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Development of a low resolution ^{1}H NMR spectroscopic technique for the study of matrix mobility in fresh and freeze-thawed hen egg yolk

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ABSTRACT

Three experiments were conducted in developing a low resolution proton nuclear magnetic resonance (^{1}H NMR) spectroscopic technique to study matrix mobility in fresh and freeze-thawed gelled yolk. The Carr–Purcell–Meiboom–Gill (CPMG) sequence was used to measure spin–spin relaxation times of proton pools representing major yolk constituents. A component identification test distinguished 3–4 pools. The least mobile pool was assigned to proteins, protein–lipid and protein–water interactions, and the most mobile to unbound water. The remaining pools were assigned to lipids, lipid–protein and lipid–water interactions. A stability test indicated that yolk had varied matrix mobility within the same sample across five days of refrigeration storage. A reproducibility test demonstrated high repeatability of fresh yolk measurements, but significant differences ($p < 0.05$) were found within gelled yolk samples. This research determined that ^{1}H NMR spectroscopy, a non-destructive technique, can identify yolk components and detect changes in the matrix.

1. Introduction

Hen egg yolk is comprised of approximately 50% water, 30% lipids, and 16% proteins. Carbohydrates and minerals are minor constituents that make up the rest of the egg yolk composition (Anton, 2007).

Low resolution ^{1}H NMR spectroscopy has been used to study molecular mobility in numerous food systems, many of which are simpler than egg yolk in structure and matrix composition. Typically they are ingredients, simple model systems of food products, or foods with extensively-studied matrices, such as starch gels and starch-based foods like bread and cake (Le Grand, Cambert, & Mariette, 2007; Lu & Seetharaman, 2013; Luyts et al., 2013; Wang, Choi, & Kerr, 2004). Simple water-based gels have also been modeled by mixed systems to study water mobility (Huang, Davies, & Lillford, 2011; Shapiro, 2011). Nevertheless, few studies utilizing low resolution ^{1}H NMR spectroscopy have been conducted on hen's egg yolk. Some researchers have studied the molecular mobility of products containing egg yolk (Le Grand et al., 2007; Luyts et al., 2013). However, in those experiments, yolk was studied as a part of a larger food system with various other molecular interactions, so the obtained Carr–Purcell–Meiboom–Gill (CPMG) spectra cannot be equated to the spectra obtained in the study of yolk itself. To date, there has been only one study on raw egg yolk (Hills, Benamira, Marigheto, & Wright, 2004) where three proton pools were distinguished and assigned, in order of increasing mobility, to (1) protons of lipids, (2) protons of proteins, and (3) protons of water. Furthermore, there have been no ^{1}H NMR studies on egg yolk that utilize equivalent instrumentation or sample material so results cannot be directly compared.

Low-resolution ^{1}H NMR spectroscopy may reveal matrix changes that occur during processing or storage. A phenomenon referred to as gelation, induced by freezing and thawing of yolk, transforms the yolk matrix and produces a very viscous and gelled material. Gelled egg yolk is undesirable to food processors because: (1) its increase in viscosity causes difficulties in mixing the yolk with other ingredients, and (2) physicochemical changes in the gelled yolk result in reduced functionality in food products (Powrie, Little, & Lopez, 1963). NMR spectroscopy can be used to observe differences in sample structure due to changes in the internal chemical environment of the food, such as those resulting from freezing and thawing. Furthermore, ^{1}H NMR spectroscopy is a non-destructive method which can be used to examine the entire yolk with all of its intact, naturally-present components.

The objective of this research was to develop a ^{1}H NMR spectroscopic technique to study the matrix mobility of fresh and freeze-thawed hen egg yolk, and thus determine if NMR can distinguish the differences in structure and matrix mobility between fresh

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and gelled yolk. A complex system such as egg yolk is difficult to study, but this technique has been proven in this study to be useful because it can show molecular mobility of several components in yolk. Yolk constituents corresponding to proton pools detected by ^{1}H NMR spectroscopy were all expected to fall within T_2 range of the CPMG pulse sequence due to the high water content (50%) of egg yolk. Therefore, only measurements from the CPMG pulse sequence were made in this study. Three experiments were conducted in this research. The first experiment was designed to identify the components in yolk which were detected in preliminary CPMG measurements. Lipid and water content in a fresh yolk system was varied in order to observe the effects of changes in yolk composition on T_2 spectra. The second experiment was a 5-d stability test using fresh and gelled yolk to determine yolk system stability as well as optimal measurement time and conditions. The main objective of this experiment was to study both yolk systems to determine if changes in the yolk matrix occurred throughout a storage period of 5 d. The third experiment was a reproducibility test using fresh and gelled yolk to determine instrument sensitivity and robustness, thus validating this method of measuring T_2 and PP for the study of yolk matrix mobility. The methodology developed in this research holds promise for studying the changes that occur in hen egg yolk as a result of processing or storage, such as freeze–thaw gelation and may be applied to other foods systems.

2. Materials and methods

2.1. Materials

Large grade A white shell eggs produced by Rose Acre Farms Inc. (IA) were purchased from retailers in Ames, IA. The shell eggs were distributed by farms located within 300 mi of the Ames retailers. Eggs were purchased at least 14 d before the sell-by date, i.e., 30 d after the packing date. They were held for no more than 16 d in refrigeration temperature (4 °C) during transportation as well as storage at the retailer and at the research laboratory.

Analytical grade di-isopropyl ether and 1-butanol were purchased from Fisher Scientific (Pittsburg, PA). Lecithin with claimed purity near 100% was purchased from Q.P. Corporation (Tokyo, Japan) and kept at −20 °C until use.

2.2. Egg yolk separation

Yolk samples were prepared from the fresh, raw eggs no more than 16 d after the packing date. Yolks were separated using a modified version of the method by Powrie et al. (1963). Yolks were separated from the albumen, and the chalazae of each yolk were carefully removed. The albumen and chalazae were discarded. The yolk with intact vitelline membrane was washed carefully in shallow basin of Milli-Q water. Milli-Q, or ultrapure water, was obtained from deionized water purified with Milli-Q® Reagent Water System (EMD Millipore, Darmstadt, Germany). Each yolk was rolled on a sheet of paper towel to remove remaining water and albumen. Once clean, the vitelline membrane was pierced and yolk was drained into a beaker. After completing the yolk harvest for all eggs, the yolks in the beaker were stirred slowly with a stir rod for 1 min to ensure sample homogeneity. Yolks were frozen immediately for the tests requiring gelled yolk samples.

2.3. Yolk freezing and thawing

For samples requiring freezing and thawing, 50 mL of yolk was poured in 10-mL portions into five 25-mL polystyrene vials with snap-on lids. Strips of plastic paraffin film, Parafilm (Beemis Company, Inc., Oshkosh, WI), were wrapped over the lid around the circumference of the vial. Vials were vacuum sealed in a vacuum bag with a FoodSaver® V222 vacuum sealing system (SunBeam Products, Inc., Jarden Consumer Solutions, Boca Raton, FL). The vacuum-sealed bags of yolk vials were submerged in the reservoir of a Haake SC 100 refrigerated/heated bath circulator (Thermo Fisher Scientific, Waltham, MA) filled with 1:1 ethylene glycol: Milli-Q water at 0 °C. The bath was then set to −20 °C. After the samples reached −20 °C at a cooling rate of 0.3–0.5 °C/min, the yolks were held in the −20 °C bath for 3 h. The frozen yolks were then stored in a −20 ± 2 °C upright freezer for 7 d. Yolks were then thawed for 1 h in a 25 °C Neslab GP-300 water bath (Neslab Instruments, Inc., Portsmouth, NH). Gelled yolks were analyzed immediately after thawing. Fresh yolks were analyzed no later than 6 h after yolk separation, and if storage was required the yolks were kept at 4 °C until analysis.

2.4. Sample preparation for component identification

Seven samples of varying compositions were prepared for the component identification experiment: fresh yolk (F); lyophilized yolk reconstituted to 25 (L-25), 50 (L-50), and 75% (L-75) water (wet basis, wb); and delipidated yolk with 0 (D), 10 (D-10), and 30% (D-30) added lecithin (wb). The natural amount of water and lipid found in yolk is about 50 and 30% (wb), respectively. Fresh yolks were freeze-dried, or lyophilized, in plastic beakers with a Labconco FreeZone 2.5-L benchtop freeze dry system (Labconco, Kansas City, MO). The lyophilized material was collected in polypropylene tubes, capped, and stored in a −20 °C freezer until use. Lyophilized yolk was reconstituted with Milli-Q water, mixed thoroughly, and stored at 4 °C for 2 h before filling the NMR tubes. Fresh yolk was defatted using a 1:1.5 (v/v) 1-butanol:di-isopropyl ether solvent system as described in the method of Cham and Knowles (1976). The delipidated yolk was collected in polypropylene tubes, capped, and stored in 4 °C until use. Egg lecithin was added to two portions of the defatted yolk to yield a sample of 10% and 30% added lecithin each.

2.5. Sample preparation for yolk system stability study

Fresh and gelled yolk were prepared in triplicate per treatment. The same three tubes of each sample were used to obtain measurements throughout the 5-d study. Proton relaxation measurements were performed at approximately the same time each of the five days. Between daily measurements, tubes were stored at 4 °C.

2.6. Sample preparation for method reproducibility study

Eggs were purchased from Ames retailers on three separate occasions at least 7 d apart. Yolks were separated, and gelled samples were stored frozen for 7 d and thawed according to the method described in Section 2.3. Fresh and gelled samples were prepared for three repeated trials. Each trial utilized a different batch of eggs purchased at different times but originating from the same farm.

2.7. ^{1}H NMR spectroscopy measurements

For all tests, flat-bottom glass NMR tubes (10 mm diameter, 180 mm length) were prepared in triplicate for each sample. Tubes were filled with yolk samples to a height of 4 cm and capped. Proton relaxation measurements were performed on a Bruker minispec mq-20 low-resolution spectrometer (Bruker, Biospin Corporation, Billerica, MA, USA) at a resonance frequency of 20 MHz. Immediately before measurement, capped samples were held at 20 °C in a Duratech TCON 2000 high precision dry bath system (Duratech, Carmel, IN) for 60–70 min. The probe head was reg-