Evaluation of climate factors affecting the quality of red huajiao (Zanthoxylum bungeanum maxim.) based on UPLC-MS/MS and MaxEnt model

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ARTICLE INFO

Keywords:
Red huajiao
UPLC-MS/MS
MaxEnt model
Climatic response
Quality evaluation

ABSTRACT

Through field investigation, UPLC-MS/MS technology and MaxEnt model were performed to predict the suitable distribution area for red huajiao (Zanthoxylum bungeanum maxim.) in China from 2021 s to 2060 s, and evaluate the effects of climate factors on the quality of red huajiao. The results demonstrated that mean temperature of the coldest quarter and min temperature of the coldest month were the most important environmental variables influencing red huajiao distribution. Suitable habitats for red huajiao were located mainly in dry and hot valley zone in the Qinba Mountains and the semi-humid and semi-arid areas of the Loess Plateau. The amides contents were higher in high suitability areas, while it was decreased in medium and low suitability areas, and temperature, wind speed and precipitation played a key role in their accumulation. This investigation was of great significance for the planting area optimization, quality control, benefit improvement and industrial development of red huajiao.

Introduction

Huajiao (Zanthoxylum bungeanum Maxim.) belongs to the Zanthoxylum genus in Rutaceae family, and includes approximately 250 species, of which red huajiao is one of most commercially popular varieties (Zheng et al., 2020; Zheng et al., 2022). Red huajiao has been widely cultivated in China with strong ecological adaptation, of which Sichuan, Shaanxi and Gansu provinces are the main planting areas (Zhao et al., 2020). The rapid increase of artificially planting areas of red huajiao has brought obvious economic and ecological benefits, and red huajiao has been a new economic increasing point in mountainous areas (Zheng et al., 2021).

The chemical constituents of red huajiao mainly include volatile oil, alkaloids, and amides. Alkaloids are ubiquitous in huajiao, and they are a class of substances with significant biological activities, mainly including quinoline alkaloids, isoquinoline alkaloids, plumerane, quinolone derivative (Wei et al., 2021; Ni et al., 2022). Specifically, they are skimmianine, dictamine, koukusagine, rutacearpine and berberine, which anti-tumor, antibacterial, antiviral, anti-inflammatory, anti-asthmatic, hypoglycemic, analgesic and other functions, and are effective components of drugs (Liu et al., 2017; Luo et al., 2022). Amide is an important material basis for the formation of unique pungent flavor of huajiao, and its content and composition are the main indicators affecting the quality (Puler et al., 2013). The chain unsaturated fatty acid amide represented by bungeanool is the main component of pungent taste, which consists of α-sanshool, β-sanshool, γ-sanshool, δ-sanshool and its derivatives (Chakthong et al., 2019; Zhang et al., 2020). Because of its unique numb taste, it also has a series of cooking applications, bringing spicy, pungent and numbing taste to food. Amide also has many physiological functions such as anesthetia, antibacterial, dispelling wind to eliminate dampness, insecticidal and analgesic (Yang 2008; Liu et al., 2020).
With the increase of economic benefit and market demands, red huajiao has been an important economic crop (Zhuo et al., 2020). However, the adaptability of red huajiao to the climate in the planting area must be taken into consideration before introduction and cultivation. Therefore, scientific selection of suitable planting areas is essential to ensure the red huajiao quality and prevent resource waste. The growth rate and quality of red huajiao are closely linked to local climate and environment. Ecological factors such as temperature, humidity, precipitation and soil nutrients have important effects on the synthesis of plant secondary metabolites (Zhao et al., 2016; Zhang et al., 2018), which are contributed to significant differences in the red huajiao quality in different climatic regions. In addition, variations in climatic conditions have a significant influence on the natural distribution ranges of species, which may lead to the habitat reduction and transfer. Therefore, establishing an effective analysis method to investigate the relationship between quality and climatic factors and select suitable planting areas are of great significance to improve the red huajiao peels quality.

In recent years, some representative chemical compounds and climate factors have been used as indicators to evaluate the impact of climate factors on the quality of plants, and to predict their climate suitable distribution. Wan et al. evaluated the effects of climate factors on the quality of Codonopsis pilosula by MaxEnt model combined with UPLC fingerprint (Wan et al., 2021). Shen et al. assessed the effects of climate change on the distribution and quality of Gentiana based on MaxEnt model and iridoid concentration (Shen et al., 2021). However, the plants quality and the active ingredients content were a comprehensive coefficient, which was controlled by various climatic factors. Therefore, a scientific and effective method should be developed to assess the relationship between climatic factors and plant active ingredients.

Geographic Information System (GIS) combined with statistical models has become an important method to evaluate the impact of various environmental factors on species distribution and the quality (Wan et al., 2021; Zhan et al., 2022). Maximum entropy model (MaxEnt) has been widely applied to the simulation of species suitable areas (Elith et al., 2011; Zhao et al., 2021), potential habitat quality assessment of endangered species (Tang et al., 2019), potential distribution area of invasive species, and the effects of global climate change on species distribution (Zhang et al., 2018). The MaxEnt model can not only provide the potential distribution range of species (Xu et al., 2022), but also provide information such as the comparison between the predicted range and the actual distribution range, the key variables affecting the species distribution, the species distribution area, the suitable grade, and the suitable range of environmental variables (Adhikari et al., 2012; Wang et al., 2021). Compared with other models, MaxEnt model is especially suitable for predicting potential species distribution and explaining limiting environmental variables (Elith et al., 2011; Tan et al., 2019).

Climate factors may affect not only the suitable cultivation areas of plants, but also affect the chemical compounds formation and accumulation. Here, UPLC-MS/MS analytical method was used to detect the amides and alkaloids in the red huajiao peels from different producing areas. Based on UPLC-MS/MS fingerprint and MaxEnt model, the aims of our research were to investigate the changes in suitable habitat for red huajiao under different climate scenarios, and explore the important environmental factors affecting the distribution of red huajiao, and evaluate the main climatic factors affecting amide accumulation. The results will provide important scientific reference for the selection of artificial cultivation area, quality control and classification of red huajiao in the future.

### Materials and methods

#### Plant material

Red huajiao (Zanthoxylum bungeanum maxim.) peels were collected by field investigation, and collected from the main producing areas at different altitude, including Gansu, Shaanxi, Shanxi, Sichuan, Henan and Shandong provinces. The collected provenance samples basically covered the main geographical distribution areas of red huajiao in China. The harvest time of red huajiao peels was from July to September 2020, and all fruits were with good appearance quality. In each location, 20 trees with similar tree age (6–7 years) and diameter were selected, and the distance between plants was more than 40 m, and 500 g of fruits were randomly picked and mixed. The detailed information of origin and morphology of red huajiao peels was shown in Fig. 1 and Table S1. The peels were dried to constant weight at room temperature (moisture content <10.5 %), and stored in −20 °C refrigerator. All samples were with three biological replicates.

#### Red huajiao distribution modeling process

### Environmental variables

The 19 bioclimatic variables were obtained from the WorldClim v2.1 database in raster format (the spatial resolution: 30 s, about 1 km²), with the year from 2021 to 2060, and. Two shared socio-economic pathways (SSP126 and SSP585) were downloaded (BCC-CSM2-MR), in which the SSP126 (radiation intensity of 2.6 W·m⁻²) is represented with the low emission scenarios (Puchalka et al., 2021), while SSP585 (radiation intensity of 8.5 W·m⁻²) is represented with the high emissions scenarios (Li et al., 2020). The two periods at 2030s (the average predicted in 2021–2040) and the 2050s (the average predicted in 2041–2060) were selected to predict the potential distribution of red huajiao under climate change scenarios (SSP126 and SSP585). ArcGIS software (version 10.7) was applied to convert the data of each ecological factor into the World Geodetic system 1984 (scale, 1:1000000; layer grid size, 1 km²). The detailed information of 19 bioclimatic variables was listed in Table S2.

#### Predicting distribution modeling

MaxEnt model was performed to obtain the suitable area range and variation of red huajiao, and before establishing the predicting distribution model, each bioclimatic variable was converted from the TIFF (tag image file format) to the ASC (action script communication format), and then the variables were imported into MaxEnt 3.4.4. software. The percentage for model validation and test is set to 25 %, and the percentage for model construction is set to 75 %, with the maximum number of background points to 10⁵. Cross validation was set to replicated run types, and logistic was set to output formats. The weight of each climate factor in suitability areas for red huajiao was evaluated by knife-cut method, and the key limiting factors affecting the distribution of red huajiao were obtained. Model accuracy was evaluated by the ROC (Receiver operating characteristic curve). As the AUC values (the area under ROC curve) were not controlled by the threshold, it was widely used to evaluate the accuracy of prediction models. The AUC value between 0.90 and 1.0 was considered as higher accuracy of the predicting model. Based on the results of MaxEnt operation model, the suitability distribution range of red huajiao in China was extracted using ArcGIS software. The suitability was divided into four grades by Jenks’ natural breaks, namely, no suitability (0–0.2), low suitability (0.2–0.4), medium suitability (0.4–0.6) and high suitability (0.6–1), so as to obtain the potential distribution area of red huajiao.

#### Amides and alkaloids metabolites analysis

#### Sample preparation and extraction

Red huajiao peels were freeze-dried in a vacuum freeze-drying...
machine (Scientz-100F, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), and then pulverized by a mixer (MM 400, Retsch, Haan, Germany) with a zirconia bead for 1.5 min at 30 Hz. With 1.2 mL 70 % methanol (Merck) as extraction buffer, 100 mg powder was vortexed once every 30 min, lasting 30 s each time with 6 times and placed at 4 °C refrigerator overnight. The extract was centrifuged at 12000 rpm for 10 min, and the supernatant was filtered with 0.22 μm-micron microporous membrane (SCAA-104, 0.22 μm pore size, ANPEL, Shanghai, China), which was stored in the sampling bottle for UPLC-MS/MS analysis.

UPLC-MS/MS analysis
Based on UPLC-MS/MS detection platform (UPLC, SHIMADZU Nexera X2, Shimadzu, Kyoto, Japan; MS, Applied Biosystems 4500 Q TRAP, Thermo Scientific), the amides and alkaloids in red huajiao peels were determined. The UPLC analytical conditions were as follows: 1) column, Agilent SB-C18 (1.8 μm, 2.1 mm * 100 mm); 2) mobile phase: ultrapure water with 0.1 % methanoic acid (Aladdin) as mobile phase A, acetonitrile (Merck) with 0.1 % methanoic acid as mobile phase B; 3) elution gradient: the starting conditions of 5 % B, a linear gradient to 95 % B was programmed within 9.00 min and 95 % B was kept for 10.00 to 11.10 min, subsequently, a composition of 5.0 % B was adjusted within 1.1 min and kept for 11.10 to 14.00 min; 4) flow velocity, column temperature, injection volume: 0.35 mL/min, 40 °C, 4 μL, respectively. Mass spectrometry conditions: 1) ESI (electrospray ionization)
temperature 550 °C; 2) IS (ion spray voltage).
5500 V (positive ion mode)/-4500 V (negative ion mode); 3) GSI (ion source gas I), GSII (gas II) and curtain gas were set at 50 psi, 60 psi, 25.0 psi, respectively; 4) CAD (the collision-activated dissociation) was high; 5) the QQQ (triple quadrupole) scan using MRM (multiple reaction monitoring) experiments; 6) the collision gas (nitrogen) was set to medium; 7) DP (declustering potential) and CE (collision energy) for individual MRM transitions was done with further DP and CE optimization. Based on the metabolites eluted in each period, a set of specific MRM ion pairs were monitored in each period.

**MS data analysis**

Metabolite data analysis was conducted by using Analyst 1.6.3 software (AB SCIEX, Concord, ON, Canada). The integration and correction of chromatographic peaks were carried out by MultiaQuant software 3.02 (AB SCIEX, Concord, ON, Canada). The relative contents of the corresponding compounds were represented as chromatographic peak area.

**Data on climate factors**

Suitable climate is a necessary condition for the distribution and growth of red huajiao, which affects its yield and quality, including light intensity, temperature, precipitation, wind speed and other factors. 10 biological climate variables (temperature, relative humidity, wind speed, sunshine duration, precipitation, sunshine percentage) of the sampling sites were provided by Yangling Meteorological Administration. The detailed information of 10 biological climate variables was listed in Table S3.

**Results**

**Predicting the habitat suitability distribution of red huajiao under modern climate conditions**

**MaxEnt model accuracy evaluation**

The ROC curve output by the MaxEnt model under modern climate conditions revealed that the AUC values of the potential distribution model of red huajiao based on the 19 environmental variables was 0.977 greater than 0.9 (Fig. 2A), which indicated that the accuracy of the model results were well excellent, indicating that the sub-prediction results can be used to study the division of the suitable area of red huajiao.
Prediction of suitable area of red huajiao under modern climate conditions

Among the 19 environmental factors (Fig. 2B) used to predict the MaxEnt model, the Jackknife test results demonstrated that, when only using a single environmental factor, the three environmental factors that had the greatest impact on the normalized training gain were bio11 (mean temperature of the coldest quarter, \(-3.358\) to \(2.409\) °C), bio6 (min temperature of the coldest month, \(-9.164\) to \(-1.161\) °C) and bio9 (mean temperature of driest quarter, \(-3.880\) to \(2.183\) °C). The top five environmental factors ranked by contribution rate were bio11, bio6, bio9, bio12 (annual precipitation, 498.479 to 795.825 mm), and bio1 (annual mean temperature, 8.454–13.701 °C). Overall, under modern climate conditions, the main environmental factors affecting the potential distribution of red huajiao were precipitation factor (bio12) and temperature factor (bio11, bio6, bio9 and bio1). The proportion of distribution points of red huajiao in different grades of suitable areas were: high suitability (1.98 %), medium suitability (2.74 %), low suitability (6.25 %) and no suitability (89.03 %), indicating that the modern potential distribution suitable area simulated by MaxEnt was basically consistent with the modern distribution point record (Table S4), and the average suitability was 0.52. Under the current climate change scenario, highly suitable areas of red huajiao were mainly located in western Sichuan (Hanyuan), eastern and southern Gansu (Wudu, Qin’an), eastern Qinghai (Xunhua), central and western Shaanxi (Fengxian and Hancheng) and southwestern Shanxi (Ruicheng) (Fig. 2C), with an area of 169850 km\(^2\) (Fig. 2D). Medium suitable areas were mainly distributed in Taihang Mountains (Hebei Shexian and Henan Lingbao) and southwest China (Fig. 2C), with an area of 223755 km\(^2\) (Fig. 2D), which were located in the peripheral areas of highly suitable habitat.

Red huajiao had high adaptability in the Huaihe River and Qinling Mountains. Most of Sichuan Province and Guizhou Province in southwest China had warm and humid climate, small temperature daily range, and sufficient rainfall, which provided suitable ecological environment for the growth of red huajiao. The results further illustrated that red huajiao could adapt to various ecological environments.

Potential distribution of red huajiao under future climate change scenarios

Environmental variables analysis under future climate change scenarios

Based on the ROC curve acquired by MaxEnt model (Fig. S1), the AUC values of the training dataset under different emission scenarios were greater than 0.978, and the AUC values of the testing dataset were greater than 0.972, and the top left corner was close to 1. The AUC values results revealed that the model simulation effect was highly effective and accurate and could be used to predict climatic suitability and potential distribution for red huajiao in China from 2021 s to 2060 s. Among 19 environmental variables, bio6 (\(-8.634\) to \(-2.296\) °C), bio11 (\(-5.837\) to \(0.189\) °C), bio9 (\(-2.554\) to \(3.433\) °C), bio1 (mean annual temperature, 8.680 to 14.254 °C) and bio12 (673.29 to 1135.089 mm) were the key variables affecting the future distribution of red huajiao (Fig. S2). In our research, the environmental variables with contribution rates ≥ 1 % were considered to be the dominant climatic factors that influenced the geographical distribution of red huajiao, and the cumulative contribution rates of the dominant climatic factors were above 98 %. The contribution rate of selected ecological factors was listed in Table S5. In general, min temperature of coldest month and annual precipitation were the main factors affecting the potential distribution of red huajiao.

Future changes in suitable habitats for red huajiao

The MaxEnt model was used for assessing the impact of future climate on the current suitable areas of red huajiao and the response of their distribution to climate change, so as to obtain the potential distribution area and the gravity center change map of red huajiao high suitable habitat area from 2021 s to 2060 s under SSP126 and SSP585 emission scenarios. The potential suitability regions of red huajiao under SSP126 and SSP585 emission scenarios was calculated and illustrated in Fig. 3. The area of different grades of suitable habitats under four emission scenarios in 2030 s and 2050 s changed in different degrees in comparison with that under modern emission scenarios.

Under the emission scenarios of 2030 s-SSP126, 2030 s-SSP585, 2050 s-SSP126 and 2050 s-SSP585, the high-suitability area of red huajiao was 173000 km\(^2\), 182475 km\(^2\), 198100 km\(^2\) and 201250 km\(^2\), respectively, which increased by 1.85 %, 7.43 %, 16.63 % and 18.49 %, respectively, compared with that under modern emission scenarios; and the medium suitability area was 237750 km\(^2\), 252375 km\(^2\), 255282 km\(^2\) and 275800 km\(^2\), respectively, which increased by 6.34 %, 12.88 %, 13.08 % and 23.36 %, respectively, compared with that under modern climate conditions (Table S5). The MaxEnt model results based on the dominant climate variables and the distribution data of red huajiao demonstrated that red huajiao was mainly located eastern Tibetan Plateau, eastern Yunnan-Guizhou Plateau, Qinling Mountains, Daba Mountains, Taihang Mountains and Dabie Mountains, which were located mainly in dry and hot valley zone in the Qinba Mountains and the semi-humid and semi-arid areas of the Loess Plateau, with good distribution continuity. The geographic coordinate of suitable distribution areas (high suitability and medium suitability) was between 100.0 and 120.0 °E and 24–38 °N. The high suitability areas were mainly in Sichuan, Shaanxi, Henan, Gansu, Shanxi and other provinces, the geographical coordinate was between 105 and 118 °E and 28–36 °N.

Under the future emission scenarios, the total suitable areas of red huajiao were present with an increasing trend. From the perspective of modern climate conditions and future emission scenarios, the moving tendency of the gravity center of the high suitability area (potential surviving probability ≥ 0.5) could be seen. Under the different emission scenarios from 2021 s to 2060 s, the gravity center of the high-suitable area of red huajiao was predicted to move to the high-latitude area. Under the emission scenario of 2050 s-SSP585, the gravity center of the high-suitable area of red huajiao was predicted to shift most obviously. In general, under the future emission scenarios, the high-suitable area of red huajiao exhibited a trend of transfer to high-latitude regions, and the area of high-suitable area displayed an increasing trend.

Changes in gravity center of the highly suitable distribution of red huajiao in the future

At present, the gravity center of current suitable habitat for red huajiao was Songjia Village, Yongshou County, Shaanxi Province (108.10237E, 34.584371 N) (Fig. S3). Under the 2030 s-SSP126 climate scenario, the gravity center of the highly suitable area was predicted to migrate eastward to 108.21721E, 34.65625 N in Fengbei Village in Qian County, Shaanxi Province, with the migration distance of 12.97 km. Under the 2050 s-SSP126 climate scenario, the gravity center of suitable habitat was forecasted to migrate to 107.91688E, 34.47543 N in Nanyang Townin Fufeng County, Baoji City, (migration distance of 20.50 km). Similarly, under the 2030 s-SSP585 scenario, the gravity center of the highly suitable area was predicted to migrate eastward to Shangzhaiji Village (108.57428 E, 308 34.45586 N) in Liju County, Shaanxi Province and the migration distance was 44.49 km. Under the 2050 s-SSP585 scenario, the gravity center moved southwestward to Shijia Village (107.94839 E, 34.45860 N) in Fufeng County, and the migration distance was 19.50 km.

Identification and quantification of alkaloid compounds in red huajiao peels by UPLC-MS/MS

To further understand the alkaloids metabolic differences, 14 red huajiao peels were selected in this study to determine the metabolites profiling. A total of 35 alkaloids compounds were detected by UPLC-MS/MS methods, and categorized into 8 classes, containing quinoline alkaloids, phenolamine, isoquinoline alkaloids, amides, alkaloids and a small amount of pyrrole alkaloids, plumerane and aporphine alkaloids.
(Fig. S4). Amides (37.14%), alkaloids (22.86%), phenolamine (14.29%) and isoquinoline alkaloids (11.43%) took over 85.71% of the alkaloid metabolites, and the main alkaloids were hydroxy-\(\alpha\)-sanshool, hydroxy-\(\beta\)-sanshool, xanthoplanine, protosinomenine, (2E, 7E, 9E)-N-(2-hydroxy-2-methylpropyl)-6,11-dioxo-2,7,9-Dodecatrienamide, dihydrobungeanool, hydro-\(\gamma\)-sanshool, pterolactam, bungeanool and \(\gamma\)-Sanshool.

Analysis of the numbing-related metabolites

Among all the detected alkaloid metabolites, amides are the important material basis for the formation of unique spicy flavor of red huajiao, and their content and composition are one of the main indicators affecting the red huajiao quality. In this study, 13 amides were identified and revealed different accumulation in peels from different habitats (Table 1), and the major amides were identified as hydroxy-\(\alpha\)-sanshool, hydroxy-\(\beta\)-sanshool, xanthoplanine, protosinomenine, (2E, 7E, 9E)-N-(2-hydroxy-2-methylpropyl)-6,11-dioxo-2,7,9-Dodecatrienamide, dihydrobungeanool, hydro-\(\gamma\)-sanshool, pterolactam, bungeanool and \(\gamma\)-Sanshool.

PCA, OPLS-DA and HCA analysis

To further illustrate the variation of red huajiao from different habitats, multivariate analysis (PCA and HCA) was attempted to analyze UPLC-MS/MS data in a more comprehensive way. The PCA results demonstrated that the red huajiao peels within same groups were clustered together, and all peels samples were divided in 2 groups: high altitude type and low altitude type (Fig. S5 A). Based on OPLS-DA results, red huajiao peels from different origins were effectively distinguished (Fig. S5 B). It could be seen from Fig. S6 that xanthoplanine (VIP = 1.465) contributed the most to the discrimination of 14 red huajiao samples, followed by hydroxy-\(\gamma\)-sanshool (VIP = 1.442), dehydro-\(\gamma\)-sanshool (VIP = 1.306), tetrahydrobungeanool (VIP = 1.300), \(\gamma\)-sanshool (VIP = 1.297), bungeanool (VIP = 1.273). VIP scores indicated that these substances were characteristic alkaloids in samples from main producing areas of red huajiao. Likewise, according to the content of alkaloid metabolites, the HCA analysis of red huajiao peels samples from different habitats was performed, and the results showed that the samples collected from eight red huajiao producing areas could be divided into two categories. The first group mainly came from high altitude areas (Gansu, Qinghai, Sichuan, western Shaanxi), and the second group mainly came from low altitude areas (eastern Shaanxi, Henan, Hebei, Shanxi, Shandong) (Fig. S5C). These results revealed that there were differences in alkaloids content among red huajiao peels from different habitats and red huajiao production could be mainly divided into east and west. Since the variables contributed by PC1 was higher than PC2, it
can be deduced that red huajiao peels in the first quadrant has better quality, so the quality of red huajiao from western China was better, which was to keep consistent with suitability areas for red huajiao.

**Variation of amides content in red huajiao peels under different suitability areas**

The results revealed that there were significant differences in the amides content in red huajiao peels under different suitable habitats. The average contents of γ-Sanshool, hydroxy-γ-sanshool, dihydrobungeanool, tetrahydrobungeanool, bungeanool and hydroxy-α-sanshool in medium suitability and high suitability areas were higher than that in low suitability areas (Fig. 4A). However, the hydroxy-β-sanshool content was just the opposite. In contrast, with the exception of hydroxy-β-sanshool, the remaining amides contents in highly suitable areas were relatively higher, and were decreased from high suitability, to medium suitability, to low suitability areas, which was consistent with those of other plants. The lower hydroxy-β-sanshool content in high suitability areas may be caused by sampling bias due to the limited sample sits in medium suitability and low suitability areas. More studies were needed to verify our findings in the future.

Finally, the spatial distribution map of amides content in 14 red huajiao peels was overlapped with the habitat suitability distribution map to obtain the quality zoning map of red huajiao. Fig. 4B exhibited that red huajiao with high quality was mainly distributed in Shaanxi, Gansu and Sichuan, and most areas of Shandong and Henan had low quality. Through the comparison of quality partition map and habitat suitability map, it could be seen that the suitable areas for the growth was also the high-quality area of red huajiao, which demonstrated that the presence of

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**Table 1**

| Samples | Yγ, Yγ, SS | Yγ, γ, SS | Yγ, Yβ, SS | Yγ, γ, SS | Yγ, SS, SS | Yγ, γ, SS, SS | Yγ, γ, SS, α, SS | Yγ, γ, SS, β, SS | Yγ, γ, SS, β, SS, SS |
|---------|------------|-----------|------------|-----------|------------|------------|------------------|------------------|-------------------|
| S1      | 1.80E+07   | 3.25E+07  | 1.69E+07   | 5.69E+07  | 2.05E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S2      | 1.76E+07   | 2.47E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S3      | 2.04E+08   | 4.63E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S4      | 1.28E+07   | 1.49E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S5      | 1.39E+07   | 1.28E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S6      | 1.33E+07   | 1.68E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S7      | 1.39E+07   | 1.28E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S8      | 1.76E+07   | 2.47E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S9      | 2.04E+08   | 4.63E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S10     | 1.33E+07   | 1.68E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S11     | 1.39E+07   | 1.28E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S12     | 1.33E+07   | 1.68E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S13     | 1.39E+07   | 1.28E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |
| S14     | 1.33E+07   | 1.68E+07  | 1.68E+07   | 4.60E+07  | 1.69E+07   | 7.56E+07   | 3.22E+07        | 9.56E+07         | 6.18E+07          |

Note: All data was reported for three replicates. Yγ, Yγ, SS = Yγ-Sanshool, Yγ, γ, SS = γ-Sanshool, Yγ, γ, SS, SS = hydroxy-γ-sanshool, Yγ, γ, SS, α, SS = dihydrobungeanool, Yγ, γ, SS, β, SS, SS = bungeanool, Yγ, γ, SS, β, SS, SS = hydroxy-α-sanshool, Yγ, γ, SS, β, SS, SS = γ-sanshool, Yγ, γ, SS, α, SS, SS = hydroxy-γ-sanshool, Yγ, γ, SS, β, SS, SS = γ-sanshool, Yγ, γ, SS, β, SS, SS = γ-sanshool.
those ecological factors that were conducive to the growth of red huajiao were also conducive to the amide compounds accumulation.

**Correlation analysis between climate factors and alkaloids metabolites**

The correlation between climatic factors and amides and alkaloids contents was described in detail in Fig. 5, which revealed that climate factors had great effects on the amides and alkaloids contents. The amide substances in the red huajiao peels were negatively correlated with the $X_{AMT}$, $X_{AMIT}$, $X_{AMAT}$ and $X_{RH}$. With the exception of dihydrobungeanool and ZP-amide N, the remaining amides exhibited a positive correlation with $X_{AAP}$. Amide substances were positively correlated with $X_{MW}$, $X_{MAW}$ and $X_{ASD}$. Dihydrobungeanool, ZP-amide N and hydroxy-β-sanshool were positively correlated with the $X_{SD}$ and $X_{ASD}$, in which hydroxy-β-sanshool had significantly positive correlation $(P < 0.01)$, while the remaining amides were negatively correlated. Dihydrobungeanool displayed a positive correlation with $X_{AMT}$ $(P < 0.05)$, tetrahydrobungeanool was positively related to $X_{MAW}$ $(P < 0.05)$, hydroxy-α-sanshool showed significant positive correlation with $X_{MW}$ $(P < 0.05)$, ZP-amide A was positively correlated with $X_{ASD}$ $(P < 0.05)$, ZP-amide N was negatively associated with $X_{AMT}$ and $X_{AMAT}$ $(P < 0.01)$, and ZP-amide M was negatively related to $X_{ASD}$ and $X_{ASD}$ $(P < 0.05)$.

**Discussion**

**Effect of climate factors on suitable areas of red huajiao**

MaxEnt and ArcGIS software were applied to establish the future distribution model of red huajiao in China, and clarify the climatic factors influencing the suitable distribution area for red huajiao. The model had high accuracy, with the AUC value greater than 0.9. Field sample collection and model prediction verification was an indispensable part of distribution suitability evaluation of Chinese medicinal materials, and also an important way to verify whether the model prediction was consistent with the actual distribution (Zhan et al., 2022). The results demonstrated that the verification area of field sampling points was all in the potential suitability distribution areas predicted by MaxEnt niche model, indicating that the MaxEnt model was reliable to predict the distribution.

Jackknife test results of 19 environmental factors to red huajiao under different climate scenarios revealed that mean temperature of the coldest quarter, min temperature of the coldest month, mean temperature of driest quarter, annual precipitation and annual mean temperature were the dominant climate factors. The annual mean temperature and precipitation suitable for the growth of red huajiao were at 8.454 to 13.701 ºC and 498.479 to 795.825 mm, respectively, which was in agreement with the research results of researchers on the range of suitable ecological factors in the distribution area. Min temperature of coldest month and mean temperature of coldest quarter limited the distribution of red huajiao, because they determined whether red huajiao could survive. Studies on the species diversity pattern and its influencing factors of Chinese gallnut plants also found that temperature and precipitation factors were the main influencing climate factors, and the results were basically consistent with red huajiao (Chakraborty et al., 2016).

The potential geographical distribution of red huajiao under modern climatic conditions was mainly located in dry and hot valley zone in the Qinba Mountains and the semi-humid and semi-arid areas of the Loess Plateau, and there appeared high quality of red huajiao in Wudu and Qin’an in Gansu, Fengxian and Hancheng in Shaanxi, Lingbao in Henan, Ruicheng in Shanxi, Shexian in Hebei and other places, which also further illustrated that the red huajiao had a strong adaptation to local environment (Yang et al., 2013; Ni et al., 2022). For example, there were varieties of red huajiao with good quality and long cultivation history in Hanyuan in Sichuan Province and Zunyi and Bijie in Guizhou (Tao et al., 2017; Hou et al., 2019). Under different emission scenarios from 2021 s

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**Fig. 5.** Correlation heatmap between alkaloids metabolites and 10 climate factors. $X_{AMT}$ (°C)-Annual mean temperature, $X_{AMAT}$ (°C)-Annual mean maximum temperature, $X_{AMM}$ (°C)-Annual mean minimum temperature, $X_{RH}$ (%)-Annual relative humidity, $X_{MW}$ (m/s)-Mean wind speed, $X_{MAW}$ (m/s)-Maximum wind speed, $X_{EW}$ (m/s)-Extreme wind speed, $X_{ASD}$ (h)-Annual sunshine duration, $X_{AAP}$ (%)-annual average sunshine percentage and $X_{ASD}$ (mm)-annual average precipitation.
Geographical variation of alkaloids metabolites in red huajiao peels

Climate factors are important factors that constitute the natural geographical environment of the producing areas of Chinese medicinal materials, and their effects are mainly reflected in the growth and development of medicinal plants, the accumulation of effective components, and the quality of efficacy (Fernandes et al., 2017; Vilkickyte & Raudone, 2021). The regional difference in the chemical composition content of medicinal plants is a specific manifestation of the genuineness of Chinese medicinal materials, which has obvious regional characteristics and is significantly correlated with altitude and geographical location (Wang et al., 2019).

13 kinds of numbing-related metabolites were detected in red huajiao peels, and the numbing flavor components content in red huajiao peels was different between different varieties and different habitats. The substance with the highest content was hydroxy-α-sanshoool, which caused numbness by activating transient receptor potential (TRPV1 and TRPA), indicating that the intensity of prickle taste was directly affected by the hydroxy-α-sanshoool content (Watanabe and Terada, 2015). The contents of 13 amide compounds in red huajiao peels from different regions had significantly different, and peels samples were divided into two groups. The samples in the first group came from high altitude areas (altitude >1000 m), including Hanyuan in northwest Sichuan, Qin’an and Wudu in Gansu, and Fengxian in Shaanxi, of which the mean amides content in this region was generally higher. The sampling in the second group were from low altitude areas, and the mean content of amide components was generally lower. It was concluded that the quality of red huajiao in high altitude areas was better, and the amides in red huajiao peels had obvious change trend with altitude and longitude. The population of red huajiao in some regions may gradually decline under the future climate change, especially in the eastern region. In addition, the amides content in red huajiao peels were gradually decreased from high suitability, to medium suitability, to the low suitability areas, indicating that the high and medium suitable areas were conducive to the amides production and accumulation in red huajiao.

Correlation between quality of red huajiao and climate factors

Plant secondary metabolites synthesis and accumulation were influenced by climate factors including precipitation, temperature, wind speed and annual sunshine duration (Ji et al., 2018; Wang et al., 2021). Appropriate precipitation had an important regulatory effect on the growth and development of plants, the accumulation of components in fruit and the formation of fruit quality. Different wind speeds had different effects, suitable wind speed could promote the circulation of carbon dioxide in the environment, which was beneficial to photosynthesis of plants and conducive to material transport and metabolism in plants through regulating canopy temperature and humidity (Zheng et al., 2021). The accumulation of alkaloids and amides in red huajiao under different habitats was regulated by climatic factors. The results of correlation analysis between climatic factors and amides substances suggested that mean wind speed, annual mean maximum temperature, annual mean minimum temperature, annual mean maximum temperature and annual average precipitation had the greatest influence on the quality of red huajiao. The amides accumulation was regulated by temperature, which affected the quality of red huajiao peels.

In our research, it provided new insights for better understanding the effects of climate change on the suitable distribution area and quality of red huajiao, and the restrictive climatic variables influencing suitable distribution area were obtained. However, the quality of red huajiao peels was affected not only by climatic factors, but also influenced by altitude, latitude and longitude, soil type, vegetation type, landscape characteristics, market demand, artificial introduction, cultivation and management. In future studies, more influencing factors should be brought into the model to enhance the accuracy of prediction.

Conclusion

The numbing flavor of red huajiao peel was mainly determined by the amide components content, which was influenced by geographical origin and growth environment. Based on UPLC-MS/MS technology and MaxEnt model, the effects of climate factors on the red huajiao peels quality was established, and the spatial distributions and their shift of suitable areas in the future climate were systematically evaluated. The main environmental variables influencing the suitable distribution area for red huajiao were mean temperature of the coldest quarter, min temperature of the coldest month, mean temperature of driest quarter, annual precipitation and annual mean temperature. Under future climate scenarios, suitable habitats for red huajiao were located mainly in dry and hot valley zone in the Qinba Mountains and the semi-humid and semi-arid areas of the Loess Plateau. Through bivariate correlation analysis, it was found that the main climatic factors affecting the content of alkaloids and amides were temperature, wind speed and precipitation. Based on ArcGIS spatial distribution model, the quality zoning map of red huajiao was described, which could intuitively exhibit the regional distribution of red huajiao with high quality. The screening of climatic factors affecting the suitable distribution area for red huajiao was consistent with their growth habits, which indicated that the prediction model was reliable. The combination of UPLC-MS/MS technology and MaxEnt model provided a new perspective and strategy for evaluating the climate factors effects on the red huajiao peels quality and optimizing the planting layout of red huajiao in China.

Funding

This article was supported by the project “The demonstration and promotion of efficient cultivation and management techniques of Zanthoxylum bungeanum in Weibei dry plateau” (2017118), “Major Science and Technology Projects in Xianyang, Shaanxi, China” (2020k01-35), and “Special fund of Technology Innovation in Shaanxi, China” (2020QFY06-02).

Author contribution statement

Tao Zheng and Shu-Ming Liu conceived and designed the experiments, Tao Zheng analyzed the data, modified the picture and wrote the paper, Jia-qian Sun, Xiao-jun Shi, Du-ling Liu, Ding-ling Zhang, Bing-yin Sun and Yuanjie Deng participated in the experiments, all authors have read and approved the manuscript for publication.

Declaration of Competing 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.

Data availability

Data will be made available on request.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100522.

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