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Content

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Effects of Nutrient Supplement and Chitosan on Microbial Population Change in Up-Flow-Anaerobic-Sludge-Blanket Reactor during Biogas Production

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Abstract

The objective of this research was to study the effects of nutrient supplement and chitosan on microbial change in an up-flow-anaerobic-sludge-blanket (UASB) reactor during biogas production. Three UASB reactors were operational in this study. All reactors were operated by feeding dilute stillage with chemical oxygen demand (COD) concentration at 10,000 mg/L and 9 days of hydraulic retention time (HRT) under anaerobic conditions. Reactor 2 and reactor 3 were supplemented with nutrient supplement and chitosan, respectively. The results of the environment and pH values of all UASB reactors showed similar conditions with total volatile acid/alkalinity (TVA/Alk) values of 0.27-0.31. The COD removal efficiency of reactor 1 (stillage), 2 (stillage and nutrient supplement) and 3 (stillage and chitosan) showed about 79%, 84% and 87%, respectively. In addition, it was found that the UASB reactors supplemented with nutrient supplement or chitosan produced higher levels of biogas than those without additives. The 16S rRNA technique by PCR reaction showed that the dominant archaea in the final fermentation of all UASB reactors and in inoculum sample were hydrogenotrophic (genus *Methanobacterium*) and acetotrophic methanogens (genus *Methanosaeta*). The methanogens population in the reactor supplemented with chitosan (18.11%) produced more biogas than the ones in the reactor supplemented with nutrient supplement (14.44%) and in the control reactor (15.95%).

Introduction

Currently, Thailand is a country considered as one which has many factories, especially agricultural-industrial factories. These agricultural processing plants discharge a large amount of wastewater which will affect the environment in the future, if not managed well. According to the study of Owusu-Agyeman et al.

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(2020), one of the most suitable and popular wastewater treatment systems for these wastewaters was the UASB system. It is effective for treating high organic wastewater, less costly to operate, and the system can produce high volumes of biogas, making it economically cost-effective (Udomsinrot, 2000; Verbyla et al., 2013), but the efficiency of the UASB system depends on the granular sludge in the system. Granular sludge consists of several groups of microorganisms such as hydrolytic, methanogens and acetogens (Xu et al., 2018). Granular sludge provides a high treatment efficiency due to its active anaerobic microorganisms as well as a good sedimentation process, thereby reducing the loss of sludge outside the system (Kiran et al., 2016). Moreover, granular sludge is resistant to various changing environmental conditions in the system, such as pH fluctuation, flow rate fluctuation and change in organic matter (Kobayashi et al., 2018).

That is why several researchers had tried to increase the efficiency of granular sludge regarding the performance of UASB reactors. Lertsitthichai (2006) studied the addition of nutrient supplement to affect the efficiency of the UASB system, and it was found that the biogas production rate was higher and the microbial pellet size was larger compared to the system without supplementation. It was shown that the supplement added resulted in a huge pellet size, which affects system efficiency of biogas production and organic removal. Jijai et al. (2015) found that the particle size of granular sludge affects system efficiency. On the other hand, many researchers tried to use chitosan to increase the granular sludge size for higher efficiency of the UASB reactor (Jijai et al., 2015). Tiwari (2005) used chitosan to improve the granular sludge for wastewater treatment in a UASB system. The result showed that 95-98% of the COD efficiency was removed and it also increased significantly the granular sludge in the UASB system. Torres (2018) used chitosan to start up their UASB system. The result showed that COD was removed about 92% within 11 days. Moreover, Khemkhao et al. (2011) have studied the effect of chitosan on UASB treating palm oil mill effluent during a transition from mesophilic to thermophilic conditions. It was found that chitosan addition showed a higher biogas production rate, decreased the washout of biomass, and increased the granular size.

As mentioned above, both nutrient supplement and chitosan led to a higher efficiency of the UASB system and also increased the pellet size of granular sludge, especially in adding chitosan. Nutrient supplement and chitosan may affect the microbial changes in the system. However, the study on the change of the microbial population and particularly the comparison between adding chitosan and nutrient supplement in an UASB reactor was limited. Therefore, this study aimed to find detailed effects of nutrient supplement and chitosan on microbial population change in UASB reactors. In addition, the organic matter removal as well as biogas production were studied.

Materials and methods

1. Cassava stillage

The effluent from a cassava ethanol production plant was used in this experiment. It was collected from the water outlet of a reservoir before entering the UASB system. Samples were preserved at a temperature below 4°C to prevent bacterial activity. The water samples were analyzed for various properties including pH, biochemical oxygen demand (BOD), COD, total solids (TS), total volatile solids (TVS), total dissolved solids (TDS), suspended solids (SS), and total Kjeldahl nitrogen (TKN). These parameters were analyzed according to the standard method of APHA (2012).

2. Nutrient supplement and chitosan

For testing nutritional supplement and chitosan regarding optimizing the removal of organic matter and biogas production the nutrient supplement was mixed with cassava stillage according to the application of Speece's formula (1996) as shown in Table 1.

Table 1 Composition of nutrient supplement

Ingredient	Concentration (mg/L)
Major	
NH_4Cl	400
KCl	400
$MgSO_4 7H_2O$	400
$Na_2S\ 9H_2O$	300
$(NH_4)_2\ HPO_4$	80
$CaCl_2\ 2H_2O$	50
Minor	
$FeCl_2\ 4H_2O$	40
$CoCl_2\ 6H_2O$	10
$NiCl_2\ 6H_2O$	0.5

The major ingredients of the supplements were ammonium, potassium, magnesium, sodium and calcium.

Minor other ingredients were ferrous, cobalt and nickel. The stillage that had been mixed with nutrient supplement was fed into the UASB every day.

Chitosan original flakes from aquacultural Yannamei shrimp. Shell with a molecular weight of 2,100 kilodalton (kDa) was used in this experiment. The long-chain of chitosan was prepared at a concentration of 2% by dissolving 100 g of chitosan powder in concentrated 150 mL acetic acid. Then, water was filled up to a net volume of 5 L. On every cycle of hydraulic retention time (HRT), 1.6 L of cassava stillage mixed with 50 mL of prepared chitosan was fed to the UASB.

3. Granular sludge and specific methanogenic activity (SMA)

Granular sludge from the same plant as the stillage sample was used in this study. Granular sludges were collected from the UASB system of a factory and stored by soaking in the effluent of the UASB system of the factory for use as a nutrient for the sludge prior to further testing. Granular sludges were analyzed by pH, moisture, total solids and total volatile solids following the APHA standard method. In the testing step, the concentrated granular sludge that was fed into the UASB reactor was approximately at 20,000 mg/L. One third of the reactor volume was fed into the reactor before the operation testing step.

The SMA test was performed in a 120 mL serum vial with a food to microorganism ratio (F/M ratio) of 0.5 using glucose as food, whereas the amount of sludge was calculated from the volatile suspended solids (VSS) (Smolder et al., 1995). The biogas was measured at least 20 days or until the biogas was at its balanced level. The biogas gas composition was then analyzed on a weekly basis using a gas chromatography-flame ionization detector (GC-FID) model SHIMADZU GC-14. The SMA was calculated from the slope of the methane production curve, divided by gram of VSS and expressed as $gCOD.gVSS^{-1}.day^{-1}$. Theoretically, 350 mL of methane was produced from 1 g of COD.

4. UASB reactor

In the experiment, all three UASB reactors used in this study were made of acrylic material. Each had a width of 15 cm., length of 15 cm, and height of 80 cm. The total volume of each reactor was 18 liters, with a working volume of 15 liters. Inside the reactor, there were 2 separated sections. The lower part was the reacting section whereas the upper part was the gas separating section (GSS). Cassava stillage was fed from the bottom of the reaction tank through 3 taps equipped with a pump. Effluent from the reactor was collected at the height of 67 cm and the biogas sampling point was located at the top of the reactor as showed in Fig 1.

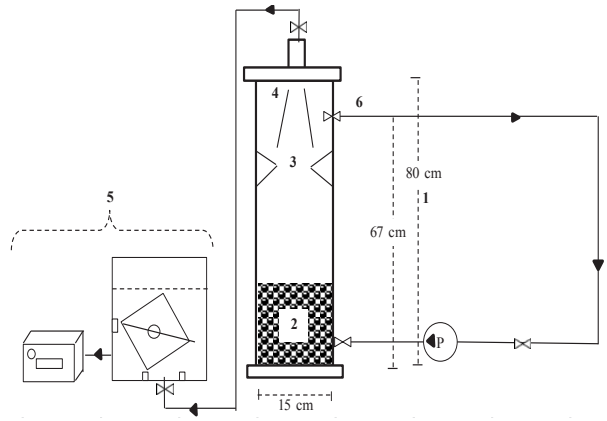

Fig. 1 Up-flow anaerobic sludge blanket reactor

5. UASB Operation

UASB reactors: reactor 1 was operated with pure cassava stillage, reactor 2 was operated with cassava stillage including nutrient supplement, and reactor 3 was operated with cassava stillage including chitosan. All UASB reactors were operated by feeding stillage diluted with COD concentration at 10,000 mg/L. They were fed at a flow rate of 1.6 L/d approximately 9 days of drolysisdraulic retention time (HRT) under anaerobic conditions. The recirculation rate of all three UASB reactors were 5L/min by a Mirano Water Pump ID8 (220 V A.C. 50 Hz 0.37 kW).

6. Analysis

The effluent and biogas production of the three UASB reactors were measured and analyzed. The effluent of each reactor was analyzed for pH, COD, Alk and TVA to assess the optimal environment and system performance. It was analyzed according to the APHA standard method (Ableling & Seyfired, 1992). The biogas production of each reactor was measured by a gas collector and counter for comparing the efficiency of each reactor.

7. Study of microbial population in UASB

Granular sludge samples from the UASB system before (inoculum source) and after adding wastewater from the cassava ethanol production plant (reactor 1),

nutritional supplements (reactor 2) and chitosan (reactor 3) were studied to extract the DNA for microbial populations with the following E.Z.N.ATM Mag-Bind Soil DNA Kit (Omega, USA). The 16S rRNA gene was increased by PCR reaction using 341F (5/TCGTCGG CAGCGTCAGATGTGTATAAGAGACAGCCTACG GGNGGCWGCAG-3/) and 805R(5/GTCTCGTG GGCTCGGAGATGTGTATAAGAGACAGGACTACH VGGGTATCTAACC-3/) primers with specific V3-V4 variable regions using sparQ HiFi PCR master mix (Quanta bio, USA) imported to a Perkin-Elmer GeneAmp PCR system 2400 thermal cycler (Perkin-Elmer, USA) by programming the DNA amplification as denaturation cycle (94°C for 3 min), 25 cycles (98°C for 20 sec, 55°C for 30 sec and 72°C for 30 sec) and final extension (72°C for 5 min). The nuclear ribosomal DNA was increased by PCR reaction by using ITS-1F and ITS-2R primers and imported to the Perkin-Elmer GeneAmp PCR system 2400 thermal cycler by programming the DNA enrichment as denaturation cycle (94°C for 3 min), 25 cycles (98°C for 20 sec, 60°C for 30 sec and 72°C for 30 sec) and a final extension (72°C for 5 min). The newly created DNA was then purified with AMPure XP beads and the specimens were classified by using 5 µl of each Nextera XT index kit. The attachment of DNA strands to classified samples was carried out by using 50 µl of PCR reaction followed by 8-10 cycles of PCR states, the final PCR products were then cleaned and collected. After the completion, the size of the DNA fragment was examined using agarose gel technique electrophoresis; 0.8% agarose gel was applied and was cut in the DNA band area of 16S rRNA at an approximate target DNA size of i.e., 550 bp and it was then diluted to the final concentration of 6 pM performing a sequencing analysis with the Illumina MiSeq genome analyzer by Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand).

FASTQC software was used to analyze the sequences' evolutionary relationships (Phylogenetic analysis) by comparing the genetic sequence using a PEAR program (Zhang et al., 2017). The FASTX-Tool kit read less than 90 % of non-quality data and would not be less than 300 bp when comparing the similarity of nucleotide sequences obtained using the UCHIME method (Edgar et al., 2011) in vsearch 1.1.1 (Rognes et al., 2016). Uchime_ref, group classification was carried out using operational taxonomic units (OTU) using pick_open_reference_otus.py command in QIIME 1.9.0. The specificity of the sequences used SortMeRNA for reference. The taxonomy was generated at 97% of Greengenes database. The error information was further read using SUMACLUST. The sequence used was 30,000 for each sample to analyze the bacterial population. Alpha diversity data was performed with the Chao1 index (Chao, 1984) and Shannon index (Shannon, 1984). Beta diversity data was estimated by calculating the weighted and unweighted UniFrac distances matrix which used principal coordinate analysis (PCoA) technique (Lozupone et al., 2011). The archaeal and bacterial 16S rRNA gene sequences obtained from inoculum samples of reactor 1, reactor 2 and reactor 3 were deposited in GenBank under Bioproject accession number PRJNA750574.

Results and discussion

1. Cassava stillage properties

The inspection and observation of the physical appearance of cassava stillage revealed that the water appeared in a light brown color with rancid odor and unclear sediment. As for the examination of the biochemical properties, relatively high values in COD and TS were found, with the average value at 42,777 ± 2,525 and 29,820 ± 1,220 mg/L, respectively (Table 2). With the consideration of the proportion of COD: N: P at 100: 1.69: 0.004, the result did not seem suitable for bacteria growth in an UASB reactor. Ableling & Seyfired (1992) found that for an anaerobic process, the appropriate COD: N: P in the wastewater is 100:0.625: 0.125. Moreover, it was found that some of the nutrients contained in the wastewater cannot be used for the growth of the bacteria. Therefore, additives that affect the growth of microorganisms under anaerobic conditions should be considered to further increase the efficiency of the treatment system. Regarding the proportion of BOD to COD in this study, a value of 0.57 was found. This means that the stillage used in this study had more than 50% of organic substances that were biodegradable, considering that any values greater than 0.5 are suitable for biodegradation in the anaerobic fermentation process (Lee & Nikraz, 2015).

2. Granular sludge and SMA

The UASB granules were characterized before they were used as inoculum in an UASB reactor (Table 3). The pH value showed a result of 7.0. The moisture content showed a high value at about 94% of the UASB wet sludge. Total volatile solid was 81% as a dry basis. From the 30 days of SMA test, it was found that the

Table 2 Characterization of cassava stillage

Property indexes	Values	Average ± S.D.
pH	4.04-4.21	4.12 ± 0.09
Alkalinity (mg/L)	42.5-112	83.17 ± 36.23
Total volatile acid (mg/L)	3,932-4,745	4,308 ± 410
BOD (mg/L)	21,500-27,180	24,428 ± 2,844
COD (mg/L)	40,250-45,300	42,777 ± 2,525
Total solids (mg/L)	28,750-31,150	29,820 ± 1,220
Total volatile solid (mg/L)	24,400-29,750	27,633 ± 2,844
Suspended solids (mg/L)	2,033-2,500	2,248 ± 236
Total nitrogen (mg/L)	678-785	724 ± 54
Phosphorus (mg/L)	1.12-1.92	1.5 ± 0.4

granular sludge could eliminate the average organic matter for about 0.28 gCOD.gVSS^{-1}.day^{-1}. These values showed that the sludge contains microorganisms capable of removing organic matter in the system. In addition, these values were used to prepare the inoculum to be suitable for the organic content in the next step of the experiment.

Table 3 Characterization of granular sludge

Property indexes	Average ± S.D.
pH	7.0 ± 0.03
Moisture (% wet basis)	94 ± 0.56
Total solids (% wet basis)	6 ± 0.065
Total volatile solid ((% dry basis)	81 ± 0.27
SMA (gCOD.gVSS^{-1}.day^{-1})	0.28 ± 0.01

3. Performance of the UASB reactors

UASB reactors: reactor 1 was operated with cassava stillage, reactor 2 was operated with cassava stillage with additional nutrient supplement and reactor 3 was operated with cassava stillage with additional chitosan.

3.1 Stability of UASB

The stability of the three UASB systems was determined by measuring Alk, TVA and pH in effluent during 60 days of operation (Fig. 2). It was found that the pH value of reactors 1, 2 and 3 were similar (6.5 ± 0.2, 6.7 ± 0.2 and 6.6 ± 0.3), showing neutral conditions suitable for microorganisms. The Alk of the reactors 1, 2 and 3 were 886 ± 151, 1,086 ± 161 and 929 ± 129 mg/L, respectively, not exceeding the reference value (range between 1,000-3,000 mg/L).

The average TVA of the three reactors also did not exceed the reference value as shown in Table 4 (Udomsinrot, 2000). The TVA/Alk ratio of the three reactors were 0.27 ± 0.02, 0.22 ± 0.05 and 0.31 ± 0.05, indicating a high buffering capability of the system. Normally, with a TVA/Alk ratio greater than 0.8, it would show an accumulation of acid, especially propionic and

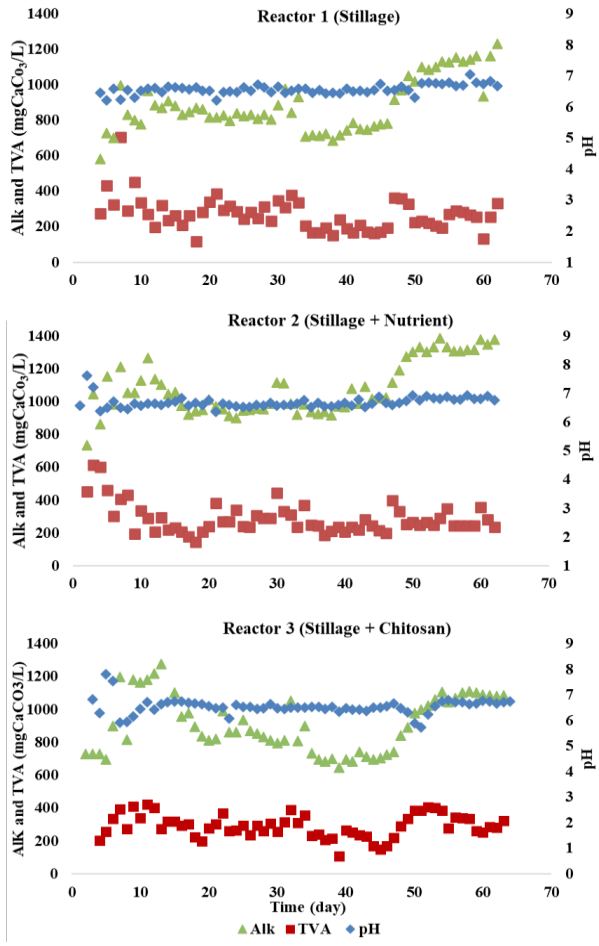

Fig. 2 Alkalinity, total volatile acid, and pH of UASB reactor

butyric, which affects the inhibition of microbial groups in anaerobic treatment (Dogan, et al., 2005). It was indicated in the study of Pooltawee regarding the inhibition of the methanogenic bacteria group that a high propionic acid level resulted in an increased removal of organic matter (Dogan et al., 2005; Pooltawee, 1994).

3.2 COD removal and biogas production

The influent and effluent of all three UASB reactors were determined by COD and SS for evaluating removal efficiency of the UASB system. It was found that the average COD concentration of effluent of the reactors 1, 2 and 3 were 2,382, 1,935 and 1,350 mg/L, respectively. Thus, the COD removal efficiency of the reactor 1, 2 and 3 showed about 79 ± 8.8%, 84 ± 6.4% and 87 ± 6.6%, respectively (Table 4 and Fig. 3). The results of the efficiency of COD removal indicated that adding nutrients to the UASB reactor causes an increase

Table 4 Stability and performance indexes of UASB

Indexes	Reactor 1	Reactor 2	Reactor 3	Reference value (Udomsinrot, 2000)
Stability				
pH	6.5 ± 0.2	6.7 ± 0.2	6.6 ± 0.3	6.6-7.6
TVA/Alk	0.27 ± 0.02	0.22 ± 0.05	0.31 ± 0.05	< 0.4
Alkalinity (mg/L)	886 ± 151.1	1,086 ± 161.4	929 ± 129.5	1,000-3,000
Total volatile solid (mg/L)	271 ± 33.4	288 ± 32.7	291 ± 30.4	< 2,000
Performance				
COD removal (%)	79 ± 8.8	84 ± 6.4	87 ± 6.6	
SS removal (%)	60 ± 7.3	61 ± 10.1	74 ± 10.6	

of the efficiency of COD removal. This may be due to the addition of nutrients that contribute to the growth of microorganisms in the system, resulting in a higher system efficiency in removing organic matter. Furthermore, adding chitosan to the UASB reactor showed a higher COD removal efficiency similar to adding a nutrient supplement to UASB. Chitosan as a coagulant helps to increase the efficiency of sludge coagulation and the flocculation process. Thus, the system UASB had a high efficiency of COD removal and biogas production including SS removal efficiency. Similar to the study of Zou et al. (2021), it was found that chitosan was beneficial in accelerating sludge granulation and reducing sludge loss in UASB systems.

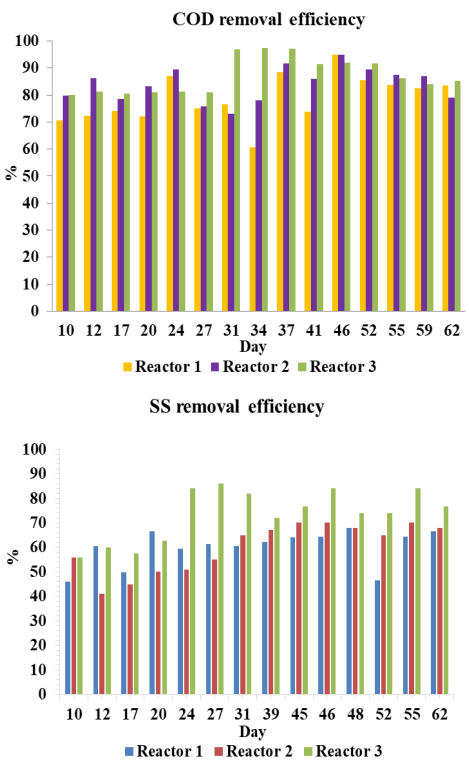

Fig. 3 COD and SS removal efficiency of UASB reactors

Considering the efficiency of SS removal of the three UASB reactors, it was found that to be consistent with the efficiency of removing COD as shown in Fig. 3. The reactor 3 had a higher SS removal efficiency (74 ± 10.6%) than the other reactors (Table 4 and Fig. 3). Chitosan filled in a reactor acted as a coagulant. The granular sludges were formed from binding of a cationic polymer of chitosan and negatively charged cell surfaces of bacteria, which leads to a higher density of sludge retained in the reactor (Khemkhao et al., 2011), as evidenced by an increase of SS removal efficiency (Table 4). Reducing the amount of SS in the system could affect the removal of organic matter, resulting in higher organic removal efficiency and higher biogas production (Fig. 4).

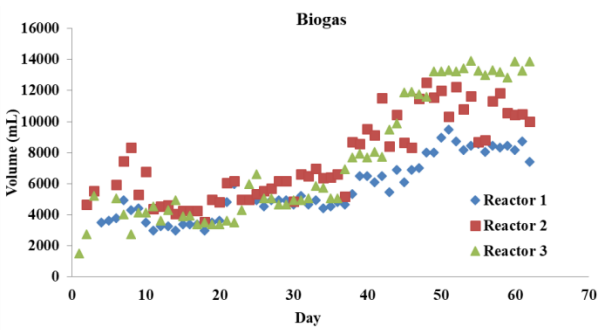

Fig. 4 Biogas production of UASB reactors

Biogas production was determined during 60 days of experimenting as shown in Fig. 4. Reactors 2 and 3 had a high average biogas production which was about 7,565 mL/day and 7,762 mL/day, respectively. While the reactor 1 produced biogas less than the two other reactors: about 5,645 mL/day. Reactor 2, which was filled with nutrient additives, had a ratio of COD:N:P, and was thus suitable for the growth of microorganisms and as a result caused a higher biogas production. Reactor 3, which was filled with chitosan, performed its biogas production at

an average level, which was not different from reactor 2. This was to be expected from chitosan, since it is a flocculant. It can reduce the loss of granular sludge from UASB reactors, which may result in a growing number of bacteria in the system or may reduce the SS out of the system (Zou et al., 2021). It was consistent with the SS removal efficiency in Fig. 3. Consequently, the result of the treatment of COD and the biogas production were higher than from the reactors 1 and 2.

4. Microbial communities

As shown in Fig. 5, a total of 78 phylotypes, which were affiliated with two phyla, Euryarchaeota and Crenachaeota, were detected in archaeal communities, and Euryarchaeota remained predominant throughout the end-up stage. These results were similar to previous findings of the archaeal communities regarding agricultural anaerobic digesters, which were dominated by members of the phylum Euryarchaeota (Langer et al., 2019).

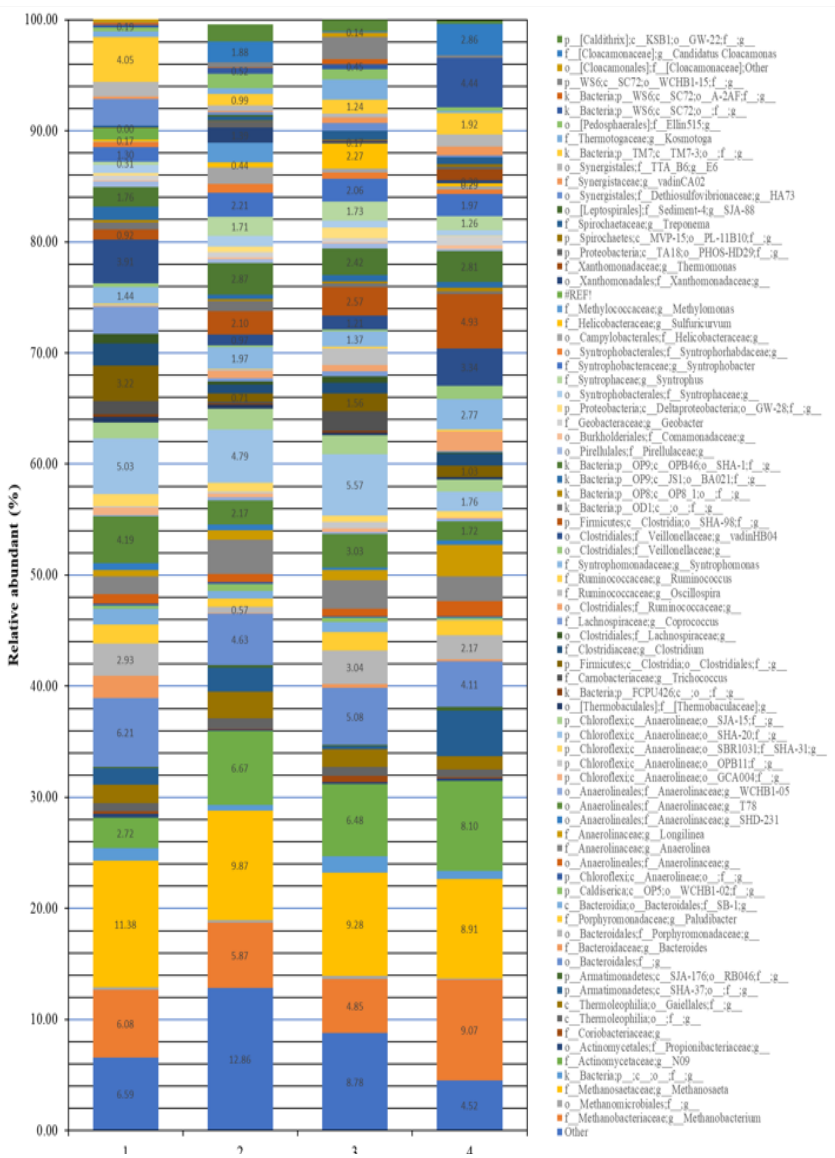

Fig. 5 Correlation of microbial content from 16S rRNA in UASB treatment tanks from factory wastewater. Cassava ethanol: (1) Inoculum sample, (2) reactor 1 with stillage, (3) reactor 2 with stillage and nutrient supplement, (4) reactor 3 with stillage and chitosan

In the methanogen populations, the acetotrophic *Methanosaeta* were highly abundant in inoculum sample (11.38%), in reactor 1 with stillage (9.87%) and reactor 2 with stillage and nutrient supplement (9.28%), while in the reactor 3 with stillage and chitosan it was the hydrogenotrophic *Methanobacterium* (9.07 %). In these findings, the hydrogenotrophic genera *Methanoculleus* and *Methanobacterium* dominated in agricultural anaerobic digesters (Langer et al., 2019). Furthermore, the acetotrophic *Methanosaeta* was the key methanogen in the digestion chamber for biogas production from a pig farm (Pampillón-González et al., 2017) and the sewage sludge from a wastewater treatment plant (Pyzik et al., 2018). The genus *Methanosaeta* acted as an acetotrophic methanogens, converted acetate into CH_4 and CO_2 and led to a higher methane production in the reactors (Pampillón-González et al., 2017). It was reported that genus *Methanoseata* favors low levels of acetic acid (Wu et al., 2016).

The predominant archaeal genus *Methanosaeta* (11.38%) and *Methanobacterium* (6.21%) and bacterial order Bacteroidales (6.21%), class Anaerolineae (5.30%), famies Anaerolinaceae (4.19%) and Veillonellaceae (3.91%) were predominant in the inoculum sample. These results indicate that the inoculum sample was highly methanogenic. Normally, the content of the inoculum sample is important for starting up biogas reactors. However, it does not seem to be the main factor for both the process performance and the overall microbial community, being dependent on an optimal organic material composition and on operating parameters.

In particular, the tendency towards an increase in the genus *Methanobacterium* (9.07%), family Actinomycetaceae (8.10%), class Clostridia (4.93%), kingdom Bacteria (4.44%), family Veillonellaceae (3.34%), kingdom Armatimonadetes (4.12%), genus *Candidatus Cloacamonas* (2.86%) and genus *Syntrophomonas* (2.77%) in reactor 3 with stillage and chitosan was higher than in reactor 1 with stillage and in reactor 2 with stillage and nutrients supplement. The bacterial populations play an important role in anaerobic digesters, such as: methane production (*Methanobacterium*); lactate, acetate and succinate production (family Actinomycetaceae); protein, cellulose, and other polysaccharides degradation (genus *Clostridium* and *Syntrophomonas*); polysaccharide degradation (family Veillonellaceae); pectin and xylan degradation (phylum Armatimonadetes) and the acidogenesis process (*Candidatus Cloacamonas*) (Langer et al., 2019; Pyzik et al., 2018; Wu et al., 2016; Ren et al., 2014; Lee et al., 2014; Esquivel-Elizondo et al., 2017). According to the report of Pachiega et al. (2019) there is a predominance of Veillonellaceae and Streptococcaceae families in UASB reactors treating brewery wastewater for hydrogen production. The Veillonellaceae family can be tolerated in high organic material and can be stabilized in the process of biogas production. The results indicated that in the reactor with chitosan there was a higher rate of archeal and bacterial communities than in the reactor with nutrient supplement and the control reactor. The granular sludges were formed from binding of a cationic polymer of chitosan and the negatively charged cell surfaces of bacteria leading to a higher density of the sludge retaining in the reactor. The chitosan addition showed a higher biogas production rate and decrease washout of biomass and increased granular size (Khemkhao et al., 2011).

Class Anaerolineae (5.57%) Order Bacteroidales (5.08%), family Porphyromonadaceae (3.40%), family Anaerolinaceae (3.03%), genus *Sulfuricurvum* (2.27%) Order Clostridiales (1.56%) and family Veillonellaceae (1.21%) were higher in reactor 2 with stillage and nutrient supplement than in the control reactor. Some bacterial members associated with the acetate production at the end of the start-up stage were Class Anaerolineae (genus *Anaerolinea*), including genera *Ruminococceae, Proteiniphillum, Syntrophomonas,* and *Coprothermobacter* (Wu et al., 2016). These bacterial populations play an important role in carbon compound degradation (order Bacteroidales and family Porphyromonadaceae) (Burns et al., 2012) and H_2S oxidation (genus *Sulfuricurvum*). Previous research has demonstrated that the bacterial families Porphyromonadaceae, Tissierellaceae, and archaeal family Methanobacteriaceae were most abundant in the slaughterhouse industry of pigs and poultry and produced high amounts of biogas/methane (Granada et al., 2018). The order Clostridiales (family Veillonellaceae) is a symbiotic gut bacteria (Pampillón-González et al., 2017) and plays an important role in the polysaccharide-degrading (Pyzik et al., 2018). Pampillón-González et al. (2017) reported that the family Clostridiaceae dominated in a sedimentation pond for biogas production at a pig farm. The microorganisms used ammonia as a nitrogen source for their metabolisms (Nsair et al., 2020). Light metals such as potassium (K), magnesium (Mg), sodium (Na) and aluminum (Al) were added in order to promote the microbial growth and enhancement of the bacterial cell immobilization (Ca).

Ca^{2+} helps genus *Sulfuricurvum* (2.27%) Order Clostridiales to degrade cellulose (Karlsson et al., 2014). Trace metals such as Fe, Ni, Co and Mo play an important role in enhancing the catalytic efficiency of enzymes in the growth of methanogenic bacteria, which leads to an increase in methane production (Feng et al., 2010).

In the comparison of biogas production in reactor 2 with stillage and nutrient supplement and reactor 3 with stillage and chitosan there were no significant differences, but the output was higher compared to the control reactor. However, the reactor with chitosan addition (18.11%) showed a higher growth of methanogen populations of genera *Methanosaeta* and *Methanobacterium* than the reactor with nutrient supplement (14.44%) or the control reactor (15.95%). This corresponds to the efficiency of SS removal. It was found that reactor 3 with chitosan had a higher efficiency than the other ones (Table 4). In connection, chitosan is a biopolymer that can be used to enhance the sludge granulation (Kaseamchochoung et al., 2006) and to decrease biomass washout from the UASB (Khemkhao et al., 2011). The abundance and diversity of the microbial community was relatively high in organic material composition. The several factors affecting the biogas production during the anaerobic digestion were temperature, pH, and organic material composition (Karakashev et al., 2006; Pampillón-González et al., 2017). These results indicate that the sludge granulation from chitosan in the reactor with added chitosan protected the methanogen cells inside an acidogenic layer and decreased washing out of the methanogen cells from the reactor better than other reactors.

Furthermore, the population of the Porphyromonadaceae family and Clostridiales order found in reactor 2 with stillage and nutrient supplement of 3.40% and 1.56%, respectively, showed a higher percentage than the control reactor which accounted for 0.57% and 0.71%, respectively. As a result, the biogas production in reactor 2 with stillage and nutrient supplement showed better performance than the control reactor. According to Su et al. (2018), order Clostridiales has been reported as a hydrogen producer whereas family Porphyromonadaceae not only hydrolyzed complex carbohydrates and proteinaceous compounds, but it also generates volatile fatty acids such as acetic, isobutyric, propionic, and isovaleric acids during the acidogenesis phase. Therefore, it promotes biogas production related to methanogens (Zhang et al., 2017; Granada et al., 2018; Kurade et al., 2020)

Conclusion

Cassava stillage in this experiment showed a high level of organic matter. However, the addition of chitosan and nutrient supplement had improved the efficiency of organic removal and biogas production of the UASB reactor. Nutrient additives in reactor 2 made the ratio of COD:N:P suitable for the growth of microorganisms and resulted in a high biogas production. The chitosan additive in reactor 3 is a substance that flocculates and thereupon reduces the loss of granular sludge from the UASB reactor, which may it result as well in a growing number of bacteria in the system or reduce the SS in the system.

The dominant archaea in the final fermentation of all reactors and in the inoculum, sample were hydrogenotrophic (genus *Methanobacterium*) and acetotrophic methanogens (genus *Methanosaeta*). The acetotrophic *Methanosaeta* and hydrogenotrophic *Methanobacterium* were the key methanogens in the reactor for biogas production from stillage of the cassava ethanol production plant with added nutrients supplement.

Among these methanogenic populations, the populations of genera *Methanosaeta* and *Methanobacterium* in the reactor with chitosan addition (18.11%) showed up in higher amounts than in those reactors with added nutrient supplement (14.44%) and also than in the control reactor (15.95%), resulting in a higher biogas production.

In addition, the results showed that there was a high abundance of other bacterial groups in all three reactors, such as the family of Actinomycetaceae, orders Bacteroidales and Anaerolinea. The functions of these bacterial groups associate with the production of methanogen, lactate, acetate, and succinate, and degrade cellulose, xylan and polysaccharide. Furthermore, other bacterial groups in the reactor with added chitosan were also highly abundant. One hydrolytic group involved polysaccharides, lipids, proteins degradation (class Clostridia, family Veillonellaceae and genera *Clostridium* and *Syntrophomonas*). Another hydrolytic group involved pectin, xylan and cellulose degradation (phylum Armatimonadetes and genus *Clostridium*) and the acidogenesis group (family Actinomycetaceae and genera *Clostridium, Syntrophomonas, Candidatus* and Cloacamonas acidaminovorans) acetogenesis group (class Clostridia, order Clostridiales and genus *Syntrophomonas*).

Chitosan, which was added to reactor 3, is a substance that helps to increase the sludge concentration and the SS removal efficiency (74%), thereby increasing the efficiency of the system. Although reactor 2 with added nutrient supplement was quite effective, it was found that the SS removal efficiency was as low as 61%, which may affect the performance of the system in the future. Moreover, compared to the nutrient supplementation option, the preparation and use of chitosan were less complicated and cheaper. Therefore, chitosan could be used to control microbial growth, to enhance the operating lifetime, the stability and the survival of the microbial cells in the anaerobic digestion process for biogas production from wastewater of cassava ethanol production plants.

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References

Ableling, U., & Seyfried, C.F. (1992). Anaerobic-aerobic treatment of high-strength ammonium wastewater-nitrogen removal via nitrite. *Water Science Technology, 26*(5-6), 1007-1015.

American public health association (APHA). (2012). *Standard methods for the examination of water and wastewater* (21st ed.). Washington, DC: American public health association/American water works association/water environment federation.

Burns, A.S., Pugh, C.W., Segid, Y.T., Behum, P.T., & Lefticariu, L. (2012). Performance and microbial community dynamics of a sulfate-reducing bioreactor treating coal generated acid mine drainage. *Biodegradation, 23,* 415-429.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics, 11,* 265-70.

Dogan, T., Ince, O., Ayman Oz, N., & Kasapgil, B. (2005) Inhibition of volatile fatty acid production in granular sludge from a UASB reactor. *Journal of Environmental Science and Health, Part A 40*(3), 633-644.

Edgas, R.C., Haas, B.J., Clemente, J.C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics, 27*(16), 2194-2200.

Esquivel-Elizondo, S., Ilhan, Z.E., Garcia-Pe, E.I., & Krajmalnik-Browna, R. (2017). Insights into butyrate production in a controlled fermentation system via gene predictions. *Applied Environmental Science, 2*(4), 1-7.

Feng, X.M., Karlssonl, A., Svensson1, B.H., & Bertilsson, S. (2010). Impact of trace element addition on biogas production from food industrial waste-linking process to microbial communities. *FEMS Microbiology Ecology, 74,* 226-240.

Granada, C.E., Hasan, C., Marder, M., Konrad, O., Vargas, L.K., Passaglia, L.M., ... Sperotto, R.A. (2018). Biogas from slaughterhouse wastewater anaerobic digestion is driven by the archaeal family *Methanobacteriaceae* and bacterial families *Porphyromonadaceae* and *Tissierellaceae. Renewable Energy, 118,* 840-846.

Jijai, S., Srisuwan, G., O-thong, S., Ismail, N., & Siripatana, C. (2015). Effect of granule sizes on the performance of up-flow anaerobic sludge blanket (UASB) reactors for cassava wastewater treatment. *Energy Process, 79,* 90-97.

Karakashev, D., Batstone, D.J., Trably, E., & Angelidaki, I. (2006). Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of methanosaetaceae. *Apply Environment Microbiology, 72,* 5138-5141.

Karlsson, A., Björn, A., Yekta, S.S., & Svensson, B.H. (2014). *Improvement of the biogas production process* (Master thesis). Linköping University, Sweden: Biogas research center.

Kaseamchochoung, C., Lertsutthiwong, P., & Phalakornkule, C. (2006). Influence of chitosan characteristics and environmental conditions on flocculation of anaerobic sludge. *Water Environment Research, 78,* 1061-4303.

Khemkhao, M., Nuntakumjorn, B., Techkarnjanaruk, S., & Phalakornkule, C. (2011). Effect of chitosan on UASB treating POME during a transition from mesophilic to thermophilic conditions. *Bioresource Technology, 102*(7), 4674-4681.

Kiran, E.U., Stamatelatou, K., Antonopoulou, G., & Lyberatos, G. (2016). Production of biogas via anaerobic digestion. In *Handbook of Biofuels Production* (pp. 259-301). Cambridge: Woodhead Publishing.

Kobayashi, T., Hu, Y., & Xu, K.Q. (2018). Impact of cationic substances on biofilm formation from sieved fine particles of anaerobic granular sludge at high salinity. *Bioresource Technology, 257,* 69-75.

Kurade, M.B., Saha, S., Kim, J.R., Roh, H.-S., & Jeon, B.H. (2020). Microbial community acclimatization for enhancement in the methane productivity of anaerobic co-digestion of fats, oil, and grease. *Bioresource Technology, 296,* 1222-1294.

Langer, S.G., Gabris, C., Einfalt, D., Wemheuer, B., Kazda, M., & Bengelsdorf, F.R. (2019). Different response of bacteria, archaea and fungi to process parameters in nine full-scale anaerobic digesters. *Microbial Biotechnology, 12,* 1210-1225.

Lee, A.H., & Nikraz, H. (2015). BOD: COD ratio as an indicator for river pollution. international proceedings of chemical, *Biological and Environmental Engineering, 88,* 89-94.

Lee, K. C.-Y., Morgan, X.C., Dunfield, P.F., Tamas, I., McDonald, I.R., & Stott, M.B. (2014). Genomic analysis of *Chthonomonas calidirosea,* the first sequenced isolate of the phylum armatimonadetes. *The ISME Journal, 8,* 1522-1533.

Lertsitthichai, S. (2006). *Effect of chitosan on efficiency of the septic tank system, anaerobic sludge layer process, up-flow type* (Master thesis). Bangkok: King Mongkut's University of Technology North Bangkok.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., & Knight, R. (2011). Unifrac: An effective distance metric for microbial community comparison. *The ISME Journal, 5,* 169-172.

Nsair, A., Cinar, S. O., Alassali, A., Qdais, H.A., & Kuchta, K. (2020). Operational parameters of biogas plants: A review and evaluation study. *Journal of Energy, 13* (3761), 2-27.

Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. (2020). Wastewater to energy: Relating granule size and biogas production of UASB reactors treating municipal wastewater. In *Frontiers in Water-Energy-Nexus—Nature-Based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability* (pp. 317-320). Cham, Switzerland: Springer.

Pachiega, R., Rodrigues, M.F., Rodrigues, C.V., Sakamoto, I.K, Maria Bernadete, A. Varesche, M.B.A., ... Maintinguer, S.I. (2019). Hydrogen bioproduction with anaerobic bacteria consortium from brewery wastewater. *International Journal of Hydrogen Energy, 44,* 155-163.

Pampillón-González, L., Ortiz-Cornejo, N.L., Luna-Guido, M., Dendooven, L., & Navarro-Noya, Y.E. (2017). Archaeal and bacterial community structure in an anaerobic digestion reactor (Lagoon type) used for biogas production at a pig farm. *Journal Microbiol Biotechnology, 27,* 306-317.

Pooltawee, J. (1994). *Effect of volatile organic acids on microorganisms related to Biogas production* (Master thesis). Bangkok: King Mongkut's University of Technology North Bangkok.

Pyzik, A., Ciezkowska, M., Krawczyk, P.S., Sobczak, A., Drewniak, L., Dziembowski, A., & Lipinski, L. (2018). Comparative analysis of deep sequenced methanogenic communities: identification of microorganisms responsible for methane production. *Microbial Cell Factories, 97,* 1-16.

Ren, J., Yuan, X., Li, J., Ma, X., Zhao, Y., & Zhu, W. (2014). Performance and microbial community dynamics in a two-phase anaerobic co-digestion system using cassava dregs and pig manure. *Bioresource Technology, 155,* 342-351.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open-source tool for metagenomics. *Peer Journal, 4,* e2584.

Shannon, C.E. (1984). A mathematical theory of communication. *Bell System Technology Journal, 27,* 623-56.

Smolder, G.J.F., van der Meij, J., Van Loosdrecht, M.C.M., & Heijnen, J.J. (1995). Model of the anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence. *Biotechnology Bioengineering, 43,* 46-470.

Speece, R.E. (1996). *Anaerobic biotechnology for industrial wastewaters.* Archae Press: Nashville, Tennessee.

Su, X., Zhao, W., & Xia, D. (2018). The diversity of hydrogen-producing bacteria and methanogens within an in-situ coal seam. *Biotechnology for Biofuels, 11*(245), 1-18.

Tiwari, M.K. (2005). Enhanced granulation by natural ionic polymer additives in UASB reactor treating low-strength wastewater. *Water Research, 39*(16), 3801-3810.

Torres, K. (2018). Granulation and microbial community dynamics in the chitosan-supplemented anaerobic treatment of wastewater polluted with organic solvents. *Water Research, 10,* 376-387.

Udomsinrot, K. (2000). *Environmental Technology Management* (Master thesis). Bangkok: Rangsit University.

Verbyla, M.E., Oakley, S.M., & Mihelcic, J.R. (2013). Wastewater infrastructure for small cities in an urbanizing world: Integrating protection of human health and the environment with resource recovery and food security. *Environment Science Technology, 47,* 3598–3605.

Wu, B., Wang, X., Deng, Y.Y., He, X.L., Li,Z. W., Li, Q., ... Yin, X.B. (2016). Adaption of microbial community during the start-up stage of a thermophilic anaerobic digester treating food waste. *Bioscience Biotechnology and Biochemistry, 80*(10), 2025-2032.

Xu, H., Liu, Y., Gao, Y., Li, F., Yang, B., Wang, M., ... Sand, W. (2018). Granulation process in an expanded granular sludge blanket (EGSB) reactor for domestic sewage treatment: impact of extracellular polymeric substances compositions and evolution of microbial population. *Bioresource technology, 269,* 153-161.

Zhang, J., Loh, K.-C., Lee, J., Wang, C.-H., Dai, Y., & Tong, Y.W. (2017). Three-stage anaerobic co-digestion of food waste and horse manure. *Scientific Reports, 7,* 1-10.

Zou, J., Yu, F., Pan, J., Pan, B., Wu, S., Qian, M., & Li, J. (2021). Rapid start-up of an aerobic granular sludge system for nitrogen and phosphorus removal through seeding chitosan-based sludge aggregates. *Science of The Total Environment, 762,* 144171.

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Effect of Chilling and Freezing Storage of Cookie Dough on Dry Roasted Pork Cookie Quality

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Abstract

Low temperature is an easy technique which preserves and retains product quality. The objectives of this research were to develop the Trang local roasted pork pieces into cookies and to study the effect of chilling and freezing of cookie dough on cookie quality. The methodology of research was conducted by studying 3 different levels (25%, 50% and 75%) of dry roasted pork suitable to be cookie and study the cookie properties: microbiological and physical quality. The results of this research indicated that addition of dry roasted pork into cookie dough at 50% level received the highest sensory score for all aspects. The 50% dry roast pork cookie dough was studied regarding chilling and freezing storage condition, and determined color, spread ratio and textural quality. The color parameters were determined through lightness (L*), redness (a*) and yellowness (b*) values of both cookie dough models stored under chilling and freezing and the results showed that these values increased with increasing retention time. In terms of cookie color from chilling and freezing cookie dough, it was found that L* and a* decreased but b* was unchanged. The spread ratio for both cookies from chilling and freezing dough decreased, the spread ratio of freezing dough was lower than chilling dough. When cookie's textural quality was investigated, hardness value from both chilling and freezing cookie dough increased after week 2 and increase rate continued until week 6. In addition, the chewiness value increased for both cookies from chilling and freezing dough. The fracturability of cookies from chilling and freezing dough slightly increased after 6-week storage. Crispiness value of both cookies from chilling and freezing dough were stable as early as 2 weeks and continually increased until last storage time at week 6.

Introduction

Trang roasted pork originated in Trang Province in Thailand's southern region. The majority of Trang residents start their day consuming roasted pork and coffee. Trang roasted pork is now a GI product, which refers to a product that is associated with a specific geographic place. As a result, it is a souvenir item for

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tourists. The cutting process causes some red meat to fall off into little pieces, which the customers do not want because it's small and hard.

A cookie is a popular snack that may be enjoyed by people of all ages and genders. It is easy to eat, usually served with a soft drink or other beverage, and it's a cheap product with a long shelf life (Olaoye et al., 2007). The major ingredients in cookie dough are wheat flour, oil, and sugar. It can be adapted in a variety of flavors, and minor additives such as nuts, dry fruit, color, and flavor can be mixed in as desired., The final step in preparing cookies is to heat the cookie dough in an oven.

Chilling and freezing are common conditions in the food sector, and they have an impact on product quality. Both ways can keep a product fresh and easily available in every home. Several studies have been performed on the effect of chilling and freezing of batter. Gupta et al. (2011) studied frozen method storage of cookie dough and it was found that cookies prepared after freezing of dough were crisper than the normal. Ávalos et al. (2016) studied the effect of refrigeration and freezing of batter in gluten free baking product and the results showed that the properties did not change when the batter was frozen.

This research was initiated to develop roasted pork pieces left over from trimming process to make cookies because roast pork tends to be compatible, easy to cook and has a long shelf life. The main objectives of this research were to develop cookie products from dry roasted pork and to study the effect of the storage condition of cookie dough comparing chilling and freezing on cookie quality. The findings offer alternatives to produce freshness of cookies from ready to bake dough, the storage of dough and contributes to the knowledge of the effects of refrigeration and freezing of dough in cookie products.

Materials and methods

1. Raw materials preparation

All the raw materials were procured from local supermarket. All purpose flour (Kite, United flour mill Ltd., Thailand), tapioca flour (Baiyok, Bangkok Inter Food co.Ltd., Thailand), sugar (Lind, Thai Roong Ruang sugar group., Thailand), butter (Orchid. Ampol Food Processing Ltd. Thailand), salt (Prung thip, Krua Pimay, Thailand) and soybean oil (Augun, Thai Vegetable Oil Public Co. Ltd., Thailand) were used in the formulation.

2. Cookie dough preparation

The dough was prepared using a kitchen aid mixer (5K5SS Model, USA.) with paddle attachment. Initially, all dry ingredients were mixed in a mixing bowl (all purpose flour, tapioca flour and salt) and the dry ingredients were sifted to aerate them and remove any possible lumps. Butter was beaten for 3 mins until well distributed and then sugar was added gradually. When the butter was mixed well with sugar, dry ingredients were added into the mixing bowl and beaten into smooth dough. The next step was to gradually add soybean oil and again mix well. The cookie dough was shaped with a cookie press with a thickness of 0.5 cm and 4.0 cm diameter. The cookie dough was transferred to baking sheet that was placed on the baking tray. Cookie dough temperature was controlled at 25°C before baking in the Zanolli oven (Teorema polis, Itali). The cookie dough was heated for 16 mins at 170°C.

Ground roast pork preparation started from selecting fresh legs pork that was cleaned with water and rinsed then set aside on sieve to drain. Pork was cut to size 2x3x1 cm pieces. The pork was then mixed with spice and herb and placed at room temperature for 2 hours to absorb the meat with the seasoning. Then Convotherm combination oven (OES 6.10 Model, Germany) was used to roast the pork at 140°C for 20 mins and then cooled at room temperature. The researcher grinded the pork mixture and spead on tray using Bos mall hot air oven (FF-68, Thailand) at 60°C for 12 hrs. Water activity (a_w) was 0.8. The result is shown in Fig.1.

Fig. 1 Dry roasted pork

The cookie formulation and cookie method were as follows according to Kun Mae Manee (2018), basic formulation of cookie was standardized after conducting

preliminary trials. There were 3 formulas used for cookie and sensory test conducted with 50 untrained panelist and scored by a 9 hedonic scale (1 = dislike very much and 9 = like very much). The formula that received the highest score was selected for further study of development of dry roasted pork cookie.

Table 1 Cookie dough formula

Ingredients	Percent (%)
All purpose flour	38
Tapioca flour	13
Sugar	18
Butter	28
salt	1
Soybean oil	2

Remark: Modified from Kun Mae Manee (2018)

2. Cookie formula selection

The researcher studied the maximum dry roasted pork quantity to cookies dough with three different levels at 25%, 50% and 75%, respectively. Dry roasted pork cookie formulas were selected by sensory evaluation. The sensory evaluation was conducted with 100 sensory panelists using a 9-point hedonic scale (1 = dislike very much and 9 = like very much) (Rawendra & Dwi, 2020). The sensory test was conducted to evaluate 5 aspects comprising of color, odor, flavor, texture and overall acceptability. All samples were contained in transparent plastic bag served at room temperature in the same time. The participants were assessed individually, drinking water at room temperature used to clean the palate during sensory test action. The data were analyzed statistically for determining the cookie formula with the highest acceptability score.

3. Cookie dough storage condition

The cookie formula with the highest score was then used to study the effects of the chilled and frozen dough storage process. Cookie dough were shaped and kept individually into plastic bags, stored in plastic containers and chilled at 8°C (System form : STF-40C, Thailand) and frozen at -18°C (System form : STF-20F, Thailand) for 6 weeks. Every week, stored cookies dough was removed from the plastic container and thawed until it reached 25°C. Fresh cookie dough from refrigeration and freezer were baked in oven at 170°C for 16 mins.

4. Quality of cookie

4.1 Microbiological quality

All storage time, microbiological analysis of chilled and frozen cookie dough were *Salmonella* spp. and *Staphylococcus aureus* by FDA, BAM (Maturin & Peeler, 2001)

4.2 Physical quality

4.2.1 Color quality was measured using Hunter Lab Color Flex (Model A60-1012-312, Hunter Associates Laboratory Inc., USA). The measurement parameters were shown as L(lightness), a*(redness) and b* (yellowness). Every week, cookie dough and baked cookie were color measurement. It was tested with 3 replicates and each replicate used 5 samples.

4.2.2 Spread ratio The spread ratio was calculated from the ratio of spread to thickness (W/T). To determine the diameter, five cookies were measured with a diameter of 0.01 mm precision vernier caliper after cooling and to determine the thickness according to Agu et al. (2007). The average of the two measurements was divided by fives and taken as diameter and thickness then calculated to spread factor. For each test, five cookies were used and tested with 2 replicates.

4.2.3 The texture was determined by texture analyzer (Model: TA-XTPlus), using blade knife probe (HDP/BS). The device setting followed Hwang et al. (2016) under the conditions of pre-test speed at 1 mm/s, test speed at 3 mm/s, post-test speed at 10 mm/s, and distance of 15 mm. The tests were conducted to determine the hardness, chewiness, fracturability and crispiness. Tests were performed with 3 replicates, each replicate used 5 samples.

5. Statical analysis

The statical analysis was conducted using SPSS software (SPSS Version 17; SPSS Inc., Chicago, USA). Data were analyzed by ANOVA and Duncan's New Multiple Range Test for identifying the different properties of cookie dough and cookie at the 95% confidence level ($P<0.05$).

Results and discussion

1. Cookie formula selection

The result of the selection of dry roasted pork cookie by testing the preference of all levels of dry roasted pork is shown on Table 2. Sensory attributes including color, odor, flavor, texture and overall acceptance of dry roasted pork cookie were determined. A 9-point hedonic scale was used as standard procedure to assess product preference. The scoring from 100 untrained individuals were shown in Table 2 above. In this study, there were no significant ($p > 0.05$) differences in the perception of the samples in terms of flavor and overall acceptance in all formulas. The appearance of roasted pork cookie at 25% and 75% had no significant differences but found

Table 2 Sensory test of dry roasted pork cookie in difference level of dry roasted pork

Characteristics	Dry roasted pork (%)		
	25%	50%	75%
Appearance	7.52 ± 0.95[b]	7.94 ± 0.88[a]	7.57 ± 1.23[b]
Color	7.61 ± 0.87[b]	7.95 ± 0.97[a]	7.71 ± 0.92[ab]
Odour	7.56 ± 1.24[b]	8.00 ± 1.19[a]	7.67 ± 1.61[ab]
Flavor[ns]	7.76 ± 1.19	7.99 ± 1.27	7.59 ± 1.67
Texture	7.90 ± 1.25[a]	7.71 ± 1.31[ab]	7.48 ± 1.38[b]
Overall acceptance[ns]	7.92 ± 0.87	7.99 ± 1.15	7.78 ± 1.32

Remark: a, b, c superscripts with different letters in the same row are different ($p < 0.05$), all values are shown as mean ± S.D.
ns refers to no statistically significant differences ($p \geq 0.05$)

significant difference in case of 50% level. The color values for 25% 50% and 75% levels were 7.61, 7.95 and 7.71, respectively. For the dry roasted pork cookie at 25% and 50%, there were significant differences ($p < 0.05$) but for 75% formula, there were no significant difference with 25% and 75%. The ground roasted pork in cookie dough was natural dark brown, so the 75% cookie sample was darker than other formula. The result of color sensory was agreement with Kumar et al. (2016), they studied the quality attributes and storage stability of chicken meat biscuit and reported that the very dark color may not be liked by the panelist. The similar results were found for odor and color and the sensory scores were 7.56,8.00 and 7.67. respectively. The roasted pork cookie at 25% and 50% had significant differences ($p < 0.05$) but the 75% formula had no significant difference with 25% and 75%. The texture score had 7.90, 7.71 and 7.48, respectively. The roasted pork cookie at 25% and 75% had significant differences ($p < 0.05$) but 50% formula had no significant difference with 25% and 75%. All aspects of roasted pork cookie at 50% had the highest scoring except for texture. The texture of roasted pork cookie at 25% which contained the less grounded roasted pork had the highest score because it was crispier. The dry roasted pork cookie at 50% needed continued study

Fig. 2 Dry roasted pork chilling cookie dough (left) and dry roasted pork freezing cookie dough (right)

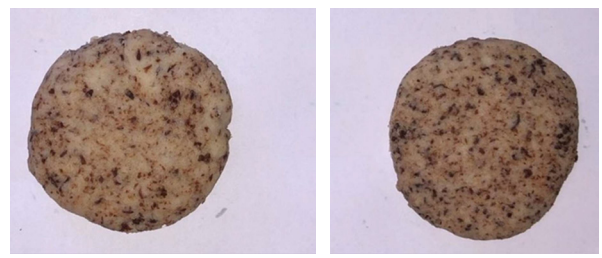

Fig. 3 Dry roasted pork cookie from chilling cookie dough (left) and dry roasted pork cookie from freezing cookie dough (right)

regarding the effect of the storage condition of cookie dough.

The dry roasted pork cookie dough at 50% formula was selected to chilling and freezing storage for 6 weeks of study. The cookie dough under chilling condition at 8°C and freezing condition at -18°C are shown in Fig. 2 and Fig. 3.

2. Microbiological quality

All storage time, the chilled and frozen cookie dough were determined for microbiological test, it found that microbiological value was in accordance with the Notification of the Ministry of Public Health No.364 B.E.2556 (Thailand Public Health, 2013) regarding food standards for pathogenic microorganisms (ready to cook food). It was determined that no *Salmonella* spp. per 25 g was found and no *Staphylococcus aureus* per 100 g of dough was found.

Table 3 Effect of chilling and freezing storage condition on cookie dough's color

Week	Chilling			Freezing		
	L	a*[ns]	b*	L	a*	b*
0	33.11 ± 0.31[a]	6.49 ± 0.21	9.82 ± 0.31[a]	33.11± 0.22[a]	6.49 ± 0.18[a]	9.82 ± 0.15[a]
1	32.90 ± 0.43[a]	6.55 ± 0.39	12.52 ± 0.19[b]	36.48 ± 0.37[b]	7.49 ± 0.24[b]	8.88 ± 0.10[b]
2	31.77 ± 0.20[b]	6.62 ± 0.24	13.03 ± 0.42[c]	34.68± 0.20[c]	7.69 ± 0.14[abc]	10.74 ± 0.26[b]
3	30.96 ± 0.11[c]	6.51 ± 0.34	14.16 ± 0.35[d]	33.37 ± 0.21[a]	7.90 ± 0.11[c]	10.97 ± 0.17[b]
4	29.86 ± 0.50[d]	6.56 ± 0.32	15.49 ± 0.32[e]	31.54 ± 0.33[d]	8.77 ± 0.17[d]	12.50 ± 0.27[c]
5	28.75 ± 0.29[e]	6.83 ± 0.29	16.39 ± 0.26[f]	30.48± 0.24[e]	8.96 ± 0.19[d]	14.59 ± 0.25[d]
6	27.76 ± 0.33[f]	6.88 ± 0.61	16.42 ± 0.29[f]	28.59 ± 0.21[f]	9.20 ± 0.19[e]	18.60 ± 0.39[e]

Remark: a, b, c, d, e, f superscripts with different letters in the same column are different ($p < 0.05$), all values are shown as mean ± S.D. for three samples. ns refers to no statistically significant differences ($p \geq 0.05$)

3. Physical quality

Physical qualities were determined important to the cookie property, namely the color of cookie dough, cookie color, spread ratio and texture.

When the color of cookie dough with different condition chilling and freezing was investigated, the color of the cookie dough was yellow due to the nature of the butter which was present in recipe. The color values are presented in Table 3, lightness (L*) redness (a*) and yellowness (b*) of chilling cookie dough increased. The same result was found for cookie dough color of freezing storage as lightness (L*) redness (a*) and yellowness (b*) increased with increasing retention time. This result was similar to the result of Ávalos et al. (2016) who reported the refrigeration or freezing of batter turned darker mainly the baked products containing dairy product, probably due to the Maillard reactions between proteins and reducing sugar. According to Patrignani et al. (2014) suggestion that lipids can suffer oxidation during storage, and the products of lipid oxidation, as ketones, can react with proteins and produce Maillard compounds. This effect was observed previously in biscuits. Moreover, Zamora & Hidalgo (2005) reported that free radicals produced during lipid oxidation could react with biscuit proteins modifying the product color. On the other hand, Dogan (2006) reported that when cookie batter (sugar snap, chocolate chip and hazelnut cookie) was stored at 4°C for 6 weeks and at −18°C for 6 months, no significant changes were observed in the physical characteristics of batter.

In Table 4 above, the results of cookie color from differences in storage condition are shown. The cookie color from cookie dough storage under chilling, lightness (L*) was constant for the first 2 weeks and gradually changed to darker until the last week of storage, redness (a*) value for the first 5 weeks were significantly different at ($P<0.05$) with week 6, and while yellowness (b*) value were not significantly different ($P\geq0.05$). The color of the cookie from freezing dough found that lightness (L*) redness (a*) decrease trend and yellowness (b*) were not significantly different ($P \geq 0.05$).

These results were consistent with Chevallier et al. (2000) that reported the color effect of cookie had many factors, in this study the appearance of main cookie was brown. Cookie's color may be influenced from Millard reactions, it occurs between reducing sugar with amino acids and caramelization of sugar. Purlis (2011) reported that browning of cookie was important quality parameter. Most bakery product's color depends both on the physicochemical characteristics of the raw dough, such as water content, pH, reducing sugars, and amino acid content. In addition, the operating conditions during processing, mainly the final baking have an effect on cookie's color.

Table 4 Effect of chilling and freezing storage condition on cookie color

Week	Chilling			Freezing		
	L	a*	b* ns	L	a*	b* ns
0	65.00a ± 0.49^{a}	9.39a± 0.80^{a}	13.23 ± 0.65	65.00^{a} ± 0.49^{a}	9.39^{a}± 0.80^{ab}	13.23 ± 0.65
1	64.98 ± 0.28^{ab}	9.24 ± 0.59^{a}	14.16 ± 0.81	64.24 ± 0.41^{b}	9.38 ± 0.69^{ab}	13.80 ± 0.48
2	64.24 ± 0.29^{b}	9.28 ± 0.69^{a}	14.00 ± 0.76	62.36 ± 0.37^{c}	9.42 ± 0.72^{ab}	14.23 ± 0.82
3	63.36± 0.61^{bc}	9.22 ± 0.60^{a}	14.23 ± 0.46	60.04 ± 0.64^{d}	9.44 ± 0.50^{b}	14.22 ± 0.51
4	62.30 ± 0.71^{c}	9.27 ± 0.65^{a}	14.41 ± 0.59	59.79 ± 0.97^{d}	8.58 ± 0.57^{a}	14.10 ± 0.46
5	60.28 ± 0.44^{d}	9.35 ± 0.72^{a}	14.71 ± 0.67	58.82 ± 0.74^{d}	8.56 ± 0.86^{a}	14.09 ± 0.58
6	59.34 ± 0.74^{d}	8.85 ± 0.62^{b}	14.30 ± 0.67	57.06 ± 0.89^{e}	8.61 ± 0.49^{a}	14.11 ± 0.86

Remark: a, b, c, d superscripts with different letters in the same column are different ($p < 0.05$), all values are shown as mean ± S.D. for three samples. ns refers to no statistically significant differences ($p \geq 0.05$)

Table 5 Effect of chilling and freezing storage condition on spread ratio

Week	Chilling			Freezing		
	Width (cm.)	Thickness (cm.)	Spread ratio (W/T)	Width (cm.)	Thickness (cm.)	Spread ratio (W/T)
0	4.29 ± 0.03^{c}	0.46 ± 0.02^{a}	9.34 ± 0.11^{e}	4.29 ± 0.03^{b}	0.46 ± 0.02^{a}	9.34 ± 0.11^{f}
1	4.40 ± 0.06^{d}	0.50 ± 0.01^{bc}	8.74 ± 0.15^{d}	4.01±0.10^{a}	0.50 ± 0.01^{b}	8.26 ± 0.27^{d}
2	4.28 ± 0.08^{bc}	0.50 ± 0.01^{b}	8.57 ± 0.13^{cd}	4.13 ± 0.10^{ab}	0.53 ± 0.02^{c}	7.75 ± 0.49^{d}
3	4.42 ± 0.02^{d}	0.53 ± 0.01^{c}	8.38 ± 0.22^{bc}	4.27 ± 0.11^{b}	0.61 ± 0.01^{d}	7.02 ± 0.10^{c}
4	4.21 ± 0.04^{abc}	0.52 ± 0.02^{bc}	8.08 ± 0.39^{b}	4.15 ± 0.08^{ab}	0.61 ± 0.01^{d}	6.84 ± 0.12^{bc}
5	4.13 ± 0.08^{a}	0.62 ± 0.02^{d}	6.71 ± 0.14^{a}	4.08 ± 0.08^{a}	0.61 ± 0.01^{d}	6.65 ± 0.22^{b}
6	4.17 ± 0.10^{ab}	0.65 ± 0.03^{e}	6.45 ± 0.29^{a}	4.19 ± 0.07^{ab}	0.65 ± 0.02^{e}	6.16 ± 0.14^{a}

Remark: a, b, c, d superscripts with different letters in the same column are different ($p < 0.05$), all values are shown as mean ± S.D. for five samples

Spread ratio has the measure of the quality index of cookies using the cookie width to cookie thickness ratio. The results of chilling and freezing storage condition had an effect on cookie quality. In Table 5 it is shown that cookies from cookie dough that were treated under chilling found that the spread ratio value in first week was highest and decreased until week 6. Cookies from freezing cookie dough decreased, spread ratio value of first 2 weeks were not significantly different at ($P \geq 0.05$) and slightly decreased from week 1 to the end of storage time in week 6.

A comparative study was conducted for the spread ratio of cookies, between chilling dough and freezing dough and it was found that the spread ratio value for all weeks of cookies from cookie dough freezing higher than cookies from cookie dough chilling. The results were consistent with Hamed et al. (2015) indicating that cookie had a significant decrease in spread factor after the first week of frozen storage followed by an insignificant reduction until the end of frozen storage time (4 weeks). Gupta et al. (2011) also reported that the thickness of the freeze-thaw cookies was higher than normal; one and diameter was almost same as that of normal ultimately spread ratio became decreased. In addition, other researchers reported that the incorporation of air was highly important in cookie development and therefore cookie spread ratio may be affected by the type of fat used in the formulation (Rogers, 2004). Sikorski (2004) gave the reason that it was probably due to the ability to retain more water during baking that increased gluten development. The enhanced gluten development leads to the decrease of the cookie spread ratio. In addition, Manaf et al. (2019) suggested that the influence of plant and animal-based fats on cookie properties was an important factor for an increase in length and decrease in weight and thickness of cookies.

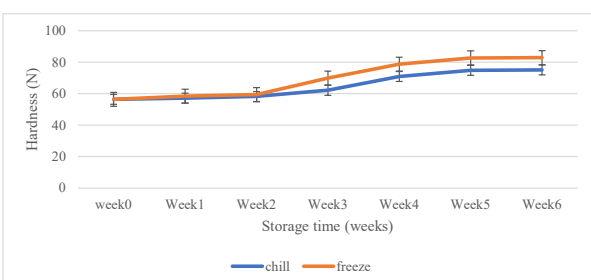

Fig. 4 Influence of chilling and freezing cooking dough storage on cookie hardness

The texture quality was important parameter of cookies, Fig. 4. shows the hardness that is summarized and values are plotted to a graph. The result obtained showed that different condition of cooking dough storage changed the hardness properties of cookies. It is evident from Fig.4 that the cookie dough from refrigerator and freezer after baking tend to have an increase hardness. The highest hardness value was obtained from freezing cookie dough. Among all weeks, cookies from cookie dough both chilling and freezing yielded the highest value in the end of storage at week 6.This was in agreement with Leray et al. (2010) who reported that a progressive increase of the hardness was observed during the aging, the changes occurred during frozen storage leads to an increase hardness of bread. Hamed et al. (2015) suggested that the type of flour, batter recipe and baking condition influenced cookie texture quality. The results were in accordance with O'Brien et al. (2003) who reported that fat was the ingredient responsible for tenderness of biscuits and it keeps the quality. Moreover, Hoseney & Rogers (1994) reported that hardness of the cookies is caused by the interaction of proteins and starch by hydrogen bonding.

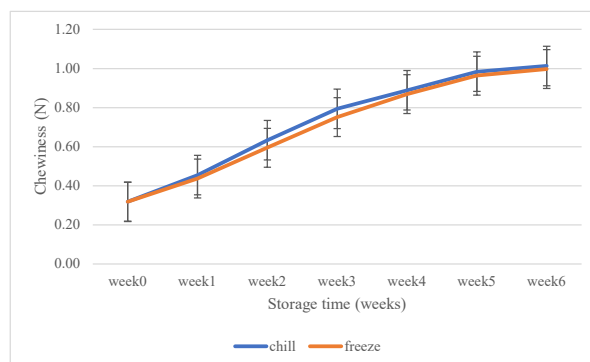

Fig. 5 Influence of chilling and freezing cooking dough storage on cookie chewiness

The chewiness was one property of cookie characteristic, commonly the chewiness is measured from resistance of chewing in the mouth. The results shows that chewiness of cookies from chilling dough and freezing dough were similar value in week 1 until week 6. It slightly increased in value range 0.45-1.01 N in cookies from chilling dough and range 0.44 – 1.00 N from freezing dough. Olewnik & Kulp (1984) reported that fat and water in the system were effective of cookie dough physical properties, If the fat distribution in the cookie system was poor, flour particles will

remain accessible to water. The result effects in the development of gluten proteins. So, the cookie chewiness was present.

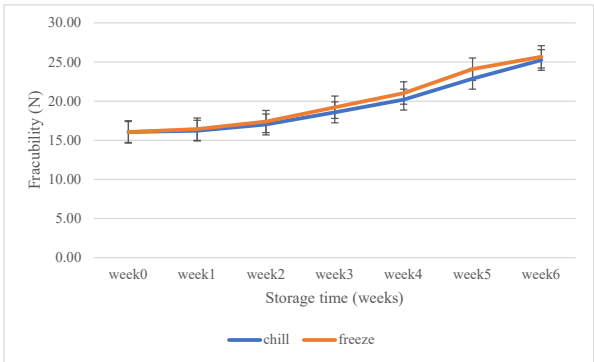

Fig. 6 Influence of chilling and freezing cooking dough storage on cookie fracturability

Fig. 6 shows the values obtained from cookies from cookie dough storage for 6 weeks. Each week and each condition contained a total of 15 samples. The results showed that fracturability of cookies from chilling and freezing dough increased, indicating that these products were more brittle when batter was treated by chilling and freezing before baking. This study provided the same results with Gupta et al. (2011) who reported that cracking was another critical factor, especially in cookies. The cracking pattern became another important physical property and did not significantly change with storage time.

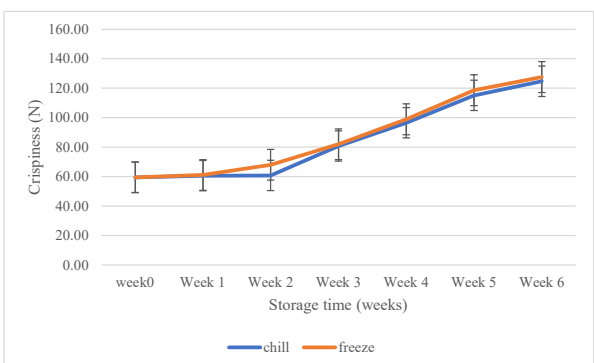

Fig. 7 Influence of chilling and freezing cooking dough storage on cookie crispiness

The results showed that crispiness of cookies from chilling and freezing dough increased. After week 2, the crispiness value of both cookies from chilling and freezing dough were high more than crispiness value at week 0 which were cookies from fresh cookie dough. These results were consistent with the findings of Gupta et al. (2011) who reported that cookies produced from batter containing barley flour and underwent freeze–thaw cycles every 4 h for 24 h were crisper than the freshly baked ones. Wheat flour (control) dough undergo increase cohesiveness and decrease adhesiveness. That also gives more crispiness to the baked cookies This indicates that frozen storage of batter produces cookies with lower breaking force, which was confirmed in the current study. It was also observed that the longer the frozen storage period of batter, the lower the breaking force of the produced cookie became. This could be attributed to the ice crystals formed during frozen storage, which weakens the hydrophobic bonds and redistributes water in the batter resulting in a physical damage to its structure (Rasanen et al., 1997).

Conclusion

The findings of this study showed that both chilling and freezing storage conditions influenced the cookie quality. In terms of cookie dough color and cookie color, L* and a* decreased and b* increased for both chilling and freezing conditions. Chilling and freezing therefore influenced cookie spread ratio. The spread ratio of cookies from freezing dough was lower than cookies from chilling dough. In sum, increasing storage time of cookie dough was related to increases in hardness, chewiness, fracturability and crispiness value.

References

Agu, H.O., Ayo, J.A., Paul, A.M., & Folorunsho, F. (2007). Quality characteristics of biscuits made from wheat and African breadfruit (*Treculia africana*). Niger. *Food J., 25*(2),19-27.

Ávalos, A., Goytiño, P., Conforti, P.A., & Lupano, C.E. (2016). Sensory and texture properties of "chipá": Influence of ingredients and storage conditions of batter. *Cogent Food & Agriculture, 2*(1), 1275394.

Chevallier, S., Colonna, P., Buleon, A., & Della Valle, G. (2000). Physicochemical behaviors of sugars, lipids, and gluten in short dough and biscuit. *Journal of Agricultural and Food Chemistry, 48*(4), 1322-1326.

Dogan, S.I. (2006). Effect of oven types on the characteristics of biscuits made from refrigerated and frozen doughs. *Food Technol. Biotechnol., 44,* 117–122.

Gupta, M., Bawa, A.S., & Abu-Ghannam, N. (2011). Effect of barley flour and freeze–thaw cycles on textural nutritional and functional properties of cookies. *Food and Bioproducts Processing, 89*(4), 520-527.

Hamed, A., Ragaee, S., Marcone, M., & Abdel, Aal, E.S.M. (2015). Quality of bread and cookie baked from frozen dough and batter containing β-glucan-rich barley flour fraction. *Journal of Food Quality, 38*(5), 316-327.

Hoseney, R.C., & Rogers, D.E., (1994). *Mechanism of sugar functionality in cookies: The science of cookie and cracker production.* American Association of Cereal Chemists: St. Paul, MN.

Hwang, H. S., Singh, M., & Lee, S. (2016). Properties of cookies made with natural wax–vegetable oil organogels. *Journal of food science, 81*(5), C1045-C1054.

Kumar, P., Chatli, M.K., Mehta, N., Malav, O.P., Verma, A.K., & Kumar, D. (2016). Quality attributes and storage stability of chicken meat biscuits incorporated with wheat and oat bran. *Journal of Food Quality, 39*(6), 649-657.

Kun Mae Manee. (2018). *Salted dry shrimp (Wang Klai Kangwon recipe)*. Retrieved January 5, 2020, from https://cooking.kapook.com/view199585.html

Leray, G., Oliete, B., Mezaize, S., Chevallier, S., & de Lamballerie, M. (2010). Effects of freezing and frozen storage conditions on the rheological properties of different formulations of non-yeasted wheat and gluten-free bread dough. *Journal of Food Engineering, 100*(1), 70-76.

Manaf, Y.N., Marikkar, J.M.N., Mustafa, S., Van Bockstaele, F. & Nusantoro, B.P. (2019). Effect of three plant-based shortenings and lard on cookie dough properties and cookies quality. *International Food Research Journal. 26*(6), 1795-1802.

Maturin, L.J., & Peeler, J.T. (2001). *Chapter 3, aerobic plate count.* Retrieved October 19, 2020, from https://www.fda.gov/food /laboratory-methods-food/bam-chapter-3-aerobic-plate-count

O'Brien C.M., Chapman D., Neville D.P., Keogh M.K., & Arendt E.K. (2003). Effect of varying the microencapsulation process on the functionality of hydrogenated vegetable fat in short dough biscuits. *Food Res Int., 36,* 215–221.

Olaoye, O.A., Onilude, A.A., & Oladoye, C.O. (2007). Bread fruit flour in biscuit making: Effect on product quality. *African Journal of Food Science, 71,* 41-16.

Olewnik, M.C., & Kulp, K. (1984). The effect of mixing time and ingredient variation on farinograms of cookie doughs. *Cereal Chemistry, 61,* 532-537.

Patrignani, M., Conforti, P.A., & Lupano, C.E. (2014). The role of lipid oxidation on biscuit texture during storage. *International Journal of Food Science and Technology, 49,* 1925–1931.

Purlis, E. (2011). Bread baking: technological considerations based on process modelling and simulation. *J. Food Eng., 103,* 92–102.

Rasanen, J., Blanshard, J.M.V., Siitari-Kauppi, M., & Autio, K. (1997). Water distribution in frozen lean wheat flour doughs. *Cereal Chem., 74,* 806–813.

Rawendra, R.D., & Dwi, G.N. (2020). Enrichment of soft ice cream with different fibrous fruit puree: Physicochemical, textural characteristics and sensory properties. In *IOP Conference Series: Earth and Environmental Science* (p. 012178). Indonesia: IOP Publishing.

Rogers, D. (2004). Functions of fats and oils in bakery products. *Journal of the American Oil Chemists' Society, 15*(9) 572–574.

Sikorski, D. (2004). Application of diacylglycerol oil in baked goods, nutritional beverages/bars, sauces, and gravies. *Diacylglycerol Oil,* 223-252.

Thailand Public Health. (2013). Notification of the Ministry of Public Health No.364 B.E.2556: Royal Thai Government Gazette 130. special issue 148.

Zamora, R., & Hidalgo, F.J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Critical Reviews in Food Science and Nutrition, 45,* 49–59.

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Evaluation of Extraction Methods of Dietary Fiber from Pomelo Juice Byproducts and Particle Size Distribution on the Physicochemical and Functional Properties

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Abstract

Pomelo (*Citrus grandis* (L.) Osbeck) is the largest citrus fruit in Thailand, which pomelo juice is commercially produced during the pomelo juice extraction process. After extraction of the juice, the number of by-products that was produced could be a potential source of functional dietary fiber.The objective of this study was to evaluate extraction methods of dietary fiber from pomelo juice byproducts for functional foods and particle size distribution (150, 180, 250 and 425 μm) by pomelo pulp powder (PPP) that was prepared by simply air drying, milling, sieving and grinding. For pomelo pulp dietary fiber powder (PPDFP), the PPP was treated with NaOH and ethanol to remove protein and fat, respectively. After that, analysis of physicochemical and functional properties of PPP and PPDFP found that the PPDFP was richer in total dietary fiber (92.04%), especially insoluble dietary fiber (91.93%). Water holding capacity (WHC) and oil holding capacity (OHC) of PPDFP were higher than those of PPP. Components that can contribute to the bitter flavor of the powders, limonin, naringin and naringenin were considerably reduced in PPDFP, particularly limonin was totally eliminated. Also, the porous structure of PPDFP may facilitate its use in food processing compared to the more sheet-like structure of PPP. The optimum particle size was 150 μm that resulted in powders with increased WHC and OHC. From the above data, it was concluded that the PPDFP was a good source for food dietary fibers that could be used as a functional ingredient in fiber rich food products.

Introduction

The citrus juice industry, for example pomelo, extraction rate for pomelo (*Citrus grandis* (L.) Osbeck) was reported to be only 38.7% juice, 8.3% flavedo, 26.5% albedo, 25.2% pulp and 1.4% seeds (Pichaiyongvongdee & Haruenkit 2009). In Thailand, and some other countries, most of these byproducts are wasted or

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underutilized, but have proven to contain useful compounds including dietary fibers (Pichaiyongvongdee & Rattanapun, 2015). Several studies have demonstrated the physical chemical and functional properties of dietary fiber extracted from residues of the citrus juice industry including orange, lime and lemon albedo, indicating their levels of insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) (Tainara de et al., 2013; Pichaiyongvongdee & Rattanapun, 2015), but not the extraction of dietary fiber from pomelo fruit pulp. In Thailand, the recommended acceptable intake of dietary fiber is 25g/ day for adults (Ministry of public health, 2020). The importance of fiber in the human diet was reviewed by Jinjin et al., 2015) who showed that adequate levels could contribute to the prevention of chronic diseases such as colon cancer, coronary heart disease, hypertension, obesity, diabetes and asthma. Also, an important functional property of dietary fiber is the prevention of loss of water and oil in processed foods and dietary fiber has been used in fruit jellies to reduce water loss (Lilian & Diego, 2019). Dietary fiber from wheat bran was reported to be added to beef burgers to reduce levels of cholesterol and improve their cooking yield, diameter and texture (Mansour & Khalil, 1999). Dietary fiber from apple pomace was used as a partial substituted for wheat flour in cakes and it was found that the volume of the cakes decreased, and their density and hardness increased (Sudha et al., 2007).

Generally, the remaining material from the pomelo, after extraction of juice, is used as animal feed or fertilizer or wasted and can be a source of environmental pollution if disposed of incorrectly. The objective of this paper were to evaluate extraction methods of dietary fiber from pomelo juice byproducts and particle size distribution on the physicochemical and functional properties.

Materials and methods

1. Samples and chemicals

The cultivars pomelo waste pulp (*Citrus grandis* (L.) Osbeck) were Kao Nampheung (KNP), which was collected from a fruit juice processing factory in the Thai province of Nakhon Pathum for the analysis. The chemicals used were gallic acid, trolox, DPPH (2-diphenyl-1-1-picrylhydrazl), naringin, limonin, quercetin, apigenin, luteolin, ferulic acid, hesperedin, naringenin, sinapic acid, p-coumaric acid and caffeic purchased from Sigma-Aldrich, Gillingham, Dorset, UK. Myricetin and kaempferol were purchased from Fluka, Loughborough, Leicestershire, UK. All the chemicals and reagents used were of analytical grade.

2. Preparation of samples

On arrival at the laboratory, the pomelo pulp samples were divided into two parts. One part was processed into pomelo pulp powder (PPP) and the other part was processed into pomelo pulp dietary fiber powder (PPDFP) using the methods described below.

2.1 Preparation of pomelo pulp powder (PPP)

The pomelo pulp was dried in a tray dryer (Memmert 400, Germany) at 70°C until its moisture content was less than 10% and then milled (Muti-function high speed disintegrator, rotation rate 25,000-28,000 RMP at 1 min, made in Taiwan) and sieved (Stainless steel sieves, by Advantech Manufacturing, Inc, U.S.A). The sieved particles were then ground to different particle sizes: 150, 180, 250 and 425 μm in order to obtain PPP and each sample sealed under vacuum in an aluminum packet and stored at 4±2 °C until analyzed.

2.2 Preparation of pomelo pulp dietary fiber powder (PPDFP)

The pomelo pulp was treated to eliminate protein by soaking in 0.01 N NaOH solution (pomelo pulp:NaOH solution, 1:10, w/v) at 37°C for 10 min, followed by washing with distilled water and pressing. Each treated sample was defatted by soaking in ethanol (pomelo pulp:40% ethanol, 1:10, w/v) for 30 min, then again washed with distilled water and pressed. Bitterness reduction was by constant soaking for 60 mins in distilled water pH 7 (adjust the pH of the distilled water from 6.8 to pH 7.0 using 0.1 N NaOH solution) (pomelo pulp: distilled water pH 7, 1:10 w/v) followed by washing twice with distilled water and pressing. Then all the samples were dried in a tray dryer (Memmert 400, Gemmany) at 70°C until the moisture content was less than 10%, followed by milled samples were ground and sieved to different particle sizes: 150, 180, 250 and 425 μm in order to obtain PPDFP and each sample was sealed under vacuum in an aluminum packet and stored at 4±2°C until analyzed.

3. Quality analysis of PPP and PPDFP

3.1 Chemical composition

Dry weight, moisture, fat, protein, ash, and total sugar contents were determined following the methods of AOAC 925.45, 922.06, 981.10, 940.26 and 982.14, respectively (AOAC, 2016).

3.2 Dietary fiber composition

Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were determined using the enzyme-gravimetric method 985.29, 985.29 and 985.29, respectively (AOAC, 2016).

3.3 Total phenolic content and antioxidant properties

3.3.1 Total polyphenol content

The total phenolic content was determined using the Folin Ciocalteu colorimetric method described by Yun et al., 2009. The sample (1 g) were extracted with methanol 20 mL (w/v), shaker at 37°C at 6,000 rpm for 30 min and then the supernatant was filtered through Whatman filter paper. The sample 0.4 mL was added to 2 mL of 10% Folin- Ciocalteu reagent and 1.6 mL of 5% Na_2CO_3 solution was added to the mixture. Incubate in darkness at room temperature for 30 min, the absorbance at 765 nm using spectrophotometer (model 1601; Shimadzu Corp; Kyoto, Japan). The sample was expressed as mg of gallic acid equivalent (GAE) per milliliter.

3.3.2 Antioxidant activity using a free radical scavenging assay

The stable DPPH radical was used for determination of free radical-scavenging activity of the pomelo albedo extracts (a modified method from Zigoneanu et al., 2007). The sample 2 mL was added to 2 mL of DPPH reagent (200 μM). After 30 min at room temperature, the absorbance at 515 nm. The antioxidant activity (DPPH) was expressed as micro molar (μM) Trolox equivalent.

3.4 Limonin and naringin

Limonin and naringin give fruit a bitter taste, therefore the dietary fibers were analyzed for both substances because they could affect their use in food. Limonin is a limonoid and is a white, crystalline substance and naringin is a flavonoid. Both occur naturally in citrus fruits and give them a bitter taste.

Extraction sample: 1 g of sample was weighed, 10 mL of methanol was added and shaken for 1 min with Vortex Mixer and then was centrifuged for 30 min at 4,500×g and solution was passed through nylon filter size 0.22 μm, and kept in a vial for analysis of limonin and naringin.

Standards of limonin by diluting the stock solution to 2, 5, 10, 20, 50 and 100 mg/100g. Standard curves were used for the calculation of limonin in the extracts. The linear regression equations for the curves for limonin were y=14726x with a correlation coefficient (r^2) value of 0.9977.

Standards of naringin by diluting the stock solution to 2, 5, 10, 20, 50 and 100 mg/100g. Standard curves were used for the calculation of naringin in the extracts. The linear regression equations for the curves for naringin were y=46918x with a correlation coefficient (r^2) value of 0.9995.

Limonin determination: the analytical method used was modified from Sun et al. (2005) using a HPLC (Water Alliance 2695, USA) system consisted of C_{18} Column (4.5x150 mm, pore size 3 μm), a C_{18} guard column, a Diode array detector (Water Alliance 2996), a computerized recorder/integrator (Waters Empower software). The mobile phase consisted of A: 45% acetonitrile and B: 55% deionized water, a flow rate of 0.7 mL/min. The auto-injection sample 10 μL by a wavelength 210 nm.

Naringin Determination: the method used was modified from Kanaze et al. (2003) using the HPLC system as limonin, but the mobile phase consisted of A: 25% acetonitrile and B: 75% deionized water, a flow rate of 0.7 mL/min and the auto injection sample 10 μL by a wavelength 280 nm.

3.5 Total flavonoid contents

Total flavonoid contents (Apigenin, Ferulic acid, Hesperedin, Kaempferol, Luleotin, Myricetin, Naringenin, Querctin, Sinapic acid, p-Coumaric acid and Caffeic acid) were determined by using a modified method of Hertog et al.1992). The 1-2 g of the sample were weighed and then added BHQ, HO and HCl in a conical flask of 250 mL and swirled. The samples for hydrolyze were put in a hot water-bath of 90°C for 2 hours (swirled occasionally). The sample was cooled for 5 min in a cool water bath. Added to each sample was 1 mL of ascorbic acid solution. Then each sample was put into a volumetric flask with MeOH and diluted to a total volume of 100 mL and sonicate for 5 min. The extract was passed through a 0.22 μm nylon filter seeping into a vial for analysis.

The system consisted of an HPLC system (Agilent 1100 Series), a column: Zorbax Eclipse XDB-C_{18} (4.6x250 mm, pore size 5 μm) were preceded by a guard column Eclipse XDB-C_{18} (4.6x12.5 mm, pore size 5 μm). The column and guard column heater were temperature controlled at 30°C. The mobile phase consisted of A: water: trifluoroacetic acid (TFA) (99.95:0.05), B: acetonitrile: TFA (99.95:0.05) and C: methanol:TFA (99.95:0.05) with a flow rate of 0.6 mL/min (isocratic). By at (1) 0-5 min: 90-85% A, 6-9 % B, 4-6% C; (2) at 5-30 min: 85-71% A, 9-17.4% B, 6-11.6% C; (4) at 30-60 min: 71-0% A, 17.4-85% B, 11.6-15 % C; (5) at 60-61 min: 0-90% A, 85-6% B, 15-4% C; and (5) at

61-66 min: 90% A, 6% B, 4% C. Auto-sample/injector were 20 µL. The detection wavelength was at 210, 280, 325, 338 and 368 nm.

3.6 Color

The color of each sample was measured using a Handy Colorimeter (Minolta Camera Co.; Osaka, Japan) following the system of the *L* a* b.**

3.7 Functional properties

Water holding capacity (WHC) was measured using the method described by Xianliang et al. (2017) 1g of dried powder of sample (W_1) was dissolved in 20 ml distilled water and shaken for 1 min with Vortex mixer and then stood at room temperature for 20 min. After centrifugation at 4,000 rpm for 15 min, the sediment was collected and weighted (W_2). The WHC was calculated as follows: WHC (g/g)=$((W_2–W_1) / W_2)$

Oil holding capacity (OHC) was measured using the method described by Xianliang et al. (2017) 1 g of dried powder of sample (W_1) was dissolved in 20 mL distilled water and shaken for 1 min with Vortex mixer and then stood at room temperature for 18 hr. After centrifugation at 4,000 rpm for 15 min, the sediment was collected and weighted (W_2). The OHC was calculated as follows: OHC (g/g)=$((W_2–W_1)/W_2)$

4. Microstructure analysis

4.1 Scanning electron microscopy (SEM)

PPP and PPDFP samples were sieved through 150 µm size sieves. Samples were coated with a layer of platinum and scanned using a SEM, Model SU3500 Hitachi, Naka Factory, Japan, at voltage of 5kv at 2000x magnification level.

4.2 Fourier-transform infrared spectroscopy (FTIR) spectral analysis

The sample was observed by using Micro-Attenuated Total Reflectance (Micro-ATR) FTIR spectroscopy (Spectrum Spotlight FTIR Imaging System, Perkin-Elmer, Illinois, USA) at a resolution of 4 cm^{-1} and 32 scans per sample in the region range at wave number from 4000-600 cm^{-1}.

5. Statistical analysis

The data were analyzed in triplicate using one-way analysis of variance with SPSS Statistic Version 20.0 (SPSS Inc., Chicago, IL, USA). Data was considered statistically significant at $p<0.05$.

Results and discussion

1. Chemical properties

The chemical properties of PPP and PPDFP is shown in Table 1. It was found that levels of total dietary fibers from PPP were only about a quarter where those from PPDFP were in excess of 90%; more than pomelo albedo dietary fiber powder. The insoluble dietary fiber content in PPDFP (91.93%) was higher than in PPP (22.72%). The levels of insoluble dietary fiber of PPDFP, increased during the extraction process which considerably reduced protein and fat levels. A high proportion of IDF (mainly cellulose, hemicellulose and lignin) in pomelo fruit processing by-products are good source of dietary fiber that are suitable for use in food processing. The proportion of IDF reported in this work were higher than those of orange peel, grapefruit peel and lemon peel (Lei et al., 2015); orange peel fiber (Tainara de et al., 2013) and albedo pomelo fiber (Pichaiyongvongdee & Rattanapun, 2015).

Table 1 Chemical properties level in PPP and PPDFP (Dry matter)

Chemical composition	PPP	PPDFP
Moisture (%)	7.30±0.01	8.98±0.07
Protein (%)	8.47±0.03	3.37±0.03
Fat (%)	2.06±0.01	0.87±0.02
Ash (%)	3.22±0.03	5.74±0.06
Total sugar (%)	47.36±0.06	n.d.
a_w	0.26±0.00	0.27±0.00
Total dietary fiber (TDF)	25.27	92.04
Insoluble dietary fiber (IDF)	22.72±0.02	91.93 ±0.06
Soluble dietary fiber (SDF)	2.55±0.06	0.11±0.01
Limonin (mg/100g)	43.87±0.14	n.d.
Naringin (mg/100g)	263.48±9.61	0.19±0.01
Total phenolic content (mg gallic/g)	4.39±0.03	1.20±0.03
Antioxidant activity (DPPH) (µM trolox/g)	37.54±0.01	25.04±0.01
Naringenin (mg/100g)	25.81±0.77	0.90±0.02
Quercetin, Apigenin, Luteolin, Ferulic Acid, Hesperedin, Sinapic Acid, Kaemferol p-Coumaric acid, Caffeic acid, Myricetin	n.d.	n.d.

Remark: nd=not detected. Means values ±Standard deviation (n=3)

Protein content decreased from 8.47% to 3.37% probably due to the denaturation of protein in contact with sodium hydroxide. The fat reduction from 2.06% to 0.87% was due to ethanol treatment and no sugar was detected in PPDFP due to the preparation period of PPDFP by several of washing, which sugar has soluble properties. (Table 1). In Meng-mei & Tai-hua (2016) study the extracted dietary fiber from cumin using 3 methods (alkali extraction, enzymatic hydrolysis, and shear emulsifying assisted enzymatic hydrolysis) these methods could decrease protein and fat more than 3 times. Clearly, modifying PPP to PPDFP could be used to greatly enhance dietary fiber for its application in food products. These additions could improve the food

products, for example, it has previously been reported that protein in dietary fiber can reduce the water holding capacity of food product and rancidity during long term storage of high fat foods. Removing protein and fats from dietary fiber, as was shown by PPDFP, was previously shown to improve texture and eating quality of coconut cake (Yajun & Yan, 2018). Fats in Chia seeds (*Salvia hispanica*) were reduced by treatment with hexane, and Vazquez-Ovando et al. (2009) found that defatting seeds of Chia improved their physicochemical properties in such a way as to improve their use in functional foods.

The bitterness in pomelo fruit may be unacceptable for use in some functional foods. The two compounds responsible for bitterness, naringin and limonin in PPDFP were almost completely reduced in Table 1. Horowitz & Gentili (1977) reported that naringin is soluble in water, which would account for its reduction. Limonin is more soluble in solvents like chloroform and only slightly in water (Maier et al., 1977), but these results showed that the ethanol plus water treatments effectively controlled levels. Naringenin was decreased from 25.81mg (PPP) to 0.90 mg/100g (PPDFP) while other components were not detected in both PPP and PPDFP. Yoon et al. (1997) reported that flavonoid can be dissolved well in pH 7 but not soluble at pH 6.5 or lower.

2. Effects of particle size on color and functional foods

2.1 Color

The color is an important factor in the applications in food which might mean that in some aspect the color of particles needs to be considered in application to the food industry because it could affect their appearance. The preparation of PPDFP could be used to greatly enhance the color by removal of sugars and another soluble component during the preparation of PPDFP by washing before drying which could be the cause due to Maillard browning reactions that was found such as *L** increased lightness, red to green *(a*)* value and blue to yellow *(b*)* value both decreased (Table 2). Reducing the particle size distribution affected the color of both PPP and PPDFP and it was found that *L** increased lightness whereas *a**and *b** decreased.

2.2 Water and oil holding capacity (WHC and OHC)

The water holding and oil holding capacity of PPDFP was higher than PPP due to PPDFP had IDF higher than PPP. IDFs have the structure of cellulose and hemicellulose that consists of several hundred to many thousands of glucose units connected by a beta acetal linkage, bond of cellulose and hemicellulose had intermolecular hydrogen bond that showed much higher WHC than that of sugar and protein in PPP. In addition, both WHC and OHC increased as particle size decreased. The increase of water holding and oil holding capacity of both PPP and PPDFP were related to particle size. The PPDFP size 150 μm could hold a higher amount of water and oil more than the size of 180, 250 and 425 μm. The PPDFP 150 μm particle exhibited the highest WHC and OHC and had 11.36 g water/g sample and 3.86 g oil/g sample, respectively (Table 3).

Table 2 Effects of particle size on color of PPP and PPDFP that had been sieved to different sizes

Dietary fiber	Sieving size (μm)	*L**	*a**	*b**
PPP	425	69.60±0.31^{h}	7.21±0.21^{a}	27.60±0.31^{a}
	250	72.71±0.24^{g}	7.00±0.15^{b}	26.50±0.26^{b}
	180	75.61±0.37^{f}	6.10±0.28^{c}	24.48±0.27^{c}
	150	77.38±0.31^{e}	3.54±0.12^{d}	21.27±0.32^{d}
PPDFP	425	80.57±0.30^{d}	1.17±0.07^{e}	14.06±0.15^{e}
	250	84.50±0.30^{fc}	0.85±0.05^{f}	13.17±0.15^{f}
	180	86.34±0.25^{b}	0.50±0.08^{g}	12.51±0.24^{g}
	150	88.85±0.31^{a}	0.32±0.05^{h}	11.28±0.15^{h}

Remark: Different letters in the same column indicate that the values have significant differences ($p < 0.05$)

Table 3 Effects of particle size on water holding capacity (WHC) and oil holding capacity (OHC) of PPP and PPDFP that were sieved to different sizes

Dietary fiber	Sieving size (μm)	WHC (g water/g sample)	OHC (g oil/g sample)
PPP	425	3.25±0.10^{h}	0.67±0.07^{g}
	250	3.76±0.15^{g}	0.88±0.02^{f}
	180	4.90±0.09^{f}	0.91±0.05^{ef}
	150	5.32±0.20^{e}	1.08±0.02^{e}
PPDFP	425	8.55±0.14^{d}	2.33±0.08^{d}
	250	9.23±0.26^{c}	2.89±0.16^{c}
	180	10.41±0.29^{b}	3.38±0.17^{b}
	150	11.36±0.18^{a}	3.86±0.05^{a}

Remark: Different letters in the same column indicate that the values have significant differences ($p < 0.05$)

Fengmei et al. (2015) reported that particle size reduction of hull-less barley bran dietary fiber for water retention capacity and oil binding capacity. Similarly, the superfine grinding increased the WHC of oat fiber (Marcin et al. 2016). Zheng & Li (2018) reported that the particle size of coconut cake dietary fiber treated by acid was reduced from 250 to 167 μm and resulted in increased WHC. Marcin et al. (2016) reported that decreasing particle size leads to reduced hydration properties, exposure of hydrophilic groups which is connected with

water absorption capacity. Increasing of surface area and pore volume as well as structural modification contribute to increasing of WHC and OHC. Schneeman (1999) said that the ability of oil holding capacity (OHC) of dietary fiber to adsorb fat or oil can be important in food applications; for example, in preventing fat loss upon cooking and in nutrition where the ability to absorb or bind bile acids and increase their excretion is associated with plasma reduction and the ability to prevent fat loss during food processing and the capacity to reduce serum cholesterol level (Navarro-González et al., 2011) Thus, characteristics of WHC and OHC of different particle sizes are important in food application or can be used for functional ingredient to modify the viscosity and texture of some formulated food and avoid syneresis in food.

2.3 SEM that had been sieved to different sizes

The microstructure of PPP and PPDFP were particle size 150 µm. The differences of their surfaces found that the PPP with sheet-like structures and the surface of the PPDFP was partially disintegrated, indicating that their cell structures were damaged (Fig 1). This effect had previously been reported by Peerajit et al. (2012) who showed that hydrolysing of hemicellulose led to collapse and distortion of the cell wall structure and increased porosity. The SEM examination was consistent with the result of the particle size 150 µm and had an affect on WHC and OHC (Table 3) indicating the increased ability in water/oil binding was mainly related to the porosity of the fibre structure.

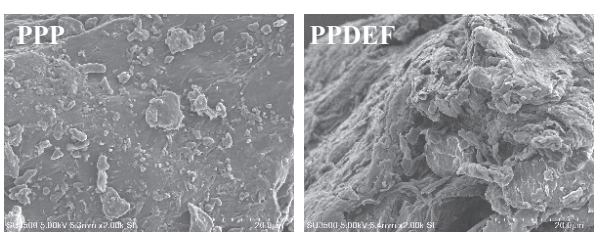

Fig. 1 Scanning electron micrographs of PPP and PPDFP were particle size 150 µm (view under 2000 magnification)

2.4 FTIR spectra of PPP and PPDFP

FTIR spectra of PPP and PPDFP are illustrated in Fig 2. It was found that most characteristic bonds of PPP and PPDFP were similar in FTIR spectra, such as bands at 3348 and 3366 cm^{-1} (O-H in stretching vibration in hemicellulose cellulose), 2932 and 2925 cm^{-1} (C-H in stretching vibration of the methylene group of the saccharide) (Liu et al., 2019; Jin-Shun et al., 2017; Jinjin et al., 2015). A band at 1739 and 1731 cm^{-1} (C=O in stretching vibration of the ester group) (Jin-Shun et al., 2017; Liu et al., 2019), 1622 and 1621 cm^{-1} (C=O in lignin benzene ring) (Liu et al., 2019), 833, 897, 1067, 1064, 1416 and 1424 cm^{-1} (due to the bending of the OH group in C-OH in hemicellulose (Jin-Shun et al., 2017), 1244 cm^{-1} (C-O in hydroxybenzene (Liu et al., 2019). All referred to the characteristic absorption peak of lignin (Jin-Shun et al.. 2017), the beading vibration absorption peak of acetyl groups (Jin-Shun et al., 2017), the beading vibration absorption peak of C-O-C (Jinjin et al., 2015) respectively, proving that the NaOH and ethanol treatment for the PPP reduced the intensity functional group and destroyed the structure of organic molecules.

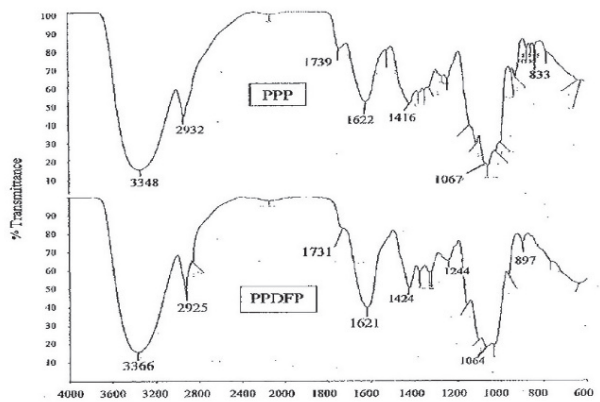

Fig. 2 FT-IR spectra of PPP and PPDFP

Conclusion

This study found that reducing the protein and fat in the pomelo pulp, by using NaOH, ethanol, and washing with distilled water prior to drying, resulted in high yielded pomelo pulp dietary fiber powder with high dry matter and low water activity. Reducing particle size to 150 µm resulted in powders with increased WHC and OHC which are more suitable for inclusion in many food products. However, the color of PPDFP must be considered in its applications to avoid a negative of the foods to which it is added. In addition, the PPDFP was richer in insoluble dietary fiber; higher than 90% which IDF are characterized by their porosity and their low density. Its favorable physical and chemical characteristics offers it to be suitable to use in food formulations such as adding to meat products to improve the texture and stability as well as increase in density and hardness in extruded cereals. In addition it can improve

water absorption and taste in baked products and increase in the water absorption of dough during the process as well as decrease water absorption and swelling of pasta product.

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References

AOAC. (2016). *Official Methods of Analysis Association of Official Analytical Chemists*. Gaithersburg, Maryland: AOAC.

Fengmei, Z., Bin, D., & Baojun, X. (2015). Superfine grinding improves functional properties and antioxidant capacities of bran dietary fibre from Qingke (hull-less barley) grown in Qinghai-Tibet Plateau, China. *Journal of Cereal Science, 65,* 43-47.

Hertog, M.G.L., Hollan, P.D.H., & Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetable and fruits. *Journal of Agricultural and Food Chemistry, 40,* 1591-1598.

Horowitz, R., & Gentili, B. (1977). Flavonoid constituents of citrus. In S. Nagy, P.E. Shaw, & M.K. Veldhuis (Eds.), *Citrus Science and Technology* (pp. 397-426). CT, USA: AVI Publishing.

Jinjin, C., Qingsheng, Z., Liwei, W., & Lijun, Z. (2015). Physicochemical and functional properties of dietary fiber from maca (*Lepidium meyenii* Walp.) liquor residue. *Carbohydrate Polymers, 132,* 509-512.

Jin-Shun, L., Xiao-Yan, L, Xiao-Pan Z., & Lin-Shuang, W. (2017). Chemical composition and functional characteristics of dietary fiber-rich powder obtained from core of maize straw. *Food Chemistry, 227,* 383-389.

Kanaze, F.I., Gabrieli, C., Kokkalou, E., Georgrakis M., & Niopas, I. (2003). Simultaneous reversed-phase high-performance liquid chromatographic method for the determination of diosmin, naringin and hesperidin in different citrus juices and pharamaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis, 33,* 243-249.

Lei, W., Honggao, X., Fang, Y., Rui, F., & Tanxiang, G. (2015). Preparation and physicochemical properties of soluble dietaryfiber from orange peel assisted by steam explosion and dilute acid soaking. *Food Chemistry, 185,* 90-98.

Lilian, E.F., & Diego, B.G. (2019). Fruit jellies enriched with dietary fibre: Development and characterization of a novel functional food product. *LWT Food Science and Technology, 111,* 423-428.

Liu, S., Jia, M., Chen, J., Wan, H., Dong, R., Nie, S., ... Yu, Q. (2019). Removal of bound polyphenols and its effect on antioxidant and prebiotics properties of carrot dietary fiber. *Food Hydrocolloids, 93,* 284-292.

Maier, V.P., Bennett, R.D., & Hasegwa, S. (1977). Limonin and other limonoids. In S. Nagy, P.E. Shaw, & M.K. Veldhuis (Eds.), *Citrus Science and Technology* (pp. 355-396). CT, USA: AVI Publishing.

Mansour, E.H., & Khalil, A.H. (1999). Characteristics of low fat beef burgersas influenced by various types of wheat fibers. *Journal of the Science of Food and Agriculture, 79,* 493-498.

Marcin, K., Jarosław, W, Monika, P., & Agnieszka, W. (2016). The effect of oat fibre powder particle size on the physical properties of wheat bread rolls. *Food Technology and Biotechnology, 54*(1), 45-51.

Meng-mei, M., &Tai-hua, M. (2016). Effects of extraction methods and particle size distribution on the structural, physicochemical, and functional properties of dietary fiber from deoiled cumin. *Food Chemistry, 194,* 237-246.

Ministry of public health. (2020). *Dietary reference intake for Thais 2020.* Retrieved August 1, 2021, from https:// www. thaidietetics.org/ wp-content/uploads/2020/04/ dri2563.pdf

Navarro-González, I., García-Valverde, V., García-Alonso J., & Jesús Periago, J. (2011). Chemical profile, functional and antioxidant properties of tomato peel fiber. *Food Research International, 44*(5),1528-1535.

Peerajit, P., Chiewcha, N., & Devahastin, S. (2012). Effects of pretreatment methods on health-related functional properties of high dietary fibre powder from lime residues. *Food Chemistry, 132*(4), 1891-1898.

Pichaiyongvongdee, S., & Haruenkit, R. (2009). Comparative studies of limonin & naringin distribution in different parts of pummelo [*Citrus grandis* (L.) Osbeck] cultivars grown in Thailand. *Kasetsart Journal Natural Science, 43*(1), 28-36.

Pichaiyongvongdee, S., & Rattanapun. B. (2015). effect of chemical treatment to reduce the bitterness and drying on chemical physical and functional properties of dietary fiber pomelo powder from *Citrus grandis* (L.) osbeck Albedo. *Kasetsart Journal Natural Science, 4* (1),122-132.

Schneeman, B.O. (1999). Fiber, inulin and oligofructose: Similarities and differences. *The Journal of Nutrition, 129*(7), 1424–427.

Sudha, M.L., Baskaran, V., & Leelavathiet, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry, 104,* 686-692.

Sun, C., Chen, K, Che, Y., & Chen, Q. (2005). Cotents and antioxidant capacity of limonin and nomilin in different tissues of citrus fruit of four cultivars during fruit growth and maturation. *Food Chemistry, 93,* 599-605.

Tainara de, M.C., Andre, J., Alessandro de, O.R., Rosane. R., & Simone, H.F. (2013) Dietary fiber from orange byproducts as a potential fat replace. *LWT Food Science and Technology, 53,* 9-14.

Vazquez-Ovando, A., Rosado-Rubio, G., Chel-Guerrero, L, & Betancur-Ancona, D. (2009) Physicochemical properties of a fibrous fraction from Chia (*Salvia hispanica* L.) *LWT Food Science and Technology, 42,*168-173.

Xianliang, L., Qi, W., Baodong, Z., Liangmei, L., Bingyan, C., Yafeng, Z, & Jianbo, X. (2017) Hydration properties and binding capacities of dietary fibers from bamboo shoot shell and its hypolipidemic effects in mice. *Food and Chemical Toxicology, 109,* 1003-1009.

Yajun, Z., & Yan., L. (2018) Physicochemical and functional properties of coconut (*Cocos nucifera* L.) cake dietary fibres: Effects of cellulase hydrolysis, acid treatment and particle size distribution. *Food Chemistry, 257,* 135-142.

Yun, S.J., Liang, J., Peng, X., Yan, L., & Jinsong, B. (2009). Total phenolice, Flaconoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science, 49,* 106-111.

Yoon, S.Y., Choi, W.J., & Park, J.M, (1997).Selective adsorption of flavonoids compounds from the leaf of *Ginkgo biloba* L. *Biotechnology Techniques, 11,* 553-556.

Zigoneanu, I.G., Willams, L., Xu, Z., & Sabilov, C.M. (2007) Determination of antioxidant component in rice brean oil extracted by microwave-assisted method. *Bioresource Technology, 99,* 4910-4918.

Zheng, Y., & Li, Y. (2018). Physicochemical and functional properties of coconut (*Cocos nucifera* L.) cake dietary fibres: Effects of cellulase hydrolysis, acid treatment and particle size distribution. *Food chemistry, 15*(257), 135-142.

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Growth and Survival of Thai Climbing Perch (*Anabas testudineus*) and Snakeskin Gourami (*Trichogaster pectoralis*) Reared in Brackish Water in Cement Pond in Salt-affected Soil

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Thai climbing perch, Snakeskin gourami, Brackish water, Aquaculture

Abstract

Thai climbing perch (*Anabas testudineus*) and snakeskin gourami (*Trichogaster pectoralis*) are freshwater fish species commercially grown in Southeast Asian countries. This research investigated survival rate and growth performances of Thai climbing perch and snakeskin gourami when cultured in brackish water (7.50 ppt) in salt affected soil. The experiment was conducted in a brackish water cement ponds for 6 months. Water quality was also measured at 2-month intervals. The results found that average daily weight gain (DWG) of Thai climbing perch and snakeskin gourami was 0.597±0.017 and 0.048±0.010 g/day, respectively. Co-culture between the fish species had a DWG of 0.592± 0.022 and 0.038±0.004 g/day for Thai climbing perch and snakeskin gourami, respectively. At the end of the study, the productivity of Thai climbing perch weight was 121.67±2.89 kg and snakeskin gourami weight was 24.15±2.10 kg. In addition, higher survival rate of 93.11±4.07% for Thai climbing perch and 52.00±17.02% for snakeskin gourami (T3) reared in brackish water was obtained in co-culture system (T3) compared with Thai climbing perch (T1, 87.33 ± 5.78%) and snakeskin gourami (T2, 43.89±15.87 %). Water quality at the end of the experimental period found the difference in each treatment. The results showed that T1 found the highest values of many water quality parameters. This study highlights the culture of freshwater fish in brackish water has advantages for fish farming in salt affected soil.

Introduction

In Nakhon Ratchasima, Thailand, the spread of salt into the land is a serious problem. The most severe of 20,480 square kilometers area is salty, 768 square kilometers found serious saline soil problem, 320 square kilometers found moderate saline soil problem, and potential saline problems might occur up to 3,360 square kilometers in a mainly agricultural area (Land Development Department of Nakhon Ratchasima Province, 2013). The phenomenon is increasing the salt concentration in freshwater (freshwater salinization), leading to significant effects on aqautic organisms.

Freshwater salinization, an increase in osmotic pressure, is one of the factors limiting the growth and development of aquatic life (Kaushal et al., 2021). In

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natural salinity water, most salts are sodium chloride where content are sodium ions and chloride ions. These cation and anion concentration are regulated by osmo-regulation and salinity stress aesthetic processes, which are the systems that maintain a balance of water and mineral salts between aquatic life and the environment. In freshwater fish, an increase in salinity results in hyperosmotic physiological changes. Therefore, fish have a great need to use energy to regulate osmosis pressure in order to maintain the balance of the body. If severe osmosis pressure occurs, it can lead to the death of the fish (Tippayada et al., 2015).

Thai climbing perch (*Anabas testudineus*) is a freshwater species that is commonly found in paddy fields, wells, irrigation channels, and drains (Yunus, 2018). Snakeskin gourami (*Trichogaster pectoralis*) is aqua cultured freshwater species in Thailand. They can survive in various aquatic ecosystems and submerge with aquatic vegetation such as rice paddies, shallow ponds, swamps and sluggish, or standing water (Sugiyama et al., 2004). These two species of fish are very common fishes in Thailand.

Thai climbing perch and snakeskin gourami are resistant to polluted water and salt tolerance. The aquaculture fish is increasing the economy in the water with high salinity and reared in clean water. The dominant feature of Thai climbing perch and snakeskin gourami is their resistance to water contamination and tolerance to salinity (Amornsakun et al., 2004; Bhaskar et al., 2015; Chotipuntu & Avakul, 2010; Khatun et al., 2019; Kazi et al., 2016; Shinsuke et al., 2010). These fishes have evolved osmoregulatory mechanisms to thrive in a variety of aquatic conditions. However, salinity tolerances of freshwater fish vary among species and with their developmental stages (Chotipuntu & Avakul, 2010). Chotipuntu (2003) reported that most adult freshwater fish can live in salinity between 7-13 ppt.

Thai climbing perch and snakeskin gourami reared in brackish water will give opportunity for fish farming located in salt affected soil. Consequently, this study investigated the survival rate, growth and performances of the fresh water fish which were cultured in brackish water.

Materials and methods

1. Experimental site and cement pond preparation

The experiment was carried out for a period of 6 months in 12 experimental ponds located at Ban Pho Subdistrict, Muang District, Nakhon Ratchasima, Thailand (15.012560N, 102.182460E). The ponds were of the same size (1.0 x 3.0 x 1.5 m) (Fig.1) and similar in shape and depth. After construction, the ponds were prepared by filling with water for 2 weeks.

Fig. 1 Cement pond used in this study

2. Brackish water preparation

The water used was pumped from a 17 m depth groundwater, in salt-affected soil, Nakhon Ratchsima, Thailand and sat for 1 week before use. Salinity was maintained at 7.50 ppt and measured with a pH meter (TLEAD, pH-009LA).

3. Fish species and maintenance

Thai climbing perch (*A. testudineus*) and snakeskin gourami (*T. pectoralis*) used in this study (Fig.2) were purchased from local farms in Nakhon Ratchsima, Thailand. Mono- and co-culture systems were carried out with 4 treatments because stocking a range of species with various eating habits enabled for more efficient use of the ecosystem's available food, boosting economics and sustainability. Average weight of Thai climbing perch and snakeskin gourami (900 fish/ species) was 11.50-12.00 g and 15.00-15.50 g, respectively. The experiment was carried out in 12 ponds with 4 treatments and 3 replicates were designed. Among them, 3 were brackish water (7.50 ppt) and another was fresh water (<1 ppt).

The following shows the treatment number, the number of fish species and water type:

Treatment 1: 300 of Thai climbing perch reared in brackish water

Treatment 2: 300 of snakeskin gourami reared in brackish water

Treatment 3: 150 of Thai climbing perch combined with 150 of snakeskin gourami reared in brackish water

Treatment 4: 150 of Thai climbing perch combined with 150 of snakeskin gourami reared in fresh water

The fish feeding operated by feed formulated diet of no less than 25% protein, 2 time a day (09.00 am and 03.00 pm) at a rate of 5% body weight. Small fish at 1.5 to 4.0 cm in size were feed during one to eight-weeks with feed pellets floating 1.0-1.5 mm. At 3-4 months when the fish were larger, the weight of 25-100 g, the feed pellets increased to floating size 2.5-3.5 mm. At 5-6 months when the fish weight gained to 100-250 g, feed pellets increased to floating size 5.5 mm. After 1 hour of feeding, fish abnormality were observed.

4. Growth performance and survival rate determination

To study the effect of salinity on the growth performance, fish were collected by a sampling of the growth of the fish. The survival rate of fish reared in cement ponds random sampling, the number of fish caught and then released again (Capture-recapture sampling) (Chao, 1989), which holds the number three times, once every two months.

The study of the fish growth and productivity by random sampling and counting the total numbers of fish were calculated by the equations as follow (Bhaskar et al., 2015):

The average daily weight gain, DWG (g/day)

DWG = (final weight (g) - initial weight (g))/ experimental time (day)

Percentage weight gain, PWG (%)

PWG = (final weight (g) - initial weight (g))/(initial weight of fish (g)) x 100

Survival growth rate, SGR (%)

SGR = (number of fish at the end of the experiment/ number of fish at an initial time of the experiment) x 100

5. Water quality analysis

The water quality in each pond was measured at 2-month intervals. pH value was measured directly by a portable pH meter (TLEAD, pH-009LA). Salinity, total dissolved solid (TDS), conductivity (EC) was measured by bench meter (Hanna, HI 4321), biochemical oxygen demand (BOD), nitrogen, chloride, and phosphate were also measured by standard methods for the examination of water and waste water (American Public Health Association, 2005).

Fig. 2 Fish species used in this study, *A. testudineus* (right), *T. pectoralis* (left)

6. Economic analysis

An economic analysis was performed to estimate the net profit by deducting the gross income from the gross cost. The analysis was based on local market prices for harvested fish and all other items in 2016. The cost of cement pond was $65.03 USD/per pond. The prices for Thai climbing perch and snakeskin gourami were $0.048 USD/ per fish and $0.065 USD/per fish and all fish feed was $117.12 USD. The selling price for Thai climbing perch and snakeskin gourami was estimated at $2.60 and $2.28 USD/kg, respectively.

7. Statical analysis

All experiments were repeated at 3 replicates and all data were analyzed by percentage, average, and standard deviation and compared mean by ANOVA.

Results and discussion

1. Growth performance and survival rate

The growth of Thai climbing perch and snakeskin gourami culture in brackish water cement ponds at the initial time of this study showed the Thai climbing perch fish averaged from 11.50 to 12.00 g in weight; width of 1.40 to 1.50 cm and length of 3.40-3.55 cm in body size. The snakeskin gourami was 15.00 to 15.50 g in weight; width of 1.75-1.80 cm and length of 3.50 to

3.85 cm in body size. After 6 months, the results shown in treatment 1 found the Thai climbing perch had an average of 3.93±0.12 cm, a length of 12.69±0.30 cm, and an average weight of 121.67±2.89 g. In treatment 2, the snakeskin gourami had a body equivalent to 2.88±0.20 cm in width and 9.73±0.35 cm in length; the average weight of the fish was 24.15±2.10 g. Treatment 3 (Thai climbing perch and snakeskin gourami) found a total average weight of 71.60±2.57 g; Thai climbing perch had an average weight of 121±4.58 g; the body was 4.46±0.23 cm in width and 14.38±0.16 cm in length. The snakeskin gourami had an average weight of 22.20±0.80 g; the body was 3.47±0.27 cm in width and 9.01±0.32 cm in length. While treatment 4 (Thai climbing perch and snakeskin gourami in freshwater) resulted in the Thai climbing perch body size of 4.23±0.23 cm in width, 13.47±0.81 cm in length; with an average weight of 121.67±0.58 g. Snakeskin gourami had a body size of 3.03±0.40 cm in width and 9.67±0.58 cm in length; a weight of 23.83±1.15 g per fish (A total weight of T4 was 72.75±0.87 g). The results of the average weight of Thai climbing perch and snakeskin gourami are shown in Fig. 3.

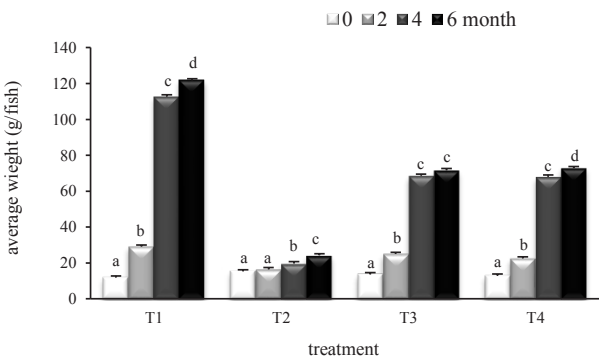

Fig. 3 The growth of fishes for 6 months. T1: Thai climbing perch, T2: snakeskin gourami, T3: Thai climbing perch x snakeskin gourami (T1-T3 cultured in brackish water) and T4 Thai climbing perch x snakeskin gourami (cultured in fresh water). Lowercase letter above the bar, values are the mean ± SD of three replicates, indicating statistically significant differences at $p < 0.05$ (DMRT) within each treatment

Average daily weight gain, DWG (g/day), the initial weight was 11.83 g forThai climbing perch, and 15.23 g for snakeskin gourami. The final weights were 121.67 g for Thai climbing perch in T1 and 24.15 g for snakeskin gourami in T2 at 6 months. Thus, the DWG of Thai climbing perch was about 00.597 g/day (T1); snakeskin gourami was 0.048 g/day (T2). The results of T3 found an average weight of 121 g for Thai climbing perch and 22.20 g for snakeskin gourami. The DWG of Thai climbing perch was 00.597 g/day and snakeskin gourami was 0.038 g/day as shown in Table 1.

Table 1 The average daily weight gain (DWG) of fishes cultured for 6 months

Treatment	Description	DWG (g/day)
T1	Thai climbing perch cultured in brackish water	0.597±0.017
T2	Snakeskin gourami cultured in brackish water	0.048±0.010
T3	Thai climbing perch Snakeskin gourami cultured in brackish water	0.592±0.022 0.038±0.004
T4	Thai climbing perch Snakeskin gourami cultured in fresh water	0.597±0.002 0.054±0.010

The survival rate at the end of the study is shown in the Table 2 below. The survival rate of 87.33±5.78% and 43.89±15.87% was observed in Thai climbing perch and snakeskin gourami in T1 and T2, respectively. Co-culture of Thai climbing perch and snakeskin gourami gave the survival rate higher than separately cultured, which was found in the T3 and T4. Hasan et al. (2010) reported that the survivable rate of Thai koi (*A. testudineus*) at 90 days of the experiment were about 73-83% when cultured in nylon hapas. Baisya et al. (2012) found that the survivable rate of snakeskin gourami (*T. pectoralis*) was 97 % of the fry weaned at day 24. Therefore, it might be due to the different culture systems and species variation.

Table 2 Survival rate of Thai climbing perch and snakeskin gourami cultured in brackish water cement ponds for 6 months

Treatment	Description	Survival rate (%)
T1	Thai climbing perch cultured in brackish water	87.33±5.78
T2	Snakeskin gourami cultured in brackish water	43.89±15.87
T3	Thai climbing perch Snakeskin gourami cultured in brackish water	93.11± 4.07 52.00±17.02
T4	Thai climbing perch Snakeskin gourami cultured in fresh water	94.44±0.96 76.67±2.89

2. Water quality

The water quality parameters of the four treatments were detected from four different times at 0, 2, 4 and 6 months during the experimental period which are shown in Table 3. A pH value in the ponds ranged from 6.5 to 7.8, which is the optimum pH levels for fish to live. The pH level outside of 6.5-9.5 could have an adverse effect on the growth and development of fish. TDS increased along with the increaseof time in all treatments especially T1 showed the highest TDS and BOD up

to 15,300 and 285 mg/L at the end of experiment, respectively. As illustrated in Table 3, the amount of time (listed in months) markedly changed the water quality, particularly TDS and BOD as well as all water quality parameters of the T1, T2 and T3, which were significantly higher than T4. Increment of nitrate-nitrogen was observed and the highest level of 132.20 mg/L was obtained in T1 at 6 months. Phosphate in the water slightly increased between 0.23 to 0.42 mg/L in T1-T3. Nevertheless, chloride and phosphate levels in all experiments gradually increased.

3. Economic analysis

Investment of cost-benefit of Thai climbing perch and snakeskin gourami culture in brackish water cement ponds had a total budget of $322.08 USD that included buying fish at $204.96 USD, and fish feed was $117.12 USD (1 USD = 30.74 Baht at CE 2021), and the cement pond cost of $65.03 USD per cement pond. The cost-benefit of Thai climbing perch culture in brackish water cement ponds found the weight of 184 kg and the total of $479.48 USD led to $2.60 USD per kilogram. The snakeskin gourami weight of 14.50 kg was $2.28 USD per kilogram, totaling $33.02 USD, and selling fish at $512.54 USD. Thus, the profit of this study was $190.46 USD (Not including the cement pond construction budget). However, if the cement pond construction budget was included, it would be profitable at the second or third fish culturing. The cost-benefit analysis found high profit occurs in the long term. This result is consistent with those of fed fish with vegetables by Patthumma (2013) as well as tilapia by Chaisuri (2012), which released the Thai climbing perch sizes starting from 27.40 to 32.25 g of 100 fish in cement circular water containing 200 liters and water volume of 332 liters of tilapia because the Thai climbing perch behavior is to jump. Thai climbing perch have respiratory organs that can live in highly polluted water. The fish fed with 30% protein, is the same with the tilapia fish but for a shorter period of 3 months. According to the study by the Pollution Control Department Ministry of Natural Resources and Environment (2006) on the amount of waste from the tilapia fish farming in cages of the Chao Phraya River at Chainat Province in 2002, these fish fed by the protein building at 32-35% and found that the FCR means rose by 1.5 and digestibility of protein in the diet of fish was 85% excreta of fish from fish production at 1,000 kg, the amount of nitrogen entering the water totaled 1,600 g and 1,400 g of phosphorus.

Table 3 Water quality parameters at 2-month intervals for 6 months

Treatments	Parameters	Duration (month)			
		0	2	4	6
T1 (Thai climbing perch cultured in brackish water)	pH	6.89	7.24	7.12	7.11
	TDS (mg/L)	609	297	542	15,300
	DO (mg/L)	1.63	0.47	2.45	0.92
	BOD (mg/L)	1.50	3.00	45.00	285.00
	Chloride (mg/L)	3.26	4.55	5.26	17.00
	Nitrate (mg/L)	1.10	2.67	20.00	132.20
	Phosphate (mg/L)	0.02	0.43	0.38	0.42
T2 (Snakeskin gourami cultured in brackish water)	pH	6.89	7.79	7.35	7.67
	TDS (mg/L)	609	268	486	6,090
	DO (mg/L)	1.63	2.75	3.00	0.97
	BOD (mg/L)	1.50	4.00	12.4	157.50
	Chloride (mg/L)	3.26	4.20	3.44	5.70
	Nitrate (mg/L)	1.10	1.60	18.54	77.30
	Phosphate (mg/L)	0.02	0.20	0.22	0.25
T3 (Thai climbing perch combined with snakeskin gourami cultured in brackish water)	pH	6.89	7.77	7.40	7.81
	TDS (mg/L)	609	368	512	8,010
	DO (mg/L)	1.63	3.82	3.42	0.97
	BOD (mg/L)	1.50	2.45	8.45	60.00
	Chloride (mg/L)	3.26	5.15	4.20	3.25
	Nitrate (mg/L)	1.10	0.67	10.00	29.70
	Phosphate (mg/L)	0.02	0.10	0.25	0.23
T4 (Thai climbing perch combined with snakeskin gourami cultured in fresh water)	pH	7.05	6.50	6.45	6.45
	TDS (mg/L)	145	245	445	445
	DO (mg/L)	6.00	4.50	4.00	4.00
	BOD (mg/L)	1.50	1.60	3.60	45.20
	Chloride (mg/L)	0.02	0.20	1.00	1.00
	Nitrate (mg/L)	0.01	0.20	1.30	12.30
	Phosphate (mg/L)	0.00	0.10	0.15	0.30

Conclusion

The study highlighted that the Thai climbing perch has tolerance to the lowest water quality (also, high salinity) more than snakeskin gourami. The productivity and survival rate of Thai climbing perch was higher than snakeskin gourami when cultured in brackish water (7.50 ppt). The result suggests that the culture of freshwater fish in brackish water has advantages for fish farming in salt affected soil. Thai climbing perch and snakeskin gourami can be cultured and has salt tolerance in brackish water cement ponds. The cost-benefit analysis found that high profit is attainable in the long term. The results on aquaculture fish in brackish water cement ponds lead to the following suggestion. Farmers would rather culture Thai climbing perch because they are resistant to salinity water and can thrive in low water quality. Local agencies involved in tackling the poverty of salinity areas can encourage fish farming with further processing of fish, including dried fish and pickled fish as professional development.

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References

American public Health Association. (2005). *Standard Methods for the Examination of water and Wastewater* (21st ed.). Washington, D.C.: American Public Health Association.

Amornsakun. T., Sriwatana, W., & Promkaew, P. (2004). Some aspects in the early life stage of Siamese gourami, *Trichogaster pectoralis* (Regan) larvae. *Songklanakarin Journal of Science and Technology, 26*(3), 347–356.

Baishya, S., Kalita, K., Phukan, B., Dutta, M.P., & Bordoloi, R. (2012) Growth performance of snakeskin gourami, *Trichogaster pectoralis* (Regan 1910) through weaning strategies. *An International Quarterly Journal of Life Sciences, 7*(3), 553-556.

Bhaskar. P., Pyne, S.K., & Ray, A.K. (2015). Growth performance study of Koi fish, *Anabas testudineus* (Bloch) by utilization of poultry viscera, as a potential fish feed ingredient, replacing fishmeal. *International Journal of Recycling of Organic Waste in Agriculture, 4,* 31–37.

Chaisuri, S. (2012). *The difference in yield ratio of tilapia with crops, the proportion of different fish and plants* (Research report). Khon Kaen: Department of Fisheries, Faculty of Agriculture Khon Kaen University.

Chao, A. (1989). Estimating population size for sparse data in capture-recapture experiments. *Biometrics, 45,* 427-438.

Chotipuntu, P. (2003). *Salinity sensitivity in early life stages of an Australian freshwater fish, murray cod (Maccullochella peelii peelii Mitchell, 1838)* (Doctoral dissertation). Australia: University of Canberra.

Chotipuntu, P., & Avakul, P. (2010) Aquaculture potential of climbing perch, *Anabas testudineus*, in brackish water. *Walailak Journal of Science and Technology, 7*(1), 15-21.

Hasan, M., Ahammad, A.K.S., & Khan, M.M.R. (2010). A preliminary investigation into the production of Thai koi (*Anabas testudineus*) reared in nylon hapas in Bangladesh. *Bangladesh Research Publications Journal, 4,* 15-23.

Kaushal, S.S., Likens, G.E., Pace, M.L., Reimer, J.E., Maas, C.M., Galella, J.G., ... Woglo, S. A. (2021). Freshwater salinization syndrome: From emerging global problem to managing risks. *Biogeochemistry, 154,* 255-292.

Kazi, B.U., Mohammad, M., Abul, B., Sanjib, B., Saiful, I., Yahia, M., Seunghan L., & Sungchul, C.B. (2016). Culture potential of Thai climbing perch (*Anabas testudineus*) in experimental cages at different stocking densities in Kaptai Lake, Bangladesh. *Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society, 9*(3), 564-573.

Khatun, D., Hossain, M., Rahman, M., Islam, M., Rahman, O., Kalam Azad, M., ... Mawa, Z. (2019). Life-history traits of the climbing perch *Anabas testudineus* (Bloch, 1792) in a Wetland ecosystem. *Jordan Journal of Biological Sciences, 12*(2), 175-182.

Land Development Department of Nakhon Ratchasima Province. (2013). *Salinity management Committee soil Conservation measures and documentation and water and soil management land development.* Nakhon Ratchasima: Department Ministry of Agriculture and Cooperatives.

Patthumma, C. (2013). *Production of cultured fish Thailand with a growing proportion of fish plants differ* (Research report). Khon Kaen: Department of Fisheries, Faculty of Agriculture Khon Kaen University.

Pollution Control Department Ministry of Natural Resources and Environment. (2006). *Fish farming is environmentally friendly.* Bangkok: Ministry of Natural Resources and Environment.

Shinsuke, M., Sayaka, I., & Shoji, K. (2010). Growth and morphological development of laboratory-reared larval and juvenile snakeskin gourami *Trichogaster pectoralis. Ichthyological Research, 57,* 24–31.

Sugiyama, S., Staples, D., & Funge-Smith, S. (2004). *Status and potential of fisheries and aquaculture in Asia and the Pacific.* Bangkok: Food and Agriculture Organization of the United. Nations Regional Office for Asia and the Pacific.

Tippayada, N., Doolgindachbaporn, S., & Suksri A. (2015). Effect of salinity levels on growth and survival of Asian redtail catfish (*Hemibagus wyckioides*). *Research Journal, 8*(1), 45-51.

Yunus, Y.B.M. (2018). *The effect of density on growth condition of Anabas testudineus. UKE ssays.* Retrieved June 19, 2020, from https://www.ukessays.com/essays/sciences/effect-density-growth-condition-climbing-1575.php?vref=1

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Effects of Chitosan Concentrations in the Chitosan-Alginate Composite on the Quality of Mulberry Caviar during Storage

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Abstract

This research aimed to study the effect of chitosan concentration in the chitosan-alginate composite on the texture profile analysis and sensory properties, the correlation between instrumental and sensory properties, and the qualitative change of mulberry juice caviar (MJC) during storage. MJC with chitosan-alginate composite containing chitosan concentrations of 0, 0.5, 1.0 and 1.5% by-weight (ALG 1.5, CHI 0.5, CHI 1.0 and CHI 1.5, respectively) were prepared by spherification process. The results showed that the MJC formed with CHI 1.5 had the best sensory acceptance scores, while which of CHI 0.5 was easier consumed with lower texture profile analysis (TPA) parameters. Meanwhile, the high positive correlation (> 0.7) between the instrumental textures and sensory scores implied that most consumers favoured MJCs with high springiness, gumminess, and chewiness. In addition, chitosan-alginate composite prolonged the shelf-life of MJCs better than alginate alone, especially refrigeration temperature. CHI 1.5 was the most effective against changes in MJC size and total phenolic content, while CHI 1.0 was the best for preserving total anthocyanin content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and microbiological quality. This product gives a special texture during chewing when adding it into jelly or similar products and it can also use to decorate foods.

Introduction

Mulberries (*Morus* spp.) derive from a fast-growing commercial plant and are cultivated around the world (Khan et al., 2013). There are many countries that cultivate the mulberry tree to focus on leaf production as a food source for silkworms (Butkhup et al., 2013). Based on its pleasant taste and nutritional value, mulberry fruit consumption has rapidly increased in recent years (Yang et al., 2016). The mulberry fruit contains various

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antioxidants, e.g. phenolics, flavonoids, and anthocyanins (Donno et al., 2015; Sánchez-Salcedo et al., 2015; Tchabo et al., 2015; Gültekin-Özgüven et al., 2015). Several studies have reported that mulberry fruit extract possesses a wide range of biological activities including neuroprotective, antioxidant, and antiobesity effects, as well as the potential to prevent cardiovascular disease, immunomodulation, and antitumor activity (Chen et al., 2005; Lim et al., 2013; Sarikaphuti et al., 2013; Seo et al., 2015; Raman et al., 2016).

It is well known that mulberry fruits can be consumed as both fresh and processed fruits. For the fresh fruit, its thin skin contributes to rot and loss during transportation and storage, mainly due to scratching and postharvest diseases. Freezing is commercially available for extending the shelf-life of mulberry fruits. However, the formation of ice in frozen fruits negatively affects the texture and disruption of cell compartments, leading to the release of chemically reactive components (Lim et al., 2004; Sirijariyawat & Charoenrein, 2012). This leads to a change in the natural texture of the mulberry fruit. Even after the texture of a frozen mulberry fruit has changed, it remains useful as an ingredient in various products including jam and other sweet recipes. One interesting alternative method to use the mulberry fruit is the creation of a new food product by transforming frozen fruits into alginate bead called fruit caviar. This leads to a new form of food. Moreover, fruit caviar can be an interesting food in order to promote essential nutrition for elderly people in the future.

Spherification is one of the culinary processes as a basic method used in recent years that is suitable for fruit caviar production. Fruit juice can be formed into the spherical shapes of caviar through the interfacial polymerization of alginate (polymers consist of 1,4-linked β-D-mannuronic and α-L-guluronic acid residues) solution after exposure to the solution of divalent ions like calcium, i.e., calcium chloride, calcium lactate, etc (Lee & Rogers, 2012; Comaposada et al., 2015; Rodríguez-Sánchez et al., 2017). Finally, a gradual gelation of edible membrane around fruit caviars occurs (Anal & Singh, 2007). Fruit caviar can be made in many sizes depending on the method, application, and consumer acceptance. Noticeably, most fruit caviar is prepared in small sizes of approximately 2 to 4 mm because of its function as a topping on sweet dishes or to add piquancy to a favourite dish. Fruit caviars can also create a special texture during chewing, to the products that added them into, such as jelly, gel, or other similar products. Clearly, the edible membrane formed by alginate helps to protect the storage loss of antioxidants existing in the fruit juice. Moreover, the addition of functions into the alginate edible membrane is increasingly challenging, especially in terms of how to improve the antimicrobial properties in order to raise the potential for extending the shelf-life of fruit caviar.

Chitosan is a natural linear polymer produced by the deacetylation of chitin, which is a major component of the exoskeleton of crustaceans, i.e., shrimp, lobsters, and crabs (Xia et al., 2011). Chitosan is non-toxic and possesses a positive charge under a low pH solution. It has been proven by prior research to possess the potential to extend the shelf-life of certain kinds of foods (Farajzadeh et al., 2016; Aquino et al., 2015; Vieira et al., 2016). Likewise, this polymer has been reported for the activity of inhibiting and killing *Staphylococcus aureus* at concentrations of 16 and 32 μg/mL, respectively (Laokuldilok et al., 2017). In addition to its antimicrobial activity, chitosan has been used as a coating agent for reducing the porosity of alginate gel surface (Gombotz & Wee, 1998). Low concentrations (2-4 g/m^3) of chitosan coating agent have also been used for shell-making on alginate and gelatin capsules (Abbaszadeh et al., 2014; Sashiwa & Aiba, 2004; Zhou et al., 1998). However, the combination of chitosan and alginate could lead to ionic crosslinking between the two polymers, which may be better for the aspect of structural bead stability.

The challenge faced by this research was to produce mulberry juice caviar (MJC) from chitosan-alginate composite. Based on the highest scores for sensory overall acceptance, one of the suitable alginate concentrations would be selected for preparing the chitosan-alginate composite with different chitosan concentrations for further use to prepare MJCs. The objectives of this research were to study the effect of chitosan concentration in the chitosan-alginate composite on the texture profile analysis and sensory properties, the correlation between instrumental and sensory properties, and the qualitative change of mulberry juice caviar (MJC) during storage. Additionally, the correlation and regression of the instrumental texture and sensory properties are discussed in this paper.

Materials and methods

1. Materials and chemicals

Frozen mulberry fruit was purchased from a local

supermarket in Chiang Mai Province, Thailand. The powders of sodium alginate and chitosan (90% deacetylation degree) were bought from Union Science, Thailand. 2,2-Diphenyl-1-picrylhydrazyl (DPPH radicals), tartaric acid, Folin–Ciocalteu's reagent, and gallic acid were purchased from Sigma-Aldrich Chemie GmbH, Germany. Potato dextrose agar, plate count agar, and peptone were purchased from Merck KGaA, Germany. Potassium chloride, sodium acetate, calcium chloride, sodium carbonate, glacial acetic acid, distilled water, and ethanol were purchased from RCI Labscan, Thailand.

2. Preparation of mulberry juice

Frozen mulberry fruits were used for preparing the mulberry juice. Firstly, the ice in mulberry fruits was left to thaw for 60 min at room temperature (25-30°C). Subsequently, the thawed fruits were blended into puree using a blender. The fraction of juice and residue was separated by filtering through a white cloth. The obtained juice was diluted to 50% using water before being investigated for physicochemical properties as follows: moisture, 88.86%; total solid content, 11.14 degree brix; total acidity, 5.5% citric acid equivalent; pH, 3.0; total phenolic content, 260.59 mg gallic acid equivalent (GAE)/L; total anthocyanin content, 120.26 mg/L; DPPH radical scavenging activity, IC_{50} = 2.25 µg/mL. The natural color of the mulberry juice was deep red, while its color parameters for L^* (lightness), C^* (chroma), $h°$ (hue angle) were 20.25, 4.20, and 80.59 respectively. The mulberry juice was used for preparing MJCs

3. Preparation of MJCs with chitosan-alginate composite

Preparation of MJCs with chitosan-alginate composite is shown in Fig. 1. Firstly, 1.5% (w/v) of alginate solution was prepared by dissolving 1.5 g of sodium alginate with distilled water (50 mL) under magnetic agitation at 80ºC for 15 min. After that, the alginate solution was left to cool (below 40ºC). Meanwhile, chitosan solutions with different concentrations were also prepared. Different amounts of chitosan powders (0.5, 1.0, and 1.5 g) were dissolved using 1.0% (v/v) acetic acid solution (25 mL) together with heating at 80°C for 15 min. The chitosan solutions were mixed with 1.5% alginate solution, after which the chitosan-alginate mixtures were stirred using an overhead stirrer (RW20, IKA Works, NC, USA) at 250 rpm for 5 min. According to the preliminary results for preparing the chitosan-alginate composite, the by-weight ratio of alginate to chitosan solution as 2:1 (w/w) was considered as the most suitable ratio based on the gel-forming properties and the mixture's viscosity. After that, mulberry juice (25 g) was added into the chitosan-alginate solutions and the weight of the mixtures were adjusted to 100 g using distilled water. These mixtures were homogenized at 12,000 rpm for 3 min using a homogenizer (Ultra-Turrax T25, IKA, Malaysia). The homogenized mixture was sonicated for 20 min to remove air bubbles prior to loading into a burette having an orifice size of 2 mm. The distance between the orifice end and the surface of 1.0% (w/v) calcium chloride solution was set at 5 cm. The mixture was allowed to form a liquid drop and dripped by gravity force for exposure with the calcium chloride solution. At this point, the MJCs were formed and immersed in a high-calcium ion environment for 30 min before being sieved through a 20-mesh sieve. The MJCs were washed with drinking water at least 5 times before being analyzed. All the obtained MJCs consisted of chitosan-alginate composite containing 1.5% (w/w) alginate and three different concentrations of chitosan (0.5, 1.0, and 1.5%, w/w). These MJCs were identified as CHI 0.5, CHI 1.0, and CHI 1.5, respectively. Several properties of the MJCs formed with chitosan-alginate composite were studied and compared to the MJC containing only 1.5% (w/w) alginate, called ALG 1.5.

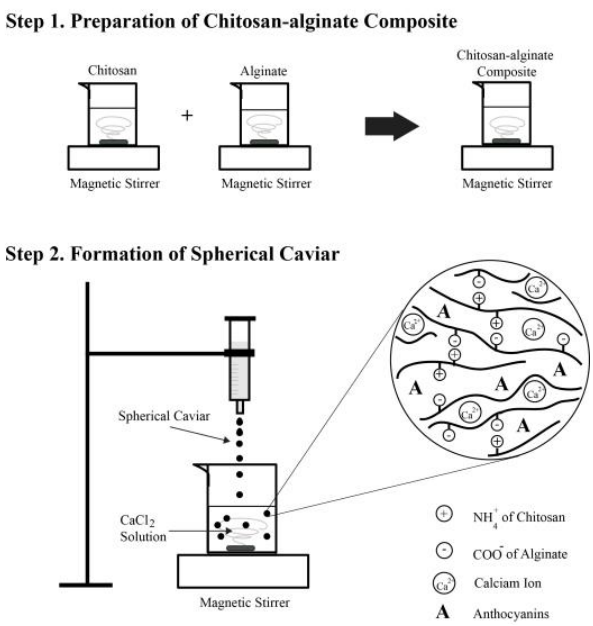

Fig. 1 The scheme shows the preparation of MJCs with chitosan-alginate composite

4. Production yield

This parameter was calculated as the ratio of the mass of collected beads (m_s) per the mass of all raw materials (m_i), as given in below equation.

$$\text{Production yield (\%)} = \left(\frac{m_s}{m_i}\right) \times 100$$

5. Determination of total phenolic content

Total phenolic content was determined using a Folin–Ciocalteu assay according to the method of Xu & Chang (2008) with some modification, while pure, authentic garlic acid was used as a standard phenolic for creating the calibration curve. Sample (50 µL), distilled water (3,000 µL), 250 µL of Folin–Ciocalteu's reagents, and 7% $NaCO_3$ (750 µL) were mixed into a 15-mL test tube and incubated for 10 min at ambient temperature. After that, distilled water (950 µL) was immediately added followed by vigorous mixing. The mixture was placed in a dark room for 2 h under ambient temperature before the absorbance of each sample was measured at 765 nm against a blank. Finally, total phenolic content was reported as milligram gallic acid equivalent per 100 g dry basis (mg GAE/100 g d.b.) by calibrating with a standard linear plot of gallic acid with linearity range of 50 to 1000 µg/mL ($R^2 = 0.992$).

6. Determination of total anthocyanin content

Total anthocyanin content was determined using the pH differential method described by Giusti & Wrolstad (2001). Briefly, the sample was diluted using potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The absorbance of both sample-buffer mixtures was read at 510 nm (A_{510}) and 700 nm (A_{700}) by a UV-visible spectrophotometer (Genesys 10, Thermo Scientific, NY, USA), which required calculating the absorbance difference (A_{diff}). The total anthocyanin content was calculated as milligram cyanidin-3-*O*-glucoside per liter (mg C3G/L) using the below equation and reported as mg C3G per 100 g d.b.

Where, MW is the molecular weight of C3G (449.39 g/mol). DF is the dilution factor, while the molar absorptivity (ε) is 26,900 l/mol.cm and the path length (l) is 1.0 cm.

7. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method described by Gülçin et al. (2006) with some modifications. Firstly, the sample (1.0 mL) was added into 99% ethanol solution (3.0 mL) of 0.1 mM DPPH solution (the absorbance at 517 nm is 0.40 ± 0.02). After that, the mixture was shaken vigorously for 30 sec and left to stand in the dark for 30 min at ambient temperature. Then, the absorbance of the sample (A_{sam}) was read at 517 nm against a blank by using a UV-visible spectrophotometer (Genesys 10, Thermo Scientific, NY, USA). For the control solution, the sample was replaced by distilled water and the absorbance of control (A_{con}) was measured against a blank. The percentage of DPPH radical discoloration of the sample was calculated using the following equation:

Finally, the antioxidant concentration inhibiting 50% radicals (EC_{50}) was calculated and reported as the radical scavenging activity

8. Microbiological analysis

Serial dilutions (10^{-1},10^{-2}, and 10^{-3}) of the sample were prepared with sterile peptone water. Volumes of each dilution sample (1.0 mL) were poured into 3 plates. Total plate counts were enumerated by using the pour plate technique on plate count agar (PCA). Incubation was done at 37°C for 48 h. Total yeast and moulds were enumerated using the surface plate technique on potato dextrose agar (PDA), plus 10% tartaric acid. Incubation for total yeast and mould counts was done at 37°C for 5 days. Each test was performed in triplicate. The results were expressed as colony-forming units (CFU) per mL.

9. Texture profile analysis

Texture profile analysis was done according the method described by Suebsaen et al. (2019) with slight modification. A texture analyser (TA-XT.Plus, Stable Micro Systems, Surrey, UK) connected to a compression probe of P/50 with 50 mm diameter was used, in which 75% strain and 2 mm/sec speed were set to determine the texture properties. The sample was stored in a cool and dry place at ambient temperature (25 ± 1°C) for 30 min before being measured. Fifty replicates were

$$A_{diff} = (A_{510} - A_{700})\ \text{pH 1.0} - (A_{510} - A_{700})\ \text{pH 4.5}$$

$$\text{Total anthocyanin content (mg C3G/L)} = \left(\frac{\text{Adiff} \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times l}\right)$$

investigated for each sample and the data for 6 texture profiles was collected, including hardness (N), adhesiveness (g.sec), cohesiveness, springiness, chewiness (g), and gumminess (g).

10. Sensory evaluation

Sensory properties were evaluated by 50 consumers using a 9-point hedonic scaling test. The samples were tasted by the consumers, who scored them using 4 parameters including color, flavor, taste, and overall acceptability.

11. Physicochemical analysis

Moisture and titratable acidity were determined according to the method described by AOAC (2000). The *L*C*h°* color space was used to describe the color properties, which were measured using a colorimeter (CR-410, Konica Minolta, Japan). Fruit caviar size was measured using a digital Vernier caliper. Bulk density was investigated according to the method of Jinapong et al. (2008), in which samples were loaded into a 10-mL cylinder to the 10-mL mark and weighed. Bulk density was calculated by dividing the weight with the volume of the sample at the 10-mL mark.

12. Changes in fruit caviar quality under different storage temperatures

To mimic the actual storage conditions, a sample (20 g) was placed into a glass clear bottle, which was stored in a refrigerator and at room temperature. All samples were stored for 12 days and analysed for their physico-chemical properties (size, total phenolic content, total anthocyanin content, and DPPH free radical scavenging activity) every 3 days. Importantly, changes in the growth of bacteria and fungi as yeasts and moulds were also investigated.

13. Statistical analysis

All analyses were performed in triplicate. Data was analysed using IBM SPSS Statistics for Windows version 20 (IBM, Armonk, NY, USA.). The differences between values were considered significant at $p \leq 0.05$. The averages were calculated by Duncan's new multiple range test. Pearson's correlation coefficients were applied to explain the relationship between parameters. The analysis was also executed using IBM SPSS Statistic version 20 software.

Results and discussion

1. Physicochemical properties of MJCs with chitosan-alginate composite

Mulberry juice was mixed with chitosan-alginate composite in order to create the MJC. All MJCs exhibited a spherical shape with different sizes depending on chitosan concentration. It is well known that a spherical shape is normal for alginate bead products produced by using the extrusion-dipping technique (Lee et al., 2006; Chan et al., 2011)

As given in Table 1, the yield of MJCs was in the range of 73.99% to 82.75%, in which CHI 1.5 was the chitosan-alginate composite providing the highest yield ($p \leq 0.05$). All of the MJCs were similar in size between 3.27 and 3.82 mm and tended to be smaller with higher chitosan concentrations. All the MJCs had bulk density in the range of 0.68 to 0.76 g/cm^3, which was higher in bulk density with smaller MJC size. These MJCs were very juicy with high moisture contents (95.28-96.50%). For analysis of total phenolic and total anthocyanin contents, 1 mg/mL aqueous MJC extract was used for this section. The result was found that these antioxidants tended to have lower content when the chitosan concentration was higher. In the case of antioxidant activity, DPPH radical scavenging activity of all MJCs (1 mg/mL aqueous MJC extract) was in the range of 110.97 to 119.30 µg/mL, which was related to the total phenolic and total anthocyanin contents for all. In addition, it was obvious that the difference in the MJC's color parameters depended on the chitosan concentrations. Noticeably, the *L** and *C** values were significantly enhanced with increasing chitosan concentrations ($p \leq 0.05$). Meanwhile, the h values of MJCs varied depending on each concentration of chitosan ($p \leq 0.05$) and expressed the different shades of red to dark red. According to the results, it was probable that the network structures of MJCs prepared with CHI 1.0 and CHI 1.5 were stronger when compared to those of CHI 0.5 and ALG 1.5.

With the stronger structure, less water inside MJCs was released due to the dense crosslinking between the interactions of both alginate-Ca^{2+} and alginate-chitosan. As a result, the strength of these interactions held each of near polymer chains together, becoming MJCs with smaller size, which also resulted in greater bulk density. However, the increased strength of the MJC structure could cause difficulty for the antioxidants in order to be leached away by the solvent during extraction, leading to lower contents of phenolics and anthocyanins as well as lower antioxidant activity.

2. Instrumental texture properties and sensory score of MJCs with chitosan-alginate composite

In this study, the texture profile analysis (TPA) of

MJCs was investigated in order to mimic human mastication and performed using a two-bite compression test to explain the textural properties during oral processing (Chen, 2009; Sharma et al., 2017). The results of the instrumental texture investigation for all MJCs are summarised in Table 2. A significant difference was clearly observed between ALG 1.5 and CHI 0.5 ($p \leq 0.05$). Most TPA parameters tended to have a lower value, except for adhesiveness. For hardness, springiness, gumminess, and chewiness, all parameters tended to have higher magnitudes when the percentage of chitosan concentrations increased from 0.5 to 1.5. Meanwhile, the adhesiveness of MJCs showed a higher negative value depending on the increasing concentrations of chitosan. A significant difference in cohesiveness was found in the MJC formed with CHI 1.5, showing the lowest value ($p \leq 0.05$).

When the switching of ALG 1.5 towards CHI 0.5 was performed, the negligible chitosan fractions might interfere with the network structure formation through the crosslinking of alginate and divalent calcium ions, leading to the significant soft-shell structure of spherical MJCs. For the hardness value, it mimics the highest force used to bite down into food during the first bite. With greater fractions of chitosan, MJCs having greater hardness were formed. Similarly, a strong network structure was created through electrostatic interaction between the residue of the chitosan's ammonium ion (NH_4^+) and the opposite alginate's carboxylate (COO^-), which were more intensified with increasing chitosan fractions (Lin et al., 2005; Pasparakis & Bouropoulos, 2006). As an interval of the first bite, the work required to overcome the attractive forces between the food and instrumental probe (mimicking the human teeth) is defined as adhesiveness (Tunick, 2000). According to the adhesiveness results, more adhesive MJCs were observed with those of greater chitosan concentrations, but still lower than the MCJ without chitosan. After the first bite, the springiness values for all MJCs with chitosan combination below the MJC without chitosan implied that the chitosan-alginate composite could strongly contribute to the ease of structural deformation during consumption. Surprisingly, the MJC formed with CHI 1.5 had the lowest cohesiveness, indicating the decreased strength of the MJC's internal bonds making it easier for disintegration into fragments while swallowing (Rosenthal, 1999). In contrast, MJCs formed with CHI 1.5 exhibited the highest values for both gumminess and chewiness, which implied that more energy was needed to chew the MJCs and make them ready for swallowing compared to that of ALG 1.5, CHI 0.5, and CHI 1.0. The lowest values for both gumminess and chewiness were found in the MJC formed with CHI 0.5, indicating that it was easily disintegrated with minimal chewing effort.

The sensory evaluation results for prepared MJCs are shown in Table 2. It was apparent that different chitosan concentrations did not affect the sensory score in terms of sourness and overall acceptance ($p > 0.05$). For appearance, color, sweetness, and texture, all samples tended to have similar scores. Noticeably, the color and texture scores for the MJC formed with CHI 0.5 were significantly lower than other concentrations ($p \leq 0.05$). This might be attributable to the instability of the bead structure (Huang & Lin, 2017; Orive et al., 2006), leading to the release of liquid to the exterior of beads in the interval of being weighed for testing a sensory attribute. As a result, the bead structure collapsed and the shriveled texture was more intensified for a darker color. This result was not acceptable among the consumers.

Table 1 Properties of MJCs prepared with chitosan-alginate composite

Chitosan concentrations (%, w/w)	Yield (%)	Size (mm)	Bulk density (g/cm^3)	Physicochemical properties						
				MC^{ns} (%)	TPC (mg GAE/100 g d.b.)	TAC (mg C3G/100 g d.b.)	EC50 (µg/mL)	*L**	*C**	*h°*
ALG 1.5	75.45^{bc}±2.42	3.82^{a}±0.04	0.68^{b}±0.03	95.35±0.00	3718.64^{a}±77.60	440.70^{a}±43.31	110.97^{b}±6.53	24.63^{c}±0.27	4.53^{c}±0.20	35.67^{a}±0.79
CHI 0.5	73.99^{c}±0.64	3.63^{b}±0.15	0.70^{b}±0.02	95.47±0.51	3360.22^{ab}±267.82	383.22^{b}±10.98	109.28^{b}±2.96	30.56^{b}±0.29	7.01^{b}±0.36	4.52^{d}±0.77
CHI 1.0	78.19^{b}±0.62	3.28^{c}±0.16	0.74^{a}±0.01	95.30±0.65	3333.33^{ab}±229.68	370.76^{b}±21.17	119.25^{a}±2.98	33.64^{a}±0.93	8.90^{a}±0.70	9.79^{b}±0.76
CHI 1.5	82.75^{a}±2.48	3.27^{c}±0.24	0.76^{a}±0.01	94.99±0.04	2912.19^{b}±388.00	342.50^{b}±16.59	119.30^{a}±2.43	33.74^{a}±0.42	8.51^{a}±0.93	7.11^{c}±0.80

Remark: MC; Moisture content, TPC; Total phenolic content, TAC; Total anthocyanin content, EC_{50}; the effective concentration of antioxidants that can scavenge 50% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals when tested by DPPH radical scavenging activity.
Data indicate the average (standard deviation) of three replicates (n = 3), except for size (n = 30).
Different superscript letters in the same column indicate significant difference at $p \leq 0.05$.
^{ns} indicates non-significant difference ($p > 0.05$).

Table 2 The instrumental texture properties and sensory attributes of MJCs with chitosan-alginate composite

	ALG 1.5	CHI 0.5	CHI 1.0	CHI 1.5
Instrumental texture properties				
Hardness (N)	1.45^{c} ±0.05	1.23^{d}±0.05	1.75^{b}±0.06	3.23^{a}±0.08
Adhesiveness (g.sec)	-1.38^{d} ±0.19	-0.35^{a}±0.04	-0.63^{b}±0.03	-1.15^{c}±0.05
Springiness	0.47^{a}±0.02	0.31^{d}±0.01	0.37^{c}±0.01	0.40^{b}±0.01
Cohesiveness	0.51^{a}±0.00	0.47^{a}±0.04	0.50^{a}±0.01	0.31^{b}±0.01
Gumminess (g)	75.59^{c}±2.98	59.31^{d}±7.21	89.14^{b}±1.38	102.99^{a}±2.49
Chewiness (g)	35.16^{b}±2.03	18.37^{c}±2.23	32.39^{b}±0.75	41.18^{a}±0.94
Sensory attributes				
Appearance	7.94^{ab}±0.59	7.11^{b}±0.38	8.06^{a}±0.42	8.44^{a}±0.35
Color	7.39^{a}±0.72	5.94^{b}±0.59	7.50^{a}±0.29	7.67^{a}±0.17
Sweetness	4.67^{b}±0.17	5.22^{ab}±0.25	5.28^{ab}±0.38	5.44^{a}±0.59
Sourness^{ns}	5.50±0.87	6.33±0.17	5.61±0.69	5.94±0.77
Texture	8.28^{a}±0.51	6.33^{b}±0.33	8.11^{a}±0.63	8.17^{a}0.29
Overall acceptance^{ns}	7.06±0.25	6.56±0.42	7.06±0.42	7.33±0.44

Remark: Data indicate the average standard deviation of fifty replicates. Different superscript letters in the same row indicate significant difference at $p \leq 0.05$.
^{ns} indicates non-significant difference ($p > 0.05$).

3. Correlation for the relationship between the instrumental texture and sensory properties of MJCs with chitosan-alginate composite prepared using different chitosan concentrations

As seen in Table 3, the instrumental texture properties including hardness, adhesiveness, springiness, gumminess, and chewiness were correlated with the sensory properties including appearance, color, texture, and overall acceptance. In this work, the correlation coefficient (r) equal to 0.7 or higher was considered a high relationship. The correlation results of all the MJCs showed that higher springiness contributed to a higher texture score ($r = 0.711$, $p \leq 0.01$). Meanwhile, high correlation coefficients were found between the gumminess and sensory scores of both appearance ($r = 0.798$, $p \leq 0.01$) and color ($r = 0.824$, $p \leq 0.01$). Likewise, chewiness was highly correlated with appearance ($r = 0.762$, $p \leq 0.01$), colour ($r = 0.863$, $p \leq 0.01$), and texture ($r = 0.825$, $p \leq 0.01$). According to the results, the high positive correlation between the instrumental textures and sensory scores implied that most consumers favoured MJCs with high springiness, gumminess, and chewiness. Apparently, small-size MJCs with high springiness, gumminess, and chewiness enhanced the joyful feeling when eating, although MJCs could be swallowed easily. Additionally, more chewing was required to be confident about safe swallowing. However, food with greater springiness and chewiness requires more chewing (Wee et al., 2018). Thus, this product may not be appropriate for older adults if considering the aspect of energy usage for chewing to get the bolus for safe swallowing. It is suggested that MJCs are of greater benefit as toppings for a favourite dish because they are rich sources of antioxidants.

Table 3 Pearson's correlation coefficients for the relationship between the instrumental texture properties and sensory attributes of MJCs with chitosan-alginate composite

Instrumental Texture profiles	Sensory attributes					
	Appearance	Color	Sweetness	Sourness	Texture	Overall
Hardness	0.640*	ns	ns	ns	ns	ns
Adhesiveness	ns	-0.644*	ns	ns	-0.662*	ns
Springiness	ns	0.595*	ns	ns	0.711**	ns
Cohesiveness	ns	ns	ns	ns	ns	ns
Gumminess	0.798**	0.824**	ns	ns	0.698*	0.635*
Chewiness	0.762**	0.863**	ns	ns	0.825**	0.642*

Remark: * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). ns is not significant ($p > 0.05$).

4. Physicochemical and microbiological changes in MJCs under different storage temperatures

4.1 Changes in physicochemical quality

Change in the size of MJCs is one of the most important indicators for determining the stability of alginate bead product, which can influence the texture and sensory properties. As seen in Fig. 2, MJCs stored under both temperature ranges tended to be smaller in size throughout the storage period. After storage, the storage of MJCs formed with ALG 1.5, CHI 0.5, CHI 1.0, and CHI 1.5 under a refrigeration temperature ranging between 5 and 10°C showed they were shrunken by 5.2, 4.3, 3.2, and 2.1%, respectively, when compared with the initial size. Storage under ambient temperature ranging between 25 and 30°C resulted in greater shrinkage of the spherical shape by 10, 6.4, 4.7, and 3.4%, respectively. Apparently, the higher storage temperatures negatively affected the MJC size through the encouragement of mass transfer of water. MJCs stored in a refrigerator were more stable than those stored at ambient temperature. Interestingly, using a greater proportion of chitosan than alginate in the chitosan-alginate composite contributed to the stability of the MJC size via less shrinkage of the sphere, likely as the result of releasing water to the exterior of beads. With the high chitosan fraction, chitosan and alginate film layer could reduce the diffusion rate of water through the crosslinking of chitosan with alginate by intermolecular bonds (e.g., electrostatic and hydrogen bonding). The network of this bonding might be increasingly dense with the increasing of chitosan concentrations, leading to fewer changes in MJC size.

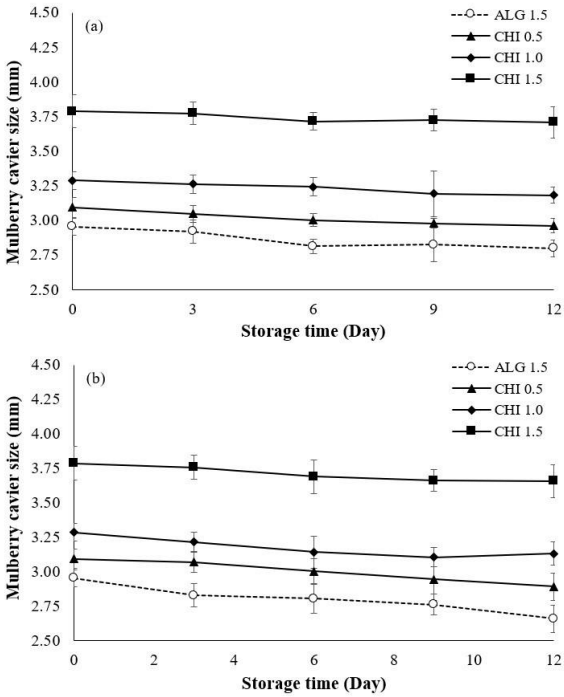

Fig. 2 Changes in size of MJCs formed with chitosan-alginate composite under (a) refrigerator (5–10°C) and (b) ambient temperatures (25–30°C)

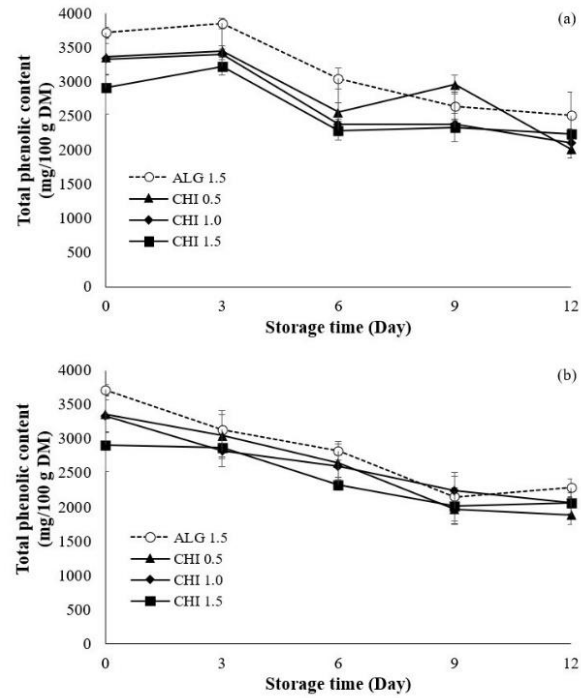

Fig. 3 Changes in total phenolic content of MJCs formed with chitosan-alginate composite under (a) refrigerator (5–10°C) and (b) ambient temperatures (25–30°C)

Total phenolic content is one of the most important indicators for explaining the content of hydrophilic antioxidants in the example. As seen in Fig 3, total phenolic contents decreased throughout the storage time. Under chilling temperature, total phenolic contents of all MJCs were reduced by 32.53, 40.00, 36.83 and 23.08%, respectively. A greater loss in percentage of phenolics was found when MJCs were stored under ambient temperature by 38.54, 44.00, 38.17, and 29.23%, respectively. Seemingly, the higher storage temperatures negatively affected the MJC size through the encouragement of the mass transfer of water. MJCs stored in a refrigerator were more stable than those stored at ambient temperature, which could activate phenolic degradation, as proven by several previous research (Serea et al., 2014; Moldovan et al., 2016; Galani et al., 2017). The MJC formed with CHI 1.5 could retard the change of total phenolic content greater than others. In this case, the strong network structure formed by high chitosan concentrations might have an important role as a barrier for reducing the negative effects associated with the temperature on phenolic compounds.

Total anthocyanin content is an important indicator for food products containing a rich source of anthocyanins, which is an antioxidant found in abundance in black mulberry fruit (Jiang & Nie, 2015). According to Fig. 4, the total anthocyanin contents of all MJCs stored under refrigeration temperature range gradually decreased when increasing the storage time (Fig. 4 (a)). Evidently, an undesirable effect was observed in all MJCs stored under ambient temperature (Fig. 4 (b)). Their total anthocyanin contents were dramatically decreased and disappeared around Day 6 for MJCs formed with all the chitosan-alginate composite of CHI 0.5, CHI 1.0, and CHI 1.5, and Day 9 for the one formed with ALG 1.5 (without chitosan). Degradation under high ranges of storage temperature implied that the storage temperature directly affected the total anthocyanin contents. It is widely recognised that anthocyanins are unstable and extremely sensitive to high temperature conditions (Ersus & Yurdagel, 2007; Kırca et al., 2007; Laokuldilok & Kanha, 2017). In addition, the change of total anthocyanin content in MJCs also depended on chitosan concentration. During storage, the MJC formed without chitosan had lower anthocyanin loss than that containing chitosan. With the ionic interaction between flavylium cations and ammonium ions, it is able to form a repulsive force (Xie et al., 2001), resulting in the instability of the

anthocyanin molecule. Therefore, chitosan might not be suitable for preparing alginate beads containing anthocyanins.

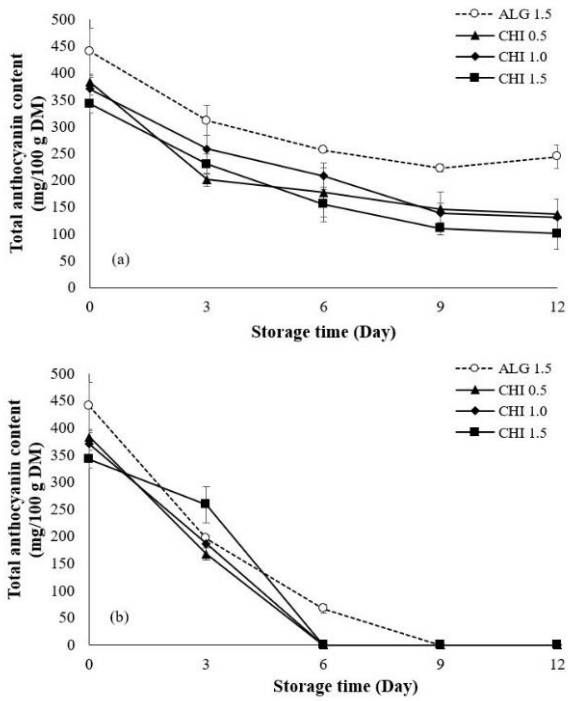

Fig. 4 Changes in total anthocyanin content of MJCs formed with chitosan-alginate composite under (a) refrigerator (5–10°C) and (b) ambient temperatures (25–30°C)

DPPH radical scavenging activity is used to explain the antioxidant activity. The results of the change in DPPH radical scavenging activity of MJCs stored at different storage temperature ranges were reported as the effective concentration of antioxidants able to scavenge 50% of all DPPH radicals, also called the EC_{50} value (Fig. 5). Before storage, MJCs prepared by ALG 1.5, CHI 0.5, CHI 1.0, and CHI 1.5 exhibited EC_{50} values of 112.80, 154.72, 149.38, and 182.19 µg/mL, respectively. After storage, all MJCs tended to have a higher EC_{50} value. The EC_{50} values of all MJCs stored under refrigeration temperature range were 124.01, 155.56, 171.02, and 230.94 µg/mL, increasing by 9.94, 0.54, 14.49 and 26.75%, respectively. Likewise, higher EC_{50} values, e.g. 277.66, 235.44, 342.11, and 331.12 µg/mL, were found for storage at higher temperature, increasing by 146.25, 52.17, 129.02 and 81.74%, respectively. According to the results, CHI 0.5 and CHI 1.0 were the chitosan-alginate composites providing MJCs with the strongest antioxidant activity under ambient and refrigerator temperatures, respectively. Possibly, the porosity and strength of the MJC's surface might provide important contributions. In addition, changes in antioxidant activity were found as a function of storage time and temperature. A higher ambient temperature than refrigeration temperature also contributed to the larger degradation of antioxidants, which could be degraded thermally. With the results of EC_{50} values among the two temperatures, the differences might be attributable to three cases including (1) total phenolic contents, (2) types of phenolics, and (3) the association of antioxidants with other molecules, *i.e.* chelation of metallic ions with anthocyanins, leading to the loss of the proton donation group.

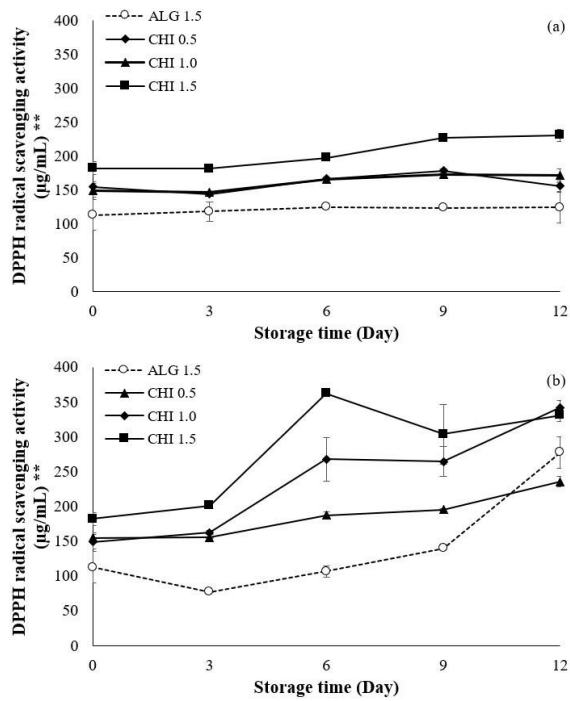

Fig. 5 Changes in DPPH radical scavenging activity of MJCs formed with chitosan-alginate composite under (a) refrigerator (5–10°C) and (b) ambient temperatures (25–30°C). **Reported as EC_{50} in the unit of µg/mL

4.2 Changes in microbiological quality

As given in Table 4, the counts for total bacteria and total yeasts as well as moulds at the initial storage time were lower than 1 CFU/mL, which confirmed that the freshly prepared MJCs were safe for consumption. At the end of storage, the microbiological changes of MJCs could be observed clearly under different storage temperatures. Bacteria could clearly grow under refrigeration temperature less than ambient temperature.

Total bacteria count below 1 CFU/mL throughout 12 days of storage was found in all MJCs with chitosan-alginate composite stored in a refrigerator. MJCs with chitosan for all concentrations could retard bacterial growth better than MJCs without chitosan under the same storage temperature. The change of total yeast and mild count during storage was found to be different from the total bacteria count, likely due to the greater growth of yeast and mild compared to bacteria (Table 4). Yeast and mild grew under a refrigeration temperature less than ambient temperature. Under the same storage temperature, MJCs with all chitosan concentrations retarded the increase of total yeast and mild count better than MJCs without chitosan. Especially for the MJC formed by CHI 1.0, the best microbiological quality resulted. This result was possible to be relevant to the weak structure of MJC with CHI 0.5 which might lead to the relaxation of the bead structure and opening of the chitosan chain's end. Thus, the growth of yeast and mould were easily inhibited by the action of chitosan during days 6 to 9 of storage before the mechanism of microbial growth would be changed back to be controlled by a function of temperature on days 9 to 12, due to the excessive microbial number. With the higher chitosan concentration, so that MJC with CHI 1.0 had the greater ability to inhibit yeast and mould compared to MJC with CHI 0.5. Meanwhile, dense cross-linking interaction in MJC with CHI 1.5 caused the difficulty of composite hydrocolloid to relax on the day 3 to 6, thus the number of the ammonium cation of chitosan for binding to negatively charged phospholipids of fungi was expected to be less than those of the other chitosan concentrations. When this bead can not inhibit microorganisms effectively, microbial growth is better even though it was considered at the same temperature range. The antimicrobial mechanism of MJCs with chitosan-alginate composite is attributable to the ionic interaction between the chitosan's cation and the bacteria's anion, resulting in increased permeability and intracellular leakage along with the loss of the function of nutrient transportation (Liu et al., 2004). The antibacterial activity of chitosan has been reported by several research (Zhang & Zhu, 2003; Liu et al., 2006; Laokuldilok et al., 2017). With the similar mechanism of chitosan against bacterial growth, the antifungal activity has been reported on by many previous research (Peña et al., 2013; Jaimun et al., 2018).Therefore, chitosan-alginate composite could prolong the shelf-life of MJCs better than using alginate alone.

Table 4 Microbiological change of MJCs with chitosan-alginate composite under two storage temperature ranges

Temperature		Storage time (Day)				
	(ºC)	0	3	6	9	12
Total bacterial counts						
ALG 1.5	4–10	< 1	3.33	3.33	13.33	1833.33
	25–30	< 1	116.67	1100	> 2000	> 2000
CHI 0.5	4–10	< 1	< 1	< 1	< 1	< 1
	25–30	< 1	< 1	10	866.67	> 2000
CHI 1.0	4–10	< 1	< 1	< 1	< 1	< 1
	25–30	< 1	< 1	< 1	10	16.67
CHI 1.5	4–10	< 1	< 1	< 1	< 1	< 1
	25–30	< 1	< 1	6.67	980	> 2000
Total yeast and mould counts						
ALG 1.5	4–10	< 1	560.00	636.67	703.33	2333.33
	25–30	< 1	> 2000	> 2000	> 2000	> 2000
CHI 0.5	4–10	< 1	6.67	33.33	73.33	806.67
	25–30	< 1	3.33	16.67	230	> 2000
CHI 1.0	4–10	< 1	< 1	6.67	20.00	70.00
	25–30	< 1	< 1	16.67	53.33	63.33
CHI 1.5	4–10	< 1	< 1	6.67	46.67	86.67
	25–30	< 1	6.67	10.00	946.67	> 2000

Remark: Data show the change in amounts of microbials (CFU/mL)

Conclusion

Mulberry juice caviar prepared from chitosan-alginate composite with different concentrations of chitosan had different characteristics in terms of physicochemical, textural, and sensory attribute properties. CHI 1.5 was the chitosan-alginate composite providing the highest yield. Chitosan concentrations importantly contributed to all physicochemical properties, which were possibly related to the dense crosslinking between the interactions of both alginate-Ca^{2+} and alginate-chitosan. Such interaction also influenced all TPA parameters. The negligible chitosan fractions might interfere the network structure formation of MJCs with CHI 0.5, leading to the softer shell and spherical structure. MJCs formed with CHI 0.5 were easier for structural deformation while eating based on the lower magnitude of hardness, adhesiveness, springiness, gumminess, and chewiness. However, CHI 1.5 possessed the best sensory acceptance scores. On the other hand, higher instrumental texture properties for springiness, gumminess, and chewiness resulted in higher sensory attributes of texture, appearance, and colour ($r > 0.711$, $p \leq 0.01$), which implied that most consumers favoured MJCs with high springiness, gumminess, and chewiness. Linear regression analysis confirmed the significant linear models ($p \leq 0.001$) with a high R^2 (>0.6) for the gumminess and chewiness as well as the sensory properties of colour and texture. In addition, the storage of MJCs under refrigeration

conditions could greatly preserve their physicochemical and microbiological properties. CHI 1.5 showed the highest effectiveness against changes in MJC size and total phenolic content, while CHI 1.0 was the most effective for total anthocyanin content and DPPH free radical scavenging activity as well as the preservation of microbiological quality. Thus, it could be confirmed that chitosan-alginate composite has the ability to prolong the shelf-life of MJCs better than using only alginate. For commercial applications, MJC is of natural raw material and it can be used to add nutrition to some products such as jelly, gel, or similar products, along with the decoration of foods and giving of a special texture during chewing. Further research should be studied more on the use of the spherification process to produce a nutrient-packing sphere for a culinary application.

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References

Abbaszadeh, S., Sharifzadeh A., Shokri, H., Khosravi, A.R., Abbaszadeh, A. (2014). Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. *Journal de Mycologie Médicale, 24,* 51–56.

Anal, A.K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology, 18,* 240–251.

AOAC (2000). *Official methods of the association of official analytical chemists.* Washington, D.C.: Association of Official Analytical Chemists.

Aquino, A.B., Blank, A.F., & Santana, L.C.L.A. (2015). Impact of edible chitosan-cassava starch coatings enriched with Lippia gracilis Schauer genotype mixtures on the shelf life of guavas (*Psidium guajava* L.) during storage at room temperature. *Food Chemistry, 171,* 108–116.

Butkhup, L., Samappito, W., & Samappito, S. (2013). Phenolic composition and antioxidant activity of white mulberry (*Morus alba* L.) fruits. *International Journal of Food Science and Technology, 48,* 934–940.

Chan, E.S., Lim, T.K., Voo, W.P., Pogaku, R., Tey, B.T., & Zhang, Z. (2011). Effect of formulation of alginate beads on their mechanical behavior and stiffness. *Particuology, 9,* 228–234.

Chen, J. (2009). Food oral processing – A review. *Food Hydrocolloids, 23*(1), 1–25.

Chen, C.C., Liu, L.K., Hsu, J.D., Huang, H.P., Yang, M.Y., & Wang, C.J. (2005). Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food Chemistry, 91,* 601-607.

Comaposada, J., Gou, P., Marcos, B., & Arau, J. (2015). Physical properties of sodium alginate solutions and edible wet calcium alginate coatings. *LWT – Food Science and Technology, 64* (1), 212-219.

Donno, D., Cerutti, A.K., Prgomet, I., Mellano, M.G., & Beccaro, G.L. (2015). Foodomics for mulberry fruit (*Morus* spp.): Analytical fingerprint as antioxidants and health properties determination tool. *Food Research International, 69,* 179–188.

Ersus, S., & Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. *Journal of Food Engineering, 80,* 805-812.

Farajzadeh, F., Motamedzadegan, A., Shahidi, S., & Hamzeh, S. (2016). The effects of chitosan-gelatin coating on the quality of shrimp (*Litopenasu vannamei*) under refrigerated condition. *Food Control, 67,* 163-170.

Galani, J. H.Y., Mankad, P.M., Shah, A.K., Patel, N.J., Acharya, R.R., & Talati, J.G. (2017). Effect of storage temperature on vitamin C, total phenolics, UPLC phenolic acid profile and antioxidant capacity of eleven potato (*Solanum tuberosum*) varieties. *Horticultural Plant Journal, 3*(2), 73–89.

Giusti, M.M., & Wrolstad, R.E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. In R.E. Wrolstad (Ed.), *Current Protocols in Food Analytical Chemistry* (pp. F1.2.1 – F1.2.13). New York: John Wiley & Sons.

Gombotz, W.R., & Wee, S.F. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews, 31,* 276–285.

Gültekin-Özgüven, M., Davarcı, F., Paslı, A.A., Demir, N., & Özçelik, B. (2015). Determination of phenolic compounds by ultra-high liquid chromatography-tandem mass spectrometry: Applications in nuts. *LWT - Food Science and Technology, 64*(1), 42–49.

Gülçin, I., Elias, R., Gepdiremen, A., & Boyer, L. (2006). Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). *European Food Research and Technology, 223,* 759–767.

Huang, S.L., & Lin, Y.S. (2017). The size stability of alginate beads by different ionic crosslinkers. *Advances in Materials Science and Engineering,* 1–7.

Jaimun, R., Sangsuwan, J., Intipunya, P., & Chantrasri, P. (2018). Active wrapping paper against mango anthracnose fungi and its releasing profiles. *Packaging Technology and Science, 31,* 421–431.

Jiang, Y., & Nie, W.J. (2015). Chemical properties in fruits of mulberry species from the Xinjiang province of China. *Food Chemistry, 174,* 460–466.

Jinapong, N., Suphantharika, M., & Jamnong, P. (2008). Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. *Journal of Food Engineering, 84*(2), 194–205.

Khan, M.A., Rahman, A.A., Islam, S., Khandokhar, P., Parvin, S., Islam, M. B., ... Alam, A.K. (2013). A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba* L. (Moraceae). *BMC Research Notes, 6*(24), 1–9.

Kırca, A., Özkan, M., & Cemeroğlu, B. (2007). Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chemistry, 101*(1), 212–218.

Laokuldilok, T., & Kanha, N. (2017). Microencapsulation of black glutinous rice anthocyanins using maltodextrins produced from broken rice fraction as wall material by spray drying and freeze drying. *Journal of Food Processing and Preservation, 41*(1), e12877.

Laokuldilok, T., Potivas, T., Kanha, N., Surawang, S., Seesuriyachan, P., Wangtueai, S., ... Regenstein, J.M. (2017). Physicochemical, antioxidant, and antimicrobial properties of chitooligosaccharides produced using three different enzyme treatments. *Food Bioscience, 18,* 28–33.

Lee, P., & Rogers, M.A. (2012). Effect of calcium source and exposure-time on basic caviar spherification using sodium alginate. *International Journal of Gastronomy and Food Science, 1,* 96–100.

Lee, H.Y., Chan, L.W., Dolzhenko, A.V., & Heng, P.W.S. (2006). Influence of viscosity and uronic acid composition of alginates on the properties of alginate films and microspheres produced by emulsification. *Journal of Microencapsulation, 23*(8), 912–927.

Lim, M.H., MeFetridge, J.E., & Liesebach, J. (2004). Frozen food components and chemical reactions. In Y.H. Hui, P. Cornillon, I.G. Legaretta, M.H. Lim, K.D. Murrell & W.K. Nip (Eds.), *Handbook of frozen foods* (pp. 67–81). New York, USA.: Marcel Dekker.

Lim, H. H., Lee, S.O., Kim, S.Y., Yang, S.J., & Lim, Y. (2013). Anti-inflammatory and antiobesity effects of mulberry leaf and fruit extract on high fat diet-induced obesity. *Experimental Biology and Medicine, 238,* 1160−1169.

Lin, Y.B., Fugetsu, B., Terui, N., & Tanaka, S. (2005). Removal of organic compounds by alginate gel beads with entrapped activated carbon. *Journal of Hazardous Materials, 120*(1–3), 237–241.

Liu, H., Du, Y., Wang, X., & Sun, L. (2004). Chitosan kills bacteria through cell membrane damage. *International Journal of Food Microbiology, 95*(2), 147–155.

Liu, N., Chen, X. G., Park, H.J., Liu, C.G., Liu, C.S., Meng, X.H., & Yu, L.J. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli. Carbohydrate Polymers, 64,* 60–65

Moldovan, B., Popa, A., & David, L. (2016). Effects of storage temperature on the total phenolic content of Cornelian Cherry (*Cornus mas* L.) fruits extracts. *Journal of Applied Botany and Food Quality, 89,* 208–211.

Orive, G., Hernández, R.M., GascÓn, A.R., & Pedraz, J.L. (2006). Encapsulation of cells in alginate gels. In J.M. Guisan (Ed), *Methods in Biotechnology: Immobilization of Enzymes and Cells* (2nd ed.) (pp. 345–355). Totowa, NJ, USA.: Humana Press.

Pasparakis, G., & Bouropoulos, N. (2006). Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate–chitosan beads. *International Journal of Pharmaceutics, 323* (1–2), 34–42.

Peña, A., Sánchez, N. S., & Calahorra, M. (2013). Effects of chitosan on *Candida albicans*: Conditions for its antifungal activity. *BioMed Research International, 2013* (29), 527549.

Raman, S.T., Ganeshan, A.K.P.G., Chen, C., Jin, C., Li, S.H., Chen, H.J., & Gui, Z.Z. (2016). In vitro and in vivo antioxidant activity of flavonoid extracted from mulberry fruit (*Morus alba* L.). *Pharmacognosy Magazine, 12,* 128−133.

Rodríguez-Sánchez, J.A., Cuatzo-Lozano, M.I., Pérez-Loredo, M.G., Abarca-Sarro, D.I., & Navarro, Y.G. (2017). Alginate encapsulation as a preservation method of pitaya fruit juice (Stenocereus spp.). *Journal of Food Science and Engineering, 7,* 127–134.

Rosenthal, A.J. (1999). Relation between instrumental and sensory measures of food texture. In A.J. Rosenthal (Ed.), *Food texture: Measurement and perception* (pp. 1–17). Gaithersburg: Aspen Publishers.

Sánchez-Salcedo, E.M., Mena, P., Garcia-Viguera, C., Martinez, J.J., & Hernández, F. (2015). Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *Journal of Functional Foods, 12,* 399–408.

Sarikaphuti, A., Nararatwanchai, T., Hashiguchi, T., Ito, T., Thaworanunta, S., Kikuchi, K., ... Tancharoen, S. (2013). Preventive effects of *Morus alba* L. anthocyanins on diabetes in Zucker diabetic fatty rats. *Experimental and Therapeutic Medicine, 6,* 689−695.

Sashiwa, H., & Aiba, S. (2004). Chemically modified chitin and chitosan as biomaterials. *Progress in Polymer Science, 29,* 887–890.

Seo, K.H., Lee, D.Y., Jeong, R.H., Lee, D. S., Kim, Y.E., Hong, E.K., ... Baek, N.I. (2015). Neuroprotective effect of prenylated arylbenzofuran and flavonoids from *Morus alba* fruits on glutamate-induced oxidative injury in HT22 hippocampal cells. *Journal of Medicinal Food, 18,* 403−408.

Serea, C., Barna, O., Manley, M., & Kidd, M. (2014). Effect of storage temperature on the ascorbic acid content, total phenolic content and antioxidant activity in lettuce (*Lactuca sativa* L.). *The Journal of Animal and Plant Sciences, 24*(4), 1173–1177.

Sirijariyawat, A., & Charoenrein, S. (2012). Freezing characteristics and texture variation after freezing and thawing of four fruit types. *Songklanakarin Journal of Science and Technology, 34*(5), 517–523.

Sharma, M., Kristo, E., Corredig, M., & Duizer, L. (2017). Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures. *Food Hydrocolloids, 63,* 478–487.

Suebsaen, K., Suksatit, B., Kanha, N., & Laokuldilok, T. (2019). Instrumental characterization of banana dessert gels for the elderly with dysphagia. *Food Bioscience, 32,* 100447.

Tchabo, W., Ma, Y., Engmann, F.N., & Zhang, H. (2015). Ultrasound-assisted enzymatic extraction (UAEE) of phytochemical compounds from mulberry (*Morus nigra*) must and optimization study using response surface methodology. *Industrial Crops and Products, 63,* 214–225.

Tunick, M.H. (2000). Rheology of dairy foods that gel, stretch, and fracture. *Journal of Dairy Science, 83*(8), 1892–1898.

Vieira, J.M., Fores-Lopez, M.L., Rodriguez, D.J., Sousa, M.C., Vicente, A.A., & Martins, J.T. (2016). Effects of chitosan-*Aloe vera* coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biology and Technology, 116,* 88–97.

Wee, M.S.M., Goh, A.T., Stieger, M., & Forde, C.G. (2018). Correlation of instrumental texture properties from textural profile analysis (TPA) with eating behaviors and macronutrient composition for a wide range of solid foods. *Food and Function, 9*(10), 5301–5312.

Xia, W., Liu, P., Zhang, J., & Chen, J. (2011). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids, 25,* 170–179.

Xie, W., Xu, P., & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. *Bioorganic & Medicinal Chemistry Letters, 11,* 1699–1701.

Xu, B.J., & Chang, S.K.G. (2008). Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. *Journal of Food Science, 73*(2), H19–H27.

Yang, C., Han, B., Zheng, Y., Liu, L., Li, C., Sheng, S., ... Wu, F. (2016). The quality changes of postharvest mulberry fruit treated by chitosan-g-caffeic acid during cold storage. *Journal of Food Science, 81*(4), C881–C888.

Zheng, L.Y., & Zhu, J.F. (2003). Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate Polymers, 54,* 527–530.

Zhou, Y., Martins, E., Groboillot, A., Champagne, C.P., & Neufeld, R.J. (1998). Spectrophotometric quantification of lactic bacteria in alginate and control of cell release with chitosan coating. *Journal of Applied Microbiology, 84*(3), 342–348.

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Microplastic Contamination in the Edible Tissues of Green Mussels Sold in the Fresh Markets for Human Consumption

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Abstract

The coastal and marine environment are currently polluted by microplastics (MPs) worldwide. The movement of MPs from land to sea and their incorporation into the food web has a significant negative impact on marine life and human health. The aim of this study was to quantify microplastics in soft tissue of green mussel *Perna viridis* (Linnaeus, 1758) sold in Nahkon Pathom and Salaya fresh market, Nakhon Pathom Province, Thailand. The total number of MPs was 30 items in mussels from Nahkon Pathom fresh market and 23 items from Salaya fresh market. The average content of microplastics was 0.51 ± 0.22 items/g (wet weight) in mussel sold in Nakhon Pathom fresh market, whereas in mussel sold in Salaya fresh market was 0.30±0.22 items/g (wet weight). Over half of the microplastics were 250-500 µm in size, and the most common shape was fibres (66%) and fragments (34%). The dominant color was blue (62%) and violet (38%). Polymer types were identified using FT-IR microscope, and the major component was polyethylene terephthalate (PET), polyvinyl alcohol (PVA) and polypropylene glycol methacrylate (PGM). Microplastic contamination was found in each soft tissue of green mussel individual. The findings indicated that the microplastic content of bivalve soft tissue was transferred to humans when they consumed whole soft tissue.

Introduction

Microplastics (MPs) contamination has become a major issue as a result of increased plastics production and poor waste management. Several researchers have investigated the presence of MPs in the air, soil, and aquatic environments in order to determine the scope of the problem. Plastic particles with a diameter of < 5 mm are considered MP, according to widely accepted definitions (Wright et al., 2013). The majority of studies on the presence of MPs in the environment have been conducted on marine ecosystems (Van Cauwenberghe & Janssen, 2014). Several researchers have found that MPs in the marine environment have an impact on a wide range of marine species (fishes, mussels, sea scallops, and seagulls) (Wegner et al., 2012; Gündoğdu et al., 2020). Because of the size of MPs, ingestion is the most typical way for MPs to enter marine species. Various studies are being conducted to better understand the presence of MPs in aquatic environments and the risks

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they pose. Mussels, oysters, and crabs were studied, as well as various methods for MPs to enter the human diet (Van Cauwenberghe & Janssen, 2014).

The composition of food in marine organisms is determined by their feeding strategies. This also determines the extent to which pollutants affect these organisms. For example, filter feeders (such as mussels and oysters) can consume everything in the surrounding water, implying that MPs can be consumed alongside microscopic life (such as copepods, decapods, and other planktons) (Walkinshaw et al., 2020). Many studies have shown that bivalves consume a significant number of MPs. However, no studies on the number of MPs in fresh market bivalves were discovered.

There is increasing concern about the impacts of MPs (< 1mm) on marine biota. The green mussel, *Perna viridis* (Linnaeus, 1758) (Mytilidae), is a commercially important tropical Indo-Pacific marine bivalve (Baker et al., 2007). Mussels are benthic extensive filter feeding organisms with a selective suspension feeding mechanism that accumulates microplastics, chemical pollutants, and microorganisms. Microplastics have been found in mussels from the wild as well as on farms in several European countries, as well as in the coastal environment of China (Mathalon & Hill, 2014; Van Cauwenberghe & Janssen, 2014; Li et al., 2015; Van Cauwenberghe et al., 2015; Li et al., 2016). According to recent reports, China's coastal and inland waters are a hotspot of plastic contamination in both biotic and abiotic factors (Qiu et al., 2015; Su et al., 2016). In the laboratory, various filter feeders were used to demonstrate the ingestion, accumulation, and translocation of synthetic microplastics (Farrell & Nelson, 2013; Van Cauwenberghe & Janssen, 2014) . Microplastics and nanoparticles are ingested or accumulated in the gut of mussels, resulting in the accumulation of plastic beads in the digestive system and haemolymph tissues. After being exposed to water, microbeads were discovered on the mussels' gills, indicating that not only sediment, but also plastic beads, could be trapped in the water column (van Moos et al., 2012). The presence of microplastics in mussel tissues and organs suggests that they are transferred to higher trophic levels, similar to what occurs in the human diet (Farrel & Nelson, 2013). In this present study, the green mussel *P. viridis* was purchased directly from two local fresh markets in Nakhon Pathom Province, Thailand. The purpose of this study was to conduct a preliminary investigation into the presence of MPs in the mussel population of a local fresh market.

Materials and methods

1. Sample collection and processing

In October 2020, a kilogram of green mussel (*Perna viridis*) was purchases directly from the two fresh markets (Nakhon Pathom fresh market and Salaya fresh market), Nakhon Pathom Province Thailand. Each market was assigned three replicates (R1, R2, and R3). Each replicate had 3 individuals (n=3) in it. In the laboratory, the wet weight of the soft tissue was determined using a precision electronic balance.

The digestion of mussels was performed as follows. In brief, each individual was placed in a labeled 100 mL glass beaker that had previously been cleaned, and then approximately 20 mL of 35% H_2O_2 was added to each glass beaker (Ehlers et al., 2019). The beaker was wrapped in parafilm and shaken at 150 rpm for seven days, until the soft tissue was completely digested. The digestion time could be adjusted based on the digestive effect, and digestion was stopped when the solution became clear.

Each solution sample was transferred to a glass separation funnel, and 99% potassium formate was added until the solution reached 1.6 g mL^{-1} saturation (Ehlers et al., 2019). For at least three hours, the samples were kept at room temperature. A saturated solution allows for the separation of less dense particles, resulting in a layer of MPs floating upwards while undissolved organic residues and inorganic matter settle at the bottom of the bottle.

The samples were filtered through a nylon membrane filter (pore size 0.45; diameter 47 mm, Whatman) using a pressure filtration unit. Each filter was placed in a clean Petri dish, covered with aluminum foil, and dried for two days in a drying cabinet (50°C).

Each filter was then visually inspected for microplastics using a stereomicroscope (Leica EZ4E) and all microplastics were identified based on their color and shape. Fiber, sphere, film (thin and small layer), fragment (part of a larger plastic item), and sphere were the shapes used to classify microplastic particles. The presence of microplastics was documented (fragments, films and fibers, and spheres).

Selected particles from the mussel specimens were manually analyzed using a Hyperion 2000 FT-IR microscope equipped with a mercury-cadmium telluride detector (Bruker) in a wavenumber range of 4,000-600 cm^{-1} with 32 co-added scans and a spectral resolution of 4 cm^{-1}. The software used was OPUS 7.5, and the

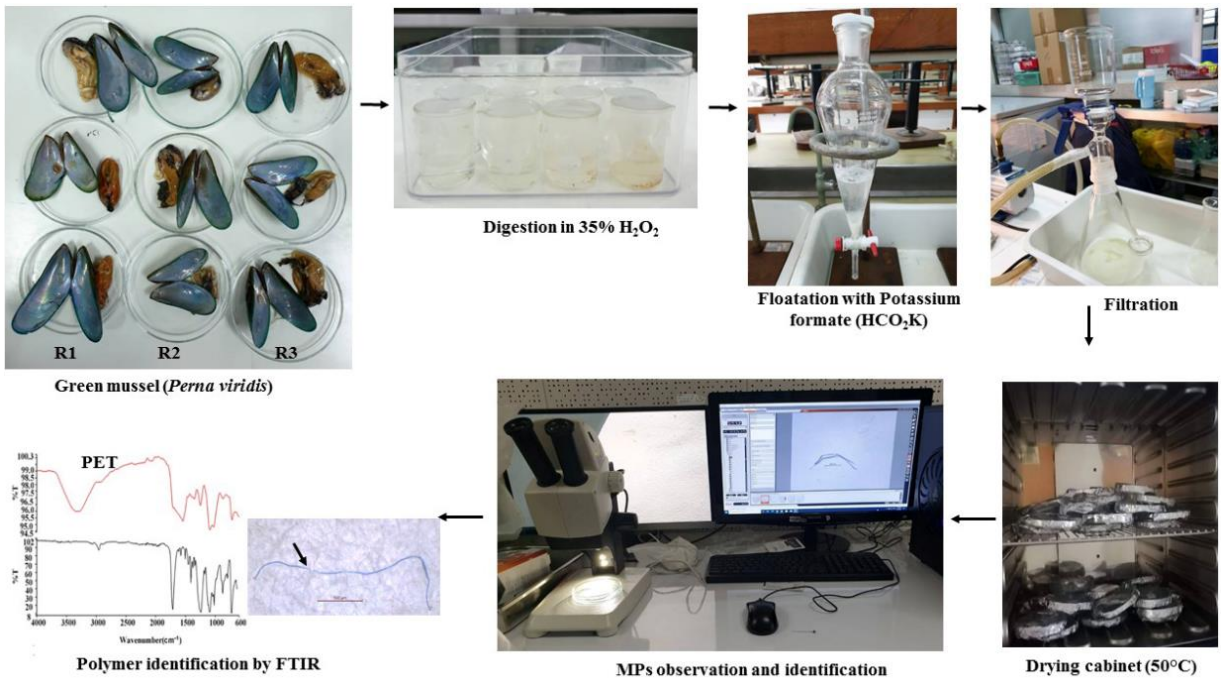


Fig. 1 Microplastics in green mussels: Sample preparation, digestion, and analytical procedures

resulting spectra were compared to the Bruker database. Only particles with a hit quality of more than 700 were considered microplastics, as in previous studies. The protocol is summarized in Fig. 1.

2. Data analysis

For each treatment, the type, size, and color of the MPs were measured and calculated. The average abundance of microplastic particles in mussel soft tissue was expressed in items/ g of tissue wet weight.

Table 1 Abundances of microplastics in the green mussels collected from two fresh markets. Three replicates were collected for each market (n=3)

Collection site	Microplastics (MPs) in soft tissues weight			
	Soft tissues weight (g)	Total MPs (items)	Average microplastic abundance (items/ individual)	Average microplastic abundance (items/g)
Nakhon Pathom fresh market	6.55±0.51	30	3.33±1.15	0.51±0.22
Salaya fresh market	8.41±1.10	23	2.56±1.90	0.30±0.22

Results and discussion

1. Abundance of microplastics in green mussels

The findings of this study confirmed that the green mussel sole in both fresh markets were heavily contaminated with microplastics (Table 1). All tested mollusk samples contained a total of 53 microplastics.

A total of 18 individual green mussel (9 individuals, 3 replicated in two fresh markets) were analyzed. All soft tissue mussel samples from the two local fresh markets contained a total of 53 microplastics, with a detection rate of 100% for each individual.

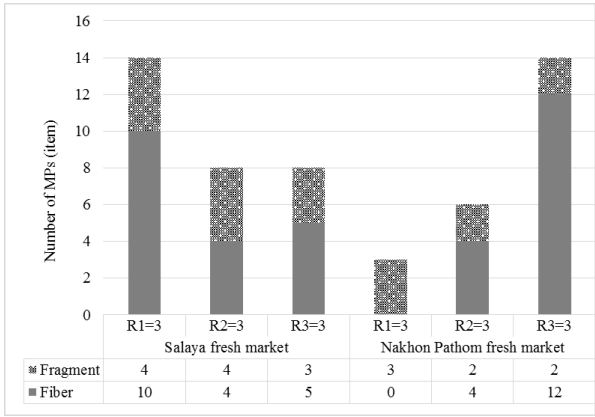
At Nakhon Pathom fresh market, the average abundance of microplastics in green mussels was 3.33±1.15 items/individual or 0.51±0.22 items/g (wet weight of soft tissue), while at Salaya fresh market, the average abundance was 2.56±1.90 items/individual or (Linnaeus, 1758) 0.30±0.22 items/g (Table 1). The bivalve green mussel *Perna viridis* is a filter feeder that absorbs microplastics in seawater while feeding. Due to the lack of data on microplastics in seawater in this study, the amount of microplastics in mussels is most likely proportional to the amount of microplastics in the water from which they are sourced. Green mussels for the two fresh markets are expected to come from the same seafood port near Bangkok. The Port's seafood market is very close to the microplastics high- value point, which may increase the likelihood of polluted local shellfish and increase microplastic abundance.

In the current study, 35% H_2O_2 was used to breakdown

the soft tissue of green mussels, and many different types of microplastics, including rich fragments and fibers, were discovered (Fig. 2). When using H_2O_2, it is critical to keep the maximum weight of soft tissue under control. Too much soft tissue in one replicate usually necessitates a longer digestion time and may even necessitate digestion repetition (Mathalon & Hill, 2014). According to these results, the addition of no more than 5 g of tissue and approximately 200 mL of H_2O_2 in a 1 L glass beaker produced a good digestion effect, as reported by Li et al. (2015).

2. Microplastic characteristics

The shape, color, and size of microplastics were used to classify them (Fig. 2). The most common shape of microplastics found in mollusks was fibers (66%), followed by fragments (34%) (Fig. 2 and Fig. 3). These results supported previous findings that fibers were the most abundant shape of microplastics in oysters captured from Chinese coastal areas (Teng et al., 2019), the Bizerte lagoon (Northern Tunisia) (Abidli et al., 2019), and the northern Persian Gulf (Naji et al., 2018). Fibers, it has been proposed, are one-dimensional materials that easily degrade into smaller pieces, possibly leading to their widespread presence in marine fish stomachs, intestines, and gills (Koongolla et al., 2020). The fibers could be derived primarily from laundry wastewater. Moreover, the pervasive use and removal of fishing gear are major sources of fiber (Browne et al., 2011). De Witte et al. (2014) discovered that quayside mussels stored a high number of fibers, which could be related to port fishing activities including boat landing, net repair, and waste disposal.



	R1=3	R2=3	R3=3	R1=3	R2=3	R3=3
Fragment	4	4	3	3	2	2
Fiber	10	4	5	0	4	12

Fig. 3 Microplastic type in edible tissue of green mussel sole in two fresh markets. R is indicated as replication in each market

Blue microplastics were the most common (62%), followed by violet (38%) (Fig. 4). The color composition varied slightly between ports, as reported by Wang et al (2021). The color of microplastic composition matched

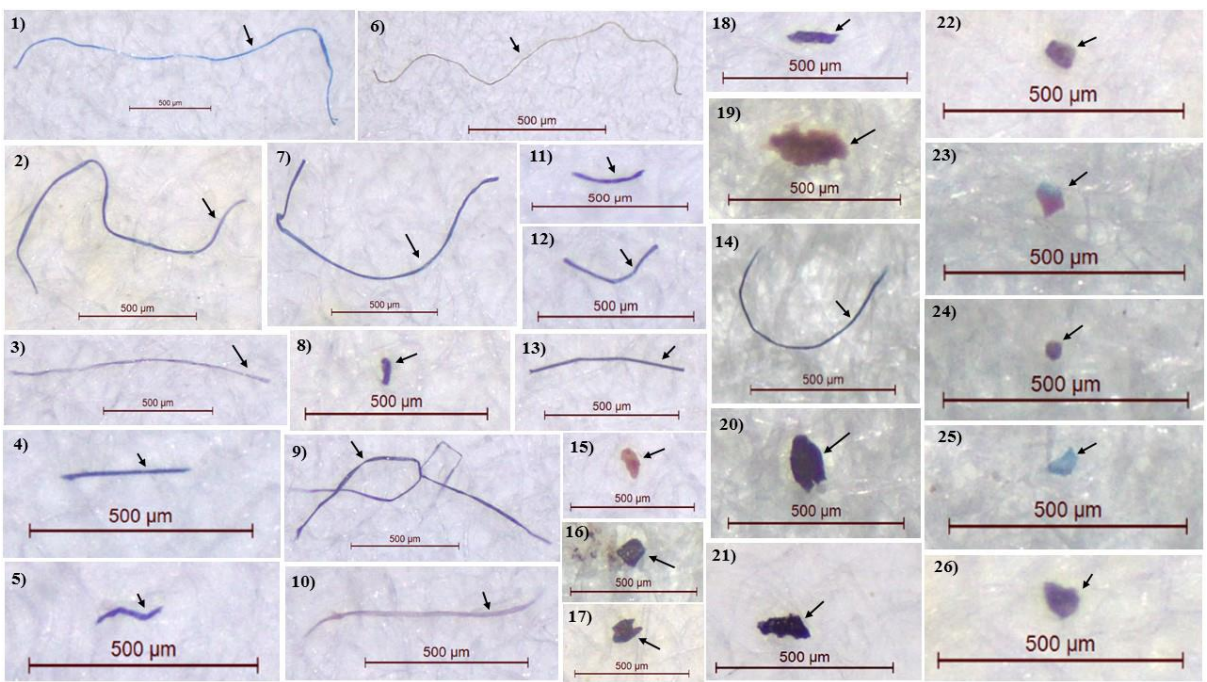


Fig. 2 Two kinds of microplastics in the filter feeding bivalve Perna viridis (1-14 = fiber; 15-16 = fragment)

that of clams collected from 21 sites (freshwater and estuarine) in the Yangtze River's middle and lower reaches (Peng et al., 2017).

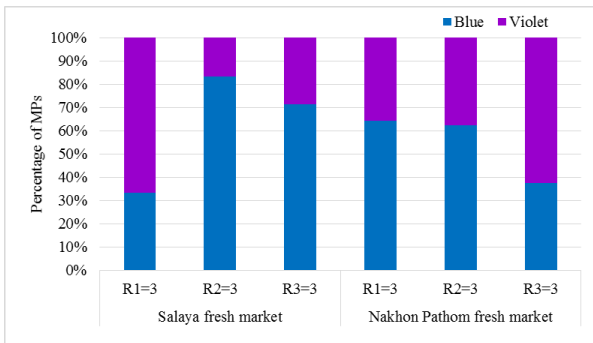


Fig. 4 Percentage of microplastic color in soft tissue of green mussel sold in two fresh markets. R is indicated as replication in each market

Particle sizes ranged from 200 to >500 µm (Fig. 4). The most common particle size range was 250-500 µm (41.6%), followed by 200-250 µm (35.8%) and large microplastics (approximately > 500 µm) (22.6%) (Fig. 5). This proportion was comparable to the amount of microplastics discovered in commercial bivalves and oysters collected from other Chinese coastal areas (Li et al., 2015; Teng et al., 2019). As particle size increased, the abundance of microplastics decreased. Among microplastics smaller than 500 µm in mussels, the size range 250-500 µm was the most abundant,

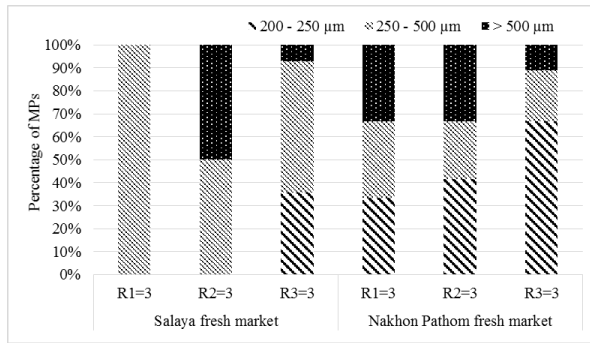


Fig. 5 Microplastic size distribution in edible tissue of green mussel sole in two fresh markets. R represents replication in each market

followed by the size range 200-250 µm (Fig. 5). Smaller particles are often more gathered in organisms than larger particles, according to various studies (Wright et al., 2013; Deng et al., 2017). Dawson et al. (2018) demonstrated that microplastics can fragment even more during digestion. This could explain why bivalves contain a higher concentration of smaller microplastics.

3. Source for microplastics

A number of 53 suspicious microplastics were chosen at random and examined using FT-IR. Mollusks contained polymers such as polyethylene terephthalate (PET), polyvinyl alcohol (PVA), and poly (propylene glycol) methacrylate (PGM) (Fig. 6). Figure 6 shows the FT-IR spectrum of each polymer. PET was the most common type of polymer. The polymer composition in this study

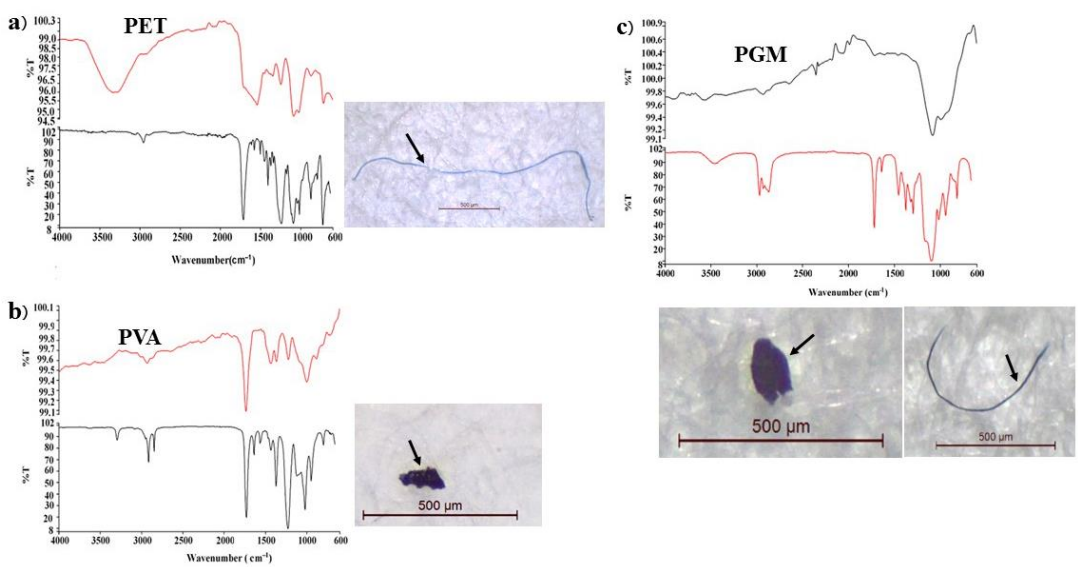


Fig. 6 FT-IR spectra of microplastics found in fresh market green mussels in Thailand. The black line represents the standard plastic spectrum, and the red line represents the microplastics in green mussels. (a) PET (Polyethylene terephthalate) (b) PVA (Polyvinyl alcohol) (c) PGM (Poly(propylene glycol) methacrylate

was dominated by polyethylene terephthalate, which was attributed to a significant proportion of the total, and PET was found in all three replicates. This finding is in accordance with microplastics found in three sessile invertebrates on Thailand's eastern coast, including *Balanus amphitrite, Saccostrea forskalii,* and *Littoraria* sp. (Thushari et al., 2017) and two bivalves (*Crassostrea gigas* and *Mytilus edulis*) on France's Atlantic coast (Phuong et al., 2017). PET is commonly used in the production of plastic bottles and food packaging bags, but it is also being used in the production of textile and garment products (Park et al., 2004). As a result, land-based sources of PET may be a significant source. PVA is used as a plastic coating in laundry and dish cleaning agents, as a sizing and finishing agent in textiles, and as a thickening or coating agent in the paper and food industries for paints, glues, meat packaging, and pharmaceuticals (DeMerlis & Schoneker, 2003).

4. Microplastic accumulation in bivalve organism world wide

Several studies have been published on the contamination of bivalves with microplastics around the world, including this one (Table 2). Because of their small size and high persistence, microplastics have the potential to be consumed by a wide range of marine organisms (Cole et al., 2011). Microplastics have also been implicated in trophic level transfer, in which higher level organisms eat other species that have already consumed microplastics, increasing the possibility of biomagnification (Farrell & Nelson, 2013).

Because of their potential impact on human health, microplastics in seafood are a source of concern. Cho et al. (2019) report that the amount of microplastics consumed by Jiangsu coastal residents through shellfish consumption is relatively low by global standards, as well as lower than the national average (6636 items/ person/ year) . The region's microplastic consumption is comparable to that of France (1139 items/person/year). Jiangsu's bivalve consumption is comparable to that of other coastal areas in China, France, and Spain. Microplastic ingestion, on the other hand, is relatively low. This is because Jiangsu bivalves have a lower concentration of microplastics. Previous research has found that the digestive tract has the highest level of microplastics (Beyer et al., 2017; Kolandhasamy et al., 2018). To decrease microplastic consumption, it is suggested that the digestive tract be removed prior to cooking mollusks (Seltenrich, 2015). Several laboratory studies have shown that microplastics or nanoplastics can enter tissues or the circulatory system, such as by passing through the gut lining or gill structures. As a result, the organism may be unable to remove these microplastics, resulting in increased accumulation and negative effects (Brennecke et al., 2015; Lu et al., 2016).

Table 2 Microplastic contamination in bivalves in each location in the world

Location	Organism	Microplastic	References
French-Belgian-Dutch coastline	*Mytilus edulis*	0.2±0.3 microplastics g^{-1}	Van Cauwenberghe et al. (2015)
Santos Estuary, Sao Paulo, Brazil	*Perna perna*	75% of mussels had ingested microplastics	Santana et al. (2016)
Belgian Coastline and Netherlands	*Mytilus edulis, Mytilus galloprovincialis, Mytilus edulis/ galloprovincialis hybrids*	2.6 – 5.1 fibers 10 g^{-1}	De Witte et al. (2014)
Commercial mussel farm - Germany	*Mytilus edulis*	0.36 ± 0.07 particles g^{-1} (ww)	Van Cauwenberghe & Janssen (2014)
Commercial mussel farm - Germany	*Crassostrea gigas*	0.47 ± 0.16 particles g^{-1} (ww)	Van Cauwenberghe & Janssen (2014)
Nova Scotia – wild mussels McCormack's Beach Rainbow Haven Beach	*Mytilus edulis*	126 particles $mussel^{-1}$ 106 particles $mussel^{-1}$	Mathalon & Hill (2014)
Nova Scotia – farmed mussels	*Mytilus edulis*	178 particles $mussel^{-1}$	Mathalon & Hill (2014)
China	*Mytilus edulis*	0.9 to 4.6 items g^{-1}	Li et al. (2016)
Thailand	*Perna viridis*	0.51±0.22 items g^{-1} (ww)	This study

The toxicity of microplastic exposure in mussels is unknown due to a lack of data. Microplastics, according to ecotoxicology studies in marine species, may cause gut inflammation by altering intestinal permeability and dysbiosis (Qiao et al., 2019).

Conclusion

As a result of this study, microplastics were discovered in the green mussel *Perna viridis*, which was obtained from local fresh markets. Microplastic fibers and fragments were found in the mussel soft tissue. This could be reflected in the abundance of microplastics obtained from filter feeder organisms. The findings of this study contribute to greater evidence that microplastics occur in the soft tissue of bivalves. Ingestion of green mussel bivalve is a route of human exposure to microplastics because these organisms are frequently eaten whole without digestion.

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References

Abidli, S., Lahbib, Y., & El Menif, N.T. (2019). Microplastics in commercial molluscs from the lagoon of Bizerte (Northern Tunisia). *Marine Pollution Bulletin, 14,* 243–252.

Baker, P., Fajans, J.S., Arnold, W.S., Ingrao, D., Marelli, D.C., & Baker, S.M. (2007). Range and dispersal of the nonindigenous green mussel, *Perna viridis,* in the southeastern United States. *Journal of Shellfish Research, 26,* 1–11.

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N., & Schøyen, M. (2017). Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environmental Research, 130,* 338–365.

Brennecke, D., Ferreira, E.C., Costa, T.M., Appel, D., da Gama, B.A., & Lenz, M. (2015). Ingested microplastics are translocated to organs of the tropical fiddler crab *Uca rapax. Marine Pollution Bulletin, 96*(1-2), 491-495.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environmental Science & Technology, 45*(21), 9175-9179.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., & Hong, S.H. (2019). Abundance and characteristics of microplastics in market bivalves from South Korea. *Environmental Pollution, 245,* 1107–1116.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T.S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin, 62,* 2588–2597.

Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., & amp; Nash, S.M.B. (2018) Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nature Communications, 9,* 1–8.

DeMerlis, C., & Schoneker, D. (2003). Review of the oral toxicity of polyvinyl alcohol (PVA). *Food and Chemical Toxicology, 41,* 319–326.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine pollution bulletin, 85*(1), 146-155.

Deng, Y., Zhang, Y., Lemos, B., & Ren, H. (2017). Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Scientific Reports, 7*(1), 46687.

Ehlers, S.M., Manz, W., & Koop, J.H. (2019). Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly *Lepidostoma basale. Aquatic Biology, 28,* 67–77.

Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastics: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution, 177*, 1–3.

Gündoğdu, S., Çevik C., & Ataş, N.T. (2020). Occurrence of microplastics in the gastrointestinal tracts of some edible fish species along the Turkish coast. *Turkish Journal of Zoology, 44,* 312–323.

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., & Shi, H. (2018) Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. *Science of the Total Environment, 610-611,* 635–640.

Koongolla, J.B., Lin, L., Pan, Y.F., Yang, C.P., Sun, D.R., Liu, S., ... Li, H.X. (2020). Occurrence of microplastics in gastrointestinal tracts and gills of fish from Beibu Gulf, South China Sea. *Environmental Pollution, 258,* 113734.

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., ... Shi, H. (2016) Microplastics in mussels along the coastal waters of China. *Environmental Pollution, 214,* 177–184.

Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution, 207,* 190–195.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., ... Ren, H. (2016). Uptake and accumulation of polystyrene microplastics in Zebrafish (*Danio rerio*) and toxic effects in liver. *Environmental science & Technology, 50*(7), 4054-4060.

Mathalon, A., & Hill, P. (2014). Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin, 81,* 69–79.

Naji, A., Nuri, M., & Vethaak, A.D. (2018). Microplastics contamination in molluscs from the northern part of the Persian Gulf. *Environmental Pollution, 235,* 113–120.

Park, C.H., Kan, Y.K., & Im, S.S. (2004). Biodegradability of cellulose fabrics. *Journal of Applied Polymer Science, 94,* 248–253.

Peng, G., Zhu, B., Yang, D., Su, L., Shi, H., & Li, D. (2017). Microplastics in sediments of the Changjiang Estuary, China. *Environmental Pollution, 225,* 283–290.

Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., & Zalouk-Vergnoux, A. (2017). Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? *Marine Pollution Bulletin, 129,* 664–674.

Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., & Lemos, B. (2019). Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in Zebrafish. *Science of the Total Environment. 662,* 246–53.

Qiu, Q.X., Peng, J.P., Yu, X.B., Chen, F., Wang, J., & Dong, F. (2015). Occurrence of microplastics in the coastal marine environment: first observation on sediment of China. *Marine Pollution Bulletin, 98,* 274- 280.

Santana, M.F.M., Ascer, L.G., Custódio, M.R., Mareira, F.T., & Turra, A. (2016). Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment. *Marine Pollution Bulletin, 106,* 183-189.

Seltenrich, N. (2015). New link in the marine food chain? Marine plastic pollution and seafood safety. *Environmental Health Perspectives, 123,* A34–A41.

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li D., & Shi H. (2016). Microplastics in Taihu Lake, China. *Environmental Pollution, 216,* 711–719.

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., ... Zhao, J. (2019). Microplastic in cultured oysters from different coastal areas of China. *Science of the Total Environment, 653,* 1282–1292.

Thushari, G.G.N., Senevirathna, J.D.M., Yakupitiyage, A., & Chavanich, S. (2017). Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. *Marine Pollution Bulletin, 124*(1), 349-355.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., & Janssen, C.R. (2015). Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environmental Pollution, 199,* 10–17.

Van Cauwenberghe, L., & Janssen, C.R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution, 193,* 65–70.

Von Moos, N., Burkhard, H.P., & Kohler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. *Environmental Science and Technology, 46,* 11327–11335.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., & Cole, M. (2020). Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicology and Environmental Safety, 190,* 110066.

Wang, T., Li, B., & Wang, D. (2021). The abundance and characteristics of microplastics in commonly consumed shellfish in the Jiangsu coastal region of China. *Environmental Science and Pollution Research,* 1-12

Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A. (2012). Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environmental Toxicology and Chemistry, 31,* 2490–2497.

Wright, S.L., Thompson, R.C., & Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution, 178,* 483–492.

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Enhancement for Microbial Safety of Peeled Shallot (*Allium ascalonicum* L.) by the Application of Hot Water and Acidified Sodium Chlorite

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Abstract

Shallot (*Allium ascalonicum* L.) is one of the most used ingredients that is commonly found in Asian cuisine preparation. However, it poses a safety risk due to the microbial contamination. The ideal conditions for peeled shallot processing were pre-treated with hot water (HW) followed by 100 ppm acidified sodium chlorite (ASC) solution and packed under vacuum packaging (VP), then stored at 5±2°C. This condition reduced the loads of aerobic bacteria, yeasts and molds during storage by 0.68-0.80 and 0.46-0.95 log CFU/g FW, respectively, which was better than the control samples. There was a slight increase in weight loss and total phenolic content during cold storage. The combination treatments of HW and ASC packed under VP had no effect on weight loss and antioxidant capacity as compared to control sample throughout the storage period.

Introduction

Shallot (*Allium ascalonicum* L.) is an edible plant that belongs to the family Alliaceae, which is similar to the onion (*Allium cepa* L.). The common name of shallots are bulbous and herbaceous plants. Shallot is a popular food ingredient due to it has a medicinal effects, distinct aroma and flavor (Dron et al., 1997). Moreover, it has a good source of antioxidants and antimicrobial properties (Leelarungrayub et al., 2006; Raeisi et al., 2016). The "minimally processed", "slightly processed", "ready to eat", and "fresh-cut" produces has increased in popularity in consumer demand (Cantwell & Suslow, 2002). However, processing techniques of fresh-cut fruits and vegetables such as washing, selecting, decorating, peeling, cutting and chopping does not impact the "fresh-like" quality of the fresh-cut produce. The shelf-life of fresh-cut produce generally declines due to the undesirable effects of physiological and biochemical changes as well as an increase of the microbial

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population. The peeling and cutting surfaces of the fruits and vegetables induce quality loss and microbial contamination rendering the products unmarketable due to their undesirable appearance. Several technologies are used to extend the shelf life of fresh-cut fruits and vegetables. For example, chlorinated water has been used extensively to eliminate foodborne pathogens and spoilage microorganisms in fresh-cut products (Guo et al., 2017; Huang & Chen, 2018). However, the use of chlorinated water raises concerns regarding the environmental and health implications of halogenated by-products such as trihalomethanes and chloramines (Ölmeza & Kretzschmar, 2009). Therefore, the alternative method has also been used to monitor undesirable physiological changes and prolong the shelf life of fresh-cut products. Especially, thermal or heat treatments have been used to control the microbial populations and extend the shelf life of the product.

Hot water (HW) treatment is a chemical-free method for reducing foodborne pathogens, delaying senescence (Dea et al., 2010; Siddiq et al., 2013; Kabelitz & Hassenberg, 2018) and inhibition of browning reaction in fresh-cut produce (Wang et al., 2014). Acidified sodium chlorite (ASC) is an effective and efficient oxidizing agent that is an alternative disinfectant to chlorine (Cruz et al., 2006). According to the Food and Drug Administration (FDA), the application of ASC is able to generate chlorine dioxide gas, which can be used as an antimicrobial agent for disinfecting water and washing fruits, vegetables and poultry (FDA, 2010). ASC is produced by decreasing the pH (2.5-3.2) of sodium chlorite ($NaClO_2$) solution with any acid that has a Generally Recognized as Safe (GRAS) status such as citric acid (FDA, 2000). The FDA has approved the use of 500-1200 ppm ASC for spraying and dipping fresh and fresh-cut produces. ASC is an antibiotic agent that prevents browning reaction in various minimally processed fruits and vegetables such as cilantro (Allende et al., 2009), pears (Xiao et al., 2011), broccoli (Renumarn et al., 2015) and rose apple (Mola et al., 2016). Furthermore, ASC does not produce carcinogenic compounds when compared with chlorine (Cruz et al., 2006). However, the effective approaches for preventing microbial contamination and maintaining the quality of peeled shallot have rarely been investigated.

Therefore, the purpose of this study was to investigate the effect of HW pre-treatment with/without ASC solution on peeled shallot processing methods to minimize the microbial loadings. Passive packaging (PP) and vacuum packaging (VP) were used to maintain the quality and antioxidant capacity of peeled shallot throughout the refrigeration storage.

Materials and methods

1. Plant material and treatment

Shallots (*Allium ascalonicum* L.) were purchased from the local marketplace in Prachinburi province, Thailand. They were selected by considering the absence of infected, damaged, and the uniformity of color, shape, and size. They were stored at room temperature (25±2°C) until the time of use in the experiments. Shallots were blanched in boiled water (95±2°C) for 30 seconds, and then immediately cooled down to 20±2°C with tap water. After that, they were immersed in 100 ppm acidified sodium chlorite solution (pH 4, citric acid) for 10 min, followed by rinsing with drinkable water. The excess water of peeled shallot was removed with sterile tissue paper. Approximately 150±5 g of minimally processed shallots were packed in a passive packaging (PP), or under vacuum packaging (VP) of polyethylene (PE) bags (150×200 mm, 30 µm thickness). The packed samples were stored at 5±2°C for 9 days. Quality attributes (weight loss), total phenolic content, antioxidant properties, and microbial populations were evaluated every three days of storage. Each treatment had three replicates (bags).

2. Microbiological analysis

Total aerobic bacteria and fungi (yeasts and molds) counts were analyzed using a spread plate method according to Renumarn & Choosuk (2020) with minor modifications. Briefly, 25 g peeled shallot sample was transferred into a stomacher bag, which contained 225 mL of 0.1% sterile peptone solution (Peptone, HiMedia, India), and homogenized for 1 min with a stomacher (Stomacher® 400 Circulator, UK). The various serial dilutions of each homogenized samples were dispersed on an agar plate. Total aerobic bacteria were enumerated on Plate Count Agar (PCA, HiMedia, India), which was incubated at 35°C for 48 h. Yeasts and molds were enumerated on Potato Dextrose Agar (PDA, HiMedia, India), which were incubated at 28°C for 7 days. Microbial populations were then enumerated by counting on agar plates that contained 25-250 colonies and microbial population were expressed as log colony forming units (CFU) /g fresh weight (FW) of shallot sample.

3. Determination of weight loss

Each shallot bags were assessed for the percentage of weight loss, by comparing the weight of samples at each sampling date and their initial weight (150±5 g).

4. Sample extraction

Approximately 10 g of peeled shallots were homogenized with 100 mL of 90% ethanol before being centrifuged at 9,000 g at 4°C for 20 min. The supernatants were collected for analysis of total phenolic content and antioxidant activity.

5. Determination of total phenolic content

Phenolic content was determined using the Folin-Ciocalteau method (Roy et al., 2009) with slight modifications. Briefly, 0.4 mL of sample extract was mixed with 2 mL of 10% Folin-Ciocalteau's phenol reagent, vortexed, and placed at 25±5°C for 4 min. The mixture was mixed with 1.6 mL of 5% (w/v) sodium carbonate solution and incubated at 25±5°C in the dark for 60 min. The absorbance was measured at 765 nm using a spectrophotometer. A calibration curve of gallic acid standards (0, 20, 40, 60, 80 and 100 mg/L) was used to determine the phenolic content of the samples, which were expressed as mg of gallic acid equivalents (GAE)/g fresh weight (FW).

6. Determination of antioxidant activity

The antioxidant activity was assayed by measuring the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical as described by Brand-Williams et al. (1995) and Thaipong et al. (2006), with some modifications. Briefly, 0.2 mL of sample extract was mixed with 0.6 mL of 0.075 mM DPPH$^{\bullet}$ and 5.2 mL of 95% ethanol mixed and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH$^{\bullet}$ radical scavenging activity was estimated following the equation below:

$$\text{DPPH}^{\bullet}\text{ radical scavenging (\%)} = (\text{Abs (control)} - \text{Abs (sample)})/(\text{Abs (control)}) \times 100$$

7. Statistical analysis

The results of all experiments were expressed in the term of mean±standard deviation (SD). The significant difference between the mean values was estimated by analysis of variance (ANOVA), followed by the Duncan's multiple range test. Statistically significant differences were informed as $p \leq 0.05$. SPSS software was used for all statistical analyses (SPSS version 21.0, SPSS Inc., Chicago, IL., USA).

Results and discussion

1. Effects of pre-treatment on microbial populations

The most important step in the minimally process of fresh-cut production during refrigeration storage are the quality of microbial loads, and sensory quality of fresh-cut produce (Artés & Allende, 2005). Microbial quality assessments also suggested that the produce was acceptable at a safe level. In this research, the results showed that pre-treatment with HW followed by ASC solution for 10 min, and packed under VP could reduce aerobic bacteria, yeast and molds counts during storage at 5±2°C (Fig. 1A). At the initial day, HW pre-treatment with ASC solution had significantly ($p \leq 0.05$) reduced aerobic bacteria counts of peeled shallot, by 0.71 log CFU/g FW as compared to HW treatment alone. Hence, the treatment of HW pre-treatment with ASC solution, and packed under VP showed the greatest significant reduction in aerobic bacteria counts (0.68-0.80 log CFU/g FW) of peeled shallots during storage as compared to the other treatments (Fig. 1A). However, the counts of aerobic bacteria slightly increased in all samples until the end of storage. At the 9th day of storage, the lowest count of aerobic bacteria was found in the peeled shallot sample treated with HW, followed by ASC, and packed under VP (4.57 log CFU/g FW), whereas it was 5.25 log CFU/g FW in the peeled shallot sample treated with HW, and packed in PP. Similarly, many previous research have shown that the application of ASC could reduce the microbial growth in various fresh-cut produce. For example, Martínez-Sánchez et al. (2006) found that ASC at the concentration of 250 ppm could reduce pathogenic bacterial growth in rocket leaves. Moreover, Allende et al. (2009) suggested that the use of 250 or 500 ppm of ASC could reduce microbial populations in fresh-cut cilantro.

The peeled shallots treated with HW pre-treatment had an initial yeast and molds count of 1.62 log CFU/g FW, whereas it was 1.16 log CFU/g FW in the samples treated with HW pre-treatment, followed by ASC solution, and packed in both PP and VP (Fig 1B). At the 9th day of storage, the lowest count of yeasts and molds was found in the sample treated with HW, followed by ASC, and packed under VP (3.35 log CFU/g FW), whereas it was 3.84 log CFU/g FW in the sample treated with HW, followed by ASC, and packed in PP, which were similar to aerobic bacteria counts (Fig. 1A). These results indicated that the pre-treatment with HW, followed by ASC solution, and packed under VP could enhance

the control of both aerobic bacteria, yeasts and molds in peeled shallot as compared to the other treatment packed in PP. Commonly, aerobic bacteria, yeasts and molds require O_2 for their growth. Therefore, the products stored under the atmosphere less than 1% O_2 (vacuum packaging) could retard the growth of aerobic microorganisms as compared to air atmosphere packaging (Masniyom, 2011).

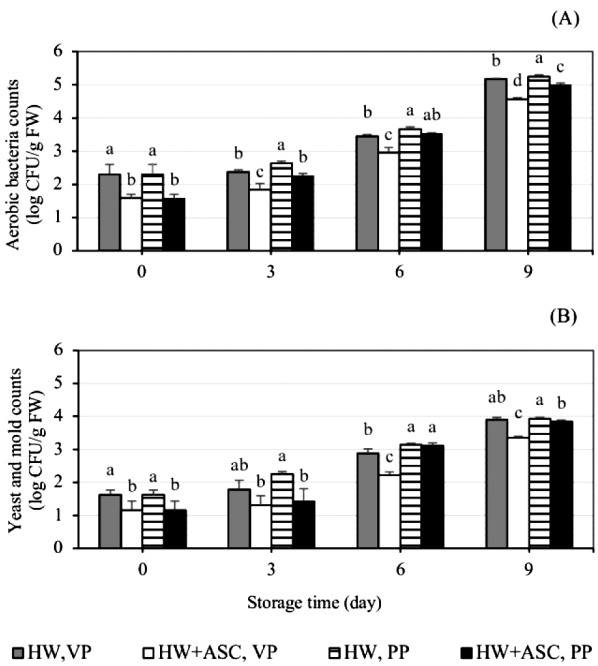

Fig. 1 Effects of pre-treatments on aerobic bacteria count (A) and yeast and mold count (B) of peeled shallots during storage at 5±2°C (HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging). The data represent mean values ± SD (n = 3). The various small letters at the top of the bars in each day indicate significantly differences ($p \leq 0.05$)

2. Change of weight loss (%)

Fresh-cut fruits and vegetables are highly perishable products that are associated with respiration, transpiration, surface dehydration, as well as the release of heat and lose of water, due to the processing of fresh-cut produces, e.g. trimming, peeling, grading, cutting and shredding, etc. (Zhang et al., 2019). In this research, weight loss of all peeled shallot samples storage at 5±2°C slightly increased over the time of storage, with ranges of 0.070-0.743% (Table 1). At the 3rd day of storage, peeled shallot sample treated with HW, and packed under VP had the highest percentage of weight loss (0.743%) with significantly difference ($p \leq 0.05$) was found when compared to the other treatments. However, no significant difference in weight loss were found in all samples ($p > 0.05$) at the 6th and the 9th day of storage. After storage for 9 days, peeled shallots treated with HW, with/without ASC, and packed under VP showed the minimum weight losses (0.183 and 0.240%, respectively) as compared to those samples packed in PP (0.390 and 0.627%, respectively). The weight loss of the vacuum-packed shallot samples during cold storage was in the highest range of 0.280-0.743%, during the first period of storage. After 6 days of storage, the weight loss was reduced to 0.183-0.240%, resulting greater in samples packed under vacuum condition. The type of packaging and packing method also affected the moisture inside the package and percentage of weight loss (Chang & Kim, 2015; Reche et al., 2019). Similarly, previous studies have found that the fruits packed in vacuum packaging increased the moisture inside the package and reduced weight loss during storage (Chang & Kim, 2015; Moradinezhad & Dorostkar, 2021). Weight loss is one of the important quality attribute that could be used to measure the shelf life of fresh-cut fruits and vegetables, which can lead to the retail value of the entire producer (Rivera-López et al., 2005; Loi et al., 2019).

Table 1 Effects of pre-treatments on the change of weight loss (%) in peeled shallots during storage at 5±2°C

Treatment	Weight loss (%)		
	Day 3	Day 6^{ns}	Day 9^{ns}
HW, VP	0.743±0.44^{a}	0.733±0.07	0.240±0.21
HW+ASC, VP	0.280±0.26^{ab}	0.363±0.04	0.183±0.16
HW, PP	0.207±0.01^{b}	0.137±0.24	0.627±0.88
HW+ASC, PP	0.070±0.35^{b}	0.173±0.21	0.390±0.34

Remark: The averages followed by the different letter in each column indicates that there are significantly differences at $p \leq 0.05$. ns, not significantly differences; HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging

3. Effects of pre-treatment on total phenolic content

Total phenolic content of all samples slightly increased with an increase in storage period at 5±2°C (Fig. 2A). On the initial day of storage (day 0), all peeled shallot samples had total phenolic content of 87.94-88.71 mg GAE/g FW. After the 9th day of storage, all peeled shallot sample had a high total phenolic content, which were in the range of 95.80-106.72%, except for the sample treated with HW, and packed under VP showed significantly the lowest ($p \leq 0.05$) total phenolic content (63.85%) that was found between the treatment conditions. This result may be associated with the induction of abiotic stress in plants metabolism when oxygen depleted in the packaging during cold storage.

These results also may indicate that the total phenolic content decreased depending on the O_2 level inside the package. Total phenolic contents of shallot in this experiment were higher than those of shallot (*Allium oschaninii* L.) in different cultivars (17.18 mg GAE/g FW) (Lu et al., 2011). According to Siddiq et al. (2013), plants stress from wounding and cutting can lead to an increase of phenolic compounds synthesis. However, improving the efficiency of postharvest handling, and the selection of suitable packaging could maintain the phenolic content of the fresh and fresh-cut produce.

4. Effects of pre-treatment on antioxidant activity

The antioxidant activity measured by the DPPH radical scavenging. At the initial day of storage, the antioxidant activity of all peeled shallot samples were in the range of 18.58-22.80% (Fig. 2B). During storage at 5±2°C, the antioxidant activity of all samples tended to slightly increase as well as total phenolic content (Fig. 2A). The antioxidant activity of peeled shallot samples packed in VP, found 18.58-25.77% (only HW) and 22.80-24.18% (HW+ASC), respectively. Whereas, the pre-treated sample packed in PP, found 18.58-25.55% (only HW) and 22.80-24.37% (HW+ASC), respectively (Fig. 2B). There was no significant (p>0.05) difference in the antioxidant activity among treatment conditions. Our results show that the application of peeled shallot samples treated with HW, followed by ASC and packed under VP did not affect the loss of antioxidant activity during storage at 5±2°C. Similar results were observed by Zudaira et al. (2020), who suggested that fresh-cut calçots (*Allium cepa* L.) stored under vacuum packaging preserved the physicochemical quality better than passive modified atmosphere after 15 days. Nevertheless, previous research has demonstrated that fresh and fresh-cut produce could be packed in the high-oxygen atmosphere ($O_2 \geq 60\%$) to enhance the antioxidant capacity as reported in blueberry fruit (Zheng et al., 2003). Furthermore, Selcuk & Erkan (2015) reported that the decrease of antioxidant activity was found in sour-sweet pomegranates cv. Hicaznar when stored under low or absent O_2 conditions in modified atmospheres packaging (MAPs). Pinela et al. (2016) also reported that the best treatment for maintaining the DPPH scavenging activity of fresh-cut watercress samples was air packaging.

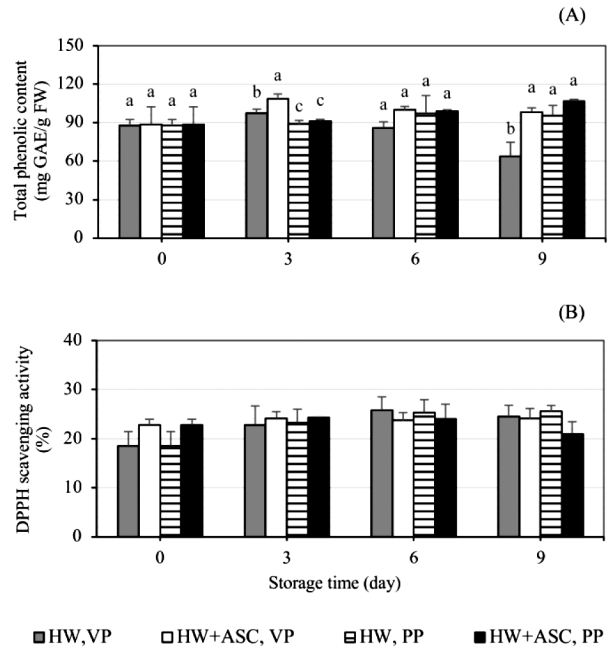

Fig. 2 Effects of pre-treatments on total phenolic content (A) and DPPH scavenging activity (B) of peeled shallots during storage at 5±2°C (HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging). The data represent mean values ± SD (n = 3). The various small letters at the top of the bars in each day indicate significantly differences ($p \leq 0.05$)

Conclusion

The combination pre-treatment with HW, followed by 100 ppm ASC solution, and packed under VP had effectively inhibit the microbial growth, maintaining the quality and antioxidant capacity of peeled shallot during cold storage. This condition could be used to prolong the shelf life of peeled shallot. Therefore, these results could be a promising procedure to extend its application for maintaining the qualities of the plant in the family Alliaceae such as onion, garlic, leek, scallion, chive and garlic chives, etc.

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References

Allende, A., McEvoy, J., Tao, Y., & Luo, Y. (2009). Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control, 20*(3), 230-234.

Artés, F., & Allende, A. (2005). Processing lines and alternative preservation techniques to prolong the shelf-life of minimally fresh processed leafy vegetables. *European Journal of Horticultural Science, 70*(5), 231-245.

Brand-Williams, W., Cuvlier, M.E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28,* 25-30.

Cantwell, M., & Suslow, T.V. (2002). Postharvest handling systems: Fresh-cut fruits and vegetables. In A.A. Kader (Ed.), *Postharvest Technology of Horticultural Crops* (pp. 445-463). Los Angeles, CA: University of California.

Chang, M.M., & Kim, G.H. (2015) Combined effect of hot water dipping and vacuum packaging for maintaining the postharvest quality of peeled taro. *Horitculture Environment and Biotechnology, 56*(5), 662-668.

Cruz, S.R., Luo, Y., Gonzalez, R.J., Tao, Y., & González, G.A. (2006). Acidified sodium chlorite as an alternative to chlorine to control microbial growth on shredded carrots while maintaining quality. *Journal of the Science of Food and Agriculture, 86* (12), 1887-1893.

Dea, S., Brecht, J.K., Nunes, M.C.N., & Baldwin, E.A. (2010). Quality of fresh-cut 'Kent' mango slices prepared from hot water or non-hot water-treated fruit. *Postharvest Biology and Technology, 56*(2), 171-180.

Dron, A., Guyer, D.E., Gage, D.A., & Lira, C.T. (1997). Yield and quality of onion flavour oil obtained by super critical fluid extraction and other methods. *Journal of Food Process Engineering, 20*(2), 107-124.

Food and Drug Administration (FDA). (2000). *"Acidified Sodium Chlorite Solutions". Code of Federal Regulations, 21 CFR173.325.* Retrieved April 14, 2019, from http://www.accessdata.fda.gov/scripts/cfdocs/cfcfr/ CERSearch.cfm

Food and Drug Administration (FDA). (2010). *"Chlorine Dioxide". Code of Federal Regulations, 21 CFR173.300.* Retrieved April 14, 2019, from http://www.gpo.gov/fdsys/pkg/CFR-2010-title21-vol3/pdf/CFR-2010-title21-vol3-sec173-300.pdf

Guo, S., Huang, R., & Chen, H. (2017). Application of water-assisted ultraviolet light in combination of chlorine and hydrogen peroxide to inactivate *Salmonella* on fresh produce. *International Journal of Food Microbiology, 257,* 101-109.

Huang, R., & Chen, H. (2018). Evaluation of inactivating *Salmonella* on iceberg lettuce shreds with washing process in combination with pulsed light, ultrasound and chlorine. *International Journal of Food Microbiology, 285,* 144-151.

Kabelitz, T., & Hassenberg, K. (2018). Control of apple surface microflora for fresh-cut produce by post-harvest hot-water treatment. *LWT-Food Science and Technology, 98,* 492-499.

Leelarungrayub, N., Rattanapanone, V., Chanarat, N., & Gebicki, J.M. (2006). Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. *Nutrition, 22*(3), 226-274.

Loi, M., Liuzzi, V.C., Fanelli, F., De Leonardis, S., Creanza, T.M., Ancona, N., Paciolla, C., & Mulè, G. (2019). Effect of different light-emitting diode (LED) irradiation on the shelf life and phytonutrient content of broccoli (*Brassica oleracea* L. var.*italica*). *Food Chemistry, 283*(15), 206-214.

Lu, X., Wang, J., Al-Qadiri, H.M., Ross, C.F., Powers, J.R., Tang, J., & Rascoa, B.A. (2011). Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. *Food Chemistry, 129*(2), 637-644.

Martínez-Sánchez, A., Allende, A., Bennett, R.N., Ferreres, F., & Gil, M.I. (2006). Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biology and Technology, 42*(1), 86-97.

Masniyom, P. (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin Journal of Science and Technology, 33*(2), 181-192.

Mola, S., Uthairatanakij, A., Srilaong, V., Aiamla-or, S., & Jitareerat, P. (2016). Impacts of sodium chlorite combined with calcium chloride, and calcium ascorbate on microbial population, browning, and quality of fresh-cut rose apple. *Agriculture and Natural Resources, 50*(5), 331-337.

Moradinezhad, F., & Dorostkar, M. (2021) Effect of vacuum and modified atmosphere packaging on the quality attributes and sensory evaluation of fresh jujube Fruit. *International Journal of Fruit Science, 21*(1), 82-94.

Ölmeza, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food Science and Technology, 42*(3), 686-693.

Pinela, J., Barreira, J.C.M., Barros, L., Antonio, A.L., Carvalho, A.M., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2016). Postharvest quality changes in fresh-cut watercress stored under conventional and inert gas-enriched modified atmosphere packaging. *Postharvest Biology and Technology, 112,* 55-63.

Raeisi, S., Sharifi-Rad, M., Quek, S.Y., Shabanpour, B., & Sharifi-Rad, J. (2016). Evaluation of antioxidant and antimicrobial effects of shallot (*Allium ascalonicum* L.) fruit and ajwain (*Trachyspermum ammi* (L.) Sprague) seed extracts in semi-fried coated rainbow trout (*Oncorhynchus mykiss*) fillets for shelf-life extension. *LWT-Food Science and Technology, 65,* 112-121.

Reche, J., M.E. García-Pastor, D. Valero, F. Hernández, M.S. Almansa, P. Legua, & A. Amorós. (2019). Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujube (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage. *Scientia Horticulturae, 258,* 108743.

Renumarn, P. & Choosuk, N. (2020). Influence of packaging and storage conditions on the quality and shelf-life of chewy santol (Kraton-Yee) candies. *E3S Web of Conferences, 141,* 1-6.

Renumarn, P., Srilaong, V., Uthairatanakij, A., Kanlayanarat, S., & Jitareerat, P. (2015). Influence of hot water treatment combined with sodium chlorite on microbial counts and antioxidant quality of fresh-cut broccoli florets. *Acta Horticulturae, 1088,* 383-388.

Rivera-López, J., Vázquez-Ortiz, F.A., Ayala-Zavala, J.F., Sotelo-Mundo, R.R., & Gonzalez-Aguilar, G.A. (2005). Cutting shape and storage temperature affect overall quality of fresh-cut papaya cv. 'Maradol'. *Journal of Food Science, 70,* s482-s489.

Roy, M.K., Juneja, L.R., Isobe, S., & Tsushida, T. (2009). Steam processed broccoli (*Brassica oleracea*) has higher antioxidant activity in chemical and cellular assay systems. *Food Chemistry, 114*(1), 263-269.

Selcuk, N., & Erkan, M. (2015). Changes in phenolic compounds and antioxidant activity of sour–sweet pomegranates cv. 'Hicaznar' during long-term storage under modified atmosphere packaging. *Postharvest Biology and Technology, 109,* 30-39.

Siddiq, M., Roidoung, S., Sogi, D.S., & Dolan, K.D. (2013) Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry, 136,* 803-806.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19,* 669-675.

Wang, Q., Nie, X., & Cantwell, M. (2014). Hot water and ethanol treatments can effectively inhibit the discoloration of fresh-cut sunchoke (*Helianthus tuberosus* L.) tubers. *Postharvest Biology and Technology, 94,* 49-57.

Xiao, Z., Luo, Y., Luo, Y., & Wang, Q. (2011). Combined effects of sodium chlorite dip treatment and chitosan coatings on the quality of fresh-cut d'Anjou pears. *Postharvest Biology and Technology, 62*(3), 319-326.

Zhang, G., Gu, L., Lu, Z., Yuan, C., & Sun, Y. (2019). Browning control of fresh-cut Chinese yam by edible coatings enriched with an inclusion complex containing star anise essential oil. *RSC Advances, 9*(9), 5002-5008.

Zheng, Y., Wang, C.Y., Wang, S.Y., & Zheng, W. (2003). Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 51*(24), 7162-7169.

Zudaira, L., Viñas, I., Abadias, M., Lafarga, T., Bobo, G., Simó, J., & Aguiló-Aguayo, K. (2020). Effects of long-term controlled atmosphere storage, minimal processing, and packaging on quality attributes of calçots (*Allium cepa* L.). *Food Science and Technology International, 26*(5), 403-412.

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Health Behaviors and Patients with Coronary Artery Disease (CAD) : Role of Self-efficacy

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Abstract

Cardiac disease is a major risk of mortality and morbidity globally. Patient with Coronary artery diseases are more likely than other chronic disease in poor health behavior either pre-treatment or post-treatment. Even though cardiac patients tried to seek the ways to promote their health, they might face with the difficulties to change the behavior. Numerous studies showed that self-efficacy plays a crucial role as a buffer to perform activity as reflect personal beliefs and confidence. We synthesize the evidences on self-efficacy and health behavior regarding smoking behavior, alcohol consumption, eating behavior, physical activity or exercise behavior and stress management.We found that most of the researchers successfully applied self-efficacy to promote physical activity or exercise among patients with coronary artery disease in short term period.Nurses should assess functional status and provide health education to promote well-being and encourage patients to perform and maintain their capability on health-promoting behaviors.

Introduction

Coronary artery disease (CAD) is a major health problem as the most common underlying cause of morbidity in the world (Chiou et al., 2009; Mosleh & Darawad, 2015). In 2013, CAD was the underlying cause of death of an estimated 17.3 million from 54 million total deaths (Benjamin et al., 2017). Globally, it is estimated that the mortality rate of CAD will increase by 34% by 2030 (Imes et al., 2016). The prevalence of CAD is 36% of the US population (Kang & Yang, 2013). In Thailand, the statistics from World Health Organization showed that cardiovascular disease are the first ranked across non-communicable diseases affecting 23% of Thai people (WHO, 2018). CAD affects life expectancy, cost of hospitality, and quality of life (Benjamin et al., 2017). Consequently, CAD have been studied across the global in which related to the various of behavioral risk factors including smoking, obesity, physical inactivity, alcohol consumption, and unhealthy diet (Benjamin et al., 2017; WHO, 2018). Moreover, CAD with poor prognosis was typically found among patients who had these behavioral risk factors. WHO has mentioned the effectiveness of health promotion to enhance physical and psychological well-being for people suffering with CAD (WHO, 2018). Nevertheless, it is challenging among CAD patients to maintain healthy lifestyle modification.

CAD patients characteristics and health promotion

CAD typically presents in several symptoms such as dyspnea, angina and fatigue which is dependent on

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the degrees of stenosis, the consequences of the vessel stenosis, plaque characteristics, and the level of myocardial ischemia (Bauersachs et al., 2019). These symptoms can develop into heart failure, hospitalization, disability, and reduced activities of daily living among CAD patients. The improvements of medical technologies and treatments have resulted in an increase life expectancy (Tinkham, 2014). However, CAD patients typically have comorbidities (Benjamin et al., 2017). Health promotion behavior is important, particularly related to maintaining physical function and quality of life (Lee et al., 2006). In fact, health promotion aims to enhance dignity, value, and life satisfaction (Tinkham, 2014). Health promotion efforts tended to focus on single behavior and condition-specific in order to facilitate health behavior change (Ryan, 2009). Patients with well-designed disease management plans the effective ways to promote behavioral health (HuynhHohnbaum et al., 2015). Nevertheless, patients with CAD are largely ignorant of health behavior and lack understanding the lifestyle modification (Chiou et al., 2009)

Self-efficacy: Concept and application for health behavior and chronic diseases

One of the concepts that described for health behavior change is self-efficacy. Self-efficacy is a psychosocial concept that expresses person's capability and confidence for behavior change and maintains healthy behavior. As a central concept of social cognitive theory, self-efficacy refers to individuals believing in their capacity to perform a specific behavior (Bandura, 1997; Grembowski et al., 1993). Bandura (1997) stated that Self-efficacy refers to the confidence in one's ability to behave in such a way as to produce a desirable outcome. Self-efficacy is an important concept because it is related to behavioral changes among individuals (Zullkosky, 2009). Perceived self-efficacy is defined as the individual's confidence to perform a behavior to reach certain expected outcomes (Bandura, 1997; Purdie & McCrindle, 2002). Gonzalo (2019) also noted that perceived self-efficacy refers to the personal capability to organize healthpromoting behavior. Perceived self-efficacy influences perceived barriers to action. Thus, a higher efficacy mediates to lowered perceptions of barriers to the healthy behavior.

Researchers have focused on self-efficacy in applying to affect certain behaviors and maintain positive outcomes (Bandura, 1997). On the one hand, focusing on self-efficacy and outcome expectation is crucial to design an effective intervention to change poor healthy behavior (Zullkosky, 2009).

Health behavior has been examined in an intervention following the theoretical model (Zanjani et al., 2006). To promote health behavior and disease management, the researchers have been integrated perceive self-efficacy as a buffer role to modify or maintain health behavioral among patients with chronic diseases (Clark & Dodge, 1999). For instance, selfefficacy has been shown to predict the individuals' confidence that relates to engaging in exercise, increasing healthy eating, and managing the symptoms (Ahn et al., 2016; Hajizadeh-Sharafabad & Alizadeh, 2016; French, 2013).

The relationship of self-efficacy on predicting health behaviors

Self-efficacy is a successful achievement of action plan (Huynh-Hohnbaum et al., 2015). A high level of self-efficacy is associated to compliance with healthy behaviors such as medication adherence, healthy diet, and regular exercise (Kang et al., 2010). Selfefficacy has been shown to decrease the rate of symptom recurrence and readmission and improve quality of life (Kang et al., 2010). Level of self-efficacy are determined for heart failure (HF) patients (Zullkosky, 2009). A low sense of self-efficacy among HF patients typically struggled with complex daily living tasks, such as sodium and water restrictions, daily weights, and proper medication administration (Zullkosky, 2009). Therefore, self-efficacy plays an important factor to predict health behavior.

The self-efficacy intervention and gap for health behavior among CAD patients

Several pieces of evidence support the idea that self-related cognition has an influence on motivation process for lifestyle modification and behavior changes among CAD patients (Banman & Sawatzky, 2017; Clark & Dodge, 1999; Grembowski et al., 1993; Kang et al., 2010). Despite the known benefits of adhering to a heart-healthy lifestyle, fewer women than men participate in prevention strategies (Banman & Sawatzky, 2017). Percieved self-efficacy as well as individual factors might be an important factor influencing individually health behaviors among CAD patients (Kang et al., 2010). Health behaviors of CAD patients and how a selfefficacy

intervention plays a crucial role to promote healthy behavior that will be discussed in this paper.

The role of self-efficacy in changing health behavior

Self-efficacy has well recognized as a beneficial effect on health behavior and health status in patients with chronic diseases (Bandura, 1997). Self-efficacy would be viewed as part of a reciprocal behavioral process (Clark & Dodge, 1999). Self-efficacy could be a predictor of specific health behaviors as well as an outcome of disease management behaviors (Clark & Dodge, 1999). Perceived self-efficacy expressed the interaction of personal, behavioral, and environmental factors that produce behavior (Clark & Dodge, 1999). Health behavior and disease management showed how self-efficacy fits into the behavioral changing process (Grembowski et al., 1993). Nevertheless, it is not clear about the summary of the effectiveness of self-efficacy for CAD health behaviors.

The level of self-efficacy and CAD health behavior

High self-efficacy for a specific health behavior allows individuals to address better without uncertainty, anxious situation, and conflict (Bandura, 1997). Exploring the specific CAD patients and their behaviors may be an important determinant to illustrate cardiac self-efficacy and intervention (Sarkar et al., 2007). For instance, dietary self-efficacy, physical activity selfefficacy, and cessation of smoking self-efficacy are the major health deviation that will be discussed how self-efficacy plays a large role in improving health behavioral change (Sol et al., 2011).

The Purpose statement

The purpose of this review is to synthesize the self-efficacy intervention strategies on health behaviors among patients with CAD regarding smoking cessation, alcohol consumption, diet /eating behavior, physical activity/exercise behavior, and stress management. We narratively synthesize the evidence indicating a relationship between self-efficacy interventions and CAD health behaviors. The findings of this scoping review may help nurses and health care providers to understand the relationship between self-efficacy and health behaviors of CAD patients.

Self-efficacy intervention on smoking cessation and addictive behavior

1. The relationship between Smoking and CAD

Cigarette smoking has been identified as a risk factor for cardiovascular disease (Sol et al., 2011; Park et al., 2015). Almost 30% of CAD patients-related deaths were smokers (Eisenberg et al., 2010). The previous studies have shown that smoking cessation can reduce the risk of heart attack and peripheral vascular disease (PAD) by one-third after 2 years (Eisenberg et al., 2010; de Hoog et al., 2016; Johnson et al., 1999).

1.1 Self-efficacy and smoking behavior

Social cognitive have been intervened to promote successful smoking cessation in general population, as well as among CAD patients (Eisenberg et al., 2010; de Hoog et al., 2016). Thus, self-efficacy in the area of smoking cessation has been widely studied. Making action and coping plans in CAD patients are the most strategies for smoking cessation (de Hoog et al., 2016). The effects of self-efficacy involved on motivating patients to quit smoking as well as predicted intention to quit smoking (de Hoog et al., 2016). For quitting smoking, self-efficacy had a significant effect on continued abstinence which was mediated by intention (de Hoog et al., 2016). Thus, the potentials factors for changing behavior among CAD smokers focused on positive attitudes, social influence belief, and selfefficacy as a result of a positive attention.

1.2 Smoking cessation intervention strategies

The smoking cessation intervention has shown the benefit for CAD patients after percutaneous coronary intervention. Post-treatment self-efficacy intervention was significantly changed for smoking behavior and increased non-smoking status among CAD patients for 6 and 12 months (Park et al., 2015). Self-efficacy strategies for smoking cessation included counseling such as brief advice, short message via telephone, and self-help materials (Park et al., 2015), and telephone support (Johnson et al., 1999). To increase actual behavior change and self-efficacy to quit smoking, physicians and nurses used verbal advice for CAD patients as a strategy (Eisenberg et al., 2010; de Hoog et al., 2016; Johnson et al., 1999). Eisenberg et al. (2010) claimed that the duration of smoking cessation intervention is varying between 20 minutes to 12 months. These results indicate that perceived self-efficacy for smoking cessation predicts short and long-term smoking cessation.

2. Alcohol and CAD

Mild to moderate amount of alcohol consumption is related to the lower risk of CAD (Emberson & Bennett, 2006). However, the type of alcohol consumed and drinking pattern may have contributing effects as a high risk of CAD (Badnardi et al., 2008). Heavy drinking behavior is not recommended as a higher risk for development of CAD (Bagnardi et al., 2008; Emberson & Bennett, 2006). Bagnardi et al. (2008) explained that irregular drinking will affect physiological response in reducing the threshold for arrhythmia and myocardial function.

For drinking behavior, self- efficacy strategies focused on drinking behavior coping strategies. Avoidant coping strategies are related to increased alcohol consumption (Hasking & Oei, 2004). Avoidant coping predicted alcohol consumption for those who had strong positive expectancies on drinking behavior (Hasking & Oei, 2004). Expectancies and refusal self-efficacy are related to the initiation and maintenance of drinking behavior (Oei & Burrow, 2000). In fact, positive and negative expectancies influence alcohol consumption. For example, individuals expected positive outcomes from drinking behavior, their alcohol consumption will increase. Holloway et al. (2007) used brief intervention (BI) in reducing alcohol consumption among drinkers in hospital. The result revealed that self-efficacy enhancement intervention reduced alcohol consumption after 6 months. It was consistent with Bartholet et al. (2009) alcohol consumption relates to cognitive dimensions of behavior change. Patients with unhealthy alcohol consumption reported a significant increase in readiness to change, importance of changing and confidence in an ability to change after completing 6 months of self-efficacy program.

In summary, the self-efficacy intervention strategies for smoking cessation have been notable while the strategies for reducing alcohol consumption are not specific in CAD patients. However, the results of this paper showed that self-efficacy intervention for changing addictive behaviors relates to the problem-solving and reinforcing patients' self-efficacy

Self-efficacy intervention and diet or eating behavior

Eating behavior is a crucial part of cardiovascular lifestyles. Poor diet quality is a risk for patients before and after cardiac events (Ma et al., 2010). For CAD patients, eating behavior affects body weight, lipid profile, blood glucose, blood pressure (Guertin et al., 2015). The recommendation for meal plan from American Heart Association includes a low-fat diet, low-carbohydrate diet, high protein diet, and low sodium diet (Ma et al., 2010). Even CAD patients should adopt the healthier eating behavior, regulation of their eating behavior is needed in order to persist and maintain well-being and health status in CAD patients (Guertin et al., 2015; Sarkar et al., 2007).

Self-efficacy and eating behavior have been studied in healthy and unhealthy people (Guertin et al., 2015). Self-efficacy can positively influence with eating habit (Kang & Yang, 2013). Researchers applied selfefficacy concept to enhance CAD patients 'confidence to modify their eating habit (Kang & Yang, 2013; Ma et al., 2010). Kang and Yang (2013) claimed that selfefficacy intervention strategies typically focused on secondary prevention as same as smoking cessation. Weight control is an example to measure how selfefficacy plays a significant role to promote eating behavior (Guertin et al., 2015). The effects of experience of receiving education, awareness of risk factors and disease knowledge increase cardiac self-efficacy and related to decrease a body mass index (Kang & Yang, 2013). Body weight is an expected outcome of eating behavior. In fact, patients' confidence in reaching their optimal goal weight, confidence in consuming a low fat diet as well as low cholesterol diet, and confidence in their ability to lose weight (Chiou et al., 2009). It is indicated that effect of self-efficacy will be moderated by outcome expectancy.

Self-efficacy intervention and physical activity/ exercise behavior

Physical activity/exercise is recognized as an important behavior in patients with CAD as part of cardiac rehabilitation (CR) (Barkley & Fahrenwald, 2013). CR programs are effective in improving the functional abilities of CAD patients who maintained adherence to regular exercise (D' Angelo et al., 2014). However, being physically active in daily life among CAD patients appears to be increasingly difficult (Warner et al., 2014). Only 50% of CR participants continue to engage exercise for 3-6 months and few meet recommended level of exercise for 12 months (D' Angelo et al., 2014). The barriers of exercise among CAD patients are beyond the type of physical activity, the duration of intervention, heart condition, fear of falling

and self-efficacy belief (Gary, 2006; Warner et al., 2014). People can have a different level of self-efficacy in specific behavior and tasks (Warner et al., 2014; Rodgers et al., 2002). Thus, CAD patients need an effective intervention to increase their confidence to produce certain action (French, 2013).

Self-efficacy plays a significant role in the adoption of adherence in exercise-related activities in CAD patients (Gary, 2006). The causal role of self-efficacy in changing health behavior is tested. French (2013) claimed that self-efficacy is purposed to be both cause and an effect of performing physical activity. Self-efficacy intervention for physical activity and exercise included behavioral intervention. Alsaleh et al. (2016) found that 6- month multicomponent behavioral change intervention increased moderate physical activity at least 600 METs- minutes per week and increased the walking level on frequency duration and intensity. Nurses used six telephone call-based consultations for 15-20 minutes per one month for 6 months. Behavioral intervention was developed by social cognitive theory and self-efficacy. The adopted behavioral change strategies consisted of goal setting, self-monitoring, and feedback that aimed to increase physical activity. Exercise self-efficacy scale increased from baseline to six-months among intervention group. Thus, the feedback on progression and tailored advice for setting the goals of CAD patients increased the level of physical activity and increased exercise self-efficacy. For older adults with CAD, home-based exercise was an effective intervention. Gary (2006) mentioned that 12-week period for walking exercise combined with education program increased excises self-efficacy, walk distance, exercise exertion. This result was consistent with Rajati et al. (2014) found that integrating the four strategies of exercise selfefficacy including learning by doing, role modeling, positive feedback, and recognition of sign and problem-solving had positive effects on confidence to initiate exercise and recover heart symptoms in short period (6 months). Therefore, self-efficacy intervention for physical activity and exercise had benefit in improving exercise behavior among CAD patients in a short-term period.

Self-efficacy intervention and stress management

Stress is one of the most common physiological responses and CAD patients' complaints (Dimsdale, 2008). The immune system is affected by the activation of the stressor that enhances cardiovascular reactivity, increases blood pressure and heart rate resulting from an excessive sympathetic nervous system activity (O' Leary, 1992). Stress affects both physical and emotional aspects (Dimslade, 2008). Most of CAD patients have experience with stress after heart attack. For example, patients with CAD feel depressed and anxious about the disease and treatment (AHA, 2017). Depression also relates to cardiac risk factor self-efficacy and stressful behaviors (Banman & Sawatzky, 2017). Thus, patients' coping strategies play a major role in determining the impact of a stressor in patients with CAD.

Self-efficacy for a particular task has been studied an integral part of the active coping process (O' Leary, 1992). The perception of self-efficacy is showed as a variable with effects prior to coping responses (Gandoy-Crego et al., 2016). The perception of self-efficacy facilitates cognitive concerning of an individual ability that influences as motivators of action (Banman & Sawatzky, 2017). Parswani et al. (2013) used mindfulness-based stress reduction program for CA patients. This program consisted of mindfulness meditation that helped patients to eliminate negative thought and enhance an ability to tolerate and the negative mood stage. Patients were encouraged to engage the activities with a sense of mastery and pleasure. It is indicated that mindfulness increases self-efficacy and help CAD patients combat low mood as well as reduce anxiety and depressive symptom (Parswani et al., 2013). Self- efficacy is also related to social support (Tkatch et al., 2016). Menne et al. (2016) found that heart health program provided the better understanding the effect stress and decision to make a healthy behavior. This program encourages patients to complete action step on their own with the support of peers (Menne et al., 2016). Thus, self-efficacy is a significant factor to support coping strategy for stress management.

Nursing implication and limitations

This review provides the evidence of the relationship between self-efficacy and health behavior. For this paper, nurses can develop the intervention regarding selfefficacy for CAD patients and other chronic diseases. However, the lack of the self-efficacy evidence on drinking behavior, diet/eating behavior and stress management is a limitation of this paper. The further study is needed to better understand how much selfefficacy influences alcohol consumption or substance

abuse among CAD patients. Also, more research is needed determining the role that self-efficacy plays in stress management and co-morbidities of CAD such as diabetes mellitus and hypertension.

Future directions

Self-efficacy intervention plays a significant role to promote a healthy behavior. Moreover, several studies used self-efficacy as a moderator and/or mediator to predict health behavior and combined with other health promotion theories such as transtheoretical model, health belief model, self-regulation, and self-determination. A lack of integrating the theories may not fully explain the lifestyle behaviors. Therefore, the further study should study the effect of a mixed model intervention on health behavior among CAD patients.

Conclusion

Self-efficacy will play as a buffer role to increase ability to perform healthy lifestyle in which focuses on positive expectation outcomes such as physiological and psychological well-being. Upon this review, selfefficacy interventions especially for physical activity or exercise have been indicated the causal role and strongly promote health-related behavior as well as predict health outcomes. The finding indicates that self-efficacy affects health behavior among CAD patients in which can promote CAD patients to modify their risky health issues. For example, self-efficacy interventions have been intervened to promote exercise and eating behavior following the expected outcome such as the body weight, and lipid profile. For further studies, researcher should conduct a psychoeducational intervention to evaluate the effectiveness of self-efficacy on health-related behavior. Moreover, engaging in healthy lifestyle is based on functional status of cardiac disease. Researchers need to assess the cardiovascular parameter such as ejection fraction to assure that the intervention is carried out in a safe manner during cardiac rehabilitation. Cardiac self-efficacy may improve health outcomes and provide a motivation for health practice for those recovering in rehabilitation period.

References

Ahn, S., Song, R., & Choi, S.W. (2016). Effects of self-care health behaviors on quality of life mediated by cardiovascular risk factors among individuals with coronary artery disease: A structural equation modeling approach. *Asian Nursing Research, 10,* 158-163.

Alsaleh, E., Windle, R., & Blake, H. (2016). Behavioral intervention to increase physical activity in adults with coronary heart disease in Jordan. *BMC Public Health, 16,* 1-11.

American Heart Association: AHA (2017).*Stress and heart health.* Retrieved November 4, 2017, from http://www.heart.org/HEARTORG/HealthyLiving/StressManagement/HowDoesStressAffectYou/Stress-and-Heart-Health_UCM_437370_Article.jsp#.WgLpu1uPKUk

Bagnardi, V., Zatonski, W., Scotti, L., La Vecchia, C., & Corrao, G. (2008). Does drinking pattern modify the effect of alcohol on the risk of coronary heart diases? Evidence from a meta-analysis. *Journal of Epidemiology & Community Health, 62,* 615-619.

Bandura, A. (1997). *Self-efficacy: The exercise of control.* New York: Freeman.

Banman, L., & Sawatzky, J.V. (2017). The role of self-efficacy in cardiovascular disease prevention in women. *Canadian Journal of Cardiovascular Nursing, 27*(3), 11-19.

Barkley, S.A., & Fahrenwald, N.L. (2013). Evaluation of an intervention to increase self-efficacy for independent exercise in cardiac rehabilitation. *Behavioral Medicine, 39,* 104-110.

Bauersachs, R., Zeymer, U., Brière, J.B., Marre, C., Bowrin, K., & Huelsebeck, M. (2019). Burden of coronary artery disease and peripheral artery disease: A literature review. *Cardiovascular therapeutics, 2019,* 8295054.

Benjamin, E.J., Blaha, M.J., Chiuve, S.E., Cushman, M., Das, S.R., Deo, R., ... Muntner, P. (2017). Heart disease and stroke statistics-2017 update: A report from the American Heart Association. *Circulation, 135*(10), e146-e603.

Chiou, A.F., Wang, H.L., Chan, P., Ding, Y.A., Hsu, K.L., & Kao, H.L. (2009). Factors associated with behavior modification for cardiovascular risk factors in patients with coronary artery disease in northern Taiwan. *Journal of Nursing Research, 17*(3), 221-230.

Clark, N.M., & Dodge, J.A. (1999). Exploring self-efficacy as a predictor of disease management. *Health Education & behavior, 26*(1), 72-89.

D' Angelo, M.E.S., Pelletier, L.G., Reid, R.D., & Huta, V. (2014). The roles of self-efficacy and motivation in the prediction of short- and long-term adherence to exercise among patients with coronary heart disease. *Health Psychology, 33*(11), 1344-1353.

de Hoog, N., Bolman, C., Berndt, N., Kers, E., Mudde, A., de Vries, H., & Lechner, L. (2016). Smoking cessation in cardiac patients: the influence of action plans, coping plans and self-efficacy on quitting smoking. *Health Education Research, 31*(3), 350-362.

Dimsdale, J.E. (2008). Psychological stress and cardiovascular disease. *Journal of the American College of Cardiology, 51*(13), 1237–1246.

Eisenberg, M.J., Blum, L.M., Filion, K.B., Rinfret, S., Pilote, L., Paradis, G., ... O'Loughlin, J. (2010). The efficacy of smoking cessation therapies in cardiac patients: a meta-analysis of randomized controlled trials. *Canadian Journal of Cardiology, 26*(2), 73-79.

Emberson, J.R., & Bennett, D.A. (2006). Effect of alcohol on risk of coronary heart disease and stroke: Causality, bias, or a bit of both? *Vascular Health and Risk Management, 2*(3), 239–249.

French, D.P. (2013). The role of self-efficacy in changing health-related behaviour: Cause, effect or spurious association? *British Journal of Health Psychology, 18*(2), 237-243.

Gandoy-Crego, M., Clemente, M., Gomez-Cantorna, C., Gonzalez-Rodriguez, R., & Reig-Botella, A. (2016). Self-efficacy and health: The SEH scale. *American Journal of Health Behavior, 40*(3), 389-395.

Gary, R. (2006). Exercise self-efficacy in older women with diastolic heart failure: Results of a walking program and education intervention. *Journal of Gerontological Nursing, 32*(7), 31-39.

Gonzalo, A. (2019). *Nola pender: Health promotion model.* Retrieved March 12, 2021, from https://nurseslabs.com/nola-pender-health-promotion-model/#perceived_self-efficacy

Grembowski, D., Patrick, D., Diehr, P., Durham, M., Beresford, S., Kay, E., & Hecht, J. (1993). Self-efficacy and health behavior among older adults. *Journal of Health and Social Behavior, 34,* 89-104.

Guertin, C., Rocchi, M., Pelletier, L.G., Emond, C., & Lalande, G. (2015). The role of motivation and the regulation of eating on the physical and psychological health of patients with cardiovascular disease. *Journal of Health Psychology, 20*(5), 543-555.

Hajizadeh-Sharafabad, F., & Alizadeh, M. (2016). Predicyors of health-promoting behaviors in patients with coronary artery disease in Iranian population. *International Journal of Nursing Practice, 22,* 486-492.

Hasking, P.A., & Oei, T. (2004). The complexities of drinking: Interactions between the cognitive and behavioral determinants of alcohol consumption. *Addiction Research and Theory, 12*(5), 469-488.

Holloway, A.S., Watson, H.E., Arthur, A.J., Starr, G., McFadyen, A.K., & McIntosh, J. (2007). The effect of brief interventions on alcohol consumption among heavy drinkers in a general hospital setting. *Addiction, 102*(11), 1762-1770.

Huynh-Hohnbaum, A.L.T., Marshall, L., Villa, V. M., & Lee, G. (2015). Self-management of heart disease in older adults. *Home Health Care Services Quarterly, 34,* 159–172.

Imes, C.C., Novosel, L.M., & Burke, L. (2016). Heart disease risk and self-efficacy in over weight and obese adults. *The Journal of Nurse Practitioners, 12*(10), 710-716.

Johnson, J.L., Budz, B., Mackay, M., & Miller, C. (1999). Evaluation of nurse-delivered smoking cessation intervention for hospitalized patients with cardiac disease. *Heart & Lung, 28*(1), 55-64.

Kang, Y., & Yang, I.S. (2013). Cardiac self-efficacy and its predictors in patients with coronary artery disease. *Journal of Clinical Nursing, 22,* 2465-2473.

Kang, Y., Yang, I.S., & Kim, N. (2010). Correlates of health behaviors in patients with coronary artery disease. *Asian Nursing Research, 4*(1), 45-55.

Lee, T. W., Ko, I.S., & Lee, K.J. (2006). Health promotion behaviors and quality of life among community-dwelling elderly in Korea: a cross-sectional survey. *International Journal of Nursing Studies, 43*(3), 293-300.

Ma, Y., Olendzki, B.C., Pagoto, S.L., Merriam, P.A., & Ockene, I.S. (2010). What are patients actually eating: The dietary practices of cardiovascular disease patients. *Current Opinion in Cardiology, 22*(5), 518-521.

Menne, H.L., Borato, L.M., Shelton, E.G., & Johnson, J.D. (2016). Promoting heart health and behavior change in a vulnerable older adult population. *Healthy Aging Research, 5*(11),1-11.

Mosleh, S.M., & Darawad, M. (2015). Patients' adherence to healthy behavior in coronary heart disease: risk factor management among Jordanian patients. *Journal of Cardiovascular Nursing, 30*(6), 471-478.

Oei, T., & Burrow, T. (2000). Alcohol expectancy and drinking refusal self-efficacy: A test of specificity theory. *Addictive Behavior, 25*(4), 499-507.

O' Leary, A. (1992). Self-efficacy and health: Behavioral and stress-physiological mediation. *Cognitive Theory and Research, 16*(2), 229-245.

Park, A.H., Lee, S.J., & Oh, S.J. (2015). The effect of a smoking cessation programme on health-promoting lifestyles and smoking cessation in smokers who had undergone percutaneous coronary intervention. *International Journal of Nursing Practice, 21,* 107-117.

Parswani, M.J., Sharma, M.P., & Iyengar, S. (2013). Mindfulness-based stress reduction program in coronary heart disease: A randomized control trial. *International Journal of Yoga, 6*(2), 111–117.

Purdie, N., & McCrindle, A. (2002). Self-regulation, self-efficacy and health behavior change in older adults. *Educational Gerontology, 28,* 379-400.

Rajati, F., Sadeghi, M., Feizi, A., Sharifirad, G., Hasandokht, T., & Mostafavi, F. (2014). Self-efficacy strategies to improve exercise in patients with heart failure: A systematic review. *ARYA Atherosclerosis, 10*(6), 319-333.

Rodgers, W.M., Hall, C.R., Blanchard, C.M., McAuley, E., & Munroe, K.J. (2002). Task and scheduling self-efficacy as predictors of exercise behavior. *Psychology & Health, 17*(4), 405-416.

Ryan, P. (2009). Integrated theory of health behavior change: Background and intervention development. *Clinical Nurse Specialist, 23*(3), 161–172.

Sarkar, U., Ali, S., & Whooley, M.A. (2007). Self-efficacy and health status in patients with coronary heart disease: findings from the heart and soul study. *Psychosomatic Medicine, 69*(4), 306–312.

So, B.G.M., Graaf, Y.V.D., Peterson, R.V., & Visseren, F.F.J. (2011). The effect of self-efficacy on cardiovascular lifestyle. *European Journal of Cardiovascular Nursing, 10,* 180-186.

Tinkham, M. (2014). Health promotion in the elderly with coronary artery disease. *Journal of Vascular Nursing, 32*(4), 151-155.

Tkatch, R., Musich, S., MacLeod, S., Alsgaard, K., Hawkins, K., & Yeh, C.S. (2016). Population health management for older adults: Review of interventions for promoting successful aging across the health continuum. *Gerontology Geriatric Medicine, 2,* 2333721416667877.

Warner, L.M., Schuz, B., Wolff, J.K., Parschau, L., Wurm, S., & Schwarzer, R. (2014). Sources of self-efficacy for physical activity. *Health Psychology, 33*(11), 1298-1308.

World Health Organization, WHO. (2018). *Noncommunicable diseases (NCD) country profiles 2018*. Retrieved March 23, 2021, from https://www.who.int/nmh/countries/tha_en.pdf

Zanjani, F.A., Schaie, K.W., & Willis, S.L. (2006). Age group and health status effects on health behavior change. *Behavioral Medicine, 32*(2), 36-46.

Zullkosky, K. (2009). Self-efficacy: A concept analysis. *Nursing Forum, 44*(2), 93-102.

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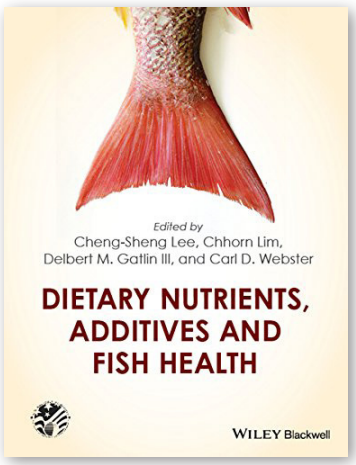
Book Review

Phukphon Munglue



Book name: Dietary Nutrients, Additives, and Fish Health
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Fish provide excellent sources of several essential nutrients to support an increasing demand for fish consumption worldwide. Many fish species have been cultivated under intensive operations. However, these practices could generate stressful conditions to cultured fish, which has often been found to be detrimental to growth performance and immune defenses. The use of antibiotics and synthetic agents in fish farms has the potential to produce the emergence and spread of drug-resistant pathogens, environmental pollution, and residues in fish products. Therefore, a variety of feed additives have been tested in the laboratory conditions and many of them have long been used by fish farmers in order to improve growth, hematological parameters, and immune components in fish. In this regard, a book entitled *Dietary Nutrients, Additives,* and *Fish Health* highlights the current knowledge of various types of feed additives such as prebiotics, probiotics, organic acids and their salts as well as plant extracts in aquaculture production written by expert international contributors. Additionally, this comprehensive text emphasizes the effects of dietary nutrients, antinutrients, mycotoxins, and nucleotides on the health and welfare of fish. The importance role of the fish immune system and the relationship between feeding practices and fish health are also described in the text. The modes of action produced by the feed additives on biochemical and molecular aspects of the growth performance and immune status of fish are completely discussed. This publication is useful for students, academics, researchers, fish farmers, and fish feed companies.

Reviewer

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5. The qualitative assessments of the expert panel returned by the manuscript's author. The author is expected to make the appropriate alterations indicated by the experts' feedback.
6. The author returns the edited document; the editorial staff examines the changes to make sure they are congruent with the experts' recommendations as well as the journal format.
7. The revised version is granted the University's recognition of "Accepted" for publication status with the Journal of Food Health and Bioenvironmental Science Stamp on every page. Information regarding publication status (Accepted) is located on the journal's website (http/ research dusit ac.th/new/e-Journal)
8. The editorial tearm conducts an accuracy check for all articles before sending the manuscripts to the printer to create a draft journal issue.
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Publication Criteria

1. The original manuscript is concise and interesting to the academic community.
2. The content of the manuscript represents quality and theory of the discipline and also possesses knowledge with practical applications.
3. The manuscript's content is consistent with the aim and scope of the journal.

4. Manuscripts submitted to Journal of Food Health and Bioenvironmental Science must not have been published previously in or actively involved in the publication process of another journal.

5. All content within the manuscript must be the product of the author himself. Any use of intellectual property within must be appropriately credited to its original authors.

6. The author must comply with the writing style established by Journal of Food Health and Bioenvironmental Science.

7. There are four levels of assessments given to reviewed manuscripts:

7.1 Requires minor or no revisions prior to publication.

7.2 Requires moderate revisions prior to publication.

7.3 Requires intensive editing and revisions followed by a future evaluation. 7.4 Unsuitable for publication

In order to be assigned the "Accepted" status, an article must be assessed as “Requires minor or no modification prior to publication” by two of the three experts from the peer review process.

Formatting Guidelines

It is the author's responsibility to format manuscripts to the standards of Journal of Food Health and Bioenvironmental Science. The details of format style are contained herein,

1. Format

1.1 Single page printing on A4 paper with a width of 19 cm and height of 26.5 cm. The vertical and horizontal spacing from the margins must be 3.5 cm and 2.5 cm, respectively.

1.2 Typefaces and layout: English must be typed using Time New Roman using Microsoft word. Specific font format guidelines are as follows.

1.2.1 The header contains the page number, aligned on the right side, in 12 pt. font.

1.2.2 The title in English languages must be 12 pt. font, bolded, and center aligned. The title should not exceed two lines of text.

1.2.3 The author's name in English language must be typed 9.5 pt. font and centered below the title. Asterisks (*) should proceed the authors' names which is correspond to the appropriate author.

1.2.4 Affiliations should match each author with their appropriate affiliated institutions and organizations. In case of different affiliations, superscript numbers should follow the surname a and affiliation a.

1.2.5 A footnote must be placed on the first page of the article with the text “*Corresponding Author”, and the next line of text should contain “e-mail”.

1.2.6 “Abstract” in English must be 9.5 pt. font, bolded, left aligned, and placed below the Thai keywords section. Abstract text must be 9 pt. font, with 1 tab indentation from left and right margins.

1.2.7 “Keywords:” should appear in English language in 9.5 pt. font, placed beneath the English abstract text and be aligned with the left margin. English keywords must be 9 pt. font, and should not exceed four words. Each keyword should be separated by a comma (,) and space.

1.2.8 Regardless of language choice, the main text headings used throughout the paper must be 9.5 pt. font, bolded, and aligned with the left margin.

1.2.9 Bulleted items must appear as 9 pt. font, bolded, and be indented 1.5 tabs from the left margin.

1.2.10 Body text must appear as 9 pt. normal font, and be indented 1 tab from the left and right margins.

1.2.11 “References” must be 9.5 pt. font, bolded, and be aligned with the left margin. Individual entries must be 9 pt. font and should follow American Psychological Association (APA) formatting guidelines. Any lines of text for a single entry that exceed the first line should use a “hanging indent” of 1.5 tabs from the left margin.

1.3 An appropriate page length for publication in the Journal is approximately 15 pages.

2. Citing

Should follow American Psychological Association (APA) formatting guidelines. Click http://jfhb.dusit.ac.th/flie/Ref%20Guidelines. pdf to see the example.

3. Ordering of Titles in Journal of Food Health and Bioenvironmental Science

The written manuscript may contain only English. The content should be easy to understand and clear. If the author uses abbreviation, full word must appear before any abbreviation.

3.1 The title should be brief, the length should not exceed 100 characters.

3.2 The authors if there are more than six authors only the first author is listed, followed by "et al."

3.3 Affiliated entities associated with the author should appear in English languages.

3.4 The abstract must be written in English language. The abstract should briefly summarize the research and not exceed 250 words or 15 lines of text.

3.5 The "Keywords" section must contain no more than four keywords that allow for appropriate searching and selection based upon the article's topic.

3.6 The "Introduction" section should provide background information relevant to the research, provide information regarding the manuscript's content and state the objectives of the work.

3.7 The "Materials and methods" section delineates the procedures, how the research was conducted, sampling method (i.e. simple random samples) and population, and the creation and development of research tools used for data collection and analysis.

3.8 The "Results" section or "Results and Discussion" presents data obtained during the research and may be displayed as tables, graphs, illustrations, and accompanying explanations. Tables should be not have left and right borders and are normally black and white printed. No more than five tables should be present in the "Results" section. Pictures within the section should be clear and use simple black and white coloring with an accompanying caption, the author wishes to use colors for any item they may do so; however, the author will be responsible for the additional costs of color printing.

3.9 The "Discussion" section or "Result and Discussion" should explore the significance of the results of the work and address whether or not the data support the research hypothesis and compare research findings to other similar research works.

3.10 The "Conclusions" section should summary of the main topic covered or a re – statement of the research problem.

3.11 The "Acknowledgements" (if any) section should provide help during the research (e.g., providing materials, laboratory, equipment, etc.) and funding.

Sending Original manuscript

1. Compose the manuscript using the format of the Journal of Food Health and Bioenvironmental Science.

2. Send the manuscript via ScholarOne website https://mc03.manuscriptcentral.com/jfhb

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- If there are conflicts of interests, reviewers should inform the editorial staff. Editors will decide whether the reviewer is appropriate for the manuscript or not.

Author Regulations

- The authors should write the manuscript related to the theme of Food, Health, biological and environmental disciplines. The research manuscript should contained relevant background information, efficient methodology, APA style citation, accurate results, and reasonable discussion.
- The authors should follow the journal guidelines strictly.
- Any opinion or perspective made in the manuscript must be explicitly highlighted as "opinion" or "perspective"
- The authors must be careful and aware that fraudulent information and omission of important information are unethical author behaviors.
- The authors must be able to provide research data if the Editor see needed.
- Authors must reference other works properly. Any work involved in the manuscript also must be well credited.
- The authors must make sure that the manuscript has not been published elsewhere before and is not currently in the publication process in other journals.

- The person must have made significant contributions to the manuscript, participate and give important efficient content during revisions and provide approval for publication in order to be listed as an author. Researchers who do not meet the above criteria should be listed in the Acknowledgements section.

- Author should identify any conflicts of interest that might have influenced the data and/or interpretations of data.

- To make the efficient revision, the authors should respond to all the given critiques and suggestions during the revision.

- If the authors find errors in their works that need to be correct, the author should inform the editors immediately.

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