Enzyme Inhibition Assays for the Determination of Insecticidal Organophosphates and Carbamates

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PRELIMINARY REMARKS

The work presented in this thesis was carried out under the supervision of Prof. Dr. W. Schwack at the Institut für Lebensmittelchemie, Universität Hohenheim, from March 2002 to July 2007. Parts of it have already been published in international peer-reviewed journals:

(1) Walz I., Schwack W.; Cutinase inhibition by means of insecticidal organophosphates and carbamates, Part 1: Basics in development of a new enzyme assay; *European Food Research and Technology* (2007) 225:593-601

(2) Walz I., Schwack W.; Cutinase inhibition by means of insecticidal organophosphates and carbamates, Part 2: Screening of representative insecticides on cutinase activity; *European Food Research and Technology*, (2008) 226:1135-1143

(3) Walz I., Schwack W.; Cutinase inhibition by means of insecticidal organophosphates and carbamates. Part 3: Oxidation of phosphorothionates by chloroperoxidase from *Caldariomyces fumago*; *Journal of Agricultural and Food Chemistry* (2007) 55:8177-8186

(4) Walz I., Schwack W.; Multienzyme inhibition assay for residue analysis of insecticidal organophosphates and carbamates; *Journal of Agricultural and Food Chemistry* (2007) 55:10563-10571

(5) Walz I., Schwack W. Reflectometric cutinase assay for rapid screening of contaminants and residues of insecticidal organophosphates and carbamates; *Journal of AOAC International*, (2008) 91: in press

or were presented in form of poster presentations at scientific conferences:

(1) Walz I., Fauser T., Schwack W.; Schnelles Screening zum Nachweis und zur Bestimmung insektizider Organophosphate und Carbamate, 32. Deutscher Lebensmittelchemikertag im Rahmen der GDCh-Jahrestagung Chemie, München, October 06 – 11, 2003; *Lebensmittelchemie* (2003) 57:161

(2) Walz I., Schwack W.; Cutinase-Hemmstoffassay für Organophosphorsäureester, 33. Deutscher Lebensmittelchemikertag, Bonn, September 13 – 15, 2004; *Lebensmittelchemie* (2004) 58:136-137

(3) Walz I., Schwack W.; Multi-Enzymassays für insektizide Organophosphate und Carbamate, 35. Deutscher Lebensmittelchemikertag, Dresden, September 18 – 20, 2006; *Lebensmittelchemie* (2007) 61:99

(4) Walz I., Schwack W.; Enzymassays für insektizide Organophosphate und Carbamate, 36. Deutscher Lebensmittelchemikertag, Erlangen-Nürnberg, September 10 – 12, 2007; *Lebensmittelchemie* (2008) 62:64

(5) Walz I., Schwack W.; Reflectometric Cutinase Assay for Screening of Insecticidal Organophosphates and Carbamates, 7th European Pesticide Residue Workshop (EPRW), Berlin, June 01 – 05, 2008

Chapters 2, 3, 4, 5, and 6 of this thesis are in form and content identical with the full publications 1, 2, 3, 4, and 5.

The contributions of the authors to the specified publications were as follows:
All experimental and analytical work, including analyses and interpretations of all obtained data, was performed by **Ingrid Walz**. Moreover, conceptions and the preparations of the original versions of the manuscripts, leading to these publications, originated from her initiative.
Professor Dr. W. Schwack was the thesis-supervisor of this work. He initiated the idea of development of a multienzyme assay for residue analysis of insecticidal organophosphates and carbamates in microtiter-plate format. Moreover, it was his proposal to study the applicability of the Reflectoquant lipase test for pesticide residue analysis. He helpfully advised in clarifying analytical questions, helped to find optimum formulations and form for the English text manuscripts and functioned as contact person throughout the whole publication processes.

ABBREVIATIONS

ε	molar extinction coefficient
λ	wavelength
AChE	acetylcholine esterase
AcOOH	peracetic acid
ARfD	acute reference dose
BCIB	5-bromo-4-chloro-3-indoxyl butyrate
BCIC	5-bromo-4-chloro-3-indoxyl caprylate
BfR	Bundesinstitut für Risikobewertung
c	concentration
ChE	choline esterase
CVUA	Chemisches und Veterinär-Untersuchungsamt
CPO	chloroperoxidase
Da	Dalton
DAD	diode array detector
DCD	dichlorodimedone
DFG	Deutsche Forschungsgemeinschaft
DIN	Deutsches Institut für Normung e.V.
DMSO	dimethyl sulfoxide
ESI	electrospray interface
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
Fsp	*Fusarium solani pisi*
GC	gas chromatography
HPLC	high performance liquid chromatography
i.b.	in the inhibition batch *(concentration)*
ISTD	internal standard
I_{50}	molar inhibitor concentration, necessary to reduce the initial enzyme activity (E_0) to 50% ($E = ½ E_0$)
k_i	inhibition constant / bimolecular inhibitory rate constant
LC	liquid chromatography
LFGB	Lebensmittel- und Futtermittel-Gesetzbuch
LOD	limit of detection
MCD	monochlorodimedone

*m*CPBA	*m*-chloroperbenzoic acid
MeCN	acetonitrile
MeOH	methanol
MRL	maximum residue limit
MS	mass spectrometry
MSD	mass selective detector
m.t.	metric ton
m/z	mass to charge ratio
n	number of determinations
NBT	nitro-blue tetrazolium chloride
PEI	polyethylenimin
PSA	primary secondary amine
RP	reversed phase
QuEChERS	quick, easy, cheap, effective, rugged, and safe
R^2	statistical correlation factor
RHmV	Rückstandshöchstmengenverordnung (German Residue Limit Order)
RSD	relative standard deviation
SAX	strong anion exchanger
SIM	selected ion monitoring mode
SPE	solid-phase extraction
t	time
t-BuOOH	*tert*-butyl hydroperoxide
TLC	thin-layer chromatography
Tris	tris(hydroxymethyl)aminomethane
U	Unit; 1 U: Amount of enzyme required to hydrolyze 1 µmol of the substrate per minute under assay conditions.

TABLE OF CONTENTS

I GENERAL INTRODUCTION 1

1 **Importance of food control during production and subsequent refinement** 1

2 **The role of insecticidal pesticides in food control** 7

2.1 Historic survey on the development of insecticides 7

2.2 Actual relevance of insecticides 9

2.3 Organophosphorus pesticides 10

2.4 Carbamate insecticides 11

2.5 Pesticide residues and their “maximum residue limit” regulations 12

2.6 Degradation of organophosphorus and carbamate insecticides 15

3 **Residue analysis** 16

4 **Studied enzymes - Esterases** 18

4.1 Cutinase 20

4.2 Rabbit liver esterases 20

4.3 Esterase BS2 20

4.4 Enzyme inhibition 21

5 **Scope of this thesis** 23

6 **Cited literature** 24

II CUTINASE INHIBITION BY MEANS OF INSECTICIDAL ORGANOPHOSPHATES AND CARBAMATES. PART 1: BASICS IN DEVELOPMENT OF A NEW ENZYME ASSAY 31

1 **Abstract** 31

2 **Introduction** 31

3 **Materials and methods** 33

3.1 Materials 33

3.2 Instruments 33

3.3 Solutions 33

3.4 Preparation of pesticide standard solutions 34

3.5 Reaction conditions of the cutinase assay 34

3.6 Performance of the cutinase assay 34

3.7 Analysis of measurements 37

4 **Results and discussion** 38

4.1 Determination of enzyme activity 38
4.2 Catalytic activity of cutinase in various solvents 38
4.3 Determination of inhibitory rate constants k_i of paraoxon and chlorpyrifos 42
5 Conclusions 45
6 References 46
III CUTINASE INHIBITION BY MEANS OF INSECTICIDAL ORGANOPHOSPHATES AND CARBAMATES. PART 2: SCREENING OF REPRESENTATIVE INSECTICIDES ON CUTINASE ACTIVITY 49
1 Abstract 49
2 Introduction 49
3 Materials and methods 51
3.1 Preparation of pesticide standard solutions 51
4 Results and discussion 52
4.1 Organophosphorus insecticides 54
4.2 Carbamate insecticides 58
4.3 Detection limits 60
5 Conclusions 60
6 References 61
IV CUTINASE INHIBITION BY MEANS OF INSECTICIDAL ORGANOPHOSPHATES AND CARBAMATES. 3: OXIDATION OF PHOSPHOROTHIONATES BY CHLOROPEROXIDASE FROM *CALDARIOMYCES FUMAGO* 65
1 Abstract 65
2 Introduction 66
3 Materials and methods 67
3.1 Reagents 67
3.2 Solutions 68
3.3 Analytical procedures 69
3.3.1 Principles and conditions of the cutinase assay 69
3.3.2 Oxidation by means of chloroperoxidase 69
3.4 Determination of chloroperoxidase activity under assay conditions 69
3.5 Sample preparation 70

3.6 Thin-layer chromatography (TLC) 71
3.7 High-performance liquid chromatography / mass spectrometry (LC/MS) 71
4 Results and discussion 72
4.1 Optimization of CPO reaction conditions 72
4.2 Application of further peroxides 75
4.3 Influence of chloride on oxidation 75
4.4 Subjection of organophosphorus thions, oxons, and carbamates to the oxidation step 77
4.5 Malathion 79
4.6 Chlorpyrifos-methyl 81
4.7 Analysis of spiked fruit samples 82
5 Literature cited 84
V MULTIENZYME INHIBITION ASSAY FOR RESIDUE ANALYSIS OF INSECTICIDAL ORGANOPHOSPHATES AND CARBAMATES 87
1 Abstract 87
2 Introduction 88
3 Materials and methods 88
3.1 Materials 88
3.2 Enzyme assay 89
3.3 Enzyme solutions 89
3.4 Pesticide standard solutions 90
3.5 Substrate solutions 90
3.6 Extract preparation of plant samples and fruit juice 90
3.7 High-performance liquid chromatography – electrospray mass spectrometry (LC/MS) 90
4 Results and discussion 91
4.1 Selection of suitable enzymes 91
4.2 Solvent tolerance of esterases 93
4.3 Inhibition effects of organophosphate and carbamate insecticides 94
4.4 Oxidation of organophosphorothionates 98
4.5 Sample extraction and cleanup 98
4.6 Pesticide residue analysis by means of multienzyme assay in plant samples 101
5 Literature cited 104

VI REFLECTOMETRIC CUTINASE ASSAY FOR RAPID SCREENING OF CONTAMINANTS AND RESIDUES OF INSECTICIDAL ORGANOPHOSPHATES AND CARBAMATES 107
1 **Abstract** 107
2 **Introduction** 107
3 **Materials and methods** 109
3.1 Materials 109
3.2 Instruments 109
3.3 Solutions 110
3.4 Cutinase microtiter-plate assay 110
3.5 Cutinase strip-assay 110
3.6 Sample preparation 111
3.7 Confirmation of indigo-blue derivative formation 112
4 **Results and discussion** 112
4.1 Microtiter-plate assay 112
4.2 Strip assay 114
5 **Conclusions** 118
6 **Literature cited** 119
VII SUMMARY 121
VIII ZUSAMMENFASSUNG 125