

Analysis of Xanthophylls in Food and their Behaviour during Human Digestion

**Dissertation zur Erlangung des Doktorgrades
der Naturwissenschaften (Dr. rer. nat.)**

**Fakultät Naturwissenschaften
Universität Hohenheim**

Institut für Lebensmittelchemie

vorgelegt von
Jörg Schlatterer

aus *Bietigheim-Bissingen*
2007

Dekan: Prof. Dr. Heinz Breer
1. berichtende Person: PD Dr. Dietmar E. Breithaupt
2. berichtende Person: Prof. Dr. Tilman Grune
Eingereicht am: 30.03.2007
Mündliche Prüfung am: 29.06.2007

Die vorliegende Arbeit wurde am 30.05.2007 von der Fakultät Naturwissenschaften der Universität Hohenheim als "Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften" angenommen.

Berichte aus der Chemie

Jörg Schlatterer

**Analysis of Xanthophylls
in Food and their Behaviour
during Human Digestion**

D 100 (Diss. Universität Hohenheim)

Shaker Verlag
Aachen 2007

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at <http://dnb.d-nb.de>.

Zugl.: Hohenheim, Univ., Diss., 2007

Copyright Shaker Verlag 2007

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Printed in Germany.

ISBN 978-3-8322-6543-4

ISSN 0945-070X

Shaker Verlag GmbH • P.O. BOX 101818 • D-52018 Aachen

Phone: 0049/2407/9596-0 • Telefax: 0049/2407/9596-9

Internet: www.shaker.de • e-mail: info@shaker.de

Preliminary Remarks

The work presented in this thesis was carried out under the supervision of PD Dr. D.E. Breithaupt at the Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany, from May 2004 to March 2007. It was supported by grant of the Konrad-Adenauer-Stiftung e.V., Rathausallee 12, 53757 Sankt Augustin, Germany.

Parts of this work have already been published in international peer-reviewed journals or were presented at international scientific conferences as oral or poster presentations.

Full Papers

1. J Schlatterer, DE Breithaupt: Cryptoxanthin Structural Isomers in Oranges, Orange Juice and Other Fruits. *J. Agric. Food Chem.* **2005**, *53*, 6355-6361.
2. J Schlatterer, DE Breithaupt: Xanthophylls in Commercial Egg Yolks: Quantification and Identification by HPLC and LC-(APCI)MS using a C₃₀ Phase. *J. Agric. Food Chem.* **2006**, *54*, 2267-2273.
3. J Schlatterer, DE Breithaupt, M Wolters, A Hahn: Plasma responses in human subjects after ingestions of multiple doses of natural α -cryptoxanthin: a pilot study. *Br. J. Nutr.* **2006**, *96*, 371-376.
4. J Schlatterer, S Maurer, DE Breithaupt: Quantification of 3*R*,3'*R*-zeaxanthin in plant derived food by a diastereomeric dilution assay applying chiral high-performance liquid chromatography. *J. Chromatogr. A* **2006**, *1137*, 216-222.

Oral Presentations

1. J Schlatterer, DE Breithaupt: Stability of xanthophylls in egg yolks during conventional cooking. 4th International Congress on Pigments in Food, Stuttgart-Hohenheim, Germany, October 9 - 12, 2006.
2. J Schlatterer, DE Breithaupt: Neue Methoden in der Carotinoidanalytik. Jahrestagung des Regionalverbandes Süd-West der Lebensmittelchemischen Gesellschaft, Gießen, Germany, March 5 - 6, 2007.

Poster Presentations

1. J Schlatterer, DE Breithaupt: Cryptoxanthinisomere - Analytik und Vorkommen in Lebensmitteln. 33. Deutscher Lebensmittelchemikertag, Bonn, Germany, September 13 - 15, 2004.
Abstract: *Lebensmittelchemie* **2005**, 29, 18
2. J Schlatterer, DE Breithaupt: Zeinoxanthin and β -cryptoxanthin: Two xanthophylls useful to distinguish fresh orange juice from reconstituted juice? 14th International Carotenoid Symposium, Edinburgh, Great Britain, July 17 - 22, 2005.
3. J Schlatterer, DE Breithaupt: Orange juice - a rich source of β -cryptoxanthin and zeinoxanthin fatty acid esters. 34. Deutscher Lebensmittelchemikertag, Hamburg, Germany, September 19 - 21, 2005; combined with: EURO FOOD CHEM XIII, September 21 - 23, 2005.
Abstract: *Macromolecules and their degradation products in food - physiological, analytical and technological aspects*. T Eklund, M Schwarz, H Steinhart, H-P Thier, P Winterhalter (Eds.), Gesellschaft Deutscher Chemiker e.V., Frankfurt am Main, **2005**, 2, 377-379.
4. J Schlatterer, S Maurer, DE Breithaupt: Quantifizierung von Zeaxanthin in Lebensmitteln mittels Diastereomeren-Verdünnungsanalyse (DIVA) und chiraler HPLC. 35. Deutscher Lebensmittelchemikertag, Dresden, Germany, September 18 - 20, 2006.
Abstract: *Lebensmittelchemie* **2007**, in print

Contents

1	Introduction	1
1.1	Carotenoids	1
1.1.1	General Information	1
1.1.2	Biosynthesis	3
1.1.3	Physiological Importance	3
1.1.4	Analysis of Carotenoids	4
1.2	Aims of the Study	6
1.3	Literature Cited	7
2	Cryptoxanthin Structural Isomers in Oranges, Orange Juice and Other Fruits	11
2.1	Abstract	11
2.2	Introduction	12
2.3	Materials and Methods.	14
2.4	Results and Discussion	17
2.5	Acknowledgement	23
2.6	Literature Cited	24
3	Xanthophylls in Commercial Egg Yolks: Quantification and Identification by HPLC and LC-(APCI)MS using a C₃₀ Phase	27
3.1	Abstract	27
3.2	Introduction	28
3.3	Materials and Methods	30
3.4	Results and Discussion	33
3.5	Acknowledgement	39
3.6	Literature Cited	39
4	Plasma responses in human subjects after ingestions of multiple doses of natural α-cryptoxanthin: a pilot study	43
4.1	Abstract	44
4.2	Introduction	44
4.3	Experimental design	46
4.3.1	Materials	46
4.3.2	Subjects	46
4.3.3	Study design	46
4.3.4	Preparation of supplements	47
4.3.5	Plasma sample preparation	48
4.3.6	Analysis and chromatography	48

Contents

4.4	Results	49
4.5	Discussion	51
4.6	Acknowledgements	52
4.7	References	53
5	Quantification of 3<i>R</i>,3'<i>R</i>-zeaxanthin in plant derived food by a diastereomeric dilution assay applying chiral high-performance liquid chromatography	55
5.1	Abstract	56
5.2	Introduction	56
5.3	Experimental	57
5.3.1	Chemicals and materials	57
5.3.2	Isolation of reference compounds	58
5.3.3	Sample preparation	58
5.3.4	High performance liquid chromatography with diode array detection (HPLC/DAD)	59
5.3.5	Calibration and quantification	60
5.3.6	Limits of detection (LOD) and quantification (LOQ)	60
5.4	Results and discussion	61
5.4.1	Optimization of the HPLC/DAD method	61
5.4.2	Application of <i>meso</i> -zeaxanthin as internal standard	62
5.4.3	Accuracy	63
5.4.4	Application of DIDA to quantification of 3 <i>R</i> ,3' <i>R</i> -zeaxanthin in plant derived food	64
5.5	Conclusions	66
5.6	Acknowledgment	66
5.7	References	66
6	Summary	69
7	Zusammenfassung	73