

ผลของสภาวะต่างต่อความสามารถในการกักเก็บลินาโลอลในเมทริกซ์สตาร์ชที่ผ่านการทำแห้ง
Effect of Alkaline Conditions on the Linalool Retention Ability of Dried Starch matrices

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Received: April 20, 2018

Accepted: June 14, 2018

บทคัดย่อ

งานวิจัยนี้ ศึกษาผลของการใช้แคลเซียมไฮดรอกไซด์ ($\text{Ca}(\text{OH})_2$) และโซเดียมไฮดรอกไซด์ (NaOH) ต่อความสามารถในการกักเก็บลินาโลอลในเมทริกซ์สตาร์ชที่ผ่านการทำแห้ง ผลการทดลองแสดงให้เห็นว่าเมทริกซ์สตาร์ชที่ผลิตโดยใช้ $\text{Ca}(\text{OH})_2$ ทั้งที่ผลิตใหม่และผ่านการเก็บรักษามีประสิทธิภาพในการกักเก็บลินาโลอลสูงที่สุด โดยในเมทริกซ์สตาร์ชที่ผลิตใหม่สามารถกักเก็บลินาโลอลไว้ได้ 34.40 ± 5.5 มก.ลินาโลอล/กรัมเมทริกซ์ มากกว่าปริมาณลินาโลอลที่กักเก็บไว้ในเมทริกซ์สตาร์ชที่ผลิตโดยใช้น้ำ 13 เท่า นอกจากนี้เมื่อเก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียส 53% RH เป็นเวลา 4 เดือน พบว่าเมทริกซ์สตาร์ชที่ผลิตโดยใช้ $\text{Ca}(\text{OH})_2$ สามารถเก็บรักษาลินาโลอลไว้ได้ ร้อยละ 92.2 ความแน่นกระชับของเมทริกซ์เนื่องจากการครอสลิงก์กันของแคลเซียมและสตาร์ช และความหนาของเมทริกซ์มีผลต่อความสามารถในการกักเก็บลินาโลอลของเมทริกซ์สตาร์ชที่ผลิตโดยใช้ $\text{Ca}(\text{OH})_2$ ในทางตรงกันข้าม เมทริกซ์สตาร์ชที่ผลิตโดยใช้ NaOH มีความสามารถในการกักเก็บลินาโลอลได้ต่ำ ผลการวิจัยนี้แสดงให้เห็นว่าการผลิตเมทริกซ์สตาร์ชในสภาวะที่เป็นต่างและมีแคลเซียมสามารถเพิ่มความสามารถในการกักเก็บสารให้กลิ่นไว้ในเมทริกซ์สตาร์ชได้

คำสำคัญ: แคลเซียมไฮดรอกไซด์ สตาร์ช เมทริกซ์ การกักเก็บลินาโลอล

ABSTRACT

This research studied the effect of calcium hydroxide ($\text{Ca}(\text{OH})_2$) and sodium hydroxide (NaOH) cooking on the retention of linalool in dried starch matrices. The results showed that the $\text{Ca}(\text{OH})_2$ -cooked starch matrix was highly effective in retaining linalool, both in freshly prepared and stored samples. In freshly cooked sample, it entrapped 34.40 ± 5.5 mg linalool/g matrix, increased 13-folds more than that entrapped in water-cooked starch matrix. Moreover, the $\text{Ca}(\text{OH})_2$ -cooked starch matrix retained 92.2% linalool after storage at 53% RH, 25°C for 4 months. The tightening of $\text{Ca}(\text{OH})_2$ -cooked starch network caused by Ca-starch crosslinks, and the matrix thickness affected the linalool retention. Conversely, the fresh NaOH -cooked starch matrix showed the poor linalool entrapment. This research suggests that cooking starch under alkaline condition with the presence of calcium increased the retention of flavor compound in dried starch matrix.

Keywords: Calcium hydroxide, Starch, Matrix, Retention, Linalool

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Introduction

Alkaline agents are important in the preparation of many traditional starch-based food products. For example, the use of a mixture of Na_2CO_3 and K_2CO_3 in the production of Chinese noodles and the use of $\text{Ca}(\text{OH})_2$ in the production of tortillas and masa [1]. Alkaline agents are used to improve and develop product characteristics such as color, firm and elastic texture [2]. The effects of alkaline agents on the properties of starch depend on the type of starch, type and concentration of alkali, treatment temperature and duration of treatment [1]. Generally, the starch hydroxyl groups have a greater propensity to ionize at high pH condition ($\text{pH} > 12$) [1, 2]. Israkarn and Hongsprabhas [3] revealed the existence of negative charges on alkali-treated starch polymers by using zeta-potential analysis. They reported that the surface charge of dilute mung bean and cassava starch heated in NaOH solution was $(-)\text{14.2} \pm 0.7$ and $(-)\text{15.3} \pm 0.5$ mV, respectively. The repulsion between these ionized starch chains can be induced the swelling of starch granule and retarded the agglomeration of starch chains [4]. In the case of $\text{Ca}(\text{OH})_2$ solution, it has been reported that the presence of Ca^{2+} ions in the solution can interact with the negatively charge starch molecules, generating Ca^{2+} -induced starch aggregation [3], as well as promoting formation of Ca-starch crosslinks [1].

In Thai dessert, a saturated solution of $\text{Ca}(\text{OH})_2$, with a pH of around 12.2, is used for making pandan sweet pudding (kanom piek poon bai toey). Not only the elastic texture is obtained by using $\text{Ca}(\text{OH})_2$ but also the prolonged

retention of pandan flavor within its matrix is observed. This phenomenon indicates a promising $\text{Ca}(\text{OH})_2$ -cooked starch matrix on entrapping flavor compounds and utilizing as a new carrier for flavor encapsulation. However, the ability of $\text{Ca}(\text{OH})_2$ -cooked starch matrix on retaining flavor compound, as well as the mechanism of interaction between flavor compound and this starch matrix, have not yet to be studied in any great detail, require further studies.

The present work investigated the retention of linalool by starch matrices. The starch matrices were prepared by casting and drying into films to ease preparation and parameter measurements. Linalool is a monoterpene alcohol found in essential oils of numerous aromatic plants such as lavender and orange. It has great commercial interest as it is widely used as a flavoring agent. Some applications include alcoholic beverages, frozen dairy products and confectionery products [5]. However, the volatility and the readiness to oxidized under normal temperature and pressure of linalool would restrict its applications in food products. Hence, the research on controlling the volatility and improve the stability of linalool by starch matrix is interesting.

Encapsulation of linalool by using starch matrix has been reported. The most common technique is inclusion complex. Keatkrai (2015) [6] have demonstrated that mung bean starch can be used to encapsulate linalool via V-type starch crystal structure provided that mung bean starch was gelatinized at 140°C for 1 h to form inclusion complex with linalool. Also, Arvisenet et al. (2002) [7] reported that linalool

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could form inclusion complex with amylose in corn starch paste prepared by cooking the mixture of starch and linalool in double jacket containing oil at 120°C for 15 min. They stated that this complexation was responsible for linalool retention in the starch matrix. Even though starch inclusion complex have attracted considerable interest within the scientific community, the new encapsulation technique is constantly looking for in order to offer encapsulated product with new functionalities.

Calcium ions reportedly have the ability to act as crosslinking agents with starch in an alkaline system. It allows the starch chains to pack closely, assists physical entrapment of active ingredient within starch matrix. This phenomenon has been utilized in the encapsulation of herbicides and pesticides [3, 8]. The application in the food industry for control flavor release, however, has not been mentioned. Our work was focused on the effect of alkaline condition (with and without Ca^{2+}) on the retention of linalool in dried starch matrices. Mung bean starch (*Vigna radiate* (L.) Wilczec) was chosen as model matrix in this experiment due to its high amylose content (31-38 %) [9], indicating possible bind greater amount of flavor compound than low amylose content starch [10]. The physicochemical properties and the linalool retention ability of starch matrices cooked in $\text{Ca}(\text{OH})_2$ (alkali with calcium, pH 12), in NaOH (alkali without calcium, pH 12) and in distilled water (neutral solution, pH 7) were examined and compared. Moreover, the interaction between starch matrices and linalool was investigated in order to gain insight on this novel approach to flavor encapsulation.

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Materials and methods

1. Materials

Commercial mung bean starch (Sithinan Co., Ltd., Bangkok, Thailand) was purchased from a local supermarket. NaOH was obtained from Merck (Darmstadt, Germany), and $\text{Ca}(\text{OH})_2$ was supplied by Ajax Finechem (Auckland, New Zealand). Linalool (purity>97%, Aldrich, St. Louis, MO, USA), was used as model flavor compound. All other chemicals were of analytical grade and solvents were high-performance liquid chromatography (HPLC) grade.

2. Gelatinization characteristics

Differential scanning calorimetry (DSC; Mettler-Toledo, Columbus, OH, USA) was used to determine the gelatinization characteristics of 2% (w/w) mung bean starch in three different dispersants containing 1.3 g/L $\text{Ca}(\text{OH})_2$, 4 g/L NaOH and distilled water. The concentration of $\text{Ca}(\text{OH})_2$ was 1.3 g/L (w/v), with a pH value of 12, similar to that of lime solution generally used in Thai households. The alkaline solution without calcium ions at the same pH level was prepared by using NaOH at 4 g/L, pH 12. Distilled water (pH~7) was used as a control.

The samples were heated at the rate of 5°C/min from 25°C - 95°C to determine transition temperatures and the enthalpy of gelatinization. The transition temperatures reported were the onset (T_o), peak (T_p) and end (T_e) temperatures of gelatinization endotherms. The enthalpy of gelatinization (ΔH) was expressed as J/g starch (d.b.).

3. Preparation of starch matrices

Starch matrices were prepared by drying into films using method described by Israkarn and Hongsprabhas (2017) [3] to ease preparation and parameter measurement. Starch (2% w/v) was gelatinized in three aqueous solutions (1.3 g/L Ca(OH)₂, 4 g/L NaOH and distilled water) at 95°C for 30 min. Linalool was added to starch suspensions to reach final concentration of 0.3% (w/v). This used linalool concentration has been reported a high enough to induce the formation of inclusion complex with starch [11]. The starch mixtures, with and without linalool, were poured in a Teflon tray (18.5 × 18.5 cm, Welch, USA) and dried at 40°C for 32 h to obtain starch films. Film samples were kept in aluminum pouches and stored at -40°C in a freezer for less than 4 weeks until further studied. For linalool entrapment measurement, freshly prepared starch matrices were used. The dried starch matrices contained 10-11% moisture content. The samples were prepared in triplicate.

4. Thickness of starch matrices

Twenty random positions of each film sample were measured for their thickness using a manual micrometer (Mitutoyo, Japan). The mean value was reported.

5. Color of starch matrices

The color of the starch matrix was determined with a colorimeter (MiniScan XE, Hunter Associates Laboratory Inc, USA) using the CIE Lab method. The colorimeter was calibrated using a standard white plate. Hunter L^* , a^* and b^* values were averaged from five points for each sample.

6. X-ray diffraction analysis

X-ray diffraction patterns of starch matrices were evaluated with a Bruker D8 Advance (Bruker Corporation, Germany). Each sample was scanned over a diffraction angle range of $2\theta = 5-30^\circ$ at 2° per min with a 0.03° step size. The relative crystallinity percentage of starch was calculated as the ratio of the area above a smooth curve of connected peak baselines to the total area over the diffraction angle range of $2\theta = 9-30^\circ$. The data were analyzed by Difffrac.Suite EVA software (Bruker Corporation, Germany).

7. Determination of the entrapped linalool in freshly-cooked starch matrix

Before quantitative analysis, the excess linalool on the surface of dried starch matrices was removed by washing with hexane [12]. Dried starch matrices (3 g) were cut into 5 mm × 5mm squares and then were washed once with 20 mL hexane for 30 min at room temperature. After washing, the samples were dried in a fume hood for 30 min and then in a desiccator overnight.

The samples (0.5 g) were dispersed in 1 N NaOH solution [12] and the entrapped linalool were extracted by hexane and quantitated by gas chromatography (GC). An internal standard, 2-methyl-3-heptanone, was added to the solvent at a concentration of 3,000 ppm. The GC (HP-6890; Agilent Technologies, U.S.) was equipped with an HP-5 column (30 m × 0.32 mm × 0.25 μ m) and a flame ionization detector. The oven temperature regime was: hold at 40°C for 2 min, heat to 180°C at the rate of 5°C/min, heat to 250 °C in 2 min, and

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hold at 250°C for 3 min. Concentration of entrapped linalool in samples was calculated using response factor and the area ratio of linalool against the internal standard. Response factor of linalool was determined using a 5-point calibration curve.

8. Determination of linalool retention during storage

The solvent-washed starch matrices (0.75 g) were put into individual 40 mL amber

vials and then kept in a tightly sealed glass container over a saturated $Mg(NO)_3$ solution (53% RH) at 25 °C for 4 months. The linalool content in starch matrices at 4 months of storage were extracted by solvent extraction technique and analyzed by GC, as previously described. The linalool retention (%) was calculated from the amount of linalool in starch matrix at 4 months of storage compared to its original quantity at the beginning of the experiment (day 0), as the following equation:

$$\% \text{retention} = \frac{\text{the amount of entrapped linalool at 4 months of storage}}{\text{the amount of entrapped linalool at the beginning (freshly prepared)}} \times 100$$

9. Statistical analysis

All response data with three replications were presented as the mean \pm standard deviation of each treatment. The experimental design was a complete randomized design (CRD) using analysis of variance (ANOVA) by the SPSS program (Version 12.0; SPSS Inc., Armonk, NY, USA). Duncan's multiple range tests were used to compare means ($p \leq 0.05$). Pearson's correlation was used to establish the relationship among different parameters.

Results and discussion

1. Gelatinization characteristic

The first step in the preparation of starch matrix is gelatinization of starch in an excess medium, resulting in a transition of the native granular structure to a starch film-forming solution. The gelatinization characteristics of mung bean starch heated in distilled water and aqueous alkalis are summarized in Table 1.

Table 1. Gelatinization characteristics of mungbean starch in different mediums

Medium	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)
Distilled water (pH~7)	70.4 \pm 0.1 ^b	73.7 \pm 0.0 ^b	81.4 \pm 0.1 ^b	1.5 \pm 0.1 ^b
NaOH solution (pH~12)	68.9 \pm 0.2 ^c	73.5 \pm 0.1 ^c	79.5 \pm 0.0 ^c	1.4 \pm 0.0 ^b
Ca(OH) ₂ solution (pH~12)	73.9 \pm 0.0 ^a	77.2 \pm 0.0 ^a	84.2 \pm 0.2 ^a	1.7 \pm 0.0 ^a

Values are expressed as mean \pm standard deviation

^{a-c} Different letters in the same column indicate significantly different between mean values ($p \leq 0.05$)

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The phase transition temperatures (T_o , T_p and T_e) and the enthalpy (ΔH) of mung bean starch heated in an alkaline solution without calcium (NaOH solution) were lower than those of mung bean starch heated in distilled water. The ability of alkaline solution in inducing gelatinization of starch is common knowledge [1]. Alkaline treatment resulted in the ionization of starch hydroxyl groups, which promoted the charge repulsion among chains. This resulted in the rupture of intermolecular hydrogen bonds and the favorable random coil conformation of amylose, as well as the initiation of starch degradation [13]. The transition temperatures and the enthalpy of starch heated in NaOH solution were consequently lowered. However, the presence of calcium in an alkaline solution ($\text{Ca}(\text{OH})_2$ solution) increased T_o , T_p , T_e and ΔH parameters ($p \leq 0.05$). The presence of Ca^{2+} ions in an alkaline solution can stabilize the starch granules by crosslinking the negatively charged starch molecules [2, 14]. As a result, there was a slight increase in the phase transition temperatures of starch treated in $\text{Ca}(\text{OH})_2$ solution. Study of the gelatinization behavior of starch is important to control processing conditions. This result revealed that a minimum of 85°C cooking temperature was required for mung bean starch to achieve complete gelatinization in all mediums. Therefore, the cooking temperature in this study was controlled at 95°C to ensure a complete gelatinization.

2. Appearance, color and thickness of dried starch matrices

The distilled water-cooked starch matrices (S-H₂O matrices), with and without

linalool, were visually colorless, smooth and flexible. Whereas, the NaOH-cooked (S-NaOH) and $\text{Ca}(\text{OH})_2$ -cooked (S- $\text{Ca}(\text{OH})_2$) starch matrices, with and without linalool, were yellowish, brittle and little shrinkage. The brittle and shrinkage of the alkaline-cooked starch matrices were probably due to the alkaline hydrolysis of starch. Chatakanonda et al. (2012) [15] reported that the lower molecular mass of starch resulted in the stronger but less extensible of film, therefore the brittle starch film was obtained when starch was treated under alkali condition. Similarly, Koskinen et al. (1995) [16] observed the less extensible of barley starch film made from acid-hydrolyzed starch.

The shrinkage of the alkaline-cooked starch matrices provided the thicker films. The average thicknesses of S-H₂O matrices, S-NaOH matrices and S- $\text{Ca}(\text{OH})_2$ matrices were around 0.16 ± 0.01 mm, 0.24 ± 0.01 mm and 0.24 ± 0.01 mm, respectively. There were no significant difference ($p > 0.05$) between the thicknesses of individual starch matrices, with and without addition of linalool, due to the low concentration of linalool used in this work.

Moreover, the alkaline cooking affected the color of dried starch matrices. A colorimetric analysis revealed the lower L^* values, higher b^* values and lower a^* values for the S-NaOH and S- $\text{Ca}(\text{OH})_2$ matrices than for the S-H₂O matrix (Table 2), attributing the decrease in the lightness and increase in greenish-yellow. It is known that alkalinity assists in the development of green and yellow colors by detaching flavone compounds that are natural pigments in starch. Asenstorfer et al. (2006) [17] reported that the flavones-C-diglycosides are the major components

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responsible for the alkali-induced development of green and yellow color in starch.

3. X-ray diffraction

X-ray diffractions were performed in order to identify the type of interaction between starch and linalool, and examine the crystallinity of the starch matrix. The results are illustrated in Figure 1. The X-ray diffraction patterns of S-H₂O matrices, with and without linalool, had the main peaks at $2\theta = 15^\circ$, 17° , 20° and 22° , indicated that their structures were the B-type crystalline [18], which is the natural structure for starch drying from a dilute solution [19]. The X-ray diffraction patterns of starch matrices made under alkaline condition (S-NaOH and S-Ca(OH)₂ matrices) attenuated the B-type characteristic peaks and showed sharp peaks at $2\theta = 9^\circ$, 18° and 29° in S-NaOH matrices and $2\theta = 29^\circ$ in S-Ca(OH)₂ matrices (Figure 1b and 1c). These additional peaks had a similar pattern to those reported by Romero-Bastida et al. (2005) [20], working with starch films prepared by cold gelatinization with NaOH, and by Lobato et al. (2015) [21], working with maize starch gelatinized in Ca(OH)₂ solution. The sharp peaks possibly attributed to the carbonate-crystals that take place during the cooking process, involving carbonation between atmospheric CO₂ and alkaline solution [21]. The X-ray diffraction data indicates that no V-amylose complex was formed in all starch matrices.

The relative crystallinities of starch matrices are shown in Table 2. It was found that the addition of linalool did not affect the

crystallinity of individual starch matrix. S-H₂O matrices had the relative crystallinity about 8.4-8.8%, while the relative crystallinity of S-NaOH and S-Ca(OH)₂ matrices, estimating after removal the non B-type crystalline peaks [21], were in ranges of 2.2-4.4% and 11.9-12.0%, respectively.

The crystallinity of starch matrix depends on reorganization capacity of starch chains during drying process and the amount of hydrogen bonds formed within the matrix [22]. From Table 2, The S-NaOH matrices exhibited the lowest relative crystallinity, possibly caused by the ionic repulsion between negatively charged starch molecules. Treating starch in NaOH solution (pH 12) resulted in the ionization of the hydroxyl groups of starch and leave negative charges on starch molecules [1, 13]. The ionic repulsion reduced starch chain availability for hydrogen bonding. Therefore, the association of starch chains in dried S-NaOH matrix was retarded. In contrast, the presence of Ca²⁺ ions in an alkaline solution increased the relative crystallinity of S-Ca(OH)₂ matrix. Ca²⁺ ions bind with the ionized starch chains and produced Ca-starch crosslinks [3]. This interaction increased the tightness of starch network and the crystallinity of S-Ca(OH)₂ matrix was consequently elevated. Rodriguez et al. (1996) [23] also reported crystallinity enhancement in corn tortillas cooked with Ca(OH)₂, suggesting calcium-induced crosslinking of starch molecules.

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Table 2. The color parameters and relative crystallinity of starch matrices

Concentration of linalool (w/v)	Matrix	Color			Relative crystallinity (%)
		L^*	a^*	b^*	
0%	S-H ₂ O	88.6 ± 0.2 ^a	-0.6 ± 0.0 ^a	3.1 ± 0.1 ^e	8.8 ± 1.6 ^b
	S-NaOH	86.7 ± 0.6 ^{bc}	-1.1 ± 0.1 ^c	9.1 ± 0.4 ^b	2.2 ± 2.0 ^c
	S-Ca(OH) ₂	84.3 ± 0.5 ^d	-0.9 ± 0.3 ^b	8.1 ± 0.9 ^c	11.9 ± 0.5 ^a
0.3%	S-H ₂ O	87.3 ± 0.4 ^b	-0.6 ± 0.0 ^a	4.0 ± 0.2 ^d	8.4 ± 3.7 ^b
	S-NaOH	85.8 ± 1.1 ^c	-1.0 ± 0.1 ^{bc}	10.1 ± 0.4 ^a	4.4 ± 0.6 ^c
	S-Ca(OH) ₂	85.9 ± 0.8 ^c	-0.5 ± 0.1 ^a	10.6 ± 0.2 ^a	12.0 ± 1.4 ^a

Values are expressed as mean ± standard deviation

^{a-c} Different letters in the same column indicate significantly different between mean values ($p \leq 0.05$)

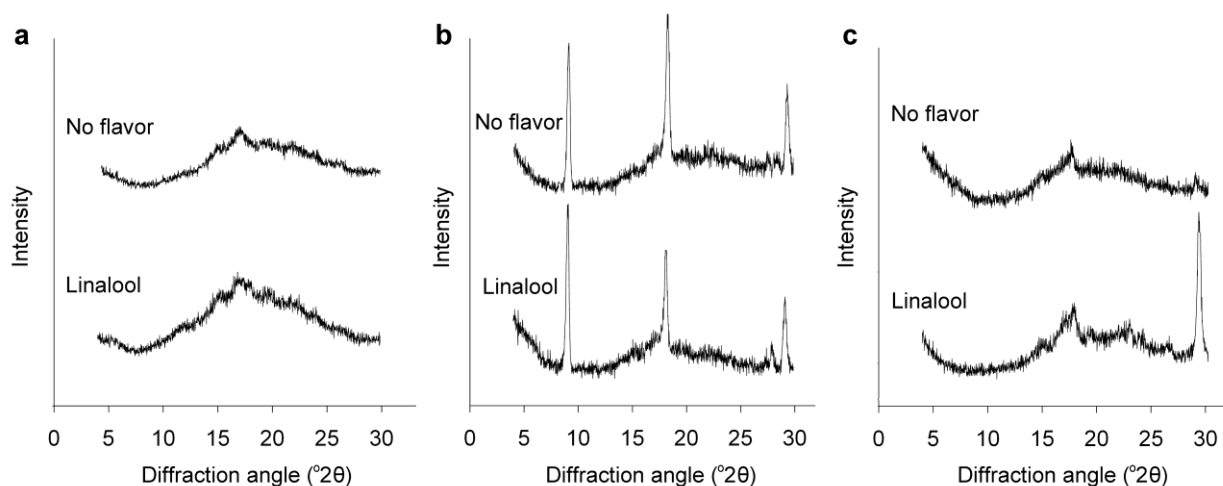


Figure 1. X-ray diffraction pattern of (a) S-H₂O matrices, (b) S-NaOH matrices and (c) S-Ca(OH)₂ matrices

4. Linalool entrapment

The linalool content in the starch matrices was determined by dissolution the starch matrices in alkaline solution before quantitated by GC. Alkaline dissolution results in extension of the amylose chains and total liberation of the aroma. The quantitative analysis of the entrapped linalool by GC is shown in Table 3. In freshly cooked samples,

the highest linalool entrapment was observed in S-Ca(OH)₂ matrix (34.4 mg linalool/g matrix), followed by S-H₂O matrix (2.64 mg linalool/g matrix) and S-NaOH matrix (0.23 mg linalool/g matrix). It is noted that the S-Ca(OH)₂ matrix exhibited a 13-fold higher linalool content when compared to the S-H₂O matrix (control sample).

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Table 3. Linalool entrapment in starch matrices

Matrix	Linalool entrapment (mg linalool /g matrix) [%Retention*]	
	Freshly cooked	4 months of storage
S-H ₂ O	2.64 ± 0.0 ^b	0.60 ± 0.1 ^b [22.8]
S-NaOH	0.23 ± 0.1 ^c	0.12 ± 0.0 ^c [54.5]
S-Ca(OH) ₂	34.40 ± 5.5 ^a	31.73 ± 1.3 ^a [92.2]

Values are expressed as mean ± standard deviation

*%Retention = ([linalool content at 4 months of storage] / [linalool content at the beginning]) × 100

^{a-c} Different letters in the same column indicate significantly different between mean values ($p \leq 0.05$)

XRD data indicated that the physical entrapment was an important interaction between linalool and all starch matrices, due to the absence of V-type crystalline. The linalool entrapment in starch matrix after drying (freshly-cooked matrix) can be explained by a selective diffusion theory [24]. Generally, when starch is heated in excess water and subsequently cooled, the disrupted amylose and amylopectin chain gradually re-associate [25]. Water evaporation during the drying process promotes starch molecular association and crystallization, while the volatiles are diffused from the suspension. The diffusion coefficients of water and volatiles are reduced as water concentration decreases. As a result of differences in the diffusivity of water and volatiles, the reduction in the diffusivity of the volatiles is more pronounced than that of water. Once the starch network has formed, volatiles are entrapped in the dried solid matrix, while water still diffuses. The dried starch matrix, therefore, effectively becomes a selective membrane [24]. The loose and slower rate of starch network formation in S-NaOH matrix, related to the low starch crystallinity from XRD data (Table 2), causing the massive linalool loss during drying and

consequently poor entrapping linalool in the matrix. On the other hand, Ca-starch crosslink decreased the release of linalool through the S-Ca(OH)₂ matrix during drying. Therefore, the highest linalool entrapment was observed in S-Ca(OH)₂ matrix. This explanation was supported by the Pearson's correlation analysis (Table 4). The linalool entrapment in starch matrix after drying showed the high correlation coefficient with crystallinity (0.74) but was not related to the thickness and color parameters of starch matrices. It could be concluded that starch crystallinity had important effect on the ability of starch matrices in entrapping flavor compounds.

In addition, the linalool retention during storage at 53% RH, 25°C was evaluated to determine the storage stability of dried starch matrices. The amount of entrapped linalool in starch matrices after 4 months of storage are shown in Table 3, and the percentage of linalool content at 4 months of storage compared to the flavor content at the beginning are shown in the square brackets, as %retention. The result indicated that S-Ca(OH)₂ matrix had the greatest ability to retain linalool during storage. This matrix retained 92.2%

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linalool after 4 months of storage, followed by S-NaOH matrix (54.5%) and S-H₂O matrix (22.8%).

The retention of a flavor compound in starch matrices during storage was inversely related to the diffusion of flavor compound from the interior of the matrix toward the surface and the loss of a flavor compound by evaporation. The Ca-starch crosslinking in S-Ca(OH)₂ might reduce pore-size as well as the available space, thereby restricting linalool diffusion [8]. Moreover, the thickness of S-Ca(OH)₂ matrix may also reduce the diffusion rate of linalool, resulting in an increase of linalool retention [7]. The highest %linalool retention, therefore, was observed in S-Ca(OH)₂ matrix. Interestingly, a loose network of S-NaOH matrix, related with the low starch crystallinity from XRD data, showed better performance in

retaining linalool during storage than that of S-H₂O matrix. It could be due to the higher thickness of S-NaOH matrix. This observation was partially supported by the Pearson's correlation analysis (Table 4). It revealed that the %linalool retention was positively correlated with the matrix thickness (0.79).

Moreover, %linalool retention showed the correlation with color parameters of starch matrices. It was positively correlated with the *b** value (0.86) and negatively correlated with **L* value (-0.74). This would be explained by the fact that the alkaline-cooked starch matrices (S-NaOH and S-Ca(OH)₂), which had more %linalool retention than that of S-H₂O matrix, exhibited the yellowish appearance. Hence, the strong correlation among **L* and *b** values with %linalool retention was obtained.

Table 4. Pearson's correlation between the film thickness, color parameters, linalool content and %linalool retention during storage in starch matrices

	Thickness	<i>L</i> *	<i>a</i> *	<i>b</i> *	Crystallinity	Linalool entrapment ^a	%Retention ^b
Thickness	1.00						
<i>L</i> *	-0.88*	1.00					
<i>a</i> *	-0.26	0.38	1.00				
<i>b</i> *	0.95*	-0.95*	-0.27	1.00			
Crystallinity	-0.46	0.20	0.18	0.14	1.00		
Linalool entrapment	0.40	-0.35	0.66	0.46	0.74*	1.00	
%Retention	0.79*	-0.74*	0.24	0.86*	0.50	0.80*	1.00

* Significant differences at $p \leq 0.05$

^a The amount of entrapped linalool in starch matrices after drying (freshly prepared)

^b The ability of starch matrices in retaining linalool after storage at 53% RH, 25°C for 4 months

Overall, the greatest retention of linalool in S-Ca(OH)₂ matrix, both in freshly cooked and stored samples, demonstrated that

cooking starch in Ca(OH)₂ solution can enhance the flavor retention ability by starch matrix. Compared to our previous research, the linalool

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entrapment in the S-Ca(OH)₂ matrix (around 3%) was nearly the same (around 4%) to that found in mung bean starch inclusion complexes prepared by heat treatment [6]. This shows that treating starch in an alkaline condition with Ca²⁺ is an interesting alternative method for flavor encapsulation. However, a major disadvantage of this technique is the use of extremely alkaline condition. Some esters compounds, for example, can be hydrolyzed under high pH and form odorless alcohol. Therefore, the preliminary test of the stability of individual flavor compound in alkaline condition before apply in this technique is needed.

Conclusion

Preparation of starch matrix by cooking in an alkaline solution containing calcium (Ca(OH)₂) solution was highly efficient in retaining flavor compound, both in freshly cooked sample and after storage at 53% RH, 25°C for 4 months. Conversely, the fresh starch matrix cooked in alkaline solution without calcium (NaOH solution) showed poor linalool entrapment. Both alkaline-cooked starch matrices had yellow appearance and were thicker than that of water-cooked starch matrices. Pearson's correlation analysis showed that the starch crystallinity had important effect on the linalool entrapment of freshly-cooked starch matrices. While, the thickness, *a** and *b** values were found to greatly correlate with the %linalool retention of starch matrices during storage. The information gained from this study expands knowledge on using of Ca-starch crosslink to flavor encapsulation application.

Acknowledgements

This work was partially supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University, under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

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