

Article - Environmental Sciences

Artisanal Vegan Soap: Preparation, Physicochemical Characterization and Bacterial Contamination Analysis for Quality Control

Larissa Maculan¹

<https://orcid.org/0009-0008-2195-1434>

Gabriela Tonello¹

<https://orcid.org/0009-0007-9376-2344>

Carlos Henrique Blum da Silva¹

<https://orcid.org/0000-0003-0596-2973>

Marcel Henrique Marcondes Sari²

<https://orcid.org/0000-0002-9913-9306>

Ana Carla Penteado Feltrin^{1*}

<https://orcid.org/0000-0001-7980-3356>

¹Faculdade Ideau, Curso de Farmácia, Passo Fundo, RS, Brasil; ²Universidade Federal do Paraná, Programa de Pós Graduação em Ciências Farmacêuticas, Curitiba, PR, Brasil.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Luiz Gustavo Lacerda

Received: 06-Sep-2023; Accepted: 24-Jun-2025

*Correspondence: anacarlafeltrin@hotmail.com; Tel.: +55-55-999017432 (A.C.P.F.).

HIGHLIGHTS

- Innovative formula for artisanal vegan soap production.
- Artisanal vegan soap presented adequate properties.
- No microbiological contamination was observed.
- The product fits the standard limits of ANVISA.

Abstract: Cosmetics made from natural ingredients have gained popularity among a new generation of consumers concerned about their environmental impact. Vegan Soap, in particular, has become a globally important product. This study aimed to produce artisanal vegan bar soap and perform physical-chemical and microbiological analyses to ensure its quality. The quality control study was based on the recommendations in the Quality Control Guide for Cosmetic Products established by ANVISA. Each soap bar weighs 90g and is made from a glycerin base (100% vegetable), lauryl glucoside, cabbage extract (powder), white clay, avocado oil, grape seed oil, and tea green essence. The soap is light green in color and has not undergone any changes in its characteristics. The results of bacterial contamination tests (*Staphylococcus aureus* and total aerobic mesophilic bacteria) were negative, and there was no evidence of free alkalinity or mechanical stress. Physicochemical analysis revealed a pH of 9.58, a density of 0.98 g/mL, a saponification value of $319.39 \pm 4.2\%$ mg of KOH/kg, a peroxide value of $9 \pm 1\%$ mEq/kg, and a total fatty acid value of $59.4\% \pm 3.2\%$. These results are within the limits set by standards, indicating that the artisanal vegan soap produced is an acceptable economic and environmental product.

Keywords: Sustainability; Biodegradable Materials; Environmental responsibility.

GRAPHICAL ABSTRACT



INTRODUCTION

Cosmetics are a part of the daily routine for many people, serving basic functions like hygiene or specific purposes like skin care. However, even the simplest or most complex products have ingredients that could result in unwanted reactions in the short or long term. Furthermore, some of these substances may harm the environment, disrupting the natural balance of aquatic life and polluting water sources [1]. In a study conducted by Rocha and colleagues [2], children exposed to cosmetics and personal care products were evaluated, revealing several components that could disrupt the endocrine system. Urine samples contained parabens, triclosan, triclocarban, and benzophenones, as well as contaminants from the packaging like bisphenol. Methylparaben, a specific type of paraben, was found in all samples.

The increasing importance of human health and environmental preservation has led to a new natural and vegan cosmetics market. This market caters to a new type of consumer, known as the "green consumer," who prioritizes the environmental impact of both production and consumption. They value biodegradable packaging, avoidance of animal-derived raw materials and dyes, and socio-environmental responsibility, and are willing to pay a premium for such products [3]. This lifestyle involves seeking products that align with ethical standards such as socio-environmental concerns, sustainability, and cruelty-free beauty [4]. In response to this demand, the cosmetics industry is transforming, focusing on conscious consumption and the emergence of biocosmetics.

Natural biocosmetics use only vegetable ingredients to stimulate skin recovery, which is expected to be less harsh on both the skin and the environment [5]. The biocosmetics market is growing, but it is essential to ensure these products are safe for human health [6]. It is important to recognize that many ingredients and processes go into creating a finished product, whether natural, organic, vegan, or synthetic, before it reaches the consumer. Even if they are handmade or natural cosmetics, they must comply with the regulations set by the responsible entity and undergo physicochemical and microbiological tests to assess important criteria such as stability [7]. Quality control is necessary regardless of the production method - industrial or artisanal - to ensure the safety and integrity of the product before it reaches the consumer. Quality control optimizes processes and resources and ensures the quality of the environment, inputs, and final product, thereby adding credibility [6].

With the increasing popularity of natural and vegan cosmetics in the market, it is crucial to ensure the safety and quality of these products. However, studies and data on quality control for homemade cosmetics are lacking. To address this issue and cater to the demands of the current consumer market, we conducted a study to produce a high-quality artisanal vegan bar soap. This involved conducting physicochemical and microbiological analyses to ensure proper quality control for this biocosmetic product.

MATERIAL AND METHODS

Reagents

To conduct this study, a range of reagents were utilized. These included: a glycerin base from Alles Soluções Químicas, corn lauryl glucoside from Alles Soluções Químicas, cabbage extract powder from Colormix Especialidades Químicas, white clay from Bioficina Superminerais, avocado oil from Distriol – Extração vegetal, grape seed oil from Distriol – Extração vegetal, green tea essence from Vollmens Fragrance LTDA, as well as ethyl alcohol, phenolphthalein, distilled water, petroleum ether, potassium iodide, acetic acid, chloroform, sodium thiosulfate, starch, potassium hydroxide, hydrochloric acid, sodium chloride, Tryptic Soy Agar, Brain Heart Infusion broth, and Baird Parker Agar culture medium. All the chemicals used in this study were of an analytical grade.

Formulation of artisanal vegan soap

The formulation used in soap production was defined based on information from suppliers, Gaboya [8] and Bortolai [9], this is described in Table 1.

Table 1. Formulation of artisanal vegan bar soap.

Feedstock	Amount (g)	Amount (%)	Function
Glycerin base (100% vegetable)	1000	100	Soap production base
Corn lauryl glucoside	172.41	17	Surfactant
Cabbage Extract (powder)	10	1	Natural dye
White clay	5	0.5	Whitening and smoothing
Avocado oil	16.59	1.6	Antioxidant
Grape seed oil	16.37	1.6	Regenerating and revitalizing
Essence (green tea)	73.35	7.3	Flavoring

Samples of artisanal vegan bar soap were produced by heating the glycerin base, not exceeding 60 °C to avoid loss of its properties, in an enameled pan next to the heating plate, until its complete melting. The cast base was removed from the plate to cool. Upon reaching 50 °C, the other components of the formulation were added and, with the aid of a silicone spatula, were incorporated into the base. After this process, the mixture was distributed in a silicone mold with 12 round cavities (65 mm wide x 30 mm high), which was stored until completely dry. After 48 h the samples were submitted for analysis.

Assessment of soap quality control

The definition of the analyzes to be used in the quality control study of the handmade soap produced was carried out, based on what is recommended in the Quality Control Guide for Cosmetic Products, established by Agência Nacional de Vigilância Sanitária (ANVISA). Sampling evaluations and organoleptic parameters, pH, density, mechanical stress, free alkalinity, and peroxide value, were determined according to ANVISA [10]. The testing was conducted three times with one replicate in each test group. The same soap solutions were stored and used in each test set and replicated to ensure that a large enough sample size was obtained for analysis.

For determining total fatty acids, 5 g of sample were weighed in a 250 mL beaker, to which 50 mL of water and 50 mL of ethyl alcohol were added. The mixture was then taken to a heating plate until complete dissolution. Then, the solubilized sample was transferred to a 0.5 L separation funnel, where 50 mL of petroleum ether was incorporated, vigorously stirred, and left to rest for a few minutes, in order to obtain phase separation. The aqueous phase was collected separately from the ether phase and the process was repeated 3 more times. After that, the ether phase collected in the extractions was transferred to a previously weighed beaker, which was taken to a heating plate until complete evaporation, and then taken to an oven at 105 °C until reaching constant weight [1]. To determine the fatty acid content, the analyzes were conducted in triplicate and the Equation 1 used:

$$AG = \frac{p \times 100}{m} \quad (1)$$

Where: AG = total fatty acid concentration (w/w), p = dry residue mass (g), m = sample mass (g).

For the analysis of the saponification value, the procedure described by the Adolfo Lutz Institute [1] was followed, which consists of adding 5 g of bar soap and 50 mL of alcoholic potassium hydroxide solution to a flat-bottomed flask with a ground mouth. (KOH). Following, the mixture was placed in reflux being counted 30 min after the start of condensation. Then, the system was cooled, and 1 mL of phenolphthalein indicator

(1%) was added to it, and titration was performed with a hydrochloric acid solution (0.5 mol/L). The results were obtained from the Equation 2:

$$IS = \frac{(V_a - V_b) \times f_c \times 28,05}{m} \quad (2)$$

Where: IS = Saponification value (mg of KOH/g of sample), V_a = Volume of HCl spent in the titration of the sample (mL), V_b = Volume of HCl spent in the titration of the blank (mL), f_c = Correction factor of HCl solution (0.5 mol/L), 28.05 = gram-equivalent of KOH (0.5 mol/L), m = sample mass (g).

Bacterial contamination assay

The microbiological analysis process aimed to confirm the absence of some microorganisms or verify the maximum limit allowed by law for the agents. The analysis processes are described below, with sample preparation, pre-incubation, and growth medium studied. The interpretation was based on the Brazilian Pharmacopoeia [13].

For total aerobic mesophilic bacteria counting, 1 g of soap sample was diluted in 9 mL of saline solution (9 g/L). Then, 1 mL of the solution was transferred onto a Petri dish with 10 mL of the Tryptic Soy Agar culture medium, previously prepared and already solidified. After that, the suspension was slightly homogenized. The analysis was performed in triplicate and a sample served as a counterproof in order to analyze whether the culture medium was contaminated. The plates were incubated in an oven at a temperature of 35 °C for a period of 48 h and then removed for counting [14].

To detect the growth of *Staphylococcus aureus*, the soap sample was enriched by adding 1 g to a test tube containing Brain Heart Infusion broth. The mixture was then placed in an oven at 35 °C for 24 hours. After removing the enrichment test tubes, seeding was carried out in Petri dishes containing Baird Parker Agar using a platinum loop. The plates were then taken to the oven at 35 °C for 48 hours. After incubation, the plates were analyzed. This technique was carried out three times, and a sample was used as a control to check for contamination of the culture medium.

Data analysis

Mean values $\pm$ standard deviation is presented in the text. All chemical analyses were done in triplicate.

RESULTS AND DISCUSSION

Artisanal soap formulation

To produce an artisanal soap, you can purchase the base mass in specialized stores or produce your own mass. It is from this base that all types of solid soaps are produced, whether decorative, therapeutic, transparent or opaque, pressed or cast. Chemically, soaps are the result of the reaction of fatty compounds (oils and fats) with a hydroxide (soda) in the presence of water, being a solid and foaming compound capable of removing dirt [15]. For the production of the bar soap under study, a 100% vegetable glycerin base was used that does not have bovine tallow or animal fats in its composition. According to Amiralian and Fernandes [16] glycerin bases of vegetable origin can be obtained from coconut, palm and palm kernel fat, and oils such as cotton, almonds, babassu, canola, soy, olive, and castor oil. As a rule, they have regular to excellent cleaning properties, and generally produce less foam than traditional ones, in addition to having a more alkaline pH, between 9 and 10.

Allied with vegetable fat, lauryl glucoside was used, which is a non-ionic surfactant, derived from corn sugar, potato, and natural oils. It has surfactant properties and provides the system with excellent detergency and foam formation, according to the supplier, there is no minimum or maximum percentage to be used, but taking into account its detergency capacity, 17% was used. According to SpecialChem [17], the recommended concentration is from 10 to 20%. Detergent surfactants routinely help clean the skin, remove dead cells and contamination, and eliminate unpleasant odors. Sodium lauryl sulfate (SLS) is an anionic surfactant commonly used in soaps, however, it is not recommended due to its high detergency, as it excessively removes the lipid layer of the skin and causes dryness. Surfactants, as well as other components, must clean while maintaining the eudermia of the skin, for this purpose mild non-ionic surfactants such as decyl(poly)glucoside and lauryl(poly)glucoside, and amphoterics derived from coconut fatty acids, such as diethylamine, monoethylamine, dimethylpropylamine, glutamic acid, and wheat amino acids can replace SLS [18].

As a natural dye, natural powdered cabbage extract was used in the formulation, providing the soap with a light green color. According to the supplier, its use for cosmetics can be from 0.5 to 1%, and 1% of the extract was used in the formulation. The percentage used is justified due to preliminary tests, in which the

desired color was obtained using 1% of the natural dye. The use of natural dyes in cosmetics is very old, and today it is a viable option to replace synthetic dyes. As well as herbal properties, some plants such as red cabbage, jambolan, blackberry, strawberry, cow's claw, azalea, lent, and many others are used for this process [19].

With the intention of incorporating a component with the potential to lighten, soften, absorb skin oiliness, and regulate sebaceous protection into the soap formulation, white clay was used. Clay is formed from the decomposition of feldspar which is a mineral constituent of rocks, and can be found in different colors, providing numerous benefits for the skin [20]. According to Amorin and Piazza [21], white clay consists of a mixture of quartz and kaolinite, in aesthetics this mixture facilitates blood circulation, has antiseptic action, high excellence in acne control, whitening, and general skin tonus. White clay also promotes a refreshing action, retains exudation from the skin, thus creating a surface for evaporation, acts to stimulate the production of collagen and elastin, and has a purifying, sanitizing, revitalizing, oxygenating, and nourishing effect on the skin [22]. According to the supplier, there is no minimum percentage of clay to be incorporated into the bar soap formulation, so 0.5% of the mineral was used for production, taking into account its exfoliating potential.

Cosmetics often contain vegetable oils which form a protective layer on the skin to prevent water loss and dryness. These oils, found in fruits and seeds, are rich in moisturizing, conditioning, and nourishing properties and comprise 90-98% triglycerides. When applied to the skin, these oils undergo enzymatic and hydrolytic processes, releasing fatty acids that improve skin appearance [23]. With its high vitamin E content, Avocado oil has anti-inflammatory and antioxidant properties, while grape seed oil has regenerating and revitalizing effects. The formulation uses 1.6% of each oil, considering other components and a blend of other vegetable oils.

The soap's fragrance was created using green tea essence, which belongs to the herbal scent family and provides a refreshing aroma that promotes well-being and freshness. According to Borges and colleagues [24], using flavorings enhances the product's sensory experience, boosts creativity, and helps establish a brand in the market. This is known as olfactory marketing, where a product is remembered not only for its quality but also for its pleasant scent. The supplier recommends using up to 10% of the essence in 1 kg of glycerin base, but for this particular formula, 7.3% was used due to its bold scent.

The vegan handmade soap's components were carefully chosen, and the formulation was innovative. To ensure high-quality standards, we conducted a relevant evaluation during product development. Additionally, we must conduct stability tests to predict the soap's shelf life and compatibility with the packaging material. In a single batch, we made 12 bars of soap, each weighing an average of 90 g.

Quality control

The quality control analysis for the soap was conducted in triplicate to ensure accuracy. The coefficient of variation was consistently less than 20%, indicating minimal deviation in the parameters analyzed. Table 2 displays the analytical findings pertaining to the soap's quality and characteristics. Additionally, the values of the parameters discovered were compared to the recommended standards set by regulatory bodies in the literature.

During soap production, pH evaluation took place at three different times: first, during production (time zero) when the soap was in a liquid state, then the next day when the soap had solidified (time one), and finally, 15 days after production (time two). This analysis was conducted to determine a preliminary stability parameter. At time zero, the pH of the soap sample was 9.18. At time one, the soap had an average pH of $9.58 \pm 0.5\%$, and at time two, it had an average pH of $9.42 \pm 0.1\%$. The pH levels observed in the final product were similar to those of Chaves and coauthors [25], who obtained pH results of 9.84 in their study. The results obtained were in accordance with the standard recommended by ANVISA.

Table 2. Determination of important parameters to quality control of soaps (n=3).

Parameter	Artisanal vegan soap	Recommended values	Reference
pH	9.58	Entre 8 e 10.4	[10]
Density	0.98 g/mL	0.9 a 1.1 g/mL	[10]
Free alkalinity	not present	Up to 0.5%	[11]
Total fatty acids	59.4%	Up to 80%	[25]
Peroxide value	9 mEq/kg $\pm$ 1%	Up to 10 mEq/kg	[30]
Saponification value	319.39 mg de KOH/kg $\pm$ 4,2%	Between 185 and 200 mg of KOH/kg	[13]

Where: mg = miligrams; g = grams; mL = milliliter; mEq/kg = milliequivalent/kilogram; KOH = potassium hydroxide.

According to Gasperi [26], a cosmetic product has its pH determined according to the region and purpose for which it will be applied. Products with a lower pH, in the range of 3.0 to 4.0, attack the epidermis, on purpose, in order to exfoliate by breaking down the keratinocytes. Soaps, tonics, and facial moisturizers have the pH range that is closest to the skin, in order to minimize irritation reactions. On the other hand, it also points out that the pH of bath bar soap, for body use, should remain between 9.0 and 10.0. This is a higher pH due to the composition of its formulation, people with very sensitive skin should be careful with possible irritation reactions.

Having a density within specified limits is crucial for quality control, as it can indicate any changes in soap composition [27]. The results obtained from a study on bar soaps were consistent, with Lage [27] reporting a range of 0.96 to 1.08 g/mL.

When analyzing free alkalinity, the samples displayed a colorless filtrate, which suggests an absence of free alkalinity. Chaves and their team [25] conducted a similar test with a comparable product and obtained the same outcome, demonstrating the absence of free alkalinity. According to Kohl [11], personal hygiene products, cosmetics, and children's perfumes may contain a maximum of 0.5% free alkalinity. Additionally, products based on surfactants not derived from petroleum are entirely free of this factor.

The concentration of total fatty acids in the artisanal soap produced resulted in $3.3\% \pm 3.2\%$ in 5 g of sample. In view of this result, and taking into account that the soap produced has 90 g, the percentage of fatty acids in the composition was 59.4%. With the aid of Table 3, it is possible to analyze the amount of vegetable oil proportional to each bar of soap. In addition to these components, it is necessary to take into account that in the manufacturing process of the glycerin base (100% vegetable), vegetable oils are added for the saponification process, thus increasing the percentage of free fatty acids present in the formulation. According to Chaves [25], high values, close to 80%, can interfere with the sensory quality of the product, altering its color, odor, and appearance, as well as its functional properties. In addition, they indicate the presence of raw materials of animal origin.

Table 3. Amount of oils added in composition of each bar of soap.

Oil	Soap mass (g)	Amount of oil (g)	Amount of oil (%)
Avocado vegetable oil	99.32	1.38	1.39
Grape seed vegetable oil	99.32	1.36	1.37
Green tea essence	99.32	6.11	6.15

Making soap involves a chemical reaction between an alkali and a fatty substance, known as saponification. Oils and fats are esters that can undergo either acid or basic hydrolysis, producing glycerol and constituent fatty acids. In the case of basic hydrolysis, the resulting salts of these fatty acids are what we call soap [28]. The International Union of Pure and Applied Chemistry (IUPAC) defines soap as a salt of fatty acid, saturated or unsaturated, containing at least eight carbon atoms or a mixture of fatty acid salts. To obtain soap, animal or vegetable fat undergoes neutralization or saponification using sodium hydroxide or KOH, which is formulated according to its intended use [27].

The soap being examined had a saponification value higher than the values of the oils used to make it, which typically ranged between 183 to 189 mg of KOH/kg. This could suggest that the soap has gone rancid. However, after comparing the saponification value with other results, it was determined that there were no signs of oxidation or rancidity in the cosmetic. Since this is a new product, no comparative studies were found in the databases.

One possible reason for the high value found could be the use of natural additives like oils, plant extracts, and natural dyes, which are commonly used by artisanal natural soap manufacturers. However, there is a shortage of data and information about how these raw materials affect the final conditions of the product and the unsaponifiable fatty acid content [29]. The saponification value is a parameter that relates to the stability of soaps. Therefore, for better exploration and monitoring of this parameter in handmade vegan bar soap production, evaluating this value for a certain period of time is recommended.

As for the peroxide value, the obtained result indicates good oxidative stability. According to RDC No. 270 [30], the maximum peroxide content allowed for refined oils and fats is 10 meq/kg. This index determines all substances that oxidize potassium iodide to iodine and are considered peroxides or similar products from the oxidation of fats. Peroxides are the primary products of lipid oxidation [31], and their presence at high levels indicates the deterioration of a given product. This parameter can also be verified by cosmetic organoleptic changes [32].

Organoleptic analysis

We selected one of the produced soaps as a standard sample to conduct an organoleptic analysis. For comparison purposes, we stored it in packaging in a cool, airy place away from light, heat, and humidity. Both the standard and the analyzed sample were kept under the same conditions. The bar soap is solid, homogeneous, and opaque, as shown in Figure 1, and no changes were observed in its appearance during the evaluation period. Due to the addition of natural extract in kale powder, the soap acquired a light green color, which remained consistent over the analysis time. The odor was as expected for the cosmetic formulation, with a characteristic herbal scent that was normal without any alterations. All the results we obtained conform to the established parameters [10].



Figure 1. Aspect of the produced artisanal vegan soap.

Mechanical stress

After centrifugation, the tubes containing the samples were visually examined, and no changes were noticed. The outcome matches the ANVISA criteria as no abnormalities were detected, including precipitation, separation of phases, formation of compact sediments (caking), and coalescence [10].

Bacterial contamination

After 48 hours of incubation, the total count of aerobic mesophilic bacteria was checked. Upon verification, no colony-forming units were observed in the sample-containing plates, only in the plate containing the culture medium, indicating possible external contamination. The findings have complied with ANVISA standards, demonstrating that the microbiological parameters were consistent with current legislation. The evaluation of the presence of *Staphylococcus aureus* yielded similar results, with no bacterial growth observed after 48 hours of sowing. This also meets ANVISA's established parameters, which specify that a product conforms to the test when there is no growth of colonies.

Personal hygiene products, perfumery, and cosmetics must follow the microbiological parameters outlined in Resolution 481/99 [33]. Investigating microbiological contamination in cosmetic formulas is vital, ensuring greater safety for consumers. When microorganisms grow, changes in color, texture, and viscosity can occur in cosmetics, as well as chemical changes such as phase separation and turbidity, among other characteristics [34].

Vegan soap: perspectives x limitations

In Brazil, cosmetics are defined by Resolution No. 211, issued on July 14, 2005. According to this regulation, cosmetics refer to products for personal use or perfumes made of natural or synthetic substances intended for external use on different body parts. Their main objective is to clean, perfume, protect and maintain them in good condition. These products are categorized into three groups: hygiene, perfumes, and baby products. They are also classified into two risk groups: Grade 1 and Grade 2. Grade 1 products are basic and do not require proof or detailed information on their use and restrictions. Conversely, Grade 2 products have specific indications, safety proof, efficacy, and information on how to use, restrictions on use, and care [30].

The findings of this study indicate that the product falls under Grade 1 risk, making it suitable for personal use and complying with current legislation. Being an artisanal product, it is produced on a small scale using 100% natural, sustainable, and biodegradable ingredients that are also vegan. Vegan cosmetics are free from animal testing and ingredients of animal origin [3]. Using such products is environmentally friendly and beneficial for the human body. They provide important nutrients and fatty acids that help maintain the skin

and its microbiota while respecting their physiology and nature [35]. Therefore, vegan cosmetics are a sustainable and natural way to care for the skin.

When creating an artisanal product, it's important to consider the limitations of using natural ingredients. These limitations include 1) the potential for restricted ingredients that could impact the effectiveness of the final product [3]; 2) the challenges with 100% natural formulation, such as limited production quantity and shortened shelf life due to microbiological activities [36]; 3) the difficulty of using synthetic preservatives, which are only allowed in certain acidic environments; and 4) higher costs for raw materials of natural origin compared to synthetic ones [3]. To ensure stability in these products, assessing them throughout the development process and until the end of their estimated validity period is recommended [37]. However, stability can vary over time depending on factors that impact product parameters.

CONCLUSION

Based on the market trends and consumers' growing concern regarding the environmental impacts of production, the study found that the soap produced can be profitable. However, further studies are needed to ensure the soap's stability over time. The artisanal soap underwent physicochemical characterization, which showed good alkalinity and fatty acid content, and demonstrated remarkable soap properties within acceptable standards. This work presents innovative formulations and results, which is noteworthy given the scarcity of references evaluating the quality control of artisanal soaps.

Funding: This research received no external funding.

Data availability statement: Research data are available in the body of the manuscript.

Acknowledgments: The authors express gratitude to the Passo Fundo Educational Development Institute (IDEAU) for all support.

Conflicts of Interest: The authors declare no conflict of interest.

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