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Applicability of Near-infrared Spectroscopic Method to Unfreezable Water Measurements in Egg White Lysozyme and Soluble Starch

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Near-infrared spectroscopy (NIR) scanning from 1100 to 2500 nm was applied to measure the unfreezable water bound to egg white lysozyme and soluble starch. Two characteristic absorption bands corresponding to water molecules were changed into the second derivative difference spectra. In the changed spectra, the peaks at each lower and higher wavelength might be attributed to the freezable and unfreezable water, respectively. When the correlation coefficients between absorbances at wavelengths with maxima in the peaks attributable to the unfreezable water and the results of differential scanning calorimetry used as a reference analysis method of unfreezable water were calculated, the correlation coefficients at 1908, 1952 and 1992 nm assigned to the combination of the stretching and bending vibrations of OH were above 0.91 in both lysozyme and starch. Therefore, it can be concluded that the NIR second derivative difference spectra, in the region assigned to the combination of the stretching and bending vibrations of OH in water molecules, have potential for the measurements of the unfreezable water bound to egg white lysozyme and soluble starch used as model samples of food materials.

Keywords: near-infrared spectroscopy; unfreezable water; egg white lysozyme; soluble starch

Introduction

The amount of unfreezable water bound to food materials can have an important effect on both the quality and the storage behaviour of the material concerned. Various analysis methods have been used to determine the amount of this unfreezable water in foods. Most workers in the field have employed differential scanning calorimetry or nuclear magnetic resonance (1–3). However, these methods are time consuming, and require highly skilled personnel.

Near-infrared (NIR) spectroscopy scanning from 1100 to 2500 nm has been used for rapid, simple and nondestructive analysis of general components such as moisture, protein, starch and oil in foods and agricultural products (4). NIR spectroscopy is especially well suited to water determination because of the relatively high absorptivity of water compared to most other substances. It measures water content by using absorption bands around 1450 and 1940 nm, assigned to the first overtone and the combination of OH in water molecules, respectively (4). Some workers suggested that these absorption bands show free and bound water. Studies on pure water reported that absorption bands at 1340 to 1590 nm in the spectra of water represented three Gaussian peaks (5). These peaks could be assigned to hydrogen bonds with 0, 1 and 2 OH groups in water molecules. Fornes and Chaussidon (6) reported that absorption bands at 1820 to 2220 nm in the spectra of water measured at –50 °C represented two Gaussian peaks assigned to free and bound water molecules. However, these studies were not carried out with food materials. To date, in studies of the application of NIR spectroscopy in the food industry, a pressing need has been noted for a simple and rapid method for the determination of unfreezable water bound to food materials.

Therefore, our objective was to confirm the applicability of NIR spectroscopy for measurements of the unfreezable water bound to egg white lysozyme and soluble starch used as model samples of food materials.

Materials and Methods

NIR spectra of water at different temperatures

NIR spectra of deionized-redistilled water were recorded at 1 °C, 5 °C and 10 °C intervals over the range from 10 to 80 °C. The spectra were obtained with an InfraAlyzer 500 K (Bran and Lübbe Co., Norderstedt,

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Germany) scanning from 1100 to 2500 nm. The sample cell was a specially designed transflectance cell, so-called gold plated alcohol drawer cell (Bran and Lübbe Co.) (7). The thickness of the water layer in the cell was about 0.1 mm, and its temperature was controlled by water circulating from a water-bath. The cell compartment of the NIR apparatus was purged with nitrogen gas in order to avoid the problem of fogging on the cell window when measuring at temperatures lower than 20 °C. Absorbance data were recorded as log 1/R (R = transflectance), and second derivative spectra data were obtained using the InfraAlyzer data analysis software (Bran and Lübbe Co.).

Preparation of samples

Egg white lysozyme and soluble starch were obtained from Seikagaku Co. (Tokyo, Japan) and Nacalai Tesque Inc. (Kyoto, Japan), respectively. The samples were placed above *di*-phosphorus pentaoxide in a vacuum desiccator, and dried to a constant weight at room temperature (8). Subsequently, these dried samples were placed above a saturated water solution of potassium sulfate in a desiccator (9), and then aspirated until the solution began to boil (10). The desiccator was kept at 30 °C in an incubator until the desired water content change. The moisture content in samples was increased by an atmosphere of high relative humidity within the desiccator.

NIR spectra of samples with different contents of water

NIR spectra of prepared samples with different contents of water were obtained with an InfraAlyzer 500 K using a specially designed diffuse reflectance cell (Bran and Lübbe Co.). This cell holds the sample between a quartz glass window and a pressure pad to maintain constant packing density (11). Absorbance data were recorded as log 1/R (R = reflectance). Second derivative difference spectra data were obtained using the InfraAlyzer data analysis software.

Measurements of freezable and unfreezable water

Contents of freezable and unfreezable water used as the reference data of the NIR method were analysed by differential scanning calorimetry (DSC). Each sample (ca. 12 mg) was weighed into aluminum pans and hermetically sealed to avoid evaporation of water (12, 13). An empty, sealed pan was used as a reference of DSC. The sample pan and the reference pan were placed in a differential scanning calorimeter DSC 7 (Perkin Elmer Co., Norwalk, CT, U.S.A.) at room temperature, and the cell block of the DSC 7 was cooled to –60 °C with a Perkin Elmer Intracooler 2 freon-based mechanical cooler. The heating and cooling temperature programme was as follows; –60 °C isotherm for 10 min, heated from –60 °C to 10 °C at 10 °C/min intervals, held at 10 °C for 3 min, cooled to –60 °C at 10 °/min intervals, and subsequently reheated and recooled twice by the same programme for reversibility.

Results and Discussion

NIR spectra of water at different temperatures

Figure 1 shows NIR spectra of water at 1, 10, 30, 50 and 80 °C. These spectra consist of two strong asymmetrical bands. Absorption bands at 1360 to 1550 nm can be assigned to the first overtone of the stretching vibration of OH, and those at 1850 to 2100 nm to the combination of the stretching and bending vibrations of OH (4). With an increase in temperature, the wavelengths with maxima in these absorption bands shifted to lower wavelengths with increased intensity. The shifts have been interpreted as breakage of some hydrogen bonds. The degree of freedom of water molecules is increased as the temperature increases (6). Degree of the shifts

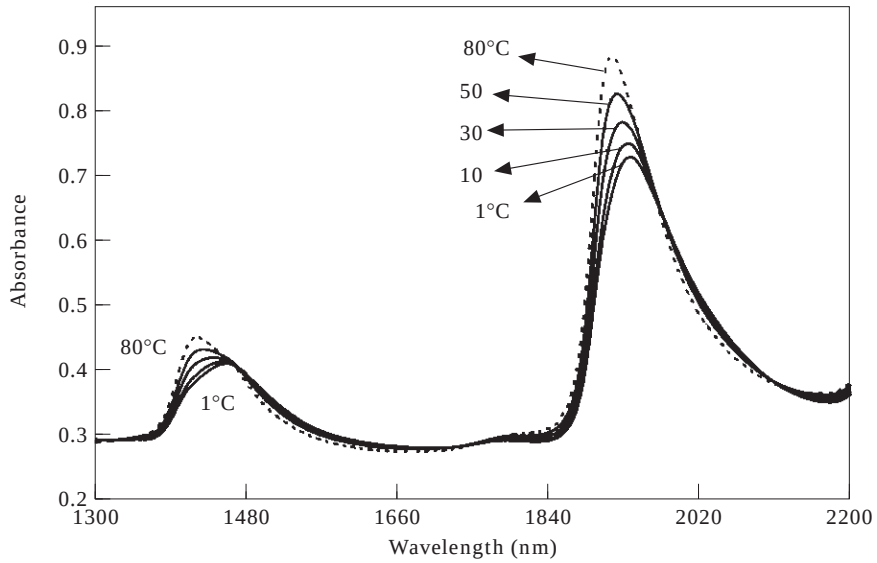

Fig. 1 Near-infrared spectra of water at 1 °C, 10 °C, 30 °C, 50 °C, and 80 °C

was 36 nm between 1456 nm at 1 °C and 1420 nm at 80 °C, and 24 nm from 1940 nm at 1 °C to 1916 nm at 80 °C. Degree of the shifts in the region of combinations was smaller than that in the region of first overtone as shown in **Fig. 1**. Libnau *et al*. (14) reported that the absorption band assigned to the bending vibration of OH did not shift with increasing temperature. These results were interpreted that the wavelengths in the region of combination of the stretching and bending vibrations of OH cannot be easily affected by temperature on the measurement of water content. There are isosbestic points at 1460 and 1960 nm in each region (**Fig. 1**). Therefore, the spectra of the two regions were changed into second derivative spectra because the isosbestic points have been known as two peaks (15, 16). **Figure 2** shows the second derivative spectra in two regions. The region of first overtone was divided into two bands with maxima at 1410 and 1462 nm, and the region of combinations was divided into two bands with maxima at 1906 and 1940 nm. Correlation coefficients (*r*) between temperatures and absorbances at these wavelengths were above 0.99. The relations at 1410 and 1906 nm toward lower wavelengths in each region appeared positive, whereas the relations at 1462 and 1940 nm toward higher wavelengths appeared negative. The positive and negative correlations could be associated with the increase and decrease of the degree of freedom of OH in water molecules with increasing temperature, respectively. Therefore, these results allowed us to conclude that the absorption bands at lower and higher wavelengths could be attributed to the free and bound water molecules, respectively.

Unfreezable and freezable water analyses by DSC

Figure 3 shows the DSC thermograms of egg white lysozyme and soluble starch with different contents of water. Prominent features of the curves from the sample series were the broad asymmetrical peaks produced as the result of the thawing of ice formed during the initial cooling process. With an increase in water contents, the temperatures with maxima in the peaks shifted to higher temperatures (–28 to –8 °C) with increases of peak area. The area of the peaks means that the heat absorption is associated with the thawing of a definite quantity of water frozen on cooling at –60 °C. The absorbed heat is the heat of fusion of ice–water phase transition for the freezable water changed to ice at –60 °C (17). However, the endothermic peaks began to disappear with water contents below 23.43 and 24.52 g/100 g in lysozyme and starch, respectively. This could be interpreted that freezable water at –60 °C did not exist below these contents of water. The results in **Fig. 3** showed a pattern similar to that reported by Duckworth (1). The enthalpy (ΔH, J/g) can be calculated from the area of the endothermic peak, and the content of freezable water can be obtained from the ΔH. The content of freezable water can be less than the total content of water. Therefore, the content of unfreezable water was calculated as the difference between total and freezable water (1, 17, 18) with the assumption that water cannot move to the outside of a sample pan in our experimental conditions. The results of the calculation are presented in **Table 1**. The contents of unfreezable water increased with increasing water contents, but the amount of that unfreezable water remained constant at 31.58 and 35.39 g/100 g for lysozyme and starch, respectively. This unfreezable water can be explained in terms of the maximum quantity of unfreezable water at –60 °C in relation to that rigidly bound to the samples.

NIR spectra of the samples with different contents of water

NIR spectra of samples with different contents of water were measured. The spectra were converted into the difference spectra on the basis of the dried samples, and then the difference spectra were changed into second derivative spectra for the separation of peaks and the correction of baselines of spectra (15). **Figure 4** shows the second derivative difference spectra. All spectra

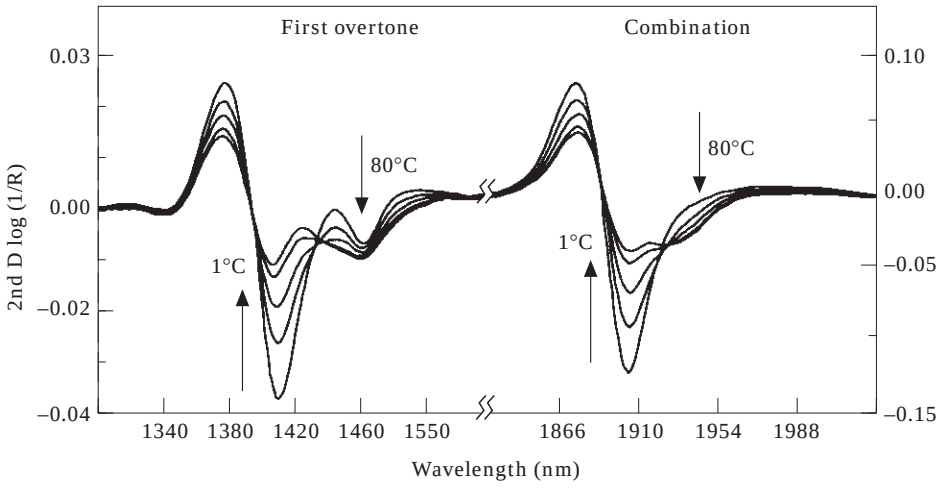

Fig. 2 Near-infrared second derivative spectra in the region of first overtone and combination of OH in **Fig. 1**

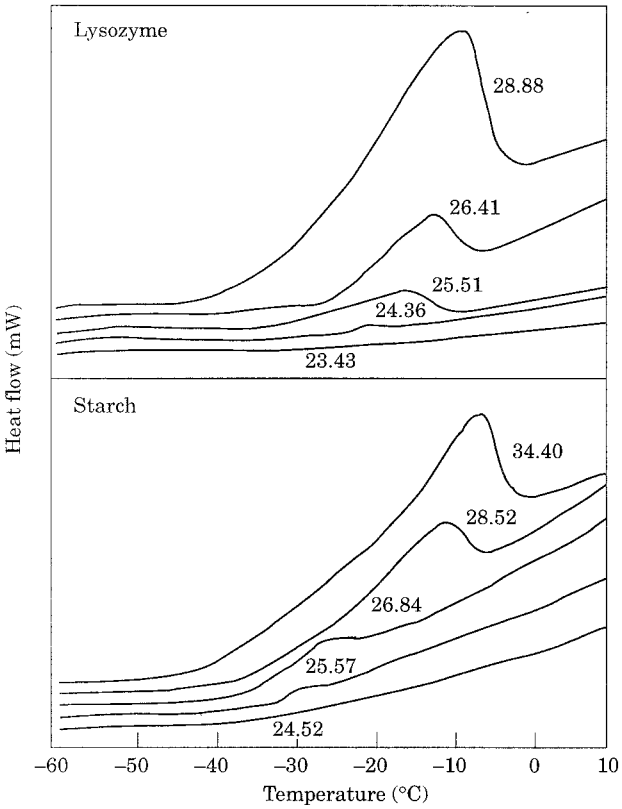

Fig. 3 Differential scanning calorimeter thermograms of egg white lysozyme and soluble starch with different contents of water (numbers written in thermograms are water contents, g/100 g)

Table 1 Freezable and unfreezable water contents calculated from differential scanning calorimeter thermograms of egg white lysozyme and soluble starch for different contents of water

Samples	Water contents (g/100g)	ΔH (J/g)	Freezable water (g/100g)	Unfreezable water (g/100g)
Soluble starch	24.52	0.00	0.00	24.52
	25.57	0.03	0.01	25.56
	26.84	0.67	0.05	26.79
	28.52	1.36	0.11	28.41
	34.52	1.36	0.11	28.41
	34.40	4.61	0.47	33.94
	42.97	62.90	7.92	35.39
Egg white lysozyme	23.43	0.00	0.00	23.43
	24.36	0.05	0.01	24.35
	25.51	1.45	0.11	25.40
	26.41	1.93	0.15	26.26
	28.88	7.30	0.62	28.26
	55.71	13.73	22.43	31.58

were divided into two bands with maxima at 1408 and 1468 nm, and 1416 and 1472 nm in the region of the first overtone of OH in water molecules for starch and lysozyme, respectively. The regions of combinations were also divided into two bands with maxima at 1908 and 1992 nm, and 1908 and 1952 nm as shown in **Fig. 4**. These results showed the same trends as shown in **Fig. 2**. Therefore, it was concluded that each absorption band at lower and higher wavelengths might be attributable to the freezable and the unfreezable water, respectively. The values of *r* between the absorbances at wavelengths with maxima in **Fig. 4** and the results of DSC in **Table 1** were calculated to investigate the

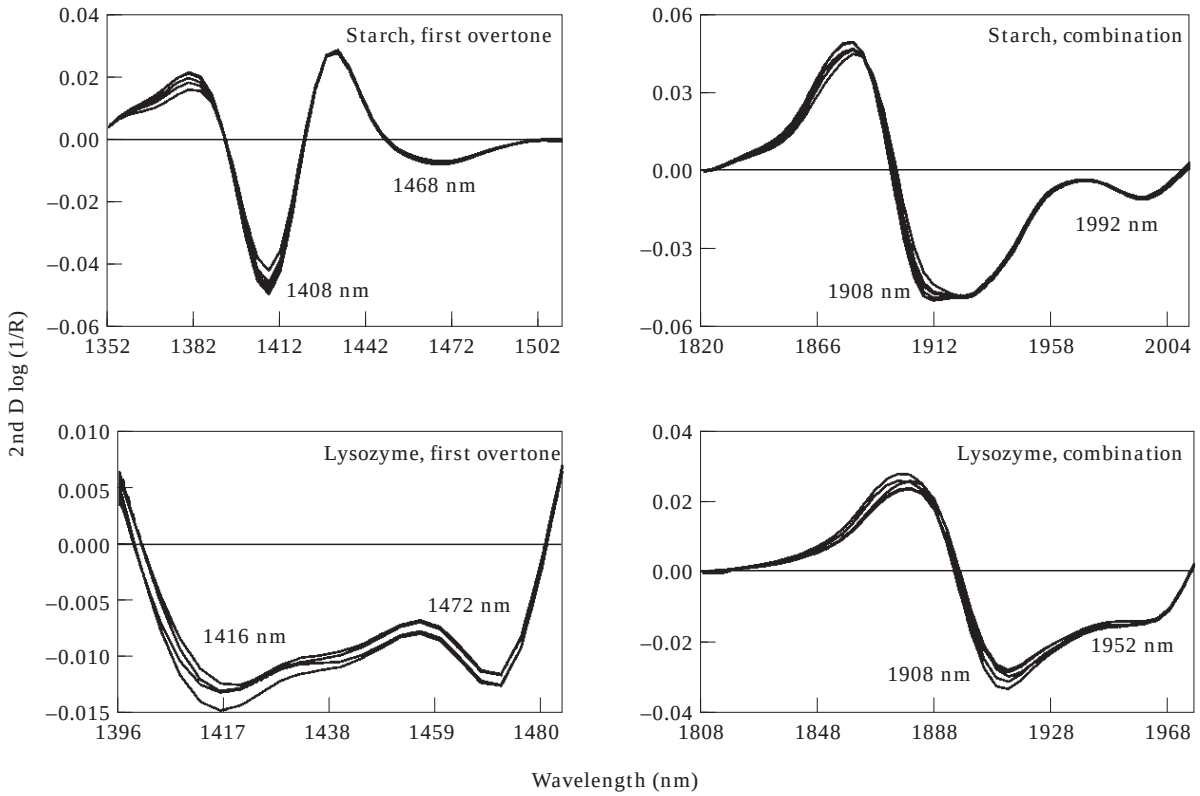

Fig. 4 Near-infrared second derivative difference spectra in the region of first overtone and combination of OH for samples used in **Fig. 3**

Table 2 Relationship between unfreezable water contents calculated from DSC thermograms and absorbances at wavelengths of NIR second derivative difference spectra in the region of first overtone and combination of OH

	First overtone		Combination	
Samples	nm[a]	r^b	nm	r
Soluble starch	1408	0.723	1908	0.964
	1468	0.926	1992	0.919
Egg white lysozyme	1416	0.792	1908	0.931
	1472	0.840	1952	0.908

[a]Wavelength.
[b]Correlation coefficient.

validity of the estimation. As can be seen from **Table 2**, the values of r at 1908, 1952 and 1992 nm in the region of combinations were higher than those of the first overtone. This may arise from the fact that the absorption bands at 1500 and 1440 nm in the spectra of the respective dried lysozyme and starch samples are overlapping with the absorption bands of the first overtone of OH in water molecules. The wavelengths in the region of combinations had values of r higher than 0.91. In conclusion, these results suggested that the NIR second derivative difference spectra, in the region assigned to the combination of the stretching and bending vibrations of OH in water molecules, has potential for measuring unfreezable water bound in egg white lysozyme and soluble starch used as model samples of food materials.

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