J. *Anim. Prod. U.A.R.* 9, No. 1, pp. 109-121 (1969).

STUDIES ON BUFFALO MILK FAT OXIDATION

II.—The Variation in Peroxide, T.B.A., and Iodine Values, and other Constituents of Buffalo Milkfat During Autoxidation.

N. S. AHMED, I. D. RIFAAT*, A. A. HOFI, AND A. M. EL-SOKKARY

The pattern of increase in the T.B.A. values, total carbonyls, conjugated dienes, and the free-mono-carbonyls contents are similar to that of the peroxide values, during the oxidation period in both butteroil and samn, while the iodine value takes an opposite trend.

There are positive significant correlations between the peroxide value and the T.B.A. values, total carbonyls, conjugated dienes, and free-mono-carbonyls classes in butteroil and samn.

Butteroil oxidizes more rapidly than samn, since it gives lower values for the peroxide, T.B.A., iodine values, total carbonyls, conjuted dienes, free-mono-carbonyls classes during autoxidation.

The corporation of atmospheric 0_2 with milkfat, known as autoxidation, causes the development of oxidized flavour in milk, butter, and other dairy products. It is well known that when fat oxidizes, carbonyl compounds are formed which contributes mainly to the oxidized flavour deffect. Most chemical tests used are termed indirect, since they do not measure the amount of the oxidized flavour compounds but mesure other chemical properties indicative of the oxidation reactions. These tests, however, do not give good correlations with the sensory tests, which are necessary in quality control purposes. Therefore, the purpose of this study is to follow the oxidation process of buffalo milkfat by determining the peroxide values, T.B.A. test, I.V., conj. fatty acids, total carbonyls, and free monocarbonyl contents which are correlated with the autoxidation reactions and involved in the off-flavour deterioration.

Experimental and Methods of Analysis

Fresh buffalo milk samples wre obtained from the herd of the Faculty of Agriculture, Ain Shams Univerity. The cream was separated, washed with hot distilled water, reseparated, and then churned to butter. A portion of the butter was melted at 55°C, washed several times with hot distilled water and the butteroil layer was separated and filtered. Another portion of the butter was converted to samn by the Boiling off-method. Both butteroil and samn were left to stand in a loosely covered flasks in a place in the laboratory where they were exposed to a maximum amount of diffused sunlight and air.

* Food Technology & Dairy Dept. N.R.C., Faculty of Agriculture, Ain Shams University and Food Industries Foundation.

The peroxide value (P.V.), conjugated fatty acids (conj. F.A.) were determined according to the AOCS method (1957), while the thiobareturic acid test (TBA) by the method of Patton, & Kurtz (1951), the iodine value (I.V.) by the British Pharmacopoeia method (1963), total carbonyls content by the method of Berry & Mc Kerrigan (1958) and the Preemonocarbonyls (F.M.C.) by the method of Keith & Day (1963).

The P.V., T.B.A., and I.V. ; T.C. and Conj. F.A. contents of freshly prepared butteroil and samn were estimated. At the end of the induction period the F.M.C. were determined simultaneously with the previous determinations every 10 days for the first 100 days, then every 30 days over a period of 380 days, in order to follow the variations that took place during the oxidation process until it was completed.

Results and Discussion

Storing the butteroil or samn samples in diffused daylight and air was necessary to induce and accelerate their autoxidation (Badings, 1960 ; Keeney and Doan, 1951 ; and Lea, 1953).

The P.V. was determined and used as a quantitative test for measuring the quantity of peroxide formed (Badings, 1960; Holman, 1954 ; and Morris, 1954). Since it measurs the peroxides ; the primary products of fat oxidations, therefore, it is not adequate in giving the history of the oxidation of the fat concerned. The T.B.A. test is more sensitive and was used to detect the early stages of autoxidation, and here or to establish its state of oxidation. Although, P.V. measure the amount of oxidation, yet, they do not estimate a specific determination of the oxidative rancidity which liable to appear at the different peroxide levels. Determining the carbonyl compounds will give more exact informations concerning the state of oxidation of the fat. The carbonyl compounds produced during oxidation are extremely complex, since they include volatile and non volatile, saturated and unsaturated compounds. However, the greatest part of them are non-volatile with high molecular weights, which are the precursors of the volatile odours substances (Berry and McKerrigan 1958 ; Gaddis, *et al.* 1959). Only the short chain volatile carbonyl compounds are the cause of the off-flavour that appear during the autoxidation of edible fats (Berry and McKerrigan, 1958 ; and Gaddis et al., 1959). The U.V. light absorption characteristics of oxidized fats indicated that most of the mono-hydroperoxides formed during autoxidation were conjugated in nature. In addition, when oils containing linoleate or more highly unsaturated systems were oxidized the conjugeated dienes systems increased parallel to the 0_2 uptake and peroxide formation (Bading, 1960 ; Holman, 1954 ; Lea, 1953 ; and Morris, 1954).

The values and trend of incease of the P.V. and T.B.A. values ; as shown in tables 1 and 2 and Figs. 1 & 2, in butteroil and samn respectively, were relatively smooth during the induction period. A sharp rise in both values was followed and the increase continued until the 100 days of storage. After this period, it was noticed that the increase in both values were fast and with wide range of variations.

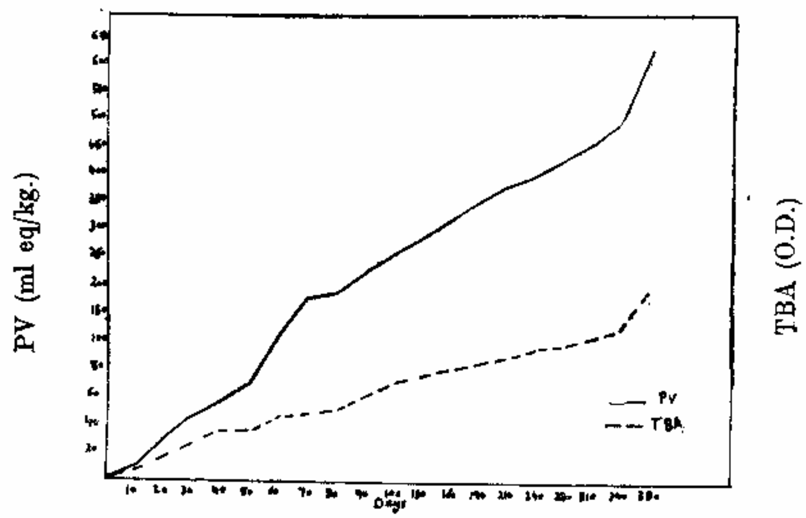

FIG. 1.—Increase of P.V. and TBA Values During Autoxidation of Burffalo Buttereil.

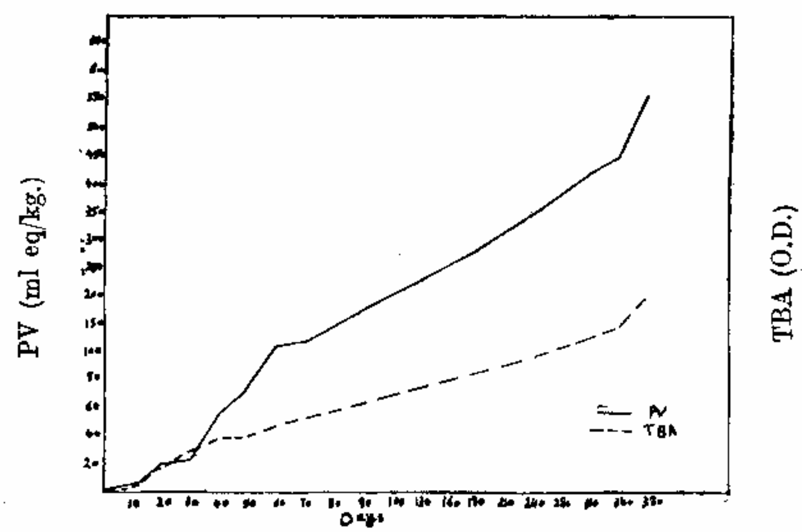

FIG. 2.—Increase of P.V. and TBA Values During Autoxidation of Buffalo Samne.

TABLE 1.—AVERAGE VALUES OF PEROXICE, T.B.A., IODINE, TOTAL CARBONYLS, CONJ. DIENE F.A., FREE-MONOCARBONYLS CLASSES OF BUTTEROIL DURING AUTOXIDATION

	P.V. ml. Eq. / kg.	TBA O.D.	I.V. %	T.C. m. mol / kg. S	U	Conj. diene F.A. %	F.M.C. m. mol. / kg. ALK.	2-Enals	2,4-Dienals
Fresh	—	—	29.8674	42.5789	—	1.0103	—	—	—
3	3.7798	.015	29.4173	—	—	—	—	—	—
5	4.2749	.020	30.1965	—	—	—	—	—	—
7	5.5750	.023	30.3105	—	—	—	—	—	—
10	7.3446	.033	30.3165	—	—	—	—	—	—
12	9.094	.038	30.1802	—	—	—	—	—	—
14	11.5449	.040	30.1818	—	—	—	—	—	—
16	17.6030	.059	30.0246	—	—	—	—	—	—
18	19.9279	.065	29.5586	—	—	—	—	—	—
20	28.1353	.080	28.9314	59.8534	2.9976	1.0721	—	—	—
30	144.8248	.116	28.6038	80.3997	7.2374	1.3102	40.4719	3.2838	1.3060
40	73.9619	.171	27.9525	94.2965	12.1373	1.3128	43.3715	7.5217	3.9413
50	90.1619	.178	28.2931	102.8069	13.7450	1.3179	57.8756	7.3673	3.7585
60	111.5140	.218	28.9969	125.1205	18.3781	1.3523	62.2852	8.0982	4.2222
70	172.5216	.241	28.5673	141.3913	21.9243	1.3849	74.7159	11.1751	5.4122
80	186.8433	.257	26.6411	152.2764	24.7858	1.4973	87.8055	12.9069	6.2051
90	226.2466	.291	26.4039	166.2929	27.7706	1.5425	101.9013	14.1769	7.0309
100	261.1199	.349	25.1771	170.6285	28.4883	1.5523	113.5479	15.0033	8.3331
130	292.0825	.380	23.8960	196.1576	32.0068	1.5696	126.4899	15.9131	9.5342
160	316.3780	.404	22.5445	213.4942	34.9066	1.6415	134.4102	17.6636	11.0849
190	354.5749	.427	21.7595	245.6823	40.6095	1.7655	149.1928	19.0290	13.5176
210	381.5710	.452	19.9204	253.3140	40.7057	1.9321	157.3548	19.3939	13.6402
240	404.6936	.476	28.2512	261.4931	43.4683	2.1270	158.2198	19.7137	14.3936
280	432.5407	.489	17.7531	281.4665	46.6104	2.1228	179.7515	21.7733	17.5588
310	460.9211	.521	17.0358	297.0165	50.1380	2.1926	192.5757	23.3019	17.9544
340	498.3474	.554	16.5218	317.1925	53.9350	2.2347	202.7296	25.1037	19.8146
380	638.6287	.701	14.2877	395.3755	58.2066	2.4769	238.4084	29.0899	22.8067

TABLE 2.—AVERAGE VALUES OF PEROXIDE, TBA, IODINE, T.C., CONG. DIENE F.A., F.M.C. CLASSES IN SAMN DURING AUTOXIDATION

	P.V. ml, Eq. / kg.	TBA O.D.	I.V. %	T.C. m. mol./kg. S	U	Conj. diene F.A. %	F.M.C. m. mol. / kg. ALK.	2-Enals	2,4-Dienals.
Fresh	—	—	30.9891	32.6676	—	1.1468	—	—	—
3	3.9034	.018	30.8443	—	—	—	—	—	—
5	4.1654	.021	30.9277	—	—	—	—	—	—
7	5.4615	.023	30.2731	—	—	—	—	—	—
10	5.8413	.033	30.8050	—	—	—	—	—	—
12	6.7487	.045	30.9799	—	—	—	—	—	—
14	9.6687	.057	30.7863	—	—	—	—	—	—
16	12.7178	.069	31.0944	—	—	—	—	—	—
18	12.3341	.074	30.7414	—	—	—	—	—	—
20	19.6702	.085	30.7890	45.4697	2.2879	1.1476	—	—	—
30	32.9662	.139	28.5439	47.7707	11.0638	1.1488	34.6419	5.2281	2.6260
40	57.0744	.186	28.5533	76.8941	18.0903	1.3181	33.4629	5.4834	3.5138
50	72.2149	.197	28.1259	90.5787	19.5722	1.4453	44.1518	5.6308	3.9458
60	106.6181	.243	28.4017	100.3650	19.6069	1.5046	53.6913	6.2748	4.0189
70	119.7631	.265	27.1685	105.9583	20.8792	1.5342	63.8387	7.3630	4.5531
80	148.9938	.283	26.0949	119.0605	21.9992	1.5685	74.5809	8.5983	5.2490
90	176.8734	.324	26.5087	131.5154	24.1771	1.6016	82.9311	9.5593	6.0360
100	201.3485	.353	25.5653	144.8713	25.3925	1.6563	91.7794	10.7802	6.7026
130	227.3307	.379	23.9071	155.8672	27.2383	1.7697	99.0908	11.5124	7.2780
160	247.0534	.402	22.8113	167.0485	29.7843	1.8191	111.0860	12.4120	7.8972
190	284.6647	.428	21.6714	182.4189	31.9530	1.8764	120.5626	13.5240	8.3166
210	314.9060	.451	20.2892	217.5154	34.0481	1.9947	142.8989	15.5541	10.4810
280	378.4298	.521	19.9077	231.5826	43.2094	2.1074	154.0296	16.5804	11.5146
310	416.6861	.551	18.8216	252.7338	45.1529	2.1393	163.0658	18.1782	12.4229
340	447.6091	.597	18.3882	271.5827	49.476	2.2473	172.8502	19.2749	13.2965
380	564.1923	.685	16.6433	337.5155	54.4625	2.2642	205.0839	20.1119	15.3219

Opposite to the P.V.; the I.V. decreased during the storage; Fig. 3. and did not show noticeable variations during the induction period. It then decreased slightly with ununiform fluctuations till the 80 days where it dropped suddenly. The decline that was followed was more pronounced during the rest of storage period.

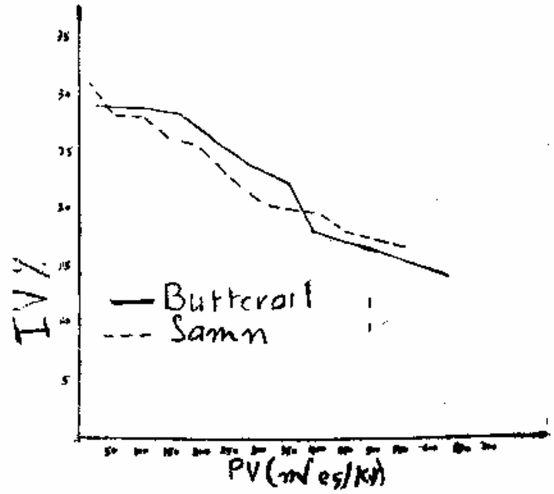

FIG. 3.—Rsletionship of P. V., and I. V. During Autoxidation of Buffalo Butteroil and Samn.

In the conjugated unsaturated fatty acids, only the variations in the conj. dienes concentrations were of value in the autoxidation of fats, since they constituted the major part of the conj. unsaturated acids. However, the increase in both butteroil and samn followed similar trend as the P.V. as shown in Fig. 4.

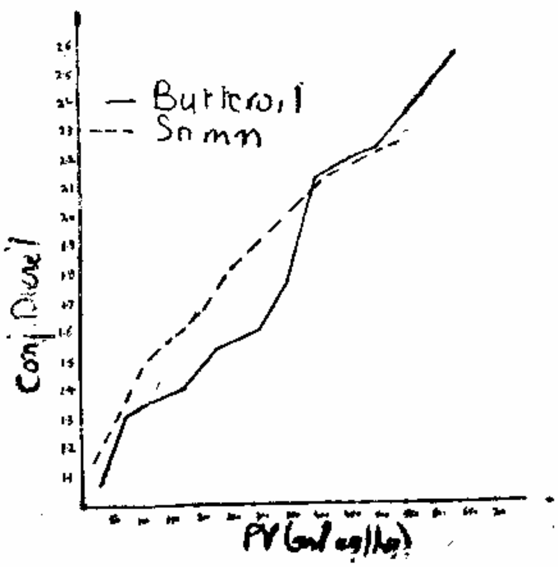

FIG. 4.—Relation ship of P.V., and Conj. Dienes During Autoxidation of Buffalo Butteroil and Samn.

Immediately after the end of the induction period, both the P.V. and T.B.A. values increased rapidly indicating a progressive and fast oxidation of the fat. Moreover; the increase in both values was significantly positive and parallel. On the other hand the I.V. showed an opposite trend as it decreased during the oxidation process. However, the decrease was slight at the early stages of storage but became more pronounced later after almost 100 days. These findings agreed with those of Achaya, 1947; Necheav, 1956; Kartha, 1957, 1958; and Glimm *et al.*, 1939), while disagreed with those of Naegamwala, *et al.*, 1954; and Nelson and Dahle, 1940), who noted that the I.V. of milkfat did not decrease during the period of greatest increase in P.V.

The relation between the increase in P.V. and the incerase in conj. dienes systems was positively significant in both butteroil and samn. This indicated that during the advancement of the autoxidation process the conj. dienes systems increased. This relation was confirmed by several investigators (Badings, 1960; Holman, 1954; Lea 1953, Morris, 1954; and Lundberg, *et al.*, 1946) as they pointed out that the increase in the conjugated unsaturated systems containing carbony groups or the conjugated polyenes formed from these systems were directly proportional to the peroxide contents during the autoxidation of fats. To the contrary, Otake (1961), claimed that the diene and triene acids of fresh butterfat were not affected by oxidation.

There were adequate evidences to suggest that measureing the amounts of the carbonyl compounds would give exact informations concerning the state of oxidation of the fat, which was found to be in the line with the development of off-flavours. The freshly prepared milkfat samples contained only saturated carbonyl compounds. After the end of the induction period the unsaturated carbonyls developed in the samples in small concentrations. Both the saturated and unsaturated carbonyls increased as the oxidation advanced. Statistical analysis of the data indicated that there was a close association between the total carbonyls and the peroxide value of the fat (Fig. 5). These results agreed with those of Gaddis *et al* (1959), who claimed that there was a linear relationship between the total, and mono-carbonyls on one hand, and the peroxide value on the other hand, in both heated and unheated Pork fat.

In the early stages of oxidation, the rate of increase in total unsaturated carbonyls in samn exceeded those of butteroil. Saturated carbonyls, on the other hand, showed an opposite trend Gaddis *et al.* (1959), found the same when they heated Pork fat, the unsaturated carbonyls increased with a more rapid rate than those of unheated fat. At the advanced stages of oxidation, however, both saturated and unsaturated carbonyls increased with approximate equal rates in butteroil and samn. This disagreed with the results of Graddis, *et al.* (1959), who found that in advanced oxidation the formation of carbonyls of unheated Pork fat exceeded that of heated fat.

In general, butteroil contained more saturated carbonyls, and increased more rapidly than in samn. The unsaturated carbonyls, however, were found in approximate equal amounts in both At the advancement of storage, they increased more rapidly in butteroil than in samn. They also constituted a higher percentage of the total carbonyls in samn than in butteroil, as they were 5 % of total carbonyls after 20 days, and increased to 14 % at the end of the storage period, but in butteroil, they were 4 % increased to 12 % at the same respective periods.

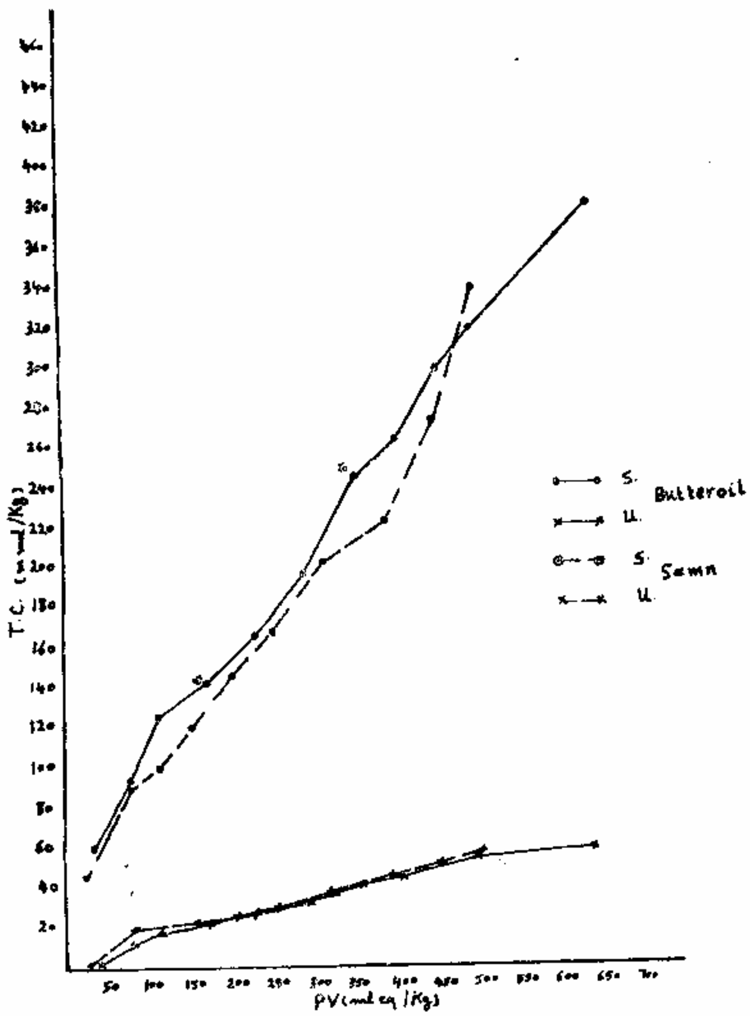

FIG. 5.—Relationship of P.V. and Total Carbonys During Autoxidation of Buffalo Butteroil and Samn.

The free mono-carbonyls ; the volatile fraction, was identified mainly to 3 classes as : (*a*) alkanals : saturated aldehydes and methyl ketones, (*b*) alk-2-enals, and (*c*) alk-2-4-dienals. The interrelationship between the peroxide value and the free-monocarbonyls was highly significant with positive correlation. (Fig. 6). These findings were confirmed by the results by Gaddis, *et al.* (1959). They reported that the peroxide-monocarbonyl relationship tended to be linear especially in the early stages of oxidation, and all the mono-carbonyl classes increased smoothly with oxidation. The unsaturated mono-carbonyl classes formed a higher percentage of the monocarbonyls fraction in samn than in butteroil. The 2-enals formed 12 % and the 2,4-dienals formed 6 % in case of samn, while they were 7 % and 3 % respectively in butteroil. With advancing oxidation, all the 3 classes increased with a more rapid rate in butteroil than in samn. Also, the ratio of the 2-enals, and 2,4-dienals were higher in butteroil than in samn ; 10 % and 9 % respectively in butteroil and 9.5 % and 7.0 % respectively in samn. These results agreed with those reported by Gaddis, *et al.* (1959), as they found that the 2,4-dienals class increased rapidly in the monocarbonyls fraction of heated fat, but it tended to decrease slightly with advanced oxidation.

In general, it is fairly clear that heating of milk fat, as in samn was accompanied by some loss in the carbonyls content, especially the volatile monocarbonyls. This was shown by the higher concentrations of these compounds in fresh butteroil than in fresh samn. This assumption aws confirmed by Gaddis, *et al.* (1961), who reported that the greater formation of carbonyls of unheated Pork fat than those of heated fat was partly due to their volatilezation and decomposition.

It was also clear that butteroil oxidized more rapidly than samn. This conclusion was reached from several facts which were noticed along the course of their autoxidation. First, the P.V. & T.B.A. values increased more rapidly than those of samn. Second, the rate of saturated carbonyls formation was faster and higher in concentration in butteroil than in samn, keeping in mind, however, that fresh butteroil bad higher concentrations of these compounds to start with. Third, the rate of increase in conjugated dienes, 2-enals, and 2,4-dienals was more in butteroil than in samn, inspite of the fact that fresh butteroil were contained less amounts of them than in fresh samn. Forth, alkanals were formed in a faster rate in butteroil than in samn. Fifth, the decrease in I.V. were more noticeable.

These results would be explained on the basis that the temperature encountered the Boiling-Off process of making samn seemed to be enough to stabilize the fat against oxidation and decomposition. Thus the component fatty acids would polymerize or isomerise and leading to the formation of conjugated systems or long chain compounds which were pobobably difficult to exidize than the non-conjugated systems present in butteroil (Badings, 1960 ; Burger and wideman, 1952 ; Lundberg, *et al.*, 1946 ; and Markeley, 1961). Also, it is possible that the temperature involved in making samn is high enough to induce the production of active sulfhydril groups which would act as antioxidants (Aurand, *et al*, 1959). Furthermore, the temperature of processing would inactive the xanthine oxidase enzymes found in butter which was considered responsible for the oxidation of the fat (Aurand *et al*, 1959 ; and Smith and Dunkley, 1962). It is a fact also that some fat soluble vitamines would act as pro-oxidants when present in evidently small amounts (Smith and Dunkley 1962[1], 1962[2] ; and El-Negoumy and Hammond, 1962). Thus, during the preparation of butteroil, little proportions of these vitamines might fiffuse and accelerate the oxidation during storage.

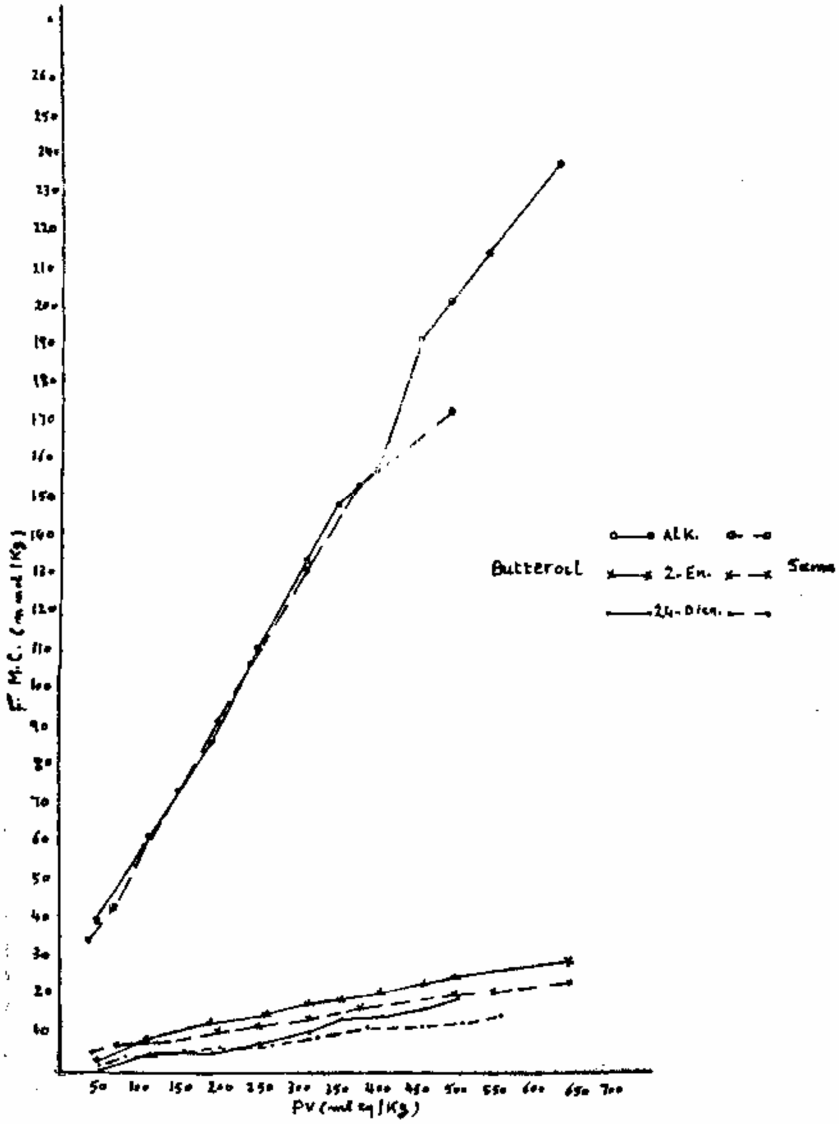

FIG. 6.—Relationship of P.V., and Free-Mono Carbonyls Classes of Buffalo Butteroil and Samn.

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دراسة على أكسدة دهن اللبن الجاموسى

٢ ــ مدى التباين فى نتائج اختبارات رقم البيروكسيد والرقم اليودى وحمض الثيوباربيتوريك وكذلك مدى التباين فى تركيز بعض المكونات خلال التأكسد الذاتى

نوال سيد أحمد ــ ابراهيم الدسوقى رفعت ــ عبد الحميد الحوفى
أمين محمد السكرى

الملخص

كانت الزيادة فى كل من رقم حامض الثيوباربيتوريك والمركبات الكربونيلية والمركبات غير المشبعة ثنائية الرابطة الزوجية المتبادلة والمركبات الكربونيلية المتطايرة تسير فى اتجاه مماثل للزيادة فى رقم البيروكسيد بينما كان الرقم اليودى يأخذ اتجاه مضاد أثناء فترة الأكسدة فى كل من دهن الزبد المسيل والسمن . وكذلك هناك ارتباط معنوى وموجب بين كل من رقم البيروكسيد من جهة وبين رقم حامض الثيوباربيتوريك والمركبات الكربونيلية والمركبات ثنائية الرابطة الزوجية المتبادلة والمركبات الكربونيلية المتطايرة خلال فترة الأكسدة . وكانت عملية الأكسدة الذاتية فى دهن الزبد المسيل تسير بمعدل أسرع من مثيلتها فى السمن وذلك يتضح من التركيزات المنخفضة فى كل من رقم البيروكسيد وحامض الثيوباربيتوريك والرقم اليودى والمركبات الكربونيلية والمركبات غير المشبعة ثنائية الرابطة الزوجية المتبادلة والمركبات الكربونيلية المتطايرة التى توجد فى السمن أثناء مراحل الأكسدة .