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Resistant starch: a functional food that prevents DNA damage and chemical carcinogenesis

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ABSTRACT. Resistant starch is formed from starch and its degradation products and is not digested or absorbed in the intestine; thus, it is characterized as a fiber. Because fiber intake is associated with the prevention of DNA damage and cancer, the potential antigenotoxic, antimutagenic, and anticarcinogenic capabilities of resistant starch

from green banana flour were evaluated. Animals were treated with 1,2-dimethylhydrazine and their diet was supplemented with 10% green banana flour according to the following resistant starch protocols: pretreatment, simultaneous treatment, post-treatment, and pre + continuous treatment. The results demonstrated that resistant starch is not genotoxic, mutagenic, or carcinogenic. The results suggest that resistant starch acts through desmutagenesis and bio-antimutagenesis, as well as by reducing aberrant crypt foci, thereby improving disease prognosis. These findings imply that green banana flour has therapeutic properties that should be explored for human dietary applications.

Key words: Green banana; Antigenotoxicity; Antimutagenicity; Anticarcinogenicity; Chemoprevention

INTRODUCTION

Researchers have reported that inter-individual genetic differences are responsible for varied responses to the environment. Diet, in particular, is a factor that interferes considerably with differential gene expression because genes are exposed to various dietary influences throughout life (Mauro et al., 2011). Intestinal and colorectal cancers are closely related to eating habits and the expression of certain genes. Thus, diet, functional foods, or both, that alter gene expression might correlate with the prevention of such neoplasms (Bergmann et al., 2006). Conversely, according to Chao et al. (2005) and the World Cancer Research Fund (1997), diets that contain high concentrations of protein, including red or processed meats, animal fats, alcohol, and carbohydrates (Stevens et al., 2007), are associated with a high risk of these tumors.

The World Health Organization estimates that approximately 21 million new cases of cancer will occur in 2030 and an estimated 13 million patients will die from cancer (Instituto Nacional do Câncer, 2014). In Brazil, the total number of current cancer cases is approximately 518,510, with colorectal cancer ranking fourth (Instituto Nacional do Câncer, 2012). These data underscore the necessity of understanding the developmental stages of this disease and relating these stages to preventative mechanisms such as consuming food compounds with potential anticancer activities (Mauro et al., 2013). This strategy would be a key alternative for residents of countries with insufficient resources to address this important and annually worsening public health issue.

Nutritional re-education based on a pre- and probiotic-rich diet of healthy foods might help prevent colorectal cancer given the considerable evidence that intestinal bacterial flora are involved in the development of intestinal and colorectal tumors (McBain and MacFarlane, 2001; Burns and Rowland, 2004). Prebiotic foods such as fibers are nondigestible foods that act beneficially by selectively stimulating the growth, activity, or both, of host bacterial flora (McBain and MacFarlane, 2001) to help prevent cancer. These foods are easily accessible, are naturally present in the diet (Roberfroid, 2005), and provide prebiotic resistant starch actions.

The resistant starch present in green banana flour can be classified as a prebiotic food because, according to Asp and Björck (1992), resistant starch is the sum of starches and starch degradation products that are not digested or absorbed in the small intestines of healthy individuals, and therefore, resistant starch is characterized as a fiber.

The scientific evidence showing that fiber prevents colorectal cancers has increased scientific interest in this food compound. In this study, the potential antigenotoxic, antimutagenic, and anticarcinogenic properties of resistant starch extracted from green bananas were evaluated in an experimental model.

MATERIAL AND METHODS

1,2-Dimethylhydrazine

The DNA damage-inducing and cancer-promoting agent 1,2-dimethylhydrazine (DMH, CAS No. 306-37-6; Sigma-Aldrich, St. Louis, MO, USA) was diluted in a 0.37 mg/mL ethylenediaminetetraacetic acid (EDTA) solution and administered intraperitoneally (*ip*) to animals following a modified version of the protocol of Rodrigues et al. (2002). DMH was administered in 4 doses of 20 mg/kg body weight (bw) twice weekly for 2 weeks.

Green banana skin and pulp drying method and flour preparation

Green banana flour was prepared according to the method described by Juarez-Garcia et al. (2006). Banana samples were prewashed and then immersed in a 50 ppm sodium hypochlorite solution.

The fruits were then cut into approximately 1-cm-thick slices that were immediately immersed in a 0.3% citric acid solution for antioxidation treatment. The fruit slices were then dried in an oven at 50°C for 72 h. After drying, the samples were ground into flour, which was stored at 25°C in plastic containers for later use.

The green banana flour was added to commercial feed (Nuvital, Curitiba, PR, Brazil) at a 10% concentration as an animal dietary supplement. This mixture was offered *ad libitum* to the animals according to the protocols and treatments described in the experimental design.

Animals and experimental design

A total of 70 male Swiss mice (*Mus musculus*) of reproductive age were divided into 7 groups (N = 10 animals). Animals were housed in propylene boxes lined with wood shavings and fed either commercial or supplemented feed; filtered water was available *ad libitum*. Temperature and lighting were controlled in a 24-h photoperiod (12 h light: 12 h dark) at a temperature of 22° ± 2°C and 55 ± 10% humidity. The experiment was conducted according to the guidelines of the Universal Declaration of Animal Rights and with the approval of the Ethics Committee on Animal Use, Universidade Federal do Mato Grosso do Sul (Protocol No. 454/2012). The animals were treated for 12 weeks according to the protocol proposed by Bolognani et al. (2001) and adapted by Mauro et al. (2013), as follows:

Negative control group: Animals were fed commercial feed *ad libitum* during the 12-week experimental period. In the third and fourth weeks of experimentation, the animals were treated with 4 doses of EDTA (0.1 mL/10 g bw *ip*). After the last administration, peripheral blood samples were collected from the animals via caudal venipuncture and used for genotoxicity and mutagenicity evaluations. Mutagenicity assessments were performed with a micronucleus assay that was performed at T1, T2, and T3, which corresponded to 24, 48, and

72 h after the last vehicle administration. Genotoxicity was assessed with a comet assay only in T1 samples.

Positive control group (DMH group): Animals were treated similarly to those in the negative control group and underwent the same blood sample collection procedure. However, instead of EDTA, the animals received DMH (20 mg/kg bw *ip*).

Resistant starch group: Animals were fed *ad libitum* commercial feed supplemented with 10% green banana flour during the 12-week experimental period. In the third and fourth weeks of experimentation, the animals were subjected to the treatments described for the negative control group and underwent the same biological sample collections.

Pre-treatment group: Animals received commercial feed supplemented with 10% green banana flour during the first 2 weeks. From the third until the twelfth weeks, the animals received commercial feed. In the third and fourth weeks of experimentation, the animals were subjected to treatments and biological sample collections described for the positive control group. The protocol for this group involved evaluation of antigenotoxicity (T1) and antimutagenicity (T1, T2, and T3).

Simultaneous group: Animals received DMH (20 mg/kg bw *ip*) in the third and fourth weeks of experimentation and also received commercial feed supplemented with 10% green banana flour *ad libitum* during that two-week period. In the first and second weeks and then from the fifth to the twelfth weeks, the animals were fed commercial feed *ad libitum*. Biological samples were collected as described above for antigenotoxicity (T1) and antimutagenicity (T1, T2, and T3) evaluations.

Post-treatment group: Animals received DMH (20 mg/kg bw *ip*) treatment in the third and fourth weeks. The animals were fed with commercial feed during the first 4 weeks and were fed *ad libitum* with commercial feed supplemented with 10% green banana flour during the last 8 weeks. Biological samples were collected as described above for antigenotoxicity (T1) and antimutagenicity (T1, T2, and T3) evaluations.

Pre + continuous group: Animals were fed *ad libitum* diets supplemented with 10% green banana flour throughout the 12-week experimental period. In the third and fourth weeks, the animals were treated with DMH (20 mg/kg bw *ip*). Biological samples for antigenotoxicity (T1) and antimutagenicity (T1, T2, and T3) evaluations were collected as described above. In the twelfth week of the experiment, the animals from all groups were killed via cervical dislocation and their intestines and colons were collected and tested for aberrant crypt foci.

Comet assay

Comet assays were performed according to the procedure described by Oliveira et al. (2009) as follows. Peripheral blood (20 μL) was collected (24 h after the last dose of EDTA or DMH) into heparinized cryotubes, homogenized with 120 μL 1.5% low-melting-point agarose at 37°C, and placed onto slides coated with standard 5% agarose. The slides were covered with glass coverslips and cooled to 4°C for 20 min. After coverslip removal, the slides from all experiments were immersed in freshly prepared lysis solution composed of 89 mL stock lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris adjusted to pH 10 with solid NaOH, 890 mL distilled water, and 1% sodium lauroyl sarcosinate), 1 mL Triton X-100 (Merck, Germany), and 10 mL dimethyl sulfoxide. The samples were lysed for 1 h at 4°C with protection from light.

The slides were then transferred to an electrophoresis tank containing buffer with a pH of >13 (300 mM NaOH and 1 mM EDTA prepared from a stock solution of 200 mM EDTA

and 10 N NaOH, pH 10.0) at 4°C and incubated for 20 min for DNA denaturation. Electrophoresis was performed at 300 mA and 25 V (1.25 V/cm) for 20 min. Subsequently, the slides were neutralized with buffer (0.4 M Tris-HCl, pH 7.5) in 3 cycles of 5 min each, air-dried, fixed in absolute ethanol for 10 min, and stored for later analysis. Next, 100 µL ethidium bromide (20 µg/mL) was used for staining. The samples were evaluated at 40X magnification under a fluorescence microscope (Bioval® Model G 2000A, Brazil) fitted with a 420- to 490-nm excitation filter and a 520-nm barrier filter.

In total, 100 cells per animal were visually analyzed, and the comets were classified as follows: class 0, undamaged cells without tails; class 1, cells with tails smaller than the nucleoid diameter; class 2, cells with tails 1- to 2-fold the nucleoid diameter; and class 3, cells with tails greater than 2-fold the nucleoid diameter. Apoptotic cells and those with totally fragmented nucleoids were not counted (Kobayashi et al., 1995). The total score was calculated by adding the results from the multiplication of the total number of observed cells per injury class by the class value. Statistical analysis was performed with analysis of variance (ANOVA) and the Tukey test ($P < 0.05$).

Peripheral blood micronucleus assay

The micronucleus assay development method described by Hayashi et al. (1990) and modified by Oliveira et al. (2009) was used. Slides were coated with 20 µL acridine orange (1 mg/mL). Next, 20 µL peripheral blood was deposited onto each slide, covered with a coverslip, and stored in a freezer (-20°C) for a minimum of 7 days. Analysis was performed at 40X magnification under an epifluorescence microscope (Bioval®, Model L 2000A, Brazil) with a 420- to 490-nm excitation filter and a 520-nm barrier filter. A total of 2000 cells/animal were analyzed, and statistical analysis was performed with ANOVA and the Tukey test ($P < 0.05$).

Testing of aberrant crypt foci

The collected mouse colons and rectums were washed in a physiological buffer solution and then dissected for subsequent fixation in 10% buffered formalin for a minimum of 24 h. For analysis, each colorectal segment was stained with 10% methylene blue solution for 10 min and placed on a slide mucosal side up. Analysis was performed at 10X magnification using a light microscope. The entire mucosa was evaluated to identify and quantify aberrant crypt foci. Aberrant crypt foci were identified using the criteria described by Bird (1987) and modified by Mauro et al. (2013) as follows. (I) In foci with a single crypt, the aberrant crypt is coated in a thick epithelial layer with an elliptical luminal opening at least 2-fold larger than those of the surrounding normal crypts. (II) In foci with 2 or more crypts, the aberrant crypts form distinct blocks and occupy an area larger than that occupied by an equal number of crypts of normal morphology. In the latter, no normal crypts separate the aberrant crypts in the foci. Comparison of the aberrant crypt foci test data (total number of foci of aberrant crypts, number of aberrant crypts per foci, and crypts/foci ratio) from the experimental groups was performed with ANOVA and the Tukey test ($P < 0.05$).

Calculation of percent damage reduction

The percentage of DMH damage reduction by green banana flour compounds in the

various experiments was calculated as suggested by Manoharan and Banerjee (1985):

$$DR\% = \left[\frac{Mean\ of\ positive\ control - Mean\ of\ associated\ group}{Mean\ of\ positive\ control - Mean\ of\ negative\ control} \right] X\ 100$$

RESULTS

The initial weights of animals from each experimental group were not significantly different ($P > 0.05$). However, an analysis of their final weights and weight gains demonstrated that banana flour supplementation in both the resistant starch and pre + continuous treatment groups caused statistically significant reductions ($P < 0.05$; Table 1).

Table 1. Mean ± standard error of initial/final weight and weight gain during the experimental period.

Experimental group	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	34.80 ± 1.27[a]	41.80 ± 1.59[a]	7.00 ± 1.37[a]
Positive control	35.40 ± 1.16[a]	39.00 ± 0.80[a,b]	3.60 ± 1.32[a,b]
Resistant starch	35.80 ± 0.96[a]	34.10 ± 1.38[b,c]	-1.70 ± 1.03[b,c]
Pretreatment	36.00 ± 2.35[a]	38.00 ± 1.66[a,b]	2.00 ± 2.10[a,b,c]
Simultaneous	35.40 ± 1.66[a]	37.20 ± 1.37[a,b]	1.80 ± 2.07[a,b]
Post-treatment	36.80 ± 1.04[a]	40.80 ± 1.27[a]	4.00 ± 1.11[a,b]
Pre + continuous	35.80 ± 1.67[a]	30.80 ± 1.20[c]	-5.00 ± 2.19[c]

Different letters indicate statistically significant differences ($P < 0.05$; analysis of variance/Tukey).

The absolute organ weight analysis revealed that lung weights were not significantly different among the groups. By contrast, the hearts, livers, and kidneys showed significant weight differences ($P < 0.05$). Nevertheless, no differences were observed in the heart weights relative to the other organ weights. In the DMH group, the lungs and livers had the lower weights ($P < 0.05$). Kidney weight reductions were observed in the DMH and post-treatment groups ($P < 0.05$; Table 2).

The results of the comet assays indicated that resistant starch supplementation had no genotoxic effects. In the antimutagenicity evaluation, resistant starch effectively reduced the frequency of damaged cells in all treatment groups and therefore demonstrated significant antimutagenic activity ($P < 0.05$). DNA damage was reduced by 50.71, 11.62, 77.74, and 28.34% in the pretreatment, simultaneous treatment, post-treatment, and pre + continuous treatment groups, respectively (Table 3).

The results of the micronucleus assay evaluation revealed that the resistant starch-rich diet was not mutagenic and had important antimutagenic activity ($P < 0.05$) in all tested groups. The damage was reduced by 70.60, 100.75, 93.23, and 87.96% at T1; 84.2%, 100.00, 92.56, and 87.60% at T2; and 95.55, 100.00, 86.66, and 100.00% at T3 in the pretreatment, simultaneous treatment, post-treatment, and pre + continuous treatment groups, respectively (Table 4).

Table 2. Mean ± standard error for absolute and relative organ weights.

Experimental group	Total weight (g)				Relative weight (g)			
	Heart	Lungs	Liver	Kidneys	Heart	Lungs	Liver	Kidneys
Control	0.2689 ± 0.0119^{c}	0.2462 ± 0.0090^{a}	2.2531 ± 0.0732^{d}	0.6340 ± 0.0300^{b}	0.0060 ± 0.0004^{a}	$0.0055 \pm 0.0033^{a,b}$	$0.0541 \pm 0.0024^{a,b}$	$0.0151 \pm 0.0013^{a,b}$
Positive control	$0.2162 \pm 0.0115^{a,b}$	0.2060 ± 0.0061^{a}	$1.7633 \pm 0.0666^{a,b,c}$	0.5229 ± 0.0233^{a}	0.0050 ± 0.0004^{a}	0.0047 ± 0.0003^{a}	0.0447 ± 0.0014^{a}	$0.0130 \pm 0.0007^{a,b}$
Resistant starch	$0.2092 \pm 0.0113^{a,b}$	0.2022 ± 0.0114^{a}	$1.6863 \pm 0.0737^{a,b,c}$	0.5066 ± 0.0234^{a}	0.0061 ± 0.0002^{a}	$0.0055 \pm 0.0004^{a,b}$	$0.0488 \pm 0.0029^{a,b}$	$0.0149 \pm 0.0008^{a,b}$
Pretreatment	$0.2219 \pm 0.0074^{b,c}$	0.2428 ± 0.0090^{a}	$1.9631 \pm 0.0881^{b,c,d}$	0.6301 ± 0.0293^{b}	0.0055 ± 0.0004^{a}	$0.0061 \pm 0.0002^{a,b}$	$0.0514 \pm 0.0017^{a,b}$	0.0162 ± 0.0010^{a}
Simultaneous	$0.2481 \pm 0.0101^{b,c}$	0.2427 ± 0.0163^{a}	$2.0811 \pm 0.0968^{b,c,d}$	$0.5747 \pm 0.0204^{a,b}$	0.0063 ± 0.0004^{a}	$0.0061 \pm 0.0005^{a,b}$	0.0559 ± 0.0029^{b}	$0.0153 \pm 0.0008^{a,b}$
Post-treatment	$0.2171 \pm 0.0115^{a,b}$	0.2440 ± 0.0096^{a}	$1.8588 \pm 0.0751^{b,c}$	0.5117 ± 0.0128^{a}	0.0048 ± 0.0003^{a}	$0.0057 \pm 0.0003^{a,b}$	$0.0452 \pm 0.0024^{a,b}$	0.0122 ± 0.0005^{b}
Pre + continuous	0.1913 ± 0.0119^{a}	0.2243 ± 0.0114^{a}	1.4546 ± 0.0605^{a}	0.5104 ± 0.0154^{a}	0.0059 ± 0.0004^{a}	0.0068 ± 0.0004^{b}	$0.0492 \pm 0.0031^{a,b}$	0.0162 ± 0.0009^{b}

Different letters indicate statistically significant differences ($P < 0.05$; analysis of variance/Tukey).

Table 3. Mean ± standard error, frequency of damaged cells, and distribution between damage classes.

Experimental group	Damaged cells	Damage class				Score	DR%
		0	1	2	3		
Genotoxicity							
Control	0.40 ± 0.22[a]	99.60 ± 0.21	0.40 ± 0.22	0.00 ± 0.00	0.00 ± 0.0	0.40 ± 0.22[a]	-
Positive control	92.50 ± 0.88[f]	7.50 ± 0.88	46.00 ± 2.74	25.50 ± 2.28	21.00 ± 2.26	160.00 ± 5.25[f]	-
Resistant starch	0.70 ± 0.39[a]	99.30 ± 0.39	0.70 ± 0.39	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.70[a]	-
Antigenotoxicity							
Pretreatment	45.80 ± 2.52[c]	54.20 ± 2.52	30.80 ±1.25	8.90 ± 0.60	6.10 ± 0.92	66.90 ± 4.70[e]	50.71
Simultaneous	81.80 ± 0.90[e]	18.20 ± 0.90	37.90 ± 1.35	33.30 ± 1.46	10.60 ± 1.48	136.60 ± 2.18[e]	11.62
Post-treatment	20.90 ± 1.41[b]	79.10 ± 1.41	10.00 ± 0.94	6.50 ± 0.60	4.40 ± 0.89	36.20 ± 2.89[b]	77.74
Pre + continuous	66.40 ± 1.23[d]	33.60 ± 1.23	29.30 ± 1.35	23.30 ± 1.16	13.80 ± 1.16	117.30 ± 3.14[d]	28.34

DR% = percent damage reduction. Different letters indicate statistically significant differences ($P < 0.05$; analysis of variance/Tukey).

Table 4. Total frequency and mean ± SE of the micronucleus assay in mouse peripheral blood cells.

Experimental group	Micronucleus frequency			Mean ± SE			DR%		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Mutagenicity									
Control	63	39	30	6.30 ± 0.59[a,b,c]	3.9 ± 0.64[a]	3.0 ± 0.39[a]	-	-	-
Positive control	196	160	75	19.60 ± 0.40[e]	16.0 ± 0.61[c]	7.5 ± 0.42[b]	-	-	-
Resistant starch	48	44	35	4.80 ± 0.32[a]	4.4 ± 0.26[a,b]	3.5 ± 0.26[a]	-	-	-
Antimutagenicity									
Pretreatment	102	58	32	9.00 ± 0.25[d]	5.8 ± 0.24[b]	3.1 ± 0.27[a]	70.6	84.29	95.55
Simultaneous	62	39	30	6.20 ± 0.38[a,b]	3.9 ± 0.31[a]	3.0 ± 0.25[a]	100.75	100.00	100.00
Post-treatment	72	48	36	7.20 ± 0.35[b,c]	4.8 ± 0.35[a,b]	3.6 ± 0.30[a]	93.23	92.56	86.66
Pre + continuous	79	54	30	7.90 ± 0.23[c,d]	5.4 ± 0.30[a,b]	3.0 ± 0.25[a]	87.96	87.60	100.00

DR% = percent damage reduction; SE = standard error. Different letters indicate statistically significant differences ($P < 0.05$; analysis of variance/Tukey).

Table 5 shows that the animals in the control and resistant starch groups showed no aberrant crypt foci in the colorectal mucosa, indicating the absence of any carcinogenic effects due to resistant starch supplementation. However, when this supplementation was administered concurrently with a cancer-inducing agent, it significantly reduced ($P < 0.05$) the frequency of aberrant crypt foci, a cancer biomarker, in all treatment groups. This chemopreventive activity was evident from the percentages of damage reduction in the intestinal mucosa, which were 62.92, 55.33, 51.12, and 67.41% in the pretreatment, simultaneous treatment, post-treatment, and pre + continuous groups, respectively. The lowest crypts/foci ratio was detected in the post-treatment group.

Table 5. Number, distribution, and percent damage reduction (DR%) of aberrant crypt foci.

Experimental group	AC foci (total)		DR%	AC (total)	AV of crypts/foci				AC/foci ratio
	AV	Mean ± SE			1 AC/foci	2 AC/foci	3 AC/foci	4 AC/foci	
Carcinogenicity									
Control	0	0[a]	-	0.00	0.00	0.00	0.00	0.00	0.00
Positive control	356	35.6 ± 0.85[e]	-	743	108	92	73	58	2.08
Resistant starch	0	0[a]	-	0.00	0.00	0.00	0.00	0.00	0.00
Anticarcinogenicity									
Pretreatment	132	13.2 ± 1.02[b]	62.92	272	43	43	33	11	2.06
Simultaneous	159	15.9 ± 0.69[d]	55.33	341	47	44	30	29	2.14
Post-treatment	174	17.4 ± 0.63[c,d]	51.12	288	68	63	33	7	1.65
Pre + continuous	116	11.6 ± 0.54[b]	67.41	238	38	36	28	11	2.05

AC = aberrant crypts; AV = absolute values; SE = standard error. Different letters indicate statistically significant differences ($P < 0.05$; analysis of variance/Tukey).

DISCUSSION

Studies suggest that diets containing prebiotics such as resistant starch plays a significant role in preventing colorectal cancer due to the high potential antigenotoxic, antimutagenic, and anticarcinogenic activities of these compounds (Liu and Xu, 2008; Clark et al., 2012). According to Champ and Faisant (1996), resistant starch is the starch fraction that provides no glucose to the body. However, resistant starch is fermented in the large intestine mainly to produce gases and short-chain fatty acids. The activities of resistant starch, as well as the effects observed in this study, are attributable to these characteristics.

Because resistant starch is not digested in the large intestine, it becomes an available substrate for fermentation by anaerobic bacteria present in the colon (Jenkins et al., 1998). Thus, this fraction shares many of the features and benefits attributed to dietary fiber in the gastrointestinal tract and thus might be a food with prebiotic potential (Muir and O'Dea, 1992).

Studies have shown that genotoxicity, mutagenicity, and carcinogenicity are correlated processes in which many chemical carcinogens can interact with genetic materials to cause cancer-inducing mutations (Vogel, 1982; Mauro et al., 2013). DMH acts similarly due to its capacity to cause DNA alkylation. Therefore, the aim of the present study was to evaluate the potential antigenotoxic and antimutagenic effects of resistant starch and relate them to the possible anticarcinogenic activity of this food compound. Resistant starch is inexpensive to acquire and prepare and could therefore be used not only in the diets of the general population, including those that are less financially privileged, but also in the diets of those seriously affected by colorectal cancer, which is an important public health issue in Brazil and worldwide.

The results of the comet assays, which were used to assess the genotoxic and antigenotoxic activities of the dietary supplements, indicated that resistant starch had no genotoxic activity but exhibited antigenotoxic effects in the pretreatment, post-treatment, and pre + continuous treatment groups. The reduced frequency of genotoxicity in these experimental groups likely occurred due to the longer supplement consumption period and therefore to the longer exposure of the lumen to fiber and the consequent improvement in the intestinal epithelium, because fiber has beneficial properties for mucin composition (glycoproteins that protect mucous membranes from environmental aggressions) (Roberfroid, 2005). However, this premise assumes that in the simultaneous treatment group, the intestinal epithelium had insufficient time to improve mucin production, leading to a greater absorption of DMH-derived xenobiotics that in turn increased the frequency of cells with genotoxic damage.

Another hypothesis that might explain the antigenotoxic activity of resistant starch is that it acts as a fiber and increases intestinal transit. Thus, xenobiotics would be in contact with the intestinal mucosa for a shorter period of time. There would also be a shorter amount of time for the intestinal flora to degrade these xenobiotics, reducing the release, absorption, or both, of products with genotoxic, mutagenic, or carcinogenic potential.

Given the antigenotoxic action observed and based on the assumption of a correlation between genotoxic and mutagenic events, we evaluated the mutagenic and antimutagenic activities of resistant starch and determined the relevant frequencies of damage-reducing effects at 3 different experimental times. This reduction was confirmed by the data obtained in the comet assay; specifically, when antigenotoxic activity occurs, a reduction in the frequency of mutagenic damage is expected (Vogel, 1982).

However, this statement was untrue for the simultaneous treatment group because antigenotoxic activity was not observed. However, in the micronucleus test, this treatment group

showed damage reduction percentages of 93.23, 92.56, and 86.66% in the T1, T2, and T3 experimental periods, respectively. These data are apparently conflicting but can be explained because genotoxic damage can be repaired, and this repair may not have been apparent in the micronucleus assay because the assay only evaluated fixed damage in the cell genome (Salvadori et al., 2003; Mauro et al., 2013), which has a higher correlation with the genetic instability that can determine cancer development.

From among the various protocols proposed in the literature, the present study incorporated pretreatment, simultaneous treatment, and post-treatment groups in addition to a pre + continuous treatment group. Whereas the results in the simultaneous treatment group indicated both desmutagenic and bio-antimutagenic activities, those in the pretreatment and post-treatment protocols indicated activity that could be inferred as bio-antimutagenic (Ferguson, 1994; Flagg et al., 1995; De Flora and Ferguson, 2005; Oliveira et al., 2009, 2013; Pesarini et al., 2013). The results of the comet and micronucleus tests suggest that the tested food compound acted through both bio-antimutagenic and desmutagenic actions.

The potential carcinogenic and anticarcinogenic activities of the resistant starch were also evaluated within the context of the correlation between mutagenic and carcinogenic events, and resistant starch was verified to be noncarcinogenic and shown to lead to a significant reduction in pre-neoplastic lesions (aberrant crypt foci). Both the absolute values and the percentages of damage reduction showed that the activity in the simultaneous and post-treatment groups was statistically similar in terms of anticarcinogenicity. However, the best damage reduction percentages were observed in the pretreatment and pre + continuous treatment groups. The lowest crypts/foci ratio index was observed in the post-treatment group.

Because the largest anticarcinogenic effects were observed in the pretreatment and pre + continuous groups, we concluded that green banana flour may be an important food compound with cancer-preventing activity. Thus, including green banana flour in the daily diet of the general population could prevent genome damage associated with cancer development or even prevent cancer development. Even if cancer is already present in the body, the continual intake of this food supplement could be beneficial.

This conclusion encourages the use of supplements in organisms that already show the presence of cancer biomarkers. This suggestion is reinforced by the crypts/foci index in the post-treatment group, which was approximately 0.8-fold lower than that observed in the positive control group. This value was significant and indicated that giving a resistant starch supplement to animals with already-initiated cancer could improve the prognosis; specifically, foci were less likely to develop into tumors. Thus, resistant starch could be sufficient to prevent full cancer development, which would correlate with improvements in the quality of life of the animals.

Given these findings, we observed no direct relationship between the time of resistant starch supplementation and its anticarcinogenic function. However, the results present strong evidence that this compound should be used continuously to address the following aspects of oncogenesis/carcinogenesis: 1) the prevention of genomic lesions that can induce cancer; 2) the prevention of intestinal mucosal changes (aberrant crypts) that are directly related to colorectal cancer development, and 3) a reduction in the crypts/foci ratio, which is related to a lower likelihood of intestinal mucosal lesions that develop into tumors. In particular, the crypts/foci ratio supports the inference of prognosis improvement in patients with existing polyps; for example, studies have concluded that in both animals and humans, the higher the crypts/foci ratio, the greater the progression of these lesions and the closer their proximities to established tumors (Cheng and Lai, 2003; Rudolph et al., 2005).

Several factors can account for the anticarcinogenic activity of resistant starch and might have reduced the number of identified aberrant crypt foci. One factor is the decrease in serum glucose levels. Studies have established that rapid tumor proliferation depends on glucose availability (Roberfroid, 2005). Consumption of resistant starch reduces the energy capacity of cells in preneoplastic lesions. Consequently, resistant starch improves resistance to colonization, prevents bacterial translocation, and ultimately helps to improve the chemical and enzymatic functions that protect the gastrointestinal tract (Roberfroid, 2005). The increased motility of the large intestine reduces the time that stool remains in the colon and relieves constipation symptoms. Increased stool transit time and constipation are among the risk factors for colorectal cancer because fecal contact with the colon and rectal walls for prolonged periods of time increases the chances of developing this disease (Roberfroid, 2005; Mauro et al., 2013). Another activity of fiber is its adsorptive capacity. Ferguson (1994) reported that dietary fibers exhibit adsorptive capacity, acting as physical barriers that lead to the elimination through adsorption of certain chemical substances, including DMH, that could induce the initiation, promotion, or progression of carcinogenesis.

Because it cannot be digested in the small intestine, resistant starch is also a substrate for probiotic organism growth and thereby a potential prebiotic agent (Haralampu, 2000). The metabolism of this type of carbohydrate by microorganisms via fermentation produces short-chain fatty acids such as acetate, propionate, and butyrate; carbon and hydrogen gases; and, in some individuals, methane; it also decreases colon pH (Englyst et al., 1992). Most of these compounds prevent inflammatory diseases of the intestine and assist in the maintenance of intestinal epithelium integrity (Jenkins et al., 1998). A study by Englyst et al. (1992) demonstrated that 59% of fermented starch is recovered as short-chain fatty acids at a molar ratio of 50:22:29 for acetate, propionate, and butyrate, respectively. Because butyrate is an important energy source for colon epithelial cells, its increased production can prevent colonic diseases (Englyst et al., 1992).

The other data obtained in this study provide evidence for weight reduction in the animals subjected to a resistant starch-containing diet, particularly for those in the pre + continuous group. Weight loss in these animals was likely due to various properties of resistant starch, including increased bowel motility, which reduces the time that fecal matter remains in the intestine and therefore decreases the absorption of food nutrients, especially fats. Fiber degradation in only this portion of the intestine reduces caloric value and decreases the glycemic rate (Roberfroid, 1993). The loss of calories also occurred because some of the energy was used to synthesize microbial biomass (Roberfroid, 1993). In addition, animals in this experimental group had the longest exposure to the resistant starch-rich diet, thus increasing the effectiveness of the various factors described above. No biologically relevant differences were observed with respect to the total and relative organ weights of animals, which suggests the absence of toxicity.

Given the relevant biological activities observed, green banana flour (resistant starch) appears to be a functional food. The concept of functional foods comes from the hypothesis that diet can control and modulate various organ functions and thus contribute to health maintenance and reduce the risk of disease development. Functional foods have biological markers for their functions in the body. These markers include, for example, increased numbers of non-pathogenic bacteria in the body, which aid in nutrient digestion and absorption, increased lactose tolerance, and immunostimulation (Borges, 2000). Other biological markers, particularly those that were evaluated in the present study of resistant starch, show antigenotoxic, antimutagenic, and anticarcinogenic functions.

For a food to be classified as functional, it must have a metabolic or physiological effect that contributes to physical health and reduces the risk of chronic disease development, be part of the usual diet, have positive effects that can be obtained from non-toxic amounts and that persist even after the cessation of ingestion, and is not used to treat or cure diseases (Milner, 1999). All of these features could be attributed to green banana flour. The results of the present study show that resistant starch might act as an antigenotoxic, antimutagenic, and anticarcinogenic agent and is nontoxic to animals that consumed it in their diet. The results obtained from the experimental protocols suggest that this prebiotic might act via 2 methods of DNA damage prevention, desmutagenesis and bio-antimutagenesis. In carcinogenesis, resistant starch is believed to reduce the incidence of cancer biomarkers and improve disease prognosis by minimizing the number of biomarkers that develop into tumors. Thus, resistant starch has therapeutic properties that could be exploited in human dietary applications.

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