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Content

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Synergistic and Counter Effect of Biocides, Amines and Emulsifier in the Combinatorial Toxicity Study

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Abstract

Pollution is one of the major environmental issues that affect human beings. Metalworking fluids are widely used in many industries. There are many chemical components such as amines and biocides in the metalworking fluids, which cannot be biologically treated, and disposal is still a problem. Often chemicals are tested for toxicity individually, however, there are interactions between combinations of chemicals. Hence, in this research, chemicals that are commonly used in metalworking fluids, are tested in combination as part of a factorial experimental design. Three types of commercially available biocides (A14, AEF, AOX - coded due to commercial rights), two amines (Monoethanolamide - MEA, Triethylolamine - TEA), and an emulsifier (blinded because of commercial rights) were tested. A bacterial biosensor *E.coli* HB101 was used to assess toxicity. A total of 63 tests were carried out. It was found that the toxic responses do not align with predictions based on the sum of the responses to individual compounds. Instead, there are interactions that cause synergistic or counter effects. For example, biocides A14 and AEF were found to be lethally toxic; biocide AOX and MEA were found to be slightly toxic. The combination of MEA, AEF, A14 was found to be the most toxic of the 63 possible combinations. However, when AOX was added, the toxicity level decreased in indication that toxicity was mitigated. This study shows that understanding the combinatorial toxicity could help to inform eco-design and promote sustainable biological treatment at the end of product life.

Introduction

Environmental issues such as air pollution, water scarcity, and waste management are a global problem. This has many impacts on lives today such as physical and mental health, resource degradation such as water and soil. The main source of those problems is human activity, such as polluting the environment, with a wide variety of chemicals including biocides. Biocides are widely employed in sectors such as agriculture, the food

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industry, manufacturing. It is used for anti-microbial applications in many industries to control the growth of microorganisms (Health and Safety Executive, 2012). They are used as antiseptics to treat infections in mucous membranes and damaged skin (Russell, 2003; Hussain et al., 2020). They are also used to control microbial contamination (Jones & Joshi, 2021) in industrial-scale applications. Because of the diversity of applications, biocides can contaminate the environment on a wide scale and result in damaged ecosystems since biocides are toxic to both microorganisms and nontarget species (Massei et al., 2018; Malhotra et al., 2021).

Current and safety regulations, require manufacturers to provide a material safety data sheet (MSDS) for each individual chemical. Chemicals are not tested in combination. Hence, a key issue is that there have been a few studies of the combined additive influence of two or more biocides in the same product, and where it has been studied the impact has been over-estimated (Liess et al., 2020). Predicting the combined effect of toxicant mixtures with distinct modes of action has yielded mixed results (Belden et al., 2007). A vast number of studies have suggested that the combined impacts of toxicants might reveal much higher effects than those expected by the Effect Addition (EA) or Concentration Addition (CA) approaches. Synergistic effects are the term for such unanticipated outcomes (Ralf & Schäfer, 2016). The counter effect is when the effect is canceled out and has a lower expected outcome. Therefore, the interaction between chemicals and the environment may lead to a synergistic or counter effect (Singh et al., 2017). For example, two or more benign compounds may result in a highly toxic reaction when comes together. Conversely, two or more toxic compounds could be benign to the environment. This approach can be applied as pollutant prevention for many industries.

Metalworking fluids are widely used in many industries for grinding, milling, drilling, and metal cutting (BP-Castrol Limited, 2012; Cheng et al., 2005; Gauthier, 2003). Metalworking fluids are composed of water, amine, emulsifier, and biocide (Canadian Centre for Occupational Health & Safety, 2005; Cheng et al., 2005; Byers, 1994; McCoy, 1994). Water sources and atmosphere deposited microorganisms cause contamination, which deterioration of metalworking fluids is caused by microbial action, this lowers the quality of metalworking fluids performance and necessitates premature disposal. Carbon, nitrogen, sulfur, and organic compounds produced from waste are nutrient-rich for the bacteria (*Escherichia coli*) to grow (Willing, 2001). Biocide or antimicrobial agent was introduced in the mid-20th century to prevent bacterial, viruses, yeasts, fungi, and protozoa growth and prolong life in use (Cheng et al., 2005). This work focuses on the bacterial growth because they are the dominant taxa in the metalworking fluids.

Fig. 1 a). Metalworking fluids employed in drilling process, b). in grinding process
Source: Health and Safety Executive (2015); Northwest Aerospace Alliance (2013)

Biocides are an important component to prevent bacterial growth in MWFs and prolong product life in the machine. However, the biocide is toxic to the environment and an eco-design approach could be implemented to understand the reaction of the chemicals both individually and in combinations. This information then applies to the product development to prevent or prepare the solution for the foreseen challenges at the design stage. Eco-design can reduce the amount of waste, dangerous chemical uses, waste disposal, and resource costs (Uapipatanakul, 2020). This research is aimed to explore the interactive toxicity effect of the biocides in different combinations. Whereby the three types of

biocides, two amines, and an emulsifier, which are the common components in the metalworking fluids were tested for combinatorial toxicity effect (Table 1).

Eco toxicity is described as the department of toxicology concerned with the consideration of poisonous impacts, caused by natural or manufactured toxins, to the constituents of biological systems, creature (counting human), vegetable and microbial, in an intrinsic context (Truhaut, 1977). Subsequently, it is critical to comprehend the concentration of chemicals which influence the environment and ecosystem recognizing that if one life form is influenced, other life forms within the web may also be impacted (Koeman, 1998; Bardgett, 2005; Procter & Gamble, 2005; Cole et al., 2006). Well-established bioassay strategies were utilized for this work. The bacterial bioassay approach used in this work is rapid reproducible, cost effective, and appropriate to a wide extent of toxins (Koskella & Stotzky, 1997; Tiensing et al., 2002).

Microscopic organisms in aqueous solutions react quickly to changes in natural conditions in their normal habitats due to their large surface to volume ratios. Whole cell bacterial biosensors, such as *E.coli* HB101 and *E.coli* dH5α strain can be utilized as a surrogate for higher living beings in harmfulness testing (Madigan et al., 2000; White et al., 1998; Kelly & Harwell, 1989). Biosensors report the aggregate impact of blended chemicals and are valuable in measuring the poisonous quality of dissolved chemicals at environmentally relevant concentrations. To perform as a biosensor the *E.coli* was transformed with a plasmid containing bacterial bioluminescence (*lux*) qualities taken from *Vibrio fischerii.* The *lux* operon was adjusted by deletion of the regulatory I and R genes so that CDABE conferring constitutive light output. The light response is directly related to intracellular adenosine triphosphate levels, the cells' medium of exchange. Lower vitality levels are in this way reflected by diminished light yield (Fig. 2). The light yield is measured in 96 well in a micro-titer plate spectrophotometer allowing high throughput screening.

Eco-design and sustainability are of interest to many researchers and industries. For instance, in northwest Europe, manufactured base stock MWF now substitute mineral oils with vegetable oils, thus reducing environmental impact and sustainability (Glenn & van Antwerpen, 1998). Understanding the relevant chemical interactions and optimizing them for in-use shelf life and natural treatment advancement. Producers plan most

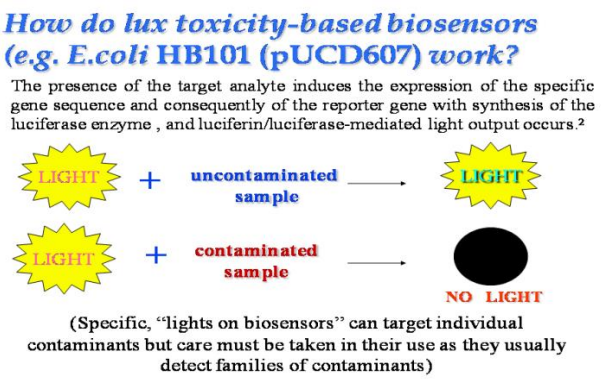

Fig. 2 Illustration of how lux based biosensors work
Source: Mwinyihija (2011)

items without considering disposal at their end-of-life, which may be a boundary to accomplishing a closed circle economy. Many technologies require high carbon footprint treatment strategies, such as chemical and physical treatment because natural treatment isn't conceivable.

Organic treatment is more energy and resource intensive than current innovations. Additionally, at the conclusion of treatment, materials are potentially reusable and recyclable (Uapipatanakul, 2020). Much waste requires high carbon footprint treatment methods, such as chemical and physical treatment because biological treatment is not available. Hence, understanding the interaction can help in considering disposal and recycling at end-of-life of products, which will challenge biological treatment development and promote the closed-loop economy (Uapipatanakul, 2020).

The overall aim of this research is to understand the toxicity of bacterial cells of individual compounds and combination mixtures employing biosensor *E.coli* HB101. The thinking was that by assembling metalworking fluids formulations based on the prior knowledge of the relative toxicity, degradability, and recalcitrance of individual components and when in combination, puts the industry in an improved position to be able to predict the durability and sustainability of end-of-life bio-treatment. Single compound responses were used to predict the fate of compounds when in combination. Combinations that were more or less toxic or biodegradable than predicted were studied in further detail. Such detailed analysis identified the pinch-point chemical components, which determine susceptibility to biodegradation and resistance to bio-deterioration.

Information can be exploited for the possible selection of biocide use in the formulation that will act as a bio-softening agent at the end-of-life biological treatment.

Materials and methods

This experiment examined combinations of common chemical components that are used in the formulation of metalworking fluids. Formulation with the choices of commonly used biocides, amines, and emulsifiers (Uapipatanakul, 2015; Azimi et al., 2017; Byers, 2006; Samuel et al., 2011). Metalworking fluid was selected as a model product and representative of many commercial products which are formulated to contain mixtures of chemical components including biocides. There are six generic chemical compounds used in formulating metalworking fluids which were studied in this experiment. These consisted of two amines (corrosion inhibitors), one emulsifier, and 3 biocides (antimicrobial agents) (Table 1).

Table 1 Chemical compound abbreviation

Type of chemical components	Code use in this research	Function
Isothiazolinone based biocide	A14	Antimicrobial
Oxazolidine biocide	AOX	Antimicrobial
Fungicide	AEF	Antimicrobial
Emulsifier	E	Emulsifier
Methanolamine	MEA	Surfactant/Emulsifier/Corrosion inhibitor
Triethanolamine	TEA	Surfactant/Emulsifier/Corrosion inhibitor

1. Preparation of biosensor

E. coli HB101, which contained *lux* genes of the plasmid vector pUCD607 (Microbial Solution Co.Ltd., Oxford, United Kingdom), was used as the biosensor in this study. The miniprep technique was used to extract plasmid pUCD607. Plasmid confirmation was done by gel electrophoresis. This strain is highly competent in terms of its ability to take up extracellular DNA. The cells energy status is reflected by the light emitted by the biosensor. The dose-dependent toxicity/light output relationship is exploited in this work as a measure of the aggregate toxicity of biocide solutions.

2. Determination of mid-exponential phase

The biosensor *E. coli* HB101 was grown to mid-exponential phase in the growth media; LB Broth (growth media) was prepared by autoclaving a mixture of 10 g/L Tryptone (OXOID), 5 g/L yeast extract (Fisher Scientific), 5 g/L sodium chloride (Fisher Scientific), 1 g/L glucose in deionised water. Ampicillin stock (25 µg/L) was also added after the solution had cooled down to prevent thermal degradation of the antibiotic. *E.coli* HB101 biosensor was inoculated into 5mL sterile LB Broth and kept overnight in a shaking incubator Innova 44, (New Brunswick Scientific, UK). at 35ºC and 120 rpm After 16 hours, the inoculum was transferred into 100 mL sterile LB Broth. Optical density (OD) and luminescence were recorded every 30 minutes using a UV spectrophotometer(UV-1800, Shimadzu, Japan) and a micro-titre plate spectrophotometer (Synergy HT, BioTek, USA). All processes were performed under aseptic conditions. Mid-exponential phase is when the membrane is at the most sensitive phase of growth, therefore, it is optimal phase to observe the effects that samples have on the cells. To identify the mid-exponential phase, typical growth and luminescence growth curves were produced. Growth and luminescence curves were obtained by incubating *E. coli* biosensor in the growth media and plot readings against time. The experiments were carried out in triplicate.

3. Toxicity testing

Toxicity was assayed using a bioluminescent bacterial sensor, which is an analytical detector of biological responses expressed as luminescence. Commercially available biocides (A14, AEF, AOX), amines (MEA, TEA), and emulsifier (E) (See Table 1) were prepared at 1.0% v/v at room temperature. With the factorial design, a total of 63 possible combinations (Equation 1, Table 2) were tested in different number of combinations from one to six as shown in Table 2.

Calculation for number of combinations

$$\binom{n}{k} = \frac{n!}{k!\,(n-k)!} = \frac{n(n-1)\ldots(n-k+1)}{1\cdot 2\ldots k}$$

n = total number of combinations; k = number of selected combinations
Source: Kreyszig (2011)

Table 2 Number of possible combinations in each complex level

No. of compounds in the combination	No. of possible combinations
1	6
2	15
3	20
4	15
5	6
6	1
Total	**63**

180 µL of test sample solutions were dispensed in a 96 well micro-titre plate (Nunc brand). *E. coli* HB101 biosensor was inoculated (20 µL) in the test samples and exposed for 15 minutes before luminescence (relative light units) were measured and recorded using a micro-titre plate spectrophotometer (Synergy HT, BioTek, USA). The chemical compounds were tested individually and in combinations employing a factorial design approach; started from two combinations and increased progressively in complexity to six combinations in 96-well microtiter plate format. This experiment was carried out at room temperature. All processes were performed under aseptic conditions. All experiments were carried out in triplicates.

Combinatorial studies were carried out to identify component interactions that produce bop "hardening" or "softening" effects, which could facilitate extended product life or effective bio-treatment, respectively. To be specific, a "hardening agent" or constituent in this respect is one, which when present on its own or in combination with another chemical constituent inhibits or slows down biodegradation activity and thus contributes to extending the life of the chemical mixtures. In contrast, a "softening agent" is defined here as one whose presence to is making the total combination more susceptible to premature biodegradation/bio-deterioration.

Results and discussion

From Fig. 3 and Fig. 4, it can be seen that the optimal time to conduct the experiment was 8 hours after inoculation, when *E. coli* HB101 biosensor was at the mid-exponential phase and was producing light.

In this toxicity testing employing an *E. coli* HB101 biosensor, deionized water was used as a control with the percentage luminescence of 100. Six samples were tested for toxicity individually and it was found that biocide A14 was the most toxic (lethal) and AOF, AEF, E, TEA, and MEA, respectively.

The experiment then further tested for toxicity

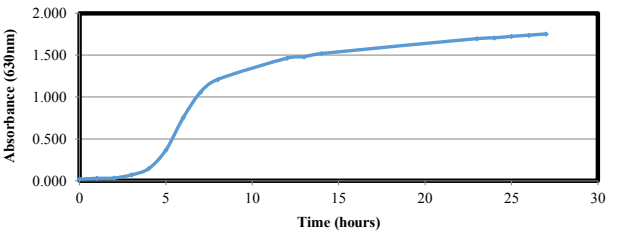

Fig. 3 Growth curve of *E.coli* HB101 biosensor

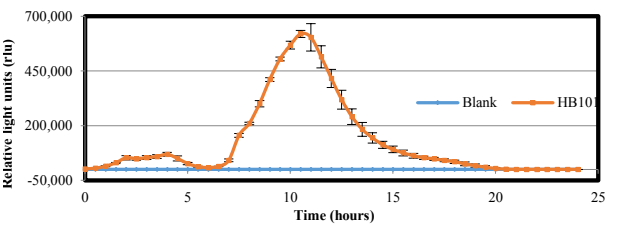

Fig. 4 Luminescence curve of *E.coli* HB101 biosensor

in 63 combinations. Fig. 5 shows four factorial data, which is 56 possible combinations. Overall results suggests that 48% of combinations were very toxic. Interestingly, it can be seen that a combination of Methanolamine (MEA), A14 biocide, and AEF biocide was the most toxic in terms of light emitted by biosensor *E. coli* HB101 (left end of the graph), but once AOX was added into the mixture; MEA, AOX, A14, and AEF, the combination was found to be in top 20% most benign. This indicated that AOX has counter toxicity effect to this combinatorial interaction, *i.e* when present with the other two components the aggregate toxicity is decreased relative to single compound toxicities.

The experiment was designed to identify these unexpected interactions using a combinatorial approach with a small panel of biocides. It aims to inform the exploitation of their combined effects to create awareness of synergistic and counter effect of the chemical release to the environment. Furthermore, it can be used to formulate future products, such as metalworking fluids, either for making them more recalcitrant or biodegradable. An understanding of the effects of interactions between specific compound combinations on bacterial growth provided the basis for designing a new generation of metalworking fluids with more predictable performance in terms of interaction of microbial cells. This includes resistance to biodeterioration of in-use fluids and biological treatment at end-of-life.

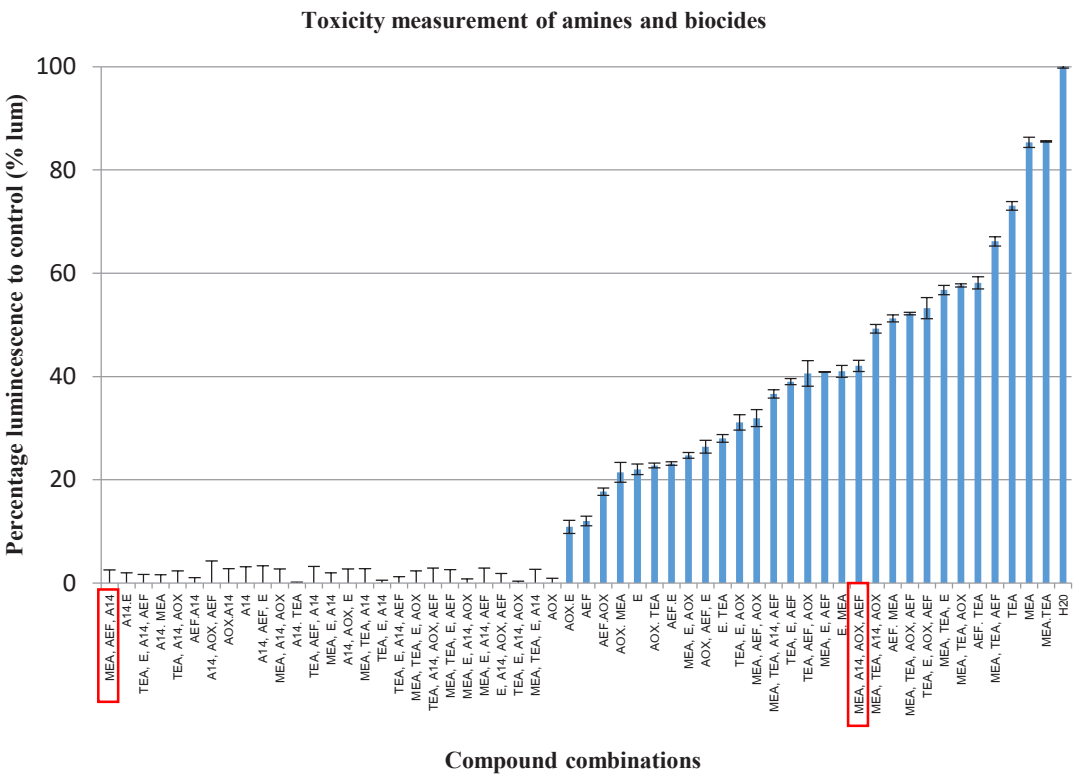

Fig. 5 Toxicity assessments of chemical combinations of increasing complexity (2, 3, and 4 compounds) composed of the following components: Methanolamine (MEA), Triethanolamine (TEA), Emulsifier (E), A14 biocide, AEF biocide, and AOX biocide. Reduce light output relative to the control is interpreted as a toxic response. Red boxes highlight one example where an additional biocide, AOX, was added to a toxic mixture resulting in a non-toxic response from the biosensor

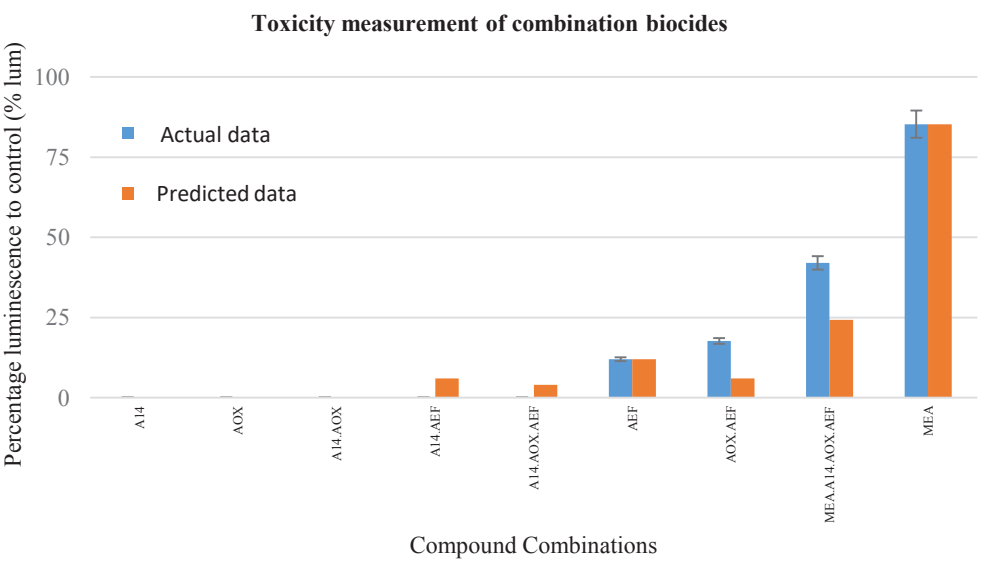

Fig.6 Toxicity measurement of full factorial combination mixture of MEA, A14, AOX, AEF employing biosensor *E. coli* HB101. Bars show the standard error for triplicate samples

From Fig. 6, it can be seen that biocide A14 and AOX are both toxic from the individual toxicity test. As predicted the combination of A14 and AOX is still toxic. However, there are cases where it does not align with the prediction. For example, biocide A14 is toxic from the individual toxicity test, while biocide AEF is mildly toxic from the individual toxicity test. When biocide A14 and AEF were combined, the toxic response to the combination was found to have a synergic effect, while the actual result is lower than the prediction. On the other hands, for combination MEA, A14, AOX, AOF, the toxic response to the combination was found to have a counter effect, where the actual result is less toxic than expected.

Toxic responses to combinations of biocides revealed that two or more toxic compounds sometime mitigated the toxic response from the biosensor to the individual compounds, such that the combination was much less toxic than when present as the single compound. Thus, in some cases observed and predicted toxicities from single compounds responses diverged for mixtures (Fig.5 and Fig 6). There is a considerable body of research that examines the toxicity and effectiveness of biocides. Usually, bacteria are killed in less than 12 hours in metal cutting fluids containing biocides. Although, biocides have been demonstrated to inhibit microbial growth in the metalworking fluids (Chazal, 1995) at lower concentration biocides have been shown to sustain growth of bacteria such as *Pseudomonas putida* and *Psuedomonas fluoresens* (BASF Agricultural Solutions – Global Website, 2022).

Conclusion

Metalworking fluids need to be designed to be safe to untimely biodegradable, arranged to draw out their working life (Cheng et al., 2005) and disposal, respectively. To attain this metalworking fluids are defined to be poisonous to microbial cells when in use. To date, the design of metalworking fluids has been directed, ease of generation, and usefulness with no thought of end-of-life treatment. This unavoidably leads to product formulations that are recalcitrant to biological treatment at the end of the product life. Consequently, until 2006, depleted metalworking fluids were disposed to landfill after energy intensive treatment by ultra-filtration or vacuum evaporation. However, chemical and physical technologies are accessible, and are widely used despite being costly in terms of running and capital costs (Chipasa, 2011; Thompson & van der Gast, 2010: Tchnobanoglous et al., 2004). Also, they create residual sludge that require incineration. Therefore, the biological solution is attractive as it is able to scale up from laboratory to business scale, and additionally water may be recycled on-site (Jiang et al., 2012; Luostarinen et al., 2009). However, the modern MWF formulations make this difficult considering they may be formulated to be proof against microbial deterioration. In order to embody those contradictory needs, it is crucial to reformulate metalworking fluids in order that they may be proof against biodeterioration while in use however they are made, preferably via way of means of a few easy chemical manipulations wherein the chemical interplay has counterintuitive responses and transfer from poisonous to benign property.

Current legislation (EU Directive 67/548/EEC) requires that toxicity and environmental assessment to be based on the single compounds and material safety data sheet. "Unless there is evidence to the contrary, authorities generally enforce regulations that assume that acceptable concentrations for pollutants can be treated independently, even when they are present in mixtures" (Walker et al., 1996; Walker et al., 1998; Strachan et al., 2001). This emphasizes the importance of individual compound and factorial toxicity screening. Furthermore, a key point raised in ecotoxicological legislation is that the particular trophic level of the organisms is of great importance, as certain pollutants interact specifically with organisms of a particular trophic level. This is not the case in a mixed system such as environmental systems. (Strachan et al., 2001). As the results in this research demonstrated, interactions between components in mixtures mean that responses to complex formulations do not necessarily correlate with the single compound assays and so an accurate picture of toxicity is not gained. Hence, the toxicology of compound mixtures is not predictive and often counter intuitive when based on single compound responses.

This work employed a factorial design to show that interactions between chemical compounds commonly found in industrial scale use can increase or decrease depending on the concentrations of other toxic components in mixtures. This knowledge can be exploited to identify combinations of effective biocides and can also be used to inform product disposal development at the end of the product life. Further work is needed to assess the toxicity across trophic levels using a broad panel of biosensors which includes

eukaryotic cells and higher trophic levels. This should be accompanied by a detailed study of the underlying chemistry to identify key component interactions which could be exploited as switches for manipulating product life and subsequent sustainable disposal. This pre-emptive design approach would allow an end-of-life product to be softened and avoid releasing pollution to the environment.

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Optimization of Roasting Temperatures on Acrylamide and Melanoidins Contents and Antioxidant Properties of Roasted Broken-Rice Infusion

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Abstract

The price of broken rice is quite low. A method to add value to this raw material of broken rice is to develop broken rice as a new product such as herbal infusion. Herbal infusion is currently the trend of functional foods and experiencing growth in the food market. Roasted broken-rice infusion (RBRI) is a source of large amounts of maillard reaction products (MRPs) relative to strong antioxidant properties. In this study, we aimed to study the effect of various roasting conditions, i.e. 100, 150, and 200°C for 20 min on the melanoidins, acrylamide (as a carcinogen), copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity of RBRI as well as the study of relationship among their parameters. The higher roasting temperatures affected the darker color in RBRI. Moreover, the highest levels of melanoidins, copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity of RBRI were obtained from the roasting rice condition at 200°C for 20 min; whereas, the estimated acrylamide content was a moderate level (120 μg kg^{-1}). Obviously, for the relationship analysis, the L*, a*, b*, melanoidins, and acrylamide presented in RBRI could be indices for the antioxidant activities. Therefore, this roasting condition was suitable for RBRI development with the high levels of melanoidins and antioxidant activities.

Introduction

Tea is a popular beverage that is widely consumed around the world, particularly in Asian and European countries (Grigg, 2002). Tea drinking has increased around the world as reported by the International Tea Committee (Dufrene, 2012). Prior research has indicated that tea drinking reduces the risks of cancer, cardiovascular disease, hyperlipidemia, diabetes, and obesity (Tanabe et al., 2008). Tea contains certain properties that avoid

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the risks such as the polyphenolic compounds (catechins and epicatechins), theaflavins, flavonol glycosides, L-theanine, caffeine, theobromine, and volatile organic substances. Hence, tea consumption promotes human health (Khan & Mukhtar, 2013).

Generally, the price of broken rice is quite low about 8-10 bath kg^{-1} (Kwak, 2010). To add value of broken rice, it should be developed as a new product such as herbal infusion. Tea is usually prepared from the cured or fresh leaves of *Camellia sinensis*. The herbal infusion prepared from cereals and/or their by-products is more interesting due to the current popular trend and previous research that has suggested their strong antioxidant properties.

Echavarria et al. (2013) suggested that the antioxidant activities were obtained from the acrylamide formation from asparagine-fructose (ASN-FRU) and/or asparagine-glucose (ASN-GLC) systems, which were found in the roasted rice products. Since the compositions of broken rice include not only histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine, and phenylalanine but also starch (Bekedam et al. 2008). For example, Kwak (2010) noted that the brown rice tea prepared from roasting temperature at 170°C for 10 min increased the highest amounts of γ-aminobutyric acid (GABA) content and Maillard reaction products (MRPs) (high antioxidant properties); whereas, the estimated reducing sugars, total soluble solid and total polyphenol depended on the roasting temperatures and times (Tian et al., 2020).

MRPs are usually produced in many kinds of foods during thermal processing via the reducing sugars interacting with available amino acids. This has an effect on the important food properties such as color, flavor, and stability (Tehrani et al., 2002). The Maillard reaction affects not only flavor and color, but also positive human health (Tamanna & Mahmood, 2015).

Melanoidins are one of the MRPs, which are present in general carbohydrate-protein diets and are of interest due to improving the various health promotion activities, e.g. antioxidant, antimicrobial, anti-inflammatory, and antihypertensive activities (Iriondo-dehond et al., 2019).The MRPs are classified into two main groups as follows: (1) the low molecular weight compounds (MW < 1000); (2) the macromolecules, also known as melanoidins (Tehrani et al., 2002). They are found in various kinds of food (e.g. coffee, polished rice, cocoa, bread, malt, and honey), and have a high molecular weight of up to 300 kDa and have more complex molecular structures (Gniechwitz et al., 2008). Besides, acrylamide (one of the MRPs) or acrylic acid amide is a chemical contaminant that is formed during the technological processes of baking, frying, and grilling for certain foods at temperatures > 120°C with low humidity conditions (Soares et al., 2015). It is a contaminant and categorized as a potential carcinogenic (class 2A) by the International Agency of Research on Cancer (IARC) (Soares et al., 2015). The latest regulation from the European Commission (EU) 2017/2158 regulates the presence of acrylamide content in foods (Iriondo-dehond et al., 2019). Although no real legal limit is defined, this regulation introduced benchmark levels with the aim to invite the food industries to implement strategies for reducing acrylamide levels in foods (Tian et al., 2020). Moreover, to assist in the control of acrylamide levels, Food Drink Europe has developed a toolbox indicating possible strategies applicable at different stages of processing of different food categories (Pedreschi et al., 2014). Lasekan and Abbas (2010) further reported that the amount of acrylamide in the food products were as follows: ~450 μg kg^{-1} for roasted coffee;~900 μg kg^{-1} for instant coffee; 272-570 μg kg^{-1} for fried potatoes; 75-1,044 μg kg-1 for bakery products; ≤149 μg kg^{-1} for breakfast cereals; ≤121 μg kg^{-1} for dried food products (Pedreschi et al., 2014).

Therefore, the aim of this research was to estimate how different roasting conditions such as 100, 150, and 200°C for 20 min will affect the acrylamide and melanoidins contents and antixoidant activities in roasted broken-rice infusion (RBRI). Their antioxidant properties were determined by the differences among *in vitro* methods such as copper chelating activity, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), copper chelating activity, and ABTS radical scavenging activity.

Materials and methods

1. Chemicals

Carrez I, Carrez II, acrylamide, $^{13}C_3$-acrylamide, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), $FeCl_3.6H_2O$, and copper(II) sulfate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2. Preparation of roasted broken-rice infusion (RBRI)

Broken rice (*Oryza sativa* L.) was obtained from a local market in Chainat, Thailand. The seeds of broken Thai jasmine rice were separated into 4 treatments and 300 g for each teatment. The seeds were roasted by an hot air oven using the roasting temperatures as follows: 100, 150, and 200°C for 20 min except the control. Then cooled at ambient temperature (32±2°C), the roasted broken-rice infusion (RBRI) samples were obtained and kept in PET/Al/LLDPE (10x15 cm) bags at ambient temperature (32±2°C) prior to the analysis.

3. Color analysis

The sample's color was evaluated with a colorimeter (Minolta CR-300, Minolta Co Ltd., Japan). The colorimeter was calibrated using a standard white plate. Minolta L indicates brightness, (0 = black, 100 = white), a redness (+value = red, -value = green), and b yellowness (+value = yellow, -value = blue). Five readings indicated the surface of the samples for color measurement. An average of five readings for L*, a*, and b* values was reported.

4. Melanoidins assay

Prior to the determination of melanoidins in the RBRI samples, a standard calibration curve of melanoidins was measured at the wavelength at 420 nm (Del Castillo et al., 2002). Because the molecular structure of melanoidins has never been determined yet, hence a melanoidin standard is not available. Therefore, the standard calibration curve was generated using the RBRI extract as a source of melanoidins. A stock solution was obtained from a ratio of the RBRI extract to distilled water, 2:1. This stock solution was diluted sequentially 5 times. For every dilution, the content of melanoidins in each dilution was evaluated using the Lambert-Beer modified formula:

$$C = \frac{A}{ba}$$

Where C = melanoidins content
A = the absorbance of extract solution
b = length of the spectrophotomer's cell (cm)
a = the specific extraction coefficient ($L\ g^{-1}cm^{-1}$)

The value for "a" was 1.1289 $L\ g^{-1}\ cm^{-1}$. The standard calibration curve was generated by the absorbance values as a function of the melanoidins content. For each sample, a 1:9 dilution was measured. Melanoidins were spectrophotometrically evaluated at 420 nm. The melanoidins content was expressed as 100 g^{-1} sample.

5. Acrylamide assay

Acrylamide was measured using a slightly modified method of Rufián-Henares et al. (2007). 450 mg of the RBRI samples was transferred to 5 mL milli-Q water. Afterwards, 100 µL of 10 mg L^{-1} [$^{13}C_3$]-labelled acrylamide methanol solution was loaded into the sample suspension and the mixture was vortexed for 1 min. Then, 750 µL Carrez I and 750 mL Carrez II were loaded into the mixture and were mixed using a vortex for 10 s. Then the mixture was left at 35°C for 10 min and was then centrifuged at 4°C for 15 min at 2400 *g*. The supernatants were individually separated into the microtubes and were frozen. Prior to acrylamide analysis, the supernatants were thawed and centrifuged at 10000 *g* at room temperature for 10 min. 1 mL of the supernatants was clarified on a pre-conditioned Oasis HLB cartridge (Waters, Milford, USA). The first seven drops were removed out and the rest was transferred into the glass vials for further LC-MS analysis. LC-MS was performed on an Agilent 1100 liquid chromatograph coupled to an Agilent Quadrupole MS detector (Agilent Technologies, Palo Alto, USA). The separation was carried out on an Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm; GLC Sciences, Tokyo, Japan) at 32°C with isocratic elution. The injection volume was 60 µL, the mobile phase at a ratio of water to formic acid, 99.8 to 0.2 and a flow rate was 0.6 mL min^{-1}. The results were obtained from a selected ion monitoring mode (SIM). Acrylamide and $^{13}C_3$-acrylamide were monitored at *m/z* 72.1 and 75.1, respectively. The calibration curve of acrylamide was carried out by the external standard solutions of acrylamide ranged between 1 µg L^{-1} and 100 µg L^{-1}. The results were expressed as µg kg^{-1} sample.

6. Preparation of RBRI extract for antioxidant activities

The sample extraction was performed using a slightly modified method of Budryn et al. (2009). The RBRI samples were extracted at a ratio of RBRI to distilled water, 1 : 100. Then, the mixture was homogenized by a sonicator (Branson sonicator 1510, NIST, UK) for 5 min and then centrifuged at 7900 *g* (IEC L31 Thermo electron) for 15 min. The supernatant were later filtered through Whatman No. 4 filter paper. The extraction was repeated twice using the method described above. Total supernatatants were then evaporated at 40°C to dryness. The RBRI extract powders were kept at -20 °C prior to

the analysis of antioxidant activities.

7. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay was applied to evaluate DPPH free radical scavenging activity of the RBRI extract powders, using a slightly modified method of Kraboun et al. (2018). 100 µL of 0.02% DPPH solution in methanol was transferred into a 96-well plate containing 50 µL of the RBRI extract at 4 mg mL^{-1}. Then incubation for 30 min in the dark, the absorbance at 510 nm was determined using a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA). The results were calculated as compared with the standard curve of ascorbic acid and expressed as an ascorbic acid equivalent value (mg AA mL^{-1}).

8. ABTS radical scavenging activity

The ability of the RBRI extract powders to scavenge ABTS radical cation was measured using the ABTS assay by a slightly modified method of Re et al. (1999). Briefly, ABTS solution contained the equal quantities of 7 mM ABTS stock solution and 2.6 mM potassium persulfate and incubation for 16 h at room temperature in the dark. Afterwards, ABTS solution was diluted to get an absorbance of 0.700±0.025 at 734 nm using a spectrophotometer. 5 µL of the RBRI extract at 4 mg mL^{-1} was transferred to react with 295 µL of ABTS solution for 6 min in the dark. The results were done at 734 nm using a microplate spectrophotometer (Epoch, BioTek) and converted to Trolox equivalents (TE) values as compared with the standard curve of Trolox. The standard curve of Trolox was plotted from 0 mM to 1 mM. Results are presented in mg $TEmL^{-1}$.

9. Ferric reducing antioxidant power (FRAP)

The FRAP was evaluated by reduction of Fe^{III+} to Fe^{II+} (Benzie & Strain, 1996). The stock solutions were separately prepared as follows: (1) 300 mM acetate buffer (pH 3.6); (2) 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl; (3) 20 mM $FeCl_3 \bullet 6H_2O$ solution. The working solution was incubated at 37°C prior to use, which was composed of 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \bullet 6H_2O$ solution. 10 µL of the RBRI extract at 4 mg mL^{-1} was transfered to react with 100 µL of FRAP solution for 4 min in the dark. The colored product, ferrous tripyridyltriazine complex, was measued at 593 nm using a microplate spectrophotometer. Ferrous sulfate was used as a positive control.

10. Copper chelating activity

The copper (II) ion chelating activity was measured using a slightly modified method of Megías et al. (2009). 30 µL of the RBRI extract at 4 mg mL^{-1}, 290 µL of 50 mM sodium acetate buffer (pH 6.0), 10 µL of copper sulfate (2 mg mL^{-1}), and 4 mM PV were transferred into a 96-well plate. The absorbance was evaluated at 632 nm using a microplate spectrophotometer. Copper chelating activity was calculated by the following the equation:

$$\text{Copper chelating activity (\%)} = \frac{(1-\text{Asample})}{\text{Acontrol}} \times 100$$

11. Statistical analysis

The analysis was performed in triplicate. The values are expressed as mean±standard deviation (SD) as shown in the tables of this paper. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. Data were analyzed by one-way ANOVA, followed by Duncan's multiple range tests. Differences were considered significant at $p \leq 0.05$. Pearson's correlation coefficient (r) was appiled to evaluate correlations between the parameters.

Results and discussion

1. Effect of roasting treatments on colors and melanoidins in RBRI

To explain the effect of the various roasting treatments for the preparation of roasted broken-rice infusion (RBRI) on the levels of colors and melanoidins, the levels of colors (L*, a*, and b*) and melanoidins in the RBRI samples are presented in Table 1. The higher roasting temperatures affected darker color in the RBRI samples. The highest L*, a* and b* values of RBRI from 200°C for 20 min were 30.27, 3.52, and 5.28, respectively. Obviously, these color values of RBRI samples increased with melanoidins content as shown in Table 1. The Maillard reaction is a chemical process without enzyme activity leading to a brown color presented in foods, which the factor significantly affecting this reaction is a high temperature (Garza et al., 1999). Hence, the development of darker color in RBRI resulted from the higher roasting temperatures (Fig. 1). Higher temperatures could accelerate the reaction between the reactive compositions presented in broken rice, including the amino acids (histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine and phenylalanine), and the reducing sugars (obtained from the high-temperature hydrolysis of starch) (Bekedam

et al., 2008) to generate the Maillard reaction products (MRPs), i.e. the polymerised proteins and brown pigment melanoidins (Del Castillo et al., 2002). Bekedam et al. (2008) suggested that higher temperatures can be related to the formation of heterocyclic derivatives in carbohydrate-protein materials. This is in agreement with Daramola (2015), who reported that the amount of melanoidins in *C. albidum* pulp through heating process at 30-70°C for 10-30 min ranged from 0.0364 to 3.6580 g 100 g^{-1}. Furthermore, the amount of coffee melanoidins was between 24.74 and 67.61 g 100 g^{-1} (Bekedam et al., 2008), which seemed to be related to higher roasting temperatures. Del Castillo et al. (2002) suggested that available water-soluble melanoidins corresponded with antioxidant and antimicrobial functions.

Table 1 Color characteristics and melanoindins content in RBRI from different roasting conditions between 100°C and 200°C for 20 min

Temperature (°C) and Time (min)	L*	a*	b*	Melanoidins (g 100g^{-1})
Control (without treatment)	80.75+4.50^{c}	0.54+0.02^{a}	0.99+0.14^{a}	n.d.
100°C and 20 min	55.11+3.85^{b}	0.75+0.06^{ab}	2.35+0.22^{b}	1.27+0.03^{a}
150°C and 20 min	45.58+4.66^{b}	1.23+0.11^{b}	3.85+0.14^{c}	5.98+0.13^{b}
200°C and 20 min	30.27+2.85^{a}	3.52+0.41^{c}	5.28+0.44^{d}	10.35+0.45^{c}

Remark: Different letters behind means within a column are significantly different ($p \leq 0.05$)
n.d.: not detected

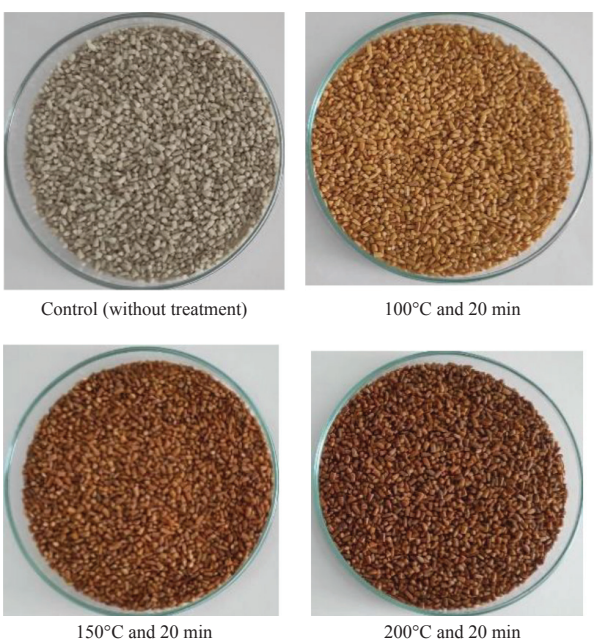

Fig. 1 Appearance of RBRI from different roasting conditions between 100°C and 200°C for 20 min

2. Effect of roasting treatments on acrylamide content in RBRI

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that acrylamide is harmful to human health (Michalak et al., 2017). Acrylamide is generated from the reaction between an α-hydroxycabonyl compound (e.g. the reducing sugars) and asparagine during the high-temperature cooking methods, e.g. frying, roasting, and baking (Gökmen et al., 2006). Acrylamide causes many kinds of cancer in animals when it is consumed at very high concentration (Bent et al., 2012).

As shown in Fig. 2, the acrylamide contents in RBRI ranged from 30 to 120 μg kg^{-1}, which were higher than those previously reported in the literature for rye bread (Fredriksson et al., 2004). The acrylamide content in RBRI prepared from 200°C for 20 min was 4-fold higher than that in the control (without treatment). This indicated that the higher amount of acrylamide in RBRI was dependent on roasting temperature. This may be a result from higher temperatures accelerating the acrylamide formation from the reaction between asparagine and the reducing sugars presented in rice (Claus et al., 2006). This is in agreement with Akkarachaneeyakorn et al. (2010), who reported that the increased acrylamide contents were related to the increased roasting times and temperatures. Lasekan & Abbas (2010) further suggested that the acrylamide formation in the heated carbohydrate-rich foods was 150-4,000 μg kg^{-1}, followed by 5-150 μg kg^{-1} for protein-rich foods (a moderate level of acrylamide), and lastly ≤ 5 μg kg^{-1} for unheated or boiled foods. Hence, the RBRI samples had a moderate level of estimated acrylamide content. Obviously, the formation of acrylamide content in RBRI was related to the melanoidins content (Table 1).

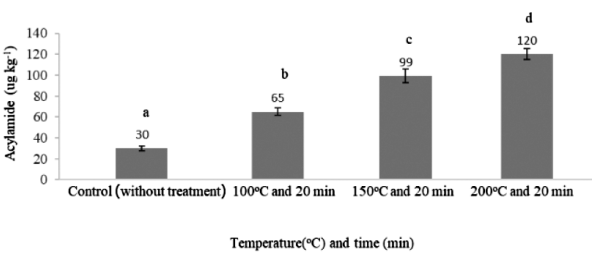

Fig. 2 Acrylamide content in RBRI obtained from roasting at different temperatures between 100°C and 200°C for 20 min

3. Effect of roasting treatments on antioxidant activities in RBRI

The DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, and copper chelating activity in RBRI extracts at 4 mg mL^{-1} increased with the roasting temperatures and are shown in Fig. 3. The DPPH radical scavenging activities, ABTS radical scavenging activities, ferric reducing capacities, and copper chelating activities in RBRI extracts were in the range of 5.12-20.25 mg AA mL^{-1}, 6.5-12.32 mg TE mL^{-1}, 2.14-8.33 mmol Fe^{2+} mL^{-1}, and 14.25-96.65%, respectively. All antioxidant activities in RBRI extracts of RBRI from 200°C for 20 min were more than 2 times higher than those from the control (without treatment) (Fig. 3A-D). This is in agreement with Yilmaz & Toledo (2005), who suggested that the Maillard reaction products (MRPs) in a histidine-glucose system at 120°C for 30 min enhanced peroxyl radical scavenging activity. Del Castillo et al. (2002) noted that higher temperatures of roasting process improved antioxidant capacities presented in a lot of the products, e.g. coffee, wheat germ, polished rice, hazelnuts, and sweet almonds. The antioxidant activities of RBRI were related to the amounts of melanoidins and acrylamide as shown in Table 1 and Fig. 2, respectively.

These resulted antioxidant activities might be obtained from the acrylamide formation from asparagine-fructose (ASN-FRU) and/or asparagine-glucose (ASN-GLC) systems, which were found in the roasted rice products (Echavarria et al., 2013), since the compositions of broken rice include not only histidine, threonine, valine, methionine, lysine, isoleucine, leucine, asparagine and phenylalanine but also starch (Bekedam et al. 2008). Wagner et al. (2002) also demonstrated that melanoidins, as a high molecular weight (HMW) product were mostly responsible for antioxidant activities. Oracz & Zyzelewicz (2019) confirmed that the HMW melanoidins formation during heating process period indicated stronger antioxidant activities. Echavarria et al. (2013) revealed that melanoidins and acrylamide had stronger antioxidant properties via FRAP, ABTS, DPPH, and oxygen radical absorbance capacity (ORAC) comparable to those of commonly used food antioxidants (Rufián-Henares et al., 2007). Furthermore, Rufián-Henares et al. (2007) indicated that the presence of some active compounds (comprising more than one active group OH or NH_2) such as phenolic compounds, quinones, and LMW melanoidins might be bound to the structure of HMW melanoidins through non-covalent bonds and improves their biological properties (Rufián-Henares et al., 2007).

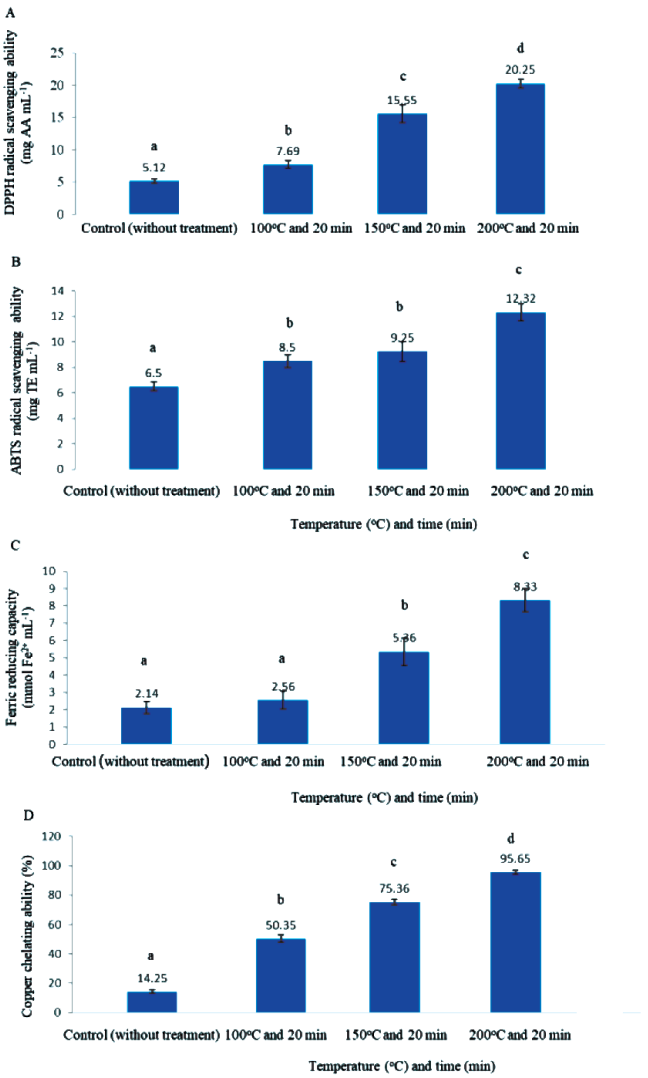

Fig. 3 DPPH radical scavenging activity (A), ABTS radical scavenging activity (B), ferric reducing capacity (C), copper chelating activity (D) of RBRI extracts at 4 mg mL^{-1} of RBRI prepared from 100°C to 200°C for 20 min

4. Relationship between color, melanoidins, acrylamide and antioxidant activities in RBRI

Pearson's correlation coefficients (r) among color, melanoidins, acrylamide, and antioxidant properties in RBRI are shown in Table 2. Positive correlations were found between melanoidins and antioxidant activities, between melanoidins and acrylamide, and between a* or b* and antioxidant activities. Whereas, negative correlations were found between L* and acrylamide, and

Table 2 Pearson's correlation among colors, melanoidins, acrylamide and antioxidant capacities of RBRI

	L*	a*	b*	Melanoidins	Acrylamide	DPPH	ABTS	FRAP	Copper chelating ability
L*	1								
a*	-0.958*	1							
b*	-0.955*	0.875	1						
Melanoidins	-0.878	0.798	0.852	1					
Acrylamide	-0.922*	0.888	0.998**	0.968**	1				
DPPH	-0.879	0.987**	0.774	0.785	0.985**	1			
ABTS	-0.754	0.989**	0.986**	0.928*	0.944*	0.898	1		
FRAP	-0.956*	0.945*	0.985**	0.988**	0.935*	0.899	0.965**	1	
Copper chelating ability	-0.911*	0.985**	0.896	0.978**	0.925*	0.874	0.945*	0.986**	1

Remark: * Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

between L* and FRAP or copper chelating ability (Table 2).

Therefore, L*, a*, b*, and melanoidins parameters could be the useful indicators for estimating the antioxidant activities and/or acrylamide (a carcinogen) in RBRI. This is in agreement with Pontis et al. (2014), who reported that a darker color in honey was shown to be associated with higher antioxidant activities and melanoidins level. Furthermore, Woffenden et al. (2001) reported that the higher antioxidant activities available in darker malts increased with the levels of reductones and melanoidins. In roasted coffee, the free radical scavenging activities occurred in the nonphenolic fraction were dependent on the accumulation of darker MRPs (Sacchetti et al., 2009).

Conclusion

The present study established the DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, copper chelating activity, and color and acrylamide and melanoidins contents in RBRI depending on higher roasting temperatures. The highest DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing capacity, and copper chelating activity obtained from the RBRI prepared from 200°C for 20 min were 20.25 mg AA mL^{-1}, 12.32 mg TE mL^{-1}, 8.33 mmol Fe^{2+} mL^{-1}, and 96.65%, respectively. To obtain the highest antioxidant potential, the suggested ratio of solid and water at 40°C should be 1:100 according to the preparation method of RBRI extract for the analysis of antioxidant activities. However, the acrylamide contents present between 30 and 120 µg kg^{-1} in the RBRI samples were observed and their amount might not be a problem for the consumption due to no real legal limit of acrylamide defined from the EU. In the relationship study, the L*, a*, b*, melanoidins, and acrylamide presented in RBRI could be the indicators for the antioxidant activities.

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Effects of Different Particle Size Distribution and Insoluble Dietary Fiber Content from Pomelo by-Products on the Quality Characteristics of Rice Noodle Products

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Abstract

Rice noodles are a widely consumed food in Asia, including in Thailand. Nevertheless, this popular food is high in carbohydrates, but low in dietary fiber. Pomelo by-products (PBP) are a highly insoluble dietary fiber (IDF) source that has been investigated for fiber-fortified rice noodles with health benefits. This study was conducted to prove the effects of IDF in PBP at different levels (10 and 12.5%) of the rice flour and different particle size distributions (180 and 150 µm) on the moisture content, water activity, cooking weight, cooking loss, textural and sensory evaluation. The addition of IDF-PBP to rice noodles was found to have significant (p<0.05) effects on the colors of a*, b*, while cooking weights were increased in all samples. Cooking loss and tensile strength also decreased as compared to the control. The moisture content and water activity of rice noodles were between 8.66-9.14% and 0.32-0.36, respectively. When considering the addition of IDF-PBP at 10 and 12.5%, the findings revealed that the sample was not significantly different (p>0.05) in the cooking weight. However, when the particle size was reduced, the cooking weight increased. Cooking loss decreased when IDF-PBP was increased, and the particle size was reduced. The texture of the particle size distribution was 180 and 150 µm at levels 10% in tensile strength and showed not significant difference (p>0.05) as compared to the control rice noodles. Rice noodles with 10% IDF-PBP particle size distribution 150 µm showed the highest overall acceptability in sensory evaluation. The results revealed that adding IDF-PBP to rice noodles increased the total dietary fiber content at 9.44% and the insoluble dietary fiber content at 8.71% as compared to the control rice noodles. Based on the results, the addition of IDF-PBP to rice noodles can lead consumers to conclude that the product is healthy.

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Introduction

Noodles are a type of food processed from rice and a traditional oriental food that is widely produced in Thailand and other parts of Southeast Asia. The main ingredient of noodles is rice flour or rice flour mixed with other components, such as tapioca flour or modified starch. Thus, rice noodles are high in carbohydrates and calories, but low in dietary fiber (DE) (Wandee et al., 2015). Therefore, the industry involved in the development of noodles is interested in developing value-added products of daily nutritional food. Several studies have been carried out recently to improve the nutritional properties of rice noodles by adding supplements such as kimchi (Kim et al. 2017), pomelo (Reshmi et al., 2020), germinated brown rice (Chung et al., 2012) and canna starch (Wandee et al., 2015). Citrus fruits are nutritious and rich in essential minerals, vitamins and dietary fiber. In addition, most of these by-products, such as the bark, seeds and pulp, are wasted or underused in the orange juice industry. The weight of fresh fruit is 50%, but findings indicate that there are useful compounds that can be extracted as dietary fiber. Studies have reported that extracted dietary fiber from citrus albedo, flavors, pulp and seeds (Pichaiyongvongdee et al., 2021; Chau & Huang, 2003; Elif et al., 2017). Pichaiyongvongdee et al. (2021) found that pomelo pulp powder prepared from pomelo juice by-products has a total dietary fiber content (TDF) of 92.04% with insoluble dietary fiber (IDF) at 91.93%, which is more than dietary fiber from citrus fruit peels (orange, grapefruit, lemon, gonggan, and ponkan). According to findings, the content of total dietary fiber (TDF) was 61.79-64.07%, and insoluble dietary fiber (IDF) was 48.49-50.32% (Lei et al., 2015).

Dietary fiber is plant carbohydrate polymers that are not digested with digestive enzymes by humans. Dietary fiber can be divided into 2 types, insoluble dietary fiber (IDF), and soluble dietary fiber (SDF). IDF includes lignin, cellulose, and hemicellulose and SDF includes pectin, gum, and mucilage. Each type of fiber has different physiological effects (Dhingra et al., 2012). IDF is related to reducing intestinal transit time with improved drainage due to bulking capacity, thereby supporting the growth of intestinal microflora with good effects on diarrhea, constipation and irritable bowel syndrome. SDF is associated with reduced cholesterol levels in the blood, delayed gastric emptying, blood glucose control and lower serum cholesterol levels. Li & Komarek (2017); Kim et al. (2017) reported in previous studies that most of the dietary fiber residue from kimchi is composed of the insoluble dietary fiber used as an ingredient in common wheat noodles. In addition, IDF has been used as an ingredient in pasta and cakes. Therefore, it can be used as an ingredient in healthy food. According to the Thai Recommended Daily Intake (Thai RDI) nutrition experts recommend that people consume at least 25 grams of fiber per day for adults for optimal health. In general, the Thai rice noodles available on the market usually contain fiber at 3g/100g and it is recommended that the level of fiber foods be increased. Therefore, IDF from pomelo by-products could be used as a functional food material.

However, dietary fiber addition influenced the noodle microstructure and the quality of texture and sensory, which limited the consumption of dietary fiber. The findings of Reshmi et al. (2020) showed that adding dietary fiber from pomelo fruit segment in noodle affected the texture and cooking loss. Kim et al. (2017) found that adding insoluble dietary fiber from kimchi had an effect of increasing cooking loss and a decrease of sensory, if added in high quantity. There are also studies on the particle size of dietary fiber to improve the product. It was found that the change in particle size of bran had a negative effect on bread quality in terms of increased water holding capacity, and better hydration properties. At the same time, Zhang et al. (2019) study on the effect of IDF wheat bran particle size in noodles showed that the particle size of rice bran influenced cooking loss, texture, and water distribution.

The main objective of this study was to investigate the quality of rice noodles by using different particle size distributions and varying the ratios of insoluble dietary fiber (IDF) from pomelo by-products (PBP). Firstly, we made IDF-PBP and then evaluated the cooking quality, color, cooking weight, cooking loss, texture and sensory evaluation of rice noodles.

Materials and methods

1. Samples and chemicals

The albeo of pomelo by-products were collected from Nakhon Pathum Province. Rice flour was obtained from Choheng rice vermicelli factory company limited (Nakhon Pathom). Tapioca flour was obtained from Thai made flour factory Bangkok Co., Ltd. (Bangkok). All the chemicals and reagents used were of analytical grade.

2. Preparation of insoluble dietary fiber from pomelo by-products (IDF- PBP)

IDF was produced by pomelo pulp according to the method of Pichaiyongvongdee et al. (2021) with minor method modification like the cleaning process and different particle size distribution of powder. Firstly, the fresh pomelo pulp was cleaned with 0.01N NaOH solution at 37°C (pomelo pulp : NaOH solution, 1:10, w/v) for 10 min, followed by washing with distilled water and then the treated sample was soaked with 40% ethanol for 30 min. Bitterness reduction was by constant soaking with distilled water at pH 7.0 for 60 min. All the samples were dried in a tray dryer (Memmert 400, Germany) at 70°C until their moisture content was less than 10%, and recorded as IDF. The milled samples were ground in a blender (Model A 327 R7; Molineux; France) and sieved to two different particle sizes distribution : labeled as 150 μm and 180 μm. The chemical composition per 100 g dry of IDF- PBP appeared as follows: total dietary fiber (TDF) was 92.04%, insoluble dietary fiber (IDF) was 91.93% and soluble dietary fiber (SDF) was 0.11% (Pichaiyongvongdee et al., 2021). IDF-PBP was blended into rice flour at 2 substitution levels (10% and 12.5%) for further tests.

3. Preparation of dried rice noodles

The rice noodles for the experiment were developed by mixing IDF-PBP with different particle size distribution (180 μm, A and 150 μm, B) and were blended into rice flour at 2 substitution levels at 10% and 12.5% of rice flour and coded as 0 (control), A10, A12.5, B10 and B12.5, respectively and shown in Table 1. Other elements like 14% tapioca starch and 22% rice flour and ingredients were mixed with 64% distilled water (modified method from Anukulwattana, 1969) The dough was incubated for 60 min at room temperature for uniform hydration and equilibrium. Each 100 ml suspension was combined and then spread on a stainless steel tray (25 x31x1.5 cm) and steamed in a steamer at 100°C for 5 min to complete gelatinization. Then the dough was cut into 5 mm-wide strands with a noodle cutter and dried in a tray dry at 70°C for 60 min to reduce the moisture content to below 10%. Finally, all rice noodle strands were packed in plastic bags and stored at room temperature until the experiment.

Table 1 The ingredient formulation of experimental rice noodles with insoluble dietary fiber-enriched fractions from pomelo by-products

Sample	Ingredients (%)			
	Rice flour	IDF- PBP	Tapioca flour	Distriilled water
control	22.00	0	14.00	64.00
A10 (180 μm)	19.80	2.20	14.00	64.00
A12.5 (150 μm)	19.25	2.75	14.00	64.00
B10 (180 μm)	19.80	2.20	14.00	64.00
B12.5 (150 μm)	19.25	2.75	14.00	64.00

Remark: IDF- PBP: insoluble dietary fiber from pomelo by-products;
A10 : fresh rice noodles with 10% IDF-PBP (180 μm) of the rice flour;
A12.5 : fresh rice noodles with 12.5% IDF-PBP (180 μm) of the rice flour
B10 : fresh rice noodles with 10% IDF-PBP (150 μm) of the rice flour;
B12.5 : fresh rice noodles with 12.5% IDF-PBP (150 μm) of the rice flour

4. Analysis of rice noodles quality

4.1 Chemical composition

Chemical composition was determined according to the AOAC, (2016); moisture content (925.45), fat content (922.06), protein content (981.10), ash content (940.26), total dietary fiber (TDF) content (985.29), insoluble dietary fiber (IDF) content (985.29) and soluble dietary fiber (SDF) content (985.29).

4.2 Color measurement

The color of samples were measured using a Hunter colorimeter (Minolta Camera Co., Osaka, Japan). Data was recorded as L^*, a^* and b^* values. (L^*=black to white); (a^*=green to red) and (b^*=blue to yellow) were recorded.

4.3 Cooking properties

Samples were prepared for cooking weight and cooking loss of rice noodles and measured according to the AACC method (1995). Dried rice noodles were cut into small pieces 5 cm in length about 10 g and boiled in 300 ml water in a beaker for 3 min or until completely cooked, rinsed with distilled water, drained for 15 min, and immediately weighed. Cooking weight was measured from the difference between noodle weights before and after cooking, and expressed as the percentage of g cooked noodle/g dried noodle. Cooking loss was measured by evaporating to dryness the cooking water and rinse water in a pre-weighed glass beaker in a hot-air oven at 105°C for 12 h, and was expressed as the percentage of solid loss during cooking.

4.4 Textural analysis

Samples prepared for textural analysis consisted of dried rice noodles boiled in 300 ml water in a beaker for 3 min or until completely cooked, rinsed with distilled water and drained for 15 min. The cooked noodles were measured for texture properties using Manual of TAXT plus Texture Analyzer (Stable Micro System Ltd., U.K.). The tensile strength of noodle settings were pre-tested at a speed of 1 mm/s, test speed 3 mm/s, post-test 10 mm/s, strain at 40% and trigger force, 5.0 g. (Wandee et al., 2015). The force of each sample at fracture, indicating the tensile strength, were recorded data.

4.5 Sensory evaluation

Samples prepared for sensory evaluation used a similar method as for textural analysis. The cooked noodles containing 5 types of noodles (including the control) were put on a plastic tray and served to the panelists. A total of 50 panelists were recruited from Suan Dusit University. All the panelists were University students in the Department of Food Technology. Before formal evaluation, the panelists were trained to obtain objective results for the sensory descriptors. The panelists evaluated the freshly cooked noodles using a 9 point-hedonic scale (1=disliked extremely, 9 =liked extremely). The attributes evaluated were whiteness, smoothness, springiness, flavor, taste and overall acceptability. The research questionnaire was examined for accuracy, appropriateness and obtained approval by the Ethical Review Subcommittee for Human Research and Development Institute, Suan Dusit University, COA.NO: SDU-RDI 2020-011.

4.6 Statistical analysis

The data were performed in triplicate as means± standard deviation (SD), using a one-way ANOVA analysis of variance and using a T-test (Independent test) for 2 samples with SPSS Statistic Version 20.0 (SPSS Inc., Chicago, IL, USA). Data were considered statistically significant at $p<0.05$ by Duncan's Multiple Range Test.

Results and discussion

1. Moisture content and water activity

Concerning the chemical properties of dried rice noodle products with the addition of different particle size distributions and content of IDF-PBP, the control raw dried noodles were placed in a dry tray at 70°C for 60 minutes to reduce the moisture content, as presented in Table 2. The moisture content and water activity of the sample noodles varied between 8.66-9.50% and 0.32-0.36, respectively. Moisture content is very important for the quality of food and can control the growth of microorganisms. Larrauri (1999) reported that dried food should have a moisture content lower than 10% and water activity value less than 0.6 to prevent microbial growth.

2. Color characteristics

Color characteristics of dried noodles with different particle size distributions and content of IDF-PBP are shown in Table 2. Increasing IDF-PBP reduced the *L** and increased the *a** and *b** colors of rice noodles. The results indicated that, as the amount of IDF-PBP increased from 10% to 12.5% at each size, the appearance of the dried noodles grew darker. The darkness of the product was caused by the Mallard reaction between reducing sugars and proteins (Mohamed et al., 2010). At the same time, in reducing the particle size distribution of the IDF-PBP addition in noodles, the L increased and the *a** and *b** of rice noodles decreased. Pichaiyongvongdee et al. (2021) reported that reducing the particle size distribution of IDF- PBP from 425 µm to 50 µm found that *L** increased in lightness, whereas *a** and *b** decreased as the particle size of the sample was reduced. Therefore, the effects of specific are as increased.

3. Cooking qualities

The cooking weight and cooking loss of noodles are important indicators for assessing overall texture quality and can be used as indicators of the structural integrity of noodles, which is defined as the amount of solids dissolved in water during cooking (Bhattacharya et al., 1999; Zhang et al., 2019). The quality of rice noodles was determined by evaluating cooking properties such as cooking weight and cooking loss, as presented in Table 2.

The addition of IDF-PBP to rice flour for rice noodle production found significant difference ($p<0.05$) in which

Table 2 Effect of IDF- PBP with different particle size distribution and content on chemical properties and functional properties of rice noodles products

Sample	Moisture (%)	water activity	Color			Cooking weight (%)	Cooking loss (%)	Tensile Strength (g force)
			*L**	*a**	*b**			
Control	8.88±0.22^{bc}	0.33±0.00^{b}	82.52±0.38^{a}	0.30±0.05^{d}	9.03±0.37^{d}	187.42±4.27^{c}	6.71±0.11^{a}	69.86± 2.73^{a}
A10	8.66±0.19^{c}	0.32±0.01^{b}	73.80±0.47^{d}	0.55±0.08^{b}	9.85±0.33^{b}	217.16±0.29^{b}	2.84±0.07^{b}	66.53± 1.24^{a}
A12.5	9.14±0.09^{ab}	0.36±0.01^{a}	73.33±0.87^{d}	0.60±0.06^{a}	9.97±0.26^{a}	218.67±0.40^{b}	2.58±0.03^{c}	47.73± 2.16^{b}
B10	8.94±0.02^{bc}	0.36±0.00^{a}	81.57±0.56^{b}	0.42±0.02^{c}	9.22±0.13^{c}	222.30±0.24^{a}	2.42±0.01^{cd}	66.62± 3.02^{a}
B12.5	9.50±0.10^{a}	0.36±0.00^{a}	80.92±0.55^{c}	0.56±0.03^{ab}	9.28±0.15^{c}	225.57±0.31^{a}	2.40±0.01^{d}	46.77± 1.42^{b}

Remark: Difference letters in the same column indicate that the values are significant difference ($p < 0.05$)
A10 : rice noodles with 10% IDF-PBP (180 µm) of the rice flour;
A12.5 : rice noodles with 12.5% IDF-PBP (180 µm) of the rice flour
B10 : rice noodles with 10% IDF-PBP (150 µm) of the rice flour;
B12.5 : rice noodles with 12.5% IDF-PBP (150 µm) of the rice flour

cooking loss decreased in all samples and cooking weight increased in all samples in comparison to the control rice noodles.

The cooking weight of noodles fortified with IDF-PBP increased from 187.42% (control) to 225.57% (experimental noodles with IDF- PBP). In addition, it was found that the rice noodles with IDF- PBP small particle size distribution of 150 µm had higher cooking weight. Pichaiyongvongdee et al. (2021) reported that IDF-PBP with a particle size of 150 µm had high insoluble dietary fiber (IDF) at 91.93%. The microstructure of the porous fiber affecting WHC indicates increased water binding capacity. Jian et al. (2019) studied the insoluble dietary fiber (IDF) of wheat bran with different particle size distributions, finding that reducing particle size distribution contributed to a rising tendency for cooking loss from 8.65% to 7.65%.

The effect of adding IDF-PBP to noodles at 10% to 12.5% in the same particle size distribution found no significant differences (p>0.05) due to insufficient volume that changed the cooking weight of the noodles. Ekthamasut (2013) studied the effects of using bambara groundnut flour at three levels (10, 20 and 30%) on noodles and found increased cooking loss.

4. Textural analysis

The texture properties could affect evaluation of the overall quality of rice noodles and the sensory evaluation by consumers. The tensile tests used to measure related attributes and elasticity were based on time and force data that can be used to determine fundamental rheological parameters (Ritthiruangdej et al., 2011). Zhang et al. (2019) noted that the addition of IDF should add the appropriate amount and size of the IDF particle size to the noodles. This can affect the hardness and chewiness of the noodles.

The main ingredients used for rice noodles are rice flour (22%) and tapioca starch (14%). Kasemsuwan et al. (1999) reported that rice flour with amylose content between 25-30% is recommended for preparing rice noodles. The amylose content produces acceptable noodle textures, i.e., stickiness, elasticity and strength. The textural parameters of tensile tests are measured and summarized in Table 2. The findings showed that the texture and tensile strength of particle size distribution at 180 µm and 150 µm at 10% of the rice flour were not significantly different (p>0.05) as compared to the control rice noodles. When considering the addition of IDF- PBP at 12.5% of the rice flour, it was found that the sample was significantly different (p>0.05), where by tensile strength was decreased due to the addition of dietary fiber, so the amount of rice flour had to be reduced. This resulted in a decrease in amylose content, which allowed the amylose to be flexible. When the amylose content decreased, the tensile strength of the noodles was reduced. These results correspond with the findings of a study by Ritthiruangdej et al. (2011) who reported that the tensile strength decreased when the banana flour content increased in wheat noodles, thereby the weakening noodle texture. The addition of banana flour, which is gluten-free, in the production of dried noodles diluted the gluten strength and interrupted as well as weakened the structure of the noodles.

Pan & Jiecheng (2019) stated that the effect of reducing the starch content in the mixture results in a reduced viscosity and gel hardness of the starch system as well as the reduced chewiness and tensile strength of the cooked noodles.

5. Sensory evaluation

The attributes evaluated were whiteness, smoothness, springiness, flavor and taste. Table 3 shows the significant differences (p<0.05) in the sensory attributes of cooked fresh noodles made by adding IDF-PBP to rice flour at different volumes and particle size distributions. Significant decreases in smoothness, flavor, and taste were discovered when IDF-PBP was added at 12.5 g/100 g in rice flour (p<0.05). Furthermore, when IDF-PBP with

Table 3 Sensory evaluation of freshly cooked noodles after addition of IDF-PBP with different volume and particle size distribution in rice flour.

Sample	Color (Whiteness)	Smoothness	Springiness	Mouthfeel	Taste	Overall Acceptability
Control	8.03±1.10^{a}	8.03±0.89^{a}	7.30 ±0.75^{a}	6.70±0.75^{a}	7.07±0.58^{ab}	7.33±0.66^{a}
A10	7.03±1.07^{b}	6.43±0.50^{b}	7.03±0.85^{ab}	5.70±0.60^{b}	6.93±0.78^{ab}	6.13±0.82^{b}
A12.5	6.57±0.82^{c}	5.57±0.90^{c}	6.83±0.75^{b}	4.63±0.85^{c}	6.87±0.57^{ab}	5.30±0.92^{c}
B10	7.83±0.79^{a}	7.77±0.86^{a}	7.13±0.78^{ab}	6.57±0.90^{a}	7.10±0.48^{a}	7.03±0.72^{a}
B12.5	7.23±0.63^{b}	6.57±0.86^{b}	6.97±0.72^{ab}	5.90±0.76^{b}	6.73±0.64^{b}	6.43±0.63^{b}

Remark: Difference letters in the same column indicate that the values are significant difference (p<0.05)
A10 : rice noodles with 10% IDF-PBP (180 µm) of the rice flour;
A12.5 : rice noodles with 12.5% IDF-PBP (180 µm) of the rice flour
B10 : rice noodles with 10% IDF-PBP (150 µm) of the rice flour;
B12.5 : rice noodles with 12.5% IDF-PBP (150 µm) of the rice flour

small particle size distribution (150 µm) was added, the scores were higher than large particle size distribution (180 µm). Thus, sensory testing, showed that rice noodles with 10% of the rice flour replaced with pomelo pulp dietary fiber powder of particle size distribution of 150 µm had the highest sensory score among the attributes used to measure the intensity of whiteness (7.83), smoothness (7.77), springiness (7.13), flavor (6.57), taste (7.10) and overall acceptability (7.03). At the same time, overall acceptability showed a positive correlation with whiteness, smoothness, springiness, flavor and taste in sensory results, even though the tensile strength of all the samples in TPA showed no significant differences ($p>0.05$) from the control and rice noodles with 10% IDF- PBP particle size distribution at 150 µm. According to the findings, the sensory scores for the preference of springiness was affected by the cooked weight and cooked loss. Therefore, rice flour mixed with IDF-PBP at 10.0 g/100 g and a particle size distribution of 150 µm, was considered to potentially produce fresh noodles with high dietary fiber content.

The chemical composition comparison between the control rice noodles and the experiment rice noodles with 10% IDF-PBP (150 µm) of the Rice flour as presented in Table 4. The rice noodles with IDF-PBP showed no significant difference ($p>0.05$) in terms of moisture and protein content in comparison to the control rice noodles. While fat, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) showed significant difference ($p<0.05$). There were differences in the composition, possibly due to the higher percentage of IDF-PBP. Similar observations were reported by Rani et al. (2019) for noodles with multigrain fortification. Furthermore, higher total dietary fiber, particularly the insoluble dietary fiber portion of rice noodles with IDF- PBP might also have been the reason for the relatively high IDF-PBP content of the raw material in which the high insoluble dietary fiber (IDF) was 91.93%. At the same time, the soluble dietary fiber (SDF) of rice noodles with IDF-PBP was lower than the control, because IDF-PBP had low soluble dietary fiber (SDF) at 0.11%. Therefore, rice noodles with IDF-PBP could be classified as a functional food due to its high total dietary fiber. Moreover, insoluble dietary fiber (IDF) is beneficial for health in reducing intestinal transit time and improving drainage due to bulk capacity, thereby supporting the growth of intestinal microflora with good effects on diarrhea, constipation and irritable bowel syndrome. This complies with European Union Commission Regulation (EU) No 1047/ 2012 on 8 November 2012 regarding the list of nutritional claims of more than 6 g of dietary fiber in each 100 g of product (Mora et al., 2013). The addition of dietary fiber to the noodles can lead consumers to conclude that a product is healthy.

Table 4 Chemical properties level in the control rice noodles and rice noodles with IDF- PBP (Dry basis)

Chemical composition	The control rice noodles	rice noodles with IDF- PBP
Moisture (%)^{ns}	8.88±0.21	8.94±0.02
Protein (%)^{ns}	4.46±0.13	4.56±0.08
Fat (%)	0.85±0.07^{b}	1.10±0.08^{a}
Ash (%)	0.20±0.00^{b}	0.54±0.01^{a}
Total dietary fiber (TDF) (%)	5.09^{b}	9.44^{a}
insoluble dietary fiber (IDF) (%)	3.37±0.26^{b}	8.71±0.17^{a}
soluble dietary fiber (SDF) (%)	1.72±0.17^{a}	0.73±0.01^{b}

Remark: Difference letters in the same row indicate that the values have a significant difference ($p<0.05$)
ns indicates that the values have not significant difference.

Conclusion

The present study investigated separating the insoluble dietary fiber from pomelo by-products (IDF-PBP) for fiber-fortified rice flour and the effects of adding IDF- PBP to rice flour at different levels and particle size distributions. The different levels of IDF- PBP in rice flour found a significant ($p<0.05$) effect on the cooking weight, which was increased, while cooking loss and tensile strength were decreased in all samples as compared to the control rice noodles. While the different particle size was reduced, the cooking weight increased, and cooking loss decreased. The particle size distribution of IDF-PBP was not significantly different ($p>0.05$) in terms of tensile strength. Rice noodles with 10% IDF-PBP with a particle size distribution of 150 µm showed the highest overall acceptability in the sensory evaluation as well as cooking weight and tensile strength. The total dietary fiber content (TDF) under these conditions was 9.44 and the insoluble dietary fiber (IDF) content was 8.71% as compared to the control rice noodles. These results suggest that IDF-PBP could be added to rice flour in the preparation of rice noodles. IDF-PBP is a good nutrition source that promotes the broad use of fruit in the human diet for potential health benefits. In addition, it will promote the production of noodles at competitive prices and expand the use of healthy fibers.

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Antimicrobial Resistance Profiles of *Salmonella* spp. Isolated from Swine Feces in Phayao Province, Thailand

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Abstract

Antimicrobial resistance in *Salmonella* spp. is a serious issue for food safety. In Thailand, antibiotics are being used more frequently for both prophylaxis and treatment as commercial swine farming expands. Antimicrobial resistance has been generated as a result of the widespread use of antimicrobials in animal agriculture. For this study, we examined the antimicrobial resistance profiles and resistance percentages of *Salmonella* spp., which were isolated from swine fecal samples in Phayao province. The results showed that the overall prevalence of *Salmonella* spp. in the fecal samples in Phayao Province was 49.17%. The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%), followed by sick swine fecal samples (65%) and adult swine fecal samples (40%). The recovered 100 *Salmonella* spp. isolated from adult swine, sick swine and piglet feces were 34, 22 and 44 respectively. After that all isolates were tested for antimicrobial susceptibility. It was found that the highest resistance rate to ampicillin, which were equal to 79.41, 81.82 and 95.45 % in isolates from adult swine, sick swine, and piglets, respectively. *Salmonella* spp. isolates were resistant to ampicillin (87%), trimethoprim-sulfamethoxazole (84%), tetracycline (62%) and chloramphenicol (61%). Interestingly, *Salmonella* spp. isolated from piglets had highest percentage of resistance. *Salmonella* spp. isolated were resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, and cefotaxime which were equal to 95.45, 95.45, 90.91, 56.82 and 52.27%, respectively. MDR *Salmonella* was observed among 87 of 100 (87%) isolates. Whereas seventeen different multidrug-resistant profiles were observed. The most frequently found antimicrobial resistance profiles was AMP-SXT-TE-CIP. Furthermore, the probability of resistance to antimicrobial agents has increased. Further description of the associations between resistance and how resistance spreads within farms, are required before effective intervention strategies can be designed to control MDR *Salmonella* in swine.

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Introduction

Salmonella spp. is the bacterial pathogen that is ubiquitously found in the human food chain. Previous studies have shown that *Salmonella* is one of the leading foodborne pathogens (Duggan et al., 2010), and plays a significant role for causing human diarrhea in various countries (Pan et al., 2018). Salmonellosis is caused by *Salmonella* contamination in food. In the epidemiology of *Salmonella* spp., food acts as the main source of infection and animal asymptomatic carriers (Denis et al, 2013). Farms are natural reservoir of *Salmonella*, especially poultry and swine (Xu et al., 2020). Pork meat is one of the major foods from an animal which is produced and consumed in Thailand. The swine production consumption has indirectly increased the risk of foodborne zoonoses. *Salmonella* could colonize the digestive tract of swine and excreted in feces and spread into the environment (Jiang et al., 2019). As a result, *Salmonella* feasibly transmitted to humans via the food chain. Whereas transmission of *Salmonella* among swine occurs mainly via the fecal–oral route. The prevalence of infection in swine on the farm that might be triggered by stress factors linked to group housing, transportation and holding pens at the slaughterhouse, as the physiological changes associated with stress could promote in carriers or increase the susceptibility of non-carriers to new infections. Thailand's livestock department in 2020 reported the primary swine-producing area is in the central region of Thailand. In the northern region. there are 1,194,042 swine and 41,931 swine farmers, which is classified as the second largest swine producing area of Thailand. For the northern region, the data on the *Salmonella* contamination in three provinces (Chiang Mai, Chiang Rai, and Lamphun) have been established (Patchanee et al., 2015a, Tadee et al., 2021). However, the data on the prevalence and antimicrobial resistance of *Salmonella* in Phayao Province have not been identified.

Antibiotic-resistant microorganisms are currently a major concern to both human and animal health. Antimicrobial resistance is a serious ongoing global concern when it comes to zoonotic *Salmonella*. This becoming more complicated due to the emergence of the pathogenic strains resistant to many antimicrobial agents simultaneously. These pathogenic strains were called multidrug resistance (MDR). MDR *Salmonella* caused foodborne illness outbreaks through contaminated pork products in 2015, which resulted in severe infection in humans. *Salmonella* isolates were resistant to multiple antimicrobial agents, including ampicillin, streptomycin, sulfamethoxazole, and tetracycline (CDC, 2015). Antimicrobial agents are used in food animal production to promote growth and to prevent, treat, and control infectious diseases (Sneeringer et al., 2015). Previous research suggested that the amount of antimicrobial agents consumed by swine outweighed the usage of antibiotics for non-therapeutic purposes. Tetracycline and sulfonamides were two antimicrobials that were frequently used in swine production to enhance productivity or as therapeutics. Also, antibiotics were found to be an effective against mortality and morbidity in piglets to diseases (Cromwell, 2002). However, excessive use and over the counter purchase of antimicrobial agents in Thailand is common in both humans and farm animals. The use of antimicrobial agents as a feed additive in farm animals is rarely carried out under veterinarian supervision. This causes a rapid increase in both the animal's resistance to certain bacteria and in the level of antimicrobial residues in animal products. Thus, antibiotic resistance has increasingly emerged and re-emerged as a major threat to public health and economy in various countries. Furthermore, the population dynamics of antibiotic-resistant *Salmonella* spp. varies in swine due to the varying selection pressure exerted by the different antimicrobial agents (Seuberlich et al., 2009). As a consequence, it is crucial to have an improved surveillance system for pathogens with antimicrobial resistance in animal-borne foods. Furthermore, it is important to note the scarcity of evidence on the epidemiology of *Salmonella* infection in the production stages, including piglets, adults, and sick swine. These phases may influence the dynamics of infection. The monitoring of MDR *Salmonella* spp. in animal are essential to effectively control antimicrobial resistance. An improved understanding is essential to evaluate the risk of *Salmonella* contamination in the swine. Furthermore, significant advancements have been achieved in understanding and prediction of antimicrobial resistance of the *Salmonella*. Because of this, we have been strongly encouraged to look into the prevalence, percentage of resistance, and antimicrobial resistance profiles of *Salmonella* spp. found in swine fecal samples in Phayao Province.

Materials and methods

1. Sample collection

During 2020, 120 Fecal samples were collected after swine excreted on the floor. The random samples were collected from a medium swine farm (21-100 swine) in Phayao, a province in Northern Thailand, for *salmonella* isolation and identification. Fecal samples were divided into 3 sample groups. Fecal samples incorporated 80 adult swine fecal samples (aged swine from 10 weeks to 24 weeks old), 20 sick swine fecal samples (swine that were diagnosed with diarrhea) and 20 piglet fecal samples (weaning swine up to 10 weeks old) (Table 1). Fecal samples were collected by cotton and transferred by Cary-Blair transport media for analysis in a laboratory at the School of Medical Science, University of Phayao.

Table 1 Number of swine fecal samples in each group

Group of sample	Number of sample
Adult Swine fecal swab	80
Sick swine fecal swab	20
Piglet fecal swab	20
Total	**120**

2. *Salmonella* spp. identification

The swine fecal sample swabs were streaked on *Salmonella*-Shigella agar and incubated overnight at 35°C. One to five black colonies were selected. Potential *Salmonella* spp. colonies on *Salmonella*-Shigella agar were confirmed by Gram staining and biochemical test. Colonies were transferred to triple sugar iron agar (TSI), sulfide indole motility medium (SIM) and motility-indole-lysine medium (MIL) and then incubated 37°C for 18 to 24 h for confirmation. Then *Salmonella* spp. isolates were stored in 20% glycerol at -80°C.

3. Antimicrobial susceptibility test

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby-Bauer disk diffusion method. Concisely, using a sterile loop, pure colonies were picked from nutrient agar and emulsified in normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of 0.5 McFarland. A sterile cotton swab was then dipped into the suspension and distributed the bacteria suspension evenly over the entire surface of Mueller-Hinton agar. The antimicrobial agents included ampicillin (AMP) 10 µg, ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg, chloramphenicol (C) 30 µg, trimethoprim-sulfamethoxazole (SXT) 1.25/23.75 µg, meropenem (MEM) 10 µg, ciprofloxacin (CIP) 5 ug and tetracycline (TET) 30 ug. The plates were then incubated at 37°C for 18 h. The zone of inhibition for each *E. coli* isolate was analyzed according to the standards and the interpretive criteria of CLSI. (Clinical Laboratory Standards Institute, 2017). *Escherichia coli* ATCC 25922, which is a recommended reference strain for antimicrobial susceptibility testing, was used as a control. MDR isolates were defined as resistant to at least one agent in three or more antimicrobial classes.

4. Statistical analysis

Data were displayed as percentages and numbers The prevalence of *Salmonella* spp. was estimated based on the number of positive samples and any associations between groups of swine were determined using the chi-square test for independence. A p value of 0.05 was required for statistical significance. Data was analyzed using SPSS Software for Windows, Version 20.0.

Results and discussion

Salmonella spp. is the major cause of foodborne gastrointestinal illnesses in humans (Herikstad et al., 2002). Food-producing swine is an important source of *Salmonella* spp. in food products (Alban et al., 2002). An estimated 23% of all cases of human salmonellosis are related to the consumption of meat (Duggan et al., 2010). Antimicrobial resistance is a great problem of public health. In recent years, a high percentage of antimicrobial-resistant *Salmonella* spp. was frequently observed in all countries. Especially, *Salmonella* spp. showed resistance to tetracycline, sulfonamides/sulfamethoxazole, and ampicillin. Moreover, an increasing number of multidrug-resistant isolates were recovered (EFSA, 2020). There is growing concern about multidrug-resistant (MDR) *Salmonella*, especially the effectiveness of important antimicrobial agents, such as fluoroquinolones and extended-spectrum cephalosporins, which are the drug of choices used for treatment of salmonellosis in human (Crump & Mintz, 2010). Reservoirs of multidrug resistance are found in swine farms in Northern Thailand. They may be affected by antimicrobial usage on the farm. Access to antimicrobial agents as a medicated feed appeared to be an important factor to consider regarding the development of drug resistance in swine farms.

In this study, a total of 120 swine fecal samples (80 adult swine fecal samples, 20 sick swine fecal

samples and 20 piglet fecal samples) were collected in Muang District, Phayao Province. Including 240 black colonies on *Salmonella*-Shigella agar were selected. All suspected colonies of *Salmonella* spp. were confirmed by Gram staining and biochemical analysis. In Gram staining, the morphology of the isolated bacteria was gram negative and rod shape (Fig. 1). For biochemical test, triple sugar iron (TSI) test of the *Salmonella* isolates showed fermentation of glucose and H2S formation. The urease and indole tests for these isolates were negative. Whereas the motility and lysine decarboxylase were positive (Fig. 2). The overall prevalence of *Salmonella* spp. in the fecal samples in Phayao Province was 49.17% (59/120) which is higher than rates identified in swine farms from the same region, Tadee et al., reported occurrence of 31% (Tadee et al., 2014) and 25% (Tadee et al., 2021). The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%; 14/20), followed by sick swine fecal samples (65%; 13/20) and adult swine fecal samples (40%; 32/80) (Table 2). We found significant differences between groups ($p \leq 0.05$). The overall higher prevalence of shedding was observed when compared to previous studies. Another potential factor influencing was related to shedding which become exacerbated by the stress associated with the transport and lairage making *Salmonella* detection possible (Arguello et al., 2013). The results showed that recovered 100 *Salmonella* spp. isolates from adult swine, sick swine and piglet feces were 34, 22 and 44 respectively (Table 2). According to the study of Vigo et al., (2009), reported that shedding of *Salmonella* spp. to peak during the nursery period and subsequently decrease over time. Besides, stress associated with travel is reported to alter the pathogen release along with a variety of other factors, including environmental contamination and dose-response parameters (Simons et al., 2016).

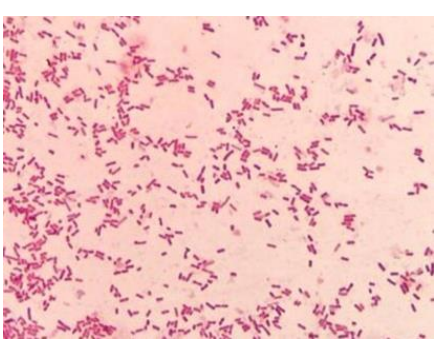

Fig. 1 Gram staining morphological observation of *Salmonella* spp. (100X)

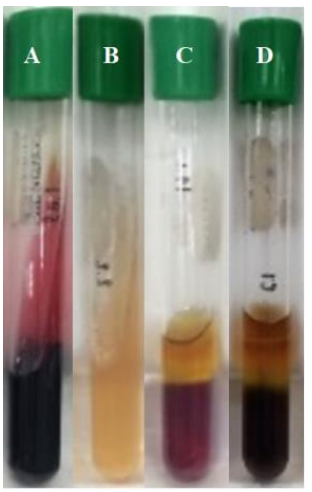

Fig. 2 Biochemical test for *Salmonella* spp. For TSI agar, Fermentation of glucose and hydrogen sulfide production (A) the urease test was negative (no color change or yellow) (B) for MIL medium, *Salmonella* spp. produces violet-colored medium (motility was positive, indole was negative, and lysine decarboxylase was positive) (C) and for SIM medium, *Salmonella* can reduce sulfur to hydrogen sulfide (hydrogen sulfide was positive, motility was positive, and indole was negative) (D)

Table 2 Results of *Salmonella* isolation from fecal swab and *Salmonella* spp. prevalence

Group of sample	No. of sample	No. of atypical Colony	No. of positive sample (%)	No. of *Salmonella* spp. isolates
Adult Swine fecal swab	80	102	32 (40%)	34
Sick swine fecal swab	20	59	13 (65%)	22
Piglet fecal swab	20	79	14 (70%)	44
Total	**120**	**240**	**59 (49.17%)**	**100**

Subsequently, we performed the antibiotic susceptibility tests by using the disk diffusion method on Muller-Hinton agar (Fig. 3). Drug susceptibility test was performed for 7 antimicrobial classes that included amphenicols (chloramphenicol), carbapenems (meropenem), cephalosporins (cefotaxime and ceftazidime), penicillin (ampicillin), quinolone (ciprofloxacin), sulfonamides (trimethoprim-sulfamethoxazole), and tetracycline. The results illustrated that *Salmonella* spp. isolates were resistant to all antibiotics used in this study, except meropenem. It was found that the highest resistance rate to ampicillin, were equal to 79.41, 81.82 and 95.45 % in isolates from adult swine, sick swine, and piglets, respectively. *Salmonella* spp. isolates were resistant to ampicillin (87%), trimethoprim-sulfamethoxazole (84%), tetracycline (62%) and chloramphenicol (61%). Based on the research of Patchanee et al., (2015b), the highest

frequency of antibiotic resistance of *Salmonella* isolates in Northern Thailand were ampicillin (83.3%) followed by tetracycline (75.7%). Simultaneously, the findings of Yue et al. (2021) in China showed that tetracycline (85.90%) and ampicillin (84.62%) had the most resistant antimicrobial agent, followed by chloramphenicol (71.80%). *Salmonella* spp. isolates from adult swine were resistant to ampicillin, trimethoprim- sulfamethoxazole, tetracycline, and chloramphenicol, which were equal to 79.41, 79.41, 64.71 and 50%, respectively. Similarly, Perron et al. (2008) reported that *Salmonella* from adult swine were resistant to common antibiotics and 65% of *Salmonella* spp. isolates showed resistance to tetracycline. Whereas *Salmonella* spp. isolates from sick swine were resistant to ampicillin, trimethoprim-sulfamethoxazole and tetracycline, which were equal to 81.82, 68.18 and 68.18%. Interestingly, *Salmonella* spp. isolates from piglets had the highest percentage of resistance. They were resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, and cefotaxime which were equal to 95.45, 95.45, 90.91, 56.82 and 52.27%, respectively (Table 3). Regarding the *Salmonella* spp., compared to previous studies found a lower prevalence of antimicrobial resistant of *Salmonella* spp. in swine in Thailand, no more than 20% (Pulsrikarn et al., 2012).

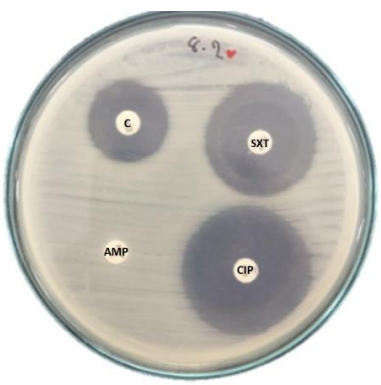

Fig. 3 Antimicrobial susceptibility test of *Salmonella* spp. performed by using the disk diffusion method

Remark: chloramphenicol (C), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP) and ciprofloxacin (CIP)

Based on results, we found that *Salmonella* spp. isolates from piglets had the highest percentage of resistance to variant of antimicrobial agents. Additionally, the probability of antimicrobial agent resistance in *Salmonella* spp. isolates from piglet has high resistance.

Table 3 Number and proportion of *Salmonella* spp. isolates resistant to different antimicrobial agents

Antimicrobial agents	Number of resistant *Salmonella* spp. isolates (%)			Total isolates (%) (n=100)
	adult swine (n=34)	sick swine (n=22)	piglet (n=44)	
Ampicillin	27 (79.41)	18 (81.82)	42 (95.45)	87 (87)
Trimethoprim-sulfamethoxazole	27 (79.41)	15 (68.18)	42 (95.45)	84 (84)
Tetracycline	22 (64.71)	15 (68.18)	25 (56.82)	62 (62)
Chloramphenicol	17 (50)	4 (18.18)	40 (90.91)	61 (61)
Ciprofloxacin	13 (38.24)	8 (36.36)	15 (34.09)	36 (36)
Cefotaxime	7 (20.59)	1 (4.55)	23 (52.27)	31 (31)
Ceftazidime	5 (14.71)	3 (18.18)	0 (0)	8 (8)
Meropenem	0 (0)	0 (0)	0 (0)	0 (0)

The study of Brun et al. (2002) speculated that young animal carry more resistant microorganisms due to increased antimicrobial exposure and physiological differences. Piglets are highly vulnerable to enteric pathogens (Lallès et al., 2007). The intestinal dysbiosis frequently seen in weaned piglets after diet change, the stress associated with changing surroundings, and the growth of swine all favor bacterial colonization by enteric pathogens. Especially, risk factors for resistance in piglets are commonly received and continuously exposed to antimicrobial agents, which raises concerns about selection for resistance (Rajic et al., 2006). Therefore, the effect of this exposure on resistance should be investigated to use in guidelines.

The *Salmonella* spp. isolates were resistant to at least one agent in three or more classes of antimicrobial agents and was defined as MDR *Salmonella*. In this study, MDR *Salmonella* was observed among 87 of 100 (87%) isolates. Seventeen different multidrug-resistant profiles were observed as shown in Table 4. Resistance to penicillin (ampicillin) and sulfonamides (trimethoprim-sulfamethoxazole) was found in MDR *Salmonella*. Furthermore, 42 (42%) isolates were resistant to antimicrobial agent in at least 4 classes. The antimicrobial resistance profiles of MDR *Salmonella* were AMP-SXT-TE-CIP, AMP-SXT-C-CTX and AMP-SXT-CTX-C-CIP which were equal to 15, 10 and 10%, respectively. Conversely, the most frequently antimicrobial resistance profiles in *Salmonella* spp. isolates from piglets were AMP-SXT-C-CTX and AMP-SXT-CTX-C-CIP. However, Phongaran et al., (2019) reported the most frequent pattern isolated from swine feces collected from slaughterhouses in nine provinces of Thailand was AMP-SXT-TET. Whereas in this study we found only 4%. In this study, we found that among the farms that recently used antimicrobials, some used antimicrobials without a prescription from

veterinarians and some farmers were unaware of the antimicrobial withdrawal time. Almost half of participates in swine farm used commercial feed. As suggested by Love et al., (2015) commercial medicated feed is likely related to the development of antimicrobial resistance. Including, the farmer was not aware of the type and dose of antimicrobial agents that was mixed in the feed.

Table 4 Antimicrobial resistance profiles of *Salmonella* spp. isolates in adult swine, sick swine, and piglet

Antimicrobial resistance profiles	Number of resistant *Salmonella* spp. isolates (%) adult swine	sick swine	piglet	Total isolates (%)
TE	2 (5.88)	2 (9.09)	-	4 (4)
C	-	-	2 (9.09)	2 (2)
TE-AMP	2 (5.88)	3 (13.64)	2 (4.55)	7 (7)
TE -CAZ-CIP	3 (8.82)	-	-	3 (3)
SXT-AMP-TE	2 (5.88)	2 (9.09)	-	4 (5)
SXT-AMP-C	5 (14.71)	-	3 (6.82)	8 (8)
SXT-AMP-CIP	-	2 (9.09)	-	2 (2)
SXT-TE-C	2 (5.88)	-	-	2 (2)
AMP-SXT-CAZ-C	3 (8.82)	3 (13.64)	-	6 (6)
AMP-SXT-TE-CIP	8 (23.53)	5 (22.73)	2 (4.55)	15 (15)
AMP-SXT-TE-C	-	2 (9.09)	2 (4.55)	4 (4)
AMP-SXT-C-CTX	2 (5.88)	-	8 (18.18)	10 (10)
AMP-SXT-TE-C-CTX	3 (8.82)	-	4 (9.09)	7 (7)
AMP-SXT-TE-C-CIP	-	-	2 (4.55)	2 (2)
AMP-SXT-CTX-C-CIP	2 (5.88)	-	8 (18.18)	10 (10)
AMP-SXT-CTX-TE-CIP	-	1 (4.55)	-	1 (1)
AMP-SXT-CTX-TE-C-CIP	-	-	3 (6.82)	3 (3)
Total number of isolates	**34**	**22**	**44**	**100**

Remark: tetracycline (TET), cefotaxime (CTX), ceftazidime (CAZ), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), chloramphenicol (C) and ciprofloxacin (CIP)

Antimicrobial resistance in *Salmonella* spp. from on-farm studies provides insight into the epidemiology of resistance in swine prior to transport and slaughter (Gebreyes et al., 2004). *Salmonella* resistance can also be impacted by dietary changes, stress from new surroundings, and the growth of swine. *Salmonella* spp. may provide particular concerns to food safety, as evidenced by the different rates of resistance in each stage of production, and this demonstrates that resistance is dynamic within farms. Therefore, in the future, we should look into agricultural resistance risk factors. Interventions to reduce antibiotic resistance in *Salmonella* spp. may result from identifying characteristics linked to variations in resistance between phases.

Conclusion

The resistance of *Salmonella* isolates from swine farms in Phayao Province, Thailand was described. The overall prevalence of *Salmonella* spp. in the fecal samples in Phayao Province was 49.17%. It is higher than rates identified in swine farms from the same region, in the previous reported an occurrence of 31% (Tadee et al., 2014) and 25% (Tadee et al., 2021). The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%), followed by sick swine fecal samples (65%) and adult swine fecal samples (40%). High resistance (>80%) was recorded toward ampicillin (87%) and trimethoprim-sulfamethoxazole (84%). MDR *Salmonella* was observed among 87 of 100 isolates (87%) and 42 isolates (42%) which were resistant to antimicrobial agent in at least 4 classes. Conversely, seventeen different multidrug-resistant profiles were observed. The most frequently found antimicrobial resistance profiles was AMP-SXT-TE-CIP. The probability of antimicrobial agent resistance in *Salmonella* spp. isolates from piglet has more resistance. Therefore, the age-specific factor study is needed to investigate reasons for differences in resistance. Likewise, further description of the associations between resistance and how resistance spreads within farms, are needed. Besides, it is time to prevent the use of antimicrobial agent in livestock to avoid the dissemination of antimicrobial resistance determinants along the food chain to avoid the transmission of foodborne pathogens to humans.

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Low-Cost Biochar Derived from Bamboo Waste for Removal of Heavy Metal in Aqueous Solution

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Abstract

This study assessed the adsorption capacity of heavy metals such as lead (Pb), copper (Cu) and zinc (Zn) in the aqueous solution of biochar. Biochar was obtained from bamboo handicraft scraps by a pyrolysis method and was used as an economical absorbent. The bamboo biochar was characterized by scanning electron microscopy coupled with energy-dispersive x-ray spectroscopy (SEM-EDS) and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The parameters such as contact time, biochar dosage, pH of the initial solution, and initial concentration of metal ions affected sorption capacity were investigated. Experiment results showed that the bamboo biochar mainly contained carbon and oxygen elements and a high number of C=O, C-O and O-H functional groups. The maximum adsorption uptake on biochar was Pb(II)>Zn(II)>Cu(II) under 1.0 g adsorbent L^{-1}, 20 mg L^{-1} initial concentration of all metal ions, pH 4, contact time 120 min and at ambient temperature. From Langmuir isotherm fitting showed the maximum adsorption capacity was 41.15, 30.21 and 34.48 mg g^{-1} for Pb(II), Cu(II), and Zn(II), respectively. Pseudo-second order kinetic model can best describe the adsorption process of all ions in solution mainly via monolayer adsorption onto a homogeneous adsorbent surface and chemisorption as ion exchange, complexation, and surface mineral precipitation of metal ions. Appling the bamboo biochar for the removal of metal ions in groundwater found it was able to eliminate manganese at more than 95% within an hour of contact time. All research findings suggest that bamboo biochar has broad potential for water purification applications.

Introduction

Heavy metals include biologically essential elements such as zinc (Zn), copper (Cu), nickel (Ni), iron (Fe), chromium (Cr), boron (B) and molybdenum (Mo), and elements that are not essential such as mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As) and silver (Ag). It is high water solubility and due to its low biodegradability

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therefore it can accumulate in the microorganisms and environment and then be further transported to the human body (Iqbal et al., 2021; Sherlala et al. 2018). They can be toxic or carcinogenic, which induced disease in the gastrointestinal, renal, and cardiovascular systems and can cause severe problems for humans and aquatic ecosystems (Balali-Mood et al., 2021; Xu et al. 2018). Surface water is threatened by humans, principally heavy metal contamination mainly from the accumulation of chemical fertilizers, pesticides and farm manure used in farming. In addition, surface water can penetrate into shallow groundwater causing heavy metals to spread throughout. They can also affect plant metabolism and growth (Ahmed et al. 2019; Sarker et al., 2022). Kheangkhum, et al., (2020) reported that heavy metals have also been recently detected in major food items, such as rice, vegetables, and meat available in large markets of Thailand. With the continuous evolution of human society and economy, human activities have caused serious pollution to water bodies (Chunhabundit, 2016). The sustainable development of a country depends on the covenant of secure, safe and renewable drinking water. Thai farmers and poor households in some parts of the country rely on groundwater as a significant source of drinking and irrigation.

Various technologies, filtration, precipitation, redox reactions, or ion exchange have been used for the elimination of heavy metals in groundwater. However, these technologies are costly to maintenance, produce harmful by-products, or energy-intensive (Jovarauskaite & Balund, 2021). The adsorption process is an interesting technique for removing heavy metals. The conventional adsorbent material as the solid material used for the adsorption consists of a porous with a high internal surface area (Da'ana et al., 2021). Activated carbon is an adsorbent derived from carbonaceous raw material. Due to their high surface area, complex pore structure (micro, meso and macro), and a high degree of surface reactivity have been used to adsorb a wide variety of substances. However, traditional activated carbon has some drawbacks, namely high production costs (Siipola et al., 2020). Consequently, research into carbon-based absorbents with high adsorption efficiency and low expense is limited and is worthwhile in practical applications. An improved low-cost adsorbent can be an appropriate alternative to expensive activated carbon for removing organic pollutants and heavy metal ions contaminating water (Nguyen et al., 2021). Biochar is an interesting option that is high porous furthermore on the surface has carboxyl (–COOH) and hydroxyl (–OH) functional groups as sorption sites for the metal ions (Murtaza et al., 2021). However, the chemical and physical properties of biochar are affected by the type of feedstock and pyrolysis technology. Biochar is a carbon material derived from biomass via a pyrolysis process in limited air. Many studies have used biochar to remove contaminants in water. For example, Iamsaard et al., (2022) reported using pineapple leaf biochar that had high efficiency for removing Ni(II), Zn(II), and Cu(II), Deng et al., (2020) used biochar derived from banana stalk to adsorb Zn(II), Mn(II) and Cu(II) from aqueous solution, Chen et al., (2019) studied the kinetics and adsorption mechanisms for adsorption of lead (Pb^{2+}) and cadmium (Cd^{2+}) with dairy manure biochar, however, the biochar were produced by muffle furnace oven. It can be easily controlled during production. Therefore, high-quality biochar production in the field is challenging. Our previous work developed a biochar from bamboo waste by a Top-Lid Updraft Drum (TLUD) equipment for the removal of dissolved organic matter in water (Angthararuk et al., 2022). The Thai Wiang Community, Hin Tung, Mueang Nakhon Nayok District, Nakhon Nayok Province (14°15′03″N 101°18′18″E) contains many bamboo plantings and bamboo is used in varies occupations. Some of them are used to produce charcoal for energy by earth kiln, pit kiln and brick kiln. The weakness of conventional charcoal production is its low quality which is not suitable for use as a water contaminant adsorbent. The Thai Wiang Community depends on groundwater for consumption and high-cost anthracite is used for treatment. Therefore, biochar derived from bamboo waste is an affordable absorbent material that can be a viable alternative to absorbing heavy metals in an aqueous solution.

In this study, biochar was produced from bamboo waste via pyrolysis process by a community low-cost portable biochar kiln to remove heavy metal ions such as Pb(II), Cu(II) and Zn(II) in an aqueous solution. The effects of variables on the initial concentration of metal ion, pH of the solution, contact time, and adsorbent dosage on the adsorption efficiencies were investigated. The kinetics and isotherms of the adsorption process were studied to elucidate this mechanism. Furthermore, the absorbent was applied to remove the heavy metals in the groundwater.

Materials and methods

1. Reagents and chemicals

All reagents and chemicals utilized in the study were analytical grades. A stock solution of Pb(II), Cu(II), and Zn(II) was prepared from $Pb(NO_3)_2$, $Cu(NO_3)_2.3H_2O$, and $Zn(NO_3)_2.6H_2O$, Sigma-Aldrich, USA. A standard solution 1.0 g L^{-1} of Pb, Cu and Zn (Merck) was used for the preparation of all standard calibration curves. Double distillation water was used to prepare all the solutions for the experiments.

2. Bamboo biochar

Biochar was converted from bamboo handicraft waste through a slow pyrolysis process under air-limiting conditions. The production used low-cost two-containers as Top-Lid Updraft Drum (TLUD) equipment with available local resources from Hin Tung, Mueang Nakhon Nayok District, Nakhon Nayok Province, Thailand (14°15′03″N 101°18′18″E). The reactive groups on surface bamboo biochar were analyzed by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (IR Tracer-100 FTIR spectrophotometer, Shimadzu, Japan), recording between wavenumber of 4000 and 400 cm^{-1} with 4 cm^{-1} resolution and a cumulative number of scans was 40. The background was collected before each measurement. The peak and band positions were obtained using the IR Tracer Software. Morphological characteristics were obtained by scanning electron microscopy (SEM analyzer JSM-6610 LV, JEOL Ltd., Tokyo, Japan) with an accelerating voltage of 20 kV and elemental composition by energy-dispersive x-ray spectroscopy (EDS, Oxford instrument X-Max 50 mm^2, England).

3. Adsorption experiments

Batch adsorption experiments were carried out with 100 mL of 20 mg L^{-1} metal ion (Pb^{2+}, Cu^{2+} and Zn^{2+}) solution mixing 0.1 g (1 g L^{-1}) bamboo biochar placed in a 250-mL Erlenmeyer flask and the mixture was shaken at 150 rpm. Adjustments to the initial pH of the metal ion solution were made using 0.1 mol L^{-1} HNO_3 or NaOH (Starter 3100 bench, Ohaus pH meter). All experiments were conducted in triplicates at room temperature. After equilibrium, the collected liquid sample was centrifuged at 3,000 rpm for 5 min before being filtered through a 0.45 μm nylon syringe filter. The filtrate was immediately acidified by 0.2% (v/v) HNO_3 for the determination of metal ions by Atomic Absorption Spectroscopy (AAS, GBC3000, Australia).

The adsorption efficiency (Q_e) of metal ions on biochar was determined by the metal ion concentration adsorbed per unit of adsorbent. The Q_e and adsorption percentage was determined by the following equations:

$$Q_e = \frac{(C_0 - C_e)V}{M} \quad (1)$$

$$\%\ Adsorption = \frac{(C_0 - C_e)}{C_o} x\ 100 \quad (2)$$

Where C_o and C_e are the concentrations (mg L^{-1}) at initial and equilibrium, respectively. M is the adsorbent amount (g) and V is the volume of solution (L).

4. Factors affecting metal ions adsorption

To investigate the effect of process parameters on adsorption efficiency of metal ions, biochar content (0.1, 0.5, 1.0, 2.0, 5.0 and 10 mg L^{-1}), contact times (15-1140 min), pH (2, 3, 4, 5, 6 and 7) and initial concentration (5, 10, 20, 30, 50, 70 and 100 mg L^{-1}) operates in parallel with the control. The experimental conditions were similar to those previously set in Section 3.

5. Removal of heavy metals in groundwater

The groundwater samples were collected from Hin Tung, Mueang Nakhon Nayok District, Nakhon Nayok Province, Thailand (14°15′03″N 101°18′18″E) during the month of September 2021 by following standard sample collection protocol. Special precautions were conducted during the collection of samples. In the initial step, metal-free sample containers were soaked overnight in 2% nitric acid and then washed with double distilled water and dried. The containers were then rinsed three times with the groundwater sample of the particular location to avoid contamination and then collection of the samples occurred. To prevent the loss of metal ions it was necessary to add 0.5 mL of 1.0 mol L^{-1} HNO_3 to the sample that was acidified solution. Batch removal of heavy metal ions in groundwater was followed by a 100 mL water sample mixing 0.1 g bamboo biochar (1.0 g L^{-1}) in 250-mL Erlenmeyer flasks with the mixture shaken at 150 rpm for 60 minutes contact time at ambient temperature. At the time, the liquid sample was centrifuged, filtered and determination of metal ions by Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES, Avio 200, Perkin Elmer, USA) was conducted.

Results and discussion

1. Characterization of bamboo biochar

The morphological characteristics of bamboo biochar obtained by scanning electron microscope (SEM) are shown in Fig.1. It is seen as obvious morphological

biochar with a large surface area, tubular shapes, rough surface structures, sharp edges and surface pore morphology as a honeycomb-like structure. An elemental analysis was carried out by energy dispersive spectroscopy (EDS). The results of EDS showed that carbon (C 77.30 %) and oxygen (O 11.93 %) were the major elements of the biochar and the mineral fractions consisted of Si (0.75 %), P (0.33 %), K (1.29 %) and Fe (8.40 %). These results demonstrated that high content C was the main skeleton with O which may come from oxygen-containing functional groups such as carboxylic acid (-COOH), hydroxyl (-OH) or metal minerals such as carbonate (CO_3^{2-}), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) (Hernández-Mena et al., 2014).

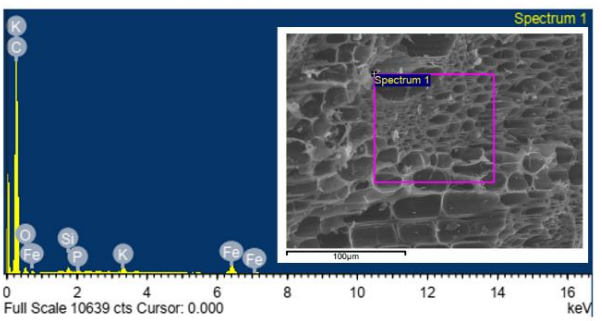

Fig. 1 SEM and EDS for morphological and elemental of bamboo biochar

The FTIR spectrum of bamboo biochar is shown in Fig. 2. It can be seen that the spectrum of adsorbents was characterized. The highest band intensities for the organic functional groups were a band of -OH stretching (3329 cm^{-1}), aliphatic CH_2 (2914, 2848 cm^{-1}), C=O stretching of carboxyl (1700 cm^{-1}), aromatic C=O or C=C ring stretching (1601 cm^{-1}) (Liu et al., 2021), C-O-C or PO_2^- asymmetric stretching (1236 cm^{-1}) (Bangaoil et al., 2020), C-O stretching (1031 and 1098 cm^{-1}) and Si-O-Si stretching (1154–1031, 798, and 471 cm^{-1}) (Cui et al., 2019).

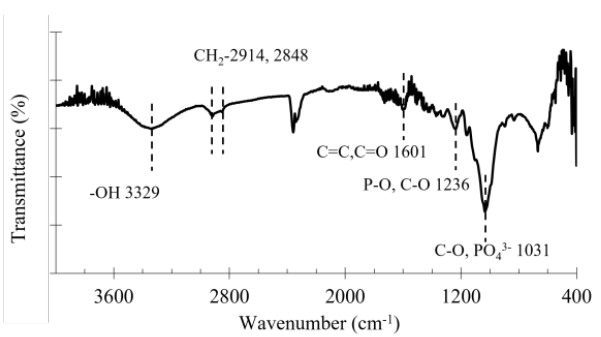

Fig. 2 FTIR spectrum of bamboo biochar

2. Effect of contact time

The contact time of metal ions on absorbent is an important factor, which greatly affects the adsorption process on the surface of biochar. The adsorption effects of Pb(II), Cu(II) and Zn(II) on biochar were determined at different time intervals as shown in Fig. 3. It was observed that the rate of Pb(II) adsorption was rapid with 83% of the ultimate adsorption occurring in the first period of 120 min, while Cu(II) and Zn(II) sorption were 60 and 70%, respectively, followed by a very slow approach to equilibrium. The result showed that after 240 min of contact time the adsorption indicated nearly achieved to the equilibrium phase which was probably the utmost adsorption capacity of the entire samples. The adsorption uptake at the equilibrium state of Pb(II), Cu(II) and Zn(II) on bamboo biochar were 18.82, 15.67 and 16.25 mg g^{-1}, respectively. Therefore, 6 hours of contact time for the rest of the sorption experiments were chosen.

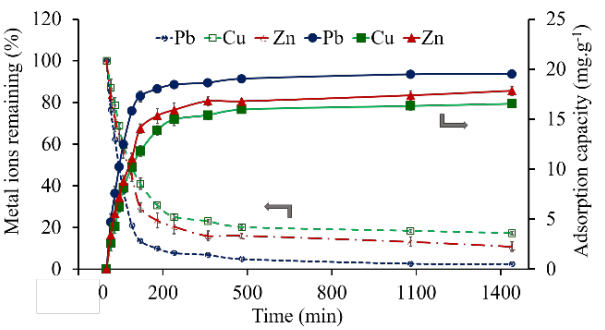

Fig. 3 Adsorption efficiencies and % remaining of metal ions in solution (20 mg L^{-1} initial concentration) on biochar (1.0 g L^{-1}) with function time

3. Effect of biochar dosage

The amount of absorbent directly affects the adsorption efficiency of metal ions, since it determines the capacity of bamboo biochar at a given initial concentration. The results of the batch experiments with various bamboo biochar dosages are presented in Fig. 4. The highest observed metal ions at 0.1 g L^{-1} biochar for Pb(II), Cu(II) and Zn(II) retention capacities were 56.35, 38.30 and 29.92 mg g^{-1}, respectively. Increasing the absorbent dosage, however, actually decreased the adsorption yield of all metal ions (Fig. 4a). For example, the adsorption efficiency of Pb(II) decreased from 43.2 mg.g^{-1} with 0.1 mg L^{-1} absorbent to 1.9 mg g^{-1} at 50 mg L^{-1} which was likely due to aggregation which was clearly visible at higher biochar concentrations. Nevertheless, this reduction in adsorption efficiency,

incremental the adsorbent concentration which did result in increased percentage removal of all metal ions (Fig. 4b). It was likely due to a function of the increased active sites, the data suggested that not all of the added sites were available for binding which is consistent with the observed aggregation (Chen et al., 2011). Whereas, it should be noted that the differences in all metal ions removal were not significantly different at 2 and 10 g biochar L^{-1}. While at 10 g absorbent L^{-1}, the efficient removal of Pb(II), Cu(II) and Zn(II) were 99.60, 98.51 and 94.61 %, respectively.

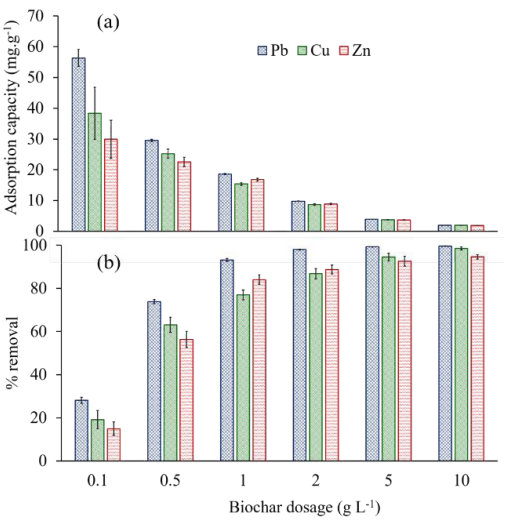

Fig. 4 Effect of biochar dosage on metal ions (a) adsorption efficiencies (b) percentages of heavy metal removal, 20 mg L^{-1} initial concentration and 6 hours contact time

4. Effect of initial pH

The pH of a solution plays a significant role in the adsorption process affecting the adsorption efficiency due to the effect of the adsorbent surface binding sites with metal ions and the metal ionization process (Qian & Chen, 2014). In this study, the pH between 2 and 7 of the initial solution was investigated and the results are presented in Fig. 5. The adsorption capacity increased with increasing pH value. The best pH of solution for the maximum adsorption capacity was pH 4 for Pb(II) (18.67 ± 0.13 mg.g^{-1}) and pH 5 for Cu(II) (16.08 ± 0.16 mg.g^{-1}) and Zn(II) (17.87 ± 0.39 mg.g^{-1}). At the lower pH of the initial solution, the competition between protons and metal ions, and a lot of hydrogen and hydronium ions in the solution obstructed the cation sorption sites on biochar (Deng et al., 2020). In addition, the surface of the biochar became positively charged and electrostatic repulsion of cations occurred resulting in low adsorption (Sakhiya et al., 2022). When the pH of the initial solution was increased, the deprotonation of functional groups (through phenolic OH dissociation) on the adsorbent surface can potentially provide more active sites for metal ions, resulting in an enhancement of adsorption effectiveness (Ding et al., 2014). At a pH above 5, the adsorption of heavy metal ions tend to decrease and was likely caused by the hydroxide complexes formation which hydroxo $M(OH)^{+}$ formed that could hinder the interaction between M^{2+} and biochar (Li et al., 2017; Oliveira et al., 2017).

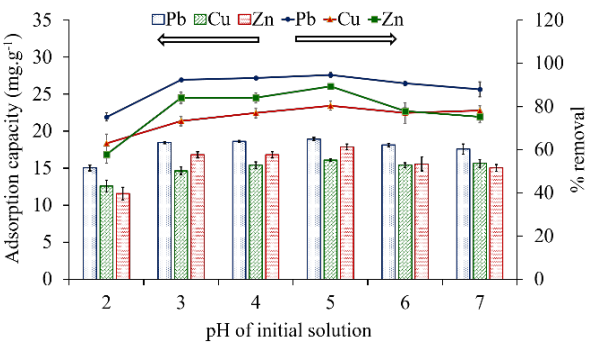

Fig. 5 Effect of initial solution pH on metal ions adsorption capacity

5. Adsorption Kinetics

Heavy metal on biochar sorption rate behaviors were evaluated using the pseudo-first-order (3) and pseudo-second-order (4) model equations as follows:

$$ln(Q_e - Q_t) = lnQ_e - K_1 t \quad (3)$$

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{t}{Q_e} \quad (4)$$

Where Q_t (mg g^{-1}) and Q_e (mg g^{-1}) are the biochar adsorption capacity at any time t (min) and equilibrium time, respectively, the adsorption rate constants K_1 (min^{-1}) and K_2 (g mg^{-1} min^{-1}) are primary and secondary constants, respectively.

The linear relationship of the kinetic model and implicated parameters of the kinetic equations were exhibited in Fig. 6 and Table 1, respectively. The adsorption capacity, Q_e of all metal ions was predicted by the pseudo-second-order kinetic model due to a correlation coefficient (R^2> 0.99) approaching 1 was proved to be more appropriate to fit the kinetics model compared with the pseudo-first-order kinetic model (R^2 0.75-0.92). The Q_e calculated value of Pb(II), Cu(II)

and Zn(II) as 20.04, 17.42 and 18.51 mg g^{-1}, which was closer to the actual adsorption capacity, 18.45, 14.97 and 15.92 mg g^{-1}, respectively, obtained from the experiment. Therefore, the adsorption process of all metal ions on bamboo biochar suggested two reactions occur; the first stage is rapid and achieves equilibrium quickly and the second is slow that can continue for a long time. It indicates that the chemisorption of metal ions was the rate-limiting mechanism and adsorbed onto the biochar surface by chemical interaction, such as ion exchange and complexation.

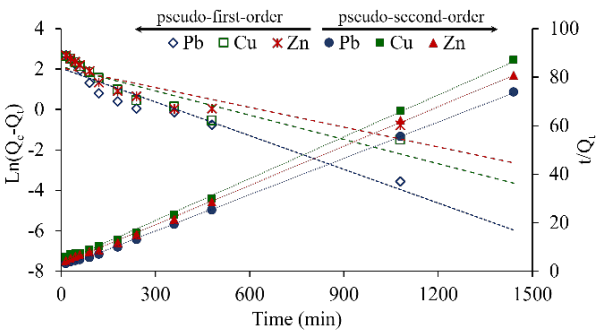

Fig. 6 Adsorption kinetic model of metal ions onto bamboo biochar (a) Pb(II) (b) Cu(II) and (c) Zn(II)

Table 1 Kinetic parameters of Pb(II), Cu(II) and Zn(II) on biochar

Metal ion	Pseudo-first-order model			Pseudo-second-order-model		
	Q_e (mg g^{-1})	K_1 (min^{-1}) x 10^{-3}	R^2	Q_e (mg g^{-1})	K_2 (g mg^{-1} min^{-1}) x 10^{-3}	R^2
Pb (II)	7.68	5.6	0.9209	20.24	1.382	0.9994
Cu (II)	8.33	4.0	0.8478	17.42	0.553	0.9985
Zn(II)	7.83	3.3	0.7599	18.51	0.514	0.9990

6. Adsorption Isotherm

Adsorption isotherms are important for describing the relation between the amounts of the adsorbate onto the adsorbent surface and the adsorbate remaining in the solution. It is a mathematical model that plots the solute concentration in the aqueous solution (x-axis) and quantity adsorbed on the adsorbent surface (y-axis) at a constant temperature. It can explain the distribution of the adsorbate molecules or ions on the surface of a material that is related to adsorbate interacting with adsorbents. (Katiyar et al., 2021). Two common models, Langmuir and Freundlich isotherms, were used to study the adsorption behavior. Langmuir's adsorption isotherm model is based on monolayer adsorption onto a homogeneous adsorbent surface and finite adsorption site assumptions without any interactions between adsorbed molecules on neighboring sites. The Freundlich isotherm model is based on multilayer adsorption that takes into account the adsorbent surface heterogeneity and an exponential distribution of the active sites. The concentrations of metal ions ranged from 5 to 100 mg L^{-1}, pH 4, 1.0 g L^{-1} adsorbent and 6 hours contact time at constant room temperature were investigated for the adsorption model. The adsorption capacity and behavior of heavy metals onto biochar were evaluated by using the experimental data fitted with linearized form Langmuir (5) and Freundlich (6) equations. The formulas of the two models are shown below:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{Q_m K_L} \quad (5)$$

Where C_e (mg L^{-1}) is the metal ion concentration in solution at equilibrium, Q_e (mg g^{-1}) is the quantity of metal ion adsorbed on adsorbent at equilibrium, Q_m (mg g^{-1}) is maximum adsorption capacity, K_L (L mg^{-1}) denotes the Empirical Langmuir constant. Plotting C_e/Q_e versus C_e (5) results in a straight line that Q_m and K_L are calculated from the slope ($1/Q_m$) and intercept ($1/Q_m K_L$), respectively.

$$logQ_e = logK_F + \frac{1}{n} logC_e \quad (6)$$

In which, K_F (L g^{-1}) denotes the Empirical Freundlich constant relating to adsorption capacity, n is Freundlich constant as the heterogeneity related to adsorption intensity. The linear plot of log Q_e versus log C_e is evaluated for Freundlich adsorption model that K_F and n are computed from intercept and slope, respectively.

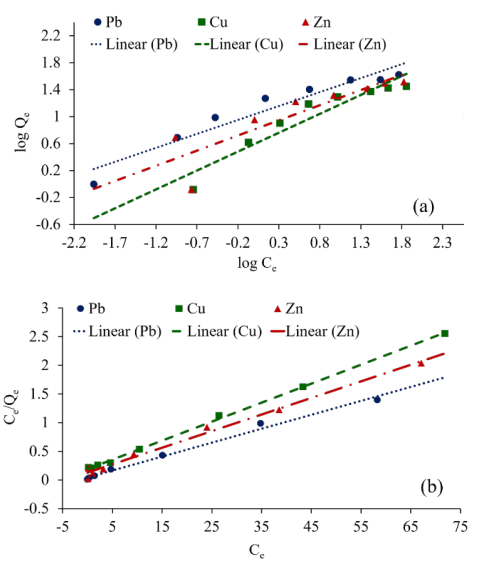

Fig. 7 Adsorption isotherm (a) Freundlich isotherm model (b) Langmuir isotherm model

The curve fitting of two isotherm models acquired in this work are presented in Fig. 7 while the isotherm data obtained are shown in Table 2. The sorption isotherms of Pb(II), Cu(II) and Zn(II) by bamboo biochar recommend, were better fitted to the Langmuir model than the Freundlich model, which can be explained by the correlation coefficients (R^2) 0.990-0.998 for Langmuir model and 0.929-0.772 for Freundlich model. According to the Langmuir model, the maximum adsorption capacities (Q_m) values of Pb(II), Cu(II) and Zn(II) were 41.15, 30.21, and 34.48 mg g^{-1}, respectively.

Table 2 Langmuir and Freundlich isotherm parameters for adsorption of Pb(II), Cu(II) and Zn(II) on biochar

Metal ion	Freundlich model			Langmuir model		
	K_F (g mg^{-1} min^{-1})	*n*	R^2	K_L (L g^{-1})	Q_m (mg g^{-1})	R^2
Pb (II)	10.76	2.415	0.929	1.835	41.15	0.991
Cu (II)	3.91	1.782	0.900	0.173	30.21	0.998
Zn(II)	2.82	2.222	0.772	0.228	34.48	0.990

A comparison of the adsorption capacity of biochar developed in this study with some other adsorbents reported in the literature is presented in Table 3. It was found that the low-cost bamboo biochar produced by the community kiln performed well in comparison with other adsorbents. As a result of the production process at a high temperature of about 600°C, that had an effect on biochar porous structure, more micropores and mesopores corresponded to a larger surface area (Angthararuk et al., 2022). However, our biochar's heavy metal adsorption efficiency was inferior compared to the commercial biochar which claims higher temperature production, meanwhile it has a high cost. The low-cost biochar derived from bamboo waste is suitable as an alternative adsorbent for heavy metals removal in water. In order to show the applicability of low-cost biochar, the investigation of adsorbents to remove metal and organic contaminants from wastewater by continuous flow experiments will be conducted in future work.

Table 3 Metal ions adsorption results from this study compared to Metal ions adsorption results in similar studies

Metal	Precursor material	Adsorption capacity (mg g^{-1})	References
Pb (II)	Bamboo waste	41.25	This study
	Bamboo*	52.12	commercial
	Corn straw modified with $MgCl_2$	5.15	Huang et al. (2020)
	Pomelo peel	21.09	Wu et al. (2021)
	Clostridium powder	9.11	Liu et al. (2021)
	Rice straw	31.15	Sakhiya et al. (2022)
Cu (II)	Bamboo waste	30.21	This study
	Bamboo*	43.29	commercial
	Bamboo modified by $FeCl_3$	27.5	Zhang et al. (2021)
	Rice husk	21.00	Zhang et al. (2011)
	Apple tree branches	11.41	Zhao et al. (2020)
	Pomelo peel	8.17	Wu et al. (2021)
Zn (II)	Bamboo waste	34.48	This study
	Bamboo*	41.65	commercial
	Bamboo	7.62	Van Hien et al. (2020)
	Rice husk	3.82	Van Hien et al. (2020)
	Wood	4.02	Van Hien et al. (2020)
	Apple tree branches	10.22	Zhao et al. (2020)
	Pomelo peel	4.41	Wu et al. (2021)
	Rice straw	32.81	Sakhiya et al. (2022)

* Bamboo biochar purchased from bambooreform, Thailand.
Source: http://www.bambooreform.com

7. Applied bamboo biochar for heavy metal removal in groundwater

Batch experiment for removal of heavy metals in groundwater with samples being collected from Hin Tung, Mueang Nakhon Nayok District, Nakhon Nayok Province, Thailand (14°15′03″N 101°18′18″E) during September 2021. In this work, the heavy metals such as arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), and zinc (Zn) in the groundwater were determined by using an ICP-OES instrument. A four-point calibration curve for each element was created with correlation coefficients of more than 0.999 for quantification. The results found that a concentration of Mn and Cu were 6.74 and 0.068 mg L^{-1}, respectively. While Pb and Zn were less than 0.01 mg L^{-1} moreover As and Cd were not detected. The results revealed that Mn levels in the water exceeded Thailand's groundwater quality standards. Manganese is not hazardous to health but manganese can cause water to have an unpleasant taste, odor, color, and brownish-black stains (Krishnakumari et al., 2018). The batch test for heavy metal removal in groundwater found that bamboo biochar was able to eliminate Mn more than 95% with an hour contact time while other heavy metals were completely eliminated. The breakthrough data obtained from this study can be utilized to design a point of using low-cost biochar as a filter that would be able to effectively remove heavy metals from groundwater.

Conclusion

Accordingly, this study demonstrates once more that the biochar derived from bamboo handicraft waste by using a simple and low-cost method can be an excellent adsorbent for removing heavy metals from aqueous solutions. The Pb(II), Cu(II) and Zn(II) were

used as representative metals in this experiment and the results showed that biochar had a greater adsorption capacity. The removal efficiency of Pb(II), Cu(II) and Zn(II) were more than 80, 60 and 70 %, respectively, at 20 mg L^{-1} initial concentration, pH 4, 120 min contact time and 1.0 g absorbent L^{-1}. The adsorption kinetics and behavior were suitably described following the pseudo-second-order kinetic and Langmuir models, respectively, indicating two reactions appear; adsorption rate was rapid in the first stage and then slow to equilibrium adsorbing via monolayer chemical adsorption. Moreover, the function group as C=O, C-O and O-H in the biochar may be essential to metal adsorption, mainly cations can form O-M (M=Pb, Cu and Zn) complexes. Applying the bamboo biochar in the removal of heavy metals in groundwater was successful. Therefore, more research is needed for the application of biochar usage in full-scale systems.

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Evaluation of *Terminalia chebula* Retz. Extract against Caries-associated Bacteria as an Alternative Compound for Oral Care Products

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Terminalia chebula Retz., *Streptococcus mutans*, Dental caries, Biofilm

Abstract

The objective of this study was to investigate the efficiency of *Terminalia chebula* Retz. fruit extract for further use as an active ingredient in oral care products. The quantification of major constituent, the antioxidant activity and antibacterial activity of an extract against *Streptococcus mutans* in both planktonic and biofilm form were measured. The results showed that ellagic acid was the major constituent of an extract and the amount of ellagic acid contained in the extract was 36.671 mg/g. The extract exhibit free radical scavenging activity with IC_{50} value of 0.44±0.01 mg/mL by DPPH assay. For antibacterial activity against *S. mutans, T. chebula* Retz. extract possess high inhibitory effect on *S. mutans* in both planktonic form and biofilm form. The MIC_{50} and MBC value of an extract against planktonic form of *S. mutans* were 0.47±0.28 and 6.25 mg/mL, respectively. For biofilm form of *S. mutans,* the extract has a high capability to preventing biofilm formation and eradicating the existing biofilm. The $MBIC_{50}$ and $MBEC_{50}$ of an extract against biofilm form of *S. mutants* were 4.47±0.32 and 9.64±0.39 mg/mL, respectively.

Introduction

Dental caries is one of the most prevalent infectious diseases which results in destruction of dental hard tissue of both adults and children. It is the most common cause of tooth loss and pain in the oral cavity. Dental caries is a multifactorial disease caused by the interaction of a dental plaque and host factor including teeth, saliva and diet containing sugar (Mathur & Dhillion, 2018; Sim et al., 2016). Among the microorganisms of the oral cavity, *Streptococcus mutans* is considered to be an important pathogen of dental caries. Several studies demonstrated that *S. mutans* is a major cariogenic microorganism especially in regard to

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disease onset. This bacteria is capable of binding to tooth surface by producing an extracellular polysaccharide from sucrose using glucosyltransferase enzyme which promote local accumulation of microbes on the teeth and leads to biofilm formation (Koo et al., 2003). Furthermore, *S. mutans* species are acid resistance and are able to produce organic acid including lactic acid, formic acid and acetic acid during metabolism of fermentable carbohydrate. The acid produced by this bacteria can cause demineralization of tooth enamel and if this process progresses long enough, the end result is a cavity (Colak et al. 2013; Lee, 2013).

Practicing a good oral hygiene routine to remove dental plaque such as brushing, flossing and rinsing is the best way to prevent dental caries and periodontal disease. Chlorhexidine is the most effective anti-plaque agent that has been widely used in antiseptic oral rinse (Brookes et al., 2020). However, several studies demonstrated that chlorhexidine caused a number of adverse effects including taste changes, tooth staining, sore mouth and/or throat, tongue irritation and wheezing/ shortness of breath (Brookes et al., 2020; Sakaue et al., 2018; Van Strydonck et al., 2012). Therefore, natural product such as plant extracts have been attracting much attention as a promising alternative substances for dental caring.

Terminalia chebula Retz. belonging to the family Combretaceae. *T. chebula* Retz. fruit has high contents of phenolic compounds including phenolic, tannin and flavonoid (Nigam et al., 2020). The chief constituents of phenolic are gallic acid, ellagic acid and hydroxy cinnamic acid (Juang et al., 2004; Lee et al., 2017b). The tannins of *T. chebula* Retz. are hydrolysable type and the main compounds among tannin are terflavin A, terchebulin, punicalagin, chebulagic acid, chebulinic acid and corilagin (Juang et al., 2004; Lee et al., 2017b; Lin et al., 1990). Flavonoid found in *T. chebula* Retz. fruit are rutin, quercetin, luteolin, isoquercitin and methylated derivative of quercetin (Kumar et al., 2012; Prakash et al., 2012).

T. chebula Retz. fruit has been used as a medicine in many Asian countries from ancient times. The use of *T. chebula* Retz. fruit for treatment includes chronic diarrhea, gastroenteritis, constipation, asthma, dyspepsia, ulcer, cough, skin disease and antiparasitic (Nigam et al., 2020). Several studies reported that *T. chebula* Retz. fruit has various pharmacological activities such as antibacteria (Bag et al., 2009; Bag et al., 2012; Kannan et al., 2009), antifungal (Barazani et al., 2003), antiinflammation (Nair, et al., 2010), antioxidant (Lee et al., 2007a), antidiabetic (Sabu & Kuttan, 2002), anticaries (Rekha et al., 2014), antiproliferative (Saleem et al., 2002) and hepatoprotective activity (Tasduq et al., 2006). A recent study reported that ethanolic *T. chebula* Retz. extract has ability to inhibit the growth of *S. mutans* and can be considered as a promising antibacterial and anti-oral inflammatory agent capable of preventing the development of gingivitis and periodontitis. However, information of the antibiofilm activity of *T. chebula* Retz. extract against *S. mutans* is still limited.

In the present study, we examined the chemical composition and biological activities of ethanolic *T. chebula* Retz. extract including free radical scavenging activity, antibacterial activity against both planktonic form and biofilm form of *S. mutans*.

Materials and methods

1. Preparation of *T. chebula* Retz. extract

The mature *T. chebula* Retz. fruit were collected in November 2018 from Loei Province, Thailand. The plant species was confirmed by the National Park, Wildlife Conservation Department, Ministry of Natural Resources and Environment, Thailand. The fruits were washed thoroughly in tap water and the seeds were separated. The pericarp of the fruit were dried in a hot airoven at 50ºC and were grounded to fine powder using a grinder. Extract of dried *T. chebula* Retz. fruit was prepared by maceration in 70% of ethanol. Briefly, 25 g of *T. chebula* Retz. fruit powder were macerated in 150 ml of 70% ethanol with occasional shaking at room temperature and kept for 24 hours and then was filtered through a Whatman No.1 filter paper. The process was repeated twice using the remaining residues. The pooled filtrate was concentrated using a vacuum rotary evaporator. The extract was then freeze dry and stored in a tight container protected from light in a refrigerator at 4ºC until further use. The yield of extract was calculated using the following equation:

Yield (%) = (Weight of *T. chebula* Retz. fruit extract x 100) / Weight of *T. chebula* Retz. fruit powder)

2. HPLC analysis

According to the previous report, phenolic compound was identified from the fruit of ethanolic *T. chebula* Retz. extract (Prompamorn et al., 2022). In the present study, the chemical constituent presented in the extract

was determined using a high performance liquid chromatography (HPLC). The separation was done in a Verticep^{TM} GES C8 column (4.6 x 250 mm, 5µ particle size), mobile phases consisted of (A) 0.05% trifluoroacetic acid in water, and (B) 0.05% trifluoroacetic acid in acetonitrile using a gradient elution and the flow rate was set at 1.0 mL/min with the controlled temperature at 40º C. Photo diode array 190-400 nm detector was set at the wavelength of 259 nm and injection volume was 10 µL for sample and reference standard (gallic acid and ellagic acid).

3. free radical scavenging activity of *T. chebula* Retz. extract

The free radical scavenging activity of an extract was performed by DPPH assay as described by Yarnpakdee et al. (2015) with slight modification. Briefly, 0.1 mM DPPH (100 µL) in 95 % ethanol was mixed with the sample solution (100 µL) at various concentrations. The mixture was incubated at room temperature for 30 minutes in the dark and the absorbance was measured at 517 nm using the microplate reader. L-ascorbic acid was used as a positive control. The percent DPPH• scavenging activity was calculated using the equation:

$$\text{Inhibition (\%)} = [(\text{Acont} - \text{Atest})/\text{Acont}] \times 100$$

Where Acont and Atest are the absorbance of control reaction and test samples, respectively. Free radical scavenging activity was expressed as IC_{50}, defined as the concentration of the sample required to inhibit 50% of the initial DPPH concentration. The test was carried out in triplicate.

4. Bacterial strain and growth conditions

S. mutans ATCC 25175T was grown on brain-heart infusion agar (BHA) supplemented with 2 % glucose at 37°C. For the preparation of planktonic cultures, colonies were picked and resuspended in brainheart infusion broth (BHB) supplemented with 2 % glucose and the culture was then incubated at 37°C in a shaker incubator for 3–6 hr. until the culture attained a turbidity of 0.5 McFarland Unit.

5. Antibacterial activity of *T. chebula* Retz. extract

The antibacterial activity of an extract was performed by agar well diffusion assay according to Ahmed & Beg (2001) with slightly modification. In brief, the broth culture of a mid-log phase of *S. mutans* (approximately 10^7 cfu/mL) was prepared using a brain heart infusion broth (BHI) supplemented with 2 % glucose at 37°C with shaking at 200 rpm. Then, the bacteria suspension was inoculated on BHI agar plate using sterile cotton swab. Subsequently, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer and a 40 µL of a different concentration of *T. chebula* Retz. extract solution was introduced into the well. The agar plate was then incubated at 37°C for 16-18 hr. 0.5% chlorhexidine was used as a positive control while BHI broth was used as a negative control. Antimicrobial activity was evaluated by measuring inhibition zone diameters. The experiment was performed in triplicate. One way ANOVA was used to calculated the significance of the difference between the experiment and the control sample. Difference were considered significant at $P < 0.05$.

6. Minimal inhibitory and minimal bactericidal concentration of *T. chebula* Retz. extract

Minimal inhibitory concentration (MIC) of *T. chebula* Retz. extract against *S. mutans* was tested using the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution assay. The test was performed in a flat-bottom 96-well microtiter plates. *T. chebula* Retz. extract solution was two-fold serially diluted with BHI ranging from 0.097-25 mg/mL and then 100 µL of each concentration of extract solution were given in each well containing 90 µL of BHI broth. Subsequently, 10 µL of working inoculum suspension ($5x10^5$ CFU/mL) was added to the wells. The negative control consisted of BHI broth and inoculum, and the blank control consisted only the BHI broth. The plate was then incubated for 24 hr. at 37ºC. To assess the cell growth, the absorbance of each sample was measured at 600 nm using a microtiter plate reader and compared with the absorbance of negative control. Then, MIC_{50} was calculated by standard plate count method. The MBC was performed by placing each sample dilution on BHA and the plate was then incubated for 24-48 hr. at 37ºC. The lowest concentration of extract that showed no visible growth was taken as the MBC. The experiments were performed in triplicate.

7. Inhibition of biofilm formation of *T. chebula* Retz. extract

The effect of a *T. chebula* Retz. extract on biofilm formation of *S. mutans* was determined using the method described by Mai Nguyen et al. (2017). In brief, 100 µL of the two-fold serial diluted of *T. chebula* Retz. extract ranging from 0.195-100 mg/mL was prepared in the flat-bottomed 96-well plates. Then, an equal volume of *S. mutans* ($1x10^6$ CFU/mL) was added into the well and the plate was incubated for 24 hr. at 37ºC. 0.1%

chlorhexidine was used as a positive control, phosphate buffered saline was used as a non-treated control and BHI broth was used as a blank control. After the incubation, supernatants were discarded and washed three times with phosphate buffer saline to remove non-adherent bacteria. The biofilms that formed in the wells were then stained with 0.1% crystal violet for 10 min. Then, the excess stain was removed by washing three times with phosphate buffered saline and the bound crystal violet was then solubilized in 200 µL of 33% acetic acid per well. The absorbance at 590 nm was then measured. The mean of the three replicates was calculated after subtraction of the blank measurement and the results were expressed as a percentage of biofilm in relation to the untreated control. 50% and 90% of minimal biofilm inhibition concentration ($MBIC_{50}$ and $MBIC_{90}$) were then calculated. Each assay was carried out independently three times.

8. Eradication of biofilm formation of *T. chebula* Retz. extract

The eradication of biofilm formation of *T. chebula* Retz. extract against *S. mutans* was determined according to Teanpaisan et al. (2014). In brief, 200 µL *S. mutans* ($1x10^6$ CFU/mL) was inoculated into each well of the flat bottom 96 well microtiter plate and incubated for 24 hr. at 37ºC to prepare the biofilm. After biofilm formation, the medium was carefully discarded. The non-adherent cells were then washed three times with sterile phosphate buffered saline. After that 100 µL of the two-fold serial diluted of *T. chebula* Retz. extract ranging from 0.195-100 mg/mL was added to the biofilms and incubated at 37ºC for 24 hr. The adherent bacteria were then washed three times with sterile phosphate buffered saline. The numbers of surviving bacteria were stained with 0.1% crystal violet for 10 min. Then, the excess stain was removed by washing three times with phosphate buffered saline and the bound crystal violet was then solubilized in 200 µL of 33% acetic acid per well. The absorbance at 590 nm was then measured. The mean of the three replicates was calculated after subtraction of the blank measurement and the results were expressed as a percentage of eradication of biofilm formation. 50% and 90% of minimal biofilm eradication concentration ($MBEC_{50}$ and $MBEC_{90}$) were then calculated. 0.1% chlorhexidine was used as a positive control, phosphate buffered saline was used as a non-treated control and BHI broth was used as a blank control. Each assay was carried out independently three times.

9. Statistic analysis

Data were expressed as mean and standard deviation (S.D.) by computational analysis from triplicate independent experiment. Statistic comparisons were made by one way ANOVA. $P < 0.05$ was considered statistically significant.

Results and discussion

1. Preparation of *T. chebula* Retz. extract

T. chebula Retz. fruit extract appeared as dark-brown powder. The crude extract was shown in Fig. 1. The yield of an extract was 55.86%.

Fig. 1 *T. chebula* Retz. fruit extract

2. HPLC analysis

The chromatograms of ellagic acid standard compound, gallic acid standard compound and *T. chebula* Retz. fruit extract ware shown in Fig. 2. The quantification of major constituent of *T. chebula* Retz. fruit extract by HPLC indicated that ellagic acid was the major constituent of an extract. From the chromatogram, the content of ellagic acid and gallic acid in the extract was 36.671 mg/g crude extract and 14.542 mg/g crude extract, respectively. The high amount of ellagic acid and gallic acid found in the extract are in agreement with previous research (Lee et al., 2017b; Saha & Verma, 2018). However, in the present study, the content of ellagic acid was higher than reported previously (Pfundstein et al., 2010). This difference may be due to the difference in the extraction process. Several studies reported that both ellagic acid and gallic acid possess various pharmacological properties such as antioxidant, antiinflammation and antimicrobial (Rios et al., 2018; Sarjit et al., 2015; Yang et al., 2015; Yan & Zhou, 2020).

3. Free radical scavenging activity of *T. chebula* Retz. extract

The free radical scavenging activity of *T. chebula* Retz. fruit extract was performed by DPPH assay which is simple, rapid, inexpensive and widely used method to

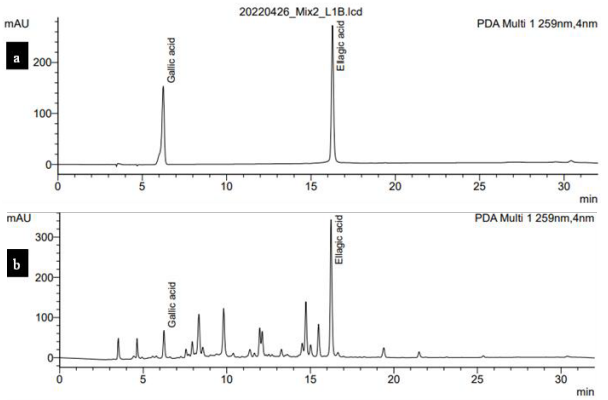

Fig. 2 High-performance liquid chromatography chromatogram of (a) gallic acid and ellagic acid standard and (b) *T. chebula* Retz. fruit extract

estimate an antioxidant activity of natural products (Kedare & Singh, 2011). According to the experiment, the extract exhibit antioxidant activity with IC_{50} value of 0.44±0.01 mg/mL while the IC_{50} value of a positive control, L-ascorbic acid 0.0586±0.002 mg/mL which are in agreement to previous studies. The antioxidant activity of an extract probably exerted by ellagic acid which is considered one of the major antioxidant molecules. The chemical structure of ellagic acid containing two lactones and four hydroxyl groups, enables scavenging a wide variety of reactive oxygen species (Ratnam et al., 2006). Several studies reported that polyphenol from extract of plants can enhance anticaries activity by elimination of oxidative stress which can lead to inflammatory processes and gingivitis (Pytko-Polończyk et al., 2021)

4. Antibacterial activity of *T. chebula* Retz. extract

A preliminary efficacy assessment of *T. chebula* Retz. fruit extract against *S. mutans* was performed using the agar well diffusion assay. The antimicrobial effect was detected by the formation of an inhibition zone around the well. Results obtained in this assay showed that *T. chebula* Retz. fruit extract exhibited strong inhibitory activity against *S. mutans* and the degree of inhibition increased with concentration (Fig. 3). The antibacterial activity of an extract are shown in Table 1. The efficiency of *T. chebula* Retz. fruit extract against *S. mutans* in planktonic form was measured by broth microdilution assay. The MIC_{50} value of an extract was 0.47±0.28 mg/mL and the MBC value of an extract was 6.25 mg/mL. The results were in accordance with previous studies which reported that ethanolic extract of *T. chebula* Retz. fruit possess anticaries activity (Aneja & Joshi, 2009; Lee et al., 2017c; Nayak et al., 2014). The exact mechanism of antibacterial of an extract is probably exerted by ellagic acid, the major constituent of *T. chebula* Retz. extract according to the present study. The mechanism of which may be associated with reducing bacterial adhesion, biofilm formation and destroying bacterial cell membrane (Loo et al., 2010; Miklasińska-Majdanik et al., 2018; Yan & Zhou, 2020).

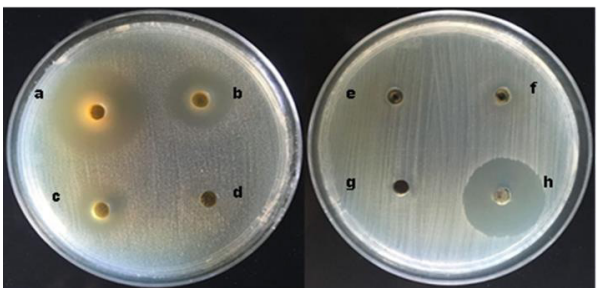

Fig. 3 Zone of inhibition of *S. mutans* tested with *T. chebula* Retz. extract at a concentration of (a) 100 mg/mL, (b) 50 mg/mL, (C) 25 mg/mL, (d) 12.5 mg/mL, (e) 6.25 mg/mL, (f) 3.125 mg/mL, (g) negative control and (h) positive control

Table 1 Antimicrobial properties of *T. chebula* Retz. fruit extract against *S. mutans* using agar well diffusion assay

Concentration of *T. chebula* Retz. fruit extract	Zone of inhibition (mm.± SD)
100	1.60±0.01[a]
50	1.37±0.02[a]
25	0.8±0.04[a]
12.5	-
6.25	-
0.2% chorhexidine	2.93±0.04[b]

Remark: (-) indicates for no inhibitory effect, experiment with 3 replicates. The different superscript letter in the same column represents significant difference when compared with each concentration in variables at $p<0.05$ (one way ANOVA)

5. Antibiofilm activity of *T. chebula* Retz. extract

Strong production of biofilm is an important virulent factor of *S. mutans*. The ability of *T. chebula* Retz. fruit extract to inhibit biofilm formation and eradication of biofilm of *S. mutans* were measured and the results are shown in Table 2. Results reveled that the extract has efficacy to inhibit the biofilm formation and eradication of biofilm of *S. mutans*. The concentration of $MBIC_{50}$ and $MBIC_{90}$ of an extract against *S. mutans* biofilm were 4.47±0.32 mg/mL and 79.32±1.13 mg/mL, respectively (Fig. 4). Whereas the concentration of $MBEC_{50}$ and $MBEC_{90}$ of an extract were 9.64±0.39 mg/mL and 151.70±0.88 mg/mL, respectively (Fig. 5). For positive control, the concentration of chlorhexidine at 0.1% showed 100% of inhibition of biofilm formation and

eradication of biofilm of *S. mutans* when compared to the control group. According to the present study, the antimicrobial effect of *T. chebula* Retz. extract on *S. mutans* was found to be less for the biofilm form than the planktonic form at approximately 10% which is in accordance with previous reports. These results are in agreement with previous researchers. The experiments conducted by Davies (2003) and Verderosa et al. (2019) indicated that bacteria in biofilms are inherently more tolerant to antimicrobial treatment when compared directly to planktonic cells of the same strain. In the biofilm form, bacteria has a limited growth rate and has lower metabolism rate than the planktonic form, therefore the bacteria are less susceptible to the antimicrobial agents and are protected from the antimicrobial action (Gilbert et al., 2002; Shemesh et al., 2007; Singh et al., 2017). Furthermore, the biofilm obstructed the penetration of antimicrobial substances from outside (Bowen & Koo, 2011). Shemesh et al. (2007) and Svensäte et al. (2001) have also demonstrated that *S. mutans* cells which form biofilm exhibit a different expression of some proteins in comparison to planktonic culture, for example, an increasing of exopolyphosphatase expression and decreasing of lactate dehydrogenase or pyruvate kinase expression.

Table 2 Inhibitory efficacy of *T. chebula* Retz. fruit extract against planktonic form and biofilm form of *S. mutans* using broth microdilution assay

***S. mutans* form**	**Inhibitory efficacy of *T. chebula* Retz. fruit extract against *S. mutans* (mg/mL)**					
	MIC_{50}	**MBC**	**$MBIC_{50}$**	**$MBIC_{90}$**	**$MBEC_{50}$**	**$MBEC_{90}$**
planktonic	0.47±0.28	6.25	-	-	-	-
Biofilm	-	-	4.47±0.32	79.32±1.13	9.64±0.39	151.70±0.88

Remark: (-) indicates for no test, experiment with 3 replicates

Conclusion

This study suggested that *T. chebula* Retz. fruit has highly effective antibacterial activity against both planktonic form and biofilm form of *S. mutans*, an important pathogen of dental caries. The extract acts as an antibiofilm agent by preventing biofilm formation and eradicating the existing biofilm. The extract also possess free radical scavenging activity. Therefore, the extract has a potential and might be further used as an active ingredient in oral care products for prevention of dental caries in terms of biofilm formation and eradication of biofilm caused by *S. mutans*. However, the toxicity and the mechanisms of action against *S. mutans* of an extract needs further study and evaluation.

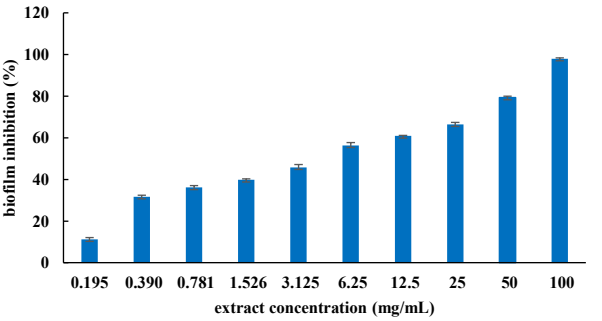

Fig. 4 Inhibition of biofilm formation of *S. mutans* by *T. chebula* Retz. extract at various concentration

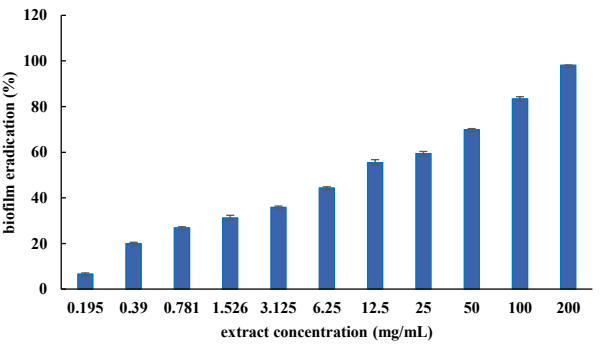

Fig. 5 Eradication of biofilm of *S. mutans* by *T. chebula* Retz. extract at various concentration

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Torch Ginger (*Etlingera elatior* (Jack) R.M. Smith.) Tea and the Effects of Brewing Time on Color, Volatile Compounds, Chemical Compositions and Sensory Quality

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Abstract

The torch ginger tea made from flower of *Etlingera elatior* (Jack) R.M. Smith. It is a type of herbal tea (tisanes) with health benefits. The composition of volatile aroma compounds is one of the factors which induce relaxation and charming scent. The purpose of this study was to profile volatile compounds of the torch ginger tea and quality on physical, chemical, and sensory properties of torch ginger tea. The torch ginger tea infusion was prepared by adding 2 and 5 g of torch ginger tea into 100 ml of hot water (95°C) infused for 5 and 10 min and is effected by quantity and brewing time. Results showed that volatile compounds of the dried petal of the torch ginger had the highest abundance that included α-pinene, 1- dodecanol and dodecanal, respectively. The odor description were fresh, sweet, pine earthy and woody. Prolonged brewing time and enrichment quantity of the dried petal of the torch ginger decreased L*(lightness) values but increased positive a* (red) and positive b*(yellow) significantly ($p < 0.05$). 5 g torch ginger with 10 min brewing time had the highest chemical content consisting of the total phenolic content (206.42 mg GAE/100 g dry weight), the total anthocyanin content (0.887 mg/100 g dry weight), the ascorbic acid (0.602 mg/100 g dry weight) and DPPH (49.38 % inhibition) were significantly ($p < 0.05$). However, 2 g torch ginger with 10 min brewing time had the chemical content (the total phenolic, the total anthocyanin, the ascorbic acid and DPPH) as well as with the 5 g torch ginger tea with brewing time at 5 min. The sensory properties showed 5 g torch ginger tea with 10 min brewing time had high scores for color, flavor, taste and over all acceptance. Finally, 5 g torch ginger and 10 min brewing time was recommended as the best condition.

Introduction

The World Health Organization (WHO) declared COVID-19 a global pandemic in March 2020. After two years, many countries were able to control the spread of COVID-19 through various measures and medical care. The COVID-19 pandemic had a great effect on people's lifestyle having to lockdown by staying and working

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from home. This situation of the lockdown was stressful for all people, effecting work routines, sociability, and transforming eating habits. The trends in coffee and tea consumption during the COVID-19 pandemic have shown that tea consumption increased to support health. (Castellana et al., 2021). Popular teas are made from the leaves of the *Camellia senenisis* plant such as green tea, oolong tea and black tea. However, Tisanes or Herbal teas do not originate from the *Camellia Sinensis* plant but are made from combinations of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical elements (Ravikumar, 2014). Tisanes are sources of natural bioactive compounds such as carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins and terpenoids, including the antioxidant that enhance the overall health (Chandrasekara & Shahidi, 2018). Tisanes has been used for its medicinal properties as method to help induce relaxation, digestive problems or curb stomachaches and also to boost the immune system. The famous herbal teas are chamomile tea, ginseng tea, peppermint tea, cinnamon tea, ginger tea (Ravikumar, 2014; Chandrasekara & Shahidi, 2018).

The torch ginger tea is made from the flower of *Etlingera elatior.* (Jack) R.M. Smith. which is kinds of herbal teas refer to Notification of the Ministry of Public Health (No.426) (2021), Thailand, Issued by virtue of Food Act, Re: Tea Infusion. *E. elatior* or the torch ginger is a plant belonging to Zingiberaceae family and recognized for aromatic plants and as a herbal plant native to South-Asian which is found in several countries such as Thailand, Malaysia and Indonesia. Thailand. It is known as Dalaa or kaalaa or kantan in Thailand, Malaysia and Indonesia Kincung. (Susanti et al., 2013). The full bloom of torch ginger may contain up to 20-25 layers (Fig. 1).

Fig. 1 The characteristics of *E. elatior* flower

The torch ginger which is widely used as a cooking herb or eaten raw for its medicinal properties and used as an ingredient for traditional food (Lachumy et al., 2010). The torch ginger flower is one of the Thai ingredients in a traditional dish called "Kao Yam Budu". The consumption of *E. elatior* in food is indicated to have high nutrients and phytochemicals such as fat (0.37%), carbohydrate (2.46%), fiber (0.96%), protein (0.44%), vitamin C content (1.05 mg/100 g), calcium (100 mg/100 g), potassium (194 mg/100 g), total phenolic (2.29 mg GAE/g extracts), total flavonoids (42.50 mg RE/g extracts), DPPH (68.70% inhibition) (Rachkeeree et al., 2018). Prior studies have reported that *E. elatior* were found to have pharmacological activities including anti-microbial, anti-hyperglycemic, anti-hyperuricemic, anti-tumor, anti-inflammatory, anti-larvae, anti-oxidant activity (Srey et al., 2014; Dewi et al., 2016; Chan et al., 2010; Aldi et al., 2020). Besides, the torch ginger inflorescence has been used as an ingredient in cosmetic for skin whitening, anti-aging, wound healing, and lipstick (Nithitanakool et al., 2014; Adliani et al., 2012; Juwita et al, 2018). In addition, tea aroma is determined by the nature of the plant that the composition of volatile aroma compounds is one of the factors that induce relaxation. The most abundant compounds in the opened torch ginger inflorescence included α-pinene, decanal and 1-dodecanol (Zoghbi & Andrade, 2005; Anzian et al., 2017). The fresh torch ginger flower was extracted with 95% ethanol and their odor description were considered fatty, woody and sweet, the most volatile compounds were dodecanal, α-humulene, decanal, β-cayophyllene and α-pinene (Kaprasob et al., 2016). Many factors are considered in preparation of good flavor tea and aroma tea infusion such as temperature of water, and quantification of tea including brewing time. The brewing time effects the diffusion of phytochemicals into the water. The report showed that prolonged brewing time increased the bioactive compounds and antioxidant capacity (Burilllo et al., 2018). The herbal tea and green tea products analyzed the polyphenols and antioxidant activity and reported that increasing volume of water used for infusion had an effect to decreasing of the phenolic compound. The concentrated tea infusion was recommended by consumers for higher antioxidant, resulting in enhancement of tea astringent (Abdullah & Mazlan, 2020). The previous report found that green teas brewed with hot water (100°C) at 10 min and oolong teas brewed with hot water (100°C) at 5 min had the best condition

for higher antioxidant (Kowalska et al., 2021). The optimum temperature and time for brewing kenaf leaves tea (*Hibiscus cannabinus* L.) was 80°C at 10 min extracting the highest antioxidants properties (Chong & Nyam, 2022). Thai herbal teas containing 2 g of each sachet for commercial use were studied for the antioxidant properties. The result showed that banaba (*Lagerstroemia speciose* L.Pers.) had the highest antioxidant properties (Chan et al., 2012). However, there is limited research on the quality of the torch ginger tea. The torch ginger tea could be used as an alternative source of phytochemical and aromatic that is beneficial for health. Thus, the objective of this study were to profile volatile compounds, quality on physical, chemical, and sensory properties of torch ginger tea as affected by quantity and brewing time.

Materials and methods

1. Plant Materials Preparation

The torch ginger flower was provided from Trang Horticultural Research Center, Thailand. The fresh flower was selected at full bloom with no physical defects, washed with water, pulled and dried the petals. The petal of the torch ginger was cut from the inflorescences, washed with water, and dried by hot air oven at 40°C for 4 hrs until moisture content reached below 7%. This drying temperature at 40°C was modified from Taufik et al. (2016). The dried petal of the torch ginger was blended using a commercial kitchen blender (Model BL 335, Waring, Selang, Thailand) then contained in a sachet (Fig. 2).

2. Analysis of Volatile Compounds

Gas chromatography- mass spectrometry (GC-MS) analysis as described by Wijekoon et al. (2013) was performed from the dried petal of the torch ginger (10 g) for analyzing volatile compound. The GC system used a mass selective detector (Agilent 7890B, USA), equipped with HP-5 (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas, injector was 250°C, split flow was 25:1, the oven temperature was maintained at 250°C for 10 min (a rate of 5°C /min), solvent was delayed 1 min, ion source and transfer temperature was 250°C and fragments from 22 to 600 Da.

3. Effect of quantity and brewing time of torch ginger tea

3.1 Physical properties

Reflected color measurement of the torch ginger tea infusion was prepared by adding 5 and 10 g of torch ginger tea into 100 mL of hot water (95°C) infused for 5 and 10 min and cooled to 25°C. Then, each torch ginger tea infusion was measured by Colorimeter (Minalta CR-400 Series, Konica Minolta, lnc., Japan). The sample in combination with the sample holder CR-A505 and specimen holder CM-A96 and a glass cell 10 mm CM-A98.This method was determined by Guzel-Seydim et al. (2021) by The CIELAB L* a* b* system. Results were reported as an average of individual values as L* (lightness), a* (+a =red, -a = green) and b*(+b = yellow, -b = blue).

3.2 Chemical properties

3.2.1 Determination of total phenolic content (TPC)

The total phenolic content in the torch ginger tea infusions were determined by the Folin-Ciocalteu's reagent this method was modified from Dian-Nashiela et al. (2016). The sample (0.1 mL) was mixed with 0.5 mL of 1 mol/l of Folin-Ciocalteu reagent and incubated at room temperature for 3 min. Then, 1.5 ml of 7.5% ($NaCO_3$) sodium carbonate and 7.9 ml distilled water were added into a test tube. The solution was mixed thoroughly and left in the dark for 2 h. The mixture was measured for absorbance at 765 nm by UV-Vis Spectrophotometer (Shimadzu, UV mini-1240, japan). The total phenolic content calculated as milligrams gallic acid equivalent (mg GAE/100g) by using a gallic acid calibration curve.

3.2.2 Determination of total anthocyanin content

Total anthocyanin content was determined using the pH difference method which was modified from Giusti & Wrolstad (2001) and Wijekoon et al. (2011). The torch ginger tea infusions 0.5 ml of the tea was mixed with 3.5 mL of 0.025 M potassium chloride buffer at pH 1. The solution was incubated at room temperature for 15 min. The absorbance was measured at wavelength of 510 and 700 nm by UV-Vis Spectrophotometer. Distilled water was used blank and according to the same method, the tea infusions was mixed with 0.025 M sodium acetate buffer at pH 4.5. The solution was left at room temperature for 15 min. The absorbance at 510 and 700 nm was measured by UV-Vis Spectrophotometer. The total anthocyanin content (mg/100 g dry weight) was calculated following equation:

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 1{,}000}{\epsilon \times l}$$

$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$
MW = the molecular weight of cyanidin-3-glucoside (449.2 g/mol)
DF = the dilution factor
ϵ = the molar extinction coefficient (26,900 L x cm^{-1} x mol^{-1})
l = the cell length

3.2.3 Determination of ascorbic acid or vitamin C content

Determination of ascorbic acid or vitamin C content was modified from Roe et al. (1948) and Sukporn et al. (2019). Briefly, 1 mL of the tea infusions was mixed thoroughly with 10 mL 5% metaphosphoric acid solution. The absorbance was measured at 540 nm by UV-Vis Spectrophotometer with a blank of distilled water. The ascorbic acid was used as standard. The experiment was reported equivalents mg/100g dry weight.

3.2.4 Determination of DPPH radical scavenging assay

The capacity of the torch ginger tea infusions on reduction of free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by Wijekoon et al. (2011). Then, the tea infusions 0.1 mL was mixed with 3.9 mL of methanolic solution of DPPH radical (25 mg/L). The solution was mixed thoroughly and incubated in the dark at room temperature for 30 min. The absorbance was measured at wavelength of 515 nm by UV-Vis Spectrophotometer. The percentage inhibition of DPPH was calculated by the following equation:

$$\text{Percentage inhibition of DPPH} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = the absorbance of the DPPH solution without the tea infusions
A_{sample} = the absorbance of the sample with DPPH solution

3.3 Sensory properties

The acceptance of the torch ginger tea was determined with a 9-point hedonic scale. Fifty untrained panelists evaluated their liking of quality attributes including color, flavor, taste, and overall liking. The 9-point hedonic scale ratings consisted of 9 like extremely, 8 like very much, 7 like moderately, 6 like slightly, 5 neither dislike nor like, 4 dislike, 3 dislike moderately, 2 dislike very much and 1 dislike extremely. The sample of the torch ginger tea was served at 65-70°C. Samples of torch ginger teas were prepared and served for amount of tea and temperature control. Samples of torch ginger tea were prepared with two methods; 2 g and 5 g of torch ginger tea were brewed with 100 mL of hot water for 10 min and 5 min, respectively (Resurreccion, 1998).

4. Statistical Analysis

Factorial in Completely Randomized Design (CRD) was performed to study the effect of quantity and brewing time on the physical and chemical qualities of torch ginger tea. The randomized completely block design (RCBD) was performed to study quality of torch ginger tea on sensory properties. The experiments were done in triplicate. The results were presented as mean values ± standard deviations (S.D.). Analysis of variance (ANOVA) with Duncan's New Multiple's Range Test (DMRT). The differences were determined to be statistically significant at $p < 0.05$.

Fig. 2 The fresh petal of the torch ginger (a), the dried petal of the torch ginger (b) and the torch ginger tea in sachet (c)

Results and discussion

1. Analysis of Volatile Compounds

Result of GC-MS analysis of the torch ginger (*E. elatior*) tea indicated a total of 6 compounds. The highest volatile compounds of the torch ginger tea were α-Pinene, 1-Dodecanol, Dodecanal, respectively. The kind of volatile compound, retention time (RT), percentage composition calculated from percentage area, chemical structure and odor description are presented in Table 1. The torch ginger odor's descriptions were fresh, sweet, pine, earthy and woody. The monoterpene group, α-pinene (54.53% area) were the major component of the torch ginger tea from the dried petal of *E.elatior*. In accordance with Zoghbi & Andrade (2005) and Wijekoon et al. (2011) dodecanol, dedecanal, α-pinene were the major component of the inflorescence oil of *E.elatior* by GC-MS. Kaprasob et al. (2016) reported the highest abundant in fresh *E.elatior* was dodecanal which in this current study the highest abundant in dry *E.elatior* was α-Pinene. Dodecanal which is a type of Aldehyde. The

amount of aldehydes from citrus essential oils was reduced by heat, sunlight, and oxygen during production and storage (Sun et al., 2014). Dodecanal in the torch ginger (*E. elatior*) tea was lower than the fresh torch ginger because the teas were dried from heat. Besides, the torch ginger odor descriptions were fresh, sweet, pine, earthy and woody. According to the results of Kaprasob et al. (2016) fatty, woody and sweet were the odor descriptions in the volatiles of the flower of *E. elatior*.

Table 1 Volatile compounds of the torch ginger (*E. elatior*) tea

Volatile compound	RT	% area	Odor description*
Furfural	3.158	5.39	Almond, bread, caramel, cinnamon, pungent, sweet
α-Pinene	4.836	54.53	Fresh, sweet, pine, earthy, woody
Bicyclo[3.1.0.] hex-2-ene, 4-methylene-1-1 (1-methylethyl)-	5.262	5.96	-
Dodecanal	16.827	13.29	Earthy, waxy, fatty, coconut like
Caryophllene	17.165	3.29	Spicy, woody, terpenic
1-Dodecanol	18.417	17.32	Earthy, soapy, waxy fatty, honey, coconut

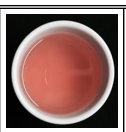
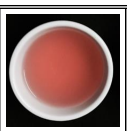
Remark: * http://www.odour.org.uk/, http://www.flavornet.org/flavornet.html

2. Quality of Torch Ginger (*Etlingera elatior*) Tea

2.1. Effect of quantity and brewing time of torch ginger tea on physical properties

Color parameter of the torch ginger (*E.elatior*) tea infusions, lightness (L* values) were significantly decreased ($p < 0.05$) with enrichment quantity of tea and brewing time.(Table 2) The result was in agreement with the L* value of blueberry juices after hot water bath treatment (5 min) were darker than blueberry juices steam treatment (3 min) (Zhang et al., 2019). The 5 g of torch ginger with brewing time at 10 min showed the positive b*(yellow) values and positive a* (red) values increased significantly ($p < 0.05$) and the positive a* (red) values related to the increasing of the total anthocyanin content. In accordance with the results of Zhang et al. (2019) showing the total anthocyanin content of blueberry juice with results showing a significant ($p<0.05$) increase with longer time of steam treatment occurred from 0 to 3 min.

Table 2 Color of the torch ginger (*E.elatior*) tea infusions

Tea infusion				
Color	2 g 5 min	2 g 10 min	5 g 5 min	5 g 10 min
L*	41.87 ± 0.10^{a}	40.61 ± 0.04^{b}	40.84 ± 0.09^{b}	29.21 ± 0.17^{c}
a*	27.05 ± 1.24^{c}	29.35 ± 0.10^{b}	30.48 ± 0.10^{b}	33.60 ± 1.20^{a}
b*	15.00 ± 0.06^{c}	17.58 ± 0.40^{b}	17.23 ± 0.04^{b}	19.94 ± 0.18^{a}

Remark: The results were expressed as average ± standard deviation. The difference letters among quantity and brewing time represented significant difference at $p < 0.05$

2.2 Effect of quantity and brewing time of torch ginger tea on chemical properties

2.2.1 Determination of total phenolic content (TPC)

In this study the total phenolic content of torch ginger tea infusions found in the range of 131.11-206.42 mg GAE/100 g dry weight and showing significant difference ($p<0.05$) among quantity and brewing time of torch ginger tea. The maximum of total phenolic content was recorded in 5 g of torch ginger with brewing time at 10 min and the minimum content was in 2 g of torch ginger with brewing time at 5 minutes. Prior studies reported higher amounts of the total phenolic content at 356 mg GAE/100 g from dried torch ginger flower extracts (Anzian et al., 2017). In this study, the total phenolic content was analyzed from torch ginger tea infusions where the dried torch ginger flower was brewed with hot water, causing this result to be lower than the previous studies where the total phenolic content was analyzed from dried torch ginger flower extracts. The results showed that quantity of torch ginger tea and brewing time significantly affected the total phenolic content ($p<0.05$).

2.2.2 Determination of total anthocyanin content

The total anthocyanin content of torch ginger tea infusion was found in the range of 0.462-0.887 mg/100 g dry weight and showing significant difference ($p<0.05$) among quantity and brewing time of torch ginger tea. The maximum of the total anthocyanin content was recorded in 5 g of torch ginger with brewing time at 10 min and the minimum content was in 2 g of torch ginger with brewing time at 5 min. (Table 3) The total anthocyanin content showed the same as the total phenolic content of torch ginger tea infusion. In accordance with the results of Zhang et al (2019), the effect of thermal pretreatment processing on juice, the total phenolic content and the total anthocyanin content of blueberry juice results showed significant ($p<0.05$) increases in the total phenolic content and the total anthocyanin content when longer time of steam treatment occurred from 0 to 3 min. For this study, the total anthocyanin content of torch ginger tea infusion was found to be higher when longer brewing time occurred. Similarly, with results of Rossi et al. (2003) and Liu et al. (2016) the extraction of anthocyanin and other phenolic compound and color density of fruit increased by heat pretreatment processing.

2.2.3 Determination of ascorbic acid or vitamin C content

The ascorbic acid content of torch ginger tea infusions was found in the range of 0.380-0.602 mg/100 g dry weight and showing significant difference ($p<0.05$) among quantity and brewing time of torch ginger tea. The maximum of ascorbic acid content was recorded in 5 g of torch ginger with brewing time at 10 min and the minimum content was in 2 g of torch ginger with brewing time at 5 min (Table 3). These values were low when compared with the study of Rachkeeree et al. (2018) when the eight edible flowers (ginger family) from Thailand were analyzed for the nutritional composition and phytochemical properties showing the vitamin C content as 1.05 mg/100 g.The vitamin C content found in the torch ginger was due to the vitamin C being analyzed in the fresh torch ginger flowers. Ascorbic acid has been indicated as sensitive to heat treatment.This was found when the fresh Cara Cara juice showed ascorbic acid as 0.47 mg/ml when heat is transferred to juice then ascorbic acid was lost at 68.09% (Lu et al., 2018). In this study the torch ginger was dried by hot air oven allowing thermal processing conditions to increase the ascorbic acid losses. Nevertheless, the ascorbic acid content of the torch ginger tea increased with longer brewing time. According to the results of Um et al. (2020) the optimum time for ascorbic acid extraction from rugosa rose fruit were 30 min. In addition, the vitamin C is beneficial to human health because it is an antioxidant that prevents humans from oxidative stress (Stevens et al., 2007).

2.2.4 Determination of DPPH radical scavenging assay

Percentage inhibition of DPPH of torch ginger tea infusion was found in the range of 29.94-49.38 and showing significant difference ($p<0.05$) among quantity and brewing time of torch ginger tea. The maximum percentage inhibition of DPPH was recorded in 5 g of torch ginger with brewing time at 10 min and the minimum content was in 2 g of torch ginger with brewing time at 5 min (Table 3). Similarly, with results of Rachkeeree et al. (2018), phytochemical properties of the eight edible flowers (ginger family) from Thailand was study that the percentage inhibition of DPPH of torch ginger flowers was found 68.70. The value of the fresh torch ginger was higher than the dry torch ginger in this study because it was heat sensitive. DPPH or the antioxidant capacity related to the total anthocyanin content and the total phenolic content. In accordance with results of Zhang et al (2019) the antioxidant capacity of blueberry juice was related to the anthocyanin content and the total phenolic content.

Table 3 The effects of quantity and brewing time on chemical properties of torch ginger tea

Chemical properties	Quantity (g) and brewing time (min) of torch ginger tea			
	2 g 5 min	2 g 10 min	5 g 5 min	5 g 10 min
Phenolic (mg GAE/100 g dry weight)	131.11±0.51^{c}	164.73±0.08^{b}	165.48±0.19^{b}	206.42±0.38^{a}
Anthocyanin content (mg/100 g dry weight)	0.462±0.0015^{c}	0.642±0.0013^{b}	0.645±0.0007^{b}	0.887±0.0039^{a}
Total ascorbic acid (mg/100g dry weight)	0.380±0.039^{c}	0.429±0.005^{b}	0.436±0.003^{b}	0.602±0.005^{a}
DPPH (% inhibition)	29.94±0.044^{c}	37.80±0.118^{b}	37.88±0.044^{b}	49.38±0.044^{a}

Remark: The results were expressed as average ± standard deviation. The difference letters among quantity and brewing time represented significant difference at $p < 0.05$

2.3 Effect of quantity and brewing time of torch ginger tea on sensory properties

Sensory evaluation of torch ginger tea infusion were prepared by adding 5 and 10 g of torch ginger tea into 100 mL of hot water (95°C) infused for 5 and 10 min, respectively and was served at 65-70°C. The score of sensory evaluation results indicated the color, flavor, taste, and over all acceptance of 5 grams' torch ginger with brewing time at 10 min and receiving the high sensory value for all attribute (Table 4).

Table 4 The effect of quantity and brewing time of torch ginger tea on sensory attribute

Sensory attribute	Quantity (g) and brewing time (min) of torch ginger tea			
	2 g 5 min	2 g 10 min	5 g 5 min	5 g 10 min
Color	6.80 ± 0.40^{b}	7.00 ± 0.40^{b}	7.04 ± 0.49^{b}	7.14 ± 0.46^{a}
Flavor	6.68 ± 0.47^{b}	6.80 ± 0.53^{ab}	6.86 ± 0.53^{ab}	6.98 ± 0.55^{a}
Taste	6.76 ± 0.43^{b}	6.92 ± 0.49^{ab}	6.94 ± 0.51^{ab}	7.10 ± 0.51^{a}
Over all acceptance	6.78 ± 0.42^{c}	6.98 ± 0.43^{b}	7.00 ± 0.45^{ab}	7.16 ± 0.42^{a}

Remark: The results were expressed as average ± standard deviation. The difference letters among quantity and brewing time represented significant difference at $p < 0.05$

Conclusion

The torch ginger (*E.elatior*) tea revealed the highest volatile compounds were α-Pinene, 1-Dodecanol, Dodecanal, respectively. Their odor description were fresh, sweet, pine, earthy and woody. The quantity and brewing time had an affect on color, chemical properties (the total phenolic content, anthocyanin content, ascorbic acid, DPPH) by 5 g of torch ginger with brewing time at 10 min showing the highest chemical content that is

beneficial for human health. Similarly, as sensory evaluation of 5 g of torch ginger with brewing time at 10 min had the best acceptability for color, flavor, taste and over all acceptance.

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Utilizing and Creating Added Value from Commercial Banana: Case Study in Phra Nakhon Si Ayutthaya Province, Thailand

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Abstract

Bananas are an interesting cash crop. The popular banana cultivars that tend to grow commercially in Thailand are Gros Michel banana, Lady Finger banana, Pisang Awak banana (Cultivated banana), and Lebmuernang banana. Banana fruit is a food source rich in nutrients and many important substances. In addition, different parts of the banana can be used and processed to create economic value. Therefore, the knowledge gained from gathering information on banana processing and the feasibility case study of the production of processed products from commercial bananas can be used as a guideline to support and extend the utilization of bananas in various aspects in order to take advantage of local resources as a method to uplift the basic economy to achieve stability, sustainability, increase careers, and income of banana growers and local enterprise.

Introduction

Banana is a tropical plant that is native to Asia. It is mainly found in Southeast Asia, such as Laos, Myanmar, Vietnam, Indonesia, Malaysia, Cambodia, and Thailand, and has continuously spread throughout the world as a cash crop (Department of Agriculture, 2018). Bananas are one of Thailand's most important economic crops. Due to the widespread utilization of different parts of bananas, 15,051,333 kg (equal to 374,388,936 Thai baht) of fresh bananas and processed bananas were exported from Thailand in 2020 (Office of Agricultural Economics, 2020). In Thailand, there are commercially valuable banana varieties such as Pisang Awak banana (Cultivated banana), Gros Michel banana, Lady Finger banana, and Lebmuernang banana (Pengpoo & Jamjang, 2016). Bananas can be eaten either raw or ripe; the fruits are a nutritious food source, content high energy due to their high starch but low-fat content, vitamins included vitamin A, vitamin B6, vitamin C, and minerals included calcium, iron, and potassium, etc. (Phukasmas, 2017). Different parts of bananas can be used in many ways,

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including being used as raw materials in both savory and sweet dishes. Including processing into beverage products, banana juice, banana wine, concentrated banana juice, banana starch, utilization from stems, bracts, leaves, banana stalks, and banana rubber, and using banana waste as raw materials for biogas production. In addition, bananas have various medicinal properties such as constipation, diarrhea, gastritis, etc. (Sangseesod, 2000).

Therefore, this paper offers a collection of knowledge about the guidelines to use and create added value from commercial bananas in Thailand is necessary for community development. A case study on the possibility of adding value to bananas in Sai Noi Sub-district, Bang Ban District, Phra Nakhon Si Ayutthaya Province, was to disseminate preliminary information to those who are interested in a guideline for further utilization of bananas in various aspects.

Characteristics of commercial banana varieties in Thailand

Bananas belong to the family Musaceae, where bananas are grown or edible are classified in section Eumusa and originated from two species of wild bananas, the wild banana (*Musa accuminata* Colla) and the Tani banana (*M. balbisiana* Colla), which are Banana genomes A and B, respectively (Mohapatra et al., 2010; Deepthi, 2016). Planted or edible bananas have 2, 3, and 4 sets of chromosomes (2X, 3X, and 4X) (Valmayor et al., 2000). There are many varieties of bananas found in each region of Thailand. There are differences in the genomes, height, size, shape, and characteristics (Fig. 1 and Table 1). The bananas studied were edible banana groups with 2 sets of chromosomes and AA or 2X (AA) genomes, such as the Sugar banana (*Musa acuminata* 'Kluai Khai, AA Group) with artificial stems no more than 2.5 m tall, approximately 16 cm in diameter (diameter more than 15 cm), the blossom is oval, pointed tip, reddish-purple. Commercial harvest occurs when 70-80% of the fruit is completed and the fruits at 40-50 days after cutting the banana blossoms (Sangudom *et al.,* 2014). There are 7 hands per bunch of bananas, and about 14 fruits per hand, the size is relatively small, 2-3 cm wide, 8-10 cm long, thin peel, bright yellow when ripe, Orange-cream-colored flesh, fragrant, sweet taste. Lebmuernang banana (*Musa acuminata* 'Kluai Lep Mu Nang', AA Group) is an edible banana 2X (AA), psuedostem not higher than 2.5 m, diameter less than 15 cm, relatively long oval, pointed tip, reddish-purple color. Harvesting period occurs when the fruit is about 60 days and after the banana blossom cutting (Youryon & Supapvanich, 2017). There are 7-8 branches per cluster of bananas, one branch has 10-16 fruits, small fruits, 2-2.5 cm wide, 11-12 cm long, fruits curved, tapered at the end, thick peel, golden yellow when ripe, fragrant, yellow flesh, sweet taste. Gros Michael banana (*Musa acuminata* 'Kluai Hom Thong', AAA Group) is an edible banana 3X (AAA) with pseudostems not higher than 2.5-3 m, diameter more than 15 cm, the blossoms are oval, quite long, pointed, reddish-purple color. Harvesting period occurs when the fruit is about 90-110 days after a banana blossom cutting (Chunwijitra, 2015). There are 4-6 branches per cluster of bananas, one branch has 12-16 fruits, large fruit, 3-4 cm wide, 21-25 cm long, thin skin (peel), golden yellow when ripe, the flesh is light orange, fragrant, sweet taste. Pisang Awak banana (Cultivated banana) (*Musa x paradisiaca* 'Kluai Nam Wa', ABB Group) is an edible hybrid banana 3X (ABB) with pseudostem not higher than 3.5 m, more than 15 cm in diameter, the blossoms are oval, pointed, reddish-brown. Harvest time occurs when the fruit is about 110-120 days after a banana blossom cutting. There are 9-12 branches per cluster of bananas, one branch has 10-16 fruits, The fruit is larger than the Lady Finger banana, 3-4 cm wide, 11-13 cm long, the peel is thicker than Lady Finger banana when ripe yellow, white meat, yellow center filling, sweet taste (Suvittawat et al., 2014; Silayoi, 2015).

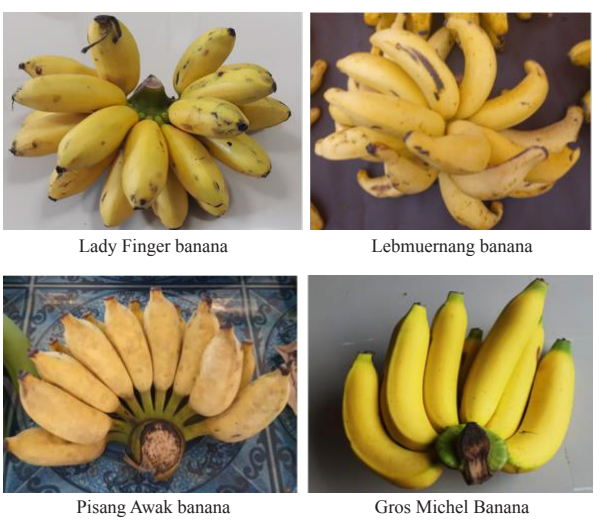

Fig. 1 Characteristics of the commercial banana varieties in Thailand

Table 1 Summarize characteristics of the commercial banana varieties in Thailand

Characteristics/ Varieties	Lady Finger banana 'Kluai Khai	Lebmuernang banana 'Kluai Lep Mu Nang'	Pisang Awak banana 'Kluai Nam Wa'	Gros Michel banana 'Kluai Hom Thong'
Set of chromosomes, Genomes	2X (AA)	2X (AA)	3X (ABB)	3X (AAA)
Harvesting period (days after blossom cutting)	40-50	60	110-120	90-110
Pseudostem high (m.)	2.5	2.5	3.5	2.5-3.5
Pseudostem diameter (cm.)	>15	<15	>15	>15
Blossom shape	oval with pointed tip	oval with pointed tip	oval with pointed tip	oval with pointed tip
Blossom color	reddish-purple	reddish-purple	reddish-brown	reddish-purple
Branches per cluster	7	7-8	9-12	4-6
Fruit per branch	14	10-16	10-16	12-16
Fruit size	small	small	medium	large
Fruit wide (cm.)	2-3	2-2.5	3-4	3-4
Fruit long (cm.)	8-10	11-12	11-13	21-25
Peel (skin)	thin	thick	thick	thin
Peel color at ripe stage	bright yellow	golden yellow	yellow	golden yellow
Flesh color	orange-cream	yellow	white with yellow center	light orange
Fragrant	fragrant	fragrant	fragrant	fragrant
Taste	sweet	sweet	sweet	sweet
References	Sangudom et al. (2014)	Youryon & Supapvanich (2017)	Suvittawat et al. (2014); Silayoi (2015)	Chunwijitra (2015)

Chemical composition from different parts of bananas

Raw bananas contain starch as the main constituent and resistant starch, which is beneficial for the digestive and circulatory systems (Table 2). The resistant starch is not digested by enzymes in the small intestine. Instead, they reach the large intestine and are fermented by microorganisms into short-chain fatty acids such as acetate, propionate, and butyrate, which are beneficial to probiotic microorganisms (Boonkong et al., 2015). Raw banana flour has important biological properties such as antioxidant activity. The analysis of the antioxidant activity of 4 types of raw banana starch, namely, Gros Michel banana, Lady Finger banana, Pisang Awak banana (Cultivated banana), and Lebmuernang banana, found that Lebmuernang banana has the most antioxidant activity, which is 38.63%. When all 4 types of bananas were processed into banana flour, it was found that the raw sugar banana flour had the highest antioxidant activity, at 31.71%. Processing resulted in a decrease in the antioxidant activity of banana starch (Pengpoo & Jamjang, 2016). The chemical composition and antioxidant activity changing when the fruits begin to ripen. The trace chemical composition of ripe Lady Finger bananas and ripe Gros Michel bananas were investigated and found that total soluble solids, the phenolic compounds, sugars, vitamin C, and antioxidant activity tended to increase as the bananas began to ripen at full maturity. After that, it tends to decrease when the bananas enter the maturity stage. A ripe Lady Finger banana has higher antioxidant activity than a ripe Gros Michel banana (Fernando et al., 2014). Other parts of bananas, including banana sheath, banana blossom, banana leaves, and stem, are primarily dietary fiber, which is not digested in the gastrointestinal tract. Therefore, does not give energy. The moisture content of fresh banana plants is 96%. Dietary fibers found in dried banana plants include cellulose, hemicellulose, lignin, and pectin (Li et al., 2010).

Methods for processing and utilizing different parts of bananas

Table 3 shows the methods for processing and utilizing different parts of bananas. Both raw and ripe bananas can be processed into a variety of food products such as fried bananas, banana chips, and sweet bananas in coconut cream. Banana flour, which can be used as a raw material in food production (Nimsung et al., 2007)

Table 2 Chemical composition from different parts of bananas

Bananas	Chemical composition (%)						References
	Moisture	Protein	Fat	Ash	Carbohydrate	Fiber	
Unripe banana flour	6.90	3.60	0.89	3.14	76.77	7.2	Menezes et al. (2011)
Ripe banana flour	9.57-8.17	4.78-4.11	0.42-0.30	4.44-4.65	80.80-82.72	NI	Alkarkhi et al. (2009)
Unripe banana peel	NI	8.6	13.1	15.25	12.78	50.25	Wachirasiri et al. (2009)
Ripe banana peel	NI	7.51	12.44	15.13	14.18	50.74	Weeragul et al. (2015)
Banana blossom	1.76-1.89	1.98-1.29	0.41-0.46	4.19-3.08	93.42-95.17	15.48-15.32	Arya & Sinija (2016)
Banana leaf	NI	11.70	9.60	31.30	NI	21.70	Yeekaew et al. (2015)
Banana pseudo stem	Cellulose 39.12%, holocellulose 72.71%, klason lignin 8.88%, acid-soluble lignin 1.90%, ash 8.20%, pectin 0.27%						Li et al. (2010)

Remark: NI means no information

including the use of bananas to produce both savory and sweet food. In addition to being consumed ripe bananas can also be used as a face mask to add moisture to the peel and reduce roughness on the peel (Hengsawadi, 2014). Banana blossoms can be processed into banana beverages, which contain phenolic compounds and have high antioxidant activity (Amornlerdpison et al., 2016). Fresh and dried banana leaves can be molded into food packaging. Fresh banana leaves are used to make a Baisri or inventions in various worship ceremonies. Factors affecting the quality of fresh banana leaves are temperature and season for harvesting banana leaves (Kwanhong et al., 2017). Banana stems are used as a raw material for both human food preparation and as an ingredient in animal feed as well as in the production of banana rope, paper, bags, or various packaging. Other waste can be used as raw materials in the production of fertilizers, bio-fermented water, and briquette fuels, etc. (Sutthiwilairatana et al., 2017).

Case study of the possibility of creating added value from commercial banana

Economic feasibility study is a study that evaluates returns and costs (Pricing of Benefit and Cost) based on the goal of efficiency of resource utilization for production. The applied price is called "efficiency price" and the collective analysis is called "Economic Cost-Benefit Analysis" (Field & Nancy, 2005).

In studying the economic suitability of a project, the expected benefits from the project are compared with the project's investment cost over the period of analysis, which is calculated in the form of the main economic index, which is discussed as follows Dellarosawati (2020).

Net Present Value (NPV) is a calculation comparing the value of Economic investment in different years with expected economic benefits during the project analysis period using the discount rate converted to the current currency.

Table 3 Example of methods for processing and utilizing different parts of bananas

Bananas	Methods for processing and utilizing different parts of bananas	References
Unripe banana flour/ Ripe banana flour	1) Unripe banana or ripe banana was washed with clean water 2) Dipped in hot water at 65-75°C for 10 mins until tender (for unripe banana) 3) Cooling banana fingers under clean water (23°C) 4) Peeling of the banana 5) Slicing (3-4 mm thick unripe and 2 mm thick ripe slices) 6) Steeping the slices in 0.2% citric acid solution for 10 min and draining of solution 7) Drying the slices in tray dryer at 60°C until the final moisture content 8-10% (wet basis) 8) Grinding and sieving (250 μ) before packaging	Pragati & Ravish (2014)
Extracted pectin from unripe banana peel	1) The unripe banana peel powder was mixed with hydrochloric acid 2) Heated (90±5°C) and stirred before filtration 3) A precipitate of pectin with ethanol and then it was recovered by centrifuge at 5000 rpm for 10 mins 4) The pectin substance was dried in a conventional oven at 65°C until a constant weight	Kamble et al. (2017)
Extraction ripe banana peel for jelly drink	1) Banana peel was cut into small pieces 2) Dried by using tray dryer at 50°C for 24 hours and crushed to make a coarse powder 3) The dried powder was extracted with water (Ratio 1:20) at 25°C for 18 hours 4) The extracts obtained were evaporated to dryness by rotary evaporator, packed and stored at 4°C 5) Jelly drink: extracted banana, milk, sugar, gelling agent, and salt were blended by blender until the mixture was homogeneous. 6) Banana jelly drink were pasteurized at 73°C for 15 second and stored in sterile containers at 4°C.	Rattanatavon et al. (2020)
Dehydrated blossom	1) Collect banana blossoms and remove the bracts 2) Cut banana blossoms and soak 0.2% lactic acid (30 mins) 3) Drain the liquid and place in trays in hot air oven 4) Dry at 60°C for 4 hours and collect dehydrated blossoms	Acharya et al. (2020)
Extracted cellulose from banana pseudo stem	1) The banana stems were cut into small pieces and left to dry in the sun. 2) The dried stems were mashed and sieved with a sieve size of 70 mesh, to obtain a 70 mesh stem powder. 3) The isolation of cellulose involved the following processes, which include liquefaction, delignification, and bleaching	Iliyin et al. (2020)
Banana leaf for food packaging	Banana Leaves are one of the most used materials for natural food packaging with its natural properties of foldable structure and its ability to hold water.	Pratama & Junianto (2021)

Benefit-Cost Ratio (B/C Ratio) is an economic index that shows the ratio of the present value of the benefit to the present value of the project investment. B/C greater than 1 means the project will provide a return that is worth the investment.

The Economic Internal Rate of Return (EIRR) is the discount rate that brings the net present value of a project to zero. This rate represents the percentage return received on the project investment as a percentage of return. It is an application of Economic Feasibility analysis theory concepts such as ENPV, EBCR, EIRR by creating added value from waste materials from upstream banana processing (banana trees, turtle-foot bananas) leading to downstream processing. Processed products from bananas to add value from waste materials The investment period is approximately 5-6 years (determined by the age of the constant factors). The main production costs consist of fixed costs such as gas stoves, pans, kitchen utensils, basket blocks, and variable costs such as raw materials, water, electricity, labor costs, research methodology. This is in line with the literature review of articles related to banana processing such as Puengpho & Boonmeephiphit (2021); Chunud et al. (2020) have chosen to use the project analysis theory as a decision-making tool and assess the worthiness of the investment.

Project sensitivity analysis is the best method for analyzing future outcomes of related events. By evaluating the rate of return-on-investment projects under risk and uncertainty, but also estimating the probability that it will occur (Probability or Expected value) which risk occurrences and economic uncertainty may affect investment decisions. For example, the production models are worthwhile. The changing of the production cost this may result in the unworthiness of production in the future that is economically unstable. Consequently, the sensitivity analysis helps in analyzing production patterns that have the potential to resist price sensitivity and the price of factors of production. Therefore, the sensitivity of the project must be analyzed as follows: 1) Costs increased by 5%, 10%, and 15%, while fixed income 2) Income decreased by 5%, 10%, and 15% while fixed costs, and 3) Income decreased by 5%, 10%, and 15% while costs increased by 5, 10 and 15% (Dellarosawati, 2020). The benefits of sensitivity analysis are to identify variables that cause volatility, net return of a project to help evaluate projects more efficiently, and that assessment should consider whether the return or benefit derived from the decision is worth enough to offset any potential risks.

Sai Noi Community, Bang Ban District, Phra Nakhon Si Ayutthaya Province is a community that has received support from government agencies in using abandoned rice fields for banana cultivation and supporting the value-adding of bananas to processed products of the community, maximizing the use of local resources, creating a career, increasing income, helping to raise the basic economy of the community. “Banana” is a native plant that has a long history within the Sai Noi Community as well as the ability to be used to add value throughout the plant from leaves, blossoms, fruits, and stems.

General information of banana farmers in Sai Noi Community, Bang Ban District

This research is an Area Based Development Research, collection of data with a structured questionnaire with a population of 53 banana farmers in Sai Noi Community. The community has a total banana plantation area of 132.71 rai, covering the area of villages 1-10, with a total of 4 banana varieties, namely Gros Michel banana 80.86 rai, accounting for 60.93%. Followed by Pisang Awak banana (Cultivated banana), 45.15 rai, accounting for 34.02%, Lady Finger banana and Lebmernang banana, 5.70 rai and 2 rai for 4.30% and 0.75%, respectively as Fig. 2.

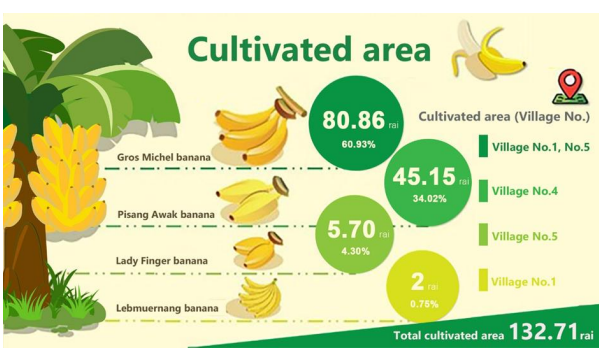

Fig. 2 Basic information on banana cultivation in Sai Noi Sub district, Bang Ban District

The majority of banana farmers were female (72.73%), average age of 61 years, primary education (49.09%), 74.55% of them use personal funds for banana business, most of them do not have any loans, the others were burdened (47.27%), liabilities between 10,000-50,000 baht. The most popular banana varieties cultivated were Gros Michel banana (76.36%), followed by Pisang Awak banana (43.63%). Most of the area was owned by

farmers 83.64%, with an average banana plantation area of 1.56 rai per household (400 plants per rai). Popular to employ 2 workers during the banana planting period, average wage 300 baht per day, 8-day average wage labor, total average wage 3,500 baht per person per production cycle. The Hom Thong banana has an average production cycle of 8 months with an average cultivation cost of 6,585.65 baht per production cycle. The average annual income is 36,352 baht, while the Pisang Awak banana has an average production cycle of 10 months with an average cultivation cost of 7,477.37 baht per production cycle. Average annual income of 72,175 baht.

Cost and return of banana cultivation

1. Gros Michel banana: The production cycle is approximately 8 months, the average cost per plant is 114.5 baht, classified as variable cost; land preparation/planting and sapling cost 25.5 baht per tree, harvesting cost 20 baht per plant, and maintenance cost 5.59 baht per plant and Fixed cost; depreciation of agricultural tools and equipment (pumps, hoses, knives, etc.) 9.5 baht per tree (Fig. 3).

The tree was classified as fresh fruit and can be sold as a whole tree, approximately 125-250 baht, if processed, can generate an additional 240 baht processed into crispy bananas for sale. Banana shoots can be sold at 15-10 baht per shoot, and banana heads at a selling price of 5 baht per kilogram (Fig. 4).

2. Pisang Awak banana (Cultivated banana): The production cycle is about 10 months, the average cost per plant is 122.5 baht, classified as variable cost; land preparation/planting and sapling cost 28 baht per tree, harvesting cost 17 baht per plant, and maintenance costs 65 baht per plant and Fixed cost; Depreciation of agricultural tools and equipment (pumps, hoses, knives, etc.) 2.5 baht per tree (Fig. 5).

The tree was classified as fresh fruit. It can be sold for around 120-200 baht. If processed, it can generate an additional income of 260 baht. The trunk can be processed into a basket and artificial flowers earning additional income of 120 and 15 baht per tree, respectively. Banana leaves can be sold for an average of 20 baht per plant, banana blossom, selling price of 6 baht per kilogram, and banana shoots selling price of 10-15 baht per shoot (Fig. 6).

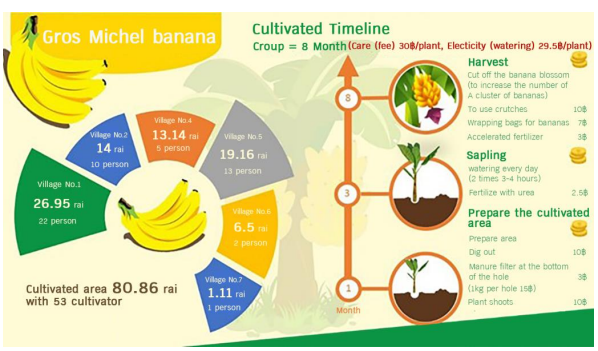

Fig. 3 Information on cultivation of Gros Michel banana in Sai Noi Sub-district, Bang Ban District

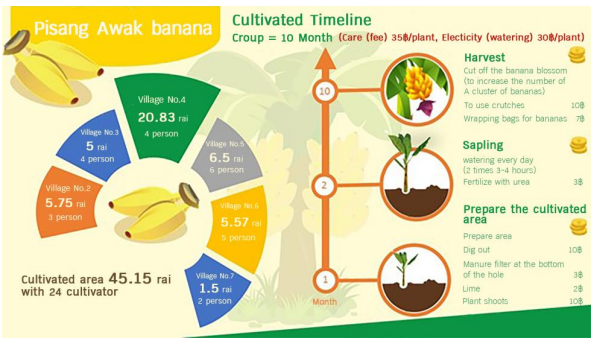

Fig. 5 Information on cultivation of Pisang Awak banana (Cultivated banana) in the study area

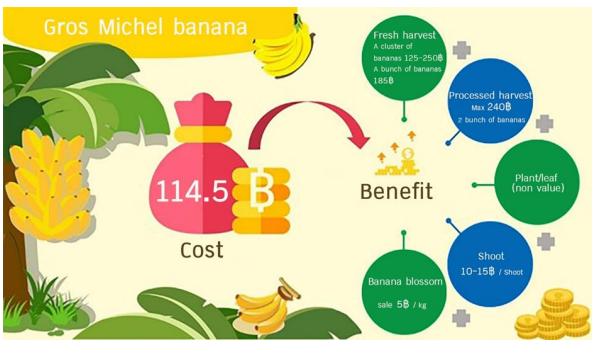

Fig. 4 Cost-benefit of Gros Michel banana production

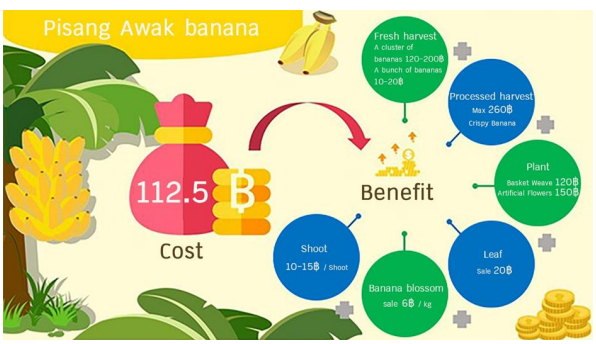

Fig. 6 Cost – benefit of Pisang Awak banana production

Possibility of adding value to banana products

Sai Noi Community prefers to bring products from Gros Michel banana (product size is not standard) and Pisang Awak banana (Cultivated banana) to add value. Including the processing of bananas into crispy banana chips with the production process as shown in Fig. 7. Undeveloped banana hand or Turtle Feet bananas are the 7-8th banana (a group of bananas will have about 8to combs). Usually, farmers eat Turtle Feet bananas for their own consumption within the household or distribute to neighbors or relatives. These bananas do not generate income or cash inflows The cost of producing crispy banana chips is classified as fixed costs such as gas stoves, pans, stainless steel trays, oven cabinets, kitchen utensils,

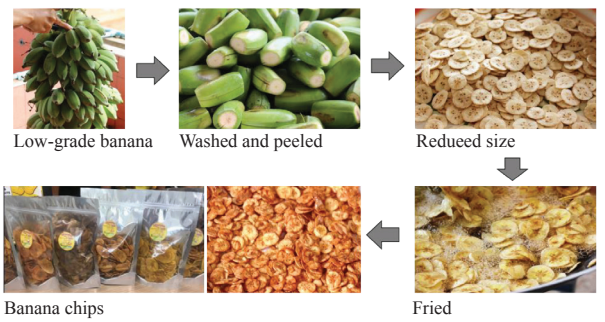

Fig. 7 Banana chip production line

Table 4 Results of banana processing cash flow

Banana Processing Crispy Banana (year)	Cash inflow	Cash outflow	Net cash flow
0		17,199.00	- 17,199.00
1	38,400.00	39,625.51	- 1,225.51
2	38,400.00	38,178.31	221.69
3	46,080.00	41,003.52	5,076.48
4	48,000.00	41,464.85	6,535.15
5	49,920.00	42,318.13	7,601.87
6	51,840.00	42,779.46	9,060.54
present value (7%)	213,797.32	197,533.67	16,263.65
Artificial Flowers (year)			
0		60.30	- 60.30
1	60,000.00	45,186.75	14,813.25
2	60,000.00	65,286.75	- 5,286.75
3	60,000.00	65,286.75	- 5,286.75
4	60,000.00	65,286.75	- 5,286.75
5	60,000.00	65,286.75	- 5,286.75
present value (7%)	246,011.85	232,676.47	13,335.38
Basket Weave (year)			
0		1,206.00	- 1,206.00
1	24,000.00	14,350.50	9,649.50
2	24,000.00	14,350.50	9,649.50
3	24,000.00	14,350.50	9,649.50
4	24,000.00	14,350.50	9,649.50
5	24,000.00	14,350.50	9,649.50
present value (7%)	98,404.74	56,117.65	42,287.09

amounting to 17,199 baht in the year 0, with a useful life of approximately 6. The years and variable costs such as water, electricity, gas, palm oil, seasoning powder, butter, sugar, oil absorbing paper, and labor cost details ares shown in the Table 4.

The banana stems were then processed into artificial flowers and basket weave. If banana stems were not used to add value such as Artificial Flowers or Basket Weave they would end up agricultural waste. Banana stems means 1 plant contains about 10-12 bracts (6 outer bracts, can be used to produce Basket Weave get 1 piece) and (6 inner cladding used for production Artificial Flowers get 100-200 flowers). Fixed factors such as dryers and irons (costs are not actually paid in the 0th year) worth about 100,000 baht, with a lifespan of 5 years. By using banana stems and not allowing them to become agricultural waste helps promote career building reduces methane emissions (mitigation) which is the cause of global warming and add value of agricultural waste. The details of banana processing is presented in Table 4.

The feasibility and sensitivity analysis of banana products' added value revealed that all three products were feasible to produce (ENPV was greater than 0, EBCR greater than 1, and EIRR greater than financial return (7%)), but economically sensitive. If the cost of production changes by at least 5% in case of value added of banana chips (Fig. 7) and artificial flowers because the cost per unit is 6.91 and 1.5 baht per unit, the selling price is 8 and 2 baht per unit, while the basket weave was able to resist economic vulnerability. In the case of production costs increased by 5% (ENPV = 39,481.21 baht, EBCR = 1.67 times and EIRR 45%). It shows that adding value to banana products is economically feasible (Table 5). There is a need for development in terms of branding and packaging to create added value for the product, resulting in more cost-effective pricing and creating better awareness or recognition of the product as noted in the study of Phetsrithong et al. (2021). They found that beautiful and distinctive packaging attracted consumers to make purchasing decisions. However, the possibility of adding product value to take advantage of local resources to raise the level of career and income of farmers need to rely on the development process "Fundamentals Economy" is the economic system of local communities which can be self-reliant under the philosophy of sufficiency economy with mutual assistance. There is a moral and economic system that encourages development

in other areas, including economy, society, people, community, culture, environment, natural resources strongly and sustainably. It is a horizontal economy system that affects and builds socio-economic relations between people in the local community. It is not only an individual vertical economy but can bring about cooperation. There is an opportunity and a good relationship between the community's collective economy and the individual economy. It is an economic system characterized by cooperation, partnership, building relationships, both in the local community and at other broader levels, and outside The Community Organizations Development Institute (2021). It shows the importance of an economic work base and community capital because it is a solid foundation that will enable community organizations to have a better quality of life and be able to sustain themselves. The key elements of a foundation economy are the integration, the management of the community's financial system, the integration of joint capital, and have a strong community fund which can be a financial mechanism for the community to develop the economy, society, occupation, culture, the environment of the community, and people in the community. The Sai Noi Community has been grouped as a tourist attraction to conserve community resources and this approach is considered an appropriate framework to continue the horizontal level to create sustainability in careers and incomes.

Table 5 Results of the economic feasibility analysis of banana processing

List	Crispy Banana	Artificial Flowers	Basket Weave
Economic Net Present Value (baht)	16,263.65	13,335.38	42,287.09
Economic Benefit Cost Ratio (Times)	1.08	1.06	1.75
Economic Internal Rate of Return (%)	10	16	51
Sensitivity analysis (cost increased 5%) :EIRR	N.A.	N.A.	45

Remark: N.A. is not worth the investment

Conclusion

Banana is an important economic crop, rich in nutrients and useful substances. All parts of a banana can be used both as food and non-food. Banana farmers or entrepreneurs should have a good and appropriate planting management process (upstream) as well as quality control of raw materials after harvesting, systematic production, and processing planning (mid-water). They should have knowledge of marketing mechanisms, including feasibility analysis in economics, transportation, and distribution to the consumer (downstream) which will result in quality products that meet the needs of consumers. The primary product is the banana fruit, and the secondary product is from different parts of the banana and by-products that can be processed to create added value. Research and development of banana products are an interesting way to improve the quality of life, livelihood, build a career and income for banana farmers. In addition, the integration of basic economic development builds community capital, extending the community towards "Local communities have a stable economic system and community capital that can be self-reliant and manage themselves sustainably". This is in line with the guidelines that the government has continuously tried to promote. For example the existing community capital is banana cultivation, extending the wisdom of the community to products and learning about the way of the community such as processing banana leaves into containers, bringing fibers from bananas to weave into bags, basket weaves, and artificial flowers, and continue to be a learning center and a source of tourism for the community, promoting community development under the base of natural resource and environmental conservation, including grouping into community enterprises to strengthen and reduce limitations. Some limitations from farmers are found including most of them are elderly people (average age 61 years), still lacking incentives to encourage the new generation to help develop and carry on. From the study finding, some farmers still have investment liabilities 47.27% have debts between 10,000 –50,000 baht. The farmer's cost of fertilizers accounted for 5% of the banana cultivation cost, 4.80% of Gros Michel banana cost plant and 5.33% of Pisang Awak banana cost plant can be shown in Fig. 3, Fig. 5 which can be reduced by using self-reliant or organic agriculture as part of the community's way of life. This study, therefore, contributes to confirming that bringing the existing community funds through the development process can create careers, incomes, and well-being, and self-reliance where communities coexist with ecosystems nature, and the environment.

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