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Determination of sensory, microbiological and antioxidant properties of tortilla added with Roselle decoction calyxes powder

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ABSTRACT

Background: Hibiscus (*Hibiscus sabdariffa* L.) is a flowering plant gaining interest for its potential health benefits due to its high content of phenolic compounds and antioxidant properties. These properties have been linked to various health improvements, including reduced risk of chronic diseases. **Aims:** The aim was to assess the sensory, microbiological, physical, and antioxidant properties of corn and wheat tortillas formulated with varying HDC concentrations. **Material and Methods:** Five formulations were prepared, incorporating HDC (5%, 20%, 50%, and 70%) into the corn and wheat flour blends. The formulation with the most favorable sensory profile was further evaluated for: microbiological analysis, tortilla quality properties (diameter, weight, yield, puffing degree, rollability, and moisture content), total soluble phenolic content (TSPCC), total flavonoid content (TFC), and antioxidant capacity (DPPH• and ABTS+• methods). **Results:** Sensory evaluation revealed that the corn and wheat tortillas with 20% HDC achieved the highest overall acceptability in terms of mouthfeel, color, and flavor attributes. The addition of HDC significantly reduced microbial growth compared to the control tortillas. All formulations displayed significant variations in quality properties. Tortillas containing HDC demonstrated significantly higher levels of TSPCC, TFC, and antioxidant capacity. **Conclusion:** The incorporation of 20% HDC flour presents a promising approach to developing functional tortillas with enhanced health benefits. These tortillas exhibit desirable sensory characteristics, improved microbiological safety, and increased antioxidant potential, potentially impacting the food industry and consumer health.

Keywords: Tortilla, roselle decoction calyxes, phenolic compounds, corn, wheat, antioxidant capacity.

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1 Introduction

Wheat (*Triticum aestivum* L.) is a monocotyledon of the genus *Triticum*. Among the cultivated wheat species, soft wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*), being the most widely grown Wheat's high prevalence in food processing stems from its favorable technological properties. Furthermore, wheat boasts a significant nutritional profile, containing 6-25% protein, 10% moisture, 2% lipids and 2% minerals. The interior of the grain is particularly rich in vitamin E, linoleic acids, phospholipids and other beneficial elements, solidifying its position as a vital source of dietary staples [1]. Additionally, wheat's unique functionality is attributed to its gluten proteins, which enable the production of a diverse array of food products, including breads, baked goods, cookies, pasta, noodles, semolina, malt, breakfast cereals, snacks, beer, whiskey, tortillas, and various processed food items [2].

Corn (*Zea mays* L.) is a cereal grain native to the Americas, archaeological evidence suggesting its cultivation commenced over 10,000 years. This grain holds significant importance as a staple food crop in Mexico, particularly due to its versatility in various food applications. The most prominent example is the tortilla, a widely consumed flatbread. While corn tortillas are a valuable source of calories due to their high starch content. They do exhibit limitations in their nutritional profile with a deficient in essential amino acids, low levels of vitamins (A, D, E and B12), essential minerals (Fe and Zn) and dietary fiber [3]. Despite these limitations, Mexico remains a leading producer and consumer of corn tortillas, with a monthly production exceeding 370,000 tons and a daily national consumption estimated at 1.4 billion tortillas/day. This translates to an average daily intake of 8 to 10 tortillas per day by adults [4]. Consequently, corn tortillas represent the most widely consumed product within the Mexican population, particularly among lower socioeconomic populations [5].

Several studies have explored the incorporation of flours derived from various plant-based sources into tortillas as a strategy to enhance their nutritional value and nutraceutical properties. These sources encompass grains [6,7], cereals [8], seeds [9], and peels [10,11]. A significant concern within the food processing industry is the generation of substantial by-products that remain untreated and unutilized. These by-products are often disposed of through incineration, leading to detrimental environmental consequences. The composition and quantity of these by-products are influenced by the source material, product type, and processing steps employed [12]. Unfortunately, the lack of comprehensive and accurate data on the volume of waste generated hinders the identification of potentially valuable nutrients and antioxidants within these by-products. The potential for incorporating these by-products represents a missed opportunity to develop commercially viable products with enhanced nutritional value and affordability. Furthermore, to being cheap and abundant. Thus, food industries are looking for the opportunity to give value to by-products to reduce their negative impact on the environment and support sustainability, so their care is an opportunity and a condition of permanence.

Hibiscus sabdariffa L. calyxes are readily available in health food stores and supermarkets, either whole dried or as tea. These calyxes are consumed as refreshing beverages or for medicinal purposes due to their content of dietary fiber, ascorbic acid, anthocyanins, β-carotene, β-cytosterol, and phenolic compounds (PC) [13,14]. Following extraction, for beverage production, the remaining hibiscus decoction calyxes (HDC) become a waste product, despite being a potential source of proteins, vitamins, minerals, dietary fiber, and PC [14]. The scientific exploration of HDC is in its early stages but is gaining interest due to its potential functional properties [13]. Therefore, incorporating HDC as an ingredient in food development presents a valuable opportunity. This approach could contribute significantly to the scientific understanding of HDC and its potential health benefits, attracting broader interest within the research community. Furthermore, it could serve as a springboard for future research to explore these benefits in greater detail. In this context, HDC offers a promising ingredient for tortilla production, potentially enhancing the product's physical, functional, nutritional, antioxidant, and microbiological properties. Additionally, it could provide consumers with a natural food product free from potentially harmful additives. However, a current limitation lies in the lack of established collection systems for spent HDC. The absence of dedicated collection centers hinders the efficient reuse of this by-product in food development applications. Consequently, this research aims to evaluate the sensory, microbiological, physical and antioxidant properties of corn and wheat tortillas fortified with HDC.

2 Material and Methods

2.1 Material and reagents

The analytical grade reagents included a gallic acid ($C_7H_6O_5$), Folin-Ciocalteu ($C_{10}H_5NaO_5S$), sodium carbonate (Na_2CO_3), (+)-catechin ($C_{15}H_{14}O_6$), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,2'-Azino-bis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), sodium monobasic phosphate (NaH_2PO_4), sodium dibasic phosphate (Na_2HPO_4), all purchased from Sigma-Aldrich (Mexico City, Mexico). Methanol and acetone were purchased from J.T. Baker (Mexico City, Mexico).

2.2 Vegetable material

Hibiscus calyxes (*Hibiscus sabdariffa* L.) of the 'China' variety (5 kg) were procured from a supplier in Juarez City, Mexico (coordinates: 18°1′4.008″ N, 95°51′34.991″ W, altitude: 1126 m). The calyxes were transported to the laboratory for further processing. Upon arrival, the calyxes were manually cleaned to remove debris such as stones, leaves and stems. Subsequently, they were subjected to a drying process in a convection oven at 85°C for 2 h (Novatech®, HS60-ED). The drying process continued until reaching a constant drying weight. For the HDC preparation, 250 g of dried calyxes were steeped in 1 L of water at 85°C for 15 min. The resulting infusion was discarded via filtration and the HDC residue was recovered. The recovered HDC was then dried at 85°C for 5 hours before being pulverized in a NutriBullet® for 3 min. The pulverized HDC was subsequently sieved through a 5 µm mesh sieve to achieve a desired particle size. Finally, the sieved HDC was stored in an airtight container at -20°C until further analysis. The selection of a 5 µm sieving granulometry aimed to facilitate blending the HDC with the dough during tortilla processing. This particle size was chosen to: (i) maintain a smooth dough consistency, (ii) promote even heat transfer during processing, and (iii) prevent the formation of lumps in the final product.

2.3 Tortilla preparation

Before the tortillas were made, eight groups were prepared: 1) corn flour with HDC (TCHDC), 2) corn flour with preservative (TCP), 3) corn flour with HDC and preservative (TCHDCP), 4) corn tortilla obtained from a local tortilleria (TCT), 5) wheat flour with HDC (TWHDC), 6) wheat flour with preservative (TWP), 7) wheat flour with HDC and preservative (TWHDCP), 8) tortillas obtained from a supermarket (TWC). Handmade tortillas were prepared for all formulations except TCT and TWC.

All dry ingredients used were sieved before being incorporated for the preparation of the tortilla. In a bowl, 800 g of flour was combined with 200 g of HDC (for TCHDC, TCHDCP, TWHDC, and TWHDCP formulations only), 30 g of salt, and 30 g of baking powder. A well was formed in the center of the dry ingredients, and 30 g of corn oil was added. Subsequently, 250 mL of 70°C water was gradually incorporated to achieve a soft dough consistency. The dough was covered with plastic wrap and allowed to rest for 10 minutes at room temperature for softening. Individual dough balls (approximately 10 g each) were formed, resembling the size of a golf ball. These dough balls were again allowed to rest at room temperature, covered only with a clean kitchen cloth. A clean and dry work surface was dusted with flour. Each dough ball was rolled out using a rolling pin to a uniform thickness of approximately 3 mm and a diameter of 18 cm. The tortillas were cooked for 30 seconds per side on a preheated conventional griddle set to medium heat. The cooking process was continued until bubbles and brown spots appeared on the tortilla surface. Sixteen tortillas from each formulation were packaged in triplicate within transparent sealed bags at room temperature to preserve their organoleptic characteristics. Control tortillas were made following the same procedure. Control tortillas were dried, pulverized, sieved and stored in airtight containers following the above process and stored at −20 °C until analysis. Following preparation, the tortilla formulations were subjected to sensory evaluation as described in a subsequent section.

2.4 Sensory evaluation of tortillas with HDC

Sensory analysis of the tortilla formulations was conducted using a consumer panel of 80 untrained individuals between the ages of 15 and 40. The sample size was determined through a statistical power analysis, considering a standard deviation of 1.96, a significance level of 0.05% and an estimated margin of error of 5%. The analysis was performed on this population because the product is designed for a mass market. Five formulations were prepared containing varying levels of HDC: 5%, 20%, 50% y 70%. Each sample was assigned a unique three-digit numbers using the random number table to ensure anonymity during evaluation. Panelists were presented with coded samples and instructed to evaluate each sample for aroma and taste. A nine-point hedonic scale was employed, ranging from "like extremely" to "dislike extremely" (Mahdi-Hassan et al., [15]). The evaluation criteria included color, odor, flavor, texture, appearance, mouthfeel, and overall acceptability of the developed formulations. Following sensory evaluation, the tortilla formulation with the highest overall acceptability was selected for further analysis as described below.

2.5 Microbiological evaluation of tortillas with HDC

Microbiological analysis was conducted to ensure the formulated tortillas met the total aerobic mesophile [16], fungi and yeast [17], and total coliform counts [18] established by the Mexican Official Standard. Eleven grams (11 g) of each tortilla sample were aseptically weighed and transferred to a sterile homogenizing flask containing 99 mL of 0.1% alkaline peptonated. The mixture was then homogenized in a blender at medium speed for 2 minutes. Serial dilutions (1:10) were prepared from the initial homogenate (10^{-1}), in sterile tubes containing 9 mL of alkaline peptonated water. The prepared dilutions were then used for plate count assays to enumerate total aerobic mesophiles, fungi and yeasts, and total coliforms. Colony forming units (CFU) per gram of sample (CFU/g) were calculated for each microbial group.

2.6 Physical and quality properties of tortillas

The total weight of each tortilla, the total weight loss during cooking (TW, %) was measured on an analytical balance (ViBRA®, VE-1000), calculating the percentage by weight of material before and after cooking (approx. 40°C). Weight loss was expressed as a percentage of the initial weight and calculated using Equation (1):

$$TW = \left(\frac{T_{BC}-T_{AC}}{T_{BC}}\right) X\ 100 \quad \ldots\ldots\ldots (1)$$

where:

TW: weight loss during cooking, g
T_{BC}: tortilla before cooking
T_{AC}: tortilla after cooking

Nine tortillas were randomly selected for diameter measurement. On each tortilla, three perpendicular diameter measurements (D, cm) were obtained and subsequently averaged. A total of eighteen observations were thus collected. A Mitutoyo Corporation Modelo CD6''C vernier caliper (Kanagawa, Japan) was employed to measure the average thickness (mm) of the tortillas. This measurement was obtained by carefully stacking twelve tortillas. The spread ratio of the tortillas was calculated using the Winstone method [19]. This ratio was determined by dividing the average diameter value by the average thickness value.

Puffing Degree

The puffing degree (PD) of the tortillas was evaluated subjectively based on the method outlined by Cuevas-Martínez et al. [20]. Ten randomly selected tortillas were assessed for their degree of inflation during preparation. A three-point scale (1-3) was employed to quantify the size of the blister observed with each point accompanied by a descriptive subgroup: 1 (0 – 25%) - No or minimal puffing, 2 (25 – 75%), Moderate puffing and 3 (75 – 100%) Complete puffing (refer to Table 1 for detailed descriptions).

Rollability

The rollability of Tortilla was measured using the method established by Cepeda et al. [21]. Thirty min after preparation, each tortilla was rolled around a glass with a diameter of 2 cm. The degree of breakage incurred during rolling was assessed subjectively using a five-point scale (1 – 5); 1: Unrollable/breaks or large cracks across most of the tortilla area (0%); 2: Half cracks over a significant portion of the tortilla or difficulty in rolling (25%); 3: Small cracks present or rolls up easily with a broken quarter of the total tortilla area (50%); 4: Rolls up easily with minimal hairline cracks in specific areas (75%) and, 5: Rolls up easily without any cracking (100%).

Table 1. Degree of inflation of tortillas

Category	Units (mm)	Score (%)
1		
- A	Null	0
- B	2-5	12
- C	6-14	25
2		
- A	15-29	25
- B	30-39	50
- C	40-49	75
3		
- A	50-59	75
- B	60-69	88
- C	70-more	100

Tortilla moisture at 24 h

The moisture content of the tortilla at 24 hours (MT24) was determined following the AOAC method 14.004 (1990) [22], with slight modifications. Samples were collected 24 h after preparation. Immediately following weighing, the cooled tortillas were bagged and stored in a refrigerator for subsequent moisture analysis. Prior to moisture testing, the samples were equilibrated at room temperature for 2 hours. Subsequently, a 2 g sample was weighed in a pre-weighed aluminum container and placed in a forced-air convection oven (Novatech®, HS60-ED) set at 100°C for 12 hours. The moisture content was determined by the gravimetric method, calculated as the difference in weight of the sample before and after drying.

Tortilla yield

The tortilla yield (TY, %) was determined from Equation 2[22]:

$$\%TY = MY(1 - \%WLC) \quad \ldots\ldots\ldots (2)$$

where:

TY: Tortilla yield, %
MY: Mass yield
WLC: weight loss during cooking, %

2.7 Extraction of total soluble phenolic compounds (TSPC)

An organic-aqueous double extraction was performed in triplicate to obtain the TSPC extract, following the procedure outlined by Mercado-Mercado et al. [14], with some modifications. Five hundred milligrams (500 mg) of sample were weighed and mixed with 20 mL of acidified methanol solution (80% v/v, 0.8 M hydrochloric acid). The mixture was subjected to constant vortex agitation for 5 min (MS1 Minishaker, IKA®). Subsequently, the extracts were centrifuged (Z 326 K, HERMLE®) at 4000 rpm for 10 min at a controlled temperature of 5°C. The supernatants were collected and 20 mL of 80% (v/v) acetone solution was added to the remaining sediments. This extraction step was repeated using the same centrifugation conditions. The combined supernatants were then adjusted to a final volume of 50 mL with a 50:50 v/v mixture of the acidified methanol-acetone solutions (80% v/v).

Total soluble phenolic compounds content

The Folin-Ciocalteu method, as described by Mercado-Mercado et al. [14] with partial modifications, was employed to determine the TSPCC. In a microplate, 25 μL aliquots of tortilla extracts or gallic acid standard were mixed with 100 μL of sodium carbonate (7.5% w/v) and incubated for 3 minutes. Subsequently, 125 μL of Folin-Ciocalteu reagent (10% v/v) was added and the mixture was incubated in a convection oven (Fisher Scientific®, Isotemp oven 637 G) at 50°C for 30 min. The absorbance of each sample was measured at 750 nm using a microplate spectrophotometer (Multiskan FC, Thermo Scientific®). The experiment was performed in triplicate. The TSPC content was expressed as milligram gallic acid equivalents per gram dry weight (mg GAE/g) using the following calibration equation: y = 3.7885x + 0.0249).

Total flavonoid content (TFC)

The TFC was analyzed using the aluminum chloride method described by Mercado-Mercado et al. [14]. Catechin (0 to 0.2 mg/mL) was employed as the standard for this analysis. Fifty microliters (50 µL) of extracts were mixed with 25 µL of distilled water, 15 µL of sodium nitrite solution (5 % w/v) and incubated for 10 min in the dark. Subsequently, 15 µL of aluminum chloride (10% w/v) and 25 µL of sodium hydroxide (0.5 M) were added. The reaction mixture was allowed to stand for 40 min in the dark and then read at 510 nm. The TFC was expressed as mg of catechin equivalents per gram dry weight (mg EC/g; y = 7.6367x + 0.3212).

Antioxidant capacity determination with ABTS method

The ability of the extracts to inhibit the ABTS radical cation (ABTS+•) was evaluated following the method described by Mercado-Mercado et al. [14]. An ABTS radical solution (7 mM) was generated by reacting ABTS with potassium persulfate (2.42 mM) in a phosphate buffer. The reaction mixture was incubated in the dark at room temperature for 17 hours. Subsequently, the ABTS radical solution was diluted to an absorbance value of 0.7 ± 0.05. The inhibition of ABTS (270 µL) by the extracts (30 µL) was determined by measuring the decrease in green color at 734 nm after 10 min of incubation in the microplate reader. The experiment was performed in triplicate. The results were expressed as millimoles of trolox equivalents per gram dry weight (mmol TE/g) using the following calibration equation: y = 0.2053x + 10.669.

Antioxidant capacity determination with DPPH method

The free radical scavenging activity of the tortillas was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical method as outlined by Mercado-Mercado et al. [14] with minor modifications. A stock solution of DPPH (1 mM) was prepared and stored at 4°C protected from light. A working solution of DPPH (230 µM) was prepared by diluting the stock solution. In a microplate, 25 µL of extract was mixed with 200 µL of the DPPH working solution. The reaction mixture was incubated for 10 minutes, and the inhibition of the DPPH radical was measured by the decrease in absorbance at 517 nm using a microplate reader. The results were expressed as mmol TE/g using the following calibration equation: (y = 0.0694x + 1.6585).

2.8 Statistical analysis

Following the completion of the analytical determinations, the data were subjected to statistical analysis using Statistica software (version 10). An analysis of variance (ANOVA) was performed to assess the existence of statistically significant differences between the obtained data points. To further explore these differences, multiple range tests were conducted using the least significant difference (LSD) criterion at a significance level of $p < 0.05$.

3 Results

3.1 Sensory evaluation of tortillas with HDC

Tortilla formulations (corn and wheat) were developed incorporating ingredients known to enhance the nutritional quality and potential health benefits of the products (TCHDC, TCHDCP, TWHDC and TWHDCP – detailed descriptions in Figure 1). These formulations were then subjected to sensory evaluation by a consumer panel. The results indicated that TCHDC and TWHDC formulations with a 20% inclusion level were the most preferred options across all evaluated parameters (color, flavor, taste, aroma, and overall acceptability) as shown in Figure 1. Conversely, formulations with 5% and 70% inclusion levels received the lowest ratings compared to the 20% formulations. Panelists' feedback revealed that the 5% inclusion level resulted in an undetectable hibiscus flavor and an unappealing visual appearance due to the red color appearing as uneven spots on the tortillas. Conversely, the 70% inclusion level led to an excessively astringent and acidic flavor profile.

The sensory evaluation results for the TCHDC and TWHDC formulations with a 20% inclusion level (as detailed in Table 2) revealed favorable attributes compared to the commercial controls. The panelists exhibited a strong preference for these formulations based on visual characteristics (color), as well as the aroma and texture were described as characteristic of a traditional tortilla. Furthermore, the chewiness was perceived to be greater, indicating a desirable textural property of softness and ease of digestion.

3.2 Microbiological evaluation of tortillas with HDC

The results of the microbiological analysis of the tortillas are presented in Table 2. The standard plate count (SPC) for mesophilic aerobes was determined according to the specifications outlined in the Mexican Official Standard

Table 2. Percentage of sensory acceptability between the comparative between commercial tortillas, tortillas of corn with hibiscus decoction calyxes (TCHDC), and wheat substituted with hibiscus decoction calyxes (TWHDC)

Sensory analysis (%)	TCHDC 20%	Commercial tortillas	TWHDC 20%	Commercial tortillas
Color	90.14	85.25	89.40	82.00
Smell	93.12	89.75	93.18	86.70
Taste	84.80	82.89	92.50	85.00
Texture	89.70	80.45	96.60	88.70
Appearance	98.30	95.17	97.48	90.48
Oral Texture	96.00	91.66	98.20	95.80
Overall Acceptability	93.40	88.73	97.00	94.00

TCDH: corn tortilla with HDC; B) TWHDC: wheat tortilla with HDC.

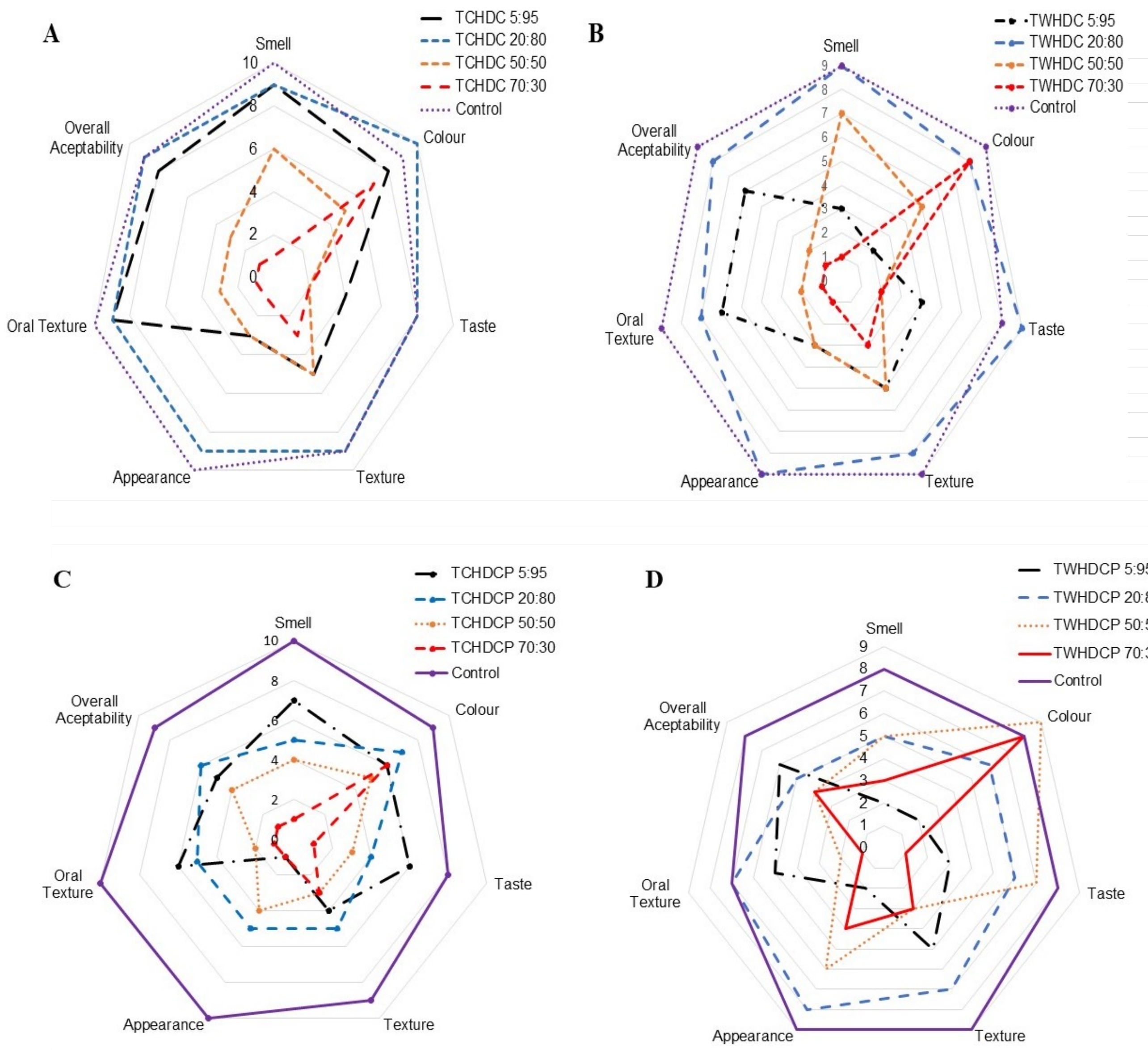

Figure 1. Sensory analysis of tortillas based on decoction calyces of Roselle (*Hibiscus sabdariffa* L). A) TCDH: corn tortilla with HDC; B) TWHDC: wheat tortilla with HDC; C) TCHDCP: corn tortilla with HDC and preservative; D) TWHDCP: wheat tortilla with HDC and preservative

NOM-187-SSA1/SCFI-2002 for "Products and Services. Dough, tortillas, toast and flour prepared for their preparation and establishments where they are processed". This standard establishes a maximum permissible limit of <30 colony-forming units (CFU) per gram for mesophilic aerobes. As shown in Table 2, the SPC values for all tortilla samples were below this limit, indicating compliance with the standard. Also, the TCAM of tortillas with hibiscus is within the maximum permitted limit set by the ¨NOM-113-SSA1/1994, Goods and services. Method for counting total coliform microorganisms on a plate". However, the samples TCT, TWP and TWC exceeded the maximum limit established by NOM-187. Regarding the count for molds and yeasts and total coliforms for all tortillas analyzed, the presence of these microorganisms was null (Table 3).

3.3 Tortilla quality

Table 3 compares the quality parameters (thickness-yield ratio (TY), diameter, puffing degree (PD), rollability, and puffiness) of corn and wheat tortillas with those containing hibiscus discards (HDC) substitution. Significant differences were observed between the groups (Table 3). The rollability values in Table 3 indicate that tortillas prepared with both corn and wheat flour did not break when rolled, demonstrating compliance with this quality parameter.

The rollability of the tortilla as indicated by the rollability value in Table 4, was sufficient to prevent breakage during rolling, demonstrating the suitability of the corn and wheat flour used in this aspect.

The analysis of MT24 revealed significant difference between tortilla formulations as shown in Table 3. Among the corn tortillas, TCHDC exhibited a statistically significant difference in MT24 compared to TCT. In contrast, all wheat tortilla formulations displayed significant differences from each other.

For all tortillas, MT24 was determined following the procedure outlined previously. TCT exhibited the highest

Table 3. Evaluation of quality characteristics and physicochemical properties of corn, wheat and tortillas substituted with hibiscus decoction calyxes (HDC)

Tortillas	Diameter (cm)	PD (%)	Rollability (%)	TW (g)	MT24 (%)	TY (Kg/Kg)
Corn tortilla						
- TCHDC	13.31 ± 0.45^{b}	84 ± 2.81^{b}	77 ± 2.19^{b}	25.48 ± 2.64^{a}	36.19 ± 2.48^{c}	1.85 ± 0.04^{a}
- TCHDCP	14.05 ± 0.33^{b}	86 ± 2.48^{b}	79 ± 3.18^{b}	24.92 ± 3.57^{a}	31.76 ± 3.84^{d}	1.74 ± 0.11^{a}
- TCP	13.66 ± 1.33^{b}	89 ± 1.29^{a}	92 ± 1.48^{a}	27.65 ± 1.17^{a}	39.48 ± 1.08^{b}	1.49 ± 0.14^{a}
- TCT	14.11 ± 1.14^{b}	91 ± 3.57^{a}	96 ± 2.49^{a}	27.45 ± 0.84^{a}	41.18 ± 1.9^{a}	1.65 ± 0.08^{a}
Wheat tortilla						
- TWHDC	18.92 ± 1.42^{b}	77 ± 3.11^{c}	74 ± 3.67^{c}	21.97 ± 1.52^{b}	32.46 ± 1.89^{c}	1.48 ± 0.07^{a}
- TWHDCP	18.24 ± 2.44^{b}	75.25 ± 3.41^{c}	74 ± 2.37^{c}	22.16 ± 1.28^{b}	33.81 ± 2.14^{c}	1.20 ± 0.21^{a}
- TWP	19.30 ± 0.49^{b}	87 ± 2.55^{b}	86 ± 1.71^{b}	24.61 ± 2.71^{a}	36.19 ± 2.48^{b}	1.22 ± 0.08^{a}
- TWC	20.44 ± 1.05^{a}	92 ± 4.19^{a}	89 ± 2.28^{a}	25.67 ± 1.88^{a}	40.53 ± 2.04^{a}	1.91 ± 0.19^{a}

HDC: decoction calyxes of hibiscus; TCHDC: tortilla of corn flour with HDC; TCP: tortillas of corn flour with preservative; TCT: handmade tortilla of corn obtained from tortilleria; TWHDC: tortilla of wheat flour with HDC; TWP: handmade tortilla of wheat flour with preservative; TWFP: wheat fluor tortilla with preservative; TWCM: wheat flour tortillas marketed in supermarket; PD: puffing degree; TW: weight of tortilla; TM24: tortilla moisture at 24 h; TY: tortilla yield: 1= fully puffing, 2= intermediate puffing, 3= non puffing. Values with lower case letters represent a significant difference between samples (p ≤ 0.05).

Table 4. Microbiological evaluation of tortillas of corn and wheat substituted with hibiscus decoction calyxes (HDC)

Samples	TCAM (CFU/g)	FY (CFU/g)	TC (CFU/g)
Corn tortilla			
TCHDC	29 ± 4^{a}	<100^{a}	<3^{a}
TCHDCP	31 ± 6^{b}	<100^{a}	<3^{a}
TCP	97 ± 5^{c}	<100^{a}	<3^{a}
TCT	118 ± 53^{d}	<100^{a}	<3^{a}
TWHDC	38 ± 7^{a}	<100^{a}	<3^{a}
TWHDCP	32 ± 6^{a}	<100^{a}	<3^{a}
TWP	67 ± 6^{c}	<100^{a}	<3^{a}
TWC	138 ± 8^{b}	<100^{a}	<3^{a}

HDC: hibiscus of decoction calyxes of; TCHDC: tortilla of corn flour with HDC; TCHDCP: tortilla of corn flour with HDC with preservative; TCP: tortillas of corn flour with preservative; TCT: tortilla of corn obtained from tortilleria; TWHDC: tortilla of wheat flour with HDC; TWHDCP: tortilla of wheat flour with preservative; TWP: handmade tortilla of wheat flour with preservative; TWFP: wheat flour tortilla with preservative; TWC: wheat flour tortillas marketed in supermarket; TCAM: total count of aerobic mesophiles bacteria; FY: fungi and yeasts; TC: total coliforms. Marketed. Values with lower case letters represent a significant difference between samples (p ≤ 0.05).

Table 5. Total soluble phenolic content (TSPCC), total flavonoids content (TFC), and antioxidant activity of wheat and maize tortillas substituted with decoction calyxes of *Hibiscus*

Samples	TSPCC (mg GAE/g)	TFC (mg CE/g)	Antioxidant capacity (mmol TE/g) DPPH	ABTS
Corn tortilla				
- TCHDC	2.98 ± 0.57[a]	2.82 ± 0.15[a]	24.79 ± 2.21[a]	27.66 ± 3.61[a]
- TCHDCP	1.85 ± 0.29[b]	2.41 ± 0.18[a]	19.42 ± 3.11[b]	25.87 ± 3.59[a]
- TCP	0.42 ± 0.07[c]	0.80 ± 0.06[b]	10.93 ± 1.09[c]	5.08 ± 0.37[b]
- TCT	0.30 ± 0.08[c]	0.73 ± 0.08[b]	10.30 ± 0.79[c]	2.66 ± 0.19[c]
Wheat tortilla				
- TWHDC	7.05 ± 0.20[a]	2.28 ± 0.26[a]	18.02 ± 0.41[a]	41.90 ± 3.39[a]
- TWHDCP	4.19 ± 0.36[b]	1.11 ± 0.19[b]	7.58 ± 0.61[b]	17.75 ± 1.95[b]
- TWP	0.22 ± 0.05[c]	0.62 ± 0.09[c]	3.15 ± 0.47[c]	3.15 ± 0.17[c]
- TWC	0.10 ± 0.09[c]	0.49 ± 0.00[c]	3.47 ± 0.47[c]	3.61 ± 6.23[c]

HDC: decoction calyxes of hibiscus; TCHDC: tortilla of corn flour with HDC; TCP: Handmade tortillas of corn flour with preservative; TCHDCP: tortilla of corn flour with HDC and preservative; TCT: tortilla of corn obtained from tortilleria; TWHDC: tortilla of wheat flour with DCH; TWP: handmade tortilla of wheat flour with preservative; TWHDCP: tortilla of wheat flour with HDC and preservative; TWC: tortillas obtained from a supermarket; TSPC: Total soluble phenolic compounds content; TFC; Total flavonoid content. Values with lower case letters represent a significant difference between samples ($p \leq 0.05$).

MT content (41.18%), followed by TCP (39.48%) and TCHDC (36.19%). A similar trend was observed in wheat tortillas, with TWCM exhibiting the highest MT24 (40.53%), and TWHDC displaying a lower MT24 content (32.46%). The thickness-yield ratio (TY) of TCHDC tortillas was the highest among all formulations, as detailed in Table 4.

3.4 Phenolic compounds content

The results of total soluble phenolic content (TSPC), total flavonoid content (TFC), and antioxidant capacity are presented in Table 4. TCHDC exhibited the highest TSPC content among all tortilla formulations. The TFC values were comparable between corn and wheat tortillas, with formulations containing hibiscus discards (HDC) demonstrating significantly greater content compared to control tortillas.

On the other hand, the antioxidant capacity of tortillas containing hibiscus surpassed that of control tortillas as determined by both the DPPH and ABTS methods (Table 5). Notably, tortillas prepared without preservatives displayed a higher antioxidant capacity (DPPH) in both corn and wheat varieties. This trend was also observed for the ABTS method in TWHDC, while no significant difference was detected between TCHDC and TCHDCP using the same method.

4 Discussion

4.1 Sensory evaluation of tortillas with HDC

Sensory evaluation plays a critical role in consumer acceptance of food product. While color received the highest ratings, the oral texture of the tortillas with HDC was negatively perceived by panelists. Specifically, comments indicated a sensation of chewing calyxes, even though they were finely ground and sieved prior to incorporation. Nonetheless, the 20% inclusion level formulations (TCHDC and TWHDC) achieved the most favorable scores across all evaluated attributes. Conversely, the 20% formulations with preservatives (TCHDCP and TWHDCP) received lower ratings for certain attributes. Panelists attributed the loss of flavor and the absence of a characteristic hibiscus aroma to the presence of preservatives in the tortillas.

The evaluation of aroma can be challenging due to inherent individual variations in preferences and olfactory abilities. These subjective factors often lead to diverse opinions during sensory assessment. Consequently, aroma can be a difficult parameter for objectively determining product quality [4].

Aroma plays a significant role in influencing the perceived quality and overall enjoyment of food. In this study, the inclusion of preservatives in the TCHDCP and TWHDCP formulations (20% HDC) resulted in a three-point decrease in overall acceptability scores, potentially due to a perceived loss of aroma. Heating wheat tortillas resulted in a noticeably browner color compared to corn tortillas. High temperatures during the cooking process can contribute to the degradation of flavor and aroma compounds. Conversely, panelists displayed a preference for the color of the hibiscus-incorporated corn tortillas (Figure 1). Color is a crucial sensory attribute that can influence both perceived quality and consumer acceptance of tortillas. Additionally, color can be used as a preliminary indicator of nutritional content [24].

The sensory acceptability scores obtained for the 20% HDC formulations (TCHDC and TWHDC) were comparable to those reported for tortillas enriched with various alternative flours, such as Maya nut flour (10% (6.99) and 20% (5.92) [25], banana flour 20% [26], moringa flour 30% [27], and broccoli flour at 4% and 8% (7.96 y 7.38, respectively) [28]. Based on the overall acceptability results from the sensory evaluation, the TCHDC and TWHDC formulations with a 20% inclusion level were identified as the most promising options for further development.

4.2 Microbiological evaluation of tortillas with HDC

Microbial growth in tortillas can be influenced by several factors. One potential contributor is the water activity, which is generally lower in corn tortillas compared to wheat tortillas [28]. This difference arises from the nixtamalization process (cooking corn at 80°C for 20-45 minutes), which dehydrates the corn and reduces the available water for microbial growth [29]. Proper hygiene practices, good manufacturing practices, and cooking in limewater further reduce the levels of molds, yeasts and total coliforms that may be present in corn dough or wheat flour dough.

4.3 Tortilla quality

During tortilla manufacturing process, corn and wheat doughs form a mixture of gelatinized starch and dispersed granules of hydrated and denatured protein. These irregular and swollen granules contribute to the dough's cohesive structure [30]. Cooking disrupts the crystallinity of native starch granules [31].

Thickness-yield ratio (TY) is an important indicator of flour quality, and is directly proportional to the water absorption capacity, which itself is associated with the degree of starch gelatinization. Notably, baking temperature and water absorption capacity are closely interrelated. Variations in these parameters impact moisture content, dough rheology and overall tortilla quality. Higher backing temperatures promote greater starch gelatinization, leading to increased water absorption capacity [32]. Conversely, the addition of other flours or fiber sources can significantly alter the degree of gelatinization [33-35].

The puffing degree (PD) and rollability of TCHDC and TWHDC formulations were lower compared to control tortillas. HDC contains dietary fiber and PC, that can accelerate starch retrogradation, a process that starts in tortillas upon cooling after cooking [35]. Studies suggest that PC binds with the starch polymers, amylose and amylopectin, reducing their water-holding capacity and causing partial starch shrinkage [31,32]. The observed quality decline in these tortillas is attributed to the faster degradation of amylose compared to amylopectin. Amylose, due to its linear structure, forms fewer hydrogen bonds with adjacent molecules (amylose and amylopectin) compared to the highly branched amylopectin. This weaker interaction leads to reduced hydration capacity and contributes to partial starch shrinkage [37,38]. Additionally, protein, dietary fiber, and PC interactions can also contribute to hardening [36].

The variations in rollability could be explained by the texture of TCHDC. While softer and more flexible than control tortillas, they were less easy to roll or bend without cracking. The addition of HDC appears to partially compromise the flexibility of both corn and wheat tortillas. Similar findings were reported by Valdez-Reyes et al. [39] for tortillas made with corn native to the Sierra de Manantlán biosphere (75% rollability) compared to our TCHDC (77%) and TWHDC (74%) formulations. Reduced rollability (7.5%) was also observed in tortillas containing Xkijit flour, leading to lower flexibility and limitations in their use for tacos [40]. Mashau et al. [41] observed a decrease in rollability with increasing *Bambara* groundnut flour concentration (15% and 20%). the rollability decreased. In contrast to our results, Moreno-Castro et al. [42] reported increased rollability with corn flour substitution using flours with higher protein content (triticale and chia seed flour). Changes in rollability are associated with decreased water retention [43,44]. The protein and CP content of THDC 20% may have hindered gluten swelling [45]. It should be underlined that water and oils function as dough conditioners, directly influencing dough formation and final structure, which in turn improves the softness and rollability of tortillas [41]. This may explain why oil was added in our tortilla, although the high rollability values of tortillas with HDC although their cohesiveness values were low compared to tortillas without HDC.

The observed differences of MT24 are consistent with findings reported by Palacios-Pola et al. [43], Ramírez-Vega et al. [46] and Salinas et al. [47], who reported similar values for TCDCH. This parameter is linked to the ability of starches to retain water after retrogradation during tortilla cooling [48]. It also influences the ease of rolling and the force required to break the tortilla [38].

The moisture content of tortillas with HDC falls within the range reported for tortillas made with lupin flour (2.5% to 7.5%) [49] and is superior to that observed in tortillas supplemented with 20% *Bambara* groundnut flour [40], those enriched with Faba-bean and White-bean flour [50] and varieties from Oaxaca, commercial corn and mixtures of hybrid corn varieties reported by Acosta-Estrada et al. [4]. However, tortillas containing oat flour retain higher moisture (59.2 – 56.5%) [51]; which aligns with data obtained in nixtamalized tortillas as reported by Gasca & Casas [52]. Therefore, the moisture in tortillas is due to the concentration of water-soluble polysaccharides capable of forming gums with little water [44].

The TY values for TCHDC formulations were identical to those reported by other researchers for native and hybrid maize varieties [53-55]. Martínez-Flores et al. [56], found yields between 1.2 to 1.5 kg of flour/kg of dough for criollo corn in central Mexico.

4.4 Phenolic compounds content

A study by Colín-Chávez et al. [57] reported higher concentrations of total soluble phenolic content (TSPC) in handmade white and blue corn tortillas (0.74 mg GAE/g and 1.21 mg GAE/g, respectively) compared to those found in the present investigation. Likewise, the reported TSPC data for the tortillas evaluated by Acosta-Estrata et al. [4] and tortillas added with *Bambara* groundnut flour [41] presented higher than our study. However, the results of commercial tortillas (0.38 mg GAE/g) (0.38 mg GAE/g) 56 are identical to those obtained in our study (0.30 mg GAE/g db). Likewise, TSPC of TCHDC and TCHDCP are within the range of blue corn tortillas reported by Colín-Chávez et al. [57], and Mexican tortillas of different grains (white, yellow, blue, red) studied by Aguayo-Rojas et al. [58] and de la De la Parra et al. [59]. However, the values for blue-grain and white-grain corn tortilla reported by Salinas-Moreno et al. [53] are higher than the data obtained in the current study with TCDCH and TCDCHP.

Our findings suggest that the content of free and bound phenolics varies as a function of the thermal processing time associated with tortilla preparation. The observed values could probably be attributed to the fact that some of the non-phenolic compounds are released and increase the phenolic content, also reacting with the Folin-Ciocalteu reagent [60]. Similarly, thermal processing likely increases the PC content by releasing bound PC, making them free and more easily quantifiable [61].

Tortillas containing Bambara groundnut studied by Mashau et al. [40] exhibited higher TFC than tortillas formulated with HDC. However, TCHDC, TCHDCP, TWHDC and TWHDCP formulations displayed higher TFC values than those reported by Mora-Rochin et al. [45]. These variations in TFC across different matrices and studies can be explained by the relationship between pericarp and endosperm, which are the structures with the highest content of phenolic compounds [61,62]. In addition, factors such as genotype, the origin of the matrix (maize, wheat, calyces) and interactions between biomolecules and other components could explain the discrepancies in PC content [53,61]. Furthermore, the content of flavonoids may be associated with the various chemical reactions leading to changes in the food's chemical composition, which were subjected to high temperature processing [63]. In this regard, PC could undergo isomerization, degradation and polymerization reactions resulting in the formation of new substances with typically weaker astringency [64]. Therefore, the astringency of hibiscus might be reduced in tortillas, potentially improving their sensory acceptability.

The antioxidant capacity of the hibiscus-containing tortillas in this study was lower compared to those reported for various corn species [58], tortillas with *Bambara* groundnut [41], tortillas with Nejayote [61]. However, the observed values were higher than those found for flour tortillas prepared with corn and wheat, respectively [59,65]. These findings suggest a potential influence of the source material and processing conditions on the antioxidant capacity of tortillas.

High temperature, in combination with oxygen availability, can exert an ambiguous effect on the antioxidant properties of foods. On one hand, it can increase the oxidation of raw materials leading to the degradation of PC [66]. On the other hand, it may also promote the formation of new compounds with antioxidant activity. Therefore, the observed antioxidant capacity (Table 5) likely reflects a balance between the loss of natural antioxidants and the generation of novel antioxidant compounds. Additionally, the total antioxidant capacity is likely influenced by the content, type, and form (free or bound) of phenolics present, which can vary depending on the origin of the flour (wheat, corn, or hibiscus). Furthermore, the specific antioxidant assays employed can also influence the measured capacity [67-69].

5 Conclusion

This study demonstrates the potential of hibiscus discards (HDC) as a functional ingredient for food applications. The results revealed statistically significant differences ($p \leq 0.05$) in most analyzed variables highlighting the impact of HDC incorporation on quality parameters, sensory characteristics, and antioxidant capacity of corn and wheat tortillas. Formulations containing 20% HDC in both corn and wheat tortillas achieved the highest sensory and microbiological acceptability. HDC flour could possess a potential antifungal effect, because the tortillas supplemented with HDC had less microbiological growth, ensuring compliance with the microbiological parameters of NOM-18/-SSA1/SCH-2002. In conclusion, the addition of HDC to wheat and corn tortillas offers promising results in terms of quality attributes, some of which hold significant value for further scientific exploration and commercial purposes.

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