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IDENTIFICATION OF THE BITTER AFTERTASTE OF SYNTHETIC SWEETENERS BY USED OF MULTIPOINT PHARMACOPHORE MODEL (MPM)

Summary

Safe and health-promoting foods are foods with appropriate sensory qualities, containing functional and nutritional ingredients, but also ones that are free of toxic and potentially toxic ingredients. Taste quality, including bitter taste, can be predicted using appropriate pharmacophore techniques. Pharmacophore modeling of bitter taste activity confirms which ingredients of food products, drugs and cosmetics are more safe for consumers. An innovative technique used to predict bitter taste was the so-called multipoint pharmacophore model (MPM). The aim of the study was to show the bitter taste and differences in its intensity for a group of synthetic sweeteners such as saccharin, acesulfame and cyclamate using a multipoint pharmacophore model (MPM). Numerous reports on the bitter aftertaste of many sweeteners do not specify clearly which sweeteners have the strongest and which the weakest bitter taste. The study showed that all of the analyzed synthetic sweeteners have a bitter taste. The ability of the group of examined sweeteners to activate bitter taste receptors was confirmed by the pharmacophore representations identified for them. The variable amount of these representations for saccharin, acesulfame and cyclamate indicates that these sweeteners have different intensities of bitter taste (IBT). Pharmacophore analysis showed that acesulfame had the strongest bitter aftertaste and cyclamate had the smallest. Based on the results obtained, it can also be assumed that the affinity of the tested sweeteners for the bitter taste receptors confirms their potential toxicity, which was indicated by the IBT value. In this approach, cyclamate should be the safest for the consumer from the examined group of sweeteners. The high IBT value for acesulfame indicates that it is potentially the most dangerous flavour additive from the studied group of synthetic sweeteners.
Keywords: *bitter aftertaste, multipoint pharmacophore model (MPM), saccharin, acesulfame, cyclamate, intensity of bitter taste (IBT), food safety*

IDENTYFIKACJA POSMAKU GORZKIEGO SYNTETYCZNYCH SŁODZIKÓW ZA POMOCĄ WIELOPUNKTOWEGO MODELU FARMAKOFOROWEGO (WMF)

Streszczenie

Żywność bezpieczna i o cechach prozdrowotnych, to żywność o odpowiednich walorach sensorycznych, zawierająca składniki funkcjonalne i odżywcze, ale także taka, która pozbawiona jest składników toksycznych i potencjalnie toksycznych. Jakość smakową, w tym również smak gorzki, można prognozować za pomocą odpowiednich technik farmakoforowych. Modelowanie farmakoforowe gorzkiej aktywności smakowej daje również odpowiedź jakie składniki produktów spożywczych, leków i kosmetyków są zdecydowanie bardziej bezpieczne dla konsumentów. Autorską techniką stosowaną do prognozowania smaku gorzkiego był tzw. wielopunktowy model farmakoforowy (WMF). Celem pracy było wykazanie gorzkiego smaku i różnic w jego intensywności dla grupy słodzików syntetycznych takich jak: sacharyna, acesulfam oraz cyklaminian za pomocą wielopunktowego modelu farmakoforowego (WMF). Liczne doniesienia nt. gorzkiego posmaku wielu słodzików nie precyzują jasno, które słodziki wykazują najsilniejszy, a które najsłabszy posmak goryczki. Przeprowadzone badania wykazały, że wszystkie z analizowanych słodzików syntetycznych wykazują smak gorzki. Zdolność grupy badanych słodzików do aktywacji receptorów smaku gorzkiego potwierdzają zidentyfikowane dla nich reprezentacje farmakoforowe. Zmienna ilość tych reprezentacji dla sacharyny, acesulfamu oraz cyklaminianu świadczy o tym, że słodziki te mają różną intensywność smaku gorzkiego (ISG). Analiza farmakoforowa wykazała, że najsilniejszy następczy smak gorzki ma acesulfam, a najmniejszy cyklaminian. Na podstawie uzyskanych wyników można również sądzić, że powinowactwo badanych słodzików do receptorów smaku gorzkiego potwierdza ich potencjalną toksyczność, której wymiernym wskaźnikiem jest prognozowana wartość ISG. W tym ujęciu najbezpieczniejszym dla konsumenta z badanej grupy słodzików powinien być cyklaminian. Duża wartość ISG dla acesulfamu wskazuje, że jest to potencjalnie najbardziej niebezpieczny dodatek smakowy z badanej grupy syntetycznych substancji słodzących.
Słowa kluczowe*: gorzki smak następczy, wielopunktowy model farmakoforowy (WMF), sacharyna, acesulfam, cyklaminian, intensywność smaku gorzkiego (ISG), bezpieczeństwo żywności*

1. Introduction

Increasing the quality of food by reducing its toxicity has always been a great technological challenge. The improvement of the composition of food products, feeds, medicines and supplements and cosmetics in terms of quality seems to be a priority for human health, agricultural production and all other animate and inanimate components of the natural environment. This research area covers issues related to bitter taste control as a qualitative differentiator for many food and pharmaceutical product ranges. Knowledge about controlling the quality of bitter taste be-

longs to the still poorly understood issues, which is mainly due to insufficient information on the quantitative and qualitative composition of the taste compositions of products. There are a number of methods of masking the bitter taste used in food, pharmaceuticals and cosmetics [1, 2, 3, 4], but in economic terms these are often time, work and material-absorbing methods [5]. Cheaper solutions are also imperfect, and its clear disadvantage are addition substances that disturb the taste compositions. As a result using taste additions, consumers are exposed to excessive consumption, among others sugars, inorganic salts, sweeteners and flavouring [6]. The primary role of bitter taste is to protect living organisms from potential poisons contained in food (these are preventive functions) [7]. At the other extreme, the importance of bitter taste are its health-promoting functions, responsible for providing the human body with small amounts of bioactive substances, including from the group of bioregulators, biostatics and biostimulators [8]. Acceptance of food with an admixture of bitter taste are very complex. However, we can say with a great approximation that she is dependent for the dose and taste activity of bitter compounds included in the food composition. The characteristics of food sensory profiles with bitter taste indicate the existence of three types of flavour mixtures with bitter taste [7]: major, moderate and minor (Fig. 1).

In addition to typical bitter compounds characterized by unchanging taste characteristics (only bitterness), there are also those that show a sweet taste initially and the bitter taste is disclosed as a second (so-called aftertaste) [9]. The secondary taste characteristics become more pronounced after a certain period of time (as a result of taste adaptation) giving food products with an intensely sweet taste a strong bitter aftertaste. The mechanism of the bitter aftertaste is not well understood. However, it is certain that some molecules, e.g. artificial sweeteners, activate the sweet taste receptors, i.e. the protein heterodimer TAS1R1-TAS1R3 [10, 11] and after a period of adaptation and taste stimulation following receptor contraction and removal of the bound molecules, they are resorbed by TAS2R receptor [11, 12], responsible for recognizing bitter taste stimuli [13]. The aim of the study was to demonstrate qualitative and quantitative interactions of a group of synthetic sweeteners such as saccharin (E-954), acesulfame (E-950) and cyclamate (E-952) with TAS2R receptors using a multipoint pharmacophore model (MPM).

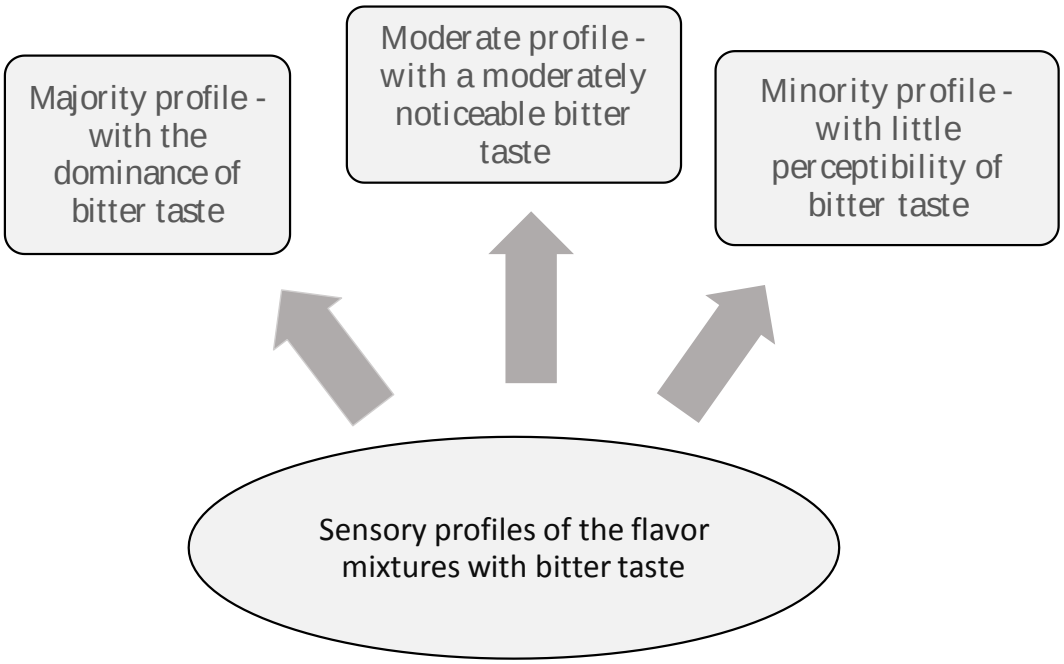
The sweeteners used for study are among the flavour additives used in different product groups. Synthetic sweeteners are most often used in food products, medicines and cosmetics, acting as replacements for traditionally used sweeteners from the group of carbohydrates [14].

2. Materials and methods

2.1. Methodology for identifying bitter taste stimuli using a multipoint pharmacophore model

Pharmacophore techniques are the tools used to prediction which product ingredients and with what intensity shape its bitter taste characteristics [15]. This tools include the original technique, referred to as the Multipoint Pharmacophore Model (MPM) [7]. This technique allows on the thorough analysis of the interaction of bitter ligands (e.g. flavour compounds contained in food) with potential molecular targets (e.g. relevant receptor protein structures) [7, 16, 17].

The multipoint pharmacophore model is a virtual sensor, obtained from superimposing dozens of molecular structures of bitter compounds (standards). The MPM model contains regionalized affinity features, such as subregions AH_1, AH_2, AH_3, B_1, B_2, B_3 and D (Fig. 2). The spatial distribution of individual subregions was illustrated on the basis of the average distances between of the areas of the model bitter compounds.



Source: own study / Źródło: opracowanie własne

Fig. 1. Types of sensory profiles found in food products with an admixture of bitter taste
Rys. 1. Rodzaje profili sensorycznych występujących w produktach spożywczych z domieszką smaku gorzkiego

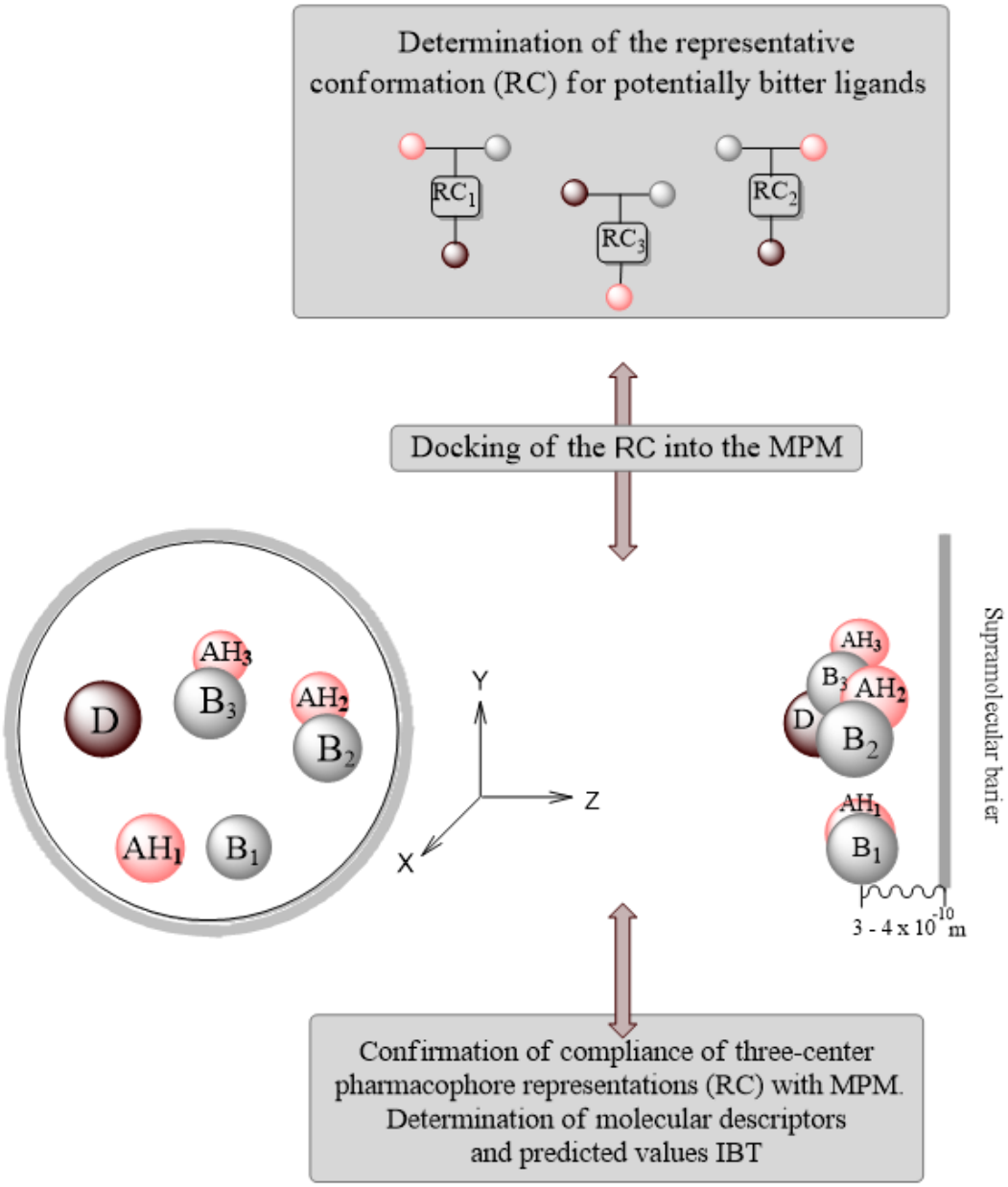


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Fig. 2. Illustration of bitter taste recognition of the potential ligands using the MPM model
Rys. 2. Rozpoznanie smaku gorzkiego potencjalnych ligandów za pomocą modelu MPM

Each of the MPM regions presented in Figure 2 has different electron characteristics to which corresponding pharmacophore functions are assigned. AH (electrophilic) regions include following pharmacophore functions: HBD-hydrogen bond donor and PI- positive ionization areas. Nucleophilic type B regions relate to pharmacophore functions such as hydrogen bond acceptors (HBA), negative ionization (NI) and represented by halogen atoms (HAL). The D (π-electron) subregion includes aromatic / hydrophobic (AR/H) areas.

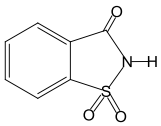
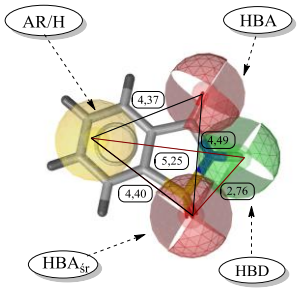
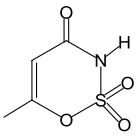
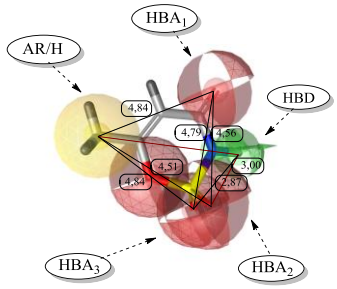
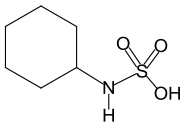
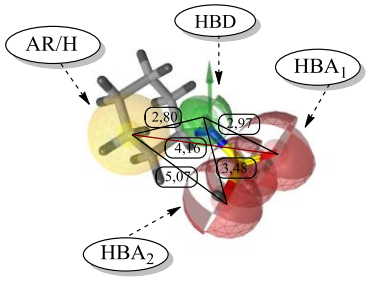
The taste identification procedure for potentially bitter molecules consists of three stages. In the first stage, the spatial structures of the studied molecules are subjected to quantum-mechanical optimization (by the method of potential density in the aqueous phase). In next step representative conformations (RC) are tested for compliance with MPM areas. All considered capable of activating RC receptor are used in descriptor analysis to determine the predicted intensity of bitter taste (IBT). The IBT value is the sum of the number of individual pharmacophore sites, so-called number of point interactions (NPI) and so-called the number of interactive stimulations (NIS), or all active (three-centre) pharmacophore representations [18].

3. Result and discussion

3.1. Study of the ability to activate bitter taste receptors by selected sweeteners

The structures of three popular sweeteners: saccharin, acesulfame and cyclamate, have been optimized by the Density Functional Theory (DFT) method with B3LYP functional and 6-31G (2d, p)) base [19]. The presence of water as a solvent was stimulated with the CPCM (Conducting Polarized Continuum Model) model [20, 21]. The obtained representative conformations (RC) of sweeteners structures were used for pharmacophore analysis, the results of which was presented in Table 1.

Table 1. Identification of pharmacophoric features and representations for studied ligands
Tab. 1. Identyfikacja funkcji i reprezentacji farmakoforowych dla badanych ligandów

Name of the ligand / No. / Structure	Location and distances between pharmacophore function (AR/H-yellow; HBA- red; HBD - green)	Pharmacophore representations identified by using docking of ligand inside the model MPM	Predicted values IBT_P (IBT_P= (∑NPI + ∑NIS) x k)
Saccharin /1 		HBA_{avg}-AR/H-HBA HBA-AR/H-HBA_{avg} HBA_{avg}-HBD-AR/H	**(4+3) x 10=70**
Acesulfame /2 		HBA_1- HBA_2- AR/H HBA_1- HBA_3- AR/H HBA_2- HBA_1- AR/H HBA_3- HBA_1- AR/H AR/H- HBA_2- HBD AR/H- HBA_3- HBD	**(5+6) x 10=110**
Cyclamate /3 		HBA_1- HBA_2- HBA_3 HBA_3- HBA_1- HBA_2	**(4+2) x 10=60**

Source: own study / Źródło: opracowanie własne

The pharmacophore analysis carried out proved that the tested sweeteners activate bitter taste receptors. The studied ligands are characterized by a variable number of pharmacophore functions and representations capable of activating receptor sites. Despite some structural similarities in the studied group of ligands, there are significant differences in their bitter taste activity. Acesulfame turned out to be the strongest sweetener in terms of IBT (IBT = 110). Other sweeteners show a much lower intensity of bitter taste. It takes IBT = 70 for saccharin and IBT = 60 for cyclamate, which can be considered as average intensities of taste stimulation.

4. Summary

The research procedure used proved that all of the analyzed synthetic sweeteners had a bitter taste. There are also significant differences in the intensity of the bitter taste in this group of sweeteners. Acesulfame has the strongest after bitter taste, which makes it the least useful and desirable (from the sweeteners tested) in flavour compositions of food products, cosmetics and medicines. It can also be assumed that the affinity of the tested sweeteners for bitter taste receptors confirms their potential toxicity, whose measurable indicator is the value of the intensity of bitter IBT taste. In this approach, cyclamates should be the safest for the consumer from the examined group of sweeteners, and acesulfame will be the most harmful. Research clearly confirms the usefulness of the multipoint pharmacophore model (MPM) as an innovative tool for assessing he bitter taste quality of food additives, drugs and cosmetics.

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