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1 Title

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19 Abstract

We measured the terahertz (THz) spectra of native, amorphous, and dried starches derived 20from corn and potato using the Fourier-transform (FT) system and compared these spectra 21to the X-ray diffraction (XRD) patterns. Both native corn and potato starches had seven 22absorption peaks in the terahertz regions, but five peaks were observed in the amorphous 23states. While spectral changes slightly occurred in corn starch even after drying, increase 24and decrease in the terahertz peak intensities were obtained in potato starch during drying. 25Similar changes in both starches during amorphization and drying were obtained in the 2627X-ray diffraction patterns, and the correlations were found between terahertz peaks and the X-ray signals. Since the intensity of the peak at 9.0 THz was correlated with 28crystallinity obtained using an X-ray diffraction ($r^2=0.98$), our data indicate that the 29Fourier-transform terahertz spectrometer can be a new analytical device to measure the 30 starch crystallinity. 31

32

33 Keywords

34 Starch, Crystallinity, Terahertz spectroscopy, X-ray diffraction

35

37 **1. Introduction**

Starch consisting of monomeric glucose is the predominant form of carbohydrate 38reserve found in diverse agricultural products, such as cereals, tubers, beans, and fruits. 3940 Starch consists of two glucans, namely amylose and amylopectin. Amylose is a primarily linear structure connected by α (1 \rightarrow 4) glycosidic linkages and is generally thought to be 41 in an amorphous state. Amylopectin demonstrates a semi-crystalline state and a highly 42branched structure connected by α (1 \rightarrow 4) and α (1 \rightarrow 6) glycosidic linkages. In 43amylopectin, the crystalline region is formed by parallel double helices and an amorphous 44 area at the branching point of the cluster chains (Blazek & Gilbert, 2011; Pérez & Bertoft, 452010). The crystalline structures of starch granules are affected by the amylose-46 amylopectin ratio, degree of branching, and chain length of the amylopectin. 47The degree of crystallinity, defined as the percentage of crystalline regions within the 48 total material, is one of the factors for determining starch function and utilization. In the 4950process of enzymatic hydrolysis of starch in humans, the crystalline regions in the starch

51 granule provide barriers to the diffusion and adsorption of the hydrolyzed enzymes, 52 resulting in a slow digestion rate. However, starch with low crystallinity is rapidly 53 digested due to the higher accessibility of enzymes (Blazek & Gilbert, 2010; Zhang, 54 Dhital, & Gidley, 2013). Thus, a correlation was found between crystallinity and the

55	digestion rate of starch (Bao, Li, Wu, & Ouyang, 2018; Carrera, Utrilla-Coello, Bello-
56	Pérez, Alvarez-Ramirez, & Vernon-Carter, 2015; Zhang et al., 2014). The crystalline
57	structure of starch is also important in industrial fields. For instance, the amylose-
58	amylopectin ratio and crystalline structure determine the strength and stiffness of starch
59	plastics (van Soest & Vliegenthart, 1997). The network structure is formed by connecting
60	starches with plasticizers during starch plastic production, but the short length of the
61	amylopectin chain weakens the materials. Starch properties are also involved in
62	bioethanol production (Yangcheng, Jiang, Blanco, & Jane, 2013). Unlike starch with a
63	high content of amylopectin with long chains and high crystallinity, starch with a high
64	content of amylose or amylopectin with short chains and low crystallinity contributed to
65	enhanced starch hydrolysis and yield of bioethanol.
66	X-ray diffraction (XRD) has been the major method used for determining the
67	crystalline structures in starch at the molecular level (long-range order) and has been used
68	to measure the crystallinity of starch. The crystallinity of carbohydrate polymers can be
69	calculated in three methods, peak height, peak deconvolution, and amorphous subtraction
70	(Park, Baker, Himmel, Parilla, & Johnson, 2010). The amorphous subtraction method has
71	been commonly used for starch measurement (Lopez-Rubio, Flanagan, Gilbert, & Gidley,
72	2008; Nara & Komiya, 1983). According to the XRD patterns, native starch granules have

73	15%–45% crystallinity and can be classified into three types depending on their botanical
74	sources (Zobel, 1988). A-type diffraction patterns are present in cereals, and B-type
75	diffraction patterns are present in the roots and tubers. Bean and specific fruit starches
76	show C-type diffraction, which is the combination of the A- and B-types. The main
77	difference between the A- and B-type starches is the structure between the double helices
78	and water molecules within the crystalline lattice (Imberty, Chanzy, Perez, Buléon, &
79	Tran, 1988a; Imberty & Perez, 1988b; Popov et al., 2009). A-type starch has a monoclinic
80	structural unit, characterized by the tightly compacted helices with fewer water molecules.
81	B-type starch has a hexagonal structural unit with ample space between the helices for
82	the accumulation of numerous water molecules. The water molecules among the double
83	helices are connected to a starch chain or other water molecules via a hydrogen bond.
84	Spectroscopy is one of the useful analytical methods for exploring the molecular
85	structure and interaction between the surrounding molecules or solvents. Conventional
86	vibrational spectroscopies (near- and mid-infrared) have been used to determine starch
87	structure in the short-range order (atomic level) (Kizil, Irudayaraj, & Seetharaman 2002;
88	van Soest, Tournois, de Wit, & Vliegenthart, 1995). However, these spectroscopic
89	techniques are not sensitive to starch crystallinity in the long-range order (Pozo et al.,
90	2018; Sevenou, Hill, Farhat, & Mitchell, 2002; Warren, Gidley, & Flanagan, 2016)

91	because the apparent vibrational modes of the functional groups and structures in the
92	short-range order are reflected in the near- and mid-infrared spectra. Nuclear magnetic
93	resonance (NMR) spectroscopy is also used to analyze starch structures in short-range
94	order because of the different chemical shift patterns of ordered and non-ordered portions.
95	Although previous studies have found a correlation between NMR data and starch
96	crystallinity (Flanagan, Gidley, & Warren, 2015), NMR cannot directly access the starch
97	structure in long-range order. Thus, direct measurement of starch crystallinity has not
98	been achieved using conventional spectroscopies.
99	Terahertz (THz) or far-infrared waves are generally referred to as the frequency range
100	from 100 GHz to 30 THz (3.3–1000 cm ⁻¹) and recent advances in optical devices have
101	made it possible to use THz spectroscopy to explore new applications (Tonouchi, 2007).
102	In THz spectra, we can obtain information about the intermolecular and the lattice
103	vibrational modes dominated by the polymorphs (King, Buchanan, & Korter, 2011; True,
104	Schroeck, French, & Schmuttenmaer, 2011). For example, crystallized molecules have
105	shown absorption bands attributed to the hydrogen bond networks, the van der Waals
106	forces, and framework vibrations, while no such bands have been observed in the
107	amorphous state due to the lack of crystalline structures in the long-range order (Otsuka,
108	Nishizawa, Fukura, & Sasaki, 2012; Strachan et al., 2004; Walther, Fischer, & Jepsen,

109	2003). Previous studies have also found correlations between the THz spectra and the
110	crystallinity of polymers (Azeyanagi & Ohki, 2018), carbohydrates (Takeuchi et al.,
111	2012; Vieira & Pasquini, 2014), and pharmaceutical materials (da Silva, Vieira,
112	Rohwedder, Pasquini, & Pereira, 2017; Strachan et al., 2005) obtained by XRD. For
113	instance, Vieira and Pasquini (2014) estimated the crystallinity of cellulose using THz
114	spectra in the range of 0.7–3.4 THz (23.3–113 cm ⁻¹) with partial least squares (PLS)
115	regression analysis. da Silva et al. (2017) used THz spectra from 0.4 to 4.4 THz (13.3-
116	147 cm ⁻¹) to determine the crystallinity of mebendazole. Compared to the near- and mid-
117	infrared spectra (da Silva, Gonçalves, Vasconcelos, Fernanda Pimentel, & Pereira, 2015),
118	THz spectroscopy provided a higher determination coefficient and fewer errors in
119	estimating the crystallinity of mebendazole. The authors remarked on the advantage of
120	using THz spectroscopy to directly access information about the crystalline structure,
121	compared to the use of near- and mid-infrared spectroscopy.
122	THz spectroscopy is commonly performed using the time-domain system (TDS) and
123	Fourier transform (FT) system. The former provides the phase directly and is suitable for
124	evaluating the optical properties in the THz region, but an expensive femtosecond pulse
125	laser system is indispensable. The latter is a system that shifts the conventional mid-
126	infrared spectrometer to the low-frequency side, and its principle is same as that of FT

127	infrared spectroscopy. While the TDS provides THz spectra in the lower frequencies
128	below 5.0 THz (167 cm ⁻¹), the broadband spectra in the higher frequency regions can be
129	obtained by the FT system. Since the region observed by the FT system is the boundary
130	between intramolecular vibration and intermolecular vibration, researchers of far-infrared
131	spectroscopy have long been interested (Han et al., 2001). Although most researchers
132	have employed the TDS (Azeyanagi & Ohki, 2018; da Silva et al., 2017; Strachan et al.,
133	2005; Takeuchi et al., 2012; Vieira & Pasquini, 2014), which is commercially obtained,
134	starch has not demonstrated any distinctive absorption peaks in the lower frequencies
135	measured by this system (Jiang, Ge, Lian, Zhang, & Xia, 2016). However, our study used
136	the FT system and identified inherent peaks in starch (Nakajima, Shiraga, Suzuki, Kondo,
137	& Ogawa, 2019). Therefore, we hypothesized that these starch peaks may be an index for
138	the starch structure in the long-range order and FT-THz spectrometer may have the
139	potential to be a new analytical method for elucidation of the crystallinity estimation.
140	The FT-THz spectrometer also has advantages for crystalline measurements, compared
141	to conventional XRD. The price of the THz device is lower than that of the X-ray
142	diffractometer. Moreover, unlike X-rays with ionizing properties, THz waves have much
143	lower energies; hence, THz spectroscopy can monitor starch structures without causing
144	ionization and damage to the structure.

145	Thus, in this study, we aimed to examine whether FT-THz spectrometer could be a new
146	analytical method for determining starch crystallinity. We measured the THz spectra of
147	corn (A-type) and potato (B-type) starches with different crystallinity and compared them
148	with the XRD patterns. Based on the obtained THz and XRD data, the model for
149	crystallinity estimation was developed. Herein, we present possible causes for the spectral
150	differences between the A- and the B-types with respect to the long-range starch structures.
151	
152	2. Materials and methods
153	2.1. Chemicals
154	We purchased standard corn and potato starches from Wako Pure Chemical Industries,
155	Ltd. Polyethylene powder with a particle size $8\pm1~\mu m$ was used to dilute samples for the
156	THz measurement. Native starches were used as the crystalline starches. To obtain stable
157	spectral data, fine particles were required, thus all starch samples were passed through a
158	32 µm mesh sieve before measurement.
159	2.2. Preparation of amorphous starches
160	Amorphous starches were prepared according to a method described in a report (Kim,
161	Kim, & Shin, 1997) with some modifications. Starches were suspended in distilled water
162	(10%, w/v) and heated at 95 °C for complete gelatinization. The gelatinized starches were

163 mixed with 99% ethanol (starch gel : ethanol = 1 : 4, v/v), thoroughly stirred, and the 164 mixed solvent was removed. The residuals were dried at 40 °C overnight until they 165 achieved a constant weight. The samples were ground into powder with a mortar and 166 pestle, and passed through a 32 μ m mesh sieve.

167 2.3. Drying process

We used a drying process to modify the starch crystallinity. While drying has slight or little influence on the crystallinity of the A-type starches, dynamic structural changes in the long-range order have been reported in the B-type starches (Gunaratne & Hoover, 2002; Jacobs & Delcour, 1998). Thus, corn starch (A-type) was dried in an air oven at 120 °C for 960 min only, while potato starch (B-type) was dried at the same temperature for 5 min, 10 min, 30 min, 120 min, and 960 min. After cooling, the starches were passed through a 32 μm mesh sieve.

175 *2.4. THz measurement*

176 Starches were mixed with polyethylene powder at 5% mass concentration (sample: 8

- 177 mg; polyethylene: 152 mg). The mixtures were pressed at 15 kN for 10 min into solid
- pellets with a 13 mm diameter and 1.25±0.05 mm thickness. The absorbance of the pellet
- 179 was then measured using an originally remodeled FT spectrometer (FARIS-1s, JASCO
- 180 Co.) with a ceramic light source from 3.0 THz to $13.5 \text{ THz} (100-450 \text{ cm}^{-1})$ at a resolution

of 0.12 THz (4 cm⁻¹) according to a method in our previous measurement (Nakajima et 181 al., 2019). To enhance the signal to noise (SN) ratio, 300 scans were recorded and 182averaged. Measurement time was approximately 5 min. After replacing a pellet, the same 183184 measurement was repeated further twice to evaluate three different points for each pellet, and the average value was used. The spectrum of pure polyethylene (152 mg) was also 185recorded as a reference spectrum. Additionally, we measured the absorbance of three 186 individual pellets for each condition and averaged the absorbance spectra. As an 187 incoherent light source was used in the FT system, we could avoid an etalon peak, which 188 189 is clearly observed in TDS equipped with a coherence laser. The peak position in the THz regions was determined from the second derivative spectra. Since the particle size and 190 pellet thickness were adjusted, the baseline spectra were stable and other spectral 191 processes, such as normalization, were not required. 192

193 *2.5. XRD analysis*

194 XRD patterns were measured using a diffractometer (Smart Lab, Rigaku Co.). Data 195 were obtained over the 2 θ range of 4°–40° at 0.05° interval with a scan speed of 3° min⁻¹, 196 using Cu-K α radiation with λ = 1.541 Å. The "d" spacings were calculated according to 197 the Bragg's law. After baseline correction and the Savitizky-Golay smoothing, the relative 198 crystallinity of samples was calculated according to the *Hermans* method reported by

Nara and Kamiya (1983). Briefly, the upper crystalline part was separated with a smooth curve connecting each point of minimum intensity of the crystalline peaks and the lower part was defined as an amorphous area, using Matlab software (See Fig. S1). Starch crystallinity was calculated by crystalline area / (crystalline + amorphous) area.

204 3. Results and discussion

205 *3.1. Crystalline and amorphous starches*



Fig. 1. Absorbance spectra of (A) crystalline (native) and (B) amorphous starches.

208 Vertical lines indicate absorption peaks, and insets represent the second derivative spectra



Fig. 2. XRD patterns of (A) crystalline and (B) amorphous starches. The values in parenthesis indicate crystallinity.

214

We first compared the THz spectra of corn and potato starches with crystalline and amorphous states (Fig. 1). Corn starch had four main peaks at 9.0 THz (300 cm^{-1}), 10.5 THz (350 cm^{-1}), 12.2 THz (407 cm^{-1}), and 13.2 THz (440 cm^{-1}), and three shoulder peaks at 4.9 THz (163 cm^{-1}), 7.9 THz (263 cm^{-1}) and 8.6 THz (287 cm^{-1}). Potato starch exhibited

similar peaks at 5.1 THz (170 cm⁻¹), 7.8 THz (260 cm⁻¹, albeit very weak), 8.5 THz (283 219cm⁻¹), 9.0 THz, 10.5 THz, 12.2 THz and 13.1 THz (437 cm⁻¹), but intensities of the four 220main peaks were lower than those of corn starch (see inset in Fig. 1A). Additionally, 221potato starch had higher absorbance in the lower frequencies. The causes for higher peak 222intensities of corn starch and higher absorbance of potato starch in the lower frequencies 223have been discussed later. 224Compared to the crystalline starches, no clear spectral difference was acquired between 225226corn and potato starches after amorphization (Fig. 1B). While the absorption peaks around 5.0 THz (167 cm⁻¹) and 8.0 THz (267 cm⁻¹) observed in the crystalline states were hardly 227228visible, amorphous starches had two overlapping peaks at 8.5 THz and 9.0 THz, and three peaks at 10.7 THz (357 cm⁻¹), 12.0 THz (400 cm⁻¹), and 13.1 THz. Although four peaks 229at 9.0 THz, 10.7 THz, 12.0 THz and 13.1 THz became less intense after amorphization, 230the peak intensity at 8.5 THz was stronger (second derivative signals of native corn, native 231potato, amorphous corn, and amorphous potato were 3.1×10^{-3} , 4.8×10^{-3} , 7.8×10^{-3} and 232 8.0×10^{-3} , respectively), suggesting that the peak was sensitive to the amorphous structure 233of starch. Noteworthy, we observed the different peak frequencies, except for peaks at 9.0 234235THz, 10.5 THz, and 12.2 THz, between native corn and potato starches, and peak shifts after amorphization. These spectral features may originate from differences in the starch 236

structure, because structural differences have been strongly reflected at peak positions in
the THz regions (King et al., 2011; Otsuka et al., 2012; Strachan et al., 2004; True et al.,
2011; Walther et al., 2003). However, in this study, it is difficult to elucidate the cause for
peak positions and shifts without a more detailed analysis.

Fig. 2 shows the XRD patterns of crystalline and amorphous starches. Crystalline corn 241starch displayed the A-type diffraction, having clear peaks at $2\theta = 15^{\circ}$, 17° , 18° , and 23° . 242243Potato starch displayed the B-type diffraction, having clear peaks at $2\theta = 5.5^{\circ}$, 17° , 22° , and 24°. Crystallinity in native corn and potato starches was 38.6% and 29.8%, 244245respectively. However, amorphous corn and potato starches exhibited no distinctive 246diffraction peaks and crystalline structures. We defined the crystallinity in amorphous starches to be 0%. The crystallinity of crystalline starches was slightly higher than 247previously reported (dos Santos, Leonel, Garcia, do Carmo, & Franco, 2016; Pozo et al., 2482018). Compared to large particles, fine starch particles contribute to higher crystallinity 249because the content of amorphous amylose decreases in fine particles (Ao & Jane, 2007; 250Wang, Tang, Fu, Huang, & Zhang, 2016). For these reasons, the higher crystallinity 251observed in this study could be due to fine starch particles. 252

253 *3.2. Dried starches*



Fig. 3. Absorbance spectra of dried (A) corn and (B) potato starches. The absorbance spectra of potato starch were offset vertically for clear spectral features, +0.1–0.45. Vertical lines indicate the absorption peaks. (C) Second derivative spectra of the dried potato starch in the range of 8.0 THz–13.5 THz (267 cm⁻¹–450 cm⁻¹). Black arrows mark

the two peaks showing the continuous changes during drying.

260



Fig. 4. XRD patterns of dried (A) corn and (B) potato starches. The values in parenthesis
indicate crystallinity.

The THz spectra of corn starch indicated slight changes even after 960 min of drying (Fig. 3A), while distinct spectral changes occurred in the potato starch during drying (Fig. 3B, 3C). The two peaks at 5.1 THz and 7.8 THz disappeared within the first 10 min, and intensities of the other five peaks were also altered during drying. Notably, the continuous

269 increase and decrease in peak intensities were found at 8.5 THz and 9.0 THz.

270Similar changes were measured in the XRD patterns of dried starches. The four clear peaks were slightly shifted to lower angles, and crystallinity decreased by 3.3% in the 271dried corn starch, compared to that in the native corn (Fig. 4A). In contrast, the 272progressive collapse of the crystalline structure and decline in the crystallinity were 273observed in potato starch during drying (Fig. 4B). The clear and small peaks became 274275weaker or disappeared during the first 10 min, and two clear peaks at $2\theta = 17^{\circ}$ and 22° were observed after 30 min. The crystallinity of potato starch decreased by 15.7% after 276277960 min of drying.



Fig. 5. Weight loss in corn and potato starches during drying (n=3).

The weight loss attributed to the evaporation of water during drying is shown in Fig. 5. The weight loss in corn and potato starches after 960 min of drying was 11.7% and 15.0%, respectively. Notably, the weight loss was constant in potato starch after 30 min, thus

potato starch lost water molecules during the first 30 min. These results indicate that water 284molecules have little influence on the crystalline structure of corn starch. Additionally, 285the decrease in crystallinity of potato starch during the first 30 min of drying could mainly 286be due to water loss or water-starch interaction, while that after 30 min could be due to 287the collapse of the starch structure. Previous XRD measurements have reported similar 288data in the A- and the B-type starches during drying, and the B-type starches enriched 289with water molecules were more easily altered than the A-type starches (Gunaratne & 290Hoover, 2002; Jacobs & Delcour, 1998). 291

292

293 3.3. THz-XRD correlation



Fig. 6. Heat map of the correlation coefficient between the THz spectra and the XRD

296 patterns. Red lines indicate the THz and XRD peaks having correlation.

Statistical heterospectroscopy is a method to investigate the correlation between 298different kinds of spectroscopic data acquired on multiple samples (Crockford et al., 299300 2006). Since the THz spectra, especially peak intensities, and the XRD patterns exhibited similar behavior during amorphization and drying, we used this analytical method to the 301 302 THz spectra and the XRD patterns in order to further elaborate the starch spectra. 303 In Fig. 6 we present the correlation coefficient between the THz spectra and the XRD patterns of the ten starch samples; the cells with low $(0.7 \le 0.8)$ and high $(0.8 \le r)$ 304 305positive values are marked in blue and red, respectively. No negative correlation was 306 found. The five THz peaks showed correlations with the XRD patterns. The shoulder peak around 5.0 THz had low correlations with $2\theta = 5.5^{\circ}$, 17°, and 35°, and these results were 307 consistent with the decrease or disappearance of THz and XRD peaks during the first 10 308 min of drying (Fig. 4B). High correlations were obtained between the four main peaks. 309 The peaks at 9.0 THz and 10.5 THz correlated with $2\theta = 17^{\circ}$, and 12.2 THz with $2\theta = 15^{\circ}$. 310 Additionally, the peak around 13.0 THz was correlated with $2\theta = 15^{\circ}$, 18° and 23°. We 311further explored the THz peaks having high correlations with the XRD patterns. Since 2θ 312= 15°-23° correspond to 3.94 Å-5.96 Å of "d" spacing base on the Bragg's law, our data 313suggests that the four peaks above 9.0 THz may be assigned to the vibrational modes of 314

these distances in the crystalline lattice.

316 *3.4. THz spectral features and starch structure*

Carbohydrates, including starch, have been found to exhibit non-covalent modes 317318 (hydrogen-bond networks and inter-crystalline forces) and covalent modes (skeletal deformations such as -C-C-C- and -C-O-C-) of interaction in the THz regions (Cael, 319 Koenig, & Blackwell, 1975; Hineno, 1977; Yang, Weng, Ferraro, & Wu, 2001; Zhbankov, 320 Andrianov, & Marchewka, 1997). Hineno (1977) investigated the peak assignments of 321322sugar in the THz regions based on the observed spectra and calculated the one-molecular 323model. The observed peak positions correlated with the calculated vibrational frequencies in the region above 7.5 THz (250 cm⁻¹), though calculations based on the model did not 324explain all the observed spectra because of intermolecular interactions in the region below 3257.5 THz. Thus, the frequency boundary of the non-covalent and covalent modes located 326 around 7.5 THz, and the THz spectra obtained in our study contained information on both 327 vibrational modes of starch. 328In starch granules, double helices are stabilized by non-covalent hydrogen bonds and 329

van der Waals forces, which are weaker than covalent forces (Lan et al., 2016; Xu et al.,
2019). During gelatinization and amorphization, weak non-covalent bonds are destroyed,

and starch granules lose their crystalline structure. The disappearance of two peaks around

5.0 THz and 8.0 THz in amorphous starches suggests that these two peaks in native starch 333 granules may be attributed to non-covalent forces connecting the helix structure. The 334other five peaks observed above 8.5 THz, however, continued to exist after amorphization 335336 and disordered the crystalline structures; thus, exhibition of these peaks could be attributed to the covalent modes of starch. The double helix structure and vibrational 337 338 modes are illustrated in the graphical abstract. The heat map (Fig. 6) further supports the assignments of four peaks observed above 339 9.0 THz. In double helices, six monomeric glucose molecules have one turn of the helix, 340 341showing 21.0 Å along the helix axis (Imberty, Buléon, Tran, & Perez, 1991; Sarko & Wu, 1978; Zobel, 1988). Thus, the spacing of 3.94 Å-5.96 Å, as shown in Fig. 6, corresponded 342343 to a distance of approximately 1.5 glucose molecules. This spacing was markedly longer than the vibrational modes of the functional groups (C-O, C-H, and O-H) in the near- and 344mid-infrared regions. Additionally, the distance spanning a hydrogen-bonded pair of 345glucose units within a double helix was 2.5 Å–3.0 Å and that of the non-covalent force 346 connecting double helices was at least 11.0 Å (the distance between the two double 347helices) (Imberty et al., 1988a; Imberty & Perez, 1988b). Magnitude of non-covalent 348 349forces in starch granules was smaller or greater than the obtained spacing of 3.94 Å–5.96 Å. These starch structures suggest that the interaction between the glucose monomers (i.e., 350

351	covalent force) may be consistent with the obtained distance. We also examined the
352	correlation coefficient between non-covalent regions below 8.0 THz and covalent regions
353	above 8.0 THz. Although THz peaks around 5.0 THz and 10.5 THz had low correlation,
354	no high THz-THz correlation was found (see Fig. S2). These results indicate that the
355	covalent and non-covalent modes in starch granule are not synchronized.
356	The A- and B-type starches have the structural differences between the double helices
357	and water molecules within the crystalline lattice, as described in the Introduction (Sarko
358	& Wu, 1978; Imberty et al., 1988a; Imberty & Perez, 1988b). Since the numerous water
359	molecules in the B-type starch are connected to a starch chain or other water molecules
360	via a hydrogen bond, dehydration during drying influences the crystalline structures and
361	XRD patterns in the B-type starches (Kainuma & French, 1972; Nara, Mori, & Komiya,
362	1978). Drying induced evaporation of water molecules from starch granules and
363	movement of double helices into the ample space originally occupied by the evaporated
364	water, resulting in the collapse of the crystalline structure (Gunaratne & Hoover, 2002).
365	A similar disruption of the crystalline structure in potato starch was observed in our study
366	(Fig. 4B).
367	The water molecules and hydrogen bond network among the helices in the B-type

starch may also be responsible for higher absorbance of potato starch in the lower 368

369	frequencies, as shown in Fig. 1A. Corn and potato starches exposed to 960 min of drying
370	showed similar absorbance spectra in the lower frequencies (see Fig. S3). Thus, water
371	molecules may be attributed as the cause of higher absorbance of potato starch, but the
372	THz spectra of starches cannot be easily interpreted. The weight loss attributed to water
373	evaporation in corn and potato starches during drying was 11.7% and 15.0%, respectively,
374	as shown in Fig. 5. If water molecules are directly reflected on the absorbance spectra in
375	the lower frequencies, the decline in absorbance should theoretically occur in both
376	starches, but this was not observed for corn starch (Fig. 3A). Although the numerous
377	water molecules among the helices are only observed in the B-type starch as described
378	above, both the A- and B-type starches have bulk and bound water (Tang, Godward, &
379	Hills, 2000), which have relatively less influence on the crystalline structure. If the THz
380	spectra in the lower frequency regions are sensitive to the vibrational modes of water
381	molecules among the helices (or interactions between the water and starch), it is
382	reasonable that a decrease in absorbance after drying would be observed only in potato
383	starch. Additionally, the evaporation of bulk and bound water could cause weight loss in
384	both corn and potato starches. Thus, we hypothesize that the absorbance of water among
385	the helices may be apparent in the THz spectra, resulting in a higher absorbance of potato
386	starch (B-type) in the lower frequency regions.

Peak (THz)	4.9 and 5.1	8.5 and 8.6	9.0	10.5	12.2	13.1 and 13.2
r ²	0.76	0.58	0.98	0.69	0.69	0.74
RMSE (%)	4.1	6.2	1.1	4.9	4.8	4.3

Table 1. The linear regression models for starch crystallinity using six peaks (n=10).

391	Previous THz studies have used linear least squares regression using a single peak
392	(Azeyanagi & Ohki, 2018; Takeuchi et al., 2012) or PLS regression using multi-spectra
393	for crystallinity estimation (da Silva et al., 2017; Strachan et al., 2005; Vieira & Pasquini,
394	2014). Since starch had absorption peaks, we used linear regression analysis in this study.
395	Table 1 shows the model performance to estimate starch crystallinity by six different
396	peaks at and around 5.0 THz, 8.5 THz, 9.0 THz, 10.5 THz, 12.2 THz, and 13.0 THz. The
397	details of these models are shown in Fig. S4. These models were obtained from second
398	derivative signals of the six peaks via comparison with the objective crystallinity values
399	calculated by the XRD ranged from 0% to 38.6% for ten different samples (corn starch
400	crystallinity: 38.6%, 35.3%, and 0%; potato starch crystallinity: 29.8%, 24.3%, 19.3%,
401	15.3%, 15.0%, 14.1%, and 0%). To correct baseline fluctuations in absorbance spectra,
402	we used the dip intensity in the second derivative spectra based on methods described by
403	a previous study (Nakajima et al., 2019). The best model was generated by using the peak

at 9.0 THz, having r^2 of 0.98 and root mean squared error (RMSE) of 1.1%. Thus, this 404 peak was found to be sensitive to structural changes in the long-range order and a useful 405index for crystallinity estimation, as hypothesized. Although the peak at 8.5 THz 406 exhibited continuous changes during drying, this peak resulted in the lowest r^2 among the 407five peaks. This result was consistent with the statistical heterospectroscopy showing no 408 correlation between peak at 8.5 THz and the XRD patterns, as shown in Fig. 6. Since the 409 410 main four peaks above 9.0 THz were correlated with crystallinity ($r^2=0.69-0.98$), crystallinity influenced these peak intensities. Thus, the reason for higher peak intensities 411 412above 9.0 THz in corn starch (Fig. 1A, inset), compared to potato starch, could be a higher 413crystallinity. Since measurement of starch crystallinity is an important aspect in various fields, 414 previous studies have investigated new analytical methods, including spectroscopy (Pozo 415et al., 2018; Sevenou et al., 2002; Warren et al., 2016), instead of XRD. However, infrared 416 417spectroscopy and THz-TDS were not useful as an alternative method for the reasons described in Introduction. In contrast, we found that the starch peak at 9.0 THz determined 418 by the FT system could be a valuable index for crystallinity estimation. The most 419 420 important finding of this study is that, in the case of starch, the peak important for crystallinity measurement appears in the frequency covered by the FT system, but not by 421

422 TDS.

423

424 **4.** Conclusions

In this study, we found synchronized data between the THz spectra and the XRD 425patterns of starches, and the useful THz peak attributed covalent vibrational modes for 426 crystallinity estimation. In XRD analysis, the extraction and purification pretreatments 427428are required to measure starch crystallinity in agricultural products due to the influences of other plant matrices. We already found that the starch peak at 9.0 THz did not overlap 429430 with the constituent saccharides (Nakajima et al., 2019). In addition, the THz spectra of dry food samples enriched with starch provided this peak, which was attributed to starch 431432and was not affected by other plant matrices. These results suggest that THz spectroscopy may measure starch crystallinity in food samples without pretreatments. To refine this 433method, further studies of THz measurements in food samples and crystallinity estimation 434will be required. 435

436

437 **Declaration of Competing Interest**

438 The authors report no declarations of interest.

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