

<b>1.</b>	<b>Intention</b> .....	<b>9</b>
<b>2.</b>	<b>Background</b> .....	<b>10</b>
2.1	<i>Camellia sinensis</i> .....	10
2.1.1	Carotenoid composition.....	11
2.1.2	Flavor composition.....	11
2.1.3	Enzymes.....	12
2.2	<i>Prunus persica nucipersica</i> .....	12
2.2.1	Flavor composition.....	13
2.2.2	Carotenoid composition.....	13
2.3	<i>Osmanthus fragrans</i> .....	14
2.3.1	Flavor composition.....	14
2.3.2	Carotenoid composition.....	15
2.4	Carotenoids.....	15
2.5	Flavor compounds formed by carotenoid degradation.....	16
2.6	Carotenoid cleavage enzymes.....	18
2.6.1	Enzymes.....	18
2.6.2	Occurrence.....	19
2.6.3	Classes and characteristics.....	20
2.6.4	Particularities.....	21
2.6.4.1	Biological functions of volatile carotenoid cleavage products.....	21
2.6.4.2	Are carotenoid cleavage enzymes monooxygenases or dioxygenases?.....	21
2.6.4.3	Structural aspects.....	22
2.6.4.4	Location of carotenoid cleavage enzymes.....	23
2.7	Characterization of the kinetics.....	24
2.8	Proteins and protein purification.....	25
2.8.1	Proteins.....	25
2.8.2	Posttranslational modifications.....	26
2.9	Extraction of proteins.....	26
2.9.1	Precipitation of proteins.....	27
2.9.2	Precipitation of proteins with organic solvents.....	28
2.9.3	Isoelectric focusing.....	28
2.9.4	Enzyme isolation by preparative chromatography.....	30
2.9.5	Size exclusion chromatography.....	30
2.9.6	Ion exchange chromatography.....	31
2.9.7	Density gradient centrifugation.....	31
2.10	Protein analysis.....	32
2.10.1	Protein quantification.....	32

2.10.2	Polyacryl amide gel electrophoresis .....	32
2.10.3	Detection of protein bands .....	33
2.10.4	Mass spectrometry.....	33
2.10.5	MALDI-TOF.....	33
2.10.6	Edman degradation.....	34
2.11	Special analytical techniques.....	35
2.11.1	CCC .....	35
2.11.2	CPC .....	37
<b>3.</b>	<b>Results and Discussion .....</b>	<b>38</b>
3.1	Enzymatic assays.....	38
3.2	Isolation of carotenoid cleavage enzymes .....	39
3.2.1	Introduction .....	39
3.2.2	<i>Prunus persica nucipersica</i> .....	39
3.2.3	<i>Camellia sinensis</i> .....	41
3.2.3.1.	Isolation of tea enzymes by centrifugal precipitation chromatography .....	43
3.2.4	<i>Osmanthus fragrans aurantiacus</i> .....	47
3.2.5	Summary.....	48
3.3	Kinetic characteristics .....	48
3.3.1	Introduction .....	48
3.3.2	Determination of the main carotenoids .....	49
3.3.2.1	<i>Camellia sinensis</i> .....	50
3.3.2.2	<i>Osmanthus fragrans aurantiacus</i> .....	51
3.3.2.3	<i>Prunus persica</i> .....	52
3.3.3	Temperature optima.....	53
3.3.4	Activation energy .....	58
3.3.5	pH-optimum .....	59
3.3.6	Time course .....	60
3.3.7	$K_m$ and $v_{max}$ .....	61
3.3.8	Summary.....	63
3.4	Structural aspects of the enzymes.....	64
3.4.1	Introduction .....	64
3.4.2	Isoelectric points .....	66
3.4.3	Structural aspects of tea samples with different harvest times .....	67
3.4.4	Determination of the molecular mass by SDS-PAGE .....	70
3.4.4.1	Nectarines.....	70
3.4.4.2	Determination of the molecular mass of enyzmes from spring and autumn tea .....	71

3.4.5	Determination of the exact molecular mass by MALDI-TOF .....	72
3.4.6	Structural information resulting from MALDI-TOF experiments after digestion with trypsin .....	75
3.4.7	Edman-degradation .....	76
3.4.8	Summary.....	77
3.5	Substrate specificity .....	78
3.5.1	Introduction .....	78
3.5.2	<i>Osmanthus fragrans</i> .....	79
3.5.3	<i>Prunus persica nucipersica</i> .....	80
3.5.4	<i>Camellia sinensis</i> .....	81
3.5.4.1	Isolation of all- <i>trans</i> -lycopene by HSCCC.....	82
3.5.4.2	Isolation of 9'- <i>cis</i> -neoxanthin by HSCCC.....	83
3.5.4.3	Specificity of the enzymes isolated from <i>Camellia sinensis</i> .....	86
3.5.4.4	Influence of the carotenoid end group ( $\alpha$ -carotene, $\beta$ -carotene, lycopene).....	90
3.5.4.5	Influence of the substitution of a $\beta$ -ring ( $\beta$ -carotene, zeaxanthin, canthaxanthin, astaxanthin).....	92
3.5.4.6	Lutein, 9'- <i>cis</i> neoxanthin .....	94
3.5.4.7	Structure activity relationship .....	96
3.5.5	Determination of reaction products.....	99
3.5.6	Summary.....	102
<b>4.</b>	<b>Experimental .....</b>	<b>104</b>
4.1	Preparation of crude enzyme extracts .....	104
4.1.1	<i>Prunus persica nucipersica</i> .....	104
4.1.2	<i>Camellia sinensis</i> .....	104
4.1.3	<i>Osmanthus fragrans aurantiacus</i> .....	105
4.1.4	Preparation of acetone powder.....	105
4.2	Isolation of carotenoid oxygenases from tea ( <i>Camellia sinensis</i> ).....	105
4.2.1	Isoelectric focusing (IEF) .....	106
4.2.2	Size exclusion chromatography on Sephadex 200.....	106
4.2.3	Centrifugal precipitation chromatography .....	107
4.2.3.1	Determination of the osmosis rate between sample and solvent channel.....	107
4.2.3.2	Determination of the critical solvent concentration .....	108
4.3	Bradford assay for the determination of the critical solvent concentration.....	109
4.4	Isolation of carotenoid oxygenases from nectarines ( <i>Prunus persica nucipersica</i> ).....	109
4.4.1	Density centrifugation with Percoll.....	110
4.4.2	Anion exchange chromatography .....	111

4.4.3	Ultra filtration.....	112
4.4.4	Isoelectric focusing .....	112
4.5	Isolation of carotenoid oxygenases from <i>Osmanthus fragrans</i> .....	112
4.6	Quantification of carotenoids by UV-spectroscopy .....	112
4.7	Determination of the purity of carotenoid standards by HPLC-DAD .....	113
4.7.1	Isolation and clean up of carotenoid standards .....	114
4.7.1.1	$\beta$ -Carotene and $\alpha$ -carotene .....	114
4.7.1.2	Zeaxanthin, astaxanthin, canthaxanthin .....	115
4.7.1.3	Zeaxanthin, astaxanthin.....	115
4.7.1.4	Canthaxanthin.....	116
4.7.1.5	Lutein .....	117
4.7.1.6	Isolation of all- <i>trans</i> -lycopene by HSCCC.....	118
4.7.1.7	Isolation of 9'- <i>cis</i> -neoxanthin by HSCCC.....	119
4.8	Enzymatic setups.....	120
4.8.1	Determination of the main carotenoids in Japanese Green Tea.....	120
4.8.2	Determination of the main carotenoids in <i>Osmanthus fragrans</i> .....	121
4.8.3	HPLC-MS.....	121
4.9	Preparation of the substrates.....	122
4.9.1	Determination of the detection wavelength for the enzymatic assay .....	123
4.9.2	Calculation of relative activities.....	124
4.9.3	Calibration of carotenoid/Tween 40 solutions .....	124
4.9.4	Determination of enzymatic activity .....	124
4.9.5	Activity screening in CPC output fractions .....	125
4.10	Michaelis-Menten Kinetics .....	125
4.11	Determination of the optimum temperature .....	126
4.12	Calculation of the activation energy .....	126
4.13	Detection of the optimal pH for the enzymatic reaction .....	126
4.14	Determination of enzyme structure.....	126
4.14.1	SDS-PAGE .....	126
4.14.2	Coomassie Brilliant Blue Staining.....	128
4.14.3	Coomassie Brilliant Blue Staining of PVDF membranes after Western blotting .....	129
4.14.4	Silver Staining.....	130
4.15	MALDI-TOF.....	131
4.15.1	In gel digest .....	132
4.15.2	Data base search.....	132
4.16	Western Blot .....	133

4.17	Determination of the reaction products .....	133
4.17.1	Determination after absorption on Bio-Beads .....	133
4.17.2	SPME .....	134
<b>5.</b>	<b>Chemicals .....</b>	<b>135</b>
<b>6.</b>	<b>Experimental Devices .....</b>	<b>137</b>
6.1	Photometer .....	137
6.2	pH-meters .....	137
6.3	Centrifuges .....	137
6.4	Fast protein liquid chromatography (FPLC) instruments .....	137
6.5	Preparative isoelectric focusing .....	138
6.6	Eletrophoresis equiment .....	138
6.7	Shaker .....	138
6.8	Blotting .....	138
6.9	High performance liquid chromatography (HPLC) instruments .....	139
6.10	Mass spectrometry .....	139
6.11	MALDI-TOF-MS .....	139
6.12	High performance liquid chromatography mass spectrometry (HPLC-MS) .....	139
6.13	High-speed counter-current chromatography (HSCCC) .....	140
6.14	Centrifugal precipitation chromatography (CPC) .....	140
6.15	Gas Chromatography (GC) .....	140
6.16	Gas Chromatography mass spectrometry (GC-MS) .....	141
<b>7.</b>	<b>Summary and Conclusion .....</b>	<b>142</b>
<b>8.</b>	<b>Zusammenfassung .....</b>	<b>145</b>
<b>Appendix</b>	<b>.....</b>	<b>149</b>
I	LC-MS chromatograms of $\beta$ -carotene at different $\text{NH}_4\text{Ac}$ concentrations..	149
II	SDS-PAGE of spring and autumn tea samples after CPC .....	150
III	Calibration of carotenoids/Tween 40 solutions $0.1 \text{ g L}^{-1}$ .....	150
	$\alpha$ -Carotene/Tween 40 .....	150
	$\beta$ -Carotene/Tween 40 .....	151
	Zeaxanthin/Tween 40 .....	151
	Lutein/Tween 40 .....	152
	Astaxanthin/Tween 40 .....	152
	Canthaxanthin/Tween 40 .....	153
	Neoxanthin/Tween 40 .....	153
	Lycopene/Tween 40 .....	154
IV	PVDF membranes used for Edman degradation .....	154

V	Data obtained by Edman degradation .....	155
VI	Mass spectra of $\beta$ -ionone.....	155
VII	Structures and abbreviations of biogene amino acids.....	156
<b>Literature Cited.....</b>		<b>157</b>