

Contents

List of Contributors XIX

Preface XXVII

1	Vitamins, Biopigments, Antioxidants and Related Compounds: A Historical, Physiological and (Bio)technological Perspective	1
	<i>Erick J. Vandamme and José L. Revuelta</i>	
1.1	Historical Aspects of the Search for Vitamins	1
1.2	Vitamins: What's in a Name	3
1.3	Physiological Functions of Vitamins and Related Compounds	6
1.4	Technical Functions of Vitamins and Related Compounds	8
1.5	Production and Application of Vitamins and Related Factors	8
1.6	Outlook	13
	References	13
	Part I Water-Soluble Vitamins	15
2	Industrial Production of Vitamin B₂ by Microbial Fermentation	17
	<i>José L. Revuelta, Rodrigo Ledesma-Amaro, and Alberto Jiménez</i>	
2.1	Introduction and Historical Outline	17
2.2	Occurrence in Natural/Food Sources	17
2.3	Chemical and Physical Properties; Technical Functions	18
2.4	Assay Methods and Units	18
2.5	Biological Role of Flavins and Flavoproteins	19
2.6	Biotechnological Synthesis of Riboflavin	21
2.6.1	Riboflavin-Producing Microorganisms	21
2.6.2	Biosynthesis of Riboflavin	22
2.6.3	Regulation of the Biosynthesis of Riboflavin	25
2.7	Strain Development: Genetic Modifications, Molecular Genetics and Metabolic Engineering	26
2.8	Fermentation Process	31
2.9	Downstream Processing	32
2.10	Chemical Synthesis	33

2.11	Application and Economics 33
	References 33
3	Vitamin B₃, Niacin 41
	<i>Tek Chand Bhalla and Savitri</i>
3.1	Introduction 41
3.2	History 42
3.3	Occurrence in Nature/Food Sources 43
3.4	Chemical and Physical Properties 44
3.4.1	Chemical Properties 44
3.4.2	Physical Properties 44
3.5	Vitamin B ₃ Deficiency Disease (Pellagra) 45
3.6	Methods Used for Determination of Vitamin B ₃ 46
3.6.1	Microbiological Methods 46
3.6.2	Chemical Methods 46
3.7	Synthesis 47
3.7.1	Chemical Process Used for Nicotinic Acid Production 47
3.7.2	Biosynthesis 49
3.7.2.1	Biological Processes Used for Nicotinic Acid Production 49
3.8	Downstream Processing of Nicotinic Acid 52
3.9	Reactive Extraction 53
3.10	Physiological Role of Vitamin B ₃ (Niacin) 53
3.10.1	Coenzyme in Metabolic Reactions 53
3.10.2	Therapeutic Molecule 56
3.10.2.1	Treatment of Pellagra 56
3.10.2.2	Treatment of Cardiovascular Diseases 57
3.10.2.3	Antihyperlipidemic Effect 57
3.10.2.4	Treatment of Hypercholesterolemia 57
3.10.2.5	Diabetes 58
3.10.2.6	Fibrinolysis 58
3.10.2.7	Treatment of Neurodegenerative Disorders 58
3.11	Safety of Niacin 59
3.12	Toxicity of Niacin 59
3.12.1	Hepatotoxicity 59
3.12.2	Vasodilation/Niacin Flush 59
3.12.3	Glucose Intolerance 60
3.13	Derivatives of Niacin 60
3.14	Application in Cosmetics, Food and Feed 61
3.15	Future Prospects 61
	References 61
4	Pantothenic Acid 67
	<i>Jesus Gonzalez-Lopez, Luis Aliaga, Alejandro Gonzalez-Martinez, and Maria V. Martinez-Toledo</i>
4.1	Introduction and Historical Outline 67

4.2	Occurrence in Natural Food Sources and Requirements	71
4.3	Physiological Role as Vitamin or as Coenzyme	74
4.4	Chemical and Physical Properties	77
4.5	Assay Methods	79
4.6	Chemical and Biotechnological Synthesis	81
4.7	Application and Economics	92
	References	98
5	Folate: Relevance of Chemical and Microbial Production	103
	<i>Maddalena Rossi, Stefano Raimondi, Luca Costantino, and Alberto Amaretti</i>	
5.1	Introduction	103
5.2	Folates: Chemical Properties and Occurrence in Food	103
5.3	Biosynthesis	105
5.4	Physiological Role	106
5.5	Bioavailability and Dietary Supplements	109
5.6	Chemical and Chemoenzymatic Synthesis of Folic Acid and Derivatives	110
5.7	Intestinal Microbiota, Probiotics and Vitamins	114
5.8	Folate Production by Lactic acid Bacteria	115
5.9	Folate Production by Bifidobacteria	117
5.10	Conclusions	120
	References	124
6	Vitamin B₁₂ – Physiology, Production and Application	129
	<i>Janice Marie Sych, Christophe Lacroix, and Marc J.A. Stevens</i>	
6.1	Introduction and Historical Outline	129
6.2	Occurrence in Food and Other Natural Sources	130
6.3	Physiological Role as a Vitamin or Coenzyme	131
6.3.1	Absorption and Transport	131
6.3.2	Metabolic Functions	132
6.3.3	Main Causes and Prevalence of Deficiencies	133
6.3.4	Diagnosis of Deficiencies	134
6.4	Chemical and Physical Properties	134
6.5	Assay Methods	137
6.6	Biotechnological Synthesis	140
6.6.1	Producing Microorganisms	140
6.6.1.1	Propionibacteria (PAB)	142
6.6.1.2	Pseudomonades	143
6.6.2	Biosynthesis and Metabolic Regulation	144
6.6.3	Engineering of B ₁₂ Production	145
6.6.3.1	Propionibacteria	145
6.6.3.2	Pseudomonades	146
6.6.4	Fermentation Process	146
6.6.4.1	Propionibacteria	146
6.6.4.2	Pseudomonades	148

6.7	Downstream Processing; Purification and Formulation	149
6.8	Application and Economics	150
6.9	Conclusions and Outlook	151
	References	151
7	Industrial Fermentation of Vitamin C	161
	<i>Weichao Yang and Hui Xu</i>	
7.1	Introduction and Historical Outline	161
7.2	Occurrence in Natural/Food Sources	162
7.2.1	Occurrence of Asc in Foods	162
7.2.2	Biosynthesis of Asc in Plants and Mammals	164
7.3	Physiological Role of Asc	164
7.4	Chemical and Physical Properties	165
7.5	Assay Methods	165
7.6	Industrial Fermentation of Asc	166
7.6.1	The Reichstein Process: The Major Industrial Asc Process until the Late 1990s	167
7.6.1.1	The Establishment of the Reichstein Process	167
7.6.1.2	Bioconversion of D-Sorbitol to L-Sorbose by <i>Gluconobacter</i>	167
7.6.1.3	The Key Enzyme of <i>Gluconobacter</i> for L-Sorbose Production	168
7.6.1.4	Oxidation of L-Sorbose to 2-KLG and Rearrangement to Asc	168
7.6.2	The Two-Step Fermentation Process for Asc Production	168
7.6.2.1	The First Step of Fermentation: Conversion of D-Sorbitol to L-Sorbose	169
7.6.2.2	The Second Step of Fermentation: Conversion of L-Sorbose to 2-Keto-L-Gulonic acid	170
7.6.2.3	Strain Development: Genetic Modification, Molecular Genetics and Metabolic Engineering	175
7.6.2.4	Fermentation Process	177
7.6.2.5	Upstream and Downstream Processing	181
7.7	Application and Economics	182
7.8	Outlook	183
	References	185
8	Direct Microbial Routes to Vitamin C Production	193
	<i>Günter Pappenberger and Hans-Peter Hohmann</i>	
8.1	Introduction and Scope	193
8.2	Principles of Direct L-Ascorbic Acid Formation: The Major Challenges	195
8.2.1	Stereochemistry of L-Ascorbic Acid	195
8.2.2	Enzymes Producing L-Ascorbic Acid and Their By-Product Spectrum	196
8.3	Direct L-Ascorbic Acid Formation via 1,4-Lactones	197
8.3.1	L-Ascorbic Acid Forming Enzymes: 1,4-Lactone Oxidoreductases	198

8.3.2	Direct L-Ascorbic Acid Formation in Heterotrophic Microalgae	200
8.3.3	Direct L-Ascorbic Acid Formation in Recombinant Yeast	201
8.3.4	Direct L-Ascorbic Acid Formation from Orange Processing Waste in Recombinant <i>Aspergillus niger</i>	203
8.3.5	Overall Conclusion on 1,4-Lactone Routes	204
8.4	Direct L-Ascorbic Acid Formation via 2-Keto Aldoses	206
8.4.1	L-Ascorbic Acid Forming Enzymes: L-Sorbose Dehydrogenases	208
8.4.1.1	Sndhak	208
8.4.1.2	Sndhai	211
8.4.1.3	Prevalence of L-Asc Forming Sorbose Dehydrogenases in Nature	211
8.4.2	L-Asc or 2-KGA from L-Sorbose: One Substrate, Several Isomers, Two Products	212
8.4.3	L-Sorbose Dehydrogenase, Accumulating L-Sorbose	215
8.4.3.1	Ssdh from <i>K. vulgare</i>	215
8.4.3.2	Sorbose Dehydrogenase Sdh from <i>G. oxydans</i>	217
8.4.4	<i>Gluconobacter</i> as Host for Direct L-Ascorbic Acid Formation	217
8.5	Outlook	219
	Acknowledgement	220
	References	220

Part II Fat Soluble Vitamins 227

9	Synthesis of β-Carotene and Other Important Carotenoids with Bacteria	229
	<i>Christoph Albermann and Holger Beuttler</i>	
9.1	Introduction	229
9.2	Carotenoids: Chemical Properties, Nomenclature and Analytics	230
9.2.1	Nomenclature	231
9.2.2	Analysis of Carotenoids	231
9.2.2.1	Handling Precautions	231
9.2.2.2	Extraction	232
9.2.2.3	Chromatography Methods for Analysis of Carotenoids	233
9.3	Natural Occurrence in Bacteria	234
9.4	Biosynthesis of Carotenoids in Bacteria	236
9.5	Biotechnological Synthesis of Carotenoids by Carotenogenic and Non-Carotenogenic Bacteria	239
9.5.1	Heterologous Expression of Carotenoid Biosynthesis Genes	240
9.5.2	Increased Isoprenoid Precursor Supply	243
9.5.3	Genome-Wide Modification of <i>E. coli</i> to Increase Carotenoid Formation	244
9.5.4	Balancing Recombinant Enzyme Activities for an Improved Synthesis of Carotenoids by <i>E. coli</i>	249

9.5.5	Production of Industrially Important Carotenoids by Other Recombinant Bacteria 252
9.5.6	Culture Conditions of Improved Formation of Carotenoids by Recombinant Bacteria 252
9.6	Conclusion 253
	References 254
10	<i>β-Carotene and Other Carotenoids and Pigments from Microalgae 265</i> <i>Borhane Samir Grama, Antoine Delhaye, Spiros N. Agathos, and Clayton Jeffryes</i>
10.1	Introduction and Historical Outline 265
10.2	Occurrence in Nature and Food Sources 266
10.3	Physiological Role as a Vitamin or as a Coenzyme 267
10.4	Chemical and Physical Properties; Technical Functions 268
10.5	Assay Methods and Units 270
10.6	Biotechnological Synthesis 270
10.6.1	Producing Organisms 270
10.6.2	Biosynthesis and Metabolic Regulation 273
10.6.3	Strain Development: Genetic Modification, Molecular Genetics and Metabolic Engineering 276
10.6.4	Downstream Processing, Purification and Formulation 276
10.7	Chemical Synthesis or Extraction 279
10.8	Process Economics 279
	References 280
11	<i>Microbial Production of Vitamin F and Other Polyunsaturated Fatty Acids 287</i> <i>Colin Ratledge</i>
	Lipid Nomenclature 287
11.1	Introduction: Essential Fatty Acids 288
11.2	General Principles for the Accumulation of Oils and Fats in Microorganisms 294
11.3	Production of Microbial Oils 297
11.3.1	Production of Gamma-Linolenic Acid (GLA; 18:3 n-6) 297
11.3.2	Productions of Docosahexaenoic Acid (DHA) and Arachidonic Acid (ARA) 300
11.3.3	Alternative Sources of DHA 302
11.3.4	Production of Eicosapentaenoic Acid (EPA n-3) 305
11.3.5	Prospects of Photosynthetic Microalgae for Production of PUFAs 307
11.4	Safety Issues 310
11.5	Future Prospects 312
	Acknowledgements 315
	References 316

12	Vitamin Q₁₀: Property, Production and Application	321
	<i>Joong K. Kim, Eun J. Kim, and Hyun Y. Jung</i>	
12.1	Background of Vitamin Q ₁₀	321
12.1.1	Historical Aspects	321
12.1.2	Definition	321
12.1.3	Occurrence	322
12.1.3.1	In Nature	322
12.1.3.2	In Food Sources	322
12.1.3.3	In Microorganisms	326
12.1.4	Functions	326
12.2	Chemical and Physical Properties of CoQ ₁₀	326
12.2.1	Chemical Properties	326
12.2.2	Physical Properties	327
12.3	Biosynthesis and Metabolic Regulation of CoQ ₁₀	327
12.3.1	Biosynthesis of CoQ ₁₀	327
12.3.1.1	Microorganisms	327
12.3.1.2	Biosynthetic Pathways	329
12.3.2	Metabolic Regulation	334
12.3.3	Strain Development	335
12.3.3.1	Mutagenesis	335
12.3.3.2	Genetic Modification	335
12.3.3.3	Metabolic Engineering	337
12.3.4	Fermentation Process	339
12.3.5	Upstream and Downstream Processing	340
12.3.5.1	Upstream Processing	340
12.3.5.2	Downstream Processing	343
12.4	Chemical Synthesis and Separation of CoQ ₁₀	345
12.4.1	Chemical Synthesis	345
12.4.2	Solvent Extraction	346
12.4.3	Purification	350
12.5	Applications and Economics of CoQ ₁₀	351
12.5.1	Applications	351
12.5.1.1	In Diseases	351
12.5.1.2	In Cosmetics	352
12.5.1.3	In Foods and Others	353
12.5.2	Economics	354
	References	355
13	Pyrroloquinoline Quinone (PQQ)	367
	<i>Hirohide Toyama</i>	
13.1	Introduction and Historical Outline	367
13.2	Occurrence in Natural/Food Sources	367
13.3	Physiological Role as Vitamin or as Bioactive Substance	368
13.4	Physiological Role as a Cofactor	373
13.5	Chemical and Physical Properties; Technical Functions	376

13.6	Assay Methods	377
13.7	Biotechnological Synthesis	377
13.7.1	Producing Microorganisms	377
13.7.2	Biosynthesis and Metabolic Regulation	378
13.8	Strain Development: Genetic Modification, Molecular Genetics and Metabolic Engineering	378
13.9	Up- and Down-stream Processing; Purification and Formulation	380
13.10	Chemical Synthesis or Extraction Technology	380
13.11	Application and Economics	380
	References	381

Part III Other Growth Factors, Biopigments and Antioxidants 389

14	L-Carnitine, the Vitamin B_T: Uses and Production by the Secondary Metabolism of Bacteria 391	
	<i>Vicente Bernal, Paula Arense, and Manuel Cánovas</i>	
14.1	Introduction and Historical Outline	391
14.2	Occurrence in Natural/Food Sources	392
14.3	Physiological Role as Vitamin or as Coenzyme	393
14.3.1	Physiological Role of Carnitine in the Mitochondria	393
14.3.2	Physiological Role of Carnitine in the Peroxisomes	394
14.3.3	Other Functions of Carnitine	394
14.4	Chemical and Physical Properties	394
14.5	Assay Methods and Units	395
14.5.1	Chromatographic Methods	395
14.5.2	MS-Based Methods	395
14.5.3	Enzymatic Methods	398
14.5.4	Automated Methods	399
14.6	Biotechnological Synthesis of L-Carnitine Microbial Metabolism of L-Carnitine and Its Regulation	399
14.6.1	Biotechnological Methods for L-Carnitine Production	399
14.6.1.1	De novo Biosynthesis of L-Carnitine	399
14.6.1.2	Biological Resolution of Racemic Mixtures	399
14.6.1.3	Biotransformation from Non-Chiral Substrates	400
14.6.2	Roles of L-Carnitine in Microorganisms	401
14.6.2.1	Protectant Agent	401
14.6.2.2	Carbon and Nitrogen Source	401
14.6.2.3	Electron Acceptor: Carnitine Respiration	402
14.6.3	L-Carnitine Metabolism in Enterobacteria and Its Regulation	403
14.6.3.1	Metabolism of L-Carnitine in <i>E. coli</i>	403
14.6.3.2	Metabolism of L-Carnitine in <i>Proteus</i> sp.	405
14.6.4	Expression of Metabolising Activities: Effect of Inducers, Oxygen and Substrates	406

14.6.5	Biotransformation with D-Carnitine or Crotonobetaine as Substrates 406
14.6.6	Transport Phenomena for L-Carnitine Production 407
14.6.6.1	Membrane Permeabilisation 407
14.6.6.2	Osmotic Stress Induction of Transporters 408
14.6.6.3	Overexpression of the Transporter caiT 408
14.6.7	Metabolic Engineering for High-Yielding L-Carnitine Producing Strains 408
14.6.7.1	Link between Central and Secondary Metabolism during Biotransformation 408
14.6.7.2	Metabolic Engineering for Strain Engineering: Feedback between Modelling and Experimental Analysis of Cell Metabolism 409
14.7	Other Methods for L-Carnitine Production: Extraction from Natural Sources and Chemical Synthesis 411
14.7.1	Isolation of L-Carnitine from Natural Sources 411
14.7.2	Chemical Synthesis 411
	Acknowledgement 412
	References 412
15	Application of Carnosine and Its Functionalised Derivatives 421
	<i>Isabelle Chevalot, Elmira Arab-Tehrany, Edouard Husson, and Christine Gerardin</i>
15.1	Introduction and Historical Outline 421
15.2	Sources and Synthesis 422
15.2.1	Occurrence in Natural/Food Sources 422
15.2.2	Chemical Synthesis of Carnosine 422
15.2.3	Enzymatic Synthesis of Carnosine 423
15.3	Physico-Chemical and Biological Properties of Carnosine 425
15.3.1	Physico-Chemical Properties 425
15.3.2	Physiological Properties 426
15.4	Biotechnological Synthesis of Carnosine Derivatives: Modification, Vectorisation and Functionalisation 427
15.4.1	Chemical Functionalisation 427
15.4.2	Enzymatic Functionalisation: Enzymatic N-Acylation of Carnosine 430
15.4.2.1	Lipase-Catalysed N-Acylation of Carnosine in Non-Aqueous Medium 431
15.4.2.2	Acyltransferase-Catalysed N-Acylation of Carnosine in Aqueous Medium 432
15.4.2.3	Impact of Enzymatic Oleylation of Carnosine on Some Biological Properties 434
15.4.3	Vectorisation 434
15.5	Applications of Carnosine and Its Derivatives 435

15.5.1	Nutraceutics and Food Supplementation	435
15.5.2	Cosmetics	436
15.5.3	Pharmaceuticals	436
	References	438
16	Metabolism and Biotechnological Production of Gamma-Aminobutyric Acid (GABA)	445
	<i>Feng Shi, Yalan Ni, and Nannan Wang</i>	
16.1	Introduction	445
16.2	Properties and Occurrence of GABA in Natural Sources	446
16.3	Metabolism of GABA	447
16.3.1	Biosynthesis and Export of GABA	450
16.3.1.1	Biosynthesis of GABA	450
16.3.1.2	Essential Enzyme for GABA Biosynthesis – GAD	451
16.3.1.3	Export of GABA	452
16.3.2	Uptake and Catabolism of GABA	454
16.3.2.1	The Uptake System of GABA	454
16.3.2.2	The Catabolism of GABA	455
16.4	Regulation of GABA Biosynthesis	456
16.5	Biotechnological Production of GABA	457
16.5.1	Fermentative Production of GABA by LAB	458
16.5.2	Production of GABA by Enzymatic Conversion	459
16.5.2.1	Production of GABA by Immobilised GAD	459
16.5.2.2	Improving GAD Activity by Rational and Irrational Designs	459
16.5.3	Fermentation of GABA by Recombinant <i>C. glutamicum</i>	460
16.6	Physiological Functions and Applications of GABA	461
16.6.1	Physiological Functions of GABA	461
16.6.2	Applications of GABA	462
16.7	Conclusion	462
	Acknowledgement	462
	References	463
17	Flavonoids: Functions, Metabolism and Biotechnology	469
	<i>Celestino Santos-Buelga and Ana M. González-Paramás</i>	
17.1	Introduction	469
17.2	Structure and Occurrence in Food	471
17.3	Activity and Metabolism	476
17.4	Biosynthesis of Flavonoids in Plants	481
17.5	Biotechnological Production	484
17.5.1	Reconstruction of Flavonoid Pathways in Plant Systems	485
17.5.2	Reconstruction of Flavonoid Pathways in Microbial Systems	487
17.5.2.1	<i>E. coli</i> Platform	487
17.5.2.2	<i>Saccharomyces cerevisiae</i> Platform	489
17.6	Concluding Remarks	489
	References	490

18	<i>Monascus</i> Pigments	497
	<i>Yanli Feng, Yanchun Shao, Youxiang Zhou, Wanping Chen, and Fusheng Chen</i>	
18.1	Introduction and History of <i>Monascus</i> Pigments	497
18.2	Categories of MPs	497
18.3	Physiological Functions of MPs	498
18.3.1	Anti-Cancer Activities	498
18.3.2	Antimicrobial Activities	508
18.3.3	Anti-Obesity Activities	509
18.3.4	Anti-Inflammation Activities	510
18.3.5	Regulation of Cholesterol Levels	510
18.3.6	Anti-Diabetes Activities	511
18.4	Chemical and Physical Properties of MPs	511
18.4.1	Solubility	511
18.4.2	Stability	511
18.4.2.1	Effects of Temperature, pH and Solvent on Stability of MPs	511
18.4.2.2	Effect of Light on Stability of MPs	512
18.4.2.3	Effect of Metal Ion on Stability of MPs	513
18.4.3	Safety	513
18.5	Assay Methods and Units of MPs	513
18.5.1	Extraction and Detection of MPs	513
18.5.2	Isolation and Purification of MPs Components	514
18.5.2.1	CC and TLC	514
18.5.2.2	HPLC	515
18.5.2.3	CE and the Others	515
18.5.3	Identification of MPs Components	515
18.6	MPs Producer – <i>Monascus</i> spp.	520
18.6.1	Brief Introduction of <i>Monascus</i> Species and Their Applications	520
18.6.2	Producing Methods of MPs	520
18.6.3	Progress of <i>Monascus</i> spp. at the Genetic Level	521
18.6.3.1	DNA Transformation	521
18.6.3.2	Citrinin Synthesis and Its Regulations	521
18.6.3.3	MK Synthesis and Its Regulations	522
18.6.3.4	MPs Synthesis and Its Regulation	522
18.6.3.5	The Regulation of Secondary Metabolism in <i>Monascus</i> spp.	523
18.6.4	<i>Monascus</i> Genomics	524
18.7	Application and Economics of MPs	524
	Acknowledgements	524
	References	526