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Gliadin Extraction for Reversed-phase High Performance Liquid Chromatography: An assesment of Methodology

By

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With 2 Figures

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Summary

GALLESCHI L., GRILLI, I., CAPOCCHI, A. & MORONI P. 1993. Gliadin extraction for reversed-phase high performance liquid chromatography: an assesment of methodology. – Phyton (Horn, Austria) 33 (1): 7–14, 2 figures. – English with German summary.

Gliadins from *Triticum durum* DESF. and *Haynaldoticum sardoum* MELETTI & ONNIS *(Poaceae)* were directly extracted with 70% ethanol or preextracted with 0.5 M NaCl to remove albumins and globulins. The extracts were chromatographed on a reversed-phase column and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Both analyses showed that the gliadin fraction directly extracted with ethanol contains additional early-eluting proteins upon reversed-phase high performance liquid chromatography, and low M_r electrophoresis bands. The early-eluted proteins were collected, concentrated and electrophoresed. Results showed that they are salt soluble protein contaminants of gliadin. We thus advise that cereal meals should be pre-extracted with NaCl prior to prolamin analysis by reversed-phase high performance liquid chromatography.

Zusammenfassung

GALLESCHI L, GRILLI I., CAPOCCHI A. & MORONI P. 1992. Gliadinextraktion für HPLC-Chromatographie: Ein Beitrag zur Methodik. – Phyton (Horn, Austria) 33 (1): 7–14, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Gliadine wurden aus *Triticum durum* DESF. und *Haynaldoticum sardoum* MELETTI & ONNIS *(Poaceae)* entweder direkt mit 70% Ethanol extrahiert oder mit 0,5 M NaCl vorextrahiert, um Albumine und Globuline zu entfernen. Die Extrakte wurden auf einer reversed-phase Säule chromatographiert und dann einer SDS-PAGE unterworfen. Beide Analysen ergaben, daß die Gliadinfraktion, welche direkt mit Ethanol extrahiert wurde, zusätzlich leicht eluierbare Proteine über HPLC und niedrige M_r Elektrophoresebanden enthielten. Die leicht eluierbaren Proteine wurden gesammelt, auskonzentriert und einer Elektrophorese zugeführt. Die Ergebnisse zeigten, daß es sich um salzlösliche Protein-Verunreinigungen von Gliadin handelt. Deshalb empfehlen wir, Getreidemehl vor der Prolamin-Analyse durch HPLC mit NaCl vorzuextrahieren.

Introduction

After starch, storage proteins are the most abundant reserve in the endosperm of cereals. They show different concentrations according to the species and to the conditions of cultivation. Cereal proteins are generally classified (OSBORNE 1885) as albumins (water soluble), globulins (soluble in dilute salt solutions), prolamins (soluble in acqueous alcohols) and glutelins (dilute acid- or alkali-soluble). The prolamin group is characterized by specific names deriving from the species of seed under consideration. Gliadin, the wheat prolamin, constitutes about one-half of the gluten and consequently of the endosperm proteins (PAYNE 1986). It is known to influence breadmaking (FINNEY & al. 1982) and it has been utilized for varietal identification of closely-related wheats by polyacrylamide gel electrophoresis (PAGE) (WRIGLEY & al. 1981) and by reversed-phase high performance liquid chromatography (RP-HPLC) (BURNOUF & BIETZ 1987, SCANLON & al. 1989).

During germination storage proteins are degraded in order to supply amino acids to a growing embryo. Different classes of proteases are involved in the proteolytic steps (SHUTOV & VAINTRAUB 1987). Since we had been studying proteases and gliadins during seed germination of *Haynaldoticum sardoum* MELETTI & ONNIS, a spontaneous wheat (GALLESCHI & al. 1989, CAPOCCHI & al. 1992), we were worried that direct extraction of gliadins with aqueous alcohol solutions (BIETZ & al. 1984, LOOKART & ALBERS 1988, VENSEL & al. 1989) could lead to albumins and globulins contaminating the gliadin fraction with a consequent possible alteration in the pattern of gliadin degradation.

This paper compares two methods for extracting gliadins from a cultivated *(Triticum durum)* and a wild *(Haynaldoticum sardoum)* wheat in order to separate them using RP-HPLC. The protein fractions and the HPLC eluates were also monitored by sodium dodecyl sulfate (SDS)-PAGE in order to characterize them and to confirm the identity of the proteins on HPLC chromatograms.

Materials and Methods

Plant material

Caryopses of two lines of *Haynaldoticum sardoum* MELETTI & ONNIS (a solid stem line, Culmo Pieno and a hollow stem line, Culmo Vuoto) (MELETTI & ONNIS 1975) and of *Triticum durum* DESF. cv. Cappelli were utilized.

Protein extraction

After surface sterilization, the hand isolated endosperms were homogenized and defatted in cold acetone (20° C) with a Polytron homogenizer. The meals were pre-extracted ×3 with 0.5 M NaCl for 1 hr at room temperature and then gliadins were extracted ×3 with 70% ethanol (EtOH). An alternative method was to directly extract ×3 the meals with 70% EtOH. The mixtures were centrifuged (4000 rpm, 10 min, 20° C) and the three supernatants for each protein class collected, pooled and lyophilized. A solvent: flour ratio of 20 : 1 (v/w) was utilized for NaCl and EtOH extractions.

RP-HPLC

A Waters chromatographic system, consisting of a Waters 820 chromatography work station and a UV detector, was used. RP-HPLC, adapted from BIETZ 1983, was performed with a Nucleosil 300-7 C_{18} (Macherey-Nagel, Düren, Germany) column (250 × 4 mm). After solubilization with 20% acetonitrile (CH_3CN) containing 0.05% trifluoroacetic acid (TFA) and filtration on 0.45 µm filters, samples (1 mg/mL) were injected with a 500 µL-loop. Proteins were eluted at a flow rate of 1 mL/min by a linear gradient formed by mixing solvent A (H_2O with 0.05% TFA) and solvent B (CH_3CN with 0.05% TFA) in which solvent B had increased from 25% of the mixture to 65% over 30 min. The column was maintained at 70° C with a column heater (CH-30, Eppendorf, U.S.A.) during all the chromatographic separations. Elution was monitored at 210 nm and the eluates, collected manually, were lyophilized prior to further investigation. The protein content of the fractions was determined according to BENSADOUN & WEINSTEIN 1976.

SDS-PAGE

Gels, according to PAYNE & CORFIELD 1979, containing 17% acrylamide were utilized to analyse the extracts. Protein samples were resuspended in 0.062 M Tris-HCl buffer, pH 6.8, containing 2% SDS and 5% 2-mercaptoethanol. They were heated in boiling water for 3 min. Low M_r calibration kits were provided by Pharmacia. Gels were run for 19 hr at 8 mA (constant current) per slab and were stained with Coomassie Brilliant Blue R solution according to FULLINGTON & al. 1983 and destained with isopropanol : acetic acid : water (10 : 10 : 80).

Results and Discussion

SDS-PAGE

The electrophoretic separation of the gliadins extracted from *Triticum durum* and *Haynaldoticum sardoum* is shown in Fig. 1. These storage proteins were extracted sequentially or directly with 70% EtOH. The former procedure initially consisted in removing albumins and globulins

with 0.5 M NaCl and then treating the residue with 70% EtOH. Comparing the electrophoretic patterns of the gliadins revealed the presence of some bands with a M_r between 28 000 and 14 000 in the EtOH extracts (Fig. 1 a_2, b_2 and c_2) which were absent in the sequentially extracted samples (Fig. 1 a_1, b_1 and c_1). We were able to demonstrate that these bands were components of the salt soluble fractions of Culmo Vuoto, Culmo Pieno and *durum* wheat (Fig. 1 a_3, b_3 and c_3).

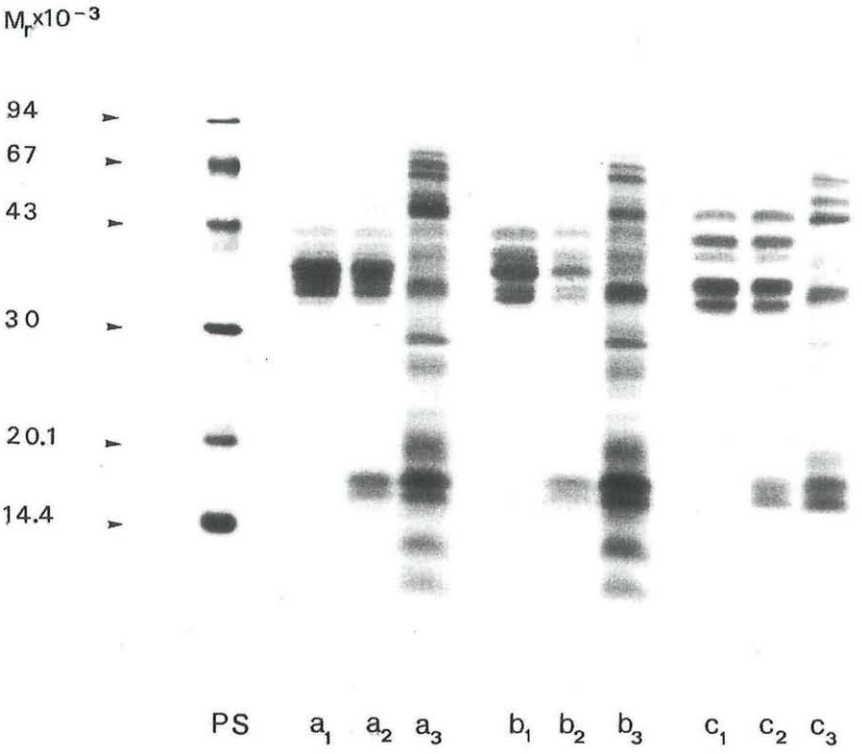

Fig. 1. SDS-PAGE of differently extracted gliadins and albumin plus globulin fractions. a, Culmo Vuoto; b, Culmo Pieno; c, *durum* wheat. PS, protein standards; 1, gliadins extracted with EtOH after pre-extraction with NaCl; 2, gliadins directly extracted with EtOH; 3, albumins plus globulins.

RP-HPLC of gliadins

RP-HPLC was first used by BIETZ 1983 to fractionate storage proteins of wheat and maize seeds. This and the PAGE technique are currently the most widely used for routine varietal identification of wheat samples. HPLC has some advantages over PAGE such as the possibility to automate

the analyses and to save time (MARCHYLO & al. 1988). HPLC has recently been utilized to isolate and characterize oat and maize prolamins (PERNOLLET & al. 1989, WILSON 1991).

Fig. 2 shows the RP-HPLC separation of gliadins extracted from *Haynaldoticum sardoum* and *durum* wheat. The resulting gliadin solutions sequentially or directly extracted were subjected to RP-HPLC for each plant material and for each type of extraction. The chromatograms generally showed, 35—40 peaks, depending on the species and type of extraction. A similar result was obtained for HPLC separation of some wheat varietals (BURNOUF & BIETZ 1987). However, the chromatograms of gliadins extracted directly with EtOH appeared more complex and revealed some early-eluted peaks, as is shown by the overlaps obtained with the two types of extraction (Fig. 2 a, b and c). These peaks had an R_t between 10.0 and 12.3, 9.5 and 12.0, 10.8 and 11.9 for Culmo Vuoto, Culmo Pieno and *durum* wheat respectively. The late-eluted peaks of each chromatogram showed minor quantitative differences in both types of extractions for the three plant materials studied (Fig. 2 a, b and c).

SDS-PAGE of protein fractions from RP-HPLC

Selected chromatographic regions (α, β and δ in Fig. 2 a, b, c) of the two types of extractions for each plant material were collected, concentrated and subjected to SDS-PAGE. The direct EtOH extracts, compared to those pre-extracted with NaCl, were shown to contain in addition proteins that gave bands which were characteristic of the salt soluble fraction of the three materials studied (Fig. 2 a′, b′, c′). This evidence clearly shows the contamination of the gliadin fraction from *Haynaldoticum sardoum* and *durum* wheat by albumins and globulins following the direct extraction of the meals with EtOH.

Conclusion

Since RP-HPLC is utilized for differentiating wheat varieties on the basis of the gliadin pattern (which reflects both pedigree and taxonomic relationships), any contamination by salt soluble proteins, whose synthesis may be more variant than that of gliadins (WRIGLEY & al. 1982), might jeopardize its use. Pre-extracting the meals with NaCl could be particularly important for those seeds with a high content of salt soluble proteins, where there might be a danger of seriously contaminating the gliadin fraction. Furthermore, the utilization of RP-HPLC to isolate and characterize gliadin fractions is severely hampered by albumin plus globulin contamination both in extractions from dry and germinating seeds. It seems impossible to follow a storage protein breakdown with dry and germinating seeds, since both gliadins and salt soluble proteins might be subjected to hydrolysis. Thus we suggest using NaCl solutions to pre-

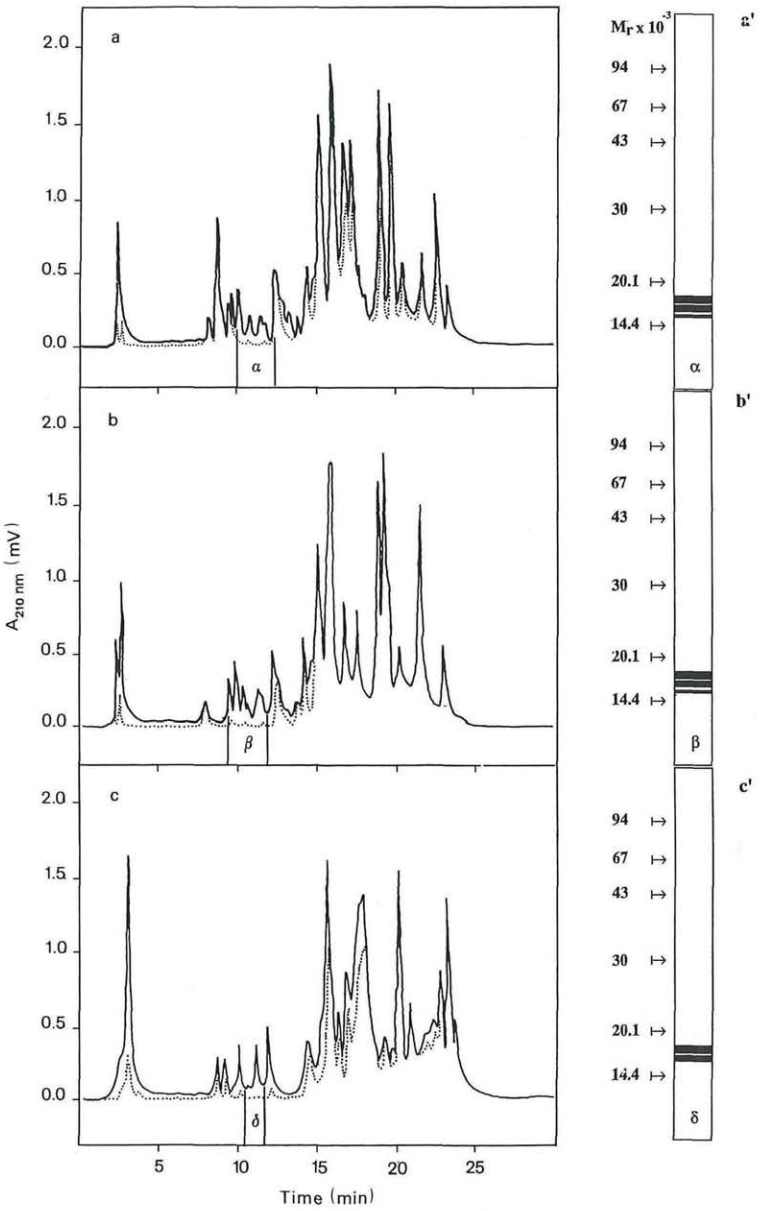

Fig. 2. Gliadin chromatograms obtained by RP-HPLC of samples extracted in different ways and SDS-PAGE of purified fractions. a, a′, Culmo Vuoto; b, b′ Culmo Pieno; c, c′ *durum* wheat. ——, gliadins directly extracted with EtOH; ....., gliadins extracted with EtOH after pre-extraction with NaCl. α, β and δ collected fractions from direct extraction with EtOH.

extract meals from wheat and other related species in order to study the characterization of gliadins by RP-HPLC.

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