



Efficacy of Microwave-Heating during Alkaline Processing of Fumonisin-Contaminated Maize

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Abstract

Background: Fumonisin (a family of foodborne carcinogenic mycotoxins) cause health hazards to humans and animals in developing countries, and has also economic implications. Therefore, the efficacy of a novel environmental friendly nixtamalization procedure to make tortillas (the main staple food for the Mexican population) was investigated.

Methods: Maize contaminated with 2136.67 ng/g total fumonisins was processed into tortillas, starting with maize grits mixed with water and calcium hydroxide that was cooked in a microwave field at 2.45 GHz during 3.75 min, and steeped 3.5 h at room temperature. The steeped maize grits (nixtamal) was stone-ground into masa (maize dough), which was then used to make tortillas. Total fumonisin content was determined using monoclonal antibody columns.

Results: Masa contained 1998.33 ng/g total fumonisins, which represents 6.5% toxin reduction. Nevertheless, fumonisin concentration was reduced significantly in tortillas (up to 985.33 ng/g) due to the cooking process, corresponding to a cumulative toxin degradation of 54%. Tortillas were below the maximum tolerated level, considering the European Union regulatory limit for fumonisins in maize (1000 ng/g). The physicochemical and technological properties of tortillas were also considered within the acceptable margins of quality.

Conclusion: Microwave nixtamalization was not a feasible method to reduce fumonisin content in masa to acceptable levels; however, an effective extra-reduction occurred when masa was baking into tortillas.

Keywords: Maize, *Fusarium verticillioides*, Fumonisin, Microwave nixtamalization, Tortillas

Introduction

Grains such as maize are invaded by fungi during their development in the field, as well as during their transport and storage. Therefore, one of the factors affecting the quality of maize-based food is mycotoxin contamination caused by the grain invasion of certain species of fungi, among them *Fusarium verticillioides*. *F. verticillioides* (Sacc.) Nirenberg (formerly *F. moniliforme* Sheldon), *F. proliferatum*, and other *Fusarium* species are fungi capable

of producing fumonisins B1, B2, and B3 (1). The most common homologue -FB1- causes leukoencephalomalacia in horses (2), pulmonary edema in swine (3), hepato- and nephrotoxicity in multiple species (4), and is a liver and kidney carcinogen in rodents (5). A higher incidence of human esophageal squamous cell carcinoma has also been linked epidemiologically with consumption of fumonisin-contaminated maize (6). The Inter-

national Agency for Research on Cancer classified the fumonisins as Group 2B carcinogens -a possible carcinogen to humans- (7).

FB1 is the most abundant of the fumonisin family and usually accounts for 70-95% of the total toxin content found in maize grains. FB1 with empirical formula $C_{34}H_{59}NO_{15}$ and molecular weight of 721 g/mol, is the diester of propane- 1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane (Fig. 1). In commercial food-grade maize the "possible" total fumonisin content usually might be at concentration range 100-3500 ng/g (8). The European Union (EU) has established regulatory limits of fumonisins in foodstuffs, based on the sum of FB1+FB2; with a maximum level of 1000 ng/g for maize used for human consumption (9). However, the recommended maximum level for total fumonisins that FDA considers is 4000 ng/g for maize intended for masa (maize dough) production (10).

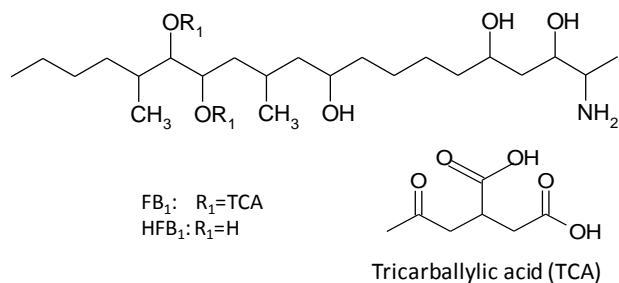


Fig. 1: Chemical structures of fumonisin B1 (FB1) and hydrolyzed fumonisin B1 (HFB1)

Given the growing food safety concerns regarding fumonisins in Mexico, it is important to understand what happens to these compounds when maize is processed. In Mexico, maize is primarily consumed as tortillas, with a *per capita* consumption of about 120 kg/year, which are traditionally made using the ancient alkaline-cooking process called nixtamalization. The traditional nixtamalization consists of the cooking of the grain in abundant water (up to 3 L/kg of maize), with 1-3% $Ca(OH)_2$ at temperatures near boiling for 35-70 min, with a steeping period of 8-16 h. After the steeping, the lime cooking solution is decanted, and the grain is thoroughly washed to leave the

grain ready for stone-milling to obtain the masa for making the tortillas. Traditional nixtamalization, though centuries old, is still used without modification. While this process implemented on a small scale did not present serious problems, it has become the cause of ecological problems on a grand scale due to the generation of large quantities of washing water. The estimated amount of lime cooking solution generated in Mexico is about 14 million m^3 /yr. Recently, a modified tortilla-making process has been developed, in which maize grits are mixed with water and lime, cooked in a microwave field, steeped, and then milled to obtain fresh masa for the tortilla making. In this process, microwave cooking offers multiple advantages including: reduced start-up time, faster heating, energy efficiency, space savings, precise process control, selective heating, reduction of water use, and food with a high nutritional quality (11). Cooking, sterilization, pasteurization, disinfection, drying, blanching, and thawing are some of the applications of microwave energy in the food industry. However, the use of this type of energy for the production of tortillas elaborated with fumonisin-contaminated maize has not been evaluated.

In Mexico, one of the problems experienced by the tortilla industry in relation to quality is fumonisin contamination of maize, either as field contamination or as a result of poor postharvest management. Traditional nixtamalization reduces up to 80% of the contamination (12-14). Therefore, it is considered crucial to evaluate the effect that the microwave nixtamalization could have on the fate of fumonisins, since this process substantially differs from the traditional process when using maize contaminated with a moderate contamination level of total fumonisins (FB1+FB2). In this study, this alternative technology was selected in order to explore the effect of other not complex heating systems to suggest alternative methods that have no greater deviation from the traditionally established nixtamalization process for reduction of fumonisin hazards in maize in an attempt to validate current practical and economical decontamination procedures.

Materials and Methods

Maize grain

Regular maize of the commercial hybrid AS-900 (Aspros, Mexico) with 10.7% moisture content (MC) was utilized. This material has a thousand-kernel weight and test weight of 283.40 ± 3.64 g and 71.59 ± 1.23 kg/hL, respectively. MC was determined by drying replicate portions of 5-10 g each of whole grain at 103°C for 72 h, with percentages calculated on a wet-weight basis.

Fungal isolate

F. verticillioides strain UNIGRAS-2509 (Culture Collection of the Grain and Seed Research Unit of the National Autonomous University of Mexico) was plated into Petri dishes containing potato-dextrose-agar medium (BD Bioxon, Becton Dickinson, Mexico) for 7 d at 28°C. This strain mainly produces FB1 and FB2.

Fungal inoculation technique

To inoculate the grain, fungal spores were removed from the Petri dishes with a spatula; a sterile-water spore suspension (1.4 L) was prepared with approximately 100,000 conidia/ml, and this suspension was used to rise the MC of the grain. The total amount of maize to be inoculated was 15 kg (5 kg per replicate). The MC of the maize was adjusted to 18% and stored in plastic bottles, which were covered with thin polyethylene film to minimize the loss of humidity from the grain. However, 10 perforations with a pin were made to each film to avoid accumulation of CO₂ generated by the respiration of grains and fungi. The bottles were incubated at 28°C during 21 d. This incubation period was chosen in order to obtain a fumonisin concentration similar to those reported in maize intended for human consumption in Mexico (15). After the incubation, the grain was put under a 1000 mg/L ethylene oxide gas atmosphere for 5 h, to stop further development of the toxigenic fungus and to avoid the dispersal of viable spores (16). Finally, the grain was dried to approximately 12% MC, transferred to plastic bags and stored at 4°C for further analysis.

Fumonisin quantification

Total fumonisin content was determined using monoclonal antibody columns for fumonisins (VICAM, Milford, MA). Samples (50 g) were extracted by blending with 100 mL methanol-water (80:20 v/v) and 5 g of sodium chloride using a laboratory blender (Mod. 51BL30; Waring, New Hartford, CT, USA). The mixture was filtered through a Whatman No. 1 filter paper and 10 mL portion was diluted with 40 mL of phosphate-buffered saline (PBS)/0.1% Tween-20 wash buffer and filtered through a 1.0 µm microfibre filter, then 10 mL were applied to an immuno-affinity column (FumoniTest; VICAM Science Technology, Watertown, MA, USA). Subsequently, the column was washed with 10 mL of PBS/0.1% Tween-20 wash buffer followed by 10 mL of PBS. The toxins were then eluted with 1 mL of HPLC-methanol and quantified in a fluorometer VICAM Series-4 (VICAM Source Scientific, Irvine, CA) after reaction with 1 ml of a mixture of developer "A" (966 µL) and developer "B" (34 µL). Fumonisins detection limit with the immunoaffinity column via fluorescence measurement is approximately 16 ng/g. When the total fumonisin concentration was greater than 250 ng/g, dilutions from the extracts were made before passed through the immuno-affinity column.

Microwave nixtamalization

Nixtamalization was performed using the procedure described by Pérez-Flores et al. (17) with minor modifications. Briefly, 1000 g fumonisin-contaminated maize sample (of each replicate) was milled in a hammer mill (Glen Mills Inc. sieve 5 mm. Clifton, NJ). The water/maize input ratio was 1:1 (w/w), and the calcium content of the suspension was 5 g of Ca(OH)₂ per kg of maize. The mixture was cooked in a microwave-resistant plastic container, and the cooking stage was carried out in a commercial microwave oven (LG Electronics Inc, model MS047GR, Korea), with an average cooking power of 100% during 3.75 min. The power output of the magnetron specified by the manufacturer was 1650 W and the operating frequency was 2.45 GHz. The alkali-cooked grain (nixtamal) was steeped 3.5 h at room

temperature (25°C) before milling (FUMASA, Model MN-400, Mexico) to provide a masa with a MC of about 57%.

Tortilla elaboration

Masa was flattened into thin disks of 12.5 cm diameter, 1.2 mm thickness, and 28 g weight, using a commercial tortilla roll machine (Model TM-G, Casa Gonzalez, Monterrey, NL). Tortillas were baked 17 s on one side (first side), 55 s on the other side, and again 17 s on the first side on a griddle at $270 \pm 5^\circ\text{C}$. The temperature was measured with a non-contact portable infrared thermometer Fluke-572 (Fluke, Melrose, MA, USA). Finally, masa (500 g), and tortillas from each treatment ($n = 20$) were oven-dried at 40°C for 48 h, milled and stored at 4°C in polyethylene bags for fumonisin analysis.

Physicochemical properties

pH

The pH was determined according to the 943.02 AOAC method (18). 10 g of sample were suspended in 100 ml of recently boiled distilled water. The suspension was shaken (1500 rpm, 25°C , 30 min) using an orbital shaker (Cole Parmer Model 21704-10, Vernon Hills, IL, USA). After 10 min, the supernatant liquid was decanted and the pH value was immediately determined using a pH meter, Model PC45 (Conductronic S.A., Puebla, Mexico).

Color

Tortillas were subjected to surface-color analysis with a MiniScan XE model 45/0-L colorimeter (Hunter Associates Laboratory, Reston, VA, USA). The colorimeter was calibrated with a white porcelain plaque ($L = 97.02$, $a = 0.13$, $b = 1.77$). Readings were made in triplicate at four positions at 90° with respect to each other. Two derived functions (ΔE and chroma) were computed from the L, a, and b readings as follows:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

$$\text{Chroma} = (a^2 + b^2)^{1/2}$$

Quality properties of tortillas

Puffing degree

Tortilla puffing was evaluated subjectively by using scores of 1-3, where: 1 = little or no puffing

(0-25%), 2 = medium puffing (25-75%), and 3 = complete puffing (75-100%).

Rollability

Rollability was evaluated by rolling a tortilla over a 1 cm diameter tube, quantifying the extent of breaking using a scale of 1-3, where: 1 = tortillas with no breaking; 2 = a partial breaking at the center and edges of the tortilla; and 3 = completely flattened tortillas.

Weight loss of tortilla during cooking

The weight loss (WL) of tortilla was determined by weighing the tortilla before and after cooking, value was reported as percentage (w/w) and computed as:

$$\text{WL} = [(W_a - W_b)/W_a] \times 100$$

Where:

W_a , W_b = weight of tortilla before and after cooking, respectively.

Statistical analysis

The experiment was conducted as a completely randomized design with three replicates. Data was assessed by analysis of variance (ANOVA) and means comparisons were performed according to the Tukey test using the Statistical Analysis System (19). A significance value of $\alpha = 0.05$ was used to distinguish significant differences.

Results

Table 1 shows some physicochemical properties of the fumonisin-contaminated maize and nixtamalized products. MC values of the inoculated maize were quite similar for the three experimental replicates, presenting an average value of $12.43 \pm 0.05\%$. In the case of masa and tortillas, MC values were $57.38 \pm 0.11\%$ and $49.61 \pm 0.21\%$, respectively. Regarding the pH, maize presented an average pH value of 6.45; however, pH values were slightly higher for masa and tortillas due to the calcium hydroxide incorporation during microwave-nixtamalization, presenting average values of 8.13 and 8.28, respectively (Table 1).

Table 1: Some physicochemical properties of maize and microwave-nixtamalized products

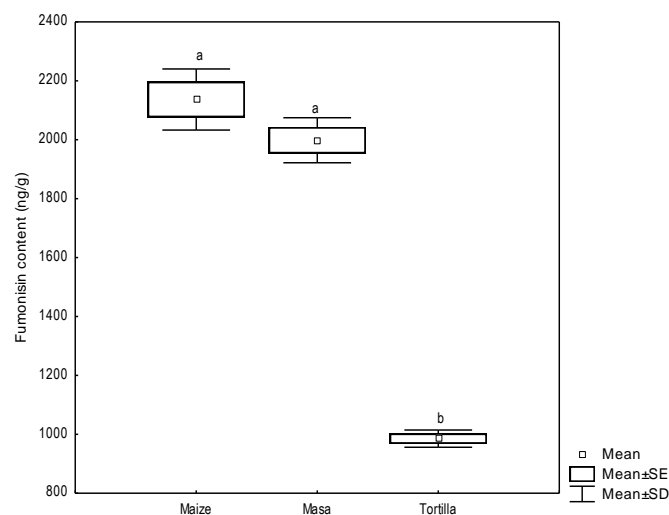
Sample	Replicate	MC* (%)	pH
Maize	R1	12.34	6.45
	R2	12.53	6.46
	R3	12.43	6.45
	Mean \pm SE [†]	12.43 \pm 0.05	6.45 \pm 0.003
Masa	R1	57.58	8.14
	R2	57.36	8.12
	R3	57.19	8.14
	Mean \pm SE	57.38 \pm 0.11	8.13 \pm 0.006
Tortilla	R1	49.20	8.29
	R2	49.89	8.28
	R3	49.74	8.28
	Mean \pm SE	49.61 \pm 0.21	8.28 \pm 0.003

* MC, moisture content/ [†] SE, standard error

Table 2 shows the surface-color analysis of maize and tortillas produced by microwave nixtamalization. In the case of luminosity (L), the maize grain presented higher values (97.13 ± 0.91) as compared to tortillas, which presented an average value of 74.18 ± 0.35 . The same trend was observed in the case of chroma, attaining values of 8.07 ± 0.34 and 5.46 ± 0.27 , for maize and tortillas, respectively. On the other hand, the total color difference (ΔE) value, significantly increased in tortillas reaching values up to 23.38 ± 0.29 (Table 2).

Some technological properties of tortillas are presented in Table 3. Tortillas produced with the three replicates showed similar puffing degree, with a subjective value of 3, which means that all tortillas had a complete puffing (75-100%). Moreover, all tortillas presented a good rollability, with a value close to 1, defined as no breaking; therefore, tortillas were also considered within the acceptable margins of quality, presenting a soft texture and rolled without breaking. Table 3 also shows the loss of weight during tortilla baking. Tortillas from the three replicates had a similar loss of weight average value ($17.20 \pm 0.09\%$ w/w). Concentrations of total fumonisins were quantified in the maize used during microwave nixtamalization, as shown in Fig. 2. The three inoculated maize replicates presented an average fumonisin content of 2136.67 ± 59.92 ng/g. Fig. 2 also shows the

fumonisin content in the products obtained during microwave nixtamalization. Results indicated that microwave nixtamalization has no significant effect on the fumonisin reduction.

**Fig. 2:** Total fumonisins content (ng/g) in maize and microwave-nixtamalized products

Consequently, masa presented an average fumonisin content of 1998.33 ± 44.17 ng/g, corresponding to a 6.5% toxin degradation; thus, statistical differences were not observed compared to the contaminated-maize grain. However, tortilla presented a fumonisin concentration of 985.33 ± 16.91 ng/g, corresponding to a cumulative reduction of 54% (Fig. 2).

Table 2: Surface-color analysis of maize and tortillas produced by microwave nixtamalization

Sample	Replicate	Color parameter				
		L	a	b	ΔE^*	Chroma
Maize	R1	98.87	1.86	-7.91	10.01	8.13
	R2	95.78	1.78	-7.24	9.24	7.46
	R3	96.75	1.34	-8.53	10.37	8.63
	Mean \pm SE [†]	97.13 \pm 0.91	1.66 \pm 0.16	-7.89 \pm 0.37	9.87 \pm 0.33	8.07 \pm 0.34
Tortilla	R1	74.48	5.54	2.06	23.18	5.91
	R2	73.49	4.42	3.23	23.96	5.47
	R3	74.58	4.97	0.45	22.99	4.99
	Mean \pm SE	74.18 \pm 0.35	4.98 \pm 0.32	1.91 \pm 0.81	23.38 \pm 0.29	5.46 \pm 0.27

* ΔE , total color difference./ [†] SE, standard error.

Table 3: Technological properties of tortillas produced by microwave-nixtamalization

Sample	Replicate	Property		
		Puffing*	Rollability [†]	Weight loss (%)
Tortilla	R1	3	1	17.11
	R2	3	1	17.12
	R3	3	1	17.38
	Mean \pm SE [‡]			17.20 \pm 0.09

* Puffing degree, 1 = little or no puffing (0-25%), 2 = medium puffing (25-75%), and 3 = complete puffing (75-100%)./ [†] Rollability, 1 = tortillas with no breaking; 2 = a partial breaking at the center and edges of the tortilla; and 3 = completely flattened tortillas./ [‡] SE, standard error.

Discussion

In the present study, the physicochemical properties of masa and tortillas were similar than those reported previously during microwave-nixtamalization of aflatoxin-contaminated maize (17) and traditional nixtamalization (20). Masa had higher MC values than tortillas, because masa lost MC during the tortilla baking process. Besides, the pH values of the products made with microwave-nixtamalization were similar to those reported previously (11, 17). Méndez-Albores et al. (16) reported a pH value of 8.33 for maize tortillas produced with the Mexican traditional nixtamalization using 3% (w/w) lime. These results are in close agreement with those obtained in this research when using 0.5% (w/w) lime concentration. In nixtamalized products, lime content represents an important factor in color, odor, shelf life, and texture characteristics. When the lime content is not sufficient to produce the characteristic alkaline flavor, tortillas are non preferred by consumers.

Likewise, if this compound is in excess, tortillas become astringent and are also rejected for consumption.

In general, tortilla color was affected by the addition of the $\text{Ca}(\text{OH})_2$, consequently tortillas presented a yellowish color, as shown by the ΔE and chroma values. Méndez-Albores et al. (11) reported Hunter L and ΔE values of 75.34 and 25.81 for tortillas produced with MASECA® (a commercial nixtamalized maize flour). The same authors reported values of 70.62 and 29.88 for Hunter L and ΔE of tortillas produced by microwave-nixtamalization using 0.125% (w/w) lime. Cuevas-Martínez et al. (20) stated that maize tortillas produced with traditional nixtamalization using 1.5% lime solution presented Hunter L values of 71.19. These color results are in close agreement with those obtained in this research. Changes in the color of tortillas are directly attributed to the amount of lime retained during the cooking of the nixtamal. Lime content affects the tortilla color

even when tortillas are produced from white maize grains, and the color intensity is closely related to carotenoid pigments, flavonoids, and pH. However, the development of color during the alkaline-cooking process is more complex, considering that the $\text{Ca}(\text{OH})_2$ reacts with the different pigments found in the grain and interferes with browning reactions such as caramelization and Maillard reactions (21).

Regarding the technological properties (rollability, puffing degree, and weight loss during cooking), all tortillas evaluated presented similar characteristics to those produced during traditional nixtamalization (22). A good puffing is obtained when two layers are formed in the tortilla; these layers, produced during the cooking process, are impermeable, retaining the steam that gives rise to the puffing during heating. Méndez-Albores et al. (11) reported loss of weight values around 18% for tortillas prepared from MASECA, and values around 19% of loss of weight for tortillas from microwave-nixtamalization using 0.5% (w/w) lime. These results are consistent with the values found in this research. The lowest loss of weight the better tortilla quality, due to the fact that MC plays an important role on tortilla yield and texture. In general, tortillas prepared with microwave-nixtamalization were stretchable, elastic, and resistant to rupture and cracking.

Fumonisin content was reduced significantly due to the complete microwave thermal-alkaline process to produce tortillas (up to 54%). The maximum reduction in the mycotoxin content was observed when masa was cooked into tortillas. In this product, the total fumonisin content (985.33 ng/g) was below the maximum limit allowed in EU for fumonisin contamination. Unfortunately, in Mexico there are no regulations established for fumonisin levels in maize and maize-based products destined for human consumption. It is well known that baking had a moderate effect on fumonisin content in the masa, a finding that is in agreement with the reported heat stability of these molecules (23). Consequently, thermal-alkaline conditions used during microwave-nixtamalization were not sufficient for fumonisin degradation at “safe” levels, so that a residual content of toxins

always remains in the tortillas. Conditions normally found in the processing of tortillas with the traditional nixtamalization process are also not adequate to completely detoxify fumonisin-contaminated maize. Consequently, several researchers pointed out that traditional nixtamalization degrade up to 80% of the initial fumonisin concentration (12-14). In relation to the previously cited fumonisin reductions, it can be said that degradation of fumonisin-contaminated maize varies considerably, depending on the nixtamalization parameters, such as: cooking time-temperature-method, lime concentration, steeping time, as well as the initial toxin concentration in the maize. It is important to note that masa MC and cooking temperature/time for baking tortillas are also factors contributing to a greater extent toward obtaining higher cumulative degradation rates.

In the case of the traditional nixtamalization, solids are lost in the lime cooking solution, and they go into the wastewater. These solids contain mainly tip-cap, pericarp, and germ; consequently, toxins present in these anatomic parts of the grain are removed and extracted to the washing water. Thus, physical removal of the toxins is a crucial step for toxin reduction during traditional nixtamalization. In the present research, no solids were lost during nixtamalization (since microwave-nixtamalization does not produce polluting effluents), and within the 3.75 minutes of nixtamalization in the microwave field plus approximately 1.5 min of cooking of the masa to produce tortillas, a considerable fumonisin degradation rate (54%) was attained.

Fumonisin B1 (FB1) is characterized by the presence of two tricarballylic acid groups esterified to a 20-carbon backbone (Fig. 1). It is relatively heat stable and remains through most of the conditions used in food processing. However, alkaline hydrolysis of FB1 -during nixtamalization- cleaves the tricarballylic side chains at C_{14} and C_{15} of the molecule to produce hydrolyzed fumonisin B1 (HFB1), as schematized in Fig. 1. FB1 has been shown to disrupt ceramide synthase, an enzyme necessary for sphingolipid metabolism (24). Consequently, hydrolysis of one or both propane-1,2,3-tricarboxylic acid side chains from the molecule diminished this effect. Hydrolysis is likely

important because hydrolyzed FB1 was significantly less toxic than FB1 when given to rats (25). These results suggest that the side chains play an important role in the effects of FB1, but that the presence of an intact primary amine is necessary for toxicity.

Conclusion

The novel process from which raw maize grits are made into tortillas reduces the level of total fumonisins to some degree. Consequently, the use of this environmental friendly process is recommended, when high quality maize is used, as it has definite several advantages. More research, however, pertaining to the possible effect of this modified tortilla-making process on the toxicity and mutagenic/carcinogenic activity of alkali-treated fumonisin sub-products, needs to be conducted.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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