J Food Sci Technol (October 2024) 61(10):1894–1904
https://doi.org/10.1007/s13197-024-05966-6

ORIGINAL ARTICLE

Exposure assessment to areca alkaloids in the Chinese populations through areca nut chewing

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Revised: 21 February 2024 / Accepted: 23 February 2024 / Published online: 10 March 2024

Abstract Chewing areca nuts is popular in China. Areca alkaloids are the major toxic compounds in areca nuts. In this study, the levels of four areca alkaloids (i.e. arecoline, arecaidine, guvacoline and guvacine) in 119 areca nut samples were analyzed and 3030 areca nut consumption questionnaires were collected to investigate the exposure to areca alkaloids in the Chinese populations through areca nut chewing. The levels of arecoline, arecaidine, guvacoline and guvacine in different areca nut products were 0.46–4.97 mg/g, 0.57–7.51 mg/g, 0.08–1.44 mg/g and 0.03–8.48 mg/g, respectively. Chewing fresh areca fruits was the main source of arecoline and the total areca alkaloids exposure. The estimated daily intake (EDI) of arecoline and the total areca alkaloids for the Chinese populations were 1.126 and 2.625 mg/kg BW/day for average exposure, 4.411 and 9.739 mg/kg BW/day for high exposure (P95th). The EDI varied with age and gender. The young male population (≤ 34 years) had the highest EDI than other populations. Concentrated and focused efforts are required to educate the general public, especially the young male population, about the risks of areca nut chewing to reduce exposure to areca alkaloids of the Chinese population.

Keywords Areca nut · Areca alkaloids · Exposure assessment · Chinese populations

Introduction

Areca nut (*Areca catechu* L.), the seed of *Areca catechu* palm, is the fourth most widely consumed substance after alcohol, nicotine, and coffee (Pan et al. 2020; Pasupuleti et al. 2022). More than 600 million people chew areca nut worldwide for its mild psychoactive effects. In China, the consumption of areca nuts is notably prevalent in the Hainan, Hunan, and Taiwan Provinces (Peng et al. 2015; Su et al. 2020). Fresh areca fruits can be chewed directly with slaked lime and betel leaf with or without tobacco in many countries and regions such as Papua New Guinea, India, Taiwan and Hainan Provinces of China (Chang et al. 2022; Lee et al. 2018; Mehrtash et al. 2017). In the Chinese mainland, fresh areca fruits can be processed into dried areca husks. Firstly, fresh areca fruits are dried either with or without smoking (Pan et al. 2020). Subsequently, the dried areca nut (unsmoking or smoking) was processed undergoes a process of blanching, soaking, coring, brining, and air-drying to produce two categories of dried areca husks, namely unsmoking-dried areca husks and smoking-dried areca husks (Sun et al. 2017; Wang et al. 2018). Dried areca husks are particularly prevalent in the Hainan and Hunan Provinces of China (Peng et al. 2015).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13197-024-05966-6.

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Epidemiological studies have demonstrated a significant association between areca nut chewing and oral cancer (Lin et al. 2022; Warnakulasuriya and Chen 2022). The International Agency for Research on Cancer (IARC) classified areca nut as a Group 1 carcinogen in 2004 (IARC 2004). The previous study reported that the risk of oral cancer from areca nut chewing was higher than that from smoking and alcohol consumption in China (Lee et al. 2019). The China Cancer Registry Annual Report 2019 showed that the oral cancer incidence rates in Hainan (5.08 per 100,000 people) and Hunan (4.22 per 100,000 people) Province were higher than the global incidence rates of oral cancer (3.72 per 100,000 people) (Hu et al. 2017; Liu 2020; Peng et al. 2019). The mutagenic mechanism of areca nut chewing is attributed to the corrosion of the slaked lime, the abrasion of areca nut fibers and the toxicity of alkaloids. Arecoline, arecaidine, guvacoline and guvacine are the major alkaloids in areca nuts (Cao et al. 2020), which contain 0.3–0.7% of the dry weight of areca nuts (Peng et al. 2015). Studies displayed that the exposure of areca alkaloids is associated with various types of cancers (Pasupuleti et al. 2022). Among all areca alkaloids, arecoline is regarded as the main carcinogenic component, which has been classified as a Group 2B carcinogen by IARC (Marques et al. 2021).

Numerous literatures have reported the contents of areca alkaloids in areca nut products from the Chinese market. Sun et al. (2017) observed that the concentrations of arecoline ranged from 0.97 to 3.60 mg/g in 30 kinds of areca nut products from different regions of China (Sun et al. 2017). Cao et al. (2020) found that the level of four areca alkaloids varied depending on the place of origin (Cao et al. 2020). However, few studies focused on the exposure of areca alkaloids to Chinese populations through areca nut chewing.

Thus, this study aimed to investigate the exposure to areca alkaloids in Chinese populations through areca nut chewing. A total of 119 samples, including fresh areca fruits, dried areca nuts and dried areca husks were analyzed to acquire the contents of four areca alkaloids in the Chinese market. Additionally, a collection of 3100 questionnaires were collected to assess the average daily intake (ADI) of fresh areca fruits and dried areca husks for the Chinese populations. The exposure levels of arecoline and the total areca alkaloids to the Chinese population were calculated based on the contents of areca alkaloids and the comprehensive survey of areca nut consumption. This study lays a foundation for the risk assessment of areca alkaloids.

Material and methods

Chemicals

Arecoline hydrobromide (purity > 98%) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd (Shanghai, China). Arecaidine hydrochloride (purity ≥ 98%) and guvacine hydrochloride (purity ≥ 95%) were purchased from Beijing Solarbio Science & Technology Co. Ltd. (Beijing, China). Guvacoline hydrochloride (purity ≥ 95%) was obtained from Chengdu DeSiTe Biological Technology Co. Ltd. (Chengdu, China). HPLC-grade formic acid was supplied by Sigma (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were provided by Balinway Chemical Technologies Co., Ltd. (Beijing, China). All the other chemicals were of analytical grade.

Areca nut samples collection

One hundred and nineteen areca nut samples were collected from the local markets in Hunan and Hainan Provinces of China from June 1st to October 30th, 2022. The samples included five fresh areca fruits (samples 1–5), eight unsmoking-dried areca nuts (samples 6–13), eight smoking-dried areca nuts (samples 14–21), five smoking-dried areca husks (samples 22–26) and 93 unsmoking-dried areca husks (samples 27–119). Twenty-two brands of commercial dried areca husks were included.

Areca alkaloids extraction

All samples were dried to a constant weight and the moisture contents were recorded. Samples were crushed and passed through a 60-mesh sieve. The sample (100 mg) was weighed into a 50 mL centrifuge tube, and extracted with 40 mL pure water under ultrasound at 30 °C for 40 min. Subsequently, the samples were centrifuged at 5000 r/min for 10 min. This process was repeated two times. The supernatant was filtered through a 0.22 μm water filtration membrane before UPLC-MS/MS determination.

UPLC-MS/MS analysis

The simultaneous analysis of arecoline, arecaidine, guvacoline and guvacine was performed on an Acquity UPLC™ I-Class system (Waters, Milford, MA, USA) coupled with a Quattro micro API triple quadrupole mass spectrometer (Micromass Company Inc, Manchester, UK) equipped with an electrospray ion source (ESI). Chromatographic separation was carried out on an Acquity UPLC™ HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters, Milford, MA, USA) at 40 °C. The binary mobile phase consisted of formic acid water (0.1%, *v*/*v*) (mobile phase A) and acetonitrile (mobile phase B). The flow rate was 0.2 mL/min and the gradient elution was carried out as follows: 0–4.0 min, 0–70% B; 4.0–4.1 min, 70–0% B; 4.1–7.1 min, 0% B. An injection volume of 5 μL was used.

Mass spectrometry analysis was operated using electrospray ionization (ESI) in the positive ion mode. The multiple

reaction monitoring (MRM) mode was used for the quantitation of target analytes. The following optimized MS instrument parameters were discovered: capillary voltage of 0.5 kV, cone voltage of 23 V, source temperature of 100 °C, desolation temperature of 500 °C, cone gas flow of 150 L/h (nitrogen, 99.9% purity), desolation gas flow of 1000 L/h (nitrogen, 99.9% purity), and argon collision gas pressure of 2×10^{-3} mbar for MS/MS. MassLynx V4.1 (Waters Co., Milford, MA, USA) was used to analyze the data.

Method validation

The characteristics of the proposed method were validated in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision and recovery. LOD and LOQ were determined as three times and ten times the observed signal-to-noise ratio (S/N), respectively. The linear regression equation was drawn by a standard curve with concentration as abscissa and response peak area as ordinate. Accuracy was evaluated by six replicate assays of standards and addressed by the % accuracy (calculated by observed amount/specified amount × 100%). The precision of the method was studied by implementing intraday (repeatability) and interday (reproducibility) precision of samples. Precision was addressed by % relative standard deviation (RSD) of six measurements. The recoveries were determined by adding three concentration mixed standards solution of arecoline, arecaidine, guvacoline and guvacine to the extracts of areca nut samples.

Exposure assessment

Questionnaire source

A face-to-face areca nut consumption questionnaire was designed to investigate the daily consumption of areca nuts by consumers. The questionnaire has twenty questions including six questions about personal basic information, seven questions about living habits and the rest about the type and quantity of areca nut consumption. Three thousand and ten questionnaires were collected in the provinces of Hunan and Hainan from June 1st, 2022 to October 30th, 2022. All participants signed the informed consent form. A total of 2151 valid participants (aged from 13 to 74 years, 1561 males and 590 females) were recovered and analyzed.

Daily exposure assessment

Simple distribution assessment method was used. The estimated daily intake (EDI) of areca alkaloids was calculated according to the average concentration of areca alkaloids in fresh areca fruits and dried areca husks, the individual consumption amount of fresh areca fruits and dried areca husks and the individual body weight using the following formula:

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{FIR}}{\mathrm{BW}}$$

where C is the concentration of areca alkaloids in the fresh areca fruit or the dried areca husk (mg/g), FIR is the daily consumption of fresh areca fruits or dried areca husks (g/day), BW is the individual body weight (kg).

2.7 Statistical analysis

All analyses were performed in triplicate with values expressed as mean ± standard deviation. Principal component analysis (PCA) was performed in SIMCA 14.1 (UMETRICS, Umea, Sweden). Statistical analyses were conducted using SPSS 27 (IBM Co., USA). Differences among experiments were obtained using Kruskal–Wallis H test ($p < 0.05$). Graphs were drawn using Origin 2022 (Northampton, MA, USA).

Results and discussion

Simultaneous determination of four areca alkaloids

The ESI positive mode was selected according to the structural characteristics of four areca alkaloids. The most abundant product ion was used for quantitation and second one was selected for qualification. The qualitative and quantitative ions were *m/z* 156.1 > 113.2 and 156.1 > 44.2 for arecoline, *m/z* 142.1 > 81 and 142.1 > 30 for arecaidine, *m/z* 142.1 > 81 and 142.1 > 30 for guvacoline, *m/z* 128.1 > 99.1 and 128.1 > 30.2 for guvacine (Table S1). The optimized MS parameters for four areca alkaloids are summarized in Supplementary Table S1. The selection of these fragment ions is consistent with others.

Different mobile phases were tested to achieve good retention and separation of four areca alkaloids. Water was chosen as the mobile phase A considering the polarity of areca alkaloids. Moreover, formic acid was added into water to improve the peak shape and sensitivity of analytes. Ideal retention, separation and response intensity of four areca alkaloids were achieved when mobile phase A was formic acid/water (0.1%, *v*/*v*) (Figure S1). Subsequently, acetonitrile and methanol were tested as mobile phase B. Compared to methanol, acetonitrile showed better separation (Figure S2). Therefore, the binary mobile phase consisting of formic acid/water (0.1%, *v*/*v*) (mobile phase A) and acetonitrile (mobile phase B) was selected.

Extraction conditions of four areca alkaloids were investigated. Water, 45% (*v*/*v*) ethanol–water, 70% (*v*/*v*)

ethanol–water and acetonitrile were selected and compared as extraction solvents. Both 45% (*v*/*v*) ethanol–water and 70% (*v*/*v*) ethanol–water led to the co-extraction of areca alkaloids and other substances, which obstructed subsequent separation and analysis (Figure S3). Although all four areca alkaloids were efficiently extracted by both water and acetonitrile, water was chosen due to its environmental sustainability and cost-effectiveness. Similarly, water was also chosen to extract areca alkaloids by Chang et al. (2022). In comparison with water bath vibration, the ultrasonic treatment showed better extraction efficiency as the ultrasound-induced cavitation facilitated the transfer of areca alkaloids from samples to the extraction solvent (Fu et al. 2020). Areca alkaloids were extracted completely after being treated by ultrasound for two times.

Method validation

The results of method validation are summarized in Table 1. The LOD and LOQ of four areca alkaloids were in the range of 0.07–1.51 μg/L and 0.25–3.85 μg/L, respectively. The intraday repeatability and interday reproducibility of four areca alkaloids were 1.23–7.85% and 1.20–2.71%, respectively. All areca alkaloids showed decent linearity with correlation coefficient (R^2) values ranging from 0.9929 to 0.9999. These results indicated that the current method had satisfactory sensitivity and stability. Meanwhile, the mean recoveries of four areca alkaloids in real areca nut samples were 76.83–111.94%, 89.54–125.92%, 87.09–123.93% in low, medium, and high markup levels, respectively (Table 1), indicating that the extraction method was effective in actual samples.

Concentration of areca alkaloids

One hundred and nineteen areca nut samples were collected and analyzed to comprehensively investigate the levels of areca alkaloids in different areca nut products (Table S2). The samples covered fresh areca fruit, dried areca nuts (smoking and unsmoking) and dried areca husks (smoking and unsmoking). The concentrations of areca alkaloids were various in different samples ($p < 0.001$). The levels of arecoline, arecaidine, guvacoline and guvacine in different areca nut products were 0.46–4.97 mg/g, 0.57–7.51 mg/g, 0.08–1.44 mg/g and 0.03–8.48 mg/g, respectively (Table 2). The P95 values were 3.21 mg/g for arecoline, 3.61 mg/g for arecaidine, 0.70 mg/g for guvacoline and 3.91 mg/g for guvacine. The average values of four areca alkaloids in five fresh areca fruit were 1.56, 0.75, 0.21 and 0.47 mg/g, respectively. While the average values of four areca alkaloids in 93 dried areca husks were 1.13, 1.77, 0.34 and 1.28 mg/g, respectively. Guvacine was the major areca alkaloid in most samples. Jain et al. (2017) reported that the level of four areca alkaloids in areca nut products were in the following order: guvacine (1.39–8.16 mg/g), arecoline (0.64–2.22 mg/g), arecaidine (0.14–1.70 mg/g) and guvacoline (0.17–0.99 mg/g) (Jain et al. 2017). This order of four areca alkaloids contents was generally consistent with our results (Figure S4). However, Cao et al. (2020) discovered that arecoline was the most prevalent areca alkaloid in areca nut products (Cao et al. 2020). Similar results were observed in samples 1–21. The difference of areca alkaloids contents in different samples was possibly due to the cultivation regions and fruit maturity (Franke et al. 2015; Srimany et al. 2016). In addition, the processing of areca nut products also affected the composition of areca alkaloids. No significant difference in guvocine level was observed in five different types of areca nut products (i.e., fresh area fruit, unsmoking-dried areca nuts, smoking-dried areca nuts, unsmoking-dried areca husks and smoking-dried areca husks). However, the contents of arecoline, arecaidine and guvacoline were significantly different in various types of areca nut products ($p < 0.001$). In general, arecoline was the most abundant areca alkaloid in fresh areca fruits, unsmoking-dried areca nuts and smoking-dried areca nuts. Guvacine and arecaidine were the most prevalent areca alkaloids in unsmoking-dried areca husks and smoking-dried areca husks, respectively.

PCA was performed to further analyze the variations of different types of areca nut products. Samples that deviated significantly from P95 were excluded. Fresh areca fruits are

Table 1 Regression equations, precision, LOD, LOQ and recovery of four areca alkaloids

Compound	Linear range (μg/L)	Intraday repeatability	Interday reproducibility	R^2	LOD (μg/L)	LOQ (μg/L)	Accuracy (%) (n = 3)[a]		
							Low level	Intermediate level	High level
		n = 6, RSD, %	n = 6, RSD, %		(n = 3)	(n = 3)			
Arecoline	100–12800	3.49	2.11	0.9999	0.07	0.25	106.00 ± 3.05	114.08 ± 2.01	117.21 ± 1.09
Arecaidine	100–12800	1.23	1.20	0.9929	1.21	4.04	115.94 ± 3.87	111.68 ± 2.61	123.93 ± 2.72
Guvacoline	100–12800	7.85	2.71	0.9999	0.18	0.59	76.83 ± 4.70	89.54 ± 4.65	87.09 ± 3.04
Guvacine	100–12800	4.53	2.67	0.9964	1.15	3.85	94.19 ± 2.41	125.92 ± 1.00	114.29 ± 0.63

[a]Low (500 μg/L), intermediate (1000 μg/L), and high concentrations (4000 μg/L)

Table 2 Arecoline, arecaidine, guvacoline and guvacine levels in five types of areca nut products

Alkaloids	Type	n	Median (mg/g)	Mean (mg/g)	P90th (mg/g)	P95th[a] (mg/g)	Range (mg/g)
Arecoline	Fresh areca fruit	5	1.59	1.56	1.64	1.65	1.39–1.65
	Unsmoking-dried areca nut	8	2.94	3.15	3.41	3.45	1.97–3.50
	Smoking-dried areca nut	8	2.51	2.52	2.74	2.78	2.26–2.81
	Unsmoking-dried areca husk	93	1.11	1.11	1.59	1.90	0.46–4.97
	Smoking-dried areca husk	5	1.27	1.49	2.22	2.53	0.99–2.85
Arecaidine	Fresh areca fruit	5	0.76	0.75	0.84	0.84	0.57–0.84
	Unsmoking-dried areca nut	8	1.47	1.60	1.84	1.87	1.01–1.91
	Smoking-dried areca nut	8	2.10	2.09	2.34	2.34	1.61–2.34
	Unsmoking-dried areca husk	93	1.52	1.74	2.22	3.01	0.65–7.51
	Smoking-dried areca husk	5	1.97	2.52	4.00	4.65	1.51–5.30
Guvacoline	Fresh areca fruit	5	0.22	0.21	0.23	0.23	0.13–0.23
	Unsmoking-dried areca nut	8	0.38	0.39	0.57	0.60	0.08–0.64
	Smoking-dried areca nut	8	0.47	0.49	0.64	0.70	0.31–0.77
	Unsmoking-dried areca husk	93	0.31	0.33	0.45	0.61	0.08–1.44
	Smoking-dried areca husk	5	0.43	0.56	0.82	0.89	0.38–0.62
Guvacine	Fresh areca fruit	5	0.55	0.47	0.58	0.58	0.17–0.58
	Unsmoking-dried areca nut	8	0.75	0.81	1.08	1.10	0.35–1.11
	Smoking-dried areca nut	8	1.51	1.46	1.66	1.67	1.13–1.68
	Unsmoking-dried areca husk	93	0.50	1.25	2.80	3.24	0.03–8.48
	Smoking-dried areca husk	5	1.03	1.74	3.33	3.92	0.75–4.51

[a]P95th (high value)

concentrated in the upper center, whereas smoking-dried areca nuts are primarily dispersed in the upper right side of the diagram (Fig. 1A). Unsmoking-dried areca husks are primarily located in the lower left of the diagram's center (Fig. 1A). The variation of arecaidine and guvacoline levels was mainly responsible for the separation of different types of areca nut products (Fig. 1B).

Fresh areca fruits and dried areca nuts (smoking and unsmoking) displayed a good separation which may be attributed to the drying process. Pre-experiments revealed that the drying process caused a water loss ranging from 61.38 to 66.42%. As a result, the levels of areca alkaloids in dried areca nuts were much higher than that in fresh areca nuts (Figure S4). On the PCA scores plot, a good separation between the smoking-dried and unsmoking-dried areca nuts was observed. It has been demonstrated that the amount of areca alkaloids in dried areca nuts could be affected by the phenols produced during the smoking process (Srimany et al. 2016). Additionally, dried areca nuts were well separated from dried areca husks, indicating that the processing significantly altered the concentration of areca alkaloids. The previous study proved the soaking and blanching processes induced the reduction of arecoline concentration (Oliveira et al. 2021). Additionally, the brining process led to a further decrease of arecoline by hydrolyzing arecoline to arecaidine (Lin et al. 2022; Wang et al. 2018). As a result, the level of arecoline in dried areca husks was much less than that in dried areca nuts ($p < 0.001$). No significant difference was observed between the smoking-dried and unsmoking-dried areca husks was observed, indicating that the smoking processing had less influence on the alterations of areca alkaloids than subsequent processing steps such as blanching, soaking, and brining.

Consumption of areca nut

From June 2022 to October 2022, 2500 and 510 questionnaires were issued in the provinces of Hunan and Hainan, respectively. Among them, 2151 questionnaires were valid. A total of 1645 valid questionnaires, 1315 (79.9%) for males and 330 (20.1%) for females, were collected throughout Hainan Province. There were 506 valid questionnaires in Hunan Province, 246 (48.4%) for males and 260 (51.8%) for females. Results showed that males chewed more areca nuts than females in both Hunan and Hunan Provinces. A similar result was found in the surveys of Taiwan Province (Peng et al. 2019). By contrast, girls were reported to consume more areca nuts than boys according to a survey of British Asian, Bangladeshi and Pakistani teenagers (Huang and Zachar 2020). The age range of areca nut consumers is extremely wide. The youngest respondent was only 13 years old, and the oldest respondent was 74 years old. The mean (± SD, range) age of respondents in Hunan and Hainan was 34.1 (± 10.4, 13–74) and 39.6 (± 7.8, 18–57), respectively.

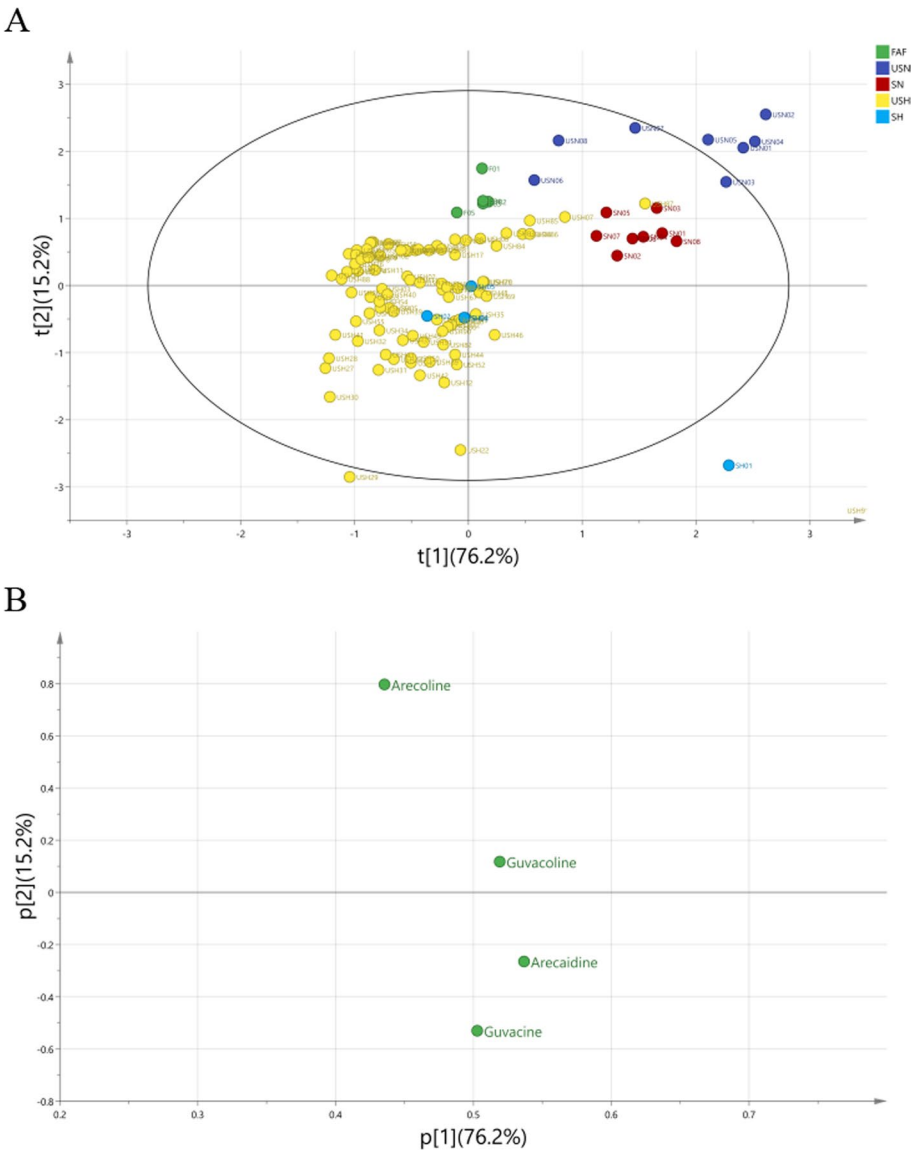

Fig. 1 **A** Scores plot of PCA analysis of five types of samples, **B** loading plot of PCA analysis of five types of samples (FAF, fresh areca fruit; USN, unsmoking-dried areca nut; SN, smoking-dried areca nut; USH, unsmoking-dried areca husk; SH, smoking-dried areca husk)

A survey of the Taiwan Province revealed that 3% of teenagers (< 18 years) had the habit of chewing areca nut (Huang and Zachar 2020). Therefore, schools should educate teenagers and protect them from the risks inducted by areca nut chewing.

The populations in Hainan Province populations chewed both fresh areca fruits and dried areca husks. Although, the ADI of fresh areca fruits (75.6 g/person) was three times higher than the ADI of dried areca husks (Table 3), the dried areca husk was more popular than the fresh areca fruit in Hainan Province: 42.2% of participants only chewed dried areca husks, 34.6% of participants only chewed fresh areca fruits, and 23.2% of participants chewed both. Du et al. thought chewing dried areca husks had a higher risk of oral disease than chewing fresh areca fruits, as chewing dried areca husks inducing more serious abrasion of the oral (Chang et al. 2022; Du et al. 2016). The major consumption methods of fresh areca fruits were fresh areca fruit + slaked lime + betel leaves (61.0%), followed by fresh areca fruit + tobacco (11.5%) and fresh areca fruit + slaked lime (11.3%) (Figure S5). Similar consumption methods were also reported in other countries and regions (Chang et al. 2022; Lee et al. 2018). Slaked lime could increase the enjoyment and excitement of chewing areca nuts by enhancing the conversion of alkaloid esters to areca alkaloids and promoting the absorption of areca alkaloids by the oral mucosa (Mehrtash et al. 2017). However, slaked lime may burn the oral and induce whitening of the oral mucosal. Chewing areca nuts with betel leaves can promote the secretion of saliva, which facilitates the dissolution of areca alkaloids (Chang et al. 2022). Chewing tobacco with areca nuts is popular in China and other countries (Peng et al. 2015) as tobacco can provide a special flavor. Nevertheless, tobacco contains high concentrations of hazardous substances, such

Table 3 The consumption ratio and average consumption of different consumption types in the province of Hainan and Hunan

Type	Hainan		Hunan	
	Consumption ratio(%)	Average consumption (g/person/day)	Consumption ratio(%)	Average consumption (g/person/day)
Fresh areca fruit	56.7	75.6	–	–
Fresh + shell powder	6.8	46.0	–	–
Fresh + tobacco	6.9	56.9	–	–
Fresh + shell powder + betel leaves	36.7	82.8	–	–
Dried areca husk	64.1	22.7	100.0	12.4
Unsmoking-dried areca husk	36.4	20.7	50.8	12.4
Smoking-dried areca husk	57.5	14.0	53.7	12.5

as tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons and aldehydes. These compounds can stimulate the oral mucosal cells and increase the risk of chewing areca nuts (Su et al. 2020).

As opposed to Hainan Province, people in Hunan Province only chew dried areca husks. The ADI of dried areca husk was 22.7 g/person and 12.4 g/person for Hainan and Hunan populations, respectively (Table 3). These levels were much higher than the ADI of the Bangladeshi populations (5–6 pieces/person/day, about 14 g/person/day) (Al-Rmalli et al. 2011). Smoking-dried areca husks were more prevalent than unsmoking-dried areca husks in both Hainan and Hunan Province. The consumption of smoking-dried areca husks accounted for 69.7% and 53.7% of the total dried areca husks consumption in Hainan and Hunan Province, respectively. Chewing smoking-dried areca husks may cause higher health risks than chewing unsmoking-dried areca husks since the smoking process result in the pollution of areca husks by polycyclic aromatic hydrocarbons which are a class of organic contaminants have carcinogenicity, mutagenicity and teratogenicity (Wu et al. 2020). Among all polycyclic aromatic hydrocarbons, benzo(α)pyrene (B(α)P) has the strongest carcinogenicity which is labeled as Group 1 carcinogen (IARC 2010). High levels of B(α)P had been detected in smoking-dried areca husks (Wu et al. 2020).

Exposure assessment

The exposure assessment of arecoline and the total areca alkaloids (i.e. arecoline, arecaidine, guvacoline and guvacine) via chewing fresh areca fruits and dried areca husks is presented in Table 4. Chewing fresh areca fruits was the main source of arecoline and the total areca alkaloids exposure. The EDIs of arecoline and the total areca alkaloids among all responders in China were 1.126 and 2.625 mg/kg BW/day for average exposure, 4.411 and 9.739 mg/kg BW/day for high exposure (P95th). The mean EDIs of arecoline and the total areca alkaloids were 1.347 and 3.138 mg/kg BW/day in Hainan Province, which is 3.3 times higher than these in Hunan Province. This may be one of the reasons that the oral cancer incidence rate in Hainan Province (5.08 per 100,000 people) was higher than that in the Hunan Province (4.22 per 100,000 people) (Liu 2020; Peng et al. 2019).

The EDIs of both arecoline and the total areca alkaloids in individuals aged ≤ 34 were the highest one among the three age groups. Moreover, the EDIs gradually decreased with the increase of age (Table 5). The exposure of the total areca alkaloids in individuals aged ≤ 34 and 35–49 was significantly higher than that in the group aged above 50 years old. Particularly, the P95th value of the total areca alkaloids assessed from individuals chewing both fresh areca fruits and dry areca husks in the 35–49 years group was 19.201 mg/kg BW/day, which was 2.4 times higher than that in over 50 years groups. It was probably because that the young (aged ≤ 34) and middle-aged people (aged 34–49) are engaged in heavy mental and physical work which makes them tend to chew more areca nuts to relieve fatigue. Young people had a higher exposure to arecoline and the total areca alkaloids, which may be one of the reasons that the age of patients with oral mucosal disease tend to be younger in China (Du et al. 2016). Meanwhile, males and females had a significant difference in the exposure of arecoline and total areca alkaloids (Table 5). The EDIs (mean, P75th and P95th) of arecoline and total areca alkaloids for males were 1.6 to 2.0 times higher than those of females in all age groups. Especially, young males aged ≤ 34 had the highest EDI. This may be one of the reasons that the morbidity of oral cancer in males was 3.80 times higher than in females based on the date of the epidemiological investigation from 2009 to 2016 in Hainan Province (He et al. 2023; Yang et al. 2021).

Overall, the exposure of arecoline and the total areca alkaloids for young male population (≤ 34 years) was higher than other populations in China. High exposure to the areca alkaloids may influence the physical health of people and induce a high medical burden. Thus, concentrated and focused efforts are required to educate the general public, especially the young male population, about the risks of areca nut chewing.

Table 4 The estimated daily intake (EDI, mg/kg BW/day) of arecoline and total areca alkaloids (i.e. arecoline, arecaidine, guvacoline and guvacine) via different consumption types of areca nut products from different age groups

Compound	Age groups	n	Consumption types[a]	Mean	P75th	P95th[b]	Maximum
Arecoline	≤ 34 years	1097	FAF	2.126	2.747	6.897	15.650
			DAH	0.469	0.521	1.410	14.100
			FAF + DAH	2.257	2.569	8.033	13.669
	35–49 years	823	FAF	1.895	2.683	5.910	9.274
			DAH	0.385	0.475	1.003	3.471
			FAF + DAH	2.139	2.057	9.092	18.676
	≥ 50 years	231	FAF	1.646	2.499	5.279	6.260
			DAH	0.287	0.408	0.765	1.611
			FAF + DAH	1.551	2.255	3.944	4.930
	All	2151	FAF	2.013	2.683	6.707	15.650
			DAH	0.416	0.487	1.180	14.100
			FAF + DAH	2.096	2.333	7.220	18.676
Total areca alkaloids	≤ 34 years[A]	1097	FAF	4.093	5.258	13.203	29.960
			DAH	1.590	1.800	4.751	56.238
			FAF + DAH	5.324	5.993	17.543	48.933
	35–49 years[B]	823	FAF	3.681	5.136	12.027	17.754
			DAH	1.138	1.322	2.839	13.843
			FAF + DAH	4.890	5.387	19.201	36.403
	≥ 50 years[C]	231	FAF	3.185	4.767	10.106	11.984
			DAH	0.934	0.990	2.758	6.427
			FAF + DAH	3.724	5.293	8.013	12.618
	All	2151	FAF	3.888	5.136	12.840	29.960
			DAH	1.337	1.500	3.905	56.238
			FAF + DAH	4.916	5.590	16.474	48.933

Age groups with superscript uppercase letters (A, B and C) are significantly different across columns using Kruskal–Wallis H test ($p < 0.01$)

[a]Consumption types including FAF (fresh areca fruit), DAH (dried areca husk), and FAF + DAH (fresh areca fruit and dried areca husk)

[b]P95th (high exposure)

Conclusion

In this study, one hundred and nineteen areca nut samples including fresh areca fruits, dried areca nuts and dried areca husks were analyzed to investigate the concentrations of four areca alkaloids (i.e. arecoline, arecaidine, guvacoline, and guvacine). The areca alkaloids levels in different types of areca nut products were various. The levels of arecoline, arecaidine, guvacoline and guvacine in different areca nut products were 0.46–4.97 mg/g, 0.57–7.51 mg/g, 0.08–1.44 mg/g and 0.03–8.48 mg/g, respectively. It indicated that the processing had a significant effect on the composition of areca alkaloids. Compared to fresh areca fruits, dried areca husks were the major consumption pattern of areca nuts in China. In addition, smoking-areca husks rather than unsmoking-areca husks were preferred, which may cause a higher risk of oral cancer in Chinese populations. The EDI of arecoline and the total areca alkaloids for Chinese populations were 1.126 and 2.625 mg/kg BW/day for average exposure, 4.411 and 9.739 mg/kg BW/day for high exposure (P95th). The EDI varied by age and gender. The young male population (≤ 34 years) had the highest EDI than other populations. Concentrated and focused efforts are required to educate the general public, especially young males and teenagers, about the risks of areca nut chewing. Follow-up epidemiological investigation should be conducted in people who only chew the fresh areca fruits and who only chew dried areca husks to clarify the association between oral abrasion and oral cancer. Moreover, the connection between chewing smoking-dried areca husk and oral cancer should be studied.

Acknowledgements This work was supported by Emergency Project for Risk Assessment of Areca Nut (Key Project of Department of Agriculture and Rural Affairs of Hainan Province & Wanning Municipal

Table 5 The estimated daily intake (EDI, mg/kg BW/day) of arecoline and the total areca alkaloids (i.e. arecoline, arecaidine, guvacoline and guvacine) via consumption of areca nut products

Compound	Age group	n	Gender	Mean	P75th	P95th[a]	Maximum	Significance between males and females in the same age group
Arecoline	≤ 34 years	263	Females	0.789	0.939	2.524	13.669	***
		834	Males	1.405	1.559	6.032	15.650	
		1097	Total	1.256	1.341	5.417	15.650	
	35–49 years	273	Females	0.787	0.839	2.828	18.676	***
		550	Males	1.121	1.250	4.087	12.460	
		823	Total	1.010	1.083	3.629	18.676	
	≥ 50 years	54	Females	0.583	0.524	2.016	4.930	**
		177	Males	1.030	1.278	3.695	6.260	
		231	Total	0.926	1.197	3.477	6.260	
	All	590	Females	0.768	0.867	2.581	18.676	***
		1561	Males	1.261	1.393	5.290	15.650	
		2151	Total	1.126	1.204	4.411	18.676	
Total areca alkaloids	≤ 34 years	263	Females	1.915	2.250	5.821	47.437	***
		834	Males	3.267	3.746	12.571	56.238	
		1097	Total	2.940	3.214	11.281	56.238	
	35–49 years	273	Females	1.776	2.115	6.084	36.403	***
		550	Males	2.606	2.960	9.487	28.922	
		823	Total	2.331	2.559	8.205	36.403	
	≥ 50 years	54	Females	1.313	1.198	4.247	9.925	**
		177	Males	2.478	3.408	7.953	12.618	
		231	Total	2.206	2.947	7.539	12.618	
	All	590	Females	1.792	2.215	5.870	47.437	***
		1561	Males	2.941	3.424	11.106	56.238	
		2151	Total	2.626	2.947	9.739	56.238	

[a]P95th (high exposure)

**Statistical analysis represented a significant difference among males and females in the same age group using Kruskal–Wallis H test ($p < 0.05$);

***Statistical analysis represented a significant difference among males and females in the same age group using Kruskal–Wallis H test ($p < 0.001$)

People's Government). The authors were grateful to all participants in the survey questionnaire.

Author contributions The contribution of each author of this manuscript are: MJ: conducted methodology, investigated, performed the analysis, data analysis, manuscript writing. LZ and H-HB: contributed data or analysis tools, investigation, wrote the review. H-MC: collected the questionnaire data, supervision. YW: methodology, investigation. X-SH and FC: funding acquisition, critical revision of the article, supervision and final approval for the publication. Y-CZ: designed the analysis data collection, supervised the work, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding Emergency Project for Risk Assessment of Areca Nut (Key Project of Department of Agriculture and Rural Affairs of Hainan Province & Wanning Municipal People's Government).

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All relevant sources are included in the Reference list and the number of references does not exceed the prescribed limit.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication The authors provide consent in publishing the data reported herewith. The manuscript has not been published before (except for some parts in the form of academic thesis). This is not also under consideration for publication elsewhere and its submission to JFST for publication has been approved by all authors.

Ethical approval The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research. All participants in this paper were fully informed why the research is being conducted, how their data will be used and if there are any risks associated with it.

References

Al-Rmalli SW, Jenkins RO, Haris PI (2011) Betel quid chewing elevates human exposure to arsenic, cadmium and lead. J Hazard Mater 190(1–3):69–74. https://doi.org/10.1016/j.jhazmat.2011.02.068

Cao M, Liu Y, Yuan H, Qiu Y, Xie Q, Yi P, Tan D, Peng Y, Wang W (2020) HPLC-based qualitative and quantitative analyses of alkaloids in chewable areca products from different geographic regions. J AOAC Int 103(5):1400–1405. https://doi.org/10.1093/jaoacint/qsaa048

Chang YJ, Muthukumaran RB, Chen JL, Chang HY, Hung YC, Hu CW, Chao MR (2022) Simultaneous determination of areca nut- and tobacco-specific alkaloids in saliva by LC-MS/MS: distribution and transformation of alkaloids in oral cavity. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2021.128116

Du Y, Sun D, Jian X, Mao Q, Cheng Y, Xu P (2016) Epidemiological investigation of chewing fresh or dried betel nut and oral mucosal disease. West China J Stomatol 34(04):391–394. https://doi.org/10.7518/hxkq.2016.04.014

Franke AA, Mendez AJ, Lai JF, Arat-Cabading C, Li XN, Custer LJ (2015) Composition of betel specific chemicals in saliva during betel chewing for the identification of biomarkers. Food Chem Toxicol 80:241–246. https://doi.org/10.1016/j.fct.2015.03.012

Fu XZ, Belwal T, Cravotto G, Luo ZS (2020) Sono-physical and sono-chemical effects of ultrasound: primary applications in extraction and freezing operations and influence on food components. Ultrason Sonochem. https://doi.org/10.1016/j.ultsonch.2019.104726

He J, Xing A, Sun B (2023) Research progress on influencing factors of oral cancer. J Environ Hyg 13(04):302–306. https://doi.org/10.13421/j.cnki.hjwsxzz.2023.04.012

Hu YJ, Chen J, Zhong WS, Ling TY, Jian XC, Lu RH, Tang ZG, Tao L (2017) Trend analysis of betel nut-associated oral cancer and health burden in China. Chin J Dent Res 20(2):69–78. https://doi.org/10.3290/j.cjdr.a38271

Huang BY, Zachar JJ (2020) Social and behavioural determinants of areca nut consumption in adolescents. Oral Dis 26(8):1820–1826. https://doi.org/10.1111/odi.13467

IARC (2004) Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum 85:1–334

IARC (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum 92:1–853

Jain V, Garg A, Parascandola M, Chaturvedi P, Khariwala SS, Stepanov I (2017) Analysis of alkaloids in areca nut-containing products by liquid chromatography-tandem mass spectrometry. J Agric Food Chem 65(9):1977–1983. https://doi.org/10.1021/acs.jafc.6b05140

Lee CH, Ko AMS, Yang FM, Hung CC, Warnakulasuriya S, Ibrahim SO, Zain RB, Ko YC (2018) Association of DSM-5 betel-quid use disorder with oral potentially malignant disorder in 6 betel-quid endemic Asian populations. JAMA Psychiat 75(3):261–269. https://doi.org/10.1001/jamapsychiatry.2017.4307

Lee YCA, Li S, Chen YJ, Li Q, Chen CJ, Hsu WL, Lou PJ, Zhu CR, Pan J, Shen HB, Ma HX, Cai L, He BC, Wang Y, Zhou XY, Ji QH, Zhou BS, Wu W, Ma J, Boffetta P, Zhang ZF, Dai M, Hashibe M (2019) Tobacco smoking, alcohol drinking, betel quid chewing, and the risk of head and neck cancer in an East Asian population. Head Neck-J Sci Spec 41(1):92–102. https://doi.org/10.1002/hed.25383

Lin HJ, Wang XL, Tian MY, Li XL, Tan HZ (2022) Betel quid chewing and oral potential malignant disorders and the impact of smoking and drinking: a meta-analysis. World J Clin Cases 10(10):3131–3142. https://doi.org/10.12998/wjcc.v10.i10.3131

Liu X (2020) Epidemiological characteristics and Trend analysis of malignanttumors incidence in Hainan, 2009–2016. Master Hainan Medical University

Marques MM, Beland FA, Lachenmeier DW, Phillips DH, Chung FL, Dorman DC, Elmore SE, Hammond SK, Krstev S, Linhart I, Long AS, Mandrioli D, Ogawa K, Pappas JJ, Morte JMP, Talaska G, Tang MS, Thakur N, Van Tongeren M, Vineis P, Grp IMV (2021) Carcinogenicity of acrolein, crotonaldehyde, and arecoline. Lancet Oncol 22(1):19–20. https://doi.org/10.1016/s1470-2045(20)30727-0

Mehrtash H, Duncan K, Parascandola M, David A, Gritz ER, Gupta PC, Mehrotra R, Nordin ASA, Pearlman PC, Warnakulasuriya S, Wen CP, Zain RB, Trimble EL (2017) Defining a global research and policy agenda for betel quid and areca nut. Lancet Oncol 18(12):E767–E775. https://doi.org/10.1016/s1470-2045(17)30460-6

Oliveira NG, Ramos DL, Dinis-Oliveira RJ (2021) Genetic toxicology and toxicokinetics of arecoline and related areca nut compounds: an updated review. Arch Toxicol 95(2):375–393. https://doi.org/10.1007/s00204-020-02926-9

Pan Y, Xu M, Guo Y, Zhang J, Li X (2020) Effect of hot water treatment on chilling injury and lignification of cold-stored fresh areca nut. J Food Sci Technol 57(12):4337–4344. https://doi.org/10.1007/s13197-020-04468-5

Pasupuleti RR, Lee CH, Osborne PG, Wu MT, Ponnusamy VK (2022) Rapid green analytical methodology for simultaneous biomonitoring of five toxic areca nut alkaloids using UHPLC-MS/MS for predicting health hazardous risks. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2021.126923

Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, Wu CJ (2015) Areca catechu L. (Arecaceae): a review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. J Ethnopharmacol 164:340–356. https://doi.org/10.1016/j.jep.2015.02.010

Peng Y, Liu J, Xu K, Liu X, Wang J, Liao X (2019) Incidence and mortality of oral cancer in registry regions of Hunan Province, 2009–2015. Bull Chin Cancer 28(9):680–688

Srimany A, George C, Naik HR, Pinto DG, Chandrakumar N, Pradeep T (2016) Developmental patterning and segregation of alkaloids in areca nut (seed of Areca catechu) revealed by magnetic resonance and mass spectrometry imaging. Phytochemistry 125:35–42. https://doi.org/10.1016/j.phytochem.2016.02.002

Su SY, Chen WT, Chiang CJ, Yang YW, Lee WC (2020) Oral cancer incidence rates from 1997 to 2016 among men in Taiwan: association between birth cohort trends and betel nut consumption. Oral Oncol. https://doi.org/10.1016/j.oraloncology.2020.104798

Sun M, Yan D, Yang X, Xue X, Zhou S, Liang S, Wang S, Meng J (2017) Quality assessment of crude and processed Arecae semen based on colorimeter and HPLC combined with chemometrics methods. J Sep Sci 40(10):2151–2160. https://doi.org/10.1002/jssc.201700006

Wang ZB, Wang X, Pei WX, Li S, Sun SQ, Zhou Q, Chen JB (2018) Chemical transitions of Areca semen during the thermal processing revealed by temperature-resolved ATR-FTIR spectroscopy and two-dimensional correlation analysis. J Mol Struct 1155:1–6. https://doi.org/10.1016/j.molstruc.2017.10.098

Warnakulasuriya S, Chen THH (2022) Areca nut and oral cancer: evidence from studies conducted in humans. J Dent Res 101(10):1139–1146. https://doi.org/10.1177/00220345221092751

Wu H, Zhou C, Ran D, Da L (2020) Study on the detection method of benzo (alpha) pyrene in areca. Food Res Dev 41(4):201–204. https://doi.org/10.12161/j.issn.1005-6521.2020.04.033

Yang Y, Zhou MG, Zeng XY, Wang CX (2021) The burden of oral cancer in China, 1990–2017: an analysis for the Global Burden of Disease, Injuries, and Risk Factors Study 2017. BMC Oral Health. https://doi.org/10.1186/s12903-020-01386-y