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Content

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Occurrence of Microplastics in Lanchester's Freshwater Prawns *(Macrobrachium lanchesteri)*

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Abstract

Microplastics (MPs) are a contemporary societal concern that have been identified as a growing environmental hazard as a result of improper disposal. The goal of this research was to study the MPs found in Lanchester's freshwater prawn, *Macrobrachium lanchesteri*, in three different sites along the irrigation canal in central Thailand. In each site, twenty individuals (n = 20) of *M. lanchesteri* were examined for MP content. A total number of MPs in site 1 was 1093 items/20 ind. (range 35-80), with an average of 60.72 ± 10.54 items/individual. Site 2 was 1001 items/20 ind. (range 34-71), with an average of 50.05±8.71 items/individual and site 3 was 1039 items/20 ind. (range 33-71), with an average of 51.95±10.62 items/individual. Among the various shapes, types and colors of MPs identified, fiber and fragment, as well as blue, were the most common. The prawn had the greatest MPs size range of 200-250 µm in each point, followed by MPs size ranges of 250-500 µm and <100 µm. FT-IR data confirmed 1 particle of ethylene propylene rubber (EPR), 4 particles of polyethylene glycol (PEG), 1 particle of polyethylene terephthalate (PET), 1 particle of bis (2-ethylhexyl) phosphate, 1 particle of cellulose acetate, 2 particles of glyceryl polypropylene glycol ether, 1 particle of polyester (PE) and 1 particle of hydroxyethyl cellulose (HEC). The findings of this study provide detailed and useful information for a better understanding of MP contamination in the region, and *M. lanchesteri* is proposed as an appropriate species for monitoring MPs in freshwater ecosystems.

Introduction

Microplastics (MPs) are pervasive pollutants of emerging concern that have recently received a lot of attention due to their widespread presence in the environment and potential negative effects on living biota and human health. Plastic production has risen dramatically worldwide, rising from 1.5 million tonnes in the 1950s to 335 million tonnes in 2016 (Kumar et al., 2021). Plastics degrade into smaller pieces in the aquatic system as a result of weathering processes such as photo-degradation, oxidation and mechanical abrasion

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(Andrady, 2011), and particles that have a length of less than five millimeters are known as microplastics (Frias & Nash, 2019). Aquatic ecosystems have been identified as being threatened by microplastics (Anderson et al. 2016). Wright et al. (2013) showed that MPs are accessible to a wide range of aquatic organisms because they are similar in size to planktonic organisms and other suspended particles. As particle size decreases, the possibility of bioaccumulation increases (Law & Thompson 2014). Contaminants that adsorb to MPs can increase their toxic effects (Rochman et al. 2013).

The genus *Macrobrachium* (Bate, 1868) is a genus of freshwater prawns of the family Palaemonidae, which are decapod crustaceans. Except for Europe, they can be found on all continents in the tropics and subtropics (Holthuis, 1993). Freshwater prawns *Macrobrachium lanchesteri* is a small but hardy Thai native prawn that can be found in a variety of freshwater habitats, including rivers, streams, rice fields, lakes, ponds and reservoirs. This prawn is a good swimmer, so it is less confined to the bottom than many palaemonids and it is commonly found in large numbers in these habitats. This species has been found in temperatures ranging from 25.5 to 36.0°C and is capable of living in shallow waters with relatively high water temperatures for several hours. *Macrobrachium lanchesteri* is one of the top five economic species in Thailand and many other Southeast Asian countries. *M. lanchesteri* is a valuable economic resource for people living in rural areas of northeastern Thailand (Uraiwan & Sodsuk, 2004). They are consumed as native foods and used in a variety of forms, including shrimp paste, crispy shrimp, koi kung and kung jom, a Northeastern Thai delicacy that generates approximately 240,000 baht per year (Rottanapradap, 2013). *M. lanchesteri* also has economic value in the aquatic environment and plays an important role in the food chain ecosystem (Thongmee et al., 2021).

Aquatic organisms can absorb MP particles easily (Li et al., 2016). The consumers or predators in the freshwater food chain, such as shrimp, can ingest MPs through prey items such as small crustaceans and arthropods, as well as fish larvae. As a result, the presence of MPs in freshwater organisms' gastrointestinal tracts has raised global concern, as freshwater food may be a major source of MPs in humans (Strungaru et al., 2019). The present study aims to assess the presence of MPs in the Lanchester's freshwater prawn (*Macrobrachium lanchesteri*) in the irrigation canal at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand. The findings will be the first of their kind, revealing microplastics in freshwater prawns.

Materials and methods

1. Sampling and preparation of samples

The freshwater prawn sample was collected in November 2020 from three different sites along the irrigation canal at Kasetsart University's Kamphaeng Saen Campus in Nakhon Pathom Province, Thailand (N 14°02′16, E 099o57′49). In each site, there are different types of vegetation coverings, and water passes through different areas. The water for this canal is supplied by the Mae Klong Dam. Irrigation canal water is a valuable water resource for the university, serving primarily as a source of drinking water and agricultural activities. Lanchester's freshwater prawns were collected in waterbodies using an aquatic dip net near macrophytes (edge habitats). There were a total of 60 prawns caught. The samples were transported in a cooler to the laboratory and stored at -20°C pending processing and analysis.

2. Extraction of microplastics from *Macrobrachium lanchesteri*

Before use, all sample processing equipment was rinsed with filtered deionized water. Within a week of being collected, prawn samples were processed. The frozen prawns were thawed in a stainless steel tray. The body weight of each specimen was measured and recorded. Individual prawns from each site was analyzed to assess MP ingestion in the prawns' whole body. To remove any microplastics from their exoskeletons, the prawns were rinsed with filtered deionized water. Individually, the prawn bodies were placed into 30 mL beaker. Following that, 20 mL of a hydrogen peroxide solution (30% H_2O_2) was added to each individual beaker to digest tissue (Ehlers et al., 2019). All beakers were then wrapped in parafilm and placed in an ES-20 environmental shaker-incubator at 150 rpm for 7 days. Microplastics with density separation were separated after 7 days. Each sample was transferred to a glass separation funnel, which was then filled with 16 g of potassium formate (99% HCO_2K). Using vacuum filtration, the solution was drained and filtered onto nylon membrane filters (pore size of 0.45 μm; diameter 47 mm). The filters were placed in small aluminum bowls, covered with aluminum foil and dried in a drying cabinet (50°C) for two days.

3. Visual observation of the microplastics

The microplastics on each filter paper were observed and photographed using a stereomicroscope (Leica EZ4E). A visual examination was also performed to identify suspected microplastics based on morphological characteristics such as color and shape (Hidalgo-Ruz et al. 2012). Microplastic particles were classified based on their shapes, such as fiber, sphere, film (a thin and small layer) and fragment (part of a larger plastic item) (Su et al., 2016; 2018). For corroboratory FTIR analysis, representative suspected particles that were visually identified as potential plastics were chosen.

4. FT-IR analyses of microplastics found in *Macrobrachium lanchesteri*

The 20 microplastic particles were selected and identified using a Perkin Elmer Spectrum-Fourier transform infrared spectrometer (FT-IR). The spectrum ranged from 4000 to 500 cm^{-1}, with an 8 cm^{-1} spectral resolution. Polymer type analysis and functional group characterization were compared with spectra databases and instrument libraries and the characteristic peaks of the functional groups were combined to determine the polymer type. As in previous studies (Bergmann et al., 2017), only particles with a hit quality of more than 700 were considered microplastics. Fig. 1 depicts a summary of the protocol.

5. Statistical analyses

The abundance of microplastics was expressed as MP/individual±SD. Levene's test was used to verify the normal variance structure. One-way analysis of variance (ANOVA) was used to compare MP concentrations, types and sizes between sites. MP concentration, type and size were the dependent variables and site was the independent variable. A post hoc HSD Tukey test (THSD) was used to identify significant differences between conditions that were established at $p < 0.05$. Statistica 20.0 software was used for all statistical analyses.

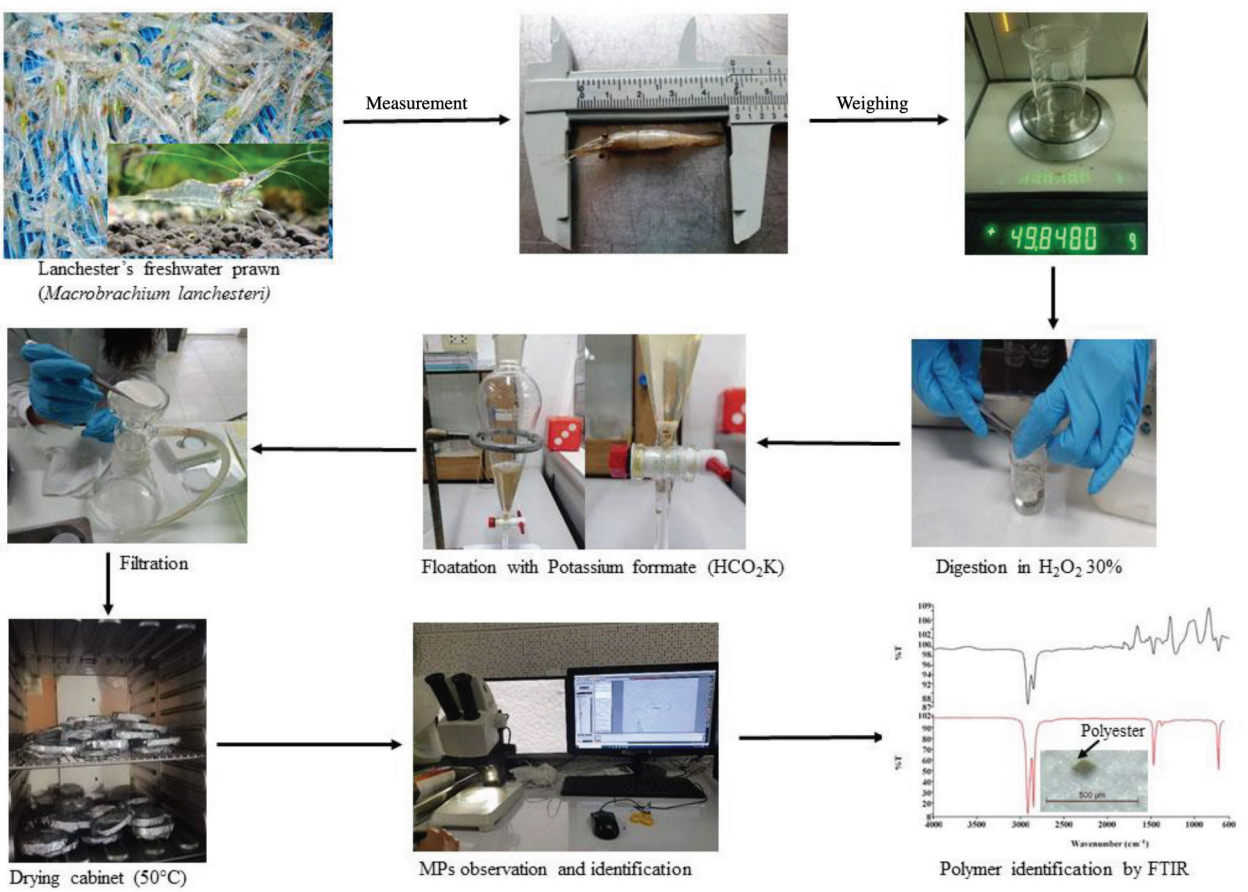

Fig. 1 Steps in the extraction of microplastics from the whole body of *Macrobrachium lanchesteri*

Results

1. Abundance of microplastics

Microplastic concentrations in Lanchester's freshwater prawn, *Macrobrachium lanchesteri* De Man, 1911, were determined for the first time. A total of n = 60 prawns were processed. The average body length of 20 prawns in site 1 was 2.96±0.82 cm, with an average weight of 0.27±0.08 g. The average body length of 20 prawns in site 2 was 3.18±0.61 cm, with an average weight of 0.30± 0.13 g. The average body length of 20 prawns in site 3 was 3.07±0.71 cm, with an average weight of 0.28±0.10 g. In site 1, the total number of MPs in prawns were 1093 item/20 ind. (range 35-80), with an average of 60.72±10.54 item/individual. In site 2, the total number of MPs in prawns were 1001 items/20 ind. (range 34–71), with an average of 50.05±8.71 items/individual. In site 3, the total number of MPs in prawns were 1039 items/20 ind. (range 33–71), with an average of 51.95±10.62 items/individual (Table 1).

Table 1 Microplastic particle mean values and standard deviations in prawns in three irrigation canal sites. Asterisks indicate significant differences in the size, type and color of microplastics in each prawn ($p < 0.05$; one-way ANOVA followed by the Tukey HSD test)

Microplastic properties	Site 1 (N =20)	Site 2 (N=20)	Site 3 (N=20)
Mean body length (cm)	2.96±0.82	3.18±0.61	3.07±0.71
Mean body weight (g)	0.27±0.08	0.30±0.13	0.28±0.10
Total number of MPs (item)	1093 (range 35-80)	1001 (range 34-71)	1039 (range 33-71)
	(60.72±10.54)	(50.05±8.71)	(51.95±10.62)
Size			
<100 µm	11.64±4.18^{a}	6.94±1.58^{a}	6.40±3.18^{b}
200-250 µm^{ns}	22.66±4.78	20.50±2.21	21.45±5.04
250-500 µm^{ns}	19.11±4.89	16.90±5.31	15.70±4.20
>500 µm^{ns}	7.94±4.00	7.11±3.77	8.84±4.07
Type			
Fiber	22.16±5.07^{a}	18.50±5.91^{a}	27.05±5.67^{b}
Fragment^{ns}	21.66±4.24	19.60±4.27	21.10±5.56
Sphere	16.88±6.41^{a}	11.95±3.60^{b}	6.06±3.47^{c}
Color			
Blue^{ns}	20.61±4.77	18.45±4.13	18.65±3.92
Violet	10.27±3.12^{a}	7.16±3.11^{b}	7.06±3.64^{b}
Red^{ns}	13.77±4.13	11.55±5.11	12.15±4.71
Transparent^{ns}	15.61±4.25	15.52±6.56	15.50±6.74

Remark: Values with different letters in each row indicate significant differences ($p \leq 0.05$). ns indicates not significant differences ($P>0.05$)

2. Microplastic particle size

The MPs were identified and categorized into four sizes: <100 µm, 200–250 µm, 250–500 µm and > 500 µm (Table 1, Fig. 2). The prawns in site 1, 2 and 3 had the greatest size MPs range of 200-250 µm, followed by MPs size range of 250-500 µm and MPs size range of <100 µm. The largest MPs size ranged over 500 µm in site 3. In site 3, the MPs size ranged from <100 µm was significantly different (Table 1, Fig. 2).

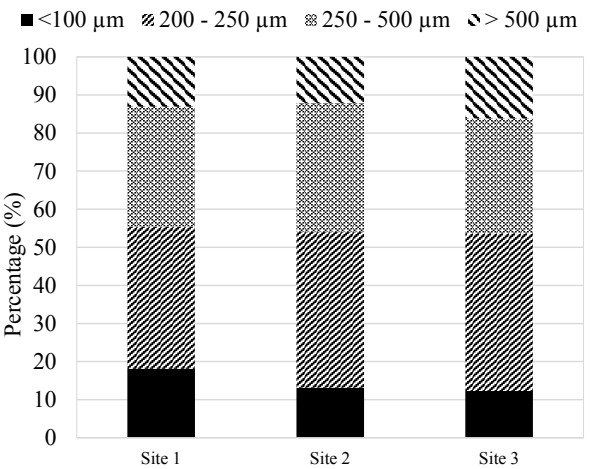

Fig. 2 Percentages of different sizes of microplastics in prawns at each sampling site

3. Color and type of microplastics

Fiber and fragment MPs were detected in the majority of prawns from all sampling sites (72–91%), with sphere MPs being less common (9–28%) (Table 1, Figs. 3-6). The relative abundance of MPs sphere shape was significantly different in all sampling sites and the abundance of MPs fiber shape was significantly different in site 3 (Table 1).

All the prawns in each sampling site were found to be of five different colors, with blue being the most common, followed by white (transparent), red, violet, and green. The green color was detected in small amounts of prawns in site 1, while not appearing in prawns from sites 2 and 3. (Fig. 3, 5). The MPs' violet color varied significantly between prawns at each sampling site (Table 1, Fig. 7).

4. Identification of polymers by FT-IR

FT-IR spectroscopy was used to characterize MPs, and the 12 items analyzed were Ethylene propylene rubber (EPR) (1 particle), Polyethylene glycol (PEG) (4 particles), Polyethylene terephthalate (PET) (1 particle), bis(2-ethylhexyl) phosphate (1 particle), Cellulose acetate (1 particle), Glyceryl polypropylene glycol ether (2 particles), Polyester (PE) (1 particle) and Hydroxyethyl cellulose (HEC) (1 particle) (Figs. 8-10).

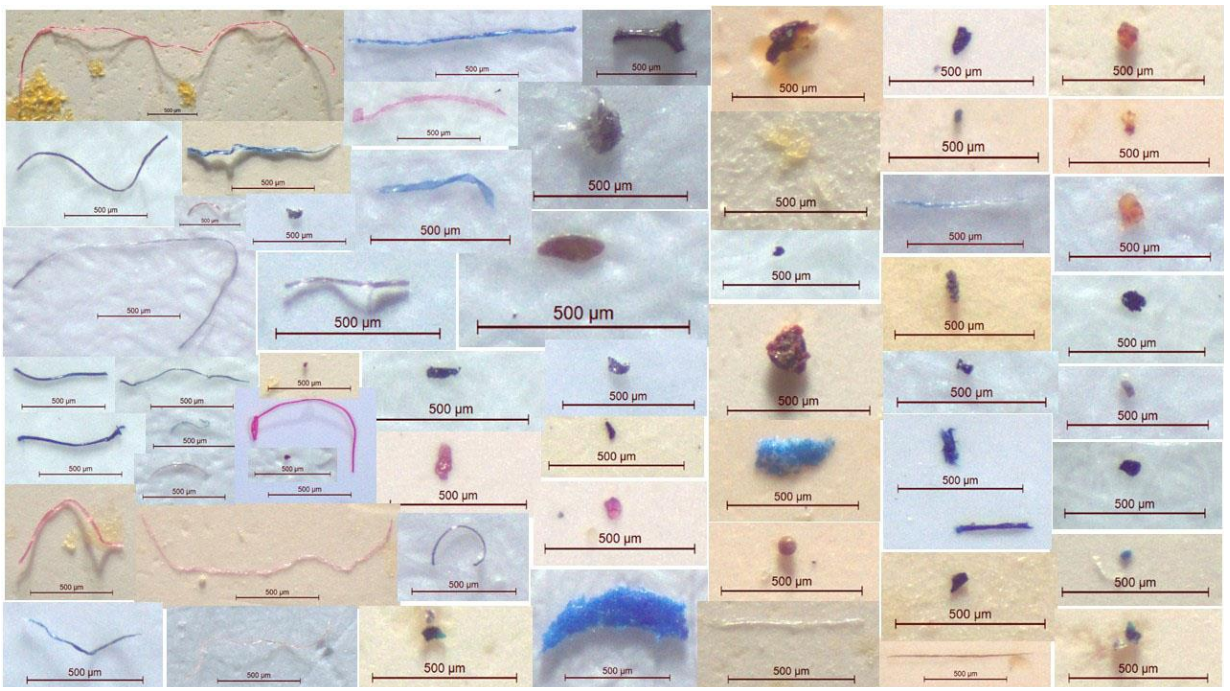

Fig. 3 An example of microplastic type, size and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 1

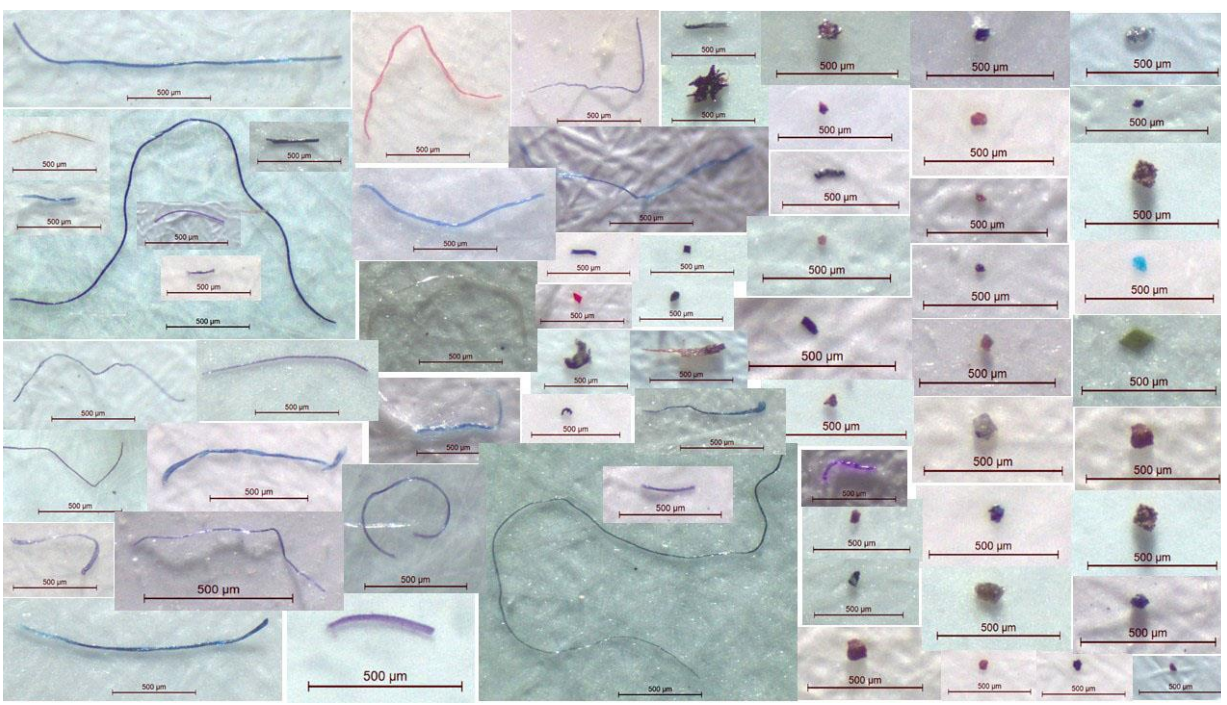

Fig. 4 An example of microplastic type, size and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 2

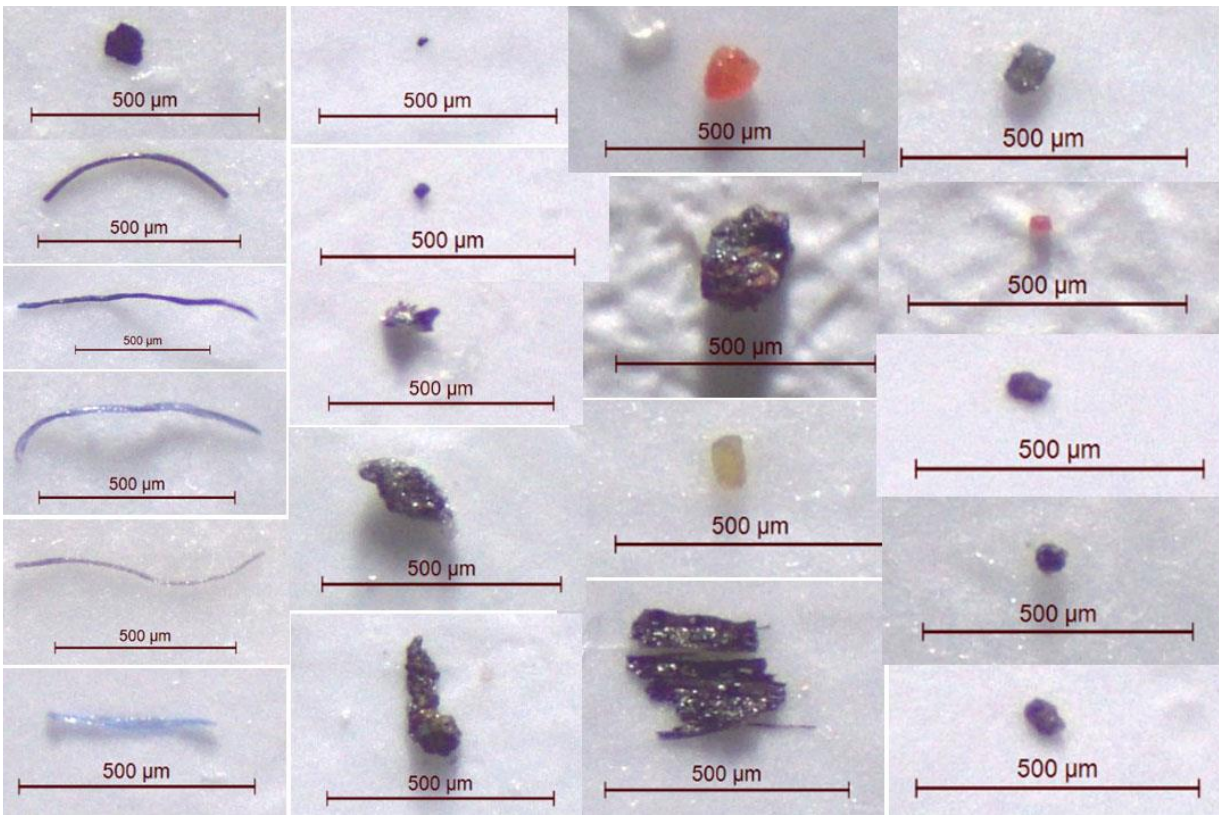

Fig. 5 An example of microplastic type, size and color in Lanchester's freshwater prawn *(Macrobrachium lanchesteri)* in site 3

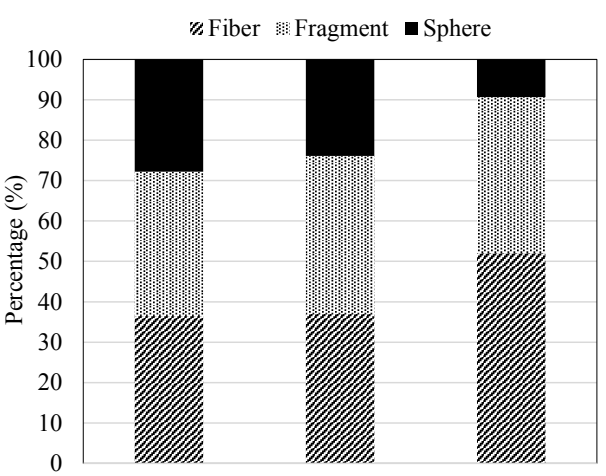

Fig. 6 Percentages of different microplastic types in prawns at each sampling site

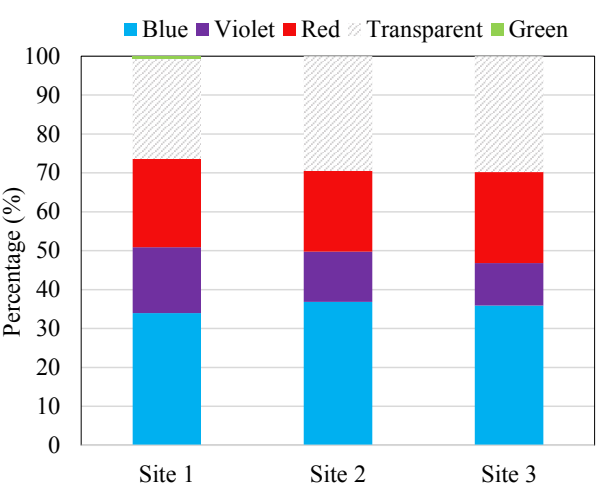

Fig. 7 Percentages of different colours of microplastics in prawns at each sampling site

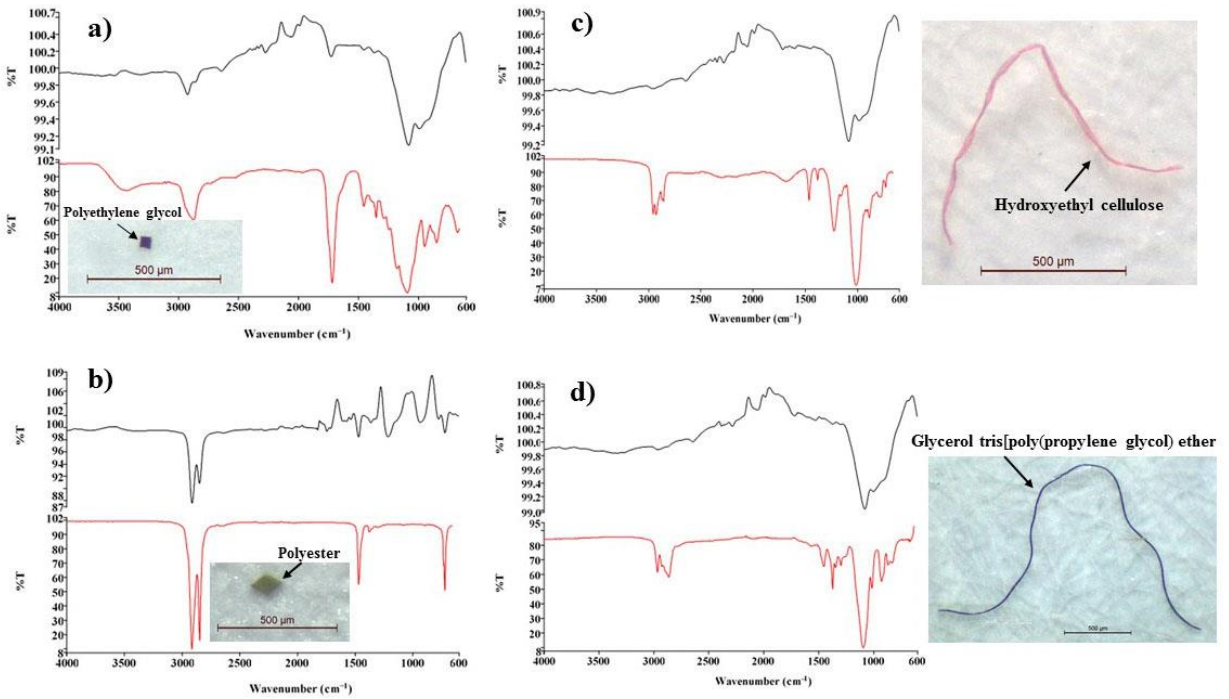

Fig. 8 FT-IR spectra of representative microplastic polymers found in prawns in site 2

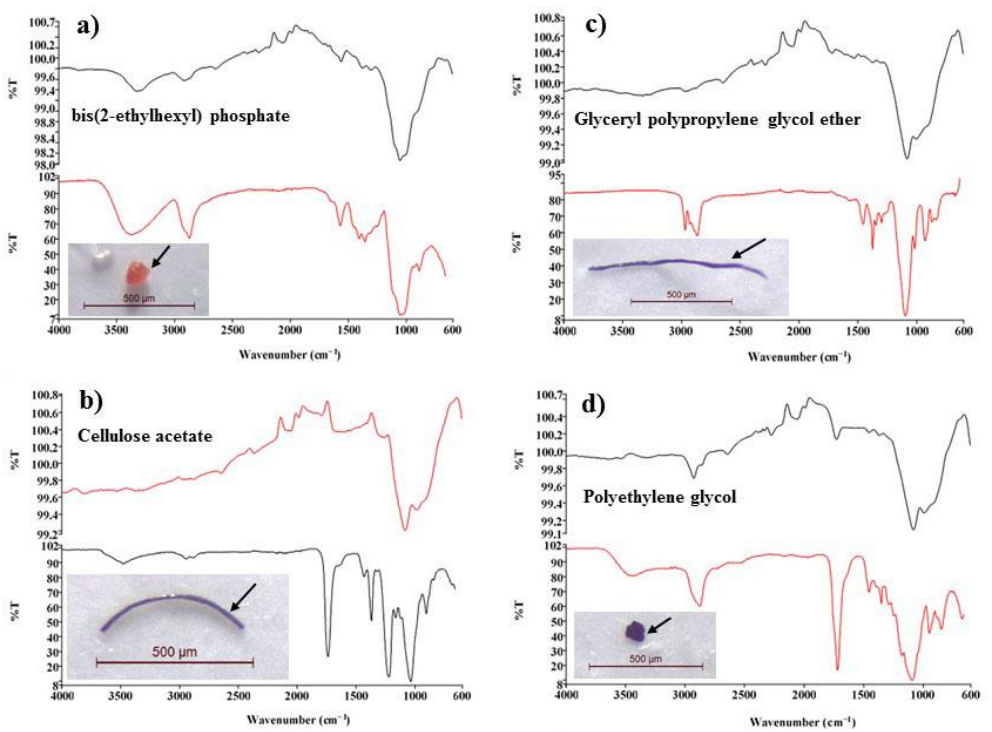

Fig. 9 FT-IR spectra of representative microplastic polymers found in prawns in site 3

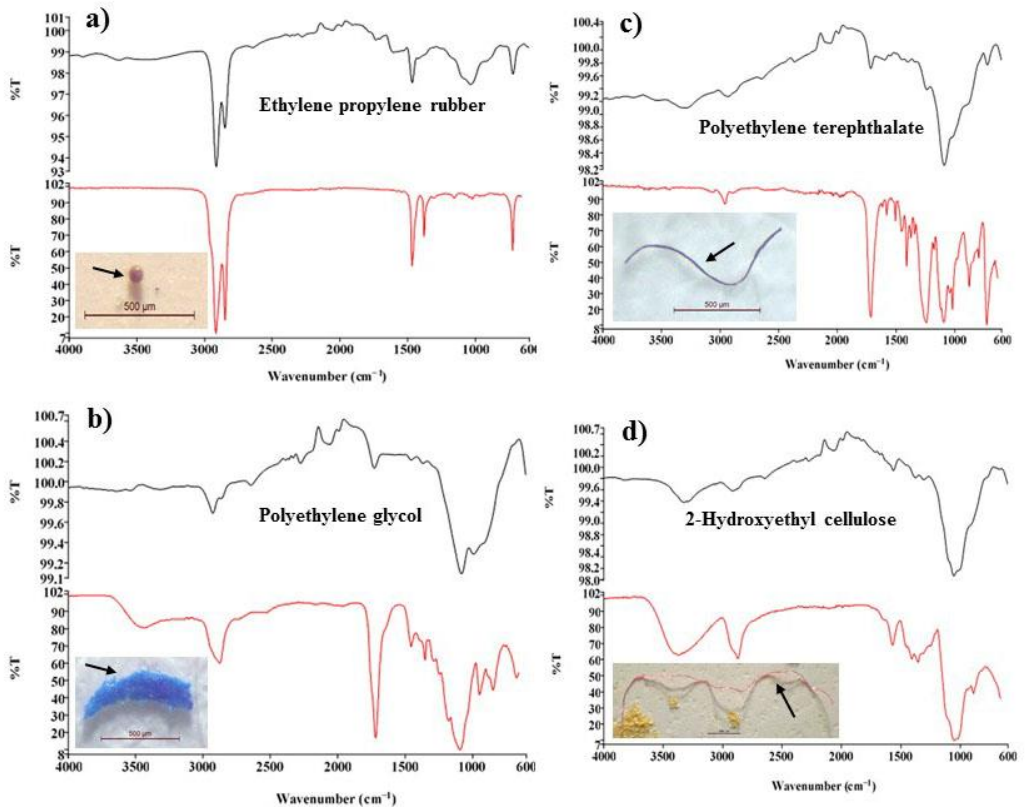

Fig. 10 FT-IR spectra of representative microplastic polymers found in prawns in site 1

Discussion

This research offers a better understanding of microplastic contamination in Lanchester's freshwater prawns as well as a new approach to conducting microplastic biomonitoring investigations in the field with healthy animals. Some species of invertebrates have been identified as potentially good bioindicators of microplastic pollution in their individual ecosystems based on their life-history strategies (Abbasi et al., 2018; Avio et al., 2017; Ory et al., 2017; Sanchez et al., 2014). There is still a deficit in understanding freshwater creatures as compared to studies on microplastic pollution in marine organisms. It's the first time a freshwater crustacean has been used as a bioindicator to measure microplastic pollution. *Macrobrachium lanchesteri* De Man, 1911, is a common crustacean in Thailand's freshwater ecosystems and could be a good bioindicator for microplastic pollution monitoring.

The results show that prawns living in the irrigation canal had MP particles in their entire body burden. This is consistent with previous reports of MP pollution in organisms from different climate zones. Microplastics, for example, were found in 63% of brown shrimp from shallow water habitats in the southern North Sea (Devriese et al., 2015). MPs were discovered in 36% of the glass shrimp *Paratya australiensis* (Family Atyidae) found in fresh waterbodies in eastern Australia (Nan et al., 2020). This study found MPs in sampling site 1 were 1093 items/20 ind., MPs in sampling site 2 were 1001 items/20 ind., and MPs in sampling site 3 were 1039 items/20 ind., indicating that prawns in the irrigation canal are not free of MP pollution. Recently, Reunura & Prommi (2022) detected microplastics in the gastrointestinal tract (GT) of *Litopenaeus vannamei* (Penaeidae) and *Macrobrachium rosenbergii* (Palaemonidae) in a cultured pond in the central part of Thailand. Female and male *M. rosenbergii* and *L. vannamei,* in particular, had an average of 33.31±19.42, 33.43±19.07 and 11.00±4.60 MP items/individual in their GTs. Furthermore, they had 32.66±5.10, 32.14±4.85 and 10.28±1.19 MP items/ gram of intestinal material, respectively.

M. lanchesteri is one of Thailand's top five economic species, as well as beingsignificant in many other Southeast Asian countries. *M. lanchesteri* is an important economic resource for local people in rural areas in Northeastern Thailand (Uraiwan & Sodsuk, 2004). *M. lanchesteri* is a valuable economic resource for those living in rural areas of northeastern Thailand (Uraiwan & Sodsuk, 2004). It is eaten as a native cuisine and is utilized in a variety of dishes, including shrimp paste, crispy shrimp, koi kung and kung jom, a local delicacy in Northeastern Thailand that generates about 240,000 baht per year (Rottanapradap, 2013). The gastrointestinal tract is not removed when these prawns are consumed because they are typically consumed fresh. *M. lanchesteri* also plays a vital part in the aquatic food chain environment, which has economic significance (Thongmee, 2021).

M. lanchesteri feeds on diatoms, small aquatic insects and detritus. The majority of these prey animals ingest MPs and contribute to trophic transfer to prawns and other predators, including fish, cetaceans, seabirds and humans (Guebert-Bartholo et al., 2011; Devriese et al., 2015; Amelineau et al., 2016; Teng et al., 2019). At sampling site 1, MP abundance in prawns was 60.72±10.54 items/individual, 50.05±8.71 items/individual at sampling site 2 and 51.95±10.62 items/individual at sampling site 3, which is comparable to MP abundance in two types of shrimp (*Metapenaeus monocerous* and *Penaeus monodon*) (Hossain et al., 2020).

Fiber and fragment were the most common shapes in the ingested MPs, accounting for 72–91% of all prawns. In Bangladesh's northern bay of Bengal, tiger shrimp were found in 57% of occurrences and brown shrimp in 32% of occurrences (Hossain et al., 2020). According to other results (Browne et al. 2011; Claessens et al. 2011), fishing nets, ropes and lines, laundry and urban debris are all likely sources of fibers and fragments in an irrigation canal.

In this investigation, five different colors of MPs were detected in prawn samples, including blue, white (transparent), red, violet and green, which is similar to Hossain et al. (2020). Other studies have reported that MP items are black (Bellas et al., 2016), white/transparent (Boerger et al., 2010) and blue (Ory et al., 2017). Fiber form MPs were also found in both shrimps (57–58%), which is consistent with previous findings in brown shrimp (Devriese et al., 2015) and decapod crustaceans (Murray & Cowie, 2011).

All MPs in the <100 µm, 200-250 µm, 250-500 µm and > 500 µm size ranges were found in all prawns. The variance in MP size could be explained by food selectivity and irrigation canal habitat variables.

Based on FT-IR examination, 12 of the 20 randomly selected particles were found to be plastic, while the remaining 8 were found to be non-plastic; of the 12 MP particles, four were polyethylene glycol (PEG), two were glyceryl polypropylene glycol ether and one was each of ethylene propylene rubber (EPR), polyethylene terephthalate (PET), bis (2-ethylhexyl) phosphate and cellulose acetate, polyester (PE) and hydroxyethyl cellulose (HEC) polymers. Polyethylene glycol, a common raw material in the production of facial foam, skin cleaners, soap and dishwashing liquid, may contribute to the accumulation of freshwater organisms. According to the findings of Steer et al. (2017) rayon particles can be found in used clothing, furniture, feminine hygiene items and nappies. However, because prawns are commonly consumed without having the gastrointestinal system or exoskeletal structures removed, MPs detected in prawns may be passed to humans through the food chain. The presence of MPs in prawn species means that future research into the effects of MPs in Thailand's freshwater environment would need to include a wider range of species and habitats.

Conclusion

This study provides preliminary information on the occurrence of microplastics (MPs) in *Macrobrachium lanchesteri,* a Thai native prawn species. All sampled prawns individually were found to have microplastics in varying abundances. The findings indicate that microplastics were widely distributed throughout Thailand. The prawns in this study were found to ingest primarily microplastics of a specific type and color, as well as a wide range of microfiber sizes. As a result, they may be a viable technique for future biomonitoring of microplastic pollution in the biota of the environment. The data from this first study could be used as reference or baseline data for future extensive research.

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Antimicrobial Effect of Deodorant Products Containing *Rhinacanthus nasutus* Extract for Reducing Armpit Odor

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Abstract

The aim of this study was to investigate the effectiveness of developing a roll-on deodorant that can reduce armpit microbiota bacteria to decrease armpit odor. The richest apocrine sweat glands in the armpit region secrete a variety of odor precursors that are transformed into volatile odoriferous substances by bacterial enzymes on the skin surface. The dominant armpits microbiota included four groups of bacteria such as *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp., and also fungi or yeasts. Two formulas of the roll-on deodorant products, RDEOF-1 and RDEOF-2 were developed. RDEOF-2 contained an ethanolic extract of *Rhinacanthus nasutus* (L.) Kurz leaves inhibited several microorganisms, including Gram-positive and Gram-negative bacteria. Sixteen healthy volunteers showed satisfactory assessment for RDEOF-2 that was greater than RDEOF-1. Armpit bacteria were collected by swab method and armpit odor was evaluated by ASTM method. Results showed dominant bacteria of two genera including *Staphylococcus* spp. and *Corynebacterium* spp. in all swab samples that mainly cause armpit odor. Armpit bacterial numbers before using deodorants were high in the range of $2x10^3$ to $9x10^5$ (CFU/mL). After applying RDEOF-1 and RDEOF-2, bacterial numbers decreased in the range of $1x10^3$ to $8x10^5$ and $3x10^2$ to $4x10^5$ (CFU/mL), respectively. Armpit bacteria were found in males more than in females. Deodorant products containing *R. nasutus* extract have been shown to reduce the bacteria that cause armpit odor. Therefore, the development of the deodorant product with natural plant extracts is warranted.

Introduction

The human body can disseminate odorous substances with the breath, saliva, sweat (from skin), urine, or reproductive organs fluids (Mogilnicka et al., 2020). The major odorants are small, volatile compounds that may either be produced in situ such as skin and oral cavity, or be carried by blood circulation from the gut, which is the main site of bacterial metabolism. Skin is the largest, most functional organ of the human body.

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A complex mixture of hundreds of chemical compounds (carboxylic acids, ketones, aldehydes, alcohols, esters, hydrocarbons, etc.), are transformed from precursors secreted by excretory skin glands (Wilke et al., 2007; Ferdenzi et al., 2020). The human armpit is covered by a dense array of glands and the area of this skin niche is colonized by a large population of bacteria or microbiome (Wilke et al., 2007; Natsch & Emter 2020). Also, the armpit has a major function in human body odor formation. The richest apocrine sweat glands in the armpit region secrete a variety of odor precursors that are transformed into volatile odoriferous substances by bacterial enzymes on the skin surface (Natsch et al., 2005; Natsch & Emter 2020). The main contributors to armpits odor are a group of acids, with 3-methyl-2-hexenoic acid (3M2H) and 3-hydroxy-3-methyl-hexanoic acid (HMHA) and also a group of sulfanylalkanols, particularly 3-methyl-3-sulfanylhexan-1-ol (3M3SH) (Troccaz et.al., 2004; Martin et al., 2010). Bromhidrosis, which is also known as osmidrosis or malodorous sweating, is a distressing condition that is characterized by offensive body odor, noticeable especially in the armpits, genital, or feet area. All three types of sweat glands (apocrine, eccrine and apoeccrine) play a role in the pathogenesis of this disease. Excessive sweating followed by decomposition of sweat constituents by bacteria results in an unpleasing smell of sweat (Semkova et al., 2015; Mogilnicka et al., 2020).

The dominant armpits microbiota inhabiting skin surface break down apocrine sweat into numerous volatiles molecules such as ammonia and short-chain fatty acids including four groups of bacteria such as *Staphylococcus, Micrococcus, Corynebacterium* and *Propionibacterium* and also fungi or yeasts such as *Malassezia* and *Candida* (Leaden et al., 1981; Wilke et al., 2007; Mogilnicka et al., 2020; Moskovicz et al., 2020; Ogbebor 2021). Deodorants and antiperspirants have been used for centuries worldwide, evolving from simple fragrances that masked aggressive odors to complex ingredients based on aluminum and zirconium chemistries that act to slow or diminish sweat production, or botanical extracts with antimicrobial activity. They help to degrade apocrine axillary secretions while antiperspirants function by reducing armpit odor and perspiration levels from the armpit making it dry and comfortable (Philip & Jack 2000; Abrutyn, 2010; Ogbebor 2021; Abrutyn 2022). Roll-on deodorant usually comes in form of a glass or plastic bottle with a ball top. The product is applied by rolling the ball over the skin and leaving a wet sensation behind the application. (Gámbaro et al., 2019).

Deodorant manufacturers have developed a variety of products employing these mechanisms including the physical methods, such as the use of silica gel or activated carbon to absorb malodorous compounds; biological methods, such as the use of sanitizers that inactivate malodor-producing bacteria; chemical methods, which involve chemical inactivation of malodorous compounds; and for sensory methods, which involve masking unpleasant odors with fragrances. Nowadays botanical extracts play an important role in deodorant products, green tea catechins are widely used in Japan and Southeast Asia (Henmi et al., 2020). Thailand has many medicinal plants such *Rhinacanthus nasutus* (L.) Kurz that contain rhinacanthin showed the effect of inhibiting many microorganisms including Gram-positive bacteria such as *Staphylococus aureus,* Gram-negative bacteria such as *Escherichia coli* and fungi such as *Candida albicans* (Puttarak et al., 2010; Kumar et al., 2021, Onvimol et al. 2021). Our previous study showed that *R. nasutus* extract had bacterial growth inhibitory activity and especially, this extract inhibited *S. aureus, S. epidermidis* and *Escherichia coli* that were found on human skin (Panichakul et al., 2017). In our previous study, *R. nasutus* extract was used to apply in the development of liquid soap and alcohol gel as presented in the petty patent (no. 1503001746 and no. 1803000589). The present study aimed to investigate the effectiveness of developing roll-on deodorant to reduce armpit microbiota bacteria in order to decrease the armpit odor.

Materials and methods

1. Participants

Sixteen healthy volunteers (male and female) aged 19-50 years were enrolled after they provided their consent to participate in this study. All volunteers had no history of allergic skin rash, no wound on the skin. Reasons for exclusion were pregnancy, hypersensitivity to cosmetics and skin diseases. The ethical and methodological aspects of this study on volunteers for odor evaluation and bacterial collection (SDU-RDI_HS 2021-002) have been approved by Ethical Review Subcommittee for Human Research in Health Science, Research and Development Institute, Suan Dusit University, Bangkok, Thailand.

2. Roll-on deodorant formulations

Two formulas of the roll-on deodorant product were developed and provided by Siam Natural Products Co.,Ltd. Khet Phaya Thai, Bangkok. Roll-on deodorant formula-1 and 2 (RDEOF-1 and RDEOF-2) were prepared with the same base formula which contained water, butylene glycol, glycerin, disodium EDTA, steareth-2, ceteareth-12, stearyl alcohol, ceteareth-20, distearyl ether, menthol, tocopheryl acetate, aluminum chlorohydrate, alum, L-glutathione, hydrolyzed collagen, niacinamide, ethyl ascorbic acid, *Aloe barbadensis* leaf extract and sodium benzoate. The difference between RDEOF-1 and RDEOF-2 was that RDEOF-2 contained 1% (w/w) of ethanolic extract of *Rhinacanthus nasutus* (L.) Kurz. For preparing *R. nasutus* extract modified from a method previously described (Panichakul et al., 2017). Leaves of *R. nasutus* were dried and extracted in 95% ethanol for 7 days at room temperature and evaporated under reduced pressure below 45°C.

3. Study design, bacterial sampling, application of formulations and odor evaluation

Briefly, participants discontinued their habitual application of deodorants, antiperspirants, or other cosmetic products to the armpits within 1 week before their first visit to the researcher (day 0) and throughout the entire trial period. At baseline, first time (day 0), the participants' right armpit cavern was swabbed by rotating with a cotton swab that was soaked in 0.85% sodium chloride repeatedly using the side of the swab for 30 s. (Capone et al., 2011; Moskovicz et al., 2020). At the same time, the participants' left armpit was odor tested using a cotton bud held in the armpit for 5 min and then an assessment of armpit malodor was evaluated by 4 investigators and scoring 1-11 range of odor (0=no malodor, 5=moderate malodor, 10=extremely strong malodor) followed by ASTM method (E 1207-87 standard practice for the sensory evaluation of axillary deodorant) (Dumas et al., 2009). The sample was carried in transfer culture or trypticase soy broth (TSB) and kept at 4°C until analyzed. For deodorant testing, participants received the RDEOF-1 or RDEOF-2 application and 1 ml of roll-on was used to apply on both right and left armpit in the morning on day 1 and after showering on the next day. On day 3, 48 h after roll-on applying, the armpit malodor was assessed and a swab sample was also collected. For RDEOF-2 testing, the same application protocol was done but RDEOF-2 was applied on day 6 and after 48 h, the armpit malodor was assessed and a swab sample was also collected. All investigators and participants were blinded. The participants were asked by questionnaire: (i) whether they had observed a difference in the odor from the armpit before and after using the products and if so, (ii) which armpit they considered being less odorous. The assessment by each participant was analyzed to compare between 2 formulas of roll-on products.

4. Microbiology: Quantification and identification of bacteria

Bacteria on the armpit were determined by total aerobic plate count. In brief, 100 µl of each sample from the armpit swab were diluted with normal saline in 10-fold dilution (10^{-1} to 10^{-4}) and each dilution was cultured on trypticase soy agar (TSA) in triplicate plates. The culture plates were incubated in ambient air for 24 h at 35°C. Bacterial growth was estimated semi-quantitatively as 0 (no growth), $1x10^2$, $1x10^3$, $1x10^4$ and $\geq 1x10^5$ CFU/ml. For identification of *Staphylococcus* spp., bacteria were cultured on selective media Baird-Parker agar (BPA), while *Corynebacterium* spp. typical colony was observed on TSA and bacterial morphology was further identified by Gram' staining and was identified under light microscopy, 100X (Leaden et al., 1981; Troccaz et al., 2004; Yamazaki et al., 2010 ; Capone et al., 2011; James et al., 2013; Urban et al., 2016; Ågren et al., 2020). The individual isolated colony of bacteria was cryopreserved in 10% glycerol at -20°C for future study.

5. Statistical analysis

Data are represented as mean±SD from three biologically independent experiments.

Results and discussion

1. Participants, product application/acceptance and bacterial swabs

Sixteen healthy volunteers, including 7 males and 9 females (age 19-50 years) were enrolled in during December 2021. Most of the female and no male participants had shaved armpits. Most of the participants had used deodorant and antiperspirant and also one man had never used both deodorant and antiperspirant before. None of the participants withdraw from the study.

Sixteen volunteers were tested by collecting bacterial swabs from the right armpit, while cotton bud insertion under left armpit following ASTM method (Dumas et al., 2009) was used to detect odor. Bacterial samples were cultured and the results of the quantitative bacteriological survey of 16 volunteers are shown in Table 1. Sixteen participant exhibit total numbers of

armpit bacteria before using deodorants in a range of $2x10^3$ to $9x10^5$ (CFU/mL) and armpit bacteria were found in males more than in females. After applying RDEOF-1 for 48 h, bacterial numbers decreased to the range of $1x10^3$ to $8x10^5$ (CFU / mL). After applying RDEOF-2 for 48 h, bacterial numbers were lower in the range of $3x10^2$ to $4x10^5$ (CFU/mL), compared to before and after using RDEOF-1. These results showed that *R. nasutus* extract could reduce bacterial growth revealing the synergistically effect of the additives in RDEOF-2. Rhinocanthin of *R. nasutus* extract (Puttarak et al., 2010; Kumar et al., 2021, Onvimol et al. 2021) may be the factor that had the additive effect on bacteria inhibition. From the results in Fig. 1, levels of armpit odor from sixteen participants were reduced after applying RDEOF-1 or RDEOF-2, compared with before using roll-on. The reduction of odor could be caused by ingredients formulated in both products relating to the study evaluated the antimicrobial activity of various multifunctional cosmetic ingredients by Youenou et al. (2022) and also zinc oxide exhibit reduced malodor and bacteria growth (Ågren et al., 2020). This study shows that RDEOF-2 reduces axillary odor more than RDEOF-1, which does not contain *R. nasutus* extract.

Table 1 Prevalence and density of armpit resident microbiota before and after using roll-on deodorants

Volunteer (N=16)	Sex	Total aerobic plate count (CFU / mL)					
		Before use	SD	After use RDEOF-1	SD	After use RDEOF-2	SD
1	M	$1x10^5$	±1.15	$5x10^3$	±1.15	$1x10^3$	±0.50
2	M	$2x10^3$	±0.50	$1x10^3$	±1.00	$1x10^3$	±0.55
3	M	$9x10^3$	±1.15	$9x10^3$	±1.50	$9x10^3$	±1.15
4	M	$2.8x10^5$	±1.00	$2.6x10^5$	±1.15	$2.4x10^5$	±1.10
5	F	$8x10^5$	±0.55	$3x10^3$	±0.65	$1x10^3$	±1.15
6	M	$6x10^3$	±0.58	$8x10^5$	±0.55	$6x10^3$	±0.50
7	F	$8x10^3$	±1.00	$8x10^5$	±1.58	$4X10^5$	±0.59
8	F	$6x10^5$	±0.55	$3.2x10^5$	±1.15	$3x10^4$	±0.55
9	F	$5x10^4$	±1.15	$5x10^4$	±0.15	$8x10^3$	±1.00
10	M	$8x10^5$	±0.65	$2x10^5$	±0.55	$3x10^2$	±0.65
11	F	$9x10^5$	±0.55	$3X10^4$	±0.63	$3X10^3$	±1.15
12	M	$3X10^3$	±0.50	$2X10^3$	±1.00	$1.5X10^3$	±1.00
13	F	$3X10^3$	±0.05	$1.2X10^3$	±0.55	$1X10^3$	±0.05
14	F	$3X10^3$	±1.15	$7.2X10^3$	±1.00	$1.8X10^3$	±1.55
15	F	$4X10^5$	±1.50	$3X10^3$	±0.65	$1X10^5$	±0.15
16	F	$5X10^4$	±1.55	$3X10^5$	±0.15	$1X10^5$	±0.55

2. Microbiology: Bacteria identification

Armpit odor showed a strong smell which may have been caused by volatile fatty acids such as 3-hydroxy-3-methylhexanoic acid (HMHA) and the sulphanylalkanol, 3-methyl-3-sulfanylhexan-1-ol (MSH). HMHA is released from a glutamine conjugate by the action of a zinc-dependent aminoacylase from *Corynebacteria* spp., whereas MSH is derived from a cysteinylglycine-S-conjugate by the action of *Staphylococcus* sp. In addition to the *S. epidermidis* strain, the most abundant microorganisms present in human axilla are *Corynebacterium* spp., possessing the aminoacylase and *Staphylococcus* spp. possessing C-S lyase activities. These substances are found in human armpits and cause armpit odor as onion-like, cheesy and rancid odor emits armpit odors (Natsch et al. 2005; Troccaz et al. 2004; Natsch et al. 2005).

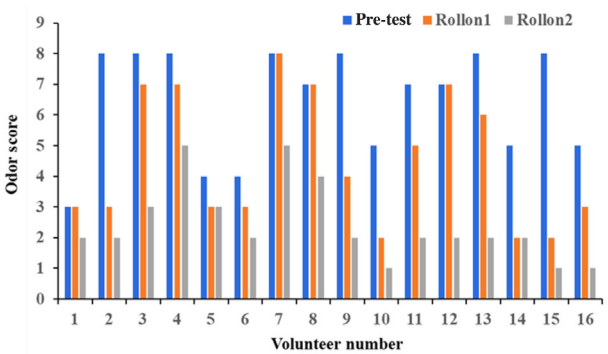

Fig. 1 Armpit odor scores in human volunteers (n = 16) by judges trained for the assessment of axillary malodor before and after the application of RDEOF-1 (Roll-on deodorant formula-1) and RDEOF-2 (Roll-on deodorant formula-2) products

In this study, the typical colony of *Staphylococcus* spp. on BPA was found to be grey-black colonies and a halo, opaque halo, clear halo around colonies and the medium became yellow as previously described (Schau, 1986) and then Gram' stain confirms to classify gram-positive, staphylococci arrangement and catalase-positive. The typical colony and morphology of *Staphylococcus* spp. were found in all 16 samples (Table 2). Moreover, *Corynebacterium* spp., was detected with colony appearance on non-selective media TSA that was a small colony with circular, convex, shiny, white to the gray colony and bacterial morphology was gram-positive, small, pleomorphic, non-sporing, catalase-positive and appeared as club-shaped, V-in Y-shaped arrangements, or in clumps that resemble Chinese letters. This suggests that *Corynebacterium* spp. (Kasper & Fauci, 2013; Bernard & Funke 2015), serves to indicate that *Corynebacterium* spp. was found in all 16 samples (Table 2). These results confirmed that both *Staphylococcus* spp. and *Corynebacterium* spp. were dominant in human armpit in both males and females, which is consistent with previous reports (Leaden et al.,

1981; Troccaz et al., 2004; Yamazaki et al., 2010; Capone et al., 2011; James et al., 2013; Urban et al., 2016; Ågren et al., 2020). However, the specific confirmation of bacterial species should be required, especially using molecular biology techniques.

Consequently, the application of roll-on deodorants with *R. nasutus* extract could decrease armpit microbiomes of *Staphylococcus* spp. and *Corynebacterium* spp. thereby generating fatty acid witch induces pH increase providing optimal microenvironment for other microbial growth. The reduction of the two dominant odor causing bacteria, *Staphylococcus* spp. and *Corynebacterium* spp. odor causing bacteria would reduce armpit odor. Furthermore, the efficacy of plant extracts show that they are suitable to be used as a substitute for synthetic chemicals that cause irritation.

Table 2 Bacterial identification

Test	Bacteria	
	Stapphylococcus spp.	*Corenybacterium spp.*
Media	Baird-Parker agar (BPA)	Trypticase soy agar (TSA)
Colony	Grey-black, opaque halo, clear halo develops around colonies	White to gray, small, circular, convex, shiny
Gram' stain	Gram+	Gram+
Light microscopy	100X	100X
Morphology	Coccus shape, staphylococci arrangement, non-sporing	Bacilli shape, club-shaped, V-in Y-shaped arrangements, or in clumps, non-sporing
Catalase	+	+

3. Satisfaction assessment

Based on Fig. 2, the satisfaction assessment results of the volunteers after using the roll-on deodorant showed that the subjects were more satisfied with RDEOF-2 than RDEOF-1 with a score of reduction of body odor (3.50, 3.25), absorption (3.31, 2.88), color (3.31, 3.25), spreading (3.25, 3.06), product texture (3.12, 3.06), smell (2.81,2.69), and overall satisfied (3.13, 3.06) respectively, while satisfaction after using the product (3.00, 3.00) and viscosity (3.13, 3.13) had the same level of satisfaction. On the other hand, regarding skin mildness (3.50, 3.44), the subjects were more satisfied with RDEOF-1 than RDEOF-2. Certainly, the effects of deodorant products containing *R. nasutus* extract are indicating a decreased growth of bacteria that cause armpit odor. Therefore, the development of the deodorant product with natural plant extracts is warranted.

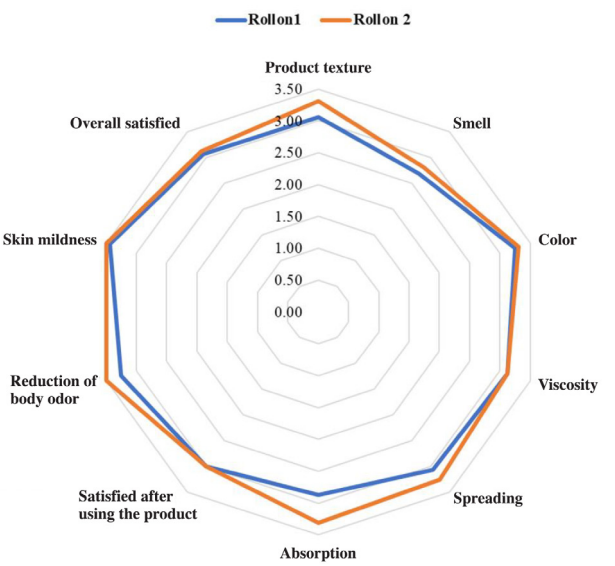

Fig. 2 Evaluation of RDEOF-1 (Roll-on deodorant formula-1) and RDEOF-2 (Roll-on deodorant formula-2) products by volunteer (n = 16) in satisfaction and product characteristics

Conclusion

We have confirmed that the two bacterial genera including *Staphylococcus* spp. and *Corenybacterium* spp. exhibit dominance in human armpit and causes axillary malodor. Using a roll-on deodorant containing *Rhinacanthus nasutus* (L.) extract could decrease armpit bacteria in order to reduce armpit odor. Satisfaction assessment results of the volunteers after using the roll-on deodorant showed that the subjects were more satisfied with RDEOF-2 than RDEOF-1.

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Local Production and Characterization of Biochar from Bamboo Waste and the Removal of Natural Organic Matter from Nakhon Nayok River, Thailand

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Abstract

The objective of this research was to produce a biochar from bamboo handicraft waste via pyrolysis process using a modified 200 L steel drum kiln. The temperature outside the kiln-producing biochar appeared around 500-600°C, closely related to the temperature of slow pyrolysis. The physical and chemical properties of bamboo biochar (BB) were characterized by using proximate and ultimate analysis, Brunauer-Emmett-Teller surface area techniques, elemental analysis, scanning electron microscopy coupled with an energy dispersive spectroscopy, Fourier transform infrared spectroscopy, Raman spectroscopy and X-ray diffraction techniques. It was found that 28.76 ± 2.22% of BB yield with 77.07 ± 1.92 % fixed carbon. As the morphology properties, its surface area and total pore were 247.5 ± 7.1 $m^2 g^{-1}$ and 0.16 ± 0.02 $cm^3 g^{-1}$, respectively. Batch test for removal of natural organic matter (NOM) in Nakhon Nayok River, by adsorbed on BB was studied. The results showed that the percentage reduction of dissolve organic matter (DOC) and absorbance at 254 nm at equilibrium were 71.33 ± 1.46 and 76.51 ± 2.01, respectively, while the adsorption capacity was 4.75 $mg.g^{-1}$ DOC. Pseudo-second order kinetic model was best suited for describing the adsorption of DOC onto BB. This suggests that interaction of NOM on BB were explored in terms of multicomponent adsorption, which the heterogeneous distribution of the adsorptive sites at biochar surfaces. It was found that biochar is suitable for the adsorption of NOM from surface water and is a low-cost effective adsorbent in the treatment of wastewater. Biochar can be applied for a variety of purposes for example: as biofuels, adsorbents and as soil amendments. In addition, the biochar kiln is small and easy to create, creates no smoke, inexpensive, easy to use, does not take much time to produce and has an eco-friendly processing.

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Introduction

High levels of natural organic matter (NOM) in surface water is a major concern for utilization of water. The presence of NOM molecules affects the color of water making the water appear yellow or brown (Ghernaout et al., 2014). NOM is an intricate mixture of organic compounds from decomposition of microbial, plants and animal waste. NOM can be a source for the formation of carcinogens such as trihalomethanes (THMs) and haloacetic acids (HAAs) that water is disinfected with chlorine. The elimination of NOM is essential by use of several physicochemical and biological methods, including, chemical precipitation, adsorption, filtration, reverse osmosis and coagulation. (Yehia & Said, 2021; Guillossou et al., 2020). However, these technologies encounter several disadvantages such as the removal efficiency of low-concentration pollutants are incomplete resulting in toxic products, high energy, chemicals and maintenance consumption (He et al., 2017). Biochar adsorption is an interesting choice, eco-friendly and low-cost material that adsorbed many pollutants from contaminated water (Srivatsav et al., 2020). Biochar is a carbon-rich solid material produced from decomposed organic matter, agricultural residue, wood waste, municipal waste and animal manures, by heating biomass precursors in an oxygen-limited environment (El-Hassanin et al., 2020; Wang et al., 2020; Yazdani et al., 2019). It has been reported to be able to ameliorate soil fertility by carbon sequestration, increasing water and nutrient retention (Dokmaingam et al., 2020; Song et al., 2019; Kätterer et al., 2019), furthermore, to reduce greenhouse gas emissions into the atmosphere (Al-Ghussain, 2019; Zhang et al., 2017). Moreover, biochar can remove various contaminants, including pathogenic organisms, organic contaminants such as dyes (Nguyen et al., 2021) and inorganics such as heavy metals (Shaheen et al., 2019).

Bamboo is a local resource that is valuable to the way of life of Thai people from the past to the present. The reason bamboo is valuable to Thai life is due to it being a fast-growing plant and multipurpose species are used from its rhizome/roots, clumps, shoots, leaves, leaf sheath, branch and culm. The study on bamboo growing in Thailand found that the commercial bamboo cultivation areas are scattered throughout the country, amounting to 91,746 rai. (approx.146.79 km^2) (Land Development Department, 2020). The Thai Wiang Community, Mueang District, Nakhon Nayok Province, Thailand (Fig. 1) has used bamboo in occupations such as handicraft, tree crutches and baked sticky rice in bamboo. The bamboo residue becomes community waste and is burnt in open fields. The burning of the bamboo waste contributes to global warming and increases the level of airborne particles (Lohan et al., 2018). Greenhouse gases (GHG) emissions caused by the burning of agricultural crops in Mae Chaem Basin, Chiang Mai Province, Thailand, was reported by Arunrat et al. (2018) and found average value emissions of CO_2, CO, CH_4, NO_x, SO_x $PM_{2.5}$ and PM_{10} were 9879.3, 253.0, 17.6, 11.7, 1.3, 29.3 and 39.1 kg ha^{-1} $year^{-1}$, respectively. This is serious air pollution that negatively affects the environment and human health.The weakness of conventional charcoal production for example, from earth kiln, pit kiln, brick kiln and horizontal 200 liter kiln, creates low quality of biochar due to the large

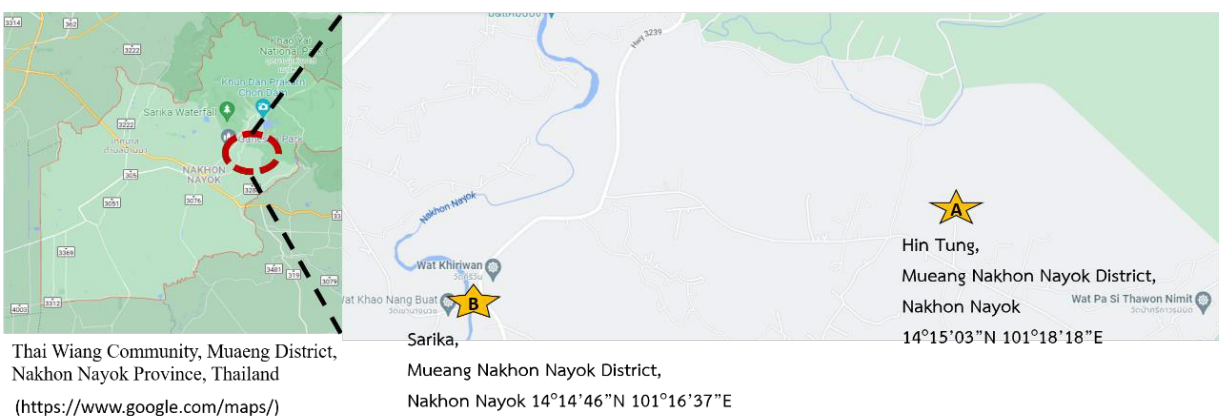

Fig. 1 Map of the Thai Wiang Community, Mueang District, Nakhon Nayok Province, Thailand; the place of (A) biochar production, (B) collected surface water from Nakhon Nayok River, by Google Earth™ mapping

amount of air entering the kiln during the burning process, long time operation of 1-3 days, smoke and inconvenient to use and move. The development of biochar kiln to produce quality biochar is interesting.

The objective of this research was to study the production of biochar from bamboo handicraft waste by local drum kiln via pyrolysis process and investigation of their properties and its application for removal of natural organic matter in Nakhon Nayok River, Thailand.

Materials and methods

1. Bamboo biomass

The bamboo handicraft waste used as feedstock for biochar production, were collected from the local area at Hin Tung, Mueang Nakhon Nayok District, Nakhon Nayok Province (14°15'03"N 101°18'18"E) showed in Fig.1 A. The dirt, sand and unwanted material from the surface of bamboo waste sample were removed and then dried in the open air for a week.

2. Biochar Production

Bamboo biochar (BB) was produced using a portable steel drum kiln made with available local resources allowing for low-cost production and easy to operate for local farmers. The steel drum kiln followed the design of O'Toole (2013) and was modified by added air vents at the bottom and upper zone as well as using 100 L of steel drum as flue for increasing air flow. In reference to Fig. 2, approximately 18 kg of bamboo materials (<10% moisture content) were filled in a small container (A) measuring 0.66 m in height and 0.37 m diameter then the lid was closed tightly to prevent oxygen entering during the pyrolysis process. The small container (A) was then placed upside down inside a larger container (B) (0.88 m height and 0.59 m diameter). The volume between the containers was filled with 20 kg of other bamboo waste, which was burnt for heating the inner container. The steel drum (C) was taken to cover the top to increase the air flow in the combustion. The combustion temperature surrounding 3 positions in each of 3 zones of the outer chamber were measured by non-contact handheld infrared thermometer (MESTEK IR01D). The biochar yield was calculated as follows: production yield (wt%) = (W_{BB}/W_{bamboo}) x 100, where W_{BB} was the weight of BB (kg) and W_{bamboo} was the weight of the bamboo biomass feedstock (kg) loaded into the kiln, both on a basis of dry weight. After the outer container wood waste had all burnt up (≈60 min) then left to cool. The BB product was taken to study the physical and chemical properties.

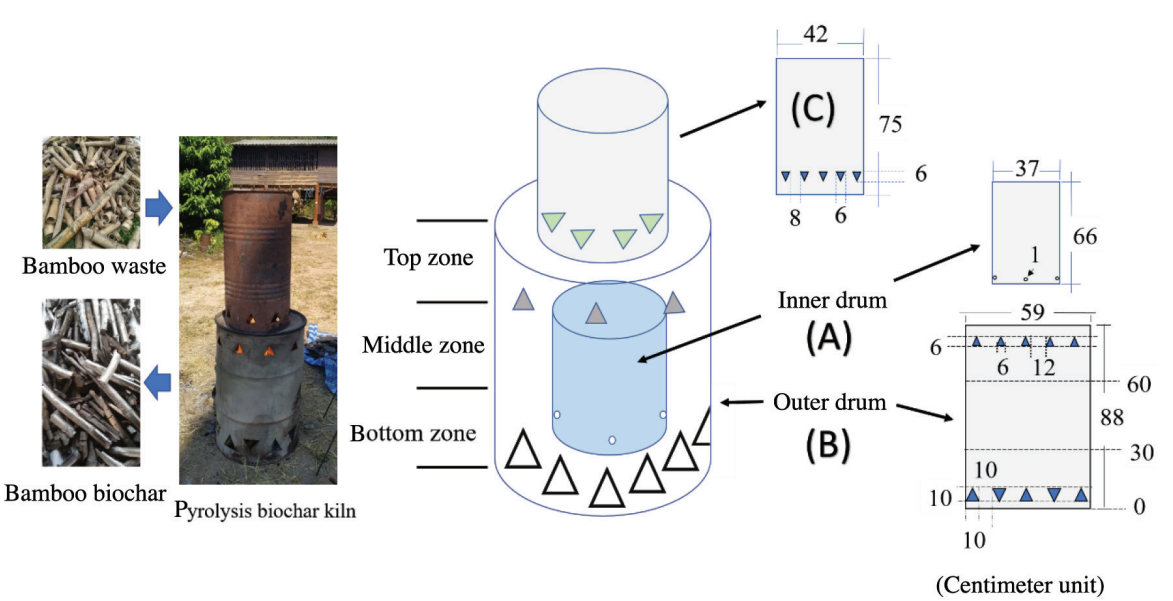

Fig. 2 Schematic diagram of bamboo biochar production

3. Characterization of BB

3.1 Proximate analysis

Proximate analysis is the composition of the moisture, ash, volatile matter and fix carbon and analyzed by modifying according to Aller, Bakshi, & Laird (2017). The moisture content of BB was determined based on weight loss after two hours at 110°C in hot air oven (Memmert, Germany) under N_2 purge. Volatile matter (VM) of BB (same sample) was carried out by heating a crucible containing the BB covered with ceramic lid placed in a stainless-steel box under nitrogen purge by muffle furnace to 950°C held 10 min at heating rate 2°C min^{-1} (THERMCONCEPT Ht40 Al, Germany). Ash content of BB was measured based on weight loss by heating the same sample to 730°C in an air atmosphere using the same muffle furnace and performed overnight (8-10 hours). After the ashing, the furnace was then switched off and let to cool before the sample was transferred to a desiccator. The moisture (% Moisture), volatile matter (% VM) and ash (% Ash) content were determined by equations 1, 2 and 3, respectively, as shown below.

$$\%\, Moisture = \left(\frac{W_I - W_D}{W_D}\right) * 100 \quad \text{(Eq. 1)}$$

$$\%\, VM = \left(\frac{W_D - W_V}{W_D}\right) * 100 \quad \text{(Eq. 2)}$$

$$\%\, Ash = \left(\frac{W_A}{W_D}\right) * 100 \quad \text{(Eq. 3)}$$

Where W_I, W_D, W_V and W_A were the weight (g) of BB of initial, oven drying at 110°C, after heat at 950°C and after combustion at 730°C overnight, respectively. Whereas, the fixed carbon is the carbon found in the biochar, which was left after moisture, volatiles and ash in the biochar were driven off. The percent fixed carbon (% FC) was calculated as equation 4 and is shown below.

$$\%\, FC = 100\% - (\%\, Moisture + \%\, VM + \%\, Ash) \quad \text{(Eq. 4)}$$

3.2 Ultimate analysis

The specific surface area and total pore volume of BB were analyzed by N_2 adsorption-desorption by Autosorb 1MP surface area analyzer (Quantachrome Instruments, USA). The BB sample was degassed under vacuum at 250°C performed overnight in order to eliminate the volatile matters before measuring. An elemental analyzer was used to determine CHN/O compositions (LECO CHNS model 932 elemental analyzer) through pyrolysis. The above techniques were analyzed by The Petroleum and Petrochemical College, Chulalongkorn University, Thailand. The oxygen content was calculated by difference. The pH value of BB was measured by a Starter 3100 bench, Ohaus pH meter. Two grams of BB was added into 50 cm^3 double distilled water, the mixture was shaken for 30 minutes at 150 rpm, then centrifuged and filtered. The filtrate was tested for the pH value.

3.3 Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) techniques allow for targeted analysis of sample surfaces that offer a direct way to observe the surface structure and mineral distribution of biochar. The BB surface morphology was investigated using a JEOL SEM analyzer JSM-6610 LV (JEOL Ltd., Tokyo, Japan) with an accelerating voltage of 20 kV. Meanwhile, the surface chemical elemental composition was determined by Energy Dispersion Spectrometry analysis (EDS, Oxford instrument X-Max 50 mm^2, England).

3.4 Infrared spectroscopy

Attenuated total reflection (ATR) - Fourier transform infrared spectroscopy (FTIR) was carried out to determine the functional groups of both the BB and feedstock. ATR-FTIR was performed using an IR Tracer-100 FTIR spectrophotometer (Shimadzu) with a diamond, zinc selenide (ZnSe) prism used in the ATR accessory. The number of scans was 40, with wide range at 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. The background was collected before each measurement. The band positions were obtained using the LabSolutions IR control software.

3.5 Raman spectroscopy

Raman spectra of BB was carried out by using a Horiba LabRAM HR Evolution Raman Spectrometer. The spectra were acquired with a 50 LWD objective that were recorded from 650-2200 cm^{-1} with an excitation laser source of 532 nm, 100 mW, 3.2 to 5% power and using 20 accumulations. The instrument was controlled using LabSpec 6 software.

3.6 X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis was used to investigate any crystallographic structure in the biochar. XRD pattern of the BB sample was identified using a Bruker D2 Phaser X-ray diffractometer (Bruker, Karlsruhe, Germany). It was operated in the 2θ range of 10-90^0 at 1^0 min^{-1} scan speed and step time 0.5^0 at room temperature.

3.7 Batch adsorption experiment

During August 2021, surface water was collected from Nakhon Nayok River, Sarika, Mueang Nakhon Nayok District, Nakhon Nayok Province, Thailand (14°14'46"N 101°16'37"E) as shown in Fig.1B. The physicochemical characteristics of raw water were as follows: pH: 7.54, DO 1.70-8.00, BOD 1.03-3.23 and N_{NH3} 0.01-1.43 mg L^{-1} (Regional Environmental Office 7, 2021). One hundred liters of surface water was collected with a black polyethylene container then brought to the laboratory. Pre-treatment included the water sample being filtered through 5 and 1 µm commercial polypropylene filter, 0.7 µm GF/C, 0.45 µm cellulose nitrate membrane (Whatman) and RO Membrane (ULTRATEK TW-1812-50 GPD) as shown in Fig. 3. Batch experiments were performed with NOM adsorption in an aqueous solution, 100 cm^3 of sample water and 1 g L^{-1} black powder adsorbent were placed in Erlenmeyer flasks (250 mL) shaken with 150 rpm at room temperature without pH adjustment. The kinetic was studied at intervals that varied between 1 minute and 48 hours. The collected samples, after batch test, were centrifuged at 3,000 rpm for 5 min before being filtered through 0.45 µm nylon membranes syringe filter to NOM measurement. In order to assess the NOM removal efficiency was analyzed for dissolved organic carbon (DOC) using Multi N/C 2100S-Direct injection TOC analyzer (Analytik Jena GmbH, Germany) and UV absorbance measurement at 254 nm (UV_{254}) wavelength in 1 cm quartz cells (Kearns et al., 2021), by a UV-1201 Shimadzu spectrophotometer. The efficiency removal of NOM and adsorption capacity were calculated by equations 5 and 6, respectively, as shown below.

$$\%\ NOM\ removal = \left(\frac{C_i - C_t}{C_i}\right) * 100 \quad \text{(Eq. 5)}$$

$$Adsorption\ capacity, Q_t(mg.g^{-1}) = \frac{(C_i - C_t)}{W} V \quad \text{(Eq. 6)}$$

Where C_i and C_t (mg L^{-1}) were DOC concentration at initial and at time t, respectively, V (L) was mixture volume and W (g) was BB weight. The NOM removal in UV_{254} absorption was calculated as: removal (%) = $[(A_0-A_t)/A_0] * 100$, where A_0 and A_t were the absorbance at 254 nm at time 0 and t, respectively.

Results and discussion

1. Temperature profile and production yield

Performance of the pyrolysis system has been defined in terms of reactor temperature profile, biochar yield, and characteristics of biochar obtained. The average temperature for different position on the outside of kiln are shown in Fig. 4. The results showed that the average of 3 zones outside temperature of the kiln obviously increased with time. The maximum temperatures of top zone, middle zone and bottom zone of the kiln, were 610.8, 605.4 and 597.3°C, respectively, as a result of heat transfer from combustion of the fuel bed at the top to bottom. It can be observed that the temperature of all zones increased from room temperature to maximum with an increase of time over 35 min and then decreased to 40 ± 1°C over 80 min. However, the inner container should not be opened, it was left for 2 hours, because biochar can ignite again. The process was observed to complete in about 4 hours. The percent average BB yield was 28.76 ± 2.21 from six pyrolysis batches. Proximate and ultimate analysis were carried out to evaluate the characteristics of the BB as shown in Table 1.

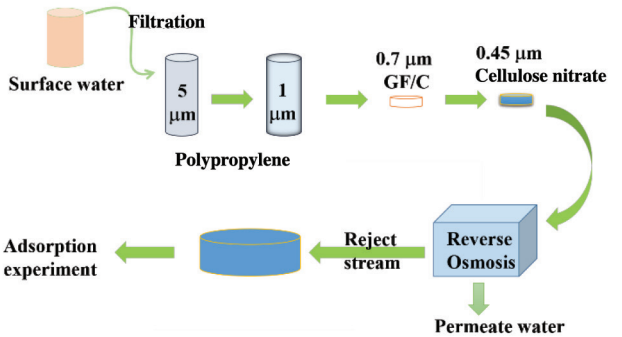

Fig. 3 Diagram prepare surface water sample to batch adsorption experiment

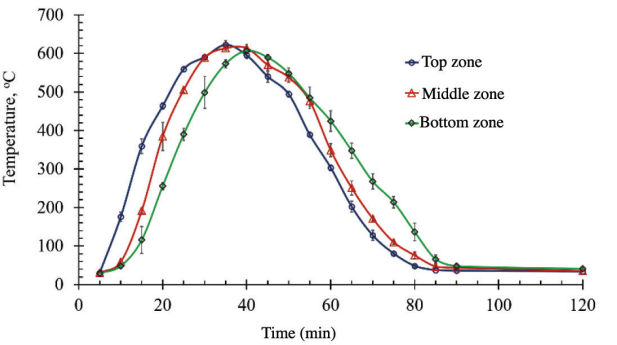

Fig. 4 The temperature profile of the outside kiln for different position

During pyrolysis process, cellulose, hemicellulose, lignin and fat of the biomass were thermally disintegrated over the temperature range between 150 and 400°C to

increase the carbon content by destroying oxygen, hydrogen and non-carbon species to gaseous products formed (Conte et al., 2021). After that, acid compounds, ketones, aldehydes, phenols, furans and guanidines were formed based on temperature between 400 and 700°C (Lewandowski et al., 2020). The weight loss of biomass from the thermal conversion to biochar was associated with decomposition of carbonates, sulfates and hydroxides, hydroxylation of some oxide compounds that affected to the surface area and pore of BB. In this study a surface analysis with specific surface area and pore volume of BB were 247.5 ± 7.1 m^2 g^{-1} and 0.16 ± 0.02 cm^3 g^{-1}, respectively. The drum kiln was developed from the traditional kiln and has been widespread to use for the biochar production in developing countries. Batch simple kiln technologies are usually the first choice for small farmers and start-up biochar producers before it can be improved into a large system that is widespread and has affordable price. In India and East Africa, forests are sustainably managed for biochar production using low-cost kiln as well as being more environmentally friendly and can reduce GHG emissions into the atmosphere by approximately 75%. Moreover, increased efficiency of biochar production was about 30%-40% better than the traditional biochar production method, which was about 10%-20% (Adam, 2009). Similarly, the kiln used in this research, had all wood gases released during carbonization and were controlled and burned as a fuel for the process to reduce emissions.

Table 1 Physical and chemical properties of the BB

Bamboo biochar properties	% Dry weight
Proximate analysisa	
Biochar yield	28.76 ± 2.22
Ash	6.58 ± 2.00
Moisture content	7.32 ± 1.00
Volatile	9.03 ± 1.77
Fix carbon	77.07 ± 1.92
pH	8.90 ± 0.51
Specific surface area (m^2/g)[b]	247.5 ± 7.1
Pore volume (cm^3/g)[b]	0.16 ± 0.02
Elemental analysis [b]	
C	65.17 ± 2.15
H	3.16 ± 0.05
N	0.77 ± 0.06
O*	30.94 ± 2.15
H/C	0.04
O/C	0.47

Remark: [a] n = 6, [b] n = 3, * By difference

2. Physical and chemical properties of the BB

Table 1 shows the elemental analysis of CHN/O chemical properties of the BB. The ratio of hydrogen to carbon (H/C) indicates aromaticity index to evaluate the degree of thermochemical change that produces fused aromatic ring structures in biochar while oxygen to carbon ratio (O/C) represents hydrophilicity index related to biochar stability. In the pyrolysis process, carbon, hydrogen and oxygen are eliminated in gases and volatile matters, as a result, the H/C and O/C ratios would decrease, corresponding to an increase in aromaticity and carbon content with an increase in pyrolysis temperature (Windeatt et al., 2014). The lower the H/C and O/C ratio used to indicate higher fused aromatic ring structure and higher stability in carbon fraction (Fernandes et al., 2020). The H/C and O/C ratio of BB in this study were 0.04 and 0.47, respectively, which indicated a high carbon content and higher stability in aromatic ring structures then would be preserved for at least 100 years in soil. This is consistent with the work done by Spokas (2010), which showed the O/C ratio in the range of 0.2–0.6. That means the dwell time of biochar in soil was about 100–1000 years.

The BB morphological characteristics obtained by scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) is shown in Fig.5. Fig.5a shows obvious morphological of BB with a large surface area, tubular shapes, rough surface structures and sharp edges, approximate porous space of 12-15 µm spread on biochar surface. Moreover, at a magnification of 5,000X, a micropore was found within the mesopores in Fig.5b. In Fig.5c the BB cross-section shows mesopores structure spread on biochar surface and the surface pore morphology is similar to a honeycomb-like structure. The results of the SEM images of BB corresponded with the results of the surface analysis in which specific surface area and pore volume were influenced by the content of water and nutrients retained (Hernández-Mena et al., 2014). The C (76.32%) and O (16.77%) content as the major elements of the BB were indicated by EDS analysis and are shown in EDS analysis Fig. 5d. Furthermore, the other minerals, such as Mg, Si, P, S, Cl, K and Ca could be detected from six pyrolysis batches appearing in Table. 2. The results implied that C was the main skeleton with O in the BB, which may be influenced from oxygen-containing compounds (e.g. carboxylic -COOH, hydroxyl -OH, carbonate -CO_3^{2-}, phosphate -PO_4^{3-}or sulphate -SO_4^{2-}).

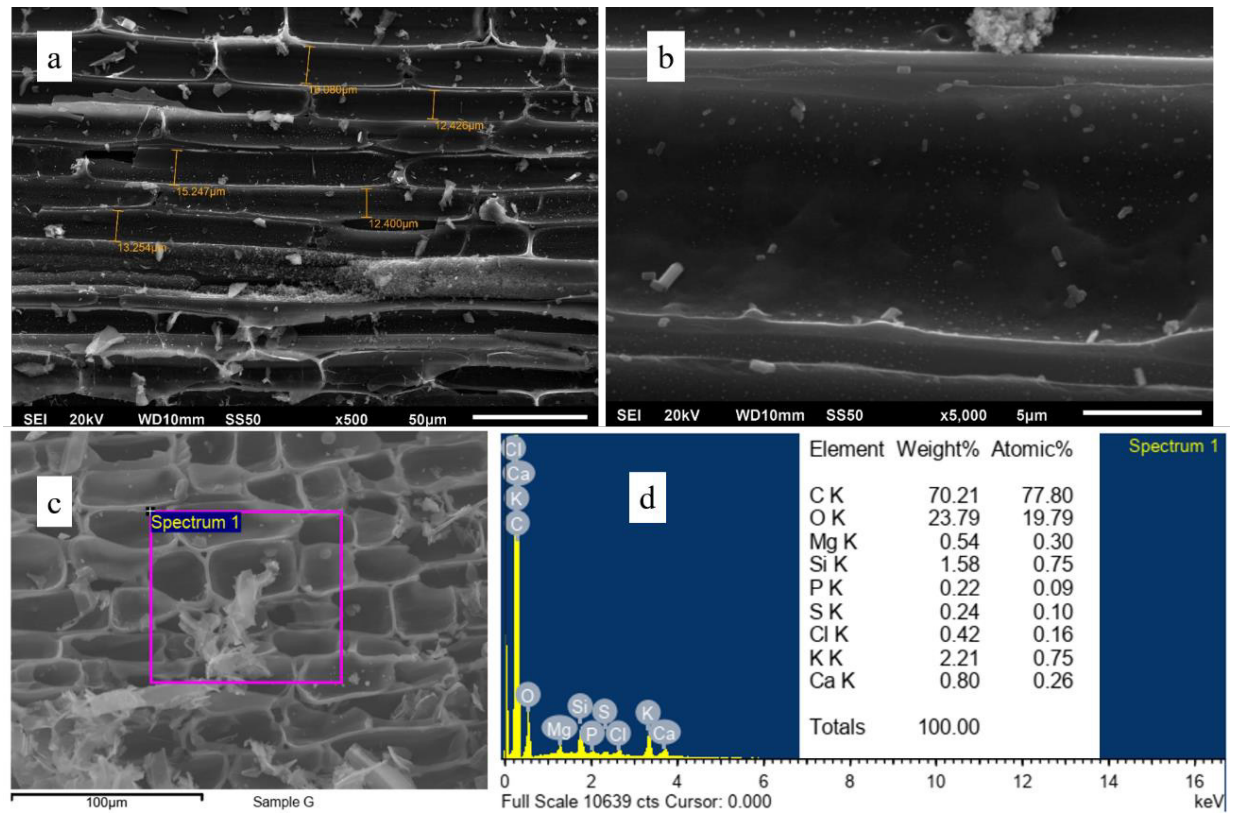

Fig. 5 SEM images of bamboo biochar: a and b were 500 and 5000 times magnification, respectively, c and d were EDS analysis

Table. 2 Element composition of BB by EDS analysis

Element	Average
C	76.32 ± 3.25
O	16.77 ± 3.02
Mg	0.43 ± 0.17
P	0.56 ± 0.16
Cl	0.61 ± 0.71
K	3.43 ± 3.28
Ca	0.64 ± 0.01
S	0.35 ± 0.14
Si	1.57 ± 1.60

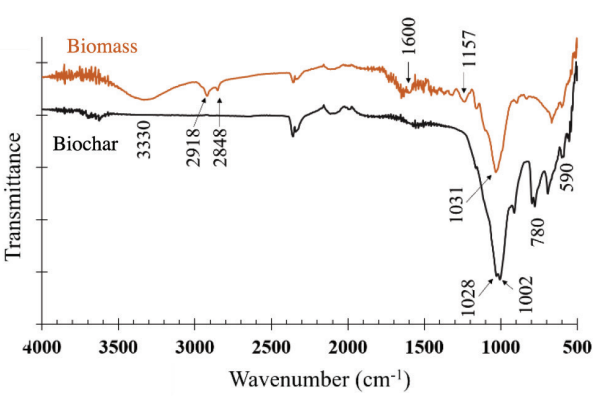

Fig. 6 ATR-FTIR spectra of raw bamboo biomass and its biochar

ATR-FTIR spectra of biomass and BB are shown in Fig.6. Lignocellulosic materials consisted of cellulose, hemicellulose and lignin indicating broad band between 3500 and 3000 cm^{-1} corresponding with O-H and N-H vibrations of phenol and amine and the C-H symmetric stretching at 2918 and 2848 cm^{-1} (Qin et al., 2020). The bamboo-derived biochar's with the band at the region of 3000-3500 cm^{-1} disappeared, hence signifying complete dehydration and de-oxygenation reactions at 600°C pyrolysis temperature. Recommending polar functional groups were reduced to hydrophobic material with very less functional groups were obtained (Ramola et al., 2014). The fingerprint region (1600 to 400 cm^{-1}) provided insight for disappearance of cellulose and hemicellulose in BB sample owing to the decomposition of the raw biomass. The spectra of biomass, a peak at 1600 cm^{-1} was assigned to the aromatic skeletal C=C and C=O vibration mode of hemicellulose and lignin (Nair et al.,

2020). The peak at 1031 cm^{-1} referred to the C-OH or C-O-C stretching vibrations of cellulose, hemicellulose and lignin (El-Sakhamed et al., 2018). Peaks for C–H bending bonds (out of plane) in the region of 900–675 cm^{-1}, which was characteristic of the aromatic substitution pattern, were clearly visible for biochars. The peak at 1000 cm^{-1} can be assigned to the C-O stretching vibration of alcohols and ester groups (Guizani et al., 2017). Silicates (Si-O) and phosphates (P-O) were displayed at the wideband around 1000 cm^{-1} that remained unchanged at temperatures below 700°C (Zhang et al., 2015; Cantrell et al., 2012). The 590 cm^{-1} peak obtained from iron oxide compounds or Fe–O–Si bond (Gotić & Musić, (2007) that forms in attendance of iron and silicate minerals at higher temperatures.

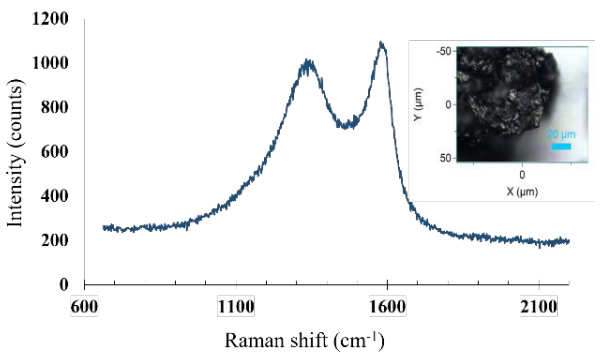

Fig. 7 Raman spectrum of bamboo biochar

Raman Spectroscopy is an outstanding method for characterizing carbon materials. Raman spectrum of the BB appeared at maximum intensity at 1348 cm^{-1} and 1588 cm^{-1}, which is in accordance with the D and G bands of the graphitic-like structures, respectively, shown in Fig. 7. Both D and G peaks are the result of vibrations of sp^2-bonded carbon atoms. The D band is due to out of plane vibrations of sp^2-bonded carbon attributed to the presence of structural defects whereas the G band is formed by sp^2 bonded crystallite carbon vibration both in rings and chains of the graphite crystalline plane. (Ferrari & Robertson, 2000). The intensity ratio between the D and G bands can be used to estimate the degree of crystallinity of carbon-containing materials. The high D/G intensity ratio of BB was 0.868 (in Fig.7) indicating the pyrolysis temperature was greater than 600°C (Gonzalez-Canche et al., 2021).

X-ray diffraction analysis is performed to assess a degree of crystalline or amorphous structure in a sample. The XRD diffractogram of BB contained mainly the

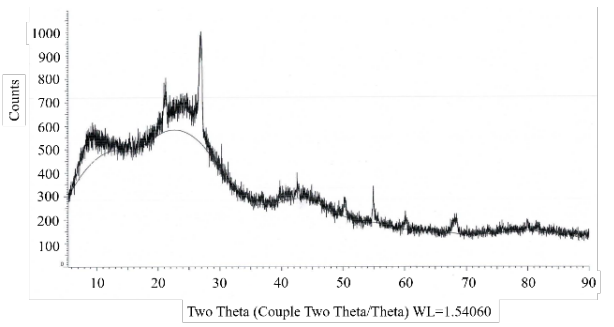

Fig. 8 XRD characterization of bamboo biochar

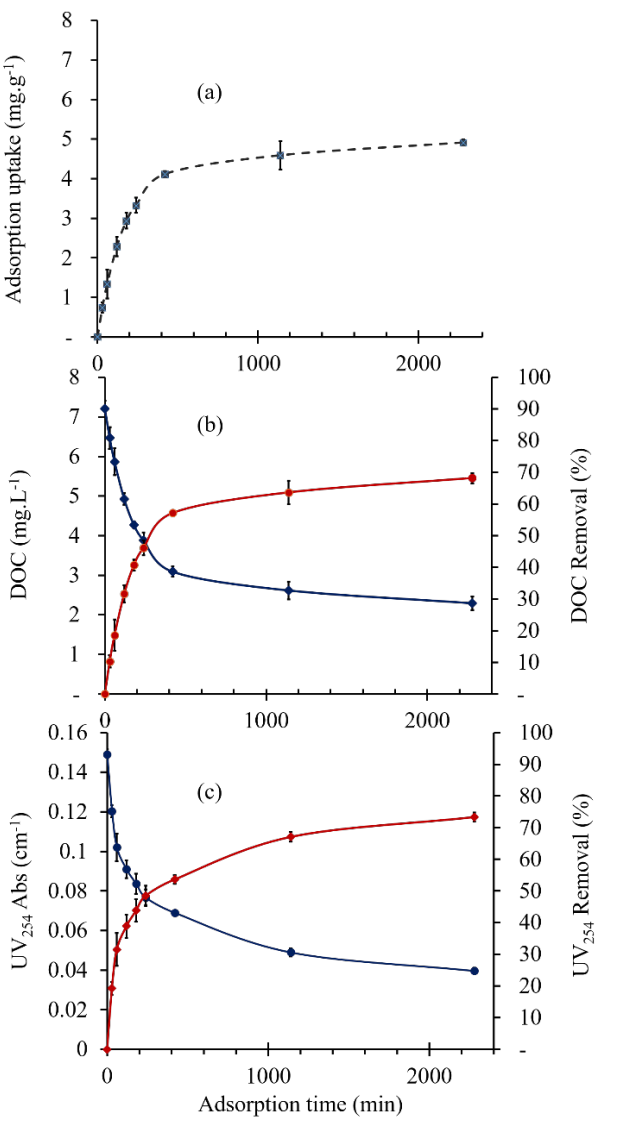

Fig. 9 NOM removal, (a) adsorption efficiency, (b) DOC reducing, (c) UV254 absorbance reducing

amorphous compounds were indicated in Fig. 8. The two theta (2θ) wide range 20˚-30˚ refers to the stacking structure of the aromatic layer (Liu et al., 2012). Sharp crystalline non-labeled peaks in BB diffractogram probably indicated the miscellaneous inorganic components, such as these peaks are consistent with the higher content of SiO_2 (quartz, 2θ = 20.86˚, 26.62˚) and $CaCO_3$ (calcite, 2θ = 50.58˚), corresponding with Sackey et al., (2021) research.

3. Removal of NOM

Application of BB for NOM removal in Nakhon Nayok River, Thailand was investigated. The amount of NOM was represented in DOC concentration and UV absorbance at 254 nm measurement. The initial content of DOC and UV_{254} were 7.21 ± 0.20 mg L^{-1} and 0.149 ± 0.002 cm^{-1}, respectively. The high values of DOC and UV_{254} were due to the samples being collected in August, which was the rainy season and soils rich in organic matter was transported into the water by run-off (Singh & Choden, 2014). The effect of adsorption time on the removal of NOM is shown in Fig. 9. The adsorption capacity of the DOC on BB increased with the extension of contact time. In the first 6 hours the adsorption capacity grew rapidly after that the adsorption tended to equilibrium gradually (Fig.9(a)) and the adsorption efficiency of equilibrium was 4.75 mg g^{-1}. Fig. 9 (b) and (c) shows that the adsorption rate of DOC and UV_{254} on BB were initially fast and then gradually reduced until equilibrium was reached. More than 50% of DOC and UV_{254} were removed within contact time 6 hours of 1 g L^{-1} BB in the first stage. A slower phase occurred thereafter with equilibrium being achieved within 48 hours, corresponding to about 70% removal.

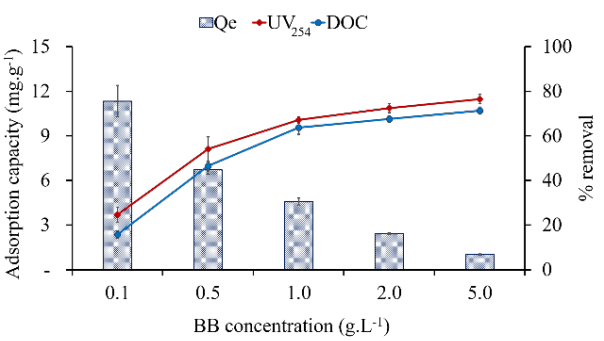

Fig. 10 Effect of adsorbent concentration on DOC adsorption

The effect of BB dose was evaluated by varying adsorbent dosage in the range from 0.01 to 0.5 g of adsorbent in 100 cm^3 water sample. It is clear from Fig. 10 that the removal percentage of the DOC and UV_{254} had a direct proportional to adsorbent dosage from 15.71 ± 1.44 to 71.33 ± 1.46% and 24.60 ± 3.31 to 76.51 ± 2.01, respectively. Whereas the adsorption efficiency of DOC on BB at equilibrium (Q_e) decreased from 11.33 ± 1.04 to 1.03 ± 0.02 mg g^{-1} with an increase in adsorbent dose 0.1 to 5.0 g L^{-1} shown in Fig.10. The increased of the adsorbent dose effected the number of active sites and thus removal percentage improved. However, the adsorbent high content may be due to aggregation of adsorbent, which would lead to a reduction in the adsorbent capacity through decreasing the total surface area causing the site to be less active thus resulting in the lower number of DOC molecules per active site.

The adsorption kinetics of BB was educated based on the above adsorption equilibrium results of DOC. The adsorption kinetic model of DOM onto BB was assessed by using pseudo-first-order and pseudo-second-order kinetic models as shown below by equations 7 and 8, respectively.

$$log(Q_e - Q_t) = logQ_e - \frac{k_1}{2.303}t \quad \text{(Eq. 7)}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e}t \quad \text{(Eq. 8)}$$

Where Q_e and Q_t are the biochar adsorption uptake (mg g^{-1}) at equilibrium time and any time t (min), respectively, the adsorption rate constants k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) are primary and secondary constants, respectively. A plotting of log (Q_e-Q_t) versus time in Eq.7 and of t/Q_t versus time in Eq.8 both yields a straight line as shown in Fig.11. The rate constant (k_1) and absorption capacity (Q_e) of the pseudo-first-order reaction can be determined from the slope of the straight line and intercept of the plot in Fig. 11 (blue line), respectively. Similarly, the rate constant (k_2) and equilibrium absorption capacity (Q_e) of the pseudo-second-order reaction were determined from the intercept and slope of a linear relationship in Fig. 11 (red line). According to the equation 7 and 8 given above, the kinetic parameters were calculated and appeared in Table 3. It was clear from the correlation coefficient, R^2 values given in Table 3 that pseudo-second-order kinetic model was greater than pseudo-first-order kinetic model,

which best describes the adsorption of DOC on BB. This suggests that the interaction of NOM on BB were explored in terms of multicomponent adsorption of its different fractions as chemical nature, functional groups present on the surface, pore filling, π–π interactions, polar/electrostatic interactions, hydrophobic effect and hydrogen bonding (Yazdani et al., 2019; Ahmad et al., 2014).

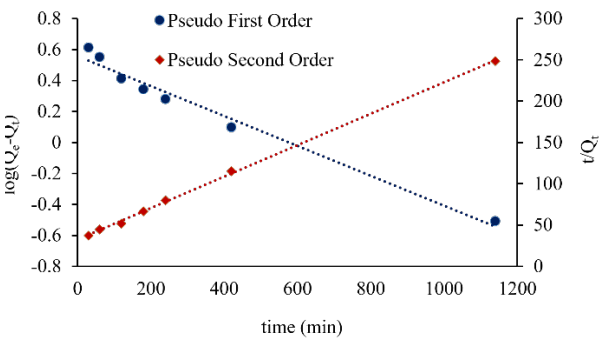

Fig. 11 Adsorption kinetic model of DOC removal

Table 3 Adsorption kinetic parameters of DOC on BB

Parameter	Pseudo-first-order	Pseudo-second-order
Adsorption rate constant, k	0.0023 (min^{-1})	0.0011 (g mg^{-1} min^{-1})
Q_e (mg g^{-1})	3.607	5.254
R^2	0.9783	0.9992

Conclusion

The production temperature (500–600°C) and heating time of about 1 hour for biochar produced in this research kiln were closely related to slow pyrolysis. The process was observed to complete in about 4 hours with the% BB yield of 28.76 ± 2.21 of dry weight. The BB high quality were characterized by BET, SEM-EDS, ATR-FTIR, Raman spectroscopy, XRD, proximate analysis and elemental composition revealed macro-meso-and micro-pore structure, high surface area was 247.5 ± 7.1 m^2 g^{-1} and 0.16 ± 0.02 cm^3 g^{-1} of pore volume, 77.07 ± 1.92% fix carbon, amorphous structure mixed of sp^2 and sp^3 carbon bonds and surface functionality. The absorbent product was applied to remove NOM in Nakhon Nayok River, Thailand, and could reduce 50% at 6 hours contact time with 1 g L^{-1} of BB. The absorption efficiency at equilibrium and maxima removal were 4.75 mg g^{-1} and about 70%, respectively following pseudo-second-order kinetic model. Suggestions for further research should study if BB may be used to eliminate heavy metals in local community groundwater. The results in this study cannot be generalized across kiln design and starting biomass feedstock, nevertheless it represents that our simple kiln can produce good quality biochar from bamboo waste. In addition, the biochar kiln is also small and easy to create and use as well as producing no smoke, inexpensive, does not take a lot of time to produce and has an eco-friendly processing.

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Low-fat, Plant-based Ice Creams Formulated with Rice Bran Oil and Rice Bran Oil Organogel

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Abstract

Low-fat, plant-based ice creams were novelty formulated by replacing milk with rice bran oil and rice bran oil organogel emulsion. The protein in milk was substituted by soy protein. The viscosity and emulsion stability of the plant-based ice cream mixtures were measured and compared with conventional milk ice cream. Frozen ice cream properties, including overrun, firmness and melt down rate were investigated as well as nutritional and sensory properties. Result found that the viscosity of the plant-based ice cream mix including organogel (398 mPa·s) and rice bran oil (363 mPa·s) exhibited higher value than those of conventional ice cream mix (289 mPa·s). The emulsion stability of milk, organogel and rice bran oil ice cream mixtures were 88.72±0.80, 88.00±0.10 and 80.12±0.65, respectively. The overrun of organogel ice cream (38.31± 0.91) showed lower quality characteristics than the milk ice cream (39.40±1.03) while rice bran oil ice cream had the lowest overrun (37.02±0.01) ($P<0.05$). The reduction of overrun related with texture of ice cream. The firmness of rice bran oil ice cream (16.4±0.91) showed lower quality characteristics than organogel ice cream (15.3± 0.87) followed by the milk ice cream (14.4±0.91). The melt down rate of plant-based ice creams (organogel: 0.46±1.75, rice bran oil: 0.45±1.65) are improved when compared with the milk ice cream (0.67±1.65) may be because of high viscous of ice cream mix and the properties of soy protein. The nutritional properties were improved for plant-based ice cream formulations. Especially, total fat content of organogel was lower than rice bran oil because of the lower fat of organogel. Sensory testing scores of the taste and flavors was decreased as well as appearance and color characteristics. Texture and body of the organogel ice creams were not different when compared to the conventional milk ice cream. Although, firmness of organogel was higher than conventional ice cream but panelists cannot perceive the difference. Nevertheless, rice bran oil ice cream had the lowest score ($P<0.05$) in texture and body which may be due to the high firmness value. Therefore, low-fat, plant-based ice cream formulated with rice bran oil organogel is a successful approach in order to obtain lower fat without compromising their qualities except the taste and flavor because of soy protein flavor.

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Introduction

Plant-based foods have gained research interest because of problems associated with saturated fat and cholesterol contents in animal-based foods. Consuming high saturated fat diet is a well-accepted cause for increasing risk of cardiovascular and coronary heart diseases (Bhupathiraju & Tucker, 2011; Willett, 2012; DiNicolantonio et al., 2016).

Ice cream is a frozen dessert which usually contains 10–12% of fat. Fat plays a significant role in ice cream mix behavior during freezing and the final structure of ice cream (Harte et al., 2003). The major structural components of ice cream are fat globules, air and ice crystals that are dispersed in a frozen solution concentrated with proteins, salts and polysaccharides (Muse & Hartel, 2004). Formulating a low saturated fat ice cream with 2–4% of fat that have comparable properties with conventional fat containing products is a challenging task. The major drawbacks of using fat replacers in ice cream is the poor sensory quality (Akbari et al., 2019). Nevertheless, numerous attempts in reducing saturated fat content of ice cream by using fat replacers or components imitating fat have been reported (Hatipoğlu &Türkoğlu, 2020).

Organogels are novel oil-based materials formed by structuring liquid oils or emulsion with organogelators such as wax, plant sterol and policosanol, rendering them as crystalline solid fats (Sawalha et al., 2021; Mandu et al., 2020; Adulpadungsak et al., 2020). Organogelation does not alter the fatty acid composition of the entrapped liquid oil/emulsion (Jaroennon et al., 2021) and no *trans* fats are generated (Stortz et al., 2012). There were few studies on the applications of organogel in low-fat ice cream. Zulim-Botega et al., (2013a, 2013b) demonstrated the potentials of rice bran wax organogel in developing fat structure in ice cream with high fat concentration (15 g/100 g) in the presence of glycerol monooleate as emulsifier. Moriano & Alamprese (2017) reported that organogels with plant sterol in ice creams with quality characteristics comparable to milk ice cream and even better overrun and melting starting time.

The aim of this research is to explore the possibility of producing plant-based and low-fat ice creams by replacing milk fat with rice bran oil and rice bran oil organogels with policosanol. The milk protein was also substituted with soy protein. The viscosity and emulsion stability of the formulated ice cream mix were measured. Overrun, firmness, melt down rate, nutritional and sensory properties of the frozen ice cream were measured and compared with conventional milk ice creams.

Materials and methods

1. Materials

The rice bran organogels with policosanol were prepared by our previous method (Manakla et al., 2020) and stored in a refrigerator at 4°C before ice cream production. Rice bran oil (Thai Edible Oil Co., Ltd., Thailand), fresh milk (whole milk 100%, Meiji, Thailand), soy protein (FITWHEY, Thailand), white sugar (Wangkanai Corporation., Ltd, Thailand) and vanilla extract (McCormick, USA) for ice cream preparation were purchased from a local supermarket. Rice bran oil utilized for ice cream and organogel production contained 31.81% of saturated fatty acid (SFA), 40.56% of monounsaturated fatty acid (MUFA) and 21.64% of polyunsaturated fatty acid (PUFA). Fresh pasteurized milk contained 3% of fat, 2% of protein and 2% of carbohydrate (Manakla et al., 2020).

2. Ice cream preparation

Low-fat, plant-based and conventional milk-based ice creams were formulated as shown in Table 1. The type of fat was varied, while the quantities of fat, protein (milk and soy protein), sugar and vanilla extract were fixed. In a typical plant-based ice cream preparation, a mixture of water and soy protein was poured into the rice bran oil-based organogel phase while homogenizing with a high-speed disperser (Ultra-Turrax T-25 basic, IKA) at 8000 rpm for 5 minutes. The resulting emulsion (240 mL) was subsequently homogenized with sugar and vanilla extract. The homogeneous ice cream mixture was allowed to cool at 5°C for 12 hours The ice cream was formed by an Ice Cream Maker (ICM-F15K, Factory Ice Cream Machine, Thailand) with a duration time of 20 minutes. All samples were kept at -18°C for 24 hours before analyzing.

Table 1 Formulations of ice creams

Ingredient (%)	Ice cream formulations		
	Conventional	Organogel	Rice bran oil
Water	0	69.28	69.28
Fresh milk	74.5	0	0
Sugar	25	25	25
Soy protein	0	2.24	2.24
Rice bran oil	0	0	2.98
Organogel	0	2.98	0
Vanilla extract	0.5	0.5	0.5

3. Ice cream mixture properties

The viscosity (in mPa·s) of the ice cream mixture was measured using a Brookfield Viscometer DV-II Pro with spindle 64S for 15 and 30 s at 120 rpm. The emulsion stability index of an ice cream mixture was measured using the reported method (Wu, 2001). Typically, a freshly prepared ice cream mixture (11.0 ± 0.5 g) was weighted in a test tube (1.5 cm in diameter and 10 cm in height) and centrifuged at 8000g for 15 minutes with a centrifuge (Universal 32, Hettich Instruments, Tuttlingen, Germany). The height of the formed free oil-phase was measured using a Vernier caliper. The emulsion stability (%) was calculated according to Equation 1.

$$\% \text{ Emulsion stability} = \frac{H_t - H_o}{H_t} \times 100 \qquad \text{(Eq. 1)}$$

where H_o is the height of oil phase and H_t is the total height of the sample in the test tubes.

4. Frozen ice cream properties

4.1 Overrun

The amount of air incorporated in the frozen ice cream samples was measured by filling a fixed volume container with the frozen ice cream immediately after finishing the total freezing/whipping time of 10.5 minutes. The density of ice cream mix was 1.1 kg/L. The container's volume and weight were 227 mL and 40.5 g, respectively (Adapa et al., 2000). The overrun percentage was calculated by the Equation 2:

$$\% \text{ Overrun} = \frac{W_m - W_f}{W_f} \times 100 \qquad \text{(Eq. 2)}$$

W_m is weight of ice cream mix and W_f is weight of frozen ice cream in the container

4.2 Melting rate

The melting rates of the ice cream samples were measured using a drip-through test, according to Soukoulis et al., (2008). The ice cream samples were kept in a freezer at −20°C for approximately 12 hours before measurement. Ice cream (50 g) were transferred to a 2 mm sieve at room temperature (approximately at 25°C). The dripped portion was evaluated for 90 minutes with a 5-minute interval. The melt down rate test was conducted in triplicate.

4.3 Firmness

The firmness was determined by a texture analyzer CT3 (Ametek, Brookfield, USA). The penetration tests on 50-mL samples were performed. A stainless-steel cylindrical probe (8 mm in diameter) connected to a 100 N load cell was used. The ice cream samples were penetrated for 18 mm using a crosshead speed of 50 mm/min. The reported results expressed as the maximum loads (N) opposed by ice creams to a 15-mm penetration were averaged values of at least 10 measurements for each ice cream formulation (Moriano & Alamprese, 2017).

4.4 Energy, protein, carbohydrate and fat content of ice cream samples

Standard methods of the Association of Official Analytical Chemists were used to determine protein (AOAC, 1990), fat (AACC, 2000) and carbohydrate content. Each analysis was carried out in triplicate. The energy content of ice cream samples were calculated by energy yielding nutrient content including fat carbohydrate and protein (Osborne & Voogt, 1978) according to the following equations:

$$\text{Food energy (kCal/g)} = (CP \times 4) + (F \times 9) + (CHO \times 4)$$

CP is crude protein (%) F is fat (%) and CHO is carbohydrate content (%)

4.5 Sensory test

Sensory characteristic and overall acceptability of ice cream samples were evaluated by 50 untrained panelists (20-35 year; 15 males, 35 females), using a sensory rating scale of 1–10 for taste and flavor, texture and body and 1–5 for appearance and color. The properties evaluated contained (a) three characteristics for appearance and color (no criticism: 5, dull color: 4–1, unnatural color: 3–1), (b) seven properties for taste and flavor (no criticism: 10, cooked flavor: 9–7, lack of sweetness and too sweet: 9–7, lack of flavor: 8–6, rancid and oxidized:6–1 and other: 5–1), and (c) seven terms describing texture and body (no criticism: 10, coarse: 9-7, crumbly: 9-7, weak: 8-6, fluffy: 8-6, gummy: 6-1, sandy: 5-1). Ice cream samples were placed into 100-mL transparent cups. The 30g ice cream samples were adequate for scooping up the teaspoonful and sensory characteristics of the samples could be evaluated. All samples were labelled with 3-digit random code numbers and randomly served to panelists.

4.6 Statistical analysis

The mean values recorded for each test were compared using analysis of variance (ANOVA). Tukey's test was applied to detect the significant differences among the ice cream samples ($P < 0.05$). The Completely Randomized Design or CRD was applied to create the different formulations of ice cream for properties measurements. Three replications were performed for each sample.

Results and discussion

1. Ice cream mix properties

1.1 Viscosity

Viscosity of ice cream mixtures have relationship with melt down rate, texture and overrun of ice cream after freezing. The viscosities of the ice creams mixtures are shown in Table 2. Viscosity of milk, organogel and rice bran oil ice cream mixtures were 289, 398 and 363 mPa·s, respectively. The conventional ice cream mixture exhibits a lower apparent viscosity than other samples. The viscosities of rice bran oil and organogel ice cream mixtures that contain soy protein were significantly different. They were higher than milk ice cream mixture that might be due to soy protein properties. Soy protein could provide several functionalities, such as water holding and emulsifying properties. In addition, soy proteins in rice bran oil ice cream mixtures might form a stable gel-like network structure, creating a greater resistance to flow (Batista et al., 2005). Our results were aligned with the study by Aboulfazli et al. (2014) which showed that soymilk ice cream increased apparent viscosity of melted ice cream.

Table 2 Viscosity and emulsion stability of ice cream mixtures

Ice cream formulation	Viscosity (mPa. s)	Emulsion stability (%)
Milk ice cream	289 ± 1.01c	88.72± 0.80a
Organogel	398 ± 2.01a	88.00± 0.10a
Rice bran oil	363 ± 1.32b	80.12± 0.65b

Remark: Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey's test)

1.2 Emulsion stability

All ice cream samples were prepared by emulsion of rice bran oil and cow milk which is oil in water emulsion and should be stable during freezing, but unstable enough so that partial coalescence occurs during the dynamic freezing (Goff et al., 1989). The emulsion stability of milk, organogel and rice bran oil ice cream mixtures were 88.72±0.80, 88.00±0.10 and 80.12±0.65, respectively (Table 2). Organogel could improve the emulsion stability by increasing the viscosity of the water phase because of its water absorption properties. Its continuity could also hold oil droplets, leading to increased stability of emulsion. The emulsion stabilities were not significantly different ($P < 0.05$) between conventional ice cream and organogel ice ceam mixture, suggesting that the emulsion stability contributed by the gelled oil in the organogel ice cream mixture was comparable to that of crystalline milk fat in the conventional ice cream mixture.

2. Frozen ice cream properties

2.1 Overrun

The overrun of conventional, organogel and rice bran oil ice cream samples were 39.40 ±1.03, 38.31±0.91 and 37.02±0.01, respectively (Table 3). Conventional milk ice cream had the highest overrun indicating larger incorporation of air. This related with the softest texture of milk ice cream. No significant difference was observed between the overrun values of conventional and organogel ice cream samples ($p>0.05$). For plant-based ice cream, organogel formulation produced higher levels of overrun when compared to rice bran oil ($p<0.05$), implying the larger incorporation of air in the organogel-based ice cream. The results suggested that the presence of organogel could improve the overrun of plant-based ice cream with respect to the use of non-structured rice bran oil. Similar results were also observed by Zulim-Botega et al. (2013a) that the application of a rice bran wax organogel instead of high oleic sunflower oil improved overrun of ice cream.

2.2 Melt down rates

The melt down rates is an indicator of the structure development and the resistance to collapse (Goff & Hartel, 2013). Melt down rates of various ice creams are presented in Table 3. The corresponding digital photographs are presented in Fig 1. All ice cream samples in this study melted within 90 minutes. The conventional ice cream had the highest melt down rate of 0.67±1.65. The rice bran oil ice cream possessed the lowest melt down rate of 0.45±1.65, which was not significantly different from the melt down rate of organogel ice cream samples, i.e., 0.46±1.75. Basically, overrun and melt down rates have a relationship because a large amount of air affected the heat transfer during melting. Hartel et al. (2004) found that higher air cooperation led to the melted ice cream having a more difficult flow, resulting in lower melt down rate. According to the overrun result, conventional ice cream incorporated more air than the plant-based ice creams and showed a higher rate of melting. Therefore, melt down rate increasing of plant-based ice cream is not related with the overrun. That could be due to the viscosity of plant-based ice creams were higher than milk ice cream. Nuwongsri et al. (2021) noted the mobility limitation of water molecules in high viscose ice cream mixture because the space between the particles in the mixture was getting narrower, resulting in lower melt down rate. Furthermore, soy protein may be adsorbed on to the surface of the droplets and emulsifiers were

effective at displacing the proteins from the droplet surfaces. The utilization of soy protein in plant-based ice creams could improve the melt down properties with respect to the milk protein.

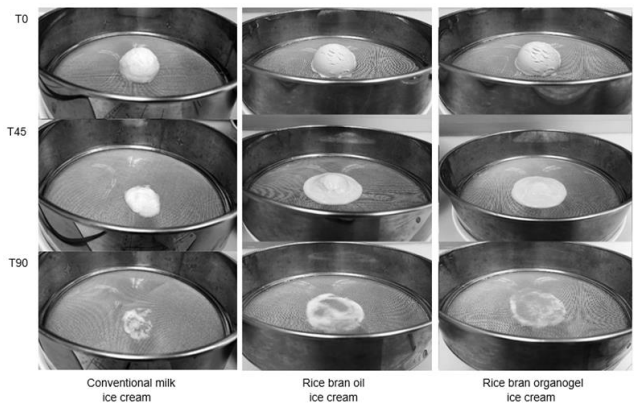

Fig. 1 Examples of pictures taken during melting test of ice creams, produced with milk, rice bran oil and rice bran oil organogel. The pictures are taken at the beginning of the test (T0) and after 45 (T45) and 90 (T90) minutes

2.3 Firmness

The results in Table 3 shows the firmness of conventional, organogel and rice bran oil ice cream were 14.4± 0.91, 15.3± 0.87 and 16.4± 0.91, respectively. The firmness values of the plant-based ice creams were significantly higher than the conventional ice cream because of high overrun value. Ice cream with air cooperated lead to softer texture found in conventional milk ice cream (Hartel et al., 2004). The rice bran oil ice cream demonstrated the highest firmness value due to the lowest overrun (Nuwongsri et al., 2021). Organogel ice cream firmness was higher than conventional milk ice cream and may be due to organogel network being recreated in the frozen ice cream, enhancing the firmness. Similar results were reported by measuring the hardness of the low-fat ice creams containing organogel (Moriano & Alamprese, 2017). It was possible the organogel accounted for an improvement in low-fat, plant-based ice cream quality characteristics when compared to the non-structured rice bran oil.

2.4 Energy, protein, carbohydrate and fat content of ice cream

Energy, fat, carbohydrate and protein contents of three ice cream formulations are presented in Table 4. Energy of conventional milk ice cream, rice bran oil and organogel ice creams (per 100 g) were 147±0.97, 135±0.67 and 126 ±0.81 kcal, respectively. Significant

Table 3 Overrun, firmness and melt down rate of frozen ice cream

Ice cream formulation	Overrun (%)	Firmness (N)	Melt down rate
Milk	39.40± 1.03^{a}	14.4± 0.91^{c}	0.67± 1.65^{a}
Organogel	38.31± 0.91^{a}	15.3± 0.87^{b}	0.46± 1.75^{b}
Rice bran oil	37.02± 0.01^{b}	16.4± 0.91^{a}	0.45± 1.65^{b}

Remark: Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey's test)

reductions in energy of 8.16% and 14.29% were observed in rice bran oil ice cream and organogel ice cream, respectively. Carbohydrate content in milk, rice bran oil and organogel ice creams were 28.01±0.23, 25.12±0.53, and 25.13±0.77 g, respectively. Replacement of milk fat with the rice bran oil and organogel resulted in decreasing total calories per serving. The higher value of carbohydrate content observed in conventional ice cream was due to lactose in milk. The protein contents of all ice creams were 2.12-2.20 g. Fat contents in conventional, rice bran oil and organogel ice cream were 3.14±1.17, 3.07±0.33 and 2.58±0.21 g, respectively.

Table 4 Energy, protein, carbohydrate and fat content of ice cream (100 g)

Nutritional composition	Milk ice cream	Rice bran oil ice cream	Organogel ice cream
Energy (kcal)	147±0.97^{a}	135±0.67^{b}	126±0.81^{c}
Protein (g)	2.20±0.17^{a}	2.12±0.05^{a}	2.12±0.90^{a}
Carbohydrate (g)	28.01±0.23^{a}	25.12±0.53^{b}	25.13±0.77^{b}
Fat (g)	3.14±1.17^{a}	3.07±0.33^{a}	2.58±0.21^{b}

Remark: Values with different letters in the same row are significantly different ($p < 0.05$) (Tukey's test)

The results revealed that the nutritional properties were improved for plant-based ice cream formulations. Especially, total fat content of organogel was lower than rice bran oil because of the lower fat organogel. Organogel is structured emulsion by organogelation of water in oil emulsion (30:70). Furthermore, according to fatty acid composition of rice bran oil and organogel rice bran. There were decrease in SFA contents for both formulations, up to 53% and 74% in rice bran oil and rice bran organogel ice creams, respectively. Moreover, the organogel was prepared by policosanol (4% (w/w)) (Manakla et al., 2020). Policosanol is long chain alcohols extracted from rice bran wax. It is used as a dietary supplement for lowering blood cholesterols. Therefore, rice bran organogel ice creams not only contained lower SFA but healthy functional compound was also added.

2.5 Sensory characteristic

The results of the sensory evaluation of the ice cream samples are shown in Table 5. Replacement of

milk by rice bran oil and rice bran oil organogel decreased scores of the taste and flavors as well as appearance and color characteristic. Conventional milk ice cream (4.77±1.17) had the higher appearance and color values than rice bran oil (3.86±1.07) and rice bran oil organogel (3.87±1.27). Taste and flavors of milk, rice bran oil and rice bran oil organogel were 8.00±0.51, 7.06±1.01 and 7.05±1.6, respectively. The rice bran oil-based ice creams exhibited higher color intensity compared to the conventional milk ice cream. This was due to the typical color of the rice bran oil used, which added a yellow note to the ice cream. The taste and flavor of conventional milk ice cream were significantly different from the rice bran oil-based ice creams ($p<0.05$). The sensory score of rice bran oil ice cream and rice bran organogel ice cream samples were decreased with replacing milk in ice creams because of their beany off flavors (Abdullah et al., 2003).

Texture and body of the organogel ice creams were not different when compared to the conventional milk ice cream. Although, firmness of organogel was higher than conventional ice cream but panelists could not perceive the difference. Nevertheless, rice bran oil ice cream had the lowest score ($P<0.05$) in texture and body that may be because it had the highest firmness value. None of the ice creams were judged to be weak, crumbly, sandy, or fluffy.

Table 5 Sensory properties

Sensory Characteristics	Ice cream formulation		
	Milk	Organogel	Rice bran oil
Appearance and color (1-5)*	4.77±1.17[a]	3.86±1.07[b]	3.87±1.27[b]
Taste and flavour (1-10)**	8.00±0.51[a]	7.06±1.01[b]	7.05±1.61[b]
Texture and body (1-10)***	7.68±1.31[a]	7.60±1.29[a]	6.72±1.60[c]

Remark: Values with different letters in the same row are significantly different ($p < 0.05$) (Tukey test)

* Appearance and color (no criticism: 5, dull color: 4-1, unnatural color: 3-1)

** Taste and flavor (no criticism: 10, cooked flavor: 9-7, lack of sweetness and too sweet: 9-7, lack of flavor: 8-6, rancid and oxidized: 6-1 and other: 5-1)

*** Texture and body (no criticism: 10, coarse: 9-7, crumbly: 9-7, weak: 8-6, fluffy: 8-6, gummy: 6-1, sandy: 5-1)

Conclusion

Low-fat, plant-based ice cream novelty formulated with rice bran oil and rice bran oil organogel is successful. The rice bran oil organogel ice creams posed similar overrun and firmness characteristics with respect to conventional milk ice cream. Significant reductions of SFA were observed in the developed plant-based ice cream formulations. Novel plant-based ice cream with organogel is a successful approach in order to obtain a healthy product without compromising their qualities except taste and flavor characteristic because of the beany flavor. Therefore, further investigations could determine the new plant protein for milk protein replacement for avoiding undesirable flavor. Moreover, the partial replacement of milk fat with organogel by keeping constant the amount of total fat should also be a focus in further studies.

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Optimization of Transethosome by Varying Dark Purple Glutinous Rice Variety Leum Phua (*Oryza sativa* var. *glutinosa*) Extracts and Rice Bran Oil under Hot Method

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Abstract

Leum Pua is native Thai glutinous rice that contains antioxidants higher than other colored rice. The purpose of this study was to develop transethosomes (TEs) extracted from Leum Phua rice by hot method. The influence of rice extract and rice bran oil concentrations was investigated on physical properties of the TEs. The formulation of TEs was performed by selecting the optimal ratio obtained from a mixture of glutinous rice extract at the concentration 20, 30 and 40% (w/v) and rice bran oil 20, 25 and 30% (w/v). Characterization of the TEs was based on results from particle size, polydispersity index, zeta potential, entrapment efficiency and stability testing. Leum Pua glutinous rice was extracted by 95% ethanol. The extracts were developed for TEs using rice bran oil as phospholipid and lecithin as permeation enhancer, Span 80 and Tween 20 as edge activator (surfactant) in their formula. Furthermore, the required size of ethosome vesicles was prepared by using sonication. Results showed that the optimized TEs formulation with particle size below 300 nm could be achieved by using rice extract at the concentration 30 and 40% (w/v) and rice bran oil 20% (w/v). At a concentration of rice extract 40% (w/v) and rice bran oil 20% (w/v) showed the highest entrapment efficiency (68.94±2.9%). The good colloidal characteristics had a particle size (PZ) of 233.0±12.9 nm, polydispersity index (PDI) of 0.314±0.078 and zeta potential (ZP) of -51.4±0.24 mV. The TEs optimized formulation was stable at room temperature and also at elevated temperature conditions (45°C/75% RH) for 3 months. Therefore, based on the current study, the potential of employing the novel carrier transethosomal-loaded nanoparticles of Leum Phua rice extracts could serve as an effective dermal delivery. The development of TEs for topical delivery can lead to value added of native Thai glutinous rice.

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Introduction

Dark purple glutinous rice has several health benefits. It contains high concentration of important bioactive compounds especially those with anti-aging properties. Other interesting properties are anti-tumor and anti-inflammatory (Seekhaw et al., 2018). Black rice extract, in particular, is a potent source of natural antioxidants, which is important for the potential to increase immune system in the body and to protect and reduce the risk of cancer and heart diseases (Pitija et al., 2013). Bioactive compounds present in pericarp include alkaloids, flavonoids and phenols. The flavonoids (particularly the anthocyanins) are basic phenolic compounds responsible for antioxidant activity (Peanparkdee et al., 2019). Leum Phua glutinous rice is a traditional purple rice known for over 100 years by the Hmong people. It is still planted in Phop Phra District, Tak Province, Thailand and can only be planted once a year. When cooked, the sticky rice is very fragrant with a soft sweet outer part. This rice has dark-purple pericarp and contains high levels of certain nutrients such as γ-oryzanol, vitamin E, omega-3-6-9 fatty acids, iron (Fe), calcium (Ca) and anthocyanins. Additionally, Leum Phua rice extracts has been reported to have antioxidant activity higher than other black rice, citing two to three times higher antioxidant activity (Wattanuruk et al., 2020; Boonsit et al., 2010). Several recent reports showed that antioxidant agents could prevent and improve many diseases such as cancer, atherosclerosis and Alzheimer's disease (Kaur & Ling, 2008; Podsedek, 2007). Therefore, with high antioxidant property, Leum Phua rice could be one of the nutraceuticals for the brain. Leum Phua rice aqueous extract (LP) has shown learning and memory enhancing effects in male mice. The effect of LP might be due to high concentration of antioxidants in Luem Phua rice (Srisuwan et al., 2013). Moreover, Leum Phua rice as an anti-aging agent due to its ability to not only counteract reactive oxygen species production induced by different challenges including UV exposure, but also to inhibit cutaneous collagenase and elastase activity (Vichit & Saewan, 2016). Although there is quite a lot of information concerning the usefulness of dark purple rice extracts available, its application as an active ingredient for cosmetics and drugs has been limited. The anthocyanins are hydrophilic and thus has poor skin permeability. Therefore, a delivery system that can facilitate the skin absorption of bioactive compounds is desirable and a formulation using nanocarrier could be among the best such vehicles for extracts-based treatment.

Nanotechnology is an increasingly widespread area of research for its various applications and advantages. Nanosystems allow for a deep skin penetration and an effective release profile of ingredients, which contributes to drug and cosmetic effects. Moreover, nanoparticle that can entrap the molecule in a physiological environment and protect it against oxidative degradation. Presently various type of vesicular nanocarrier systems is available such as liposomes, ethosomes, transferosomes, niosomes, transethosomes etc. Transethosomes (TEs) are the new generation of ethosomal systems and were first reported in 2012 (Song et al., 2012). The tranethosomal system contains the basic components of classical ethosomes and an additional compound, such as a penetration enhancer or an edge activator (surfactant) in their formula. These novel lipid vesicles were developed to combine the advantages of classical ethosomes and deformable liposomes (transfersomes) in one formula to produce TEs. TEs may contain advantages of both transfersomes and ethosomes. The mechanism of skin penetration might be a fusion of both mechanisms that can improve penetration through the skin due to their deformable ability, but they are unable to penetrate deep into the stratum corneum. TEs are composed of phospholipid (PC), water, edge activator or permeation enhancer (oleic acid) and are characterized by having a high content of ethanol (up to 30%). Due to presence of ethanol the intercellular space between the corneocytes increases which increase the permeation (Ascenso et al., 2015). The PC component of the vesicles tends to evade a dry environment. Thus, to stay fully swollen, the vesicles follow the local hydration gradient and penetrate more strongly the hydrated layers of skin, reaching the epidermis and dermis (Kumar et al., 2012). Recently, the efficacy of TEs have been successfully developed for both pharmaceuticals and cosmeceuticals in enhancing the dermal and transdermal delivery (Ascenso et al., 2015; Meng et al., 2013). TEs can cross intact skin by transcutaneous hydration gradient. Drying and partial dehydration of vesicles are the initial events in skin permeation by the vesicles after topical application. As a result, the vesicles become compressed or curved. This fact may be due to the combination of ethanol and edge activator that causes a rearrangement in the lipid bilayer of these vesicles (Gondkar et al., 2017). Few studies have investigated the nanoparticle of black rice extract

(Bulatao et al., 2017). To the best of our knowledge, there is no published research about the use of TEs to enhance the transdermal delivery of black glutinous rice extract. Further, many methods have been made for formulating TEs. In hot method, a dispersion of phospholipid and water is subjected to heating at a temperature 40-80°C on a magnetic stirrer. Depending on the ability and affinity of active ingredient to bind with hydrophilic and hydrophobic solvent, it either gets dissolved in water or in ethanol. In addition, the required size of ethosome vesicles can be easily prepared by using methods like extrusion or sonication (Pandey et al., 2015; Garg et al., 2017).

This study prepared TEs from Leum Phua glutinous rice extracts. The resulting formulation of TEs is a development of a form that produces sufficiently the particles' size and ensures stability. Therefore, this affects the transdermal delivery. Thereafter, the optimized formulation was analyzed for particle size (PZ), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency and stability properties.

Materials and methods

1. Preparation of crude extracts

Crude rice extract was prepared according to the methods of Plaitho, (2016) with some modifications. The glutinous rice of Leum Phua rice was purchased from Tak Province, Thailand and were dried in the oven at 60°C for 24 hours. Dried rice samples were extracted with 95% ethanol under stirring in a shaker at 120 rpm for 24 hours. The ethanol extracts were separated in the centrifuge (Becton Dickinson Dynac Centrifuge, Sparks, MD, USA) at 6,000 g for 10 min and were filtered through a paper filter (Whatman No.1). The waste from the previous extraction was re-extracted with the same method and then combined with the extract obtained from the first and mixed well. The extracts were transferred to a flat-bottomed flask. The solvents were evaporated by a rotary evaporator (Buchi R-124 Rotary Vap System, New Castle, USA) at 45°C until the samples were dry. All crude extracts were stored at -10°C in storage vials.

2. Preparation of TEs

The TEs from Leum Phua glutinous rice was prepared by hot method in 100 ml batches (Nandan & Shivalik, 2016). In a beaker the Leum Phua glutinous rice extracts was placed in specified amount of 95% ethanol, rice bran oil, lecithin, Sorbitan Oleate (Span 80) and Ceteary Alcohol (and) Ceteary Glucoside (Montanov 82) and dissolved at 80°C. Stirring was continuing through the magnetic stirrer at 300 rpm. Later, Polysorbate 20 (Tween20), Poloxamer and glyceral was dissolved in distilled water in a separate beaker at 80°C using the magnetic stirrer at 300 rpm. All chemicals' reagents were from Namsiang, Ltd., Bangkok,Thailand (analytical grade). Aqueous phase was added into the organic phase and stirring was continuing through the magnetic stirrer at 500 rpm for 30 min at 70°C. After the stirring process, sonication was done by the probe sonicator (Sonics Vibra Cell, Sonics & materials, Inc., Newtown, USA) at 8,000 rpm for 10 min (Fig 1). Finally, the TEs were prepared. For this experimental, different variable influencing vesicles' characteristics were prepared using four different amounts of Leum Phua glutinous rice extracts such as 20, 30 and 40% (w/v) and rice bran oil (20, 25 and 30 (w/v)). Among the various designs available for the purpose, the central composite design (CCD) has extensively been employed in optimization practice for identifying the best formulation (Singh et al., 2009). The compositions of each TEs formulation are presented in Table 1.

Fig. 1 Probe sonicator

Table 1 The compositions of TEs formulation

Material	Formular% (w/v)				
	TEs 1	TEs 2	TEs 3	TEs 4	TEs 5
Leum Phua glutinous rice extract	40	30	20	20	20
Rice bran oil	20	20	20	25	30
Lecithin	1	1	1	1	1
Span 80	5	5	5	5	5
Montanov 82	3	3	3	3	3
Tween 20	5	5	5	5	5
Poloxamer	3	3	3	3	3
Glycerol	2	2	2	2	2
Distilled water	increase to 100	increase to 100	increase to 100	increase to 100	increase to 100

3. Characterization of TEs

The mean particle size (PZ), polydispersity index (PDI) and zeta potential (ZP) of vesicles dispersions were measured by Dynamic light scattering technique using Malvern Zetasizer 2000 (Malvern Instruments Ltd., Malvern, UK). Physical properties were performed after 30 days, storage at 25°C. The measurements were performed after dilution. The dilute sample (50 µl) was added to 950 µl of distilled water to obtain a light-scattering substance of sufficient light intensity. (Abdelbary et al., 2015). Zeta potential evaluation was carried out by monitoring the electrophoretic movement of the particles in the electrical field using Malvern Zetasizer 2000 (Malvern Instruments Ltd., Malvern, UK). The ZP indicates charge present on the surface of TEs which is responsible for stability of the formulation and interact with membrane (Souto et al., 2004) All measurements were performed in triplicate. The optimized formulation was selected on the basis of size, extract entrapment and other colloidal characteristics like zeta potential and polydispersity index (PDI).

4. Entrapment efficiency

The percentage entrapment of the rice extracts added is called entrapment efficiency. The amount of rice extracts entrapped in the nanoparticles was determined by the separation of nanoparticles of the rice extracts from the freely suspeded rice extracts by centrifugation. Free unentrapped from TEs was separated by centrifugation at 20,000 g for 1 h at 4°C using a cooling centrifuge. The pellets that were formed after centrifugation were washed twice with 5 ml of phosphate buffer (pH 7.4) and re-centrifuged again for 1 h. The encapsulation efficiency of the extract was determined after the breakdown of the pellets with 5 ml of methanol and sonication for 10 min. The concentration of extract in methanol was determined using UV-Visible spectrophotometer (Bio-Tek, Vermont, UK) at 268 nm. Entrapment was determined using the following equation (Ramachandran & Shanmughavel, 2010).

$$\text{Entrapment Efficency (\%)} = \frac{\text{Total extract added} - \text{Free unentraped extract}}{\text{Total extract Added x 100}}$$

The entrapment efficiency or percentage of the content was estimated as the difference between the initial extract quantity and the free or unentrapped quantity of extract in the supernatant with respect to the total quantity incorporated in the nanocarrier preparation.

5. Accelerated stability study

The optimum physical stability testing of TEs were exposed to various temperatures like 45 ± 2°C and room temperature at 25°C±2°C for three months. The humidity level was kept at 75% in dark conditions. Optimized TEs were evaluated mainly for their physical characteristics at 0 and 3 months to check for the storage physical stability. Vesicles were examined visually for aggregation and change in their appearance such as texture and phase separation (Li et al., 2012).

6. Data and statistical assessments

The experiments were performed in triplicates and the results were presented as mean ± standard deviation. The statistical differences were determined at $P \leq 0.05$. Data were subjected to Duncan's post hoc test and the differences were detected for homogenous subsets. All statistical analyses were performed using SPSS® software 22.0.

Results and discussion

TEs extracted from Leum Phua glutinous rice extracts were effectively produced by using the hot method. This method is simple and does not require highly specialized equipment or extreme conditions. Various TEs formulations were produced by varying the amount of Leum Phua rice extracts and rice bran oil in their compositions. (Table 1) The optimized formulation was selected based on size (below 300 nm), entrapment efficiency and other colloidal characteristics like zeta potential and polydispersity index (PDI). The size of the colloidal carrier system is an important parameter of investigation as it affects the penetration of carrier into the skin especially in deeper layers. Prior research on transdermal systems revealed that vesicular carrier having a size below 500 nm effectively penetrated the skin and formed holes in the deeper layers of the skin (Zakir et al., 2010). Size of all TEs formulations was found in the range of 141–280 nm (Kohli & Alpar, 2004). Table 2 and graphically illustrated in Fig. 2 describes particle size (PZ), polydispersity index (PDI), zeta potential (ZP) and% entrapment efficiency of different TEs formulations.

The present work developed TEs using rice bran oil as phospholipid and lecithin as permeation enhancer. The size of TEs extracted from Leum Phua rice increased with the increase amount of rice bran oil. The vesicular size of ethosomal systems plays an important parameter that should be considered in the preparation of

Table 2 PZ, PDI, ZP and Entrapment efficiency of formula variables

Formular	PZ (nm)	PDI	ZP (mV)	% Entrapment efficiency
TEs 1	233.0±12.9[a]	0.314±0.078	-51.41±0.24	68.94±2.9
TEs 2	256.7±18.9[a]	0.364±0.036	-52.08±2.13	66.21±3.2
TEs 3	391.2±.21.9[b]	0.427±0.029	-57.35±2.93	59.63±2.5
TEs 4	415.2±14.9[c]	0.450±0.064	-58.62±1.15	56.07±2.8
TEs 5	435.8±15.5[c]	0.474±0.026	-58.69±2.38	50.58±2.2

Remark: Mean values for each parameter followed by a different letter within each column are significantly different ($p \leq 0.05$)

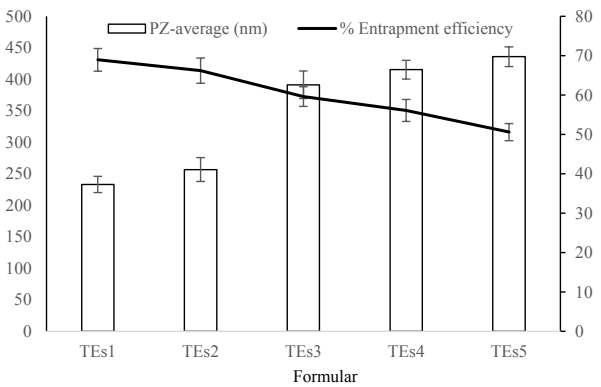

Fig. 2 PZ-average diameter and entrapment efficiency of formula variables

nanocarriers. Smaller vesicular size facilitates the TEs to pass through the small pores of the skin leading to more enhanced skin permeation. It has been reported that the vesicular size should be <300 nm to be suitable for this route of administration (Verma & Pathak, 2012). The optimized TEs formulation with particle size below 300 nm with low PDI could be achieved by using rice extract at the concentration 30 and 40% (w/v) and rice bran oil 20% (w/v) (TEs1 and TEs2). There was no significant difference in the particles size of all vesicles in both formulations ($p<0.05$) . Vesicle size of TEs1 and TEs2 formulation had an optimized range of 233.0±12.9 nm and 256.7±18.9 nm, respectively. It was observed that rice bran oil concentrations >20%(w/v), the size of the vesicles was >300 nm. The vesicular size of the TEs increased significantly ($p<0.05$) by increasing the rice bran oil concentration. The polydispersity of TEs1 and TEs2 formulation was 0.314±0.078 and 0.364±0.036, respectively. PDI≤0.3 are considered ideal and indicate a narrow size distribution (Pathak & Nagarsenker, 2009). Moreover, TEs1 and TEs2 formulations showed high entrapment efficiencies (more than 65%) indicating their capability to produce the desired therapeutic effect. The%entrapment efficiency of TEs1 and TEs2 formulation was found to be 68.94±2.9% and 66.21±3.2%, respectively. The rice extracts of TEs3, TEs4 and TEs5 formulation were also excluded from the selection due to the associated increase in the vesicular size. Therefore, formulation were TEs1 and TEs2 formulation considered to be optimized. The phospholipid type had a significant effect on the ethosomal size, but not the entrapment efficiency. Increasing phospholipid concentration will increase vesicular size slightly or moderately but will increase entrapment efficiency significantly (Prasanthi & Lakshmi, 2012). The selection of a proper edge activator or penetration enhancer is a critical step in the formulation of TEs, as they have profound effects on the properties of the ethosomal system.

The results of the zeta potential analysis revealed that all the prepared TEs had negatively charged zeta potential, ranging from −51.41 to −58.69 mV. All the prepared TEs nanovesicles showed zeta potential values that were significantly affected by the percentage of rice extract and rice bran oil. As the concentration of rice extract was decreased and rice bran oil was increased, the obtained zeta potential value was increased owing to further deposition of the charge-inducing agent on the vesicles' outer surface. Zeta potential involved with surface charges of particles can be positive or negative. Ogiso et al., (2001) reported that the negatively charged vesicles had better skin permeation properties than the positively charged ones. The zeta potential of vesicles in formular TEs1 and TEs2 showed higher negative value compared to other vesicles (TEs3, TEs4 and TEs5). Zeta potential of TEs1 and TEs2 formulation was found to be -51.41±0.24 mV and -52.08±2.13 mv, respectively. Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. High zeta indicates high repulsive force, resulting in prevention of particle aggregation. In general, nanoparticles with good physical stability should have zeta potential higher than +20 mV or lower than -20 mV due to the electric repulsion between particles. However, some studies mentioned that this was not always true and the stability studies should be established (Das et al., 2012).

TEs represent novel lipid formulation which contains phospholipids, ethanol in high concentration of 30 to 40% (Prasanthi & Lakshmi, 2012). The negative charge of the zeta potential of ethosomal systems is attributed mainly to the high ethanol content in these nanocarriers. Ethanol is a central character of TEs system giving unique identity to it as a vesicular system. Presence

of high content of ethanol imparts a negative charge on the surface of vesicles which promotes reduction of its size, thereby avoiding aggregation of the vesicular system due to electrostatic repulsion (Lopez-Pintoet et al., 2005). Additionally, ethanol was also reported to have stabilizing effect and efficient penetration enhancer (Finnin & Morgan, 1999; Dubey et al., 2007) Ethanol also has a significant effect on ethosomal system entrapment efficiency and in general increasing ethanol concentration will increase entrapment efficiency (Bhadra et al., 2004).

Bragagni et al., (2012) introduced Tween 20 in an ethosomal system of celecoxib at 15% of the total phospholipid concentration. It was found that Tween 20-containing TEs had smaller vesicular size (258.4±3.3 nm), higher entrapment efficiency (54.4%) and better ex vivo skin permeation through human skin compared to TEs containing Tween 80. The effects of Tween 20 on the ethosomal system are mainly due to its solubilizing property and the prevention of vesicle fusion. In another study, addition of Tween 20 formed an unstable formulation. Morever, Spans 20 were successful in producing homogeneous and stable TEs (Ascenso et al., 2015)

The optimized formulation (TEs1 and TEs2) was evaluated after storage at room temperature and after accelerated stability studies at elevated temperature (45°C/75% RH) in stability chamber. Stability profile of TEs loaded with Leum Phua rice extracts were checked by determining their appearance for 3 months. The results of stability studies show that the formulation was stable at room temperature and at elevated temperature conditions. The formulation TEs1 and TEs2 revealed its excellent colloidal stability. Physical appearance showed no change in color and odor on TEs both before and after of storage. It was found that the appearance of the TEs1 and TEs2 formulation was unchanged during the 3 months storage (Fig. 3). However, Optimum TEs were stored at 4-25°C. The TEs can be explored for transdermal delivery of various bioactive molecules due to their high entrapment efficiency and excellent colloidal stability (Verma & Utreja, 2018).

Fig. 3 Colloidal stability of TEs1 formulation (left) and TEs2 formulation (right)

Conclusion

Leum Phua glutinous rice has been studied and concluded for its high nutrition value. TEs are elastic vesicles composed of phospholipid, ethanol, and edge activator (surfactant). Preparation of TEs from Leum Phua glutinous rice extracted by heating method to be effective can be explained by setting the appropriate formula ratios, the resulting particle characteristics and the stability test. The optimized formulation had suitable characteristics for the transdermal delivery of extracts, such as small vesicular size, negatively charged ZP and high% entrapment efficiency. The TEs formulation was optimized using rice bran oil as phospholipid. The study found that the ratio between the glutinous rice extract at 40% (w/v) and rice bran oil at 20% (w/v) was the most suitable optimization. The results suggested that vesicle size and% entrapment efficiency of the optimized TEs formulation was found to be 233.0±12.9 nm and 68.94±2.9%, respectively. Polydispersity index and zeta potential of the optimized TEs formulation was found to be 0.314±0.078 and -51.4±0.24 mV, respectively. Nevertheless, The TEs optimized formulation was stable at room temperature and at 45°C for at least 3 months.

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The Effect of Health Literacy on Self-Management Related to Food Consumption among Older Adults with Hypertension in the Community

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Abstract

The study aimed to study the effect of the health literacy program on self - management related to food consumption among older adults with hypertension in the community. The study design was quasi-experimental study with pre and posttest groups. The participants consisted of 64 older adults with hypertension who were randomly assigned to an experimental and control group. Participants in experimental group received health education and six sessions of health literacy. Each session lasted 20-60 minutes within eight weeks. Control group received usual care and health education from health provider. self-administration questionnaire (nutrition literacy, food consumption pattern and self-management) was used to collect the data. Data were analyzed using independent t-test. Group reflection was analyzed using content analysis. Results showed that mean score of nutritional literacy, food consumption and self-management of the experiment group was significantly higher than the mean score of the control group (p-value <.001). Results from the reflection in the experimental group suggested some issues, including the understanding, access to information and applying knowledge. Participants suggested the methods to enhance health literacy for self-management on food consumption including providing knowledge, communication, sharing experiences and using photographic media. In sum, health literacy program can be used as a tool to enhance knowledge related to health.

Introduction

The older adult population in Thailand is at risk resulting in Thailand's ageing society. It has been predicted that Thailand will become an aged society in 2030 (National Committee on the Older adult, Department of Older adults, Ministry of Social Development and Human Security, 2020). This will inevitably affect the shift of social services towards the older adult population as an important target group for health promotion. At an older age, changes occur both physically and mentally. The changes in the body reduce the ability to care for themselves and subsequently, they have to rely more on family care in terms of economic, social and daily activities. However, the requirement for older adult care varies according to health conditions.

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Normal and at-risk older adult people can participate in outdoor or community activities due to less or no physical limitations. Moreover, the community can put their effort to participate in care for the older adult with complex illnesses. Care activities associated with health conditions, can be focused on health promotion and surveillance of risks to severity or complications of such diseases.

The significant health problems among the older adult include suffering from chronic diseases such as high blood pressure, diabetes, heart disease, etc. Chronic diseases are related to many modifiable risk factors such as unhealthy diets, physical inactivity, consumption of tobacco and alcohol and being overweight or obese (Aekplakorn, 2021). Behavior changes particularly in the retirement age group (from 60 onwards) prevent any further damage to their health. Moreover, any retirees without any health problem can also prevent serious illnesses later on in life. In 2020, the Thai National Health Examination Survey (NHES VI) reported that the prevalence of older adult with hypertension was 60.7% and prevalence of hypertension in Bangkok metropolitan was 27.2%. Division of Non-communicable Diseases 2022 in Thailand also reported the number of deaths from uncontrolled hypertension at 9.303 per 100,000 population.

High blood pressure, or hypertension, is a major health problem in older adults. The vascular system changes with age resulting in stiffer arteries. This causes increasing of blood pressure. Hypertension is known as "the silent killer," and often does not show any signs of illness. If hypertension cannot be controlled with lifestyle changes and medication, it can lead to serious health problems, including cardiovascular disease such as heart disease and stroke, vascular dementia, eye problems and kidney disease (WHO, 2021). These complications have an impact on older adult such as morbidity and mortality. The effects lead to dependency on family and community (Kanjanapibulwong et al., 2020; Aekplakorn, 2021. In addition, consumer behavior can also affect health conditions in those who are considered well. It can cause changes in biomarkers to indicate a person is at risk of developing the disease. Therefore, blood pressure control requires self-management. WHO (2013) recommended the involvement of patients through their own self-management surveillance such as smoking cessation, weight management, low-sodium and low-fat diet to better control high blood pressure. Self-management also applies to health promotion and the distal outcomes are health status and quality of life. In addition, a systematic review found that the majority of hypertensive individuals with higher health literacy tend to have better blood pressure control (Mohd Isa et al., 2021)

Self-management is the intrinsically controlled ability of an active, responsible, informed and autonomous individual to live with the medical condition, role and emotional consequences of their chronic conditions in partnership with their social network and the healthcare providers (Van de Velde et al., 2019). Therefore, the adjustment of consumer behavior is a good guideline for the self-management of the older adult. Meanwhile, advances in telecommunication technology have given the older adult access to information through various channels and a mass of health information. If older adults do not have sufficient health literacy, they could potentially struggle to comprehend an overflow of information and to make decisions on healthy choices. The latter could lead to an inappropriate decision on healthy behaviors. On the other hand, the older adult with chronic illnesses manages their consumption appropriately with health literacy through various information and communication channels that will offer them a good quality of life according to their health condition. The development of health literacy and self-management mutually complemented one another (Norris et al., 2002; Kim et al., 2004; Chodosh et al., 2005; Chao et al., 2013; Wang et al., 2017).

Health information education is a model of action to change consumer behavior and the outcome is the number of people who consumes that information. However, Nutbeam (2010) argues that the results of health information education should be the skill of individuals to make decisions or improve their behavior. This is known as "health literacy". Health literacy is considered as one of the most important skills to control people health. Health literacy is the set of patients' cognitive and social skills that allows them to access, understand and use information in ways that promote and maintain their health. However, one specific form of health literacy is nutrition literacy. Nutrition literacy reflects the ability to access, interpret and use nutrition information and exactly focuses on health literacy skills related to food consumption (Velardo, 2015). Food consumption is a periodic behavior. It is triggered at various moments of the day by a number of converging factors (time of day, need state, sensory stimulation, social context, etc.). As eating progresses, inhibitory influences of many origins (sensory, gastric, hormonal,

neural, as well as cognitive) develop and finally bring the meal to an end (Bellisle, 2019). Previous research evaluated the relationship between health literacy and food consumption among adult population. The results showed that high food literacy was associated with increased consumption of fruits and vegetables (Namdar et al., 2021)

The educational program is blended with sharing and learning and self-talk. It helps the older adult manage themselves to change dietary habits under their health condition. Developing health literacy in ways that builds confidence in decision-making contributes to better health literacy than education alone. The reasons mentioned above lead to the main objective of this research to develop health literacy of the older adult with the expectation that literacy will lead to appropriate decision-making and self-management. Health literacy is associated with self-management abilities, therefore, early recognition of low health literacy among older person together with interventions to improve health literacy might be very beneficial for older adults (Geboers et al., 2016).

Therefore, the objective of this research was to study the effectiveness of a health literacy program for self-management on food consumption among hypertensive older adult. The research framework employs the concept of health literacy (Nutbeam, 2000), namely access to information and knowledge, data analysis skills, utilizing information and knowledge, guiding of knowledge contributes to the health of others and concept of self-management by assessing health needs, selecting use sources and behavioral changes under health needs (Creer, 2000). There have been research supporting the study of the relationship between health literacy and self-management, health literacy and food consumption behavior (Geboers et al., 2016; Namdar et al., 2021). In this research, we explored these elements with the aim of viewing the effectiveness of the health literacy program.

Materials and methods

1. Study design

This quasi-experimental study was conducted using two groups and a pre-posttest design. The study period was from September 2019 to October 2020. Intervention conducted at eight weeks and measured nutrition literacy, self-management on food intake and food consumption pattern.

2. Population and sample

The reference population was the older adult, both male and female, aged between 60 and 65 years, who lived in the Bang Phlat District, Bangkok, Thailand. We selected this particular age group for our implementation because this is an early retirement age group and chronic diseases starts to affect their health. Hence, changing behavior prior to serious health consequences is therefore an imperative measure for disease prevention.

Samples were drawn from the reference population, who met the inclusion criteria as follows: 1) between the ages of 60 - 65 years, male or female 2) have a blood pressure ≤140/90 mmHg 3) fluency in reading and writing Thai language 4) participate in the project voluntarily and 5) have lived in the community for more than three months.

The sample size calculation used power analysis. A one-tailed test model was selected and a method for comparing the mean scores used two independent group tests. The influence effect size was 0.80 (Meethien et al., 2011; Sawekwan et al., 2019), the confidence level was 0.05 and the test power was 0.95 (Cohen, 1992). The results of the calculation were a sample group of 70 people, 35 in the experimental group and 35 in the control group.

The sampling technique used a stratified multistage sampling method. Bang Phlat district consists of 4 sub-districts, namely Bang Phlat Sub-District, Bang Yi Khan Sub-District, Bang Ao Sub-District and Bang Bamru District. Then communities from all four districts were selected to be represented at the provincial level using a probability proportional to size. The communities that were in the area of the sample 4 communities were Daowadung Temple community (A), Bang Yi Khan Temple community (B), Chatkaew Jongkolnee Temple community (C), Khong Makham community (D). Community A and C were assigned experimental group using a non-random method. A total of 64 older adults met the inclusion criteria and were enrolled into our study. The number in the experimental was equal to the control group (32 in each group). Three people from each group withdrew during the course of the study. It is to be noted that sample of 32 from each group still provided 90% of study power.

3. Research instruments

Experimental research tool: Health literacy program. The activities design was developed by researcher and used the concepts of health literacy of Nutbeam (2000), which aimed to increases the subjects' health literacy and

self-management and food consumption behavior. The details of the activities were making relationships, education, practicing communication skills by self-talk techniques, sharing experiences and learning and evaluating the program by reflection from the group. The program was conducted in eight weeks and it took 20 - 60 minutes to organize each activity (Table 1). Kaewdamkerng (2019) stated that in general, habit is formed by completing a task continuing within 66 days (approximately 2 months) or 21 days at least. Moreover, previous research applied 6-8 weeks (Patipattarakul et al., 2018).

The program was conducted by researchers and held at each community. The program for the experimental group comprised as follow:

Building relationship and health education: Before starting the health education program, we built the relationship within the group to create trust, empowerment and encouragement within the group. These activities took 20 minutes. Also, during the week the health education was scheduled during the 1st week which lasted 60 minutes for small group teaching and discussion, via use of flip chart to demonstrate knowledge on hypertension, regarding: definition, causes, symptom, treatment, prevention and risk factors (WHO, 2021; Thai Hypertension Society, 2019).

Nutrition education: The nutrition education was scheduled during 2nd week and lasted 60 minutes for small group teaching. We delivered the nutrition information related to healthy eating, a nutritional recommendation based on dietary approaches to stop hypertension (DASH) (NIH, 2021), Thai Food Pyramid Guide, Dietary Guidelines and Nutrition Facts Labels for Thai elders; essential nutrients (Nutrition Division, 2020) via picture media. We provided information on how to access resources and use of information (20 minutes for this session).

Individual practice skill: We demonstrated process of decision making by using a tree diagram. Evaluation of decision-making was captured by asking questions about advantages or benefits and disadvantages or risks of decisions. In addition, we demonstrated how to use such information, select resource and verify the reliability of the information for example reading food label. After demonstration, they practiced their skill and showed their results to researchers. This was scheduled during the 3rd week of the program in small groups and took 60 minutes. The intervention materials included food labels, leaflets, drug labels etc.

Practice self-communication skills: Activities were scheduled during 4th week and took 60 minutes. The activities consisted of an overview of self-talk technique (Kross et al., 2014; Health Direct, 2022) and practice of self-talk by telling own messages to encourage and motivate subjects healthy eating and behavior modification. Example of dialogues included: “I can do it. I have accomplished something more difficult”, “Can I eat salty food?” and “I have to read food labels before buying”. The dialogues were developed by researchers based on review of existing information (Nutrition Division, 2020; Health Direct, 2022)

Monitoring health eating: Activities were scheduled during 5th and 6th weeks. Subjects reported food consumption and self-talk technique to the researcher. It took approximately 20 minutes. We advised older adults whenever they were not appropriately performing healthy eating behavior.

Sharing and Learning: We delivered the activities during the 7th week in small groups and took approximately 60 minutes. Subjects shared experience and learning about self-talk technique, how to access health information and how decisions were made and applied.

Program evaluation: An evaluation by reflection was held during the 8th week in small groups. This session took approximately 40 minutes.

4. The intervention for control group

Like those in the experimental group, subjects in the control group received the same and usual health care and routine health education activities from their respective community health care providers. However, subjects in the control group did not receive the 8 weeks health literacy program that was administered to those in the experimental group. Their usual health care and routine health education activities included; curative care, home health care and health disease prevention information

The instrument for data collection comprised of four questionnaires and developed by literature review as follows:

1. The personal data questionnaire consisted of seven items, namely sex, age, BMI, congenital disease, cigarette smoking, alcohol drinking and physical activity.

2. The nutrition literacy questionnaire consisted of 15 closed-ended questions. The answers were five-point on the Likert scale, i.e., never practice, seldom practice, sometimes practice, often practice, and always practice (75 full marks).

Table 1 Health literacy program for self-management on food consumption

Session/Week	Activities	Relation between health literacy and self-management
Week 1 Session 1: 20 mins Session 2: 60 mins	1. Building relationship within the group to create trust and empowerment and encouragement within the group 1. Providing health education	Functional health literacy (empowerment)
Week 2 Session 3: 60 mins Session 4: 20 mins	1. Providing knowledge about nutrition for hypertension, DASH diet, diet control guidelines 2. Knowledge about how to access resources and use of information	Functional health literacy (access and understand)
Week 3 Session 5: 60 mins	1. Training about how to decision making 2. Practice skills in selecting resources, and verifying the reliability of the information	Functional health literacy (access and understand)
Week 4 Session 6: 60 mins	1. Overview and introduction self-talk 2. Practice self-communication skills by using the self-talk technique by telling own messages (internal dialogue) for behavior modification	Interactive health literacy (analytic and problems solving) Self-management
Week 5 – 6 Session 7: 20 mins by telephone	1. Individually reported on food consumption and self-talk technique to the researcher	Critical health literacy (decision making and application) Self-management
Week 7 Session 8: 60 mins	1. Experience sharing and learning (Sharing and Learning)	Functional health literacy (understand)
Week 8 Session 9: 40 mins	1. Program evaluation	

3. Food consumption patterns for the older adult questionnaire consisted of 13 closed-ended questions. The answers were five-point on the Likert scale, i.e., never practice, seldom practice, sometimes practice, often practice and always practice (65 full marks).

4. The questionnaire for self-management food intake pattern consisted of 16 closed-ended questions. The answers were five-point on the Likert scale, i.e., never practice, seldom practice, sometimes practice, often practice and always practice (80 full marks).

The determination of benchmarks to classify health literacy scores, dietary patterns and self-management behaviors on food consumption used a 4-level classification based on the criteria for measuring the health quotient of the Health Education Division, Department of Health Service Support, Ministry of Public Health (2018): Poor <60% of the full score, Fair ≥ 60% - <70% of the full score, Good ≥ 70% - <80% of the full score and Very Good ≥ 80% of the full score.

5. Measurement instrument

Health literacy program was validated by three experts in nutrition, adult and geriatric nursing and community health nursing. By checking how well the results corresponded to established theories and other measures of the same concept, security and the possibility of using the program. Health literacy program was revised according to the recommendations before applying to the sample group. The Index of Item-Objective Congruence (IOC) was used so as to find the content validity. In this process, the questionnaires were checked by three experts including, one expert in nutrition, two experts in adult and geriatric nursing and community health nursing field. The items that had scores lower than 0.5 were revised. On the other hand, the items that had scores higher than or equal to 0.5 were reserved. All of questionnaires had IOC between 0.8 to 1. The reliability of the questionnaires was determined to ensure that the responses collected through the instrument were reliable and consistent. The questionnaire was tested with 30 older persons that were not in the sample group. The reliability value was calculated by using Cronbach's alpha to ensure whether there was internal consistency within the items. According to the pre-test, the Cronbach's Alpha was 0.78, so the questionnaire was accepted for reliability (Tavakol & Dennick, 2011).

6. Ethical approval

This study was approved by the Ethical Review Committee for Human Research, Research and

Development Institute, Suan Dusit University, Thailand (SDU-RDI 2020-008). Each patient who participated in the study was informed of the nature and objectives of the study. A written consent form was obtained before data collection from each participant.

7. Data Collection

Once the older adults, in both the experimental and controls groups, were identified, they were informed the purpose of the research. Appointments were made during the first week to collect data in their communities. Data were obtained from the older adults via administration questionnaires. The health literacy development program was then conducted. The experimental group received the health literacy program, along with the usual health care and routine health education activities provided by their health care providers. The control group received only the usual health care and routine health education activities provided by their health care providers. During the 9th week, the questionnaires were administered to each older adults in each group. During the 10th week (9 weeks after the experimental group completed the health literacy program and all data were collected), the researchers provided the control group members the same health literacy program. Details of the protocol timeline is shown in Table 2.

8. Data analysis

Descriptive statistics were used to summarize demographic data. Continuous variables were presented as mean ± standard deviation and categorical variables were shown as frequencies and percentages. Chi-square, Fisher's exact test was used to compare the demographic data between groups and the independent t-test was used to examine differences between intervention and control groups, based on the assumptions of each statistic. Paired t-test was used to analyze in the difference between pre and posttest for the same subject. P values less than 0.05 were considered as statistical significance. Content analysis was used to summarize the results from the reflection.

Results

A total of 64 participants completed the study, with 32 in the experimental and 32 in the control group. All characteristics of participants in both groups were similar in gender, age, cigarette smoking, alcohol drinking and physical activity except BMI (Table 3).

The general characteristics of the experimental group was that most were female (84.4%). Participants' ages ranged from 60 to 65 years old, with a mean age of 63.34 years old (SD =1.89). Half the participants had BMI at the level of obesity (50.0%). Twenty five percent of the participants had congenital diseases such as high blood pressure and diabetes. The majority (93.8%) were non-smokers and did not drink alcohol (84.4%). Only one person exercised daily (3.1%). The majority of the control group was also females (68.8%). Their age ranged from 60 to 65 years old, with a mean age of 62.78 years

Table 2 Research protocol timeline

Week	0	1	2	3	4	5	6	7	8	9	10
Experimental group											
measurement	T1	-	-	-	-	-	-	-	-	T_2	
intervention	-	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	-	-
		Group					Individual		Group			
Control group											
measurement	T1	-	-	-	-	-	-	-	-	T_2	HL
intervention	-	P_0	P_0	P_0	P_0	P_0	P_0	P_0	P_0	P_0	P_0	

Remark: 0 = Before beginning the experimental intervention
T_1 = Pre-test; measurement of the nutritional literacy, food consumption, self-management before the experimental intervention
T_2 = Post-test; measurement of the nutritional literacy, food consumption, self-management at 9th week
P_0 = Usual care
$P_1 – P_6$ = Group session 1-6
P_7 = Individual session
$P_8 – P_9$ = Group session 8-9
HL = Provision of the health literacy program

old (SD = 1.93). The BMI at the level of obese was 43.8%. With regards to the control group, there were 37% of congenital diseases such as high blood pressure and diabetes. The majority of the participants were non-smokers and did not drink alcohol (84.4% and 65.6%, respectively). Only 9.4% exercised every day (Table 3). No significant differences were found between the demographic of the two groups (p-value>0.05), except BMI and congenital diseases (p-value<0.05).

The overall pre-test nutritional literacy of the experimental group and the control group was at a fair level meaning sufficient nutrition literacy and ability to practice it correctly. After the experiment, results found that most of the experimental groups had very good nutrition literacy and had proper and consistent practice. For the majority of the control group, there was a fair level of nutrition literacy.

The overall food consumption behavior of the experimental group and the control group was at a fair level. In other words, participants had a minority of correct dietary consumption. After the experiment, results found that most of the experimental groups were at a good level of dietary behavior. The majority of the control group had food consumption behavior at a fair level.

The pre-test self-management behavior on food consumption in the experimental group showed a majority of subjects at a fair level. They were able to

Table 3 Demographic characteristics of participants

Characteristics	Experimental group (n = 32)		Control group (n = 32)		P-value
	n	%	n	%	
Gender					0.637
male	5	15.6	10	31.3	
female	27	84.4	22	68.8	
Age (years)					0.187
60 - 61	8	25.0	10	31.3	
62 - 63	5	15.7	7	21.9	
64 – 65	19	59.3	15	46.9	
	Mean =63.34 SD.=1.89		Mean =62.78 SD.=1.93		
BMI (Kg./m.^{2})					0.000
< 18.5	1	3.1	0		
18.5 – 22.9	8	25.0	6	18.8	
23.0 – 24.9	7	21.9	12	37.5	
≥ 25	16	50.0	14	43.8	
	Mean = 25.22 SD.=3.54		Mean = 24.87 SD.=2.62		
Congenital diseases					0.03
Yes	8	25.0	12	37.5	
No	24	75.0	20	62.5	
Cigarette smoking					1.00
Yes	1	3.1	1	3.1	
Recovering smoker	1	3.1	4	12.5	
No	30	93.8	27	84.4	
Alcohol drinking					0.762
Yes	1	3.1	1	3.1	
Recovering drinker	4	12.5	10	31.3	
No	27	84.4	21	65.6	
Physical activity					0.119
Everyday	1	3.1	3	9.4	
> 2 days - ≤ 5 days /week	15	46.9	12	37.5	
≤ 2 days /week	16	50.0	17	53.1	

Table 4 Level of nutrition literacy, food consumption and self-management behaviors

	Scores	Level	Pre-test n (%)		Post -test n (%)		p-value
			Experimental group (n=32)	Control group (n=32)	Experimental group (n=32)	Control group (n=32)	
Nutrition literacy							0.000
(75 full marks)	< 60% of full marks	Poor	10 (31.3)	12 (37.5)	0	11 (34.4)	
	≥ 60% - <70% of full marks	Fair	12 (37.5)	17 (53.1)	2 (6.3)	19 (59.4)	
	≥ 70% - <80% of full marks	Good	10 (31.3)	3 (9.4)	11 (34.4)	2 (6.3)	
	≥ 80% of full marks	Very good	0	0	19 (59.4)	0	
Food consumption behavior							0.346
(65 full marks)	< 60% of full marks	Poor	11 (31.4)	7 (21.9)	2 (6.3)	7 (21.9)	
	≥ 60% - <70% of full marks	Fair	14 (43.8)	20 (62.5)	8 (25.0)	20 (62.5)	
	≥ 70% - <80% of full marks	Good	7 (21.9)	5 (15.6)	13 (40.6)	5 (15.6)	
	≥ 80% of full marks	Very good	0	0	9(28.1)	0	
Self-management behaviors							0.451
(80 full marks)	< 60% of full marks	Poor	7 (21.9)	18 (56.3)	0	6 (18.8)	
	≥ 60% - <70% of full marks	Fair	17 (53.1)	13 (40.6)	1 (3.1)	19 (59.4)	
	≥ 70% - <80% of full marks	Good	8 (25.0)	1 (3.1)	6 (18.8)	7 (21.9)	
	≥ 80% of full marks	Very good	0	0	25 (78.1)	0	

manage themselves on food consumption well. The majority of the control group was poor. They could not manage their food consumption well. However, after the experiment, results suggested that most of the experimental group had a very good level of self-management behavior. In the majority of control group, the level of self-management behavior of food consumption was moderate (Table 4).

The experimental group had the mean score of all components (nutritional literacy, food consumption and self-management) significantly higher than the control group with statistical significance at level of <0.001 (Table 5).

Table 5 Mean score of nutritional literacy, food consumption behavior, and self-management behavior between experimental and controlled groups by using independent t-test

variables	Experimental group		Controlled group		p-value
	mean	SD	mean	SD	
Before					
nutritional literacy	47.72	6.0	45.31	5.29	0.940
food consumption behavior	40.06	4.85	40.40	3.32	0.742
self-management behavior	51.91	6.26	66.66	5.77	0.001
After					
nutritional literacy	60.25	5.63	45.38	5.19	<0.001
food consumption behavior	47.53	5.58	40.81	3.27	<0.001
self-management behavior	66.65	5.77	52.06	5.69	<0.001

The experimental group had the mean score of all components significantly higher than before the experiment with statistical significance level of <001 (Table 6).

Table 6 the mean score of nutritional literacy, food consumption behavior, and self-management behavior between before and after intervention of experimental group by suing dependent t-test

variables	Before experiment		After experiment		p-value
	mean	SD	mean	SD	
nutritional literacy	47.72	6.0	60.25	5.63	<0.001
food consumption behavior	40.06	4.85	47.53	5.58	<0.001
self-management behavior	51.91	6.26	66.65	5.77	<0.001

Evaluation of the program by reflection from the experimental group

The results from the reflection of the experimental group are summarized as follows: the understanding of the information or health literacy for self-management on food consumption must be studied and understood well; read more; do not believe in delivered information without factual information from knowledgeable people; or from advertising with solicitation to buy. People and communities with access to information and health literacy need to seek further knowledge and build empowerment, community and social support and effective communication at the family level, as well as expand widely at the community level. People must interpret information before they can analyze and make food consumption decisions. Therefore, having academics come to suggest and help develop community leadership skills results in an analytical thinking process to be a leader in community development and improve people in the community to gain skills. The methods of developing health literacy for self-management on food consumption were health education from academics giving direct knowledge. People can transmit the knowledge correctly to the community. In addition, there are self-talk techniques, communication in small groups and communication at the community level. Self-talk is a method that helps people be mindful before doing anything. It serves as a reminder to them every time they do something, until the behavior becomes second nature for them. For example, they read a nutrition label before buying a product or searching for information. Moreover, sharing experience and learning support health literacy and self-management, such as exchanging knowledge on how to self-manage and make decisions on consumption. The visual teaching materials are important to enhance health literacy for self-management in groups with learning restrictions. Therefore, the use of illustrations and explanations is suitable for the older adult, such that pictures can provide more understanding.

Discussion

All characteristics of participants in both groups were similar in gender, age, cigarette smoking, alcohol drinking and physical activity. However, BMI showed significant difference at level of <0.001 and the mean score of BMI experimental group was greater than the control group. The finding suggested that the experimental group showed a great self-management. This indicated that BMI factor could have an effect on self-management. Vinkers et al. (2014) suggested that good self-management intervention in overweight individuals could improve outcomes in behavioral and anthropometric. This indicated that older adult's obesity might have greater interest in program to improve their health.

The level of nutrition literacy and self-management behavior in the experimental group

increased after the experiment. It has been suggested that the development of health literacy, guidelines and appropriate activities can develop and promote health literacy (WHO, 2009). Good health literacy will improve health behaviors (Ginggaew & Prasertsri, 2016). Likewise, Rattanawarang & Chanta (2018) reported a study that health literacy is related to self-care behavior in chronic disease and the testing program showed the mean scores on nutrition literacy increased significantly after testing of the experimental group (p-value < 0.001). Our study findings were supported by several previous studies (Thepin, 2019; Boontanon et al., 2019; Visscher et al., 2018). An appropriate activity for the group will improve health literacy (WHO, 2009). Methods and guidelines for development in organizing the learning process led to learner memorization learning (Sheridan et al., 2011; Visscher et al., 2018; Kaewdamkeeng, 2018). The results of the mean score before and after the experiment of food consumption behavior and self-management behavior on food intake of the experimental group showed a significantly higher score than before the test (p-value < 0.001). The results could be explained by knowledgeable individuals, having a variety of information which helps them in making decisions about behavior improvement (Pongkiatchai & Wongwiseskul, 2018). Moreover, Santo et al. (2005) stated that the development of health literacy, especially health education followed by group or individual practices, will help promote decision-making skills on health behaviors and good self-management. Prior studies have found that health literacy is associated with positive self-management and health behaviors (Ginggaew & Prasertsri, 2016; Wang et al., 2017; Ratanawarang & Chantha, 2018). The study by Jayasinghe et al., (2016) found that low health literacy had a significant effect on health behaviors among Australian patients (p-value < 0.001). Therefore, in this study the effects of the health literacy development program on the older adults for self-management in food consumption influenced the experimental group to gain more nutrition literacy, consumption behavior and self-management behavior. The experimental group's mean scores were significantly different from the control group's (p-value < 0.001). Our findings suggested that health literacy and health education, including activities to promote knowledge, can empower individuals to gain self-management in terms of health and the ability to make decisions about their health more appropriately.

Interestingly, the evaluation of reflections toward the activities among experimental group indicated that perspectives of older adults on nutritional health literacy appeared to be associated with understanding of the information, information accessibility, the ability to transform the information to daily life practice. Likewise, older adults reflected the emphasized strategies to augment health literacy helped them with self-management on nutritional habits to improve their health. These strategies included education, communication, sharing experiences and infographic or illustration. Moreover, the ability to read and write are important for older adults to gain and understand the information on basic health literacy, especially in health education and cognitive appraisal on health information such as the predominated risk, medical information and nutrition labels (Nutbeam, 2000; Sorensen, et al., 2012; Pongkiatchai & Wongwiseskul, 2018). Health information is typically available and older adults could seek the information resources. However, the complexities of the different data structures had an influence on the decision to evaluate and apply information (Harzheim et al., 2020). World Health Organization (WHO) implied that readability, transformation skills and an insightful understanding are the principal factors for enhancing health literacy and improving an effective communication among individuals (WHO, 2009). Besides, information accessibility is an interactive health literacy (Nutbeam, 2000; Sorensen et al., 2012; Pongkiatchai & Wongwiseskul, 2018). It is the ability to find a strategy to promote self-management by seeking and classifying information across the different sources. Ghaffari-Fam et al. (2020) studied health information and blood pressure control. They found that the accessibility to seek health information could predict blood pressure control among Iranian hypertensive clients (p-value < 0.05). In sum, the availability, accessibility of the information and communication are crucial to generate nutritional health literacy.

The ability to apply knowledge skills are critical to the health literacy level (Nutbeam, 2000; Sorensen et al., 2012; Pongkiatchai & Wongwiseskul, 2018). The older adult's ability to interpret data, analyzing and decision-making lead to decision-makings in self-management. The results of the activity assessment showed that knowledge and understanding of the older adult helped decision-making and ability to apply information. This study findings are in keeping with the results of Boontanon et al., (2019). That reported the

older adult with good health literacy led to nutrition decision making and behavioral change. Besides, a study by Ghaffari-Fam et al. (2020) found that people with healthy literacy were able to control high blood pressure.

When discussing the promotion of health literacy for self-management in food consumption, the older adult noted that educating, communicating, sharing experiences and using infographic illustration could improve health literacy. This suggestion is in line with the Kwanmuang's study (Kaewdamkereng, 2019) which reported that the strategies of promoting health literacy are varied based on the health literacy level such as the functional health literacy level, the strategies of promoting health literacy, the learning process development and the educational tool selection. Moreover, the interactive health literacy level, such as the questioning skills and creating a friendly atmosphere for learning and the critical health literacy level, such as the method of promoting health literacy, the training in decision-making skills, identifying problems, searching for information to help evaluate and decide, self-assessment and self-management are also included. According to the health education study results from Santo et al. (2005), that mentioned the development of health literacy, particularly health promotion as well as individual and group practice will enhance the decision-making skills toward health behaviors and self-management. Allen et al. (2017) stated that health lifestyle will be improved by the individual or group health promotion as well as launching group activities, sharing experiences and discussing topics related to communication channel where the information is dependable and of high-quality. The communication in this research is intrapersonal communication or self-talk, following mindfulness in the manner of Buddhist psychology which was used amongst the athletes. Tod et al. (2011) suggested that positive self-talk can motivate self-empowerment. It also helps the athletes to be more concentrated during competitions. The findings are supported by the psychologist as self-talk helps people to consolidate positive thoughts, feelings, emotions in stressful situations. (Kross et al., 2014). It is similar to the Buddhism (constant mindfulness) as it induces precepts, meditation and reduce stress. For the instructional tools, our results are consistent with the study of Kaewdamkereng (2019) in that the process of promoting health literacy, is essential to choose appropriate and standard instructional medias. To our knowledge, there are several instructional tools available, however these are used in many ways based on the type of study objectives and participants. In this current study, we employed image and explanation for the older adult, as this type of tool allows the older adult to ask questions as well as helps the researcher to clarify any points that the older adult may not fully understood. There was an agreement between researchers and participants on using images as a teaching media as well.

This study has some limitations including measured food consumption behavior and self-management behavior for only at a short period. Secondly, this study did not follow blood pressure control, which is an important clinical outcome. Therefore, it could not be assured whether this program was effective in blood pressure control. The potential effectiveness of health literacy program and health outcomes would be a relevant topic for future research with an extended period of study.

Conclusion

In conclusion, a health literacy development program for self-management on food consumption of the older adult can increase the level of nutrition literacy, dietary behavior, and self-management. Besides, the method to enhance health literacy for self-management of food consumption is educating, communicating, sharing experiences, and use of the Fotonovela technique. It could develop health literacy in the older adult as well. Therefore, we recommended that it would be suitable to implement at primary public health center to improve health literacy among older adults. They could also use self-talk technique and sharing learning to improve decision making and applying skills.

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Preparation, Characterization and Evaluation of Octyl Methoxycinnamate (OMC)-Loaded Solid Lipid Nanoparticles (SLNs) by Using a Microemulsion Technique

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Abstract

This research prepared the solid lipid nanoparticles (SLNs) based on microemulsion to load octyl methoxycinnamate (OMC) as a UV absorber for instability protection. The optimal microemulsion formulation was selected by visual assessment according to a transparent and thermodynamically system form. The optimal microemulsion obtained from the experiment composed of 10% w/w glyceryl monostearate (GMS, solid lipid), 10% w/w Tween 80 (surfactant), 15% w/w PEG-40 hydrogenated castor oil (surfactant) and 20% w/w ethanol (co-surfactant). Various processing parameters for the preparation of SLN was carried out using three factors including, 1) warm microemulsion (mL) and iced water ratio (mL), 2) homogenization speed (rpm) and 3) homogenization time (min) and two responses assessed were particle size and polydispersity index (PDI) to obtain a SLNs batch with smaller particle size and optimum PDI. The OMC-loaded SLN had 693.07± 0.05 nm mean particle size and 0.56±0.04 PDI, prepared by optimal processing parameters. The surface morphology of the SLNs was assessed by transmission electron microscopy (TEM), the result found that OMC-loaded SLN showed a smooth surface and spherical shape. Entrapment efficiency of OMC-loaded SLN was found to be 99.89±0.020% . The stability testing of the prepared SLN was studied. Result found that OMC-loaded SLN showed slightly lower stable compared with the initial condition. All obtained results indicated that this method and processing parameters can prepare the SLNs which was suitable for cosmetic application.

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Introduction

UV light is divided into UVA (320-400 nm), which can penetrate to reach the dermis, causing damage of the skin such as immediate and delayed tanning reactions, loss of collagen and skin photosensitization, UVB (280-320 nm) in the cause of sunburn (erythema) and skin cancer and UVC (100-280 nm), which is totally absorbed by the ozone layer (Gromkowska-Kępka et al., 2021). Thus, UV protection is very important to avoid harmful effects to the skin. The parameter for evaluation of the UV protection degree is the sun protection factor (SPF) rating. The substances with SPF have been widely used as a photoprotective agents which are known as sunscreens (Young et al., 2017).

Sunscreens have been divided into chemical absorbers and physical blockers on the basis of their mechanism of action, namely absorbance and reflection. Octyl methoxycinnamate (OMC) or 2-ethylhexyl 4-methoxycinnamate is currently the most popular chemical sunscreen, with good UVB absorption, solubility in oils and insolubility in water, so that it is suitable for use in most waterproof sunscreen formulations. Although this compound can be easily formulated in the lipid phase of cosmetic products, the concentrations of use as a cosmetic ingredient recommended by US and European Union (EU) was 7.5-10% because exposure of OMC to UV radiation generates reactive chemical species including free radicals which can give rise to adverse skin effects as well as to limit the UV-protected molecules to penetrate to the skin layer especially viable layer and presence on stratum corneum which are the two most important properties (Butt & Christensen, 2001; Andreani et al., 2020). That is why OMC is a good candidate for loading into nanoparticles by using a novel carrier since it can both reduce the adverse effects of sunlight and enhance UV protection (Xia et al., 2007).

Nowadays, the nanocarrier systems represent a mild way in order to enhance the penetration degree and increase the performance of a cosmetic product. Solid lipid nanoparticle (SLNs) is a novel drug delivery system for cosmetics and dermatologic formulations (Sastri et al., 2020). SLNs systems present some advantages including, improved stability of a chemical molecule which act as an active ingredient. It is able to provide a carrier system with controlled release and shows occlusive properties which created a film on the skin (Farboud et al., 2011). The use of SLNs as a carrier system for UV absorbers has been introduced because it showed that lipid nanoparticles present a high potential to inhibit the UV radiation and act as a physical UV sunscreen by scattering or reflection of light, thus able to improve the sun protection effect (Andreani et al., 2020). Chemical sunscreens incorporated into the solid lipid matrix of the SLNs can prevent penetration of the skin and resulting side effects (Andréo-Filho et al., 2018).

Therefore, this investigation is focused on the preparation of SLNs using a microemulsion. The microemulsion method is simple, contains no toxic organic solvents, reproducible and can be scaled up. For large-scale production, the microemulsion can be prepared in a large temperature-controlled tank and pumped into another tank containing cold water for lipid precipitation (Gasco, 1993). In order to optimize microemulsion for OMC-loaded SLN preparation, different formulation variables; types of solid lipid, quantity of selected solid lipid and type and concentration of surfactants were evaluated. The concept of this method is the optimal microemulsion dispersed in a cold water under homogenizer mixing and time for mixing. SLNs with reduced mean particle size and narrow size distribution can be obtained after dilution in cool water of the hot microemulsion (Gasco, 1993; Gasco, 1997). For this research, the optimization of processing parameters for the SLNs preparation was investigated. The smaller particle size and optimum polydispersity index (PDI) which were two responses from each SLN batch and were assessed for selection of the optimal parameter. This is the first study that examined the various parameters (type of solid lipid, quantity of lipid, type and concentration of surfactant and co-surfactant concentrations) for the preparation of the first oil in water microemulsion to select microemulsion formulations to prepare solid lipid nanoparticle evaluated by visual appearance in terms of a transparent and thermodynamically system form. Further, we studied the various processing parameters for the preparation using three factors; 1) warm microemulsion (mL) and iced water ratio (mL), 2) homogenization speed (rpm) and 3) homogenization time (min) and two responses assessed were particle size and polydispersity index (PDI) to obtain a SLNs batch with smaller particle size and optimum PDI. The characterization of OMC-loaded SLN; particle size, polydispersity index (PDI), entrapment efficiency and morphology were studied. The physicochemical stability of OMC-loaded SLN was evaluated.

Materials and methods

1. Raw materials

Glyceryl monostearate (GMS), Tween 80, PEG-40 hydrogenated castor oil, Palmitic acid, Stearic acid were purchased from Namsiang (Thailand). Ethanol (AR grade) was purchased from Labscan (Thailand). Octyl methoxycinnamate was purchased from Chemico group (Thailand).

2. Preparation of oil in water microemulsion components

The microemulsions are transparent, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. Preparing the first hot microemulsion, the solid lipid was melted as oil phase, and the water phase was prepared by a mixture of water, surfactant and co-surfactant(s) and heated at the same temperature as the solid lipid. The water phase was then added under mild stirring to the oil phase. After that, a transparent, thermodynamically stable system was formed since the component were mixed in the correct ratio. Finally, obtained SLNs was made by dilution of the hot microemulsion in cold water under constant stirring. The volume ratios of the hot microemulsion to cold water were in the range of 1:25 to 1:50 (Gasco, 1997). In this study, the selection of the optimal microemulsion formula, the parameters; solid lipids, surfactants and co-surfactants were examined for SLN preparation.

2.1 Types of solid lipid

Three solid lipids; stearic acid, palmitic acid and glyceryl monosterate (GMS) were used for formation of microemulsion. Solid lipids were melted at 60-70°C. The concentration of each solid lipid was 10% w/w for microemulsion preparation. The solid lipid was selected from a transparent form of microemulsion (Ramteke et al., 2012; Ratcharin et al., 2012).

2.2 Type and concentration of surfactant

This, two surfactants; Tween 80 and PEG-40 hydrogenated castor oil were used for microemulsion preparation. The concentration of surfactant was optimized; Tween 80 (10% w/w, 15% w/w and 20% w/w) and PEG-40 hydrogenated castor oil (10% w/w, 15% w/w and 20% w/w). The concentration of the surfactant was selected based on the transparent of the microemulsion form after 24 h storage (Ramteke et al., 2012; Ratcharin et al., 2012).

2.3 Co-surfactant concentrations

The concentration of ethanol used for the microemulsion formation were 10% w/w, 15% w/w and 20% w/w. The suitable of ethanol concentration was performed based on the transparent of the microemulsion form after 24 h (Ramteke et al., 2012; Ratcharin et al., 2012).

3. Optimization processing parameters for OMC-loaded SLN

The SLN for loading the OMC (10% w/w) was prepared by using microemulsion technique. The optimal microemulsion was then dispersed into cold water (2-4°C) under homogenizer mixing by using a high-speed homogenizer (Silverson L5M, England). Optimized processing parameters for the SLNs preparation was carried out using three factors; (i) microemulsion (mL) and iced water ratio (mL), (ii) homogenization speed (rpm) and (iii) homogenization time (min) and two responses assessed were particle size and polydispersity index (PDI) to obtain a SLN batch with lesser particle size and optimum PDI (Ratcharin et al., 2012).

(i) different ratio of microemulsion (mL) and iced water (mL) were 1:20, 1:25, 1:30 and 1:35,

(ii) homogenizer speeds were performed at 4000, 6000, 8000 and 10000 rpm,

(iii) homogenization time were done for 5, 10, 15 and 20 min.

4. Characterization of SLN

4.1 Particle size and size distribution

The mean particle size and size distribution of SLNs were assessed by dynamic light scattering (DLS) method using Zetasizer (Malvern, ZEN 3600, England).

4.2 Morphology

The surface morphology of SLN was characterized with scanning electron microscope (SEM) (Hitachi, S-3400N, Japan). The air-dried SLN were then coated with conducting materials using gold sputter and visualized under SEM. The morphology of SLN were observed under transmission electron microscopy (TEM) (Jeol, JEM-2100, Japan). SLNs were diluted with water and placed on a carbon-coated copper grid and the excess water was wiped off with filter paper. Then, 20 µL of 2% w/v uranyl acetate in water was placed on SLN and wiped off by another filter paper. The grid was dried at room temperature and assessed by TEM.

4.3 Entrapment efficiency (EE)

The entrapment efficiency (% EE) was determined by measuring the concentration of entrapped OMC. Briefly, 2.0 g of OMC-loaded SLN was dispersed in ethanol 1.0 mL and then placed into a centrifuge tube which was centrifuged at 8000 rpm for 10 min at 25°C (Gemmy, PLC-05, Taiwan) (Prombutara et al., 2012).

The supernatant was analyzed for encapsulated OMC at 348 nm using a UV-Vis spectrophotometer (Shimadzu, UV-2401PC, Japan). Then, the percent of entrapment efficiency in SLN was calculated according to the following equations:

$$\text{Entrapment efficiency (\% EE)} = (W_{\text{intial drug}} - W_{\text{free drug}} / W_{\text{intial drug}}) \times 100 \quad [1]$$

where $W_{\text{initial drug}}$ is the amount of OMC, $W_{\text{free drug}}$ is the amount of free OMC detected in the aqueous phase after isolation of the dispersion

5. Storage stability of SLNs

The stability of OMC-loaded SLN were evaluated at different stability conditions including room temperature (RT, ≈30±5°C), 4°C and 40°C for 30 d. Evaluating parameters included any change in physical appearance, particle size, PDI and entrapment efficiency (% EE) that were assessed compared with initial condition.

6. Statistical analysis

Data were reported as mean ± SD values of three different experiments. Statistical comparisons were analyzed by a one-way analysis of variance (ANOVA) using Microsoft Excel 2010. $P<0.05$ was considered statistically significant.

Results and discussion

1. Oil in water microemulsion preparation

As shown in Table 1, formulations with different solid lipids (stearic acid, GMS, palmitic acid) and a fixed amount of surfactant and co-surfactant. The result indicated that GMS gave a transparent microemulsion whereas stearic acid and palmitic acid showed slightly turbid microemulsion. Furthermore, we examined the effect of GMS at 5, 10 and 15% w/w on microemulsion form. The result clearly showed that GMS at 10% w/w gave a transparent microemulsion, whereas at concentration lower than 10% w/w could not formed a microemulsion and at concentration higher than 10% w/w could not be achieved due to highly viscous lipid phase (Yingngam et al., 2007). This investigation was in agreement with the results obtained by previously reported which found that GMS increased the solubility of drugs, therefore the concentration of 10% w/w of GMS was chosen for the preparation of solid lipid nanoparticles and concentration of GMS was not more than at 10% w/w showed a good SLNs (Mulla et al., 2009; Shah et al., 2009). The effect of type and concentrations of surfactant on the formation of microemulsion were investigated using 10, 15 and 20% w/w of Tween 80 and 10, 15 and 20% w/w of PEG-40 hydrogenated castor oil. This study was in good agreement with results previously reported which found that the amount of surfactant used to prepare o/w microemulsions should be between 8 and 30% w/v however, in this range, a concentration of 12–20% was the best (Gasco, 1997). From our preliminary study it was found that the mixture of nonionic surfactants, Tween 80 and PEG-40 hydrogenated castor oil, could give a transparent microemulsion which are more stability than single surfactant (data not shown), so that, in this research we examined the effect of surfactants mixtures on the formation of microemulsion. The results showed that Tween 80 at all concentration gave a transparent microemulsion, whereas PEG-40 hydrogenated castor oil at concentration of 15 and 20% w/w showed a transparent microemulsion. The mixture of surfactants can reduce surface tension and facilitate the particle partition. This result obtained from the experiment was similar to previous reports (Olbrich, & Muller, 1999). Thus, the lower concentration of Tween 80 and PEG-40 hydrogenated castor oil were chosen to use as a mixture surfactant in microemulsion formulation. The hydrophilic co-surfactant of the microemulsion could distribute very rapidly into the aqueous phase and have a critical role in the formation of lipid nanoparticles (Caboi et al., 2005). Ethanol was chosen as co-surfactant in this study.

Table 1 Various formulation parameters for the preparation of the first oil in water microemulsion

Formulations	Variables	Quantity (% w/w)	Visual appearance of O/W microemulsion
Type of solid lipid			
F1	Stearic acid	10	Slightly turbid
F2	GMS	10	Transparent
F3	Palmitic acid	10	Slightly turbid
Quantity of solid lipid			
F4	GMS	5	Slightly turbid
F5	GMS	10	Transparent
F6	GMS	15	Turbid
Type and concentration of surfactant			
F7	Tween 80	10	Transparent
F8	Tween 80	15	Transparent
F9	Tween 80	20	Transparent
F10	PEG-40 hydrogenated castor oil	10	Slightly turbid
F11	PEG-40 hydrogenated castor oil	15	Transparent
F12	PEG-40 hydrogenated castor oil	20	Transparent
Co-surfactant concentrations			
F13	Ethanol	10	Turbid
F14	Ethanol	15	Slightly turbid
F15	Ethanol	20	Transparent

Obtained result found that ethanol at a concentration of 20% w/w gave a transparent solution. This finding was in agreement with the previous work (Caboi et al., 2005).

According to Table 1, the optimal microemulsion composed of 10% w/w glyceryl monostearate (solid lipid), 10% w/w Tween 80 (surfactant), 15% w/w PEG-40 hydrogenated castor oil (surfactant) and 20% w/w ethanol (co-surfactant). The result was transparent and thermodynamically stable and this optimal formulation is visually represented in Fig 1. This optimal microemulsion was used for the preparation of SLNs for loading OMC.

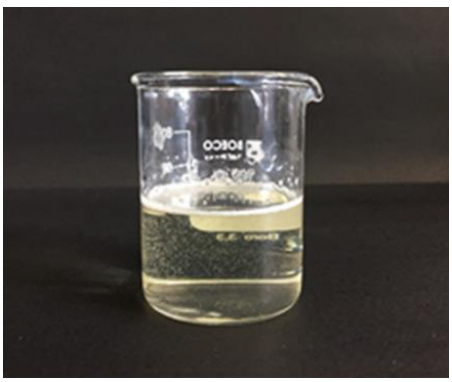

Fig. 1 Visual appearance of the optimal microemulsion (oil in water, O/W) formulation

2. Optimization processing parameters for OMC-loaded SLN

The results of various process variables for preparation SLNs are shown in Table 2. According to Table 2, we investigated the ratio of warm microemulsion (mL) and iced water (mL) using 1:20, 1:25, 1:30 and 1:35 which was modified from previous research (Ratcharin et al., 2012) and nowadays, few research have reported on this design. The size of the ratio of warm microemulsion (mL) and iced water (mL) using 1:25 significantly ($p<0.05$) decreased a mean particle size of 840.90±4.20 nm with the polydispersity index of 0.66±0.05 compared with each formulation in the same variables. Afterward, the homogenizer speeds (rpm) were examined using 4000, 6000, 8000 and 10000 rpm which was modified from previous research (Ratcharin et al., 2012). The homogenizing speed and time for mixing at 8000 rpm and 15 min, respectively, could significantly ($p<0.05$) reduce the particle size which was 219.77±1.82 nm with the polydispersity index of 0.51±0.04 compared with each formulation in the same variables. This might be due to the amount of surfactant and co-surfactants affect to SLNs particle size. Additionally, the use of proper homogenizing speed can reduce the free energy at the interfacial surface between the internal and external phase, thus making the particles smaller than lower speed.

Table 2 Various process variables for preparation solid lipid nanoparticles (SLNs)

Formulations	Variables	Particle diameter (nm)	Polydispersity index (PDI)
Microemulsion (mL):Iced water (mL)			
F16	1:20	1973.00±26.67^a	0.30±0.14^a
F17	1:25	840.90±4.20^b	0.66±0.05^a
F18	1:30	1028.67±24.9^c	0.52±0.07^a
F19	1:35	1239.33±7.37^d	0.39±0.01^a
Homogenizer speeds (rpm)			
F20	4000	840.90±4.20^a	0.66±0.05^a
F21	6000	878.50±9.66^a	0.50±0.04^a
F22	8000	219.77±1.82^b	0.51±0.04^a
F23	10000	333.77±1.89^c	0.54±0.03^a
Homogenization time (min)			
F24	5	414.53±5.50^a	0.64±0.03^a
F25	10	322.17±4.04^b	0.49±0.03^a
F26	15	219.77±1.82^c	0.51±0.04^a
F27	20	233.73±1.87^d	0.49±0.04^a

Remark: Values are given as mean ± S.D of triplicate. The different superscript letter in the same column represents significant differences when compared with each formulation in variables at $p<0.05$

Table 2 presents a summary of the optimum process for the preparation of SLNs that were mixed by glyceryl monostearate (GMS), Tween 80, PEG-40 hydrogenated castor oil and ethanol (10:10:15:20% w/w), respectively, then, the warm microemulsions were dispersed into a cold water at 2 to 4°C at the ratio of 1:25 (warm microemulsion, mL: iced water, mL), after that, the mixture was blended with a high-speed homogenizer at 8000 rpm for 15 min. After freezing, the obtained SLN showed a white fine powder as shown in Fig 2.

Fig. 2 The appearance of SLN loaded OMC

3. The characterization of SLNs

The characterization of prepared SLNs were investigated. Particle mean diameter of SLNs and polydispersity index (PDI) were determined by DLS. Obtained results showed the empty SLNs had the particle size of 219.77±1.82 nm with a minimum polydispersity index (PDI) of 0.51±0.04 (Fig.3A). OMC-loaded SLN was significant ($p<0.05$) resulting in larger particle size than empty SLNs which was 693.07±0.05 nm with a minimum polydispersity index of 0.56±0.04 (Fig.3B). This is possibly due to the presence of OMC inside of the solid lipid core of SLNs. However, mean diameters of OMC-loaded SLN showed particle in nanosize range without aggregation.

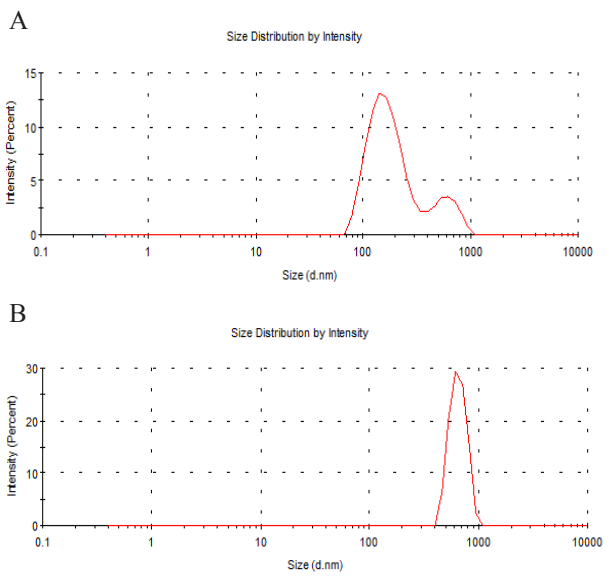

Fig. 3 The particle size and distribution of empty solid lipid nanoparticles (SLNs) (A) and solid lipid nanoparticles (SLNs) loaded octyl methoxycinnamate (10% w/w) (B)

The SEM and TEM analysis of OMC-loaded SLN are shown in Fig. 4. SEM micrograph showed a surface smooth and TEM image seen to be spherical in shape. The particle size as given by SEM and TEM were in line with that found using DLS. The result of entrapment efficiency (% EE) of OMC-loaded SLN was 99.89±0.02%. Obtained result was higher than previously report (Liu et a., 2015; Xu et al., 2021). This might be the sufficiently high solubility of the OMC in the lipid melt. High encapsulation of lipophilic agents is usually recorded for poorly water-soluble in lipid nanoparticles (Andreani et al., 2020).

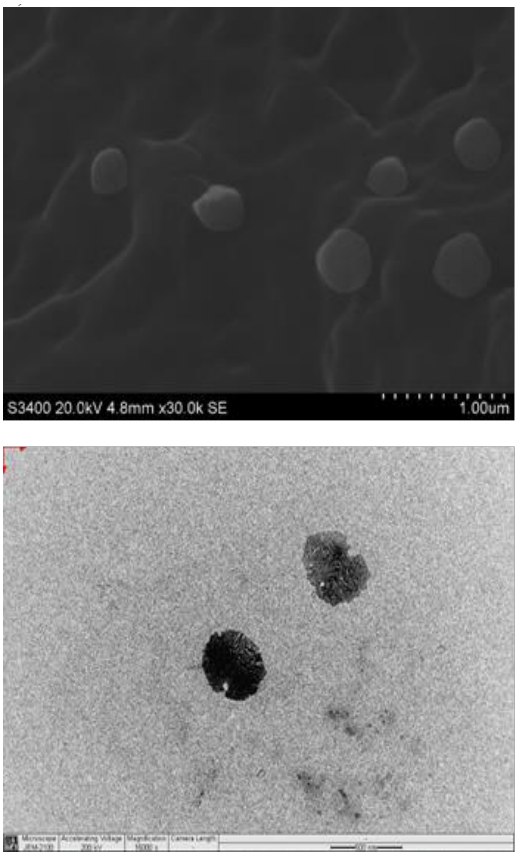

Fig. 4 Images of octyl methoxycinnamate (OMC)-loaded solid lipid nanoparticles (SLNs); scanning electron microscope (SEM) at x30000 magnification (A) and transmission electron microscopy (TEM) at x15000 magnification (B)

4. The physicochemical stability of OMC-loaded SLN

The physicochemical stability of OMC-loaded SLN were determined under three storage conditions; RT, 4°C and 40°C for 30 d. Results showed that the physical appearance of SLN remained as white powder under all conditions of testing. The particle size of OMC-loaded SLN under storage were found to be larger than initial condition ($p<0.05$). The PDI of OMC-loaded SLN under 4°C and 40°C showed no significant differences ($p>0.05$) when compared with the initial condition. Entrapment efficiency (% EE) of OMC-loaded SLN at all conditions tested were found to be statistically significant ($p>0.05$) compared with initial condition as presented in Table 3. This may be attributed to a rearrangement of the solid lipid (GMS) core, leading to changes in particle size and the presence of emulsifier that may lead to drug expulsion from solid lipid nanoparticles (Mulla et al., 2009; Radaic et al., 2014). Therefore, lowered entrapment

efficiency may be due to expulsion during lipid modification.

Table 3 Physicochemical stability of octyl methoxycinnamate (OMC)-loaded solid lipid nanoparticles (SLNs)

Conditions	Particle diameter (nm)	Polydispersity index (PDI)	Entrapment efficiency (% EE)
Initial	693.07±0.05[a]	0.56±0.04[a]	99.89±0.02[a]
Room temperature	821.93±2.87[b]	0.70±0.04[b]	98.07±0.17[b]
4°C	869.60±4.23[c]	0.47±0.05[a]	98.44±0.25[c]
40°C	879.70±6.68[d]	0.65±0.08[a]	98.76±0.22[d]

Remark: Values are given as mean ± S.D of triplicate. The different superscript letter in the same column represents significant differences when compared with initial condition at $p<0.05$

Conclusion

The findings of the study showed that OMC-loaded SLN were prepared successfully by microemulsion method. The preparation of the first oil in water microemulsion were investigated. Optimal microemulsions composed of glyceryl monostearate (GMS) as solid lipid, Tween 80 and PEG-40 hydrogenated castor oil as surfactant and ethanol as co-surfactant which gave the transparent form. The OMC-loaded SLNs prepared by the warm microemulsions were dispersed into a cold water at the ratio of 1:25, mixed with a high-speed homogenizer at 8000 rpm for 15 min, according to various process variables for SLNs investigated. The mean diameter of OMC-loaded SLNs was 693.07±0.05 nm, PDI was 0.51±0.04 and very high entrapment efficiency was achieved at about 99.89% . The TEM and SEM studies confirmed the particle size analysis and showed spherical shape morphology. The OMC-loaded SLN showed a good physicochemical stability during the shelf life. No obvious changes of color, degradation, or phase separation were observed. The mean particle size and PDI of OMC-loaded SLN showed larger than initial condition, however, they showed particle in nanosize and without aggregation. The entrapment efficiency was found to be slightly decreased at the range of 98.07-98.76% from initial condition. A further study is, planned to examine the OMC release and application to sunscreen formulation including a stability study.

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Empowering Preparation for Old Age: An Application of the SDU Health Care Project

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Abstract

This article aims to explore the concepts of empowerment in personal preparation for middle and late adults as well as to describe the application of empowerment within the SDU health project as an empowerment mechanism. Empowerment might play as mediator to enhance an optimal preparation for old age in a completely age society among staffs at Suan Dusit University. The results showed that the empowerment to prepare for old age consists of five aspects: physical, emotional, economic, social and housing and includes the processes of self-assessment, problem identification, problem analysis, planning and implementation, which help to improve individuals' awareness and preparation for old age, leading to active aging in an era of rapid change in which technology has become a part of people's daily lives.

Introduction

This article is written based on data derived from the development of the SDU Health Care Project. This project was conducted for Suan Dusit University adults and elderly staffs. The main purpose of the project was to promote quality of life during a working life which was crucial to become a successful aging. Staffs should be able to augment their empowerment. Therefore, the preparation before retirement implies to reduce physical and mental deveriation and psychosocial problems in the future. The concept of this project was used empowerment theory in preparation for retirement. The objectives of this study were 1) explore the concepts of the empowerment of personnel preparation for middle and late adults and 2) to describe the application of empowerment on SDU health care project.

Across health promotion concepts, empowerment is a concept that researchers applied the framwork into the research studies to understand the process and consequences of efforts to exert control and influence over the decisions that affect one's life, including perceptions of personal control as behaviours to realize control. Empowerment focuses on internal motivation and derive to promote interpersonal relationship. In addition, the transfer of power with the outcome of liberation, emancipation, energy and power-sharing can determine several perspectives, including social and developmental strengths (Shearer et al., 2010).

Situation of the Thai elderly

Thailand is an ageing society with an ageing population accounting for 16.73% of a total population

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of 66,558,953. The population over 60 years of age consists of more females than males and resides mostly in the north-eastern region, followed by the central region (Department of Older Persons, 2019). The number of older people in Thailand has increased rapidly, exceeding the previous forecast of 10.8 million people or 15.2% of the total population in 2020 (Nunsupawat, 2009). Preparing for an ageing society is vital to adults enabling older adults to age actively (Purakom, 2016), live valuable lives and enjoy a good quality of life. The preparation for an ageing population requires both policy and management planning to ensure proper development. To broaden the scope of policy planning, the United Nations (2013) has divided an ageing society into 4 different levels: 1) an ageing society in which the population is getting older, 2) an aged society in which the share of people aged over 60 exceeds 10% of the total population or more than 7% of the entire population is aged 65 and above, 3) a completely over-aged society, in which the proportion of people over 60 exceeds 20% of the total population or more than 14% of the entire population is aged 65 and above and 4) a super-aged society in which the share of people aged over 60 exceeds 28% of the total population or more than 20% of the entire population is aged 65 and above (Purakom, 2016; Thaniwattananon, 2016; Siriphanich, 2018; Chewasopit, 2019). The above information indicates that Thailand has become an aged society in nearly two years (Department of Older Persons, 2020). Therefore, the government policies must be implemented promptly to support the country's forthcoming transition to a completely aged society and individuals should seek knowledge and prepare themselves for active ageing in a complete-aged society.

Many researchers are interested in exploring the enhancement of the preparation for an ageing society. Statistics show that 67 million or 10% of ASEAN's total population of 654 million in 2018 were elderly. Thailand has the second largest share of elderly people in ASEAN next to Singapore. The older population in Thailand accounts for 18% of the entire population, making it an aged society. The country is predicted to become a complete-aged society in 2022 (Siriphanich, 2018). In addition, the ageing process of individuals is induced by internal and external factors (Othaganont, 2011). It is thus necessary to prepare individuals for ageing in a complete-aged society to ensure their good quality of life and active ageing.

Preparing for elderly

As a result of social structural changes induced by economic growth and advances in medical and public health technology, Thailand has transitioned to an ageing society and the process of becoming a completely aged society. The government has realized the importance of social transformation and formulated a master plan under the 20-year national strategy (2018-2037) for driving the development and enhancement of human resources, including a national economic and social development plan to guarantee equality and social protection by developing and preparing citizens for an ageing society and enhancing senior citizens' quality of life and security by protecting and promoting the welfare rights of senior citizens (Phitayanoraseth, 2020). In short, the Thai government is preparing the country for an ageing society.

Self-preparation is also necessary. Individuals should start preparing for retirement while still in working age in order to achieve active ageing with a good quality of life and happiness. The preparation should begin with physical and mental training, active fostering of social contacts and ensuring economic security (Touhy & Jett, 2018; Siripanich, 1995; Chuenwattana, 2012). Evidences have hown that promoting well-being on physical, mental, psychosocial, as well as housing preparation is warranted to enhance active aging. That Physical, mental, economic aspects as well as housing and social preparation should be made for an individual to pursue active ageing, which will allow them to enjoy a good quality of life in old age by being able to perform daily activities with little or no assistance or, in other words, being more independent (Meechana et al., 2017; Bunchai & Wongthanavasu, 2012; Chuenwattana, 2012; Sukchot & Hongwityakorn, 2016; Ratana-Ubol et al., 2009; Pattrapagdekul et al., 2011).

Promoting physical well-being is needed. Ageing causes a decline in physical function, i.e. regarding vision, hearing, muscles, skin, bones, heart, respiratory system, gastrointestinal system, urinary system, immune system and nervous system which can result in memory problems or even dementia. Starting caring for physical health at a young age by practicing the 3 E's (eating healthy food, exercising and emotional management) and having an annual check-up can help maintain physical fitness, enhance functional capacity, prevent illnesses and diseases that hasten physical deterioration, manage stress and anxiety and avert potential accidents and dangers (Cress, 2017; Ratana-Ubol et al., 2009; Chuenwattana,

2012; Bunchai & Wongthanavasu, 2012; Pattrapagdekul et al., 2011; Sukchot & Hongwityakorn, 2016; Meechana et al., 2017). Factors that affect individuals' self-care behavior are marital status, membership of aging clubs, participation in aging clubs' activities and application of self-care manuals for seniors (Silangirn, 2017).

Regarding mental preparation, it can be claimed that older people often have mental health issues caused by the loss of the ability to do things independently, isolation, anxiety over physical decline and dependency which cause emotional changes, moodiness, sleep problems and low self-esteem that can lead to depression. Early mental preparation has an impact on adaptability and readiness for change. Mental health and physical health are connected in other words, good physical health leads to good mental health. Maintaining the aging's mental health requires family support. Family members can help keep seniors mentally healthy by encouraging their self-esteem, doing activities together and choosing words carefully (Cress, 2017; Ratana-Ubol et al., 2009; Chuenwattana, 2012; Bunchai & Wongthanavasu, 2012; Pattrapagdekul et al., 2011; Sukchot & Hongwityakorn, 2016; Meechana et al., 2017). In summary, good physical preparation leads to positive mental health.

Pertaining social preparation, it is crucial that there have been changes in Thai society and family structure such as a shift from extended families to nuclear families. The way people live and communicate has particularly changed. As a result of ageing, people lose their social status and roles, financial stability, friends and ways of living. Social preparation for old age should be done by planning for social participation, utilizing one's knowledge and skills, accepting change, preparing for ageing with a caregiver, engaging in social activities for seniors and creating a safe living environment that allows the elderly to live independently (Cress, 2017; Ratana-Ubol et al., 2009; Chuenwattana, 2012; Bunchai & Wongthanavasu, 2012; Pattrapagdekul et al., 2011; Sukchot & Hongwityakorn, 2016; Meechana et al., 2017).

Economic preparation, the shift from working age to old age results in sudden changes in household economies (Cress, 2017; Ratana-Ubol et al., 2009; Chuenwattana, 2012; Bunchai & Wongthanavasu, 2012; Pattrapagdekul et al., 2011; Sukchot & Hongwityakorn, 2016; Meechana et al., 2017). Therefore, economic preparation, i.e. financial planning, investments and backup career plans, should be made to ensure financial stability in old age. Some studies have also mention a reported significance of housing preparation for seniors.

According to Table 1, the studies showed that most people made physical and economic preparation, followed by social and housing preparation. Adaptation, mental preparation, and knowledge preparation have also been explored in some studies. The studies on preparation for old age aimed to improve the ageing's quality of life and investigated personal factors associated with preparation for ageing, such as age which affects an individual's awareness, status, education, income and

Table 1 Synthesis of studies on preparation for old age

Studies on preparation for old age	Physical preparation	Economic preparation	Mental preparation	Adaptation	Knowledge preparation	Social preparation	Housing preparation
Ratana-Ubol et al., 2009	✓	✓	✓	✓	✓		
Pattrapagdekul et al., 2011	✓	✓	✓			✓	✓
Chuenwattana & Beadnok, 2012	✓	✓	✓			✓	✓
Bunchai & Wongthanavasu, 2012	✓	✓	✓	✓		✓	✓
Junphet, 2015	✓	✓				✓	✓
Sukchot & Hongwityakorn, 2016	✓	✓				✓	
Meechana et al., 2017	✓	✓				✓	✓
Wanitchakham, 2019	✓	✓				✓	✓
Jirojanakul et al., 2019	✓	✓				✓	✓

financial situations (Pattrapagdekul et al., 2011; Yommana, 2008; Thammatawat, 2011). Sukchot & Hongwityakorn (2016) have described happy ageing as one's sense of independence in terms of not being a burden to others, and the ability to spend more time alone, spend time with family and support the community.

In conclusion, preparation for old age is an essential factor to achieve active ageing. Therefore, people should make efforts in the fields of physical, mental, social, economic/financial and housing preparation in order to enhance their future happiness and independent living.

Empowerment

According to the profiles of Suan Dusit University (SDU) staffs SDU is an educational institute, which recruits middle and late adults. The faculty of nursing has a strategy for preparing for healthy ageing among SDU workers. We conducted a pilot study to allocate workers, who were willing to participate in this project and empowerment was used as the main concept to design activities on this project.

Empowerment has been studied and applied in various fields. It has been particularly applied to processes to enhance individuals' awareness and self-confidence in self-management, especially in terms of healthcare, or as a mechanism for helping individuals direct their behavior towards their goals based on different attitudes and beliefs (Freire, 1972; Gibson, 1991; Zimmerman, 2000). Empowerment is the opposite of powerlessness which has negative impacts on the body and mind.

Individual powerlessness is caused by 1) allowing others to make oneself feel powerless; a person feels that they are not able to do anything, 2) allowing oneself to feel powerless; a person believes that they are not capable, so they do what others tell them and refuse to develop themselves and 3) being prevented from developing by systems or others (Anderson, 1986). The empowerment process is thus necessary for enhancing an individual's self-care agency for preparing for ageing. A summary of the empowerment process as a diagram is following (Vajhollah & Naghavi, 2019).

Empowerment is a mechanism for promoting one's self-control, ability to understand and analyze problems (Zimmerman, 2000) and self-preparation for old age. It is an endless learning process (learning spiral) in which one analyzes their experience to understand causes and related factors, leading to planning and implementation for preparing oneself for ageing. Empowerment can be used to enhance an individual's ability to self-assess, leading to solution planning consisting of 5 steps: 1) experiencing, 2) naming experience, 3) analyzing, 4) planning, and 5) doing. These steps will bring about positive changes and help individuals gain self-control and move towards their goals. Therefore, the Faculty of Nursing, Suan Dusit University has employed the "SDU Health Care Project" as an active mechanism for empowering the university personnel's preparation for old age.

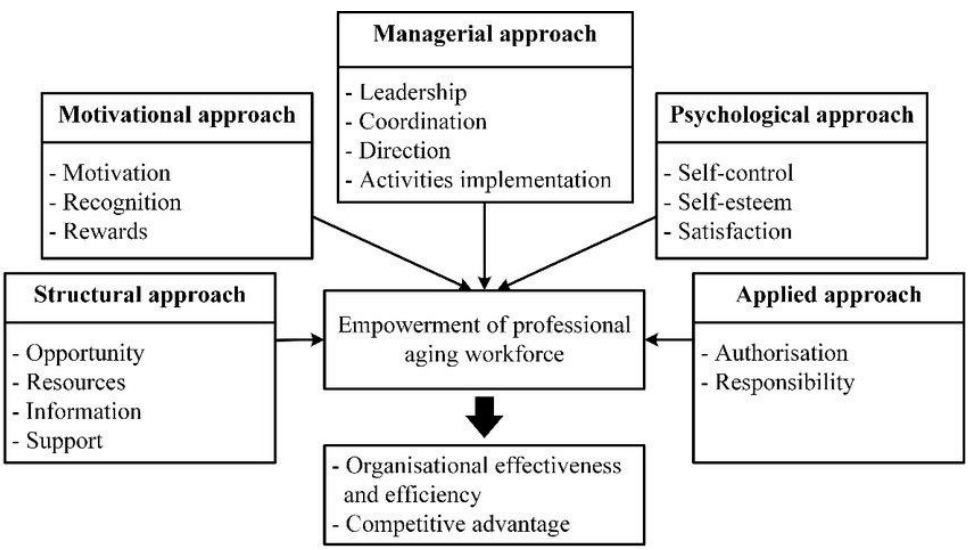

Fig. 1 Conceptual model of empowerment of the professional aging workforce
Source: Vajhollah & Naghavi (2019)

Suan Dusit University's personnel for quality ageing through "SDU health care project"

The Faculty of Nursing, Suan Dusit University has realized the importance of preparation for old age and invited the employees from all departments to participate in the "SDU Health Care Project". This project is applied to foster their understanding, awareness and cooperation in practising self-care for active ageing. The concept of the empowerment process has been applied to the enhancement of the university personnel's awareness of the preparation for quality ageing., focusing on five aspects: physical preparation, mental preparation, economic preparation, social preparation and housing preparation. This project is for the conservation of health and preparation for ageing by bringing knowledge, preparation for being the ageing and the empowerment of personnel. This activity recruited small group of participants. Therefore, the target audience is those who voluntarily participate from Suan Dusit University. The activities consist of basic health screening tests such as taking the history of congenital disease, blood pressure measurement, height, waist circumference and making a diary for participants to record their data. The activities provided knowledge about health care in high-risk diseases for the ageing, including cardiovascular disease, diabetes, eye disease, osteoarthritis, etc. In addition, Manivet Tara therapy, eye exercises are an activity suitable for the elderly. A Line group has been created in order to ensure continuous communication, knowledge sharing and to be used as a tool for monitoring and communicating to stimulate the group continuously. The details of five aspects of preparation are as follows:

1. Physical preparation. The project has organized activities to promote the participants' self-care by teaching them how to take care of their bodies to prevent physical decline e.g. caused by infections, cardiovascular diseases, degenerative bone and joint diseases (especially knee osteoarthritis), diabetes, dementia and visual degenerations and providing participants with knowledge and training in self-care exercises such as knee exercises, eye exercises, brain exercises for preventing and improving dementia symptoms, Maneevej exercises, yoga and aquatic therapy. It has also conducted activities to promote healthy eating behaviors among participants, encourage participants to keep a health journal, help check participants' blood pressure and advise participants on annual check-ups.

2. Mental preparation. The project has organized activities to foster participants' self-esteem and motivate them to build their own self-esteem, encourage teamwork among participants, teach participants how to balance their emotions and feel positive and happy on a daily basis and how exercise can help improve their physical appearances and moods, help participants manage stress and embrace change and reality through meditation and promote participants' positive thinking by encouraging them to look on the bright side.

3. Economic preparation. The project has organized activities to teach participants how to save and make money in retirement and prepare for retirement expenses, including emergency funds and expenses for life, health and accident insurance. It has also organized group activities for participants to analyze their present financial situations (income, sources of income and expenses), learn about financial management and planning, investment and backup career planning for pre-and post-retirement and practice financial planning in a given situation, i.e. shopping at the market.

4. Social preparation. The project has organized activities to provide knowledge about teamwork, encourage participants to exchange their knowledge and experience, promote the use of modern technology and set up social networks for sharing self-care knowledge to enhance participants' self-care knowledge and ability and self-esteem through community activities, social clubs, and meetings.

5. Housing preparation. This project derives supportive activities related to housing environmental supports such as house repairs and renovations, environmental household safety and functional activity promotion as well as physical ability and activities of daily living enhancing. These activities can reduce risk of the household accidents and motivate older adults to perform their physical performances.

Conclusion

Based on a systematic review, the preparation for the aging focused on physical, psychological, economic, social and environmental aspects. Moreover, we applied empowerment concepts into the activities to motivate individuals. Suan Dusit University's target audience consists of employees ranging from working age to the elderly. Thus, the Faculty of Nursing has promoted personnel to prepare for the aging among adult age by using comprehensive activities to get ready for healthy

aging and enhance empowerment of staffs. The outcomes showed that the "SDU Health Care Project" has been used as a mechanism for empowering and encouraging the university personnel to start preparing for ageing by encouraging them to prepare how to promote physical and mental wellness as well as economical sustainability. We expected that the participants will be able to take care of their physical and mental health, monitor their eating and exercise habits constantly, prepare mentally for old age by seeking advice from their close seniors, etc., create a savings plan and spend money carefully, find a backup job to cover retirement expenses and participate in community activities, to ensure that they achieve active ageing and have a good quality of life in old age so that they will not become a burden on their families or society in the future. Moreover, there are several fruitful suggestions toward this project: 1) empowering personnel's preparation for old age to enhance a level of self awareness in preparing for old age. Young workers need to emphasize their health at the beginning of their employment; 2) enhancing organizational strength which apply a strategic health plan to approach an empowering personnel's preparation for old age. Also, this project may apply the empower concept to focus on promoting health in workplace for successful aging in a gradually disruptive society.

Acknowledgment

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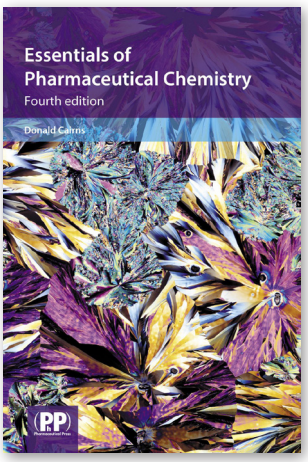
Book Review

Christoph Sontag



Book name: Essentials of Pharmaceutical Chemistry 4th edition
Author: Donald Cairns
Publisher: Pharmaceutical Press, London
Published: 2012
Language: English
Paperback: 295 pages
ISBN: 978 0 85369 979 8

This textbook is an excellent compendium of the various topics concerning chemical, physical and biological properties of organic molecules relevant for pharmaceutical applications. A basic understanding of chemistry on a high-school level would be necessary to profit from the text. It is a helpful tool to study the most relevant chemical and physio-chemical aspects of drug molecules as well as to serve as a great reference book.

Typical chemical properties explained include acid/base behavior, stereochemistry and stability towards hydrolysis and oxidation and the kinetics behind. Physiochemical aspects discussed are partition coefficients/absorption, chromatographic isolation methods and several case studies for typical drug molecules. Two chapters deal with analytical methods: volumetric/titration methods and spectroscopic analysis. Biological aspects of drug molecules described are metabolism and enzyme/receptor-drug interactions. The book concludes with drug licensing, development and actual medical issues in today's society. A minor limitation of the text is the exclusive focus on organic molecules; interesting developments in inorganic/metalorganic drug chemistry are omitted. Nevertheless, those topics could be found in more specialized literature. The covered topics allow the reader to get a good fundamental understanding how drug molecules are developed, characterized, isolated and how they actually work.

The layout of each chapter is consistently simple and clean. Each chapter can be studied independently, using various examples and ending with case studies and problems to solve. This enhances the learning process significantly. Theoretical aspects for each topic are briefly explained like atomic structure, chemical kinetics, nomenclature, etc. without going into distracting details (which are covered by numerous general chemistry textbooks). A drawback in the layout is the limited use of pictorial illustrations, which would have made the reading less tedious.

This compendium may not be only interesting for pharmaceutical students but also for learners of medicine and bio- or environmental chemistry. It is also a great reference book for professionals in pharmacy, biology and medicine. For all these groups this textbook is highly recommended.

Reviewer

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Guidelines for Writing and Submitting Original Manuscripts for Publication in Journal of Food Health and Bioenvironmental Science

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4. Articles that are deemed appropriate for publication are subjected to peer review by a panel of three experts in the appropriate field. In order to be deemed appropriate for publication, an article must be by recommended two of the three experts via the double-blinded review system.
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.
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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)
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3. The manuscript's content is consistent with the aim and scope of the journal.

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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.

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

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- 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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Original Articles

Review article

Book Review

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