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 REVIEW

Phytochemistry and Alternative use of Sweeteners in Metabolic Diseases

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Abstract. The plants native to tropical climates and even southern Africa (5/8) are considerable, which served as a matrix for the isolation and identification of natural sweeteners. These compounds of plant origin have become essential in many fields. From the agronomic industry to the pharmaceutical industry, their use is aimed at combating the supply of glucose and additional calories to **consumers** who wage a fierce fight against metabolic diseases. In this context, that vascular plants capable of harboring new molecules with similar sweet principles are of interest to researchers. Indeed, these molecules of global interest mainly belong to the terpene, flavonoid (phenolic) and protein structural classes, but are mainly of protein nature (7/9) whose use would not promote an increase in calories or blood sugar in the subjects who would use them. Therefore, this review aims to understand the information regarding, the phytochemistry and the impact of the use of these sweeteners on those affected by food conditions such as diabetes and obesity. All with the aim of finding other sources of sweeteners in our Burkinabe ecosystem, in order to provide local relief to the affected populations.

Key words: Sweetener, protein, glycaemia, calories, obesity, diabetes.

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Introduction

Sweeteners refer to a substance other than sugar, of natural or synthetic origin, used as a food additive. Their sweetening power is defined in relation to that of sucrose. A sweetener must have as characteristics; a sweet flavor without leaving an aftertaste, playing the same functions as the sugar it is supposed to substitute while having the lowest possible caloric load, to be chemically stable and physiologically inert, to be non-toxic, to provide the same tastes and appearances on the finished product as traditional products to be adopted more easily by the consumer. Sweetener molecules are used as an alternative to the consumption of sugar as a food, or drug excipient, by those affected by the thorny issue of metabolic disorders, including diabetes. They are used a lot in the management of chronic pathologies or nutritional disorders such as diabetes, hypertension, obesity [1] [2]. According to Jean-Michel Lecerf, these intense sweeteners reduce the carbohydrate content and at the same time the calorific intake of food while providing a taste similar to that provided by sucrose [3]. These molecules are of natural origin, extracted from plants and animals. Nowadays many of them are produced in the chemical industries by synthesis or in genetic engineering laboratories by biosynthesis.

According to, Amouyal and Andeelli, sweeteners are compounds that impart a sweet taste after binding with receptors in the lingual mucosa. There are different categories of sweeteners. The best known are the intense sweeteners which have a sweetening power much greater than that of sucrose (approximately 30 to 500 times more if sucrose is taken as a reference and has a sweetening power of 1). The sweeteners concerned are saccharin, cyclamates, aspartame, sucralose and rebaudioside (or Stevia). Nutritional sweeteners have a limited sweetening power compared to that of sucrose (1.5 times). Among these, mention may be made of the best known of the polyols which are sorbitol, xylitol and mannitol. Sweeteners are part of our daily lives as their use has become widespread in the food and pharmaceutical industry. [4].

Many proteinaceous, terpenoid and phenolic compounds derived from sweet vascular plants of tropical origin are prototypes of biosynthetic and chemically synthesized sweeteners. Today, some sweeteners are accepted in some and some are not. Also, others are accused of being responsible for metabolic diseases [5]**.**

The present study provides a general review of the natural intense sweetening molecules available today and particularly those extracted from plants. Also, this review will focus specifically on the phytochemistry of these sweeteners, their impacts on metabolic disorders, on cariogenic bacteria and on the behavior of people who consume them.

Today, sweeteners which are molecules with a low energy value are used by people with diabetes and also by people who are overweight.

Methodology

Scientific literature was collected using bibliographic search engines such as PubMed, Science Direct, Scopus and Google Scholar. The bibliographic survey covered the period 1969 to 2015. The keywords and expressions used were: artificial sweeteners; Glycemic control; intense sweeteners; sweetener and metabolism disorder; non-caloric sweeteners, physicochemistry of sweeteners, sweeteners and cariogenic bacteria, impact of sweeteners on behavior, plants and traditional knowledge of sweeteners. This methodology allowed us to collect data through sixty-four references.

Phytochemistry of sweeteners

It alludes to the study of the structure, metabolism, as well as methods of analysis, extraction and purification of natural sweetening molecules from plants such as terpenoids, phenolics and proteins.

Protein chemical class compounds

Pentadine and brazzeine are both sweet active ingredients extracted from the pulp of the fruit of pentadiplandra brazzeana which illustrates their name successively [6]. These sweet ingredients were isolated by extraction in water, followed by ultrafiltration and gel filtration. Subsequently, a characteristic analysis of the absorption spectrum with ultraviolet rays coupled with a positive staining reaction with the Coomassie blue of their amino acids made it possible to specify the protein nature of these sweet ingredients [7]. Regarding the characteristics of brazzéin, its taste has been estimated to be 2000 times sweeter than that of sucrose diluted to 2%. This taste would be closer to that of saccharin and thaumatin, both successively artificial and natural intense sweeteners. The 54 amino acid residues identified in the primary structure of brazein thus give it a molecular mass of 6473 Da [8]. Their calorie intake is therefore equivalent to proteins of identical mass and of zero glycemic power, because they are not carbohydrate. Brazein which has been identified as a monomeric protein and the smallest of the sweet tasting proteins already identified, appears to be the basic sound structure of pentadine which is a dimer made up of two units of brazzein [9]. Its sequence having already been identified (appendix 1) [10], brazzéin in terms of chemical and physical property remains stable in a wide pH range from 2.5 to 8 and withstands high temperatures up to 98 ° C. for 2 hours and 80 ° C over 4 hours. Its solubility in water has been shown to exceed 50 mg / ml [11][6].

Curculin: It is a proteinaceous molecule with a sweet taste and was extracted from the fruit of Molineria latifolia a Hypoxidaceae. Curculin is a homodimer in which the two monomers of 114 amino acids and of molecular weight 12.5 kDa each are linked by two disulfide bridges. Indeed, this sweet principle was extracted using 0.5 M NaCl from the fruits of Curculigo latifolia and purified by fractionation with ammonium sulfate, followed by ion exchange chromatography on CM-Sepharose and then by gel filtration. The complete sequence of these amino acids was determined by automatic Edman degradation [12]. The sweetening power of curculin is difficult to assess because it varies with the concentration of the latter and the pH of the solution. However, it was estimated to be between 430 and 2070 [12]. Moreover, its sweet flavor seems to be reduced in the presence of divalent cations (Ca2 + and Mg2 +) in solution and at neutral pH. However these do not seem to have any effect on the sweetness enhancing property of curculin in acidic solution. [13]. This protein is degraded from 50 ° C. It can therefore only be used in products having a temperature below 50 ° C to give it its expected sweetness. [13].

Mabinlines, they are sweet tasting proteins extracted from Capparis masaikai Levl. At present, four homologous proteins have been identified there and named mabinline-1, 2, 3, and 4. Mabinline-2 was the first to be isolated and characterized by Z Hu and M He in 1983 and thereafter. by X Liu et al in 1993 [14].

Indeed, mabinlin-2 was extracted using a 0.5 M NaCl solution from the seeds of Capaparis masaikai Levl. And purified by ammonium sulfate fractionation followed by

ion exchange chromatography on carboxymethylcellulose-Sepharose followed by gel filtration.

The molecular weights of Mabinline-1, Mabinline-3 and Mabinline-4 are, respectively, 12.3 kDa, 12.3 kDa and 11.9 kDa [15]. With a molecular weight of 10.4 kDa, mabinline-2 is the lightest. Its tertiary structure consists of the assembly of 2 protein chains, A and B, linked by 2 intermolecular disulfide bridges. The amino acid sequences of the A chain and the B chain were also determined by Edman's automatic degradation method. The two chains consist of residues of 33 and 72 amino acids, respectively [16]. The protein is the most heat resistant compared to its 3 homologues but also one of the most stable compared to other known sweetening proteins [17], and this is due to the presence of disulfide bonds. According to Y. Kurihara [15], the differences in heat stability are due to the presence of arginine or glutamine at position 47 on the B chain. The sweetening power of mabinline-2 has been estimated at 100 (at equal weight) and 375 (at equal concentration [18]) is significantly less than thaumatin but with a similar sweetening profile [19]. The homologous mabinlines (-1, -3 and -4) have a sweetening power equivalent to mabinline-2. The sweetening power of mabinline-2 remains unchanged after incubation at 100 C for 48 hours [18]. The four proteins identified have a similar tertiary structure (2 chains linked by disulfide bridges) whose amino acid sequences are practically identical [20][21][22][23].

Monellin is a sweet tasting protein extracted from Dioscoreophyllum volkensii (En) [24]. Using conventional methods of protein purification by precipitation in the presence of inorganic salts, followed by dialysis and ion exchange chromatography on substituted celluloses, a protein from the fruit of *Dioscoreophyllum cumminsii* could be obtained which is homogeneous. by disc gel electrophoresis [25]. It is 800 to 2,000 times sweeter than equal weight sugar, and resistant to heat. Monellin has a molecular weight of 10.7kDa, similar in size to mabinlines (12 kDa) [24]. Monellin is a heterodimer, with monomer A composed of a sequence of 44 amino acids linked by a covalent bond to monomer B composed of a sequence of 50 amino acids [26]. The secondary structure of monellin is made up of five antiparallel beta sheets and an alpha helix comprising 17 amino acids. In its natural form, monellin does not have a disulfide bridge, which makes it thermolabile and not very stable in an acidic environment [27] [28][29].

Thaumatin, or more generally thaumatin, designates a family of proteins with a sweet taste. Thaumatin refers to the mixture of two proteins. Thaumatin I and II, predominant in the seeds of the fruit of *Thaumatococcus danielli* (Benth) [30] also called katemfe [31]. Indeed, thaumatin I can be purified from an extract of the fruit pulp by ion exchange chromatography followed by filtration by gel chromatography [31]. Thaumatin is the natural sweetener with a sweetening power estimated between 2000 and 3000 times sweeter than sucrose (table sugar) at equal weight [30]. Thaumins I and II, predominant in the seeds, have a molecular weight of 22kDa, which is twice as large as the sweetening proteins mabinlines (12kDa). They are made up of a chain of 207 amino acids.

They are very similar and only differ on 5 amino acids: in position 46 (N instead of K), 63 (S instead of R), 67 (K instead of R), 76 (R instead of Q) and 113 (N instead of D). Also, they have 4 alpha helices and 11 beta strands. The peptide chain contains 8 internal disulfide bridges, responsible for its remarkable heat stability. In terms of tertiary structure thaumatin consists of three distinct domains including 11 beta strands in the beta

sheet for domain I; a larger region rich in disufure bridges for domain II; a region rich in disufures bridges for domain III. Thaumatin is very stable to heat in an acidic medium, but it is not in an alkaline medium [30]. Indeed, after 4 hours spent at 80 ° C and pH 2, or at pH 4 for 2 hours, the protein is still sweet. On the other hand, after 15 minutes at pH 7, still at 80 ° C, the protein lost its sweet taste [31]. The protein is very soluble in a wide variety of liquids and solvents. It is very soluble in water, up to 20% (mass percentage) at pH 3 [32]. It is soluble up to 5% by mass in 60% ethanol and propylene glycol[33]. Dissolution in more concentrated ethanol (up to 90%) is also possible when the protein is pre-hydrated. The protein is also soluble in glycerol and sugar alcohols [34]. However, it is insoluble in acetone [35]. Once consumed, thaumatin is metabolized and ingested by the body as amino acids [36]. It thus produces 4 kcal / g like any other protein; however, the calorific intake is negligible given its very low level of use (0.5 to 10 ppm) in food [37] with a negligible glycemic index. The primary amino acid sequences of the sweetening proteins Thaumatin I (TI) and thaumatin II are identified [38]: Miraculin is a protein that has the unusual property of changing sour taste into sweet taste. The complete amino acid sequence of miraculin purified from miracle fruits was determined by Edman's automatic degradation method [39]. Miraculin was extracted using 0.5 M NaCl solution. The extracted solution is colorless and shows strong inducing activity. It was purified from the extracted solution by ammonium sulfate fractionation, ion exchange chromatography on CM-Sepharose and affinity chromatography on concanavalin A-Sepharose. The miraculin thus purified gives a single sharp peak in reverse phase high performance liquid chromatography, indicating its very high purity. The same sample also gives a single band having a molecular weight of 28,000 Da in sodium dodecyl sulfate-polyacrylamide gel electrophoresis [39]. Miraculin is a single polypeptide with 191 amino acid residues. Its molecular weight calculated on the basis of its amino acid sequence and its carbohydrate content (13.9%) is of the order of 24,600 Da. The amino acids at position Asn-42 and Asn-186 are linked to carbohydrate chains by an N-glycosidic bond. There is a strong homology between the amino acid sequences of miraculin and the soybean trypsin inhibitor [40]. Sequence analysis of the purified miraculin indicated that it is composed of a single pure polypeptide and of which the 20 amino-terminal amino acids have been identified. These carbohydrates consist of glucosamine, mannose, galactose, xylose and fructose in a molar ratio of 3.03: 3.00: 0.69: 0.96: 2.12 [39]. It can be noted that the studies by S. Theerasilp et al do not clearly establish the primary structure of miraculin.

The sweet compounds of the terpenoids type that are the glycoside diterpenes of the type Steviosides rebaudiosides A on the one hand.

These are heterosides or major sweetening chemical molecules extracted from the leaves of *Stevia rebaudiana* Bertoni, a plant of the Asteraceae family [41]. two osidic groups; a glucose unit on the hydroxyl group 19 and a glucose disaccharide (sophorose) on the hydroxyl group 13. Its semantic name is therefore 19-O-β-D-Glucopyranosyl-13-O- [β-D-glucopyranosyl (1 → 2) - β-D-glucopyranosyl] -steviol. It has a sweetening power of between 250 to 300 times greater than sucrose [41]. Stevioside is heat stable, acidic and basic in pH, soluble in water (5). The acceptable daily intake for rebaudioside A is based on that of steviol, i.e. 0-4 mg / kg body weight (7). Its crude chemical formula would correspond to C38H60O18 with a molar mass of 804.8722 ± 0.04 g / mol

representing C 56.71%, H 7.51%, O 35.78% [42]. Its melting temperature was evaluated at 198 ° C with estimated solubility of 1250 mg / l in water[43]. On the other hand, an ammoniated derivative of the triterpene glycoside of the oleanane type, which is glycyrrhizin (glycyrrhizic acid) is the compound conferring a sweet taste, of the extract of Liquorice which is a plant still called Glycyrrhiza glabra of the family of legumes or Fabaceae, of the subfamily of Faboideae whose roots, rhizomes and stolons are used in traditional medicine [44]. Glycyrrhizic acid is a heteroside, the aglycone part of which (an acidic terpene) is linked to an acidic acid which is dimeric of glucuronic acid. The semantic name is: (3-beta, 20-beta) -20-carboxy-11-oxo-30-norolean-12-en-3-yl2-O-beta-D-glucopyranuronosyl-alpha-D-glucopyranosiduronic. Glycyrrhizic acid is not specially soluble in water, but its ammonium salt is in water with a pH above 4.5. The sweetening power of glycyrrhizic acid is 30 to 50 times greater than that of sucrose at equal weight. However, its sweet profile is different from that of sugar, the sensation of sweetness is later in the mouth and is persistent with a characteristic aftertaste [45]. The active ingredients extracted from the roots of licorice are glycyrrhizic acid and glycyrhizic acid ammonium salt by the HPLC method. The chemical formula and the molecular weight of glycyrrhizic acid are respectively $C_{42}H_{62}O_{16}$ and 822.9321 ± 0.0427 g / mol for percentages of C 61.3%, H 7.59%, O 31.11%, as well as its melting temperature of 220 ° C [46][45][47][48].

Sweetener and metabolic disorders (Cancer, diabetes and cardiovascular disease)

Most intense sweeteners are protein or steviol in nature which are non-energetic will not inherently provide calories [49], but the use of these sweeteners to provide palatability to already high-energy foods does not. will not contribute favorably to the fight against obesity and increases in plasma glucose levels in subjects who abuse it [49] [50]. This same observation is established with intense artificial sweeteners and polyols [49]. In addition, fructose, which is said to have a low glycemic index, is used to maintain sweetness while limiting hyperglycemia and energy intake [51] [52]. In general, these sweeteners have no documented benefit in diabetic and obese patients; because most of the studies make case of their combined use as additives in products themselves already very caloric. The ideal would be to consider prospective studies randomized with these sweet active ingredients only to be able to better locate their implication in the metabolic disorder.

Sweeteners and cariogenic bacteria

The episodes of dental caries are attributed to acids resulting from the breakdown of sugars ingested during meals by cariogenic bacteria. These acids produced contribute to the degradation of dental plaque by demineralizing the calcified tissues of the tooth. Thus, the use of sweeteners that are not a substrate for these bacteria would prevent this damage to the teeth [53]. In addition, polyols would be a weapon in the prevention of dental caries [53]. In fact, dental caries is a multifactorial infection involving the ingestion of fermentable carbohydrates, the presence of cariogenic bacteria and the patient's ability to defend themselves, particularly against the acidity produced by these cariogenic bacteria [54] [55]. Thus, sugars would participate directly in the development of cariogenic dental plaque while promoting the co-aggregation of bacteria with a favorable prognosis for the

development of caries in proportion to the amount of sugar ingested [54]. Sugar is therefore at the beginning of the chain of the occurrence of dental caries. To fight against this infection it is prudent and imperative to establish barriers in the availability of the substrate for cariogenic bacteria, by removing that by brushing or by replacing it with non-fermentable substitutes such as sweeteners [56] [57].

Impact of sweeteners on behavior (mood, short- and long-term memories)

The sense of taste and the sense of smell is one of the most important senses involved in perceiving food. The sense of taste is dedicated to evaluating the nutritional content of foods. Detection of sugary molecules and amino acids helps identify energy-rich nutrients [58]. The sweet taste being for most men a source of pleasure. It has been reported that these substances, which include sweeteners, are nutritionally active and have an impact on the metabolism. A study comparing sugar and sucralose by functional cerebral imaging shows that the pleasure of sweet taste is very dependent on the activation of the left insula for the two substances. [4]. Indeed, sugar would induce a stronger activation of the anterior part of the insula, the striatum and the dopaminergic areas. With regard to behavioral modification, we observe a Pavlovian reflex more than a precise physiological mechanism. [59] [60].

In recent decades, the use of sweeteners has experienced a meteoric rise, especially with the concerns that nutritional diseases have caused among populations. Changes in the use and consumption patterns of sugar substitutes are seen around the world. Indeed, people who experience a suspension of consumption of table sugar have the possibility of finding the sweet taste in their food. This is not without consequences for the eating habits of these people, who for a large part end up having an addiction to the products they use as additives and who are however initially very hyperglycemic and caloric. It is in this sense that obesity is thought to be a heterogeneous clinical entity that results from the interaction of biological determinants, mainly but not exclusively genetic, behavioral and environmental [61]. This is why the dietary approach should favor behavioral aspects, ensure that the caloric density of the diet is reduced [61]. Especially those which enhance their sweet taste with sweeteners.

Plants and traditional knowledge of sweeteners

Some plants have always been used to enhance the sweetness of food in traditional societies around the world. This is how many South American populations incorporated the use of plants to sweeten food into their dietary habit. Indeed, the leaves of Bertoni stevia rebaudiana were used to sweeten drinks by the Indians of Paraguay. Subsequently, its leaves were used to produce natural sweeteners (steviol glycosides), non-nutritive and non-toxic, but with very strong sweetening power, capable of replacing sugar and other artificial sweeteners [62].

In West Africa, plants have traditionally been used by indigenous people to enhance the sweetness of their food. Indeed, the fruit of *thaumatoccocus denielli* was used by the populations of West Africa to improve the taste quality of sour fruits and palm wine. People in Ghana, Côte d'Ivoire, Togo, Sierra Leone and Nigeria have been cultivating the fruit and using its seeds for decades to sweeten foods and drinks. [29]. Thaumins were subsequently extracted [30] [63]. The fruit of *pentadiplandra brazzeana* is part of the

eating habits of monkeys in Gabon and is known and consumed by local people for its extremely sweet taste [29]. Pentadine and brazein were subsequently extracted there [64] [65]. The red fruit of *Synsepalum dulcificum* or Richadella dulcifica has been used in West Africa to improve the taste of acidic foods. From this fruit, miraculin was extracted [66]. As yet documented uses of several organs of certain plants by indigenous people in Burkina Faso have been reported. This is how runners of Imperata cylindrica and stolons of Abrus precatorius L. are used to enhance the sweet taste of food.

Conclusions

Nutritional pathologies are one of the major public health challenges around the world today. The research community being a bulwark to find solutions to the challenges of the moment is called upon to research new natural molecules to facilitate the management of these pathologies. Thus, many sugar substitutes have been indefied to play this role. Our investigation confirms that the southern African region is a hope for the search for new sources of active sweet ingredient involved in the management of these metabolic pathologies. Most of these identified sweet molecules are protein in nature and therefore low in calories and non-glycemic.

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