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Research Article

ASSESSMENT OF ORGANIC ACID CONTENT, AND BRIX VALUE OF REPRESENTATIVE INDIGENOUS FERMENTED PLANT BEVERAGES OF THAILAND

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ABSTRACT

Objective: In Thailand, people believe that the fermented plant beverages (FPBs) have the pharmaceutical effects and consumption of FPBs cure the diseases. This study was conducted to detect and quantify the organic acids, pH, and Brix value of the commonly using FPBs of Thailand.

Methods: The samples were collected from all the region of Thailand. The total acidity, pH, and organic acid content were measured by titration, pH meter, and high-performance liquid chromatography methods, respectively. The sugar content of the sample was assessed by Brix value determination using a refractometer.

Results: All the tested FPBs showed the pH value of 2-4 except sample no. 64 (pH 6.49) and 65 (pH 5.72). The acidity of the samples showed a range of ~0.1-2.5% of lactic acid equivalent. The acidity of sample no. 64 and 65 was not in detectable level. As per the Thai Community Product Standard (TCPS) - No. 481/2004, all the samples were qualified for the human consumption except sample no. 64 and 65. About 34, 17, 58, 53, 8, 6, 12, and 1 samples were detected with 0.018-0.241, 0.015-0.389, 0.028-1.244, 0.052-0.550, 0.024-0.267, 0.049-0.373, 0.025-0.980, and 0.31 mg/mL of as tartaric, malic, lactic, acetic, citric, sorbic, fumaric, and propionic acids, respectively. The sugar content of the sample no. 9 and 49 was >55 % of Brix value, while other tested samples were <50%.

Conclusion: All the tested FPBs were harmless to human regarding organic acid content, pH, and Brix value except sample no. 64 and 65 as per the TCPS. Further studies are mandatory to explore the microbial and pharmaceutical nature of traditional FPBs and other fermented foods of Thailand.

Keywords: Brix value, Fermented plant beverages, *Morinda*, Organic acids, pH.

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INTRODUCTION

The fermented plant beverages (FPBs) are considered as functional food, in particular, among the people of Asian countries. FPBs are an aid to prevent and cure the illness. In general, traditionally FPBs are being prepared by natural fermentation, in some cases controlled fermentation process has been employed to achieve the best quality. The lactic acid bacteria (LAB) based controlled fermentation improves the nutritional value and bioactivity of active ingredients [1,2].

In Thailand, FPBs were initially produced by local Thai people for their consumption; later FPBs were commercially manufactured throughout the country. Many Thai people are the regular consumers of FPBs. In Thailand, consumption of FPBs is one of the traditional ways of keeping the body healthy. The preparation of a typical FPB is comprised of plant materials, sugar, and water in the ratio of 3:1:10 with or without the addition of starter culture (10%) [3]. The fermentation process was carried out at room temperature for 6 months to 1 year depends on the raw materials used and the type of fermentation (with the starter or without starter). The seed stock of fermented juice from the previous batch can be used as a starter. For the formulation or production of fresh FPBs, people employed the spontaneous fermentation process by the involvement of naïve residing microbes of raw materials.

Morinda citrifolia Linn., commonly known as Noni, is one of the widely used raw material for FPBs preparation in Thailand and it has the history of 2000 years in folk medicine. The major constituents of Noni have been reported [4]. Many people are consuming the noni beverages, and they believe that the noni juice has disease curing ability, even before any scientific pieces of evidence. The studies proved that the noni has some therapeutic properties such as anticonstipation, antihypertension, antimicrobial activity, and even promising candidate for autoimmune disorder and cancer treatments [4-6]. Moreover, *in vivo* diuretic activity of noni juice and phytochemical components were reported [7-9].

Another frequently used raw material for the preparation of FPBs in Thailand is black galingale (BG) (*Kaempferia parviflora*), a native Thai herbaceous plant, used in the traditional medicines of Southeast Asia to treat gastrointestinal related diseases and people believe that BG has testosterone-like activity but it was proved that BG does not have such an activity [10]. The principle compounds of the BG were already reported, and the bioactivities - such as antibacterial, and antigastro ulcer activities - were studied [11,12].

Many fermented plant products are prepared with mixed fruits and vegetables in Thailand to gain the combined benefits from several raw materials. The preparation of FPBs is a vast area of discussion, and several secret traditional and ancient fermentation methods are being practiced by the native producers of FPBs. Even though high throughput industrial and commercial techniques are available for the production, packaging, and marketing of fermented foods, especially FPBs, still many of the small and medium scale manufacturers following the traditional way of preparing and processing of FPBs in Thailand. To ensure the safety of the commonly used FPBs of Thailand, we have previously studied the major chemical constituents of FPBs [1]. The factor affecting methanol formation during noni-based FPB preparation was described earlier [3].

Table 1: List of FPBs evaluated in the study

S. No.	Type of sample
Morinda based FPBs	
1	Concentrated *Morinda* juice
2	*Morinda* juice mixed with Indian gooseberry and other fruit
3	*Morinda* bio extract (concentrated)
4	*Morinda* juice leavening
5	Concentrated *Morinda* juice (Enzyme)
6	*Morinda* juice leavening
7	Pasteurized *Morinda* herb juice with honey
8	Concentrated *Morinda* herb juice
9	*Morinda* herb extraction
10	Concentrated *Morinda* herb extraction
11	*Morinda* herb juice
12	Concentrated *Morinda* juice (Concentrated leavening)
13	*Morinda* juice-1
14	Fermented *Morinda* juice-1
15	Fermented *Morinda* juice-2
16	*Morinda* juice (99%)
17	*Morinda* juice-2
18	Pasteurized sour *Morinda* juice
19	*Morinda* juice-3
20	*Morinda* juice with leavening-1
21	*Morinda* juice with leavening-2
22	Fermented *Morinda* juice-3
23	130 type fermented fruit juice
24	*Morinda* and banana juice
25	*Morinda* and banana juice with honey
26	Fermented *Morinda* juice-4
27	Fermented *Morinda* juice-5
28	Concentrated enzyme *Morinda* juice
29	Fermented *Morinda* juice-6
30	Fermented *Morinda* juice-7
31	Fermented *Morinda* juice-8
32	Fermented *Morinda* juice-9
33	Fermented *Morinda* juice-10
Mixed fruits based FPBs	
35	Concentrated mixed fruit juice (sour)
36	Concentrated mixed fruit juice with honey
37	Bio extract juice
38	Fermented mixed fruit juice (sour)
39	Concentrated fruit juice leavening
40	Fermented sour fruits juice
41	Fermented sweet fruits juice
42	Mixed herb juice
43	Fermented mixed fruit juice
44	Fermented enzyme bio extract
45	Mixed fruit enzyme juice
46	Mixed fruit enzyme-1
47	Premium fruit enzyme
48	Mixed fruit enzyme-2
BG based FPBs	
49	BG herb juice-1
50	BG herb juice mixed with *Morinda* juice
51	BG leavening
52	BG juice with honey and brown sugar
53	Concentrated BG herb juice
54	BG bio extract
55	Fermented BG juice
56	BG herb juice-2
Other tested FPBs	
57	Bio-fermented juice
58	Cha-tu-pa-la ti-ka herb juice
59	*Curcuma xanthorrhiza* Roxb. herb juice
60	Fermented banana juice
61	Fermented longan juice
62	Concentrated 9 herbals juice
63	Pasteurized and fermented herb juice
64	Healthy drinking Kefer 100%
65	Healthy drinking EM.X
66	Fermented banana juice
67	Fermented Indian goose berry juice
68	Worm wood juice
69	Syrup and concentrated juice
70	Fermented passion fruit juice

The FPBs were classified based on the raw material. FPB: Fermented plant beverages

This is the primary paper explaining about the pH, organic acid, and brix values of the representative commonly used FPBs of Thailand. The samples were carefully collected and excluded the imported FPBs to figure out the status of Thai ethnic products.

METHODS

Sample collection

The representative FPBs with various composition, raw materials, making procedures, and manufacturing unit (homemade, small-scale, and medium scale) were collected from all the main cities of Thailand. The list of samples was tabulated (Table 1).

Measurement of pH and titratable acidity

The acidity of the samples was measured by pH meter (Inola, pH level 2, Weilheim, Germany) and titration technique. In the case of titration procedure, acidity was expressed as a percentage of lactic acid. Briefly, 100 ml of hot water was mixed with 0.5 ml of phenolphthalein (act as an indicator), and 2.5 ml of degassed sample (samples were kept open for 5 minutes before analysis) was added. The samples were titrated with 0.1 M sodium hydroxide (NaOH) until the formation of pink color (endpoint). Then, the percentage of lactic acid was calculated by following formula 1.

$$\%\text{Lactic acid} = \frac{M \times V \times 0.09 \times 100}{V \text{ of sample}} \quad (1)$$

Where,

M is the concentration of NaOH in M or mol/L,

V is the volume of NaOH used in mL,

0.09 is the constant which come from molecular weight of lactic acid,

V of the sample is the volume of sample in mL.

Determination of organic acid content

The organic acid content in the samples was evaluated by high-performance liquid chromatography (HPLC). About 20 µL of filtered (syringe filter, 0.45 µm) samples were used for analysis, and the separation was attained using Phenomenex C8 column (250 mm × 4.6 mm, 5 µm) (Phenomenex, Darmstadt, Germany). The phosphate buffer (0.1 M, pH 2.1) was used as mobile phase (flow rate of 0.8 mL/min). The concentration of organic acids was calculated by comparing the peak area of standards (lactic acid and acetic acid) and samples. The values lower than the limitations of detection were described as not detected (ND) (Table 2). The values equal and higher than the limitation of quantity were also reported [2].

Measurement of brix value

The Brix value of samples was calculated by refractive index using ABBE precision refractometer (Atago, 3T Model, Honshu, Japan).

Statistical analysis

All the values were denoted as mean±standard deviation. Data were evaluated with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance. Differences were considered as significant at $p<0.05$.

RESULTS AND DISCUSSION

A sum of 70 samples was collected, among which, 33 samples were *Morinda* based FPBs. The collected samples preserved at −20°C for the further analysis. The exact composition of the samples was reported. The FPB samples were classified based on the principal raw material used for the preparation and are listed in Table 1. We found that about ~47% of commonly used FPBs of Thailand were made out of *Morinda.* The samples were collected with the notion that to study the organic acid content of the domestic fermented products, thus imported beverages were not included in the sampling procedure.

The pH of FPB samples was measured, and the results were represented (Fig. 1). All the samples exhibited the pH value of 3-4 except sample no. 64 (pH 6.49) and 65 (pH 5.72). The acidity of samples was represented as a percentage of lactic acid equivalent. There was no consistency in the acidity of the samples, and ~0.1-2.5% of acidity was recorded. The acidity of samples 64 and 65 was not in detectable level (Fig. 2). The pH and acidity of the FPBs are close associated with the type of fermentation process. Some of the producers use specific LAB as a starter for making FPBs while others rely on natural fermentation by residing microbes of raw materials. The duration of the fermentation process also played a significant role in pH and acidity of the product. The pH of ≤4.3 is acceptable for fermented beverages as per the Thai Community Product Standard (TCPS) - No. 481/2004. All the tested FPBs passed the TCPS regulation except sample no. 64 (healthy drink Kefer 100%) and 65 (healthy drink EM.X). The composition of the products was not available. Moreover, the acidity, regarding lactic acid content, was ND in sample no. 64 and 65.

Table 2: Stable values of LOD and LOQ of organic acid in FPBs.

S.No.	Organic acid	LOD (mg/mL)	LOQ (mg/mL)
1	Tartaric acid	0.015	0.038
2	Malic acid	0.015	0.037
3	Ascorbic	0.08	0.201
4	Lactic acid	0.028	0.069
5	Acetic acid	0.041	0.102
6	Citric acid	0.021	0.051
7	Sorbic acid	0.016	0.04
8	Fumaric acid	0.024	0.06
9	Propionic acid	0.038	0.094

LOD: Limit of detection, LOQ: Limit of quantitative, FPB: Fermented plant beverages

The formation of organic acid during the fermentation process is due to the hydrolysis, biochemical, and microbial metabolism. Even though the presence of organic acids prevents or diminish the growth of potent foodborne pathogens during fermentation, it affects the color, flavor, and stability of the finished product [13].

The presence and amount of the primary organic acids in FPBs were measured by HPLC (Tables 3 and 4). The organic acids, such as tartaric acid, malic acid, lactic acid, acetic acid, citric acid, sorbic acid, fumaric acid, propionic acid, ascorbic acid, and shikimic acid, were quantified in FPB samples. About 34, 17, 58, 53, 8, 6, 12, and 1 samples were detected with 0.018-0.241, 0.015-0.389, 0.028-1.244, 0.052-0.550,

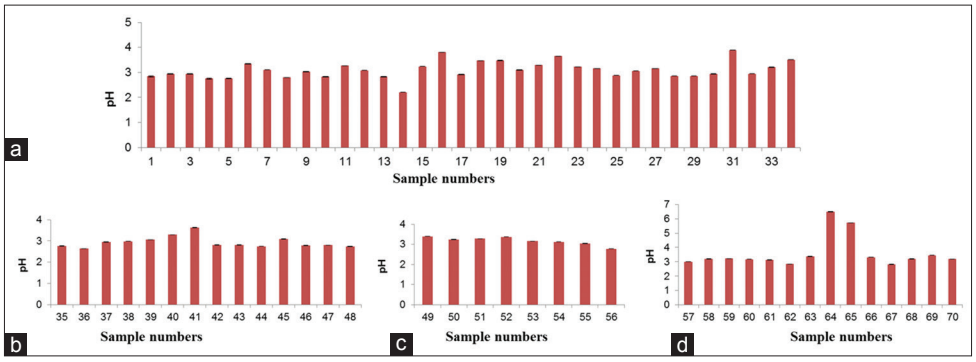

Fig. 1: The pH profile of the tested fermented plant beverages. (a) *Morinda* based fermented plant beverages (FPBs), (b) Mixed fruits based FPBs, (c) Black galingale based FPBs, (d) Other tested FPBs

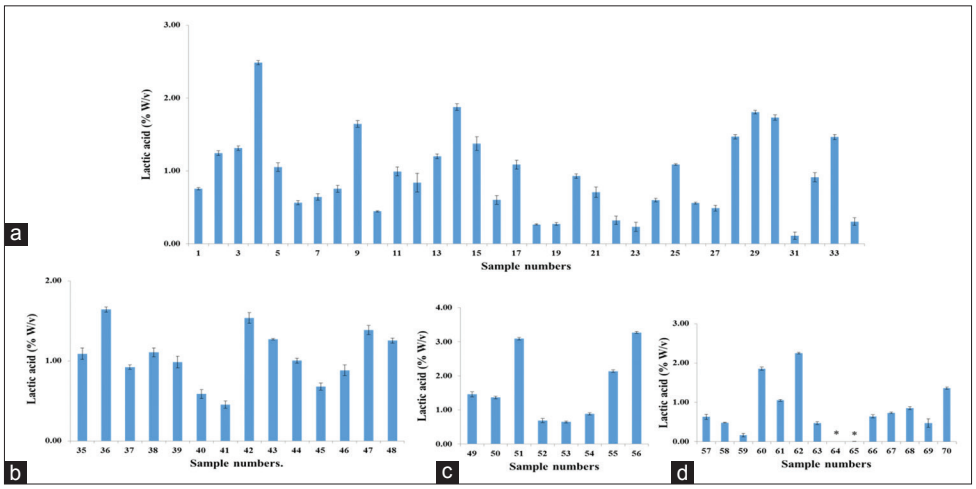

Fig. 2: The acidity of the tested fermented plant beverages. The values were represented as a percentage of lactic acid equivalence of acidity. (a) *Morinda* based fermented plant beverages (FPBs), (b) Mixed fruits based FPBs, (c) Black galingale based FPBs, (d) Other tested FPBs

Table 3: The presence of difference types of organic acid in FPBs

Sample no.	Tartaric	Malic	Lactic	Acetic	Citric	Sorbic	Fumaric	Propionic
1	ND	NF	0.36	0.095	NF	NF	NF	NF
2	ND	NF	0.13	0.21	0.037	NF	ND	0.31
3	NF	NF	0.2	0.12	ND	NF	NF	NF
4	NF	NF	0.33	0.27	ND	NF	NF	NF
5	NF	NF	0.25	0.052	NF	NF	NF	NF
6	0.019	0.045	0.06	0.064	0.03	0.145	ND	NF
7	0.124	0.034	0.19	0.063	ND	0.373	NF	NF
8	0.107	NF	0.137	0.276	NF	NF	0.072	NF
9	0.061	0.021	0.122	0.116	NF	0.049	NF	NF
10	NF	NF	0.29	0.42	NF	0.129	NF	NF
11	0.085	NF	0.166	0.106	NF	NF	0.039	NF
12	ND	NF	0.078	0.057	NF	NF	NF	NF
13	ND	NF	0.206	0.16	NF	NF	NF	NF
14	NF	NF	0.32	0.17	ND	NF	0.074	NF
15	ND	NF	0.29	0.153	ND	NF	0.028	NF
16	0.212	NF	0.37	0.26	NF	NF	0.049	NF
17	0.165	0.101	0.13	0.066	NF	NF	ND	NF
18	NF	NF	0.12	0.076	NF	NF	0.98	NF
19	NF	NF	0.1	0.21	NF	NF	NF	NF
20	0.089	NF	0.23	0.094	NF	NF	NF	NF
21	0.025	NF	0.053	NF	NF	NF	NF	NF
22	NF	NF	NF	NF	NF	NF	0.398	NF
23	NF	0.021	0.33	0.36	NF	NF	NF	NF
24	ND	NF	0.28	0.166	NF	NF	ND	NF
25	NF	NF	0.05	0.55	0.024	NF	ND	NF
26	NF	NF	0.09	0.146	NF	NF	0.106	NF
27	NF	NF	0.51	0.275	NF	NF	0.178	NF
28	0.018	NF	0.22	0.059	NF	NF	NF	NF
29	0.052	0.017	0.052	0.1	NF	NF	NF	NF
30	NF	0.389	0.152	NF	NF	NF	NF	NF
31	NF	0.027	0.213	NF	NF	NF	0.173	NF
32	NF	0.014	0.049	NF	NF	NF	NF	NF
33	NF	0.018	ND	NF	NF	NF	NF	NF
34	NF	0.015	0.031	NF	NF	NF	NF	NF
35	0.066	0.102	0.036	NF	0.267	NF	NF	NF
36	0.055	NF	0.072	0.062	NF	NF	NF	NF
37	NF	NF	0.208	0.191	NF	NF	NF	NF
38	0.126	0.032	0.028	NF	NF	NF	NF	NF
39	0.028	NF	NF	NF	NF	NF	NF	NF
40	0.18	NF	NF	NF	NF	NF	NF	NF
41	0.07	0.043	NF	NF	0.057	NF	NF	NF
42	0.067	NF	0.078	0.09	NF	NF	NF	NF
43	0.049	NF	0.059	0.115	NF	NF	NF	NF
44	0.17	0.048	ND	NF	NF	NF	NF	ND
45	0.1	NF	0.35	0.068	NF	NF	0.025	NF
46	0.106	0.033	ND	NF	NF	NF	NF	NF
47	0.12	NF	0.165	0.121	NF	NF	NF	NF
48	0.241	0.014	0.219	0.189	NF	NF	NF	NF
49	0.122	NF	0.131	0.11	NF	NF	NF	NF
50	0.116	NF	0.133	0.043	NF	NF	0.047	NF
51	0.195	NF	0.058	0.048	NF	NF	NF	NF
52	0.019	NF	0.88	0.139	0.143	NF	NF	NF
53	0.14	0.031	0.565	0.094	NF	0.101	NF	NF
54	0.134	ND	1.244	0.161	0.118	NF	NF	NF
55	0.115	ND	0.449	0.246	NF	NF	NF	NF
56	NF	NF	0.062	0.059	NF	NF	NF	NF
57	NF	NF	0.185	0.235	NF	NF	NF	NF
58	NF	NF	0.327	0.159	NF	NF	NF	NF
59	NF	NF	0.17	0.142	NF	NF	NF	NF
60	0.057	NF	0.133	0.137	NF	NF	NF	NF
61	0.057	NF	ND	0.143	NF	ND	NF	NF
62	NF	NF	0.187	0.115	NF	NF	NF	NF
63	0.046	0.108	ND	0.059	0.053	0.08	NF	NF
64	NF	NF	0.18	0.27	NF	NF	NF	NF
65	ND	NF	NF	ND	NF	NF	NF	NF
66	NF	NF	0.034	0.22	NF	NF	NF	NF
67	NF	NF	0.088	0.304	NF	NF	NF	NF
68	0.082	NF	0.104	0.338	NF	NF	NF	NF
69	NF	NF	NF	NF	NF	NF	NF	NF
70	NF	NF	NF	NF	NF	NF	NF	NF

The values are represented as mg/mL of sample. The values are expressed as a percentage of organic acids in tested samples. Ascorbic acid and shikimic acid content were not found in any of the tested samples. NF: Not found, ND: Not detected, FPB: Fermented plant beverages

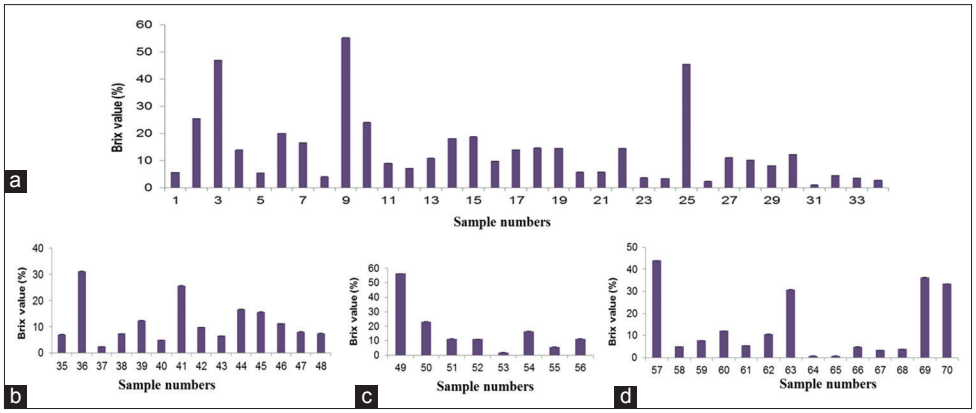

Fig. 3: The Brix values of the tested fermented plant beverages. (a) *Morinda* based fermented plant beverages (FPBs), (b) Mixed fruits based FPBs, (c) Black galingale based FPBs, (d) Other tested FPBs

Table 4: The organic acid content in FPBs samples

S.No.	Organic acid	Content (mg/mL)	Number of samples
1	Tartaric acid	0.018-0.241	34
2	Malic acid	0.015-0.389	17
3	Lactic acid	0.028-1.244	58
4	Acetic acid	0.052-0.550	53
5	Citric acid	0.024-0.267	8
6	Sorbic acid	0.049-0.373	6
7	Fumaric acid	0.025-0.980	12
8	Propionic acid	0.31	1
9	Ascorbic acid	The content was lower than LOD (0.102 mg/mL)	
10	Shikimic acid	Not found	

LOD: Limit of detection, FPB: Fermented plant beverages

0.024-0.267, 0.049-0.373, 0.025-0.980, and 0.31 mg/mL of as tartaric acid, malic acid, lactic acid, acetic acid, citric acid, sorbic acid, fumaric acid, and propionic acid, respectively. The concentration of ascorbic acid was less than limit of quantitation, while shikimic acid was not found (Table 4).

The breakdown of lactic acid and formation of acetic acid were observed after 12 days of fermentation [14]. The formation of acetic acid was due to the oxidation of lactic acid [15]. It is known that the organic acid content of fermented avocado juice was mainly composed of malic and tartaric acids [16]. The results of this study indicated that most of the tested FPB samples are enriched with lactic acid (58), acetic acid (53) and tartaric acid (34), possibly due the presence of LAB.

The Brix value of the samples was studied to determine the sugar level, and the mean percentage of Brix values of tested FPBs were represented (Fig. 3). The sample No. 9 and 49 showed more than 55% of Brix value, which were prepared with *Morinda*, and BG as primary raw materials, respectively. The sugar content of the product was varied based on the raw materials and the addition of a different amount of sugar sources like honey.

CONCLUSION

All the tested FPBs were safe for the human consumption regarding organic acid content, pH, and Brix value except sample no. 64 and 65 as per the TCPS. Further studies are required to explore the microbial and biochemical nature of traditionally FPBs and other fermented foods of Thailand.

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