Analytical Chemistry: An Indian Journal

ISSN: 0974-7419

Opinion| Vol22Iss8

Rapid Screening Methods for Wheat Lines with Modified *Gliadin* Compositions: MALDI-TOF-MS and RP-HPLC Comparison

Erick Straw*

Managing Editor, Analytical Chemistry: An Indian Journal, Hampshire, UK

***Corresponding author:** Erick Straw, Managing Editor, Analytical Chemistry: An Indian Journal, Hampshire, UK, E-mail: janalchem@theresearchpub.com

Received: 05-Aug-2022, Manuscript No. TSAC-22-76235; **Editor assigned:** 07-Aug-2022, PreQC No. TSAC-22-76235 (PQ); **Reviewed:** 21-Aug-2022, QC No. TSAC-22-76235 (Q); **Revised:** 23-Aug-2022, Manuscript No. TSAC-22-76235 (R); **Published:** 27-Aug-2022; DOI: 10.37532/0974-7419.2022.22(8).212

Abstract

A complex set of flour proteins called wheat *gliadins* can cause celiac disease and severe food sensitivities. As a result, new wheat lines with lower immunogenic potential are being created using mutation breeding and biotechnology techniques. The creation of quick, high-throughput technologies that can be applied as a first step in choosing lines with altered gliadin content is essential to these efforts. In this study, we improved Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) methods for the isolation of *gliadins* from *Triticum aestivum cv.* Chinese Spring (CS). Using the entire set of *gliadin* gene sequences recently obtained from this cultivar as well as a collection of aneuploid lines, we assessed the quality of the generated profiles in CS. By using MALDI-TOF-MS, the gliadins were divided into 13 peaks. There were multiple peaks of-or-gliadins, which are plausible targets for efforts to reduce the immunogenicity of flour and include a lot of celiac disease epitopes. However, other peaks, including one with as many as 12 distinct gliadins, contained several- and -*gliadins.* In contrast, 28 *gliadin* peaks were obtained from the separation of proteins using RP-HPLC, including 13 peaks that contained *gliadins* and 8 peaks that included gliadins. Even while RP-HPLC was able to separate gliadins more well than MALDI-TOF-MS, it was not possible to connect specific peaks to specific protein sequences. MALDI-TOF-MS and RP-HPLC both successfully separated gliadins. Although MALDI-TOF-MS is quicker and may be advantageous in investigations that focus on particular gliadins, an efficient technique that can be used more widely to identify changes in *gliadin* composition is RP-HPLC.

Keywords: Proteins; Spectrometry; Biotechnology

Introduction

Triticum aestivum L., also known as wheat, is a key staple cereal grain that people consume around the world and a significant source of protein in the diet. About 70% of the total grain protein is made up of gluten proteins. *Gluten* proteins, which are made up of 70 proteins-100 distinct proteins, have a significant impact on how well flours combine dough and determine whether they are suitable for creating bread. Additionally, some *gluten* proteins cause serious illnesses in people, such as food allergies and Celiac Disease (CD). Traditionally, *gluten* proteins have been divided into *glutenins* and *gliadins*. High-molecular-weight *glutenin* subunits make up polymeric *glutenins*. Disulfide linkages connect the High-Molecular-Weight Glutenin Subunits (HMW-GS) and Low-Molecular-Weight Glutenin Subunits (LMW-GS). About 40% of the *gluten* proteins are monomeric proteins called *gliadins*, and they are particularly immunogenic. Based on their electrophoretic mobility in acid polyacrylamide gel electrophoresis, *gliadins* are often divided into a complex classes i.e. *gliadin*. Based on the size and repeating motifs, the *gliadins* are further split into *-5 gliadins* and *-1,2 gliadins*. While *-5 gliadins* contain several epitopes that cause the food allergy, Wheat-Dependent Exercise-Induced Anaphylaxis (WDEIA), *-1, 2 gliadins* have clusters of epitopes that are active in celiac disease.

In hexaploid wheat, the genes encoding the and-*gliadins* are located at the *Gli-1* loci on the short arms of the group 1 homologous

Citation: Straw E. Rapid Screening Methods for Wheat Lines with Modified *Gliadin* Compositions: MALDI-TOF-MS and RP-HPLC Comparison. Anal Chem Ind J. 2022;22(8):212.

chromosome and are connected to the LMW-GS, whereas the genes encoding the gliadins are located at the *Gli-2* loci on the short arms of the group 6 chromosomes. A full set of *gliadin* and *LMW-GS* genes, including *47-gliadin*, *14-gliadin*, *5-gliadin*, *19-gliadin*, and 17 *LMW-GS* genes, was assembled and annotated. Recent genome sequencing efforts in the reference wheat Chinese Spring (CS) have revealed the complexities of the genome. Of these, full-length proteins are encoded by the genes encoding *26-gliadins*, *11-gliadins*, *2 LMW-GS*, *7 LMW-GS*, and *10 LMW-GS*. Further transcriptomic research indicated large variations in gene expression levels within the families. Additionally, linked specific protein spots in a total protein extract from CS flour to 16 of *26-gliadin*, 10 of 11, one of two-*gliadin*, and six of seven-*gliadin* genes using Two-Dimensional Gel Electrophoresis (2-DFE) coupled with tandem Mass Spectrometry (MS). In transcriptome studies, the majority of the genes that weren't linked to protein spots encoded proteins that were either highly comparable to other proteins or expressed in small amounts.

Many research organizations are currently removing gliadins that include immunogenic sequences from wheat flour using conventional breeding methods or biotechnology approaches to minimize the immunogenic potential of wheat flour. Mutation breeding methods like Ethyl Methanesulfonate (EMS) or ray-irradiation, gene silencing via RNA interference, and genome editing methods like CRISPR/Cas9 can all be employed to inactivate or delete specific genes, groups of homologous genes, or entire chromosomal regions of genes. The availability of high-throughput screening techniques that can be employed as a first step in choosing lines with altered gliadin levels is crucial for these endeavors.

In this study, we improved Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) methodologies for the analysis of *gliadins*. We were able to assess whether the approaches have adequate resolution to expose the complexity of this group of proteins by using the reference wheat CS in this investigation, which also allowed us to take into consideration the recently published set of *gliadin* sequences. Additionally, the analysis of aneuploid lines from CS allowed for the confirmation of the chromosomal positions of *gliadins* detected in various chromatogram peaks as well as the assessment of the ability to distinguish between lines lacking chromosomal areas with several gliadin genes. In this article, we compare and contrast the benefits and drawbacks of MALDI-TOF-MS and RP-HPLC for usage in preliminary screening tests.