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Molecular interactions between green tea catechins and cheese fat studied by solid-state nuclear magnetic resonance spectroscopy

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ABSTRACT

Molecular integrations between green tea catechins and milk fat globules in a cheese matrix were investigated using solid-state magic angle spinning nuclear magnetic resonance spectroscopy. Full-fat cheeses were manufactured containing free catechin or free green tea extract (GTE), and liposomal encapsulated catechin or liposomal encapsulated GTE. Molecular mobility of the carbon species in the cheeses was measured by a wide-line separation technique. The ^{1}H evolution frequency profile of the ^{13}C peak at 16 ppm obtained for the control cheese and cheeses containing encapsulated polyphenols (catechin or GTE) were similar, however, the spectrum was narrower for cheeses containing free polyphenols. Differences in spectral width indicates changes in the molecular mobility of –-CH_3– or –C–C–PO_4– species through hydrophobic and/or cation–π associations between green tea catechins and cheese fat components. However, the similar spectral profile suggests that encapsulation protects cheese fat from interaction with catechins.

1. Introduction

Polyphenols are structurally diverse secondary metabolites of plants, and contribute to the sensory and nutritional properties of plant-derived foods (Cheynier, Tomas-Barberan, & Yoshida, 2015). Polyphenols have practical applications due to antioxidant properties. A wide range of health benefits, such as protection against human immunodeficiency virus, and anticarcinogenic and anti-cardiovascular effects have been reported for green tea polyphenols, such as catechins (Lewandowska, Szewczyk, Hrabec, Janecka, & Gorlach, 2013; Nath, Bachani, Harshavardhana, & Steiner, 2012; Okamoto, 2013; Rathore & Wang, 2012). There is growing interest in the use of green tea antioxidants as food additives to reduce oxidative instability and increase the shelf-life of food products (Wambura, Yang, & Mwakatage, 2011). These benefits have increased the interest in incorporating tea catechins into dairy products, such as cheese (Giroux et al., 2013; Han et al., 2011; Rashidinejad, Birch, & Everett, 2016a). There are challenges due to chemical interactions between milk proteins and green tea catechins (Haratifar & Corredig, 2014; Ozdal, Capanoglu, & Altay, 2013; Ye, Fan, Xu, & Liang, 2013), and association between milk fat and green tea catechins (Rashidinejad, Birch, & Everett, 2016b), which can impact enzymatic activity for flavour compound generation.

Free catechins from green tea affect pH and microstructure of low-fat (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2013) and full-fat (Rashidinejad et al., 2016a) cheeses. Giroux et al. (2013) reported negative effects of green tea extract (GTE) on the colour, hardness, and flavour of Cheddar cheese during ripening. These authors demonstrated that the effect was dose-dependent and can result in astringency in the cheese. The addition of tea catechins to pasteurized milk decreases the pH of the corresponding rennet gels (Han et al., 2011). However, there was no significant effect ($P > 0.05$) of green tea catechins or GTE on either pH or microstructure of full-fat cheese when green tea catechins were added to milk in nanoliposomal encapsulated form (Rashidinejad, Birch, & Everett, 2016c). Moreover, it was found that when catechins were incorporated into milk in a free form to manufacture full-fat cheese, a greater proportion partitioned into the whey compared to when catechins were encapsulated and incorporated into milk prior to cheese manufacture (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014; Rashidinejad et al., 2016a, 2016c). Encapsulation also increased the retention of catechins in cheese curd (Rashidinejad et al., 2014), and increased the recovery of

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catechins from both low- and full-fat cheeses after digestion in a simulated gastrointestinal digestion system (Rashidinejad et al., 2016a, 2016c).

Molecular dynamic studies of biophysical interactions between the lipid bilayers of cell membranes and green tea catechins revealed a strong affinity of catechins for the bilayer through hydrogen bonding (Sirk, Brown, Sum, & Friedman, 2008). Using transmission electron microscopy (TEM), it was shown that free catechins from green tea altered the microstructure of full-fat cheese such that it became less homogenous, with destabilisation of the milk fat globule membrane (Rashidinejad et al., 2016a), whereas, encapsulated catechins did not disrupt the cheese microstructure (Rashidinejad et al., 2016c). TEM micrographs showed that green tea catechins may attach to the surface of the milk fat globule membrane (Rashidinejad et al., 2016b). Hydrophobic interactions between green tea catechins (free form) and milk fat globules in suspension or in a cheese matrix were also revealed by Fourier transform infrared (FTIR) spectroscopy (Rashidinejad et al., 2016a, 2016b). These interactions were not found in full-fat cheese containing encapsulated catechins (Rashidinejad et al., 2016c).

Among the suitable nuclei, the most extensive ones employed in NMR studies of dairy products have been ^{1}H, ^{13}C, and ^{31}P (Kaffarnik, Ehlers, Gröbner, Schleucher, & Vetter, 2013; Mazzei & Piccolo, 2012; Sacco et al., 2009). Nuclear magnetic resonance (NMR) spectroscopy provides a useful tool to examine molecular interactions. ^{1}H nuclei are more abundant and have higher obsolete sensitivity than other nuclei. This enables shorter acquisition times. But in solids, peak broadening and narrow chemical shift spectral widths reduce the ability to resolve the peaks of different nuclei. ^{13}C has a much broader chemical shift field, and therefore, many more individual nuclei peaks can be resolved in solid-state NMR (Belloque & Ramos, 1999). Solid-state NMR spectroscopy has been employed for investigations in cheese (Kakalis, Kumosinski, & Farrell, 1994). By placing the sample at the magic angle (54.7°), spinning at high speed (>∼2 kHz), and with high-powered decoupling, the peaks can be narrowed to a point where the peaks of individual nuclei can be resolved. Cross-polarisation-magic angle spinning (CP-MAS) is the polarisation transfer from abundant nuclei (^{1}H) to less abundant nuclei (^{13}C). The CP-MAS experiment acts as a filter for detecting only the 'rigid' components in a heterogeneous sample. For this study, 'rigid' is defined as being molecules that do not have any viscous properties; i.e. are not able to reorient their position within a millisecond time period. CP-MAS observes only rigid components because the cross-polarisation relies on a strong static component of the dipolar interaction between the proton and other kind/type of nucleus. This means if the molecule is able to re-orientate in space more than once during the cross-polarisation period (contact time for magnetisation transfer), the net magnetisation transfer will be zero. Hence, CP-MAS is efficient for immobile, rigid molecular species, but is inefficient for mobile, fluid-like species (Kolodziejski & Klinowski, 2002). Direct polarised (DP)-MAS observes all species, both rigid and highly mobile. Acquiring both DP- and CP-MAS spectra enables observation of the molecular state of each component in the cheese.

The objective of this work was to obtain information about molecular interactions between green tea catechins and cheese components using solid-state carbon-13 (^{13}C NMR) spectroscopy. Molecular interactions of solute and solvent can be detected by observing the mobility of solvent in the presence and absence of the solute. The mobility of the solvent (protein, fat, and water in cheese) on a molecular level will change (either decreasing or increasing in motion) when in contact with the solute (catechins). The individual mobility of carbon nuclei can be monitored with 2D NMR methods, where the first dimension identifies the species (carbon spectra) and the second is encoded with the mobility of protons in close proximity (<10 nm). One such method is wide-line separation (WISE) NMR (Paris, Bizot, Emery, Buzare, & Buleon, 2001), which was used in this study.

2. Materials and methods

2.1. Materials and reagents

Pasteurized full-fat milk (3.3% fat) was purchased from a local supermarket (Dunedin, New Zealand). Freeze dried mesophilic *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* starter culture (R-704) and rennet were obtained from Chr. Hansen (Hørsholm, Denmark) and Renco (Eltham, New Zealand), respectively. (+)-Catechin was purchased from Sigma Aldrich (Auckland, New Zealand). Green tea extract was obtained from Invita (Auckland, New Zealand). Soy lecithin was provided by Hawkins Watts (Auckland, New Zealand).

2.2. Cheese manufacture

Full-fat cheeses containing either free or encapsulated forms of catechin and GTE were manufactured in 500 mL aluminium containers placed in a water bath set at 31 °C (Rashidinejad et al., 2016a, 2016c). Cheeses were packed and ripened at 8 °C for 90 days. For encapsulating catechin and GTE in liposomes, the aqueous solutions of these catechins were prepared in 0.25 M acetate buffer (pH 3.8) and coated with soy lecithin (Rashidinejad et al., 2014). The prepared liposomes were then stored at 4 °C for 24 h and afterwards were incorporated into milk for cheesemaking. Compositional information about the green tea extract is reported in Rashidinejad et al. (2016b). Five different cheeses were manufactured: (1) full-fat cheese with no catechin (control), (2) full-fat cheese containing 500 ppm free catechin, (3) full-fat cheese containing 1000 ppm free GTE, (4) full-fat cheese containing 500 ppm liposomal encapsulated catechin, and (5) full-fat cheese containing 1000 ppm liposomal encapsulated GTE. For NMR analysis, the cheese samples were grated and freeze-dried. Preliminary tests on the wet cheese were also carried out.

2.3. Nuclear magnetic resonance spectroscopy

Solid-state NMR spectra were acquired on a Bruker BioSpec spectrometer (Elektronik GmbH, Rheinstetten, Germany) operating at a ^{1}H frequency of 200.32 MHz and a ^{13}C frequency of 50.39 MHz. The experiments were carried out with a Bruker 7-mm double resonance H/X SB-MAS (magic angle spinning) probe at a regulated temperature of 22 °C. Typically, 150 mg of sample (dry weight) was packed into a 7 mm rotor with a water tight cap. The 90° pulses for ^{1}H and ^{13}C were both set to 5.54 μs. During all acquisitions, a 45 kHz dipolar proton decoupling was employed. Recycle delays of 2 s for cross-polarised spectra (CP-MAS) and 40 s for direct-polarised spectra (DP-MAS) were used. The rotor spinning speed was maintained at 4000 Hz ± 10 Hz. All ^{13}C chemical shifts were referenced externally to glycine. Spectra were zero-filled to 4096 data points and processed with a 10–70 Hz Lorentzian line broadening and a 0.010 s Gaussian broadening. Wide-line separation (WISE) NMR measurements were done with an increment time of 3 μs for the 2D experiments corresponding to a 100 kHz ^{1}H sweep width and a cross-polarisation contact time of 0.1 ms. The ^{1}H-^{13}C Hartmann-Hahn cross-polarisation kinetics were measured with the TORQUE (T One Rho Quenching) method (Tekely, Gérardy, Palmas, Canet, & Retournard, 1995). A total spin-lock time of 4 ms was used and fourteen cross-polarisation contact times ranging from 0.1–3.5 ms were used. The proton $T_{1\rho}(H)$