

# Table of Contents

- Preface to the New Edition..... 3**
- Information about the MEBAK Website..... 4**
- Contributors ..... 5**
- List of Abbreviations..... 7**
- Table of Contents ..... 9**
- 1            Monitoring Brewhouse Operations ..... 19**
- 1.1            Monitoring Brewery Grist..... 19**
- 1.1.1        Grist Composition..... 19
- 1.1.2        Husk Volume..... 22
- 1.2            Mashing..... 23**
- 1.2.1        Mashing Intensity..... 23
- 1.2.2        Visual Iodine Test..... 24
- 1.3            Turbidity Measurement during Lautering..... 26**
- 1.4            Spent Grains..... 28**
- 1.4.1        Sample Collection..... 28
- 1.4.2        Moisture Content (EBC) ..... 29
- 1.4.3        Soluble Extract..... 30
- 1.4.3.1      Soluble Extract in Wet Spent Grains Obtained  
By Pressing (Rapid Method)..... 30
- 1.4.3.2      Soluble Extract in Wet and Dry Spent Grains  
Obtained By Rinsing (EBC)..... 31
- 1.4.4        Available Residual Extract..... 32
- 1.4.4.1      Available Residual Extract (EBC)..... 32
- 1.4.4.2      Available Residual Extract (Diastase)..... 34
- 1.4.5        Iodine Value of Brewery Spent Grains..... 36
- 1.4.6        Calculation of Extract in Spent Grains as Extract in Malt..... 39
- 1.5            Brewhouse and Extract Yield ..... 41**
- 1.5.1        Brewhouse Yield ..... 41
- 1.5.2        Cold Wort Yield..... 42
- 1.5.3        Fermentation Cellar Yield..... 42

<b>1.6</b>	<b>Solids</b> .....	<b>44</b>
1.6.1	Solids in Wort (Labor Veritas Method).....	44
1.6.2	Solids or Trub Material (Field Method).....	45
1.6.3	Cold Trub.....	47
<b>2</b>	<b>Wort and Beer</b> .....	<b>51</b>
<b>2.1</b>	<b>Sample Collection</b> .....	<b>51</b>
<b>2.2</b>	<b>Odor and Flavor of Wort</b> .....	<b>52</b>
<b>2.3</b>	<b>Photometric Iodine Test</b> .....	<b>53</b>
<b>2.4</b>	<b>Thiobarbituric Acid Index (TBI)</b> .....	<b>56</b>
<b>2.5</b>	<b>High Molecular Weight <math>\beta</math>-Glucan</b> .....	<b>59</b>
2.5.1	Enzymatic Method.....	59
2.5.2	Fluorimetric Method (EBC).....	64
2.5.3	Modified Fluorimetric Method (HPLC).....	68
2.5.4	Colorimetric Method (EBC).....	70
2.5.5	Determination of $\beta$ -Glucan Gel.....	72
<b>2.6</b>	<b>Nitrogenous Substances</b> .....	<b>73</b>
2.6.1	Total Nitrogen.....	73
2.6.1.1	KJELDAHL Method (EBC).....	73
2.6.1.2	DUMAS Combustion Method (EBC).....	76
2.6.2	Coagulable Nitrogen (Thermal Coagulation of Protein).....	78
2.6.3	Nitrogen Fractionation.....	81
2.6.3.1	Precipitation with Magnesium Sulfate.....	81
2.6.3.2	Precipitation with Phosphomolybdic Acid.....	83
2.6.4	Low Molecular Weight Nitrogenous Substances.....	86
2.6.4.1	Free Amino Nitrogen (FAN).....	86
2.6.4.1.1	Ninhydrin Method (Photometric Method, EBC).....	86
2.6.4.1.2	Determination of Amino Acids in Wort and Beer.....	89
2.6.4.1.3	Amines in Wort and Beer.....	95
2.6.4.2	Nitrosamines in Wort and Beer.....	101
2.6.5	Prolamins in Beer (ELISA Method).....	106

<b>2.7</b>	<b>Carbohydrates</b> .....	<b>110</b>
2.7.1	Sugar Spectrum.....	110
2.7.2	Fermentable Carbohydrates (EBC).....	113
2.7.3	Total Carbohydrates.....	117
2.7.4	Determination of Carbohydrates – Enzymatic Method.....	119
2.7.4.1	Glucose and Fructose.....	121
2.7.4.2	Sucrose.....	123
2.7.4.3	Maltose (and Maltotriose).....	124
2.7.4.4	Starch.....	125
2.7.5	Sugar Analysis with HPAEC-PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection).....	127
<b>2.8</b>	<b>Degree of Attenuation</b> .....	<b>131</b>
2.8.1	Limit of Attenuation in Wort (Fermentation Tube Method).....	131
2.8.2	Limit of Attenuation in Wort and Beer (Reference Method – EBC).....	134
2.8.3	Limit of Attenuation in Wort and Beer (Rapid Method – EBC).....	136
2.8.4	Attenuation of Maturing Beer and Finished Beer.....	137
<b>2.9</b>	<b>Original Gravity and Alcohol</b> .....	<b>139</b>
2.9.1	Degassing a Sample (EBC).....	140
2.9.2	Density Measurement.....	141
2.9.2.1	Pycnometer.....	141
2.9.2.2	Hydrometer.....	143
2.9.2.3	Oscillating U-tube Density Measurement (EBC).....	144
2.9.3	Apparent Extract (EBC).....	145
2.9.4	Distillation Analysis (Reference Method – EBC).....	146
2.9.5	Refractometric Analysis.....	150
2.9.6	Multifunctional Devices.....	153
2.9.6.1	Oscillating U-tube and Sound Velocity Measurements.....	154
2.9.6.2	Oscillating U-tube Density and Alcohol Content Measurements.....	154
2.9.6.3	Oscillating U-tube Density and NIR Measurements.....	155
2.9.6.4	Thermal Analysis Method – Fermento-Star.....	157
2.9.7	Alcohol (Ethanol).....	158
2.9.7.1	Enzymatic Analysis (EBC).....	158
2.9.7.2	Ethanol (Chromatographic).....	160

<b>2.10</b>	<b>Nutritional Analyses</b> .....	<b>161</b>
2.10.1	Energy Value or Caloric Value.....	161
2.10.2	Measuring Osmolality with an Osmometer.....	162
2.10.3	Recommended Analysis for Highly Attenuated Beer (Formerly, Analysis of Dietetic Beer).....	164
2.10.3.1	General Analyses.....	166
2.10.3.2	Determination of Carbohydrate Content Based on Nutritional Value (Total Glucose).....	167
2.10.3.2.1	Hydrolysis.....	167
2.10.3.2.2	Enzymatic Determination of Glucose.....	168
2.10.3.3	Fermentable Extract.....	168
2.10.3.4	Calculation of Dextrin Content.....	169
2.10.3.5	Calculation of Carbohydrate Content.....	170
2.10.3.6	Calculation of Utilizable Carbohydrates.....	170
2.10.3.7	Energy Value or Caloric Value.....	170
2.10.3.8	Bread Units.....	170
2.10.4	“Big Four” or “Big Eight” Declaration of Nutrition Information for Beverages.....	171
<b>2.11</b>	<b>Determination of Vitamin B<sub>1</sub> and B<sub>2</sub> in Wort and Beer</b> .....	<b>184</b>
<b>2.12</b>	<b>Color</b> .....	<b>189</b>
2.12.1	Visual Determination of Color by Comparison.....	189
2.12.2	Spectrophotometric Color (EBC).....	191
<b>2.13</b>	<b>pH (EBC)</b> .....	<b>194</b>
<b>2.14</b>	<b>Haze Formation (Protein Stability)</b> .....	<b>198</b>
2.14.1	Haze.....	198
2.14.1.1	Visual Method.....	198
2.14.1.2	Optical Method.....	199
2.14.2	Predicting Chemical-physical Stability (Protein Stability).....	201
2.14.2.1	Forced Aging.....	201
2.14.2.2	Alcohol Chill Haze Test, CHAPON (Cold Sensitivity).....	203
2.14.2.3	Formaldehyde Test.....	205
2.14.2.4	Precipitation with Ammonium Sulfate.....	207
2.14.2.5	Esbach Reaction Test.....	209

<b>2.15</b>	<b>Reduction Potential</b> .....	<b>211</b>
2.15.1	Spectrophotometric Method.....	211
2.15.2	Indicator Time Test (ITT).....	213
2.15.3	Endogenous Antioxidative Potential (EAP Value) and Radical Generation (T Value) in Beverages (ESR Spectroscopy).....	214
2.15.4	Characteristics of Malt and Malt Mixtures during the Process of Mashing-in (Tendency for Oxidation): Measurement of Oxidation Reactions while Mashing-in through Chemiluminescence.....	225
<b>2.16</b>	<b>Phenolic Compounds</b> .....	<b>229</b>
2.16.1	Total Polyphenols (EBC).....	229
2.16.2	Anthocyanogens, HARRIS and RICKETTS Method.....	232
2.16.3	Tannoids.....	234
2.16.4	Steam-volatile Phenols.....	237
<b>2.17</b>	<b>Bitter Substances</b> .....	<b>240</b>
2.17.1	Bittering Units (EBC).....	240
2.17.2	Iso- $\alpha$ -Acids and $\beta$ -Acids (Spectrophotometry).....	243
2.17.3	Determination of Hop Bitter Substances in Wort and Beer (EBC).....	245
2.17.4	Determination of Xanthohumol Using HPLC.....	249
<b>2.18</b>	<b>Foam</b> .....	<b>254</b>
2.18.1	Foam Stability, ROSS and CLARK.....	254
2.18.2	Foam Stability, NIBEM.....	257
2.18.3	Foam Stability, Lg-Foam Tester.....	262
2.18.4	Foam Stability, Steinfurth Foam Stability Tester.....	264
2.18.5	NIBEM Cling Meter (Haffmans).....	265
<b>2.19</b>	<b>Proof of Pasteurization</b> .....	<b>270</b>
<b>2.20</b>	<b>Filterability of Beer</b> .....	<b>273</b>
2.20.1	Membrane Filter Test (ESSER).....	273
2.20.2	Filterability Test (RAIBLE).....	277
<b>2.21</b>	<b>Fermentation By-Products (General)</b> .....	<b>280</b>
2.21.1	Highly Volatile Fermentation By-Products (Headspace Method).....	280

2.21.2	Fermentation By-Products.....	286
2.21.2.1	Fermentation By-Products (Distillation Method).....	286
2.21.2.2	Fermentation By-Products (Dichloromethane Extraction).....	290
2.21.3	Aromatic Alcohols and Phenolcarboxylic Acids.....	296
2.21.3.1	Aromatic Alcohols.....	296
2.21.3.2	Coumaric Acid and Ferulic Acid.....	300
2.21.3.3	4-Vinyl Guaiacol and 4-Vinyl Phenol (HPLC).....	303
2.21.4	Short Chain Fatty Acids (Distillation Method).....	307
2.21.5	Vicinal Diketones.....	311
2.21.5.1	Vicinal Diketones (Headspace Method).....	311
2.21.5.2	Vicinal Diketones (EBC).....	314
2.21.5.3	Vicinal Diketones (Distillation Method).....	319
2.21.5.4	3-Hydroxy-2-butanone (Acetoin) (Headspace Method).....	324
2.21.6	Higher Alcohols and Esters in Beer.....	326
2.21.7	Organic Acids.....	335
2.21.7.1	Enzymatic Determination of Organic Acids.....	335
2.21.7.1.1	Formic Acid (Formate).....	336
2.21.7.1.2	L-Malic Acid (Malate).....	337
2.21.7.1.3	Ascorbic acid (Ascorbate).....	338
2.21.7.1.4	Pyruvic Acid (Pyruvate).....	340
2.21.7.1.5	Citric Acid (Citrate).....	341
2.21.7.1.6	Acetic Acid (Acetate) (EBC).....	342
2.21.7.1.7	L-Lactic Acid (EBC)/D-Lactic Acid (Lactate).....	344
2.21.7.1.8	Oxalic Acid (Oxalate) and Formic Acid (Formate).....	346
2.21.7.2	Organic Acids in Beer and Upstream Intermediary Brewery Products, Non-alcoholic Beverages, Water und Wastewater Using Ion Chromatography.....	347
2.21.8	Sulfur Dioxide.....	350
2.21.8.1	Enzymatic Method.....	351
2.21.8.2	Distillation Method (EBC).....	353
2.21.8.3	Detection with Continuous Flow Rate Analysis (CFA).....	356
2.21.8.4	Ion Chromatographic Detection.....	360
<b>2.22</b>	<b>Anions.....</b>	<b>364</b>
2.22.1	Chloride, Sulfate, Nitrate and Phosphate in Beer (EBC).....	364
2.22.2	Bromide, Chloride, Fluoride, Nitrate, Nitrite, Oxalate, Phosphate and Sulfate in Water, Malt, Hops, Wort and Beer.....	367
2.22.3	Nitrate, Enzymatic Determination.....	371

2.22.4	Chloride .....	373
2.22.5	Sulfate .....	375
<b>2.23</b>	<b>Special Chromatographic Methods.....</b>	<b>377</b>
2.23.1	Dimethyl Sulfide and Its Precursor.....	377
2.23.1.1	Free DMS in Wort and Beer .....	377
2.23.1.2	DMS Precursors in Wort.....	380
2.23.2	Determination of Long-Chain Fatty Acids in Wort and Beer by Gas Chromatography.....	381
2.23.3	Hydroxymethylfurfural in Wort and Beer by HPLC .....	384
2.23.4	Determination of Aging Indicators in Beer.....	386
2.23.5	Determination of Steam-volatile Aroma Compounds in Wort.....	395
2.23.6	Determination of Steam-volatile Aroma Compounds in Beer.....	405
<b>2.24</b>	<b>Minerals.....</b>	<b>415</b>
2.24.1	Sodium (EBC).....	416
2.24.2	Potassium (EBC).....	419
2.24.3	Magnesium (EBC).....	422
2.24.4	Calcium (EBC).....	424
2.24.5	Manganese.....	427
2.24.6	Iron.....	429
2.24.6.1	Iron (EBC).....	430
2.24.6.2	Iron (Spectrophotometric Method – EBC).....	432
2.24.7	Nickel.....	435
2.24.7.1	Nickel (Graphite Furnace AAS).....	435
2.24.7.2	Nickel (EBC).....	438
2.24.8	Copper.....	440
2.24.8.1	Copper (EBC).....	440
2.24.8.2	Copper (ZDBT – EBC).....	443
2.24.8.3	Copper (Cuprethol Rapid Method – EBC).....	446
2.24.9	Zinc (EBC).....	447
2.24.10	Aluminum.....	450
2.24.11	Tin.....	452
2.24.12	Multi-element Determination Using Inductively Coupled Plasma Emission Spectrometry (ICP-OES) in Beer and Wort.....	454

<b>2.25</b>	<b>Viscosity (EBC) in Cast-out Wort and Beer</b> .....	<b>459</b>
2.25.1	HÖPPLER Falling Ball Viscometer .....	461
2.25.2	Rotational Viscometer.....	464
2.25.3	Capillary Viscometer.....	464
<b>2.26</b>	<b>Carbon Dioxide</b> .....	<b>469</b>
2.26.1	Manometric Method.....	469
2.26.1.1	Measuring Device for Carbon Dioxide in Tanks or Lines, Haffmans.....	470
2.26.1.2	Rapid Determination of Carbon Dioxide in Bottled Beer, STADLER and ZELLER.....	471
2.26.1.3	CO <sub>2</sub> Measuring Device, Steinfurth System.....	472
2.26.1.4	Carbon Dioxide in Bottled Beer, ZAHM and NAGEL .....	474
2.26.1.5	Carbon Dioxide in Packaged Beverages or At-line with CarboQC, Anton Paar.....	475
2.26.2	Carbon Dioxide in Beer in a Bottle or Tank, BLOM and LUND (Titrimetric Method).....	477
2.26.3	Orbisphere CO <sub>2</sub> Analyzer (Thermal Conductivity).....	481
2.26.4	CO <sub>2</sub> Determination Using a TOC Analyzer.....	482
<b>2.27</b>	<b>Nitrogen</b> .....	<b>485</b>
<b>2.28</b>	<b>Oxygen and Air</b> .....	<b>486</b>
2.28.1	Dissolved Oxygen.....	487
2.28.1.1	Electrometric Methods.....	487
2.28.1.1.1	Oxygen Determination with Clark Electrodes (Measurement of Electrical Current).....	487
2.28.1.1.2	Oxygen Determination, TÖDT and TESKE (Digox Analyzer).....	490
2.28.1.1.3	O <sub>2</sub> Measurement Device Orbisphere 3650 (Hach Lange).....	494
2.28.1.2	Oxygen Measurement with Optochemical Sensors.....	495
2.28.1.2.1	CO <sub>2</sub> /O <sub>2</sub> Meter, Model c-DGM and O <sub>2</sub> Meter, Model o-DGM (Haffmans).....	497
2.28.1.2.2	O <sub>2</sub> Measuring Device OxyQC (Anton Paar).....	498
2.28.1.2.3	O <sub>2</sub> Measurement Device 3110 (Hach Lange).....	500
2.28.2	Air in Headspace and Total Air in Bottles and Cans Oxygen in Headspace.....	501
2.28.2.1	Underwater Funnel Method.....	501
2.28.2.2	Underwater Funnel Method with Auxiliary Burette.....	504



2.28.2.3	Total Air in Bottles and Cans, ZAHM and NAGEL .....	506
2.28.2.4	Gases in Packaging .....	508
2.28.2.4.1	Inpack TPO/CO <sub>2</sub> Meter, Type c-TPO (Haffmans).....	508
2.28.2.4.2	Orbisphere 6110 Package Analyzer (Hach Lange).....	511
2.28.2.4.3	Package Analyzer Model 76060 (MecSens).....	513
2.28.3	Total Oxygen in Bottled and Canned Beer.....	515

### **3 Non-alcoholic Beverages and Beer-based Beverages, 518**

#### **3.1 Beer-based Beverages, 518**

3.1.1	Original Gravity and Alcohol.....	518
3.1.2	Color.....	518
3.1.3	pH.....	518
3.1.4	Turbidity.....	519
3.1.5	CO <sub>2</sub> Content.....	519

#### **3.2 Non-alcoholic Soft Drinks and Malt Beverages, 520**

3.2.1	Dry Matter (Extract) – Refractometric Determination.....	520
3.2.2	Carbohydrates.....	523
3.2.2.1	Sugars.....	523
3.2.2.1.1	Glucose, Fructose and Sucrose (Enzymatic Method).....	523
3.2.2.1.2	Glucose, Fructose and Sucrose (HPLC).....	523
3.2.2.1.3	Maltose (Enzymatic Method).....	523
3.2.2.2	Sugar Alcohols.....	524
3.2.2.2.1	D-Sorbitol (Enzymatic Method).....	524
3.2.2.2.2	Sorbitol (Ion Chromatography Method).....	526
3.2.3	Titrateable Acid.....	528
3.2.4	Volatile Acids.....	530
3.2.5	Organic Acids.....	534
3.2.5.1	Ion Chromatography Analysis.....	534
3.2.5.2	Enzymatic Analysis.....	535
3.2.5.2.1	L-Malic Acid.....	535
3.2.5.2.2	Citric Acid.....	535
3.2.5.2.3	DL-Lactic Acid.....	535
3.2.5.2.4	Total D-Isocitrate According to WALLRAUCH.....	536
3.2.5.2.5	D-Gluconate.....	538
3.2.6	Determination of Fruit Juice Content.....	540
3.2.7	Vitamins.....	542
3.2.7.1	Ascorbic Acid.....	543
3.2.7.1.1	Ascorbic Acid (HPLC).....	543

3.2.7.1.2	Ascorbic Acid (Enzymatic Method).....	545
3.2.7.1.3	Ascorbic Acid (Titrimetric Method), TANNER and BRUNNER.....	545
3.2.7.2	Carotenoids.....	547
3.2.7.2.1	Total Carotenoids (Photometric Method).....	547
3.2.7.2.2	Determination of Total Carotene and HPLC Analysis (Wesergold Rapid Method with Carrez Extraction).....	550
3.2.7.3	Niacin (HPLC).....	555
3.2.7.4	Vitamin B <sub>1</sub> (Microbiological Assay on Microtiter Plates).....	557
3.2.7.5	Vitamin B <sub>2</sub> (Riboflavin) (HPLC).....	558
3.2.7.6	Vitamin B <sub>6</sub> (Pyridoxine) (HPLC).....	560
3.2.7.7	Vitamin E (HPLC).....	562
3.2.7.8	Pantothenic Acid (Microbiological Assay on Microtiter Plates).....	564
3.2.7.9	Folic Acid (Microbiological Assay on Microtiter Plates).....	565
3.2.8	Taurine (IC Method).....	566
3.2.9	Glucuronolactone (IC Method).....	568
3.2.10	Caffeine and Theobromine (HPLC).....	571
3.2.11	Quinine (HPLC).....	574
3.2.12	Sweeteners.....	576
3.2.12.1	Aspartame, Acesulfame and Saccharin (HPLC).....	576
3.2.12.2	Neohesperidin Dihydrochalcone (HPLC).....	579
3.2.12.3	Cyclamate (Photometric Method).....	581
3.2.13	Hydroxymethylfurfural (HMF).....	583
3.2.13.1	Hydroxymethylfurfural (HPLC).....	584
3.2.13.2	Hydroxymethylfurfural (Photometric Method).....	586
3.2.14	Preservatives.....	588
3.2.14.1	Benzoic Acid and Sorbic Acid (HPLC).....	588
3.2.14.2	Total Sulfurous Acid (Distillation Method).....	592
3.2.15	Dimethyl Dicarbonate (DMDC) – Dosage Determination.....	593
The Fundamentals of Statistics.....		mebak.org
Index.....		594
List of Advertisers.....		602