

Dynamics of Water Molecules in Sago Starch Determined by ^1H NMR

Masanori Okazaki^{1*}, Naoya Katsumi¹, Koyo Yonebayashi¹, Tomoe Nishi¹,
Yuji Honda¹ and Masato Igura²

¹Ishikawa Prefectural University, 1-308, Suematsu, Nonouchi, Ishikawa 921-8836 Japan

²Tokyo University of Agriculture and Technology, 2-24-16, Nakacho, Koganei, Tokyo 184-8588 Japan

*Corresponding author

Abstract: To study the behavior of water molecules in the sago (*Metroxylon sagu*) starch (CA type starch) structure, a ^1H solid state cross polarization (CP) magic angle spinning (MAS) nuclear magnetic resonance (NMR) technique has been applied using corn (type A starch) and potato (type B starch) as references. The exchange of H_2O molecules on/in the sago starch structure to D_2O based on the H-D isotopic exchange was analyzed with time from 1 to 24 hrs. The amount of water-molecule exchange on/in sago starch increased with the increasing contact time with D_2O , which was similar to the exchange amount of water-molecule of corn starch and smaller than that of potato starch. This suggested that the type B starch holds a larger channel space of a hexagonal crystalline structure for water molecules than does the type A starch and that the type CA starch showed the dynamics of water molecules, similarly to type A.

Keywords: ^1H solid state CP MAS NMR, isotopic exchange, sago starch, water molecules

Introduction

Sago palm (*Metroxylon sagu*) is one of the starch crops grown in the lowlands of Southeast Asia (Karim et al., 2008). Sago starch shows a temple bell shape, 35 μm in diameter and with a CA-type structure, which has the characteristics of both types A and B (Tang and Hills, 2003). The type A crystalline structure is composed of double helices with monoclinic arrangements, while the type B crystalline structure has another double helix with hexagonal arrangements including the channel, which was elucidated by electron microscopy, X-ray diffraction, and high-resolution solid state NMR (Gallant et al., 1997). The structure of amylose and amylopectin of wheat starch granules has been studied with cross polarization (CP) magic angle spinning (MAS) nuclear magnetic resonance (NMR) (Morgan et al., 1995), showing three distinct components: highly crystalline regions (amylose and amylopectin), solid-like regions (lipid inclusion complexes of starch), completely amorphous regions (associated with amylopectin branching regions). Among the previous researches on amylopectin determined by ^1H -NMR,

Falk and Stanek (1997) suggested that the signal at 5.11 ppm was assigned to H_1 linked to the C_1 carbons of glucan units, the signal at 3.33 ppm to H_2 linked to C_2 carbons, and the signal at 3.68 ppm to the H_3 linked to C_3 carbons. Dragunski and Pawlicka (2001) also assigned 3.3 ppm to the hydrogens linked to the C_6 carbon of $\text{CH}_2\text{-O}$ and $\text{CH}_2\text{-OH}$; 3.6 ppm to the hydrogens linked to the C_1 and C_4 carbons of CH-O ; 3.9 ppm to the hydrogens linked to the C_2 , C_3 , and C_5 carbons of CH-OH ; and 5.0 ppm to the hydrogens linked to the R-OH hydroxyl groups.

^1H NMR is the most effective technique for the bound water of starch (Mousseri et al., 1974; Leckert, 1976; Fan et al., 2013). The movement of water molecules in a short time, 10^{-12} to 10^{-6} seconds, was able to be detected by NMR. Wu et al. (1992) studied the heterogeneity in proton mobility in the water/starch systems using a magic-angle-spinning, wide-line, and high-resolution ^1H NMR. The bound water of corn starch was determined by ^1H NMR, and their signals of corn starch were related to moisture content (Mousseri et al., 1974). ^1H and ^2H NMR have been used for the identification of different water

molecule groups in native, acid hydrolyzed, and gelatinized starches (Hills et al., 1989; Hills et al., 1990; Tang et al., 2000; Tang et al., 2001; Choi and Kerr, 2003). Tang and Hills (2000) assigned the water molecule populations to the water in the amorphous growth rings, the water in the semi-crystalline lamellae, and the water in the channel. The 50-millisecond relaxation time peak was attributed to bulk water in external interstitial spaces between starch granules. The relatively short relaxation time was due to the fast ^1H exchange with starch hydroxyl protons (Schmidt, 2004). Fan et al. (2013) reported that water populations in starch granules were observed by the analysis of proton relaxation curves. The water populations in the type A starch showed more mobility than did those in the type B starch. Thereafter, Tang et al. (2001) identified four distinct water molecule populations, among which a fraction of mobile water in the granule can only be frozen at a temperature as low as -50°C . To show the mobility of water in starch, Li et al. (1998) studied unfreezable and freezable water (Mousseri et al., 1974; Noguchi and Nakazawa, 1979; Botlan et al., 1998) in waxy corn starch using ^1H and ^2H NMR and concluded that most of the water in waxy corn starch was highly mobile (liquid-like) even though the material was in a solid, semi-crystalline, glassy state (9–24% water content). Recently, Fan et al. (2013) investigated the starch and water interactions by ^1H NMR to show the effect of microwave heating on water distribution and dynamics in rice starch. Meanwhile, Chatakanonda et al. (2003) found that increased irrigation (water supply) caused a looser packing of amylose and amylopectin chains in cassava (type A) starch using a ^1H NMR relaxation. However, there are few reports on the dynamics of water molecules in type C starches (sago, kuzu, sweet potato, and so forth).

In this research, we tried to study water molecules in a sago starch structure using ^1H NMR to investigate the mobility of water molecules presented in type CA starch, compared to that of the water molecules in type A (corn) starch and type B (potato) starch.

Materials and Methods

1. Starch samples

The sago starch sample used in this study was collected from Leyte in the Philippines in 2006. The sago starch from the log was extracted by macerating the pith in water in a blender. The starch was separated from the pulp by squeezing the pulp in a cotton cloth and then air-dried. Corn and potato starch samples were produced by Kosakai Pharmaceutical Co., Ltd., and by Miyazawa Pharmaceutical Co., Ltd., which were certificated under Japanese Pharmacopoeia. The starch samples were dried in a Taitec VA-810 freeze-dryer and stocked in the starch sample box. The moisture content of the starch samples was 13.6% for sago, 12.0% for corn, and 15.3% for potato.

2. Electron and light microscope observation

The appearance of air-dried starch samples was analyzed by a scanning electron microscope (SEM) (Hitachi, Miniscope TM-1000) and a light microscope (Meijitech, MT 9000). Before SEM analysis, air-dried starch samples were carried out without any treatments.

3. H-D exchange on/in starch samples

Starch samples were vacuum-dried at 40°C for 24 hrs using a Sibata BV-001, and each 0.5 g of them (moisture content: 4.6% for sago, 3.7% for corn, and 5.1% for potato) was taken in a 4 mL glass bottle. D_2O (0.25 mL) was added to it and the mixture was allowed to stand for 1, 5, 12, and 24 hrs to exchange H with D isotopically after sealing with Parafilm to prevent water loss. After the exchange of H and D, the starch samples were stored in a desiccator, which held the silica gel in the space below the platform at 4°C for 24 hrs at room temperature. Accordingly, a part of H_2O and D_2O in/on the starch samples was lost due to the desiccation by silica gel. However, there was a possibility that a part of water molecules was added from the air in the desiccator. The starch samples were fully taken into a zirconium sampler (70 μL) to determine the ^1H in/on the starch structure.

4. ^1H CP MAS NMR analysis

^1H was analyzed by a Jeol Datum ECA/ECX CP/MAS NMR spectrometer operated at a ^1H resonance frequency of 400 MHz, spinning rate 10 kHz, point number 2048, using scanning number 256, at room temperature. The ^1H signal of starch samples exchanged by D_2O was determined in the probe to investigate the H-D exchange on/in sago starch. The D_2O -exchanged cellulose powder containing the percentages of H_2O similar to those in the starch samples was used as an external standard. The determination of ^1H NMR abundance was duplicated. The relative integrated abundance of ^1H NMR was obtained by the equation $X/Y \times 100$, where X is the integrated abundance of ^1H NMR of corn, sago, and potato starches at 1 to 24 hrs and Y is the integrated abundance of ^1H NMR of potato starch at 1 hr.

Results and Discussion

1. Sago starch

Sago starch granules show the spherical and temple-bell shape with a diameter of 30 – 40 μm (mean diameter: 37.59 μm) and a uniform distribution of diameter (Fig. 1), which coincided with the results of Kobayashi (1993) and Karim et al. (2008). Corn and potato starch granules were the polygon shape and oval shape, 15–20 μm in diameter (mean diameter: 14.76 μm) and 20 – 40 μm in diameter (mean diameter: 23.70 μm), respectively. The microscope images of sago, corn, and potato are shown in Figs. 2 – 4, which show the Maltase cross under a polarized microscope clearly. This provides the crystallinity structure of the starch samples.

2. Dynamics of water molecules on/in sago starch

The abundance of ^1H signals of the starch samples is shown in Fig. 5. The signal at 5.12 to 5.18 ppm was assigned to the ^1H of water molecules. Meanwhile, the signals at 30.3 to 33.5 ppm and -19.5 to -20.0 ppm were attributed to the spinning side band signals of the ^1H of water molecules. The signals of ^1H linked to the C_1 to C_6 of amylopectin were found at 3.3 to 3.9 ppm, and the

signals of ^1H of the R-OH hydroxyl groups affected by hydrogen bonds appeared at 5.0 ppm (Dragunski and Pawlicka, 2001). The presence of hydrogen bonds provided a slightly larger peak than the 5.0 ppm peak. Holmbum et al. (2000), however, suggested that the starch backbone protons of sago starch glutarate occurred as a complex multiplet in the region of 3 to 6 ppm. Therefore, the peak at 5.12 to 5.18 ppm of water molecules slightly overlapped with the hydrogens linked to sago starch.

In addition, the water molecules in poly (vinyl butyral) (PVB) studied using ^1H MAS NMR by Jeong and Han (2007) were detected at 5.0 and 4.9 ppm for the ^1H of free water A and B (Schaefer et al., 1983) due to inherent nanoscale inhomogeneity. Furthermore, the ^1H of hydroxyl and bound water was found at 4.4 ppm (Jeong and Han, 2007). They indicated that more water molecules in PVB with 9 weight % water content were tightly bound to PVB than those in PVB with 18

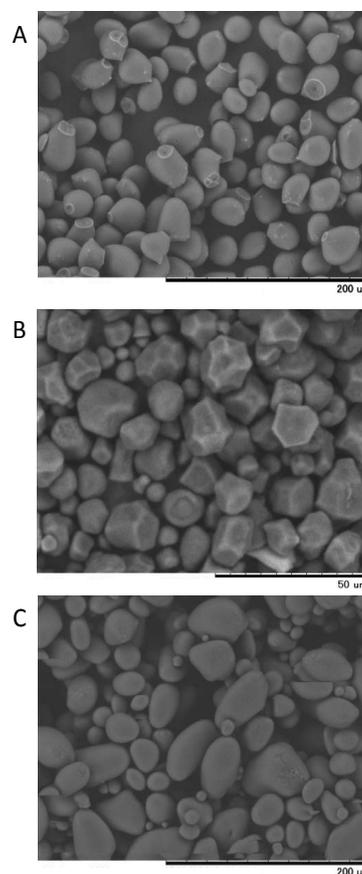


Fig. 1. Scanning electron microscope image of starch A: Sago ($\times 500$), B: Corn ($\times 1500$), C: Potato ($\times 500$)

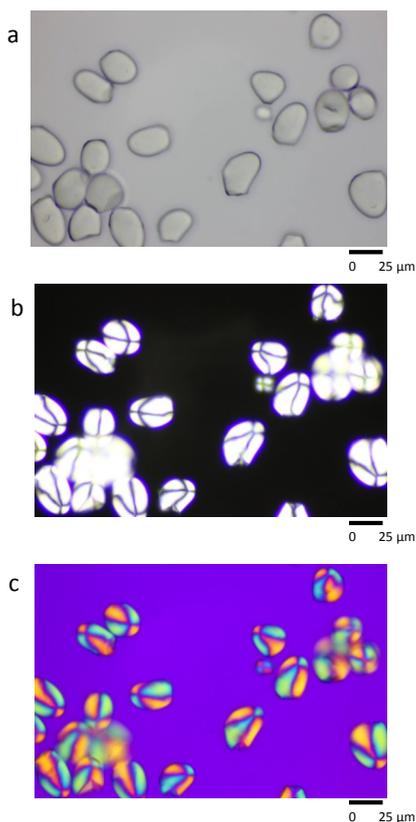


Fig. 2. Microscopic image of sago starch ($\times 400$)
 a: Open nicol, b: Cross nicol,
 c: Cross nicol with a 530 μm retardation plate

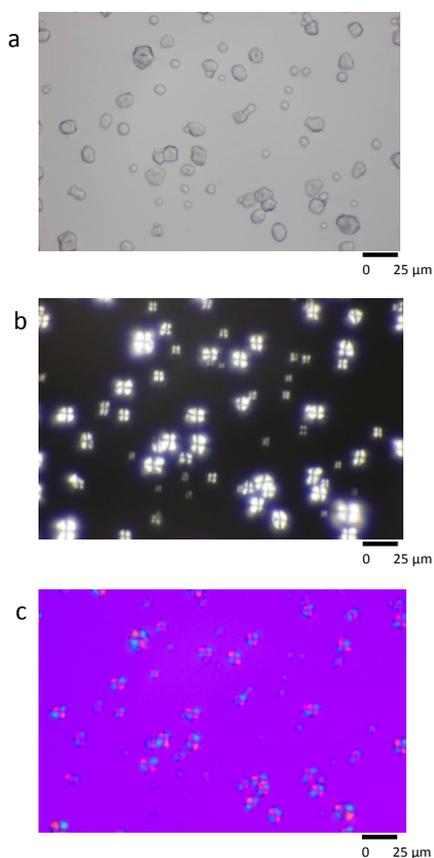


Fig. 3. Microscopic image of corn starch ($\times 400$)
 a: Open nicol, b: Cross nicol,
 c: Cross nicol with a 530 μm retardation plate

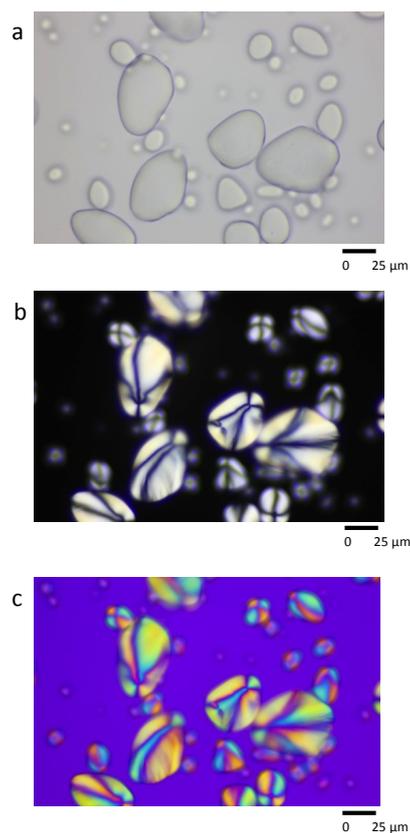


Fig. 4. Microscopic image of potato starch ($\times 400$)
 a: Open nicol, b: Cross nicol,
 c: Cross nicol with a 530 μm retardation plate

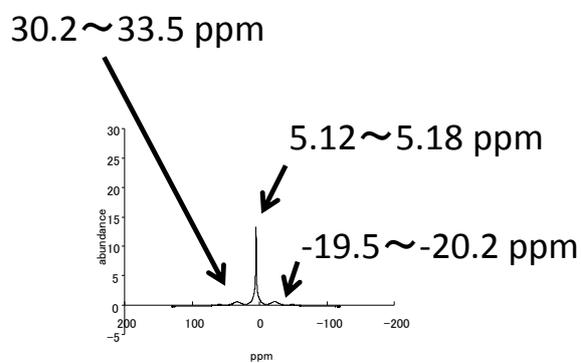
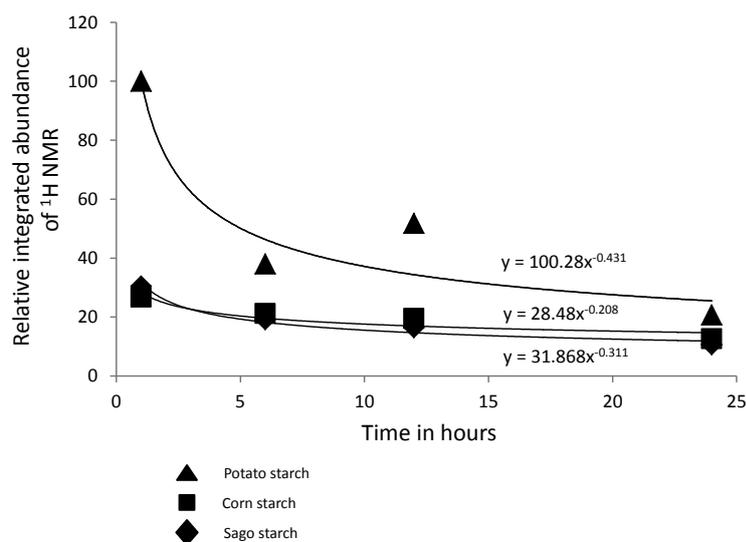


Fig. 5. Abundance of ^1H NMR of sago starch treated with D_2O
 Spinning side band: -19.5–5.18 ppm, 30.2–33.5 ppm

weight % water content, which caused the phenomenon of less water loss in PVB with 9 weight % water content.

The abundance of the ^1H signal linked to the water molecules in starch samples determined by the integration provided a higher value in potato starch than those in sago and corn starches (Fig. 6). Based on the ^1H signal at 5.12 to 5.18 ppm, the relative



The relative integrated abundance of ^1H NMR was obtained by the equation of $X/Y \times 100$, where X is the integrated abundance of the ^1H NMR of corn, sago, and potato starches at 1 to 24 hrs and Y is the integrated abundance of the ^1H NMR of potato starch at 1 hr.

Fig. 6. Relative integrated abundance of ^1H NMR

abundance of the ^1H signal decreased as the isotopic exchange time increased from 1 to 24 hrs with D_2O , owing to the isotopic exchange of H-D. This shows that the reduction of peak dominantly means the isotopic exchange and that it also contains micro loss of water molecules from starch samples during the transfer of starch samples from a small container to the zirconium sampler for the determination of ^1H NMR. The apparent exchange speed of water molecules calculated was 0.68 abundance per hr (a/hr) for sago starch, 0.54 a/h for corn starch, and 2.6 a/h for potato starch.

The water molecules in the starch structure consisted of water molecules in the amorphous growth rings associated with amylopectin branch points, water molecules in the crystalline lamellae formed from amylopectin double helices, and water molecules in the hexagonal channel of type B starch (Tang et al., 2000; Yano et al., 2005). The mobility of water molecules in type B starch may be higher than that in type A and C starches. The exchange of water molecules in type B starch expected by this study was 4 to 5 times higher than that in type A and C starches. This explains, using two-dimensional solid-state NMR study, that the mobility of water molecules in

type A corn starch was higher than that in B type corn starch (waxy corn starch) (Kulik et al., 1994) because of its channel water molecules.

Conclusion

The water molecules were mobile in spite of high arrangements of the starch crystalline structure. The exchange of water molecules in potato starch expected by this study was 4 to 5 times larger than that in sago and corn starches, based on the internal and external space of the crystalline structure of starch.

Acknowledgments

The authors would like to thank the support in part by the Tojuro Iijima Foundation for Food Science and Technology and the useful discussion of the analysis using a solid state ^1H NMR by Dr. Nobuaki Ishida, Ishikawa Prefectural University.

References

- Botlan, D. L., Y. Rugraff, C. Martin and P. Colonna 1998 Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy. *Carbohydrate Research* 308: 29-36.
- Chatakanonda, P., P. Chinachoti, K. Sriroth, K. Piyachomkwan, S. Chotineeranat, H. R. Tang and B. Hills 2003 The influence of time and conditions of harvest on functional behavior of cassava starch – a proton NMR relaxation study. *Carbohydrate Polymers* 53: 233-240.
- Choi, S. G. and W. L. Kerr 2003 ^1H NMR studies of molecular mobility in wheat starch. *Food Research International* 36: 341-348.
- Dragunski, D. C. and A. Pawlicka 2001 Preparation and characterization of starch grafted with toluene poly(propylene oxide) diisocyanate. *Materials Research* 4: 77-81.
- Falk, H. and M. Stanek 1997 Two-dimensional ^1H and

- ^{13}C NMR spectroscopy and the structural aspects of amylose and amylopectin. *Monatshefte für Chemie* 128: 777-784.
- Fan, D. M., S. Y. Ma, L. Y. Wang, H. F. Zhao, J. X. Zhao, H. Zhang, and W. Chen 2013 ^1H NMR studies of starch-water interactions during microwave heating. *Carbohydrate Polymers* 97: 406-412.
- Gallant, D. J., B. Bouchet and P. M. Baldwin 1997 Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers* 32: 177-191.
- Hills, B., K. Wright and P. Belton 1989 Proton NMR studies of chemical and diffusive exchange in carbohydrate systems. *Molecular Physics* 67: 1309-1326.
- Hills, B., S. Takacs and P. Belton 1990 A new interpretation of proton NMR relaxation time measurements of water in food. *Food Chemistry* 37: 95-111.
- Holmbom, B., A. V. Pranovich, A. Sundberg and J. Buchert 2000 Charged groups in wood and material pulps. *In: Celulosic Pulps, Fibers and Materials: Cellucon '98 Proceedings*, ed. (Kennedy, J. F., G. O. Phillips, G. O. and Williams, P. A. eds.) Woodhead Publishing Limited (Oxford) 322-327.
- Jeong, S. Y. and O. H. Han 2007 A solid-state NMR study of water in poly (vinyl butyral) by magic angle spinning. *Bulletin of the Korean Chemical Society* 28: 662-666.
- Karim, A. A., A P-L Tie, D. M. A. Manan and ISM. Zaidul 2008 Starch from sago (*Metroxylon sagu*) palm tree – Properties, prospects, and challenges as a new industrial source for food and other uses. *Comprehensive Reviews in Food Science and Food Safety* 7: 215-228.
- Kobayashi, S. 1993 Utilizing characteristics of sago palm. *SAGO PALM* 1: 25-32.
- Kulik, A. S., J. R. C. de Costa and J. Haverkamp 1994 Water organization and molecular mobility in maize starch investigated by two-dimensional solid-state NMR. *Journal of Agriculture and Food Chemistry* 42: 2803-2807.
- Leckert, von H. 1976 Possibilities and limits of pulsed NMR spectroscopy for the investigation of problems of starch research and starch technology. *Starch/Stärke* 28: 369-373.
- Li, S., L. C. Dickinson and P. Chinachoti 1998 Mobility of “unfreezable” and “freezable” water in waxy corn starch by ^2H and ^1H NMR. *Journal of Agriculture and Food Chemistry* 46: 62-71
- Morgan, K. R., R. H. Furneaux and N. G. Larsen 1995 Solid-state NMR studies on the structure of starch granules. *Carbohydrate Research* 276: 387-399.
- Mousseri, J., M. P. Steinberg, A. I. Nelson and L. S. Wei 1974 Bound water capacity of corn starch and its derivatives by NMR. *Journal of Food Science* 39: 114-116.
- Noguchi, S. and F. Nakazawa 1979 Pulsed NMR and its application to food science. *Journal of Cookery Science of Japan* 12: 8-16
- Schaefer, J., R. A. McKay, E. O. Stejskal and W. T. Dixon 1983 Dipolar rotational spin-echo ^{13}C NMR of polymers. *Journal of Magnetic Resonance* 52: 123-129.
- Schmidt, S. J. 2004 Water and solids mobility in foods. *In: Advances in Food and Nutrition Research*, Vol. 48. Elsevier Academic Press (Amsterdam) 53-55.
- Tang, H. R., J. Godward and B. Hills 2000 The distribution of water in native starch granules – a multinuclear NMR study. *Carbohydrate Polymer* 43: 375-387.
- Tang, H. R., A. Brun and B. Hills 2001 A proton NMR relaxation study of the gelatinization and acid hydrolysis of native potato starch. *Carbohydrate Polymers* 46: 7-18.
- Tang, H. and B. P. Hills 2003 Use of ^{13}C MAS MR to study domain structure and dynamics of polysaccharides in the native starch granules. *Biomacromolecules* 4:1269-1276.
- Wu, J. Y., R. G. Bryant and T. Eads 1992 Detection of solidlike components in starch using cross-relaxation and fourier transformed wide-line ^1H NMR methods. *Journal of Agricultural and Food Chemistry* 40: 449-455.
- Yano, S., N. Suzuki and Y. Kanzaki 2005 Pulse NMR study on the mobility of water molecules in various starch samples. *Japanese Journal of Food Chemistry* 12: 40-45.