CHEMICAL CHANGES IN THE LIPID FRACTION OF WHEAT GERM DURING STORAGE

A Thesis

by

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INTRODUCTION

The germ, or embryo, of cereal grains is the part of the berry from which the new plant develops. In wheat the germ is readily distinguished as the small wrinkled portion of the grain on the opposite side of the crease. It makes up about 1.2 percent of the weight of wheat. Structurally, the scutellum is closely associated with the germ, both being separated from the endosperm by a layer of compressed cells, the hyaline layer. The scutellum and embryo together make up about 2.7 percent of the weight of the wheat kernel (27). Bailey (7) reports 2.23 percent in hard wheat. The embryo consists essentially of a plumule and a radicle portion, which become the leaves and roots respectively, of the new plant.

Many analyses of wheat germ are to be found in the literature. Frequently, however, the source of the germ is not specified, nor is the degree of contamination with non-germ portions of the wheat indicated. Dissection of the wheat manually would be the ideal method of preparing samples, but this has not generally been undertaken, although Hinton has resorted to this method (27). Germ as produced by ordinary methods of milling contains a certain amount of the scutellum. It is always contaminated with some bran, although this may be reduced to a relatively low figure by careful screening and aspirating. The range of analytical values reported is shown in Table I.

TABLE I - Composition of Wheat Germ According to Various Workers. Values Expressed as Percent of the Fresh Weight.

	Kalning(32)	Osborne(42)	Grewe(25)	Bull(11)	Schuette(35)
Moisture	Nil	---	9.3	11.9-12.4	---
Total protein	40.75	31.00	28.9	33.7-35.0	23.6-44.7
Water-soluble protein	19.76				
Carbohydrates	39.25		44.2		
Sucrose		7.71			
Reducing sugar (as glucose)	4.07				
Dextrin		7.50			
Total fat	12.00	10.44	11.0	10.4-24.5	6.0-13.5
Fiber	2.50	2.35	2.0	2.0	1.6- 4.8
Pentosans	11.55				10.92
Ash	5.50	4.91	4.6	4.63-5.20	3.9- 4.6

Ash Analysis (as % of ash)	Sullivan & Near (58)	Grewe (25)
P_2O_5	56.90	57.50
MgO	12.50	-----
CaO	1.92	2.04
Fe_2O_3	0.192	0.267
ZnO	1.037	
CuO	0.024	
K_2O	26.49	
Mn_2O_3	0.240	
Al_2O_3	0.094	
SiO_2 and C	0.80	

Among the published analyses of wheat germ the chief point of agreement is in the low content of crude fiber, high fat and nitrogen values. There is a wide variation in the values reported for the various carbohydrates. Sucrose, raffinose, dextrin, starch, pentosans, cellulose, and reducing sugars have been reported present in varying amounts.

Grewe and LeClerc (25) report the percentage nitrogen in the germ of various types of wheat as follows:-

Hard Red Spring Wheat	5.82
Durum	5.45
Hard Red Winter Wheat	5.42
Soft Red Winter Wheat	4.54
White Wheat	4.38

Sherwood and co-workers (53) analyzed both commercial wheat germ and pure, hand separated wheat germ and found large differences in the amount of ether extract, i.e.

	Commercial germ (% dry weight)	Pure germ
Moisture	-----	-----
Protein (N x 6.25)	35.40	37.48
Ether extract	11.76	16.10
Crude fiber	2.00	1.40
Ash	4.95	-----

Attention has been focused on the food value of wheat germ largely because of the high biological value of the protein, and the B vitamins content. The high protein content of the germ was of course recognized before the concept of biological value was developed. The unusually high biological value of the protein has been noted by Chick (16), Plimmer (47), Hove and Harrel (29), Stare and Hegsted (54), and others. A comparison of the essential amino acid content of wheat germ as compared to other proteins has been made by Block and Bolling (10). The difference in amino acid distribution between the endosperm and germ proteins of wheat is shown in Table II.

TABLE II - Amino Acid Content of Wheat and Its Products, According to Block and Bolling (10). Values Expressed as Percent of Protein (N x 6.25)

	Wheat	Wheat Germ	Wheat Endosperm
Arginine	3.0	6.0	3.9
Histidine	1.2	2.5	2.2
Lysine	2.7	5.5	1.9
Tyrosine	3.8	3.8	3.8
Tryptophane	1.0	1.0	1.0
Phenylalanine	5.7	4.2	5.5
Cystine	1.3	0.6	1.8
Methionine	3.0	2.0	3.0
Threonine	3.3	3.8	2.6
Leucine	13.0	7.0	12.0
Isoleucine	4.0	3.0	4.0
Valine	3.4	3.5	3.0
Glycine	---	---	8.0

The high concentration of thiamin in wheat germ is well known. Other members of the B complex are present in the following amounts (5):

	Milligrams/100 grams
Thiamin	1.9-4.7
Riboflavin	0.5-1.5
Niacin	3.4-7.7
Pyridoxine	0.9-1.5
Pantothenic acid	1.5-3.5
Biotin	0.045
Inositol	250
Choline	410
Para-amino benzoic acid	0.10

As pointed out above, the wide range in the published figures is due to contamination with bran and endosperm and to varietal differences.

The lipid fraction of wheat germ has attrached a great deal of attention due to its notably high content of tocopherol. In fact, tocopherol was first isolated from wheat germ oil (22). The oil has been examined for its physical constants by many investigators. The variations in the values reported are probably due to the same factors as affect the composition of the germ itself. The most reliable and representative figures for the oil from American wheat germ appear to be those of Jamieson and Baughman (31), and Radlove (49) which are recorded to Table III.

TABLE III - Analytical Constants of Wheat Germ Oil.

	Jamieson & Baughman	Radlove
Density at 25°	0.9268	0.9268
Acid value	7.6	8.25
Thiocyanogen Number	79.7	82.0
Acetyl Number	9.9	
Polenske Number	0.35	
Saturated Acids	13.3%	
Iodine Value of Unsaturated acids	160.7	
Refractive Index	1.4762	1.4737
Saponification Number	186.5	184.
Iodine Value	125.6	
Hexabromides	Trace	
Reichert-Meissl Number	0.2	
Unsaponifiable	4.7%	4.04%
Unsaturated acids	75.3%	
Iodine Value of Unsaponifiable	97.3	
Thiocyanogen Number of Unsaponifiable	62.0	

The composition of the fatty acids has been painstakingly determined by Sullivan and Bailey (56). Their values are included in Table IV.

TABLE IV - Fatty Acid Content of Wheat Germ Oil Expressed as Percent of Total Fat

	Sullivan & Bailey (56)	Jamieson & Baughman(31)	Radlove(49)	Thaler & Groseff(62)
Oleic acid	28.14	27.8	25.5	12.7
Linoleic acid	52.31	40.9	52.5	59.2
Linolenic acid	3.55	10.0	6.3	3.4
Palmitic acid	11.77	12.8		
Stearic acid	3.05	0.9		
Lignoceric acid	1.18	0.3		
Total saturated acids	16.00	14.0	15.5	18.5

The exceptionally high content of unsaponifiable matter in wheat germ oil has also attracted the attention of several investigators. In addition to tocopherols, it contains a number of sterols, and at least one hydrocarbon has been reported (61).

The phosphatide fraction of wheat germ has been investigated by Channon and Foster (14), Rewald (50) and Diemair, Bleyer, and

Schmidt (18, 19). Channon and Foster note the presence of phosphatidic acid, and give a phosphatidic acid : lecithin : kephalin ratio of 4:4:1. Rewald extracted germ first with petroleum ether, then with an 80:20 mixture of benzol and ethanol. The total lecithin found was 0.49%, and total kephalin 0.115%; this proportion agrees well with those of Channon and Foster. Alpers (2) reports that the lecithin content of wheat embryos is 2.49%, the germ oils extracted with benzine having a higher lecithin content than pressed oils. Diemair and co-workers find the fatty acids of the phosphatides from wheat to contain a higher proportion of saturated acids than the glycerides themselves, also the linoleic and possibly linolenic acids comprise a larger fraction of the fatty acids than in the glycerides.

Stout, Schuette, and Fischer (55) report that the amount of "oil" extracted from rye germ varies from 11.5% with petroleum ether, 14.1% with ethylene dichloride, 15.5% with chloroform, to 17.2% with acetone. The amount of lecithin (P x 26) in these extracts is 1.0, 3.8, 7.3 and 1.7% respectively. There is no correlation between yield of oil, lecithin, and unsaponifiable matter in the extract.

Sullivan and Near (59) studied the changes in the crude lipids of wheat germ, bran, meal, and patent flour during storage. The amount of extract obtained with various solvents and the nitrogen and phosphorus content of these extracts varied markedly during storage. The N:P ratio of all the extracts is greater than unity, indicating the presence of nitrogen-containing lipids other than lecithin. This contrasts with the findings of Diemair and co-workers (18, 19) that the only base present is choline.

Sherwood and co-workers (53) determined the change in acidity of stored wheat germ by the Greek method, which involves the

titration of an 85% alcoholic extract of the material, and found the increase in acidity to rise much more rapidly at 29° than at -10°, -1°, or 6°. This determination of acidity would include fatty acids from the breakdown of glycerides, alcohol-soluble acids from carbohydrate fermentation and other sources of substances with an acid reaction. These investigators did not report on the effect of moisture content on the rate of rise of acidity.

All fat-containing foods undergo deterioration of several types. Lipids are relatively well protected as long as they remain within intact cells, but hydrolysis with the liberation of free fatty acids takes place more rapidly when the cell walls are broken. In the milling of wheat the germ is removed, and in the process it is flattened by passage between rolls, and the cell walls broken. This explains why commercial wheat germ is unstable. Intact germ removed from wheat before milling remains organoleptically fresh for a longer time than the germ produced in the usual way. Even intact wheat kernels may undergo a type of deterioration known as "sick wheat". A study of this phenomenon was recently made by Carter and Young (12). They find that the changes taking place are characterized by a loss of viability, a marked increase in fatty acid content, and a characteristic change in the appearance of the germ. Although the occurrence of "sick wheat" has not yet been fully explained, it is known that high storage temperatures and a high moisture content favor its development.

A type of food deterioration which gives rise to fluorescence in dried eggs has recently received attention on account of its application as a criterion for the acceptability of this product. Recent work by Olcott (41) indicates that the changes responsible

for the increase in fluorescence of potassium chloride extracts of eggs, and the increased color are due to the reaction between protein and sugar present. It is interesting to note that Pearce (44) found an increase in the fluorescence of potassium chloride extracts of wheat germ during storage. Wheat germ contains large amounts of both soluble sugars and protein, so there is a strong possibility that the same type of deterioration takes place in wheat germ especially if Olcott's hypothesis is correct.

Oil bearing plant material usually has fat-splitting enzymes associated with it, and wheat germ is no exception. Pett (46) made a study of the lipase of wheat embryo. He found the scutellum had a very high esterase content which dropped rapidly on germination. The greatest part of the lipolytic activity of the embryo is in the cotyledon, which increases on 12 hours germination, then decreases. Pett used a synthetic substrate, and employed a hydrogen-ion concentration of pH 8.2, although he noted that the enzyme had a broad optimum range of hydrogen-ion concentration; Sullivan and Howe (57) also record similar findings.

Sumner's (60) discovery of "lipoxidase" prompted a great deal of investigation on this enzyme. However, Lusena (37) failed to find a correlation between lipoxidase activity and off-odors in wheat germ; the proteolytic enzymes in wheat germ could be inactivated by the use of ethylene dichloride vapor and re-activated by the use of glutathione or cyanide. Treatment with ethylene dichloride vapor followed by steam inactivated the protease irreversibly. Previously, as noted above, Pearce had postulated that proteolysis was responsible for the increase in fluorescence of potassium chloride extracts of wheat germ. Olcott gives a more plausible explanation

of this phenomenon, and no enzyme system need be postulated to explain the changes observed by Pearce.

Several methods of treating germ to prevent or retard deterioration have been proposed. For example, the momentary raising of the temperature to a point sufficiently high to inactivate enzymes has been used. (48). Passing ethylene dichloride vapor through the germ has already been mentioned. Storage under refrigeration and at reduced pressure and under an inert atmosphere have also been proposed as methods of preservation. Levin (36) removes practically all the fat from germ with a chlorinated solvent at low temperature. This treatment not only renders the defatted germ stable, but also produces a stable oil. In this connection it is interesting to note that Pearce postulates the presence in wheat germ of an anti-oxidant for fats, which is insoluble in petroleum ether. The existence of such an antioxidant is proven in the experiments to be described below.

Altschul and his co-workers, in a series of papers (3,4,33) discuss the theoretical and practical aspects of preventing the increase of fatty acids in the fat of stored cottonseed. This is an important economic problem, as cottonseed decreased in value on storage, due to the lower price obtainable for the oil from old seed, which yields a high-acid oil. These workers studied the effect of various factors such as moisture and temperature on the rate of increase of fatty acids in stored cottonseed. They found that a small change in the hydrogen-ion concentration of the seeds markedly retards the rate of fatty acid formation. This is explained as being due to some change in the protein; inhibition of lipase activity is not believed to be involved.

The studies recorded in this thesis are primarily concerned with the effect of various conditions and treatments on the lipid fraction of wheat germ during storage.

METHODS

It is well known that different solvents extract varying fractions of the fatty substances from most biological material. In general, chlorinated solvents extract more lipid than hydrocarbon solvents, although in some cases the total fat cannot be extracted unless extraction is preceded by hydrolysis to liberate the fatty acids. For example, Grossfeld and Hess (26) find that light petroleum or a mixture of alcohol and benzene remove all the fatty material from yeast only after decomposition with hydrochloric acid. On the other hand, Rewald (51) obtained phosphatides by extraction with a non-polar solvent, followed by extraction with a mixture of chloroform and alcohol. Milhorat (39) has investigated the physiological properties of the lipids extracted from fresh wheat germ by ethylene dichloride, after a preliminary extraction with petrol ether. This fraction gives positive results in the treatment of muscle disorders; the petrol ether extract is inactive.

Serial extraction of soybeans has been employed to obtain a phosphatide-rich fraction. Lebedev (35) uses a gasoline-alcohol mixture to extract the phosphatide after extracting the fat with gasoline alone. The procedure is claimed to give a commercial lecithin of good quality.

Simple adaptations of these procedures were employed in this investigation to extract three lipid fractions from wheat germ:

1. A petroleum ether extract, obtained by extracting the germ in a Soxhlet apparatus for 24 hours. The solvent was removed from the oil by vacuum distillation at a low temperature.

2. The residue from the petroleum ether extract was extracted with ethylene dichloride for 24 hours.

The solvent was removed from the lipid fraction in the same manner as before, but it was found advantageous to remove the last of the solvent by repeatedly adding a small amount of petroleum ether and evaporating under vacuum. In this way it was easy to remove the last trace of solvent odor from the ethylene dichloride extract and it was unnecessary to maintain a high vacuum for long periods of time as is ordinarily the case with this type of solvent.

3. The residue from the ethylene dichloride extraction was extracted with a mixture of 35 parts of isopropanol and 65 parts of ethylene dichloride (by volume) for 24 hours.

This solvent removed not only a lipid fraction, but also a solid carbohydrate fraction. It was necessary to remove the mixed solvent by vacuum distillation and then extract the fatty material by repeated treatment with petrol ether. Four extractions by decantation were sufficient to remove all the petrol ether soluble material.

The first fraction was a clear, orange-colored oil, with a characteristic pleasant odor. The second fraction was a brown gummy material, the viscosity of which rapidly decreased as the temperature was raised. It also had a characteristic odor. The third fraction was somewhat similar in appearance to the second but was even more viscous and sticky at room temperatures.

The brown, water-soluble material in the third extraction appeared to consist largely of non-reducing disaccharides.

In the storage tests, it was originally planned to adjust the moisture content of the germ samples by placing them in desiccators over appropriate solutions of known vapor pressure, as was done by Larmour (34), when studying the respiration of flaxseed and sunflowerseed. To establish the equilibrium-moisture relationship of the wheat germ the method of Coleman (17) was tried. This comprises drawing air through sulphuric acid solutions of known

strength and then through the sample. Several days were required to reach equilibrium, especially at the higher relative humidities. A longer time would be required if a static method was used to bring the germ to a known moisture content, so the method finally adopted was to draw water-saturated air very rapidly through the germ until suitable moisture levels were reached, and then determining the moisture content, by the official A.A.C.C. method (13). The data obtained on equilibrium-moisture contents are quite different from those of either wheat or flour..(see experimental section).

Other analyses were conducted by standard official methods, e.g. the A.O.A.C. volumetric method for phosphorus (1) and semi-micro nitrogen analyses; the Parker-McFarlane (43) modification of the Emmerie-Engel method for determination of tocopherol and the A.O.C.S. method for the determination of free fatty acid, employing hot isopropanol as the solvent.

To adjust the hydrogen-ion concentration of germ to desired values, it was useful to know how much ammonia or hydrogen chloride would be required. Buffer curves were therefore constructed from data obtained by the addition of standard hydrochloric acid and ammonium hydroxide to suspensions of germ, and allowing an hour for equilibrium to be reached. The addition of hydrogen chloride or ammonia was effected by drawing the gas through the germ while the latter was vigorously agitated. The pH was measured at periodic intervals until the desired hydrogen-ion concentration was obtained.

Free and total choline determinations were made by the method of Chapman (15). This method is similar to that of Glick (23) but it differentiates between free and bound choline.

Samples in storage were always packed in tightly stoppered glass containers. To investigate the effect of storage temperature, three series of samples were stored : at room temperature, $4^{o}\pm1$, and $43^{o}\pm1$, respectively.

EXPERIMENTAL

The equilibrium moisture content of wheat germ was measured by the dynamic method of Wilson (63). Sulphuric acid solutions of appropriate strengths were made up and the concentrations checked by specific gravity measurements and by titration. Freshly milled germ was placed in U-tubes and air was drawn first through the acid, then through the sample at as rapid a rate as possible without forcing the germ out of the tube. Equilibrium was attained in varying lengths of time depending on the relative humidity of the air being drawn through. At the highest relative humidity - 83% - the time required was 9 days. The data on equilibrium moisture are given in Table V.

TABLE V - The Moisture Content of Wheat Germ Equilibrated at Varying Relative Humidities.

Relative Humidity (%)	Moisture Content (%)	Dry Basis
24.5	4.3	4.5
40.0	6.9	7.4
60.0	10.5	11.7
83.0	15.7	18.6

It is interesting to compare the results in Table V with similar data for wheat or wheat flour; this comparison is illustrated graphically in Fig. I. While the proportion of protein in germ is more than twice that in wheat, the moisture content at the same relative humidity is strikingly lower. The relative water-binding capacity of the proteins in germ and endosperm may account, at least in part, for this result.

To determine the approximate quantities of gaseous acid or base which would be required to change the hydrogen-ion concentration of

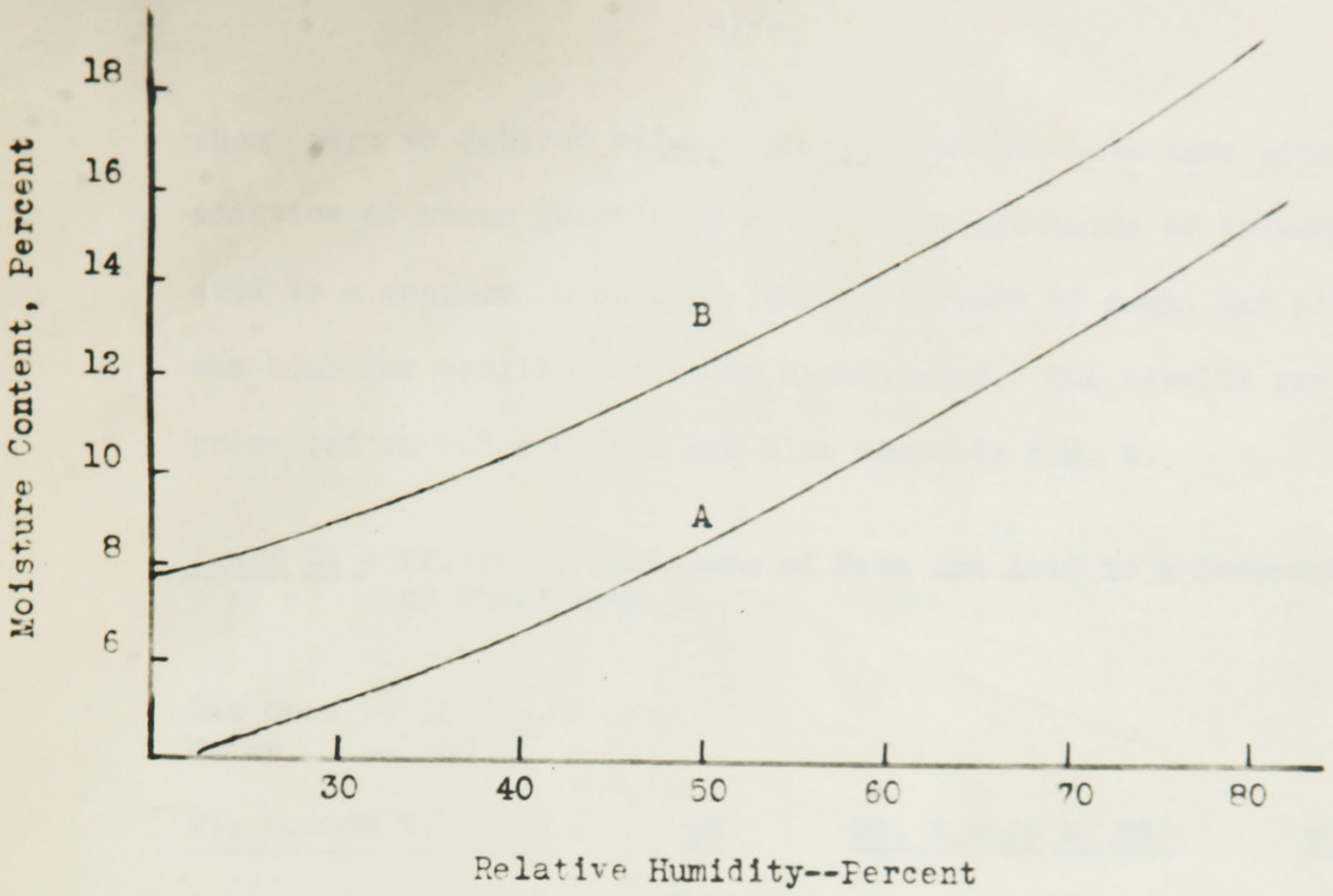

Figure 1. Equilibrium Moisture Content of Wheat Germ
A. Wheat Germ
B. Wheat (Data of Coleman)

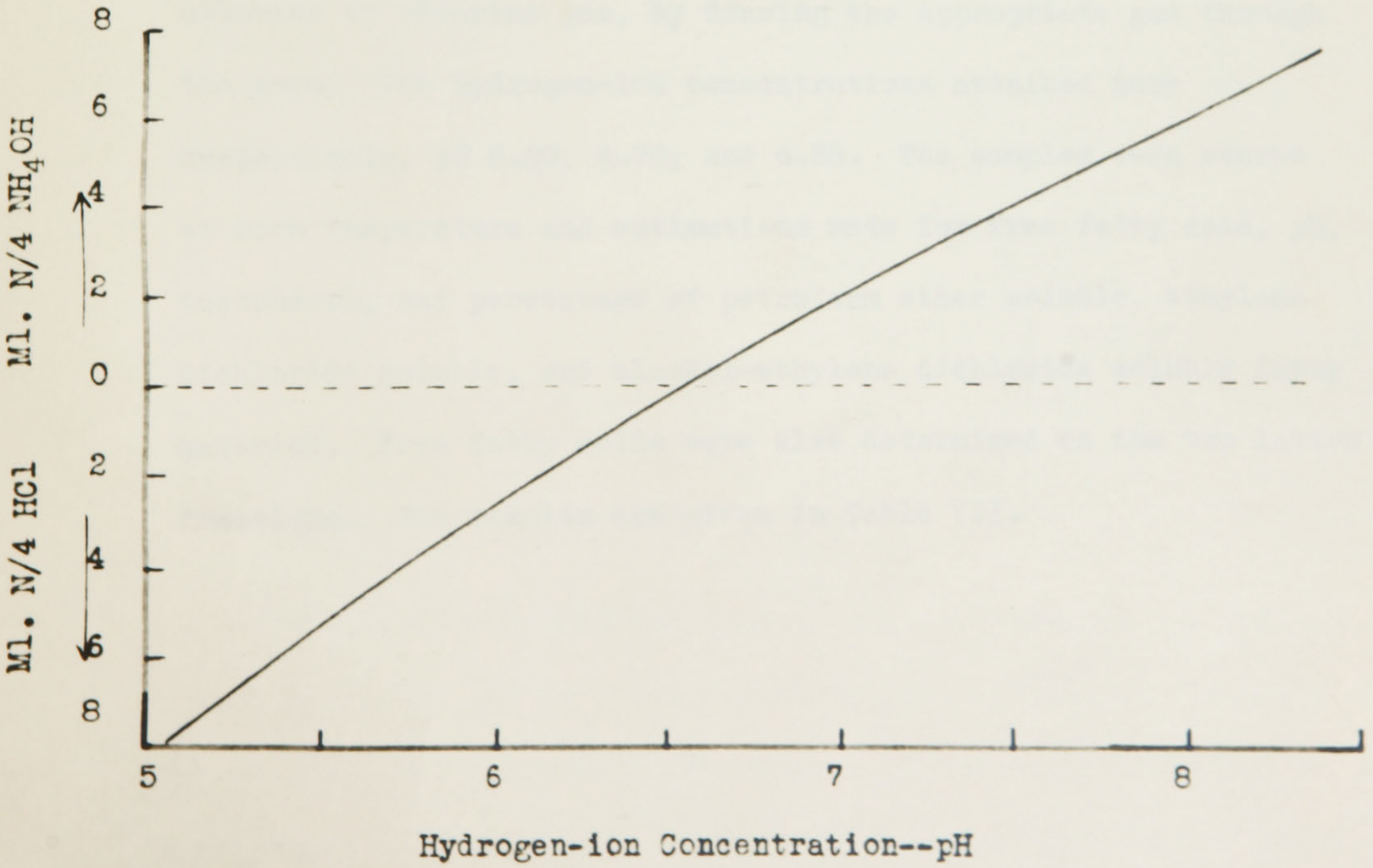

Figure 2. Buffer Curve of Wheat Germ--10 g. Germ in 100 ml. H_2O

wheat germ to desired values, pH measurements were made after the addition of known quantities of ammonium hydroxide or hydrochloric acid to a suspension of germ (ground to pass 20 mesh) and allowing one hour for equilibrium to be established. The results are presented in Table VI and are also shown in Fig. 2.

TABLE VI - Effect of Additions of Base and Acid to a Suspension of Wheat Germ

Raw Germ -- 10.0 g.
Water -- 100 ml.

Ml. 0.2508 N. NH_4OH	pH	Ml. 0.2526 N. HCl	pH
1.00	6.85	1.00	6.30
3.00	7.32	3.00	5.93
4.00	7.51	4.00	5.81
6.00	7.98	6.00	5.42
8.00	8.38	8.00	5.08

Three lots of germ were next treated with ammonia, hydrogen chloride or chlorine gas, by drawing the appropriate gas through the germ. The hydrogen-ion concentrations attained were respectively, pH 8.20, 4.70, and 4.85. The samples were stored at room temperature and estimations made for free fatty acid, pH, tocopherol, and percentage of petroleum ether soluble, ethylene dichloride soluble, and alcohol-ethylene dichloride soluble fatty material. Free fatty acids were also determined on the two latter fractions. The results are given in Table VII.

TABLE VII - Effect of Hydrogen-ion Concentration on the Development of Free Fatty Acid in Stored Wheat Germ. (Moisture 6.2%; Germ 89.0% Pure)

A. Untreated Sample

Time (weeks)	0	4	11
pH	6.60	6.65	6.58
Petrol ether extract	12.0%	12.1%	12.0%
Tocopherol content of petrol ether extract	3.0 mg./gm.	3.0 mg./gm.	3.0 mg./gm.
F.F.A. in petrol ether extract	2.6%	4.3%	5.2%
Ethylene dichloride extract	1.64%	1.66%	2.27%
F.F.A. in ethylene dichloride extract	16.7%	16.9%	16.3%
Alcohol-ethylene dichloride extract	2.1%	2.0%	0.92%
F.F.A. in alcohol-ethylene dichloride extract	28.4%	29.2%	32.8%

B. Ammonia-treated

Time (weeks)	0	4	11
pH	8.20	7.22	6.96
Pet. Ether Ext.	12.0%	12.1%	12.1%
Tocopherol	3.0 mg./gm.	2.9 mg./gm.	2.8 mg./gm.
F.F.A.	1.0%	5.1%	5.0%
Ethylene dichloride extract	1.64%	1.68%	2.48%
F.F.A. in E. D. extract	15.9%	15.8%	15.1%
Alcohol-E. D. extract	2.1%	1.9%	0.85%
F.F.A. in Alcohol-E.D. extract	28.9%	32.0%	34.9%

C. Hydrogen Chloride-treated

Time (weeks)	0	4	11
pH	4.70	4.73	4.85
Pet. Ether Ext.	12.1%	12.1%	12.0%
Tocopherol	3.0 mg./gm.	2.8 mg./gm.	2.4 mg./gm.
F.F.A.	3.0%	3.8%	3.9%
Ethylene dichloride extract	1.66%	1.69%	2.16%
F.F.A. in E.D. extract	21.9%	23.9%	20.0%
Alcohol-E.D. extract	2.0%	1.6%	0.70%
F.F.A. in Alcohol-E.D. extract	28.9%	28.4%	28.2%

The chlorine-treated sample was discarded because the fat was chlorinated, the iodine value and tocopherol content of the petrol ether extract being reduced to one-half of the control value. It was not possible to reach a satisfactory phenolphthalein end-point in the F.F.A. determination on the chlorinated fat; in addition the

petroleum ether extract was very light in color, indicating destruction of the carotenoid pigments.

The relation of temperature to the rate of increase of free fatty acid was investigated by analyzing three samples of germ stored at 43±1°, room temperature, and 4±1°, all at 7.9% moisture content. The same analyses were made as in the above investigation of the effect of hydrogen-ion concentration, and the results are shown in Table VIII.

TABLE VIII - Effect of Storage Temperature on the Changes in Lipid Fractions of Wheat Germ.

A. High-temperature

Time (weeks)	0	10
Pet. ether extract	12.1%	12.1%
Tocopherol	3.2 mg./gm.	1.6 mg./gm.
F.F.A.	1.95%	16.0%
Ethylene dichloride extract	1.91%	0.94%
F.F.A. in E.D. extract	12.4%	31.4%
Alcohol-E.D. extract	1.0%	0.58%
F.F.A. in Alcohol-E.D. extract	28.4%	32.3%

B. Room-temperature

Time (weeks)	0	10
Pet. ether extract	12.1%	12.2%
Tocopherol	3.2 mg./gm.	3.1 mg./gm.
F.F.A.	1.95%	4.1%
Ethylene dichloride extract	1.91%	1.33%
F.F.A. in E.D. extract	12.4%	20.3%
Alcohol-E.D. extract	1.0%	0.89%
F.F.A. in Alcohol-E.D. extract	28.4%	29.9%

C. Refrigerated

Time (weeks)	0	23
Pet. Ether extract	12.1%	12.1%
Tocopherol	3.2 mg./gm.	3.2%
F.F.A.	1.95%	2.3%
Ethylene dichloride extract	1.91%	1.71%
F.F.A. in E.D. extract	12.4%	17.0%
Alcohol-E.D. extract	1.0%	0.93%
F.F.A. in Alcohol-E.D. extract	28.4%	29.9%

In the same way the changes as correlated to the moisture content of the germ were studied. Water-saturated air was drawn through a sample of germ until a suitable moisture level was reached. The moisture levels were 7.9% and 11.5%, and both samples were stored at 43°. The results are shown in Table IX.

TABLE IX - Effect of Moisture Content on the Changes in the Lipid Fractions of Wheat Germ.

Moisture 7.9%

Time (weeks)	0	10
Petrol ether extract	12.1%	12.1%
Tocopherol-petrol ether extract	3.2 mg./gm.	1.6 mg./gm.
F.F.A. in petrol ether extract	2.0%	16.0%
Ethylene dichloride extract	1.91%	0.94%
F.F.A. in ethylene dichloride extract	12.4%	31.4%

Moisture 11.5%

Time (weeks)	0	10
Petrol ether extract	12.1%	12.1%
Tocopherol-petrol ether extract	3.2 mg./gm.	1.1 mg./gm.
F.F.A. in petrol ether extract	2.0%	28.9%
Ethylene dichloride extract	1.91%	0.95%
F.F.A. in ethylene dichloride extract	12.4%	32.2%

The effect of ethylene dichloride vapor and of papain on the rise in acidity of the petrol ether extract of germ was next investigated as follows:-

Samples were stored in bottles of 275 ml. capacity, and the amount of ethylene dichloride to saturate this amount of air at the temperature used was calculated. The required amount of ethylene dichloride was placed in a small crucible placed on top of the sample and allowed to evaporate spontaneously into the closed bottle. The amount of ethylene dichloride to produce a vapor-saturated atmosphere was 0.40 grams; two other similar samples were run at the same time, with one half and twice this quantity of ethylene dichloride respectively. The papain was added at a level of 0.5% of the weight of the germ and thoroughly mixed. The moisture content of the germ was 13.5%, purity 88.7%, temperature 43°. The original F.F.A. of the petrol ether extract was 3.1%. After 8 days storage the Free Fatty Acid was determined on the petrol ether extracts. The results are shown in Table X.

TABLE X - Effect of Ethylene Dichloride Vapor on the Increase of Free Fatty Acid in Stored Wheat Germ.

Treatment	F.F.A. On Petrol Ether Extract
Control	11.4%
Half Saturation with ethylene dichloride vapor	11.4%
Saturation with ethylene dichloride vapor	10.9%
Twice saturation with ethylene dichloride vapor	9.8%
Papain	78.2%

It would appear that papain very greatly accelerates the breakdown of the glycerides, while ethylene dichloride vapor had a slight retarding action. This indicated the desirability of trying the effect of known inhibitors of papain-like enzymes in wheat flour. For this purpose, nitrogen trichloride and potassium bromate are frequently used in flour and their effect in germ was tested. The germ was 93% pure, moisture 13.1%, original F. F. A. on the petrol ether extract 2.9%. One sample was treated with 200 p.pm. of potassium bromate and the other with an unmeasured, but very high quantity of nitrogen trichloride. The samples were stored 12 days at 43°. The following results were obtained:-

Treatment	F.F.A. In Petrol Ether Extract
Control	9.2%
Bromate	8.8%
Nitrogen trichloride	9.2%

It will be seen that these enzyme inhibitors had no appreciable effect on the breakdown on the glycerides.

Woolley (64) showed that commercial soybean lecithin contains an appreciable amount of a complex phospholipid-like material which he names lipositol. An attempt was therefore made to isolate lipositol from the fraction of wheat germ insoluble in petrol ether, but

soluble in ethylene dichloride. After extracting 330 g. of germ with petroleum ether the residue yielded 7.84 g. of ethylene dichloride soluble material. This was dissolved in chloroform, lipositol precipitated by pouring into methanol, redissolved in chloroform and reprecipitated with acetone. This precipitate was washed with acetone and dried. It had a light yellow color but rapidly darkened on drying. The yield was only 2.7% of the ethylene dichloride soluble fraction. Woolley repeated the solution and reprecipitation several times, and obtained an over-all recovery of approximately 50%, as calculated from the amount of inositol present. It seems probable, therefore, that if lipositol is present in this fraction the proportion is less than 5.4%. The inositol content of this fraction has not yet been determined.

Antioxidant Activity of the Lipid Fractions of Wheat Germ

Phospholipid determinations were made on the three fatty fractions by the method of Baldwin and Longenecker (8). This method involves an acetone precipitation of the phosphatides, using a small amount of magnesium chloride to bring about complete precipitation. The phospholipid content of the three fractions was found to be as follows:

Petrol ether extract	1.1%
Ethylene dichloride extract	62.5%
Alcohol-ethylene dichloride extract	74.0%

These three fractions were used as antioxidants in lard, the procedure being to add the fraction to a sample of lard which was then placed in an open crystallizing dish and stored in an oven at 63°C. Peroxide values were determined iodimetrically at intervals and the time required to reach a peroxide value of 50 m.e./kg. was arbitrarily taken as the "keeping time". The keeping times are shown in Table XI.

TABLE XI - Antioxidant Activity of the Lipid Fractions of Wheat Germ.

Antioxidant	Keeping Time (hours)
None (control)	43
0.05% commercial lecithin	48
0.05% ethylene dichloride extract of wheat germ	67
0.05% ethylene dichloride-alcohol extract	58
0.5% petrol ether extract	63

The acetone insoluble material obtained from the lipid fractions mentioned above were also tested as antioxidants. To determine whether all of the antioxidant activity was due to the phosphatide the concentrations used were adjusted so that there was the same amount of phosphatide (acetone-insoluble) as in the first instance. That is, the levels of purified material used were 0.031% and 0.037% of the ethylene dichloride soluble and alcohol-ethylene dichloride soluble fractions respectively. The results are given in Table XII.

TABLE XII - Effect of Acetone Fractionation on the Antioxidant Activity of Lipid Fractions of Wheat Germ.

Antioxidant	Keeping Time (hours)
Crude ethylene dichloride extract (0.05%)	67
Acetone-insoluble fraction of 1 (0.031%)	48
Crude alcohol-ethylene dichloride extract (0.05%)	58
Acetone-insoluble fraction of 3 (0.037%)	74

These results are difficult to interpret, since in one case the antioxidant effect is increased and in the other decreased.

Choline Fractionation of Germ Lipids

The choline content of wheat germ has been reported by Engel (20) and by Glick (23). Assuming all the choline to be combined as lecithin, the lecithin content of wheat germ would be approximately two percent. Since the fatty fraction of germ not soluble in petrol

ether is considerably larger than this, a study was made of the distribution of choline in the fatty fractions. It was necessary first to determine if any free choline was present, and for this purpose the method of Chapman (15) was used. This method fractionates free and combined choline by removing combined choline with a calcium-hydroxide suspension. Free choline remains in solution, and is determined on the filtrate, while the combined choline is calculated by the difference between total and free choline. Instead of Chapman's rapid extraction in a Waring Blendor, a 20-hour extraction was made in the Soxhlet apparatus, using absolute methanol. No trace of free choline could be found in fresh germ, or in germ of 5.9% moisture stored at 43° for 21 days.

Choline estimations were made on the absolute methanol extracts of raw germ of 93.6 purity and on the residue of germ after extraction with petrol ether, ethylene dichloride, or ethylene-dichloride-alcohol mixture. The nitrogen and phosphorus contents of the last two extracts was also determined. The choline content of the germ after the various extractions was calculated back to the raw germ basis. The results were as follows:-

Treatment	Choline content (mg./g. of germ)
Nil, raw germ	3.73
Petrol ether extracted	3.58
Ethylene dichloride extracted	2.07
Ethylene dichloride-alcohol extracted	0.87

The choline content of the extracts was not determined, but the nitrogen and phosphorus content was checked. The following results indicate that there was more nitrogen in the extracts than could be accounted for on the basis of the choline disappearing from the germ.

	N, %	P, %	N/P
Ethylene dichloride extract	1.46	3.04	1.06
Ethylene dichloride-alcohol extract	1.60	2.45	1.45

On the basis of 100 g. of raw germ the nitrogen distribution appears thus:-

	Nitrogen in extract	Choline nitrogen disappearing from germ
Ethylene dichloride extract	28.9 mg.	15.7 mg.
Ethylene dichloride alcohol extract	27.8 mg.	12.0 mg.

Thus, the choline accounts for only about half of the nitrogen in the extracts. The molecular ratio of nitrogen to phosphorus also indicates the presence of some phosphatide other than lecithin.

The change in the N/P ratio during storage was measured with the results shown in Table XIII.

TABLE XIII - Effect of Storage on the N/P Ratio of Lipid Fractions of Wheat Germ.

Ethylene dichloride extract	Original	Stored 21 days
Nitrogen	1.46%	1.36%
Phosphorus	3.04%	3.10%
N/P	1.06	0.96

Ethylene dichloride-alcohol extract	Original	Stored 21 days
Nitrogen	1.60%	2.20%
Phosphorus	2.45%	2.81%
N/P	1.45	1.73

DISCUSSION AND SUMMARY

The rate of increase of free fatty acids in stored wheat germ is affected by temperature, water content, and hydrogen-ion concentration of the germ. In all respects these findings are similar to those of Altschul and co-workers with cottonseed. Increase of temperature markedly increases the rate of breakdown of the glycerides. The free fatty acid content of a sample of germ held at 4° for 23 weeks increased but slightly, while at the same moisture level the fatty acid concentration increased over two-fold and eight-fold at room temperature and 43° respectively.

The very marked effect of papain in increasing the rate of formation of free fatty acid in wheat germ lends weight to the theory of Altschul that the action of ammonia and hydrogen chloride in decreasing the rate of formation of free fatty acid in cottonseed is due not to the inactivation of the lipase, but to the destruction of a protease which in turn liberates the lipase. Although oxidizing agents such as bromate or nitrogen trichloride are effective in counteracting the undesirable effects of protease in wheat flour they are not effective in lessening the lipase activity of wheat germ.

Treatment of wheat germ with either hydrogen chloride or ammonia gas, does not prevent entirely the increase in free fatty acids but does decrease the rate at which free fatty acids are formed. From a practical standpoint neither of these methods shows much promise, because of other undesirable effects. Both treatments produce color changes in the germ, the former producing a reddish, branny appearance, and the latter a yellow coloration caused by the

indicator-like substance present in germ. Furthermore, both treatments accelerated the destruction of tocopherol, particularly the hydrogen chloride treatment.

The fraction extracted by ethylene dichloride but not by petroleum ether appears to be largely phospholipid in nature, but only a part of the nitrogen is present as choline. Its breakdown to fatty acids is extremely slow in comparison with the breakdown of glycerides under the same conditions.

The fraction extractable with alcohol-ethylene dichloride after the removal of the ethylene dichloride soluble fraction contains a much higher proportion of free fatty acid than the ethylene dichloride soluble fraction. If it were not for the consistently higher free fatty acid content of this fraction it would resemble the ethylene dichloride soluble fraction. The acetone-insoluble portions comprise approximately the same amount of each fraction, and the antioxidant effects, although slight, are comparable. As in the case of the ethylene dichloride soluble fraction, some, but not all, of the nitrogen is accounted for by the disappearance of choline from the extracted germ. The nitrogen:phosphorus ratio is appreciably higher in this fraction than in the ethylene dichloride soluble fraction; in the ethylene dichloride extract the ratio is close to unity, while in the alcohol-ethylene dichloride soluble fraction it approximates 3:2 in freshly milled germ.

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