FOOD

RESEARCH

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-gluconate solution at temperature of $80^{\circ}C$ for 20 mins. The rice types used in this study were low-, medium- and high amylose rice, represented by Memberamo, Ciherang 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-gluconate), Ca retention after dialysis was between 37.49% to 44.13% (Ca-lactate) and 37.40% to 42.86% (Ca-gluconate). The addition of Ca-lactate or Ca-gluconate 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 Ca^{2+} after washing and dialysis, the Ca^{2+} 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 (Ca^{2+}) 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). Ca^{2+} loss after washing was only 5% and Ca^{2+} retention after dialysis was 60%. It was assumed that Ca^{2+} was trapped in a gel formed when steaming and there was an interaction of Ca^{2+} with starch molecules. The high retention of Ca^{2+} in rice was assumed because Ca^{2+} 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 Oscik (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 amylose 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 amylose content, rice is divided into three types, namely low amylose rice (12-20%), medium amylose (20- 25%) and high amylose (25-33%) (Juliano, 1993). Wariyah et al. (2010) stated that the calcium absorption rate into rice grain was influenced by the amylose content. The higher the rice amylose content, the lower the absorption rate of calcium and the higher the activation energy. In addition, the amylose straight-chain facilitates crosslinking with other molecules (Tester, 2004). In rice with a high amylose content, the gelatinization temperature is lower than that of low amylose rice (Lii et al., 1996). The gelatinization temperatures of high amylose rice (IR-42), medium amylose (Ciherang) and low amylose (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, amylose will leach together with the formation of gel (Lii et al., 1996). In high amylose 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- amylose 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 (Kotterman 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- amylose 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)

	Low amylose rice	Medium amylose rice	High amylose rice
	(Memberamo)	(Ciherang)	(IR-42)
Normal rice	4.92±0.04	4.36±0.03	5.02±0.02
Ca-fortified rice added with :			
Ca-lactate	108.21±3.74	101.38±1.37	109.40±5.92
Ca-gluconate	102.77±5.75	109.87±1.50	119.55±1.95

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.
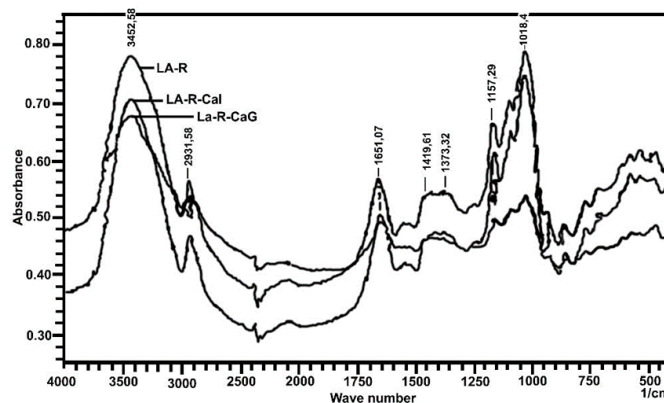

Figure 1. Calcium fortified-rice spectra of low amylose rice, LA-R: Normal Low Amylose Rice, LA-R-CaL: Low Amylose Rice with added Ca-Lactate, and LA-R-CaG: Low Amylose Rice with added Ca-Gluconate
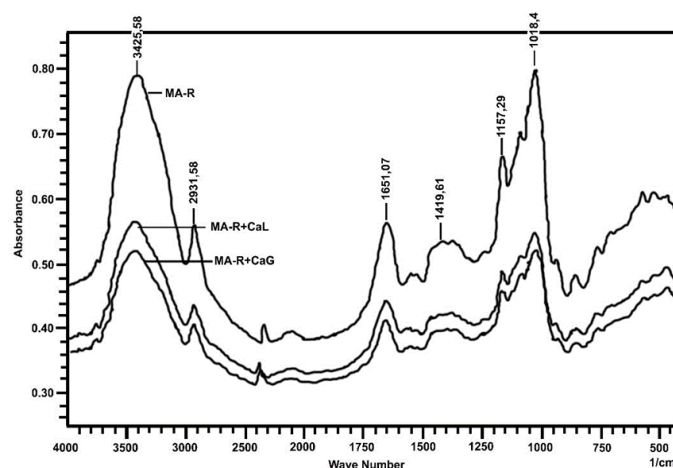

Figure 2. Calcium fortified-rice spectra of medium amylose rice, MAR: Normal Medium Amylose Rice, MA-R-CaL: Medium Amylose Rice with added Ca-Lactate, and MA-R-CaG: Medium Amylose Rice with added Ca-Gluconate

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

| Rice type	Wavenumber (cm⁻¹)			
	1018.41	1026.13	1157.29	3425.58
Low amylose (normal rice)	79.814	-	54.823	481.900
Low amylose (+Ca-lactate)	-	73.744	45.454	414.376
Low amylose (+Ca-gluconate)	-	57.691	40.333	404.157
Medium amylose (normal rice)	79.616	-	49.995	482.402
Medium amylose (+Ca-lactate)	-	58.375	-	323.568
Medium amylose (+Ca-gluconate)	55.632	-	39.736	299.171
High amylose (normal rice)	64.931	-	41.624	385.472
High amylose (+Ca-lactate)	-	54.702	-	353.494
High amylose (+Ca-gluconate)	-	55.283	37.123	318.108
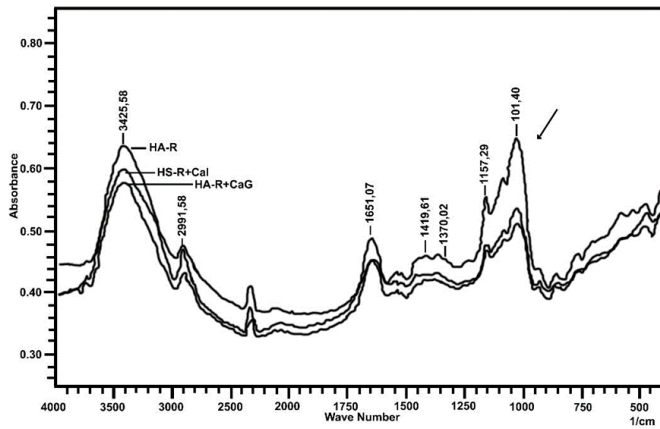
Figure 3. Calcium fortified-rice spectra of high amylose rice, HA-R: Normal High Amylose Rice, HA-R-CaL: High Amylose Rice with added Ca-Lactate, and HA-R-CaG: High Amylose Rice with added Ca-Gluconate

The main component of rice is starch, which is composed of amylose and amylopectin molecules. Amylose and amylopectin molecules constitute glucose polymers ($C_6H_{12}O_6$), 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-C-H bending mode (curve); C-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 Ca^{2+} 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 Ca^{2+} was hydrated and water was diffused into the rice grains. Ca^{2+} ions were hydrated and bound to about 12 water molecules, namely $Ca(H_2O)_{12}$ (Bush *et al*., 2008). Possible interactions between Ca^{2+} and starch were physical adsorption between Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} retention from washing was about 86.23 - 94.39% which indicated the presence of Ca^{2+} trapped or bound to the components of rice. Ca^{2+} retention during dialysis was between 38.42 - 44.13%. This showed the presence of Ca^{2+} which was bound to the starch component and was well trapped in the rice component. This means that there was a binding relationship of Ca^{2+} to rice from the results of testing FT-IR spectra of calcium-fortified rice and rice.

According to Kariper and Kaya (2014), metal ions can act as receptors of electron pairs, or as Lewis acids, which can form co-ordination compounds, whereas glucose molecules composed of amylose and amylopectin have many -OH groups and CO bonds which can act as Lewis acids that could also act as donor electron pairs, or Lewis pairs, and as ligands. The interaction between Ca^{2+} and rice starch molecules may also involve chemical interactions with the formation of

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^{2+} complexes with starch through ionic-dipole bonds, especially in helical amylose 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^{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^{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^{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_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^{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^{-1} (-OH group) and 1300-1000 cm^{-1} (CO group).

Conflict of interest

The authors herewith declare no conflict of interest.

Acknowledgments

References

Anugrahati, N.A., Pranoto, Y., Marsono, Y. and Marseno, D.W. (2017). Physicochemical properties of rice (*Oryza sativ*a L.) flour and starch of two Indonesian rice varieties differing in amylose content. *International Food Research Journal*, 24(1), 108–113.

AOAC. (1990). Officials Methods of Analysis of AOAC International. 15th ed. Arlington, Virginia: Association of Official Analytical Chemists, Inc.

Bauernfeind, J.C. and P. Lachance. (1991). Nutrient Additions to Food: Nutritional, Technological and Regulatory Aspect in Food and Nutrition. , p. 49. Connecticut, Trumbull, USA: Humana Press.

Bryant, C.M. and Hamaker, B.R. (1997). Effect of lime on gelatinization of corn flour and starch. *Cereal Chemistry,* 74(2), 171–1757. https://doi.org/10.1094/CCHEM.1997.74.2.171

Bush, M.F., Saykally, R.J. and. William, E.R. (2008). Infrared action spectra of $Ca^{2+}(H_2O)_{11-69}$ exhibit spectral signatures for condensed-phase structures with increasing cluster size. *Journal of American Chemical Society,* 130, 15482 – 15489. https://doi.org/10.1021/ja804621r

Champagne, E.T. (2004). Rice: chemistry and technology. 3rd ed. St Paul, USA: American Association of Cereal Chemists.

Cooper, J.W. (1980). Spectroscopic Techniques for Organic Chemist. New York: John Wiley and Sons.

Desai, M., Kolla, P.K. and Reddy, P.L.V.P. (2013). Calcium unresponsive hypocalcemic tetany: gitelman syndrome with hypocalcemia. *Case Report in Medicine*, 2013, 197374. https://doi.org/10.1155/2013/197374

Domagala, W.U. (2012). The Use of the Spectrometric Technique FTIR-ATR to Examine the Polymers Surface, p. 86. In Farrukh, M.A. (Ed.) Advanced Aspects of Spectroscopy. InTech Open E-book.

FAO/WHO. (2001). Human Vitamin and Mineral Requirement. FAO-WHO Food and Nutrition Division. FAO Rome. Retrieved on July 10, 2020 from FAO website: http://www.fao.org/3/a-y2809e.pdf

Fennema, O.R. (1996). Principles of Food Science. New York, USA: Marcell Dekker Inc.

Hardjono, S. (2001). Spectroscopy. Yogyakarta, Indonesia: Liberty.

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Harrison, P. (2017). Dietary Calcium Intake Poor in much of the World. Retrieved on July 5, 2020 from Medscape website: https://www.medscape.com/viewarticle/888206.

Hettiarachchy, N., Gnanasambandam R. and Lee, M.H. (1996). Calcium fortification of rice: distribution and retention. *Journal of Food Science*, 61(1), 195–1976. https://doi.org/10.1111/j.1365-2621.1996.tb14758.x

Juliano, B. (1993). Rice in Human Nutrition. Food And Agriculture Organization of the United Nations. Retrieved on July 5, 2020 from FAO website: http://www.fao.org/3/t0567e/T0567E00.htm.

Jung, K.G.H., Lee, S.H. and Kim, J.C. (2016). Retrogradation of heat-gelatinized rice grain in sealed packaging: investigation of moisture relocation. *Food Science and Technology*, 37(1), 97–102. https://doi.org/10.1590/1678-457x.07816

Kariper, S.E. and Kaya, C. (2014). Effect of some electron donor and electron acceptor groups on stability of complexes according to the Principle of HSAB. *Journal of New Results in Science*, 4, 82-89.

Lee, M.H., Hettiarachchy, N.S., McNew, R.W. and Gnanasambandam, R. (1995). Physicochemical properties of calcium-fortified rice. *Cereal Chemistry*, 72(4), 352–355.

Lii, C.Y., Tsai, M.L. and Tseng, K.H. (1996). Effect of amylose content on the rheological property of rice starch. *Cereal Chemistry*, 73(4), 415–420.

Lizuka, K. and Aishima, T. (1999). Starch gelation process observed by FT-IR/ATR spectrometry with multivariate data analysis. Journal Food Science, 64(4), 653–658. https://doi.org/10.1111/j.1365-2621.1999.tb15104.x

McCarthy, J.T. and Kumar, R. (2004). Divalent Cation Metabolism: Calcium. Retrieved on March 29, 2009 Kidney Atlas website: from www.kidneyatlas.org.

Oscik, J. (1982). Adsorption. New York: E. Horwood, Halsted Press Chichester.

Pu, F., Chen, N. and Xue, S. (2016). Calcium intake, calcium homeostasis and health. *Food Science and Human Wellness,* 5(1), 8–16. https://doi.org/10.1016/j.fshw.2016.01.001

Reddy, C.K., Kimi, L. Haripriya, S. and Kang, N. (2017). Effects of polishing on proximate composition, physico-chemical characteristics, mineral composition and antioxidant properties of pigmented rice. *Rice Science*, 24(5), 241–252. https://doi.org/10.1016/j.rsci.2017.05.002

Ruan, R.R. and P. Chen, P. (1998). Water in Food. New York, USA: CRC Press.

Tako, M., Tamaki, Y., Teruya, T. and Takeda, Y. (2014). The Principles of starch gelatinization and retrogradation. *Food and Nutrition Sciences*, 5(3), 280–291. https://doi.org/10.4236/fns.2014.53035

Tester, R.F., Karkalas, J. and Qi, X. (2004). Starch structure and digestibility Enzyme-Substrate relationship. *World's Poultry Science Journal*, 60(2), 186–195. https://doi.org/10.1079/WPS20040014

Walker, A.F. and Rolls, B.A. (1992). Nutrition and the Consumer: Issues in Nutrition and Toxicology. London and New York: Elsevier Applied Science.

Wariyah, C., Anwar, C., Astuti, M. and Supriyadi. (2007). Kinetics of water absorption in rice. *Agritech*, 27(3), 112–117.

Wariyah, C., Anwar, C., Astuti, M. and Supriyadi. (2008). Physical properties and acceptability of calcium fortified-rice. *Agritech*, 28(1), 34-42.

Wariyah, C. and Supriyadi. (2010). Moisture Sorption Isotherm of Calcium-Fortified Rice. *Agritech*, 30(4), 199–203.

Wariyah, C., Astuti, M., Supriyadi and Anwar, C. (2010). Calcium absorption kinetic on Indonesian rice. *Indonesian Journal of Chemistry*, 8(2), 252–257. https://doi.org/10.22146/ijc.21630

Wariyah, C., Anwar, C., Astuti, M. and Supriyadi. (2014). Pasting properties of calcium-fortified rice. *International Food Research Journal*, 21(3), 989–993.