THE EFFECT OF SEVERAL ANTIOXIDANTS UPON FAT WHICH HAS BEEN EXTRACTED FROM TURKEYS FED VARIOUS DIETS

by

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INTRODUCTION

Flavor deterioration in poultry due to extended storage periods is a problem of considerable economic importance. Turkeys and chickens bought by the armed forces and placed in cold storage until needed for consumption were often unpalatable by the time they were eaten. Since then this problem has received consideration by many workers in an attempt to stabilize the fat and prevent or at least delay the occurrence of rancidity and the accompanying unpleasant odors and tastes.

It has been the policy of packers in recent years to eviscerate poultry before freezing it. As a result of this, the larger tissue area thus exposed to the air leaves a greater chance for oxidation of the meat both before and after it is frozen. Wagoner, Vail, and Conrad (16) have shown that the main cause of flavor deterioration is due to oxidative rancidity, thus it would seem that a modification of the packing procedure would be in order. Wagoner et al. (17) found that holding dressed poultry at chill room temperature for at least a day before evisceration or freezing or thawing the birds before evisceration decreases the stability of the poultry in subsequent cold storage. Later work has shown, however, that rancidification continues at freezing temperature thus indicating the need for an antioxidizing substance present within the tissue of the stored carcass itself.

Barnes, Lundberg, Henson, and Burr (1) proposed two basic factors controlling the resistance of natural fats to rancidification, (1) the composition of component glycerides and

(2) the amount and the nature of the existing natural antioxidants. Studies on the composition of component glycerides by Cruickshank (5) and Hilditch et al. (6) showed that the degree of unsaturation of dietary fat has great influence on the character of depot fat. Wagoner, Vail, and Conrad (16) fed chickens alfalfa and fish oil two weeks before slaughter and found that the stability of the carcass fat was decreased greatly due to the change in composition of the depot fat. Since the iodine number of the depot fat showed a substantial increase, the instability was apparently caused by the increase in unsaturation of the stored fat glycerides. The importance of this unsaturation is evident when the findings of Holman and Elmer (7) are considered. They found that an increase in the number of double bonds in a fatty acid by one increased the rate of oxidation of a fatty acid or ester by a factor of two. Thus to inhibit this increase in susceptibility to oxidation, one of two things would be necessary: (1) alter the type of fatty acids deposited in the tissues of the turkey, or (2) find an antioxidant which when deposited in a tissue would inhibit the oxidation of the highly unsaturated fatty acids.

Kummerow et al. (8) fed chickens a diet in which the fat consisted of 25 per cent hydrogenated fat (Spry). The birds developed crooked legs, oily feathers, and didn't gain as much weight as did the control birds which received 25 per cent corn in their ration. The percentages of oleic and linoleic acids were not greatly different than were those of the control group, and the undesirable nutritional effects incurred by the absence

of the "essential" linolenic and arachidonic acids indicated that the highly saturated diet, as used, was not the answer to the rancidity problem. Other investigators to date have not been very successful in their attempts to stabilize fat by feeding a highly saturated diet deficient in or without the highly unsaturated "essential" fatty acids.

Several naturally occurring compounds which possess or are suspected of possessing antioxidant activity have been applied to the rancidity problem. Barnes and co-workers (1) fed tocopherols to rats in quantities larger than the ordinary dietary amounts, and found that the stability of the rat fat was not increased proportionately. Overman et al. (15) found hydroquinone and ascorbic acid unsatisfactory as antioxidants in their experimentation. According to Wittka (19), lecithins and cephalins are known to promote fat stabilization. Since they are present in the tissues, it would appear that a maximum amount of phospholipids would be desirable. Kummerow et al. (9) fed turkeys ethanolamine and choline in order to determine their effect upon the synthesis in vivo of phospholipids and the subsequent stabilization from their presence in tissue fat. Induction period data and organoleptic tests indicated the storage time of turkeys could be lengthened by the feeding of those compounds. Lea (10) stated that some workers have obtained antioxidation protection factors of 1.5 to 3.0 and 1.7 to 1.8 when concentrations of 0.1 per cent to 0.2 per cent phospholipid were used. Other workers, however, haven't obtained any stabilizing effects by addition of relatively large amounts

of partially purified phospholipid.

In order to find a solution to the fat stabilization problem, other factors in fat metabolism should be considered. Birch and co-workers (3) in 1938 suggested that the physiological function of vitamin B_6 was concerned with the utilization of unsaturated fatty acids, and since then many workers have confirmed and extended their suggestion. Another factor, pantothenic acid was suggested by Lipmann (11) to participate as part of an enzyme in protein and fat synthesis as well as other processes requiring the activation of a carboxyl group as a preliminary to condensation.

The supplements of the present experiment were planned in an attempt to check the antioxidant activities of several compounds when added to a ration. Of the compounds used, ethanolamine has been shown to have stabilizing activity, mono ethanol ammonium gallate has been used successfully with lard stabilization, in vitro, and of course the effects of vitamin B_6 and pantothenic acid could be synergistic in type or by some other indirect antioxidative mechanism. Utilization of spectrophotometric analysis as developed by Mitchell, Kraybill and Zscheile (12) would reveal composition of the fats, and coupled with organoleptic and induction period data, the general effectiveness of the antioxidents could be evaluated.

EXPERIMENTAL

The turkeys used in this experiment were a Broadbreasted Bronze breed of the Kansas State College strain. Seventy-two poults from the same hatch were started on the basal ration shown in Table 1 when one day old. At eight weeks of age, the poults were divided into 6 groups of 12 birds each and fed different supplements. They were kept off the ground in a wire enclosed shelter house and had access to sunlight and fresh air at will.

One group of 12 turkeys was used as a control, these turkeys received only the basal ration. Other groups received various dietary supplements as listed in Table 2. Weights of the birds were taken at 9, 18, and 28 weeks of age, Table 3.

The turkeys were killed at the age of 28 weeks and dressed using the methods of Wagoner, Vail and Conrad (16, 17, 18). The whole skin of the turkeys in each group was removed and the skin was pooled in each group before being placed in storage at -13°C. The livers and gizzards of the turkeys also were combined within groups and the groups of the respective organs were stored at -13°C. until analyzed.

Two methods were used to extract the fat of the skin tissue, one involved saponification and the other solvent extraction. The fat from the skin tissue was extracted by both methods, that from the gizzard and livers by saponification only. The percentage of fat laid down in the respective tissues, and its composition were determined. The determinations are described

under Experimental Procedures.

EXPERIMENTAL PROCEDURES

Extraction of Fat from Tissue by Saponification

The turkey skin, liver and gizzards were cut into small pieces and a representative 50 g sample of the respective tissues were placed in beakers. After the addition of 50 ml of 30 per cent potassium hydroxide, the tissues were allowed to digest on a steam bath. One fifth volume of ethyl alcohol was added to the mixture at the end of six hours, and the digestion was allowed to continue for at least another two hours. The saponification mixture was then cooled and poured into large separatory funnels. The non-saponifiable material was removed by two extractions with one third volume of Skellysolve F and discarded. After removal of the non-saponifiable material, the solution was cooled under tap water and acidified with dilute 1:1 hydrochloric acid. The freed fatty acids were extracted three times with one third volumes of Skellysolve F. The Skellysolve layer, consisting chiefly of crude fatty acids, was washed twice with distilled water to remove any excess hydrochloric acid or potassium chloride and dried over anhydrous sodium sulfate. The fatty acids were partially freed from the solvent under vacuum, and the residue poured into a weighed flask. The last traces of solvent were removed in a vacuum oven, the flask reweighed, and the percentage of free fatty acids calculated.

Iodine Value

Approximately 0.1 g of the mixed fatty acids was weighed into a glass stoppered iodine flask. Five ml of chloroform and exactly 15 ml of Wij's solution was added. The flasks were stoppered and placed in the dark. At the end of one hour, 10 ml of 15 per cent potassium iodide solution was added and the solution mixed thoroughly. The glass stopper and sides were rinsed with distilled water into the flask and the contents were mixed thoroughly. The sample was then titrated with N/10 sodium thiosulfate solution to a faint yellow. A few drops of starch solution were added and the mixture shaken well to free all iodine. The solution was then titrated to a colorless end point. Two blank determinations were also titrated with the unknowns (20).

Wij's Solution. Thirteen g of iodine was dissolved in a liter of glacial acetic acid. The solution was heated until all the iodine crystals were dissolved and then cooled. Chlorine gas was bubbled into the solution until the color was orange brown. At this point, the titration of the original iodine solution was doubled when titrated with N/10 thiosulfate solution.

Potassium Iodide Solution. Fifteen g of potassium iodide was dissolved in 85 ml of distilled water.

Standard Sodium Thiosulfate Solution. Twenty four and eight tenths g of sodium thiosulfate were dissolved in one liter of distilled water and the exact normality determined by the following procedure: Exactly 10 ml of N/10 potassium

dichromate was measured into an iodine flask and 5 ml of concentrated hydrochloric acid added. Ten ml of a 15 per cent potassium iodide solution were added and the mixture was titrated at once to a greenish color with thiosulfate solution. Starch solution was then added and the titration continued slowly and with occasional shaking until the solution turned bright, clear green in color. The formulas used in calculating the iodine value were:

$$\text{Normality factor} = \frac{1.26 \times .1 \text{ (normality of dichromate)}}{\text{ml thiosulfate used}}$$

$$\text{Iodine value} = \frac{\text{normality factor (blank tit.-sample tit.)}}{\text{sample weight}}$$

Spectrophotometric Analysis

The method of Mitchell, Kraybill, and Zscheile was used in this analysis (12). Approximately 0.1 g samples of mixed fatty acids completely freed of solvent were pipetted into small weighing vessels. These vessels were placed in marked ignition tubes. Four ml of freshly prepared ethylene glycol containing 7.5 per cent potassium hydroxide was added to each sample. Two blanks were run along with the unknowns. Nitrogen was blown into each tube to replace the air, and the apparatus was arranged so that air was excluded during the course of the isomerization. The tubes were lowered into an oil bath heated to about 190°-200°C. so that upon the introduction of the tubes the temperature of the bath would not go below 180°C. (± 2°) for 30 minutes.

After 30 minutes, the tubes were removed, wiped clean,

and cooled in tap water. The contents of the tube were transferred quantitatively to 100 ml flasks and made up to the mark using absolute alcohol. The solutions were then thoroughly mixed and were read on a Beckman spectrophotometer against blanks which had been diluted in the same manner. Readings were taken at 2320, 2340, 2620, 2660, 2740, 3100, 3160 and 3220 Å. If readings were not between 0.2 and 0.8 current density, suitable dilutions were made and the proper readings obtained. Blanks were diluted in accordance with the unknowns.

The Ethylene glycol solution was prepared from 15 g of ground potassium hydroxide dissolved in 180 ml of ethylene glycol.

The formulas used for calculating the fatty acid compositions were as follows:

$$K_2 = \frac{K_{2320}}{\text{wt. sample per liter}} - 0.04$$

$$K_3 = \frac{2.8}{\text{wt. sample per liter}} \left(K_{2660} - \frac{K_{2620} - K_{2740}}{2}\right)$$

$$K_4 = \frac{2.5}{\text{wt. sample per liter}} \left(K_{3160} - \frac{K_{3100} - K_{2740}}{2}\right)$$

The preceding formulas were those used for unisomerized samples which were used as correction factors.

$$K_2' = \frac{K_{2320}}{\text{wt. sample per liter}} + 0.07$$

$$K_3' = \frac{4.1}{\text{wt. sample per liter}} \left(K_{2660} - \frac{K_{2620} - K_{2740}}{2}\right)$$

$$K_4' = \frac{2.5}{\text{wt. sample per liter}} \left(K_{3160} - \frac{K_{3100} - K_{3220}}{2}\right)$$

$$K_2'' = K_2' - K_2$$

$$K_3'' = K_3' - K_3$$

$$K_4'' = K_4' - K_4$$

In the foregoing formulae, K_{2320}, K_{2620}, etc. designate the spectrophotometric readings obtained at the particular wave length indicated.

Per cent linoleic acid = $1.125 \times K_2'' - (1.27 \times K_3'') + .04 K_4''$

Per cent linolenic acid = $1.87 \times K_3'' - 4.43 \times K_4''$

Per cent arachidonic acid = $4.43 \times K_4''$

Per cent oleic acid = Iodine number x 100 - (181.5 x per cent linoleic acid - 273.5 x per cent linolenic acid - 333.5 x per cent arachidonic acid) / 90

Per cent saturated acids 100 - per cent linoleic acid - per cent linolenic acid - per cent arachidonic acid - per cent oleic acid.

Organoleptic Procedure

The method of Schreiber et al. was used for this procedure (15). The unskinned turkey legs to be used in the organoleptic tests were washed with cold water, cleaned and placed in casseroles. One cup of water was added and the samples were cooked, covered, in a pre-heated rotary oven at 350°F. for one and one-half hours. The covered casseroles were removed from the oven

and allowed to cool for 15 minutes. The palatability committee judged the odor and flavor of the cooked turkeys while hot and recorded the ratings. A scale for evaluations from 1 to 10 was used. These numerical values carried the ratings of:

10. extremely good	5. medium, minus
9. very good	4. fair
8. good	3. poor
7. medium, plus	2. very poor
6. medium	1. extremely poor

Extraction With Acetone, Alcohol, Skellysolve F

The skin tissue was cut into small pieces and representative 60 gram samples were weighed into Erlenmeyer flasks. Enough acetone was then added to cover the tissue and the contents of the flask were refluxed for one hour. The acetone was removed by filtering through a Buchner funnel. The acetone extraction was repeated and the extracts combined. Enough 95 per cent ethyl alcohol to cover the tissue was then added and the mixture was allowed to reflux for one hour. The alcohol was removed from the tissue as before and the extraction was repeated. The alcohol fractions were combined and filtered through sodium sulfate into a weighed round bottom flask. The solvent from the alcohol fractions was removed under vacuum leaving a residue which contained the phospholipid.

The tissue was extracted twice with Skellysolve F using the same procedure. The acetone and Skellysolve extracts were combined in a separatory funnel and shaken. The Skellysolve

layer was then drawn off and the acetone layer was washed with fresh Skellysolve F several times. The Skellysolve extracts were combined and washed three times with water. Extreme care must be taken at this point to avoid emulsions. The extracts were dried by filtration through sodium sulfate.

The Skellysolve extract was poured into the weighed flask containing the extracted phospholipids and other fats, and the solvent removed under vacuum. The flask and fat was then weighed to determine the amount of fat extracted from the tissue. The per cent of fat in the tissue was calculated by the following equation:

$$\text{Per cent crude fat extract} = \frac{\text{wt. extract} \times 100}{\text{wt. tissue}}$$

Phospholipid Precipitation

Samples of approximately 20 g of the total fat which had been previously freed from solvent were poured slowly and with stirring into a 250 ml centrifuge bottle containing 200 ml cold acetone. The mixture was then centrifuged until the acetone was clear and the phospholipid completely precipitated. The acetone fraction was decanted, and the remaining phospholipid precipitate was transferred quantitatively to a beaker using Skellysolve F. The remainder of the solvent was removed under vacuum and the beaker containing the phospholipid was weighed in order to obtain the weight of phospholipid. The percentage of phospholipid was calculated by the following equation:

$$\text{Percentage of phospholipid} = \frac{\text{wt. phospholipid} \times 100}{\text{wt. crude fat extract}}$$

Induction Period Procedure

One gram samples of fat were weighed into 125 ml iodine flasks with funnel type necks. Each flask was stoppered with a small mercury manometer constructed on a male joint which fit the ground glass neck of the flask. The flasks were suspended into a constant temperature water bath and when the flask and water bath temperatures became equal, the system was closed by a stopcock. The manometric system was constructed so that when enough oxygen was absorbed by the fat in the flask to lower the pressure in the system an amount equivalent to 1 cm of mercury, the circuit would be broken. An electrical contact from one arm of the manometer completed a circuit through a recorder as long as the mercury levels in the arms remained equal. A clock making contact with each manometer circuit once an hour was used to measure the time to the nearest hour by actuating an electronic relay. The time required to break the manometer circuit was used as the length of the induction period.

Table 1. Basal ration.

Ingredients	Pounds per 100 pounds basal ration
Corn	30.0
Shorts	20.0
Oats, ground	10.0
Bran	7.0
Alfalfa meal, dehydrated	7.0
Meat scraps	11.5
Soya bean meal	12.0
Calcium carbonate	2.0
Sodium chloride	.5
Vitamin A, D, G mixture	1.0
Calcium phosphate	.5
Manganese	15 G

Table 2. Diet supplements.

Group	Supplements	
	Pounds per 100 pounds feed	Grams per 100 pounds feed
1. Alfalfa	15	
Ground wheat	15	None
2. Alfalfa	15	
Ground wheat	15	
Pantothenic acid		3.0
Vitamin B_6		1.5
3. Alfalfa	15	
Ground wheat	15	
Ethanolamine-Hydrochloride		100
4. Alfalfa	15	
Ground wheat	15	
Ethanolamine		100
Pantothenic acid		3.0
Vitamin B_6		1.5
5. Alfalfa	15	
Ground wheat	15	
Monoethanol-Ammonium gallate		6
6. Corn	30	None

Table 3. Weight gain and skin fat induction period data.

Group	Supplement	Average weight of birds			Induction period of extracted fats
		9wks.	18wks. (pounds)	28wks.	Hours
1.	Alfalfa, ground wheat	2.32	10.02	16.99	5.5
2.	Alfalfa, ground wheat, pantothenic acid, B_8	2.45	11.02	18.12	8
3.	Alfalfa, ground wheat, ethanolamine	2.37	11.12	19.18	9
4.	Alfalfa, ground wheat B_8, pantothenic acid, ethanolamine	2.29	10.06	16.82	7
5.	Alfalfa, ground wheat, monoethanolammonium gallate	2.36	10.20	19.89	8.5
6.	Ground corn	2.28	9.94	18.38	9

Table 4. Organoleptic tests.

Group No.	Fresh turkey		Turkey stored 6 months	
	Odor	Flavor	Odor	Flavor
1	8	8.4	5.3	5.3
2	7.2	7.8	5.3	5.0
3	7.2	8	5.0	5.3
4	6.8	7.6	5.3	4.0
5	7.2	6.8	5.3	5.3
6	8	8	5.0	5.0

Table 5. Iodine values.

	Group No.					
	1	2	3	4	5	6
Skin tissue	91.2	86.2	88.6	85.2	85.9	88.0
Liver tissue	100.3	97.9	121.4	97.4	119.9	104.2
Gizzard fat tissue	83.3	84.5	85.7	85.0	84.3	84.8

Table 6. Percentage of total fat in turkey skin, liver and gizzard fat tissue, and percentage of phospholipids in skin tissue.

	Group No.					
	1	2	3	4	5	6
Skin tissue	36.7	55.7	37.7	55.4	53.5	47.4
Liver tissue	2.13	4.28	2.64	3.31	2.94	3.66
Gizzard fat tissue	68.3	70.6	60.4	67.6	60.4	56.4
Phospholipids	0.65	0.55	0.45	0.36	0.55	0.67

Table 7. Fatty acid composition of skin tissue, fat, saponification extraction.

	Group No.					
	1	2	3	4	5	6
Per cent Linoleic	26.99	16.51	17.57	22.43	21.73	25.45
Per cent Linolenic	2.38	2.66	2.78	2.33	2.35	1.58
Per cent Arachidonic	0.67	0.54	0.44	0.40	0.40	0.40
Per cent Oleic	42.75	53.31	54.58	42.43	45.70	42.26
Per cent Saturated	27.21	26.98	24.63	32.41	29.82	30.31

Table 8. Fatty acid composition of skin tissue fat, alcohol-acetone-skellysolve F extraction.

	Group No.					
	1	2	3	4	5	6
Per cent Linoleic	19.14	18.63	21.62	19.43	21.09	27.55
Per cent Linolenic	2.34	2.53	2.26	2.37	2.14	1.45
Per cent Arachidonic	0.62	0.58	0.53	0.44	0.40	0.53
Per cent Oleic	48.80	48.38	42.41	44.97	42.29	33.80
Per cent Saturated	29.10	29.88	33.18	32.79	34.08	36.67

Table 9. Fatty acid composition of gizzard fat tissue saponification extraction.

	Group No.					
	1	2	3	4	5	6
Per cent Linoleic	24.14	18.99	24.30	26.30	22.93	27.69
Per cent Linolenic	2.13	2.60	2.21	2.29	2.32	1.34
Per cent Arachidonic	0.60	0.43	0.41	0.48	0.43	0.44
Per cent Oleic	35.22	46.16	36.77	32.64	38.80	32.70
Per cent Saturated	37.91	31.82	35.71	38.29	35.52	37.83

Table 10. Fatty acid composition of liver tissue fat saponification extraction.

	Group No.					
	1	2	3	4	5	6
Per cent Linoleic	12.16	16.77	23.46	16.05	24.51	23.00
Per cent Linolenic	0	0	0	0	0	0
Per cent Arachidonic	8.50	9.97	19.25	10.29	15.65	13.16
Per cent Oleic	55.48	38.07	16.11	37.77	25.72	20.70
Per cent Saturated	23.86	35.19	41.18	35.89	34.12	43.14

RESULTS AND DISCUSSION

As a result of the turkeys being fed a diet planned to eliminate any nutritional deficiencies, there was little difference in the weight and growth of the groups of turkeys. However the effect of the antioxidants was apparent when the composition of the fat was noted, and especially when the induction period data was evaluated.

In previous work of Kummerow et al. (9), it was shown that in turkeys fed a normal amount of unsaturated fat in the diet, the induction period of the control group was about half that of the groups which had been supplemented with ethanolamine. In the present study, the groups which received a supplement of ethanolamine and corn, respectively, were of the same order of effectiveness. The other supplements were effective, but to a lesser degree. The control group receiving supplement of ground wheat and alfalfa only had an induction period of 5.5 hours, Table 3, while the group receiving ethanolamine in addition to the alfalfa and wheat had an induction period of 9 hours. The group receiving ground corn only as a supplement also had an induction period of 9 hours. This would be explainable by the fact that there is no linolenic acid in corn and also because of the presence of tocopherol in the corn.

The organoleptic tests failed to show any significant differences using either fresh turkey, or turkey that had been stored for six months. All of the groups showed deterioration after storage of six months with group four on the verge of unpalatability in regard to flavor. More significant results

possibly would have been apparent at a longer storage time. While oranoleptic tests do not yield any specific information in regard to changes in composition, they do offer an overall picture of the degree of rancidification resulting from those changes.

The iodine values indicated that in all cases the liver tissue was more unsaturated than either the skin tissue or gizzard tissue. This is possibly due to the fact that many of the unsaturated fatty acids are bound in the form of phospholipids in the skin and gizzard fat, while the liver is the seat of incorporation of the unsaturated fatty acids into phospholipids. Since it is impracticable to remove the turkey skin tissue, during evisceration, it would be desirable to have as little unsaturation as possible in the skin tissue. The data showed little difference in the unsaturation of the groups of skin tissue with the exception of group one in which the supplement consisted only of alfalfa and ground wheat. Group one was significantly more unsaturated, and this was in agreement with the induction period findings where the first group had a much lower induction period than the rest of the groups.

The gizzard tissue had the highest percentage of total fat deposited, with a fairly constant agreement within the groups. The percentage of fat deposited in the liver tissue was also relatively constant, with an average of only 3.16 per cent fat being laid down by the birds.

No significant difference was noted in the percentages of precipitated phospholipids of the skin tissue. This is as ex-

pected, considering the findings of Kummerow and Hite (9) who found no reflection in the phospholipid content of the stabilization induced by the ethanolamine. In the present experiment, the high iodine value and low induction period of the group fed the supplement of ground wheat and corn only, was not followed by a correspondingly small phospholipid content as compared to those fed the other supplements. This would seem to indicate that the stabilizing action of the ethanolamine in the case of Kummerow and Hite was caused by a larger number of the more highly unsaturated fatty acids being incorporated into a constant amount of phospholipid rather than much more phospholipid being formed.

The fatty acid composition of the turkey skin fat was determined using two methods of extraction as described previously in Experimental Procedures. There was general agreement in most of the values, between the two methods and where the percentages differed to any extent the value obtained using the saponification extraction should have preference over those obtained from the solvent extraction method. The saponification extraction percentages should be somewhat more accurate because of the absence of some non-saponifiable, fat soluble compounds which could be present in solvent extracted fat. These compounds could in some cases interfere with the spectro photometric analysis. In the light of the finding of the present experiment, however, the presence of the foreign compounds did not in most cases produce errors larger than the experimental errors. This supports the previous work of Kummerow and Hite (9), who used the

solvent method of extracting the turkey tissue fat.

Groups one and six showed more total unsaturation than the other groups, the main differences, however, being in the percentages of linoliec acid where group one had a percentage of 26.99 and group six had a percentage of 25.45 (Table 7): The percentages of linolenic acid and arachidonic acid were relatively constant, thus explaining the lack of differences in the induction period, iodine value and organoleptic data. Small changes in the percentage of linoleic acid would be noticeable, but the differences in the iodine values, organoleptic data, and induction periods are much more apparent with the larger number of double bonds in the arachidonic and linolenic acids. The control group had a larger total percentage of unsaturated fatty acids as would have been predicted by the induction period and iodine value for this group. In all cases in the skin tissue where the percentage of linoleic acid was low, there was a correspondingly higher content of oleic acid and vice versa, making little difference in the sums of the two acids among the different groups.

The gizzard fat, being depot fat, had a very uniform composition. All four of the unsaturated acids for which the analysis was carried out were found, and were in a constant ratio with no significant variation among the different groups (Table 9).

The turkey liver tissue was found to contain no linolenic acid, Table 10. Randolph (14) found no linolenic acid in the livers of rats fed a fat free diet plus the methyl ester of

linolenic acid and suggested that the animals synthesized linoleic and arachidonic acids from the linolenic acid. Some indirect support for this idea might be found in the present data. It is generally assumed that part of the linolenic acid from the supplements is incorporated into phospholipids for transfer from the liver to other tissues. Since, however, 92.3 per cent of the total arachidonic acid present in the liver, gizzard and skin tissue was found in the liver, and the amounts found were considerably higher than would be predictable by the diet, the idea of conversion to arachidonic acid seems possible. The role of linoleic and arachidonic acids in fat metabolism is not clear, but perhaps linolenic acid is detoxified through synthesis by the body into these acids.

There was wide variation in the percentages of arachidonic acid and also linoleic acid in the liver, however, when the amount of linoleic acid was high, the amount of arachidonic acid was high also. This data suggests perhaps a stimulation of the synthesis and maintenance of these fatty acids in the liver by some of the supplements fed.

Since the differences in the present experiment were not great enough to properly evaluate the effectiveness of the antioxidants used, further study is being planned which will include a more unsaturated diet by the addition of 2 per cent linseed oil to the basal ration. This greater unsaturation should make the group differences much more pronounced, and should show more clearly the differences in effectiveness of the antioxidants.

SUMMARY

In an attempt to stabilize the fat of turkeys placed in cold storage, several antioxidants and synergistic combinations were added to a basal ration similar to that used commercially in growing turkeys.

The control group fed a diet containing a normal amount of unsaturated fatty acids and no antioxidants was found to be less stable in general than the other groups. The general effectiveness of the antioxidants fed was nearly the same, with no supplement showing a marked superiority in stabilization. The group supplemented only by ground corn compared favorably with the other groups in stability, indicating the effectiveness of the natural antioxidants of the corn, such as tocopherols, in stabilizing frozen poultry. Percentages of component fatty acids, iodine values, induction periods, and organoleptic tests were used in evaluating the stability of the fat analyzed.

The induction period of the group fed ethanolamine had nearly twice the induction period of the control group.

The two methods of extraction of turkey skin tissue produced results which showed little variation in the methods of extraction.

Indirect evidence was found to support the proposal that linoleic acid could be converted by the animal body to linoleic and arachidonic acids.

Low percentages of linoleic acid were accompanied by correspondingly higher percentages of oleic acid and vice versa, giving sums of the two acids which varied little among the groups.

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LITERATURE CITED

1. Barnes, R. H., W. O. Lundberg, H. T. Hanson, and G. O. Burr.
   The effect of certain dietary ingredients on the keeping quality of body fat. Jour. Biol. Chem. 141:313-322, 1943.

2. Beadle, B. W. and H. R. Kraybill.
   The spectrophotometric analysis of fats. Amer. Chem. Soc. Jour. 66:1232, 1944.

3. Birch, T. W.
   The relation between vitamin B_6 and the unsaturated fatty acid factor. Jour. Biol. Chem. 124:775-793, 1938.

4. Bradley, T. F. and David Richardson.
   Alkali-induced isomerization of drying oils and fatty acids. Indus. and Engg. Chem. 34:237, 1942.

5. Cruickshank, Ethyl M.
   Studies in fat metabolism in the fowl. Biochem. Jour. 28:965, 1934.

6. Hilditch, T. P., E. C. Jones and A. J. Rhead.
   The body fats of the hen. Biochem. Jour. 28:786, 1935.

7. Holman, R. T. and Otto C. Elmer.
   The rates of oxidation of unsaturated fatty acids and esters. Amer. Oil Chem. Soc. Jour. 24:127-29, 1947.

8. Kummerow, F. A.
   Unpublished data. Dept. of Chem., Kansas State College, Manhattan, Kans.

9. Kummerow, F. A., J. P. Hite, and S. E. Kloxin.
   Fat rancidity in eviscerated poultry IV. Poultry Sci. 28:249-253, 1949.

10. Lea, C. H.
    Rancidity in edible fats. Food Investigation Board, special report No. 46, London, 1938.

11. Lipmann, F.
    Chem. and Engg. News. 26:860, 1948.

12. Mitchell, J. H., H. R. Kraybill and F. P. Zscheile.
    Quantitative spectral analysis of fats. Indus. and Engg. Chem. Analyt. Ed. 15:1, 1943.

13. Overman, Andrea.
    Influence of some dietary factors on the development of rancidity in the fat of the white rat. Jour. Biol. Chem. 142:441-444, 1942.

14. Randolph, P. M.
The metabolism of linolenic acid. Unpublished M. S. thesis, Kansas State College, Manhattan, Kansas, 1949.

15. Schreiber, M. L., Gladys E. Vail, R. M. Conrad and L. F. Payne.
The effect of tissue fat stability on deterioration of frozen poultry. Poultry Sci. 26:14-19, 1947.

16. Wagoner, C. E., Gladys E. Vail and R. M. Conrad.
The effect of premortal fast on deterioration of frozen poultry. Poultry Sci. 26:167-169, 1947.

17. Wagoner, C. E., Gladys E. Vail, and R. M. Conrad.
The influence of preliminary holding conditions on deterioration of frozen poultry. Poultry Sci. 26:170-172, 1947.

18. Wagoner, C. E., Gladys E. Vail, and R. M. Conrad.
The effect of degree of surface exposure on deterioration of frozen poultry. Poultry Sci. 26:173-175, 1947.

19. Wittka, F.
Antioxidants and stabilizers for fats. Chem.-Ztg. 61:386-388, 1937. Chem. Abs. 31:5189, 1937.

20. Yasuda, M.
The determination of the iodine number of lipids. Jour. Biol. Chem. 94:401, 1931.