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REVIEW ARTICLE

Miraculin-based sweeteners in the protein-engineering era: an alternative for developing more efficient and safer products

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ABSTRACT

The current sweeteners available are very efficient in providing sweet taste. However, they are associated with several chronic diseases. Some glycoproteins, such as miraculins, are extremely interesting from a biotechnological point of view because they perform the bitter into sweet taste modifying function excellently, in addition to being safer as food. In contrast, purifying and synthesizing these proteins represents a major challenge for the food industry, as these proteins are large and complex molecules, which would make the final product expensive and economically unviable. In this context, emerging techniques from computational biology and molecular modelling have been promoting a remarkable revolution in protein bioengineering. Bioinspired peptides can provide many possibilities in sweeteners development through rational design. Once these peptides are smaller molecules than an entire protein, its synthesis on a large scale tends to be much easier and more economical, besides presenting a potential for better bioavailability in the organism. The techniques discussed here allow, through sophisticated pipelines and algorithms, to perform the rational design of mimetic peptides and with smaller size, which can carry out the activation of sweet taste of miraculins and to be more viable for industrial production. In this review, the premises and tools for the elaboration of synthetic peptides bioinspired in proteins with sweetening activity that mimic this action will be emphasized.

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Introduction

Bioactive molecules as sweeteners

The main bioactives are chemical compounds present in various foods and natural products that can perform important biological functions in the human body. Among these molecules, sweeteners are a relevant group, as they have been widely used as sugar substitutes in foods and beverages, seeking to meet the needs of individuals who aim to reduce caloric intake or avoid sugar consumption for health reasons. One of the widely studied and used sweeteners is aspartame, a dipeptide composed of phenylalanine, aspartic acid and methanol. Aspartame, for example, has significantly greater sweetening power than regular sugar and is found in a variety of commercial products, including diet drinks, confectionery and low-calorie foods.

Bioactive molecules, especially those with sweetening properties, have garnered significant attention in the field of food science and nutrition due to their potential to provide healthier alternatives to traditional sugar-based sweeteners. As concerns over the adverse health effects of excessive sugar consumption have grown, researchers have turned to natural compounds that possess sweet tastes without the caloric burden or metabolic drawbacks associated with sugars. Recent articles in the scientific literature highlight the exploration and utilization of various bioactive molecules sourced from plants, fruits, and other natural sources as potential sweeteners.

In a study by the authors investigated the sweetening potential of steviol glycosides extracted from the leaves of *Stevia rebaudiana* (Smith et al., 2022) The research demonstrated that steviol glycosides not only possess intense sweetening properties but also exhibit a minimal impact on blood glucose levels, positioning them as promising candidates for addressing concerns related to diabetes and obesity. Similarly, an study explored the sweetening capabilities of mogrosides extracted from Siraitia grosvenorii, also known as monk fruit (Chen et al., 2021). The researchers revealed that mogrosides not only offer a natural sweetness but also present antioxidant properties, further contributing to their appeal as a potential sugar substitute.

Continuing this trend, a work delved into the sweetening effects of thaumatin, a protein derived from the katemfe fruit (*Thaumatococcus daniellii*) (Garcia et al., 2023). The researchers demonstrated that thaumatin elicits a sweet taste sensation, often described as clean and well-rounded, making it a desirable alternative for enhancing the palatability of various food and beverage products. Collectively, these recent studies underscore the ongoing exploration of bioactive molecules as sweeteners, offering a glimpse into the potential of

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these compounds to reshape the landscape of sugar alternatives in the quest for healthier dietary options.

Miraculins (Mir) are glycoproteins from plant origin, primarily isolated from the miracle fruit, scientific name *Richardella dulcifica,* a shrub native to West Africa. Its ability to modify flavour was associated with a miracle, because it is responsible for the conversion of sour and bitter taste into sweet, when miraculins meets the taste buds (Garcia et al., 2023; Misaka, 2013).

The search for natural compounds that have sweetening properties, such as miraculin, is of great interest to society in view to the relevant growth of chronic increasing diseases such as diabetes, and the high consumption of simple sugars is related to the increase in the disposition of metabolic syndrome (Ronchillo & Joivin, 2019). There is a great demand in the industry for better alternatives focused on the sweeteners production, or flavour modifying compounds, since the synthetic sweeteners available in the current market, are linked with the development of several types of cancers and degenerative diseases (Castiglia et al., 2018; Han et al., 2019).

Therefore, miraculin proteins have great potential for biotechnological applications in the food industry as sweeteners or flavour enhancers in various products, which can be a good associate with dietary prescriptions, in view of low-calorie concentrations and minimal glycaemic load (Garcia et al., 2023). However, miraculin production is scarce and its purification is limited.

Therefore, to aim biotechnological applications for miraculins is necessary to design analogues (mimetic) peptides, capable to interact with the active site from human sweet taste receptor. In this review, potential applying of miraculin as sweeteners using protein-engineering techniques is highlighted.

Miraculin protein

Some plants have ability to produce sweet-tasting compounds, such as glycoproteins. Among them, stands out Miraculin (*Synsepalum dulcificum*), belonging to the *Sapotaceae* family. The plant was recognized in 1968 in West Africa because of its red berry fruits (Campos & Gonzáles, 2015; Ohkubo et al., 2015).

The fruit has been studied since the 1980s by several countries in the world and has different names or terms such as Magic Berry, Miracle fruit, Miracle Berry, Miracle Fruit, Miraculous Berry, Sweet Berry and Miraculous Berry (Ronchillo & Joivin, 2019).

Miraculins are characterized as glycoproteins from plant origin or N-glycoproteins, belonging to the family of Kunitz trypsin-inhibiting proteins. The native miraculins has a molecular weight of 42,000 Da, with a polypeptide chain structure containing about 191 residues, which have a flavor-modifying function when they are in homodimeric format, each monomer has N-glycosylated polypeptide chains, which are interconnected by a disulfide bridge in asparagine residues (Asn42 and Asn186) and by interchain interactions of cysteine residues, mainly CYS138 (Figure 1), which is directly linked to the other subunit. It is also known that the

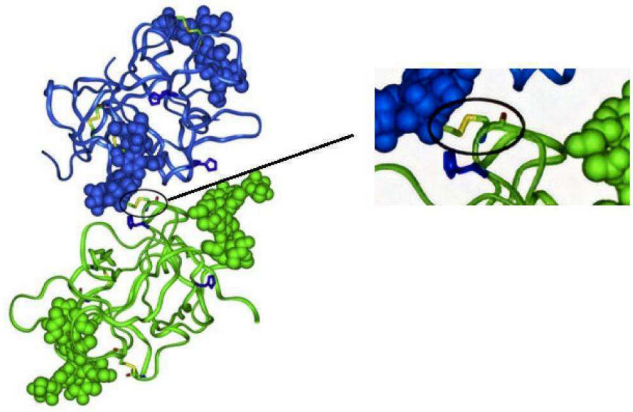

Figure 1. Miraculin homodimeric structure linked by a disulfide bridge. Source: Adapted from Paladino et al. (2008).

dimerization and prevalence of histidine residues (His30 and His60) are crucial for protein functionality (Ohkura et al., 2018; Theerasilp & Kurihara, 1988).

In the presence of acidic substance, miraculin can interact with the palatability receptor cells located on the tongue, causing a chemical reaction, synapsing with the brain, and causing a sensation of flavour modification, resulting in a sensation of strong sweet taste, masking acidic flavour and completely bitter flavour. Even after contact with acidic substances, its effect on sweet taste buds can last for about 1 to 2 h (Igeta et al., 1991).

The mechanisms of interaction with the receptor (T1R2/T1R3), is not yet fully known, studies show that miraculin behaves as an agonist at acidic pH and inactive at neutral pH, binding antagonistically to the receptor under these conditions. Over the years, proteins homologous to miraculin has been discovered, isolated in several plants belonging to the family Rutacea, Solanaceae, Rubiaceae. Based on sequence and similarity, these proteins are called miraculin-like proteins (MLPs), when they exhibit 30 to 95% identity. Due to the amino acid sequence, MLPs are classified within the family of soybean trypsin inhibitors of the Kunitz type (IST), exhibiting approximately 30% identity with IST (Cevallos-Muñoz et al., 2007; Ito et al., 2007).

The orange tree species (*Citrus sinensis*) is included in the *Citrus* genus, and in the biological family Rutacea. Among the genes found naturally in this plant, there are miraculin type genes, which were related to the potential bioinsecticide capacity (Sanematsu et al., 2014; Takai et al., 2013).

The sweet taste in human food

The preference for sweet flavour has an innate and historical origin, specialized taste cells begin to be developed in the intrauterine phase (approximately at 7–8 wk), they reach maturity at around 13th weeks and already begin to transmit palatability information to the brain, the stimuli continue in breastfeeding and lasting for the rest of life (Mondego et al., 2011; Sun et al., 2007).

The sweet taste receptor, located in taste buds, is formed by two types of receptors: T1R2 and T1R3, coupled to G protein (GPCRs), and they are responsible for sending sweetness

signals to the central nervous system. Five basic tastes are identified: bitter, sour, sweet, umami and salty. Historically, two tastes have shown greater relevance to the evolution of species, bitter and sweet (Bachchu et al., 2011; Silva & Teles, 2013).

Sweet taste was important for survival, as the availability of substances rich in carbohydrates is associated to good sources of energy, and consequently energy storage, in addition to familiarity, as well as greater exposure throughout life, makes it preferable for the sweet taste. The bitter taste was associated with foods with toxic components, existing in the natural environment (Cul & Col, 2006; Ventura & Mennella, 2011).

One of the first strategies to sweeten food was the use of honey, used since ancient Greece and China, but with the development of the sugar industry, honey was replaced by common sugar, sucrose. However, the overuse of sucrose has relation to the increase of chronic diseases, such as obesity and diabetes. Which led society to seek new products and alternatives to replace sugar. Currently, the industry offers several substances that can confer or intensify the sweet taste in foods; they are called sweeteners (Moreira, 2005; Ohtsu & Col, 2014).

Sweeteners are basically divided into two major groups: nutritive sweeteners (NS) and non-nutritive sweeteners (NNS), NS add caloric value to foods, within this group are sucrose, lactose, fructose, and polyols, whereas NNS are sweet flavour components, which offer insignificant amounts or no calories in food, such as: cyclamate, saccharin, aspartame, stevia and others. NNS, like dietary sweeteners, are food additive, which can totally or partially replace sugar in a range of products, and can be of high intensity, which are those that exert up to a hundred times greater capacity to sweeten than sucrose and low intensity ones. They are generally used in combination with another type of sugar or compound (Chattopadhyay et al., 2014; Mahar & Duizer, 2007).

Sweeteners can also be classified according to their origin into: (i) natural, composed of organic substances found in nature that cause sweetness, and (ii) artificial, which are synthetic substances, prepared in the laboratory, which are also responsible for to give sweet taste to foods (Weihrauch & Diehl, 2004).

The synthetic sweeteners most used by the industry are sucralose, saccharin, aspartame, cyclamate and acesulfame. However, due to side effects of long-term consumption, linked to psychological problems, brain tumours, the development of glucose intolerance, papillae sensation loss, heart failure, and severe cancer has led to a great concern of use in consumers and health professionals. That is why natural compounds or derivatives are of great interest for industrial applications in the sweeteners manufacture, such as for example miraculin, which has a natural sweet feeling property (Misaka, 2013; Ronchillo & Joivin, 2019; Yang, 2010).

Biotechnological applications of miraculin

Due to the high sugar consumption and its probable contribution to the appearance of chronic non-communicable diseases, such as diabetes, cancer, coronary heart disease, and even other comorbidities such as obesity; the search for other alternatives to sweeten food has increased. The demand for sweeteners from natural sources, such as miraculous ones only tend to grow, what led the biotechnological community to develop products, or dietary sweeteners with miraculin, due to the high intensity, persistence, and prevalence of sweetness (Igeta et al., 1991; Shibao et al., 2009).

In this sense, biotechnological use of miraculins can be diverse, covering different areas of application, but mainly linked in the food and pharmaceutical industry areas. Regarding food and flavour modifying capacity, miraculins had been used since the nineteenth century, to improve the palatability of acidic foods and sweeten beverages, by the native West African Indians (He et al., 2015; Mooradian, 2019).

The miraculous fruit application in commercial acidic drinks, which contained in its composition great amount of citric acid, had better effects on the perception of sweet taste, compared to other types of acid, such as acetic acid, demonstrated in the study (Gnanavel & Peddha, 2011).

The application of the miracle fruit was performed in acidic drinks based on lemon, in relation to sensory characteristics, the results were like sucralose, which is one of the most used sweeteners in the population. It provided a promising alternative as a substitute for sugar for a natural component. What connoted in an indication for the potential use as a flavour enhancer in *Citrus*-based drinks, or other products like soft drinks, where this type of acid is usually added for the balance of flavour, odour, and quality maintenance (He et al., 2015).

The pulp and seed of the miraculous fruits were introduced in the elaboration of a functional yogurt, the flavour modifying activity makes it possible to introduce sweet characteristic foods, as well as savoury foods, such as cheeses, as well as in milk derivatives, acting as a flavour moderator (Akinmoladun, 2016; Igarashi et al., 2013; Muller et al., 2018). Furthermore, a research (Freitas & Araújo, 2010) describes alternatives for replacing sugar with natural compounds including miraculin, influencing the manufacturing potential for use as a sweetener, considering that the available synthetic sweeteners indicate having undesirable clinical effects. The miraculins property as an alternative sweetener and sugar substitute is commonly recognized and can be applied mainly to people affected by diabetes (Garcia et al., 2023).

Once diabetes is a chronic disease, a change in eating habits is necessary such as decreased consumption of carbohydrates and restriction to the use of refined sugar directed the individual to the use of sweeteners. The use of miraculin would be of great value, since its effect is not linked to sweet molecules, but rather to the sensation of the sweet taste, when the active compound, miraculin binds with sweet taste receptors (Smith et al., 2022; Igarashi et al., 2013).

The safety of new proteins use was evaluated, where it was demonstrated by means of proteomic analysis, and by *in vitro* digestibility assay, that miraculin is quickly and completely digested by pepsin, which indicates that these proteins may not be absorbed, reducing the risk of toxicity, and

the release of insulin by the pancreas (Fazilah et al., 2019). In addition, they also analysed *in silico* the allergenic or toxigenic effect of Mir, showing that it did not represent such risks. Miraculin can also be used in the pharmaceutical industry to increase the palatability of medicines, such as syrups (Chattopadhyay et al., 2014).

Industrial production limitations

The production of miraculin by means of the native plant on a large scale is limited, considering that large plantations would be necessary to obtain small amounts of purified miraculin, in addition to which, for the protein displays a potential flavour-modifying effect, it must present structural conformation suitable to bind to the sweet taste receptors, T1R2 and T1R3. In addition, to being a high molecular weight glycoprotein, its production in the original format would have great costs (Cevallos-Muñoz et al., 2007; Holloway et al., 1996; Shibao et al., 2009).

Regarding cultivation, the *Synsepalum dulcificum* plant has a prolonged youthful period and slow growth rate, which hinders industrial production. Furthermore, it is an endangered species, seen as likely recalcitrant species, that is, the seeds drastically decrease viability when air-dried, little is known about the storage and conservation of the germinative activity of the seeds, which hinders conservation, storage and planting (Agblekpe et al., 2016; Holloway et al., 1996).

Miraculins extracting and purifying methods from *Synsepalum dulcificum* were analysed (Shibao et al., 2009). For the extraction of total protein, some buffers were compared (phosphate buffer, saline, NaCl and tris-HCL), and for the protein purification, used immobilized metal ion affinity chromatography (IMAC) with nickel-NTA, and demonstrated that this method of purification in a single step process is satisfactory, however the total volume of purified miraculin was still unsatisfactory.

What becomes an impasse for industrial production, in addition to the miraculous fruit, outside the native environment is difficult to cultivate. In this way, some studies have moved towards the heterologous production of proteins like native miraculin, both artificially inserting the gene in organisms such as bacteria, yeasts, however the recombinant Mir expressed through these transgenic organisms has not shown satisfactory results in inducing the modifying activity of flavour (Agblekpe et al., 2016; Holloway et al., 1996). The expression is also found in plants with easier cultivation, such as tomatoes (*Solanum lycopersicum*), lettuce (*Lactuca sativa*) and grapes (*Vitis vinifera*), to express and obtain greater amounts of purified miraculin (Leal et al., 2019). A study (Ohkura et al., 2018) was developed of heterologous production of miraculin in grapes (*Vitis vinifera*), where it presented a homology of 61%, suggesting that this recombinant miraculin could have flavour-modifying activity, he also evaluated and confirmed that protein produced managed to demonstrate inhibitory activity against trypsin.

However, its production by heterologous expression is still economically expensive and needs to be in the proper conformation as a homodimer to trigger the flavour modification, which would be an obstacle for mass production. In addition, the original miraculin molecule has a molecular weight around 90 KDa, which makes production in the laboratory more difficult and more expensive (Agblekpe et al., 2016; Ohkura et al., 2018). Therefore, the production of functional peptides analogous to miraculin would be of great use for biotechnological production.

Computational tests for selection and rational design of industrial molecules

The advancement and improvement in the computers data processing capacity, enabled the bioinformatics development, where the use of computational tools is focused on the study of biological systems, and possible applications for problems related to human health, such as molecule design for drugs or bioactive substances (Tafazoli et al., 2019).

The study of bioinformatics aimed at rational design, can be applied both in the discovery of potential substances, for the preparation of drugs, but also, it can be used to maximize the uniformity and functionality of existing substances, both obtained by the rational design itself, as well as also of substances found naturally. In this way, the methods of development of bioactive compounds have moved from the laboratory to computational studies with greater intensity, because the activity models generated have been showing efficiency and fidelity in the prediction of biological target molecules (Matsuyama et al., 2009; Tchokponhoué et al., 2019).

By providing rationality, the techniques used can previously rank the essential components and delete the inactive ones. In addition to that, the computational techniques can explore faster many sequences, simplifying the process of identification and selection of molecules, connoting improvement in efficiency and research performance, reducing time and cost with experimental methods. After demonstrating high selectivity and affinity with the molecule of interest, studies should go in partnership with experimental (*in vitro* and *in vivo*) techniques (Ezura & Hiwasa-Tanase, 2016; Matsuyama et al., 2009; Tchokponhoué et al., 2017).

Rational peptide design

For the rational design of peptides, there are four types of methodological procedures most used, which are the (i) methods based on a template sequence, (ii) physicochemical methods, (iii) evolutionary methods or (iv) *de novo* methods (Muttenthaler et al., 2021).

Methods based on a template sequence is usually chosen when there are studies on a known sequence, which presents considerable activity, but which still needs to be improved, and can be in the perspective of size reduction, increased selectivity, greater stability, reduction, or inhibition cytotoxic effects. The construction takes place through the identification of the key amino acids (aa), replacement of aa residues and deletion of aa that has no crucial activity for the stability and action of the molecule (Pearce et al., 2019). In an interesting study (Zhang & Skolnick, 2004), it was

designed a peptide rich in alanine (Pa-MAP2), based on a peptide synthetically produced and obtained from fish *P. americana*, the substitutions of aa led to greater specificity and less toxicity for mammals.

The physical-chemical methods are based on the mechanism of action, this method is generally chosen for peptides that have α-helix conformation, as this method easily analyses a spectrum of activities and properties, such as total charge, hydrophobicity, generally its information is coupled to the method based on model sequence or other rational design methods (Muttenthaler et al., 2021).

The two methods that need a high computational power are the evolutionary methods and de novo methods. For the design through evolutionary forms, it is necessary the aid of genetic algorithms, which analyse a group of sequences, deletion of aa in the evolution and similarity. The methods again, use aa patterns or frequency, and positioning preferences, to generate a variety of analogous sequences (Arodola & Soliman, 2017; Muttenthaler et al., 2021).

EvoDesign is an online server (Bazzoli et al., 2011; Menetti, 2006), which uses evolution-based profiles to direct the search simulation sequence; it searches for complete atomic models of a PDB scaffold, produces a list of projected sequences and indicates the identity percentage of the sequences generated based on the scaffold structure.

Scaffold is the structure of the specific chain of a complex, which serves to increase affinity, fixation with another complex or binding partner and improve stability, since most protein functions are mediated by protein-protein interactions. The scaffold is part of the interface design protocol, the protocol can be applied for several purposes, one of the applications is the design of bioactive proteins, which can be redesigned to increase the affinity to a fixed receptor, this methodology showed satisfactory results to the redesign of various protein systems. This indicates a great potential for the synthesis of new therapeutic molecules, enzymes and other active compounds (Fjell et al., 2011).

For the user to perceive the quality of the generated sequences, the server also provides information on solvent accessibility, torsion angle of the backbone and possible relative errors normalized for the projected secondary structure. In addition to the server being based on evolutionary profiles, the method shows an advantage for coupling knowledge based on physics. After the structured profiles are produced from the protein fold and protein interfaces, the physical-chemical chain and the main chain are accommodated through a force field. The final sequences produced are organized through a grouping by an algorithm of all sequence decoys designed during the Monte Carlo (MC) simulation of replica exchange (Arodola & Soliman, 2017; Fjell et al., 2011).

EvoDesign server features two design options, "Monomer Design", which uses monomeric proteins, and features "Interface Design", which can work with more complex, protein-protein interaction structures. Both options are worked in three stages, the pre-processing, simulation, and analysis of the data produced during the simulation, which will result in the final projection of the sequences (Figure 2).

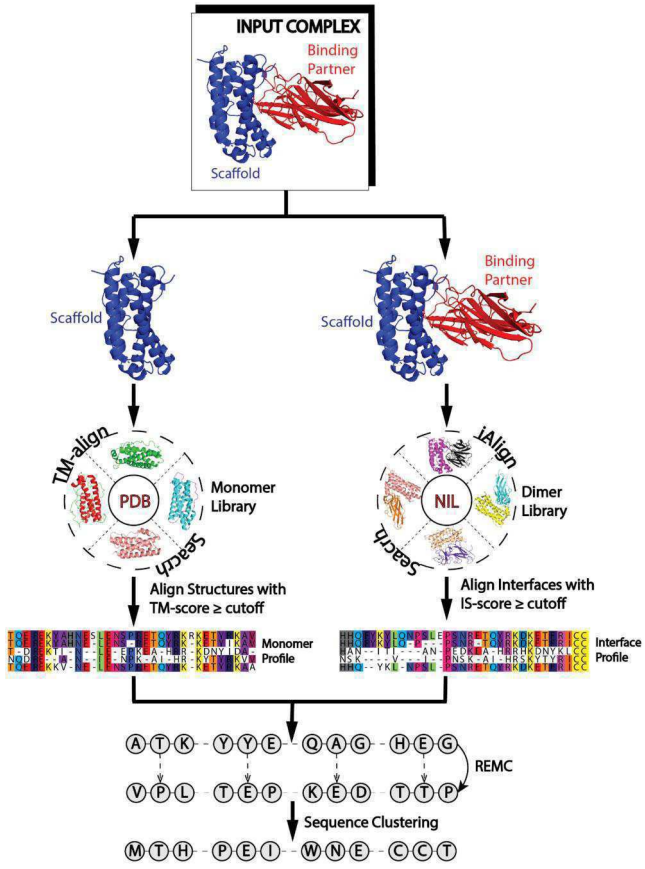

Figure 2. Overview of EvoDesing server strategies. Source: Pearce et al. (2019).

The high-scale design, with foldable molecules, indicates that the combination of evolutionary profiles with the knowledge of physical energy, is more satisfactory than the protocols that are based only on physics (Costa, 2018; Holloway et al., 1996).

Molecular modelling

Due to limitations of experimental techniques, computational tools have been increasingly developed, with constant improvement of the base algorithms used, using methodologies based on physical equations and theories and computer skills. According to this perspective studies of molecular modeling, molecular dynamics and molecular docking are crucial to a better understanding of molecules functions and applications. Molecular modelling, for example, analyses and predicts the three-dimensional (3D) structure of a protein by simulations, enabling important information about the location of aa, through the development of computational codes (Muttenthaler et al., 2021; Porto et al., 2012).

Computational modelling methods have been a good alternative for the study of protein structures, as they are cheaper than the approaches of X-ray crystallography and NMR (Magnetic Resonance) spectroscopy; in addition to that, in computational models it is possible to predict results experimental models. Modelling can be done using methods that do not depend on a template free template structure – prediction *ab initio* and again, and those that do are template based – comparative modelling and threading.

The *ab initio* (template free) methods are based on the knowledge of thermodynamics, which seeks to find the minimum global free energy, corresponding to the energies of native protein structures. As for methods based on comparative modelling, or model based on homology (template based), it is based on the use of a sequence of template amino acids, which is aligned with the aa sequence that one aims to project, it is one of the most used methods to study the structure of proteins (Ahn et al., 2006; Migliolo et al., 2016; Mitra et al., 2013).

Threading modelling is also a methodology (template based), where a sequence of aa considered as a template is crossed with the data from a topology bank, resulting in a new structure. If there is an evolutionary relationship, it is determined that the modelling is by homology, if there are no structural characteristics of the evolutionary relationship, the modelling should be by threading (Ahn et al., 2006; Mitra et al., 2013).

Molecular docking

The term docking is used to check a set of computational techniques that try to determine, through atomic coordinates, the best association or fit between two structures, being normally applied to a receptor and a ligand (Dorn et al., 2014; Pearce et al., 2019).

In summary, docking perceives the connection mode by the orientation and conformation of a small molecule that is the ligand, with the connection site of a large molecule that is the receptor, allowing the coupling and formation of a stable complex (Dorn, 2008; Halperin et al., 2002; Pearce et al., 2019).

The connection affinity of the complex can be measured using punctuation functions; the layout of the molecular docking is shown in. The ability to complex a molecule with another target structure depends on the ability to interact positively with a specific binding site, thus, the structures that share these positive interactions, are probably capable of exerting active biological effects (Santoyo et al., 2013).

What makes protein-ligand or protein-protein binding stable are intermolecular or non-covalent interactions as interactions of hydrogen bonds, electrostatic, hydrophobic, and other interactions. A range of possibilities for available docking software is available, the most used ones are AutoDock, DOCK, LUDI, Glide, FlexX, GOLD and AutoDock Vina (Dorn, 2008; Grinter & Zou, 2014).

An important factor in choosing the possibilities is the protein-mooring model. The approaches related to rigidity are divided into three, flexible docking, semi-flexible docking, and rigid docking. Flexible docking is when the receptor and the ligand are adaptable and flexible bodies, analysed by the degrees of freedom of both molecules. Semi-flexible docking is when one element of the complex is considered rigid, and the other is not. The algorithms generally consider that the molecular surface of the ligand is flexible, where all degrees of freedom of the ligand, rotational, translational, and conformational are considered, due to being a protein sequence with smaller dimensions, with greater probabilities of changing the shape, this model is applied only in protein-ligand docking, mainly in the design and development of drugs (Muttenthaler et al., 2021).

In rigid docking, the molecular surfaces of both structures are considered as rigid in their entirety; only the rotational and translational degrees of freedom of the ligand are considered, being fixed in a rigid structure. It is understood that, even if the surfaces are rigid, one of the proteins in the complex will penetrate the other (Hung & Chen, 2014).

There are other theories and variation of the rigid docking, called soft flexible docking, where variations in the surface of both structures (receptor/ligand) are allowed, provided they are between a surface atom and a central atom or both surface atoms (Muttenthaler et al., 2021).

Ab initio methods

The *ab initio* methods are very precise and consistent because they are semi-empirical, they are based only on the laws of quantum mechanics, electron masses and charges, atomic nuclei, and fundamental physics constants, speed of light and constant of Plank, used in computational chemistry. From Latin, the expression means “from the beginning”. The methods are based on two principles: the first is based on the theory that the information of the amino acid sequence is responsible for producing the three-dimensional structure of the protein, occurring an envelope of structures to a native state, which is, found with the minimum of global energy (Sliwoski et al., 2014; Yunta, 2016).

These methods are intended to solve the Schrödinger equations for the system, resulting in energy and wave function data. However, this equation is not well applied to molecules with more than one electron, so, for simulations with larger systems, it is considered a harmonic approximation system to facilitate calculations (Muttenthaler et al., 2021; Yunta, 2016).

With the development of methods that use approximations, such as those based on electronic density, Density Functional Theory (DFT), Local Density Approximation (LDA) and Generalized Gradient Approximation (GGA), associated with the improvement of computational power in recent times, the analysis of complex biological systems has been showing satisfactory results, with a high processing rate when performing the calculations, and low computational cost (Matta, 2010; Vakser, 2014).

Molecular fractionation with conjugated caps (MFCC)

To enhance the studies of biological systems with many atoms, the Molecular Fractionation with Conjugated Caps (MFCC) method was developed, which provides results at the ab initio level of interaction energies between a protein and another structure. Its results are based on the division of the three-dimensional molecular structure, in the groups of amino acids, thus demonstrating, as a result, the energy generated between the protein and the ligand through the combinations between the small fragments or groups analysed (Ewars, 2011; Matta, 2010).

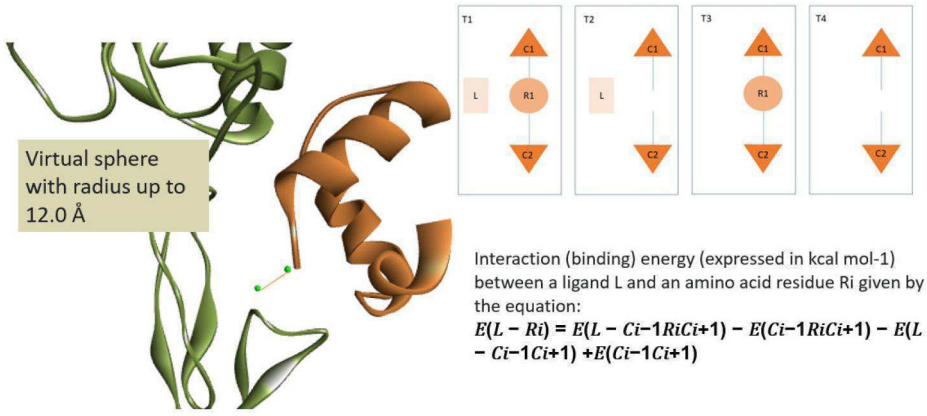

Figure 3. Schematic representation of the MFCC method.

This technique is based on the thermochemical theory. The energetic description of a given microsystem is calculated from Schrödinger's Hamiltonian equation, which consists in the fragmentation of a three-dimensional structure (macromolecules) into smaller individual fragments. Based on amino acids that presented a close interaction, this strategy has the purpose of conserving the orbital properties of the analysed residues. The neighbouring amino acids (caps) and their respective peptide bonds serves to simulate and not break the valences that would naturally occur in the complete system or protein (Gordon et al., 2012; Vakser, 2014).

The calculation of the interaction energy E (L - Ri) is expressed in kcal mol-1, between a ligand L and an amino acid residue Ri of the protein, being defined by the equation:

$$E(L-Ri) = E(L-Ci-1RiCi+1) - E(Ci-1RiCi+1) - E(L-Ci+1) + E(Ci-1Ci+1)$$

When ligand L is aa residue ($L = Rj$) from another protein, it binds to aa from the receptor, the equation can be given, as follows:

$$\begin{aligned} E(Rj-Ri) \quad &= E(Cj-1RjCj+1-Ci-1RiCi+1) \\ &-E(CJ-1Cj+1-Ci-1RiCi+1) \\ &-E(Cj-1RjCj+1-Ci-1Ci+1) \\ &+E(Cj-1Cj+1-Ci+1) \end{aligned}$$

where E ($Cj - 1RjCj + 1$ - $Ci - 1RiCi + 1$) corresponds to the total energy of the system produced by the two residues (ligand and receptor), that interact mutually together with the hoods; the term E ($Cj - 1Cj + 1$ - $Ci - 1RiCi + 1$) is equivalent to the energy of the residue Ri with its conjugated hoods $Ci - 1Ci + 1$ and $Cj - 1Cj + 1$ of Rj. Corresponding to E ($Cj - 1RjCj + 1$ - $Ci - 1Ci + 1$) there is the energy system formed by Rj and other hoods. The fourth term E ($Cj - 1Cj + 1$ - $Ci - 1Ci + 1$) suggests the energy of the fragment formed only by the de and Rj hoods (Mota, 2016). The simplified layout of the MFCC method is shown in Figure 3:

The term (T1) da refers to the total energy of the system, formed by the residue of the ligand and residue of the receiver with its caps. In the second term (T2) the total energy of the waste of the receiver with its caps is calculated, in the third term (T3) the total energy of the residue of the ligand with the caps is denoted, and finally, the fourth term (T4) corresponds to only caps energy.

Final considerations

Glycoproteins such as miraculins are complex biomolecules that perform essential biological functions. The size of miraculins is approximately 190 amino acids, which hinders their industrial production. This opens a gap for studies that focus on the rational design of mimetic peptides, that is, smaller peptides that simulate the action of these proteins.

The use of computational techniques for protein engineering allowed incredible advances in almost all related areas. In the specific case of miraculins as sweeteners, protein engineering is an incredible opportunity to generate useful products, with commercial demand, with benefit and safe for human consumption. In the future, where sweeteners possibly will be produced and consumed in the order of consumption of certain commodities, it is extremely interesting that they use emerging techniques to develop better products. The computational design of proteins represents, in this sense, an innovative, fast, safe, and incredible potential technique to be applied in the food field. More research is needed for this new field of knowledge.

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References

Agblekpe, A. K., Osseyi, E. J., & Dossou, J. (2016). Potential nutritional values of skin, pulp and seed of miracle fruit (*Synsepalum dulcifium*). *Applied Scientific Research*, *4*, 1–7.

Ahn, H. S., Cho, W., Kang, S. H., Ko, S. S., Park, M. S., Cho, H., & Lee, K. H. (2006). Design and synthesis of novel antimicrobial peptides on the basis of alpha helical domain of Tenecin 1, an insect defensin protein, and structure-activity relationship study. *Peptides*, *27*(4), 640–648. https://doi.org/10.1016/j.peptides.2005.08.016

Akinmoladun, A. C. (2016). Effect of *Synsepalum dulcificum* berry extract on oxidative stress and hepatotoxicity indexes, after subacute administration in normal rats. *Futa Journal of Research in Sciences*, *12*, 167–177.

Arodola, O. A., & Soliman, M. E. (2017). Quantum mechanics implementation in drug-design workflows: Does it really help? *Drug Design, Development and Therapy*, *11*, 2551–2564. https://doi.org/10.2147/DDDT.S126344

Bachchu, M. A. A., Jin, S. B., Park, J. W., Boo, K. H., Jin-Sun, H., Kim, Y. W., Lee, H. Y., Riu, K. Z., & Kim, J. H. (2011). Functional expression. Of miraculina, a taste-modifying protein, in transgenic Miyagawa wase satsuma mandarin (*Citrus unshiu* Marc.). *Journal of the Korean Society for Applied Biological Chemistry*, *54*(1), 24–29. https://doi.org/10.3839/jksabc.2011.003

Bazzoli, A., Tettamanzi, A. G. B., & Zhang, Y. (2011). Computational protein design and large-scale evaluation by structure assembly simulations I-TASSER. *Journal of Molecular Biology*, *407*(5), 764–776. https://doi.org/10.1016/j.jmb.2011.02.017

Campos, G. M., & Gonzáles, M. A. S. J. (2015). Use of sugars and sweeteners in children's diets. Recommendations of the Nutrition Committee of the Spanish Association of Pediatrics. Pedriatría. *Espanha*, *83*, 353.

Castiglia, D., Leone, S., Tamburino, R., Sannino, L., Fonderico, J., Melchiorre, C., Carpentieri, A., Grillo, S., Picone, D., & Scotti, N. (2018). High-level production of single chain monellin mutants with enhanced sweetness and stability in tobacco chloroplasts. *Planta*, *248*(2), 465–476. https://doi.org/10.1007/s00425-018-2920-z

Cevallos-Muñoz, L. A., Andrade-Mena, S. R., Singh, B. K., & Arce-Portuguéz, J. A. (2007). Estudio de la fruta milagrosa (*Synsepalum dulcificum Daniell*) como posible edulcorante natural. Development of a natural sweetener/acid blocker from miracle fruit (*Synsepalum dulcificum Daniell*). *Tierra Tropical: Sostenibilidad, Ambiente y Sociedad*, *3*, 71–80.

Chattopadhyay, S., Raychaudhuri, U., & Chakraborty, R. (2014). Artificial sweeteners – A review. *Journal of Food Science and Technology*, *51*(4), 611–621. https://doi.org/10.1007/s13197-011-0571-1

Chen, L., Wu, X., & Shi, Y. (2021). Mogrosides: A review of their biosynthesis, extraction, and applications. *Journal of Agricultural and Food Chemistry*, *69*(12), 3517–3526.

Costa, M. F. D. (2018). *Docking, molecular dynamics and artificial neural networks in the search for anti-RSV flavonoids* [Doctoral thesis]. Microbiology, Institute of Biosciences, Letters and Exact Sciences.

Cul, M., & Col, J. M. (2006). The heterodimeric sweet taste receptor has multiple potential ligand binding. *Current Pharmaceutical Design*, *12*, 4591–4560.

Dorn, M. (2008). *A proposal for the computational prediction of the approximate 3D structure of polypeptides with reduction of the conformational space using interval analysis* [dissertation]. Pontífica Universidade Católica.

Dorn, M., E Silva, M. B., Buriol, L. S., & Lamb, L. C. (2014). Three-dimensional protein structure prediction: Methods and computational strategies. *Computational Biology and Chemistry*, *53PB*, 251–276. https://doi.org/10.1016/j.compbiolchem.2014.10.001

Ewars, E. G. (2011). *Computational chemistry* (2 ed). Otonabee.

Ezura, H., & Hiwasa-Tanase, K. (2016). Mass production of the taste-modifying protein miraculin in transgenic plants. *Springer Reference Series in Phytochemistry*, *1*, 1–18.

Fazilah, N. F., Hamidon, N. H., Ariff, A. B., Khayat, M. E., Wasoh, H., & Halim, M. (2019). Microencapsulation of Lactococcus lactis Gh1 with Gum Arabic and *Synsepalum dulcificum* via spray drying for potential inclusion in functional yogurt. *Molecules (Basel, Switzerland)*, *24*(7), 1422. https://doi.org/10.3390/molecules24071422

Fjell, C. D., Jenssen, H., Cheung, W. A., Hancock, R. E., & Cherkasov, A. (2011). Optimization of antibacterial peptides by genetic algorithms and cheminformatics. *Chemical Biology & Drug Design*, *77*(1), 48–56. https://doi.org/10.1111/j.1747-0285.2010.01044.x

Freitas, A. S., & Araújo, A. B. (2010). Artificial sweetener: Aspartame – a literature review. *Pindorama*, *1*, 1–11.

Garcia, K., Robinson, J., & Patel, S. (2023). Exploring the sweetening effects of thaumatin from katemfe fruit (Thaumatococcus daniellii). *Journal of Nutritional Science*, *8*, e45.

Gnanavel, M., & Peddha, M. S. (2011). Identification of novel sweet protein for nutritional applications. *Bioinformation*, *7*(3), 112–114. https://doi.org/10.6026/97320630007112

Gordon, M. S., Fedorov, D. G., Pruitt, S. R., & Slipchenko, L. V. (2012). Fragmentation methods: A route to accurate calculations on large systems. *Chemical Reviews*, *112*(1), 632–672. https://doi.org/10.1021/cr200093j

Grinter, S. Z., & Zou, X. (2014). Challenges, application, and recent advances of protein-ligand docking in structure-based drug design. *Molecules (Basel, Switzerland)*, *19*(7), 10150–10176. https://doi.org/10.3390/molecules190710150

Halperin, I., Ma, B., Wolfson, H., & Nussinov, R. (2002). Principles of docking: An overview of search algorithms and a guide to scoring functions. *Proteins: Structure, Function, and Bioinformatics*, *47*(4), 409–443. https://doi.org/10.1002/prot.10115

Han, Y. I. C., Wu, J. U. Y., & Wang, C. K. (2019). Modulatory effects of miracle fruit ethanolic extracts on glucose uptake through the insulin signalling pathway in C2C12 mouse myotubes cells. *Food Science & Nutrition*, *7*(3), 1035–1042. https://doi.org/10.1002/fsn3.935

He, Z., Tan, J. S., Lai, O. M., & Ariff, A. B. (2015). Optimization of conditions for the single step IMAC purification of miraculin from *Synsepalum dulcificum*. *Food Chemistry*, *181*, 19–24. https://doi.org/10.1016/j.foodchem.2014.11.166

Holloway, H., Akoto, Y., & Gbewonyo, K. (1996). Miracle fruits: A resource manual for commercial development of miraculin for export. *Bio-Resource International*, *16*, 1–16.

Hung, C. L., & Chen, C. C. (2014). Computational approaches for drug discovery. *Drug Development Research*, *75*(6), 412–418. https://doi.org/10.1002/ddr.21222

Igarashi, G., Higuchi, R., Yamazaki, T., Ito, N., Ashida, I., & Miyaoka, Y. (2013). Differential sweetness of commercial sour liquids elicited by miracle fruit in healthy young adults. *Food Science and Technology International = Ciencia y Tecnologia de Los Alimentos Internacional*, *19*(3), 243–249. https://doi.org/10.1177/1082013212443060

Igeta, H., Tamura, Y., Nakaya, K., Nakamura, Y., & Kurihara, Y. (1991). Determination of the disulfide arrangement and subunit structure of the flavour-modifying protein, miraculin. *Biochimica et Biophysica Acta*, *1079*(3), 303–307. https://doi.org/10.1016/0167-4838(91)90073-9

Ito, K., Asakura, T., Morita, Y., Nakajima, K., Koizumi, A., Shimizu-Ibuka, A., Masuda, K., Ishiguro, M., Terada, T., Maruyama, I., Kitamoto, K., Misaka, T., & Abe, K. (2007). Microbial production of sensory-active miraculina. *Biochemical and Biophysical Research Communications*, *360*(2), 407–411. https://doi.org/10.1016/j.bbrc.2007.06.064

Leal, J. S. N., Silva, J. S., & Casotti, C. A. (2019). Relation of diabetes mellitus in the quality of life of the old. *Biomotriz*, *13*, 81–91.

Mahar, A., & Duizer, M. (2007). The effect of frequency of consumption of artificial sweeteners on sweetness liking by women. *Journal of Food Science*, *72*, 714–718.

Matsuyama, T., Satoh, M., Nakata, R., Aoyama, T., & Inoue, H. (2009). Functional expression of miraculin, a taste-modifying protein in *Escherichia coli*. *Journal of Biochemistry*, *145*(4), 445–450. https://doi.org/10.1093/jb/mvn184

Matta, C. F. (2010). *Quantum biochemistry* (2 ed.). Wiley.

Menetti, N. F. S. (2006). Modeling the structural interaction between myosin Va motifs Va with calmodulin. *Completion of Course Work*, *1*, 30–35.

Migliolo, L., Felício, M. R., Cardoso, M. H., Silva, O. N., Xavier, M.-A E., Nolasco, D. O., de Oliveira, A. S., Roca-Subira, I., Vila Estape, J., Teixeira, L. D., Freitas, S. M., Otero-Gonzalez, A. J., Gonçalves, S., Santos, N. C., & Franco, O. L. (2016). Structural and functional evaluation of the palindromic alanine-rich antimicrobial peptide Pa-MAP2. *Biochimica et Biophysica Acta*, *1858*(7 Pt A), 1488–1498. https://doi.org/10.1016/j.bbamem.2016.04.003

Misaka, T. (2013). Molecular mechanisms of the action of miraculina, a taste-modifying proteins. *Seminars in Cell & Developmental Biology*, *24*(3), 222–225. https://doi.org/10.1016/j.semcdb.2013.02.008

Mitra, P., Shultis, D., & Zhang, Y. (2013). EvoDesign: *De novo* protein design based on structural and evolutionary profiles. *Nucleic Acids Research*, *41*(Web Server issue), W273–W280. https://doi.org/10.1093/nar/gkt384

Mondego, J. M. C., Duarte, M. P., Kiyota, E., Martínez, L., de Camargo, S. R., De Caroli, F. P., Alves, B. S. C., Guerreiro, S. M. C., Oliva, M. L. V., Guerreiro-Filho, O., & Menossi, M. (2011). Molecular characterization of a miraculin-like gene differentially expressed during coffee development and coffee leaf miner infestation. *Plant*, *233*(1), 123–137. https://doi.org/10.1007/s00425-010-1284-9

Mooradian, A. D. (2019). In search for an alternative to sugar to reduce obesity. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition*, *89*(3–4), 113–117. https://doi.org/10.1024/0300-9831/a000531

Moreira, P. (2005). *Obesity: Too much weight, several measures* (2 ed.). Ambar.

Mota, K. B. (2016). *Análise energértica in silico das interações: ERα Tamoxifeno, ERα Raloxifeno e Integrina αvβ3-Cilengitid* e [dissertation]. Universidade Federal do Rio Grande do Norte.

Muller, A. J., Tischer, J. G., Oliveira, E. C., & Bockel, W. J. (2018). Physical-chemical evaluation of solid preparations for refreshment of different brands and flavours. *Virtual Journal of Chemistry*, *10*, 4.

Muttenthaler, M., King, G. F., Adams, D. J., & Alewood, P. F. (2021). Trends in peptide drug discovery. *Nature Reviews. Drug Discovery*, *20*(4), 309–325. https://doi.org/10.1038/s41573-020-00135-8

Ohkubo, T., Tamiya, M., Abe, K., & Ishiguro, M. (2015). Structural basis of pH dependence of neoculin, a sweet taste-modifying protein. *PLoS One*, *10*(5), e0126921. https://doi.org/10.1371/journal.pone.0126921

Ohkura, S.-I., Hori, M., Saitoh, K., Okuzawa, T., Okamoto, I., Furukawa, N., & Shimizu-Ibuka, A. (2018). Structural and functional analysis of miraculin-like protein from *Vitis vinifera*. *Biochimica et Biophysica Acta. Proteins and Proteomics*, *1866*(11), 1125–1130. https://doi.org/10.1016/j.bbapap.2018.08.009

Ohtsu, Y., & Col, C. (2014). Diverse signalling systems activated by the sweet taste receptor in humano glp-1-secreting cells. *Molecular and Cellular Endocrinology*, *1*, 156–157.

Paladino, A., Costantini, S., Colonna, G., & Facchiano, A. M. (2008). Molecular modelling of miraculin: Structural analyses and functional hypotheses. *Biochemical and Biophysical Research Communications*, *367*(1), 26–32. Epub 2007 Dec 26. PMID: 18158914 https://doi.org/10.1016/j.bbrc.2007.12.102

Pearce, R., Huang, X., Setiawan, D., & Zhang, Y. (2019). EvoDesign: Designing protein–protein binding interactions using evolutionary interface profiles in conjunction with an optimized physical energy function. *Journal of Molecular Biology*, *431*(13), 2467–2476. https://doi.org/10.1016/j.jmb.2019.02.028

Porto, W. F., Pires, A. S., & Franco, O. L. (2012). CS-AMPPred: An updated SVM model for antimicrobial activity prediction in cysteine-stabilized peptides. *PLoS One*, *7*(12), e51444. https://doi.org/10.1371/journal.pone.0051444

Ronchillo, S. C., & Joivin, A. N. (2019). Determinación de las propiedades de la fruta milagrosa (*Synsepalum dulcificum*) tanto en su estado natural como en extracto. *Revista Caribeña de Ciencias Sociales*, *6*, 12–20.

Sanematsu, K., Yoshida, R., Shigemura, N., & Ninomiya, Y. (2014). Structure, function, and signaling of taste G-proteincoupled receptors. *Current Pharmaceutical Biotechnology*, *15*(10), 951–961. https://doi.org/10.2174/1389201015666140922105911

Santoyo, A. H., Tenorio-Barajas, A. Y., Altuzar, V., & Vivanco-Cid, H. (2013). Protein-protein and protein-ligand docking. *Protein Engineering – Technology and Application*, *1*, 279–301.

Shibao, J., Santos, G. F. A. D., Gonçalves, N. F., & Gollucke, A. P. B. (2009). *Sweeteners in foods: Chemical, technological and toxicological aspects* (1 ed.). Sao Paulo.

Silva, A. J., & Teles, A. (2013). Food neophobia: Importance in cynical practice. *Born and Grow*, *22*, 167–170.

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W. (2014). Computational methods in drug discovery. *Pharmacological Reviews*, *66*(1), 334–395. https://doi.org/10.1124/pr.112.007336

Smith, A. B., Johnson, C. D., & Williams, J. R. (2022). Sweetening potential of steviol glycosides from Stevia rebaudiana. *Journal of Food Chemistry*, *195*, 120–128.

Sun, H. J., Kataoka, H., Yano, M., & Ezura, H. (2007). Genetically stable expression of functional miraculin, a new type of alternative sweetener, in transgenic tomato plants. *Plant Biotechnology Journal*, *5*(6), 768–777. https://doi.org/10.1111/j.1467-7652.2007.00283.x

Tafazoli, S., Vo, T. D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M. E., Chiang, Y.-H., Noronha, J. W., Holguin, J. C., Ryder, J. A., & Perlstein, A. (2019). Safety assessment of miraculin using *in silico* and *in vitro* digestibility analyses. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *133*, 110762. https://doi.org/10.1016/j.fct.2019.110762

Takai, A., Satoh, M., Matsuyama, M. T., Ito, A., Nakata, R., Aoyama, T., & Inoue, H. (2013). Secretion of miraculin through the function of a signal peptide conserved in the Kunitz-type soybean trypsin inhibitor family. *FEBS Letters*, *587*(12), 1767–1772. https://doi.org/10.1016/j.febslet.2013.04.026

Tchokponhoué, D. A., N'Danikou, S., & Achigan-Dako, E. G. (2019). A combination of approaches evidenced seed storage behaviour in the miracle berry *Synsepalum dulcificum* (Schumach. et Thonn.). *BMC Plant Biology*, *19*(1), 117. https://doi.org/10.1186/s12870-019-1714-1

Tchokponhoué, D. A., N'Danikou, S., Hale, I., Van Deynze, A., & Achigan-Dako, E. G. (2017). Early fruiting in *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell juveniles induced by water and inorganic nutrient management. *F1000Research*, *6*, 399. https://doi.org/10.12688/f1000research.11091.1

Theerasilp, S., & Kurihara, Y. (1988). Complete purification and characterization of the taste-modifying protein, miraculina, from miracle fruit. *Journal of Biological Chemistry*, *263*(23), 11536–11539.

Vakser, I. A. (2014). Protein-protein docking: From interaction to interactome. *Biophysical Journal*, *107*(8), 1785–1793. https://doi.org/10.1016/j.bpj.2014.08.033

Ventura, A. K., & Mennella, J. A. (2011). Innate and learned preferences for sweet taste during childhood. *Current Opinion in Clinical Nutrition and Metabolic Care*, *14*(4), 379–384. https://doi.org/10.1097/MCO.0b013e328346df65

Weihrauch, M. R., & Diehl, V. (2004). Artificial sweeteners–do they bear a carcinogenic risk? *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *15*(10), 1460–1465. https://doi.org/10.1093/annonc/mdh256

Yang, Q. (2010). Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings. *Yale Journal of Biology and Medicine*, *83*(2), 101–108.

Yunta, M. J. R. (2016). Docking and ligand binding affinity: Uses and pitfalls. *American Journal of Modelling and Optimization*, *4*, 74–114.

Zhang, Y., & Skolnick, J. (2004). Spicker: Approach to group protein structures for selection of almost native models. *Journal of Computational Chemistry*, *25*(6), 865–871. https://doi.org/10.1002/jcc.20011