

Contents

Chapter 1 Crop and Steviol Glycoside Improvement in Stevia by Breeding	1
<i>S. Tavarini, B. Passera and L. G. Angelini</i>	
1.1 Introduction	1
1.2 Objectives of Stevia Selection and Breeding	2
1.2.1 Dry Leaf Yield per Plant and Unit Area	2
1.2.2 Leaf-to-stem Ratio	3
1.2.3 Growth and Photosynthetic Activity	3
1.2.4 Wider Crop Adaptability, Yield Stability and Abiotic Stress Resistance	3
1.2.5 Biotic Stress Resistance	4
1.2.6 Photoperiod Insensitivity	4
1.2.7 Self-compatibility for Viable Seed Production	5
1.2.8 Steviol Glycoside Composition and Yield	5
1.3 Variability of Steviol Glycosides Content and Composition in Stevia Leaves	11
1.3.1 Source of Variation	11
1.3.2 Phenotypic and Genetic Variation in <i>S. rebaudiana</i> : The Genetic Control of SVgly Composition	11
1.3.3 Character Association and Heritability in <i>S. rebaudiana</i>	14

1.4	The Chromosome Number in <i>Stevia</i> Genus	14
1.5	Plant Breeding Programs in <i>Stevia</i>	15
1.5.1	Selection Programs in <i>Stevia</i>	16
1.5.2	Recurrent Selection	17
1.5.3	Synthetic Cultivars	17
1.5.4	Changing the Number of Chromosomes in Plant Cells	18
1.5.5	Marker-assisted Selection	19
1.5.6	New Biotechnological Tools	20
1.6	Conclusions	23
	References	24
Chapter 2	Biosynthesis of Steviol Glycosides and Related Diterpenes in Leaves and Glandular Trichomes of <i>Stevia rebaudiana</i> Bertoni	32
	<i>A. Oehme, W. Schwab and M. Wüst</i>	
2.1	Introduction	32
2.2	Morphology of <i>Stevia rebaudiana</i> Bertoni	34
2.2.1	Foliar Trichomes of <i>Stevia rebaudiana</i> Bertoni	35
2.3	Biosynthesis of Steviol Glycosides	41
2.3.1	UDP-glycosyltransferases in <i>Stevia rebaudiana</i>	43
2.3.2	MVA or DXP Pathway?	45
2.3.3	Site of Biosynthesis	48
2.4	HS-SPME-GC-MS and LC-MS/MS-analysis of Foliar Trichomes and Exudates	50
2.4.1	HS-SPME-GC-MS	50
2.4.2	LC-MS/MS	52
2.5	Outlook	53
	References	53
Chapter 3	Steviol Glycosides Production: Traditional Versus New Technologies	59
	<i>J. Perret</i>	
3.1	Introduction	59
3.2	Steviol Glycosides Production Technologies	60
3.2.1	Objectives	61
3.2.2	Process Steps	61
3.2.3	Steviol Glycosides Primary Extraction	62
3.2.4	Steviol Glycosides Separation	70
3.2.5	Steviol Glycosides Final Purification	72

3.3 Alternative Steviol Glycosides Production Techniques	74
3.3.1 Enzymatic Modification	75
3.3.2 Genetic Engineering and Fermentation Techniques	76
3.4 Production Technologies vs. Naturalness	77
3.5 Conclusion	78
Acknowledgements	79
References	80
Chapter 4 Analysis of Steviol Glycosides	84
<i>U. Wölwer-Rieck</i>	
4.1 Introduction	84
4.2 Sample Preparation	87
4.2.1 Isolation of Steviol Glycosides from Leaves	87
4.2.2 Isolation of Steviol Glycosides from Stevia Extract Preparation and Food	89
4.3 Separation of Steviol Glycosides by High-performance Liquid Chromatography (HPLC)	89
4.3.1 Separation on Normal Phase Columns	90
4.3.2 Separation on C 18-columns	92
4.3.3 Separation on Further Columns	94
4.3.4 Separation Using Two-dimensional Systems	94
4.4 Detection in HPLC	95
4.4.1 UV-detection	95
4.4.2 MS-detection	95
4.4.3 Applying Alternative Detectors	101
4.5 Alternative Techniques for Steviol Glycosides Determination	102
4.5.1 Thin Layer (TLC) and High Performance Thin Layer Chromatography (HPTLC)	102
4.5.2 Capillary Electrophoresis (CE)	102
4.5.3 ¹ H-NMR Spectroscopy	104
4.5.4 Near-infrared Reflectance Spectroscopy (NIRS)	104
4.5.5 Raman Spectroscopy	104
4.5.6 Detection of Steviol Glycosides as Sum Parameter	104
4.5.7 Detection of the Aglycon Steviol	105
4.6 Conclusions	106
References	106

Chapter 5	Presentation and Analysis of Other Constituents in the Leaves: Polyphenolics in <i>Stevia rebaudiana</i> Leaves	113
	<i>N. Kuhnert and H. Karaköse</i>	
5.1.	Introduction	113
5.1.1	Polyphenolics in Stevia	114
5.1.2	Quantification of Polyphenols in Stevia	116
5.1.3	Further Lessons from Stevia Polyphenolic Analysis	119
5.1.4	Plant Polyphenols and UV Exposure	120
5.1.5	Correlation Analysis of Polyphenol Concentrations	121
5.1.6	Multivariant Statistical Analysis of Stevia Phytochemical Profiles	122
	Acknowledgement	123
	References	124
Chapter 6	Presentation and Analysis of Other Constituents in the Leaves: Analysis of Lipids and Volatile Terpenes in <i>Stevia rebaudiana</i>	125
	<i>H. Karaköse, A. Golon, D. Sirbu and N. Kuhnert</i>	
6.1	Introduction	125
6.2	Analysis of Lipid Fraction	126
6.2.1	Analysis of the Fatty Acid Profile	126
6.2.2	Analysis of Intact Lipids	128
6.2.3	Analysis of Terpenoid Lipophilic Volatiles	129
	Acknowledgements	131
	References	131
Chapter 7	Antioxidant Capacity of Stevia Leaves	132
	<i>C. Bender and B. F. Zimmermann</i>	
7.1.	Definition and Significance of Antioxidant Capacity for Food and Health	132
7.1.1	Definition	132
7.1.2	Significance of Antioxidant Capacity for Food	133
7.1.3	Significance of Antioxidant Capacity for Health	133
7.2	Determination of Antioxidant Capacity	135
7.2.1	Extraction of the Antioxidants	135

7.2.2	Methods for Determination of Antioxidant Capacity	135
7.2.3	Bioassays for the Determination of Antioxidant Capacity	139
	References	145
Chapter 8	<i>Stevia rebaudiana</i> Bertoni: Beyond Its Use as a Sweetener. Pharmacological and Toxicological Profile of Steviol Glycosides of <i>Stevia rebaudiana</i> Bertoni	148
	<i>Lara Testai and Vincenzo Calderone</i>	
8.1	Introduction	148
8.2	Regulatory Committee Position	149
8.3	Low Calorie Sweeteners	149
8.4	The Pharmacokinetic Profile of SGs	150
8.5	The Pharmacodynamic Profile of SGs	150
8.6	Cardiovascular Benefits	151
8.6.1	Antidiabetic Activity	151
8.6.2	Antihypertensive Activity	153
8.6.3	Cardioprotective Activity	154
8.7	Non-cardiovascular Benefits of SGs	154
8.7.1	Anticariogenic Activity	155
8.7.2	Antimicrobial Activity	155
8.7.3	Anticancer Activity	156
8.7.4	Antioxidant and Anti-inflammatory Activity	156
8.8	Toxic and Adverse Effects	156
	References	158
Chapter 9	Steviol Glycosides in Dentistry	162
	<i>M. Frentzen, P. Besrukow, A. Ackermann, S. Pierog, B. Schiermeyer, J. Winter, U. Wölwer-Rieck and D. Kraus</i>	
9.1	Introduction	162
9.2	Oral Diseases	163
9.3	Oral Biofilm/Dental Plaque	164
9.4	Biofilm Management in Oral Diseases	166
9.5	Steviol Glycosides in Dental Care	167
9.5.1	Literature Overview	167
9.5.2	Current Research	172
9.5.3	Modulation of Bacterial Growth	173
9.5.4	Effects of Steviol Glycosides on Bacterial Sugar Consumption and Acid Production	174

9.5.5	Suppression of Oral Biofilm Formation	178
9.5.6	Decomposition of Steviol Glycosides by Oral Bacteria	180
9.6	Interaction of Steviol Glycosides to Oral Tissues	181
9.7	Perspectives	182
	References	182
Chapter 10	Sensory Effects of Steviol Glycosides: Taste Perception and Beyond	185
	<i>Koenraad Philippaert, Caroline Wuyts, Caroline Simoens and Rudi Vennekens</i>	
10.1	Introduction	185
10.2	Taste Aspects of Steviol Glycosides and Their Interaction with the Sweet-taste Receptor	186
10.2.1	Taste Perception	186
10.2.2	Taste Receptors	186
10.2.3	Taste Perception of Stevioside	187
10.3	Steviol and Steviol Glycosides Act as a Taste Amplifier through Interaction with TRPM5	190
10.3.1	TRPM5	190
10.3.2	TRPM5 in Taste Sensation	190
10.3.3	TRPM5 and Steviol Glycosides	191
10.4	Anti-diabetic Effects of Steviol Glycosides through Their Interaction with TRPM5	192
10.4.1	Diabetes	192
10.4.2	TRPM5 and Diabetes	192
10.4.3	Steviol Glycosides and Diabetes	193
10.5	Anti-hyperglycaemic Effects of Steviol Glycosides in Humans	195
10.5.1	Studies with Healthy Volunteers	195
10.5.2	Studies with Individuals Diagnosed with Type-2 Diabetes Mellitus	195
10.6	Concluding Remarks	199
	Acknowledgements	200
	References	200
	Subject Index	204