Water loss and status in sponge cake: Impact of *Eucheuma* as a flour replacement

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Highlights

- *Eucheuma* increased the water content of sponge cake after baking.
- Mathematical models described the water loss process in more detail.
- Crust formation and crumb structure prevented water evaporation.
- LF-NMR and MRI indicated the water status in the final products.
- A proposed schematic diagram based on the qualitative analysis of the transfer mechanisms was developed.

Graphic abstract:
Abstract

The impact of *Eucheuma* on water loss and status in sponge cakes was measured and analyzed in this study. *Eucheuma* was used to replace 0%, 10%, and 20% of wheat flour to make sponge cakes, coded as the control, EP10, and EP20, respectively. The initial water content of batters showed no significant differences (around 57.0%, dry basis), whereas the final EP10 and EP20 products had higher water content. Three stages were found during baking in control sample and these three stages were fitted by linear, linear, and exponential models with root mean squared error (RMSE) of 0.016, 0.018, and 0.133, respectively. *Eucheuma* addition decreased the water loss rate and changed the water loss stages, which were fitted by linear, linear, and linear models in EP20 sample (RMSE = 0.027, 0.047, and 0.108, respectively). The crust formation and crumb structure analysis showed that the formation of cracks and the disappearance of pore structures hindered the water evaporation. The low field proton nuclear magnetic resonance (LF-NMR) and magnetic resonance imaging (MRI) results showed that the water status in the final EP20 products was not as tightly as that in the control samples. A proposed schematic diagram was developed based on the qualitative analysis of the transfer mechanisms to explain the total effect of *Eucheuma* on the water loss rate and status. These results aid our understanding of the water loss process of sponge cakes and promote the potential application of *Eucheuma* in bakery products.

Keywords: baking; seaweed; nuclear magnetic resonance (NMR); crust color; modeling.

Practical Application: *Eucheuma* as a flour replacement can improve the contents of dietary fiber and minerals like potassium of sponge cake. The impact of *Eucheuma* on water loss and status in sponge cake was measured and analyzed in this study. The results can promote the potential application of *Eucheuma* in bakery products and predict the quality attributes of baking products.
1. Introduction

Sponge cakes are typical foam cakes, which are made by whipping egg and sugar into a thick foam first and then adding sifted flour (Eslava-Zomeño, Quiles, & Hernando, 2016; Wilderjans, Luyts, Brijs, & Delcour, 2013). Baking is a principal step for sponge cake making. During baking, sponge cake batter is transformed into a fine and soft solid due to a series of physical, chemical and biochemical reactions. The key factors affecting these reactions are water transport, water status, and water distribution (Guillard, Broyart, Bonazzi, Guilbert, & Gontard, 2003; Lodi, Abduljalil, & Vodovotz, 2007). The movement of water can be limited by starch gelatinization and affected by expansion of gas cells during baking, and then plays an important role in bread texture and nutrition (Zhang, Datta, & Mukherjee, 2005; Zhang, Vanin, Lucas, Doursat, & Flick, 2017). The water distribution can substantially affect the organoleptic properties and the shelf life of the final product (Vanin et al., 2017).

For bakery products such as bread, water transport and loss mechanisms, e.g., evaporation–condensation–diffusion mechanisms, have been studied widely (Ureta et al., 2019; Zhang et al., 2005; Zhang et al., 2017). However, few studies measured the water loss and status in sponge cakes. Only Lostie, Peczalski, Andrieu, and Laurent (2002b) divided the sponge cake baking process into two stages: the heating period, when water migrates from the core to the surface by diffusion in the liquid phase; and the crust and crumb period, when water vapor migrates by convection. Thus, further studies are needed to show the water loss process and water status in sponge cakes.

*Eucheuma* is used as flour replacer to develop a healthier sponge cake in our previous study (Huang & Yang, 2019). *Eucheuma* is a seaweed with high amount of dietary fiber and minerals (Huang, Theng, Yang, & Yang, 2021; Jumaidin, Sapuan, Jawaid, Ishak, & Sahari, 2017), and it is usually eaten raw in some Asian coastal countries. The appearance of *Eucheuma* in sponge cake changed the texture such as hardness. These macroscopic changes are caused by the microscopic variables, such as water status and distribution (Lodi et al., 2007; Sommier, Chiron, Colonna, Della Valle, & Rouillé, 2005).
Thus, the influence of *Eucheuma* on water loss and water status in sponge cake should be studied to better understand the potential mechanisms, which could aid our understanding of water loss in sponge cakes and promote the application of flour replacer e.g. *Eucheuma* in bakery products.

Low field proton nuclear magnetic resonance (LF-NMR) is a non-invasive and quantitative way to study water status and distribution in food products (Assifaoui, Champion, Chiotelli, & Verel, 2006; Botosoa, Karoui, Chèné, & Blecker, 2015). The spin-spin relaxation time ($T_2$) is mainly used to measure molecular mobility and show the interaction between water and macromolecules in food products (Luyts et al., 2013; Shao et al., 2016). The higher $T_2$ values represent the higher water mobility and the lower $T_2$ values indicate the lower water mobility. Usually, three or four proton populations can be observed in bakery products according to the transverse relaxation information (Assifaoui et al., 2006; Luyts et al., 2013; Wang, Choi, & Kerr, 2004). For instance, the water mobility in biscuit dough is studied using LF-NMR and the proton populations around 1 and 10 ms are attributed to the internal water in starch and gluten, and the inter-granular water, respectively (Assifaoui et al., 2006). The proton populations in the pound cake are assigned systematically to non-exchanging protons, exchanging protons, and protons of lipids (Luyts et al., 2013). The effect of water content on water mobility in white bread crumb is studied and found different moisture content influenced the $T_2$ values significantly (Wang et al., 2004). Thus, $T_2$ values are good indicators to measure the water status in bakery products.

In the present study, the water content of cake batters and final products was measured first to confirm the water changes before and after baking on a macro level. Then the water loss was described in a mathematical way to illustrate the baking process in more detail. The factors which can affect the water loss such as crust formation and crumb structure were analyzed as well. The water status of the final products was investigated by LF-NMR. Finally, a water loss pathway was proposed to explain the water loss process and the effects of *Eucheuma* on sponge cake.
2. Materials and methods

2.1 Ingredients

*Eucheuma* was purchased from Indonesia via a local supplier (Tam Kah Shark's Fin, Singapore) and pre-treated as described in Huang and Yang (2019). After thoroughly water-washing, *Eucheuma* was dried at 50 °C for 24 h using a cabinet dryer and then ground to sieve through a 200-mesh sieve. Cake flour (12.1% moisture, 8.0% protein, and 0.5% ash) was provided by PRIMA RND (Prima Group, Singapore, Singapore); the cake emulsifier (Liang San, Singapore, Singapore) including emulsifier, sorbitol, propylene glycol, potassium hydroxide, and permitted coloring was obtained from Liang San Food Industry Company (Pantech Industrial Complex, Singapore, Singapore); the remaining ingredients (eggs, sugar, and canola oil) were obtained from a local supermarket (Fairprice, Singapore, Singapore).

2.2 Preparation of sponge cake batter and baking

The sponge cake recipe and preparation process were the same as those described by Huang and Yang (2019). The recipe of the control sponge cake was: 100 g cake flour, 200 g whole eggs, 100 g sugar, 8 g cake emulsifier, and 25 g canola oil. The EP10 and EP20 samples were prepared with 10% and 20% replacement of cake flour using *Eucheuma* powder, respectively. The EP10 and EP20 were chosen as representative samples because EP10 is the most acceptable replacement cake, and EP20 is the highest replacement sample with unacceptable texture and used as a negative reference here (Huang & Yang, 2019). The cake batter (250 g) was measured into a 6" baking tin and baked in a convection oven (Fabricant Eurfours® , Gommegnies, France) which was pre-heated at 180 °C for 30 min.

2.3 Water contents of batters and sponge cakes

The water content of batters and baked cake samples were analyzed according to AOAC method 925.10 (AOAC, 2002). The baked cake was kept at room temperature to cool for 120 min, and then the crust was removed and cut. The cube of the core (2×2×2 cm³) was remained for further analysis. Samples were dried in a hot air oven at 105 °C to a constant weight for 12 h and placed in a desiccator.
for another 12 h. The moisture content was calculated using the equation below.

\[
\text{Water content (dry basis)} = \frac{M_0 - M}{M} \times 100\% \quad \text{Eq. (1)}
\]

where \(M_0\) (g) is the initial mass, \(M\) (g) is the constant mass.

**2.4 Mathematical model fitting of water loss during baking**

The mass of a sponge cake at various times was recorded with an interval of two minutes. Additional sets of data (at the 3rd, 9th, 19th, and 29th minute) were also measured for validation. The water loss \((WL)\) was calculated using Eq. (2) according to Ureta et al. (2019). We assumed that water evaporation was the only component that affected mass loss during baking.

\[
WL(\%) = \frac{M_0 - M_t}{M_0} \times 100\% \quad \text{Eq. (2)}
\]

where \(M_0\) and \(M_t\) are the mass of the sponge cake at time zero and after baking for time \(t\), respectively.

The water loss \((WL)\) process was described mathematically using two models as follows:

- **Linear model:** \(WL = mt + n\) \quad \text{Eq. (3)}
- **Exponential model:** \(WL = ae^{-kt}\) \quad \text{Eq. (4)}

Letters \(m\) and \(k\) represent the water loss coefficients/model parameters in the linear and exponential models, respectively. Letters \(n\) and \(a\) are a constant of each group.

The goodness of model fitting was tested by R-square \((R^2)\) and root mean squared error (RMSE) based on Eq (5) using MATLAB R2014b (The Mathworks Inc., Natick, MA, USA).

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=0}^{n}(C_{t,\text{Mod},i} - C_{t,\text{Exp},i})^2}{N}} \quad \text{Eq. (5)}
\]

where \(C_{t,\text{Mod},i}\) is modelled value; \(C_{t,\text{Exp},i}\) is experimental value; \(N\) is the number of experimental value.

Furthermore, the Akaike Information Criterion (AIC) values were calculated to analyze overfitting:

\[
\text{AIC} = N_d \ln(\text{SSE}) + 2p \quad \text{Eq. (6)}
\]
where \( N_d \) is the number of data points used, SSE is the sum of squares for error, and \( p \) is the parameter number used in each model.

**2.5 Crust color measurement**

The surface color of the sponge cake crust was measured using a color measurement spectrophotometer (3nh, YS3010, Shenzhen, China) with the D65 standard illuminate and the 10° standard observer, recording the CIE \( L^* \ a^* \ b^* \) parameters. The color parameters \( L^* \), \( a^* \) and \( b^* \) represent lightness, redness/greenness, and yellowness/blueness, respectively (Geng et al., 2017; You, Zheng, Regenstein, Zhao, & Liu, 2012).

The total color change (\( \Delta E^* \)) was determined using Eq (7) according to literature (Sinthusamran, Benjakul, & Kishimura, 2014). The batter color before baking was measured and used as a reference for each sample.

\[
\Delta E^* = \left[ (L^* - L_{ref}^*)^2 + (a^* - a_{ref}^*)^2 + (b^* - b_{ref}^*)^2 \right]^{1/2} \quad \text{Eq. (7)}
\]

**2.6 Image analysis**

A Nikon DS5100 digital camera (Nikon Corporation, Tokyo, Japan) was used to capture images of the surfaces of samples at different time. For the crumb images, cakes were cut into 1 cm thick cross-section according to literature (Lin, Tay, Yang, Yang, & Li, 2017; Rodríguez-García, Puig, Salvador, & Hernando, 2012). An Olympus SZ61 stereomicroscope (Olympus, Melville, NY, USA) with a camera attachment was used. ImageJ2 software (National Institutes of Health, USA) was used to get a black and white image of the pore skeleton and calculate the percent of pore area.

**2.7 LF-NMR analysis of sponge cakes**

The baked cake was kept at room temperature to cool for 120 min and cut into cubes (2x2x2 cm\(^3\)) carefully. The cube of the core was used for LF-NMR analysis immediately. The relaxation time measurements were performed on a Niumag Pulsed NMR analyzer (Suzhou Niumag Analytical Instrument Corporation, Suzhou, China) equipped with a 0.5 T permanent magnet corresponding to a proton resonance frequency of 23.2 MHz at 30.00 ± 0.02 °C. The transverse relaxation time (\( T_2 \)) was
determined using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences. The main parameters for $T_2$
relaxation time measurements were: $TW$ (time waiting) = 2000 ms, $TE$ (time echo) = 1.0 ms, $NECH$
(number of echoes) = 4000, $NS$ (number of scans) = 16. To verify the proton populations, the crumb
cubes were dried in a 105 °C oven for 36 h and performed the LF-NMR analysis after cooling in a
desiccator.

For the magnetic resonance imaging (MRI) measurements, SPIN ECHO sequence was used to
obtain proton density weighted images. The main parameters were: $TR$ (time repetition) = 1200 ms,
$TE$ (time echo) = 5.885 ms, Average =1.

### 2.8 Statistical analysis

Each group was tested in triplicates independently. Each value represents the mean of triplicate
measurements, with the associated standard deviation. Differences between treatments were analyzed
using the Student-Newman-Keuls (SNK) procedure, as implemented in the SPSS statistical software
package (IBM Corp., Armonk, NY, USA). Differences with a $P$ value < 0.05 were considered
statistically significant.

### 3. Results and discussion

#### 3.1 Water content of different sponge cakes

Table 1 shows the water content of the different batters and sponge cakes. There was no significant
difference for all batters in terms of their water contents (Table 1). However, the water content of the
sponge cakes changed markedly after baking. Samples containing *Eucheuma* had higher water contents,
with increases of 3.67% and 6.37% for EP10 and EP20 sample, respectively. *Eucheuma* used in this
study had much higher water holding capacity than cake flour (Huang & Yang, 2019). During the
baking process, the addition of *Eucheuma* powder trapped more water, thereby preventing evaporation
and reducing water loss. Hence, how *Eucheuma* changed the water loss process and the final water
status of sponge cake is of great interest for better understanding the related food materials used in bakery products.

3.2 Model fitting of water loss during baking

To investigate the water loss process during baking, the mass of the sponge cakes before and after baking for various time was measured and the water loss during baking is shown in Figure 1. In previous studies, a linear model was used to describe water loss during microwave cake baking (Megahey, McMinn, & Magee, 2005) and bread baking (Ureta et al., 2019; Zhang et al., 2017). The water loss rate during baking presented first order kinetics and an exponential model was used to predict the process of bread baking (Ureta et al., 2019). Thus, in the present study, a linear and an exponential model were used and fitted to describe the baking process in more detail. The related parameters are shown in Table 2.

Generally, the water loss during baking could be represented by different successive stages according to previous studies (Lara, Cortés, Briones, & Perez, 2011; Lostie et al., 2002b; Megahey et al., 2005; Zhang et al., 2017). In this study, the water loss of the control sample showed three obvious stages (Figure 1a). Stage I, from 0 to 4 min, was a short period with slow weight loss and fitted well by linear model with very small RMSE values (Table 2). The batter temperature started to rise, and the surface moisture began to evaporate. Stage II, from 6 to 12 min, was a period of water loss at a higher rate and also fitted well by linear model. In this stage, more free water evaporated from the batter below the surface as the temperature increased. Stage III, from 14 to 30 min, had the highest loss rate and was fitted well by exponential model. The crust on surface had already formed in this stage and the opening and stiffening of the batter increased its permeability (Lostie, Peczalski, Andrieu, & Laurent, 2002a; Megahey et al., 2005).

For EP10 and EP20 samples, the final water loss values decreased as increasing the amount of Eucheuma powder content (Figure 1 b, c), consisting with the former water content results. In addition, the duration of the different stages was affected by the high amount of Eucheuma powder. The duration
of Stage I for the EP20 sample persisted to the 6th minute with a lower water loss rate, and the intervals of Stage II and Stage III were between 8 and 20 min, 22 and 30 min, respectively. All of the three stages of the EP20 sample were fitted well by the linear model, which was different from the control sample. Thus, the water loss rate became slower because of the addition of *Eucheuma* powder. *Eucheuma* had a better water holding capacity because of its high carrageenan content (Huang & Yang, 2019; Senthil, Mamatha, & Mahadevaswamy, 2005). During the baking process, carrageenan absorbed water and swelled, thus more water molecules were trapped, and the water loss rate decreases.

Additional experiments were conducted to validate the models. The water loss of the different samples at 3, 9, 19, and 29 min was measured. Figure 1d shows the comparison between the experimental data and the predicted values of water loss. It can be observed that the experimental data could fit into the models very well and had a good linear correlation with the predicted values. All values of the determination coefficient ($R^2$) were greater than 0.99.

### 3.3 Crust and crumb formation analysis

Crust formation is also a factor that can influence water loss (Mondal & Datta, 2008; Therdthai, Zhou, & Adamczak, 2002). During baking, the maximum evaporation occurs at the product surface. The surface is dried and becomes smooth in the first few minutes and crust browning occurs when the temperature reached 110 °C (Mondal & Datta, 2008; Purlis & Salvadori, 2007). Thus, in this study we measured the $\Delta E^*$ values to reflect the formation of crust.

Figure 2 shows the total color change ($\Delta E^*$) of all samples at different time. For the control sample, the $\Delta E^*$ values increased slightly at first and then increased sharply after 14 min. For the EP10 and EP20 samples, the trends of $\Delta E^*$ values were similar with the control sample at the beginning. However, the EP10 and EP20 samples showed much lower $\Delta E^*$ values at the end of the baking. Figure 3 shows the surface of all cake samples at specific times. For the control sample, many large cracks formed already at the 14th minute. These cracks developed and became larger as the baking proceeding. For the EP10 and EP20 samples, the cracks opening behavior was not as significant as that of the
control sample. Very small cracks and poles can be observed at the end of baking. Thus, the crust formation was hindered during baking because of the appearance of *Eucheuma*. Actually, the crust acts as a barrier towards weight loss (Wählby & Skjöldebrand, 2002). However, the crust formation accompanies by the surface crack opening, which could favor the water evaporation (Sommier et al., 2005). In the baking process of control cake, many cracks formed on the surface due to the earlier crust formation, and water molecules can be escaped easily. But for the EP10 and EP20 samples, the crust formation was hindered and the cracks became smaller. The water molecules cannot escape as easily as those in control sample. Thus, more water was trapped and resulted in higher water content in the final EP10 and EP20 products.

As for the crumb, many circular pores can be observed in the crumb of the control cake (Figure S1). These pore structures could form water escape channels during baking, resulting in lower water content and higher water loss. However, these pore structures became fewer and smaller in the EP10 and EP20 samples due to the addition of *Eucheuma* (Figure S1). The reduced pore area made it harder to form water escape channels in the crumb, resulting in less water loss during baking.

### 3.4 Water status analysis of different cakes

LF-NMR was used in this study to show the water status of the sponge cakes. The transverse relaxation time ($T_2$) (referred to as the spin-spin-proton relaxation time) can provide information about the water status, as well as the interaction between water and the other macromolecules (Luyts et al., 2013; Xu, Jin, Zhang, & Chen, 2017). Figure 4 shows the CPMG spectra of fresh cake crumbs and crumbs after thorough drying. The control sample showed four CPMG proton populations (Figure 4a). According to previous studies, proton population A is assigned to non-exchanging CH protons of amorphous starch and gluten, as well as the protons of bound water (Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2014). Proton population B contains exchanging protons of water, starch, proteins, and sucrose; and proton population C contains non-exchanging CH protons of egg yolk lipids and oil lipids. The very small proton population D had extremely high mobility ($T_2 \approx 464$ ms) and it might represent...
water protons expelled from the cake system (Luyts et al., 2013). To verify the ratio of the hydrogen proton signals produced by water molecules, the CPMG spectra of crumbs after thorough drying are also shown in Figure 4a as red dotted lines. Proton population A and B mostly disappeared, indicating that these two proton signals mainly comprised the protons of water. Proton population C changed to a continuous double peak signal, which was also the typical signal of lipids (Luyts et al., 2013). During the drying process, protons of lipids might be influenced and changed to another type of peak. Thus, proton population C mainly contains the protons of lipids.

No significant change can be observed for the peaks of proton population A. The relaxation time $T_{21}$ (peak time of proton population A) did not change in the different cake samples. The proton population D had the same phenomenon as the proton population A in different cake samples (Figure 4). However, it is worth noting that the peaks of proton population B cannot separate from the peaks of proton population C completely in EP10 sample, and the former one almost covered the later one in EP20 samples (Figure 4b & c). This indicated that the mobility of proton varies continuously in this region of relaxation time. Meanwhile the relaxation time $T_{22}$ (peak time of proton population B) was 7.055 ms in both control and EP10 samples, but this value changed to 8.111 ms in EP20 sample (Figure 4). This indicated that some water molecules in the final EP20 sample did not bind with macromolecules as tightly as they did in the control sample, although EP20 sample had the highest water content (Table 1). These water molecules did not escape from the cake during baking. This might be caused by the obstruction of crust and the disappearance of water escape channels in crumb. Thus, the addition of Eucheuma powder changed the water status in the sponge cake system.

To visualize the spatial distribution of water, MRI images were used to show the water status of the center part of different sponge cakes (Figure 5). Different color brightness represents the distribution of water and the color bar provides a relative scale for the moisture content (Xu, Jin, Zhang, & Chen, 2017). It could be observed that water distribution inside the sponge cakes was influenced by Eucheuma a lot. Samples with Eucheuma showed brighter brightness when comparing with the control
cake, indicating higher moisture contents. This is consistent with the results of the initial water content and the LF-NMR results.

### 3.5 Schematic diagram of water loss

Based on the above results, a pathway was proposed to illustrate the water loss process of different sponge cake samples and shown in Figure 6. The initial water content in all samples showed no significant differences (Table 1). During the baking process, water molecules can be trapped in the bakery products by starch, gluten, and other components (Izadi Najafabadi, Le-Bail, Hamdami, Monteau, & Keramat, 2014). Actually, in a cake system, flour plays a critical role because of its main component—starch. Starch granules swell and gelatinize, which accompanied by water absorption, and then form the “building bricks” of the final crumb (Donovan, 1977; Wilderjans et al., 2013). *Eucheuma* had a higher ability to absorb water because of its high carrageenan content. Thus, EP10 and EP20 samples could trap more water during the baking process. Meanwhile, limited opening cracks on the crust and smaller pores in the crumbs caused the disappearance of the water escape channel and hindered the water evaporation (Figure 3 & S1). The addition of *Eucheuma* powder changed the water status in the sponge cake system. Consequently, the EP10 and EP20 samples had higher water contents after baking (Table 1).

### 4. Conclusions

In the present study, the water contents of different batters and sponge cakes were measured. The initial water contents of cake batters showed no significant differences, while the final products showed significant differences. Different mathematical models were used to describe the water loss process in more detail, and *Eucheuma* changed the model type and water loss process. The crust formation and crumb structure analysis showed that the formation of cracks and the disappearance of pore structures hindered the water evaporation. The LF-NMR and MRI results showed that the water status in the final
EP20 products was not as tightly as that in the control samples. A proposed schematic diagram based
on the qualitative analysis of the transfer mechanisms was developed to explain the total effect of
*Eucheuma* on the water loss rate and status. The high water holding capacity of the high amount of
carrageenan in this ingredient, the obstruction of crust, and the disappearance of water escape channels
in crumb were all responsible for the changes of water content and status. These results aid our
understanding of the water loss process of sponge cakes and promote the potential application of
*Eucheuma* in bakery products.

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**Author Contributions**

Min Huang designed and performed the experiments, analyzed the data, and drafted the
manuscript. Hongshun Yang provided guidance on experimental design and improvement. Hongshun
Yang and Lin Zhao helped revise the manuscript.

**Conflicts of Interest**

There are none to declare

**References**


biscuit dough using a low-field $^1$H NMR technique. *Carbohydrate Polymers, 64*, 197–204.


Figure 1 Water loss rate of (a) control, (b) EP10, and (c) EP20 sponge cakes at various time during baking. (d) Validation of the mathematical models of water loss. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively.
Figure 2 Crust color change of sponge cakes at various time during baking. ■, Control; ▼, EP10; ♦, EP20. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with Eucheuma powder, respectively.
Figure 3 Images of the surface of all cake samples at specific time during baking. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively.
Figure 4 Carr-Purcell-Meiboom-Gill spectra of fresh cake crumbs (---) and crumbs after thorough drying (-----). (a) Control; (b) EP10, (c) EP20. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively.
Figure 5 MRI images of sponge cakes. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively. The color bar provides a relative scale for the moisture content.
Figure 6 Schematic diagram of the water loss process during sponge cake baking. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with Eucheuma powder, respectively.
Table 1 Effect of *Eucheuma* replacement on the moisture contents of batter and final cake samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Batter (dry basis, %)</th>
<th>Cake (dry basis, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EP10</td>
<td>57.1 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.7 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EP20</td>
<td>57.7 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.1 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively. Values are means ± standard deviations of triplicates. Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).
<table>
<thead>
<tr>
<th>Sample</th>
<th>Model</th>
<th>$R^2$</th>
<th>RMSE</th>
<th>SSE</th>
<th>AIC</th>
<th>$m$</th>
<th>$n$</th>
<th>$a$</th>
<th>$-k$</th>
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<tbody>
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<td>Control</td>
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<td>Linear</td>
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*EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with *Eucheuma* powder, respectively.
Figure S1 Images of the crumbs of sponge cakes under an optical microscope. The black color indicates the pores in cake crumb, and the white color indicates the solid matrix of the cake crumb. (A) Control cake, (B) EP10, (C) EP20, (D) The percent of pore area in different samples. EP10 and EP20 represent cakes prepared with 10% and 20% replacement of cake flour with Eucheuma powder, respectively.