

Berichte aus der Chemie

Benno F. Zimmermann

**Proanthocyanidins in barley and malt analyzed
by pressurized liquid extraction, solid-phase
extraction and HPLC**

D 98 (Diss. Universität zu Bonn)

Institut für Ernährungs- und Lebensmittelwissenschaften,
Fachgebiet Lebensmittelchemie I

Shaker Verlag
Aachen 2005

Bibliographic information published by Die Deutsche Bibliothek

Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the internet at <http://dnb.ddb.de>.

Zugl.: Bonn, Univ., Diss., 2005

Copyright Shaker Verlag 2005

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 3-8322-4723-8

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 • eMail: info@shaker.de

Proanthocyanidins in barley and malt analyzed by pressurized liquid extraction, solid-phase extraction and HPLC

BENNO F. ZIMMERMANN

Aim of this work is to develop a convenient method for the determination of proanthocyanidins in barley and malt. In a second step this method is applied to 61 barley and malt samples of different varieties, proveniences and growing years.

In the brewing industry proanthocyanidins are of special interest. Main activities of the proanthocyanidins are related to undesired formation of chill haze and to the positively valued augmentation of the antioxidative capacity of beer. The detailed mechanisms are still under discussion. It is clear, that the positive and negative effects of proanthocyanidins depend on their quantity and quality. So determination of the sum of proanthocyanidins does not give sufficient information to discuss their action.

Selective analysis of proanthocyanidins is time and labour consuming. Especially sample preparation requires a lot of manual work. Thus, this work presents a fully automated and therefore fast and reliable method for sample preparation of barley and malt followed by HPLC-UV. The here described method bases on extraction using pressurized liquid extraction (PLE). Essentially it is a static solid/liquid extraction with high pressure and eventually high temperature in stainless steel extraction cells. Using the *Accelerated Solvent Extractor* (ASE) by Dionex, up to 24 samples in a series can be extracted automatically.

The second step of sample preparation is clean-up by solid-phase extraction (SPE). For the first time, commercially available polyamide cartridges are used for proanthocyanidins. SPE is accomplished automatically by a liquid handling robot, the *Automated Sample Preparation with Extraction Cartridges*-device (ASPEC) by ABIMED and Gilson. The ASPEC takes the extracts from the ASE and carries out the complete SPE procedure. The resulting solution is ready to inject into the HPLC, that separates and quantifies six proanthocyanidins and catechin in one run of 90 min.

Sample extraction and extract clean-up are coupled online. This coupling was developed by ABIMED and Dionex and is tested and established under real laboratory conditions for the first time. Within 24 hours 16 samples can be analyzed, about 6 hours of manual work is needed. Recovery of the overall method is 70 - 91 %, reproducibility is 2.3 - 6.4 %. With this method 61 barley and malt samples of the growing years 1998 - 2001 from four locations including summer and winter barley varieties are analyzed. The annual and local variation of absolute contents of proanthocyanidins appears to interfere varietal differences, so differentiation between the samples is not possible. The ratio of several pairs of proanthocyanidins (the relative quantitative polyphenolic fingerprint) is characteristic for the variety and can be used to control authenticity.

In addition, the here presented method is supposed to be applicable to samples taken during the brewing process and to other food samples. Two examples are given: monitoring beer filtration and analyzing proanthocyanidins in the seeds of the açai fruit from northern Brazil. Since proanthocyanidins are discussed to have positive effects on health, there is a market for functional food with naturally high or enriched content of proanthocyanidins. Hence it is necessary to control such products.