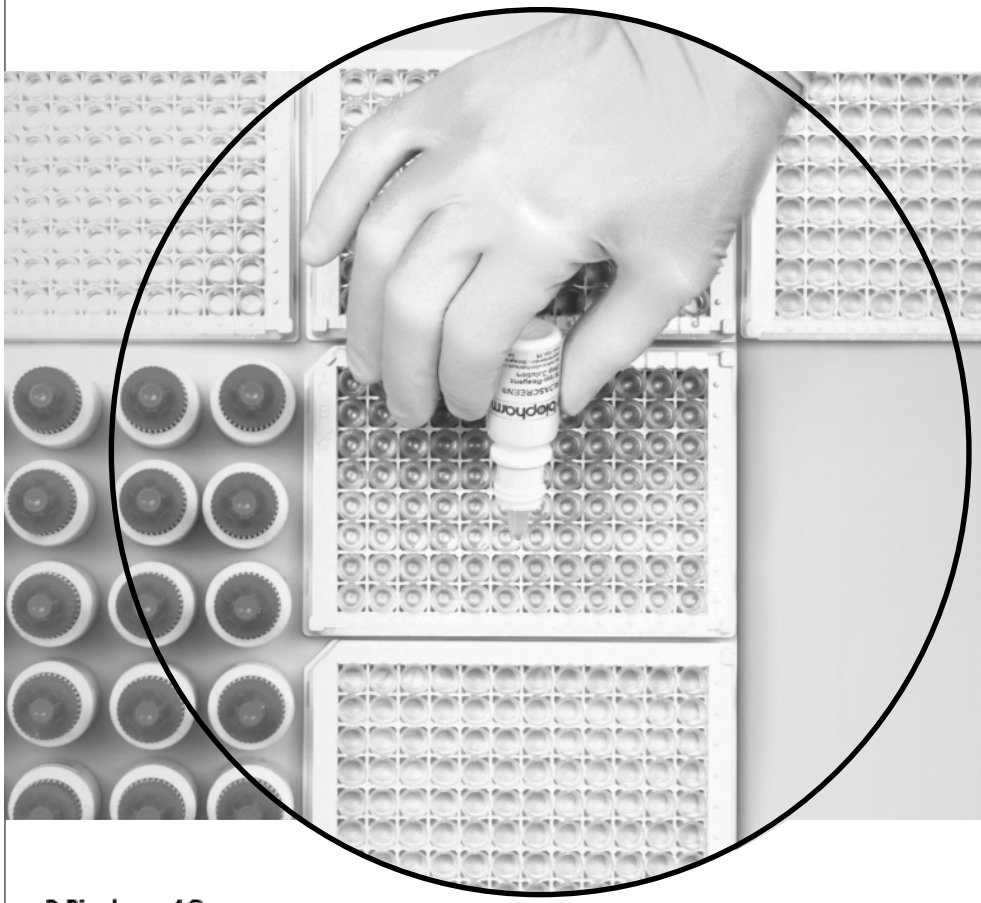

Sugars in Feeds

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Sugars

Determination of various sugars in feedstuffs by enzymatic analysis

Enzymes are biocatalysts in the metabolism of all living cells. This key function as specific catalysts makes enzymes especially suitable for the analysis of complex food materials. Enzymatic analysis is a valuable tool for identifying sugars, acids, alcohols and many other food constituents.

“Boehringer Mannheim Enzymatic BioAnalysis and Food Analysis” test kits contain selected and multi-tested reagents in appropriate quantities. The advantages of using test kits of this nature (i.e. ready-to-use reagents) include time saving aspects, their high degree of analytical safety (certification is on hand) and the use of safe reagents.

Sugars

10 feeds with unknown sugar content were analyzed for **Maltose,Sucrose, Glucose, Fructose, Lactose** and **Galactose.**

In order to provide data on all sugars, three distinct test kits were utilized.

1)Maltose/Sucrose/D-Glucose
2)Sucrose/D-Glucose/D-Fructose
3)Lactose/D-Galactose

A common extraction procedure was followed for all ten samples: Homogenize, deproteinate with Carrez reagents, adjust pH and dilute with water (1:10 or 1:100)

Sugars

Maltose Analysis

Principle

Maltose is hydrolyzed in the presence of the enzyme α -glucosidase (maltase) at pH 6.6 to two molecules of D-glucose

$$\text{Maltose} + H_2O \xrightarrow{\alpha\text{-glucosidase}} 2\ \text{D-glucose}$$

The enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) under simultaneous formation of adenosine-5'-diphosphate (ADP).

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

The formed D-glucose-6-phosphate (G-6-P) is oxidized by nicotinamideadenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate(NADPH).

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{D-gluconate-6-phophate} + \text{NADPH} + \text{H}^+$$

The amount of NADPH formed in this reaction is stoichiometric to the amount of sucrose, D-glucose and half the amount of maltose. The increase in NADPH is measured by means of its light absorbance at 334, 340 or 365 nm.

Maltose: Test Procedure

Pipette into cuvettes	Blank maltose sample	Maltose sample	Blank sucrose sample	Sucrose sample	Blank D-glucose sample	D-Glucose sample
solution 1*	0.200 ml	0.200 ml	-	-	-	-
solution 2*	-	-	0.200 ml	0.200 ml	-	-
sample solution**	-	0.100 ml	-	0.100 ml	-	0.100 ml
Mix*, and incubate for 20 min at 20-25°C. Add:						
solution 3	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
redist. water	1.800 ml	1.700 ml	1.800 ml	1.700 ml	2.000 ml	1.900 ml
Mix***, read absorbances of the solutions after approx. 3 min (A_1). Start reaction by addition of:						
suspension 4	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix***, wait for the completion of the reaction (10-15 min) and read absorbances of the solutions (A_2). If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbance increases constantly.						

Maltose: Calculating Results

$C_{maltose} = (5.169 \times \Delta A_{maltose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.120	0.155				
A	0.430	0.902	0.437	**3.6**	**0.36**	10
B	0.230	1.615	1.35	**11.1**	**1.11**	10
C	0.334	1.682	1.313	**10.8**	**1.08**	10
D	0.166	0.44	0.267	**21.9**	**2.19**	100
E	0.186	0.592	0.399	**32.7**	**3.27**	100
F	0.171	0.416	0.238	**19.5**	**1.95**	100
G	0.263	0.503	0.205	**1.7**	**0.17**	10
H	0.269	0.592	0.288	**2.4**	**0.24**	10
I	0.213	0.857	0.609	**5.0**	**0.50**	10
J	0.219	0.301	0.047	**0.4**	**0.04**	10

Sucrose

$C_{sucrose} = (10.34 \times \Delta A_{sucrose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.143	0.15	0.007			10
A	0.442	1.287	0.845	**13.8**	**1.38**	10
B	0.156	0.370	0.206	**33.8**	**3.38**	100
C	0.331	1.494	1.163	**19.0**	**1.90**	10
D	0.288	2.153	1.865	**30.5**	**3.05**	10
E	0.184	0.478	0.286	**46.9**	**4.69**	100
F	0.168	0.388	0.212	**34.8**	**3.48**	100
G	0.273	0.504	0.231	**3.7**	**0.37**	10
H	0.27	0.565	0.295	**4.7**	**0.47**	10
I	0.215	0.816	0.601	**9.7**	**0.97**	10
J	0.222	0.298	0.076	**1.1**	**0.11**	10

D-Glucose

$C_{glucose} = (5.441 \times \Delta A_{glucose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.140	0.145				10
A	0.270	0.764	0.489	**4.2**	**0.42**	10
B	0.227	0.727	0.495	**4.3**	**0.43**	10
C	0.328	0.687	0.354	**3.1**	**0.31**	10
D	0.280	1.254	0.969	**8.4**	**0.84**	10
E	0.546	1.073	0.522	**4.5**	**0.45**	10
F	0.331	1.028	0.692	**6.0**	**0.60**	10
G	0.289	0.342	0.048	**0.4**	**0.04**	10
H	0.300	0.356	0.051	**0.4**	**0.04**	10
I	0.210	0.254	0.039	**0.3**	**0.03**	10
J	0.218	0.230	0.007	**0.1**	**0.01**	10

D-Fructose

$C_{fructose} = (5.477 \times \Delta A_{fructose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	A3	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.139	0.139	0.139				
A	0.125	0.918	1.579	0.661	**5.7**	**0.57**	10
B	0.168	0.302	0.428	0.126	**10.9**	**1.09**	100
C	0.102	1.163	1.999	0.836	**7.3**	**0.73**	10
D	0.161	0.400	0.630	0.23	**20.0**	**2.00**	100
E	0.119	1.351	2.206	0.855	**7.4**	**0.74**	10
F	0.161	0.404	0.669	0.265	**23.0**	**2.30**	100
G	0.073	0.147	0.326	0.179	**1.6**	**0.16**	10
H	0.078	0.133	0.357	0.224	**1.9**	**0.19**	10
I	0.078	0.13	0.229	0.099	**0.9**	**0.09**	10
J	0.080	0.097	0.162	0.065	**0.6**	**0.06**	10

Lactose

$C_{lactose} = (11.3 \times \Delta A_{lactose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.083	0.085				10
A	0.076	0.128	0.05	**0.90**	**0.09**	10
B	0.036	0.116	0.078	**1.40**	**0.14**	10
C	0.030	0.107	0.075	**1.34**	**0.13**	10
D	0.062	0.224	0.16	**2.87**	**0.29**	10
E	0.058	0.119	0.059	**1.06**	**0.11**	10
F	0.059	0.101	0.04	**0.72**	**0.07**	10
G	0.013	0.02	0.005	**0.09**	**0.01**	10
H	0.009	0.029	0.018	**0.32**	**0.03**	10
I	0.015	0.023	0.006	**0.11**	**0.01**	10
J	0.027	0.032	0.003	**0.05**	**0.00**	10

D- Galactose

$C_{galactose} = (5.945 \times \Delta A_{galactose} \times \text{dilution factor}) \div \varepsilon$

Sample ID	A1	A2	ΔA	Result (g/kg)	Result (%)	Dilution
Blank	0.080	0.081				
A	0.081	0.119	0.037	**0.35**	**0.04**	10
B	0.034	0.107	0.072	**0.68**	**0.07**	10
C	0.023	0.090	0.066	**0.62**	**0.06**	10
D	0.052	0.214	0.161	**1.52**	**0.15**	100
E	0.059	0.116	0.056	**0.53**	**0.05**	100
F	0.059	0.100	0.040	**0.38**	**0.04**	100
G	0.005	0.010	0.004	**0.04**	**0.00**	10
H	0.006	0.009	0.002	**0.02**	**0.00**	10
I	0.018	0.021	0.002	**0.02**	**0.00**	10
J	0.018	0.024	0.005	**0.05**	**0.00**	10

Summary of Sugar Content in 10 Unknown Feed Samples

Sample ID	Sugar Content (g/kg)						Total Sugars	
	Maltose	Sucrose	Glucose	Lactose	Galactose	Fructose	(g/kg)	(%)
A	3.6	13.8	4.2	0.9	0.3	5.7	28.5	2.9
B	11.1	33.8	4.3	1.4	0.7	10.9	62.2	6.2
C	10.8	19.0	3.1	1.3	0.6	7.3	42.1	4.2
D	21.9	30.5	8.4	2.9	1.5	20.0	85.2	8.5
E	32.7	46.9	4.5	1.1	0.5	7.4	93.1	9.3
F	19.5	34.8	6.0	0.7	0.4	23.0	84.4	8.4
G	1.7	3.7	0.4	0.1	0.0	1.6	7.5	0.8
H	2.4	4.7	0.4	0.3	0.0	1.9	9.7	1.0
I	5.0	9.7	0.3	0.1	0.0	0.9	16.0	1.6
J	0.4	1.1	0.1	0.1	0.0	0.6	2.3	0.2