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Prediction of Flavor Potential of *Ocimum basilicum* L. Side-Stream Phytoconstituents, Using Liquid Chromatography–Tandem Mass Spectrometry Analysis and In Silico Techniques

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Abstract: Although post-distillation side-streams of basil (*Ocimum basilicum* L.) pose significant economic and environmental challenges, they also bring forth new opportunities in the flavor industry. Thus, the objective of the current study was to assess the phenolic profile of basil side-stream extracts to identify key compounds and to evaluate their taste properties, using liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, flavor prediction tools and molecular docking. In particular, 52 phytoconstituents, mainly phenolic acids, salvianolic acids, flavonoids and fatty acids derivatives, were elucidated in the side-streams of two different basil varieties (*Minimum* and *Genovese*) harvested and distilled in early and late autumn, highlighting the effect of pre-harvest factors on basil's phenolic fingerprint. Furthermore, the results of tests undertaken using taste prediction tools showed that most of the identified compounds were very likely to taste bitter, while six of them (caffeoylferuloyltartaric acid, isoquercetin, lithospermic acid A, sagerinic acid, salvianolic acids C and F) presented a high bitterant capacity (70–90%). Moreover, according to molecular docking studies, these compounds exhibited a stronger binding affinity to the *h*TAS2R46 bitter receptor compared to its known agonist, strychnine. This outcome and consequently their bitterness were mainly attributed to interactions with Glu265, Thr180 and/or Trp88 through the formation of direct hydrogen bonds. Therefore, the present results provide insights into the taste profiles of basil side-streams, leading to more sustainable and innovative uses of aromatic herbs residues.

Keywords: *Ocimum basilicum* L.; basil; post-distillation side-streams; phenolic fingerprint; liquid chromatography–mass spectrometry (LC-MS); in silico techniques; taste prediction; TAS2R46 bitter receptor

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1. Introduction

Basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family, is characterized as an aromatic herb that is well known for its significant culinary and medicinal applications. In the food industry, basil serves as a natural preservative and flavor enhancer in processed foods, and it is also a prominent ingredient in culinary dishes [1]. Beyond its culinary value, basil and its components are gaining widespread attention owing to their multiple beneficial health effects, including their antimicrobial, antifungal, antioxidant, anti-inflammatory, cardioprotective, neuroprotective and anti-cancer activities, and also their potential to assist

with the treatment of chronic obstructive pulmonary diseases, such as asthma, and other health benefits [2–4]. These diverse applications underscore the importance of basil not only as an edible plant but also as a medicinal one, making the exploration of its bioactive compounds highly relevant for both the food and health industries. By focusing on the phytochemical composition and taste properties of basil side-streams, the present study aimed to enhance the understanding of how these compounds can be utilized to develop innovative and health-promoting products. The overall demand for basil extract is expected to increase at a compound annual growth rate (CAGR) of 3.3% from 2023 to 2033, with a market value predicted to reach approximately USD 82.0 million by the end of this period (https://www.futuremarketinsights.com/reports/basil-extract-market) (accessed on 15 July 2024).

Besides the abovementioned uses, basil, which is the most widely grown aromatic herb globally, has garnered significant attention due to the essential oils it contains [5]. However, the distillation process for the recovery of essential oils generates a vast amount of biomass, which comprises various bioactive phytoconstituents, such as phenolic compounds, carotenoids, vitamins, etc. Nonetheless, these solid residues are usually discarded without implementing dedicated waste management strategies, posing significant environmental challenges (i.e., the generation of greenhouse gases, accumulation of pathogenic microorganisms, emission of toxic gases, biodiversity loss, etc.) and socioeconomic risks (i.e., proliferation of diseases, decrease in land values, increased waste management and crop production costs, loss of potential revenues from the exploitation of valuable compounds, etc.) [6,7]. Thus, discovering sustainable methods to manage and utilize post-distillation side-streams of aromatic herbs, through the extraction and elucidation of their bioactive components, is of the utmost importance to diminish these arguably hazardous impacts. When it comes to basil side-streams and their future use in the food industry, the mapping of their phytochemical profile using non-targeted liquid chromatography–mass spectrometry (LC-MS)-based approaches [8–10] is critical to identifying the taste compounds of these extracts in order to pinpoint specific molecules with flavor properties.

Notably, taste constitutes a fundamental sensory element that influences consumer preferences, product development and the overall success of food products in local and international markets [11]. Therefore, understanding and predicting taste are crucial for developing attractive and innovative food products that cover diverse consumer preferences. To this end, in silico techniques have gained great attention as a powerful tool that gives researchers the opportunity to simulate and predict taste interactions and profiles. These tools combine computational techniques, food databases and machine learning algorithms to model the chemical and sensory properties of taste compounds, providing insights into how different ingredients and formulations will be perceived through taste [12–14]. Recent studies showed that taste receptors [15,16] located on the taste buds of the tongue and in other areas of the oral cavity not only detect fundamental taste sensations but are also related to human health through their impact on dietary behaviour/preferences, metabolic processes and disease management [17–19].

The aim of the present study was to elucidate the phenolic profile of post-distillation side-streams of *Ocimum basilicum* L. to identify key bioactive compounds and evaluate their gustatory properties. Utilizing liquid chromatography–tandem mass spectrometry (LC-MS/MS), 52 phytoconstituents, including phenolic acids, salvianolic acids, flavonoids and fatty acid derivatives, were characterized from two basil varieties harvested in early and late autumn. This study further employed advanced taste prediction tools to assess the bitterness potential of these compounds, while molecular docking studies were performed to examine the compounds' binding affinity to the *h*TAS2R46 bitter receptor. The findings provide insights into the taste profiles of basil's side-stream phytoconstituents, promoting their sustainable and innovative applications in the flavor industry.

2. Materials and Methods

2.1. Plant Material

The investigated plant material included the distilled basil side-streams of two different varieties—Greek Basil, also known as *Ocimum basilicum* var. *Minimum* and curly leaf basil, and Genovese Basil, also known as *Ocimum basilicum* var. *Genovese* and broadleaf basil—obtained from local producers in different collection/harvest periods (mid-September 2022, mid-October 2022). The *Genovese* variety was selected as a standard among sweet basil varieties used globally, mainly in culinary practices, since it is used for the preparation of the famous Genovese pesto sauce. On the other hand, *Minimum* is a less commonly studied, yet chemically distinct, variety, which nonetheless presents a similar taste to the *Genovese* variety. Moreover, *Minimum* basil is important for the Greek herb industry since it is widely cultivated in Greece. Therefore, it is important to study this variety in order to improve strategies for its cultivation and to bolster its share in the global market.

Basil biomass, which is what remains after the hydrodistillation of raw plant material for the recovery of its essential oils, was lyophilized in order to acquire dry material. The dried side-streams were stored at −20 °C until further analysis.

2.2. Extraction of the Phenolic Fraction of Basil Side-Streams

Ultrasound-assisted extraction (UAE) was applied to the basil side-streams post-distillation to recover their phenolic components. The UAE process was carried out using an ultrasonic probe system (Sonoplus HD 4400, Bandelin Sonoplus, Berlin, Germany) with a maximum ultrasonic nominal power of 400 W. For the extraction, 0.5 g of dried material was mixed with a 80:20% v/v water–ethanol solution, which was used as an extraction solvent in order to provide a final extract that is rich in phenolic compounds whilst also being acceptable for human consumption. The extaction time was set at 10 min, while the solvent/material ratio was adjusted at 50 mL/g and the ultrasonic power at 80% of the nominal value (320 W).

During the extraction process, the vessels containing the samples were placed in an ice-cold bath to keep the temperature constant at 20–25 °C. Continuous sonication was applied to all samples. After sonication, the extracts were centrifuged at 3500 rpm for 15 min, and the supernatant was collected for further analysis.

2.3. Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) Information-Dependent Acquisition (IDA) for the Screening of Phenolic Compounds

The identification of basil by-products' phytoconstituents was conducted by constructing a suspect ion list based on literature data and on experimental spectra stored in online compound databases, such as the Human Metabolome Database (https://hmdb.ca/) (accessed on 25 April 2024) and Mass Bank of North America (MoNA) (https://mona.fiehnlab.ucdavis.edu/) (accessed on 25 April 2024). In order to elucidate a compound on the list, it needed to present (a) the same precursor ion and (b) a similar fragmentation pattern when compared to the data of the suspect ion list. The compounds presenting 3 or more similar fragment ions to those on the suspect list were identified with a high reliability score. The ionization was performed in the negative mode, where the phenolic compounds are usually ionized.

For the LC-MS/MS analysis, 1 mL of basil extract was lyophilized, and the dry residue was weighted and then reconstituted in LC-MS-grade methanol containing 0.1% v/v formic acid. Prior to the analysis, all samples were filtered using Chromafil Xtra PET 0.45 μm filters (Macherey-Nagel, Düren, Germany).

Chromatographic System:

The chromatographic system included an Agilent Eclipse Plus C-18 reversed-phase column (50 mm × 2.1 mm inner diameter, 3.5 μm particle size) connected to an RRLC in-line filter kit (2.1 mm, 0.2 μm filter) from Agilent Technologies (Santa Clara, CA, USA). The mobile phase binary solvent system consisted of water with 0.2% v/v formic acid

(Solvent 1) and acetonitrile with 0.1% v/v formic acid (Solvent 2). The gradient elution program and flow rate adjustments were described in detail by Kavga et al. (2019) [20]. The autosampler and column temperatures were maintained at 25 °C, and the injection volume was set to 5 μL.

Mass Spectral Analysis:

The mass spectral analysis was performed using a 3200 Q TRAP triple-quadrupole linear ion trap mass spectrometer (Sciex, Framingham, MA, USA) with an electrospray ionization (ESI) source operating in negative ionization mode. The formation of fragment ions and thus the identification of phenolic compounds in the basil were carried out by applying information-dependent acquisition (IDA)-triggered MS/MS with a mass tolerance of 5 ppm for MS/MS analysis. The applied MS conditions are described in previous work by our group [21]. The m/z range in MS was set at 100–1000 amu, while the m/z range in MS/MS was tuned to 100–700 amu. Data processing was performed using Analyst software (version 1.6.2) (Sciex, Framingham, MA, USA)

2.4. Flavor Prediction Tools

2.4.1. Organoleptic Profile Prediction

All phytochemicals identified by LC-ESI(−)-MS/MS analysis (52 compounds) (Table S1, Supplementary Materials) were sketched in 2D format, and the derived smiles files were imported into the publicly accessible web platform "Virtuous" (https://virtuoush2020.com/) (accessed on 22 May 2024) in an effort to predict their taste [22]. In particular, Virtuous Multitaste (https://virtuous.isi.gr/#/multitaste) (accessed on 22 May 2024), a machine learning-based web tool, was used to predict the four tastes of the studied compounds based on their chemical structures [23]. The analysis of the results showed the likelihood of the compounds presenting bitter, sweet, umami and other (sour and salty) taste perceptions [23].

2.4.2. Molecular Docking Studies

Molecular docking studies were subsequently employed to explore the potential binding affinity of basil by-products' phytoconstituents to the hTAS2R46 bitter receptor. For the present study, the cryo-electron microscopy (cryo-EM)-elucidated structure of the human TAS2R46-mini$G_{s/gust}$ receptor complexed with the potent agonist strychnine (PDB ID: 7XP6, resolution: 3.01 Å) was downloaded from the Protein Data Bank (https://www.rcsb.org) (accessed on 20 May 2024) and prepared by applying the Protein Preparation Wizard [24] of the Maestro interface [25]. In particular, Guanine nucleotide-binding protein subunits and water molecules were removed, all missing residues and hydrogen atoms were added, bond orders were assigned, and finally the complex was minimized, using the OPLS3 force field. Simultaneously, all examined phenolic compounds were prepared at pH = 7.0 ± 0.5, using LigPrep [26] of the Maestro interface [24].

Afterwards, a grid box with the dimensions 10 × 10 × 10 Å was created and molecular docking studies were carried out on all examined phytochemicals, using the Standard Precision (SP) mode of Glide [27]. The maximum number of docking poses was set equal to 10, and all poses were visually inspected and analyzed. The docking validation process was based on the similarity between the superimposed cryo-EM and the docked strychnine, showing an RMSD value equal to 0.1418 Å. According to the results, 6 of the identified phytochemicals could be considered as promising bitterants.

2.4.3. Bitter Taste Prediction Tool

BitterX (http://mdl.shsmu.edu.cn/BitterX) (accessed on 10 June 2024), an open-access web server on bitter compound identification and potential bitter target prediction for small molecules, was used as an additional bitter taste filter [28]. Hence, the chemical molecules of the 6 phytochemicals (smiles files) were submitted as the input field, and the BitterX

server was used to estimate the probability that the compounds would possess a bitter taste, while simultaneously identifying their potential bitter taste receptors.

3. Results and Discussion

3.1. Determining the Phenolic Profile of Post-Distillation Basil Side-Streams Using Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) Information-Dependent Acquisition (IDA)

The non-targeted screening of basil side-stream extracts resulted in the identification of 52 compounds, mainly phenolic acids (12 compounds), phenolic glycosides (14 compounds) and salvianolic acids (7 compounds). The LC-MS characteristics of the elucidated compounds are presented in Table 1.

Table 1. Characterization of basil phytochemicals by LC-MS/MS in negative ionization.

Compounds	Precursor Ion $[M-H]^-$	Product Ions MS/MS	Chemical Group
1-octen-yl pentosyl glucoside	421.20	259.22	O-acyl carbohydrate
3-(3,4-dihydroxyphenyl) lactic acid glucoside	359.10	310.10, 219.10, 197.10, 161.10, 145.10	Hydroxy monocarboxylic acid glucoside
Caffeic acid	179.04	135.04, 117.03, 107.05	Phenolic acid
Caffeoyl-dihydroxyphenyllactoyltartaric acid	491.12	329.03, 293.04, 251.04	Phenolic acid derivative
Caffeoylferuloyltartaric acid (cichoric acid methyl ether)	487.08	325.60, 310.70, 291.50	Phenolic acid derivative
Caftaric acid (Caffeoryl-tartaric acid)	311.04	179.03, 149.01, 135.04	Phenolic acid
Chicoric acid	947.10 *	473.10, 341.10, 311.10, 293.10, 149.10	Phenolic acid
Chlorogenic acid	353.09	191.05, 179.04, 161.02	Phenolic acid
Dihydroxy dimethoxyflavone	313.07	298.10, 283.11	Flavone
Dihydroxybenzoic acid-O-pentosyl pentoside	447.11	429.10, 403.10, 297.10, 153.10, 137.10	Phenolic acid glucoside
Dihydroxy-octadecadienoic acid	311.22	275.21	Hydroxy fatty acid
Dihydroxy-octadecatrienoic acid	309.21	273.20	Hydroxy fatty acid
Dihydroxy-oleanenoic acid	471.34	399.30	Hydroxy fatty acid
Ethyl caffeate	207.07	179.03, 161.02, 135.04	Phenolic acid ester
Ethyl protocatechuate	181.05	153.02, 108.02	Phenolic acid ester
Fertaric acid (feruloytartaric acid)	325.06	193.10, 134.04, 149.10	Phenolic acid
Ferulic acid	193.05	178.03, 149.06, 134.04	Phenolic acid
Gallic acid			Phenolic acid
Galloylglucose	331.07	271.04, 211.02, 169.01, 151.00	Tannin
Hydroxy jasmonic acid-O-glucoside	387.17	207.20, 163.20	Sesquiterpene derivative
Hydroxy-octadecatrienoic acid	293.21	275.20, 211.20, 161.20	Lineolic acid derivative
Hydroxy-oxo-phytodienoic acid	307.19	289.20, 265.20, 223.20	Derivative of fatty acid
Isocitric acid			Tricarboxylic acid
Isoquercetin	463.09	300.04, 286.90, 243.90	Tetrahydroxyflavone
Lithospermic acid A	537.10	493.10, 358.10, 339.50, 293.50, 135.40	Phenolic acid
Methyl gallate	183.03	168.00, 124.02	Phenolic acid ester
Nepetoidin glucoside	475.12	323.10, 313.10, 161.10, 151.10	Caffeic acid derivative
O-caffeoyl rosmarinic acid (isomelitric acid A)	537.10	493.10, 427.10, 377.10, 339.10, 161.10	Catechol
Palmitic acid	255.23	182.20	Fatty acids
p-Coumaric acid	163.04	133.03, 119.05, 121.01	Phenolic acid
p-Hydroxybenzoic acid	137.02	108.2, 93.04, 90.90	Phenolic acid

Table 1. *Cont.*

Compounds	Precursor Ion $[M-H]^-$	Product Ions MS/MS	Chemical Group
Protocatechuic acid	153.11	135.10, 132.91, 123.04, 109.03	Phenolic acid
Quercetin 3-O-glucoside	463.10	301.04	Flavonol derivative
Quercetin-3-O-apiosyl (1–2) galactoside	595.10	445.10, 300.04	Flavonol derivative
Quercetin-O-pentosyl-glucoside	595.13	463.10, 445.10, 301.10	Flavonol derivative
Rosmarinic acid	359.08	223.04,197.05, 179.04, 161.03, 135.03	Phenolic acid
Rosmarinic acid glucoside A	521.12	359.10, 197.10, 179.04, 161.04, 135.06	Phenolic acid derivative
Rosmarinic acid glucoside B	521.12	359.10, 323.10, 197.04, 179.04, 161.04, 135.06	Phenolic acid derivative
Rosmarinic acid-O-glucoside	521.14	359.10, 341.10	Phenolic acid derivative
Rutin	609.5	463.04, 301.10, 272.05, 256.03, 179.10, 151.10	Flavonol derivative
Sagerinic acid	719.10	359.60, 197.70, 179.70, 161.10	Lignan
Salvialinic acid (danshensu)	197.04	161.06	Phenolic acid
Salvianolic acid A	493.10	359.10, 313.10, 295.10, 185.10	Phenolic acid
Salvianolic acid B	717.14	519, 10, 339.10, 321.10, 293.10, 277.10	Phenolic acid
Salvianolic acid C	491.10	311.90, 293.50, 179.30, 135.20	Phenolic acid
Salvianolic acid F	313.07	269.60, 254,.50, 227.70	Phenolic acid
Salvianolic acid G	717.14	555.10, 537.10, 519, 10, 339.10, 321.10, 295.10	Phenolic acid
Salvianolic acid H/I	537.10	493.10, 339.04, 313.05, 295.10, 197.10, 179.10	Phenolic acid
Salvianolic acid K	555.11	537.10, 493.10, 295.04	Phenolic acid
Salvigenin (5-Hydroxy-6,7,4′-trimethoxyflavone)	327.21	311.12, 277.10, 215.04	Flavone
Trihydroxy-octadecendic acid	329.07	315.10, 299.10	Linoleic acid derivative
Vanillic acid	167.03	152.01, 125.87, 108.02	Phenolic acid

* Precursor ion: $[2M-H]^-$.

While all the elucidated compounds were detected in both curly and broadleaf basil extracts, their normalized content, as estimated by the values of their m/z intensities using a semiquantitative approach, varied (Figure 1, Figure S1). These variations show that plant variety, geographical origin, climatic conditions, pre-harvest management practices and harvest time play a significant role in the phytochemical profile of the extracts [29,30].

When thoroughly reviewing the results depicted in Figure 1, it became apparent that phenolic acids were present in relatively high contents in the basil side-stream extracts. This outcome may be attributed to the fact that shorter extraction times (10 min), combined with a high US power (80%) and the higher content of water (80% v/v) in the extraction solvent, favor the recovery of free non-conjugated molecules of higher polarity, such as phenolic acids [31,32].

A varietal comparison of the two examined basil cultivars revealed differences in their composition, with the broadleaf basil showing a greater diversity of compounds than the curly leaf basil (Figure 1). Salvianolic acids and derivatives are characteristic phytoconstituents of the broadleaf basil, as they were detected in all samples of this genotype, regardless of the pre-harvest factors or the harvest period [33]. Moreover, rutin and chlorogenic acid were also present in higher contents, mostly in broadleaf basil. On the other hand, rosmarinic acid and its derivatives were determined to be signature phenolic compounds for the curly leaf basil [34].

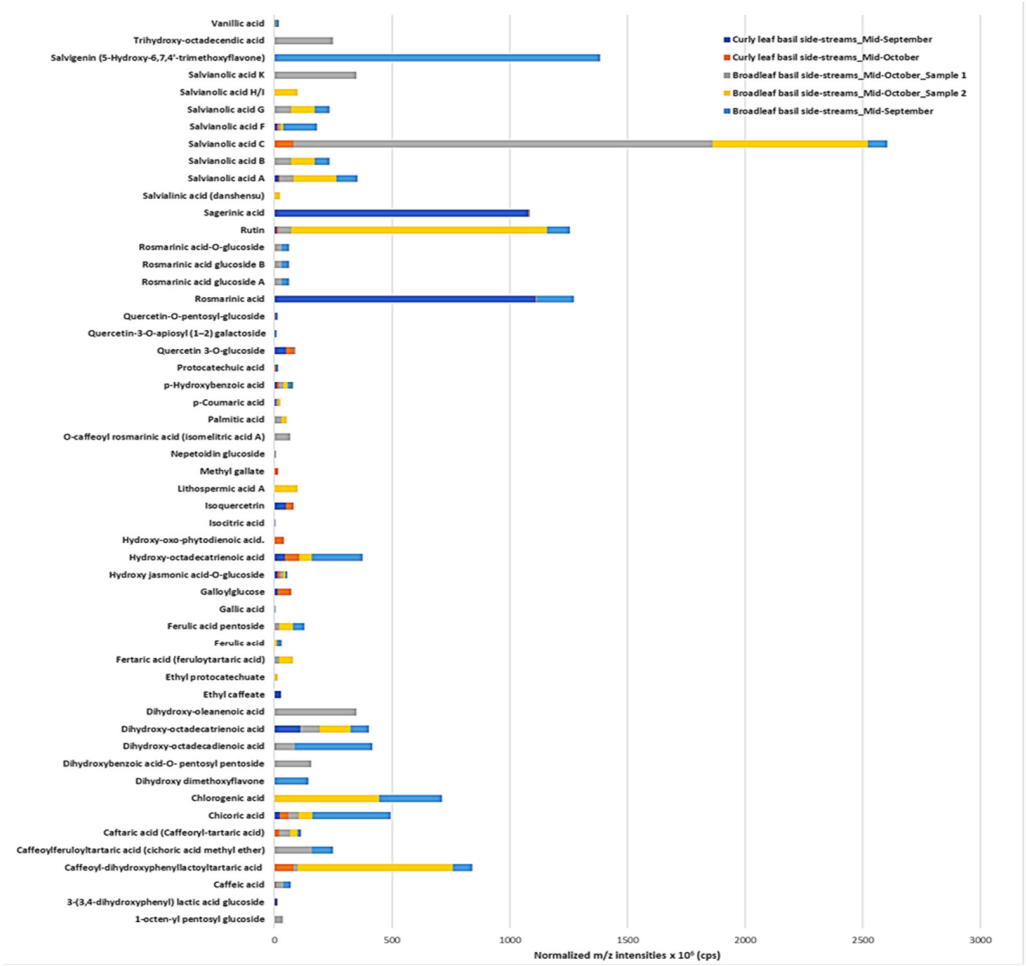

Figure 1. Comparison of the normalized contents of annotated compounds in basil side-stream extracts.

Further, the harvest date emerged as an essential factor affecting the phytochemical profile of basil extracts since it is firmly connected to environmental conditions [34]. In fact, salvianolic acid C was dominant in the mid-October samples of broadleaf basil by-products. Additionally, the flavonoid salvigenin exhibited extremely high normalized intensities in the mid-September extract. Although the samples harvested and then distillated in early autumn (mid-September) did not present a wide variety of identified compounds, the phytoconstituents that were detected were found in relatively high concentrations compared to the mid-October samples. For instance, rosmarinic acid was found nearly exclusively in mid-September curly leaf basil samples. Conversely, side-streams derived from basil samples harvested in late fall (mid-October) appeared to exhibit a greater variety of compounds, and thus a richer phytochemical profile (Figure 1). Recent scientific research has reported that basil harvested in October yields higher levels of phenolic compounds compared to that harvested in the summer or early autumn months [35]. This observation could be explained by the fact that when basil, a thermophilic plant (growth temperature 25–30 °C), experiences conditions outside its optimal environment in terms of temperature, soil drought, solar radiation and/or soil pH, it undergoes abiotic stress, which triggers the defense system of the plant. As part of these defensive mechanisms, plants synthesize and accumulate a wider range of secondary metabolites, such as phenolic compounds, which serve numerous protective functions (antioxidant agents, UV screening agents, etc.) to mitigate the adverse effects of stress [36].

Thus, when it comes to phenolic compounds, the richness of basil's and basil side-streams' phytochemical profile needs to be accounted for in order to better record the chemical composition and to explore the sensory attributes of this important culinary plant.

3.2. In Silico Tools for Ocimum basilicum Side-Stream Phytochemical Flavor Prediction

The taste profile of all phytochemicals identified by LC-ESI(−)-MS/MS was predicted using the freely accessible machine learning-based web tool, Virtuous Multitaste (https://virtuous.isi.gr/#/multitaste) (accessed on 22 May 2024). This tool calculates the percentage probability of different tastes (sweet, bitter, umami and other, which includes sour and salty) for the examined compounds. Results analysis (Table S1, Supplementary Materials) revealed that 38 out of the 52 phytochemicals of the basil extracts were highly likely to present a bitter taste, with probabilities exceeding 40%. Additionally, 21 of these compounds displayed a bitter taste, with a probability of more than 50%, and 6 compounds exhibited a bitter taste, with probabilities ranging from 70% to 90%. The above observation can be considered as a strong indication that the overall taste of basil extract is characterized as bitter. Furthermore, the taste prediction results indicated that only quercetin-O-pentosyl-glucoside, isocitric acid, rutin and quercetin-3-O-apiosyl (1–2) galactoside demonstrated significant probabilities for sweet, umami and other tastes, respectively. Quercetin-O-pentosyl-glucoside, rutin and quercetin-3-O-apiosyl (1–2) galactoside are glycosylated forms of quercetin, and their flavour profile may be influenced by the sweetness of the attached sugars [37]. Several studies have demonstrated that citric acid, a structural isomer of isocitric acid, effectively reduces the bitter taste of low-sodium salts containing potassium chloride when used to mask the bitterness of drugs dissolved in solution [38,39]. This finding may offer a putative explanation for its low bitter taste percentage prediction.

In humans, bitter taste sensing is mediated by type 2 taste receptors (TAS2Rs), which represent a distinct class of G-protein-coupled receptors named the T GPCR subfamily [40]. TAS2Rs can recognize thousands of diverse bitter molecules, even though humans only possess about 25 different TAS2Rs [41]. Normally, TAS2Rs are expressed on the functional gustatory transduction units, specifically the taste buds on the tongue, which are located within the gustatory papillae. It is important to note that TAS2Rs are not only found in the taste buds of the tongue; they are also expressed in several extra-oral tissues, including the heart, skeletal and smooth muscle, upper and lower airways, gut, adipose tissue, brain and immune cells. Therefore, these extra-oral bitter taste receptors play a role in various physiological processes, and they are linked to a variety of diseases [42]. Among them, TAS2R46, which is located on the motile cilia of human airway epithelial cells, constitutes a potential target for asthma treatment [43].

Taking into account the twofold role of the *h*TAS2R46 receptor, the compounds (21 in total) that, in the previous step, were found to have a bitterant capacity greater than 50% (Table S1, Supplementary Materials), were subjected to molecular docking studies in an effort to study not only their potential bitter taste by activation of the *h*TAS2R46 receptor but also their relevance for asthma treatment. For this study, the selected compounds were docked at the orthosteric binding pocket of the *h*TAS2R46 receptor (PDB ID: 7XP6, resolution: 3.01 Å) complexed with the potent agonist strychnine [44]. The results evaluation was based not only on the predicted binding affinity values (glide gscore) but also on the compounds' interaction pattern (Table S2, Supplementary Materials).

Therefore, the outcome showed that all molecules tested, except hydroxy-octadecatrienoic acid and palmitic acid, exhibited similar or greater binding affinity based on their glide gscore compared to the known agonist strychnine (gscore = -5.44 kcal·mol^{-1}), ranging from -7.00 to -3.00 kcal·mol^{-1}. It is critical to mention that although hydroxy-octadecatrienoic acid and palmitic acid presented satisfactory (57% and 58%, respectively) bitter taste probability, they did not bind to the TAS2R46 receptor. A putative explanation for this could be that fatty acids are the sixth basic taste modality, as recently proposed. However, the classification of taste bud-mediated fat detection as a distinct taste modality remains a contentious issue [45]. Also, the glide gscores of the phenolic compounds caffeoylferuloyltartaric acid, isoquercetin, sagerinic acid and salvianolic acids (including A, B, C, F and K) were significantly higher than that of strychnine, providing strong evidence for their binding to the *h*TAS2R46 receptor and consequently bitter taste.

When evaluating the docked poses of the aforementioned compounds, it is obvious that all compounds interact via a direct hydrogen bond with Glu265 and with Thr180 and/or Trp88, as strychnine, and present a fruitful interaction pattern that reinforce their binding stability (Table S2, Supplementary Materials). In particular, caffeoylferuloyltartaric acid (gscore = 7.21 kcal·mol^{-1}), a hydroxycinnamic acid derivative, formed direct hydrogen bonds with Glu265 and pi–pi interaction with Tpr88, similarly to strychnine (gscore = 5.44 kcal·mol^{-1}), and also formed direct hydrogen bonds with Asn176 and Ala84. In the case of isoquercetin (gscore = −6.48 kcal·mol^{-1}), a naturally occurring flavonoid also known as isoquercitrin, binding mode analysis exhibited hydrogen bond interactions with Glu265, Thr180, Lys156 and Asn65, indicating strong binding to the *h*TAS2R46 receptor. Furthermore, the interaction pattern of sagerinic acid, the dimeric form of rosmarinic acid, included the formation of direct hydrogen bonds with Ala268, Glu265, Thr180, Lys156, Arg81 and Asn65 as well as pi–pi interaction with Tyr85, suggesting a high affinity for *h*TAS2R46 receptor binding. According to the literature, it is known that a variety of non-volatile dietary compounds, including saponins, phenolic compounds, tannins and alkaloids, are characterized as bitter and activate different taste receptors [46,47]. Several examples of bitter phytonutrients in common plant foods are naringin, tangeretin, quercetin, (−)-epicatechin, catechin and epicatechin [46–48], confirming our in silico results.

Salvianolic acids, a group of bioactive compounds found primarily in the roots of *Salvia miltiorrhiza*, commonly known as Danshen, present a variety of interactions that stabilize the binding (Table S2, Supplementary Materials). According to recent studies, the taste of Danshen is characterized as bitter, and this fact may offer a putative explanation for salvianolics acids' interesting in silico results [49,50].

Additionally, docking results showed that lithospermic acid A (gscore = −5.70 kcal·mol^{-1}), a polyphenolic compound commonly found in certain plants and closely related to salvianolic acids, exhibited a rich interaction motif that involves the formation of hydrogen bonds with Glu265, Thr180, Lys15, Val61 and p-p with Trp88. Consequently, it is reasonable to infer that it may be a *h*TAS2R46 activator and therefore a bitter flavor compound.

Considering the results analysis of the taste prediction tool and molecular docking, six phytochemicals, including caffeoylferuloyltartaric acid, isoquercetin, lithospermic acid A, sagerinic acid and salvianolic acids A and F (Figure 2), were selected as the most promising bitterants. Also, representative binding poses of the selected phytochemicals at the orthosteric binding site of the *h*TAS2R46 bitter receptor are illustrated in Figure 3, while their chromatographs and mass spectra are presented in Figure S2 (Supplementary Materials). It is also worth mentioning that caffeoylferuloyltartaric acid and salvianolic acid C were mainly present in broadleaf basil side-streams, while isoquercetin and sagerinic acid were the main components of curly leaf basil. Moreover, the post-distillation by-products of early-fall broadleaf basil seem to be a rich source of salvianolic acid F.

In the last step, the web server BitterX (http://mdl.shsmu.edu.cn/BitterX) (accessed on 10 June 2024) was used in an effort to provide further information related not only to the probability of the six phytochemicals presenting a bitter taste, but also their ability to bind to the *h*TAS2R46 bitter receptor. The BitterX prediction results indicated that all selected compounds were bitterants, and moreover, they may bind to *h*TAS2R46 at a rate greater than 50%, confirming the applied in silico pipeline. The bitter taste prediction percentage derived from the Virtuous Multitaste tool, and also the probability of the selected compounds to bind to the *h*TAS2R46 bitter taste receptor, are illustrated in Table 2.

To conclude, our in silico methodology was used to determine six *Ocimum basilicum* side-stream phytoconstituents that may possess a bitter taste and act as *h*TAS2R46 bitter receptor agonists. The exploitation of these compounds represents a multifaceted opportunity for the food industry and human health, as they can serve as natural preservatives and also as an alternative potential target for asthma treatment.

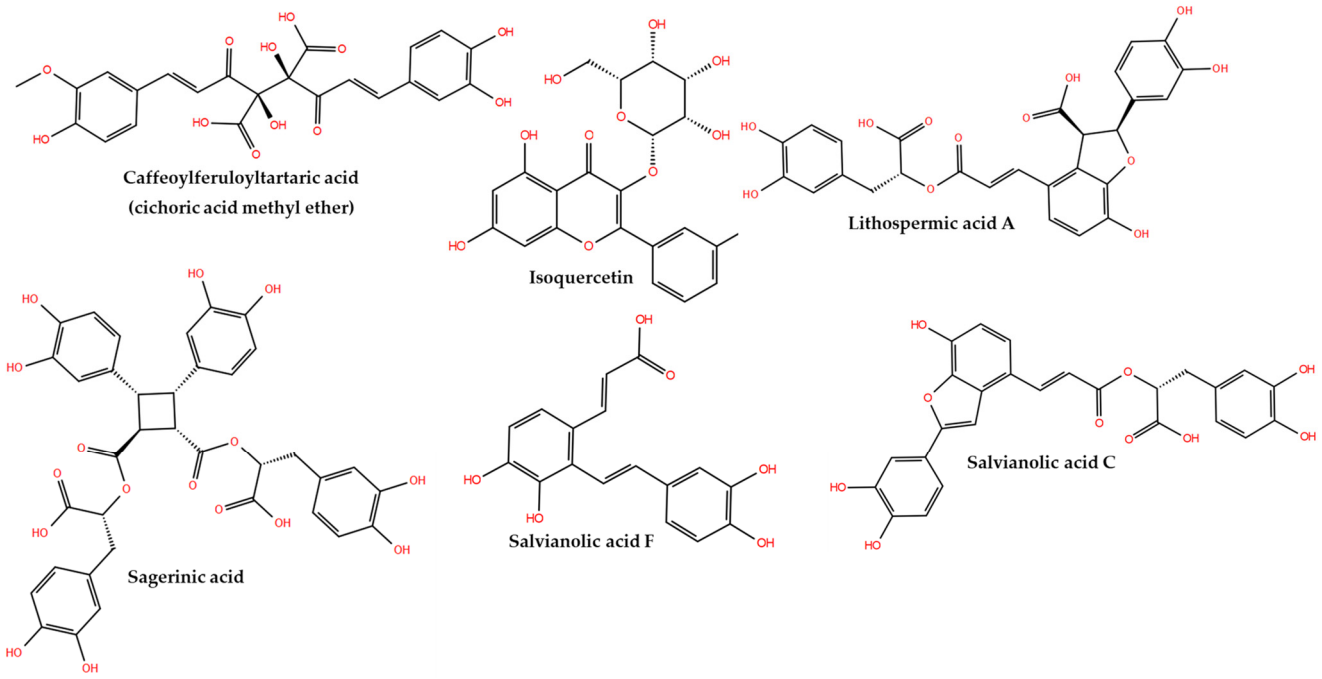

Figure 2. The chemical structures of the selected *Ocimum basilicum* side-stream phytoconstituents, as elucidated by in silico studies.

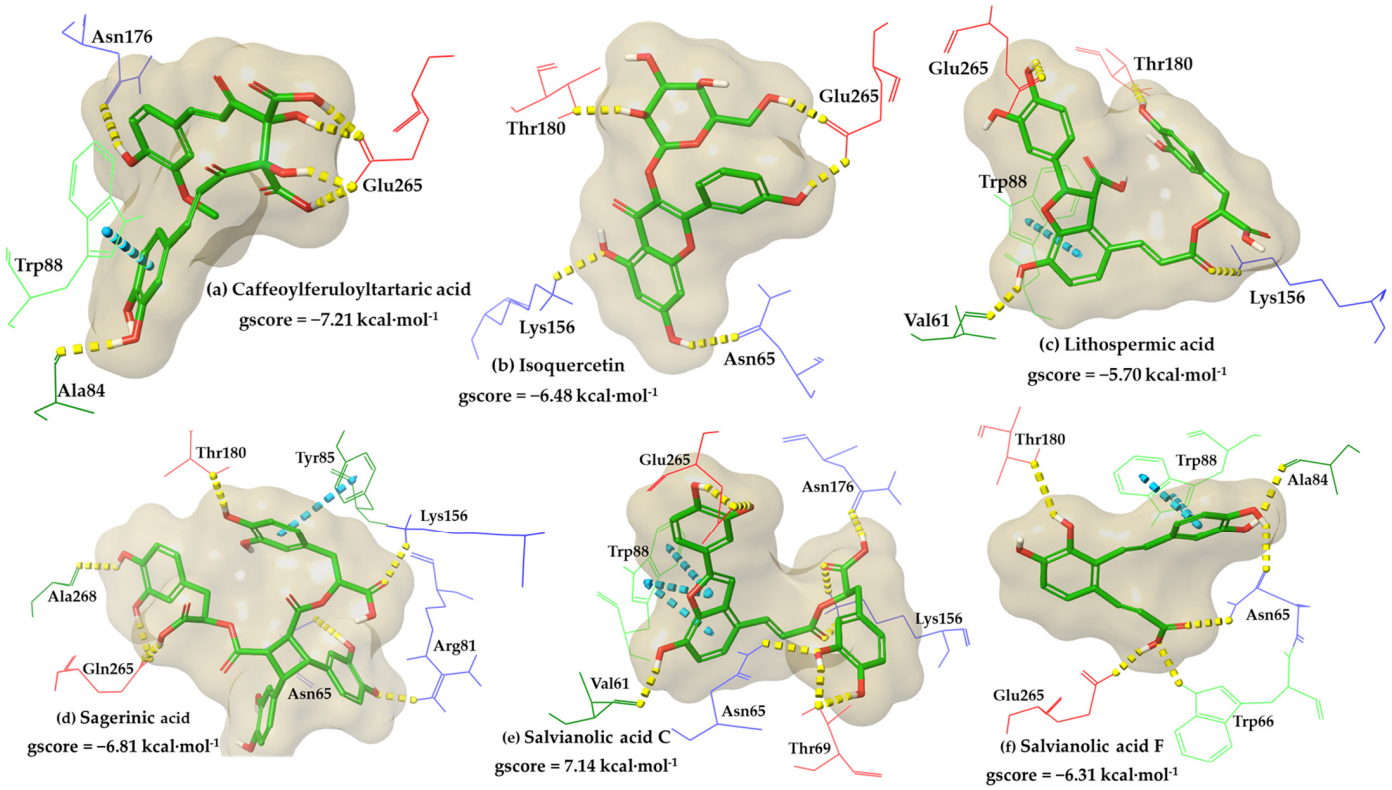

Figure 3. Representative binding poses of (**a**) caffeoylferuloyltartaric acid, (**b**) isoquercetin, (**c**) lithospermic acid A, (**d**) sagerinic acid, (**e**) salvianolic acid C and (**f**) salvianlolic acid F to the *h*TASR46 bitter receptor.

Table 2. Bitter taste prediction and TAS2R46 binding probability of the most promising compounds derived from Virtuous Multitaste and BitterX tools, respectively.

Compounds	%Bitter (Virtuous Multitaste)	TAS2R46 Probability (BitterX)
Strychnine	75%	74.34%
Caffeoylferuloyltartaric acid	55%	58.26%
Isoquercetin	86%	60.99%
Lithospermic acid A	53%	70.83%
Sagerinic acid	52%	51.78%
Salvianolic acid C	64%	74.46%
Salvianlolic acid F	78%	65.81%

4. Conclusions

The current study surveyed the phenolic profile of basil (*Ocimum basilicum* L.) side-stream extracts, focusing on the elucidation of key compounds and the evaluation of their bitter properties using LC-MS/MS analysis, flavor prediction tools and molecular docking techniques. A total of 52 phytoconstituents, predominantly phenolic acids, salvianolic acids, flavonoids and fatty acid derivatives, were detected in the side-streams of two basil varieties (curly leaf and broadleaf basil), both of which were harvested and distilled in mid-September and mid-October. This analysis underscored the significant influence of pre-harvest factors on the phenolic composition of basil, since the curly leaf variety contained mostly rosmarinic acid derivatives, while salvianolic acids were the major components of the broadleaf variety. Moreover, the late-fall extracts exhibited a richer phenolic profile than the early autumn samples due to the abiotic stress induced by the environmental conditions, which at this period of time (mid-October) were not optimal for basil growth.

Furthermore, the Virtuous Multitaste prediction tool revealed that the majority of the identified compounds were likely to have a bitter taste, with six of them (caffeoylferuloyltartaric acid, isoquercetin, lithospermic acid A, sagerinic acid, salvianolic acids C and F) indicating a high probability of bitterness that ranged from 70% to 90%. These phenolic compounds demonstrated a stronger binding affinity to the TAS2R46 bitter receptor than the known agonist strychnine, primarily through interactions implicating Glu265, Thr180 and/or Trp88 via direct hydrogen bonds. Hence, the findings of the present research offer a comprehensive understanding of the taste profiles of basil side-streams, underscoring the significant potential of these compounds as well as basil extracts as bitterants in the flavor industry. By determining the bitter properties and interactions of specific phytoconstituents with the *h*TAS2R46 receptor, this work lays the groundwork for the sustainable and innovative utilization of aromatic herbs biomass to enhance the development of new flavor agents that can serve as natural preservatives. Furthermore, it could facilitate the economic and environmental sustainability of basil cultivation and processing (a) by informing the development of high-quality basil varieties rich in selected target compounds and (b) by ensuring consistency and standardization in culinary applications. Also, the correlation of the *h*TAS2R46 bitter receptor with asthma treatment paves the way for advancements that could benefit both the food industry and public health.

Future studies will include (a) the application of more advanced tools (i.e., an electronic tongue and nose); (b) the comparative study of different extraction techniques or solvents (natural deep eutectic solvents, NADESs), in order to pinpoint the optimal use of basil extracts; and (c) the integration of multi-omics data with machine learning algorithms to predict the plant metabolic pathways and to explore their relationship to stress factors, would drive further advancements in this research area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations11090261/s1, Figure S1: annotated compounds with low normalized contents in basil side-stream extracts; Figure S2: LC-MS/MS spectra of the six potential bitterants; Table S1: taste prediction results of *Ocimum basilicum* side-streams characterized by phytochemicals, derived from the Virtuous Multitaste tool (https://virtuous.isi.gr/#/multitaste) (accessed on 22 May 2024); Table S2: the glide score (gscore) and the interaction pattern of the co-crystallized ligand strychnine and examined phytochemicals bound to the *h*TAS2R46 binding site.

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