Bitter taste: prediction, relation to toxicity, and effect on emotions

Ayana Dagan-Wiener, Ido Nissim, Nitzan Dubovski and MASHA Y. NIV
The Institute of Biochemistry, Food and Nutrition, The Robert H Smith Faculty of Agriculture, Food and Environment, The Hebrew University, 76100 Rehovot and The Fritz Haber Center for Molecular Dynamics, The Hebrew University, Jerusalem, 91904, Israel

Abstract

Bitter taste sensation is considered a signal of toxicity and is elicited by molecules of widely varying chemical structures, as summarized in BitterDB (http://bitterdb.agri.huji.ac.il). We developed a machine-learning (decision trees-based) tool BitterPredict, and showed that only 60% of the toxic compounds are known or predicted to be bitter, similar to the predicted bitter abundance in FDA-approved drugs and lower than in natural compounds. This suggests that there are many non-bitter toxic compounds. Interestingly, bitter mouth-rinse leads to lower mood scores and the effect depends on perceiving the solution as bitter.

Introduction

Bitter taste is one of the basic taste modalities and is typically considered a sentinel of toxicity. Yet, several examples of tasteless poisons or bitter non-toxic molecules are known. Intriguingly, the molecules that elicit taste sensation are numerous and chemically diverse [1] (Figure 1).

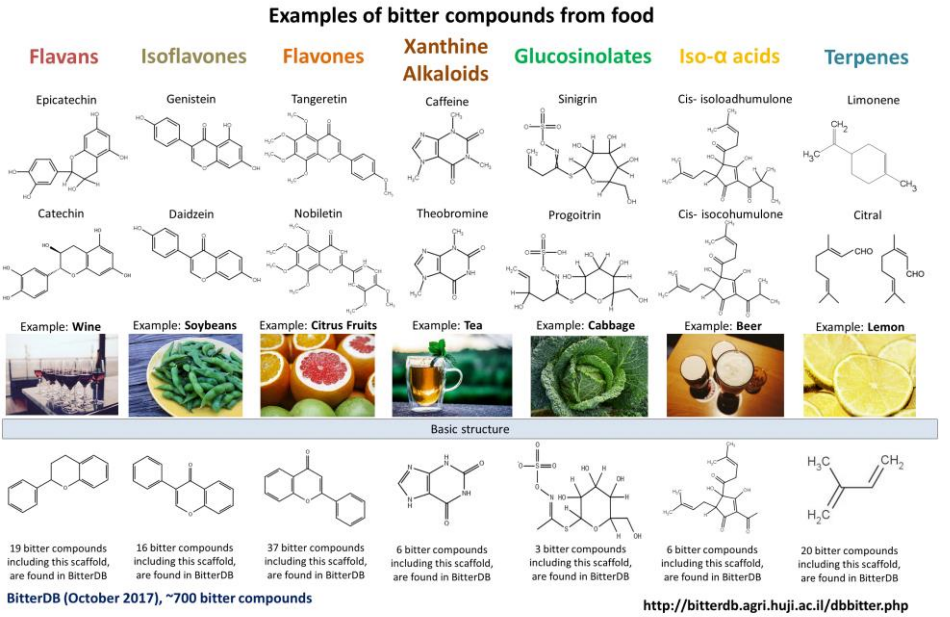

Figure 1: Examples of bitter compounds from food

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-56, CC BY-NC-ND 4.0

To facilitate the study of bitter taste, we have established the BitterDB (http://bitterdb.agri.huji.ac.il) [2], which has served over 30,000 users so far. The BitterDB contains data on molecules that were reported as bitter or were shown to activate at least one bitter taste receptor (T2R). Close to 700 bitter molecules have been gathered in the BitterDB, but clearly many additional bitter compounds exist. Some additional bitter molecules can be unraveled using a combination of computational, cell-based and sensory techniques [3]. Machine-learning approaches are proving to be extremely powerful in many areas of research and engineering, including sensory science [4]. Here we describe BitterPredict [5], a machine learning adaptive boosting program to classify molecules as bitter or non-bitter, and apply it to datasets of toxic and other compounds [6]. Finally, complementing the vast literature on emotional effects of odors, we present the effects of bitter (quinine or 6-n-propylthiouracil (PROP) dissolved in water) and sweet (sucrose in water) mouth rinse on mood [7].

Experimental methods

I. BitterPredict [5]

14 physicochemical properties and 47 Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME/T) descriptors from the QikProp package (Schrödinger, LLC) were calculated for the molecules. BitterPredict is an AdaBoost model constructed from 200 decision trees. AdaBoost is an ensemble method, where the final prediction uses the weighted average of the predictions given by each of the decision trees in the ensemble. The data was divided into a training set (70%) and a test set (30%). The model was trained only on the training set. Additionally, 3 external validation sets (molecules which were not used for training or testing) were collected and used to assess the performance of BitterPredict.

II. Toxicity datasets [6]

Two datasets were created to represent toxicity: FocTox is a focused, relatively small dataset aptly named FocTox, comprised of ~40 compounds from the *FAO/WHO food contaminants list* and ~350 compounds from the *List of extremely hazardous substances* defined in *Section 302* of the *U.S. Emergency Planning and Community Right-to-Know Act*. CombiTox is a broad dataset, combining two publicly available datasets: T3DB (The Toxin and Toxin-Target Database, contains ~3000 compounds) and DSSTox (Distributed Structure-Searchable Toxicity Database, contains ~140,000 compounds).

III. Sensory panel testing [7]

Participants tasted a solution without swallowing, and then had to: identify the taste they perceive (by choosing either sweet, sour, salty or bitter as the main taste modality), rank the taste intensity, perform seemingly unrelated behavioral tasks, and fill a standard *Positive and Negative Affect Schedule* (PANAS) [8] mood questionnaire. The questionnaire consists of 10 positive and 10 negative affect items. Each item was rated on a Likert scale of 1 (not at all) to 5 (very much). The total score was calculated by subtracting the sum of the 10 negative items, from the sum of the 10 positive items. T2R38 genotype was determined by collecting saliva samples from the participants, using OG-500 Saliva collection kits (Pronto Diagnostics Ltd). Nucleotides and amino acid codons for the two alleles of each panelist were carried out in The Monell Chemical Senses Center.

Results and discussion

Bitter compounds are very diverse in their chemical structures. Various chemical classes (terpenes, flavones, glucosinolates and more) are found among bitter compounds in food (Figure 1). So what are the chemical properties of bitter compounds, and can these be used for bitterness prediction?

A principle component analysis (PCA) of the bitter set, non-bitter set and 2000 random molecules, using physicochemical properties suggests that the non-bitter sub-sets are capturing different narrow chemical spaces (Figure 2). Most of the bitter molecules (97%) have molecular weight below 700 and range in -3 < AlogP < 7. This range was defined as the applicability domain for BitterPredict, which was then trained using the physicochemical and the ADME/T descriptors of the bitter and non-bitter set.

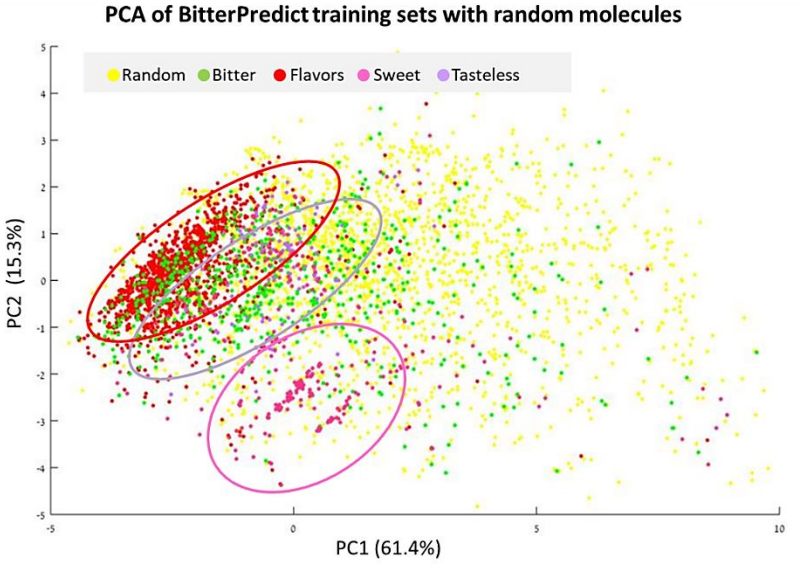

Figure 2: PCA of the negative sets (flavors, sweet and tasteless molecules), positive set (bitter) and random molecules within the Bitter Domain. The bitter molecules (green) spread widely inside the Bitter Domain. Each non-bitter sub set covers distinct sub-space (red, pink and purple); however, the combined non-bitter set covers almost all the domain, though not uniformly distributed. Principle components (PC) PC1 and PC2 explain ~61% and ~15% of the variation, respectively.

BitterPredict outputs a numerical score, positive for bitter and negative for non-bitter. Higher absolute values indicate higher confidence scores. Score > 0.6 can selected as a high confidence bitter score (leading to a false positive rate lower than 0.05) and < -0.7 can be selected as a high confidence non-bitter score (leading to a false negative rate lower than 0.1). BitterPredict separates well between the bitter and non-bitter molecules, with sensitivity (true positive/true positive+false negative) of 0.77 and specificity (true negative /true negative+false positive) of 0.85 on the test set (Table 1).

Table 1: BitterPredict performance on train set, test set and validation sets
Sensitivity = (true positive) / (true positive + false negative)
Specificity = (true negative) / (true negative + false positive)

		Sensitivity	*Specificity*	*Accuracy*
	train set	0.9	0.94	0.93
	Test set	0.77	0.85	0.83
Negative subsets	Non-Bitter flavors		0.83	
	sweet		0.82	
	tasteless		0.86	
Validation Sets	BitterNew	0.75		0.75
	Phyto	0.98	0.69	0.88
	UNIMI	0.78	0.85	0.82

The high performance of BitterPredict was confirmed via external sets, sensory evaluation, and datamining of prospective predictions [5] and enabled us to estimate the abundance of bitter compounds in toxic, random, natural and other datasets (Table 2). Interestingly, only 60% of the toxic compounds were predicted as bitter. This prediction is higher than in food compounds, but lower than in natural products and in approved drugs, suggesting existence of many toxic compounds that are not bitter. All in all, the number of predicted bitter compounds in the entire chemical space may be higher than initially thought.

Table 2: Approximate percentage of molecules predicted by BitterPredict as bitter/non-bitter with different confidence levels, in datasets with defined orientation

	FooDB	DrugBank Approved	DrugBank Experimental	Natural Products ZINC15	ChEBI	CombiTox	FocTox
	FooDB	DRUGBANK	DRUGBANK	ZINC15	ChEBI	EPA T3DB	World Health Organization
% Molecules predicted as bitter	**38.36%** (7,926 / 20,661)	**65.94%** (1,024 / 1,553)	**49.93%** 2,506/5,019	**77.21%** (21,786 / 28,217)	**43.71%** (16,188 / 37,033)	**55.84%** (77,712 / 139,165)	**60.18%** (192 / 319)
% Molecules predicted as bitter with 0.6 cutoff	**29.05%** (6,001 / 20,661)	**52.54%** (816/ 1,553)	**33.35%** 1,674/5,019	**62.39%** (17,604 / 28,217)	**30.01%** (11,115 / 37,033)	**37.33%** (51,960 / 139,165)	**37.30%** (119 / 319)
% Molecules out of Bitter Domain (considered non bitter)	**34.23%** 7,703 / 20,661)	**11.46%** 178/1,553	**14.05%** 705/5,019	**2.88%** (813 / 28,217)	**19.84%** (6,688 / 37,033)	**7.95%** (11,050 / 139,165)	**15.99%** (51 / 319)
% Molecules predicted as non-bitter in bitter domain	**27.40%** 5,662/ 20,661	**22.60%** 351/1,553	**36.02%** 1,808/5,019	**20.16%** (5,688 / 28,217)	**29.24%** (10,827 / 37,033)	**36.22%** (50,403 / 139,165)	**23.82%** (76 / 319)
% Molecules predicted as non-bitter with -0.7 cutoff	**14.99%** 3,098 / 20,661	**10.56%** 164/1,553	**17.99%** 903/5,019	**9.24%** (2,606 / 28,217)	**19.84%** (13,53/ 37,033)	**17.20%** (23,932 / 139,165)	**8.46%** (27 / 319)

Bitter taste may be generally associated with unpleasant and difficult situations [9], and thus may evoke negative emotions. A direct negative change in PANAS score compared to water baseline score, was induced by oral exposure to quinine or to PROP, but not to sucrose. PROP taster/non-taster status was determined by the participants' genotype of T2R38, which underlies the ability to taste PROP as bitter-. The negative affect caused by exposure to PROP depends on the taster/non-taster status of the participants (Figure 3A). Furthermore, the mean PANAS score for a group of participants

that tasted quinine solution was significantly lower than for groups of participants that tasted water, sweet or bitter-sweet mixture solutions (Figure 3B). However, the reverse effect – positive mood changes as a result of sweet solution – was not observed.

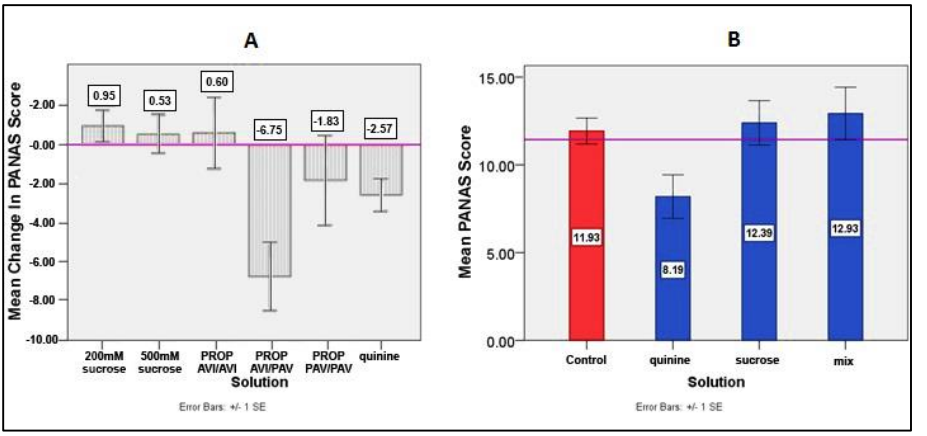

Figure 3: (A) Mean change in PANAS score, after exposure to the examined solutions, compared to water baseline. Bars indicate standard error. The horizontal line represents 0 (no change). PAV and AVI represent PROP tasters and non-tasters, respectively. PAV/PAV homozygotes have high sensitivity to PROP compound, PAV/AVI heterozygotes have intermediate sensitivity, while AVI/AVI homozygotes are PROP non-tasters. **(B)** Mean PANAS score of the examined solutions (blue), compared to the water group (red). Bars indicate standard error. The horizontal line represents the mean for all participants.

Summary

Bitter ligands can be accessed via BitterDB (http://bitterdb.agri.huji.ac.il). The BitterPredict bitter/non-bitter classifier works well despite tremendous chemical diversity of bitter compounds and can be applied to drug repurposing and bitterness prediction. Many random compounds may be bitter and only 60% of toxic compounds are predicted to be bitter. The high percentage of predicted bitter compounds in the datasets tested – including food-derived compounds – suggests that bitter taste may not be a strong marker for toxicity [6]. Mood scores were decreased by quinine solution that was perceived as bitter. PROP mouth rinse lead to negative mood change among in PROP tasters only. Conversely, while sweet mouth rinse ranked higher hedonic scores, it did not positively affect mood scores.

References

1. Meyerhof, W., Batram C., Kuhn C., Brockhoff A., Chudoba E., Bufe B., Appendino G. and Behrens M., (2010) Chem Senses 35: 157-170
2. Wiener, A., Shudler, M., Levit, A. and Niv, M. Y. (2012) Nucleic Acids Res 40: D413-419
3. Bahia, M. S., Nissim, I. and Niv, M. Y., (2017) Int J Pharm, 2017.03.076
4. Keller, A. R.C. Gerkin, Y. Guan, A. Dhurandhar, G. Turu, B. Szalai, J.D. Mainland, Y. Ihara, C. Wen Yu, R. Wolfinger, C. Vens, L. Schietgat, K. De Grave, R. Norel, G. Stolovitzky, G.A. Cecchi, L.B. Vosshall and P. Meyr, (2017) Science 355: 820-826
5. Dagan-Wiener A., I. Nissim, N.B. Abu, G. Borgonovo, A. Bassoli and M.Y. Niv. (2017) Sci Rep 7: 12074
6. Nissim, I., Dagan-Wiener, A. and Niv, M. Y. (2017) IUBMB life 69: 938-946,
7. Dubovski, N., Ert, E. and Niv, M. Y. (2017) Food Quality and Preference 60. 154-164
8. Watson, D., Clark, L. A. and Tellegen, A. (1988) Journal of personality and social psychology 54: 1063-1070
9. Reed, D. R. and Knaapila, (2010) Prog Mol Biol Transl Sci 94: 213-240