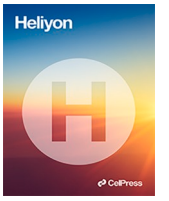
Heliyon 10 (2024) e37133

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Non-targeted metabolomics reveals the characteristics of the unique bitterness substances in quinoa

Junqi Huo [a,b], Tingting Feng [a,e,*], Heting Shang [a,b], Chen Guo [a,b], Tianyu Wu [e], Mingjuan Chu [e], Huixin Zhao [e], Erbin Wu [d], Hui Li [a,b,c], Shuo Wang [f], Dong Wei [a,b,**]

[a] Hebei North University Hebei Key Laboratory of Analysis and Testing for Quality and Safety of Agricultural Products and Food, Hebei North University, Zhangjiakou, 075000, China
[b] Hebei North University College of Agriculture, Forestry and Technology, Zhangjiakou, 075000, China
[c] Hebei North University Zhangjiakou City Key Laboratory of Quality and Safety of Special Agricultural Products, Zhangjiakou, 075000, China
[d] Zhangjiakou Animal Health Supervision Institute, Zhangjiakou, 075000, China
[e] Hebei North University College of traditional Chinese Medicine, Zhangjiakou, 075000, China
[f] School of Medicine, Nankai University, Tianjin, 300350, China

ARTICLE INFO

Keywords:
Quinoa
Sensory analysis
Bitter metabolites
Untargeted metabolomics

ABSTRACT

Bitterness is a key factor that affects the consumption of quinoa products, even if they are nutritious. In this study, a non-targeted metabolomics approach based on UHPLC-Orbitrap-MS was applied to comprehensively profile the characteristic metabolites of twenty-two quinoas. A total of twenty key metabolites were identified correlated with bitterness, among which, fifteen were triterpenoid saponins. In addition, these metabolites bind to the active site of the human bitter taste receptor and are the main compounds that produce the bitter taste of quinoa. Our results contribute to a deeper understanding of the origin of quinoa bitterness and provide directions for optimizing its flavor to improve market acceptance.

1. Introduction

Quinoa (Chenopodium quinoa Willd) is an annual herb classified as a pseudocereal. It is originally from the Andean region of South America, including Peru, Ecuador, Colombia, Bolivia, and Chile [1,2]. Quinoa has been grown as a new alternative crop in many places because of its high nutritional properties and environmental adaptability [3]. Quinoa exhibits remarkable adaptability and plasticity to diverse environmental conditions due to its extensive genetic diversity. This diversity enables quinoa to tolerate frost, salinity, and drought, and allows it to thrive in marginal soils [4–6]. With the global population growing and the demand for increased production, quinoa production has been steadily increasing. With the expansion of quinoa cultivation, research on quinoa has been gradually increasing; however, it is still at a relatively early stage. Sensory evaluations of quinoa are just beginning. A study in 2006 only indicated that quinoa has characteristics recognized by humans as food, such as texture and mouthfeel. It wasn't until 2009 that the

* Corresponding author. Hebei North University Hebei Key Laboratory of Analysis and Testing for Quality and Safety of Agricultural Products and Food, Hebei North University, Zhangjiakou, 075000, China.
** Corresponding author. Hebei North University Hebei Key Laboratory of Analysis and Testing for Quality and Safety of Agricultural Products and Food, Hebei North University, Zhangjiakou, 075000, China.
E-mail address: 17888739812@163.com (J. Huo).

https://doi.org/10.1016/j.heliyon.2024.e37133
Received 1 August 2024; Received in revised form 27 August 2024; Accepted 28 August 2024
Available online 2 September 2024

internal nutritional components of quinoa were explored, focusing only on protein and starch content. Consequently, research on quinoa's flavor is limited, and investigations into quinoa's flavoromics are virtually nonexistent [7].

Quinoa, a food with high starch content, is consumed in a manner similar to traditional grains. It can be used in the production of various food products, such as bread and crackers [8]. Quinoa is a nutrient-rich grain that contains fiber, protein, vitamins, unsaturated fats, and minerals [9]. Moreover, the content of each substance is similar to the normal nutrient demand intake of the human bodies [10]. It is known for its exceptional balance of essential amino acids. Additionally, quinoa is marketed as a gluten-free grain, making it a valuable addition to the diet of individuals with celiac disease [11,12]. Variations in nutrient composition and bioactive components, such as protein, starch, fiber, polyphenols, flavonoids and saponins, have been observed among quinoa from different varieties and regions [13,14]. There is limited research on the causes of bitterness, however the presence of bitterness limits the consumption of quinoa [15]. Saponins are mainly found in the seed husk of quinoa and may contribute to the bitter flavor. However, recent research suggests that the bitter taste of quinoa actually originates from flavonoids and polyphenols, and that saponins may contribute to the fresh flavor. Further research is therefore needed to investigate what factors contribute to the bitter flavor [16].

Untargeted metabolomics approaches are capable of detecting a wide range of metabolites and provide extensive insights into the metabolite profiles of biological samples [17]. As such, they have been used to conduct medical, botanical and microbiological studies. Regarding quinoa, there has been a gradual increase in the number of studies based on non-targeted metabolomics, including antioxidant activity of different varieties of quinoa [18], metabolite changes in quinoa genotypes [6], and mitigating effects of quinoa saponins on hyperlipidemia [19]. In this study, key metabolites contributing to the bitter taste of quinoa were investigated from 22 quinoa samples rated for bitter intensity using an untargeted metabolomics approach using ultra-high performance liquid chromatography coupled with Orbitrap mass spectrometry (UHPLC-Orbitrap-MS).

2. Materials and methods

2.1. Quinoa material

A total of 22 quinoas produced from different manufacturers to obtain the chemical profiling of quinoa. The products were carefully stored away from light to ensure their quality. The surface roughness and diameter of each sample were recorded by observation with a 40 x microscope and photographed one by one.

2.2. Chemicals & instruments

UPLC-grade formic acid, acetonitrile, methanol, were purchased from Fisher Scientific (Waltham, MA); BX43 microscope purchased from Olympus Japan (Shenzhen, China); Thermo Scientific Barnstead system (Thermo Fisher Scientific, Suzhou, China); small pulverizer (Aenaeder, Foshan, China); MARS 7 flash microwave disintegrator (PNYY, Boston, USA). THZ-C-1 full temperature oscillator (Peiving Experimental Equipment Co., Suzhou, China); SIGMA D-37520 centrifuges (SIGMA, Germany); Metabolic profiling adopted an UltiMate 3000 ultra-high-performance LC system (Thermo Fisher Scientific, Waltham, MA, USA) ACQUITY UPLC® BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters Corp.), coupled with Orbitrap Fusion Lumos high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Sample preparation

Based on a previous microwave extraction method with modifications [20,21]. To prepare the samples, 50 g of each of the 22 samples was pulverized using a pulverizer andsieved through a 40 mesh sieve. For the extraction process, 5 g of quinoa flour was dissolved in a 15 mL solution of 70 % ethanol, with a ratio of ethanol to quinoa of 1:3(W:V). The mixture was placed on a homogenizing shaker and shaken at 120 rpm for 20 min to ensure thorough contact between the ethanol and the quinoa flour. The mixture was then transferred to a dedicated reaction tube of the Mars 7 microwave digestion system and heated at 220 W of microwave power for 330 s to ensure complete extraction.The mixture was centrifuged at 10000 g for 20 min and the supernatant was collected. The separated supernatant was packed into a reservoir bottle and labeled to be kept in the refrigerator at 4 °C for cold storage. The supernatants were collected with crystal syringes, passed through 0.22 μm microfilters, and transferred to LC vials for UHPLC-MS analysis.

2.4. Evaluation of quinoa bitter intensity

Sensory evaluation experiments on bitter taste were carried out in the functional food laboratory of Hebei North University to ensure adherence to laboratory standards and confirm compliance with ethical committee guidelines, as well as relevant protocols and laws. All quinoa samples used were food grade and the measurements of caffeine intake were within safe limits for human consumption. The experiment received approval from the Medical Ethics Committee of Hebei North University (No. 20230820WD008) and followed appropriate protocols to protect the rights and privacy of all participants. These protocols included voluntary participation, full disclosure of study requirements and risks, written consent from participants, confidentiality of participant data, and the ability to withdraw from the study at any time. All participants consented to participate in the experiment by means of a written signature.

The sensory assessment was conducted according to the previous methodology with minor modifications [22,23]. To enhance the texture of quinoa in the mouth, 22 original samples weighing 50 g each were soaked for 1 h and then transferred to small tin foil bowls.

Once the quinoa was soaked and softened, it was steamed in a steamer for 30 min. Ten graduate students (5 males and 5 females, aged 22–25 years) specializing in food processing and safety were recruited as tasters. Before the sensory experiments, the panelists underwent training on using a 0–10 point evaluation scale, where 0 represented 0.08 % caffeine, 2.5 represented 0.06 % caffeine, 5.0 represented 0.04 % caffeine, 7.5 represented 0.02 % caffeine, and 10.0 represented 0.01 % caffeine. Sensory evaluators sampled various concentrations of caffeine solutions, with the highest concentration being a 0.08 % caffeine solution. This equates to a human intake of 1.6 mg of caffeine, resulting in a body concentration of about 0.02 mg/kg. This concentration is significantly lower than the World Health Organization's (WHO) recommended daily limit of 400 mg The quinoa sensory evaluation experiment also employed the same 0.0–10.0 scale, with lower scores indicating less acceptance of the bitter flavor and vice versa. Subsequently, 10 tasters evaluated the bitterness of 22 quinoa samples in duplicate over two days in two sessions, assessing 10 samples each time. Nose clips were used to prevent the influence of olfactory perception on the bitter taste evaluation. Panelists rinsed their mouths between samples using salt-free crackers and water, with a mandatory 90-s break between each sample.

2.5. UPLC–MS conditions

Chromatographic conditions: UitiMate 3000 system (Thermo Scientific); analytical column: CORTECS T3 Column, 1.6 μm, 2.1 mm × 100 mm; mobile phase A: 0.1 % formic acid (FA), mobile phase B: acetonitrile (containing 0.1 % FA); elution gradient: 0–2 min, 2 % B; 2–18 min, 2–68 % B; 18–29 min, 68–95 % B; 29–33 min, 95 % B; 33–36 min, 95-2% B; 36–38 min, 2 % B; flow rate: 0.2 mL min^{-1}. Mass spectrometry conditions: Mass spectrometric analysis was performed using an Orbitrap Fusion Lumos in positive ion mode. The electrospray ionization source parameters were set as follows: scanning in positive mode, with a primary scanning resolution of 120,000 and a secondary mass spectrometry resolution of 15,000. High-energy collision-induced dissociation (HCD) was set at 30 %. Dynamic data-dependent acquisition (DDA) was employed for data collection, and data acquisition and processing were carried out using Xcalibur 4.0 software.To ensure the accuracy and reliability of the analytical results, quality control samples were prepared. A small amount of samples from each experimental group was mixed to create quality control samples, with each quality control sample containing a mixture from different samples. To ensure the reliability of the experimental data, the sample analysis sequence was randomized to minimize potential systemic bias. The analysis sequence was shuffled before the experiment began and remained unchanged throughout the process. Each sample was analyzed in triplicate to enhance the repeatability and reliability of the data. Blank samples (including solvents and water) were injected as controls, with a total of 5 blank injections, to monitor possible background noise and system contamination.

2.6. Data anlaysis

The preprocessing of liquid chromatography-mass spectrometry (LC/MS) data was performed using Progenesis QI 3.0 software (Waters Corporation, Milford, USA), resulting in a three-dimensional data matrix which was exported in CSV format. This matrix comprised sample information, metabolite identities, and mass spectrometric response intensities. Initially, internal standard peaks and known false positives, such as noise, chromatographic column bleed, and derivatization reagent peaks, were removed. Simultaneously, background subtraction was conducted to mitigate the influence of instrumental and environmental baselines, leveraging an automated platform tool from Majorbio(cloud.majorbio.com) for optimal correction. Subsequently, noise was eliminated from the mass spectral data to enhance the signal-to-noise ratio of the dataset [24]. Data analysis was carried out using the Majorbio cloud platform, retaining metabolite features detected in at least 80 % of samples. For metabolites with levels below the quantification limit, a minimal metabolite value was assigned to avoid missing data. To reduce variability stemming from sample preparation and instrumental instability, each metabolite feature was subjected to sum normalization, as was the response intensity of the chromatographic peaks. Following normalization, all variables were subjected to statistical analysis to ensure data uniformity and reliability. During the quality control phase, variables with relative standard deviations (RSDs) exceeding 30 % in the quality control samples were removed to ensure the stability and accuracy of the data. Ultimately, a log10 transformation was applied to the data matrix to further standardize the dataset, laying a solid foundation for subsequent analyses [25]. The metabolite identification was performed using the Majorbio tool by analyzing the fragment peaks of the secondary spectra for each sample. The molecular formula was inferred by obtaining the precise mass, isotope distribution, and fragment ion formulas of the metabolites. The possible molecular formulas were then compared with structural information and matched against public databases(HMDB, http://www.hmdb.ca/; Metlin, https://metlin.scripps.edu/).

Compounds with similar structures and precise molecular masses were further compared using mass spectra, leading to the final identification of the substance.

2.7. Multivariate statistical analysis

Variance analysis was performed on the matrix file after data preprocessing. A Venn diagram was used for between-group analysis, and principal component analysis (PCA) and orthogonal least squares discriminant analysis (OPLS-DA) were conducted using the R package 'ropls' (version 1.6.2). Model stability was assessed through interactive validation. Metabolites that had a predictor importance (VIP) greater than 1 and a p-value less than 0.05, as determined by the Student's t-test, were identified as significantly different metabolites. Differential metabolites were annotated by metabolic pathways through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/kegg/pathway.html) [26].

Bitterness was grouped by sensory assessment and the components were compared using the Majorbio Analytics Cloud Platform to

screen for different metabolites. Information on the differential metabolites was compared with a public bitter taste database (BITTER DB, The Hebrew University of Jerusalem, Israel) to screen for bitter metabolites [27].

2.8. Molecular docking of bitter taste receptors

To further verify whether the compounds have bitter taste, the public bitter taste database was first used to query the bitter taste receptor proteins in human body, and the receptor information was obtained from the Receptor Protein Database (PDB, https://www.rcsb.org/), and then from Pub Chem (https://pubchem.ncbi.nlm.nih.gov/) 2D structures of the screened compounds. Simulated binding was performed using an online molecular docking platform (http://www.swissdock.ch/) to analyze whether the metabolites had good affinity with the receptors.

3. Results

3.1. Bitterness score

In the sensory evaluation of bitterness intensity for the 22 quinoa samples, a bitterness intensity of 10.0 indicates almost no bitterness, a bitterness intensity of 5.5 indicates a recognizable bitterness perception, and a bitterness intensity of 0.0 indicates a strong bitterness perception. The graph below shows that a lower percentage of green color indicates a lower level of acceptability (Fig. 1). Based on this, the 22 quinoa samples can be divided into two groups:BK and KW (BK refers to the less bitter group,KW refers to the more bitter group), and there is a significant difference between these two groups ($p < 0.01$). Further analysis can be conducted to identify the specific substances that differentiate these groups using multivariate analysis.

3.2. Microscopic view of quinoa appearance

Microscopic observation revealed that some quinoa samples exhibited a rough surfaceand others displayed a smooth surface. The smooth texture of the quinoa might be attributed to treatments such as grinding or washing. This disparity in surface characteristics loosely corresponds to the sensory rating categories. This situation is consistent with previous studies, and the presence of saponins in the bitter compounds found on the outer surface of quinoa suggests that the majority of its bitter substances are derived from its outer skin [15]. The comparison can be seen in Fig. 2, where we selected three sets of quinoa micrographs. Below is a statistical chart of the sensory scores, roughness, and color of all the samples (Fig. 3).

3.3. Overview of metabolic profiles

A total of 3071 features were identified from the raw data. After data processing, 1063 compounds were identified in positive ion mode. Among them, 332 metabolites were annotated by KEGG (Fig. 4)and 989 metabolites were annotated by HMDB (Fig. 5). The majority of these substances are lipids and lipid-like molecules (50.5 %). They also include Organoheterocyclic compounds (9.20 %), Phenylpropanoids and polyketides (8.49 %), Benzenoids (7.89 %), Organic acids and derivatives (6.17 %), Organic oxygen compounds (5.76 %), Nucleosides, nucleotides, and analogues (1.11 %), as well as Lignans, neolignans, and related compounds (0.3 %).It can be

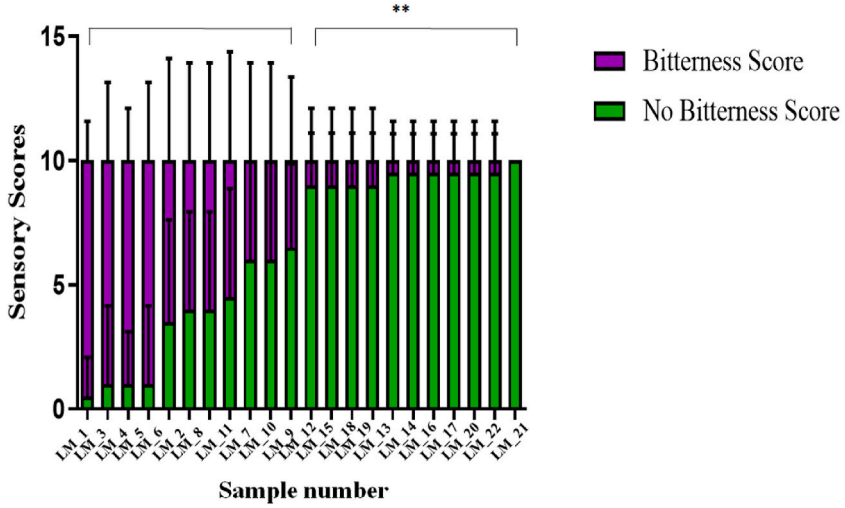


Fig. 1. Sensory scores of 22 quinoa samples. ($\bar{x}\pm s$, n=10) $^{**}P < 0.01$ compared with Bitterness Scores group. The lower the percentage of green color means the more bitter the quinoa is. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

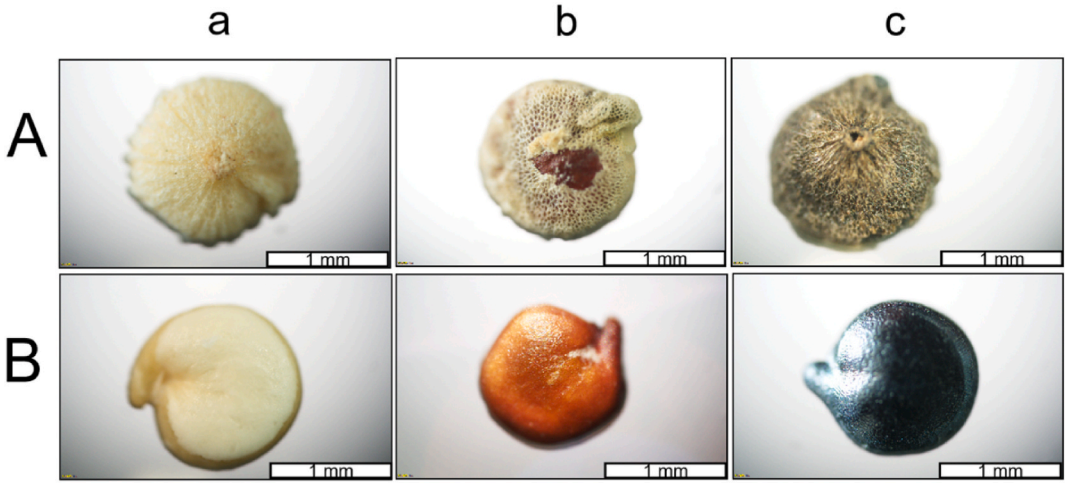


Fig. 2. Comparison of surface roughness of quinoa under microscope, A is quinoa with rough surface and B is quinoa with smooth surface. (a) White quinoa, (b) Red quinoa, (c) Black quinoa. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

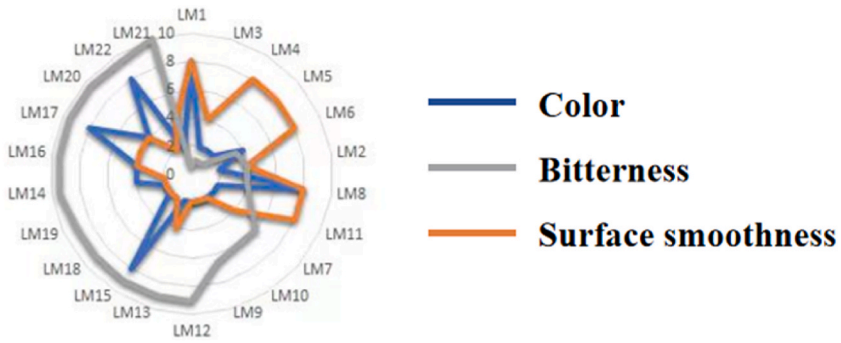


Fig. 3. A chart analyzing the color, surface smoothness, and bitterness of all quinoa samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

seen that quinoa contains a high amount of lipid compounds, as well as some glycoside compounds. The majority of starch is found in the interior of quinoa, so bitter substances are likely to be primarily concentrated on the surface. This aligns with the microscopic imaging results, where untreated quinoa displays a rough surface due to coating, while processed quinoa has a reduced presence of surface substances, resulting in a decrease in the water content of lipid and glycoside compounds. This is similar to the findings obtained from sensory analysis experiments.

3.4. Metabolite cluster analysis

Given the substantial number of metabolites, effective visualization of distribution and discrepancies arising from experimental data often requires clustering of samples and generating heatmaps to present the expression patterns of metabolites in a visually informative manner. Heatmaps provide the ability to aggregate a significant volume of data while enabling spatial frequency, high and low, of data to be visualized [28,29]. From the cluster analysis plot (Fig. 6), it can be seen that the 11 samples in the bitter group showed similar expression patterns The samples in the less bitter group were also expressed similarly, which indicates good reproducibility between the samples. This allows for further analysis. In addition, the plot shows significant differences in metabolites between the two groups. About half of the metabolites showed an up-regulation pattern (indicated by red bars), while most of the remaining metabolites showed a down-regulation pattern (indicated by blue bars). Overall, it appears that the metabolite expression patterns are significantly different between the two groups.

3.5. Multivariate statistical modeling

Volcano plots are a common visualization tool used to show the differences between two sets of samples. The horizontal coordinate is the value of the fold change of the difference in metabolite expression between the two groups, i.e. log2FC, and the vertical

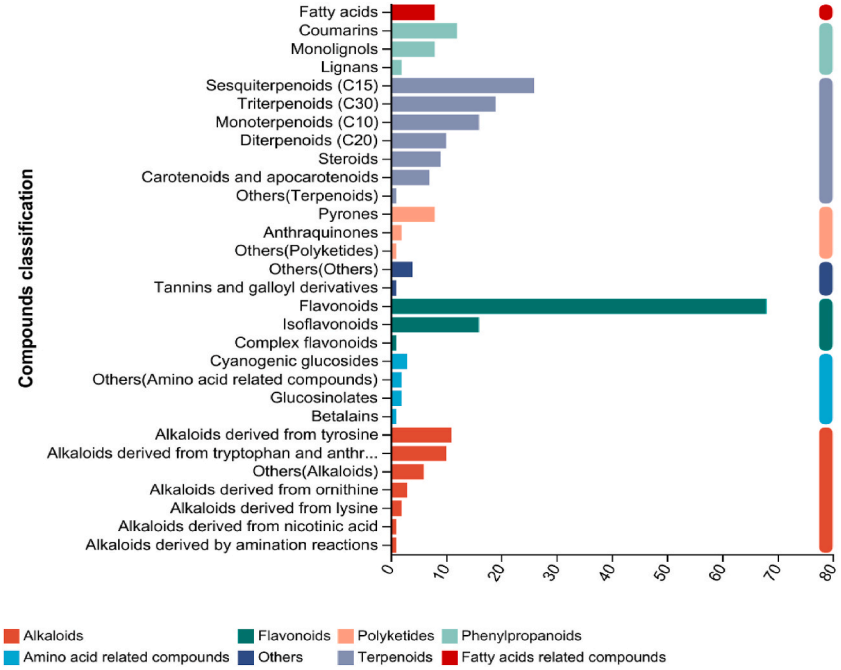


Fig. 4. KEGG compound annotation chart.

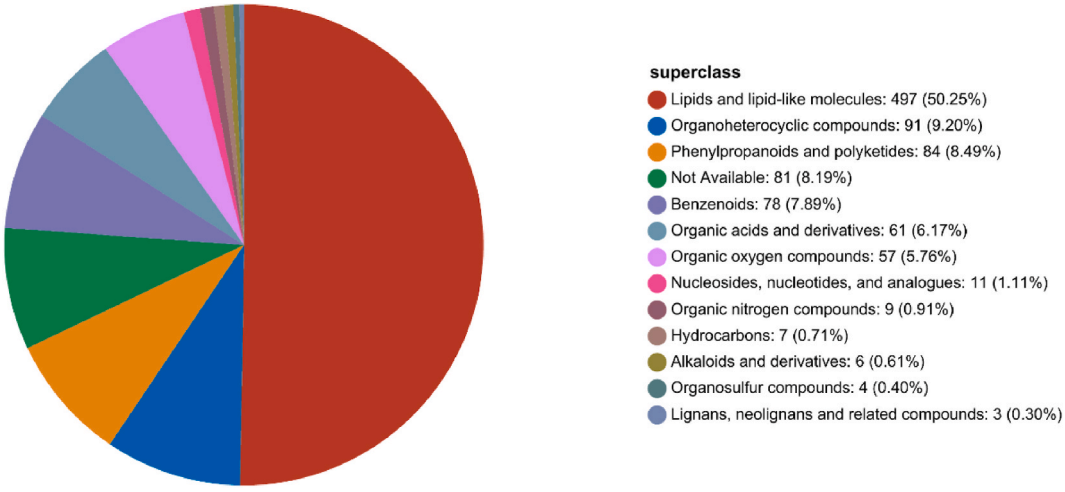


Fig. 5. Taxonomic map of the 1063 metabolites identified by the initial processing of the data.

coordinate is the value of the statistical test for the difference in the change in metabolite expression, i.e. -log10 (*p*_value) value, where the higher the value is the more significant the difference in expression, and the values of the horizontal and vertical coordinates are logarithmized. Each point in the graph represents a specific metabolite, and the size of the point indicates the Vip value. The points on the left are metabolites with down-regulated expression differences, and the points on the right are metabolites with up-regulated expression differences, and the closer to the left, right, and upper sides of the points, the more significant the expression differences are. From the figure (Fig. 7A), it is evident that 309 metabolites exhibited up-regulation, while 11 metabolites showed down-regulation. PCA is a multidimensional statistical analysis method used to elucidate the relationship between data. Through dimensionality reduction, the samples are transformed into relative coordinate points on the principal components p1 and p2. The distance between each coordinate point reflects the degree of aggregation or disaggregation between the samples. A shorter distance indicates higher similarity, while a longer distance indicates higher dissimilarity [30]. The PCA plot (Fig. 7B) indicates that the samples are mostly within the 95 % confidence interval. PC1 contributes 33.60 %, and PC2 contributes 17.60 %, suggesting that PC1 and PC2 contain information about the metabolites in the samples. The graph further demonstrates that the two groups of samples are individually clustered and exhibit a tendency to separate, indicating significant differences between the metabolites of the two groups. This

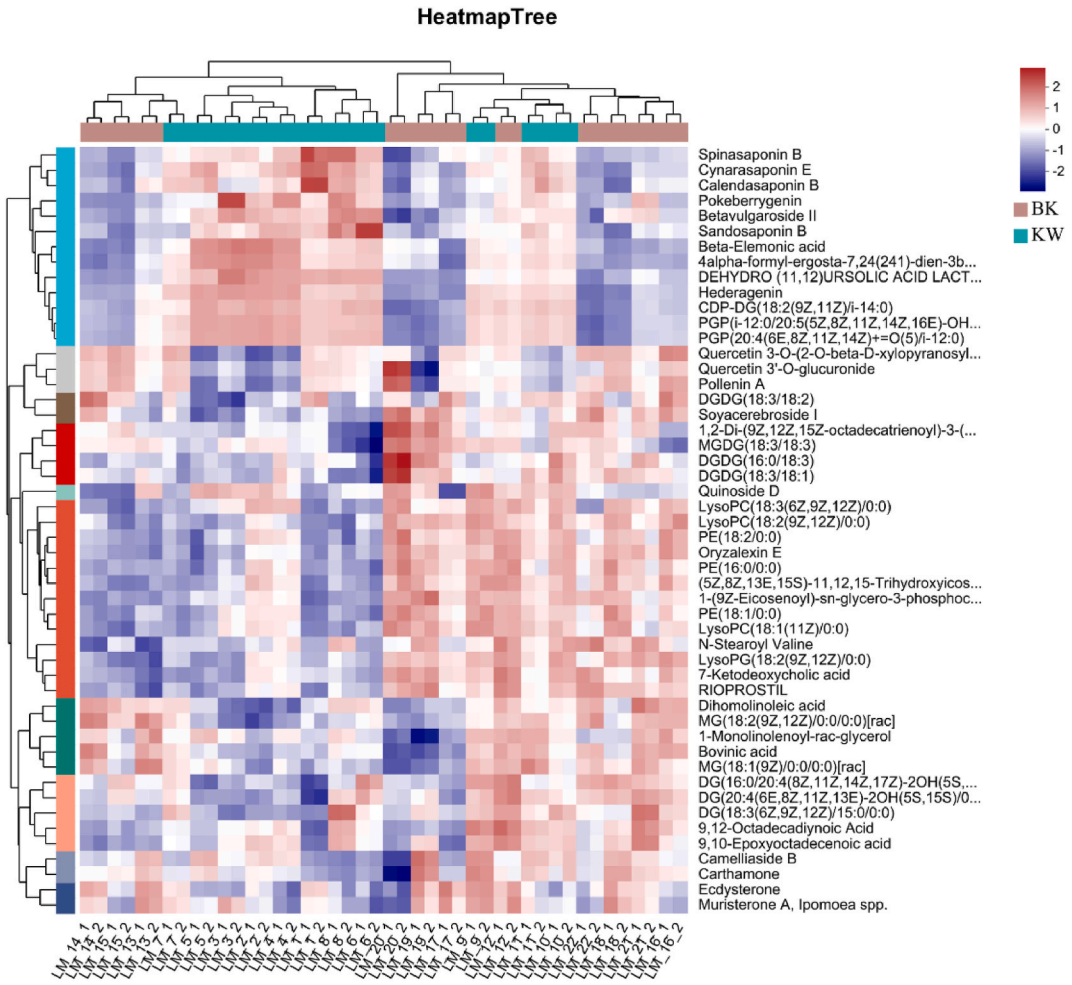


Fig. 6. Heatmap of cluster analysis of initially identified metabolites, with red expressing up-regulated relationships and blue expressing down-regulated relationships. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

validates the reliability and validity of the PCA model in depicting overall metabolite differences. The utilization of the OPLS-DA model allows for the optimal display of between-group variations and effectively compensates for PCA's limited sensitivity towards less relevant variables. In the OPLS-DA plot (Fig. 7C), distinct experimental groups are represented by different colored scatters, all falling within the 95 % confidence interval. Notably, samples from the bitter and non-bitter groups are clearly separated, indicating high data reproducibility and significant differences in metabolites relevant to quinoa between these two groups. To ensure the accuracy of the analytical model (Fig. 7D), we conducted 100 replacement experiments and obtained R2Y = 0.992 and Q2 = 0.993, which are close to 1. The Q2 intercept is less than 0, indicating that the data model is not overfitted and aligns well with the real situation of the sample data. So, the model demonstrates good predictive ability. Further analysis of these metabolite differences was conducted using class cluster analysis, as depicted in Fig. 8. The results revealed significant differences between the two groups, thereby confirming the rationality of the screening criteria.

3.6. Screening and analysis of potential bitter substances in quinoa

Based on conditions such as the rate of difference (FC KW/BK) and mass spectrometry abundance, and then compared with public bitter taste databases, Chemspider, and related literature, the TOP20 key bitter substances were found from the 50 compounds with the most pronounced differences and contents.The results were found to be consistent with previous inferences (see Table 1). The majority of the 20 potential bitter substances examined for comparison were triterpenoid saponins (15), followed by steroids (2), flavonoids (1), fatty acids (1), and esters (1). All these 20 substances exhibit an up-regulation pattern with significant differences. In addition to the substances corresponding to those present in the BITTER DB database, it is evident that most saponin metabolites are typical five or four-membered ring structures, clearly belonging to terpenoids. Since terpenoids usually contain a bitter taste, it is hypothesized that these substances may produce a bitter taste [31]. During the comparison, metabolites such as coumarins and alkaloids were also found, but the differences were not significant or their abundance was low. Although quinoa subgroups are divided into bitter and non-bitter,

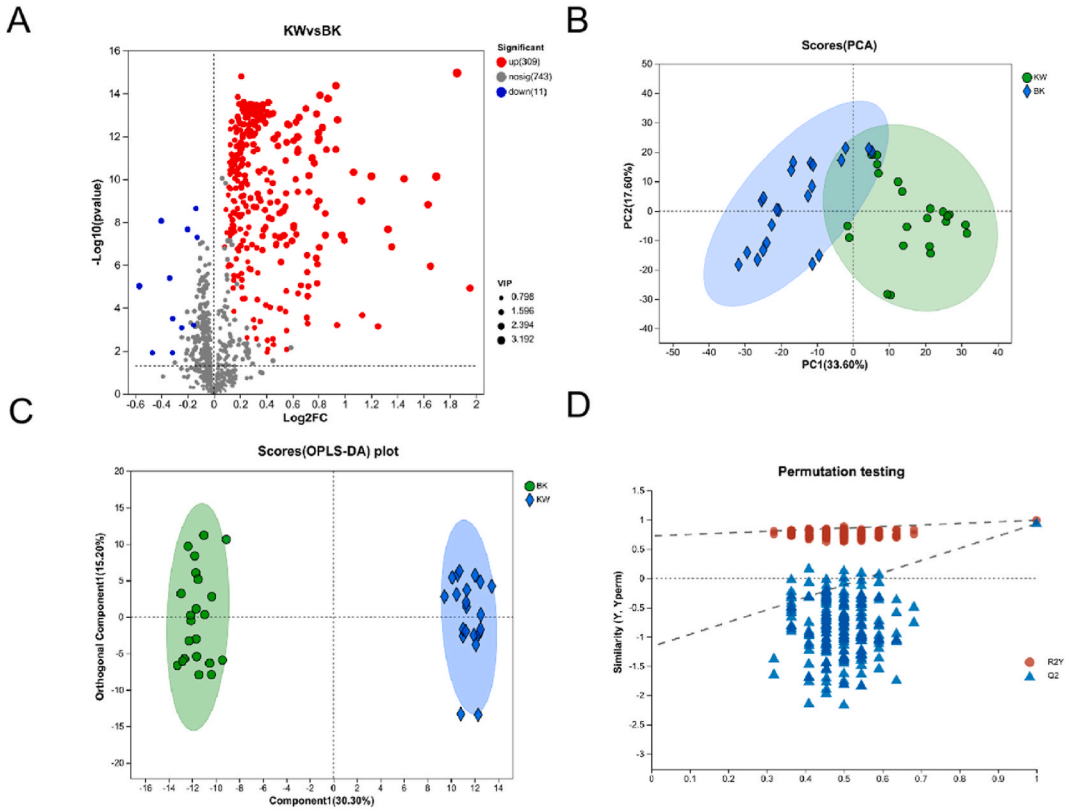


Fig. 7. A: Volcano plot between two groups of quinoa. B: Plot of PCA analysis between the two groups of quinoa. C: Plot of OPLS-DA analysis between the two groups of quinoa. D: Data model replacement test plot.

in reality, quinoa-based products are almost always bitter. However, the difference in bitterness between different quinoa subgroups is evident, so the metabolites responsible for this difference were screened. Therefore, it is expected that bitter metabolites with minor differences must be present. However, these less varied bitter metabolites did not make a significant overall sensory contribution. To validate the accuracy of the results, molecular docking experiments were conducted, which revealed that all 20 compounds exhibited good binding affinity to the human TAS2R10 bitter taste receptor. The affinity values ranged from 6.8 to 8.5. The molecular structures of the metabolites were precisely matched with the active site of the bitter taste receptor (Fig. 9), and various potential non-covalent bonding interactions, such as hydrogen bonding and hydrophobic interactions, were observed between them. These interactions play a crucial role in stabilizing the binding of the compound to the receptor. Therefore, based on the docking results, it is reasonable to conclude that these compounds are likely to induce a bitter flavor under physiological conditions.

4. Discussion

It has been reported that the majority of bitter substances found in plants are derived from amino acids, peptides, alkaloids, terpenes, and glycosides [32–34]. Therefore, it is more appropriate to utilize 70 % ethanol as the extraction solvent. Furthermore, we observed that quinoa powder tends to precipitate rapidly during the extraction process, leading to inadequate extraction efficiency. Additionally, some quinoa may adhere to the walls of the microwave extraction tube, resulting in incomplete extraction. To address these issues, we pre-mixed the quinoa powder with ethanol and thoroughly shook the mixture for 20 min using a shaker. Moreover, we employed magnetic stirring at a slower speed during the microwave extraction process to enhance extraction efficiency [35].

Since food components are often complex and diverse, it becomes challenging to qualitatively and quantitatively analyze the target compounds directly using instrumental methods. Therefore,.a sensory difference comparison approach associated with a non-targeted metabolomics analysis method was employed for bitterness analysis. The use of UPLC-MS/MS for qualitative and quantitative analysis of metabolites is attributed to its high resolution, sensitivity, and increasing application in metabolomics research. Different groups of quinoa were evaluated using a 10-point scale, which proved to be a simple and effective method. It should be noted that the ratings of some quinoa samples as less bitter or more acceptable do not imply the absence of bitterness entirely, but rather indicate a variation between the two groups [36]. The aim was to identify the substances that exerted the most significant influence on the taste of quinoa. Additionally, the quinoa used in this sensory analysis was steamed to align with its practical application. This also helped exclude some substances that could potentially shift from bitterness to coffee or nut aromas due to high temperatures. During the analysis of over 300 differential metabolites, it was observed that nearly all substances exhibited up-regulation, while 11 substances displayed

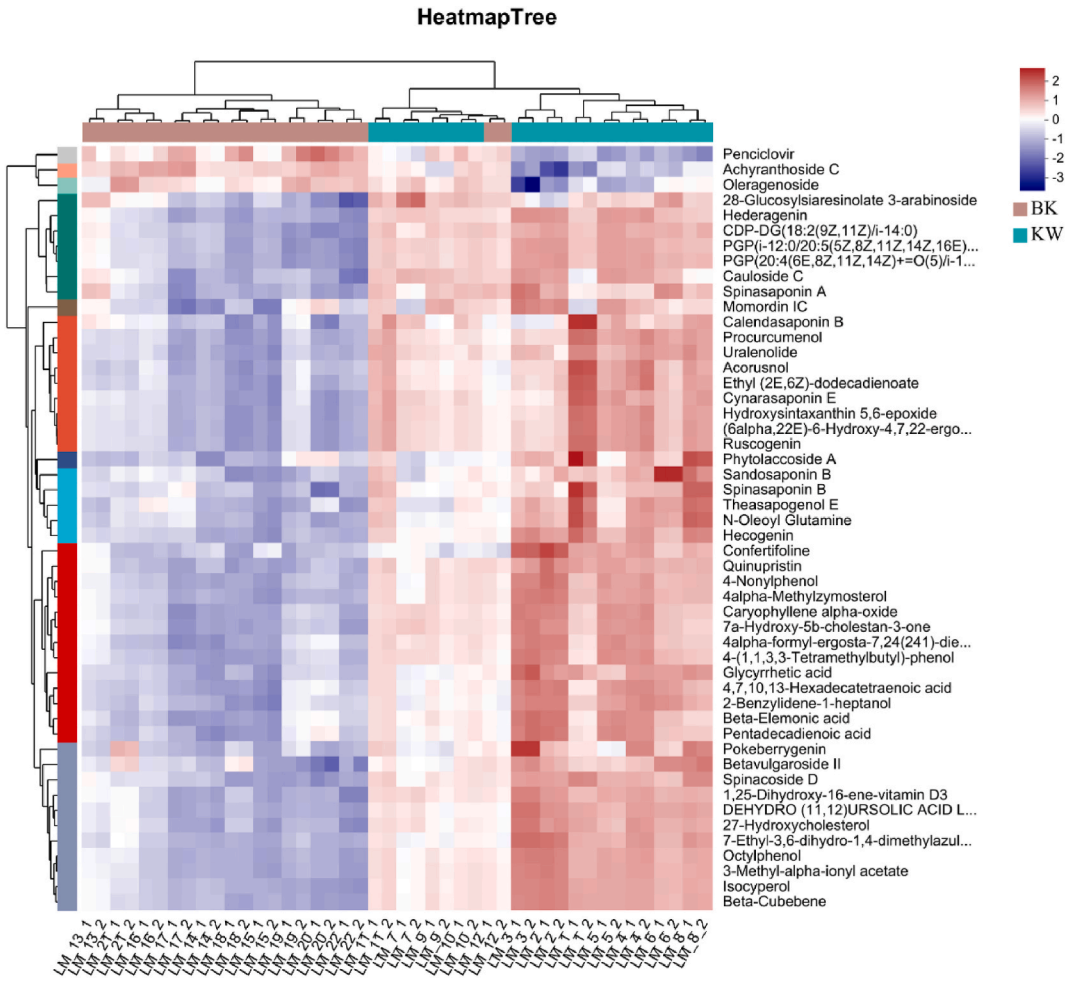


Fig. 8. Heat map of differential metabolite clustering in two groups of quinoa, with red indicating up-regulated relationships and blue indicating down-regulated relationships. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Compounds found after screening that may have a bitter flavor and their specific information.

Metabolite	Type of compound	Regulate	CAS ID	Quant Mass	Formula
Momordin IC	Triterpenoid saponin	up	–	782.4684578	C41H64O13
Momordicoside I	Triterpenoid saponin	up	81371-55-3	641.4024629	C36H58O8
Bassic acid	Triterpenoid saponin	up	465-01-0	487.3417596	C30H46O5
Hederagenin	Triterpenoid saponin	up	–	437.3412909	C30H48O4
Allodeoxycholic acid	Steroid	up	1912-55-6	357.2787555	C24H40O4
Androsterone	Steroid	up	53-41-8	255.2106739	C19H30O2
Octadecatrienoic acid	Fatty acid	up	–	243.2106597	C18H30O2
Eugenin	Flavone	up	480-34-2	189.0544546	C11H10O4
3-Phenylpropyl 2-methylpropanoate	Ester	up	103-58-2	171.1167506	C13H18O2
Calendasaponin B	Triterpenoid saponin	up	358732-32-8	995.4816825	C48H76O20
Achyranthoside C	Triterpenoid saponin	down	168111-48-6	974.495581	C47H72O20
Cynarasaponin C	Triterpenoid saponin	up	117804-09-8	817.4343463	C42H66O14
Spinacoside D	Triterpenoid saponin	up	182322-58-3	782.431986	C40H60O14
Monoglucuronylglycyrrhetinic acid	Triterpenoid saponin	up	34096-83-8	669.3618841	C36H54O10
Cauloside C	Triterpenoid saponin	up	–	767.4574472	C41H66O13
Vinaginsenoside R14	Triterpenoid saponin	up	156398-73-1	847.4445363	C41H70O15
Arvensoside D	Triterpenoid saponin	up	58231-97-3	965.5077518	C48H78O18
Spinasaponin A	Triterpenoid saponin	up	25406-56-8	817.4344355	C42H66O14
Cynarasaponin E	Triterpenoid saponin	up	117804-11-2	811.4474466	C42H66O15
Betavulgaroside II	Triterpenoid saponin	up	168010-05-7	810.4269012	C41H60O15

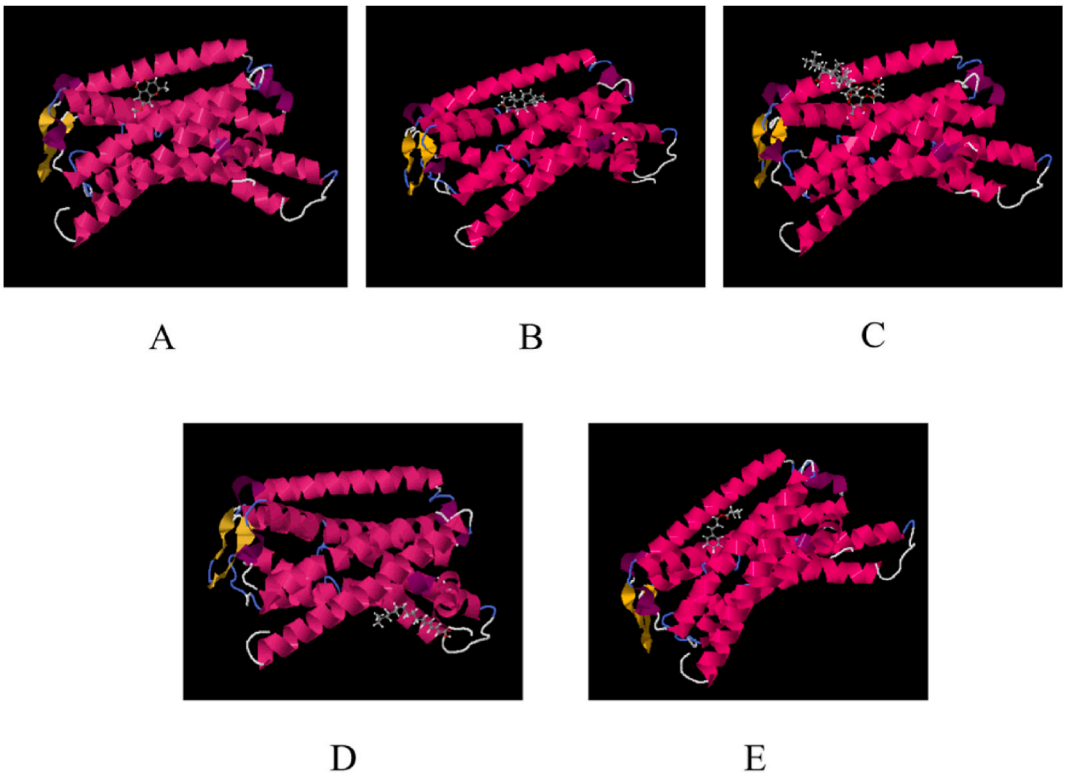


Fig. 9. Binding map of five classes of metabolites to the human bitter taste receptor (TAS2R10). **A: Eugenin, flavonoid; B: Androsterone, steroid; C: Momordin IC, triterpenoids; D: Octadecatrienoic acid, fatty acid; E: 3-Phenylpropyl 2-methylpropanoate, ester.**
Note: After molecular docking simulations, a lower binding energy is better. When the binding energy is less than −4, it indicates a strong binding affinity between the two entities. When the binding energy is less than −7, it indicates a very strong binding affinity.

down-regulation patterns. The up-regulated substances directly reflected the degree of difference between the two groups. On the other hand, the down-regulated substances might have inhibited certain reactions, resulting in a reduced bitterness. Nonetheless, a certain level of bitterness was still present due to the down-regulated expression. Further research investigating the metabolic pathways and targets of these down-regulated substances is warranted.Although some argue that saponins in quinoa may not be the primary cause of its bitterness, certain saponins can enhance its flavor. Nonetheless, our sensory analysis results demonstrate significant metabolic differences between the two groups, mainly attributed to saponins. On the other hand, terpenoids and alkaloids are common secondary metabolites in plants, serving as natural insecticides to resist predator invasion and prevent pathogen infection. These substances also inhibit the growth of larvae and prevent microbial reproduction, so most of these substances, which play a protective role in the epidermis, have a bitter taste and are toxic. The search for bitter substances not only supports the enhancement of the taste of quinoa, but also the antioxidant, antimicrobial, hypoglycemic and antihypertensive effects of saponins and phenols in quinoa, which support the further development of quinoa extracts [37].We also found that the substances in the bitter database were not comprehensive and the degree of bitterness of many substances was not reported. Many of the differential metabolites are terpene structures, which cannot be retrieved in the database. It is worthwhile to pay attention to the bitter substances speculation program that has been constructed based on the structures of the compounds in the Bitter DB database, which can expand the scope of bitter substances retrieval.

Most current quinoa products are ground and washed, which can effectively remove some bitterness. However, these methods may not fully eliminate the bitter taste and are traditional approaches without established standards or further research. Excessive grinding and washing might lead to issues such as particle fragmentation, loss of nutrients, and waste of water. By screening bitter substances, we can continue to explore their synthetic pathways and forms of expression, or their relationship with human bitter taste receptors. Genetic engineering to modify the expression of bitter genes is also a popular development direction. Addressing the root cause of quinoa's bitterness and enhancing its taste could further promote the quinoa industry and support the development of high-value, deep-processed quinoa products.

5. Conclusions

In this study, the surface of quinoa was observed using micrographic techniques to assess the roughness of the quinoa skin, followed by sensory analysis. Twenty-two quinoa cultivars from different varieties or production areas were divided into two groups for non-

targeted metabolomics analysis. More than 1000 substances were identified through database searches. Subsequently, differential metabolites were searched in public bittering databases, ChemSpider and related literature. Ultimately, 20 potential bittering compounds were selected that showed significant differences between the bitter and non-bitter groups. These compounds included 15 triterpene saponins, 2 steroidal compounds, 1 fatty acid, 1 ester, and 1 flavonoid. These substances have a better affinity for human bitter taste receptors, indicating that these compounds are likely to exhibit bitter taste under physiological effects. This study provides theoretical support for improving the taste of quinoa and lays the foundation for future research on synthetic methods and genetic engineering to inhibit the expression of bitter taste genes from the source.

Ethical approval

This study was conducted in accordance with relevant ethical guidelines, legal regulations, and laboratory protocols. Informed written consent was obtained from all participants involved in the sensory evaluation experiments. The study was approved by the Ethics Committee of Hebei North University (Approval No. 20230820WD008). All procedures performed in this study comply with relevant regulations.

Funding

This study was funded by the Performance Subsidy Funds for the Key Laboratory of Analysis and Testing of Agricultural Products and Food Quality and Safety in Hebei Province (22567613H) Zhangjiakou Basic Research and Talent Cultivation Program Project (2311001A), Zhangjiakou Municipal Scientific and Technological Program Self-funded Project (2221005A), Science and Technology Research Project of Basic Scientific Research Operating Expenses of Hebei North University (JYT202204), Hebei North University Research Project (XJ2023041)and Doctoral Scientific Research Fund Project of Hebei North University (BSJJ202317).

Data availability statement

The data supporting the findings of this study are not publicly available but can be obtained from the corresponding author upon reasonable request. Please contact Junqi Huo at 17888739812@163.com for access to the data.

CRediT authorship contribution statement

Junqi Huo: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation, Validation, Visualization. **Tingting Feng:** Writing – review & editing, Software, Validation, Methodology, Conceptualization, Funding acquisition. **Heting Shang:** Data curation. **Chen Guo:** Data curation. **Tianyu Wu:** Formal analysis. **Mingjuan Chu:** Formal analysis. **Huixin Zhao:** Formal analysis. **Erbin Wu:** Conceptualization. **Hui Li:** Supervision, Project administration. **Shuo Wang:** Conceptualization. **Dong Wei:** Conceptualization, Project administration, Resources, Supervision, Writing – review & editing, Funding acquisition.

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.

Acknowledgements

We thank Mr. Zhou, Director of the Quinoa Research Institute of the Academy of Agricultural Sciences, Zhangjiakou City, Hebei Province, China, for providing some of the quinoa samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37133.

References

[1] H.Y. Mu, S.P. Xue, Q.R. Sun, J.H. Shi, D.Y. Zhang, D.D. Wang, J.T. Wei, Research progress of quinoa seeds (Chenopodium quinoa wild.): nutritional components, Technological treatment, and application, Foods 12 (10) (2023) 21, https://doi.org/10.3390/foods12102087.

[2] S. Ranjan, S. Sow, M. Ghosh, S.R. Padhan, S. Kumar, H. Gitari, D. Nath, Nutraceutical properties and secondary metabolites of quinoa (Chenopodium quinoa Willd.): a review, Int. J. Food Prop. 26 (2) (2023) 3477–3491, https://doi.org/10.1080/10942912.2023.2286895.

[3] G.X. Ren, C. Teng, X. Fan, S.Y. Guo, G. Zhao, L.Z. Zhang, P.Y. Qin, Nutrient composition, functional activity and industrial applications of quinoa (Chenopodium quinoa Willd.), Food Chem. (2023) 410, https://doi.org/10.1016/j.foodchem.2022.135290.

[4] X. Chen, Y.Y. Zhang, B.E. Cao, X.A. Wei, Z.G. Shen, N.A. Su, Assessment and comparison of nutritional qualities of thirty quinoa (Chenopodium quinoa Willd.) seed varieties, Food Chem. X (2023) 19, https://doi.org/10.1016/j.fochx.2023.100808.

[5] X.L. Zhu, B.Q. Wang, W.Y. Liu, X.H. Wei, X. Wang, X.F. Du, H.X. Liu, Genome-wide analysis of AP2/ERF gene and functional analysis of CqERF24 gene in drought stress in quinoa, Int. J. Biol. Macromol. 253 (2023) 19, https://doi.org/10.1016/j.ijbiomac.2023.127582.
[6] X.L. Zhu, M.J. Zhang, B.Q. Wang, X.R. Song, X. Wang, X.H. Wei, Non-targeted metabolomics analysis of metabolite changes in two quinoa genotypes under drought stress, BMC Plant Biol. 23 (1) (2023) 19, https://doi.org/10.1186/s12870-023-04467-6.
[7] L.A. Scanlin, Production and Functionality of Quinoa (Chenopodium Quinoa) Protein Concentrate, Colorado State University, 2006.
[8] H. Kaur, R. Shams, K.K. Dash, A.H. Dar, A comprehensive review of pseudo-cereals: nutritional profile, phytochemicals constituents and potential health promoting benefits, Applied Food Research 3 (2) (2023) 12, https://doi.org/10.1016/j.afres.2023.100351.
[9] X.Y. Zhou, T. Yue, Z.F. Wei, L.Y. Yang, L.H. Zhang, B.M. Wu, Evaluation of nutritional value, bioactivity and mineral content of quinoa bran in China and its potential use in the food industry, Curr. Res. Food Sci. (2023) 7, https://doi.org/10.1016/j.crfs.2023.100562.
[10] A. Agarwal, Rizwana, A.D. Tripathi, T. Kumar, K.P. Sharma, S.K.S. Patel, Nutritional and functional new perspectives and potential health benefits of quinoa and chia seeds, Antioxidants 12 (7) (2023) 23, https://doi.org/10.3390/antiox12071413.
[11] I.M.A. Ahmed, F. Al Juhaimi, M.M. Özcan, Insights into the nutritional value and bioactive properties of quinoa (Chenopodium quinoa): past, present and future prospective, Int. J. Food Sci. Technol. 56 (8) (2021) 3726–3741, https://doi.org/10.1111/ijfs.15011.
[12] X. Fan, X.M. Ma, R. Maimaitiyiming, A. Aihaiti, J.Y. Yang, X.A. Li, L. Wang, Study on the preparation process of quinoa anti-hypertensive peptide and its stability, Front. Nutr. 9 (2023), https://doi.org/10.3389/fnut.2022.1119042.
[13] Y.C. Feng, X. Fan, S. Zhang, T. Wu, L. Bai, H.Y. Wang, H.Z. Yang, Effects of variety and origin on the metabolic and texture characteristics of quinoa seeds based on ultrahigh-performance liquid chromatography coupled with high-field quadrupole-orbitrap high-resolution mass spectrometry, Food Res. Int. 162 (2022), https://doi.org/10.1016/j.foodres.2022.111693.
[14] Y.J. Liu, Z.Y. Kong, J.N. Liu, P. Zhang, Q.C. Wang, X.J. Huan, P. Qin, Non-targeted metabolomics of quinoa seed filling period based on liquid chromatography-mass spectrometry, Food Res. Int. (2020) 137, https://doi.org/10.1016/j.foodres.2020.109743.
[15] D. Suárez-Estrella, L. Torri, M.A. Pagani, A. Marti, Quinoa bitterness: causes and solutions for improving product acceptability, J. Sci. Food Agric. 98 (11) (2018) 4033–4041, https://doi.org/10.1002/jsfa.8980.
[16] L.M. Song, Y. Yu, L.D. Du, X.Y. Ji, H. Gao, Y.Q. Cai, P. Xue, Does saponin in quinoa really embody the source of its bitterness? Food Chem. (2024) 437, https://doi.org/10.1016/j.foodchem.2023.137872.
[17] A. Karnovsky, S. Li, Pathway analysis for targeted and untargeted metabolomics, Computational methods and data analysis for metabolomics (2020) 387–400, https://doi.org/10.1007/978-1-0716-0239-3_19.
[18] S. Wang, G. Liu, C. Xie, Y. Zhou, R. Yang, J. Wu, K. Tu, Metabolomics analysis of different quinoa cultivars based on UPLC-ZenoTOF-MS/MS and investigation into their antioxidant characteristics, Plants 13 (2) (2024), https://doi.org/10.3390/plants13020240.
[19] Y. Yu, X.Y. Ji, L.M. Song, Y.Q. Cao, J. Feng, R.Y. Zhang, P. Xue, Saponins from Chenopodium quinoa Willd. husks alleviated high-fat-diet-induced hyperlipidemia via modulating the gut microbiota and multiple metabolic pathways, J. Sci. Food Agric. (2023) 12, https://doi.org/10.1002/jsfa.13127.
[20] M.G. Li, C.S. Zhou, B. Wang, S.Y. Zeng, R.Y. Mu, G.H. Li, W.Q. Lv, Research progress and application of ultrasonic- and microwave-assisted food processing technology, Compr. Rev. Food Sci. Food Saf. 22 (5) (2023) 3707–3731, https://doi.org/10.1111/1541-4337.13198.
[21] Y. Zhang, Y. Yan, W.Q. Li, K. Huang, S. Li, H.W. Cao, X. Guan, Microwaving released more polyphenols from black quinoa grains with hypoglycemic effects compared with traditional cooking methods, J. Sci. Food Agric. 102 (13) (2022) 5948–5956, https://doi.org/10.1002/jsfa.11947.
[22] C.Y. Gao, E. Tello, D.G. Peterson, Identification of compounds that enhance bitterness of coffee brew, Food Chem. 415 (2023), https://doi.org/10.1016/j.foodchem.2023.135674.
[23] Y.Z. Huang, Y. Liu, Z. Jin, Q.F. Cheng, M. Qian, B.W. Zhu, X.P. Dong, Sensory evaluation of fresh/frozen mackerel products: a review, Compr. Rev. Food Sci. Food Saf. 20 (4) (2021) 3504–3530, https://doi.org/10.1111/1541-4337.12776.
[24] L. Yao, A.M. Sheflin, C.D. Broeckling, J.E. Prenni, Data processing for GC-MS- and LC-MS-based untargeted metabolomics, High-Throughput Metabolomics: Methods and Protocols (2019) 287–299, https://doi.org/10.1007/978-1-4939-9236-2_18.
[25] C.Z. Zeng, H.Y. Lin, Z.X. Liu, Z.H. Liu, Analysis of young shoots of "Anji Baicha' (Camellia sinensis) at three developmental stages using nontargeted LC-MS-based metabolomics, J. Food Sci. 84 (7) (2019) 1746–1757, https://doi.org/10.1111/1750-3841.14657.
[26] Y. Ren, G. Yu, C. Shi, L. Liu, Q. Guo, C. Han, H. Huang, Majorbio Cloud: a one-stop, comprehensive bioinformatic platform for multiomics analyses, iMeta 1 (2) (2022), https://doi.org/10.1002/imt2.12.
[27] A. Dagan-Wiener, A. Di Pizio, I. Nissim, M.S. Bahia, N. Dubovski, E. Margulis, M.Y. Niv, BitterDB: taste ligands and receptors database in 2019, Nucleic Acids Res. 47 (D1) (2019) D1179–D1185, https://doi.org/10.1093/nar/gky974.
[28] L. Lalaleo, D. Hidalgo, M. Valle, W. Calero-Cáceres, R.M. Lamuela-Raventós, E. Becerra-Martínez, Differentiating, evaluating, and classifying three quinoa ecotypes by washing, cooking and germination treatments, using H NMR-based metabolomic approach, Food Chem. 331 (2020) 8, https://doi.org/10.1016/j.foodchem.2020.127351.
[29] T. Siddharth, N.E. Lewis, Predicting pathways for old and new metabolites through clustering, J. Theor. Biol. 578 (2024) 111684, https://doi.org/10.1016/j.jtbi.2023.111684.
[30] I. Sofrenic, B. Andelkovic, D. Godevac, S. Ivanovic, K. Simic, J. Ljujic, S. Milosavljevic, Metabolomics as a potential chemotaxonomical tool: application on the selected Euphorbia species growing wild in Serbia, Plants-Basel 12 (2) (2023), https://doi.org/10.3390/plants12020262.
[31] I.G. Medina-Meza, N.A. Aluwi, S.R. Saunders, G.M. Ganjyal, GC-MS profiling of triterpenoid saponins from 28 quinoa varieties (Chenopodium quinoa Willd.) grown in Washington state, J. Agric. Food Chem. 64 (45) (2016) 8583–8591, https://doi.org/10.1021/acs.jafc.6b02156.
[32] A.J. Akinboye, K. Kim, S. Choi, I.H. Yang, J.G. Lee, Alkaloids in food: a review of toxicity, analytical methods, occurrence and risk assessments, Food Sci. Biotechnol. 32 (9) (2023) 1133–1158, https://doi.org/10.1007/s10068-023-01295-0.
[33] S.P. Jiang, X.D. Wang, M.S. Yu, J.X. Tian, P. Chang, S.J. Zhu, Bitter peptides in fermented Soybean Foods-A review, Plant Foods Hum. Nutr. 9 (2023), https://doi.org/10.1007/s11130-023-01077-3.
[34] Y.P. Timilsena, A. Phosanam, R. Stockmann, Perspectives on saponins: food functionality and applications, Int. J. Mol. Sci. 24 (17) (2023) 13538, https://doi.org/10.3390/ijms241713538.
[35] A.S. Lozano Pérez, J.J. Lozada Castro, C.A. Guerrero Fajardo, Application of microwave energy to biomass: a comprehensive review of microwave-assisted technologies, optimization parameters, and the strengths and weaknesses, Journal of Manufacturing and Materials Processing 8 (3) (2024) 121, https://doi.org/10.3390/jmmp8030121.
[36] G.V. Bonfiglio, R.V. Wierna, N.A. Bonini, M. Armada, M.C. Goldner, Study of bitterness perception of quinoa (Chenopodium quinoa wild.) saponin extracts, J. Cereal. Sci. 95 (2020) 103032, https://doi.org/10.1016/j.jcs.2020.103032.
[37] J.B. Johnson, Mechanism of Action of Natural and Biorational Insecticides (Master's Thesis, Iowa State University, 2021.