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SUMMARY

The determination of skimmed milk powder (s.m.p.) based on the enzimatic coagulation of the para-casein has been applied to samples of buttermilk, pure s.m.p. and compound feedingstuffs. Interferences likely to be introduced by the presence of starch, various proteins or whey into the abovementioned sample have been investigated.

Accuracy and repeatability of the analytical method when applied to the different samples are reported.

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I. INTRODUCTION

In order to increase the use of the skimmed milk powder (s.m.p.) the production of which is very high in the European Community (two million tonnes in 1979), the addition of 60 to 70% of s.m.p. in feedingstuffs is financially supported through EEC regulations (1).
Buttermilk is considered by the EEC regulations (2) as s.m.p. to this purpose.
In addition to milk and buttermilk various proteins of vegetable, animal or microbiological origin as well as whey and other ingredients are often found in compound feedingstuffs (3). The relatively high price of the s.m.p. compared with the substitute products (3) can sometimes lead to incorrect declarations of the s.p.m. content for which financial support is granted, the substitution being masked by the similarity of certain physical and chemical properties of the substitute components to those of s.m.p.

Determination of casein, a constant specific component of milk, offers a way of checking skimmed milk content. Several analytical methods are reported in literature i.e.: electrophoresis (4) (5) (6) (7), immunoelectrophoresis (8), determination of sialic acid (9) or selective precipitation of para-casein by the action of rennet enzimes.(10)
The last analytical procedure (10), is the analytical method of choice (3) due to its semplicity.

By the request of the "Milk Products Division of the General Directorate for Agriculture" an experimental work has been undertaken aiming at:

1. Application of the adopted analytical method to samples of s.p.m. and buttermilk of known composition.
2. Test of the method in presence of some substitute products likely to be added to feedingstuffs.
3. Test of the method on compound feedingstuffs.

All the the samples have been furnished by Prof. Sadini, Milk Products Division, Brussels.

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II. EXPERIMENTAL*

2.1. Principle of the method

Quantitative determination of skimmend milk powder (s.m.p.) in feedingstuffs is carried out by enzimatic coagulation of para-casein.
The sample is extracted with sodium citrate solution.
The casein in the extract is precipitated as para-casein by adjustment of the calcium ion concentration to the required level and addition of rennet.
The nitrogen content of the para-casein precipitate is determined by the Kjeldhal method (11). The quantity of s.m.p. is calculated on the basis of a minimum casein content of 27,5% (10) (3).

2.2. Reagents

Reagents used are of analytical grade . Use either distilled water or water of an equivalent quality level. With the exception of the rennet all the reagents and solutions must be free of nitrogenous substances.

2.2.1. Trisodium citrate, dyhydrate (1% w/v solution).

2.2.2. Calcium chloride saturated aqueous solution at 20°C.
Dissolve about 90 g of anhydrous calcium chloride in 100 ml of distilled water by slightly warming and stirring. Leave overnight at 20°C (a deposit of cristals should be formed on the bottom, otherwise some calcium chloride should be added and the procedure repeated),filter the liquid next day and storage the filtered solution a well closed bottle at 20°C.

2.2.3. 0,1 N sodium hydroxide.

2.2.4. 0,1 N hydrochloric acid.

2.2.5. Liquid of calf rennet-standard strength of 1 : 10000 ;store in a refrigerator.

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* This chapter has been derived from the final report of the working group on the det. of s.m.p. in animal feedingstuffs (3).

2.2.6. Reagents for the quantitative determination of nitrogen according to the Kjeldahl method as described (11).

2.3. Materials

Current material used in laboratory and especially :

2.3.1. Mortar or homogenizer

2.3.2. Analytical balance

2.3.3. Bench-top centrifuge (2000 to 3000 rpm) with 50 ml tubes

2.3.4. Magnetic stirrer with (10-15 mm) followers

2.3.5. 150-200 ml beakers

2.3.6. 250 ml and 500 ml Erlenmeyer flasks

2.3.7. Glass funnels of ø 60-80mm

2.3.8. Fast-filtering ashless filters of ø 150mm (S.S.589^{2} S.S. 595$^{\frac{1}{2}}$)

2.3.9. Pipettes of various nominal volume

2.3.10. Water bath at 37°C

2.3.11. pH meter

2.3.12. Kjeldahl digestion an distillation assembly with fittings

2.3.13. 25 ml graduated burette

2.3.14. Plastic wash bottle for distilled water

2.3.15. Stainless steel spatulas

2.3.16. Thermometers

2.3.17. Temperature-controlled drying oven

2.4. Procedure

2.4.1. Preparation of the sample.
Grind in the mortar or homogenize in the mill 10-20 g of the sample to obtain as far as possible, a homogeneous mixture.

2.4.2. Dissolution of milk powder and separation of the insoluble residue.

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1. Weigh 1,000 ± 0,002g of well-homogenized compound feedingstuffs directly into a 50 ml centrifuge tube and add 30 ml of a 1% w/v aqueous solution of trisodium citrate (2.2.1.) previously heated to 45°C; mix with the aid of the stirrer and magnetic follower for at least 5 min.
2. Centrifuge the mixture immediately at 500g (2000-3000 rpm) for 10 min. and decant the clear aqueous supernatant into a 150-200 ml beaker taking care that no loose material on the bottom goes over.
3. Carry out two further extractions on the residue, according to the same procedure, adding the extracts to the first one.
4. If a layer of oil forms at the surface, it can be separated by cooling the beaker with extracts in the refrigerator until the fat solidifies and removing the solid layer with a spatula.

2.4.3. Coagulation of casein with rennet.

1. While stirring continuously, add dropwise 1 ml of a saturated solution of calcium chloride (2.2.2.) at 20°C to the total aqueous extract (about 100 ml), adjusting the pH to 6.4 -6.6 with solutions of NaOH (2.2.3.) or HCl (2.2.4.). Place in the thermostatically-controlled water bath at 37°C for 15-20 min. to obtain saline balance. It becomes more evident by the formation of a light turbidity.
2. If a precipitate has been formed, it must be removed by further centrifugation at 1000 rpm during 5 min. and removal of the supernatant without washing the sediment.
3. Immediately after taking out from the bath at 37°C add dropwise to the equilibrated extract, while stirring 0,5 ml of the liquid rennet (2.2.5.). Coagulation appears in 1-2 min.
4. Leave at a temperature of 20-37°C for 10-15 min. Break the coagulum and then filter immediately when still warm through the fast ash-less filter (2.3.8.) in the funnel, transferring the precipitate quantitatively to the filter and washing three times with 15 ml of distilled water.

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2.4.4. Blank test

A blank test shall be made regularly using an ashless filter paper (2.3.8.) moistened with a mixture of 90 ml 1% w/v (2.2.1.) sodium citrate, 1 ml saturated solution of calcium chloride (2.2.2.), 0.5 ml of liquid rennet (2.2.5.), and washed with 100 ml of distilled water before mineralization by the kjeldahl method,(11).
The volume of acid used for the blank test must be subtracted from that used for titration of the sample.

2.6. Expression of results

The percentage of skimmed milk powder in the compound feedingstuffs is calculated by means of the following formula (10):

$$\% \text{ SMP} = \frac{N \times 6.38}{27.5} \times 100$$

where N is the percentage of para-casein nitrogen and N x 6.38 the percentage of para-casein formed from the casein in the product, calculated according to the formula in the Commission Directive 72/199/EEC of April; 27.5 is the factor for converting the casein percentage of skimmed-milk powder.

III. RESULTS OBTAINED

3.1. Application of the method to samples of pure skimmed milk and buttermilk

Four samples of pure s.m.p. (A-1,A-2,A-3 and A-4) have been analysed ten times by the same operator on different days.
No preliminary drying of the samples was performed.
Taking into account that the maximum s.m.p. content in feedingstuffs is 70%, 0.7 g sample has been used for each determination.

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The mean values obtained (table I) for the four samples show that an accuracy within $\pm$ 0.3% can be achieved.
The application of the "t" test (t_{theor} = 2.26 for 9 degrees of freedom and a probability of 95%) shows that the obtained mean values are not significantly different from the theoretical value.
The repeatability of the method is 1.72%.

Using a similar procedure five different buttermilk samples (B-1, B-2, B-3, B-4 and B-5) have been analysed. Between four and six replicates for each samples were carried out. The mean values obtained for the five samples (table II) are ranging from 80 to 89%; these values are systematically lower than the expected s.m.p. contents (100%). It must be pointed out that similar results have been found by other laboratories (3) (10).
The sample B-4 is acid buttermilk. The repeatability of the method is 1.18%.
Ash contents obtained following AOAC method (12) and total protein contents for the investigated s.m.p. and buttermilk samples are reported in table III.
Total protein contents are lower in buttermilk samples, whereas ash contents are similar in both types of samples.
The fat contents in these buttermilk samples range from 6 to 8% (3).
Low contents of total proteins and incomplete dissolution of these samples in the citrate solution (2.2.1.) caused by the presence of the fat could lead to low results (3).

3.2. Testing of the method in presence of other substances (whey, starch, proteins)

Amongst the possible substitute products, the behaviour of the additions of whey, starch and various proteins to pure s.m.p. and buttermilk samples has been investigated in order to check the possible interferences in the analytical method for the determination of s.m.p.

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The influence of the whey has been studied by analysing the original sample of whey powder (C1) and various mixtures of the same whey powder with varying but known amounts of both s.m.p. or buttermilk. Deviations of about $\pm$ 1% rel.between the mean values obtained and the corresponding theoretical contents have been found when the whey powder C-1 is added up to 60% to s.m.p. samples or up to 50% to buttermilk samples (table IV). The theoretical s.m.p. contents of the various mixtures can be easily calculated taking into account the s.m.p. content of the samples (Table II).
The obtained repeatability is similar to that obtained operating on pure samples (table II).

In a similar way the behaviour of the starch addition has been investigated. Soluble starch normally used in the chemical laboratories as iodine indicator was employed.
For starch contents up to 30% the deviations of the mean values obtained for the various mixtures from their theoretical ones (table V) are always less than 1% relative. If the starch content exceeds 30% the results obtained are systematically lower (from 8 to 16% rel.)
In such cases the analytical procedure is difficult to be correctly applied because the solutions obtained are always cloudy and the filtration is extremely slow.

Proteins derived from hydrocarbons with the aid of bacteria (F-1) or yeast (G-1), maize proteins (H-1), potato proteins with a protein contents of 48,5% (D-1) or soya proteins with a protein contents of 75.6% (E-1) or (E-2) were employed in order to study their behaviour when added to s.m.p. or buttermilk samples.
In the case of maize or derived from hydrocarbons proteins added to s.m.p. samples even up to 60% deviations of ca 1% rel. of the mean values of the various mixtures from the theoretical values have been found.
In the case of potato or soya proteins the same behaviour was observed when their contents was not exceeding 20% (table VII). However when the contents of these proteins were increased to 30% the results obtained were considerably higher (table VII).
By applying the described procedure to the various proteins an appearent s.m.p. content ranging from 0.31% to 1.34% was

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found (table VI and VII) . Co-precipitation of non-milk proteins, particularly potato or soya proteins, could occur when the addition of these proteins are higher than 20%

3.3. Testing of the method on compound feedingstuffs

In order to test the method on compound feedingstuff samples seven different samples of unknown composition were replicated six times by the same operator on different days. The declared content of s.m.p. was notified to us subsequently. The results obtained are reported in tables VIII and X . The deviations between the mean values for each sample and the declared content range from + 0.16 to-1.35. The comparison by the t test (five degrees of freedom and a probability of 95%) shows that these deviations are not significant for six out of seven samples (table X). However for the anomalous sample (K-13) the deviation from the declared content is only 2.2% rel.
The repeatability of the method based on the results obtained for the seven samples is 1.40%.

The additions of soya protein (E-2) or whey up to 20% or soluble starch up to 10% do not seem to interfere practically. Indeed the deviations of the theoretical contents of s.m.p. of these mixtures based on the declared values (table IX)for the two feedingstuffs employed from the analytical values are less than $\pm$ 1% rel.

IV. CONCLUSIONS

The proposed analytical method for the determination of skimmed milk powder ,based on the enzimatic coagulation of para-casein (10) ,has been applied to samples of pure s.m.p.,buttermilk and compound feedingstuffs. Possible interferences due to the presence of the substitute products as whey, starch and proteins have been investigated.
With regard to the accuracy and repeatability of the method the following results have been obtained :

The mean values obtained on four different samples of pure skimmed milk powder, each analysed ten times, are not signifi-

cantly different from their theoretical values (100%), the repeatability being 1.72%.

The mean values obtained (80-89%) on five different samples of pure buttermilk are distinctly lower than the expected theoretical value (100%).
Total proteins are lower (30-34% than those of the s.m.p. samples analysed (36.6-36.8%). The proposed analytical method could be furtherly developed to be applied to buttermilk samples.

The mean values obtained on seven different compound feedingstuffs each analysed six times are not, six out seven significantly different from their declared contents, the repeatability being 1.40%.

With regard to the possible interferences due to the addition of other products, the following points must be stressed :

The additions up to 50% of whey powder or up to 20% of soluble starch or various proteins (maize,potato, soya, hydrocarbon) to samples of pure s.m.p. or buttermilk do not cause practical interferences. Relevant positive interferences have been noted if quantities higher than 50% are added.

In the case of compound feedingstuffs the additions of soya proteins or whey up to 20% or of soluble starch up to 10% do not appear to cause practical interferences.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Prof. V.SADINI for his advices , suggestions and for the helpful discussions of this work.

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TABLE I

REPEATABILITY TEST APPLIED TO SKIMMED MILK POWDER SAMPLES

SAMPLE	SKIMMED MILK CONTENT %	AVERAGE	STANDARD DEVIATION
A-1	98.2-97.6-97.7-97.8-100.8 102.3-99.7-101.5-99.4-102.0	99.70	1.85
A-2	101.0-99.7-97.6-100.3-102.8 101.4-97.3-102.3-98.8-101.4	100.26	1.89
A-4	100.0-99.8-102.1-98.2-100.2 98.4-101.6-99.0-102.1-98.4	99.98	1.52
A-5	98.0-99.9-101.6-100.2-98.6 102.1-100.6-98.4-98.4-101.6	99.94	1.59

TABLE II

REPEATABILITY TEST APPLIED TO BUTTERMILK POWDER SAMPLES

SAMPLE	SKIMMED MILK CONTENT %	AVERAGE	STANDARD DEVIATION
B-1	87.0-88.0-88.0-86.8	87.45	0.64
B-2	86.0-88.0-84.6-85.6	86.05	1.43
B-3	90.6-88.8-88.2-89.6	89.30	1.04
B-4	79.8-80.5-81.1-81.0-79.7 81.4-79.4	80.41	0.79
B-5	85.2-85.2-86.7-84.2-84.8 84.3-86.4	85.26	0.97

TABLE III

ASH AND TOTAL PROTEINS IN SAMPLES OF SKIMMED MILK AND BUTTERMILK

SAMPLE	ASH (%)	N (%)	TOTAL PROTEINS (6.38 x %N) (%)
A-1	7.25	5.73	36.6
A-2	7.44	5.76	36.7
A-4	7.35	5.77	36.8
A-5	7.40	5.73	36.6
B-1	6.90	5.02	32.0
B-2	9.30	5.12	32.7
B-3	7.90	5.37	34.3
B-4	6.41	4.66	29.7
B-5	7.44	4.75	30.3

TABLE IV

EFFECT OF ADDITION OF POWDERED WHEY TO SAMPLES OF SKIMMED MILK AND BUTTERMILK POWDER

SAMPLES	SKIMMED MILK CONTENT (%)	AVERAGE	STANDARD DEVIATION
WHEY C1	0.05–0.12–0.00–0.23–0.12–0.00–0.12–0.19	0.10	0.08
40%A_1+60%C_1	38.4–39.7–38.4–39.9–40.4–40.8–39.8–40.0	39.68	0.86
60%A_1+40%C_1	57.4–59.0–60.0–58.4–59.9–60.1–61.2–60.0	59.50	1.18
40%A_2+60%C_1	39.7–40.0–41.0–39.8–39.0	39.90	0.72
60%A_2+40%C_1	60.5–59.8–60.0–58.9–59.4	59.72	0.61
70%A_2+30%C_1	68.3–69.3–69.9–69.1	69.15	0.66
50%A_4+50%C_1	50.9–50.2–51.0–49.8	50.48	0.57
60%A_4+40%C_1	62.0–60.0–60.0–61.0	60.75	0.96
50%A_5+50%C_1	51.6–50.0–49.8–51.4	50.70	0.93
60%A_5+40%C_1	61.0–59.5–62.5–62.5	61.38	1.44
50%B_1+50%C_1	43.5–43.0–42.5–44.0	43.25	0.65
60%B_1+40%C_1	53.0–52.6–52.3–53.4	52.83	0.48
50%B_2+50%C_1	42.5–42.1–42.0–43.6	42.80	0.70
50%B_3+50%C_1	45.0–46.1–45.8–44.0	45.82	0.94

TABLE V

EFFECT OF ADDITION OF STARCH TO SAMPLES OF SKIMMED MILK POWDER

SAMPLE	SKIMMED MILK CONTENT (%)	AVERAGE	STANDARD DEVIATION
A_2 95%+ Starch 5%	95.0-94.1-96.8-95.1	95.25	1.13
A_2 90%+ Starch 10%	88.8-90.2-89.1-91.3	89.85	1.14
A_4 40%+ Starch 20% + B1 40%	75.2-76.5-76.0-75.2	75.72	0.64
A_4 70%+ Starch 30%	70.0-71.5-70.5-71.0	70.75	0.65
A_5 70%+ Starch 30%	69.8-69.5-70.5-70.8	70.15	0.60
A_4 60%+ Starch 40%	56.3-54.4-55.5	55.40	—
A_5 60%+ Starch 40%	54.8-53.2-52.0	53.30	—
A_4 50%+ Starch 50%	41.6-42.6-42.2	42.13	—
A_5 50%+ Starch 50%	42.6-42.9-44.7	43.40	—

TABLE VI

EFFECT OF ADDITION OF ALKANE -BASED PROTEINS (F-1)AND (G-1) AND MAIZE PROTEINS (H-1) TO SAMPLES OF SKIMMED MILK POWDER

SAMPLE	SKIMMED MILK CONTENT %	AVERAGE	STANDARD DEVIATION
F-1	0.75-0.55-0.85-0.90-0.90	0.79	0.15
G-1	0.40-0.40-0.30-0.20-0.25	0.31	0.09
H-1	0.55-0.60-0.65-0.70-0.80	0.66	0.096
60%A_2-40%F-1	61.5-62.8-61.0-60.0-59.5-61.1	60.98	1.16
60%A_2-40%G-1	61.8-60.8-61.5-60.0-59.5-59.8	60.57	0.97
60%A_2-40%H-1	60.8-60.8-59.5-62.0-61.5-69.0	60.60	1.15
40%A_2-60%F-1	40.0-40.2-41.0-39.5-39.2-41.0	40.15	0.75
40%A_2-60%G-1	41.0-40.7-39.5-39.0-40.0-41.0	40.20	0.84
40%A_2-60%H-1	42.3-42.5-40.0-39.5-40.5-41.0	40.97	1.22

TABLE VII

EFFECT OF ADDITION OF POTATO PROTEINS (D-1) and SOYA PROTEINS (E-1) and (E-2) TO SAMPLES OF SKIMMED MILK OR BUTTERMILK POWDER

SAMPLE	SKIMMED MILK CONTENT (%)	AVERAGE	STANDARD DEVIATION
D-1	1.10-1.07-1.15-1.20-1.30-1.25	1.18	0.09
E-1	0.93-1.07-1.30-1.24-1.07-1.00	1.10	0.14
E-2	1.20-1.60-1.20-1.40-1.30-1.34	1.34	0.15
90%A_2+10% D-1	90.4-89.6-89.1-90.5-89.9	89.90	0.58
80%A_2+20% D-1	79.5-80.4-81.0-79.4-81.6	80.38	0.95
70%A_2+30% D-1	72.0-75.0-74.9-72.2-73.1	73.44	1.44
90%A_2+10% E-1	90.0-89.4-88.9-91.5-90.4	90.04	1.00
80%A_2+20% E-1	79.9-81.1-80.9-79.5-80.2	80.32	0.67
70%A_2+30% E-1	78.7-78.4-77.3-75.6-79.2	77.84	1.44
95%A_1+ 5% E-2	93.8-94.7-95.8-95.8-94.2	94.86	0.92
90%A_1+10% E-2	88.8-89.6-89.3-90.5-91.2	89.88	0.96
80%A_1+20% E-2	79.8-79.3-81.2-80.5-80.6	80.28	0.74
70%A_1+30% E-2	77.5-76.8-79.4-76.5-77.2	77.48	1.14
95%B_2+ 5% E-2	82.4-81.5-80.7-81.0	81.40	0.74
90%B_2+10% E-2	78.4-77.5-76.8-77.4	77.52	C.66
80%B_2+20% E-2	69.4-68.2-70.4-69.4	69.35	0.90

TABLE VIII

-FEEDINGSTUFFS ANALYSED - COMPARISON WITH DECLARED CONTENTS

SAMPLE	SKIMMED MILK (DECLARED CONTENT) %	SKIMMED MILK CONTENT %	AVERAGE	STANDARD DEVIATION
K-11	60.0	58.8-57.8-58.9-60.5-57.7-59.8	58.92	1.10
K-12	70.0	69.6-71.4-71.4-69.0-69.4-70.2	70.16	1.03
K-13	60.6	59.2-59.5-58.4-58.6-59.6-60.2	59.25	0.67
K-14	60.6	59.0-60.0-60.8-59.8-60.5-60.5	60.10	0.65
K-15	64.5	64.0-63.8-62.5-63.0-64.5-65.0	63.80	0.93
K-16	71.0	71.2-71.0-71.2-69.8-69.1-70.0	70.38	0.88
K-17	76.0	74.9-74.0-75.0-76.1-76.6-74.0	75.10	1.07

TABLE IX

-EFFECT OF ADDITION OF SOYA PROTEINS(E-2),POWDERED WHEY (C-1)TO FEEDINGSTUFFS

SAMPLE	SKIMMED MILK CONTENT %	AVERAGE	STANDARD DEVIATION
95%K16+5% E-2	67.8-67.9-66.2-66.1	67.00	0.98
90%K16+10%E-2	64.3-62.5-64.8-64.5	64.03	1.04
80%K16+20%E-2	56.8-56.2-57.9-58.2	57.27	0.94
95%K15+ 5%E-2	61.0-59.4-61.6-61.0	60.75	0.94
90%K15+10%E-2	57.6-57.4-60.0-58.0	58.25	1.19
80%K15+20%E-2	51.0-52.8-52.5-51.5	51.95	0.84
95%K15+ 5%C-1	61.0-60.0-60.5-61.0	60.62	0.48
90%K15+10%C-1	57.6-58.4-56.8-57.5	57.57	0.66
80%K15+20%C-1	50.5-51.8-51.2-52.1	51.40	0.71
95%K16+ 5% Starch	67.9-66.1-67.2-66.9	67.02	0.75
90%K16+10% Starch	64.6-63.5-63.0-63.0	63.52	0.75

TABLE X

REPEATABILITY AND DEVIATIONS OF THE MEAN VALUES OBTAINED FROM THE DECLARED CONTENTS

SAMPLE	SKIMMED MILK (DECLARED CONTENT) % (A)	SKIMMED MILK (MEAN VALUES) % (B)	NUMBER OF	REL.STAND.DEV.	DEVIATION (B-A)	PERCENT DEVIATION	t_{exper}
K-11	60.0	58.92	6	1.87	-1.08	-1.80%	2.40
K-12	70.0	70.16	6	1.47	+0.16	+0.23	0.38
K-13	60.6	59.25	6	1.13	-1.35	-2.23	4.93*
K-14	60.6	60.10	6	1.08	-0.50	-0.83	1.88
K-15	64.5	63.80	6	1.46	-0.70	-1.08	1.84
K-16	71.0	70.38	6	1.25	-0.62	-0.87	1.74
K-17	76.0	75.10	6	1.42	-0.90	-1.18	2.06
				1.40 (pooled value)			t_{theor}= 2.57

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