

# Utilization of Cation-Exchange FPLC Technique for Partial Purification of Wheat Gliadin

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**Abstract:** Cation-exchange chromatography was performed in conjunction with fast protein liquid chromatography (FPLC) on 70% ethanol soluble crude wheat gliadin by using strongly HiPrep SP Sepharose HP 16/10 column. Two different buffers were used for column equilibration and protein binding, buffer A (2 M urea; 0.05 M sodium acetate pH 4.6) and buffer B (2 M urea; 0.05 M sodium acetate; 0.2 M NaCl pH 4.6), respectively. Wheat gliadin sample was dissolved in buffer A and column was equilibrated in respective buffers, after binding eluted via NaCl concentration gradient of buffer B (2 M urea; 0.05 M sodium acetate; 0.2 M NaCl pH 4.6). Cation-exchange chromatogram was segregated into total 14 peaks.

**KEYWORDS:** Chromatography, Column, Equilibration, Wheat gliadin, Gradient

## I. INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important arable crops because of its wide spread food applications. Wheat grain is a staple food in most of the world and the special rheological properties of gluten allows wheat flour to be processed into a multitude food as well as non-food products [1]. It is well established that the bread making potential of wheat flour depends largely on its gluten proteins [2]. Gluten is a complex of heterogeneous protein components that form a three-dimensional network of linearly cross-linked glutenin subunits and gliadin components through hydrogen, hydrophobic, and disulfide bonds [3, 4], which significantly affect the technological quality of flour and dough, especially the elasticity and viscosity of dough during the bread-making process [5]. Aggregated glutenins and gliadins are able to form large complexes (gluten) of approximately several million Daltons. Gliadins are mainly monomeric proteins with molecular weights (MWs) around 28,000–55,000 and can be classified according to their different primary structures into the  $\alpha/\beta$ -,  $\gamma$ - and  $\omega$ -type. Disulphide bonds are either absent or present as intra-chain crosslinks. The glutenin fraction comprises aggregated proteins linked by interchain disulphide bonds. After reduction of disulphide bonds, the resulting glutenin subunits show solubility in aqueous alcohols similar to gliadins. Based on primary structure, glutenin subunits have been divided into the high-molecular-weight (HMW) subunits (MW 67,000–88,000) and low-molecular-weight (LMW) subunits (MW 32,000–35,000) [5, 6]. The HMW subunits account for about 25–35% of the total glutenins, the remainder comprising LMW subunits and influence the baking quality. Nevertheless, low *M*<sub>w</sub> glutenin subunits and gliadin polypeptides also play important roles in influencing rheological properties and baking quality of wheat flours [7]. Each gluten protein type consists of two or three different structural domains; one of them contains unique repetitive sequences rich in glutamine and proline. Native glutenins are composed of a backbone formed by HMW subunit polymers and of LMW subunit polymers branched off from HMW subunits. Non-covalent bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds are important for the aggregation of gliadins and glutenins and implicate structure and physical properties of dough [6]. Under this investigation we reported the use of FPLC cation exchange column (HiPrep SP Sepharose HP 16/10 column), for the separation of wheat gliadins under acidic conditions in quantities large enough for further experimentation and the resulted chromatograms' applicability for varietal identification were reviewed.

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## II. MATERIAL AND METHODS

### Materials

Commonly cultivated Indian wheat variety HW2004 was tempered and milling was done to an extraction rate of 65% using Chopin laboratory mill (Model CD1, Villeneuve la Garenne, France).

### Sample preparation

Wheat flours were defatted according to MacRitchie [8] by successive extraction with chloroform. Gluten was extracted on Glutomatic (Model GM 2200, Perten Instruments) from defatted flour as per standard ICC method. Resulted gluten was dried by freeze-drying and powdered, subjected to gliadin extraction by using 70% (v/v) as per modified Osborne [9] method and freeze-dried. The extraction was repeated thrice. The supernatant was subjected to rotary evaporator at 30 °C to evaporate ethanol to recover the gliadins. For chromatography, gliadin sample was weighed, dissolved in respective buffer and before injection sample was filtered through 0.22 µm Millipore syringe filter.

### Ion-exchange chromatography on HiPrep SP Sepharose HP 16/10 column on FPLC system

Procedure was applied according to Booth and Ewart [10] with certain modifications. The same strongly cationic column was equilibrated with buffer A (2 M urea; 0.05 M sodium acetate pH 4.6 with acetic acid) and 100 mg gliadin sample was injected into the column. Elution was completed using NaCl concentration gradient of buffer B (2 M urea; 0.05 M sodium acetate; 0.2 M NaCl pH 4.6 with acetic acid) with a flow rate of 1 ml/min. Buffer B was run for more 40 ml to elute remaining proteins in the column. The fractions were collected and dialyzed with 2 % acetic acid.

### Electrophoresis

The molecular composition of eluted gliadin fractions after dialysis (10 µl) were estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [11].

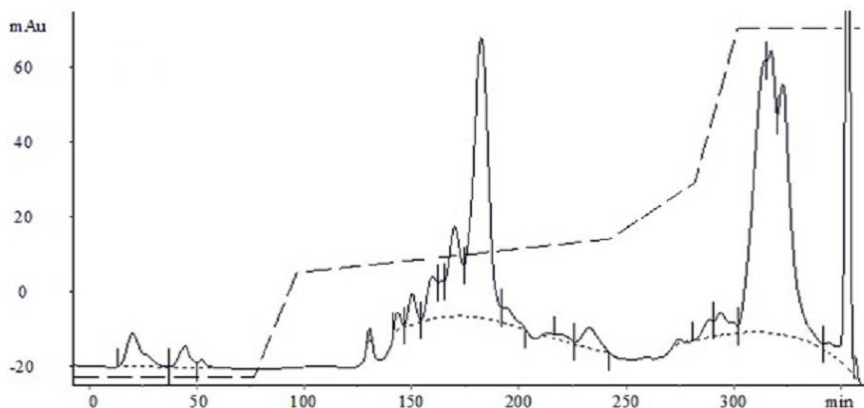
### Data Analysis

Data analysis was carried out using SPSS software version 16.0 (SPSS Inc.). The mean comparison of area % of various peaks were evaluated using Duncan's multiple range test at a statistical significance of  $p < 0.05$ . All the tests were carried out in triplicates.

## III. RESULTS AND DISCUSSION

### Fractionation of gliadins and identification of eluted fractions

**Figure 1** depicts the results estimated under mild acidic conditions by utilizing a preparative scale HiPrep SP Sepharose HP column on FPLC with different gradient composition. Out of 100 mg sample loaded onto the column, around 1-2 % of protein was eluted unbound from the column, although about 20 % injected sample eluted only with the 1M NaCl solution which was bound very strongly to the column. **Table 1** representing the mean values of area % of various 14 peaks produced from the chromatogram resulted after fractionation of gliadin. The relative proportion of gliadin fractions was denoted as the area % of respective peaks. This procedure resulted in considerably good resolution after separation of the peaks.



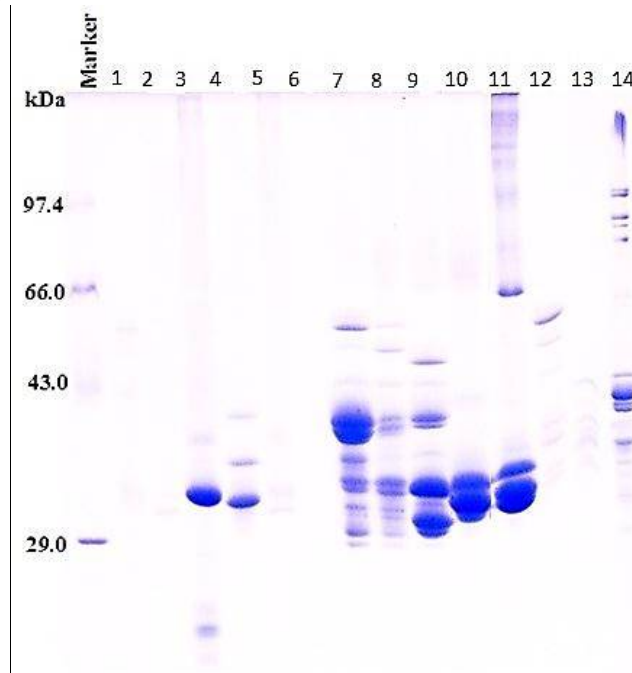
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**Figure 1** Partial fractionation of gliadin sub-fractions from common Indian wheat variety HW 2004 by utilizing FPLC. Experimental conditions -buffer A 2 M urea; 0.05 M sodium acetate pH 4.6 with acetic acid; buffer B 2 M urea; 0.05 M sodium acetate; 0.2 M NaCl pH 4.6 with acetic acid at a flow rate of 1ml/min. The baseline is indicated by the dotted line and gradient is indicated by the dashed line.

SDS-PAGE gels of five eluted fractions are shown in **Figure 2**. Under specific gradient condition all the subgroups of gliadin were eluted in a particular sequence of elution but some peaks eluted the mixed subgroups of gliadins. The order of elution of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ - gliadin was quite different by using procedure I and procedure II as in procedure II almost same kinds of proteins got eluted in more or less quantity at different retention time. Initially eluted unbound fraction was found devoid of gliadins and when the NaCl gradient was initialized, the sequence was followed by  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ - gliadin. The whole remaining and strongly bound gliadin was eluted in the end, under 100 % NaCl buffer solution. **Figure 3** is showing the SDS-PAGE of eluted fractions.



**Figure 2** SDS PAGE patterns of separated sub-fractions of gliadin.

### *Effect of quantity injected*

**Table 1** is showing the resulted mean of area % of chromatographic peaks after loading gliadin sample in varied quantity as 100 mg, 200 mg and 500 mg crude gliadin by using same buffer A and fractionated by incorporating the five step NaCl concentration gradient at a flow rate of 1 ml/ min. With the increase in the sample concentration, the area % of respective peaks was differed significantly very less or not differed significantly. Although only peak 6, 13 and 14 showed significant differences when sample concentration was increased. Increase and decrease in area % of individual peaks in each run with different quantities did not follow the same trend as the quantity increased. Due the increased quantity of sample minor change in the relative area % and resolution of the peaks was also noticed. SDS-PAGE patterns represented that the peaks under the chromatogram related to particular kind of gliadin were remained same.

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Peak No.	100 mg	200 mg	500 mg
1	0.75±0.33 <sup>a</sup>	0.66±0.21 <sup>a</sup>	0.69±0.32 <sup>a</sup>
2	1.08±0.35 <sup>a</sup>	1.05±0.19 <sup>a</sup>	0.99±0.17 <sup>a</sup>
3	2.87±0.54 <sup>a</sup>	2.86±0.19 <sup>a</sup>	2.71±0.17 <sup>a</sup>
4	0.91±0.27 <sup>a</sup>	0.57±0.28 <sup>a</sup>	0.76±0.21 <sup>a</sup>
5	6.21±0.72 <sup>c</sup>	7.26±0.16 <sup>ab</sup>	6.73±0.12 <sup>abc</sup>
6	26.22±0.61 <sup>c</sup>	21.01±0.13 <sup>a</sup>	22.04±0.26 <sup>b</sup>
7	1.33±0.37 <sup>a</sup>	1.61±0.37 <sup>a</sup>	1.47±0.37 <sup>a</sup>
8	0.68±0.19 <sup>a</sup>	0.39±0.16 <sup>a</sup>	0.38±0.22 <sup>a</sup>
9	1.93±0.15 <sup>a</sup>	2.81±0.18 <sup>b</sup>	2.51±0.43 <sup>b</sup>
10	1.19±0.31 <sup>a</sup>	2.32±0.07 <sup>b</sup>	2.45±0.07 <sup>b</sup>
11	2.03±0.21 <sup>a</sup>	2.09±0.08 <sup>ab</sup>	2.11±0.20 <sup>ab</sup>
12	20.45±0.41 <sup>a</sup>	22.68±0.91 <sup>a</sup>	23.24±0.71 <sup>c</sup>
13	14.10±0.79 <sup>c</sup>	11.79±0.27 <sup>b</sup>	11.85±0.23 <sup>a</sup>
14	19.42±1.20 <sup>a</sup>	22.90±0.65 <sup>b</sup>	22.07±0.60 <sup>a</sup>

Means with the same letter are not significantly different at P<0.05

**Table 1** Area % of peaks eluted after loading varied quantity of gliadin proteins

## IV. CONCLUSIONS

HighPrep SP-sepharose HP column on FPLC fairly separated gliadin sub-fractions by using preparative scale chromatography. Results demonstrated that in a cation-exchange system acidic pH levels are suitable for fractionation of gliadin. Increase in concentration of gliadin sample to be loaded onto the column gave rise to noticeably resolved peaks. Very less or no significant difference between the area % of peaks was noticed in runs with different concentration.

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