Calcium fixation on fortified rice made with various rice varieties

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Abstract

The aim of this study was to evaluate calcium fixation and calcium binding in calcium-fortified rice. Calcium-fortified rice was made by soaking (infusing) rice in a Ca-lactate or Ca-glucanulate solution at temperature of 80°C for 20 mins. The rice types used in this study were low-, medium- and high amylase rice, represented by Memberamo, Ciheringan and IR-42 rice varieties. Calcium retention in rice was tested by washing and dialysis, and calcium levels in the rice were determined by Atomic Absorption Spectroscopy. Calcium fixation was determined by using an FT-IR infrared spectrometer based on the changes in infrared spectra of the functional groups of -OH and C-O. The research showed that calcium retention in rice after washing was between 86.23% - 94.38% (Ca-lactate) and 89.37% - 90.15% (Ca-glucanulate), Ca retention after dialysis was between 37.49% to 44.13% (Ca-lactate) and 37.40% to 42.86% (Ca-glucanulate). The addition of Ca-lactate or Ca-glucanulate to rice caused a decrease in the absorbance value and the absorption band area square at a wave numbers 3425 cm⁻¹ (-OH group) and 1300-1000 cm⁻¹ (C-O stretching vibration). Based on these data and the retention of Ca²⁺ after washing and dialysis, the Ca²⁺ in fortified-rice was bound by hydrogen bonding to form Ca-hydrate and by ionic-dipole bonding with –OH group of starch molecules, and/or trapping in gelatinized starch.

1. Introduction

Calcium (Ca²⁺) is an essential macromineral in the body that functioned as bone structural component, blood acid-base balance regulator (McCarthy and Kumar, 2004), nerve impulse transmission, muscle contraction, blood coagulation, hormone secretion and intercellular adhesion (Walker and Rolls, 1992). Therefore, calcium deficiency can cause bone abnormalities such as osteoporosis, rickets (Pu et al., 2016), and tetani (Desai et al., 2013). Currently, calcium intake in humans, especially in Asia, is still less than 400 mg/day (Harrison, 2017), whereas the Recommended Daily Intake of calcium is about 1000 mg (FAO/WHO, 2001). The low calcium intake of Asian due to lack of calcium-rich food consumption such as milk, cheese and eggs. Therefore, fortification of calcium in food that consumed many people or staple food such as rice was important. The calcium content of rice was between 4.25 and 6.32 mg/100 g polished-rice (Reddy et al., 2017). Therefore, fortification was carried out on rice to improve its nutritional quality.

Fortification is a part of nutrification, i.e. the addition of one or more nutrients to food commonly consumed to obtain, control or increase the dietary intake of groups, communities or populations (Bauernfeind and Lachance, 1991). Calcium fortification of the rice was done by soaking in a Ca-lactate 0.5% - 3% solution then steaming for 10 mins. The results showed that the increase of calcium in rice was about 50-100 mg/100 g of rice (Lee et al., 1995; Hettiarachchy et al., 1996). Ca²⁺ loss after washing was only 5% and Ca²⁺ retention after dialysis was 60%. It was assumed that Ca²⁺ was trapped in a gel formed when steaming and there was an interaction of Ca²⁺ with starch molecules. The high retention of Ca²⁺ in rice was assumed because Ca²⁺ acts as a crosslinker and was held inside the gelatinized starch matrix by ionic-dipole bonds. Wariyah et al. (2014), found that the texture of calcium-fortified rice
was harder than that of normal rice. This showed that during the process of making calcium-fortified rice, the rice underwent gelatinization and became hard after drying, so that Ca\(^{2+}\) trapping was stronger.

According to Osciak (1982), the adsorption of liquids into solids could take place physically or chemically. Hettiarachchy et al. (1996) stated that calcium fortification by immersion at high temperatures allowed physical adsorption and capillary condensation because hydrated Ca\(^{2+}\) was able to form weak hydrogen bonds and ionic-dipole bonds with starch molecules. In addition, the high temperatures used in rice soaking could also cause calcium fixation by Ca\(^{2+}\) trapping in a three-dimensional network from gelatinized starch.

The main component of rice is starch, a compound consisting of amylase and branched amylopectin molecules in molar ratios of 15% to 25% and 85% to 75%, respectively (Tako et al., 2014). Amylose and amylopectin of the starch molecules have many hydroxyl groups (-OH) (Champagne, 2004), so the potential for interactions with Ca\(^{2+}\) is high. According to the amylase content, rice is divided into three types, namely low amylase rice (12-20%), medium amylase (20-25%) and high amylase (25-33%) (Juliano, 1993). Wariyah et al. (2010) stated that the calcium absorption rate into rice grain was influenced by the amylase content. The higher the rice amylase content, the lower the absorption rate of calcium and the higher the activation energy. In addition, the amylase straight-chain facilitates crosslinking with other molecules (Tester, 2004). In rice with a high amylase content, the gelatinization temperature is lower than that of low amylase rice (Lii et al., 1996). The gelatinization temperatures of high amylase rice (IR-42), medium amylase (Ciherang) and low amylase (Memberamo) are 63.0°C; 72.5°C and 63.4°C, respectively (Wariyah et al., 2014). Rice gelatinization during soaking at high temperatures had a more open structure, making it easier to bind Ca\(^{2+}\). However, when heating reaches a certain gelatinization level, amylase will leach together with the formation of gel (Lii et al., 1996). In high amylase rice, after gelatinization, retrogradation would immediately occur (Jung et al., 2016), so that absorbed substance trapping is stronger. The aim of this study was to evaluate the calcium-binding mechanism in rice that underlies calcium stability in Ca-fortified rice.

2. Materials and methods

2.1 Materials

Low-, medium- and high- amylase rice grains represented by Memberamo, Ciherang and IR-42 varieties were obtained from The Rice Research Institute, Sukamandi, Subang, West Java, Indonesia. The grains were hulled and polished twice with a Da ichi blower rice polisher. The whole rice was then used as research material. Calcium salts used Ca lactate (Calcium lactate pentahydrate, Sigma Aldrich Chemie, Gm) and Ca gluconate (Brataco Chemika), dialysis membrane MWCO 7000 from SnakeSkinTM Pleated Dialysis Tubing (Pierce Chemical Company) and deionized water were used as solvents.

2.2 Instrumentation

Da ichi blower rice polisher (type N50 from Da ichi Engineering Co., Ltd.) was used to hull and polish the rice grains, and shaker waterbath (Koterman D-3162) was used for infusing calcium solution into rice, temperature controlled by thermocouple (Hanna Instruments HI 92704C K-Thermocouple). Fluidized Bed Drier (Armfield series 1253-2) for rice drying and calcium content on the fortified-rice and normal rice (control) were analyzed by using an Atomic Absorption Spectrometer (AAS, GBC 932 AA). The infrared spectra of the calcium-fortified rice and the normal rice were analyzed using an Infrared (FT-IR) mid-infrared Fourier Transform-Infrared spectrometer (FT-IR, Shimadzu, Prestige-21).

2.3. Procedure

Calcium-fortified rice made from low-, medium- and high- amylase rice was processed by the infusion method Wariyah et al. (2008), at 80°C using a shaker waterbath and dried with a fluidized bed drier and temperature controlled by thermocouple. Calcium-fortified rice and normal rice (control) were analyzed for their calcium content using an atomic absorption spectrometer and moisture content measured using the static gravimetric method (AOAC, 1990). The infrared spectra of the calcium-fortified rice and the normal rice were analyzed using an Infrared (FT-IR) mid-infrared. Samples for testing with the FT-IR were prepared as pellets made with 0.6% sample (flour form) mixed with KBr, refer to Hardjono (2001). The pattern of calcium fixation in the rice was determined by mid-infrared at frequencies between 4000-400 cm\(^{-1}\), based on the changes of Ca\(^{2+}\) binding spectra which were estimated in the -OH group of the starch molecules and C-O which vibrated. The group could be detected at wavenumbers around 3300 cm\(^{-1}\) and 1200-900 cm\(^{-1}\) (Lizuka and Aishima, 1999). If the group interacted with Ca\(^{2+}\), it would cause changes in the absorption bands from the resulting FT-IR spectra.
3. Results and discussion

3.1 Calcium content of normal and Ca-fortified rice

The Ca$^{2+}$ content of normal rice and calcium-fortified rice of low-, medium- and high amylose rice is shown in Table 1. The results of this study show the Ca$^{2+}$ of normal rice to be between 4.36 - 5.02 mg/100 g dry matter, while the Ca$^{2+}$ of fortified-rice was between 101.38 - 109.40 mg/100 g (with Ca-lactate fortificant) and between 102.77 - 119.55 mg/100 g dry matter (with Ca-gluconate fortificant). The Ca$^{2+}$ normal rice content was about 4.25 - 6.32 mg / 100g polished rice (Reddy et al., 2017). According to Wariyah et al. (2010), calcium in rice was influenced by processing conditions, such as the ratio of rice to calcium solution and the varieties of rice, but the range of calcium content in rice was similar, i.e. between 101.38 - 119.5 mg/100 g of rice (dry matter).

Table 1. Ca$^{2+}$ content on calcium fortified-rice (mg/100 g dry matter)

<table>
<thead>
<tr>
<th>Rice type</th>
<th>Low amylose rice (Memberamo)</th>
<th>Medium amylose rice (Ciherang)</th>
<th>High amylose rice (IR-42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rice</td>
<td>4.92±0.04</td>
<td>4.36±0.03</td>
<td>5.02±0.02</td>
</tr>
<tr>
<td>Ca-fortified rice added with:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca-lactate</td>
<td>108.21±3.74</td>
<td>101.38±1.37</td>
<td>109.40±5.92</td>
</tr>
<tr>
<td>Ca-gluconate</td>
<td>102.77±5.75</td>
<td>109.87±1.50</td>
<td>119.55±1.95</td>
</tr>
</tbody>
</table>

3.2. Spectra FT-IR and Ca$^{2+}$ fixation

Calcium fixation on rice was determined in both normal rice and calcium-fortified rice types with low amylose (Memberamo), medium amylose (Ciherang) and high amylose (IR-42) varieties which were fortified with Ca-lactate or Ca-gluconate. The rice was soaked in a calcium solution at 80°C which caused rice starch gelatinization (Wariyah et al., 2014). Hettiarachchy et al. (1996) and Bryant and Hamaker (1997) stated that starch gelatinization caused Ca$^{2+}$ trapping in a three-dimensional network of starch gel and interaction of Ca$^{2+}$ with starch molecules. Figure 1 - 3 shows the spectra of normal rice with low amylose (LA-R), medium amylose (MA-R) and high amylose (HA-R) and calcium-fortified rice with Ca-lactate (LA-R-CaL, MA-R-CaL, HA-R-CaL) or Ca-gluconate (LA-R-CaG, MA-R-CaG, HA-R-CaG). Recapitulation of the FT-IR band area of the spectral absorption of rice and calcium-fortified rice is shown in Table 2.

Table 2. FT-IR spectra of normal rice and calcium fortified-rice

<table>
<thead>
<tr>
<th>Rice type</th>
<th>Wavenumber (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1018.41</td>
</tr>
<tr>
<td>Low amylose (normal rice)</td>
<td>79.814</td>
</tr>
<tr>
<td>Low amylose (+Ca-lactate)</td>
<td>73.744</td>
</tr>
<tr>
<td>Low amylose (+Ca-gluconate)</td>
<td>57.691</td>
</tr>
<tr>
<td>Medium amylose (normal rice)</td>
<td>79.616</td>
</tr>
<tr>
<td>Medium amylose (+Ca-lactate)</td>
<td>58.375</td>
</tr>
<tr>
<td>Medium amylose (+Ca-gluconate)</td>
<td>55.632</td>
</tr>
<tr>
<td>High amylose (normal rice)</td>
<td>64.931</td>
</tr>
<tr>
<td>High amylose (+Ca-lactate)</td>
<td>54.702</td>
</tr>
<tr>
<td>High amylose (+Ca-gluconate)</td>
<td>55.283</td>
</tr>
</tbody>
</table>
The main component of rice is starch, which is composed of amylose and amylopectin molecules. Amylose and amylopectin molecules constitute glucose polymers (C₆H₁₂O₆), therefore there are many -OH groups and C-O bonds. According to Lizuka and Aishima (1999), FT-IR spectra from rice starch showed 4 spectral peaks at 3300, 1610, 1350 and 1000 cm⁻¹. The spectra at 3300 and 1610 cm⁻¹ were from water molecules, while the peak of 1350 cm⁻¹ was the O-H bending mode (curve); C-H and C-O-H had peaks at 1200 and 900 cm⁻¹ and showed strong absorption of stretching mode (stretch) of C-C and C-O groups. According to Cooper (1980) and Hardjono (2001), spectra between 3400-2400cm⁻¹ showed the -OH group (hydroxyl), while the spectra between 1280-1000 cm⁻¹ showed a C-O group.

This study, with FT-IR spectrometers on LA-R, MA-R and HA-R rice and calcium-fortified rice (LA-R-CaL, MA-R-CaL, HA-R-CaL and LA-R-CaG, MA- R-CaG, HA-R-CaG), showed 4 specific spectral peaks at wave numbers of 3425.0 cm⁻¹ and 1157.29 cm⁻¹; 1026.13 cm⁻¹; 1018.14 cm⁻¹. Stretching vibration at wave number 3425 cm⁻¹ showed the -OH group, and at the wavenumbers of 1157.29 cm⁻¹; 1026.13 cm⁻¹; 1018.14 cm⁻¹ was a C = O group. According to Anugrahati et al. (2017), rice flour spectra (normal rice) had functional groups similar to those of Menthik Susu and Ramos Sentra rice varieties. The group included –OH in spectra with wave numbers 3749.62 cm⁻¹, 3425.58 cm⁻¹ and 3387 cm⁻¹; C = O at 1157.29 cm⁻¹ and C-OH at bending vibrations at 856.39 cm⁻¹ and 578.64 cm⁻¹. These groups were mainly on glucose molecules that comprised amylose and amylopectin and these results were in accordance with this study. However, when seen at the peak area (Table 2) it appeared that the addition of Ca-lactate and Ca-gluconate caused a decrease in the absorption band area or a decrease in absorbance of the group at wavenumbers 1018.41 cm⁻¹; 1026.13 cm⁻¹; 1157.29 cm⁻¹ and 3425 cm⁻¹. According to Domagala (2012), the absorption band intensity in infrared spectra was expressed in absorbance (A) namely A = -log₁₀ (I/Iₒ), (Io and I are light intensities before and after interacting with the sample). Strong interactions were indicated by high absorbance values. In rice without added calcium, absorbance and absorption band areas were high, meaning that there was a high interaction between specific functional groups at certain frequencies in the sample, while the reduction in absorbance value, or area of the band, indicating a decrease in the number of functional groups. The addition of calcium salts can cause a part of the -OH group to bind to Ca²⁺ and the C-O bond in rice starch to vibrate so that the absorbance or area of the band area decreases.

According to Wariyah et al. (2007), water absorption in rice is influenced by temperature. The higher the temperature, the greater the water absorption and at 85°C the increase in water absorption was very high, together with the occurrence of starch gelatinization. In this research, the fortification was carried out by soaking rice in a calcium solution, so that Ca²⁺ was hydrated and water was diffused into the rice grains. Ca²⁺ ions were hydrated and bound to about 12 water molecules, namely Ca(H₂O)₁₂ (Bush et al., 2008). Possible interactions between Ca²⁺ and starch were physical adsorption between Ca²⁺ which had undergone hydration with the O atoms of starch molecules through hydrogen binding. In addition, when the rice was soaked in a calcium solution at high temperatures, gelatinization or formation of 3-dimensional networks occurred, so that water and Ca²⁺ became trapped in the starch gel. The strong calcium interaction in calcium-fortified rice had been demonstrated in the results of previous studies which showed that high Ca²⁺ retention from washing was about 86.23 - 94.39% which indicated the presence of Ca²⁺ trapped or bound to the components of rice. Ca²⁺ retention during dialysis was between 38.42 - 44.13%. This showed the presence of Ca²⁺ which was bound to the starch component and was well trapped in the rice component. This means that there was a binding relationship of Ca²⁺ to rice from the results of testing FT-IR spectra of calcium-fortified rice and rice.
a coordination complex. Based on the band absorption changes in the -OH group and -C=O bonds (Table 2), the addition formed hydrogen bonds, and, it was estimated, formed Ca\textsuperscript{2+} complexes with starch through ionic-dipole bonds, especially in helical amyllose molecules and cross-linked between amylose molecules. The size of the Ca atom is around 0.2 nm, which allows binding in the helical amylose structure with a diameter of 1.4 nm. According to Oscik (1980), physical adsorption occurs through weak bonds, so it is reversible and can break up by decreasing the solute concentration or increasing vapor pressure. In chemical adsorption, an adsorption energy of around 80 - 650 kJ/mol or 19 - 155 kcal/mol is needed. Hydrogen bond energy is 0.5 - 9.6 kcal/mol (Fennema, 1996). In this study, there was no known energy of Ca\textsuperscript{2+} adsorption on rice. What was known was the water-binding energy in the monolayer, which was only about 87.87 - 190.93 kcal/mol or 0.09 - 0.19 kcal/mol, so that the Ca\textsuperscript{2+} was weak (Wariyah and Supriyadi, 2010).

The addition of Ca-gluconate resulted in a band area and absorbance value smaller than Ca-lactate. This showed that the interaction of infrared light with the -OH group and the vibration of the C=O bond was lower. The structure of Ca-gluconate is more complex and there are more -OH groups than Ca-lactate. With a high molecular weight, the amount of Ca\textsuperscript{2+} of Ca-gluconate in the same amount with another calcium salt is more, so the possibility of interacting with the starch molecules is greater. According to Ruan and Chen (1998), hydrophilic substances such as proteins, carbohydrates (e.g. glucose) have side chains that could form hydrogen bonds such as dipole-electric forms -OH, COOH, NH\textsubscript{3}+ and COO-. Therefore, the more groups in Ca-gluconate that are able to interact with starch molecules, the lower the absorbance value after the addition of calcium.

4. Conclusion

From this study it can be concluded that calcium fixation in rice can be through the formation of hydrogen bonds between starch and Ca-hydrated ionic-dipole bonds between Ca\textsuperscript{2+} and -OH of starch molecules and trapping in gelatinized starch matrix, shown by decreases in the absorbance and the absorption band area at wave numbers of 3425 cm\textsuperscript{-1} (-OH group) and 1300-1000 cm\textsuperscript{-1} (CO group).

Conflict of interest

The authors herewith declare no conflict of interest.

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