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An Overview of Biotransformation for the Sustainability of Sweet-Tasting Proteins as Natural Sugar Replacers †

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Abstract: According to WHO, sugar intake rates should be reduced due to the connection between sugar and diseases. However, reducing sugar in foods is a challenge both for food manufacturers and consumers. Therefore, sweet-tasting proteins may solve this problem with a sweet taste, health benefits, and without caloric contents. Thus far, known natural sweet-tasting proteins are brazzein, curculin, thaumatin, monellin, miraculin, and mabinlin. Nevertheless, natural sources of sweet proteins might be extinct in the future due to overconsumption. Thus, biotransformation studies of sweet proteins are promising as they produce high yield rates, quality, fewer by-products, and more sustainable solutions.

Keywords: sugar overconsumption; natural sugar substitutes; sugar replacement

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1. Introduction

Sugar is a crucial compound for food processing with characteristics of texture, stability, mouthfeel, flavour, colour, and preservation features [1]. Moreover, sugar is an energy source for our body, but excessive sugar consumption is an issue that causes obesity [2]. According to the World Health Organization [3], less than 10% of total energy should be intaken from free sugars for adults. However, nowadays, sugar overconsumption is a challenging issue because of caused disorders in the body such as weakening of immunity [4], diabetes, cardiovascular diseases, and cancer [5].

On the other hand, consuming sweet foods is a genetically evolutionary survival mechanism for human-beings because of psychological necessity [6]. Nevertheless, sweetness causes addiction with tooth decay, weight gain, obesity, type 2 diabetes mellitus, high blood cholesterol, depression, and cancer [7–9]. Thus, due to the side effects of sugar overconsumption, it has been suggested to remove sugar from the GRAS (generally regarded as safe) list of the FDA (U.S. Food and Drug Administration) by Lustig et al. [10].

In conclusion, this review aims to discuss natural, sweet-tasting proteins in combination with health-promoting activities and sustainability features. Thu far, the known natural sweet proteins are brazzein, curculin, thaumatin, monellin, miraculin, and mabinlin. Thus, the scope includes identifying sweet-tasting proteins as natural food ingredients.

2. Sweet-Tasting Proteins

2.1. Brazzein

Brazzein is a derivative of *Pentadiplandra brazzeana* Baillon, which is found in African tropical forests naturally [11]. Brazzein is the smallest sweet-tasting protein with a 54 amino acid structure [12]. The sweetness of brazzein is 2000 times higher than a 5% sucrose solution [13], and the stability of brazzein maintains up to 80 °C [14], which is an important feature for food manufacturing.

Because of original plant location and limited brazzein production, the alternative method of bioconversion is the best way to manufacture brazzein close to natural. The

first brazzein biotransformation study was made via *Escherichia coli* in 2000. However, following brazzein biotransformation studies in *E. coli*, it exhibited a lower sweet taste than the product of the original plant. Later, sweet brazzein manufacturing was achieved with *Pichia pastoris*. *Pichia* cells released about 120 mg/L of brazzein in 6 days. Nevertheless, *Kluyveromyces lactis* produced about 104 mg/L of brazzein into the cultured medium in a short period, and recombinant brazzein's sensory characteristics were similar to the original plant product [11]. Recently, *Bacillus licheniformis* was applied for brazzein extraction, due to its fast growth, high secretion, and low cost [12]. Thus, the rbrazzein genes were expressed and 57 mg/L of brazzein was produced at 36 h. Ebrazzein and Bbrazzein demonstrated 400 and 266 times more sweetness characteristics than sucrose, respectively.

For plant biotransformation studies of brazzein, the most applied mediums are maize, corn, rice, and lettuce [15,16]. Moreover, brazzein was achieved when produced in about 400 μg/g of corn seeds, and corn brazzein allows for industrial production, which can solve issues related to the sustainability of the original brazzein in the future [11]. Thus, the sweet taste of brazzein is felt slower than sucrose and may replace sugar in food processing with novel food applications.

2.2. Curculin

Curculin is extracted from *Molineria latifolia* (Dryand. ex W.T.Aiton) Herb. ex Kurz, which is native to Malaysia [11]. Dried fruits of Molineria are used by local people against the bitter taste of black tea and sour foods [17]. Therefore, the compound is a promising ingredient for future food production as a novel material.

Indeed, curculin demonstrates 550 times more sweetness than sucrose on a weight basis [18]. Moreover, water solutions of the curculin exhibit a strong sweet taste at low pH [17]. Thus, the feature might be applied for innovative food productions.

Thus far, gene expression studies of curculin have been made via *E. coli*, but homodimeric forms of the compound have not exhibited any sweet taste; heterodimeric forms of curculin have demonstrated characteristics of sweet taste [19]. Furthermore, the natural source of curculin is unsustainable, and biotransformation studies of curculin have exhibited valuable results for flavour enhancement and sweet-taste features [20]. Therefore, the characteristics of curculin are attractive and promising.

2.3. Mabinlin

Mabinlin is found in the seeds of *Capparis masaikai* Levl., which comes from the Yunnan region in China [11]. Mabinlin possesses four isoforms, which are mabinlin I-1, mabinlin II, mabinlin III, and mabinlin IV [21]. Mabinlin II is the only compound that is heat stable and the sweetness is maintained following 48 h of incubation at 80 °C. Therefore, the sweetness of mabinlin is 400 times higher than sucrose on a molar basis [22].

Mabinlin II is difficult to extract from the *Capparis* plant, but biotransformation studies via *E. coli* and *Lactococcus lactis* provide availabilities to produce mabinlin in wide spectrums for food applications [23]. Moreover, biotransformation studies of mabinlin in plants researched in potato, where mabinlin II had an astringent sweet taste and the amount was 1 mg/mL [19]. Therefore, the sweetness characteristics of mabinlin provide possibilities to apply to vegan foods to mask the bitterness of plant-based ingredients.

2.4. Miraculin

Miraculin is found in *Richardella (Synsepalum) dulcifica* (Schumach. & Thonn.) Baehni, and demonstrates an unsweet feature but can transform a sour taste into a sweet feeling. Miraculin consists of 191 amino acids and N-linked oligosaccharide [24], which is solely extracted from the *Richardella* fruit after 6 weeks of pollination, following the fruit colour change from green to dark red [25]. The miraculin provides abilities to use for the taste enhancement of acids [26]. Thus, miraculin solutions may enhance the flavour characteristics of acids in food products for more than 1 h [27].

The first biotransformation study of miraculin was made via *E. coli* [28] without sweet-taste characteristics after recombinant miraculin was produced in transgenic lettuce, and the amount of miraculin was between 33.7 and 43.5 μg/g of fresh weight with sweet-taste feeling characteristics [29]. Following this, miraculin was produced in a transgenic tomato and strawberry as well [30]. Thus, biotransformation of miraculin causes low cost, and it is genetically stable [25].

2.5. Monellin

Monellin contains 44 amino acids in one chain and 50 amino acids in another chain as polypeptides [19]. Monellin is a sweet-tasting protein of *Dioscoreophyllum cumminsii* Diels, and the plant grows naturally in African forests [11]. The sweetness of monellin is 4000 times higher than sucrose on a weight basis [31].

Cultivation studies of *Dioscoreophyllum* have not been achieved except in natural habitats to obtain stabile monellin [32]. For this reason, biotransformation studies have been implemented, and a specific form of monellin provides flexibility for biotransformation. For instance, the transformation of monellin via *E. coli* supports a sweet flavour during heating, with pH stability better than the original compound [19]. Moreover, biotransformation of monellin via *S. cerevisiae* yielded about 54 g of purified monellin [33].

Transgenic plant studies of monellin were made in transgenic tomato and lettuce [34]. Therefore, an ethylene-applied transgenic tomato provided about 23.9 μg/g of fresh weight of monellin with high heat stability and elevated sweet taste [35].

To conclude, biotransformation studies of monellin will be carried on to find sustainable solutions for broad applications of the component in food manufacturing. As monellin possesses a zero glycemic index, it can be applied to the diets of diabetic people [36]. Additionally, any adverse effects of monellin have not been reported for food applications thus far [37]. Thus, the compound may find varied applications in food processing forthcoming.

2.6. Thaumatin

The arils of the African species *Thaumatococcus daniellii* Bennett include the sweet-tasting thaumatin proteins; the amount of thaumatin in a ripe fruit is about 30–55 mg/g of fresh weight [38]. The sweetness level of thaumatin is 3000 times more than sucrose without caloric values [39].

The sweetness characteristics of thaumatin attract researchers to find alternative production ways due to sustainability issues. Therefore, biotransformation studies of thaumatin exhibit promising results for future implementations. Thus far, thaumatin gene expressions were made in rice [40], strawberry, barley, tomatoes, potatoes [41], cucumber, and pear to enhance the taste of fruit and vegetables [19]. Hence, plant gene expression studies of thaumatin demonstrate advantages such as low toxicity and a rise in economical incomes.

On the other hand, biotransformation of thaumatin by bacteria and fungi provides much faster growth, control of the pathway, and a high yield of thaumatin [42]. For instance, *E. coli* is the most used bacteria for protein expression due to well-understood genomics. However, the production of thaumatin via *E. coli* has supplied low amounts of total thaumatin [43]. Faus et al. [44] applied synthetic genes of *E. coli* to express thaumatin proteins, and the study provided a similar molecular weight with original thaumatin. Following those studies, in 2000 Daniell et al. [45] achieved producing about 40 mg of pure thaumatin with similar sweetness characteristics of the original compound. Nevertheless, the disadvantage of *E. coli* is that it is toxic with by-products [41]. For this reason, *Lactococcus lactis*, which has been recommended for the gene expression of thaumatin, has been approved as GRAS [46].

Moreover, thaumatin was produced also by yeast, where the yield was about 100 mg/L [19], and *Pichia pastoris* is a good example for commercial thaumatin production without toxins [41]. Hence, thaumatin might be utilized for varied food products with forthcoming sustainability features due to biotransformation studies.

3. Conclusions

Natural sugar substitutes promote activities against obesity, type 2 diabetes, and cardiovascular diseases. Forthcoming, we may see many more applications of natural sugar replacers with wide utilities. However, huge interest in natural sugar replacers may create extinctions of the sources of sugar substitutes. Therefore, biotransformation studies may bring solutions for issues related to sources of natural sugar substitutes, and with extra advantages, such as how biotransformation creates fewer environmental issues and is more sustainable for production [47].

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