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Review

Current Advances and Future Aspects of Sweetener Synergy: Properties, Evaluation Methods and Molecular Mechanisms

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Abstract: Sweetener synergy is the phenomenon in which certain combinations of sweeteners work more effectively than the theoretical sum of the effects of each components. It provides benefits in reducing sweetener dosages and improving their sweetness. Many mixtures of sweeteners with synergistic effects have been reported up to now. Both artificial high-intensity sweeteners and natural sweeteners are popularly used in sweetener mixtures for synergism, although the former seem to display more potential to exhibit synergy than the latter. Furthermore, several evaluation methods to investigate sweetener synergy have been applied, which could lead to discrepancies in results. Moreover, structurally dissimilar sweeteners could cooperatively bind at the different sites in the sweet taste receptor T1R2/T1R3 to activate the receptor, and their hydration characters/packing characteristics in solvents could affect their interaction with the receptor, providing the preliminary explanations for the molecular basis of sweetener synergy. In this article, we firstly present a systematic review, analysis and comment on the properties, evaluation methods and molecular mechanisms of sweetener synergy. Secondly, challenges of sweetener synergy in both theory and practice and possible strategies to overcome these limitations are comprehensively discussed. Finally, future perspectives for this important performance in human sweet taste perception are proposed.

Keywords: sweetener; synergy; evaluation methods; sweet taste receptor; molecular basis; interaction; mixture

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1. Introduction

Sweetener is an important food additive that provides a pleasant sense for humans and other animals. Some sweeteners such as sugars are important nutrient and energy sources [1,2]. According to the original producing methods of the sweeteners, they can be divided into natural sweeteners (e.g., sugars, sugar alcohols and sweet-tasting proteins) and artificial sweeteners (e.g., sucralose, saccharin and aspartame) [3,4]. With the development of diet and nutrition in human beings, high standards for sweeteners in food and beverages are needed [5].

A single sweetener often has the disadvantage of high calories (e.g., sucrose) that are a potential inducer for obesity, oral health problems, diabetes and hyperlipemia, or has an aftertaste and bitter taste (e.g., stevioside) [6,7]. Furthermore, artificial sweeteners have long been controversial for their safety, which requires their use only under strict control and below specific dose levels [8]. Therefore, different sweeteners are often used together to provide certain sensory properties and to take advantage of the cooperative effect that occurs in certain sweeteners combinations [9]. Based on the comparison of the sweet intensities of the mixed sweeteners with that of the sum of the unmixed components, the effect of sweetener mixtures can be categorized as addition (equal), synergism (greater) and suppression (less). Until now, many sweetener mixtures with synergistic effects have been reported [10–12].

Mixtures of sweeteners can be divided into binary and polynary combinations, according to the categories of sweeteners used. Generally, the polynary mixtures can lead to more complex and pleiotropic effects than the binary mixtures, accounting for their distinct properties in sweeteners' synergism [13]. In this paper, we summarize, analyze and comment on the findings for sweeteners' synergism reported up to now as well as their evaluation methods. Furthermore, insight into the molecular basis of sweeteners' synergism has seen substantial progress in recent years, although it is still limited due to several technological constraints [14]. We then describe several explanations reported so far for understanding the molecular mechanism of sweeteners' synergism, especially the consequent interaction between the sweeteners and sweet taste receptors due to the mixture of sweeteners, and analyze their scientific merit and deficiency in a broad context. Moreover, the future challenges and perspectives of sweeteners' synergism in performance improvement, development and application are proposed. This article provides meaningful and helpful guidelines for further research on sweetener synergy and promotion of its applications in the food industry.

2. Methods to Determine the Synergistic Effect of Sweetener Mixtures

Different methods have been applied in the evaluation of the synergistic effect of sweetener mixtures. Besides the long- and popularly used sensory evaluation, some novel methods have emerged in recent years, such as biosensors and electronic tongues [15,16]. However, most of the results for sweetener synergy were obtained by sensory evaluation. Basically, the discrepancy among these methods lies mainly in the comparison modes for the sweet intensity between the mixed sweeteners and the sum of the unmixed components. On the other hand, variable outcomes in the evaluations of specific sweetener mixtures could be obtained when different evaluation methods were adopted. We briefly describe the main points of these methods as follows.

2.1. Sensory Evaluation Method I

The first method is the most common and simple approach that has been popularly used [11,17]. In this method, which is called the simple additive model, the perceived sweetness of the mixture is directly compared to the sum of the sweetness of the unmixed components. If the sweetness of the mixture is equal to, greater or less than the summed sweetness of its components, then it is regarded as having additive, synergistic and suppressive effects, respectively. However, this method has been questioned by many researchers because it does not consider the effect of sweetener concentrations on the results; thus, contradictory findings in the research on sweeteners' synergism could often arise when different doses of the components were added in the sweetener mixtures [18]. Furthermore, it should be noted that in this method, the concentrations of used sweeteners were usually expressed as a % (weight of sweetener/weight of solvent) rather than a molarity (moles of sweetener/volume of solvent), which cannot address the fact that sweeteners have different molecular weights and thus are not scientifically rational for studying the mechanical interactions between the sweeteners as well as the sweeteners and receptors [17].

2.2. Sensory Evaluation Method II

Because the perceived sweetness of some sweeteners show a sigmoidal rather than linear relationship with their concentrations, so many researchers assessed the sweet intensity of self-mixtures (mixtures of each individual component with itself) to compare it with the sweet intensity of blend mixtures to determine the interactive effect of different sweeteners, which is essential to discriminate between superadditivity (which means that the self-mixture of a sweetener produces more sweetness than the summed sweetness of this sweetener in isolation) and synergism [19]. Based on this principle, the sweetness of sweetener mixtures is compared with the average of the sweetness ratings of the two pure components in the mixture (e.g., comparing the sweetness of a 3%A + 3%B mixture with the average sweetness of a 3%A + 3%A and 3%B + 3%B). This self-mixture model accounts for

the concentration-dependent and non-linear psychophysical functions and considers the effect of interactions between sweeteners on their synergism [20,21]. Although this method has been popularly used, it was thought by some researchers to be too straightforward to elucidate the complex response toward the interactive sweet stimulus [22].

2.3. Sensory Evaluation Method III

A modified isobole analysis method was proposed by Sühnel in 1993, in which evaluating the effect of sweeteners' interactions was based on the comparison between the observed effect of the sweetener mixtures and what would be expected from each sweetener in isolation [23]. This method has been widely used to evaluate drug combinations with synergistic action [24]. Specifically, an equation (cA/CA) + (cB/CB) = I is adopted in this approach, where cA and cB are the concentrations of sweeteners A and B in the mixture, respectively, and CA and CB are the concentrations of A and B that would individually produce the same sweet intensity as the mixture, respectively. A value of I less than 1 indicates synergy, more than 1 indicates suppression and around 1 indicates no interaction. This method has been suggested to have the advantage of demonstrating the empirical concentration–effect relationships, which is completely independent of the mechanism of interaction [22,25].

2.4. Other Evaluation Methods

Besides the traditional sensory evaluation methods, other methods have also been devised to assess the sweeteners' synergism. For instance, Fujiwara et al. carried out a cell-based Ca^{2+} mobilization assay that represented the elicited signal upon sweet-taste-receptor activation to investigate the synergistic effects of sweetener mixtures, and the results were in good agreement with that of sensory evaluations [26]. Moreover, the electronic tongue and bioelectronic tongue technologies, also called sensors and biosensors, respectively, have made significant advances in recent years, which show their great potential in evaluations of sweeteners' synergism [15,16,27,28]. For example, an in vitro binding assay with cloned human sweet taste receptors could well reflect the taste properties of sweeteners, which indicates that the taste-cell- and receptor-based biosensors can exhibit great advantages in obtaining taste-related qualitative/quantitative information of ingredients in food, thus enabling a more logical recognition of sweet substances in complex systems [28,29]. In this regard, with the improvement and development of the performance of these taste biosensors, their future practical application in evaluations of sweeteners' synergism could be greatly prospective [27,28].

3. Synergism in Mixtures of Sweeteners

3.1. Binary Mixtures of Sweeteners

Initially, research on sweeteners' synergy was mainly focused on high-intensity artificial sweeteners such as acesulfame-K, aspartame, cyclamate and sucralose. For example, early in 1969, Stone and Oliver reported that cyclamate was synergistic with sucrose and saccharin [30]. In 1989, Wells summarized the sweetener combinations exhibiting synergism, most of which were high potency sweeteners [31]. The most popular high-intensity sweeteners in these mixtures were acesulfame-K, cyclamate and saccharin. For instance, both acesulfame-K and cyclamate were synergistic with alitame, aspartame, stevioside and sucralose, respectively, while saccharin was synergistic with aspartame, cyclamate, neohesperidin dihydrochalcone (NHDC) and the sweet-tasting protein thaumatin. Based on these findings, it could be presumed that the high-intensity artificial sweeteners could have more potency to elicit synergy than natural sweeteners. Many other sweetener combinations showing synergism have also been reported [11,17]. However, there is no physicochemical or structural patterns or principles found in these mixtures of sweeteners that exhibit synergism.

Later, it was found that natural sugars and proteins could also elicit synergistic effects. In 1995, Schiffman et al. systematically examined the presence and degree of synergism among all binary mixtures of 14 sweeteners including 3 sugars (fructose, glucose and sucrose), 2 polyhydric alcohols (mannitol and sorbitol), 2 natural glycosides (rebaudioside-A

and stevioside), 2 dipeptide derivatives (alitame and aspartame), 1 sweet-tasting protein (thaumatin), 1 sulfamate (sodium cyclamate), 2 amides (acesulfame-K and sodium saccharin) and NHDC [32]. Notably, the results of the synergism assessment were obviously concentration-dependent. Specifically, when two sweeteners with a sweetness equal to 3% sucrose were respectively added in the mixture, significant synergistic effects were found. However, only a few mixtures of sweeteners with a sweetness equal to 5% sucrose exhibited synergism, and no synergy was found for mixtures of sweeteners with a sweetness equal to 7% sucrose. Furthermore, two evaluation methods (Method I and II) were separately used in their investigation but led to some different conclusions, especially for mixtures of two sweeteners with a sweetness equal to 5% and 7% sucrose, respectively.

Reyes et al. advanced sweetener synergy research through an isobole analysis using Method III in 2019 [22]. They used 15 representative sweeteners at 3 different concentrations equal to 4%, 6% and 8% sucrose in sweet intensity, respectively, to investigate their synergism. However, no obvious effect of the concentrations on the results was found with this evaluation method. Furthermore, as expected, the highly synergistic mixtures ($I < 0.6$, see the equation in 2.3 Method III) included most high-intensity artificial sweeteners such as acesulfame-K and NHDC. For instance, the long-tested combinations acesulfame-K/aspartame and acesulfame-K/rebaudioside-A exhibited full synergistic effects, and NHDC showed significant synergism with acesulfame-K, sucralose, thaumatin and rebaudioside-A, respectively. The natural fructose showed partial synergy ($I = 0.6–0.9$) with acesulfame-K or rebaudioside-A. However, a few findings in their study, such as the mixture of acesulfame-K/rebaudioside-A, showed full synergy conflicts with the previously reported corresponding data that were revealed with Method I or II [22,32].

A lot of results about binary sweeteners' synergism have been reported up to now [11,17,33–39], which cannot be elaborated herein due to the page limit. In addition, alternative models were also proposed to account for the interaction between sweeteners in binary mixtures [40]. However, these models seem to be too mathematically complicated to be popularly used. Table 1 summarizes the previously reported most popular binary mixtures of sweeteners showing significant synergism, as well as their concentrations and the evaluation methods applied [20,22,32].

Table 1. The popular binary mixtures of sweeteners showing significant synergism reported up to now.

Sweetener 1	Sweetener 2	Concentration of 1	Concentration of 2	Evaluation Method	References
Acesulfame-K	Aspartame	Equal sweetness to 3% or 5% sucrose [1]	Same as sweetener 1	I	[32]
Acesulfame-K	Cyclamate	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Alitame	NHDC [2]	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Aspartame	Saccharin	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Aspartame	Stevioside	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
NHDC	Fructose	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Fructose	Sorbitol	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
NHDC	Glucose	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Glucose	Sorbitol	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Cyclamate	Stevioside	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Saccharin	Rebaudioside-A	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
NHDC	Rebaudioside-A	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
NHDC	Stevioside	Equal sweetness to 3% or 5% sucrose	Same as sweetener 1	I	[32]
Cyclamate	Sucrose	5.6/12.5/22.6 mM [3]	0.1/0.25/0.5 M	II	[20]
Cyclamate	Aspartame	5.6/12.5/22.6 mM	0.7/2.7/4.9 mM	II	[20]
Cyclamate	Acesulfame-K	5.6/12.5/22.6 mM	1.0/4.0/6.0 mM	II	[20]
Cyclamate	Glucose	5.6/12.5/22.6 mM	0.39/0.88/1.69 M	II	[20]
Cyclamate	Xylitol	5.6/12.5/22.6 mM	0.2/0.6/1.0 M	II	[20]
Stevioside	Aspartame	0.25/0.62/1.1 mM	0.7/2.7/4.9 mM	II	[20]
Stevioside	Acesulfame-K	0.25/0.62/1.1 mM	1.0/4.0/6.0 mM	II	[20]
Saccharin	Sucrose	0.39/1.95/4.87 mM	0.1/0.25/0.5 M	II	[20]
Saccharin	Aspartame	0.39/1.95/4.87 mM	0.7/2.7/4.9 mM	II	[20]

Table 1. *Cont.*

Sweetener 1	Sweetener 2	Concentration of 1	Concentration of 2	Evaluation Method	References
Acesulfame-K	Sucrose	1.0/4.0/6.0 mM	0.1/0.25/0.5 M	II	[20]
Acesulfame-K	Glucose	1.0/4.0/6.0 mM	0.39/0.88/1.69 M	II	[20]
Acesulfame-K	Fructose	1.0/4.0/6.0 mM	0.15/0.39/0.74 M	II	[20]
Acesulfame-K	Xylitol	1.0/4.0/6.0 mM	0.2/0.6/1.0 M	II	[20]
Acesulfame-K	Aspartame	1.0/4.0/6.0 mM	0.7/2.7/4.9 mM	II	[20]
Acesulfame-K	Aspartame	Equal sweetness to 4%, 6% or 8% sucrose [4]	Same as sweetener 1	III	[22]
Acesulfame-K	NHDC	Equal sweetness to 4%, 6% or 8% sucrose	Same as sweetener 1	III	[22]
Acesulfame-K	Rebaudioside-A	Equal sweetness to 4%, 6% or 8% sucrose	Same as sweetener 1	III	[22]
NHDC	Sucralose	Equal sweetness to 4%, 6% or 8% sucrose	Same as sweetener 1	III	[22]
NHDC	Thaumatin	Equal sweetness to 4%, 6% or 8% sucrose	Same as sweetener 1	III	[22]
NHDC	Rebaudioside-A	Equal sweetness to 4%, 6% or 8% sucrose	Same as sweetener 1	III	[22]

[1] Each sweetener with specific concentrations in the mixtures displayed a sweetness intensity equal to that of 3% or 5% sucrose (*w*/*w*), respectively. [2] NHDC, neohesperidin dihydrochalcone. [3] Three molar concentrations divided by "/" were applied for each sweetener in the mixtures, respectively. [4] Each sweetener with specific concentrations in the mixtures displayed a sweetness intensity equal to that of 4%, 6% or 8% sucrose (*w*/*w*), respectively.

3.2. Polynary Mixtures of Sweeteners

The only detailed study on the synergism of polynary mixtures of sweeteners was performed by Schiffman et al. in 2000, who extended their binary mixture study to investigate the ternary mixture synergism with the same 14 sweeteners previously used [41]. Each sweetener in the mixture had a sweetness equal to 2% sucrose, and interactive effects were analyzed by comparing the sweetness of each ternary mixture with the average sweetness of the self-mixtures of its three constituent sweeteners (Method II). The results indicated that no obvious trend was found to predict the combinational effect in ternary mixtures of sweeteners, although the ternary combinations exhibited relatively less synergism than the binary combinations of their constituent sweeteners. Similar to the binary mixtures, the highest synergistic effect in ternary mixtures was found with three high-intensity sweeteners (alitame + NHDC + rebaudioside-A), whereas less synergistic effects in ternary mixtures were found with natural sugars (e.g., glucose and fructose). Considering the lower amount of information on the synergism of polynary mixtures, we proposed that further investigations should be performed to reveal their unique properties [35,41], such as studying the quaternary or polynary mixtures with multifarious concentrations, evaluation methods and sweeteners to be applied.

4. Mechanism for Synergism in Mixtures of Sweeteners

Why can combinations of two or more sweeteners exert synergism? It is well known that sweet taste perception is mediated by a class C G protein-coupled receptor (GPCR)—the heterodimer T1R2 (taste type 1 receptor 2)/T1R3 (taste type 1 receptor 3) [42,43]. Accordingly, this question certainly leads to elucidating the sweet taste elicitation process in which the sweeteners in mixtures cooperatively interact with and then activate the human sweet taste receptor T1R2/T1R3 [44–46]. However, due to the lack of structural information on the receptor, mechanical insights into the interplay between sweeteners and receptor are still elusive [47]. Nevertheless, molecular simulations and functional mutagenesis/chimera analysis can provide meaningful guidance for understanding the sweetener–receptor interaction as well as sweeteners' synergism [48–50].

In principle, different sweeteners have different weights, shapes and configurations, which definitely affect their specific interactions with the sweet taste receptor. In this regard, compared with the separate sweeteners, the mixing of sweeteners can result in a change or modification of their contact properties with the receptor, thus leading to activation of the receptor and eliciting their distinct sweetness [51]. Indeed, many previous studies have revealed that there are multiple binding sites in the sweet taste receptor for various sweeteners [47,48]. For example, the artificial high-intensity sweeteners aspartame, neotame, saccharin and acesulfame K interact with the VFTM (Venus flytrap module) of human T1R2, while natural sugars (e.g., glucose and sucrose) and sucralose bind to the

VFTMs of both T1R2 and T1R3 [52–54]. However, cyclamate and NHDC act on the TMD (transmembrane domain) of human T1R3 [55,56]. The sweet-tasting protein thaumatin was proposed to bind into the CRD (cysteine rich domain) of T1R3 [47,57]. We have demonstrated that the intense sweeteners perillartine and saccharin (at high concentrations above 3 mM) can bind to the TMD of human T1R2 (Figure 1) [58–60].

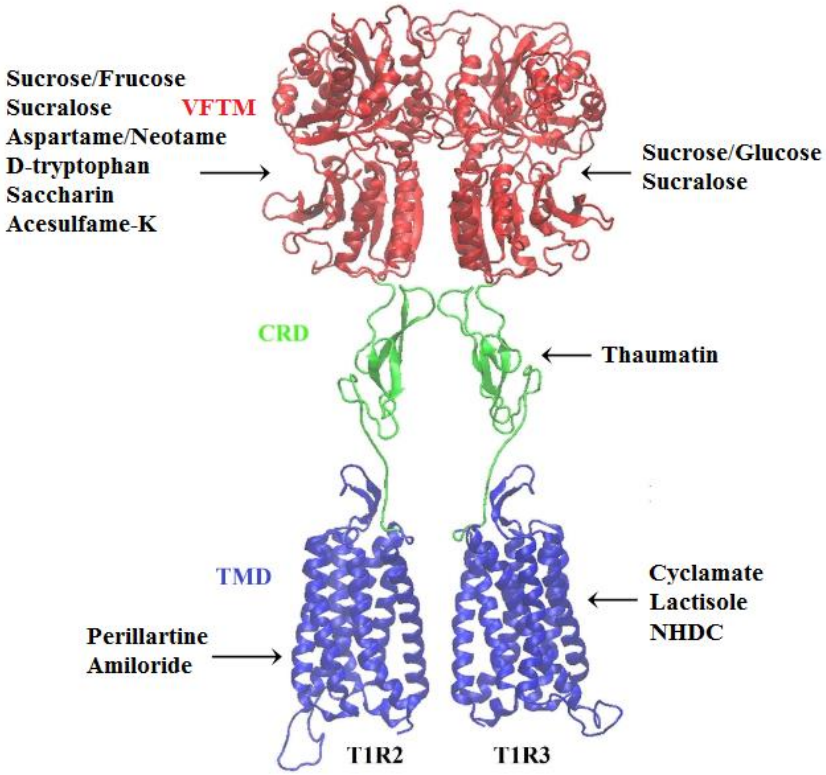

Figure 1. Graphic representation of the modeled structure of the human sweet taste receptor T1R2/T1R3 and its binding sites for different sweeteners. The extracellular VFTM (Venus flytrap module), CRD (cysteine rich domain) and TMD (transmembrane domain) are colored in red, light-green and blue, respectively. This figure was made with the PyMOL software. The binding sites of various sweeteners in the receptor are indicated by arrow lines.

According to these findings, it was postulated that the different sweeteners in the mixture could bind at different sites in the receptor to concertedly increase the efficiency of receptor activation, thus displaying synergism. This hypothesis has been supported by some sweetener combinations with synergistic effects that interact with different monomers/domains of human T1R2/T1R3, as demonstrated by a cell-based receptor function analysis [26]. Specifically, two high-intensity artificial sweeteners, NHDC and cyclamate, which have been identified as binding at the TMD of T1R3, significantly enhance the sweetness of various sweeteners including sucrose, aspartame, neotame, rebaudioside-A, saccharin, sucralose and thaumatin, which have been identified as interacting with the VFTM or CRD of T1R2/T1R3. Figure 1 shows the distinct binding sites of various sweeteners in the human sweet taste receptor. Based on these results, the authors suggest that NHDC and cyclamate act as positive sweet modulators to exert their synergistic effects with other sweeteners [50,61]. This finding is in line with the previous results that in mixtures, structurally dissimilar sweeteners interacting with different sites in the receptor have the potential to exhibit synergy, whereas structurally similar sweeteners interacting with parallel sites in the receptor have the potential to show suppression [22,32]. Furthermore, the relatively less effective synergism in polynary mixtures than binary mixtures could be partly explained by the distinct binding sites for various sweeteners, as the more sweeteners appear in the mixtures, the more possibilities for overlapping binding and contact with the re-

ceptor, resulting in an overall counteracted or alleviatived effect in sweeteners' synergism [41]. Moreover, a recent study has used molecular dynamics simulations to illuminate the potential structural basis of receptor activation by full synergistic combinations of sweeteners [14].

It was also proposed that synergy occurs when the components in the sweetener mixtures have identical hydration characters (e.g., hydrophobic or hydrophilic), which was explained by increases in the mobility of water molecules and reductions in the volume of hydrated sweetener molecules, but its link to the receptor activation and origin of sweetener synergy has not been elucidated [62]. Another study investigated the packing characteristics of blend sweetener molecules in water to account for their synergistic effect [63]. The results indicate that sweetener–sweetener, sweetener–solvent and solvent–solvent interactions have essential effects on the properties of binary sweeteners. The bulk sweeteners (sucrose and maltitol) appear to dominate the properties of the mixtures due to their molar excess over the intense sweeteners (acesulfame K, aspartame and cyclamate), while the intense sweeteners play an important role in modifying the structure of water in solution. The molecular volumes, hydrophobicity, ionic character and isentropic compressibility of the sweeteners in the binary mixtures are of obvious relevance to their interaction as well as the solvent's packing, thus affecting their accession to the sweet taste receptor and their sweet intensities [63]. Another interesting study pointed out that in some sweetener mixtures, across-adaption and synergism display opposite relationships, with the most across-adaption combinations showing the least synergism, and vice versa, suggesting a complex interplay between the sweetener components [64,65]. It is worthy to note that the sweeteners' synergism at the level of molecular interactions are sweetener-dependent, which should be analyzed carefully when specific sweetener combinations are considered or applied [63,65].

Other explanations of the molecular mechanisms of sweetener synergy have also been proposed. For instance, because some artificial sweeteners exhibiting synergism often have a bitter side taste, some researchers suggested that synergy may partly be due to the suppression of the bitter taste of one sweetener by another, whereas the bitterness of components could reduce their sweetness in isolation [66,67]. Furthermore, some researchers proposed that sweeteners' synergism may result from their simultaneous stimulation of multiple receptors, but no experimental evidence supports this hypothesis [68,69]. Moreover, it was suggested that different sweeteners could induce different downstream signal transduction cascades, leading to sweet taste signal convergence in a broadly tuned TRC (taste receptor cell) [70]. However, the mechanisms of sweetener synergy at the level of cellular signaling are still unknown, which should be explored in further investigations.

5. Factors Affecting the Results of Sweeteners' Synergism

As previously stated, there are usually obvious discrepancies among the results in evaluating the synergism of sweetener combinations [11,32]. A lot of factors may affect the evaluation results of the sweeteners' synergism. Firstly, the sweeteners used in these investigations may differ in source, purity and concentration, and the latter has been shown to be crucial for the resulted conclusions [19]. Furthermore, as described above, the evaluation methods have an important influence on the outcome, which has been shown in many previous investigations [22,32]. Based on their design principles, we propose that method III is the most logical approach for evaluating sweeteners' synergism. Moreover, the participants in the sensory tests were variable in age, nationality and health conditions, and different taste propensities in different populations have been reported [71]. All these factors could individually or cooperatively affect the results of synergism in mixtures of sweeteners, so researchers and manufacturers should pay attention to their conditions and background and use these data carefully.

6. Applications of Sweeteners' Synergism in Food and Beverages

Mixing of sweeteners has been widely used in food and beverage such as candy, chutty, biscuits and drinks [72]. Both natural bulk sweeteners (e.g., fructose, erythritol and stevioside) and artificial intense sweeteners (e.g., sucralose, aspartame and acesulfame-K) can be mixed to achieve optimization of the acceptability, functionality and economics of the products [73]. These formulations not only reduce the amount of some sweetener components to conform to their dosage restrictions, but also have healthy and nutritional advantages (e.g., the popular and welcomed sugar-free coke nowadays) [74]. It should be noteworthy herein that the aim of sweeteners' synergism applied in practice is not only to take advantage of the synergism of sweet intensities, but also to acquire a specific taste or flavor in certain sweetener mixtures. However, the latter topic is beyond the scope of the present paper and could be discussed elsewhere.

7. Challenges, Strategies and Outlooks

Since the phenomenon of sweeteners' synergism became of academic concern around 80 years ago, a great deal of data have been reported on this interesting topic. However, there seems to be confusion in some ways when analyzing and categorizing this information. This is largely due to the varied aims, conditions, methods or experimental designs in these different investigations [11,22,32]. In the future, it is necessary to set up relatively unified criterions effective both in research and practice for the sweeteners' synergism. For instance, specific dose ranges for certain sweeteners, generally acknowledged evaluation methods and standards requirements for various sweeteners, test panels and conditions should be normalized to better define the sweeteners' synergism. Furthermore, new evaluation methods (e.g., biomimetic sensors and biosensors) could be optimized to improve the accuracy of the results of sweeteners' synergism [15,16,27–29]. Moreover, emphasis should be taken on the functional performances of sweeteners' synergism (e.g., decreasing the usage of specific sweeteners, improving the sensory quality and reducing the calories of sweetener mixtures) to design the distinct and optimal combinations of chemically diverse sweeteners.

Despite several considerable proposals having been raised, the molecular mechanisms of sweeteners' synergism are still obscure, which limits the further development of the design and application of sweetener mixtures. The molecular basis for the onset of sweet perception that is dependent on the interaction between the sweetener (or mixture of sweeteners) and the sweet taste receptor is elusive due to the lack of structure of the receptor and its complexes with various sweeteners [75]. The recent breakthrough of AlphaFold in predicting GPCR structures could facilitate research on the mechanisms of interaction between the cooperative sweeteners and receptors, especially for molecular simulation studies [76]. Furthermore, with technological innovations such as cryo-electron microscopy, more and more structures of GPCRs have been solved in recent years, which could greatly promote the deciphering of the mechanisms of sweeteners' synergism at a structural basis [77,78]. Lastly, because sweeteners' synergism is a complex psychophysical process and involves many signal transduction pathways, elucidating the molecular mechanisms of sweeteners' synergism needs further multidisciplinary cooperation of scientists in many fields such as food, structural biology, neuroscience, physiology and biochemistry [79,80]. These efforts could jointly achieve the theoretical and practical development of sweeteners' synergism in the future, which will ultimately benefit the diet and health of human beings.

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