The importance of the catalyst and salt/acid ratio in Kjeldahl analysis

In the Nitrogen / Protein determination according the Kjeldahl method use of catalyst is essential for the result. The choice is often done according tradition, price, efficiency and influence on the environment. The most efficient catalyst, Mercury, nowadays is banned in almost all parts of the world due to its effect on the environment. Today Copper and to some extent also Selenium are used as catalyst. Both are available as tablets in different concentrations. The tablets make the addition of salt/catalyst easy and more accurate.

To find out if there is any difference between some of the most used tablets available on the market different samples were digested for times between 45 and 90 minutes. To keep the salt / acid ratio as close as possible two tablets and 12 ml Sulphuric Acid was used for this comparison. Please note that for the "Universal Copper" the salt amount used was 6.65g (one tablet of each size)

Catalyst Tablets

Order No.	Name	Weight	Composition
KT-211-A	0.3% Cu / "Missouri"	3.50 g	99.7% K_2SO_4 + 0.3% $CuSO_4 x 5H_20$
KT-220-A	9% Cu / "Universal Copper"	1.65 g	91% K_2SO_4 + 9% $CuSO_4 x 5H_20$
KT-230-A	9% Cu / "Universal Copper"	5.00 g	91% K_2SO_4 + 9% $CuSO_4 x 5H_20$
KT-240-A	Ti + Cu / "Titanium"	3.71 g	47.17% K_2SO_4 + 47.17% Na_2SO_4 + 2.83% $CuSO_4 x 5H_20$ + 2.83% TiO_2
KT-250-A	Se / "Selenium"	3.50 g	99.9% K_2SO_4 + 0.1% Se

Equipment

For digestion; 20 position KjelROC Auto Digestor with manual Rack, Exhaust and Scrubber

For distillation/titration and calculation; KjelROC Analyzer (Manufactured by Opsis LiquidLine in Sweden)

Samples

(ground and well homogenised) Wheat flour, Fish meal, Cat feed and Glycine

Digestion

Approximately one gram sample was weighed, the exact weight was recorded, (Glycine just ≈250mg) and put into dry test tubes. Kjeldahl tablets and 12ml Sulphuric Acid was added to each tube. To make sure the sample was wet by the acid the tube was swirled before placed into the Tube Rack. The samples were randomly placed in the Tube Rack to avoid any eventual influence from the built in heater and or the ventilation in the fume hood.

After placing the Exhaust above the tubes the Rack with all 20 test tubes was put into the pre-heated Digestor, +420°C. The Scrubber was adjusted to full suction during the first 10 minutes, thereafter reduced to a minimum during the remaining time. After completed digestion the Scrubber was adjusted to full suction just during that the Tube Rack was raised to the cooling position. The tubes were allowed to cool for approximately 10 minutes with the Exhaust still in place.

Distillation

The sample weights were entered into the KjelROC Analyzer and each tube was put in place. The liberated ammonia was collected and titrated with 0.2000N HCl in the Titration Vessel during the distillation. After analysis the test tube was automatically drained and the result presented on the Touch Screen. When the Rack, 20 tubes, was completed all results were transferred via WiFi to a PC.

For all tests the KjelROC was set to give;

30ml 1% Boric Acid as receiver solution, 70ml dilution water, 50ml 40% NaOH, Delay time 5 seconds and a distillation volume of 100 ml.

Result and Conclusion

Catalyst

Most results were stable already after 60 minutes, only Glycine, a common internal standard in many laboratories needed slightly longer time. This is often the case pure amino acids are more difficult to digest than “normal” samples. When samples needing different time for completion are mixed in the same Tube Rack the time has to be adjusted to the longest.

Table 1. shows the performance for each catalyst/sample type.

As can be seen there is no significant difference between the catalysts tested.

The choice can therefore be done according the preferences most important for each laboratory, price, environment etc.

0.3% Cu tablets most likely have the lowest environmental influence.

Salt / acid ratio

During the tests we noticed that there always was plenty of acid left after completed digestion. The reason is most likely the design of the fume removal system. Still it is efficient in removing evaporated gases it gives a nice reflux when the air flow is reduced. Table 2. shows that by reducing the Sulphuric Acid volume from 12 to 10 ml we got slightly higher results. The comparison was only done using the 0.3% Copper tablets.

A higher salt / acid ratio normally gives a higher result at the same digestion time as the boiling point is higher. All possible reductions of chemicals are welcome as long as it does not have a negative influence on the result. For dry samples as used in this study a well working digestion procedure is to use two 0.3% Cu tablets and just 10 ml H_2SO_4 at 420 during one hour.

It is important to consider that other samples e.g. if containing fat might need more acid. In practical work when mixing several sample types in the same rack the “most difficult sample” decides for acid volume and total time. A good thumb rule is that when raising the tube rack after completed digestion the acid/salt mixture has to be a liquid. After cooling it is no problem if the residue forms a soft crystal cake.

Table 1.
Results after 60 minutes digestion using
12 ml H_2SO_4 and ≈7g indicator/salt mixture

Sample	Catalyst	%N	std	% rel	max	min	n
Wheat flour	0.3% Cu	1.862	0.009	0.49	1.881	1.836	20
	9% Cu	1.890	0.009	0.48	1.906	1.870	12
	Ti+Cu	1.922	0.009	0.46	1.939	1.914	6
	Se	1.898	0.006	0.33	1.906	1.891	6
Fish meal	0.3% Cu	11.093	0.037	0.33	11.178	11.028	20
	9% Cu	11.102	0.103	0.39	11.170	10.790	11
	Ti+Cu	11.098	0.034	0.31	11.158	11.055	6
	Se	11.064	0.040	0.36	11.122	10.991	6
Cat feed	0.3% Cu	4.683	0.025	0.54	4.727	4,648	11
	9% Cu	4.741	0.027	0.56	4.783	4,694	12
	Ti+Cu	4.754	0.025	0.53	4.801	4,716	6
	Se	4.749	0.025	0.52	4.794	4,722	6
Glycine (18.66% N)	0.3% Cu	18.395	0.024	0.13	18.425	18.362	7
	9% Cu	18.510	0.106	0.57	18.643	18.348	4
	Ti+Cu	18.522	0.035	0.19	18.556	18.487	2
	Se	18.441	0.026	0.14	18.467	18.415	2

Table 2.
Results after 60 minutes digestion using
0.3% Cu and 10 or 12 ml H_2SO_4

Sample	ml acid	%N	std	% rel	max	min	n
Wheat flour	12	1.862	0.009	0.49	1.881	1.836	20
	10	1.903	0.012	0.61	1.925	1.882	12
Fish meal	12	11.046	0.060	0.54	11.133	10.950	20
	10	11.153	0.057	0.51	11.253	11.048	12
Cat feed	12	4.683	0.025	0.54	4.727	4.648	11
	10	4.733	0.036	0.75	4.781	4.651	11
Glycine (18.66% N)	12	18,395	0.024	0.13	18.425	18.362	7
	10	18.506	0.033	0.18	18.539	18.473	2

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