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Confocal Raman microscopy of frozen bread dough

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ABSTRACT

The use of freezing technology is well established in industrial and craft bakeries and is still gaining importance. In order to optimize recipes and processes of frozen baked goods, it is essential to be able to investigate the products' microstructure. Especially ice crystals and their interaction with the other components of the frozen products are of interest. In this study, frozen wheat bread dough was investigated by confocal Raman microscopy. The Raman spectra measured within the dough were compared with spectra of the main components of frozen dough, i.e. ice, liquid water, starch, gluten and yeast. In this way, the spatial distribution of the single components within the dough was determined and corresponding images of the frozen dough microstructure were generated. On these images, ice appears as a continuous network rather than as isolated crystals. We suggest that this method may be appropriate for characterizing crystallization phenomena in frozen baked goods, allowing to better understand the reasons for quality losses and to develop strategies for avoiding such losses.

1. Introduction

As the bakery business is being concentrated and rationalized, increasing use is made of freezing technology in production and distribution (Le Bail et al., 2012). Freezing allows a separation in time and space of process operations that would traditionally be performed in one run and in one place.

In bread-making, freezing is used at several stages of production: for non-fermented or partly-fermented dough, for partly or fully baked products (Le Bail et al., 2012). Depending on the application, the products are kept frozen for a few hours or for several weeks or months. A large variety of equipment, including shock-freezers, fermentation interrupters, climatic chambers, and cold storage rooms are used for realizing the operations of freezing, cold storage and thawing.

Although the intention when using freezing is to keep the product in a steady state, in practice a number of physical and chemical phenomena occur, affecting the quality of the final product in a mostly negative way. Among these phenomena, the formation of ice crystals is believed to be of primary importance for two main reasons (Berglund et al., 1991; Baier-Schenk et al., 2005a): (1) Ice crystals are made of pure water which is being separated from the product matrix. Cryoconcentration occurs in the liquid phase, which may influence the solubility of proteins and the activity of enzymes. During storage, ice crystals grow due to recrystallization, especially in the pores, thus further modifying the distribution of water in the product. (2) Ice crystals may mechanically damage the dough components, especially the gluten network and the yeast cells, because the freezing front exerts stress on the surrounding material. This effect is believed to be more pronounced as the crystal size increases due to recrystallization.

In order to optimize the recipes and the production processes of frozen baked goods, it is essential to be able to monitor the phenomena occurring in the products in the frozen state. Differential scanning calorimetry (DSC) allows quantitative investigations of ice crystallization. For monitoring the size and the distribution of the ice crystals as well as their mechanical interactions with the other components of the dough, imaging techniques are required. So far, scanning electron microscopy in the frozen state (cryo SEM, Zounis et al., 2002, Esselink et al., 2003; Baier-Schenk et al., 2005a) and confocal laser scanning microscopy (CLSM, Baier-Schenk et al., 2005b) have been used for that purpose. Cryo SEM has allowed demonstrating the growth of ice crystals within the pores over storage time and CLSM to identify regions of preferential nucleation. However, in both techniques, a difficulty is the limited possibility to unambiguously differentiate the ice crystals from the

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other components of the dough. In cryo SEM, this differentiation is performed based on the regular shape of the crystals – but this is only valid in the pores, where ice crystals can grow without spatial constraints. In CLSM, changes in the reflection properties were attributed to ice crystal growth. However, this method did not allow for generating precise images of the ice crystal structure. Due to these limitations, little is known about the structure of the ice crystals that are entrapped in the dough matrix, which yet represent the main part of the frozen water.

Raman spectroscopy belongs to the group of vibrational spectroscopies (Smith and Dent, 2005). It utilizes the inelastic scattering of light photons on molecules or molecular groups, called Raman effect. If the molecule (or group) has suitable vibrational modes, a photon can transfer a fraction of its energy to the vibration (Stokes scattering). The positions of the Raman bands directly give the energy of the detected vibrations. The ensemble of Raman active vibrations is characteristic for each compound and can range from single bands to very complex multi-band spectra. Raman spectroscopy is a non-destructive method requiring very little sample preparation and it is suitable for a wide range of materials. If high-quality reference spectra are available, it is a very sensitive tool for phase identification.

With the implementation of Raman spectroscopy in confocal microscopy in the late 1990s, it became possible to use Raman data for microimaging purposes. Applications were developed in a variety of scientific fields including mineralogy, petrography, polymer science, pharmaceutical research (Dieing et al., 2011), biomedical diagnostics (Krafft et al., 2012) and glaciology (Weikusat et al., 2012). In agricultural and food science and more specifically in cereal science, only little use has been made of this technique so far. Piot et al. (2000, 2001, 2002) used confocal Raman microscopy for exploring the spatial distribution of starch, gluten, arabinoxylan and ferulic acid in wheat grains. Recently, Jääskeläinen et al. (2013) performed similar investigations with higher (sub-μm) spatial resolution on barley and wheat grains.

Based on the fact that confocal Raman microscopy has shown to be suitable for characterising both ice crystals and the main components of cereals, our objective was to develop a measurement method appropriate for investigating the microstructure of frozen bread dough.

2. Experimental

2.1. Raw materials and equipment

The following ingredients were used in the experiments: Wheat flour type 550 (Roland Mühle, Germany), compressed yeast (Frischhefe, Deutsche Hefewerke GmbH, Germany), and salt (Suprasel fine, Suprasel, The Netherlands).

Raman measurements were performed on a WITec Alpha 300R microspectroscopy system equipped with a frequency-doubled Nd:YAG laser (λ = 532 nm), an UHTS300 Raman Spectrometer (grating: 600 grooves/mm, pixel resolution <0.09 nm) with a Peltier-cooled DV401A-BV CCD detector (peak quantum efficiency at ~550 nm and −60 °C: >95%) and a 50x LWD objective, operated in a cold laboratory at −15 °C at the Alfred-Wegener Institute. The laser power on the sample was <30 mW.

2.2. Assessment of Raman spectra of single dough components

The Raman spectra of ice, liquid water, starch, gluten, and yeast were assessed using the following procedure.

2.2.1. Sample preparation

A 3.5% (w/v) salt solution in bidistilled water was prepared. One droplet of this solution (20 μL) was placed on a microscope slide, covered with a cover slip using a 2 mm spacer to standardize thickness, and frozen at −20 °C. In this way ice crystals and a liquid phase (cryoconcentrated salt solution) were formed. The salt present in the liquid phase is expected to influence the Raman spectrum only to a minimal extent, as its main component NaCl (≥99.8% according to the supplier's specification) has no molecular vibration.

Wheat flour was hydrated and separated into a starch suspension and a wet gluten piece using a Glutomatic 2200 from Perten Instruments, Sweden. One droplet of the starch suspension was placed on a microscope slide, covered with a cover slip using a 2 mm spacer and frozen at −20 °C. The same was done with a small portion of the wet gluten piece and of the compressed yeast block.

2.2.2. Measurement

The Raman spectrum of each of the samples representative for the individual dough components was measured at 10 different points, with 20 accumulations of 1 s each per point, and the average spectrum was calculated for each component.

2.3. Dough sample preparation

Three frozen dough samples were prepared at three different days in the following way: 50 g of wheat flour, 28 g of bidistilled water, 1.5 g of compressed yeast and 1 g of salt were mixed and kneaded to a dough in a Brabender Farinograph AT at 20 °C. The mixing time was 2 min at 36 rpm and the kneading time 4 min at 63 rpm. After kneading, the dough was allowed to rest for 15 min at room temperature. Subsequently, a small piece (approx. 250 mg) of the inner part of the dough was cut out, placed on a microscope slide, covered with a cover slip using a 2 mm spacer and frozen at −20 °C.

2.4. Confocal Raman microscopy of frozen dough samples

On the day following preparation, the samples were transferred to the microscopy laboratory at −15 °C. Before measurement, the samples were kept for at least one hour at −15 °C to stabilize at that temperature.

For each of the 3 frozen dough samples, an area of 100 × 100 μm was measured with a resolution of 200 x 200 points and an integration time of 1 s per point, resulting in a measurement time of approx. 12 h.

2.5. Confocal Raman microscopy: data processing and imaging

The data from the area scans were processed in two different ways to produce images showing the spatial distribution of the single dough components (ice, liquid water, starch, gluten and yeast).

In the first method, single Raman bands characteristic for each component were integrated. Monochrome images were generated representing the intensity of the individual bands at each measurement point. The spectral ranges of the chosen bands are given in Table 1 and are marked in blue in Fig. 1.

The second method considered the full Raman spectra instead of single bands. In that method, the Raman spectrum measured at each point of the sample was assumed to be a linear combination of the spectra of the single dough components. After performing a 3rd order polynomial background subtraction on all spectra, a multiple linear regression was completed using the function *Basis Analysis* of the WITec Project software (release 2.10, WITec GmbH, Ulm,