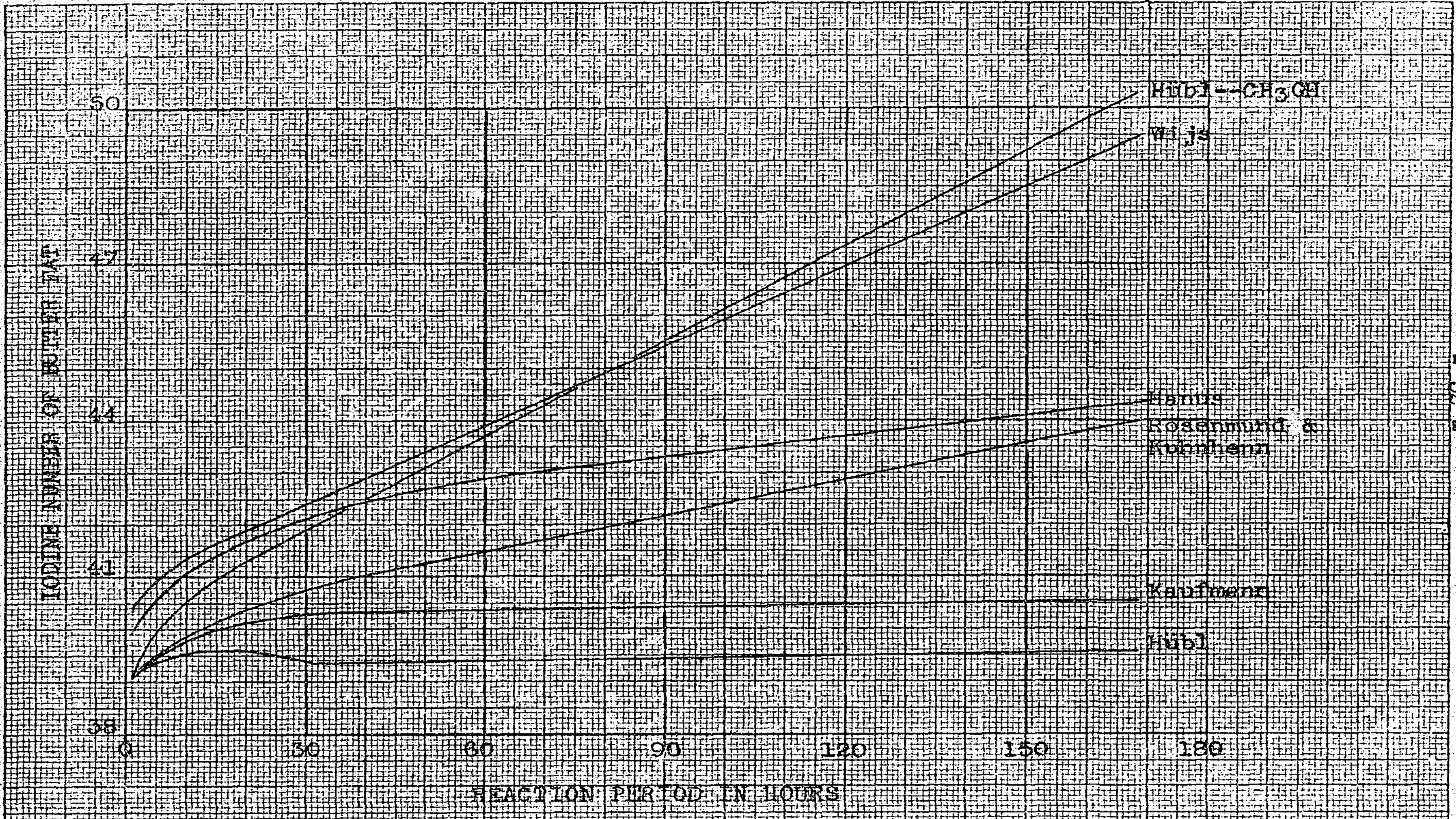

IODINE NUMBER OF BUTTER FAT
50
47
44
41
38
REACTION PERIOD IN HOURS
0
30
60
90
120
150
180
Hübl--CH$_3$OH
Wijs
Hanus
Rosenmund &
Kuhnhenn
Kaufmann
Hübl

Fig. 1.

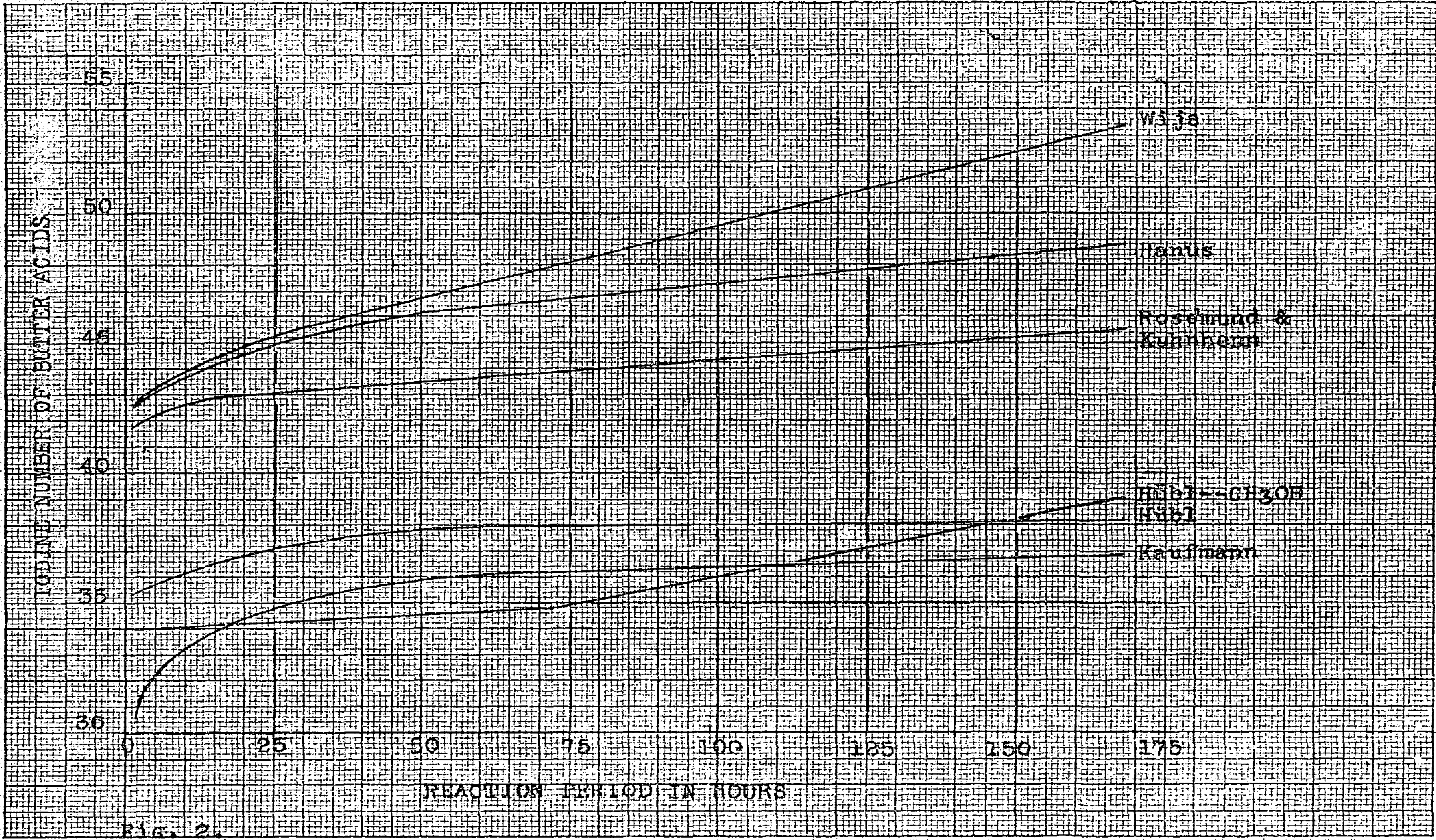

IODINE NUMBER OF BUTTER ACIDS
55
50
45
40
35
30
0
25
50
75
100
125
150
175
REACTION PERIOD IN HOURS
Wijs
Hanus
Rosenmund &
Kuhnhenn
Hübl--CH3OH
Hübl
Kaufmann

Fig. 2.

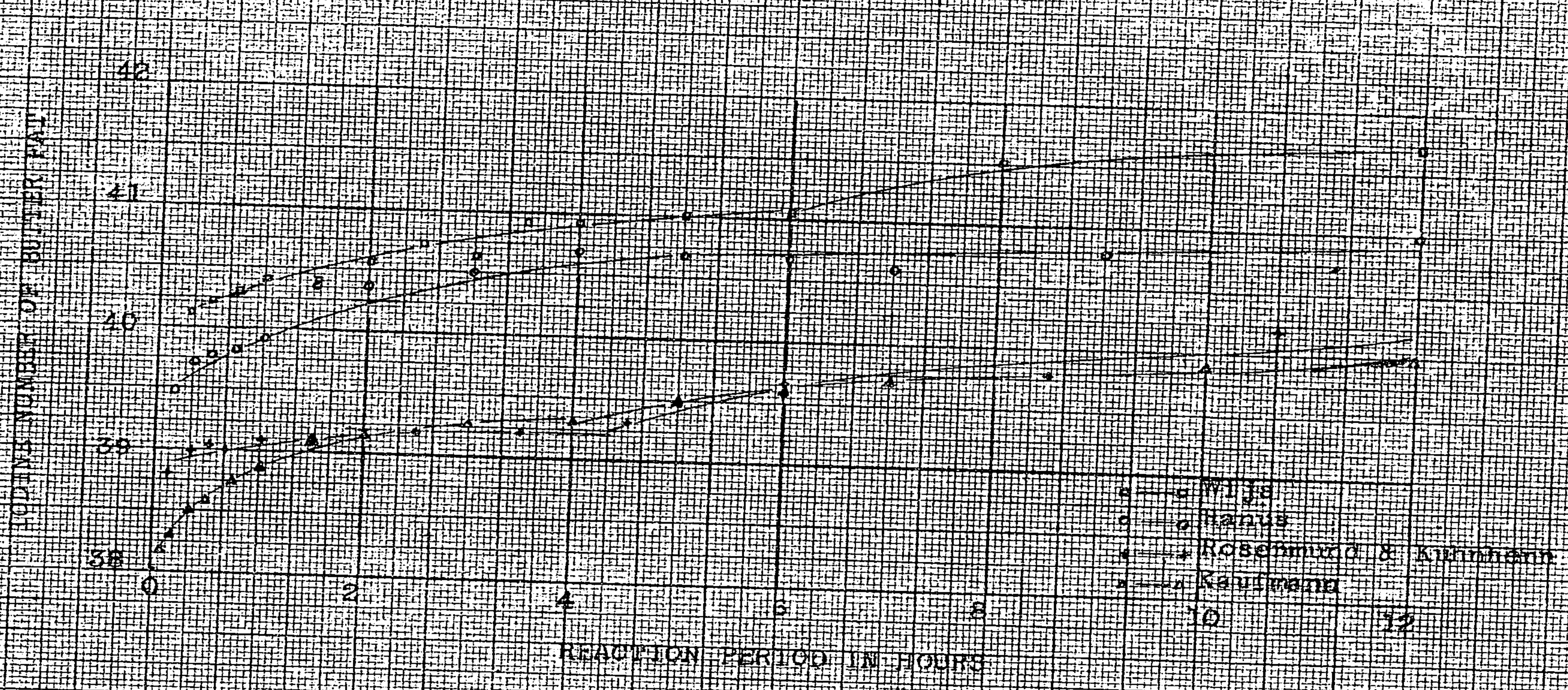

IODINE NUMBER OF BUTTER FAT
38
39
40
41
42
0
2
4
6
8
10
12
REACTION PERIOD IN HOURS
Wijs
Hanus
Rosenmund & Kuhnhenn
Kaufmann

Fig. 3.

a slight tendency to become flat in the same region. Again the Wijs and Hanus values increase with increasing reaction period and show less tendency to flatten than do the other two curves.

The data just presented and others that were in agreement with them indicate that the Rosenmund and Kuhnhenn reagent yielded an "equilibrium period" (2 to 4 hours) that was more pronounced than any of the other methods. For this reason it should give more easily reproducible results than the other methods. It was considered, therefore, that it warranted further study.

Influence of experimental conditions on iodine numbers obtained with the Rosenmund and Kuhnhenn reagent. Chloroform is the fat solvent which Rosenmund and Kuhnhenn (74) recommended for their method. The data in table 10 were secured to determine whether or not other solvents might react to a lesser extent with the reagent.

Table 10 shows that acetic acid and carbon tetrachloride react less with pyridine sulfate dibromide than do the other solvents. Carbon tetrachloride appeared to be the best solvent to use because fats are not readily soluble in acetic acid. When carbon tetrachloride was tried, difficulty was encountered with the end points. When the flasks were shaken,

Table 10

Reaction of Pyridine Sulfate Dibromide on Some Organic Solvents. (Reaction Period 5 Hours; 5 ml. Pyridine Sulfate Dibromide; 2 ml. Solvent)

| | ml. $Na_2S_2O_3$ soln. approx. 0.012N | | |
|---|---|---|---|
| Solvent | a | b | average |
| None | 40.71 | 40.73 | 40.72 |
| Acetic acid | 40.70 | 40.60 | 40.65 |
| Carbon tetrachloride | 40.70 | 40.76 | 40.73 |
| Chloroform | 40.42 | 40.46 | 40.44 |
| Ether | 38.50 | 38.52 | 38.51 |
| Petroleum ether (b.p. 30-60°C.) | 34.82 | 34.92 | 34.86 |
| Tetrachloroethane | 39.55 | 39.67 | 39.61 |
| $\beta,\beta'$-dichloroethyl ether | 39.56 | 39.54 | 39.55 |

a fairly stable white emulsion was formed. This emulsion seemed to adsorb the starch-iodine color compound and to cause a lag in the end point. It was very difficult to determine when all of the blue color had disappeared. In order to alleviate this difficulty, an excess of sodium thiosulfate solution was added before pouring the starch indicator into the solution. The excess thiosulfate was then back titrated with iodine in potassium iodide (approximately 0.005N) to the appearance of the blue color. The end point thus determined was quite sharp (0.02 ml. of 0.005N iodine in potassium iodide gave a definite color change).

To determine the amount of excess sodium thiosulfate that could be used without causing a decrease in iodine numbers, the excesses were varied from 0.0 to 10.0 ml. with both the blank and sample titrations. The results of this trial are shown in table 11. They indicate that practically constant values are obtained under these conditions.

Table 11

Effect of Different Amounts of Excess Sodium Thiosulfate on the Iodine Number of a Sample of Butter Fat. Semi-micro Rosenmund and Kuhnhenn Method.

| ml. excess $Na_2S_2O_3$ | ml. $Na_2S_2O_3$ blanks | ml. $Na_2S_2O_3$ halogen consumed | Iodine number |
|---|---|---|---|
| 0.0 | 46.22 | 16.05 | 34.60 |
| 0.5 | 46.22 | 15.97 | 34.51 |
| 1.0 | 46.22 | 15.99 | 34.55 |
| 3.0 | 46.22 | 16.00 | 34.57 |
| 5.0 | 46.22 | 15.98 | 34.52 |
| 10.0 | 46.22 | 15.98 | 34.52 |

Each value is the average of duplicate determinations. Sample weight, 0.06094 gm.; normality of $Na_2S_2O_3$, 0.01037; reaction period, 2 hours.

The iodine in potassium iodide solution was standardized as follows: 5 ml. of standard sodium thiosulfate were diluted with 50 ml. of oxygen-free distilled water, 5 ml. of acetic acid and 5 ml. of the starch solution were added and the solution of iodine in potassium iodide was added from a semi-micro burette, until the blue color of the starch end point was reached. The strength of the iodine solution was calculated as ml. sodium thiosulfate equivalent to 1 ml. of the iodine solution.

The amount of potassium iodide in excess of that required to react with the bromine at the end of the reaction period, apparently has little influence on the iodine number of butter fat. The data in table 12 do not show a very definite trend and all of the values are within the range of experimental error of the method. In all subsequent work with this method 2 ml. of 10 per cent potassium iodide were used.

The semi-micro Rosenmund and Kuhnhenn method, modified by using carbon tetrachloride as the fat solvent and determining the end point by back titration of the excess sodium thiosulfate, was compared with the method used for obtaining the data for figures 1, 2 and 3 in which chloroform was used as the fat solvent. These data which are presented

Table 12

Effect of the Amount of Potassium Iodide on the
Iodine Number of a Sample of Butter Fat.
Semi-micro Rosenmund and Kuhnhenn Method.

| ml. 10% KI | 0.9 | 1.0 | 1.3 | 1.5 | 1.7 | 2.0 | 2.5 | 3.4 | 5.1 | 6.8 |
|---|---|---|---|---|---|---|---|---|---|---|
| Iodine no. | 33.70 | 33.63 | 33.60 | 33.64 | 33.68 | 33.74 | 33.66 | 33.71 | 33.66 | 33.70 |

in table 13 indicate that there is essentially no difference in the maximum variations among replicate determinations with the two methods. The average iodine number was 0.22 higher when chloroform was the fat solvent than when $CCl_4$ was employed.

The carbon tetrachloride modification as described for table 13 was employed in obtaining the data of table 14. In this case the amount of reagent was constant, but the sample weights were varied over a wide range. These variations are expressed in terms of excess halogen and are calculated as percentages of the total halogen added. These results show that there was a little greater variation among the average values for the acids than for the fats from which they were prepared. The averages for the two groups with greatest halogen excesses show surprisingly little variation with the

## Table 13

### Effect of Fat Solvent on Iodine Number of Butter Fat. Semi-micro Rosenmund and Kuhnhenn Method. Reaction Period Two Hours

| Sample number | Fat solvent | | Sample number | Fat solvent | |
|---|---|---|---|---|---|
| | $CHCl_3$ | $CCl_4$ | | $CHCl_3$ | $CCl_4$ |
| | I no. | I no. | | I no. | I no. |
| 1 | 37.30 | 37.05 | 2 | 35.64 | 35.47 |
| | 37.36 | 37.05 | | 35.64 | 35.47 |
| | 37.23 | 37.01 | | 35.64 | 35.51 |
| | 37.23 | 37.05 | | 35.64 | 35.46 |
| | 37.23 | 37.01 | | 35.71 | 35.42 |
| | 37.23 | 37.05 | | 35.60 | 35.42 |
| | 37.34 | 37.05 | | 35.82 | 35.46 |
| | 37.34 | 37.10 | | 35.59 | 35.46 |
| | 37.30 | 37.06 | | 35.71 | 35.52 |
| | 37.32 | 37.01 | | 35.66 | 35.51 |
| Max. var. | 0.13 | 0.09 | Max. var. | 0.22 | 0.10 |
| 3 | 36.59 | 36.27 | 4 | 36.47 | 36.00 |
| | 36.50 | 36.22 | | 36.36 | 36.00 |
| | 36.53 | 36.27 | | 36.42 | 35.92 |
| | 36.50 | 36.25 | | 36.35 | 35.94 |
| | 36.46 | 36.25 | | 36.35 | 36.00 |
| | 36.46 | 36.18 | | 36.40 | 35.94 |
| | 36.43 | 36.24 | | 36.33 | 35.92 |
| | 36.45 | 36.15 | | 36.29 | 36.01 |
| | 36.43 | 36.24 | | 36.33 | 36.05 |
| | 36.50 | 36.20 | | 36.33 | 36.01 |
| Max. var. | 0.16 | 0.12 | Max. var. | 0.14 | 0.13 |
| 5 | 36.26 | 36.30 | Average maximum variation | 0.154 | 0.128 |
| | 36.28 | 36.22 | | | |
| | 36.28 | 36.33 | | | |
| | 36.28 | 36.13 | Average iodine number | 36.418 | 36.191 |
| | 36.38 | 36.27 | | | |
| | 36.28 | 36.27 | | | |
| | 36.28 | 36.20 | | | |
| | 36.28 | 36.25 | | | |
| | 36.28 | 36.18 | | | |
| | 36.38 | 36.22 | | | |
| Max. var. | 0.12 | 0.20 | | | |

## Table 14

### Effect of Variable Amounts of Excess Halogen on the Iodine Numbers of Butter Fat and Insoluble Butter Acids. Semi-micro Rosenmund and Kuhnhenn Method. Reaction Period Two Hours

| Sample | Excess % of total halogen | Iodine number | Excess % of total halogen | Iodine number | Excess % of total halogen | Iodine number |
|---|---|---|---|---|---|---|
| Butter fat<br>1 | 93 | 37.46 | 55 | 37.00 | 14 | 36.79 |
| | | 37.46 | | 37.00 | | 36.83 |
| | | 37.32 | | 37.16 | | 36.84 |
| | | 37.19 | | 37.11 | | 36.48 |
| | av. | 37.36 | | 37.07 | | 36.74 |
| 2 | 94 | 35.64 | 57 | 35.21 | 17 | 35.04 |
| | | 35.64 | | 35.21 | | 35.07 |
| | | 35.82 | | 35.17 | | 35.13 |
| | | 36.10 | | 35.36 | | 35.22 |
| | av. | 35.80 | | 35.24 | | 35.12 |
| 3 | 92 | 36.33 | 60 | 36.12 | 18 | 35.82 |
| | | 35.97 | | 36.05 | | 35.86 |
| | | 35.97 | | 36.09 | | 35.94 |
| | | 36.09 | | 36.16 | | --- |
| | av. | 36.09 | | 36.11 | | 35.87 |
| 4 | 91 | 36.33 | 55 | 36.03 | 16 | 35.80 |
| | | 36.11 | | 36.05 | | 35.75 |
| | | 36.11 | | 36.12 | | 35.76 |
| | | 36.33 | | 36.10 | | 35.60 |
| | av. | 36.22 | | 36.08 | | 35.73 |
| 5 | 91 | 37.26 | 49 | 36.47 | 14 | 36.17 |
| | | 37.26 | | 36.55 | | 36.19 |
| | | 37.03 | | 36.53 | | 36.23 |
| | | 37.61 | | 36.60 | | 36.28 |
| | av. | 37.29 | | 36.54 | | 36.22 |
| Average | 92.2 | 36.552 | 55.2 | 36.205 | 15.8 | 35.937 |
| Butter acids<br>1 | 94 | 39.39 | 59 | 40.78 | 14 | 40.45 |
| | | 39.86 | | 40.69 | | 40.40 |
| | | 40.02 | | 40.67 | | 40.47 |
| | | 39.55 | | 40.67 | | 40.43 |
| | av. | 39.71 | | 40.70 | | 40.44 |
| 2 | 94 | 39.28 | 59 | 38.83 | 17 | 38.62 |
| | | 39.12 | | 38.87 | | 38.66 |
| | | 39.28 | | 38.85 | | 38.61 |
| | | 38.81 | | 39.02 | | 38.64 |
| | av. | 39.12 | | 38.89 | | 38.63 |
| 3 | 92 | 40.37 | 57 | 39.12 | 13 | 38.62 |
| | | 40.27 | | 39.16 | | 38.77 |
| | | 40.27 | | 39.14 | | 38.78 |
| | | 40.27 | | 39.16 | | 38.80 |
| | av. | 40.30 | | 39.15 | | 38.75 |

Rosenmund and Kuhnhenn Method.

Reaction Period Two Hours

| Sample | Excess % of total halogen | Iodine number | Excess % of total halogen | Iodine number | Excess % of total halogen | Iodine number |
|---|---|---|---|---|---|---|
| Butter fat 1 | 93 | 37.46 | 55 | 37.00 | 14 | 36.79 |
| | | 37.46 | | 37.00 | | 36.83 |
| | | 37.32 | | 37.16 | | 36.84 |
| | | 37.19 | | 37.11 | | 36.48 |
| | av. | 37.36 | | 37.07 | | 36.74 |
| 2 | 94 | 35.64 | 57 | 35.21 | 17 | 35.04 |
| | | 35.64 | | 35.21 | | 35.07 |
| | | 35.82 | | 35.17 | | 35.13 |
| | | 36.10 | | 35.36 | | 35.22 |
| | av. | 35.80 | | 35.24 | | 35.12 |
| 3 | 92 | 36.33 | 60 | 36.12 | 18 | 35.82 |
| | | 35.97 | | 36.05 | | 35.86 |
| | | 35.97 | | 36.09 | | 35.94 |
| | | 36.09 | | 36.16 | | --- |
| | av. | 36.09 | | 36.11 | | 35.87 |
| 4 | 91 | 36.33 | 55 | 36.03 | 16 | 35.80 |
| | | 36.11 | | 36.05 | | 35.75 |
| | | 36.11 | | 36.12 | | 35.76 |
| | | 36.33 | | 36.10 | | 35.60 |
| | av. | 36.22 | | 36.08 | | 35.73 |
| 5 | 91 | 37.26 | 49 | 36.47 | 14 | 36.17 |
| | | 37.26 | | 36.55 | | 36.19 |
| | | 37.03 | | 36.53 | | 36.23 |
| | | 37.61 | | 36.60 | | 36.28 |
| | av. | 37.29 | | 36.54 | | 36.22 |
| Average | 92.2 | 36.552 | 55.2 | 36.205 | 15.8 | 35.937 |
| Butter acids 1 | 94 | 39.39 | 59 | 40.78 | 14 | 40.45 |
| | | 39.86 | | 40.69 | | 40.40 |
| | | 40.02 | | 40.67 | | 40.47 |
| | | 39.55 | | 40.67 | | 40.43 |
| | av. | 39.71 | | 40.70 | | 40.44 |
| 2 | 94 | 39.28 | 59 | 38.83 | 17 | 38.62 |
| | | 39.12 | | 38.87 | | 38.66 |
| | | 39.28 | | 38.85 | | 38.61 |
| | | 38.81 | | 39.02 | | 38.64 |
| | av. | 39.12 | | 38.89 | | 38.63 |
| 3 | 92 | 40.37 | 57 | 39.12 | 13 | 38.62 |
| | | 40.27 | | 39.16 | | 38.77 |
| | | 40.27 | | 39.14 | | 38.78 |
| | | 40.27 | | 39.16 | | 38.80 |
| | av. | 40.30 | | 39.15 | | 38.75 |
| 4 | 92 | 41.12 | 44 | 39.67 | 5 | 39.12 |
| | | 41.01 | | 39.64 | | 39.09 |
| | | 41.01 | | 39.64 | | 39.12 |
| | | 41.01 | | 39.70 | | 39.13 |
| | av. | 41.04 | | 39.66 | | 39.12 |
| 5 | 92 | 40.79 | 59 | 39.99 | 20 | 39.79 |
| | | 40.31 | | 39.90 | | 39.78 |
| | | 40.11 | | 39.99 | | 39.79 |
| | | 40.00 | | 39.97 | | 39.82 |
| | av. | 40.30 | | 39.96 | | 39.80 |
| Average | 92.8 | 40.093 | 55.6 | 39.943 | 15.8 | 39.345 |

exception of the no. 5 fat sample.

The semi-micro Hanus method was used under the same conditions of variable sample weights with the no. 5 sample of fat and its acids. The excess halogen does not vary over as great a range with the Hanus as it does with the Rosenmund and Kuhnhenn reagent because the former reagent contains twice as much total halogen. The Rosenmund and Kuhnhenn reagent gave much less variation with the insoluble acids than did the Hanus, and if similar excesses of halogenating agent are compared, the values for the fat sample are practically identical by the two methods.

The age of the pyridine sulfate dibromide solution is a factor which was considered as a possible source of variation in iodine number determinations. A sample of fat was tested with a solution which was at least six months old and another solution which had been prepared only a few hours. The values obtained were 37.27 and 37.17 respectively. It is assumed, therefore, that an equilibrium is quickly established between bromine and pyridine sulfate and that it does not change appreciably with time.

<u>Iodine number as a function of the reaction period of pyridine sulfate dibromide.</u> In the work presented thus far with the semi-micro Rosenmund and Kuhnhenn method, it appeared that a reaction period of two hours was optimum for

Table 15

Comparison of the Iodine Numbers of a Sample of Butter Fat and the Insoluble Acids From That Fat When the Excess of Halogen is Varied. Semi-micro Hanus and Semi-micro Rosenmund and Kuhnhenn Methods.
(Reaction Periods 0.5 and 2.0 Hours Respectively.)

| Sample | Excess as % of total halogen | Iodine number | Excess as % of total halogen | Iodine number | Excess as % of total halogen | Iodine number |
|---|---|---|---|---|---|---|
| Butter fat | 96 | 37.74 | 78 | 37.92 | 59 | 37.32 |
| Semi-micro | | 37.95 | | 37.71 | | 37.32 |
| Hanus | | 37.95 | | 37.92 | | 37.30 |
| | | 37.95 | | 37.92 | | 37.32 |
| | Average | 37.90 | | 37.87 | | 37.32 |
| Semi-micro | 91 | 37.26 | 49 | 36.47 | 14 | 36.17 |
| Rosenmund | | 37.26 | | 36.55 | | 36.19 |
| and | | 37.03 | | 36.53 | | 36.23 |
| Kuhnhenn | | 37.61 | | 36.60 | | 36.28 |
| | Average | 37.29 | | 36.54 | | 36.22 |
| Butter acids | 96 | 42.84 | 78 | 40.20 | 59 | 39.20 |
| Semi-micro | | 41.62 | | 40.24 | | 39.16 |
| Hanus | | 41.62 | | 40.06 | | 39.20 |
| | | 41.62 | | 39.89 | | 39.11 |
| | Average | 41.93 | | 40.10 | | 39.17 |
| Semi-micro | 92 | 40.79 | 59 | 39.99 | 20 | 39.79 |
| Rosenmund | | 40.31 | | 39.90 | | 39.78 |
| and | | 40.11 | | 39.99 | | 39.79 |
| Kuhnhenn | | 40.10 | | 39.97 | | 39.82 |
| | Average | 40.30 | | 39.96 | | 39.80 |

reproducible values. The curves of iodine numbers against reaction period appeared to have the least slope at the point.

It is well known that iodine numbers of butter fat vary considerably with season and with the feed consumed by the cows. It seemed necessary to determine the effect of these conditions on the type of curves obtained when iodine numbers were plotted against reaction periods, if a correct reaction period were to be established for this method.

Samples of fat were prepared from butter of different grades from several sources during the period from July to December, 1937. The iodine numbers of these fats were determined at five minutes and at hourly intervals thereafter up to eight hours. Insoluble fatty acids were prepared from these fats and their iodine numbers were determined at the same time intervals. The data are presented in figures 4 and 5 respectively.

The technique used in these trials differed from that previously described for this method in one detail, namely, that possible volatilization of the reagent was largely prevented by lubricating the stoppers with one drop of syrupy phosphoric acid and then fitting them tightly into the flasks. Other materials, such as, 10 per cent potassium iodide, saturated calcium chloride, concentrated sulfuric acid and mineral oil had been tried previously but none was satisfactory. The phosphoric acid maintained a tight seal in nearly all cases, it did not interfere with the end points and it did not oxidize the potassium iodide added at the end of the reaction period. Furthermore, it did not react with the halogenating reagent and could be washed into the reaction flask so that any adsorbed halogen could be titrated. The

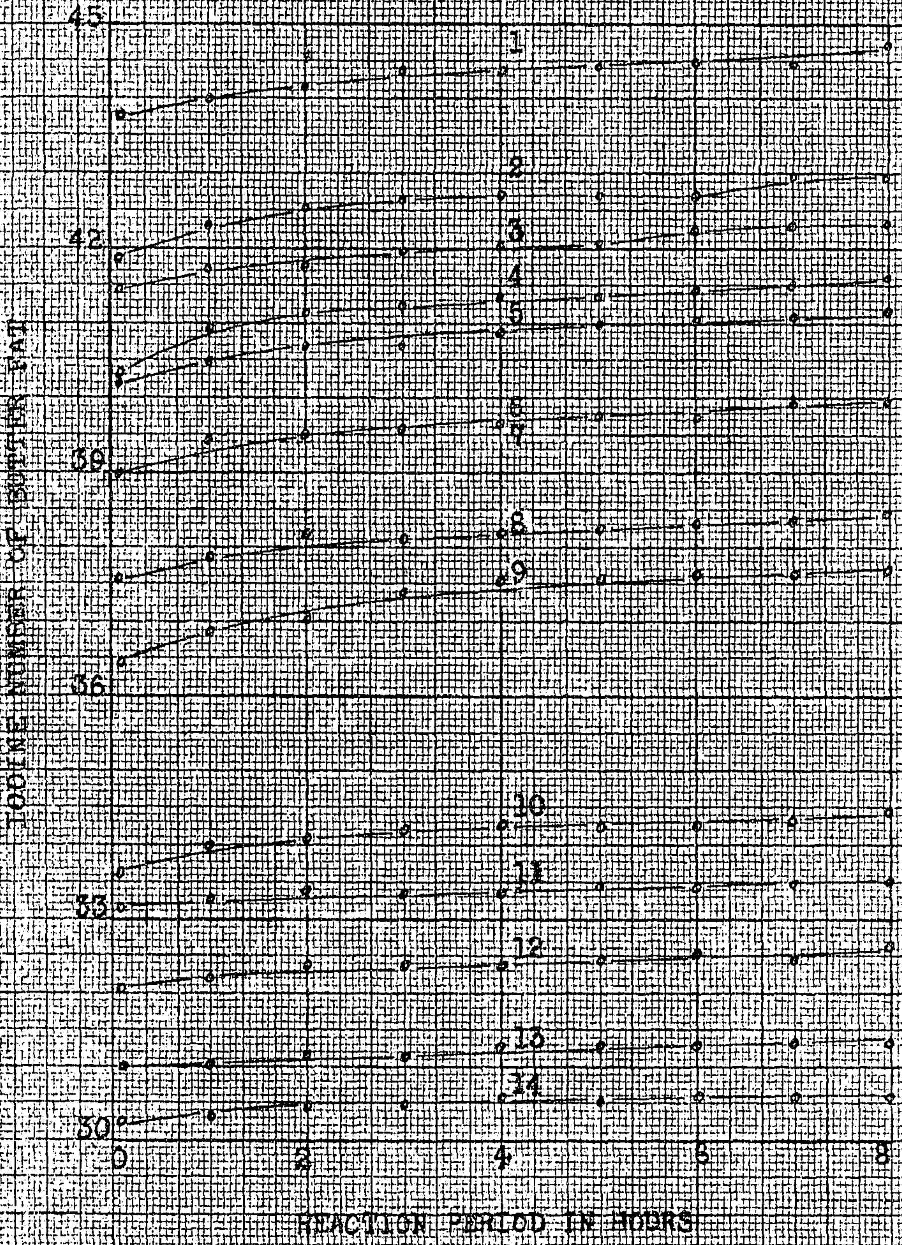

IODINE NUMBER OF BUTTER FAT
45
42
39
36
33
30
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
2
4
6
8
REACTION PERIOD IN HOURS

Fig. 4.

Form E-3

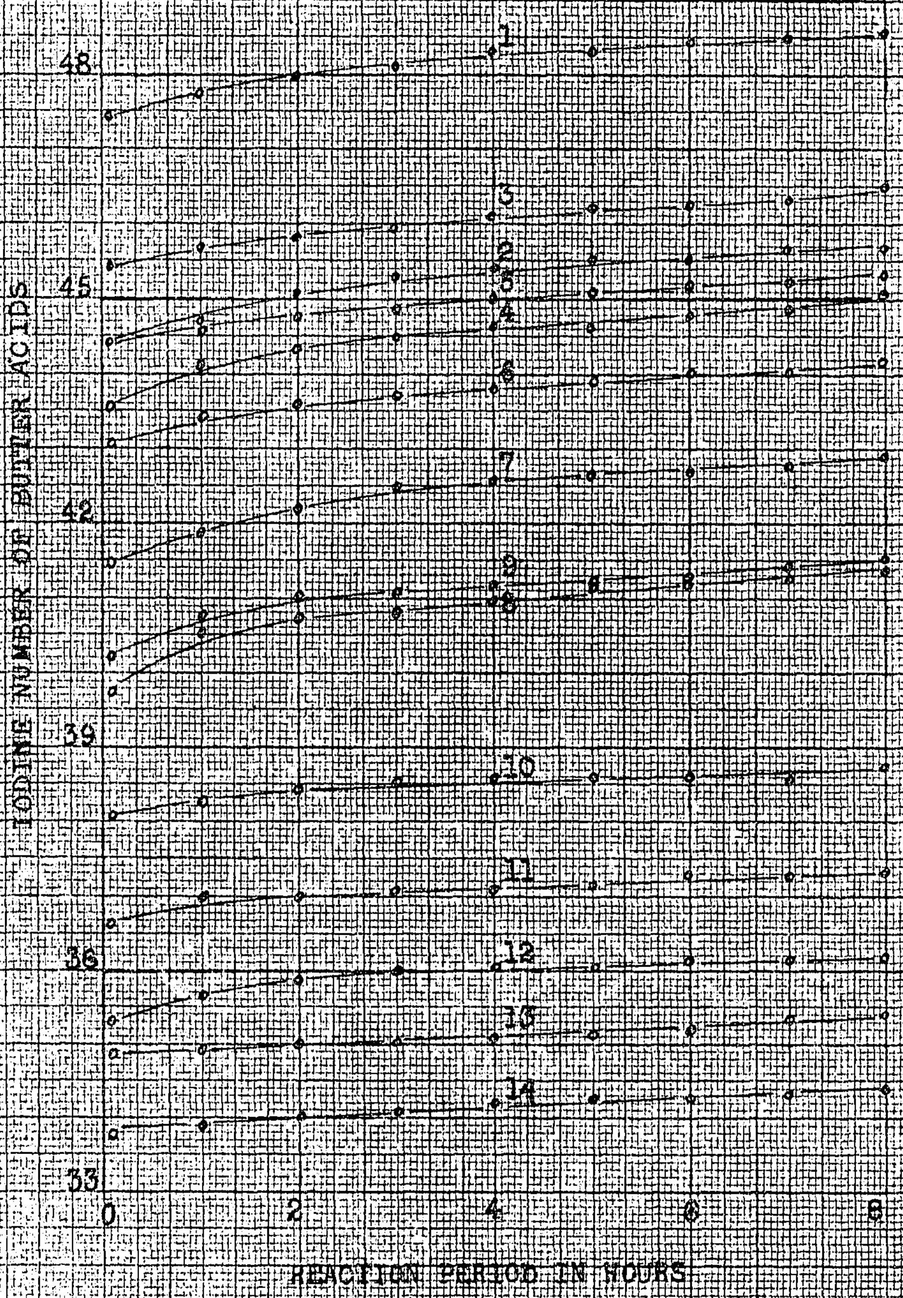

IODINE NUMBER OF BUTTER ACIDS
48
45
42
39
36
33
0
2
4
6
8
REACTION PERIOD IN HOURS
1
3
2
5
4
6
7
9
8
10
11
12
13
14

Fig. 5.

other substances failed to meet all of these requirements.

A critical inspection of figures 4 and 5 reveals that there is much similarity between the iodine number curves of the fats and of the insoluble acids obtained from them. In both sets of curves, the iodine numbers increase slowly during the entire reaction period although the rate of increase is greater with some samples than with others. It is assumed that if some compounds which take up halogen very slowly are present in the fat these compounds are not removed in the preparation of the acids.

Another rather interesting observation is that the type of curve varies somewhat with the magnitude of the iodine number. The samples of summer fats and acids, which have the higher iodine numbers, show a greater rate of increase in iodine number during the first two hours of the reaction than during the remaining part of the reaction period. The samples of late fall and early winter fats and acids (nos. 11 to 14) increase at a slower rate during the entire reaction period than do the summer samples. This is especially marked during the first two hours of the reaction period.

With the majority of fat and acid samples it appears that a four-hour reaction period would be the shortest that would represent the portion of the curves having slopes most nearly approaching zero. On the basis of these curves it seems logical to establish a four-hour reaction period for routine analyses with this method.

An attempt was made to determine whether or not the reaction of pyridine sulfate dibromide on a sample of butter fat would reach an equilibrium, if continued for several days with the stoppers sealed in the flasks with phosphoric acid. Sample number 12 from the previous experiments was used. The results are recorded in table 16.

There was some diffusion of bromine through the phosphoric acid and an inspection of the titration data indicates that this was not entirely uniform. Although all of the stoppers had been re-ground into the flasks, it seems to be nearly impossible to obtain absolute uniformity. In order to minimize this source of variation, the titration values for both blanks and samples were plotted against reaction period. A smooth curve was drawn through these points. The titration values at the different time intervals were then read from the curve and the iodine numbers were re-calculated on the basis of these figures.

It cannot be stated definitely that an equilibrium was reached in this reaction. The re-calculated values, however, show an increase of only 0.2 iodine unit from 68 to 146 hour reaction periods.

Table 16

Variation of the Iodine Number of a Butter Fat Sample
With Change in the Duration of the Reaction Period.
Semi-micro Rosenmund and Kuhnhenn Method*.

| Reaction period in hours | ml. $Na_2S_2O_3$ halogen in blanks | ml. $Na_2S_2O_3$ halogen in samples | Iodine number | ml. $Na_2S_2O_3$ (read from curve) halogen in blanks | ml. $Na_2S_2O_3$ (read from curve) halogen in samples | Iodine number |
|---|---|---|---|---|---|---|
| 2 | 44.19 | 30.81 | 32.60 | 44.19 | 30.81 | 32.60 |
| 5 | 44.18 | 30.72 | 32.80 | 44.18 | 30.73 | 32.77 |
| 20 | 44.14 | 30.48 | 33.28 | 44.14 | 30.50 | 33.24 |
| 25 | 44.13 | 30.45 | 33.33 | 44.13 | 30.45 | 33.33 |
| 33 | 44.10 | 30.36 | 33.48 | 44.11 | 30.37 | 33.48 |
| 45 | 44.08 | 30.30 | 33.58 | 44.09 | 30.28 | 33.65 |
| 54 | 44.13 | 30.18 | 33.99 | 44.07 | 30.21 | 33.77 |
| 68 | 44.08 | 30.15 | 33.92 | 44.04 | 30.16 | 33.82 |
| 96 | 43.98 | 30.08 | 33.87 | 43.99 | 30.06 | 33.92 |
| 122 | 43.93 | 29.97 | 33.02 | 43.92 | 29.99 | 33.92 |
| 146 | 43.70 | 29.92 | 33.58 | 43.83 | 29.87 | 34.02 |

*Stoppers sealed with $H_3PO_4$; sample weight, 0.05560 gm.; normality of $Na_2S_2O_3$, 0.01065; butter fat sample no. 12.

Study of factors which might contribute to the gradual increase of iodine numbers of butter fat by the semi-micro Rosenmund and Kuhnhenn method. It was considered that the presence of carotene, cholesterol, phospholipins, or hydroxy acids might give slow reactions with pyridine sulfate dibromide and be responsible for the gradual rise in the curves presented in figure 4. Another possible explanation is the substitution of hydrogen of the fatty acid radicals.

According to the literature reviewed by Associates of Rogers (70) butter fat contains from approximately 0.2 mg. (winter) to 1.0 mg. (summer) carotene per 100 gm. of fat. If it is assumed that each of the 11 double bonds in carotene reacts completely with halogen, then the iodine number would be:

$$\frac{253.84 \times 11}{536.45} \times 100 = 520.6$$

The amount of iodine which could be absorbed by the carotene contained in a 50 mg. sample of summer butter fat would be:

$$0.00001 \times 0.05 \times 5.206 = 0.0000026 \text{ gm. iodine}$$

The limit of sensitivity of the starch-iodine end point in the iodine number determinations by this method is about 0.02 ml. of 0.005N iodine corresponding to 0.0000127 gm. of iodine. Unless carotene and/or vitamin D are present in butter fat greatly in excess of the quantities reported in the literature (70) these compounds could not have a measurable influence on the iodine number of butter fat.

According to Associates of Rogers (70), approximately 75 mg. of cholesterol are present in 100 gm. of butter fat. Cholesterol contains only one double bond and has a calculated iodine number of 65.7. The amount of iodine which could be absorbed by the cholesterol contained in 50 mg. of butter fat would be:

$$0.00075 \times 0.05 \times 0.657 = 0.0000246 \text{ gm. iodine}$$

This would be equivalent to about 0.05 iodine units. Cholesterol can not be considered as contributing greatly to the increase in iodine numbers encountered between two and eight hour reaction periods.

Figure 6 presents iodine number curves plotted against reaction period for cholesterol (from Eastman Kodak Co.), buttermilk extract (obtained with a macro Röese-Gottlieb extraction), butter fat and insoluble butter acids. The last three substances were obtained from the same lot of cream. They yield curves having similar slopes. The buttermilk extract is comparatively rich in phospholipins and although its iodine number is higher than those of the fat or acids, the rate at which it increases is not appreciably greater. The curve for cholesterol is different from the others. Its slope is much greater even though the ordinate is plotted on a smaller scale. Highly purified samples of cholesterol yielded similar curves although the initial values were more nearly the calculated values.

It was considered that if hydroxy acids cause an increase in the iodine number of butter fat with increasing reaction

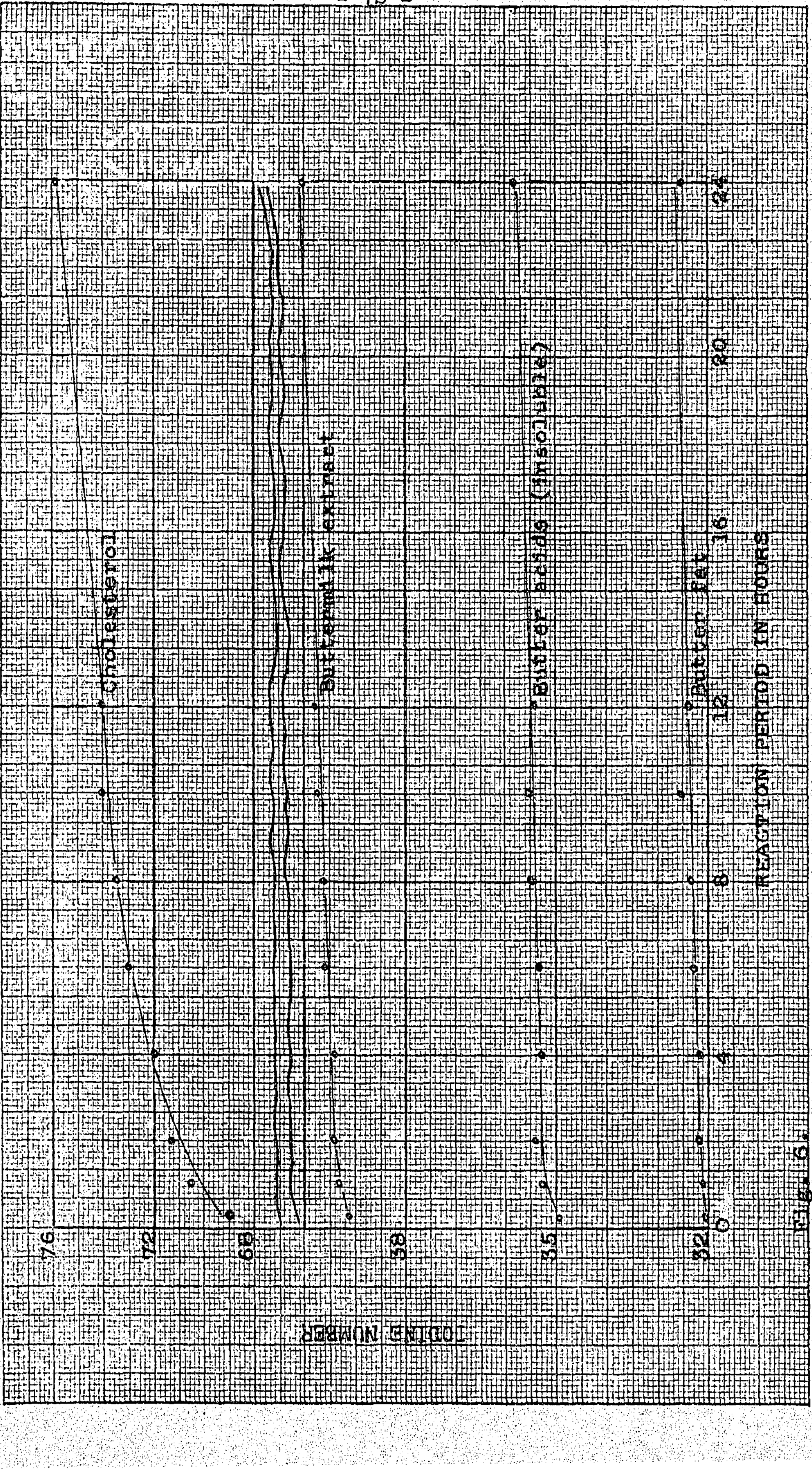

Cholesterol
Buttermilk extract
Butter acids (insoluble)
Butter fat
REACTION PERIOD IN HOURS
0
4
8
12
16
20
24
IODINE NUMBER
76
72
68
38
35
32

Fig. 6.

periods, that it would occur to a much greater degree with castor oil. Ricinoleic acid (12-hydroxyoleic acid) is the chief acid constituent of castor oil. Iodine numbers were determined for a sample of castor oil over a reaction period of 24 hours by the semi-micro Rosenmund and Kuhnhenn method. These values ranged from 83.36 at the 15 min. period to 83.78 at the 24 hour period. These iodine numbers are as constant as those usually obtained with butter fat.

It is possible that pyridine sulfate dibromide substitutes hydrogen and thereby causes the iodine numbers to gradually increase. It was considered that if it could be shown that substitution occurred with a pure fatty acid or a pure triglyceride, it would be logical to assume that this type of reaction also took place with butter fat and mixed insoluble acids from butter fat. Iodine numbers of stearic acid and tristearin (both Eastman products) were determined by the semi-micro Rosenmund and Kuhnhenn method with reaction periods up to eight hours. The iodine number with each of these products was less than 2.0 units; there was no tendency for these values to increase with increasing reaction periods. No substitution was indicated by these results. It cannot be concluded from these trials that substitution does not occur in the reaction of pyridine sulfate dibromide on butter fat, but, if it does occur, it is probable that groups other than the fatty acid radicals are attacked.

Agreement of replicate determinations with the semimicro Rosenmund and Kuhnhenn method. As a final check on the method with regard to the constancy of results which could be expected in routine analyses, iodine numbers of a few samples of butter fat and the corresponding insoluble acids were determined in quadruplicate. These samples were weighed (individually), were dissolved in 2 ml. of carbon tetrachloride, 5 ml. of pyridine sulfate dibromide reagent were added and the reactions were allowed to take place in the dark at 25°C. for 4 hours. The excess bromine at the end of the reaction period was determined by the back titration method previously described. The results are recorded in table 17.

The maximum variations among replicate iodine numbers are somewhat greater in table 17 than in table 13. This was expected because weighing and sampling errors were practically negligible for the data in table 13 in which aliquot samples were pipetted into the reaction flasks.

Precise weighings can be obtained more rapidly when the light weight glass boats are used than when the iodine flasks are weighed. Although these data are too few in number to permit drawing sweeping conclusions, it seems reasonable to expect that replicate iodine numbers, with about the same

## Table 17

Variation Among Replicate Iodine Numbers of Some Samples of Butter Fat and the Insoluble Acids From These Fats, When the Samples Were Weighed Individually. Semi-micro Rosenmund and Kuhnhenn Method.

| Sample | Sample weight | Iodine number | Sample | Sample weight | Iodine number |
|---|---|---|---|---|---|
| Butter fat no. 3 | 0.05482 | 41.44 | Butter acids no. 3 | 0.05513 | 45.16 |
| | 0.05241 | 41.54 | | 0.05043 | 45.10 |
| | 0.05165 | lost | | 0.05429 | 44.96 |
| | 0.05333 | 41.76 | | 0.05352 | 44.97 |
| | Max. var. | 0.32 | | Max. var. | 0.20 |
| Butter fat no. 13 | 0.05401 | 31.12 | Butter acids no. 13 | 0.05140 | 34.60 |
| | 0.05364 | 31.28 | | 0.05019 | 34.76 |
| | 0.05290 | 31.01 | | 0.05000 | 34.64 |
| | 0.05464 | 31.35 | | 0.05045 | 34.63 |
| | Max. var. | 0.34 | | Max. var. | 0.16 |
| Butter fat no. 1 | 0.05455 | 43.93 | Butter fat no. 1* | 0.05640 | 44.19 |
| | 0.05680 | 43.95 | | 0.05517 | 44.07 |
| | 0.05720 | 43.90 | | 0.05441 | 43.99 |
| | 0.05660 | 44.06 | | 0.05273 | 44.16 |
| | Max. var. | 0.16 | | Max. var. | 0.20 |

*Samples were weighed in small glass weighing boats. These boats weighed approximately 0.1 gm. All other samples were weighed directly into the iodine flasks. These flasks weighed about 55 gm.

sample weights, should agree within 0.5 of a unit in all cases and generally within 0.3 of a unit.

Attempts to measure the amount of substitution reaction by titration of acids. Many halogenation experiments have been described in the literature in which the acids formed in the reaction have been determined either with alkali or with sodium thiosulfate after the addition of potassium iodate. When a neutral and non-reactive solvent, such as carbon tetrachloride, is used it has been considered that the acid formed must be a result of hydrogen substitution. The following general reaction is considered to take place:

$$RH + X_2 \longrightarrow RX + HX$$

If the halogen, which is equivalent to the HX is doubled and subtracted from the total amount of halogen used, the value thus obtained should represent the halogen consumed in the addition reaction. The majority of previous investigators, however, do not agree whether or not this halogen acid results from substitution or from hydrolysis of the addition product after the addition of potassium iodide and water.

In attempts to use this method in the present investigation, it was observed that (a) a pungent odor suggestive of a halogen acid was noticeable upon removal of the stopper from the iodine flask and (b) sometimes the rims of the iodine flasks were cloudy resembling a deposit of ammonium chloride on laboratory glassware. These facts suggested that some of the halogen acid formed in the reaction was escaping and was not being determined in the usual procedure.

Preliminary experiments indicated that the solubility of dry HCl gas in carbon tetrachloride was very low and that this gas gradually escaped from the solution. It was considered, therefore, that with a properly designed reaction

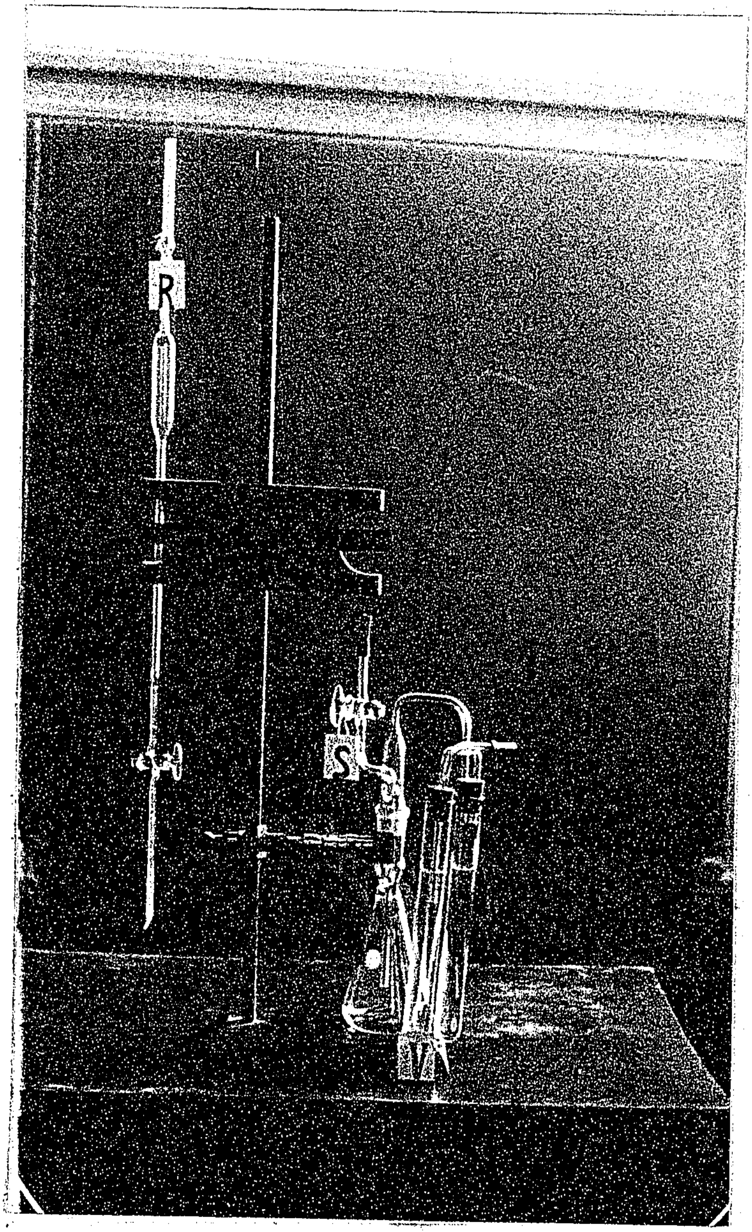


Plate 2. Equipment used to determine acids formed in halogenation reactions.

flask which would prevent the escape of any gas formed in the reaction of halogens on fat, it might be possible to calculate a "true" addition value.

The special flask shown in plate 2 was fabricated for this purpose. The outer section of a standard taper Pyrex joint was sealed to a 250 ml. Erlenmeyer flask to form the reaction chamber. The inner portion of the standard taper Pyrex joint formed the closure for the flask. The outlet from the system was sealed to the top of this closure and was bent twice at right angles so that any halogen acid not in solution in the flask could be swept into the gas-washing tubes for absorption. The intake (with stopcock (S)) passed through the side of the closure by means of an inner seal and extended to within 3 cm. of the surface of the reaction mixture in the flask. The two test tubes (V) contained 50 ml. of 1 per cent potassium iodide solution. The tube above the stopcock was connected to a cylinder of nitrogen while the outlet tube from (V) was connected to an aspirator bottle.

The pipette (R) was calibrated to deliver 25 ml. at 25°C. It was used to measure the halogenating solution into the reaction flask. The stopcock and ground-glass joint were lubricated with a highly refined mineral oil. The iodine number of this oil is approximately 0.10.

Fat sample no. 3 (fig. 4) was used for the experiments recorded in table 18 and presented graphically in figure 7. Sample weights of approximately 0.3 gm. were dissolved in 10 ml. of carbon tetrachloride, 25 ml. of 0.2N iodine monobromide in the same solvent were added and the reactions were allowed to take place in the dark at 25°C. for different periods. At the end of the reaction period, four liters of nitrogen were forced through the system in about 20 minutes. The stopcock was closed, the reaction flask was disconnected from the rest of the system, 10 ml. of 10 per cent potassium iodide were added to the reaction flask and well mixed with its contents and then 50 ml. of oxygen-free water were added and mixed with the solution. The excess iodine was titrated with an approximately 0.11N solution of sodium thiosulfate. Next, 5 ml. of 3 per cent potassium iodate were added and the iodine liberated by acids was titrated with a 1 : 10 dilution of the thiosulfate previously used. The iodine and halogen acid present in the test tubes containing potassium iodide were determined in the same fashion.

It can not be stated definitely whether or not an equilibrium was reached in the reaction period of one week (table 18, trial 2 and figure 7). The end points of the last two acid determinations of this experiment were not sharp which indicated that hydrolysis of the fat with the liberation

Table 18

Attempted Determination of the "True" Iodine Number by Correcting for the Amount of Halogen Acid Formed in the Reaction of 0.2N Iodine Monobromide on Butter Fat Sample No. 3

| Reaction period in hours | Iodine number not corrected for HX | Iodine number corrected for HX* | HX expressed as iodine number |
|---|---|---|---|
| | Trial 1 | | |
| 0 | 41.41 | 40.60 | 0.81 |
| 24 | 47.44 | 44.21 | 3.23 |
| 48 | 48.39 | 44.72 | 3.67 |
| 72 | 48.61 | 45.34 | 3.27 |
| 96 | 49.15 | 46.01 | 3.14 |
| | Trial 2 | | |
| 0 | 41.05 | 40.25 | 0.80 |
| 24 | 47.91 | 44.53 | 3.38 |
| 72 | 49.15 | 45.72 | 3.43 |
| 120 | 49.82 | 46.65 | 3.17 |
| 168 | 50.34 | 46.34 | 4.00 |

---

*Corrections were made by subtracting the iodine equivalent to 2(HX) from the total halogen consumed.

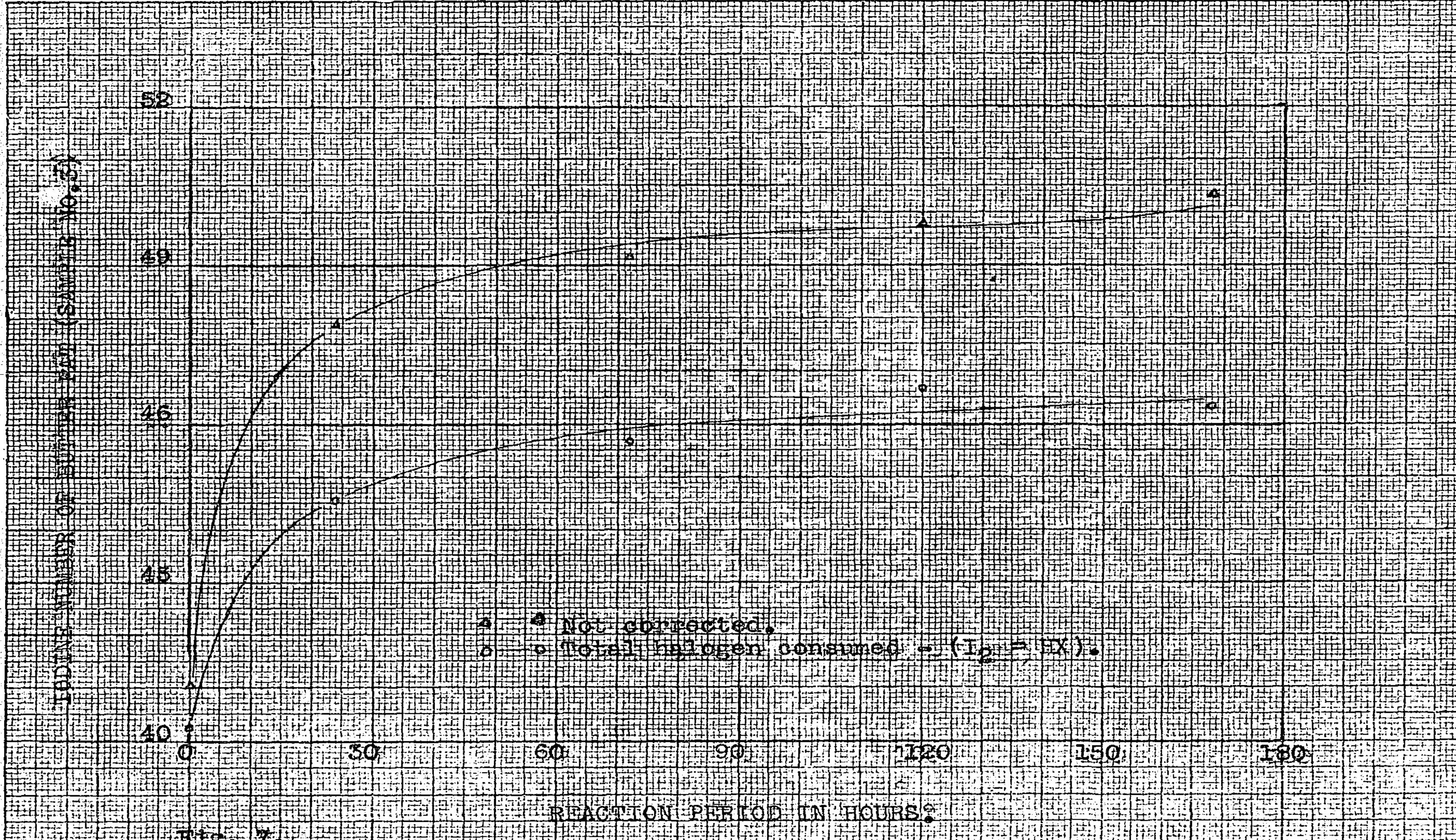

IODINE NUMBER OF BUTTER FAT (SAMPLE No.3)
52
49
46
43
40
0
30
60
90
120
150
180
REACTION PERIOD IN HOURS.
△ Not corrected.
○ Total halogen consumed - $(I_2 = HX)$.

Fig. 7.

of weak fatty acids might have taken place. With the last determination of trial 2 there was a considerable increase in acidity although the total halogen consumed was almost identical with that of the preceding titration. In other words, if the acidity had not increased, the corrected iodine number would have been constant.

The gravimetric bromine vapor method. The results of other investigators indicate that (a) bromine vapor adds to ethylenic linkages quantitatively (conjugated as well as not conjugated systems); (b) substitution is not encountered at room temperature in the absence of light regardless of the excess bromine present and (c) the chief difficulty in using the method is the complete removal of the excess bromine not chemically bound. Previous investigators have not reported any values for butter fat by this method. Preliminary results in this laboratory with make-shift equipment indicated that the method could be used for butter fat satisfactorily.

The "brominator" illustrated in plate 3 was constructed to meet the following requirements: Complete elimination of light from the bromination tube (T), adequate gas washing system (G) to absorb the excess bromine and keep it from escaping into the laboratory, protection of the bromine reservoir (C) from light and possible breakage, precise

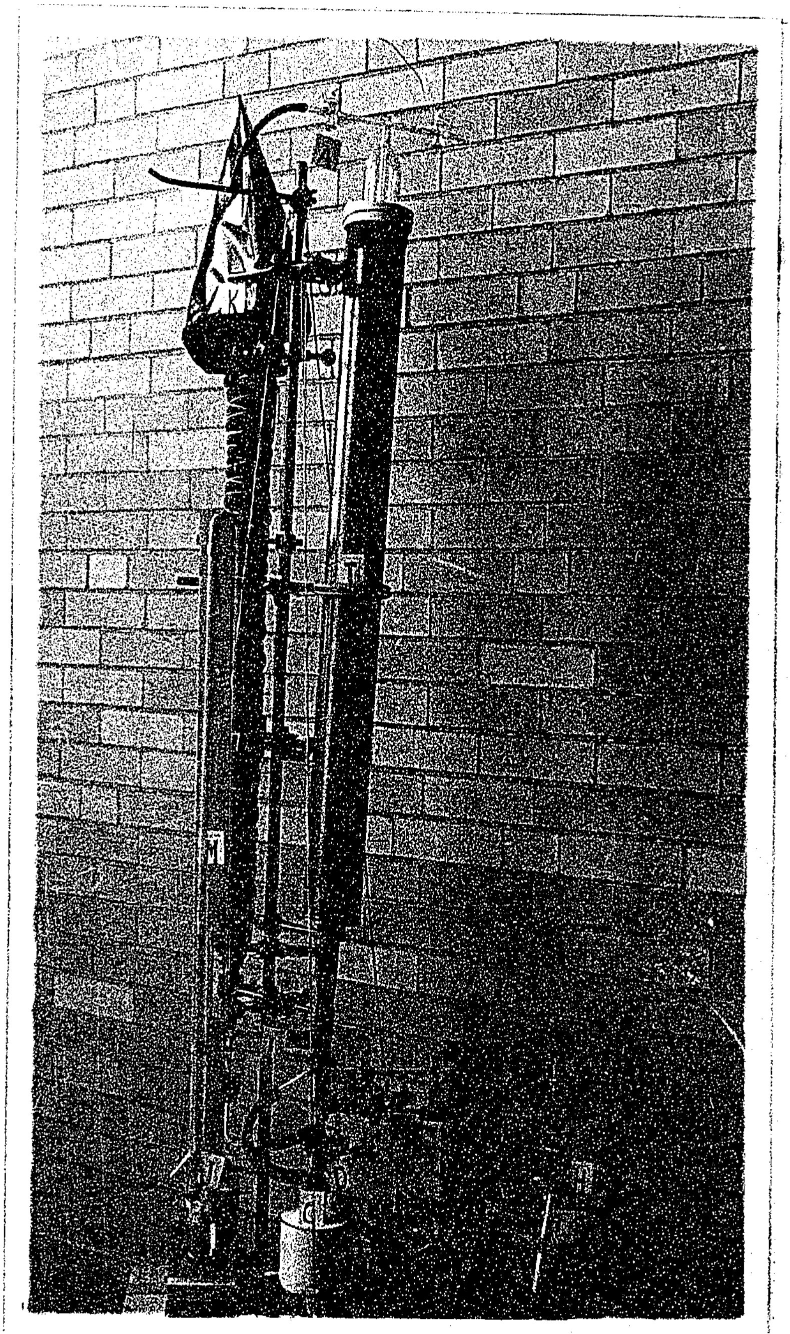


Plate 3. Apparatus used with bromine vapor method.

control of the partial pressure of bromine in the tube (T) by use of stopcocks (D and B) and the manometer (M) and facilities for obtaining constant weight of the samples and brominated samples (tube covered with black oilcloth bag (K)).

The "brominator" and "debrominator" tubes are 46 mm. internal diameter and about 90 cm. in length. Standard taper $\frac{55}{50}$ ground-glass joints are used at the tops of the tubes. The caps contain inner-sealed tubes which serve to admit nitrogen into the systems and as hooks from which the glass rod support, on which the sample cloths were suspended, was hung. The samples were placed on strips of glass cloth about 2.5 by 2.5 cm. in size. The edges of these cloths were fused in a small flame to prevent loss of small particles of glass. Glass hooks were placed in the cloths so that they could be suspended on the rod in the "brominator". Before these cloths were used they were thoroughly cleaned in hot sulfuric and nitric acids, rinsed with water several times, dried, boiled in carbon tetrachloride and again dried. Following this they were subjected to 15 to 17 cm. bromine pressure for 8 to 10 hours, rinsed with ether, boiled out with carbon tetrachloride and weighed.

A 1 : 10 solution of the fat sample in petroleum ether (fraction distilling at 32° to 34°C.) was used to impregnate the cloths with fat. From 10 to 20 drops of this solution were evenly distributed on each side of the weighed cloths. To prevent oxidation they were suspended on a rod in a desiccator in an atmosphere of nitrogen as soon as the fat solution was added. When all of the samples had been prepared, the cloths were transferred to the "debrominator". This tube was evacuated for 15 min. (at 6 to 8 mm. pressure), filled with nitrogen and the nitrogen allowed to flow through it for 3 min. This process was repeated and the samples were weighed by the method of swings.

The samples were then placed in the "brominator" and the tube was evacuated to 1.0 to 1.5 cm. pressure. Bromine vapor was then allowed to flow into the system by slowly turning stopcock (D) until the pressure within the system increased 4 to 5 cm. as indicated on the manometer (M). For butter fat a reaction period of 2 to 4 hours is sufficient with this concentration of bromine.

Excess bromine was removed by forcing nitrogen through the system for 3 min., evacuating for 5 min., sweeping out the system with nitrogen for 3 min. and again repeating this process. The excess bromine was removed by washing the nitrogen-bromine gases in a solution which was a mixture of 70 ml.

water containing 40 gm. anhydrous sodium thiosulfate and 130 ml. of water containing 100 gm. sodium hydroxide.

The samples on the glass cloths were transferred to the "debrominator". Bromine was further removed by twice evacuating for 15 min. at 6 to 7 mm. pressure and then sweeping out the system with nitrogen for 3 min. The bromine was absorbed in a saturated solution of a mixture of sodium thiosulfate and sodium hydroxide in the ratio of 1 : 4 by weight.

The samples were then transferred to a 100°C. oven, held at that temperature for 1½ to 2 hours, cooled and weighed. It was found that the glass cloths (without any sample) retained a small amount of bromine that was not removed by the evacuation and heating processes, that is, they were heavier at the end of the bromination process than they were before bromination. For this reason no fat was placed on the cloth at each end of the tube; these served as controls. Since the cloth at the lower end of the tube retained more bromine than did the cloth at the upper end, a linear correction determined graphically was applied to the cloths containing the samples. From the weights of bromine and fat the iodine numbers were calculated as follows:

$$\text{I no.} = \frac{\text{Wt. Br}}{\text{Wt. sample}} \times \frac{\text{I}}{\text{Br}} \times 100$$

The following iodine numbers were obtained with butter fat sample no. 3 when different exposures to bromine vapor were used:

| Sample no. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Trial 1. bromine pressure, 2.0 cm.; reaction period 30 min. | | | | | | |
| Sample wt. | 0.03149 | 0.03094 | 0.02382 | 0.04485 | 0.06454 | 0.04826 |
| I no. | 39.89 | 40.18 | 40.71 | 40.82 | 41.04 | 41.43 |
| Trial 2. bromine pressure, 3.0 cm.; reaction period, 1 hour | | | | | | |
| Sample wt. | 0.03168 | 0.07977 | 0.04461 | 0.04877 | 0.03485 | 0.06449 |
| I no. | 41.63 | 41.59 | 41.40 | 41.91 | 42.04 | 42.11 |
| Trial 3. bromine pressure, 4.5 cm.; reaction period, 3 hours | | | | | | |
| Sample wt. | 0.03261 | 0.06236 | 0.04718 | 0.04577 | 0.05894 | 0.03095 |
| I no. | 42.22 | 42.47 | 42.14 | 42.26 | lost | 41.82 |

The sample numbers correspond to the relative position of the samples in the bromination tube with number 1 at the top. The data of the first two trials indicate that the bromine concentration was higher at the bottom of the tube than it was at the top. Although the tube had been evacuated to a pressure of 1.0 to 1.5 cm. before admitting bromine, apparently considerable time is necessary for the bromine concentration to become uniform. The data in the third trial are more nearly constant and of about the same magnitude as the last two samples of trial 2.

There is little doubt but that an equilibrium value was reached in trial 3. It is believed that the value thus obtained (average 42.25) represents the total amount of unsaturation in this sample.

Rossmann (73) found that the methyl ester of $\beta$-elaeostearic acid gave an iodine number of 259 against the calculated value of 261 when brominated for one hour (concentration of bromine not given). This compound contains a conjugated system of three double bonds and should be many times more difficult to saturate with bromine than butter fat. He, likewise, obtained an iodine number of 506 for carotene (calculated value, 521) in 105 min.

There is the possibility that the value of 42.25 is slightly too high since other investigators (72,73,13) have found that bromine vapor reacted slowly with hydroxyl groups in castor oil. They are not in agreement with regard to the magnitude or the mechanism of the reaction. With the gravimetric bromine vapor method Rossmann (73) found that the iodine number of castor oil increased from 91 with a reaction period of 30 min. to 135 with a reaction period of 20 hours.

Hafner, Swinney and West (25) reported that a sample of butter fat contained hydroxylated fat equivalent to 2.7 to 3.0 mg. of acetyl per gram of fat. If it is assumed that hydroxyl groups bind one atom of bromine (13), the 3.0 mg. of acetyl would be equivalent to 0.88 iodine unit and if two atoms of bromine are bound (72), the iodine number would increase by 1.76 units.

Considering the conditions of the experiments on the bromination of butter fat, it is believed that the error resulting from reaction with hydroxyl groups is very small--probably less than 0.5 iodine unit.

It is interesting to note that the iodine number obtained with the semi-micro Rosenmund and Kuhnhenn method for the same sample of butter fat (fig. 4, sample no. 3) with a four-hour reaction period is in excellent agreement with the bromine vapor value. This supports the previous contention that the Rosenmund and Kuhnhenn is the best of the volumetric methods.

## SUMMARY

The preliminary work dealt with the Hanus and Hübl reagents. The Hanus method, especially with the insoluble acids of butter fat, depended too greatly on conditions of the experiment, such as sample weight, excess of reagent, etc. Reaction of the Hübl reagent with its solvent (ethyl alcohol) caused variations in the hydriodic acid content of the solution with age and changed the iodine numbers somewhat. There was likewise some indication that during long reaction periods the Hübl reagent might cause hydrolysis of fats, although with the methods available it was impossible to demonstrate this.

The variation of sample weight caused the iodine numbers to be lower the higher the sample for the semi-micro Hanus method. When sample weights were varied with the Hübl reagent, the iodine number decreased with increase in sample weight when the values were calculated using the average of the beginning and end blanks; they increased with increasing sample weight when calculated by the Schmidt-Nielsen and Owe formula.

An attempt to find a solvent for the Hübl reagent less reactive with it than ethyl alcohol showed that methyl alcohol was better. It caused greater variations in iodine numbers of fats when compared with ethyl alcohol solutions.

The Wijs, Hanus, Rosenmund and Kuhnhenn, Kaufmann, Hübl and Hübl reagent in methyl alcohol were studied from a reaction rate-time standpoint. It was considered that at equilibrium conditions a flat region should appear in the graphs obtained when iodine numbers were plotted against reaction period. These data (fig. 1 and 2) indicate that no equilibrium over a long period is attained either with butter fat or with its insoluble acids. When, however, the portions of these graphs (fig. 3) that represent 0 to 12 hours reaction periods are considered the Kaufmann, Hanus and Rosenmund and Kuhnhenn methods seem to show portions approaching zero slope. The Rosenmund and Kuhnhenn method between 1 and 4 hours appeared to be the best of the methods in this respect.

Certain peculiarities were shown between the iodine numbers of butter fat and its insoluble acids (fig. 1 and 2). The Wijs, Hanus and Rosenmund and Kuhnhenn methods yielded higher iodine numbers for the acids than their corresponding fat. The reverse was true for the Hübl, the methyl alcohol Hübl and the Kaufmann reagents. No definite explanation can be given for the anomalous behavior of the latter group of reagents.

It was considered that the results thus far indicated that further study was warranted with the Rosenmund and

Kuhnhenn reagent. As a result of this study the following conditions were worked out for its use: Weigh from 10 to 100 mg. of butter fat or insoluble butter acids into a 125 ml. iodine flask and dissolve in 2 ml. of carbon tetrachloride. Add 5 ml. of 0.1N pyridine sulfate dibromide in acetic acid freed of reducing compounds, and close the flask with a glass stopper on the surface of which one drop of syrupy phosphoric acid was spread to prevent escape of reagents. Rotate the contents of the flask a few times and allow the reaction to take place in the dark at 25°C. for 4 hours. After the reaction period, add 2 ml. of 10 per cent potassium iodide to the rim of the flask, cautiously turn the stopper in order that small gas bubbles will go through the potassium iodide. Thoroughly mix the potassium iodide with the reactants and then add 25 ml. of oxygen-free water and mix well. Titrate the iodine with sodium thiosulfate (approximately 0.011N) to the complete disappearance of the yellow color (a slight excess of thiosulfate). Stopper the flask and shake violently, rinse liquid adhering to stopper into the flask and add 4 to 5 ml. of 0.5 per cent starch indicator. Back titrate the excess of sodium thiosulfate with iodine in potassium iodide (approximately 0.005N) from a burette graduated to 0.02 ml.

When sample weights are approximately alike, the semimicro Rosenmund and Kuhnhenn method yields replicate determinations which agree within 0.3 of an iodine unit.

It was considered that as final checks on the semi-micro Rosenmund and Kuhnhenn method the following factors should be studied: 1. effect of excess reagent on the iodine number obtained for butter fat and its insoluble acids, 2. agreement of replicate determinations on weighed samples, 3. comparison of the value by this method with the value obtained with a carbon tetrachloride solution of iodine monobromide conducted in such fashion that substitution should have been detected and 4. comparison of the value by this method with that obtained by the bromine vapor method.

It is considered from these studies that excess reagent from 14 to 90 per cent will cause a variation of 1.0 unit in the iodine number of butter fat, while variation of excess reagent from 20 to 90 per cent will cause a variation of approximately 0.5 unit with the insoluble acids from butter fat.

Replicate determinations on weighed samples should check within 0.5 unit.

The attempted determination of a "true" iodine number by measuring substitution and correcting the iodine number for it did not prove satisfactory nor did it afford the desired comparison.

The comparison with the bromine vapor method which should give values very closely representing only the degree of unsaturation of butter fat indicate that for this fat the Rosenmund and Kuhnhenn method, used as described above, gives very nearly the correct iodine number.

The bromine vapor method was studied with regard to its adaptation for use with butter fat and its acids and was considered a satisfactory research method, although it is long and tedious.

## CONCLUSIONS

1. The relationship between the iodine numbers of butter fat and its acids was shown by some iodine number methods to be as was expected, i.e., higher for the acids than for the fats. These were in about the correct relationship with methods deemed reliable.

2. The Hanus, Wijs, Rosenmund and Kuhnhenn, Kaufmann, Hübl and Hübl reagent in methyl alcohol methods were studied. The Rosenmund and Kuhnhenn method was deemed the most reliable of those studied.

3. An attempt to determine "true" iodine numbers by measuring the substitution that occurred and correcting for this did not yield satisfactory results.

4. The bromine vapor method was shown to be a satisfactory research method for use in studies with butter fat.

# LITERATURE CITED

(1) André, E. Sur le pouvoir d'addition de l'iode vis-a-vis des composés éthyléniques. Bull. soc. chim. (4), 33: 1641-1647. 1923.

(2) Arup, P. The iodine and thiocyanogen values of Irish butter. Analyst 57: 610-615. 1932.

(3) Association of Official Agricultural Chemists. Official and tentative methods of analysis. 4th ed., p. 410-412. Washington, D. C. 1936.

(4) Auguent, M. Étude comparative de la méthode de Hübl et de la méthode de Wijs pour la détermination de l'indice d'iode des corps gras. Ann. fals. 5: 459-475. 1912.

(5) Babkine, M. Determination microchimique de l'indice d'iode d'après Hübl. Maslob. Jirov. Del. 9, no. 5: 32-33. 1933. Original not seen. Abstracted in Chimie & industrie 31: 639-640. 1934.

(6) Bankston, H. J. and Vilbrandt, F. C. Influence of several solvents on the Hanus iodine values of cottonseed and coconut oils. Ind. Eng. Chem. 16: 707-708. 1924.

(7) Bauer, H. Zur Kenntniss der Kohlenstoffdoppelbindung. Addition von Brom. Ber. 37: 3317-3321. 1904.

(8) Bauer, H. and Moser, H. Zur Natur der Kohlenstoffdoppelbindung. Addition von Brom. Ber. 40: 918-924. 1907.

(9) Becker, P. Über die Bromzahl. Z. angew. Chem. 36: 539. 1923.

(10) Biilmann, E. Sur la formation et la décomposition de quelques composés halogénés organiques. Rec. trav. chim. 36: 313-328. 1917.

(11) Böeseken, J. and Blumberger, J. S. P. L'action de l'iode en solution chloriformique sur quelques hydrocarbures éthyléniques. Rec. trav. chim. 44: 96-98. 1925.

(12) Böeseken, J. and Gelber, E. T. Betrachtungen über die Jodzahlbestimmungen. I. Rec. trav. chim. 46: 158-171. 1927.

(13) Böeseken, J. and Pols, P. Sur la détermination microchimique de l'insaturation par exposition aux vapeurs de brome. Rec. trav. chim. 54: 162-166. 1935.

(14) Bolton, E. R. and Williams, K. A. The composition and polymerisation of Chinese wood (tung) oil. Analyst 55: 360-364. 1930.

(15) Caldwell, B. P. and Piontkowski, F. A. The addition of iodine and thiocyanogen to unsaturated compounds. J. Am. Chem. Soc. 56: 2086-2089. 1934.

(16) Chargaff, E. Charakterisierung von Fetten in geringen Substanzmengen. Z. physiol. Chem. 199: 221-224. 1931.

(17) Dam, H. Jodzahlbestimmungen an Cholesterin. Biochem. Z. 152: 101-110. 1924.

(18) Dawson, H. M., Burton, D. and Ark, H. The dynamics of the action of halogens on aliphatic aldehydes. Keto-enol isomerism of the aldehydes. J. Chem. Soc. 105: 1276-1290. 1914.

(19) Dawson, H. M. and Wheatley, R. The reactivity of ketones towards iodine and the relative rates of tautomeric change. J. Chem. Soc. 97: 2048-2054. 1910.

(20) van Duin, C. F. The action of inorganic iodides on dibromo-compounds. I. The mechanism of the reaction. Rec. trav. chim. 43: 341-358. 1924.

(21) van Duin, C. F. The action of inorganic iodides on dibromo-compounds. II. The general character of the reaction and the influence of substituents. Rec. trav. chim. 45: 345-362. 1926.

(22) Ellis, B. A. and Jones, R. A. The condensation of maleic anhydride with tung oil: a new "constant" for oils. Analyst 61: 812-816. 1936.

(23) Fahrion, W. Zur Hübl'schen Jodadditions methode. Chem. Z. 15: 1791-1792. 1891.

(24) Godbole, N. N., Ketkar, V. V., Sharma, K. V. J. S. and Kamath, H. R. Comparative study of the determination of iodine values. Current Sci. 5: 361-364. 1937. Original not seen. Abstracted in Chem. Abs. 31: 3719. 1937.

(25) Hafner, P. G., Swinney, R. H. and West, E. S. Hydroxylated acids of fats: an improved method of determination. J. Biol. Chem. 116: 691-697. 1936.

(26) Hanus, J. Die Anwendung von Jod monobromid bei der Analyse von Fetten und Oelen. Z. Nahr. Genussm. 4: 913-920. 1901.

(27) Hawley, H. The determination of iodine values by the pyridine sulfate bromide method. Analyst 58: 601. 1933.

(28) Hehner, O. On the determination of the bromine absorption of fats gravimetrically. Analyst 20: 49-53. 1895.

(29) Herz, W. and Mylius, B. Die Geschwindigkeit der Addition von Brom an Zimmtsäure. Ber. 39: 3816-3820. 1906.

(30) Herz, W. and Mylius, B. Die Geschwindigkeit der Addition von Jod an Allylalkohol. Ber. 40: 2898-2904. 1907.

(31) Ho, K., Wan, C. S. and Wen, S. H. Iodine value of tung oil. Effect of time, excess of Wijs reagent and temperature. Ind. Eng. Chem. Anal. Ed. 7: 96-101. 1935.

(32) Holde, D. and Gorgas, A. Über Additionsprodukte von Jodmonobromide und unterjodiger Säure an ungesättigte Körper. I. Ber. 58: 1071-1074. 1925.

(33) Hübl, A. Eine allgemein anwendbare Methode zur Untersuchung der Fette. Dinglers polytech. J. 253: 281-295. 1884.

(34) Hunt, F. W. A comparison of methods used to determine the iodine value of oils. J. Soc. Chem. Ind. 21: 454-456. 1902.

(35) Hunter, L. and Hyde, F. F. A new reagent for the determination of iodine values. Analyst 58: 523-526. 1933.

(36) Ingle, H. The origin and nature of the free acid formed during Hübl's reaction with unsaturated compounds. J. Soc. Chem. Ind. 21: 587-595. 1902.

(37) Ingle, H. The "iodine value" of unsaturated organic compounds. J. Soc. Chem. Ind. 23: 422-431. 1904.

(38) Jamieson, G. S. Report on fats and oils. J. Assoc. Official Agr. Chem. 6: 440-445. 1923.

(39) Kaufmann, H. P. Weitere Versuche zur Bromometrie der Fette. Z. Untersuch. Lebensm. 51: 3-14. 1926.

(40) Kaufmann, H. P. Die Rhodanometrie von Fettem und Fettgemischen. Z. Untersuch. Lebensm. 51: 15-27. 1926.

(41) Kaufmann, H. P. Studien auf dem Fettgebiet. p. 18, 32, 33. Maurer and Dimmick, Berlin. 1935.

(42) Kaufmann, H. P. Dienometrie und Dien-Zahl der Fette. Bemerkungen zu der M.-A.-Zahl von B. A. Ellis und R. A. Jones. Ber. 70: 900-902. 1937.

(43) Kaufmann, H. P. and Baltes, J. Dien Synthesen auf dem Fettgebiet, II.: Die Zusammenset;zung des chineseschen Holzöls (Studien auf dem Fettgebiet, XXX Mitteil.). Ber. 69: 2676-2679. 1936.

(44) Kaufmann, H. P. and Baltes, J. Dien-Synthesen auf dem Fettgebiet, III.: Über das Oiticica-Öl (Studien auf dem Fettgebiet, XXXI Mitteil.). Ber. 69: 2679-2683. 1936.

(45) Kaufmann, H. P. and Baltes, J. Die Errechnung der Zusammensetzung von Fetten mit Hilfe ihrer Kennzahlen (Studien auf dem Fettgebiet, 46. Mitteil.). Ber. 70: 2545-2549. 1937.

(46) Kaufmann, H. P. and Baltes, J. Die Bestimmung der Hydrierjodzahl und ihre Anwendung bei der Analyse des Essangöls (Studien auf dem Fettgebiet, 45. Mitteil.). Ber. 70: 2537-2544. 1937.

(47) Kaufmann, H. P., Baltes, J. and Büter, H. Dien-Synthesen auf dem Fettgebiet, IV: Die Bestimmung der Dien-Zahl auf jodmetrischem Wege. Dien-Zahlen verschiedener Fette und ihre Auswertung (Studien auf dem Fettgebiet, XXXV. Mitteil.). Ber. 70: 903-907. 1937.

(48) Kaufmann, H. P. and Grosse-Oetringhaus, H. Über das Jodrhodan und seine Addition an ungesattigte Fettsauren (Studien auf dem Fettgebiet, XXIX. Mitteil.). Ber. 69: 2670-2676. 1936.

(49) Kaufmann, H. P. and Grosse-Oetringhaus, H. Die quantitative Bestimmung ungesättigter Kohlenwasserstoff in Gemischen. Jodrhodan in der Massanalyse. Ber. 70: 911-915. 1937.

(50) Kaufmann, H. P. and Hansen-Schmidt, E. Bromometrische Versuche. Arch. Pharm. 263: 32-50. 1925.

(51) Kaufmann, H. P. and Hartweg, L. Meso-und mikroanalytische Methoden auf dem Fettgebiet, I: Jodzahl und Dienzahl (Studien auf dem Fettgebiet, 48. Mitteil.). Ber. 70: 2554-2559. 1937.

(52) Kaufmann, H. P. with Lutenberg, C. Partielle Halogen-Addition an mehrfach ungesättigte Fettsäuren. β-Eloeostearinsäure-glycerid und Holzöl (Studien auf dem Fettgebiet. 13 Mitteilung). Ber. 62: 392-401. 1929.

(53) Kolthoff, I. M. and Furman, N. H. Volumetric analysis. Vol. I, p. 190-200. John Wiley and Sons, New York. 1928.

(54) Kolthoff, I. M. and Sandell, E. B. Textbook of quantitative analysis, p. 584-607. The Macmillan Co., New York. 1936.

(55) MacLean, I. S. and Thomas, E. M. Observations on abnormal iodine values, with special reference to the sterols and resins. Biochem. J. 15: 319-333. 1921.

(56) Mahle, A. Ueber die Hübl'sche Chlorjodadditionsmethode und Vorschläge ju deren Verbesserung. Chem. Z. 19: 1786-1787. 1895.

(57) Margosches, B. M., Friedmann, L. and Tschörner, W. Zur chemischen Natur der Fette I. Die überjodzahl fetter Öle und ungesättigter Fettsäuren. Ber. 58: 794-797. 1925.

(58) Margosches, B. M. and Hinner, W. Über die Reaktionsfähigkeit des Jods gegen Fette. II. Verhalten wasseriger Jodlösungen. Z. angew. Chem. 37: 202-205. 1924.

(59) Margosches, B. M. and Hinner, W. Über die Reaktionsfähigkeit des Jods gegen Fette. IV. Verhalten von Jod-Jodsäurelösungen. Z. Nahr. Genussm. 47: 349-355. 1924.

(60) Margosches, B. M., Hinner, W. and Friedmann, L. Über die Einwirkung alkoholischer Jodlösungen auf ungesättigte Fettsäuren und fett Öle; Einfluss von Wasser und Kaliumjodide auf dem Reaktionsverlauf. Ber. 57: 996-1001. 1924.

(61) Margosches, B. M., Hinner, W. and Friedmann, L. Über eine Schnellmethode zur Bestimmung der Jodzahl fetter Öle mit Jod und Alkohol. Z. angew. Chem. 37: 334-337. 1924.

(62) Margosches, B. M., Ludwig, F., Scheinost, E. and Tschörner, W. Zur chemischen Natur der Fette II. Die überjodzahl fetter Öle und ungesättigter Fettsäuren mit gleicher Jodzahl. Ber. 58: 1064-1067. 1925.

(63) Marshall, A. The iodine value of oils. J. Soc. Chem. Ind. 19: 213-215. 1900.

(64) Michael, A. Zur Kenntniss des Additions-Abspaltungs-Gesetzes. Ber. 34: 4215-4226. 1901.

(65) Netto, I. d'O.C.C. A study of the methods of determining the iodine value of oils. Ministerio da Agr. Lisbon, Estudos Quimicos Bol. No. 21: 1-71. 1936. Original not seen. Abstracted in Chem. Abs. 31: 6491-6492. 1937.

(66) Page, I. H., Pasternack, L. and Burt, M. L. Über den Transport von Fetten und Lipoiden durch Blut nach Öleingabe. Biochem. Z. 223: 445-456. 1930.

(67) Pelikan, K. A. and Mikusch, J. D. Comparison of two methods for the determination of conjugated double bonds. Oil & Soap 14: 209-210. 1937.

(68) Ralls, J. O. Factors giving rise to the abnormal iodine absorption number of cholesterol. J. Am. Chem. Soc. 55: 2083-2094. 1933.

(69) Ralls, J. O. A micro method for the determination of iodine numbers. J. Am. Chem. Soc. 56: 121-123. 1934.

(70) Rogers, Associates of. Fundamentals of dairy science, p. 74, 100. Reinhold Publishing Corp., New York. 1935.

(71) Rossmann, E. Bromdampf addition nach P. Becker. Mikronachweis aktiver und inaktiver Doppelbindungen. Ber. 65: 1847-1851. 1932.

(72) Rossmann, E. Titrimetrische Jodzahlbestimmung durch Bromdampfaddition. Angew. Chem. 48: 223-226. 1935.

(73) Rossmann, E. Zur Bestimmung der Zahl der Doppelbindungen bei Ölen und Harzen. Angew. Chem. 50: 187-190. 1937.

(74) Rosenmund, K. W. and Kuhnhenn, W. Eine neue Methode zur Jodzahlbestimmung in Fetten und Ölen unter Verwendung von Pyridinsulfatdibromid. Z. Nahr. Genussm. 46: 154-159. 1923.

(75) Ruziczka, W. Zur Bestimmung der Jodzahl nach der Schnellmethode. Mikrochemie 17: 215-221. 1935.

(76) Schmidt-Nielsen, S. and Owe, A. W. Die Bestimmung der Jodzahl. I. Vergleichende Untersuchungen über die Jodzahl der Fette. Skrifter Videnskapsselskapet. I. Kristiania. Mat. natur. Klasse. No. 15. 1923.

(77) van der Steur, J. P. K. Jodaddition der ungesättigten Öle, Fette und Fettsäuren in einigen organischen Lösungsmitteln. I. Rec. trav. chim. 46: 278-283. 1927.

(78) van der Steur, J. P. K. Jodaddition der ungesättigten Öle, Fette und Fettsäuren in einigen organischen Lösungsmitteln. II. Die quantitative Bestimmung der Ölsäure und der Elaidinsäure nebeneinander. Rec. trav. chim. 46: 409-413. 1927.

(79) van der Steur, J. P. K. Jodaddition der ungesättigten Öle, Fette und Fettsäuren in einigen organischen Lösungsmitteln. III. Rec. trav. chim. 46: 414-416. 1927.

(80) Sudborough, J. J. and Thomas, J. The addition of bromine to unsaturated compounds. Part I. J. Chem. Soc. 97: 715-720. 1910.

(81) Sudborough, J. J. and Thomas, J. The addition of bromine to unsaturated compounds. Part II. J. Chem. Soc. 97: 2450-2453. 1910.

(82) Toms, H. Oil bromide films and their use in determining the halogen absorption of oils. Analyst 53: 69-77. 1928.

(83) Toms, H. Notes on the bromine vapor method for the determination of the halogen absorption of oils. Analyst 61: 177-178. 1936.

(84) Tolman, L. M. and Munson, L. S. Iodine absorption of oils and fats. A comparison of methods. J. Am. Chem. Soc. 25: 244-251. 1903.

(85) Volmar and Samdahl. Dosage de l'acide oléique et de l'acide linoléique contenus dans une huile. Détermination de son indice de brome. Bull. soc. chim. (4), 39: 1505-1507. 1926.

(86) Volmar and Wagner. L'indice de non saturation des composés éthyléniques. Bull. soc. chim. (5) 2: 826-844. 1935.

(87) Waller. Ueber die Hübl'sche Chlorjodadditionsmethode und Vorschläge zu deren Verbesserung. Chem. Z. 19: 1831-1832. 1895.

(88) Werner, H. Über die Einwirkung von Chlorjod auf Cholesterin, ein Beitrag zur Methode zur Bestimmung der Jodzahl. Z. Untersuch. Lebensm. 61: 321-337. 1931.

(89) Wijs, J. J. A. Zur Hübl'schen Jodadditions-Methode Z. anal. Chem. 37: 277-283. 1898.

(90) Wijs, J. J. A. Zur Hübl'schen Jodadditionsmethode. Z. angew. Chem. 11: 291-297. 1898.

(91) Wijs, J. J. A. Zur Jod-Additionsmethode. Ber. 31: 750-752. 1898.

(92) Wijs, J. J. A. Zur Jodzahlbestimmung. Z. Untersuch. Lebensm. 56: 488-490. 1928.

(93) Wollschitt, H. Die Jodzahl als universelle biologische Konstante (gravimetrische Schnellmikrobestimmung der Jodzahl durch Bromdampfanlangerung). Arch. exp. Path. Pharmkol. 179: 260-265. 1935.

(94) Yamaguchi, E., Matsumura, T. and Takagi, T. Bromine value of some fatty oils. Waseda Applied Chem. Soc. Bull. 13, No. 4 (29): 7-11 (Abstracts in English p. 61). 1936. Original not seen. Abstracted in Chem. Abs. 31: 2459. 1937.

(95) Yasuda, M. The determination of the iodine number of lipids. J. Biol. Chem. 94: 401-409. 1931.

(96) Yoshiyuka, T. and Ishikawa, T. On the iodine values of hydrogenated castor oil. J. Soc. Chem. Ind., Japan. Suppl. binding. 39: 300-302. 1936.