Gerhardt
Analytical Systems

CASE STUDY

How to surpass significantly the high demands of ISO 8968-1 for protein with a simple Kjeldahl method

Abstract

With the target to optimise the range of variation in Kjeldahl protein analysis of milk according to official norms like AOAC 991.20 or ISO 8968-1:2001, digestion conditions were varied to obtain lowest variation in protein content between duplicates (significantly below 0,038 % protein) with highest possible recovery .

It has been found that digesting for 2 hours at 390 °C with a KJELDATHERM® digestion block KBL20s gives very convincing results, especially in the range of variation the maximum allowed value of 0,038 % protein between duplicates was significantly lower, the maximum range could be even kept in series of 20(!) samples.

The only difference found compared to the mentioned official norms is the status of the sample after digestion. Although the norms describe a clear solution, we could obtain very good results with the parameters mentioned below, even with samples which were still dark after digestion. The quality of the results justify this small difference.

Recovery was checked with L-Tryptophane (AppliChem, purity min. 99 %). Average recovery found was 99,494 % with the described method below which is significantly higher as 98 % as demanded for L-Tryptophane by the norms.

Method Data and Results

Sample type:	Milk
Submission no.:	3590, 3667
Date:	20.09.-19.10.2012

Analytical details:

Chemicals

Water:	demineralised or distilled
Sulphuric acid:	H_2SO_4 98 % at least
Catalyst tablets:	Kjeltabs CX analog Kjelcat Cu
Caustic-Soda:	NaOH 32 %
Boric acid:	H_3BO_3 2 %

Standard Solution:

Hydrochloric acid: HCl c=0.1 mol/l **or**
Sulphuric acid: H_2SO_4 c=0.05 mol/l

All chemicals applied have p.a. grade.

Instruments

Analytical Balance (0.0001 g)
Kjeldahl Digestion Block KJELDATHERM® KBL20s, TURBOSOG-Scrubber
Distillation system VAPODEST® 50 s c

Sample preparation

Heat up the milk in a water bath of 40 °C to a temperature of 38 °C, stir up carefully and let cool down to room temperature. The initial sample weight of liquid samples is determined with a disposable syringe by differential weighing. Sulphuric acid is used to wash down any sample residue, which might remain at the glass walls.

Sample:	~4g
Catalyst:	2 Kjeltabs type CX or analog 2 Kjelcat Cu
H_2SO_4:	20 ml min. 98 %

Parameters KJELDATHERM® KBL 20 s:

Time [min]	Temperature [°C]	Remarks
	390	Samples are put into preheated block
120	390	After 2 hours let the samples cool down and analyse.

Foaming during the digestion has to be expected, however, the foaming should not exceed two-thirds of the glass. During the digestion black particles are washed back from the glass walls by condensing sulphuric acid. The sample should be usually translucent after digestion in order to obtain good results.
However, it could be very likely that individual samples have dark colour and/or debris particles after digestion step.

Practical experience has shown that it had no negative effect on the accuracy of results.
The results shown below contained some samples as well not being completely clear and translucent green,
but provided very good results.

Parameters VAPODEST® 50 sc:

H_2O Addition	~90 ml
NaOH Addition	~80 ml
Reaction Time	0 s
Distillation Time	240 s
Steam Power	100 %
Suction Sample	30 s
H_3BO_3 Addition	~80ml
Suction Receiver	30 s
Titration	C_{eq} = 0,1

RESULTS

Method	Series Position	Date	Sample	Weight [g]	Blank value [ml]	Consumption [ml]	N [%]	P [%]	Duplicate determination	
Milch	1	20.09.2012 13:39	3590	3,9856	0,1563	14,9352	0,5194	3,3137		
Milch	2	20.09.2012 13:45	3590	4,0618	0,1563	15,2158	0,5193	3,3133	0,0004	
Milch	3	20.09.2012 13:52	3590	3,9331	0,1563	14,7559	0,5199	3,3172		-0,0039
Milch	4	20.09.2012 13:58	3590	3,7237	0,1563	13,9711	0,5197	3,3154	0,0018	
Milch	5	20.09.2012 14:05	3590	3,8159	0,1563	14,2849	0,5186	3,3088		0,0066
Milch	6	20.09.2012 14:12	3590	3,9434	0,1563	14,8061	0,5204	3,3199	-0,0111	
Milch	7	20.09.2012 14:18	3590	4,2094	0,1563	15,7451	0,5187	3,3095		0,0104
Milch	8	20.09.2012 14:24	3590	4,0130	0,1563	15,0824	0,5210	3,3239	-0,0144	
Milch	9	20.09.2012 14:31	3590	3,7047	0,1563	13,8856	0,5191	3,3118		0,0121
Milch	10	20.09.2012 14:38	3590	4,1220	0,1563	15,4467	0,5196	3,3149	-0,0032	
Milch	11	20.09.2012 14:44	3590	4,2383	0,1563	15,9304	0,5213	3,3260		-0,0110
Milch	12	20.09.2012 14:50	3590	3,8869	0,1563	14,6354	0,5218	3,3289	-0,0030	
Milch	13	20.09.2012 14:57	3590	4,0947	0,1563	15,3363	0,5193	3,3129		0,0160
Milch	14	20.09.2012 15:03	3590	4,0010	0,1563	15,0150	0,5202	3,3188	-0,0058	
Milch	15	20.09.2012 15:10	3590	3,9217	0,1563	14,6804	0,5188	3,3097		0,0091
Milch	16	20.09.2012 15:16	3590	3,8691	0,1563	14,5212	0,5200	3,3179	-0,0082	
Milch	17	20.09.2012 15:23	3590	4,0271	0,1563	15,0520	0,5181	3,3055		0,0124
Milch	18	20.09.2012 15:30	3590	4,1710	0,1563	15,6061	0,5188	3,3102	-0,0047	
Milch	19	20.09.2012 15:36	3590	4,1738	0,1563	15,6212	0,5190	3,3112		-0,0010
Milch	20	20.09.2012 15:43	3590	4,2412	0,1563	15,8801	0,5193	3,3131	-0,0019	
						Maximum value	0,5218	3,3289		
						Average value	0,5196	3,3151		
						Minimum value	0,5181	3,3055		
						Range of Variation	0,0037	0,0235		

Method	Series Position	Date	Sample	Weight [g]	Blank value [ml]	Consumption [ml]	N [%]	P [%]	Duplicate determination	
Milk	1	21.09.2012 10:53	3590	4,1874	0,1560	15,5790	0,5159	3,2914		
Milk	2	21.09.2012 10:59	3590	5,3487	0,1560	19,9050	0,5172	3,2996	-0,0082	
Milk	3	21.09.2012 11:06	3590	4,2927	0,1560	16,0820	0,5197	3,3154		-0,0158
Milk	4	21.09.2012 11:12	3590	4,0159	0,1560	15,0300	0,5188	3,3099	0,0056	
Milk	5	21.09.2012 11:19	3590	3,7109	0,1560	13,8520	0,5170	3,2983		0,0116
Milk	6	21.09.2012 11:25	3590	4,1427	0,1560	15,4550	0,5173	3,3001	-0,0019	
Milk	7	21.09.2012 11:31	3590	3,9277	0,1560	14,6950	0,5185	3,3080		-0,0078
Milk	8	21.09.2012 11:37	3590	3,9669	0,1560	14,8390	0,5184	3,3076	0,0004	
Milk	9	21.09.2012 11:44	3590	3,7556	0,1560	14,0450	0,5180	3,3049		0,0026
Milk	10	21.09.2012 11:50	3590	3,8642	0,1560	14,4520	0,5182	3,3060	-0,0011	
Milk	11	21.09.2012 11:57	3590	4,2394	0,1560	15,9070	0,5204	3,3201		-0,0142
Milk	12	21.09.2012 12:03	3590	3,6609	0,1560	13,7220	0,5190	3,3114	0,0087	
Milk	13	21.09.2012 12:10	3590	3,8367	0,1560	14,3120	0,5168	3,2972		0,0142
Milk	14	21.09.2012 12:17	3590	3,9995	0,1560	14,9060	0,5166	3,2958	0,0014	
Milk	15	21.09.2012 12:23	3590	3,9099	0,1560	14,6550	0,5194	3,3139		-0,0182
Milk	16	21.09.2012 12:29	3590	3,8849	0,1560	14,5140	0,5177	3,3027	0,0113	
Milk	17	21.09.2012 12:36	3590	4,1005	0,1560	15,2740	0,5164	3,2946		0,0080
Milk	18	21.09.2012 12:43	3590	3,9932	0,1560	14,9290	0,5182	3,3061	-0,0115	
Milk	19	21.09.2012 13:12	3590	4,1403	0,1560	15,4080	0,5160	3,2920		0,0141
Milk	20	21.09.2012 13:19	3590	4,0088	0,1560	14,9780	0,5179	3,3041	-0,0121	
						Maximum value	0,5204	3,3201		
						Average value	0,5179	3,3040		
						Minimum value	0,5159	3,2914		
						Range of Variation	0,0045	0,0288		

Method	Series Position	Date	Sample	Weight [g]	Blank value [ml]	Consumption [ml]	N [%]	P [%]	Duplicate determination	
Milk	1	16.10.2012 13:07	3667	4,0717	0,2023	15,2790	0,5187	3,3090		
Milk	2	16.10.2012 13:16	3667	3,7255	0,2023	14,0800	0,5218	3,3289	-0,0199	
Milk	3	16.10.2012 13:23	3667	4,1337	0,2023	15,6018	0,5218	3,3292		-0,0003
Milk	4	16.10.2012 13:29	3667	4,1002	0,2023	15,4660	0,5214	3,3268	0,0024	
Milk	5	16.10.2012 13:36	3667	3,9186	0,2023	14,7417	0,5197	3,3158		0,0110
Milk	6	16.10.2012 13:42	3667	4,1053	0,2023	15,4515	0,5203	3,3195	-0,0037	
Milk	7	16.10.2012 13:49	3667	4,2131	0,2023	15,8274	0,5195	3,3143		0,0052
Milk	8	16.10.2012 13:56	3667	3,6923	0,2023	13,9341	0,5209	3,3235	-0,0092	
Milk	9	16.10.2012 14:02	3667	3,8203	0,2023	14,3501	0,5187	3,3095		0,0141
Milk	10	16.10.2012 14:09	3667	3,9688	0,2023	14,8952	0,5186	3,3084	0,0011	
Milk	11	16.10.2012 14:16	3667	4,0099	0,2023	15,0945	0,5202	3,3189		-0,0105
Milk	12	16.10.2012 14:22	3667	3,9603	0,2023	14,9183	0,5205	3,3207	-0,0018	
Milk	13	16.10.2012 14:29	3667	4,1726	0,2023	15,6498	0,5186	3,3084		0,0123
Milk	14	16.10.2012 14:35	3667	4,1243	0,2023	15,5270	0,5205	3,3205	-0,0121	
Milk	15	16.10.2012 14:42	3667	4,1194	0,2023	15,4634	0,5189	3,3107		0,0099
Milk	16	16.10.2012 14:49	3667	4,1454	0,2023	15,5674	0,5192	3,3123	-0,0017	
Milk	17	16.10.2012 14:56	3667	4,1413	0,2023	15,5274	0,5183	3,3070		0,0053
Milk	18	16.10.2012 15:02	3667	3,8252	0,2023	14,4547	0,5219	3,3297	-0,0227	
Milk	19	16.10.2012 15:09	3667	4,2073	0,2023	15,7720	0,5184	3,3071		0,0226
Milk	20	16.10.2012 15:16	3667	3,9366	0,2023	14,7825	0,5188	3,3098	-0,0028	
						Maximum value	0,5219	3,3297		
						Average value	0,5198	3,3165		
						Minimum value	0,5183	3,3070		
						Range of Variation	0,0036	0,0227		

Method	Series Position	Date	Sample	Weight [g]	Blank value [ml]	Consumption [ml]	N [%]	P [%]	Duplicate determination	
Milk	1	18.10.2012 12:11	3667	3,4133	0,2023	12,8085	0,5173	3,3005		
Milk	2	18.10.2012 12:25	3667	3,6963	0,2023	13,8524	0,5173	3,3002	0,0003	
Milk	3	18.10.2012 12:32	3667	3,5943	0,2023	13,5570	0,5204	3,3204		-0,0202
Milk	4	18.10.2012 12:39	3667	3,8219	0,2023	14,2929	0,5164	3,2947	0,0257	
Milk	5	18.10.2012 12:46	3667	3,6595	0,2023	13,7306	0,5178	3,3036		-0,0089
Milk	6	18.10.2012 12:53	3667	3,7314	0,2023	14,0334	0,5192	3,3125	-0,0089	
Milk	7	18.10.2012 13:00	3667	3,5626	0,2023	13,3612	0,5174	3,3008		0,0117
Milk	8	18.10.2012 13:07	3667	3,8268	0,2023	14,3613	0,5183	3,3065	-0,0056	
Milk	9	18.10.2012 13:15	3667	3,8050	0,2023	14,2601	0,5175	3,3016		0,0048
Milk	10	18.10.2012 13:21	3667	4,4999	0,2023	16,8589	0,5185	3,3079	-0,0062	
Milk	11	18.10.2012 13:28	3667	4,0541	0,2023	15,2076	0,5184	3,3076		0,0003
Milk	12	18.10.2012 13:35	3667	3,9354	0,2023	14,8308	0,5207	3,3218	-0,0142	
Milk	13	18.10.2012 13:42	3667	3,5699	0,2023	13,4644	0,5204	3,3199		0,0019
Milk	14	18.10.2012 13:48	3667	3,9336	0,2023	14,7559	0,5182	3,3063	0,0135	
Milk	15	18.10.2012 13:55	3667	3,9511	0,2023	14,8226	0,5183	3,3068		-0,0004
Milk	16	18.10.2012 14:02	3667	3,8191	0,2023	14,3456	0,5187	3,3095	-0,0027	
Milk	17	18.10.2012 14:09	3667	3,9122	0,2023	14,6109	0,5159	3,2913		0,0182
Milk	18	18.10.2012 14:16	3667	3,8360	0,2023	14,4045	0,5186	3,3086	-0,0173	
Milk	19	18.10.2012 14:22	3667	3,8617	0,2023	14,5230	0,5194	3,3140		-0,0054
Milk	20	18.10.2012 14:29	3667	3,9812	0,2023	14,9423	0,5186	3,3086	0,0054	
						Maximum value	0,5207	3,3218		
						Average value	0,5184	3,3071		
						Minimum value	0,5159	3,2913		
						Range of Variation	0,0048	0,0305		

Method	Series Position	Date	Sample	Weight [g]	Blank value [ml]	Consumption [ml]	N [%]	P [%]	Duplicate determination	
Milk	1	19.10.2012 12:13	3667	3,7384	0,2023	14,0532	0,5190	3,3110		
Milk	2	19.10.2012 12:19	3667	3,9010	0,2023	14,7057	0,5208	3,3225	-0,0115	
Milk	3	19.10.2012 12:26	3667	3,8669	0,2023	14,5834	0,5209	3,3235		-0,0010
Milk	4	19.10.2012 12:33	3667	3,8625	0,2023	14,5464	0,5202	3,3187	0,0048	
Milk	5	19.10.2012 12:40	3667	3,9185	0,2023	14,7356	0,5195	3,3144		0,0043
Milk	6	19.10.2012 12:47	3667	3,7295	0,2023	14,0657	0,5207	3,3219	-0,0075	
Milk	7	19.10.2012 12:53	3667	4,1781	0,2023	15,7384	0,5208	3,3230		-0,0011
Milk	8	19.10.2012 13:00	3667	4,0103	0,2023	15,1072	0,5206	3,3214	0,0016	
Milk	9	19.10.2012 13:07	3667	4,0094	0,2023	15,0517	0,5188	3,3097		0,0116
Milk	10	19.10.2012 13:14	3667	3,9677	0,2023	14,8945	0,5187	3,3091	0,0006	
Milk	11	19.10.2012 13:21	3667	3,9054	0,2023	14,7275	0,5210	3,3237		-0,0146
Milk	12	19.10.2012 13:28	3667	4,1788	0,2023	15,7528	0,5212	3,3255	-0,0018	
Milk	13	19.10.2012 13:34	3667	4,1184	0,2023	15,5028	0,5204	3,3200		0,0055
Milk	14	19.10.2012 13:59	3667	4,0040	0,2023	15,0695	0,5201	3,3182	0,0018	
Milk	15	19.10.2012 14:06	3667	4,0282	0,2023	15,1327	0,5192	3,3123		0,0059
Milk	16	19.10.2012 14:13	3667	4,1692	0,2023	15,6593	0,5193	3,3131	-0,0009	
Milk	17	19.10.2012 14:20	3667	3,9925	0,2023	15,0162	0,5197	3,3158		-0,0027
Milk	18	19.10.2012 14:27	3667	4,1946	0,2023	15,8242	0,5217	3,3282	-0,0124	
Milk	19	19.10.2012 14:33	3667	3,9430	0,2023	14,9214	0,5229	3,3360		-0,0078
Milk	20	19.10.2012 14:40	3667	4,2586	0,2023	16,0484	0,5212	3,3252	0,0107	
						Maximum value	0,5229	3,3360		
						Average value	0,5203	3,3197		
						Minimum value	0,5187	3,3091		
						Range of Variation	0,0042	0,0268		

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