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Abstract

Automated Allergen Sample Preparation and Detection via Centrifugal Microfluidic Lateral Flow Assay †

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Abstract: Food allergies are a severe burden for affected individuals and healthcare systems. To tackle the need for simple food allergen detection, we developed a system for the detection of the soy protein glycinin via a centrifugal microfluidics-assisted lateral flow immunoassay (LFIA). Glycinin is a complex allergen requiring extensive sample preparation. The presented workflow includes a manual denaturing extraction, followed by automated centrifugal microfluidic desalting, metering and detection via LFIA. The functionality of the microfluidic cassettes was tested on prototypes produced via microthermoforming before an injection molding tool was designed, which added a cylindrical lens to improve the readout. Overall, this system aims to aid in food allergen detection with high sensitivity and minimized manual steps.

Keywords: lateral flow assay; centrifugal microfluidics; sample preparation; allergen detection

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1. Introduction

Food allergies are on the rise worldwide, which places a burden on healthcare systems and can lead to a severely deteriorated quality of life for affected individuals [1]. Since the main way of coping with food allergies is avoidance of foods containing the allergens, consistent and reliable labelling of products is of great importance. This leads to a demand for easy-to-use analytical tools for the detection of allergens for the food industry. Glycinin is a storage protein in soy and one of the allergenic moieties causing adverse reactions in individuals sensitized to soy [2]. It is a complex and variable allergen and can be affected by food processing. This necessitates extensive sample preparation and makes it a challenging analyte for a quantitative assay. Innovative technological solutions, which automate sample preparation while maintaining test performance, can enable time and cost savings in providing safe food.

2. Materials and Methods

Rabbit monoclonal and polyclonal antibodies (mAbs and pAbs) against denatured glycinin were developed and a lateral flow immunoassay (LFIA) was established. Ground-up food samples containing glycinin were treated with extraction buffer containing 6 M urea for 30 min followed by centrifugation. The samples were desalted with disposable spin desalting columns, 7K MWCO. A centrifugal microfluidic cassette was designed to

automate the sample preparation and detection steps (Figure 1) and was produced as a prototype by microthermoforming as described previously [3]. The prototype was tested on the LabDisk-Player2 (Rhonda version). After a functional model was established, the design was transferred to injection molding, where a cylindrical polymer optic lens was integrated to optimize the fluorescent readout of the system.

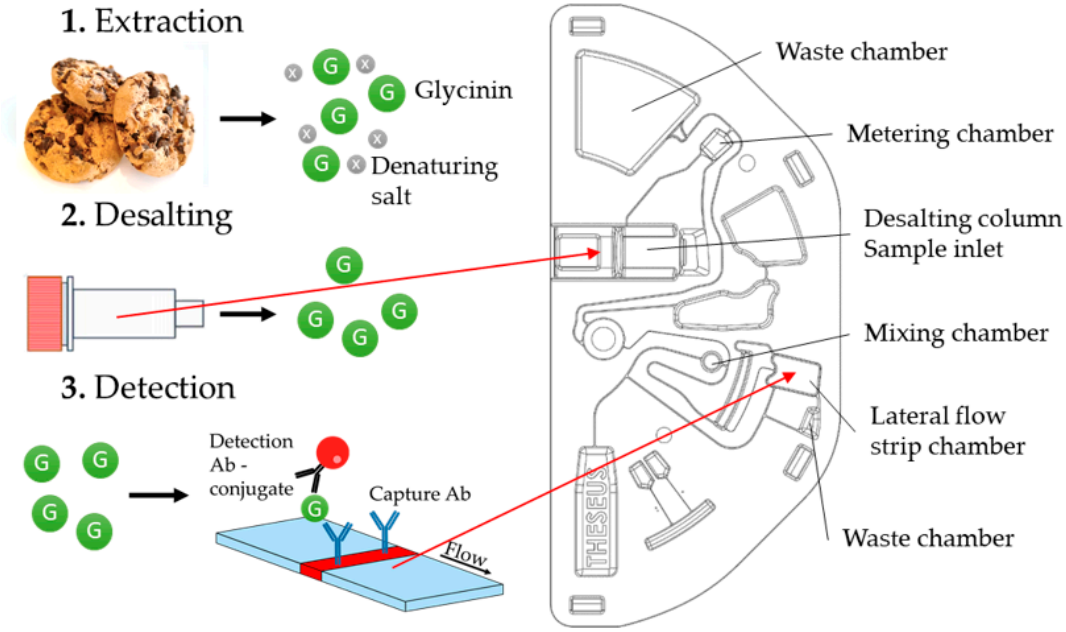

Figure 1. Depicted is the workflow of the glycinin extraction, desalting and detection with the corresponding automated steps for the design of the centrifugal microfluidic cassette.

3. Results and Discussion

A workflow for the extraction and detection of glycinin was established (Figure 1). The denaturing extraction was implemented outside of the microfluidic cassette and optimized for maximum glycinin yield. The desalting, metering, mixing with assay components and detection on the lateral flow strip were integrated into the cassette. Rabbit anti-glycinin Abs were developed and a sandwich-format LFIA was established with the pAb on the test line and the mAb functionalized with fluorescent microspheres as the detection Ab. The injection-molded cassette can be run in the commercially available LabDisk-Player2 (Rhonda version) point-of-care device and benefit the user by reducing the time to result and the number of manual steps.

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