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The Development of Local Based Riceberry Rice Standard Recipes to Promote Commercialization and Tourism in the ASEAN Community

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Abstract

This research is in the sequence of the innovative utilization from a previous study on developing standard food recipes utilizing Riceberry rice as the key ingredient under the context of the communities of Uttaradit province, Thailand along with the context of the ASEAN Community. The 10 standard recipes from the previous study were used in real commercialization that aimed to promote the utilization of local food raw materials of Uttaradit province, to increase the competitiveness of local products in the ASEAN Community's market, and to promote commercialization and tourism. The recipes were tested on sensory acceptance evaluation with 100 tourist samples and nutritional values assessment. The evaluation data were used to indicate the potential of the recipes to meet tourists' satisfaction, which was eventually used together with the recipes' nutritional data as the reference for the consideration of and selection for one month of real commercialized trial testing by seven food business entrepreneurs in Uttaradit province. After finishing the commercialized test, the food business entrepreneurs evaluated the appropriateness of the recipes to use for real commercialization. The result of the average sensory acceptance evaluation scored on all 10 recipes using a 5-point hedonic scale evaluation form indicated that, all 10 recipes were accepted by the tourists and had the potential to be utilized for serving to tourists. The seven food business entrepreneurs selected four out of the 10 recipes for the commercialized test according to the reference data of each recipe provided for their consideration, flexibility, convenience and capability to adopt the recipes. The reasons that the entrepreneurs selected only four recipes were because of their consideration on the available raw materials during the testing period, their capability to cook the food according to the instruction of the recipes, and the conforming of the theme of the recipes to their business theme that mostly sold healthy food. The results of the recipes' appropriateness for real commercialization using a Likert-type scale appropriateness evaluation form have shown that the selected recipes were appropriate for use in a real commercialization situation.

Keywords: Riceberry rice, Standard food recipe, Community-context, Commercialization, ASEAN Community, Tourism promotion

1. Introduction

After the official establishment of the ASEAN Economic Community (AEC) in 2015, member countries consisting of Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam have been affected by many major changes, especially on the economic situation. Consequently, the regional economic growth rate is rapidly increasing due to many changes of economic policies and agreements; such as, exemption of import and export tariffs between member countries, the free transfer policy for skilled labour in the region, and tourist visa exemption policy for the citizens of the member countries. The tourist visa exemption policy is one of the most effective policies that has had an effect on the growth in tourism markets. Therefore, the number of ASEAN tourists who travel to other ASEAN countries has been increasing greatly in recent years (Association of Southeast Asian Nations, 2015). Hence, the rapid expansion in the number of tourists has largely affected the increasing demands of tourist accommodation businesses, which has led to an increasing demand of food service businesses as a part of the tourist accommodation businesses as well.

It is known that rice is one of the most important staple foods of all ASEAN nations and is also one of the widely accepted staple grains of people of all cultures around the world. In 2015, the Mintel Group Ltd. (2015), one of the leading marketing research companies, revealed a study on the present global food trend showing that global consumers are currently concerned about healthy food and food with cultural background stories. A study by Bunnak (2016) on tourist consumption behavior found that tourism services that offered

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local food would receive positive satisfaction to the local tourism services. Furthermore, Kawanobe's (2001) study about tourists' point of interests stated that tourists showed an interesting trend in local culture and traditions of the local community during their trips. Accordingly, these studies about tourist behavior conform to the study of the Mintel Group Ltd. (2015) revealing the present trend of consumers' food behavior that gives priority to the cultural background stories of the food.

Riceberry rice, a product of a natural cross-breeding process between Dok Mali 105 white rice and Hom-Nil rice from the collaborative research of the National Research Committee of Thailand and Kasetsart University, has higher nutritional benefits than normal white rice according to the Rice Science Centre of Kasetsart University (2016), and the study of Wanassanan et al. (2011) also showed that Riceberry rice had the potential to prevent Alzheimer's disease and other neurodegenerative syndromes. From the above-mentioned, the researchers have considered rice, which has special nutritional properties, to correspond to the current global food trend.

Uttaradit province has a rich cultural background, as it borders the three diverse cultural regions of Central Thai, Lanna, and Lan Xang (Sukha et al., 2012); which has resulted in the people in different areas of Uttaradit have their own ways of living, local language and local traditional ceremonies, including distinctive food cultures (Chaima & Toosaranon, 2012). This distinctive integration of cultures has made it possible for Uttaradit to have the suitable potential for further tourism promotion.

In 2016, the researchers developed 10 new food recipes utilizing the Riceberry rice and local community-based food and raw ingredients of Uttaradit province to meet the current consumer demand trend that concerns about health issues and the cultural background stories of the food. All of these 10 recipes have already been approved and modified by internationally recognized food experts. However, for actual commercialization opportunities, the potential of these recipes still needs to be proved (Panyayong & Chatjaroenchaikul, 2016).

In order to prove this potential of the developed community-based Riceberry rice standard recipes, the researchers aimed to study the potential of the recipes utilization in real commercialization and tourism promotion purposes.

The study would show the possibility of local wisdom and resource utilization for tourism purposes. It would also assist to serve the local Uttaradit raw material markets, especially local Riceberry rice supplies due to the expansion of the cultivated area in Uttaradit province (Bank of Agriculture and Agricultural Co-operatives, Uttaradit Branch. 2014).

This result might be adapted to be used as a model for other regions to enhance the local competitiveness of the tourism industry as well as prepare for joining in the ASEAN Community at the present time.

2. Objective

This research aimed to study the acceptance of tourists on 10 previously developed Riceberry-based recipes using a 5-point hedonic evaluation with 100 tourists, and each recipe was analyzed for its nutritional value. Therefore, the result would be used as reference data for local food business entrepreneurs to select the recipes for a one-month trial to study the appropriateness for commercialization by using a modified Likert-type appropriateness evaluation form.

3. Study procedures

There were six procedures conducted during this study as follows:

1. All 10 developed recipes from the previous study (Panyayong & Chatjaroenchaikul, 2016), which were already approved and modified by internationally recognized food experts, were evaluated on a sensory acceptance by 100 samples of tourists at popular tourist destinations in Uttaradit province during the Songkran Festival using a 5-point hedonic scale sensory evaluation form to assess the potential of the recipes to match the tourists' needs and to be used as reference data for the food business entrepreneurs to select for commercialized testing.

The list of the 10 recipes is shown as follows:

- Recipe 1: Riceberry Coconut Crepe with Fruity Tropical Sauce.
- Recipe 2: Crispy Fruit Salad with Spicy Riceberry Dressing.
- Recipe 3: Northern-style Herbal Rice with Black Herbal Sauce.
- Recipe 4: Thai Crepe with Herbal Salad on a Riceberry Crispy Crust.
- Recipe 5: Tha-Nueh Riceberry Cheese Tart.
- Recipe 6: Tha-Nueh Amok Tria with Rice Berry Rice.
- Recipe 7: Khi Lek Cashew Nut Dal with Riceberry Masala Pakora.
- Recipe 8: Uttaradit Riceberry Cracker with Herbal Mango Chutney and Berry Salsa.
- Recipe 9: Fresh Water Escargot Riceberry Cream Soup with Vegetable Puree Sauce.
- Recipe 10: Double Berry Cashew Nut Macaron.

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2. All 10 developed recipes were analyzed for the nutritional value of each recipe by using trusted food nutritional databases to determine the nutritional value for each recipe in one serving size. These nutritional data of the recipes were used as reference data for the food business entrepreneur samples to select the recipes for the commercialized testing.

3. The detailed data of all 10 recipes along with the sensory evaluation results and nutritional value data were presented to seven food business entrepreneurs in Uttaradit province, to use as information for selecting the recipes for the commercialized test.

4. The cooking techniques of the selected recipes were demonstrated and taught to the food business entrepreneurs.

5. After one month, all seven entrepreneurs were further investigated to find the results of the commercialized trial test to evaluate the appropriateness of the recipes to use in real commercialization using the adapted Likert-type appropriateness evaluation form (Vagias, 2006).

6. The data were processed, analyzed, and summarized for writing the study's report.

4. Results

The sensory evaluation results of 100 tourist samples of all 10 recipes using a 5-point hedonic scale sensory evaluation form are shown in the table 1 below. (The organization of the recipe numbers are arranged as shown in the Study Procedures section.) (Full marks = 5). From the evaluation result of each sensory characteristic, it is shown that for the appearance characteristic, recipe no. 10 had the highest score of 4.68±0.56, and recipe no. 7 had the lowest score of 4.27±0.71. For the color characteristic, recipe no. 10 had the highest score of 4.64±0.57, and recipe no. 7 had the lowest score of 4.15±0.85. For the smell characteristic, recipe no. 10 had the highest score of 4.52±0.73, and recipe no. 7 had the lowest score of 3.90±0.89. For the taste characteristic, recipe no. 10 had the highest score of 4.61±0.65, and recipe no. 7 had the lowest score of 3.95±0.80. For the texture characteristic, recipe no. 10 had the highest score of 4.55±0.70, and recipe no. 7 had the lowest score of 3.90±0.89. For the overall acceptance, recipe no. 10 had the highest score of 4.70±0.55 and recipe no. 7 had the lowest score of 4.15±0.7.

Table 1. The sensory evaluation results of 100 tourist samples (N=100)

Sensory Characteristics	Recipe Number 1	2	3
Appearance	4.44±0.59	4.36±0.78	4.34±0.71
Colour	4.44±0.63	4.34±0.71	4.34±0.83
Smell	4.12±0.60	4.09±0.86	4.07±0.87
Taste	4.29±0.68	4.20±0.79	4.16±0.86
Texture	4.02±0.85	4.11±0.84	4.07±0.79
Overall Acceptance	4.39±0.54	4.39±0.75	4.39±0.69

Sensory Characteristics	Recipe Number 4	5	6
Appearance	4.46±0.55	4.46±0.55	4.34±0.71
Colour	4.29±0.72	4.44±0.59	4.34±0.83
Smell	4.17±0.80	4.24±0.70	4.07±0.87
Taste	4.27±0.81	4.37±0.62	4.16±0.86
Texture	4.24±0.86	4.37±0.73	4.07±0.79
Overall Acceptance	4.37±0.70	4.46±0.64	4.39±0.69

Sensory Characteristics	Recipe Number 7	8	9	10
Appearance	4.27±0.71	4.41±0.82	4.34±0.71	4.68±0.56
Colour	4.15±0.85	4.52±0.63	4.30±0.76	4.64±0.57
Smell	3.90±0.89	4.43±0.79	4.20±0.85	4.52±0.73
Taste	3.95±0.80	4.43±0.79	4.36±0.81	4.61±0.65
Texture	3.90±0.89	4.43±0.76	4.41±0.62	4.55±0.70
Overall Acceptance	4.15±0.73	4.57±0.73	4.43±0.62	4.70±0.55

The overall sensory evaluation results of the 100 tourist samples of all 10 recipes, using the 5-point hedonic scale sensory evaluation form, were shown as follows: (Full marks = 5)

Table 2. Overall sensory evaluation results of all 10 recipes by 100 tourist samples (N=100)

Sensory Characteristics	Average Score	Score Interpretation
Appearance	4.44±0.12	Like
Colour	4.38±0.14	Like
Smell	4.21±0.18	Like
Taste	4.32±0.19	Like
Texture	4.25±0.21	Like
Overall Acceptance	4.44±0.15	Like

From the evaluation results, it is shown that the overall evaluation results in all sensory characteristics were to the level of "Like".

The results on the nutritional value analysis for one serving of all 10 recipes obtained by using the comparison method of the food nutritional value databases of the Bureau of Nutrition, Thailand's Ministry of Public Health and the United States Department of Agriculture (USDA) are shown in Table 3.

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Table 3. Nutritional value of all 10 recipes obtained by using the comparison method with the food nutritional value databases (Bureau of Nutrition, Ministry of Public Health, Thailand 2001; United States Department of Agriculture 2016)

Recipes	Energy	Protein	Fat	Carbohydrate	Dietary Fiber	Calcium	Phosphorus	Dietary Iron	Sodium	Vitamin E	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin C
	Kcal	G				Mg.				Mg.				
1	267.61	2.32	11.78	42.24	2.81	20.74	56.71	1.29	217.14	0.61	0.25	0.24	0.992	57.08
2	210.04	5.899	5.94	36.69	2.68	55.22	104.4	1.79	1215.83	0.78	0.17	0.12	2.09	55.36
3	373.30	9.26	5.18	75.14	3.63	435.89	136.71	7.25	1754.10	0.38	0.41	0.87	3.39	10.41
4	270.82	4.22	6.76	50.32	1.15	58.47	62.54	2.19	263.97	0.54	0.14	0.07	0.51	1.95
5	75.58	0.86	4.78	6.72	0.21	4.78	15.96	0.86	76.76	0.16	0.05	0.02	0.044	0.15
6	654.41	21.57	46.87	44.87	4.93	115.71	375.10	8.34	1232.8333	0.52	0.34	0.25	4.47	11.09
7	446.68	11.41	29.53	38.88	4.06	57.55	261.62	8.47	1442.58	0.59	0.41	0.31	2.16	2.48
8	151.92	7.11	2.89	26.78	2.02	106.80	117.999	1.73	893.77	0.56	0.08	0.23	0.92	30.72
9	397.28	10.17	28.66	26.34	1.35	79.41	1448.06	9.04	1152.557	2.42	0.16	0.29	2.333	4.08
10	63.36	1.29	2.69	9.10	0.23	6.34	27.87	1.41	32.33	0.11	0.04	0.03	0.08	1.29

The serving size of each recipe was different, which depended on the serving type of the recipes; the appetizers and desserts might have a smaller serving size than the main courses. The nutritional assessment data were used for the consideration of the food business entrepreneurs to select the recipes; not for comparing which recipe was better than another.

There were four recipes selected by the seven food business entrepreneurs, which are listed below.

- Recipe 2: Crispy Fruit Salad with Spicy Riceberry Dressing.
- Recipe 3: Northern-style Herbal Rice with Black Herbal Sauce.
- Recipe 8: Uttaradit Riceberry Cracker with Herbal Mango Chutney and Berry Salsa.
- Recipe 10: Double Berry Cashew Nut Macaron.

The reasons the entrepreneurs chose these four recipes to use for the commercialized test were 1) the availability of the raw materials during the test-run season, 2) the theme of the food conformed to their styles of food, which they currently serve to customers, 3) the skill of the cooks in their business, 4) the tourist's sensory test result of the recipes, and 5) the nutritional properties that responded to the market demand for healthy food. After one month of commercialized test of the selected recipes by the seven food business entrepreneurs, the evaluation results of the appropriateness of the recipes to use in actual commercialization are shown in the table below.

Table 4. Evaluation result of the appropriateness of the recipes to use in actual commercialization. (Full marks = 7)

Evaluation Issues	Average Score	Score Interpretation
Cost	5.86±0.90	Appropriate
Customer Satisfaction	6.57±0.53	Absolutely Appropriate
Cooking Process	7.00±0.00	Absolutely Appropriate
Raw Materials	6.86±0.38	Absolutely Appropriate
Market Capability	6.29±0.95	Appropriate
Overall Appropriateness	6.57±0.53	Absolutely Appropriate

According to the outcome, the evaluation results by the seven food business entrepreneurs were to the level of "Absolutely Appropriate" in four evaluation issues and the level of "Appropriate" in two evaluation issues. The overall satisfaction was at a level of "Absolutely Appropriate".

5. Discussions and conclusions

The overall processes of this study aimed to utilize local food wisdom in the context for the local community integrated with the ASEAN and international food context to create Riceberry rice recipes that have the capabilities to utilize for commercialization and tourism promotion. The overall process concept of this study are shown in the figure below.

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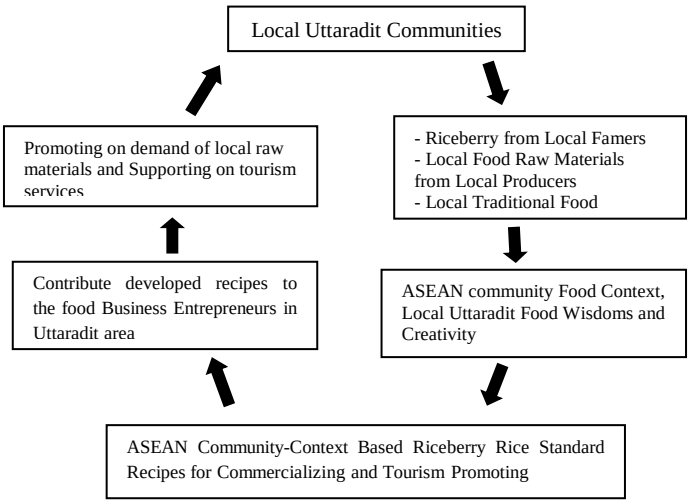

Figure 1. Overall process of the study

From the 100 tourist samples sensory evaluation results of all 10 ASEAN community-based Riceberry rice standard recipes, the results have shown that the average scores of all 10 recipes were to the criteria level of "Like" in all sensory characteristics. These can be concluded that the overall acceptance of tourists to the ASEAN community-based Riceberry rice standard recipes were to a satisfactory level, which indicates that these recipes were acceptable to tourists and had the potential to be utilized for serving to tourists. From these data accompanied with the nutritional data of the recipes, the seven food business entrepreneur samples selected four out of 10 recipes to use for the commercialized test.

After one month of the commercialized trial testing of the selected recipes, the food business entrepreneurs evaluated the appropriateness of the recipes to use in real commercialization. The results showed that most of the evaluation topics were to the level of "Absolutely Appropriate". According to the evaluation results from a previous study on all of these recipes (Panyayong & Chatjaroenchaikul, 2016), the overall sensory acceptance of these recipes were evaluated to the level of "Moderately Like" and the overall appropriateness of these recipes to promote to international markets were evaluated to the level of "Moderately Appropriate". This can be concluded that the evaluation results from the experts, tourist samples, and the entrepreneur samples conformed with one another and would indicate that the ASEAN community- based Riceberry rice standard recipes were really appropriate for utilizing in actual commercialization. The reasons behind the conformation of the evaluation results from the experts, tourist samples, and the entrepreneur samples are because the recipes are aligned to the recent real food trend on healthy food and food with background stories of local cultures. These results are in accordance with the study of Mintel Group Ltd. (2015) and also meet the tourists' requirements for local food with local cultural uniqueness (Bunnak, 2014; Kawanobe, 2001). Therefore, these can also show an important key to develop successful recipes that are able to be utilized in real commercialization. The recipes should be developed based on the current demands of the market to achieve the customers' demands by studying the present market trend before developing the recipes. In this case, it could lower the risk of investment in developing a recipe or a product for business purposes.

The results of this study have also shown the possibilities for developing healthy and high nutritive food; such as, Riceberry rice and local community raw food materials to standard food recipes with high potential in commercialization by local food business entrepreneurs, as well as promote the local capability for supporting the increase in the demand of the tourism sector. In addition, the developed recipes could contribute to increasing the demand to local raw food materials, which could also help to support the local raw material producers and local farmers. Moreover, the results of this study could be applied to the food business sectors in other provinces to increase the country's competiveness in supporting the expansion of the tourism sector.

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Development of Spray-Dried Lime Juice Powder with Improved Bioactive Compound Retention

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Abstract

Lime juice powder was developed with 20% of combined maltodextrin/gum Arabic at a ratio of 4:1. A high shear homogenization was applied to encapsulate phytochemical compounds before spray drying with the purpose of protecting bioactive compounds from thermal degradation. The effect of high shear homogenization on physicochemical properties and antioxidant activity of spray-dried lime powder was studied. The particle size, morphology, moisture content, color, solubility, hygroscopicity, ascorbic acid, total polyphenol and flavonoid contents, and antioxidant activity of the spray-dried powder undergoing the homogenization were compared with those of the non-homogenized powder. The higher yield (30%) was obtained in the homogenized powder compared with the control (28%) with no significant difference. The average particle diameter of the homogenized powder was 0.1-4 micrometers, smaller than 18-26 micrometers of the untreated powder. Morphological study revealed that the powder without homogenization was densely packed compared with the homogenized powder. Moisture content of homogenized powder and that of the particles without homogenization were 3.91% and 5.42%, respectively. Higher solubility and less hygroscopicity values and color after spray drying were observed in reconstituted lime powder with high shear homogenization. Folin-Ciocalteu and aluminum trichloride ($AlCl_3$) assays were used to determine total phenolic and flavonoid compounds, respectively. 2, 2-diphenyl-1-picryl hydrazyle-hydrate (DPPH) assay was used to determine total antioxidant activity of the powders. The retention of ascorbic acid and total phenolic contents, and antioxidant activity was significantly better in the homogenized powder, but not the flavonoid contents. The antioxidant activity derived from total phytochemical compounds in the treated powder was preserved by the homogenization before spray drying. This study indicated that the application of high shear homogenization with combined drying agent before spray drying could prevent phytochemical compounds in lime from thermal degradation.

Keywords: Lime powder, Spray drying, Physicochemical properties, Bioactive retention, Antioxidant activity

1. Introduction

Lime (*Citrus aurantifolia*) is a popular ingredient used to accent flavors of Thai foods and beverages. Several species of citrus limes were investigated in vitamin C and phenolic compounds including flavonoid compounds which are all sources of natural antioxidants (Ghafar, Prasad, Weng, & Ismail, 2010). Due to the antioxidant properties of these compounds, lime has many functional health benefits such as prevention of vitamin C deficiency, heartburn and nausea, some forms of cancer, and relief of fever, coughs, and various respiratory disorders (Attaway, 1993; Theansuwan, Triratanasirichai, & Tangchaichit, 2008). It is of great interest to transform lime juice into dried powder with desirable properties to have high nutritional value of lime juice powder available throughout the year.

In order to enhance the nutritional quality of processed lime juice, the processing techniques must be carefully selected. Of various processing methods, spray drying is a technique applied to efficiently dehydrate sugar and acid-rich fruit juice using different drying agents with advantages of heat short-contact time, microbial growth inhibition, and storage stability (Largo Avila, Cortes Rodríguez, & Velásquez, 2015). By contacting hot air to evaporate water from small droplets, some heat-sensitive compounds in lime juice are degraded resulting in reduced total antioxidant capacity (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012).

The entrapment of antioxidant compounds inside appropriate food-grade drying agents such as maltodextrin, gum Arabic, pectin, modified starch, or cellulose etc. combined with spray drying technique has been pronounced to protect the entrapped core against caking and stickiness, temperature and enzymatic changes. As mentioned by Chuacharoen and Sabliov (2016), the stability of bioactive compounds was improved and its total antioxidant activities were enhanced when encapsulated into the particles. In previous studies, the temperature of 170°C with the addition of drying agents (maltodextrin, gum Arabic, and sucrose syrup) of approximately 10-20% w/v was proposed to increase the product mass of the spray-dried lime juice powder (Footrakul, Mawimol, & Boonyasupa,

2003; Shihawong, 2003). Caking of dried powder was observed when using only maltodextrin (Paterson & Bröckel, 2015). Therefore, a combination of maltodextrin and gum Arabic was proposed as a good protection after spray drying (Frascareli, Silva, Tonon, & Hubinger, 2012). Gum Arabic has become an entrapment aid for essential oils due to its emulsifying properties. The addition of high shear homogenization to stabilize the droplets before spray drying is challenge to influences good re-constitutional characteristics in terms of particle size, water solubility, and hygroscopicity as well as physicochemical and functional properties of the spray-dried powder. Thus, drying operations must be carefully performed to minimize the loss of the powder's properties.

The aims of the present study were to develop lime powder utilizing a combination of maltodextrin/gum Arabic as drying agents processed with a high shear homogenization prior to spray drying and evaluate the physicochemical properties and functionality in terms of color, solubility, hygroscopicity, vitamin C, total phenolic and flavonoid contents, and total antioxidant capacity of lime powder.

2. Materials and methods

2.1 Materials

Fresh lime (*Citrus aurantifolia*) was purchased from a local market. It was immediately processed without further storage. Maltodextrin (Dextrose Equivalent, DE=10), gum Arabic (AG), aluminum trichloride ($AlCl_3$), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical research grade.

2.2 Preparation of lime juice

Fresh lime (*Citrus aurantifolia*) was hand squeezed, and lime juice was filtered to remove seeds. The average amount of lime juice per kilogram, nutrition values, and total soluble solids of the lime juice were analyzed for references. Drying materials were separately rehydrated overnight and then gently heated at 60°C in a water bath to allow complete dissolution. The obtained juice was mixed with 20% blended drying agent of maltodextrin and gum Arabic as encapsulating carriers at the ratio of 4:1 (w/w). The mixture was homogenized in an Ultra Turrax Model T25 basic high shear homogenizer (UT-HSH) (IKA, Works Inc., Wilmington, NC, USA) for 10 min at 12,000 rpm until complete dispersion obtained. A control was made in parallel without homogenization. Then all mixtures were spray-dried as described in the following section.

2.3 Spray drying of lime powder

The prepared mixtures were diluted and filtered to remove insoluble solids before spray drying. Subsequently, the solutions were spray-dried through two-fluid nozzle using a Mini spray dryer B-290 (Büchi Labortechnik AG, USA). The feed flow rate (6 mL/min), air flow (580 L/min), inlet temperature (120°C), outlet temperature (65°C), pressure (0.0038 MPa), and spray percentage (90%) were kept constant for all treatments. The reconstituted powders were stored at 4°C for further analyses. The spray drying experiment was carried out in triplicate. The yield of the spray drying process was calculated by considering the total solid content of the feed sample with maltodextrin/gum Arabic and weight of the final dried powder followed the equation below:

$$\text{Yield (\%)} = \frac{\text{Obtained spray dried powder (g)}}{\text{Lime juice (g)} + \text{drying agent (g)}} \times 100$$

2.4 Particle size characterization and morphological study

The particle size of sample was measured using a laser diffraction particle size analyzer, Mastersizer 3000 (Malvern Instruments, Malvern, UK) equipped with a wet sample unit. A small amount of obtained spray-dried lime powder was suspended in ethanol under agitation, and the particle size was measured successively. The data acquisition was presented in micrometers (μm) (Tonon, Brabet, & Hubinger, 2010). The morphological study was done with a Scanning Electron Microscope (SEM) (JSM-6610LV, JEOL Ltd. Japan). The sample was mounted on aluminum SEM stubs and then coated with gold: palladium (60:40) in an Edwards S150 sputter coater. Then, it was observed with 1000×magnification. All measurements were performed in triplicate.

2.5 Physical properties of lime powder

Spray-dried lime powder was analyzed for moisture content (MC) following the AOAC method 930.15 (AOAC, 1999). Color of the samples determined using a chroma meter LABSCAN XE (Hunterlab, VA, USA) was reported in CIELAB color scales. L* value is the degree of lightness to darkness, a* value is the degree of redness to greenness, and b* value is degree of yellowness to blueness. Chroma and hue angles were calculated using the equation below:

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{1/2}$$

and

$$\text{Hue} = \tan^{-1}(b^*/a^*)$$

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2.6 Solubility and hygroscopicity

The solubility of the powder was evaluated according to the method described by Chau, Wang, and Wen (2007). Briefly, sample was added with distilled water (1:10 w/v) and stirred for 1 hour at room temperature and centrifuged at 1,500 rpm for 10 min. Then, the supernatant was collected, dried and weighed. The solubility was calculated using the equation below:

$$\text{Solubility (\%)} = \frac{\text{weight (g) of supernatant after drying}}{\text{weight (g) of sample}} \times 100$$

The hygroscopicity property of the powder samples was determined according to Cai and Corke (2000) with some modifications. Briefly, 2 g of samples were placed in pre-weighed glass vials and placed in a desiccator containing saturated salt solution of sodium chloride (relative humidity of 75%) maintained at 30°C and kept for 7 days. After the incubation, sample vials were weighed, and hygroscopicity was presented as g absorbed moisture/100 g solids.

2.7 Determination of ascorbic acid, total phenolic, and flavonoid contents

L-ascorbic acid content was measured in powder samples by AOAC method 967.21 (AOAC, 2006) and the value was expressed as mg of ascorbic acid/100 g of dry solid. Total phenolic compounds in the sample were determined by the Folin–Ciocalteu assay described by Singleton, Orthofer, and Lamuela-Raventós (1999) with slight modifications. Briefly, 20 µL each of extract, gallic acid standard or blank were taken in separate test tubes, and then 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well, and set for 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately, and incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Aquarius 7400, Cecil, Cambridge, England). The results were expressed as gallic acid equivalent (mg GAE/100 g). The flavonoid content was determined by aluminum trichloride ($AlCl_3$) method (Chang, Yang, Wen, & Chern, 2002). Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against deionized water blank in a UV-Vis spectrophotometer (Aquarius 7400, Cecil). Results were expressed as quercetin equivalent (mg QE/100 g).

2.8 Antioxidant activity

DPPH radical scavenging assay was performed according to Ghafar et al. (2010). Spray-dried samples (500 mg) were dissolved in 4 mL of 90% ethanol solution and stirred for 30 min. Samples (200 mL) were reacted with 2.8 mL of 100 µM DPPH (dissolved in 80% ethanol) for 30 min in the dark. A control containing only DPPH solution and 80% ethanol was used as a blank. The absorbance was recorded at 515 nm using a UV-Vis spectrophotometer (Spectronic™ GENESYS, Thermo Fisher Scientific, Waltham, MA). Samples were analyzed in triplicate and reported as trolox equivalents.

2.9 Data analysis

All data were analyzed using SAS software version 9.2 (SAS Institute Inc., 2008). Means and standard deviations of the data were presented at the significant level of $P < 0.05$.

3. Results and discussions

3.1 Lime juice composition

The average amount of lime juice per kg of lime was 621.8 mL or 0.59 kg. Vitamin C of lime juice per 100 g was 29.1 mg. The total soluble solids (TSS) of lime juice measured using a digital Refractometer (AR 200, Reichert, USA) was 11.7% Brix. A pH of lime juice was 2.1 which is sufficient to inhibit microbial growth due to its high acidity.

3.2 Morphology and particle size analyses

The spray drying conditions was kept constant for both systems. The average particle size (Table 1) of spray-dried lime powder obtained from homogenization ranged from 0.1 to 4 µm which was smaller than that of the non-homogenized powder (18-26 µm). It can be assumed that the homogenization of lime juice applied before spray-drying led to the formation of smaller and stable droplets due to the increased pressure (McClements, Decker, & Weiss, 2007) and the effect of gum Arabic by forming a thin film over the surface of the particles to consequently stabilize and reduce the surface adhesiveness among the droplets (Janiszewska, Jedlińska, & Witrowa-Rajchert, 2015).

Table 1. Particle size of spray-dried lime powder with and without homogenization

Samples	Average diameter (µm)
Non-homogenized lime powder	18-26
Homogenized lime powder	0.1-4

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The morphological characteristics of spray-dried lime powder with and without high shear homogenization are shown in Figure 1. The surface morphology study showed different shape and sizes between two systems. In non-homogenized powder, the particles were clumped together and no individual particle was formed. While small and large spherical particles were observed in homogenized lime powder at 1,000x magnification, which is due to the effect of high shear homogenization on smooth surface, small size, and spherical shape made a difference from non-homogenized powders (Janiszewska et al., 2015).

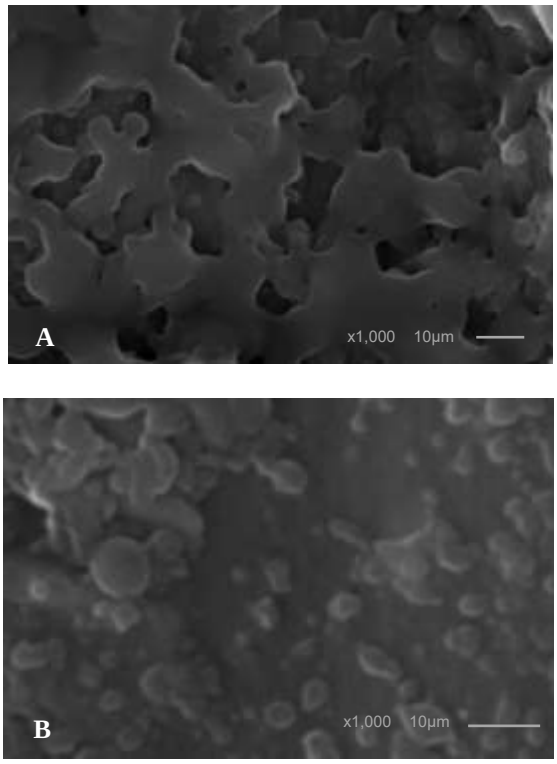

Figure 1. SEM images of (A) non-homogenized lime powders and (B) homogenized lime powders

3.3 Lime powder analyses

The same conditions of spray drying were performed for all treatments. There was not statistically significant difference between yields in the homogenized and untreated powders (Table 2). Moisture contents regardless of drying agent are affected by the air flow rate, drying air temperature of the spray dryer (Goula & Adamopoulos, 2005), and the evaporation rate of the droplet (Chranioti, Chanioti, & Tzia, 2016). When the small droplet is contacted with hot drying air, the evaporation of moisture occurs more rapidly compared with the bigger one (Bhattarai, Tran, & Duke, 2001). This caused low moisture content reported in homogenized powder (Table 2). It is consistent with Finney, Buffo, and Reineccius (2002) stated that larger particles lead to higher moisture content because it can block the spray-disc and form insufficiently dried large droplet.

Table 2. Physical analyses of spray-dried lime powder with and without homogenization

Analyses	Non-homogenized lime powder	Homogenized lime powder
Yield (%)	28.72±2.31[a]	30.11±0.29[a]
Moisture content (g/100g powder)	5.42±0.61[a]	3.91±0.14[b]
Color variables		
*L**	79.5±0.01[a]	84.1±0.01[a]
Chroma	12.55±0.01[a]	8.91±0.008[b]
Hue angle	74.5±0.01[a]	78.8±0.07[b]
Solubility (%)	59.54±0.24[a]	76.84±0.32[b]
Hygroscopicity (g/100g powder)	12.99±0.11[a]	11.68±0.27[b]

Mean ± standard deviation of triplicate analysis. [a,b] means with same letters in each row are not significantly different ($p<0.05$).

Both types of lime powder had a whitish color, in which the color of homogenized lime powder was significantly lighter than that of the non-homogenized powder (Table 2). Chroma measurement indicates the vividness of color and the value of homogenized lime powder is lower. A hue angle indicates the color shade based on a degree angle of 0°C, 90°C, 120°C, and 240°C representing red, yellow, green, and blue colors, respectively. The homogenized lime powder had significantly ($p<0.05$) higher hue angle values as seen with stronger yellow color.

3.4 Solubility and hygroscopicity

The size of particles affects not only its physical properties, but may also contribute to solubility and hygroscopicity of the spray-dried powder. The sample treated with homogenization treatment showed the significantly higher solubility (76.84%), whereas the non-homogenized system showed the lower solubility (59.54%) (Table 2). The results indicated that the solubility of spray-dried powder increased with reducing size of particles, hence increasing the total surface area available for water binding (Fazaeli et al., 2012).

Hygroscopicity was decreased from 12.99 to 11.68 g absorbed water/100 g dried sample with reducing particle size of the powder. Such that the small particle powder containing low moisture content indicated lower hygroscopicity. It is clear that the moisture content considerably influenced the powder's hygroscopicity (Santhalakshmy, Don Bosco, Francis, & Sabeena, 2015). Thus, the small particles with low moisture content contributed to increased solubility and less hygroscopicity which was observed in the system with homogenization.

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3.5 Phytochemical contents and antioxidant activity analyses

Fresh lime juice contained 7.74 mg Gallic acid/g solid, 2.67 mg Quercetin/g solid, and 19.85 μmol Trolox/g solid. The antioxidant activity derived from total phytochemical compounds in the powders was reported in Table 3. Maltodextrin and gum Arabic created a strong impermeable film around the droplets (Frascareli et al., 2012) that helps protect phytochemical compounds from hot drying air. Phytochemical contents and antioxidant activity of lime powders were decreased after the hot-air spray drying operation.

Table 3. Ascorbic acid, gallic acid, quercetin, and trolox equivalents of spray-dried lime powder with and without homogenization

Analyses	Non-homogenized lime powder	Homogenized lime powder
Ascorbic acid (mg/g solid)	0.19 ± 0.01^{a}	0.41 ± 0.02^{b}
Gallic acid equivalents (mg/g solid)	1.49 ± 0.12^{a}	2.51 ± 0.34^{b}
Quercetin equivalents (mg/g solid)	0.32 ± 0.01^{a}	0.37 ± 0.02^{a}
Trolox equivalents (μmol/g solid)	7.93 ± 0.12^{a}	13.48 ± 0.22^{b}

Mean ± standard deviation of triplicate analysis. ^{a,b} means with same letters in each row are not significantly different ($p<0.05$).

The initial ascorbic acid in fresh lime juice (29.1 mg /100 g fresh lime juice) was degraded approximately 77% (0.19 mg/g dry solid calculated based on 30.11% yield) and 48% (0.41 mg/g dry solid calculated based on 28.72% yield) in lime powder without and with homogenization, respectively. The results showed that the entrapment process with combined drying agents and homogenization before spray drying help prevent some ascorbic acid loss. The similar results were observed with total phenolic content, which showed the higher amounts in homogenized powder, but total flavonoid content did not significantly differ between the two powders. In addition, the antioxidant activity measured by DPPH assay was significantly higher in the homogenized lime powder than the untreated powder. The variation in the phytochemical levels preserved by the homogenization may be due to the different degree of tolerance to heat of individual phenolic compounds and the difference in structural degradation during the hot drying process diversely affects the phenolic content and antioxidant activity of lime powder. Thus, the change in phenolic and flavonoid contents and antioxidant activity depends upon individual phenolic acid constituents and their susceptibility to heat and conformational changes. In summary, this study indicated that application of drying agents with the high shear homogenization helps protect some phytochemical compounds from heat during spray drying.

4. Conclusions

The homogenization of lime juice prior to spray drying leads to formation of smaller powder particles. The surface morphology between the powders with and without homogenization showed differences in shapes and sizes, which was smaller in the homogenized powder than the untreated powder. A reduced size of particles resulted in low moisture content due to faster diffusion rates. Both lime powders had a whitish color, in which the color of homogenized lime power was significantly lighter than that of non-homogenized powder. The smaller particles with low moisture content resulted in increased solubility and less hygroscopicity of the powder, which implies the better powder quality during storage. The antioxidant activity derived from total phytochemical compounds was higher when the sample was homogenized before spray drying. Thus, application of encapsulating agents combined with high shear homogenization was a promising process to improve retention of phytochemical compounds in spray-dried powder during spray drying processing.

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Species Checklist and Abundance of Birds in Salt Field Areas and Aquaculture Areas along the Coastal Land, Bang Kaew Sub-district, Muang District, Samut Songkhram Province

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Abstract

The objectives of this research were to study species checklist, abundance, feeding behavior, status of birds and group of migratory birds in the research area. The data were carried out by field survey of species checklist and abundance of birds which foraged in land-use areas namely salt field areas, aquaculture areas, shrimp pond and cockle pond in Bang Kaew Sub-district, Muang District, Samut Songkhram Province from August 2015 - July 2016. The data were analyzed to find out abundance of birds. The results showed that 10 orders, 24 families and 51 bird species were found in salt field areas and aquaculture areas of coastal land. 9 orders, 17 families and 35 bird species were found in salt field. 10 orders, 22 families and 44 bird species were found in shrimp pond. 7 orders, 16 families and 32 bird species were found in cockle pond. According to bird abundance, 6 bird species were in level 5 of bird abundance. It was 11.76% of all bird species. According to feeding behavior, there were 12 insectivorous birds, 20 piscivorous birds, 12 aquatic, benthic invertebrate feeding birds, 4 granivorous birds, 1 frugivorous bird, 1 carnivorous bird, 1 omnivorous bird and 1 predator bird. According to bird seasonal status, there were 24 resident birds, 7 resident and migratory birds and 20 migratory birds. According to migratory bird group, there were 12 shore birds, 4 terrestrial birds and 3 sea birds. According to IUCN threatened Status, Painted Stork, Eastern Black-tailed Godwit, Red-necked Stint and Eurasian Curlew were near-threatened (NT). So the communities should give their hands to conserve salt field areas and aquaculture areas of coastal land and biodiversity continuously, because the birds are indicators of natural balance and plenty of food in local ecosystem.

Keywords: Species checklist of birds, Abundance of birds, Coastal land

1. Introduction

Samut Songkram Province is located at central region of Thailand along the Inner Gulf of Thailand. There are natural areas and land-use areas along the coastal land in Bang Kaew Sub-district, Muang district, Samut Songkhram Province. There are mangrove forest, mudflat, salt field, cockle pond, shrimp pond and planted mangrove forest. Tourists are interested in birding in salt field, cockle pond and shrimp pond in the research area. The salt field, cockle pond and shrimp pond are rich of invertebrates which are foods of both resident and migratory birds in the winter (November - February) and in the summer (March - May). Three areas are near Don Hoi Lod wetland which is the 1099th Ramsar Site. The research areas of Bang Kaew situate near the Inner Gulf of Thailand which is foraging place for many shorebirds and sea birds every year. The shorebirds can be found mostly in the summer (March - May) and in the winter (December - February). The Inner Gulf of Thailand is surrounded by flooding mudflat, seacoast shrimp farms, salt fields, cockle ponds and mangrove forests. Most of migratory birds found in Thailand used East-Asian Australasian Flyway which started from Arctic to Southeast Asia, Australia and New Zealand (Erftemeijer & Jugmongkol, 1999). In Bang Kaew Sub-district, 13 migratory bird species and 20 resident and migratory bird species were found (Meewattana, Chamchoi, & Sooksawang, 2013). In the nature trail at Bang Kaew Sub-district, 31 migratory bird species and 7 resident and migratory bird species were found (Plailek, Chitman, Charoenpokaraj, 2016). So the researchers were interested in studying species checklist and abundance of birds in salt field areas and aquaculture areas along the coastal land in Bang Kaew Sub-district, Muang district, Samut Songkhram Province.

The aim of the research is to study the species checklist, abundance, feeding behavior, status of

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birds and group of migratory birds in the research areas.

2. Methodology

The research methodology was shown as follows;

2.1 Area study

Salt field areas and aquaculture areas along the coastal land around the Mangrove Natural School of Bang Kaew Sub-district, in Muang district, Samut Songkhram Province, Thailand.

2.2 Data collection and analysis method

Step 1: Survey the birds

Observe and survey the birds and their behaviors by using binoculars and camera then identified the birds as found according to the books "A Guide to the Birds of Thailand" by Boonsong Lekagul and Philip D. Round (1991), " A Field Guide to the Birds of Thailand and South-East Asia" by Craig Robson (2008). The survey areas were Area 1 (salt field), Area 2 (shrimp pond) and Area 3 (cockle pond). The survey was conducted from 7 a.m. - 6 p.m. depending on high and low tide. The survey was made once a month from August 2015 - July 2016.

Record the species of birds and their behaviors sighted directly and from their calls by using point transects method.

Step 2: Classify the bird species, feeding behaviors, status, group of migratory birds and threatened status according to the checklist of the birds of Thailand by Bird Conservation Society of Thailand (2016).

Step 3: Analyze the abundance of birds (Pettingill, 1969).

Step 4: Conclusion and report the results.

3. Results

The showed found that 10 orders, 24 families and 51 species of birds were found in the research area of coastal land. 13 bird species in order *Charadriformes* and *Passeriformes* were found the most. 9 orders, 17 families and 35 bird species were found in Area 1 (salt field). 10 orders, 22 families and 44 bird species were found in Area 2 (shrimp pond). 7 orders, 16 families and 32 bird species were found in Area 3 (cockle pond).

In December, bird species were found the most in Area 1 (salt field). In February, bird species were found the most in Area 2 (shrimp pond) because it was migratory season for many migratory birds in the research area, as shown in Figure 1. Bird abundance in the research area was grouped into five categories namely level 5 abundant, level 4 common, level 3 moderately common, level 2 uncommon and level 1 rare.

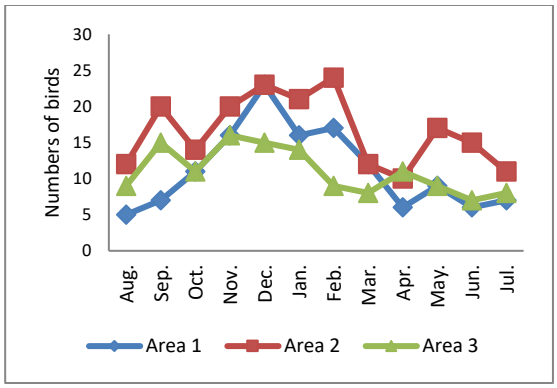

Figure 1. Bird species found in 3 areas, in Bang Kaew Sub-district, Samut Songkram Province in each month.

Six bird species were in level 5. It was 11.76 % of all bird species. 6 bird species were in level 4, 11.76 %. 17 bird species were in level 3, 33.34 %. 12 bird species were in level 2, it was 23.53 % and 10 bird species were in level 1, 19.61 %. The highest number of bird species is 36 species in December and 27 species in February accordingly because it was migratory season of migratory birds in salt field areas and aquaculture areas. Abundance of birds in salt field areas and aquaculture areas were shown in Figure 2.

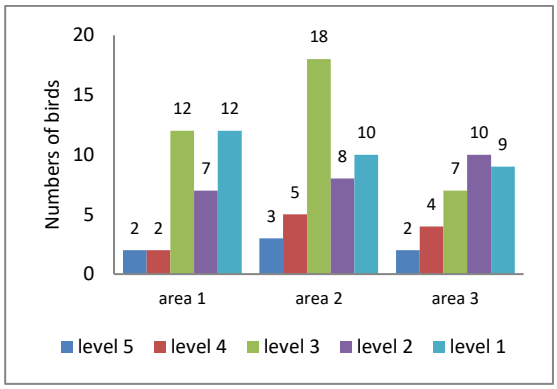

Figure 2. Abundance of Birds in salt field areas and aquaculture areas in Bang Kaew Sub-district, Samut Songkram Province.

Feeding behaviors: it was found that there were 20 species of piscivorous birds such as Caspian Tern (*Hydroprogne caspia*), Common Kingfisher (*Alcedo atthis*) and so on. There were 19 species of aquatic, benthic invertebrate feeder birds such as Little Ringed Plover (*Charadrius dubius*), Long-toed Stint (*Calidris subminuta*) and so on. There were 12 species of insectivorous birds such as Green Bee-eater (*Merops orientalis*), Lanceolated Warbler

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(*Locustella Lanceolata*) and so on. There were 4 species of granivorous birds such as Chestnut Munia (*Lonchura atricapilla*), Zebra Dove (*Geopelia striata*) . There were 1 frugivorous bird namely Streak-eared Bulbul (*Pycnonotus blanfordi*). There are 1 carnivorous bird namely Brown Shrike *(Lanius cristatus)* and 1 omnivorous bird White-breasted Waterhen (*Amaurornis phoenicurus*) was found.

As for birds' seasonal status, there were 24 resident birds, 7 resident and migratory birds, and 20 migratory birds. In Area 1 (salt field), there were 12 resident birds, 7 resident and migratory birds, and 16 migratory birds. In Area 2 (shrimp pond), there were 21 resident birds, 7 resident and migratory birds, and 16 migratory birds. In Area 3 (cockle pond), there were 13 resident birds, 6 resident and migratory birds, and 13 migratory birds because this area is rich of invertebrates which are foods of migratory birds.

According to the group of migratory birds, there were 12 species of shore birds such as Pacific Golden Plover (*Pluvialis fulva*), Whimbrel (*Numenius phaeopus*). There were 4 species of terrestrial birds such as Black-capped Kingfisher (*Halcyon pileata*) , Common Kingfisher (*Alcedo atthis*) . There were 3 species of sea birds such as Caspian Tern (*Hydroprogne caspia*), Whiskered Tern (*Chlidonias hybrida*). There were 1 species of predator namely Western Osprey (*Pandion haliaetus*).

As for IUCN Threatened Status, Painted Stork(*Mycteria leucocephala*), Eastern Black-tailed Godwit (*Limosa melanuroides*), Red-necked Stint (*Calidris ruficollis*) and Eurasian Curlew (*Numenius arquata*) were near-threatened (NT), as shown in Figure 3-5.

Figure 3. Eastern Black-tailed Godwit

According to this research, near-threaten bird species, Painted Stork (*Mycteria leucocephala*), Eastern Black-tailed Godwit (*Limosa melanuroides*), and Eurasian Curlew (*Numenius arquata*), were found. These bird species were attractive for birders and tourists who were interested in birding activity because the research areas were in the area of Don Hoi Lot which was the 1099th Ramsar Site. Moreover, these research areas were valuable for being birding site because a group of local people worked hard for protecting mangrove forest and birding foraging areas and could be a local guide for those who wanted to watch the birds, and there were lots of accommodation for tourists.

Figure 4. Eurasian Curlew

Figure 5. Painted Stork

4. Discussions

Most of migratory birds found in Thailand used East Asian Australasian Flyway which started from Arctic to Southeast Asia, Australia and New Zealand (Erftemeijer and Jugmongkol, 1999). A migration of birds is an important circle of life in living among season change. Most of birds will migrate to the place that is plenty of foods by heading south to tropical region and migrate back home for nesting when the winter is gone (Jukmongkol 2002; Kober 2004). So the result of this research indicated that the land used area in salt field, cockle pond and shrimp pond in Bang Kaew Sub-district participated in the coastal land conservation by hosting mangrove plantation activity, local fishery and Meder's mangrove crab releasing. It encouraged species checklist of migrant shorebirds foraging in the land used area such as salt field, cockle pond, and shrimp pond and so on. So these areas played an important

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role as birding sites for tourists in local community's eco-tourism and knowledge center for local communities. It is related to Chuchat (2001) who said that ecotourism is to get a chance defined as close and experience the nature and local people's life style, acknowledge the value of resources and offering jobs and income to local people.

5. Conclusion

It was found that there were 10 orders, 24 families and 51 species of birds. According to abundance of birds, 6 bird species were in level 5, 6 bird species in level 4, 17 bird species level 3, 12 bird species in level 2 and 10 bird species in level 1. According to their feeding behavior, there were 20 species of piscivorous birds, 12 species of aquatic and benthic invertebrate feeder birds, 12 species of insectivorous birds, 4 species of granivorous birds, 1 species of frugivorous birds, 1 species of omnivorous bird, 1 species of carnivorous birds and 1 predator bird. As for bird seasonal status, there were 24 resident birds, 7 resident and migratory birds, and 20 migratory birds. According to the group of migratory birds, there were 12 shore birds, 4 terrestrial birds and 3 sea birds.

6. Acknowledgements

This research was supported by Suan Sunandha Rajabhat University. Special thanks also extended to people of Samut Songhkram Province who helped with field survey in the research areas.

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Scavenging Capacity and Antibacterial Activity of Roselle Aqueous Extract and Wine Production

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Abstract

Roselle is an herbaceous medicinal plant where being native to Asia. The calyx of roselle action as well as its bioactive compounds and natural pigments. In this work, total phenolics, total flavonoids, scavenging capacity and antibacterial activity of roselle aqueous extract were investigated and applied as a substrate for wine production. Scavenging capacity of roselle aqueous extracts was increased dependently on dose of total phenolic compound in the extract. Roselle wine was produced and evaluated on pH, alcohol (%), total acids (%), total solids (°Brix), total viable counts of bacteria and yeast/mold for 10 days of fermentation. pH and total solids were significantly decreased from 0 day of fermentation, while alcohol, total acids and total viable counts of bacteria and yeast/mold were significantly increased until the end of process. Roselle aqueous extract at the concentration of 0.5 and 1 mg/ml inhibited the growth of *Escherichia coli*, *Salmonella* sp., *Bacillus* sp. and *Staphylococcus aureus* while wine products have no effect on antibacterial activity.

Keywords: Roselle, Roselle wine, Scavenging capacity, Antibacterial activity

1. Introduction

Roselle is the popular name of *Hibiscus sabdariffa* Linn. which belongs to *Malvaceae* family (Alarcon-Alonso et al., 2012). It was cultivated in many area, including Africa and Central America despite being native to Asia (Barhe & Tchouya, 2015). The outer ring of the fruit called calyx is commonly used in beverages and foods such as tea, syrup, jams and jellies (Mahadevan, Shivali & Kamboj, 2009; Borras-Linares et al., 2015). In many countries, the calyx is also applied as a traditional medicine to prevent hypertension, inflammation and liver disorders (Alarcon-Alonso et al., 2012). The aqueous extract of dried roselle flowers containing a high concentration of polyphenol which is prospective for treatment of leukemia and gastric carcinoma, hypolipiddaemia (Hopkins et al., 2013), antihypoglycaemia (Sachdewa, Nigam & Khemani, 2001) and antioxidant (Fernandez et al., 2012). Moreover, several reports are available on the consumption of the dried or fresh calyces, seeds and leaves of roselle in the preparation of beverages, fermented drinks (Da-Costa-Rocha et al., 2014). The non-alcoholic beverages have been made from a hot water extract of Roselle calyx and the product is usually sweetened with sugar and may be flavored with flavorings (Omemu et al., 2006). Other potentials of roselle calyx were the antibacterial action. The aqueous and ethanol extracts can inhibit food spoilage bacteria such as *Salmonella* Typhimurium DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* (Chao & Yin., 2008) and *Klebsiella pneumoniae* (Liu et al., 2005).

Roselle calyx can also be used in wine production in Thailand according to its red variety and due to the very high duty on imported wine. The beneficial properties of wine, mainly polyphenols, are well established (Rosenzweig et al., 2017). Wine polyphenols are derived from the extraction of the skins or seeds of fruits during the maceration process. While the color components are readily extracted into the fermenting wine, and come to equilibrium before the end of fermentation, tannins and other polymeric polyphenols continue to be extracted as long as the skins are in contact with the wine (Sacchi, Bisson & Adams, 2005). Red wines have not been generally produced from tropical fruits because of the low content of extractable red pigments in red varietal grapes (Okoro & Emeka., 2007). Efforts towards producing red wine by adding synthetic red colorants or dyes are usually controlled, as their use and quantities are regulated by law to prevent toxicity in humans (USFDA, 1993). Thus, roselle is a potential red wine raw material according to the report of Ifie et al. (2012) During fermentation, physico-chemical analysis of the wines

indicated decrease in specific gravity, soluble solids, pH and color intensity. Sensory evaluation of the aged roselle wine in terms of color, flavor, taste and overall acceptability showed no significant difference compared to commercial wine samples.

The multifunctional properties (colorant and bioactive properties) of roselle can be explored in the food and pharmaceutical industry, as natural ingredients to be incorporated into the food products (e.g. as a multifunctional ingredient) and pharmaceutical industries (e.g. as a natural colorant and medication for its bioactive properties) (Jabeur et al., 2017). From the application and medicinal values of anthocyanins, polyphenols of roselle can propose the antioxidant and antibacterial action through the wine products. Therefore, the aim of this work is to evaluate the antioxidant activity of aqueous extract of roselle, to estimate the chemical changes in wine production, to determine the antibacterial activity on roselle wine and aqueous extracts, and to prove the effect of roselle as a powerful natural plant.

2. Materials and methods

2.1 Preparation of roselle aqueous extract

Air-dried of roselle calyces were purchased from Muang, Nakhonsithammarat local market. A 50 g edible portion of roselle calyx was chopped and mixed with 100 ml of sterile distilled water at 25ºC for 12 hours, and then homogenized with blender. After filtration through a Whatman No. 1 filter paper, the filtrate was sterilized by passing through a 22-mm pore size and further freeze dried to fine powder (Chao & Yin., 2008). The powder was stored at 4 ºC until used. The substock solution of 10, 20, 30, 40 and 50 mg/ml were prepared by diluting absolute ethanol for total phenolic, total flavonoid and scavenging capacity test and 0.5 and 1 mg/ml of substock solution were prepared for antibacterial activity test.

2.1.1 Determination of total phenolics and total flavonoid contents of roselle aqueous extract

The amounts of total phenolics in roselle aqueous extract at a concentration of 10, 20, 30, 40 and 50 mg/ml were determined with the Folin-Ciocalteu reagent using the modified method of Lister and Wilson (2001). Twenty microliter of each concentration (three replicates), 100 µl of 2 N Folin-Ciocalteu's reagent were added and incubated at room temperature for 5 min. Thirty hundred microliter of sodium carbonate (Na_2CO_3) (25% w/v) was mixed and incubated at 45 ºC for 30 min. The absorbance of all samples were measured at 765 nm using UV–visible spectrophotometer. Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al., 2002). One milliliter of sample (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. Then it was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm with UV-visible spectrophotometer.

2.1.2 Scavenging capacity of roselle aqueous extract

ABTS radical-scavenging activity of the extract was determined according to Re et al., (1999) The $ABTS.^+$cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution were diluted with ethanol to get an absorbance of 0.700±0.020 at 734 nm. The roselle aqueous extract at various concentrations with 1 ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µL of $ABTS.^+$ solution and 50 µl of BHT. The inhibition percentage of ABTS radical was calculated using the following formula: ABTS scavenging activity (%) = (A0 – A1)/A0 ×100 where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

2.2 Roselle wine production

Roselle wine was prepared follow by modified method of Ifie et al., (2012). 5 g of roselle powder were dissolved in 1800 ml of distilled water into 2000 ml flask. Sucrose was added to the juice to adjust the soluble solid to 22 ºBrix. 250 ppm of sodium metabisulphite was added to inhibit the growth of bacteria and wild yeast. Dried wine yeast (*Saccharomyces cerevisiae*) was pitched in to the juice at 27±2 ºC (room temperature). Racking was done at room temperature immediately after the evolution of gases terminated; remaining yeast cells were removed from the fermenting to prevent further fermentation. Second racking was done with the introduction of bentonite slurry to aid racking and clarification. The second racking lasted for 10 days. The chemical and microbiological changes during wine production were evaluated on pH, titratable acidity, total solid, total alcohol, total bacteria and total yeast and fungal on 0, 2, 4, 6, 8 and 10 days of production.

pH and titratable acidity were determined by the method of AOAC (2000). Total titratable solid was expressed as percentage citric acid after titrating 10 ml of wine with 0.1 N sodiumhydroxide (NaOH) with phenolphthalein as an indicator. Total soluble

solids was determined using refractometer, while the alcohol percentage was estimated using ebulliometer. Total bacteria, yeast and fungal counts were determined by pour plate method (Harrigan and McCance, 1976). Total viable bacteria counts were carried out using plate count agar (PCA) and total yeast and fungal counts were grown on potato dextrose agar (PDA).

2.3 Antibacterial activity of roselle wine and aqueous extract

Four food spoilage bacteria, *Escherichia coli*, *Bacillus* sp., *Staphylococcus aureus*, and *Salmonella* sp. were supplied by Microbiology Laboratory of Science and Technology Faculty of NSTRU University, Nakhon Si Thammarat, Thailand. The roselle wine and aqueous extracts were subjected by disc diffusion assay (Jorgensen et al., 1999) with minor modifications. Briefly, four bacterial strains were grown in trypticase soy broth (TSB) at 37ºC for 16 h and cells were suspended in TSB to get 10^8 cfu/ml by using McFarland No. 0.5. Each bacterial test strains were swab onto mueller-hinton agar (MHA) medium. Then, 6 mm diameter filter paper discs with 50 µl of various concentrations of crude extracts were placed onto MHA. After incubation at 37ºC for 24 h, the antibacterial activity was measured in the diameter (mm.) of clear zone of growth inhibition.

2.4 Statistical analysis

Total phenolics, total flavonoids, scavenging capacity and antibacterial activity were expressed as mean±SD. Chemical and microbiological analysis during the roselle wine production was carried out using one way analysis of variance (ANOVA) using spss program at $p \leq 0.05$.

3. Results and discussions

3.1 Total phenolics, total flavonoid content and scavenging capacity of roselle aqueous extract

A large and diverse class of compounds, many of which occur naturally in a wide range of food and plants, is polyphenols and flavonoids, the largest and best studied group among polyphenols (Nishaa et al., 2012). A range of plant polyphenols is either being actively developed or currently sold as dietary supplements and/or herbal derived medicines. Although, many of them have properties including antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome (Furguson, 2001). Total phenolics and total flavonoid contents of roselle aqueous extracts were clearly increased with concentrations (Figure 1). Scavenging activity of roselle aqueous extract is presented in Figure 2. The extract exerted a concentration dependent scavenging and showed a maximum activity of 40.00±2.52 % inhibition at a concentration of 50 mg/ml. Roselle or *H. sabdariffa* revealed the presence of several interesting compounds, such as tocopherols, phenolic acids and flavonoids, including three different anthocyanins, 5-(Hydroxymethyl) furfural was the most abundant non-anthocyanin compound, while delphinidin-3-O-sambubioside was the major anthocyanin (Jabeur et al., 2017).

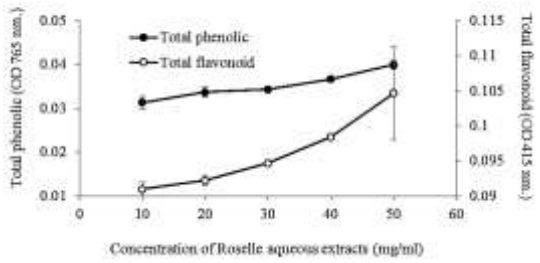

Figure 1. Total phenolics and total flavonoid contents of roselle aqueous extract**.**

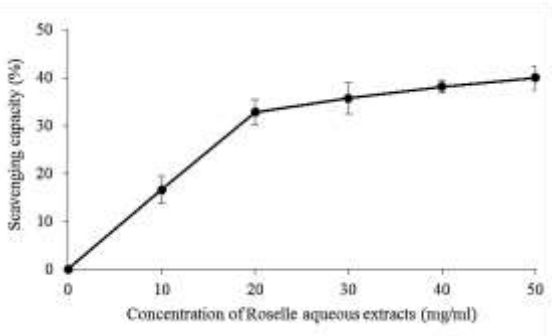

Figure 2. Scavenging capacity of roselle aqueous extract.

3.2 Chemical and microbiological changes during roselle wine production

Table 1 shows the results of chemical and microbiological changes during wine processes. The results displayed the changes in pH during fermentation which led towards the acidic range. The results equally show that the pH significantly decreased from an initial value of 2.74±0.06 on 0 day to pH of 2.54±0.06. Theoretically, sugars are converted to alcohols, then alcohols to aldehydes, aldehydes to ketones, and ketones are finally converted to acids during fermentation (Opara & Rexford, 2012) and the percentage of total acid of roselle wine was increased from 0.46±0.02 to 1.07±0.08 during the fermentation. Total solids were significantly decreased during the fermentation progress. The initial value of 22.5±0.70 ºBrix was obtained on 0 day and 16.35±0.49 ºBrix at the end of the fermentation. The decrease in total solid values

may be attributed to utilization of the sugars for growth and other metabolic activities by the organism. There was a steady increase in percentage of alcohol from an initial value which was not detectable at day 0 to a value of 6.40±0.07% at the 10th day. The increasing alcohol in the wine is due to the conversion of sugar, which has been shown to reduce as fermentation proceeded.

Table 1. Chemical and microbiological changes during the roselle wine production

Days	Chemical changes			
	pH	Alcohol (%)	Total acids (%)	Total solids (°Brix)
0	2.74±0.06	0.00	0.46±0.02	22.5±0.70
2	2.65±0.07*	2.60±0.14*	0.75±0.01*	18.90±0.71*
4	2.64±0.03*	3.55±0.07*	0.81±0.03*	18.40±0.28*
6	2.61±0.01*	4.75±0.07*	0.86±0.06*	17.65±0.77*
8	2.56±0.04*	5.60±0.14*	1.04±0.10*	16.85±0.63*
10	2.54±0.06*	6.40±0.07*	1.07±0.08*	16.35±0.49*
Days	**Microbiological changes**			
	Total bacteria (10^4 cfu/ml)		**Total yeast and fungi (10^4 cfu/ml)**	
0	1.54±4.42		1.27±3.53	
2	62.00±8.73*		3.50±4.59	
4	48.50±9.19*		2.82±1.41	
6	31.82±8.48*		4.12±1.41	
8	48.15±9.49*		41.50±6.00*	
10	34.57±9.44*		39.40±4.77 *	

Values are mean±SD for three replicates; *$P \leq 0.05$ as compared to the 0 day.

Microbiological changes in roselle wine production were observed from 0 to 10 days. There was a steady increase in viable bacteria and yeast counts from initial values of 1.54±4.42x10^4 cfu/ml and 1.27±3.53x10^4 cfu/ml to maximum values of 34.57±9.44x10^4 cfu/ml and 39.40±4.77x10^4 cfu/ml respectively, on the last day of wine production. However, from the report of Opara and Rexford, (2012), the population density started to decrease on the 6th day and continued until the 11th day. The decrease in population density could be a result of depletion of some nutrients especially nitrogen and phosphate which have been reported to enhance growth of yeast during wine fermentation. These elements were not added to the culture medium. Another reason may be due to the accumulation of toxic metabolites. It is known that consumption of glucose by yeast results in acidic metabolites.

3.3 Antibacterial activity of roselle wine and aqueous extract

Roselle wine and aqueous extracts (0.5 and 1 mg/ml) were evaluated on the antibacterial activity by disc diffusion method. Both concentrations of aqueous extract of roselle were inhibited all tested microorganisms (*E. coli*, *Bacillus* sp., *S. aureus* and *Salmonella* sp.). The highest clear zones of 0.5 and 1 mg/ml of aqueous extract of roselle were found to inhibit *E. coli* at 3.58 and 4.08 mm., respectively, which was greater than ethanol control. However, roselle wine product has no effect on antibacterial activity (Table 2). Similar to the report of Chao and Yin (2008), roselle calyx aqueous and ethanol extracts and protocatechuic acid effectively and dose-dependently inhibited the growth of *S.* Typhimurium DT104, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *B. cereus* in ground beef and apple juice. The mechanism of this action is not completely understood but it has been proposed that the extract contains phenolic compounds including flavonoids and cyaniding which have been reported to exhibit antimicrobial activities (Diarra et al., 2013).

Table 2. Antibacterial activities of roselle aqueous extract and wine product

Sample	Inhibition zone (mm.)			
	EC	***BC***	***SA***	***SM***
Roselle wine product (6.4% alcohol)	-	-	-	-
Aqueous extract of roselle (0.5 mg/ml)	3.58	3.42	3.3	3.25
Aqueous extract of roselle (1 mg/ml)	4.08	3.92	3.5	3.5
Ethanol (99.99%)	3.42	2.58	2.66	3.25
Absolute solution alcohol (6.4%)	-	-	-	-
Distilled water	-	-	-	-

- no inhibition zone; EC=*E. coli*; BC=*Bacillus* sp.; SA=*S. aureus* and SM=*Salmonella* sp.

4. Conclusions

Part of health benefits, plants are a source of pigments and pharmaceutical compounds that can be used as natural food and drug colorants. Today, the nutritional and bioactive components of plants gain much interest not only among scientists, but also in people's life styles. *H. sabdariffa* or roselle calyx is one of the plants which is commercially applied into food products. From our report, roselle was claimed to have a strong effect on scavenging and antibacterial activity due to polyphenol compounds. When roselle calyx was included in the raw material of wine production, the wine produced from roselle has been found to be a very good raw material as it can give great color and flavor. Also, high acidity of roselle wine gives it an edge in terms of storability and its resistance to microbial spoilage. In addition, roselle is readily available and cheap. Thus, it can be a good raw material for wine industry.

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6. References

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