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Microbial hydrolysis of steviol glycosides

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ABSTRACT

A review of the role of gut microbiota in the metabolism of the steviol glycosides, stevioside and rebaudioside A, indicates that they are not absorbed intact but undergo hydrolysis by the intestinal microflora to steviol. Steviol is not metabolized by the intestinal flora and is absorbed from the intestine. The rate of hydrolysis for stevioside is greater than for rebaudioside A. Recent studies using mass spectrometry have shown that steviol-16,17-epoxide is not a microbial metabolite of steviol glycosides. Bacteroides species are primarily responsible for hydrolysis via their β-glucosidase activity. Fecal incubation studies with both human and animal mixed flora provide similar results, and this indicates that the rat is an appropriate model for studies on steviol glycosides. Given the similarity in the microbial metabolism of stevioside and rebaudioside A with the formation of steviol as the single hydrolysis product that is absorbed from the intestinal tract, the toxicological data on stevioside are relevant to the risk assessment of rebaudioside A.

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1. Introduction

Stevia glycosides are a group of intensely sweet compounds that includes stevioside and rebaudioside A. Stevioside has been subject to extensive safety studies that have been evaluated by national and international regulatory agencies. In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2005) assessed the toxicology data on stevioside and allocated a temporary acceptable daily intake (ADI) for steviol glycosides of 0–2 mg/kg body weight expressed in terms of steviol equivalents. The ADI was expressed as steviol equivalents since this is the common potentially toxic metabolite of the different glycosides, which can exist in different proportions in different stevioside preparations.

In principle, the JECFA temporary ADI expressed as steviol equivalents applies to rebaudioside A providing that the safety data on stevioside can be extrapolated to this structurally-related glycoside. The comparative metabolism of stevioside and rebaudioside A to steviol is therefore critical to the use of data for stevioside in the safety assessment of rebaudioside A. This paper reviews the published data on the metabolism of these glycosides by the intestinal microbiota and the rates and extents of formation of steviol.

2. The role of the gut microbiota in the metabolism of stevioside and rebaudioside A

2.1. Inherent enzyme activities in the intestinal microbiota

The intestinal microbiota represent an important site of possible metabolism for compounds which are poorly absorbed form the gut, or which are excreted into the gut via the bile. The composition of the intestinal microflora shows differences between host species, both in the numbers of organisms present, their distribution along the gastrointestinal tract and their metabolic potential. In addition, the composition and metabolic activity of the gut microbiota can be influenced by the nature of the diet.

Rowland et al. (1986) compared the metabolic activity of the hind gut contents of experimental animals and humans for a range of substrates and metabolic reactions, and concluded that it may not be valid to extrapolate the results of bacterial metabolism studies across host species. Their work clearly showed that no single mammalian species showed a quantitatively similar overall pattern of metabolism to that found with human feces. However, Rowland et al. (1986) found similar β-glucosidase activity in the cecal contents/feces of rats, mice and humans. This is not surprising because β-glucosidase activity has been reported for most of the major groups of intestinal organisms (Hawksworth and Hill, 1971) such as enterococci, lactobacilli, clostridia, bacteroides and bifidobacteria. These organisms represent the major types of

Abbreviations: ADI, acceptable daily intake; FAO, Food and Agriculture Organization of the United Nations; g, gram; h, hour; HPLC, high performance liquid chromatography; JECFA, Joint FAO/WHO Expert Committee on Food Additives; kg, kilogram; LC–DAD-MS, liquid chromatography/diode array detection mass spectrometry; LC/MS/ESI, Liquid chromatography/mass spectrometry/electrospray ionization; mL, milliliter; UV, ultraviolet; WHO, World Health Organization.

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bacteria in the gastrointestinal tract of most species (Hawksworth and Hill, 1971) and although there are species differences in the numbers of bacteria in different parts of the intestine, there are similar numbers of enterococci, lactobacilli, bacteroides and bifidobacteria in the large intestine of rats, mice and humans. Not all strains of a particular type of bacterium show the same activity (Hawksworth and Hill, 1971); for example, only two out of nine strains of bifidobacteria studied were able to hydrolyze sennoside B (Akao et al., 1994). Both α- and β-glucosidase activities were higher than β-glucuronidase for all types of intestinal bacteria, apart from enterobacteria, and these activities would be even more uniform across species than β-glucuronidase, which is recognized as a common intestinal hydrolytic reaction. In consequence, the basic intestinal metabolic activity towards glucosides would be similar in rats, mice and humans, and data from the two rodent species would be relevant to humans. This conclusion is supported by the studies of Tamura et al. (1980) who showed that there were similar glycosidase activities towards a variety of xenobiotic glycosides present in enzyme preparations from human feces (fecalase) and rat cecum (cecalase) (these preparations were being proposed for *in vitro* bioactivation of chemicals to mutagens comparable to a S9 mix, which is a cell-free system prepared from the livers of rats pre-treated with enzyme inducers).

2.2. Hydrolysis of stevioside and rebaudioside A by the intestinal microbiota

Wingard et al. (1980) showed that the glycosidic sweeteners stevioside and rebaudioside A are degraded to the diterpenoid aglycone steviol by rat intestinal microflora *in vitro*. The aglycone, steviol, was virtually completely absorbed from the lower bowel of the rat after oral or intracecal administration, and is eliminated by biliary excretion. Stevioside was completely transformed into steviol within 2 days on incubation with whole-cell suspensions, while similar conversion of rebaudioside A required 6 days. Hydrolysis by cell-free extracts was much slower with steviol being generated from stevioside and rebaudioside A in yields of only 50% and 2%, respectively, after 7 days. These results indicate that both stevioside and rebaudioside A are degraded to steviol by microbial action in the mammalian lower bowel. The rebaudioside A to steviol degradation rate is in the range of 0.4–0.8 mg converted/h/g cecal contents. The flora of the rat cecum and the lower human bowel are quantitatively and qualitatively similar, and the metabolic fate in humans can be predicted to be similar to that in rats. The human large bowel contains about 10^{12} organisms/g fresh weight and has an average total weight of 1 kg (Steer et al., 2000). From these data, it was estimated that *in vivo,* the human bowel could hydrolyze over 0.4 g rebaudioside A to steviol per h (Wingard et al., 1980).

In vitro work by Hutapea et al. (1997) and Ishii-Iwamoto and Brache (1995) demonstrated that the digestive enzymes α-amylase, pepsin and pancreatin, and hepatic tissue from isolated perfused rat liver were unable to hydrolyze stevioside or rebaudioside A. In contrast, these glycosides were hydrolyzed to their aglycone steviol by rat intestinal microflora (Hutapea et al., 1997; Wingard et al., 1980). Unlike other studies, Hutapea et al. (1997) reported that stevioside was metabolized to steviol (89%) and also to steviol-16,17-epoxide (13.5%) after 48-h incubation, and that the steviol-16,17-epoxide was reduced to steviol by human intestinal microflora after 96 h of incubation under anaerobic conditions. The formation of an epoxide by the anaerobic intestinal microflora is an unlikely reaction, while the time course for its formation and subsequent loss would require that nearly all stevioside is initially converted to the epoxide, which is then reduced more slowly to steviol. This reported reaction is discussed further below.

The most comprehensive study on the hydrolysis of steviol glycosides by the human intestinal microbiota was conducted by Gardana et al. (2003) who investigated (i) the *in vitro* transformation of stevioside and rebaudioside A after incubation with human microflora, (ii) the identity of organisms able to metabolize stevioside and rebaudioside A, and (iii) the influence of these sweeteners on the human fecal microflora.

The experiments were carried out under strict anaerobic conditions in batch cultures inoculated with mixed fecal bacteria from 11 healthy human volunteers (six men and five women), ages 20–50 years. Feces from five subjects were tested with stevioside or glucose, and feces from six subjects were tested with rebaudioside A or glucose. Forty milligrams of stevioside or rebaudioside A were added to 40 mL of the fecal suspensions under agitation, and in order to supply the same sugar quantity derived from these two compounds, 27 or 30 mg of glucose were also incubated under anaerobic conditions for 72 h. At fixed intervals (1 h) and for 48 h, 0.5 mL of fecal suspensions from both the test compounds and glucose controls were transferred into 0.5 mL of methanol to stop microbial action and then analyzed by HPLC coupled to photodiode array and mass spectrometric detectors. Stevioside was completely degraded to its aglycone steviol after 10 h incubation with human intestinal microflora. Hydrolysis proceeded via formation of steviolbioside, the concentration of which peaked after 2–4 h of incubation, and then decreased rapidly to zero. Steviol was detected only after 3–4 h of incubation, and subsequently, its concentration increased rapidly. Initial hydrolysis to steviolbioside suggests that the *j3* 1:19 bond is hydrolyzed more rapidly than the α 1:13 linkage. Rebaudioside A was also completely metabolized to steviol by human microflora, but a longer time was required (24 h): steviolbioside was detected after an initial lag phase of 6–7 h, reached a peak concentration at 12–15 h, and was then rapidly converted to steviol. Steviol, the final microbial metabolite of stevioside and rebaudioside A, remained unchanged during 72 h incubation with human microflora, indicating that bacterial enzymes are not able to cleave the steviol structure.

Hydrolysis studies have also been performed on a mixture of stevia glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni, consisting mainly of stevioside and rebaudioside A (Koyama et al., 2003). The study used LC/MS/ESI to investigate the *in vitro* metabolism by the human intestinal microflora of stevia mixture, enzymatically modified stevia, stevioside, rebaudioside A, x-monoglucosylstevioside, x-monoglucosylrebaudioside A and the aglycone, steviol. The compounds were incubated under anaerobic conditions for 0, 8 and 24 h with pooled human fecal homogenates obtained from five healthy volunteers. Stevia mixture, stevioside and rebaudioside A (0.2 mg/mL) were completely hydrolyzed to steviol within 24 h, whereas no degradation of steviol (0.08 and 0.2 mg/mL) occurred during the incubation period. The hydrolysis of rebaudioside A by human fecal homogenates tended to be slower in the presence of other components (stevioside, rebaudioside C) than in their absence, suggesting competition for the same enzyme(s). The metabolism of rebaudioside C (which has a (1 > 2)-linked rhamnose on the 13-position) was more rapid than that of rebaudioside A (which has (1 > 2)-linked glucose at 13-position) when both compounds were present in a stevia mixture. Therefore, there is similar metabolism of steviol glycosides by human and rat gut microflora.

Epoxide formation reported by Hutapea et al. (1997) was not detected by either Gardana et al. (2003) or Koyama et al. (2003), and the latter study provided two possible explanations. Firstly, it is possible that the experimental protocol of Hutapea et al. (1997) was not sufficiently anaerobic, allowing oxidation of the steviol to occur: some aerobic organisms, especially soil organisms, can oxidize organic compounds, but usually when the compound is present as the sole source of carbon. A second, and more likely

Table 1
Stevioside ($n = 5$) or rebaudioside A ($n = 6$) hydrolysis by isolated human fecal bacterial groups

Sample	Incubation time (h)	Stevioside incubation (% present)			Rebaudioside A incubation (% present)		
		St[a]	Sv[a]	Sb[a]	Ra[a]	Sv[a]	Sb[a]
Blank[b]	24	100.0 ± 0.7	0.0	0.0	102.1 ± 0.8	0.0	0.0
	48	100.1 ± 0.2	0.0	0.0	99.9 ± 1.0	0.0	0.0
Bacteroidaceae	24	60.9 ± 2.5	14.5 ± 1.9	9.5 ± 3.1	82.6 ± 6.6	16.8 ± 4.4	6.2 ± 2.7
	48	26.0 ± 3.9	61.6 ± 6.8	18.0 ± 4.3	0.0	73.4 ± 4.9	30.9 ± 3.2
Bifidobacteria, clostridia coliforms enterococci lactobacilli	24	99–105	0.0	0.0	97–103	0.0	0.0
	48	91–104	0.0	0.0	100–104	0.0	0.0

Data adapted from Gardana et al., 2003. Stevioside or rebaudioside A were studied at added concentrations of 40 mg in 40 mL of fecal suspension.
[a] St, stevioside; Sv, steviol; Sb, steviolbioside; Ra, rebaudioside A.
[b] Blank: media + St or Ra. St: $n = 5$. Ra: $n = 6$.

explanation, is that HPLC-UV as used by Hutapea et al. (1997) is not highly specific, so that another HPLC peak produced after incubation, possibly not even a metabolite of stevioside, might have been erroneously reported as steviol-16,17-epoxide.

2.3. Hydrolysis of stevioside and rebaudioside A by different groups of intestinal bacteria

β-Glucosidases are produced by most of the common types of intestinal bacteria of both animals and humans (Hawksworth and Hill, 1971). Quantitative, but not qualitative differences have been reported in different host species and following dietary manipulation, however, these are generally minor in extent. β-Glucosidases are constitutive enzymes in the lower gastrointestinal tract of animals and humans, and there have been no reports of the induction of unique glucosidases which are not expressed constitutively.

To evaluate which microbial groups were involved in the metabolism of stevioside and rebaudioside A, bacterial colonies grown on different selective media were separated and suspended in the incubation medium with added stevioside and rebaudioside A (1 mg/mL) (Gardana et al., 2003). The cultures of selected microbial groups, obtained after 24 and 48 h of anaerobic incubation, were analyzed by LC–DAD-MS to monitor the biotransformation of stevioside and rebaudioside A. Colonies of bacteroides, bifidobacteria, clostridia, coliforms, enterococci, and lactobacilli were isolated, cultured and incubated with stevioside and rebaudioside A. Only the bacteroides (the most prevalent of the intestinal bacteria) were able to hydrolyze either glycoside, and the extents of hydrolysis of rebaudioside A and stevioside were similar under the incubation conditions (Table 1). Only limited inter-subject variation in hydrolysis in humans would be expected *in vivo*, because bacteroides are by far the most numerous bacterial species present in the human bowel.

3. The potential for metabolic adaptation on continuous intake of steviol glycosides

Microbial adaptation caused by prior treatment with a potential substrate could be in the form of a change in the numbers of relevant bacterial species or a change in enzyme activity without change in bacterial numbers (or a mixture of both). This issue revolves around three important questions.

3.1 Would there be a metabolic advantage to the metabolizing organism (bacteroides) that would create a stimulus for adaptation?
3.2 Can β-glucosidases be induced by dietary manipulation?
3.3 Does stevioside or rebaudioside A alter the composition of the microbiota?

3.1. Would there be a metabolic advantage to the metabolizing organism (bacteroides) that would create a stimulus for adaptation?

Metabolic adaptation by the gut microbiota has been shown following the chronic administration of high dietary concentrations of cyclamate (Renwick 1985, 1986) or of monosaccharides such as sorbitol, xylitol, and xylose (Krishnan et al., 1980). The induced metabolism results in the formation of sulphur from cyclamate, which is then available to the bacteria, while monosaccharide hydrolysis provides increased availability of energy. The data for cyclamate differ from those for the monosaccharides, because there was no detectable cyclamate hydrolysis by the gut flora from untreated animals (Drasar et al., 1972; Renwick and Williams, 1972). Administration of up to 20% of the monosaccharides in the diet caused up to a 40-fold increase in monosaccharide metabolism by the cecal flora above the activity which was inherent in the microflora of untreated animals.

The sennoside hydrolyzing glucosidase produced by Bifidobacterium SEN (Akao et al., 1994) is suppressed *in vitro* by glucose and induced *in vitro* by the substrate (Yang et al., 1996); which indicates that the enzyme is expressed in order to take advantage of an available carbon source. However, this is unlikely to be of significance *in vivo* because (i) bacteriodes rather than bifidobacteria show high hydrolyzing activity with stevioside and rebaudioside A, (ii) the extensive hydrolysis of stevioside and rebaudioside A by mixed fecal and cecal cultures means that the carbohydrate units are rapidly released in the lower bowel via a mechanism not dependent on enzyme induction and (iii) the availability of various carbon sources in the lower bowel means that there would not be a large metabolic advantage to the inducing organism.

It is therefore theoretically possible that the rate of hydrolysis of either stevioside or rebaudioside A might increase during chronic administration. This would be most likely in animal studies where the amount of monosaccharide released from the test compound could represent a significant source of carbon within the lower bowel. Increased hydrolysis could result in higher peak concentrations of steviol for the same total amount released in the lower gut. In reality, a greater rate of hydrolysis of either stevioside or rebaudioside A would probably not be of major consequence in a sub-chronic or chronic toxicity test.

3.2. Can β-glucosidases be induced by dietary manipulation?

There has been considerable research on the effects of diet on the human gut microbiota, largely in relation to the causes of colon cancer. These have shown that the composition of the adult gut flora is relatively stable in terms of the numbers of the major genera such as bacteriodes present.

The enzymes involved in the degradation of polysaccharides by bacteroides isolated from human feces were all inducible when the

Table 2
Quali-quantitative values (log10 colony-forming units per g of feces (dried weight) of some isolated fecal bacterial strains grown in the presence or absence of stevioside ($n = 5$) or rebaudioside A ($n = 6$)

Fecal bacteria	t_0[a]	Blank[b]	Rebaudioside A[c]	t_0[a]	Blank[b]	Stevioside[c]
Total aerobes	7.8 ± 0.8	8.4 ± 0.7	7.7[d,e] ± 0.9	8.8 ± 1.8	8.7 ± 1.9	8.2 ± 2.1
Total anaerobes	10.1 ± 0.6	10.4 ± 0.5	10.2 ± 0.8	10.4 ± 0.4	10.2 ± 0.4	9.1[d,e] ± 0.6
Bacteroidaceae	10.6 ± 0.3	10.6 ± 0.1	10.5 ± 0.2	10.9 ± 0.4	10.6 ± 0.3	10.1[d,e] ± 0.3
Bifidobacteria	8.4 ± 2.0	8.4 ± 2.0	8.3[d,e] ± 1.7	9.1 ± 0.7	8.8 ± 0.8	8.8 ± 0.5
Clostridia	8.0 ± 0.2	7.9 ± 0.7	7.9 ± 0.8	7.0 ± 1.1	6.9 ± 1.0	6.9 ± 0.7
Coliforms	7.8 ± 0.6	8.2 ± 0.5	7.5[e] ± 0.9	7.4 ± 1.4	8.4 ± 1.8	7.3 ± 1.6
Enterococci	6.8 ± 0.6	6.5 ± 0.8	6.2[d] ± 0.8	7.3 ± 1.6	7.2 ± 1.5	6.9 ± 1.5
Lactobacilli	6.6 ± 2.0	6.4 ± 2.0	5.9 ± 1.9	7.1 ± 1.3	6.7 ± 1.4	6.3[d] ± 1.4

Data adapted from Gardana et al., 2003. Stevioside or rebaudioside were studied at added concentrations of 1 mg/mL of the incubation medium.
[a] t_0 h = microflora at time zero.
[b] Blank: (media + fecal suspension) after 24 h of incubation time.
[c] Rebaudioside A or stevioside: (media + fecal suspension + rebaudioside A or stevioside) after 24 h of incubation time. Repeated-measures ANOVA with treatment as an independent factor found that some of the differences to be statistically significant.
[d] $p < 0.05$ for rebaudioside A or stevioside vs. t_0 h.
[e] $p < 0.05$ for rebaudioside A or stevioside vs. blank.

bacteria were grown *in vitro* on specific substrates, indicating that the activity for polysaccharide hydrolysis *in vivo* will depend on the substrates available within the lower bowel (Salyers et al., 1978). Similarly, β-glucosidases can be induced in soil organisms when they are grown on cellobiose as the sole source of carbon (Busto et al., 1995).

The effects of dietary manipulation on the β-glucosidase activity of the intestinal contents of experimental animals have been reported in a number of studies. The β-glucosidase activity of the rat intestinal contents was increased by guar gum and locust bean gum (Mallett et al., 1984a) and by plant cell–wall constituents (Rowland et al., 1983), but decreased by feeding carrageenan (50 g/kg diet) (Mallett et al., 1985), high dietary fats (Mallett et al., 1984b) and apple pectin (Ohkami et al., 1995). Other enzymes such as β-glucuronidase and nitroreductase have shown small changes arising from quite major changes in diet composition (Goldin et al., 1978). The reported changes in activity have been quantitatively minor and not qualitative in nature.

3.3. Does stevioside or rebaudioside A alter the composition of the microbiota?

The influence of stevioside and rebaudioside A on intestinal microflora was tested under anaerobic conditions in batch cultures inoculated with fecal samples from healthy human subjects (Gardana et al., 2003). Bacterial groups that represented the predominant populations in the human colon were investigated by quali-quantitative microbiological analyses of the influence of the stevioside, rebaudioside A, and glucose on the human fecal microbial community. Bacterial counts were recorded at 0 and 24 h after incubation for control cultures (the medium plus fecal suspension) and the test cultures (the medium containing stevioside, rebaudioside A or glucose plus fecal suspension). The results are presented in Table 2. High inter-individual variability was observed in the counts of the subdominant microbial groups (total aerobes, clostridia, coliforms, enterococci, and lactobacilli); however, these data are in agreement with those previously reported (Finegold et al., 1971). In control cultures, the counts of the tested microbial groups after 24 h of anaerobic incubation were maintained at the initial level; this indicates that the incubation medium was effective in maintaining the fecal microbial balance and that it did not significantly stimulate the growth of any microbial group. The results on the fecal microbial composition on incubation in the presence of stevioside or rebaudioside A were substantially similar to those recorded in the control and glucose cultures. The data (Table 2) suggest that stevioside exerted a weak inhibition against anaerobic bacteria, whereas rebaudioside A showed a weak inhibitory activity on aerobic bacteria. These effects became significant ($p < 0.05$) when data from 24 h incubations of rebaudioside A and glucose were compared.

4. Conclusions

There is an extensive database on the microbial hydrolysis of stevioside and rebaudioside A, which allows confidence in several conclusions. Both stevioside and rebaudioside A undergo hydrolysis by mixed intestinal flora to steviol and the rate of hydrolysis of stevioside is slightly greater than that of rebaudioside A (Wingard et al., 1980; Koyama et al., 2003). Hydrolysis proceeds via initial formation of steviolbioside with steviol as the final product of hydrolysis. Of importance, steviol-16,17-epoxide has not been detected using specific and sensitive analytical methods.

The bacteroides are primarily responsible for the hydrolysis of either stevioside or rebaudioside A, as negligible activity was found in other major bacterial groups. Incubation of fecal samples with either stevioside or rebaudioside A did not result in biologically significant changes in the bacterial populations. Rats represent a suitable model for human metabolism of stevioside and rebaudioside A because of the overall similarities in the bacterial composition of rats and humans with respect to the importance of bacteroides and the extensive *in vitro* hydrolysis of stevioside and rebaudioside A by fecal/cecal samples from both species.

Finally, data on the toxicological effects of stevioside can be extrapolated to rebaudioside A because of the overall similarities in the metabolic fates of stevioside and rebaudioside A on incubation with the intestinal microflora with essentially quantitative formation of steviol, the comparable rates of hydrolysis, and the negligible changes in flora produced by prolonged incubation with these glycosides. Although the rate of release of steviol from stevioside appears to exceed that from rebaudioside A, suggesting that stevioside might show greater toxicity than rebaudioside A, the lack of any adverse pathological findings from sub-chronic or chronic studies of stevioside indicate that this is not of toxicological significance (Toyoda et al., 1997; Xili et al., 1992; Yamada et al., 1985).

Conflict of interest statement

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References

Akao, T., Che, Q.M., Kobashi, Y., Yang, L., Hattori, M., Namba, T., 1994. Isolation of a human intestinal anaerobic *Bifidobacterium* sp. strain SEN, capable of hydrolysing sennosides to sennidins. Appl. Environ. Microb. 60, 1041–1043.

Busto, M.D., Ortega, N., Perezmateos, M., 1995. Induction of beta-glucosidase in fungal and soil bacterial cultures. Soil Biol. Biochem. 27, 949–954.

Drasar, B.S., Renwick, A.G., Williams, R.T., 1972. The role of the gut flora in the metabolism of cyclamate. Biochem. J. 129, 881–890.

Finegold, S.M., Sugihara, P.T., Sutter, V.L., 1971. Use of selective media for isolation of anaerobes from humans. In: Shapton, D.A., Board, R.G. (Eds.), Isolation of Anaerobes. Academic Press, London, UK, pp. 99–108.

Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., Pietta, P., 2003. Metabolism of stevioside and rebaudioside A from *Stevia rebaudiana* by human microflora. J. Agr. Food Chem. 51, 6618–6622.

Goldin, B., Dwyer, J., Gorbach, S.L., Gordon, W., Swenson, L., 1978. Influence of diet and age on fecal bacterial enzymes. Am. J. Clin. Nutr. 31, S136–S140.

Hawksworth, G.M., Hill, M., 1971. Intestinal bacteria and the hydrolysis of glycosidic bonds. J. Med. Microbiol. 4, 451–459.

Hutapea, A.M., Toskulkao, C., Wilairat, P., Glinsukon, T., 1997. Digestion of stevioside, a natural sweetener, by various digestive enzymes. J. Clin. Biochem. Nutr. 23, 177–186.

Ishii-Iwamoto, E.L., Brache, A., 1995. Stevioside is not metabolized in the isolated perfused rat liver. Res. Comm. Mol. Path. 87, 167–175.

JECFA, 2005. Steviol glycosides. In: 63rd Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland. WHO Technical Report Series, vol. 928, pp. 34–39, 138. World Health Organization (WHO), Geneva, Switzerland. <http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf>.

Koyama, E., Kitazawa, K., Ohori, Y., Izawaa, O., Kakegawa, K., Fujino, A., Ui, M., 2003. In vitro metabolism of the glycosidic sweeteners, stevia, stevia mixture and enzymatically modified stevia in human intestinal microflora. Food Chem. Toxicol. 41, 359–374.

Krishnan, R., Wilkinson, I., Joyce, L., Rofe, A.M., Bais, R., Conyers, R.A.J., Edwards, J.B., 1980. The effect of dietary xylitol on the ability of rat caecal flora to metabolise xylitol. Aust. J. Exp. Biol. Med. 8, 639–652.

Mallett, A.K., Wise, A., Rowland, I.R., 1984a. Hydrocolloid food additives and rat caecal microbial enzyme activities. Food Chem. Toxicol. 22, 415–418.

Mallett, A.K., Wise, A., Rowland, I.R., 1984b. Dietary fat and cecal microbial activity in the rat. Nutr. Cancer 6, 86–91.

Mallett, A.K., Rowland, I.R., Bearne, C.A., Nicklin, S., 1985. Influence of dietary carageenans on microbial biotransformation activities in the cecum of rodents and on gastrointestinal immune status in the rat. Toxicol. Appl. Pharm. 78, 377–385.

Ohkami, H., Tazawa, K., Yamashita, I., Shimizu, T., Murai, K., Toyama, K., Fujimaki, M., 1995. Effects of apple pectin on fecal enzymes in azoxymethane-induced rat colon carcinogenesis. Jpn. J. Cancer Res. 86, 523–529.

Renwick, A.G., 1985. Intense sweeteners and the gut microflora. In: Rowland, I.R. (Ed.), Role of the Gut Flora in Toxicity and Cancer. Academic Press, New York, pp. 175–206.

Renwick, A.G., 1986. The metabolism of intense sweeteners. Xenobiotica 16, 1057–1071.

Renwick, A.G., Williams, R.T., 1972. The fate of cyclamate in man and other species. Biochem. J. 129, 869–879.

Rowland, I.R., Wise, A., Mallett, A.K., 1983. Metabolic profile of caecal micro-organisms from rats fed indigestible plant cell–wall components. Food Chem. Toxicol. 21, 25–29.

Rowland, I.R., Mallett, A.K., Bearne, C.A., Farthing, M.J.G., 1986. Enzyme activities of the hindgut microflora of laboratory animals and man. Xenobiotica 16, 519–523.

Salyers, A.A., Palmer, J.K., Wilkins, T.D., 1978. Degradation of polysaccharides by intestinal bacterial enzymes. Am. J. Clin. Nutr. 31, S128–S130.

Steer, T., Carpenter, H., Tuohy, K., Gibson, G.R., 2000. Perspectives on the role of the human gut microbiota and its modulation by pro- and prebiotics. Nutr. Res. Rev. 13, 229–254.

Tamura, G., Gold, C., Ferro-Luzzi, A., Ames, B.N., 1980. Fecalase: a model for activation of dietary glycosides to mutagens by intestinal flora. Proc. Natl. Acad. Sci. USA 77, 4961–4967.

Toyoda, K., Matsui, H., Shoda, T., Uneyama, C., Takada, K., Takahashi, M., 1997. Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem. Toxicol. 35 (6), 597–603.

Wingard, R.E., Brown, J.P., Enderlin, F., Dale, J., Hale, R., Seitz, C.T., 1980. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 36, 519–520.

Xili, L., Chengjiany, B., Eryi, X., Reiming, S., Yuengming, W., Haodong, S., Zhiyan, H., 1992. Chronic oral toxicity and carcinogenicity study of stevioside in rats. Food Chem. Toxicol. 30, 957–965.

Yamada, A., Ohgaki, S., Noda, T., Shimizu, M., 1985. Chronic toxicity study of dietary stevia extracts in F344 rats. Shokuhin Eiseigaku Zasshi 26, 169–183.

Yang, L., Akao, T., Kobashi, K., Hattori, M., 1996. A sennoside hydrolysing beta-glucosidase from *Bifidobacterium* sp. Strain SEN is inducible. Biol. Pharm. Bull. 19, 701–704.