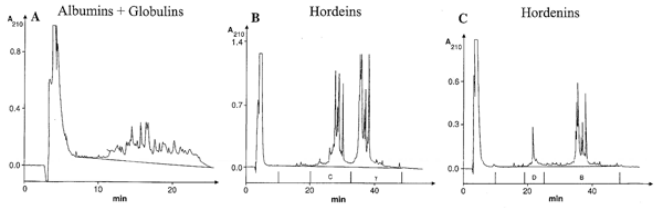
Application Number	124290
Method	HPLC
Results	



Legend:

Protein fractions in nacked barley [Lawina]:

figure A: albumins + globulins (total)
figure B: C-hordeins; γ-hordeins; total hordeins (prolamins)
figure C: D-hordenins; B-hordenins; total hordenins (glutelins)

Substances	albumins; globulins; hordeins; hordenins

Product(s)

Phase	REF	Webshop
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Matrix

Sample(s)

Protein extraction from 100 mg flour samples (naked barley):

1. step: 1 mL extraction with solution A (phosphate buffer, pH 7.6) - two times; vortexing for 2 min and shaking at RT (ambient) for 10 min; sample was centrifuged

for 20 min; supernantants filled up to 2.0 mL => A: fraction of albumins + globulins

2. step: the pellet was extracted with 0.5 mL solution B (60 % ethanol, v/v) - three times; vortexing for 2 min and shaking at RT (ambient) for 10 min; sample was centrifuged for 20 min; supernantants filled up to 2.0 mL => B: fraction of hordeins (prolamins)

3. step: the remaining pellet was extracted with 1 mL solution C (50 % 1-propanol (v/v) and 0.05 mol/L Tris/HCl (pH 7.5) containing 2 mol/L urea as well as 1 % dithioerythriol) under N2 - two times; vortexing for 2 min and shaking at 60 °C for 30 min; sample was centrifuged for 20 min; supernantants filled up to 2.0 mL => C: fraction of hordenins (glutelins)

All fractions are filtered with 0.45 µm cellulose acetate filter (e.g. CHROMAFIL Xtra CA-45/25, REF 729227) before HPLC injection.

Conditions	Eluent A: 0.1 % TFA in water (v/v) Eluent B: 0.1 % TFA in acetonitrile (v/v) Flow rate: 1.0 mL/min Temperature: 50 °C Injection volume 1 (albumins + globulins): 150 µL Gradient 1: 20-60 % B in 20 min, 60-90 % B in 1 min, 90 % B for 5 min, 90-20 % B in 11 min Injection volume 2 (hordeins, hordenins): 50 and 100 µL Gradient 2: 24-56 % B in 50 min, 56-90 % B in 1 min, 90 % B for 5 min, 90-24 % B in 11 min
Detection	UV, 210
Note	

Author	Pawelzik, E.; Wieser, H.; Eggert, K.
Source	Eur Food Res Technol (2010) 230, 893-902
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AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17 • D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de