Antimicrobial activity of a crude peptide extract from lablab bean (*Dolichos lablab*) for semi-dried rice noodles shelf-life

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Abstract

This study provides an application of new, natural source of crude peptide extract from Lablab bean (CPL). Use of additive such as benzoate in the rice noodle industry is a common practice and has several beneficial effects on quality and shelf-life. However, the shelf-life of semi-dried rice noodles can be extended by crude peptide extract with acceptable quality when compared to other additives. This research aimed to extract CPL and determine its effect on the growth of microorganisms. The use of the CPL to extend the shelf-life of semi-dried rice noodles was compared with other natural extracts (chitosan and thymol) and benzoic acid. The CPL samples were extracted using 5% pepsin and incubated for up to 24 h. CPL at 200 mg/mL could be used as the growth inhibitor for *Bacillus cereus* but not for *Staphylococcus aureus* and *Escherichia coli*. It was found that noodles with CPL had the highest cooking loss (4.69) and lowest tensile strength (22.6 g.force). Overall-liking scores showed slightly liked (6.0 out of 9) and 68% of the consumers accepted the CPL-treated noodles. Shelf-life testing showed that CPL could extend the shelf-life of the noodles for 3 days than the control (1 day). Using 200 mg/mL of CPL could extend the shelf-life more than 3 times when compared to the control noodle (no additive). The CPL was nearly as effective as benzoic acid that could be kept for 3 days. Hedonic score in overall-liking showed a slightly like (6.2) for the noodles with CPL. This study suggests the application of adding CPL could be used as new natural additive and seems to be promising to apply in many food products such as pasta or sausages.

Keywords: antimicrobial activity; *Dolichos lablab*; lablab bean; rice noodles; shelf-life

Introduction

Rice noodles are the second most consumed food in Thailand (Saikhunthod and Peerattana, 2015). However, fresh and semi-dried rice noodles have a short shelf-life. With high moisture content (37%), semi-dried rice noodles can be stored for only 1–2 days before microbial spoilage. More than 80% of spoilage is caused by microorganisms such as mold, yeast and bacteria (Berthold-Pluta *et al*., 2019; Zhang *et al*., 2016). To extend the shelf-life, preservatives and chemicals such as benzoic or sorbic acid or calcium propionate have been used (Wang *et al*., 2018). However, when high amounts of preservative and chemicals are consumed, liver and kidney functions are reduced (Inetianbor *et al*., 2015). Therefore, Codex (2009) limits the use of benzoic acid in noodles to < 1000 mg/kg. According to the Thai Ministry of Public Health, a consumer weighing approximately 50 kg should not ingest more than 250 mg/kg body weight of benzoic acid following the Codex advisory specification for the identity and purity of food additives (Ministry of Public Health, 2016). The use of

50–100 g of cooked noodles for one meal with maximum benzoic acid use is equivalent to 226–451 mg of benzoic acid per day. The daily dose of benzoic acid should not exceed 2000 mg. With the problems mentioned above, natural extracts such as chitosan, thymol, grape seeds, and lemon extracts have been used to extend the shelf-life of pasta (Li *et al*., 2014). Again, Zhang *et al*. (2020) also found that bamboo leaf extract can be used as a shelf-life extension ingredient. Peptide extracts have been shown to inhibit both Gram-positive bacteria and Gram-negative bacteria (Gasu *et al*., 2018). Such microbial inhibited peptides have been extracted from some types of beans. It has been reported that peptides with the four amino acids, namely, leucine, valine, methionine, and serine, can be used to inhibit Gram-positive microorganisms (Tsutsumi and Tsutsumi, 2014). For example, Wong and Ng (2005) studied the peptides from lima beans and groundnuts, and used them to inhibit *Mycobacterium phlei*, *Bacillus megaterium*, *Bacillus subtilis*, and *Proteus vulgaris*. The concentrations need for a 50% reduction (IC_{50}) were 96, 115, 98, and 81 μM, respectively, with lima beans and 87, 105, 98 and 75 μM, respectively, with groundnut.

Lablab bean **(***Dolichos lablab***)** is commonly grown in areas of Southeast Asia as it has a high nutrient content and is an inexpensive source of protein (Borijindakul and Phimolsiripol, 2013; Jaisankar and Manivannan, 2018). Lablab beans typically have 24.9–26.5% protein with a good balance of essential amino acids such as lysine, histidine, etc. Some published data have shown small antimicrobial and antifungal effects. Seeds of the lablab bean contain lectin proteins, which have significant antimicrobial activity on different bacterial strains such as *Vibrio mimicus, S. aureus, B. cereus, Salmonella typhi,* and *Shigella dysentery* (Rahman and Akhter, 2018; Saha *et al*., 2014). El-Araby *et al*. (2020) also found that bean lectins showed antimicrobial activity against *S. aureus* and *Pseudomonas aeruginosa*. However, the information on lablab bean extract and application in the shelf-life of noodles is very poor. Therefore, this research aimed to extract crude peptides from lablab beans (CPL) and determine the ability to inhibit microorganisms in semi-dried rice noodles compared to two natural materials (chitosan and thymol) and chemical preservative (benzoic acid). The qualities of noodles, including physical, chemical, microbial, and sensory properties, were investigated in the production and shelf-life testing.

Materials and Methods

Materials

Dried lablab beans (*Dolichos lablab* L.) were purchased from Mae-Sot District, Tak Province, Thailand. Chemical compositions of lablab bean including moisture (AOAC 950.46), protein (AOAC 976.06), fat (AOAC 960.39), ash (AOAC 942.05), crude fiber (AOAC 962.09), and carbohydrate were measured following the standard methods of AOAC (2007). Pepsin enzyme (porcine gastric (EC 3.4.23.1), Merck, Darmstadt, Germany) was used in this study. The cultures of *B. cereus*, *S. Aureus*, and *E. coli* were purchased from the Thailand Institute of Scientific and Technological Research (TISTR) culture collection (Bangkok, Thailand). Rice flour (Jade Leaf brand, Bangkok Inter Food Co., Ltd., Bangkok, Thailand) and tapioca starch (Fish brand, ETC International Trading Co., Ltd., Bangkok, Thailand) were used for rice noodle preparation. Benzoic acid (Lobachemie PVT Ltd., Mumbai, India), chitosan (King Crab Chitosan Oligomer Type 100 mesh pass, Taming Enterprises, Samutsakon, Thailand) and thymol (Lobachemie PVT Ltd., Mumbai, India) were used as commercially available preservatives.

Crude peptide preparation

A factorial experiment was used to study three enzyme concentration levels (1, 3, and 5% (w/v) of the protein content in the lablab beans), which corresponded to 4.65, 14.0, and 23.36 U/g according to the method of Wang *et al*. (2007), and three hydrolysis times (0.5, 6, and 12 h). Hydrochloric acid 37% with water (pH 2) was added to ground lablab beans (1:10 w/v). Then, the samples were incubated in a water bath at 37°C for 0.5, 6, and 12 h. The reaction was stopped by heating to 75°C for 10 min. Then, it was centrifuged (Universal 320R, Hettich, Westphalia, Germany) at 11,700 × *g* at 4°C for 15 min. After that, the supernatant was dried in a hot air oven at 40°C for 6 h (Cheison *et al*., 2012). The dried CPL was stored in a desiccator before use.

Molecular weight of protein in CPL

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done by preparing 6–8% acrylamide gels (Ramos *et al*., 2019). The samples or the standard proteins were prepared by mixing the sample to buffer at the ratio of 3:1. The buffer contains 0.2 M Tris-HCl, pH 6.8, 8 mM EDTA, 40% glycerol, 4% SDS, and 0.4% bromophenol blue. For reducing conditions, 1% *ß*–mercaptoethanol was added and boiled for 2 min. The sample solution and the standard solution were placed in each of the channels. The electrophoresis buffer was 0.025 M Tris-0.192 M glycine and 0.1% SDS at pH 8.3. Then, turn on the electric current at 130 Volts for 1.5 h until the color of bromophenol blue moved to the bottom of the gel. Then, the gel was dyed. Size of protein was identified as a color band relative to the reference color band.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was measured using the method of Vipra *et al*. (2013) and Surin *et al*. (2018). The CPL was dissolved in 200 mg/mL distilled water and then mixed using a vortex mixer (G560E, Scientific Industries, New York, USA) for 30 min and centrifuged at 5,530 × *g* at 4°C for 15 min. Then, the samples were serially diluted twofold serial to 25, 50, 100, and 200 mg/mL with nutrient broth (NB) (MM244, HiMedia Laboratories Pvt. Ltd., Mumbai, India). The samples were incubated at 37°C for 24 h. Next, 1 mL of the sample was added to 50 mL Trypticase Soy Broth (LQ508, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37°C by shaking for 3 h. One mL of the sample was pipetted into 0.85% sodium chloride and the turbidity was adjusted to 0.5 McFarland units which equate with bacteria 1.5×10^8 CFU/mL. The control tubes had distilled water and a mixture of distilled water and NB. The samples were incubated at 37°C for 24 h. The result was read by observing the turbidity in each tube and comparing them with the control by comparison to the turbidity measurement paper.

Effect of addition CPL on rice noodle properties

Noodle preparation

A completely randomized design (CRD) was used to study the effect of the additives on noodle properties. Four types of additives were studied including chitosan (1% v/v in lactic acid solution) at 2000 mg/kg of noodle, thymol (50% v/v) at 2000 mg/kg of noodles, benzoic acid at 1000 mg/kg of noodle, and CPL at 200 mg/mL. Noodle preparation used the method of Liu *et al*. (2014) with slight modification. The ingredients including 125 g of rice flour, 24 g of tapioca starch, and 282 g of water were mixed in a bowl to prepare noodle slurry. The additives were then added to the noodle slurry. About 70 g of slurry was spread evenly on a stainless-steel tray, size 6 × 9 inch, and steamed in a steamer for 3 min to form a noodle sheet. After that, the noodle sheet was dried at 35°C for 10 min, and then cut into strips of 1 cm width before drying at 60°C for 10 min to obtain semi-dried rice noodles.

Quality measurement of semi-dried rice noodles

CIE L*, a*, and b* of the uncooked and cooked noodles were measured using a Chroma meter (CR-410, Konica-Minolta, Osaka, Japan). The cooking time, cooking loss, and water absorption of noodle were measured according to AACC standard methods 66-50 (AACC International, 2010). Sensory properties of cooked noodle from different additives were evaluated using a 9-point hedonic scale with 1, dislike extremely, and 9, extremely like the semi-dried rice noodles with crude peptide extract from lablab bean using the method described by Phimolsiripol *et al*. (2017) and Wangtueai *et al*. (2020). A total of 50 panelists participated in individual booths in this study which, was held in the Chiang Mai University Sensory Research Unit (Chiang Mai, Thailand). For shelf-life testing, the noodle samples were kept at 30°C and tested every day for up to 6 days. Microbial properties, including total plate count, and yeasts and molds, were determined following the methods of AOAC (2007) (methods 999.11 and 997.02 respectively).

Statistical analysis

One-way analysis of variance (ANOVA) was applied to analyze the data ($P < 0.05$). Duncan's multiple range test (DMRT) was used for *post hoc* multiple comparisons. The experiments were done in triplicates.

Results and Discussion

Chemical properties of lablab bean

Chemical compositions of lablab bean including moisture, fat, ash, crude fiber, and carbohydrate were 10.8, 0.6, 3.5, 0.2 and 61.5%, respectively. Lablab bean had 23.26% protein. Likewise, Borijindakul and Phimolsiripol (2013) reported that protein content in lablab bean was 20–25%. This agrees with another study including 20–25% crude protein in *Dolichos lablab* seed (Hossain *et al*., 2016; Kala *et al*., 2010). It was found that the main five components of the amino acids in lablab bean were lysine, phenylalanine, leucine, histidine, and tyrosine as listed in Table 1. For CPL, the main five components of amino acid were glutamic acid, lysine, phenylalanine, leucine, and tyrosine. Table 1 shows that 100 g of CPL had 2642 mg of lysine and 949 mg of histidine. According to the amount of the amino acids found in CPL, it can be expected that the peptide can possibly inhibit microorganisms. This was due to these essential amino acids having cations that enhance their ability to resist microorganisms (Cantor *et al*., 2019). Intorasoot (2013) also indicated that amino acids that can inhibit microorganisms are the positively charged amino acids, lysine and histidine. The mechanisms of the antimicrobial activity of amino acids were confirmed by Saha *et al*. (2014). Almost all microorganisms express surface-exposed carbohydrates, which may be covalently bound. Every surface-exposed carbohydrate is a potential lectin-reactive site, giving lectins the ability to form complexes with microbial glycoconjugates.

Protein molecular weight of CPL

SDS-PAGE showed that the CPL had molecular weight ranging from 30–45 kDa (Figure 1). This was consistent

Table 1. Amino acid profiles found in the lablab bean and CPL.

Amino acids	Lablab bean (mg/100 g)	CPL (mg/100 g)
Lysine	5278	2642
Phenylalanine	3860	2178
Leucine	2927	1801
Histidine	2256	949
Tyrosine	1759	1421
Isoleucine	1578	752
Glutamic acid + Glutamine	1342	2979
Valine	858	739
Aspartic acid + Asparagine	574	856
Proline	393	431
Alanine	332	572
Glycine	309	431
Tryptophan	226	152
Serine	153	181
Methionine	137	212
Threonine	124	173
Cysteine	116	131
Arginine	<5	<5

with the results of Sukamto *et al*. (2019) who reported that the range of protein in lablab bean has a molecular weight between 25 and 41 kDa, while Saha *et al*. (2014) found that the molecular weight of hydrolyzed lablab seeds ranged from 18 to 45 kDa. From the results of SDS-PAGE, all tested samples were found to have the same patterns of proteins. Thus, 5% pepsin enzyme with 0.5 h of hydrolysis time was chosen to be used in further shelf-life experiments in rice noodles due to lower cost of production and time saving.

Minimum inhibitory concentration

MIC is measured the lowest concentration completely inhibited the bacterial growth as widely used for determining the antimicrobial properties. (Laokuldilok *et al*., 2017). It was found that the CPL could inhibit the growth of the microorganisms. The concentration of 200 mg/mL of the CPL was found to be able to stop the growth of *B. cereus* as presented in Table 2. Saha *et al*. (2014) also found that the crude extract from lablab seeds inhibited the growth of *B. cereus*. El-Araby *et al*. (2020) confirmed that leguminous seed lectins provided antibacterial and antifungal activities. This is probably due to the exchanges of cations and anions between amino acid and cell membrane, and it causes damage to the cell walls of the microorganisms and stops their growth (Lei *et al*., 2019). In addition, the cell structure of *B. Cereus*, which is a Gram-positive bacteria, is less complex than that of gram-negative bacteria (Dia *et al*., 2014; Reshmi *et al*., 2012). However, the CPL cannot inhibit the growth of *S. aureus* because *S. aureus* is resistant to adverse conditions (Patel *et al*., 2012). Moreover, some species of *S. aureus* can produce mucilage-like capsules that are resistant to antibiotics (Arciola *et al*., 2019; Kuipers *et al*., 2016). For *E. coli*, it was also found that the CPL could not stop the growth of *E. coli*. It is because *E. coli* is a Gram-negative bacteria, which has more complex cell walls than gram-positive bacteria. Gram-positive bacteria have only a single lipid membrane that is surrounded by a thick layer of peptidoglycan. In contrast, gram-negative bacteria have an inner and outer membrane, so antimicrobials cannot penetrate the bacterial membrane in order to exert their effects (Kuipers *et al*., 2016). Rahman and Akhter (2018) also confirmed that the presence of the outer membrane of Gram-negative bacteria acts as

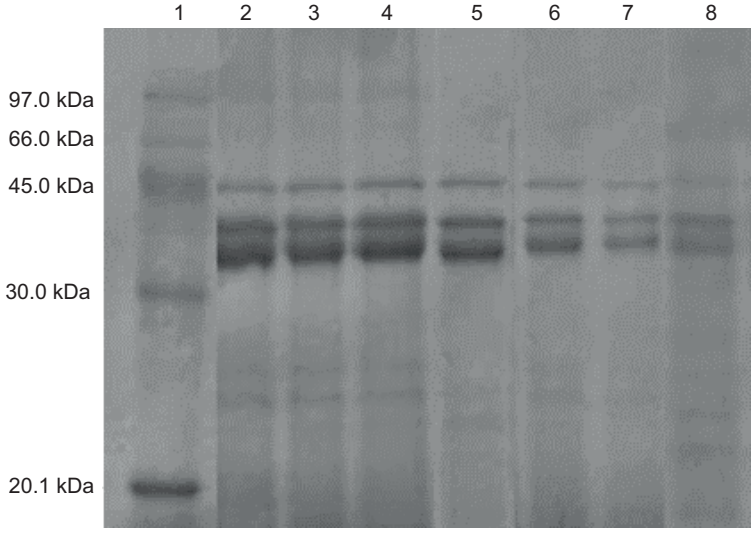

Figure 1. Molecular weights of the CPL: 1 =References; 2 = 5% hydrolysis, 12 h; 3 = 3% hydrolysis, 12 h; 4 = 1% hydrolysis, 12 h; 5 = 5% hydrolysis, 6 h; 6 = 3% hydrolysis, 6 h; 7 = 1% hydrolysis, 6 h; and 8 = 5% hydrolysis, 0.5 h.

a barrier against numerous antibiotic molecules and the enzymes of the periplasmic spaces. Ratnayani *et al.* (2017) indicated that protein hydrolysates from beans had no antimicrobial effects on *E. coli* and *S. aureus*. This is because protein hydrolysates are mixtures of different types of amino acids and are of different sizes. As mentioned by Mirzapour-Kouhdasht *et al.* (2020), peptide concentration is an important factor in the biological and functional properties of the hydrolyzed protein. However, the bioactive peptides should have molecular weight of <3 kDa for exhibiting antimicrobial activities. Moreover, it was possible that the function of the enzyme inappropriately cut the peptide bond. In other words, the ends of the peptide chains are not positively charged, so they cannot inhibit the growth of the microorganisms. As positive charge facilitates the initial association of CPL with the negatively charged cell membranes, their hydrophobicity allows subsequent insertion of peptide into the hydrophobic cores of the cell membrane, resulting in membrane rupture and cell lysis (Wang and Vermerris, 2016). These findings correspond to the results of Duangmal and Saetongtae (2014) who found that longer duration of protein hydrolysis by enzymes led to inappropriate bond cutting of protein, resulting in less effectiveness of anti-microorganisms (Guinane *et al.*, 2015).

Table 2. Minimum inhibitory concentration of CPL from different hydrolysis conditions.

Hydrolysis time (h)	Enzyme concentration (%)	Minimum inhibitory concentration (MIC) (mg/mL)		
		B. *cereus*	S. *aureus*	*E. coli*
0.5	1	–	–	–
	3	–	–	–
	5	200	–	–
6	1	200	–	–
	3	200	–	–
	5	200	–	–
12	1	200	–	–
	3	200	–	–
	5	200	–	–

–, indicates no inhibition.

Color and cooking quality of semi-dried rice noodles

The appearance characteristics of the noodles with different additives were shown in Figure 2. The lightness (L* value) of the cooked noodle was in the range of 65.5–70.6, which was equivalent to standard noodles with L* values between 60 and 80 (Chung *et al.*, 2012). The noodle of CPL had the highest a* value (redness) and b* value (yellowness), which is shown in yellow shades (Figure 2c). For cooking loss, the noodle with CPL had the highest cooking loss which was 4.69%, while the noodle with chitosan had 1.76% cooking loss. These results were similar to Wandee *et al.* (2014) who found that the cooking loss in noodles containing fibers from the pomelo peel increased from 1.3 to 2.4%. Similarly, Tiboonbun *et al.* (2011) also found that the noodles mixed with banana flour had cooking loss ranging between 1.0 and 2.5%. In addition, Thomas *et al.* (2014) reported that the cooking loss in flat rice noodles was between 5.9 and 7.14%. Since the CPL has a high ability to absorb water, the noodles with added CPL had less cooking time (0.5 min) than the other samples (1.5 min) as shown in Table 3. Regarding water absorption, the weights of the noodle with CPL was similar to the control samples, which was 24.99 and 24.87 g, respectively. Texture measurement showed that the tensile strength of the noodle with CPL was 22.61 g. force, the tensile strength of the noodle with chitosan was 24.36 g.force, and the tensile strength of the noodle with thymol was 30.02 g.force. For the noodles with no additive and benzoic acid, their tensile strengths were 37.39 and 37.23 g.force, respectively. These are equivalent to general semi-dried rice noodles that were between 39 and 43 g.force (Cham and Suwannaporn, 2010). Similarly, Qazi *et al.* (2014) reported that the addition of sweet potato starch to general semi-dried rice noodles ranged between 18 and 43 g.force.

Sensory properties of cooked noodles

Hedonic scores of the developed noodle with different additives (0-day storage) are presented in Table 4. The hedonic score for appearance, aroma, taste, flavor, texture, and overall-liking of noodles with CPL were slightly like (6.1, 6.0, 6.1, 6.5, 6.5, and 6.0, respectively). The noodle with thymol was unacceptable for consumers with the

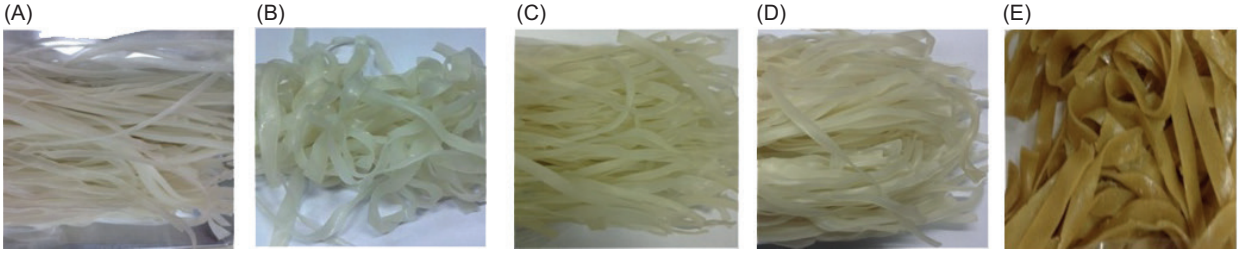

Figure 2. Characteristics of the noodles with different additives. (A) Control. (B) Benzoic acid. (C) Chitosan. (D) Thymol. (E) CPL.

Table 3. Effect of different additives on the quality of the semi-dried rice noodles.

Samples	L*^{ns}	a*	b*	ΔE*	Cooking time (min)	Cooking loss (%)	Water absorption (%)	Tensile strength (g.force)
Control	68.2 ± 0.4	−1.8 ± 0.1^{c}	0.9 ± 0.2^{d}	–	1.5	0.98 ± 0.01^{a}	24.9 ± 0.03^{c}	37.4 ± 0.5^{a}
Benzoic acid	65.5 ± 0.4	−1.8 ± 0.1^{cb}	3.4 ± 0.1^{b}	3.67	1.5	0.80 ± 0.02^{a}	31.9 ± 0.04^{a}	37.2 ± 0.5^{a}
Chitosan	67.6 ± 0.2	−1.7 ± 0.1^{b}	2.5 ± 0.6^{c}	1.68	1.5	1.76 ± 0.01^{b}	30.4 ± 0.1^{b}	24.4 ± 0.5^{c}
Thymol	69.9 ± 0.1	−1.6 ± 0.1^{b}	3.9 ± 0.1^{b}	3.39	1.5	1.14 ± 0.01^{c}	33.5 ± 0.03^{a}	30.0 ± 0.6^{b}
CPL	70.6 ± 0.4	1.1 ± 0.1^{a}	17.5 ± 0.3^{a}	16.7	0.5	4.69 ± 0.12^{a}	25.0 ± 0.01^{c}	22.6 ± 0.3^{d}

Values are the mean and standard deviation. Mean values in the same column with different letters are significantly different ($P < 0.05$). ^{ns} indicates not significant ($P \geq 0.05$). The cooking time was measured by sampling every 0.5 min.

overall-liking score below 5. This may be because of the strong flavor of thymol, resulting in an unpleasant smell and taste of the noodles.

Microbiological quality evaluation of semi-dried rice noodles

Based on food safety, microbial property is a major concern. Table 5 shows the result of total plate count during storage. For standard value, the total plate count must be no higher than 1×10^3 CFU/g. It was found that the noodle with benzoic acid and CPL could be kept for 3 days while the noodle with thymol could be kept for only 2 days because total plate count exceeded the standard. Moreover, the total plate count of the control noodle (no additive) and noodle with chitosan was rejected after 1−day storage due to over standard value (1×10^3 CFU/g) for semi-dried noodle as mentioned by FDA. Considering the appearance of noodles with no mold that could be detected by the naked eye, the standard amount was set at no higher than 10 CFU/g. The amount of yeasts and molds are shown in Table 6. The noodle with thymol could be kept for 6 days, while the noodle with benzoic acid and CPL could be kept only for 4 days.

Table 4. Hedonic score using 9-point scale of 50 consumers on cooked rice noodle with different additives.

Sensorial attributes	Control	Benzoic acid	Chitosan	Thymol	CPL
Appearance	7.8 ± 0.8^{a}	6.5 ± 1.0^{c}	7.0 ± 0.8^{b}	6.5 ± 1.0^{c}	6.1 ± 1.3^{c}
Aroma	7.5 ± 1.1^{a}	6.6 ± 1.2^{b}	7.1 ± 0.7^{a}	5.7 ± 1.5^{c}	6.0 ± 1.1^{c}
Taste	7.4 ± 1.1^{a}	6.8 ± 1.0^{b}	7.0 ±0.8^{ab}	3.6 ± 1.5^{d}	6.1 ± 1.5^{c}
Flavor	7.4 ± 0.8^{a}	6.9 ± 0.9^{a}	6.3 ± 1.0^{b}	3.2 ± 1.5^{d}	6.5 ± 1.5^{c}
Texture	7.4 ± 0.9^{a}	7.2 ± 0.9^{a}	7.0 ± 1.1^{a}	6.1 ± 1.4^{b}	6.5 ± 1.4^{c}
Overall-liking	7.7 ± 0.9^{a}	7.5 ± 1.0^{a}	7.3 ± 0.9^{a}	4.2 ± 1.5^{c}	6.0 ± 0.8^{b}

Values are the mean and standard deviation. Mean values in the same row with different letters are significantly different ($P < 0.05$).

Table 5. Total plate count of semi-dried rice noodles with different additives during storage.

Storage (day)	Total plate count (CFU/g)				
	Control	Benzoic acid	Chitosan	Thymol	CPL
0	$\underline{2.9 \times 10^2}$	1.2×10	$\underline{2.9 \times 10^2}$	1.0×10^2	1.1×10
1	3.5×10^3	4.3 ×10	3.8×10^3	3.0×10^2	2.4 ×10
2	nd	3.0×10^2	nd	$\underline{6.0 \times 10^2}$	1.3×10^2
3	nd	$\underline{1.0 \times 10^3}$	nd	2.0×10^3	$\underline{1.0 \times 10^3}$
4	nd	1.0×10^5	nd	nd	3.0×10^8
5	nd	nd	nd	nd	nd
6	nd	nd	nd	nd	nd

nd indicates that the samples were not determined due to molds in physical appearance.
The underline represents that the day that the total plate count were not higher than the standard amount set (1×10^3 CFU/g).

Table 6. Yeasts and molds of semi-dried rice noodles with different additives during storage.

Storage (day)	Amounts of yeasts and molds (CFU/g)				
	Control	Benzoic acid	Chitosan	Thymol	CPL
0	<1.0	<1.0	<1.0	<1.0	<1.0
1	<1.0	<1.0	<1.0	<1.0	<1.0
2	5	<1.0	8	<1.0	<1.0
3	12	8	11	<1.0	7
4	nd	11	nd	3	14
5	nd	nd	nd	7	nd
6	nd	nd	nd	13	nd

nd indicates that the samples were not determined due to molds in physical appearance.
The underline represents the day that yeasts and molds were not higher than the standard amount set (<10 CFU/g).

In addition, the control samples (no additive) and the noodles with chitosan were found to have a short shelf-life since they could be kept only for 3 days. However, the deterioration of noodle that detect by total plate count occurred before the defect from yeast and molds. Therefore, the shelf-life of noodles was based on total plate count analysis.

Conclusions

Using 5% of the pepsin enzyme to extract peptides from Lablab can be effective to inhibit the growth of *B. cereus*. However, it cannot inhibit the growth of *S. aureus* and *E. coli.* For application in noodles, adding CPL resulted in high yellowness and low cooking time, compared to other noodles. For shelf-life of noodles, 200 mg/mL CPL could be extended more than 3 times when compared to the control noodle (no additive), which could be kept only for 1 day. The CPL was nearly as effective as benzoic acid that could be kept for 3 days. Furthermore, hedonic score in overall-liking showed a slightly like (6.2) for the noodles with CPL. It is suggested that the CPL could be used as the new natural additive and seems to be promising to apply in food products. However, future work is required to do more in molecular purification, testing and another toxicity of the extracts for finding more mechanisms of its applications.

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Conflict of Interest

The authors declare no conflict of interest associated with this research.

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