

8-1-1936

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## Recommended Citation

Jacobsen, D. H., "The Relation of Amino Nitrogen Content to Quality of Cream and Butter" (1936). *Bulletins*. Paper 304.  
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**The Relation of  
Amino Nitrogen Content  
to Quality of  
Cream and Butter**

**By**

**D. H. Jacobsen**

**Department of Dairy Husbandry  
Agricultural Experiment Station  
South Dakota State College of  
Agriculture and Mechanic Arts  
Brookings, S. D.**

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# **The Relation of the Amino Nitrogen Content to Quality of Cream and Butter**

**By D. H. Jacobsen**

Grading or evaluating the quality of cream for buttermaking has been based largely on the age, acidity, and flavor of the cream. The classification on the basis of flavor is necessarily indefinite, and results of such grading vary with different graders. Age and acidity are important, but wide variations in the flavor and condition of cream may be found at each stage of holding and within each acidity range. The study of the amino nitrogen content of cream is suggested as an indication of the stage of cream deterioration and consequently an indication of the quality of cream for buttermaking. Age, acidity, and bacterial contamination of cream are recognized as prime factors in causing flavor deterioration in cream, and these factors also operate to increase the rate and extent of protein decomposition. Consequently, a measure of amino nitrogen in cream should give some indication of the extent to which such factors have operated.

## **Review of Literature**

A number of investigators have shown that amino nitrogen is present in increasing quantities in cream as acidity development progresses. Ferris (4) studied the soluble nitrogen compounds of cream and butter treated in various ways. He found that proteolysis in cream, as shown by increase in amino acid nitrogen and nitrogen not precipitated by phosphotungstic acid in the corresponding butters, begins as soon as the cream develops acidity of 0.2 to 0.3 per cent. Butters held in storage showed an increase in soluble nitrogen compounds, the increase in butters made from sweet cream being slight, while the butters made from the neutralized sour cream showed a somewhat greater increase. The greatest per cent of soluble nitrogen when the butter was fresh, and also the greatest increase during storage, was in butter made from cream which had been allowed to sour before it was pasteurized. In a later paper, Ferris (5) observed that the percentage of total nitrogen occurring as amino nitrogen and ammonia was generally lower in first grade cream than in second grade cream. The butters from these grades of cream showed much the same relationship.

That increasing amino nitrogen content in butter is accompanied by lowered score in storage is indicated by Brown (1), Rahn, Brown, and Smith (7), Hunziker and Spitzer (6), Brown, Smith, and Ruehle (2), and Ferris (5). Although different methods were used by these investigators for determining the degree of proteolysis, their general conclusions were in close agreement.

Decomposition of casein in salted and unsalted milk and butter was studied by Brown (1). The butter was held in storage at 0° C. (32° F.) for 240 days. The casein in both salted and unsalted butter was slowly broken down into amino acids and ammonia. The average increase in amino acid and ammonia nitrogen in 20 samples of unsalted butter was



from 5.71 per cent to 7.59 per cent while the average increase in 20 samples of salted butter was from 5.71 per cent to 8.19 per cent. These percentages represent the amino acid and ammonia nitrogen as per cent of the total nitrogen in the butter. Milk to which 5 per cent salt had been added also showed significant increases in amino acid and ammonia nitrogen content over a seven day period.

The experiments of Rahn, Brown, and Smith (7) on the keeping quality of butter showed that an increase in amino nitrogen occurred in all samples during storage. The greatest increase occurred in the samples of lowest quality at the end of the storage period. Bacteriological analysis of the butter at intervals during storage showed no apparent correlation with the depreciation in score.

That common off-flavors of butter in cold storage may be caused by protein decomposition was also indicated by Dyer (3). He reported that "common off-flavors in cold storage butter may be due to the chemical change expressed thru slow oxidation progressing in some one or more of the non-fatty substances occurring in the buttermilk. The extent of such chemical change is directly proportional to the acid present."

Extensive investigations on butter made from pasteurized and unpasteurized cream were reported by Hunziker and Spitzer (6). A chemical study of proteolysis in the butter showed that there was a gradual increase in soluble nitrogen during storage. This increase was closely related to the quality of the butter as shown by the score when fresh and again after storage for five months. They concluded that protein decomposition was greatest in the raw cream butter.

Brown, Smith, and Ruehle (2) showed that the total nitrogen content of butter decreased during storage and that this decrease was accompanied by an increase in soluble nitrogen not precipitated by copper sulphate. The per cent of amino acid and ammonia nitrogen showed a steady increase on long storage.

Spitzer and co-workers (9) studied the proteolysis of specific organisms in graded cream and concluded that the quality of butter decreased in proportion to the protein hydrolysis. Proteolytic action progressed at a greater rate in the presence of proteolytic organisms. Salting of butter had no influence in retarding hydrolysis although the growth of microorganisms was retarded according to this report.

Ruehle (8) reported that the flavor termed metallic was produced by metals, bacteria, and amino acids according to experiments in which milk samples were treated with these agents. The amino acids added were those which result on hydrolysis of casein by microorganisms and enzymes.

Results of these investigations indicate a general relationship between flavor deterioration in cream and butter and the condition with regard to the nitrogenous constituents. This relationship is studied in the present work to show what aid in cream classification or grading may be evolved from the use of amino nitrogen content.

### Method of Procedure

The cream used in this study was selected from a number of different sources for the purpose of studying the nitrogen content relationships of cream showing different degrees of deterioration. Churnings were made from cream which was graded as second or undergrade, first grade sour,

and fresh sweet cream. An effort was made to obtain a representative market range of butter scores.

All cream was pasteurized by heating to 145° F. and holding for 30 minutes. Churnings with acidities above .25 per cent were neutralized to .23 per cent and no starter was used. The churning was carried on in a churn of 400 pounds capacity according to regular plant methods. The butter was salted at the rate of 2 per cent in the finished butter and moisture content was limited to 16 per cent.

Samples of the cream, buttermilk, and butter were taken at churning time for amino nitrogen analysis. From each churning two 10-pound tubs of butter were saved; one of which was held in the local cold storage rooms and the other shipped to a Federal-State butter grader\* for scoring. The butter stored locally was sampled at the end of one, three, and six-month storage periods for the amino nitrogen analysis. The butter submitted for scoring was scored when fresh and after three and six months storage at 32° F. These scores were then used in classifying the butters for the comparison of the amino nitrogen contents with the flavor scores of butter of different grades.

### Chemical Analysis

Total nitrogen in cream, buttermilk, and butter was determined by the Kjeldahl method. The determination of total nitrogen in butter was made on 50 gram samples from which the fat had been extracted with petroleum ether. It was found that a fairly complete removal of fat from the butter sample was necessary before digestion could be carried on successfully.

Amino nitrogen was determined by the Sorensen formol titration method as described in A.O.A.C. Methods of Analysis 2nd edition, (1925). The cream and buttermilk samples for analysis consisted of 20 grams weighed in duplicate into Erlemeyer flasks and diluted with equal volumes of distilled water. This dilution aided materially in showing a sharper end point in the titration. The Sorensen method for amino nitrogen was applied on butter using duplicate 50-gram weighed samples. An equal volume of warm distilled water (approximately 110° F.) was added to each sample and the entire charge titrated with N/20 NaOH allowing time for the aqueous and fat layers to separate so that the color developed in the aqueous layer could be noted.

The titrable acidity of cream was determined by titrating 9 grams of cream with N/10 NaOH using phenolphthalein as the indicator. The titrable acidity of butter was determined in connection with the Sorensen formol titration method in which a 50-gram sample was titrated in a water suspension. The values obtained represent the acidity in the serum and the acidity washed free from the fat globules by shaking the butter in the warm water suspension.

### Experimental Results

Although it is recognized that any classification made on the basis of cream grade or butter score is not entirely satisfactory, it was used

\* The butter was stored by the Land O'Lakes Creameries, in Minneapolis., Minn., and scored by C. W. Fryhofer.



because it is the method of classification employed for these products in the trade.

The values recorded as amino nitrogen in this work refer to the nitrogenous products commonly referred to as amino acid and ammonia nitrogen. The designation "amino nitrogen" is used throughout for the sake of brevity.

The results on 27 churnings have been arranged in various ways for the purpose of showing the relationships which exist between amino nitrogen content and quality of cream and butter. Table I presents the flavor, acidity, and amino nitrogen content of 27 churnings of cream and the flavor criticisms, flavor scores, and amino nitrogen content of butters made from this cream. In general, it may be noted that cream of low acidity showed a low percentage of amino nitrogen and produced butter of a high flavor score. Conversely, the high acid cream showed higher amino nitrogen content and lower butter score. Exceptions, however, may be noted such as churnings 20 and 24 where feed flavors were responsible for the low butter flavor scores received. It is of interest to note that the churnings termed stale generally showed relatively high amino nitrogen content in both the cream and butter.

TABLE 1.—Amino Nitrogen Content and Quality of Cream and Butter

Churn No.	Cream			Butter		
	Acidity %	Flavor Criticism	Amino Nitrogen	Amino* Nitrogen	Flavor Score	Flavor Criticism
1	.40	Clean sour	4.90	4.42	36	Sl. old cream
2	.48	Clean sour	5.40	5.13	35	Sl. alkaline
3	.40	Clean sour	5.71	4.21	36	Sl. old cream
4	.48	Sour, slightly fermented	5.76	5.92	35	Sl. bitter
5	.16	Sweet, fairly clean	4.23	6.02	35	Sl. bitter
6	.54	Clean sour	4.81	5.76	36	Heated
7	.46	Clean sour	5.76	4.83	36	Sl. old cream
8	.55	Slightly stale, rancid	5.84	6.44	34	Unclean
9	.40	Sour old cream	5.63	6.55	35	Old cream
10	.76	High acid, stale, putrid	9.94	7.54	31	Very rank, metallic
11	.62	High acid, stale, metallic	6.36	8.22	32	Stale metallic
12	.54	High acid, stale	6.19	6.00	34	Kerosene taint
13	.19	Sweet clean	4.63	4.73	37	Clean
14	.46	Clean sour	4.31	5.10	35	Old Cream
15	.17	Sweet clean	5.22	4.45	36	Weedy
16	.58	Sour, stale, oily	5.76	7.89	34	Metallic
17	.70	High acid, stale, oily	6.51	6.52	33	Unclean, sl. cheesy
18	.71	High acid, cheesy	7.03	6.22	32	Very stale, oily
19	.14	Sweet clean	5.18	5.39	36	Sl. unclean
20	.20	Low acid, feed	5.18	8.56	34	Stale, alkaline
21	.72	High acid, stale	6.20	7.91	33	Stale cream
22	.72	High acid, stale	6.47	6.79	32	Very stale cream
23	.83	High acid, oily	6.30	7.09	32	Very stale alkaline
24	.20	Stale feed	6.08	8.55	31	Barny, stale, very unclean
25	.29	Weedy, bitter	4.67	6.66	33	Very unclean, barny
26	.16	Sweet clean	4.59	3.87	38	Clean
27	.16	Sweet clean	5.13	5.65	37	Coarse salt

\* Expressed as per cent of the total nitrogen.

The apparent lack of agreement between the amino nitrogen values for cream and butter of the same churnings may be explained by the fact that the washing of the butter in the churning process may have removed certain types of nitrogenous constituents. The nitrogenous products present in the cream, no doubt, were transferred to the butter in varying proportions according to the form in which they were

present and to the thoroughness with which washing was carried out.

**Amino nitrogen content and cream grade**—In Table 2 the churnings were classified according to the flavor and acidity of the cream. The butter resulting from the cream in these three grades ranged in flavor score as follows: I.—36 to 38; II.—34 to 36; and III.—31 to 34. The average amino nitrogen content showed an increase from one cream grade to the next, but the difference was not great enough to be of significance for grading. Cream of low acidity without stale flavor showed low amino nitrogen content while cream of stale flavor regardless of acidity showed high amino nitrogen content. This may indicate that the flavor commonly termed stale may be caused by increased amino nitrogen content in the cream.

TABLE 2.—Amino Nitrogen Content of Cream of Three Grades

Cream Grade	Cream Description	Number Churnings	Av. Amino Nitrogen		Av. Butter Flavor Score
			Cream	Butter	
1	Sweet, clean, below .20% acidity	5	4.82	4.95	36.8
2	Slightly off-flavored, below .60% acidity	10	5.17	5.65	35.3
3	Marked off-flavored, above .60% acidity or both	12	6.45	7.15	32.6

The wide variation in the amino nitrogen content of churnings within each grade makes definite conclusions impossible. Although the lower flavor grades in general showed a higher per cent of amino nitrogen, there were exceptions to this rule. For instance, churning 25 was low in amino nitrogen content but was placed in grade III because of a feed flavor.

Data on amino nitrogen content of cream and butter agree in general with the figures given by Ferris (5) who compared the amino acid and ammonia nitrogen content of first and second grade cream and butter. His work showed "first" grade cream with a range from 1.6 to 9.6 per cent and "second" grade with a range of 8.1 to 12.6 per cent. The classification used in the present work includes an intermediate grade. The ranges for the three grades as shown in Table 3 were I.—4.59 to 5.22, II.—4.28 to 5.76, and III.—4.67 to 9.94. The absence of the extremely high and low values may possibly be explained by the fact that a limited number of churnings are included in this work. Butter made from the "first" grade cream according to Ferris ranged from 1.6 to 6.3 per cent amino nitrogen while "second" grade showed 4.6 to 6.7 per cent. The present work failed to show churnings below 3.87 and ranged upward to 8.56 per cent.

**Relationship of butter flavor grade to the amino nitrogen content of cream, buttermilk, and butter**—The 27 churnings were divided into three grades on the basis of the butter flavor score as follows: Grade I.—36 or above; Grade II.—34 to 36; and Grade III.—below 34. A comparison is presented in Table 3 of the average flavor score in each grade and the average amino nitrogen content of cream, buttermilk, and butter in these grades.



The amino nitrogen values in the arbitrary grades set up increased from the first to the third cream grade, but the difference between grades was not great. The range of values in each grade was too wide to allow definite conclusions in regard to the utility of amino nitrogen

TABLE 3.—Amino Nitrogen in Cream, Buttermilk, and Butter

Butter Grade	Number Churnings	Flavor Score On Butter	Per Cent Amino Nitrogen					
			Cream		Buttermilk		Butter	
			Av.	Range	Av.	Range	Av.	Range
1	11	36.36	5.09	(4.28-5.76)	5.44	(4.71-6.14)	5.02	(3.87-6.02)
2	7	34.43	5.47	(4.31-6.19)	5.82	(4.80-7.84)	6.85	(5.10-8.56)
3	9	32.11	6.62	(4.67-9.94)	6.54	(4.88-8.53)	7.08	(4.86-8.55)

values for grading cream. Considerable overlapping was shown when the amino nitrogen values of the three grades were compared. Similar overlapping was shown with the values obtained for the buttermilk and butter.

**Amino nitrogen content as a basis for grading cream**—A general relationship between cream grade and amino nitrogen content was shown in Table 2, when cream was classified according to its acidity and flavor. The difference in amino nitrogen content of the three grades suggested that a division of churnings on the basis of amino nitrogen content of the cream might give additional information. The results of such a classification are shown in Table 4.

TABLE 4.—Butter Flavor Score Resulting from Cream of Three Grades Based on Amino Nitrogen Content

Per Cent Amino Nitrogen	Number of Churnings	Butter Flavor Score	
		Av.	Range
Less than 5%	7	35.9	33-38
5 to 6%	12	35.3	34-37
over 6%	8	32.2	31-34

Cream containing more than 6 per cent of the total nitrogen as amino nitrogen made butter of significantly lower flavor score, but butter from cream of lower amino nitrogen content showed considerable variation in quality. This lack of agreement might be expected, however, because off-flavors apparently not related to protein decomposition caused the low score in some cases.

**Changes in amino nitrogen content of butter in storage**—A number of investigators have indicated that a gradual increase in amino nitrogen content of butter occurs in storage. The data reported in Table 5 bear out this conclusion and in addition show the rate of change in three grades of butter. The butter was divided into three grades on the basis of flavor score.

The amino nitrogen values of the three grades of butter maintained the same general relationship to each other throughout the storage period. The difference between classes, however, became smaller as the storage period advanced.

There appears to be no direct correlation between the increase in amino nitrogen and loss in flavor score of butter in storage. In fact,

a slight improvement in average score of butters in grade II was accompanied by a significant increase in amino nitrogen. It will be noted, however, that at each storage interval the lowest score is associated with the highest amino nitrogen content.

TABLE 5.—Increase in Amino Nitrogen Compared With Loss in Flavor Score of Three in Storage

Butter Grade	Number of Churnings	Fresh		3 Months		6 Months	
		Av. Flavor Score	Av. Amino Nitrogen	Av. Flavor Score	Av. Amino Nitrogen	Av. Flavor Score	Av. Amino Nitrogen
1	11	36.36	5.02	35.45	7.26	35.41	8.65
2	7	34.43	6.85	34.08	8.19	34.57	9.27
3	9	32.11	7.08	31.44	8.59	31.00	9.60

The greatest increase in amino nitrogen was recorded in the highest grade butter, which was made largely from cream of low acidity. This is not in agreement with Ferris (4) who found that the increase in soluble nitrogen compounds was somewhat greater for the sour cream butter. The highest per cent of amino nitrogen, however, was found in the lowest grade butter when fresh and at each stage of the storage period.

**Titration acidity in cream and butter**—Table 6 presents a summary of the acidity tests on cream and butter in three classes based upon the cream acidity. The results on butter were obtained in connection with the Sorensen formol titration and represent the acidity obtained by titration of a water fat suspension. The highest acidity in the fresh butter

TABLE 6.—Relationship Between Titrable Acidity of Cream and the Butter Made from It

Class	Number of Churnings	Per Cent Acidity					
		Cream		Butter after Storage			
		Range	Average	Fresh Butter	1 Mo.	3 Mo.	6 Mo.
1	8	Below .20	.16	.043	.048	.052	.062
2	12	.20 to .60	.45	.038	.045	.051	.055
3	7	.60 or above	.72	.062	.076	.079	.104

and the greatest increase in acidity during storage occurred in the butter made from cream of over .60 per cent acidity even though this cream was neutralized before churning. There was no significant difference in the acidity of the butter from the unneutralized cream in Class I and the butter from the neutralized cream in Class II. The cream of acidity above .60 per cent was also relatively high in amino nitrogen and low in flavor quality as has been noted in Table 2.



### Summary

The amino nitrogen content of cream and butter was compared with the flavor score of butter of 27 churnings. The butter was scored and analyzed when fresh and after one, three, and six months in storage at 32° F.

When the 27 churnings were graded according to the flavor and acidity of cream, the amino nitrogen content increased as the quality of cream decreased.

Although there were a few exceptions, in general high amino nitrogen content in cream and butter was associated with low butter flavor scores. Classifications of churnings on the basis of amino nitrogen content of cream alone, however, failed to divide the churnings into satisfactory butter flavor grades.

When the churnings were classified according to the butter flavor score received on the market, some definite trends were shown. The highest percentage of total nitrogen occurring as amino nitrogen was found in the lowest score butter both when fresh and at each stage of the storage period up to six months.

From these results it appears that the amino nitrogen content of cream is an aid in cream grading but only when used in combination with other tests such as flavor and acidity.



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