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INVESTIGATION OF CELLULAR LEVEL WATER IN PLANT-BASED FOOD MATERIAL

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Abstract: Water in plant tissue is generally distributed in three different spaces namely, intercellular, intracellular, and cell wall. For hygroscopic material, these three types of water state should be considered for understanding the actual heat and mass transfer during drying. However, with the author's best knowledge, the proportion of the three types of water in plant-based food tissue has not been investigated yet. The present study was performed to investigate the proportion of intercellular water, intracellular water, and cell wall water inside the plant-based food material. In this study, experiments were performed for two different plant-based food tissues namely, granny smith apple and potato tissue. H^1-NMR relaxation measurement is a unique method of investigating the physical state of tissue water compartments by using the physics of T_2 relaxometry. Different types of water component were calculated by using multicomponent fits of the T_2 relaxation curves. The experimental results confirmed that plant-based food materials contain about 80 to 92 % loosely bound water (LBW), 6 to 16 % free water and only about 1 to 6 % strongly bound water (SBW). Attempt was made to establish the relationship between physical properties of fruits and vegetables and the proportion of different types of water. Interestingly, it was found that SBW strongly depends on the proportion of solid in the sample tissue whereas FW depends on the porosity of the material.

Keywords: Plant-based food material, Intracellular water, Intercellular water, Cell wall water, food drying

INTRODUCTION

The understanding of actual heat and mass transfer during thermal processing of plant-based food materials are not well understood yet. This is because of plant-based food materials are complex in structure and their porous, amorphous and hygroscopic in nature (Khan et al., 2016). Generally, plant tissue contains about 80-90 % of water, which is distributed in three different environments namely, intercellular environments, intracellular spaces, and cell wall spaces.

The water residing in the intercellular space is known as capillary water or free water (FW). The water inside the cell is referred to as intracellular water and the cell wall water occupies the fine space inside the cell wall, as shown in Fig. 1. These two types of water (intracellular water and cell wall water) are known as physically bound water or simply bound water (Karel, 2003). According to the mobility of water, bound water present inside the cells (intracellular water) is referred to as loosely bound water (LBW) while cell wall water is termed as strongly bound water (SBW) (Caurie, 2011).

The plant-based food materials that are subjected to dehydration can be treated as hygroscopic, porous and amorphous media with multiphase transport of heat and mass during drying process (Srikiatden and Roberts, 2007). Most of the existing food drying models consider all of the water inside the food material as transportable i.e. bulk water as shown in Fig. 2.

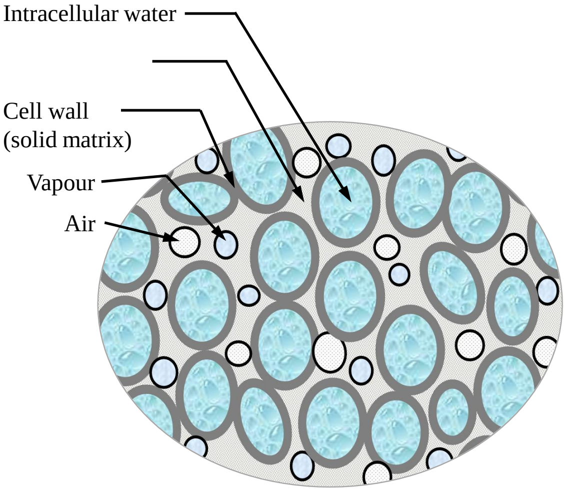

Fig. 1. Actual domain geometry of plant-based food material.

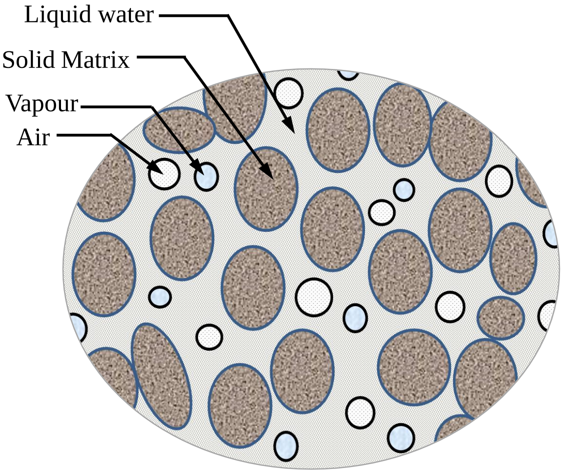

Fig. 2. The Domain that considered in existing literature (Datta 2007, Halder 2007, Feyissa, Gernaey et al. 2013, Mercier, Marcos et al. 2014, Gulati and Datta 2015, Kumar et al., 2015)

Figure 2 shows the domain that is considered for current model development. It can be seen that current models consider three phases inside food structure: water, gas (vapour and air), and solid matrix (Saber Chemkhi, 2009; Yamsaengsung and Moreira, 2002; Datta, 2007; Halder and Datta; 2012; Feyissa Gernaey et al., 2013; van der Sman; 2013; Gulati and Datta; 2015; Kumar et al., 2015). In this type of modelling, all the water is considered as intercellular water (free water); therefore, it assumes that there is no water inside the solid matrix. Due to the hygroscopic nature of plant-based food materials, bound water transport has a great effect on material shrinkage during food drying (Prothon et al., 2003). Joardder et al. (2015) suggested that migration of free water during drying has no effect on material structure whereas; the migration of LBW contributes to cellular shrinkage, pore formation and collapse of the cell. Furthermore, all of the food tissues are deformed due to the migration of SBW. Whereas, Prothon et al. (2003) showed that the migration of three types of water causes overall tissue shrinkage, cellular shrinkage as well as structure collapses.

Therefore, without considering bound water transport, a food drying model cannot provide a realistic understanding of the heat and mass transfer mechanism as well as material deformation (shrinkage) during drying.

Very few researchers have attempted to investigate the proportion of different water environments in the plant-based food material (Honikel KO, 1994, 2000; PS., 2009; Halder et al.; 2011). However, the literature has not described the amount of the three different types of water environment. Therefore, it is necessary to accurately measure the proportion of FW, LBW and SBW in plant-based food material for developing an accurate heat and mass transfer model.

There are many methods for measuring the proportion of the different types of water, including differential scanning calorimetry (DSC), differential thermal analysis (DTA), dilatometry, bioelectric impedance analysis (BIA), nuclear magnetic resonance (NMR), etc.

Among all the methods, proton nuclear magnetic resonance (^{1}H-NMR) relaxometry studies have proven to be valuable in the study of plants and plant-based food materials submitted to stress reflecting anatomical details of the entire tissue and the water status in particular (Van Der Weerd et al., 2002; Gambhir et al., 2005). The water exchange rates between different compartments are controlled by the water proton relaxation behaviour T_2 that strongly depends on the water mobility in the microscopic environment of the tissue and the strength of the applied magnetic field. The spin-spin T_2 relaxation is the transverse component of the magnetization vector, which exponentially decays towards its equilibrium value after excitation by radio frequency energy. It can be expressed as

$$M(t) = \sum_{i=1}^{n} A_i \times \exp(-t/T_2^i) \qquad (1)$$

Where, M(t) is the function of relaxation time, A is the relative contributions of sets of protons, T_2 is the relaxation time of water proton, and *i* is the number of contributing component.

Many studies used this technique for investigating differences in tissue composition. For instance, literatures investigated the free and bound water in animal tissue for lung (Cutillo et al., 1992; Sedin et al., 2000), brain (Furuse et al., 1984; Inao et al., 1985; Berenyi et al., 1998; Vajda et al., 1999; Sulyok et al., 2001), liver (Moser et al., (1996); Moser et al., 1992), red blood cells (Besson et al., 1989). In the case of plant-based food material, T_2 relaxation theories have been applied to the investigation of different things such as sugar content in the fruit tissue (Delgado-Goni et al., 2013), the quality of fruits and vegetables (Chen, 1989; Van de Velde et al., 2016), and the maturity of fruits and vegetables (Chen et al., 1993; Ruan et al., 1999). The literature for measuring free and bound water using T_2 relaxometry in plant-based food materials is very rare. Most of the NMR experiments have used T_2 relaxometry to study the development of the water core (Clark et al., 1998; Cho et al., 2008; Melado-Herreros et al., 2013), internal browning (Clark and Burmeister, 1999; Gonzalez et al., 2001; Cho et al., 2008) and microstructural heterogeneity (Defraeye et al., 2013; Winisdorffer et al., 2015) in plant tissue. However, these studies have not investigated the proportion of FW, LBW and SBW.

To the author’s knowledge, the proportion of the three types of water (free, LBW, and SBW) in plant-based food tissue has not yet been properly investigated. It is essential to develop an accurate heat and mass transfer model for use in food processing.

Therefore, the primary aim of this study is to investigate the proportion or the percentage of three types of water namely free water, LBW (intracellular water), and SBW (cell wall water) for two different plant-based food materials using ^{1}H-NMR method that can be used for developing accurate food processing models.

MATERIALS AND METHODS

Sample Preparation

Experiments were performed for two different materials, one is highly porous (Apple) and another is very low porous (Potato). The samples were collected from a local market in Brisbane, Australia. Samples were stored in a refrigerator at 4°C prior to NMR measurement. At the start of each experiment, the materials were washed and cut into cylindrical shapes of 30 mm length and 10 mm radius and then weighed on digital electronic balance (model BB3000; Mettler-Toledo AG, Grefensee, Switzerland) with an accuracy of 0.01 g.

NMR measurement

The prepared samples were then placed in a 25 mm diameter NMR tubes, sealed with a standard NMR tube cap and incubated at 22°C for 5 min to reach thermal equilibrium. Measurement were made with a Bruker DRX wide-bore spectrometer (Bruker Bio-spin, Karlsruhe, Germany) operating at 300 MHz for hydrogen with a micro-imaging (micro 120) gradient set was used for measuring the spatial T_2 relaxation times. Data were collected by Paravision 4 softwaer (Bruker). T_2 relaxation times were measured by a Multi-Slice-Multi-Echo (MSME) sequence using the following acquisition parameters: 64 averages, 1000 echoes with 10 ms echo time and 5.0 sec repetition time. The slice thickness and the matrix size were 3 mm and 64, respectively. For each sample, two ROIs (regions-of-interest) were defined manually from the MSME images. The mean value of these ROIs was computed for all the images. A third ROI was assessed outside the sample for determining the signal-to-noise ratio. Noisy T_2-signals were eliminated from the original signal. The data were transferred to a personal computer for storage and further analysis.

Mathematical analysis

The water mobility in different plant-based food tissues was investigated by assessing the water fractions quantitatively using multicomponent analysis of the T2 relaxation decay curves. The nonlinear least-squares method was applied for data analysis (Mulkern et al. 1989). Generally, an exponential decay curve is followed by the free induction decay of the proton relaxation process. A multi-exponential equation can describe this function. Each tissue compartment corresponding to a different environment has a distinct relaxation time constant (T_2). We assumed that these compartments were not inter-reliant at the time of the measurements. The multi-exponential nature of the T_2 decay curve relates to the different water compartments in the tissue, the protons of water molecules do not undergo rapid exchange. However, when water proton relaxation follows a pattern of mono-exponential decay, there is a fast exchange of protons between tissue water and macromolecules, showing that the water compartments are symbiotic (Shioya et al., 1990; Cole et al., 1993).

In this study, we applied Tri-exponential fitting of T_2 relaxation time curve, as shown in Figure 3 for estimation of the free water (FW), LBW and SBW in the plant-based food material. Three components of the T_2 relaxation curve were derived from the following expression:

$$Y = A_1 \times \exp^{-t/T_2^1} + A_2 \times \exp^{-t/T_2^2} + A_3 \times \exp^{-t/T_2^3} \qquad (2)$$

Where, Y is the function of T_2 relaxation time constant, A_1, A_2 and A_3 represent the relative contributions of the three proton environments, and T_2^1, T_2^2 and T_2^3 are the relaxation time constants of the different components. The multicomponent T_2 relaxation curve was fitted with user defined MATLAB code (MathWorks, Natick, MA).

The experiments were replicated three times for each sample, and the average of the relative contribution was taken for analysing the results.

Statistical analysis

Data are expressed as mean $\pm SD$ of the mean. For statistical analysis least-squares linear regression analysis was used where the P value was.

RESULTS AND DISCUSSION

In this study, the bound water of two different plant-based food materials was investigated using ^{1}H-NMR relaxometry. The T_2 relaxation decay curve as shown in Fig. 3 was fitted by the tri-exponential decay equation (Eq. 2). After fitting the tri-exponential decay curve with the different T_2 relaxation intensity data, three different proton components (slow, medium and fast) of water were described in Table 1, with their corresponding standard deviation and goodness of fit.

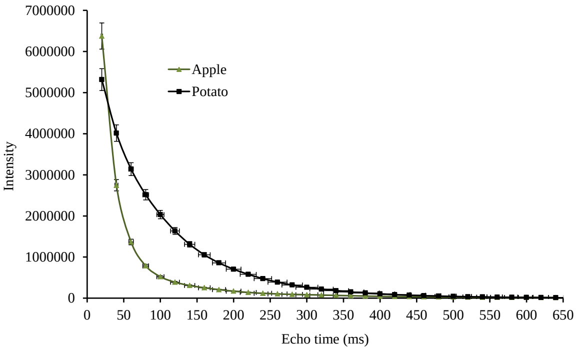

Fig. 3 T_2 Relaxation decay curve for Apple and Potato

Table 1. The different T2 component of Apple and Potato with their SD

Component	Potato (R^2 = 0.9994)		Apple (R^2 = 0.9997)	
	T_2 (ms)	%	T_2 (ms)	%
Slow	141.0±11.2	81.3±3.8	116.6±12.2	87.8±4.2
Medium	35.9 ± 9.6	12.9±2.5	36.1± 6.6	9.7± 3.2
Fast	7.3 ± 2.6	5.8± 2.1	8.5 ± 3.6	2.5± 2.1

Three types of signal component namely, slow, medium and fast were categorised as LBW, FW, and SBW respectively. This categorisation is mainly based on the pore size and water mobility within the sample (Saeedi, 2012). In NMR measurements, T_2 relaxation depends on water mobility within the sample. Fast T_2 relaxation occurs in that space where the water mobility is strongly restricted. In case of plant-based food material, the cell wall is composed of solid matter where the water mobility of very few water molecules is very low. Therefore, proton with the faster T_2 relaxation time is most likely to relate to SBW. On the other hand, it is well established that the majority of water is present in intracellular space with only water-water interface (Honikel and Hamm, 1994). In this region, water mobility is much higher; therefore, slow T_2 relaxation time is expected.

In intercellular space, water interacts with air, solid and also water molecule. The mobility of this water is less than the intracellular water and the T2 relaxation time is faster than LBW. Hence, this type of water is categorised as FW in this study.

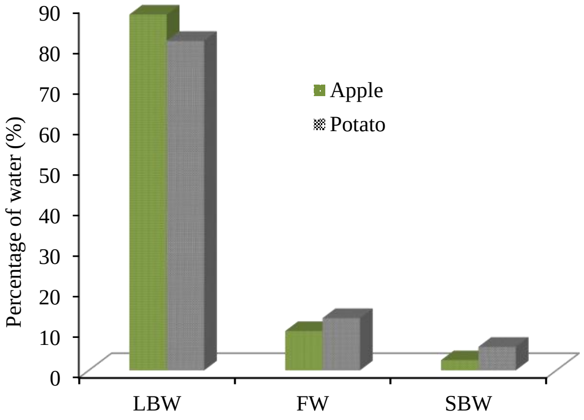

Fig. 4. The percentage of different types of water in plant-based food material

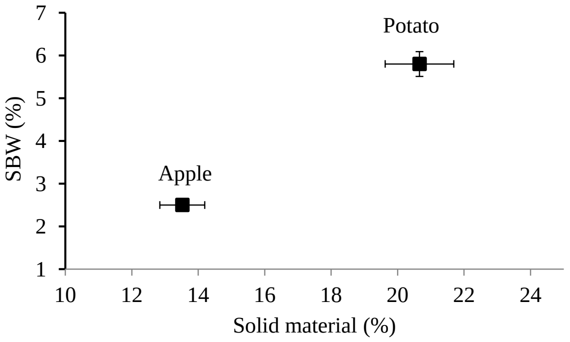

Fig. 5. The relationship between SBW and percentage of solid for highly and low porous material

Figure 4 shows a comparison of the percentage of different types of water in two different food materials. Depending on the type of food and its cell structure, approximately 80-90% LBW, 2-5% SBW and 10-20 % FW was observed. Due to diversified food properties, cell structure orientation and the solute content, the proportion of SBW, LBW and FW differs for various plant-based food materials.

It can also be seen from the illustration that between the two different materials, apple contains the highest amount of LBW (about 90%). This result shows a good agreement with study of Halder and Datta (2011); they were investigated the intracellular water using BIA method and found about 90% water of apple tissue exists in the intracellular space.

On the other hand, the SBW of potato is higher than that of apple. This may cause solely due to its high proportion of solid material content, as shown in Fig. 5. The SBW of both of potato and apple is proportional to the amount of solid.

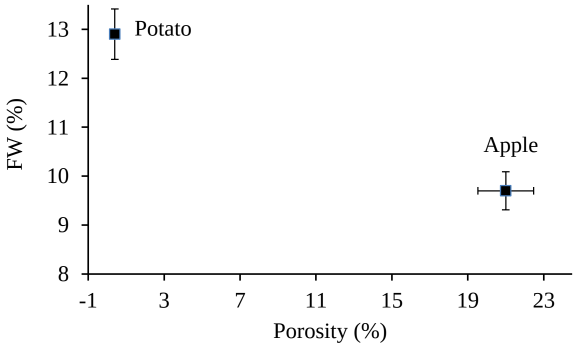

Fig. 6. Effect of porosity on free water (results are mean ±SD)

Fig. 6 shows the effect of initial porosity on FW. It can be seen that the percentage of free water illustrates an inverse relationship with the percentage of porosity. Therefore, in hygroscopic porous materials, air and water mutually stay within intercellular spaces (Woolley, 1983). Highly porous material contains a higher amount of air inside intercellular space. From Fig. 6, it can be observed that potato contains the highest amount of free water because of its least amount of porosity. On the other hand, apple, with the greatest porosity, contains the lowest amount of free water.

CONCLUSION

^{1}H NMR measurements have been performed to investigate the proportion of free water, loosely bound water and strongly bound water in two different plant-based food materials. The experimental results show that based on multiple component T_2 relaxometry data, the proportion of FW, LBW and SBW presenting in plant-based food materials is about 80-92%, 6-16 % and 1-5% respectively. Between the two different materials, apple showed the highest fraction of LBW. Depending on the solid portion and porosity of those two materials tissue, SBW and FW both were found highest in potato. This study also confirmed that the significant effect of porosity on the free water measured. The findings of this study enhance the understanding of plant-based food tissue that may contribute to a better understanding of potential changes occurring during food processing and may also contribute to develop an accurate and reliable heat and mass transfer models, hence providing better prediction of deformation.

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