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Study on release suppression of cinnamaldehyde from κ -carrageenan gel by HR-MASNMR and pulsed field gradient NMR (PFG-NMR)

Yasumasa Yamada, Ph.D.^{a,*}, Motohiro Shizuma, Ph.D.^b

^a Department of Food Science and Human Nutrition, Faculty of Food Culture and Human Nutrition, Baika Women's University, 2-19-5 Shukunoshō, Ibaraki City, Osaka, 567-8578, Japan

^b Department of Biochemistry, Osaka Research Institute of Industrial and Scientific Technology (ORIST), 1-6-50 Morinomiya, Joto-ku, Osaka, 536-8553, Japan

ARTICLE INFO

Keywords:

Flavor release
Cinnamaldehyde
 κ -Carrageenan gel
Cinnamon
HR-MAS NMR
Pulsed field gradient (PFG) NMR

ABSTRACT

Aiming toward the production and characterization of delicious and functional gel foods, this communication studies the flavor release from cinnamon-containing κ -carrageenan gel. Cinnamaldehyde, which provides the flavor of cinnamon, was released in a trace amount from the gel and detected by flame ionization detector gas chromatography. The retention of cinnamaldehyde in κ -carrageenan gel and the interaction between flavor and polysaccharide were investigated by high-resolution magic-angle spinning nuclear magnetic resonance (HR-MAS NMR) and pulsed-field gradient NMR (PFG NMR). The intact cinnamaldehyde in the gel was also observed by HR-MAS NMR. The relative mobility difference of the flavor and polysaccharide molecules was observed from the diffusion-ordered NMR spectrum of PFG NMR.

1. Introduction

Cinnamon (*Cinnamomum zeylanicum*) is a folklore medicinal spice in many Asian countries, such as China, Japan, and India. Owing to its possible antidiabetic effects, cinnamon has been utilized as an ingredient in functional foods that manage Type 2 diabetes mellitus (T2DM). However, whether cinnamon is clinically effective against T2DM has not been verified. This short communication investigates the release of cinnamaldehyde from κ -carrageenan gel (a polysaccharide found in seaweed) and the retention of cinnamaldehyde in the gel by flame ionization detector gas chromatography (FID-GC), high-resolution magic-angle spinning nuclear magnetic resonance (HR-MAS NMR), and pulsed-field gradient NMR (PFG NMR).

Cinnamon (*Cinnamomum zeylanicum*) and cassia (*C. cassia*) are well-known spice species with a long medicinal history rooted in folklore, especially in Asian countries such as China, Japan, and India. Recently, the management of Type 2 diabetes mellitus (T2DM) has emerged as a serious medical problem in developed countries. Cinnamon might exert antidiabetic effects that improve glycemic control in diabetic patients. For this reason, cinnamon supplements are often used as adjuvants to

standard hypoglycemic medication and other lifestyle therapies (Lu 2012). However, Costello et al. (2016) concluded that cinnamon supplements only modestly affect fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) levels. At present, whether cinnamon supplements are clinically meaningful in T2DM management is unclear. Toward the design of hydrocolloids with food functionality, this communication investigates κ -carrageenan gel containing cinnamon powder. For continuous daily intake by patients and consumers, the functional hydrocolloid gel must be palatable. Palatability relies on the texture and the flavor-release degrees of the taste and smell compounds from the gel. This communication investigates the phenomena concerning the release of cinnamaldehyde (a major flavor component of cinnamon) from κ -carrageenan gel by FID-GC, HR-MAS NMR and PFG NMR.

2. Materials and methods

2.1. Materials

κ -Carrageenan was purchased from Tokyo Chemical Industry (TCI Co. Ltd., Tokyo, Japan) and was used without further purification.

Abbreviations: HR-MAS NMR, High-resolution Magic-angle Spinning Nuclear Magnetic Resonance; PFG NMR, Pulsed-field Gradient Nuclear Magnetic Resonance; T2DM, Type 2 Diabetes Mellitus; FPG, Fasting Plasma Glucose; HbA1c, Hemoglobin A1c; FID-GC, Flame Ionization Detector Gas Chromatography; ICP-OES, Inductivity Coupled Plasma Optical Emission Spectrometer.

* Corresponding author. Department of Food Science and Human Nutrition, Faculty of Food culture and Human Nutrition, Baika Women's University, 2-19-5 Shukunoshō, Ibaraki city, Osaka, 567-8578, Japan.

E-mail address: y-yamada@baika.ac.jp (Y. Yamada).

<https://doi.org/10.1016/j.foodhyd.2020.106130>

Received 30 December 2019; Received in revised form 22 June 2020; Accepted 24 June 2020

Available online 9 July 2020

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Table 1

Amounts of cations.

Sample	Cation (mg/sample 1.0 g)			
	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺
κ -carrageenan	70.90	3.62	4.78	2.29
cinnamon powder	6.81	0.15	11.76	0.66

Cinnamon powder was purchased from Amari-koshin Shokuhin Co. Ltd. (Kyoto, Japan).

The effects of cations on the rheological and thermal properties of κ -carrageenan gels are known. Especially, potassium cations play an important role in the gelation of κ -carrageenan. (Hermansson, 1989; Mangione et al., 2005).

Cation contents of κ -carrageenan and cinnamon powder were measured by ICP-OES. Analytical results are shown in Table 1. The amounts of K⁺ were 70.90 mg and 6.81 mg, respectively in 1.0 g of κ -carrageenan and cinnamon powder.

For the κ -carrageenan gel preparation and NMR solvent, authentic cinnamaldehyde (*trans*-3-phenyl-2-propenal) and deuterium oxide (D₂O) were purchased from Fujifilm Wako Pure Chemical Corporation Co., Ltd. (Tokyo, Japan). These chemicals were of reagent grade.

2.2. Instruments

Cations of κ -carrageenan and cinnamon powder were measured by ICP-OES (iCAP 6000, Thermo Fischer Scientific Inc., Waltham, Massachusetts, USA.).

2.2.1. FID-GC

The volatile compounds were analyzed by FID-GC (Shimadzu GC-2014) using a ZB-FAME column (Phenomenex Zebron Capillary GC Column, 0.25 mm ID × 30 m). The carrier gas was He flowing at 1.3 mL/min. The injection temperature was 250 °C, and the oven temperature was increased from 45 to 250 °C at 15 °C/min. The detector temperature was 260 °C.

2.2.2. HRMAS-NMR and PFG-NMR

The HR-MAS NMR spectrum was measured in a nanotube (40 μ L) using a VARIAN VNMR5600 (600 MHz).

The PFG NMR spectrum for diffusion-ordered spectroscopy (DOSY) was measured by a JEOL RESONANCE JNM-ECA600 (600 MHz) NMR spectrometer using Shigemi symmetrical NMR microtubes matched with D₂O (BMS-005TV, Shigemi Co., Ltd.).

2.3. Methods

Analysis of cation contents of the κ -carrageenan and cinnamon powder by ICP-OES.

The K⁺, Na⁺, Ca²⁺, and Mg²⁺ contents of the κ -carrageenan and cinnamon powder were measured by ICP-OES. Wave lengths of measuring amounts of cations were set as following. K; 766.490 nm, Na; 589.592 nm, Ca; 422.673 nm, Mg; 280.770 nm.

2.3.1. Dynamic headspace gas analysis of flavor

For the purpose of gel destruction, a closed system of a homogenizer (20 mL of gel containing flavor) and the bubbler to collect volatile compounds under nitrogen gas was assembled. κ -Carrageenan gel

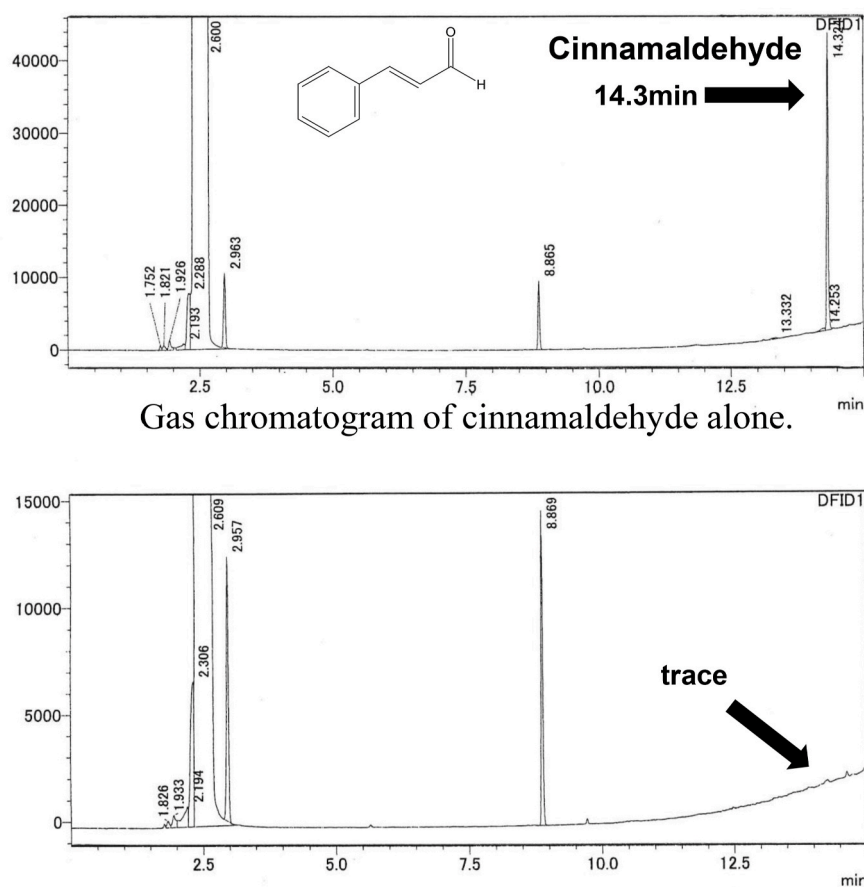


Fig. 1. (Upper) Gas chromatogram of cinnamaldehyde alone. (lower) Gas chromatogram of volatiles after homogenization of κ -carrageenan gel containing cinnamon powder. The scale of lower chromatogram was magnified about three times to upper one.

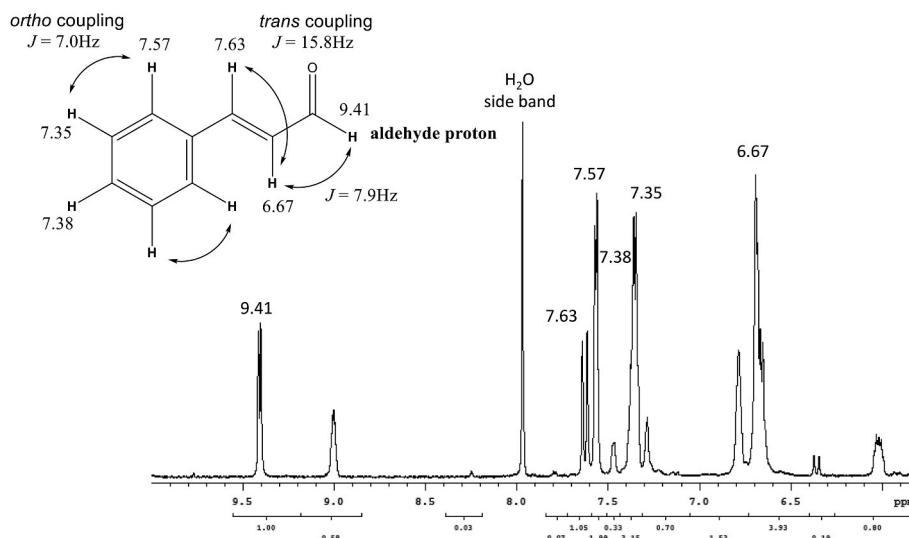


Fig. 2. ^1H NMR spectrum of cinnamaldehyde in κ -carrageenan gel prepared with D_2O and analyzed by HR-MAS NMR.

(1.0%, w/v) containing cinnamon powder (1.0%, w/v) was prepared in the closed cell system. A homogenizer tube was surrounded with a water jacket for circulation at 37°C . The homogenizer was operated at 4,000 rpm for 7 min. After release of flavor from homogenized gel, identification and quantitative analyses of trapped volatile compounds in acetone solution within the bubbler under nitrogen gas flow (150 mL/min) were executed by FID-GC.

2.3.2. Analysis of cinnamaldehyde by HR-MAS NMR and PFG-NMR

κ -Carrageenan powder was dissolved in deuterium oxide (0.8% w/v) at 90°C . After cooling to 60°C , temperature-volatile cinnamaldehyde (10 μL) was added to sol (10 mL) through a microsyringe and was immediately dispersed by a vibrator. The resulting κ -carrageenan D_2O gel containing cinnamaldehyde was reserved for NMR spectral analysis. The spectra were acquired by two types of NMR instruments. The HR-MAS NMR spectrum was measured in a 40- μL nanotube by a VARIAN VNMR5600, and the PFG NMR spectrum for DOSY was measured by a JEOL JNM-ECA600 using symmetrical Shigemi NMR microtubes matched with D_2O .

3. Results and discussion

The upper part of Fig. 1 is a gas chromatogram of cinnamaldehyde alone. The lower part is a chromatogram of the trapped volatiles analyzed by FID-GC after destructing the κ -carrageenan gel containing cinnamon powder. Only a trace of cinnamaldehyde remained after a retention time of 14.3 min, suggesting that the cinnamaldehyde was retained in the homogenized κ -carrageenan gel rather than released. To observe the presence of cinnamaldehyde in the κ -carrageenan gel, the NMR spectrum of the flavor in the gel state was measured. Tavel et al. (2010) investigated the interaction between aroma compounds and proteins such as β -lactoglobulin by NMR spectroscopy. In the present experiment, the NMR spectrum of cinnamaldehyde contained in κ -carrageenan gel was analyzed by the magic-angle spinning method. The HR-MAS NMR approach removes the interaction between the chemical shift anisotropy and the magnetic susceptibility (Alam & Jenkins, 2012; Polenova et al., 2015). The gel-state sample was spun at a “magic angle” gradient ($\theta = 54.7^\circ$) of the rotor spinning axis with respect to the magnetic field. The HR-MAS NMR spectrum of cinnamaldehyde contained in the κ -carrageenan gel is shown in Fig. 2. The two olefin protons presenting at 6.67 ppm and 7.63 ppm were *trans*-coupled ($J = 15.8$ Hz) to each other. In contrast, the two pairs of magnetically equivalent aromatic protons at 7.57 ppm and 7.35 ppm were *ortho*-coupled ($J = 7.0$ Hz) to each other. A single *para*-aromatic

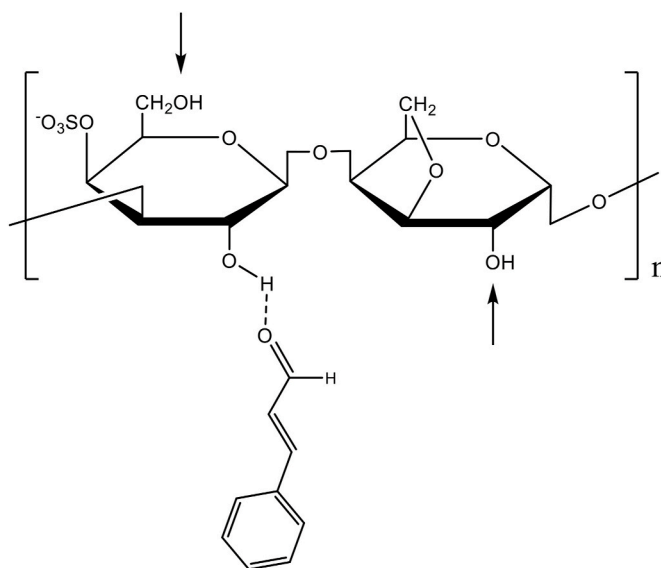


Fig. 3. Hydrogen bonding between κ -carrageenan and cinnamaldehyde. Arrows indicate the possible positions of the hydrogen bonds.

proton presented at 7.38 ppm. The doublet signal of one aldehyde proton coupled with the olefin proton at 6.67 ppm ($J = 7.9$ Hz) appeared at 9.41 ppm. Detecting the aldehyde proton in the gel was considered important because aldehyde readily reacts with other organic compounds and biomolecules. In particular, the aldehyde group forms hydrogen bonds with the polar functional groups on other molecules. The HR-MAS NMR spectral data confirmed the existence of cinnamaldehyde in the gel state of the polysaccharide (6.67 ppm (1H, dd, $J = 7.9, 15.8$ Hz), 7.35 ppm (2H, d, $J = 7.0$ Hz), 7.38 ppm (1H, brs), 7.57 ppm (2H, d, $J = 7.0$ Hz), 7.63 ppm (1H, d, $J = 15.8$ Hz), 9.41 ppm (1H, d, $J = 7.9$ Hz) in D_2O gel).

The cinnamaldehyde was not oxidized to carbonic acid, and reacted neither with the D-galactosyl moiety nor the 3, 6-anhydro-D-galactosyl moiety of κ -carrageenan. In other words, no covalent bonds between cinnamaldehyde and κ -carrageenan were formed under the present conditions. Few of the cinnamaldehyde molecules were released from the destroyed gel, suggesting that the cinnamaldehyde was hydrogen-bonded to κ -carrageenan (see Fig. 3).

In the next experiment, the self-diffusion behavior of

cinnamaldehyde in the κ -carrageenan gel was probed by PFG NMR. In a similar PFG NMR study, [Kato, Saito, Nabeshima, Shimada, and Kinugasa \(2006, 2008a, 2008b\)](#) assessed the self-diffusion coefficients of general solvents and later characterized nanoparticles in aqueous solution. [Zhao and Matsukawa \(2012\)](#) estimated the hydrodynamic screening length in κ -carrageenan solutions from NMR diffusion measurements. [Shimizu, Brenner, Liao and Matsukawa \(2012\)](#) measured the diffusion of probe polymer in gellan gum by PFG NMR.

Before evaluating the self-diffusion behavior of cinnamaldehyde in the κ -carrageenan gel, we measured the self-diffusion coefficient (D) of water at 25 °C by PFG NMR. The result ($D = 2.16 \times 10^{-9} \text{ m}^2/\text{s}$) almost coincided with that of [Holz, Heil and Stacco \(2000\)](#), who reported $D = 2.29 \times 10^{-9} \text{ m}^2/\text{s}$ at 25 °C. By confirming the reproducibility of D of water, we could prepare the NMR condition for measuring the D of cinnamaldehyde. The water signals at 4.7 ppm clearly decreased with increasing g (T/m) (see [Fig. 4](#)). Monitoring the aldehyde-proton signals at 9.4 ppm, the D of cinnamaldehyde at 25 °C was determined as $0.85 \times 10^{-9} \text{ m}^2/\text{s}$. The D value was calculated by the modified [Stejskal and Tanner, 1965](#) equation (1) on the basis of decreasing the relative signal intensity (S/S_0) against the field gradient magnetic power (g) under fixed conditions: temperature (25 °C), FG irradiation time (δ), and

diffusion time (Δ).

$$E(\delta, g, \Delta) = S/S_0 = \exp(-\gamma^2 g^2 \delta^2 D(\Delta - \delta/3)) \quad (1)$$

γ : gyromagnetic ratio ($\text{s}^{-1} \text{ T}^{-1}$)

g : FG power (T/m)

δ : FG irradiation time (s)

Δ : diffusion time (s)

D : self-diffusion coefficient (m^2s^{-1})

To our knowledge, we report the first self-diffusivity measure of cinnamaldehyde in κ -carrageenan gel. Next, focusing on the aliphatic-proton signals at 3.5 ppm in a higher magnetic field (3.2–5.2 ppm), the D of κ -carrageenan was determined as $0.26 \times 10^{-9} \text{ m}^2/\text{s}$. These self-diffusion coefficients revealed that cinnamaldehyde diffuses faster than κ -carrageenan. Accordingly, the two-dimensional DOSY spectrum showing the mobility differences between cinnamaldehyde and κ -carrageenan was obtained ([Fig. 5](#)). The self-diffusion coefficients D of cinnamaldehyde and κ -carrageenan ranged from 0.7 to $1.0 \times 10^{-9} \text{ m}^2/\text{s}$ and from 0.1 to $0.4 \times 10^{-9} \text{ m}^2/\text{s}$, respectively shown in [Fig. 5](#). The DOSY spectrum clarifies the relative molecular mobility and the interaction

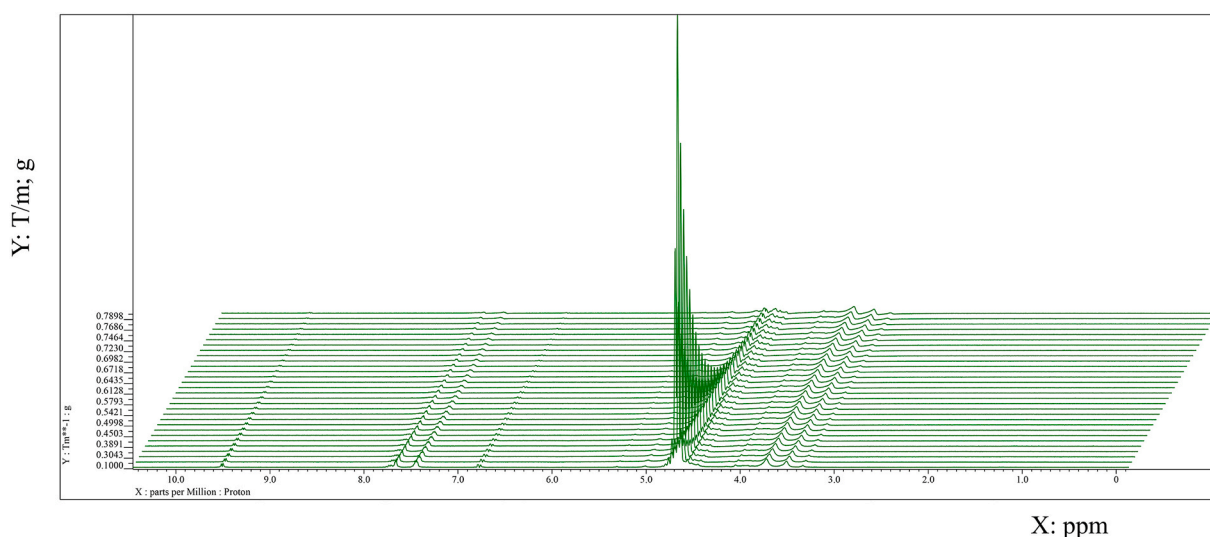


Fig. 4. Decrease of g with increasing field-gradient strength (G) at 25 °C, determined by PFG NMR.

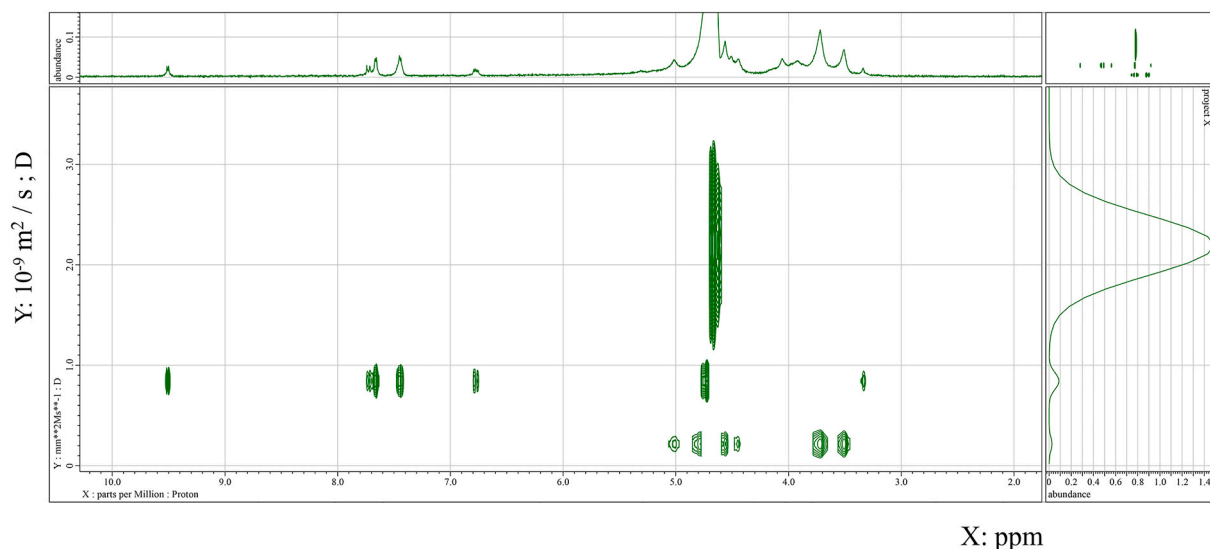


Fig. 5. DOSY spectrum of cinnamaldehyde in κ -carrageenan gel at 25 °C, determined by PFG NMR.

between the flavor molecules and polysaccharide. When the flavor molecules and polysaccharide show almost the same behaviors in the DOSY spectrum, both molecules might be bound restrictively within the gel. On the contrary, when the relative molecular diffusion mobilities are widely different, the flavor molecule and polysaccharide might interact to a limited extent. The present DOSY spectrum suggests that after the gel destruction, the flavor release was regulated not only by the total surface area of the decomposed small-size gel fragments, but also by chemical interactions such as hydrogen bonds between the flavor and polysaccharide molecules.

Suppressing the undesirably strong smell of gel foods is an important part of food science and food manufacture. Conversely, attractive aromas should be released from the hydrocolloid gel during mastication. To produce a delicious gel food with an approved taste and healthy functionality, further studies on the flavor release and texture of gel foods are expected in the near future.

Funding

This work was supported by a Grant-in-aid for Scientific Research [grant number (A) 22240076] from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MECSST).

Author statement

Editor-in-Chief: Food Hydrocolloids, Professor Pete Williams, I greatly thank you for your kind support and editing article.

Journal Manager, Research Content Operations Dear Dr. Thiyagarajan Sivakumar, I also thank you for kind permission to postpone sending a revised article under restricted social conditions by influence of covid-19.

Declarations of competing interest

None.

Acknowledgments

The authors express our gratitude to Dr. Nakauma M., San-Ei Gen F. F.I., Inc. for measurements of cations of the κ -carrageenan and cinnamon powder by ICP-OES.

The authors would like to thank MARUZEN-YUSHODO Co., Ltd. for

proof reading the article.

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