

COMPLEX METHOD FOR NUTRITION LABELING OF BEER BEVERAGES

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AIM OF STUDY

- Recapitulation of list of macronutrients requiring nutrition labeling of beer.
- Review of current methods for determination of macronutrients and EV of beer.
- Development of a new method for total carbohydrate in beer determination.
- Modification of a method for EV determination in non-alcoholic beer and beer mix.

METHODS and EXPERIMENTS

- **Proteins** Method EBC 9.9.1.
- **Alcohol** Method EBC 9.26
- **Extract** Method EBC 9.4
- **EV** Method EBC 9.45
- **Carbohydrate** see results
- **EV_{modif}** see results
- **Sugars** see results
- **Glycerol** see results

INTRODUCTION – WHY new methods?

Definition of selected nutrients and EV according to Regulation (EU) Number 1169/2011 in general

- Carbohydrates** means any carbohydrate which is metabolized by humans, and includes polyols
- Sugars** means all monosaccharides and disaccharides present in food, but excludes polyols
- Polyols** means alcohols containing more than two hydroxyl groups
- Proteins** means the protein content calculated using the formula: protein = total Kjeldahl nitrogen × 6.25
- Fat** means total lipids, and includes phospholipids
- Salt** means the salt equivalent content calculated using the formula: salt = sodium × 2.5

EV = sum of all nutrients multiplied by the respective conversion factors

Nutrients and EV according in beer

- Carbohydrates** oligomers and polymers of glucose with degree of polymerization DP>2
- Sugars** glucose, maltose (fructose)
- Polyols** glycerol
- Proteins** protein = total Kjeldahl nitrogen × 6.25
- Fat** fatty acids are neglected
- Salt** salt = sodium × 2.5
- EV_{calculating}** **EV (kJ/100 ml) = (A × 29) + (C × 17) + (P × 17) + (G × 10)**
or **EV (kcal/100 ml) = (A × 7) + (C × 4) + (P × 4) + (G × 2.4)**
- EV_{direct}** **EV (kJ/100 ml) = ρ (A × 29) + (Er × 15)**

EU 1169/2011

EBC 9.45

A - alcohol, C - carbohydrates without polyols, P - proteins, G - glycerol, Er - real extract, ρ - density

Why is a current method for total carbohydrates content in beer insufficient?

- The fermentable carbohydrates are simply measured by an official EBC 9.27 method with refractometric detection (RI) using Aminex column in Ca²⁺ form and MEBAK 2.7.2, where packed column Aminex HPX-87 and RI detector are used.
- However, this method is not suitable for determination of carbohydrates with higher polymerization degree (DP4 – DP10 and higher).
- The total content of carbohydrates in beer is at present determined by EBC 9.26 and MEBAK 2.7.3, based on the hydrolysis and dehydration of carbohydrates with 85 % v/v sulfuric acid, result in the formation of 5-hydroxymethylfurfural, which reacts with anthrone to produce a blue-green color measured at 625 nm.
- These methods do not meet the requirements of the Regulation (EU) 117/2010, which requires the enzymatic conversion of polymers and oligomers of carbohydrates to glucose using amylase or amyloglucosidase followed by a high-performance liquid chromatography (HPLC) determination.

What is the weakness of a current direct method EBC 9.45 for a total energy of beer?

Contribution of components to the total EV of beer

Comparison of EU 1169 and EBC 9.45			
Type of beer	EV dir kJ/100 mL	EV calc kJ/100 mL	Δ %
Lager 1	144	147	2.0
Lager 2	181	186	2.6
Non alc 1	64	73	12.4
Non alc 2	60	67	10.8
Beer mix 1	128	145	11.5
Beer mix 2	118	134	11.8

➤ EBC method 9.45, which is designed for the EV determination in beer, uses a simplified alternative, where an estimated EV can be calculated from the alcohol and real extract of the beer.

➤ However, this method does not provide accurate results for some types of beer (e.g., nonalcoholic beer or low-alcoholic beer mix manufactured by mixing beer and sweetened soft drinks). It is caused by the fact that in this type of beer there is a different proportion of alcohol and carbohydrates comparing with lager or ale resulting in inconvenient conversion factor of extract "15".

RESULTS AND DISCUSSION

ENZYMATIC CLEAVAGE

- degassing of beer sample (25 mL), temperation (15 min, 60 °C)
- addition of 1.2 mL of enzyme solution (0.12 g of amyloglucosidase in 10 mL of deionized water)
- incubation of the mixture for 120 min at 60 °C.
- inactivation of enzyme (15 min incubation at 85 °C)
- cooling of the sample, centrifugation (15 min, 10,000 rpm, 20 °C)

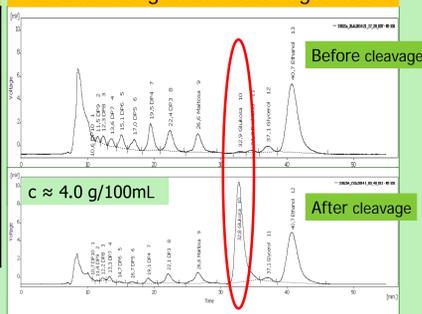
Recovery of the method

Alcohol (% vol.)	Recovery (%)
0 – 0.5 %	100
0.5 – 1 %	98
1 – 2 %	97
2 – 4 %	95
4 – 6 %	92
6 – 10 %	90

Profile of sugars before and after cleavage

Ethanol addition	Original samples		Cleaved samples	
	0% g/100 mL	5 % g/100mL	0% g/100 mL	5% g/100 mL
Fructose	0.04	0.04	0.06	0.04
Glucose	0.01	0.01	3.84	3.58
Maltose	1.97	1.97	0.10	0.14
DP3	0.61	0.61	0.04	0.06
DP4	0.19	0.19	0.01	0.02
...
DP10	0.01	0.01	< 0.01	< 0.01
Sum	3.07	3.05	4.08	3.87

HPLC-RI of original and cleaved beer



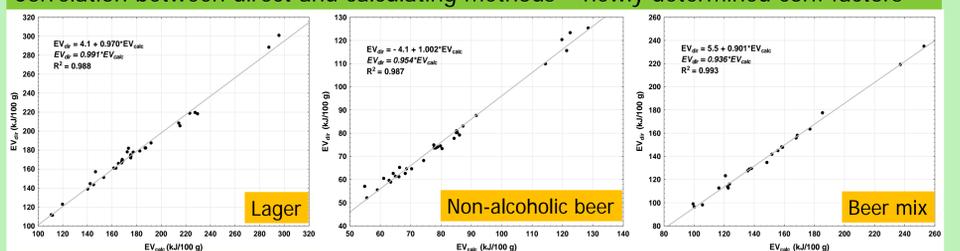
HPLC CONDITIONS
 Rezex RSO-Oligosaccharide ion exchange column in Ag+ mode (200×10 mm; Phenomenex, USA); MF: deionized water (isocratic); flow rate: 0.3 mL/min; column temperature: 80 °C.

Correlation between total carbohydrates C_{tot} and real extract Er

Mean (n = 15)	Er	C _{tot} (g/100 mL)	Rel. diff. (%)	C _{tot} /Er (%)
Nonalcoholic	4.27	3.70	4.5	95.5
Alcoholic	4.58	4.21	13.5	86.5

- More than 25% of polysaccharides DP>10.
- A good correlation between total carbohydrates and real extract.
- Dependence of enzyme activity on alcohol concentration.
- The method was suggested to EBC as a reference method.

Correlation between direct and calculating methods – newly determined corr. factors



$EV_{dir} = \rho \times (A \times 29 + Er \times 15.2)$ $EV_{dir} = \rho \times (A \times 29 + Es \times 15.9)$ $EV_{dir} = \rho \times (A \times 29 + Es \times 16.5)$

Results of Statistical Analysis	Lager	Non-alcoholic beer	Beer mix
Paired t-test (EV _{calc} vs. EV _{dir}) before modification mean difference (kJ/100 mL)	-1.3	-3.9	-9.2
Standard error of mean difference (kJ/100 mL)	4.6	4.9	4.8
Paired t-test (EV _{calc} vs. EV _{dir}) after modification mean difference (kJ/100 mL)	-0.4	0.3	0.0
Standard error of mean difference (kJ/100 mL)	4.4	2.7	3.7

- The direct EBC 9.45 method based on simply measurement of extract and alcohol is less expensive and time consuming than calculating method EU 1169/2011.
- EBC 9.45 is correct only for lagers, ales... (~ 0.75% of alcohol and 25% of carbohydrates).
- When a new correction factors are used, the agreement with calculation method is excellent also for the other types of beer.

References:

- [1] Olšovská J., Štěřba K., Pavlovič M., Čejka P.: Determination of the Energy Value of Beer. J. Am. Soc. Brew. Chem. 73(2):165-169, 2015.
- [2] Jurková M., Čejka P., Štěřba K., Olšovská J.: Determination of Total Carbohydrate Content in Beer Using Its Pre-column Enzymatic Cleavage and HPLC-RI. Food Anal. Methods 7:1677–1686, 2014.



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