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Probing the distribution of gliadin proteins in dough and baked bread using conjugated quantum dots as a labeling tool

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ABSTRACT

In this study, Quantum dots (QDs) were conjugated to gliadin antibodies and used as fluorescent probes to track gliadin proteins in dough and baked bread samples. QDs conjugated to gliadin antibody were specifically bound to gliadin to determine its molecular distribution in two different unleavened flat bread samples prepared at various baking times and compared with the uncooked dough. The specificity of gliadin antibody to gliadin was shown successfully with a Western Blot experiment excluding binding to all other hard wheat flour proteins.

Confocal laser scanning microscopy (CLSM) was used to monitor QDs-gliadin antibody conjugates and obtain images of the distribution of gliadin in the dough and flat bread matrix as a function of baking conditions. The mean intensity value of gliadin for each sample was calculated and plotted. CLSM images showed significant changes in the distribution of fluorescence intensity generated by the gliadin-QD conjugate with baking time. The analysis of variance (ANOVA) was used to validate the statistical significance of the variation in mean intensity values of gliadin in different samples and sections. Based on all the aggregate intensity data gathered, we concluded that gliadin is distributed non-uniformly in different layers (top, center, and bottom) that were analyzed as well as between dough and flat bread samples baked at different times. Baking time and the location of layers in flat bread play a significant role in the distribution of gliadin proteins.

1. Introduction

Wheat storage proteins gluten and their fractions, gliadins and glutenins are closely related to flour quality and baking performance (Hoseney et al., 1970; Kokini et al., 1994; Micard and Guilbert, 2000; Orth and Bushuk, 1972; Schofield et al., 1983; Shewry and Tatham, 1990; Wrigley, 1970; Wrigley et al., 1982, 2006; Wieser, 2007 Wrigley, 1970). Gliadins are generally considered to contribute to the viscosity of gluten proteins and plasticize and improve the flowability of glutenin proteins. The molecular mass of gliadin polypeptides are in the range of 25–40 kDa (Wrigley et al., 2006) and they have a low content of charged amino acids with the presence of only intramolecular disulfide bonds. Therefore, they are assumed to exist largely as monomeric molecules in their native states (Wrigley et al., 2006).

Gliadins are subdivided into α, β, γ and ω gliadins (Li et al., 2008) according to their relative mobilities and the composition and quantity of gliadin and glutenin proteins are very important to wheat quality. They account for about 50% of the protein content in wheat grain and glutenins account for 35% of total proteins (Leszczynska et al., 2008). Gliadins may also be divided into sulfur rich (α-, β- and γ-gliadins) and sulfur poor (ω-gliadin) based on the difference in sulfur containing cysteine content. Thiol groups and disulfide bonds play an important role in determining gluten and dough properties (Lasztity, 1996). The disulfide bonds in gluten and dough form a dynamically changing system. The changes are related to both the quantity and the distribution of the S–S linkages and affect their rheological properties (Madeka and Kokini, 1994).

Abbreviations: ANOVA, analysis of variance; CLSM, confocal laser scanning microscopy; FCS, fluorescence correlation spectroscopy; FITC, fluorescein-5-isothiocyanate; PEG, polyethylene glycol; PMT, photomultiplier tube; QD, quantum dots; SH, sulfhydryl group; SMCC, succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate.

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Li et al. (2004) studied the dynamic surface and rheological properties of purified gliadin and glutenin with a fluorescence labeling technique and located gluten protein components and polar and nonpolar lipids in dough using CLSM. By using tetraethylrhodamine B as a labeling tool, they observed that gliadin was found not only in strands of dough but also in gas cell walls. Varriale et al. (2007) measured the fluctuations of fluorescein-labeled gliadin peptides with fluorescence correlation spectroscopy (FCS) in the absence and presence of anti-gliadin antibodies. They labeled gliadin peptides with fluorescein-5-isothiocyanate (FITC). They obtained a detection limit of 0.006 ppm, a sensitivity higher than previous studies with ELISA.

In all previous studies so far, organic dyes such as RhB or FITC were used as a labeling tool to investigate and locate gliadin proteins in the dough matrix. These have been very helpful in advancing the understanding of the role of gliadin in dough and baked product functionality. However, one of the drawbacks of using organic dyes is their higher sensitivity to laser illumination, causing bleaching. Also during imaging of food structure by organic dyes only one fluorescence channel is available which does not allow multiple illuminations (Sozer and Kokini, 2014).

Developments in molecular biology, cell biology and medical immunology have resulted in major improvements in fluorescent labeling techniques using quantum dots. Quantum dots are nanometer sized semi conductor crystals and are stable, bright and multicolored fluorescent labeling probes where the emission wavelength depends on size. The high brightness, long-lasting, size-tunable and narrow luminescence of QDs set them apart from organic dyes (Rosenthal et al., 2011). The first attempt to use QDs with wheat flour dough and a model baked flat bread gave a clearer understanding of how gluten proteins are organized and distributed in dough (Sozer and Kokini, 2014).

There are many methods for binding biomolecules to QDs including covalent bonding, physical adsorption and hydrophobic interaction (Walling et al., 2009). Covalent attachment of biomolecules to quantum dots is achieved through direct linkage to the quantum dot surface coating or via small molecule cross-linkers. Covalent attachment is a simple, effective way of linking biomolecules to quantum dots and contributes minimally to the overall bioconjugate size (Sozer and Kokini, 2014).

Highly specific labeling with QDs requires the conjugation of quantum dots with gliadin antibodies. The method works well when there is an antibody for an extracellular epitope as a target. Bentzen et al. (2005) used antibodies for Fusion (F) and attachment (G) proteins on two sizes of QDs, to detect and follow the progression of respiratory syncytial viral infection in vitro. Gao et al. (2004) used antibody-QD conjugates to target a prostate-specific membrane antigen in tumors in vivo in mice. Anti-HER_2 QD conjugates have been reported in imaging breast cancer cells in vitro (Wu et al., 2002) and in vivo (Tada et al., 2007). A wide selection of antibody-QD conjugates are commercially available making the technique convenient to use. However, one faces challenges with their selectivity and affinity (Walling et al., 2009).

In this study, we used immunoglobulins (gliadin antibodies) that are tagged with fluorescent quantum dots (QDs) as a labeling tool to detect gliadin proteins in dough and flat bread. We conducted western blot experiments to demonstrate that gliadin antibodies are specific to gliadin. QDs were conjugated to gliadin antibodies and visualized in 3D by confocal laser scanning microscopy (CLSM) to identify the changes in the distribution of gliadin during baking in comparison to dough. While a few prior studies (Bugusu et al., 2002; Lindsay and Skerritt, 2000; Li et al., 2004) have used commercial dyes conjugated to gliadin antibodies in dough, this is the first study to our knowledge that utilizes antibody conjugated quantum dots to detect and study the distribution of intrinsic gliadins in dough and in a model baked flat unleavened bread.

2. Materials

Wheat flour, enriched and bleached (Gold Medalbrand, manufactured by General Mills, Minneapolis, MN) was purchased from a supermarket. The approximate protein content was 10.5%, carbohydrate content was 79.3%, dietary fiber was about 3.5% and the fat content was negligible.

Commercially available, native and heat-treated polyclonal gliadin antibody was purchased from Sigma–Aldrich, St Louis, Mo. Quantum dots with a CdSe core coated with ZnS and polyethylene glycol (PEG) approximately in the range of 15–20 nm in size were purchased from Invitrogen, Carlsbad, CA. in the form of a kit (Qdot-625 antibody conjugation kit). The quantum dots were amine activated for crosslinking to enable conjugation with gliadin antibody.

For SDS-Page Gel Electrophoresis and Western Blot, Precision plus protein, ladder, Tris–Cl pH 8.8 0.5 M and 1.5 M), 30% Acrylamide/Bis solution, Immun-Blot PVDF membrane, Criterion Cell for Gel Electrophoresis and Criterion Blotter with Plate Electrodes were purchased from Bio-Rad. SDS, Glycine, Glacial Acetic Acid, Methanol, Glycerol, Bromophenol blue and Tris buffer pH 6.8 (1 M) were purchased from Fisher Scientific. TEMED, APS and Anti-rabbit HRP secondary antibody were purchased from Thermo Scientific.

PBS buffer concentrate was purchased from Sigma Aldrich and was diluted 10 times to reach the pH of the phosphate buffer saline solution needed. The hydrophobic positively charged microscope slides were obtained from Fisher Scientific through the Institute of Genomic Biology of the U of I and were used to appropriately fix the dough or baked model flat bread sample on them for CLSM studies.

3. Methods

3.1. Preparation of wheat flour dough

Dough was prepared following the procedure used by Sozer and Kokini (2014). Briefly, 50 g of flour was hand mixed with 32 ml of distilled water. This water content was that needed to reach a 500 BU value with a Farinograph. The dough sample was shaped into a ball, pressing it down and reshaping it continuously for 5 min and allowed to rise for 5 min at room temperature in a wrapped plastic (Sozer and Kokini, 2014). 57 g of dough was weighed. Flour was sprinkled over the dry flat surface to prevent the dough from becoming sticky and it was sheeted on a flat surface using a rolling pin in all directions until its thickness was reduced to 1.87 mm which was measured using a caliper.

A circular 4 inch cutting die was used to cut the dough with an accurate diameter with a thickness of 1.87 mm. Then the dough sample was stored in a closed container in a freezer at 20 °C temperature. Fig. 1A shows an example of the dough sample.

3.2. Preparation of the flat breads

Flat bread samples were cooked at oven temperature of 375 °F for 5 and 9 min. The samples were then allowed to equilibrate at room temperature and 5 mm × 5 mm square pieces were cut for QD antibody conjugation.

3.3. Conjugation of gliadin antibody to quantum dots (QDs)

Conjugation of gliadin antibody to quantum dots (QDs) was conducted based on the protocol by Invitrogen. The QD antibody conjugation kit contains 4 μM amine-derivatized, PEG-coated QDs,