BIOTECHNOLOGICAL DEVELOPMENT OF ANTIOXIDANT REJUVENATING SERUM CONTAINING LIPID ACTIVE INGREDIENT FROM RANA CATESBEIANA SHAW

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ABSTRACT

This article proposes an innovative approach in the field of skin care, focusing on the biotechnological development of an antioxidant rejuvenating serum, incorporating a lipid active ingredient from Rana catesbeiana Shaw. In addition to exploring the antioxidant properties of this bioactive compound, the research emphasizes the process of pharmacotechnical development of the serum. In this context, physicochemical and microbiological tests were carried out to validate its efficacy and safety. The physicochemical assays cover crucial aspects including analysis of peroxides, titratable acidity, unsaturations, as well as antioxidant, viscosity and density assay. These not only validate the stability and quality of the formulation, but also highlight the careful selection and combination of ingredients. At the same time, microbiological tests were conducted to ensure the absence of unwanted microorganisms, reinforcing the safety of the product. The consolidated results of these assays support the scientific basis of the serum, highlighting not only its potential rejuvenating action, but also its microbiological integrity and physicochemical stability. This research thus offers a significant contribution to cosmetic biotechnology, promoting sustainable innovation in the skincare industry.

Keywords: Cosmetic Engineering. Facial Rejuvenation. Frog oil. Serum. Biotechnology.

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INTRODUCTION

The aging process is something natural to human beings, however, there are factors that corroborate the anticipation of phenomena related to it (BOISMAL *et al*., 2020). In this sense, the skin is the organ in which the first signs of this process usually become notorious, allowing us to denote the precocity of the advent of aging in an individual (ELLIS; KHAVKIN, 2011).

Within the organic scope, as a person advances in age, there is a reduction in metabolic activity, and consequent physiological dysfunctions. These processes will result in the deficiency of certain inputs and structures, such as collagen and elastin (ELLIS; KHAVKIN, 2011). Therefore, the physiological priority will direct a large part of them to regions of greater importance, such as joints, resulting in a deficit in the skin (BONTÉ *et al*., 2019). Consequently, there is a reduction in cell regeneration and a deficiency in support structures, which generates the presence of wrinkles, sagging and a decrease in the volume of the skin layers (CSEKES; RAČKOVÁ, 2021; ELLIS; KHAVKIN, 2011).

In addition, environmentally-induced factors are associated with photoaging. Frequently, human beings are exposed, not only to one factor, but to a combination of these, which interact with each other, resulting in the enhancement of skin aging. Among these agents, solar radiation, air pollution, exposure to cigarette smoke, food, and others stand out (KRUTMANN *et al.,* 2021).

The clinical findings between these two types of aging are distinct. While intrinsic aging can be observed throughout the organ, in extrinsic aging the involvement occurs predominantly in the parts exposed to the environment, such as the face, back of the hands and neck. Furthermore, the traits evidenced on the skin are also distinct. The intrinsic results in fine lines, sagging, dryness of the skin, and the gradual atrophy of the skin, while the extrinsic is marked by the appearance of thick lines, rough texture, loss of elasticity, irregular pigmentation, and lentigo (KRUTMANN *et al.,* 2021, ZHANG; DUAN, 2018).

Considering that the beauty of the skin is an important indicator of human well-being, several strategies are developed to circumvent photoaging, such as cosmeceuticals (SHANBHAG *et al.,* 2019). These products are responsible for exerting a pharmacotherapeutic benefit on the skin, attenuating wrinkles, expression lines and

improving the appearance, which are the main marks of aging, and also treating other aesthetic dysfunctions (PANDEY; JATANA; SONTHALIA; 2022).

In addition, with the influence of the media, warning about the benefits of bioactive compounds, the cosmetic industry has increasingly bet on the elaboration of formulations with natural products (ALVES *et al*., 2020). And with the advancement of technologies applied to pharmacotechnics, several innovative pharmaceutical forms have been developed and applied in the market, such as serums, which consist of a viscous fluid product, easy to apply and spreadable, containing high levels of active ingredients (SASIDHARAN; JOSEPH, 2014).

In this context, the oil extracted from *Rana catesbeiana* Shaw (Bullfrog) has been studied due to its therapeutic properties, mainly from the presence of fatty acids. Some effects already elucidated point to action on the skin due to the interaction of linoleic and linolenic acid, which guarantee elasticity, skin integrity, preventing trasepiddermic dehydration (BELDA; POURCHET-CAMPOS, 1991).

In addition, a recent study indicated activity against melanomas, by pathways of action against free radicals (ROS), highlighting the antioxidant potential of the lipocomponents of this oil, protecting cellular DNA and cite structures (MACHADO *et al.,* 2019). Therefore, bullfrog (BO) oil may present itself as a promising active ingredient in the development of formulations for facial rejuvenation.

In view of the above, the objective of the present work is to develop an emulsified serum with a rejuvenating approach, containing a characterized lipid active ingredient, from the bullfrog, with commercial viability.

METHODOLOGY

The study consisted of an exploratory, quantitative and qualitative research, with an experimental nature. To assist in its elaboration, a bibliographic review survey was established in the PubMed, Scientific Electronic Library Online (SciELO), and other informational databases. For this purpose, the descriptors "Rana catesbeiana" were used; "Skin Aging"; "Cosmetic Technology", all duly standardized as descriptors in health sciences (DeCS/MeSH). In addition, analysis, extraction, purification and development protocols were also adopted, which will be listed in the sequence of the work in question.

OBTAINING THE RAW MATERIAL

The frogs were obtained in a frog farm located in the municipality of Jerônimo Monteiro, with certification in the specific production of the species described in this work. In the same place, these were submitted to slaughter under controlled conditions, aiming to mitigate possible metabolic impacts that interfere with the raw material intended for processing.

After slaughter and evisceration, the fatty body of the animal was deposited in a thermal box, refrigerated with the help of ice plates, at approximately -5°C, followed by the laboratory facilities of the research institution, where the raw material was conditioned in a freezer, at a temperature of approximately -20°C.

EXTRACTION AND PURIFICATION

In order to enable the extraction of BO, the sample was processed in a food multiprocessor, after being thawed in an environment with a temperature of approximately 5°C, and then 500g of crushed material was obtained, which was conditioned in a 2000 mL beaker, being immersed in the organic solvent Hexane. Subsequently, the aforementioned glassware was deposited in the cooling chamber of a chiller, at approximately -20°C, with an inverted ethanol water bath, for one hour, in a constant mechanical agitation system.

After this time extension, the extract obtained was drained, and with the remaining solids of the fat body, the process was repeated two more times, however, with shorter time intervals, which were thirty-fifteen minutes. With the obtaining of the lipid extracts of the amphibian, a simple filtration was conducted, aiming at the retention of any solid particles originating from the fatty body.

Soon after the discussion, the crude extract was sent to a rotary evaporator, coupled to a vacuum pump, and to a refrigeration system with ethanol at -20°C, aiming at its purification. Subsequently, the primary extract was filtered in an analytical filter soaked in saline solution, and later in an activated carbon filter. With this, a purified extract was obtained.

2.3 CHEMICAL AND PHYSICAL CHARACTERIZATION

After thawing, the fat body was processed, weighed (5 g) and submitted to the Goldfish assay, in order to determine the macrobiochemical quantity of total lipids in its structure. For this, 120 mL of Petroleum Ether were used, deposited in an extractor cup, for the sequence of the method.

To determine the chemical constituents, a gas chromatography device coupled to a mass spectrometer (GC/MS) was used, planning the sample run as a function of time, on a given heating ramp. This process had as its purpose the separation and identification of the fatty organic structures that make up the BO, following the device's library.

In addition, 5 mL volumetric flasks were used to determine the density, and the results were obtained by gravimetric methods. For the viscosity analysis, an adaptation of the Ostwald method was used, where the flow time of 10 mL of the oil in a cylinder with volumetric demarcation was evaluated (ADOLFO LUTZ, 2008).

PHYSICOCHEMICAL QUALITY CONTROL OF BULLFROG OIL

As for the physicochemical analyses, the peroxide index, titratable acidity, unsaturations were evaluated using the methods described in the Manual of Analytical Methods of the Adolfo Lutz Institute (2008), and all analyses were conducted in triplicates. To obtain the peroxide index, 5 g of BO diluted in acetic acid and chloroform solution were used, in a 3:2 ratio, 0.5 mL of saturated solution of potassium iodide, and 0.5 mL of 1% starch indicator solution, and then titration was performed, using the 0.1 M sodium thiosulfate solution as the titrating agent.

The acidity index was also obtained by means of the titration technique, using 5 g of BO diluted in a solution of Ethyl Ether and Ethanol, in a 2:1 proportion, and 2 drops of 1% Alcoholic Phenolphthalein as an indicator, with the 0.1 M solution of Potassium Hydroxide as a titrant. For the analysis of unsaturations, 0.22g of the 500 mL Erlenmeyer BO was weighed, along with 15 mL of Chloroform and 25 mL of the Wijs Reagent, and was subsequently capped and stored under the light for one hour. After that, 15 mL of 15% Potassium Iodide and 100 mL of distilled water were added, followed by titration with 0.1 M Sodium Thiosulfate, with pipetting of 2 mL of 1% Starch indicator solution during the process, and the process continued until the disappearance of the dark hue.

MICROBIOLOGICAL QUALITY CONTROL

As for the microbiological assay, the culture of MacConkey Agar, Blood and Sabouraud was carried out in order to evaluate the bacterial and fungal growth of the sample. For this, the medium was inverted in a culture plate, being performed in triplicate, and when it was already at room temperature, 1 mL of bullfrog oil was added to each of the identified plates and then taken to the greenhouse for 24 hours to evaluate the microbiological growth. Next, the visual count of UFC was performed. The same protocol for the analysis of the developed serum was also executed.

ANTIOXIDANT ASSAY

For the antioxidant assay, the N,N-diethyl-1,4-phenylenediamine (DPD) free radical capture method was used. The mentioned radical was obtained through the reaction of DPD (Ether:Ethanol Solution 2:1 at 2 mmol $^{L-1}$) with Iron III, at 0.2 mmol $^{L-1}$. Soon after, 1 mL of the solution containing the radical was added to 45 test tubes. For the assay, 5, 10, 50, 100 and 500 µL/mL were pipetted in a triplicate system, 15 with the BO, 15 with the positive control of BHT (0.1 M alcohol solution), and 15 with the negative control of Ether:Ethanol solution 2:1. The light was waited for about 10 minutes to rest, and then the reading was performed in a spectrophotometer, at 540 nm, and the calculations were performed based on the absorbance (FOGLIANO et al., 1998; HSBD, 2023; SANTOS et al., 2015)

PHARMACOTECHNICAL DEVELOPMENT

The developed product consists of an emulsified serum, containing oily and water-soluble bases, as well as emulsifying agents, preservatives and pharmacotechnical adjuvants. For its execution, the Quality by Designer (QbD) methodology was used, performing the risk assessment and experimental planning based on this assessment.

After primary tests, it was possible to obtain the formula described in Table 01, which was prepared using classical pharmacotechnical techniques, being homogenized via a mechanical stirrer, without any kind of heat injection, obtaining 300 mL of serum. And after obtaining the formula, it was conditioned in amber glass bottles.

Table 01: Formula of BO serum

SERUM FORMULA			
PHASE	**RAW MATERIAL**	**INCI NAME**	**(%)**
1	WATER	AQUA/WATER	What happens
1	ARISTOFLEX® AVC	Ammonium Acryloyldimethyltaurate/VP Copolymer	0.300
1	GLYCERINE	Glycerol	5.000
2	BULLFROG OIL	Bullfrog Oil	2.000
2	VITAMIN E	Tocopheryl Acetate	0.500
2	ESSENTIAL OIL BERGAMOTA	Citrus aurantium bergamia Fruit Oil	0.700
3	PAYOUT	Methylparaben	0.150
3	BHT	Butylated Hydroxytoluene	0.200
4	TWEEN 80®	Polysorbate 80	1.000

Source: AUTHORS, 2025

QUALITY, STABILITY AND DEGRADATION STUDIES

Several tests were used to evaluate the quality, stability and degradation. The first consists of centrifugation, where the product was rotated in a centrifuge for 10 minutes, with 3000 RPM, and a microscopic evaluation was made. In addition, the ice-thaw cycle test took place, where the serum was conditioned in an environment with an estimated temperature of -5°C for 24 hours and then taken to an oven with 50°C over the same period of time. This cycle was repeated for 4 days, and in the fifth day, conductivity and pH were evaluated, by potentiometric techniques, density and peroxide index and titratable acidity, following the aforementioned methodology (BRASIL, 2008).

The formulated product was also left at room temperature for 4 months, in the *Shelf-Life assay*, and each month the peroxide index and acidity present, conductivity, pH and density were evaluated. This will allow you to assess the shelf potential of the product over that duration. The methodologies described are contained in the Guide for Quality Control of Cosmetic Products, developed by ANVISA (BRASIL, 2008).

RESULTS AND DISCUSSION

CHEMICAL AND PHYSICAL CHARACTERIZATION OF BULLFROG OIL

When conducting the Goldfish assay, aiming to detect the percentage of macrobiochemical composition of lipids in the fatty body, a value of 83.843 ± 0.991% was

obtained. And the extraction with Hexane, in an inverted water bath, reached a utilization of 67.9%, presenting a yield similar to those described by Perina (2016), in different replicates between 62.78 ± 0.44% and 68.17 ± 0.05%, with thermal variation between 25 °C and 80 °C.

Subsequently, with the characterization of lipomolecular microcomponents, using the gas chromatography technique coupled to mass spectrometer, the composition of the extracted input was elucidated, as can be seen in table 02. In this case, there is also a comparison with other studies, where Lopes et al. (2010) did not find the presence of Eicosapentaenoic Acid, and these authors, as well as Silva and partner researchers (2004), detected the presence of Arachidonic Acid. Only Méndez et al (1998) presented exactly the same fatty components, although there was a quantitative variation.

Table 02: Chemical composition of BO

COMPOSITION	**CHEMISTRY**	**OF OIL**	**TAURUS FROG**	
GRAPHIC COMPONENTS	**AUTHORS**	**MENDEZ et al., 1998**	**SILVA et al., 2004**	**LOPES et al., 2010**
Myristic Acid (14:0)	1,6	2,7	2,77	2,8
Palmitic Acid (16:0)	17,52	18,1	11,91	18,5
Stearic Acid (18:0)	3,21	4,1	2,34	3,2
Oleic Acid (18:1 n-9)	34,96	31,7	37,6	36,3
Linoleic acid (18:2 n-6)	21,8	12,9	23,78	25
Linolenic Acid (18:3 n-3)	1,27	1,4	1,97	2,1
Palmitoleic acid (16:1 n-7)	6,28	8	17	9,4
Acid Eicosapentaenóico-EPA (20:5 D-3)	0,12	1,5	0,46	-
Acid Docosaexaenóico-DHA (22:6 N-3)	0,27	4,7	0,91	0,1
Arachidonic Acid AA (20:4 n-6)	-	-	0,74	0,6

Fonte: AUTORES, 2025 – Adaptado de: LOPES et al., 2010; SILVA et al., 2004; MENDEZ et al.,1998

It should be noted, however, that the composition of BO is interfered with by the diet used in frog farms, since these lipids do not come from the intrinsic metabolism of frogs (Filho et al., 2008). Thus, as Hayashi and study partners (2004) point out, the breeding of frogs with feeding protocols using diets rich in fatty acids will directly influence the obtaining of lipophilic extracts with greater rigor, with regard to composition.

The last characterization tests were focused on physical property profiles, with the results expressed in Table 03. As for the density of the input, the value of 0.905 ± 0.006 g/mL was obtained, which is in quantitative proximity to the characterizations of other oils, carried out by Sahasrabudhe et al (2017), which were 0.913 ± 0.007 g/mL; 0.915 ± 0.007g/mL; 0.909 ± 0.007g/mL; 0.912 ± 0.007g/mL; 916 ± 0.007g/mL, referring to canola (*Brassica napus*), corn (*Zea mays*), olive (*Olea europaea* L.), peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) oils, respectively.

Table 03: Physical characterization of the BO

DETERMINATION D	**AND DENSITY AND VIS**	**COSITY OF BULLFROG OIL**
REPLICA	**DENSITY**	**KINEMATIC VISCOSITY**
1	0.898 g/mL	62.474 mPas. S
2	0.906 g/mL	61.871 mPas. S
3	0.912 g/mL	62.989 mPas. S
	0.905 ± 0.006 g/mL	62.445 ± 0.457 mPas. S

Source: AUTHORS, 2025

In addition to the density, the viscosity was also evaluated, and a value of 62,445 ± 0.457 mPas. S. This also maintains a profile similar to other oils, as expressed in the results obtained by Silva et al. (2012), which are 59.0 mPas.s; 67.6 mPas.s; 58.3 mPas.s; 73.8 mPas.s, for soybean (*Glycine max* L.), corn (*Zea mays*), sunflower (*Helianthus annuus* L.) and rice (*Oryza sativa*), respectively. Thus, both the density and the viscosity of BO are established in similarity with physicochemical profiles of oils used in the daily life of society, for various purposes.

PHYSICOCHEMICAL QUALITY CONTROL OF BULLFROG OIL

According to Silva et al. (1999), the analysis of peroxides allows quantitatively measuring the initial process of lipid oxidation, denoting the impacts that various agents can influence in an oil. Therefore, high rates indicate a greater degradation of fatty matrices, which directly impacts the biological effects of bioactive compounds, in addition to transforming these molecules into structures capable of inducing oxidative stress (PIZZINO et al., 2017; PERCÁRIO et al., 2020).

Table 4 shows the data related to the analysis of the peroxide index, where a value of 21.703 ± 0.384 mEq/Kg was obtained, which is in line with those described in the literature. Machado (2015) and Coutinho et al (2023), when conducting the same analysis, with the oil obtained by extraction with Hexane, found a value of 35.87 ± 1.021 mEq/kg and 23.52 ± 0.684 mEq/kg, respectively. In view of this, it becomes noticeable that the extracted matrix has a low rate of degradation by oxidation.

Table 04: Physicochemical quality control of BO

	WED ANALYSIS	PHYSICOCHEMICAL LITY OF THE	BULLFROG OIL
REPLICA	**INDEX OF PEROXIDES**	**TITRATABLE ACID VALUE**	**UNSATURATION ANALYSIS**
1	21.78 mEq	1.39 mg de KOH/g de BO	145.16 mEq O2 active/1000g BO
2	21.20 mEq	1.39 mg de KOH/g de BO	144.70 mEq O2 active/1000g BO
3	22.13 mEq	1.86 mg the KOH/g the BO	145.28 mEq O2 active/1000g BO
	21,703 ± 0,384 Meq	1.547 ± 0.222 mg de KOH/g BO	145,047 ± 0.250 mEq O2 active/1000g bo

Source: AUTHORS, 2025

As for the titratable acidity profile, according to Costa and partner authors (2006), it refers to the degradation index of lipocomponents, allowing the evaluation of the quality of the raw material. With the titration, a result of 1.547 ± 0.222 mg of KOH/g of BO was obtained, which is close to studies exposed in databases. Coutinho et al. (2023) described a value of 2.956 mg of KOH/g ± 0.784 mg of KOH/g of BO, while Machado (2015) described a value of 2.9 ± 0.044 mg of KOH/g of BO. In this sense, the analyzed sample also presented a higher quality profile than the secondary data compared.

On the other hand, the analysis of unsaturations, by titration, generates iodine addition reactions in unsaturated carbons, causing the breaking of π bonds between these homoatoms, allowing the analysis of the molecular structures in their bonding profiles (FREIRE et al., 2013). Through this method in volumetry, an Iodine index of 145.047 ± 0.250 mEq O2 active/1000g BO was obtained, thus, it had a higher unsaturation profile than the sample described by Machado (2015), which recorded a value of 78.604 ± 0.810 mEq O2 active/1000g BO, demonstrating a higher quantity of unsaturated fatty acids.

MICROBIOLOGICAL QUALITY CONTROL OF BULLFROG OIL

Microbial contamination of a non-sterile product, in addition to causing a risk of infection for the user, can lead to its deterioration from the associated physical and chemical changes. Consequently, oral and topical pharmaceutical products, which are not sterile, must be subjected to microbiological controls. Therefore, for each type of pharmaceutical ingredient there is an acceptable limit for the presence of bacteria and fungi. With regard to products of animal and mineral origin for topical use, the CFU limit for bacteria is up to 2 x 10^2 and for fungi up to 2 x 10^1 (BRASIL, 2019).

In this sense, the values found after the microbiological analysis of bullfrog oil are in accordance with what is recommended by the Brazilian Pharmacopoeia (2019, 6th ed), given that the results obtained were 0.33 x 10 ± 2.08 CFU for bacteria and 1.4 x 101 ± 2.52 CFU for fungi.

IN VITRO ANTIOXIDANT ACTIVITY ASSAY

Validating the biological effect of pharmaceutical ingredients is a fundamental step in product development, allowing the measurement of application possibilities (SANTOS et al., 2015). Thus, in table 05 it is possible to observe the results of the antioxidant activity index (IAA), in percentage. This evaluation clarified that BO had higher levels than the control group, where the 0.1 M BHT solution was used, in all concentrations tested.

This finding corroborates those described by Machado et al (2019), who evidenced the action of BO in mitigating markers of inflammation and oxidation in cell cultures. Thus, the therapeutic value attributed to the input addressed becomes notorious, highlighting it as a cosmetic active with potential against damage caused by oxidative stress, which culminates in skin aging.

Table 05: BO Antioxidant Assay

ANTIOXID ASSAY	**ANTE OF BULLFROG OIL**	
CONCENTRATION	**%IAA**	
	BO	**BHT**
5 µg/mL	3.91 ± 0.37%	2.89 ± 0.49%
10 µg/mL	10.04 ± 0.64%	6.21 ± 0.34%
50 µg/mL	29.51 ± 0.21%	17.34 ± 0.67%
100 µg/mL	40.28 ± 1.04%	28.71 ± 0.81%
500 µg/mL	61.77 ± 1.41%	43.34 ± 1, 69%

Source: AUTHORS, 2025

COSMETIC ENGINEERING BY QBD

In view of the above, biotechnological techniques in cosmetic engineering are presented as a viable alternative for the development of BO-based products, and in contemporary times, Quality by Designer (QbD) strategies are the main guidelines for the elaboration of these formulations (YU *et al.,* 2014). This technique is based on the construction of products already anticipating possible impasses, such as instability of assets, degradation in specific environments, chemical and physical interactions between formula components and packaging materials, as well as extractable and leachable potentials (SWAIN *et al.,* 2019; BRAZIL, 2020).

Therefore, a thorough study of all the components of the product is necessary, from its formula to its packaging, outlining methodologies that mitigate or inhibit possible damage to it. Thus, it is necessary to analyze possible factors related to BO that may interfere with formulations that have it as a basis (YU *et al.,* 2014).

In the first analysis, it is notorious that some inclement weather conditions are obstacles to the acceptability of BO by the consumer market, such as certain organoleptic characteristics, especially with regard to its odor. In view of this reality, pharmacotechnical strategies need to be outlined, aiming to enable the acceptability of formulas with this compound (ALENCAR, 2013).

According to the studies developed by Machado (2015), the emulsification of BO is a viable technique to reduce the characteristic odor of this input, which associated with the incorporation of fragrances in the formulation generates a market viability. In this, the formula bergamot essential oil was added, which associated with the emulsion generated a neutralization of the aroma of the BO.

In addition to the odor, the low skin permeation manifests itself as an impasse for the use of BO, however, this was solved with the addition of glycerin, which will allow a better absorption of the active ingredient, in addition to enabling the wetting of the skin layers. The low spreadability of such a product also characterizes a problem (ALENCAR, 2013), which was also solved with the structuring of emulsion in the form of serums.

Another criterion that must be taken into account concerns the stability of the BO, considering that it is a lipid matrix, susceptible to the propagation of oxidative reactions,

which can trigger its rancidity and loss of bioactive function. Rutckeviski and his collaborators (2017) demonstrated that BHT was the most effective agent in controlling lipid oxidation of this input. With this in mind, the formula of the developed serum was applied.

Furthermore, dealing with the induction of oxidation, the importance of the material used for the packaging of the final product is emphasized, as the use of certain polymers can enable the permeation of oxygen and photons into the bottle (BALAN; BÓCOLI, 2018). Therefore, tempered amber glass presents itself as a raw material that will provide better protection for the serum, reducing peroxide propagation factors, which is why it was adopted for the conditioning of the product.

FORMULATION PHARMACOLOGY

With regard to the lipid active ingredient used for the formulation of the rejuvenating serum, the main chemical compounds found in it stand out: Oleic Acid, Linolenic Acid (ALA) and Linoleic Acid (LA). Some studies have already demonstrated the application of these fatty acids for the treatment of skin-related diseases and their use includes therapies for photoaging, dermatitis, hyperpigmentation, cancer and wound healing, including some of these components have already been approved for clinical use or are in the study phase for therapeutic or preventive purposes (HUANG et al, 2022).

When it comes to photoaging, connective tissues are damaged by sun exposure resulting in the classic signs of aging, such as wrinkles, sagging, hyperpigmentation and dermal thickening. In addition, the production of ROS, induced by solar radiation in the skin, activates MAP kinase signaling pathways and, consequently, stimulates the secretions of inflammatory cytokines. This overexpression of ROS can result in the degradation of collagen and elastic fibers (CHOI et al, 2020).

According to Huang et al. (2022), these fatty acids can act in associations for skin protection. Omega-3 PUFAs, such as Linolenic Acid (ALA), may decrease the suppression of pro-inflammatory eicosanoids through direct competition with Arachidonic Acid (AA) metabolism. In addition, these compounds can decrease the damage to keratinocytes induced by UV radiation, through the regulation of COX-2, NF-Kb, and MAP kinase pathways.

Linoleic Acid (LA), classified as omega 6 PUFAs, is the richest fatty acid in the epidermal layer, and is even a precursor of the synthesis of ceramide, a predominant input of the lipid matrix of the intercellular stratum corneum, producing the skin's skin barrier. This substance acts as a modulator of the cell membrane, protecting the skin against chemical and enzymatic agents. It also acts as an important tissue restorative agent, since it promotes chemotaxis and angiogenesis, in addition to being responsible for maintaining the moist environment and accelerating the tissue granulation process, regulating the permeability of the skin's water barrier and providing local cellular nutrition (LI et al., 2020)

Oleic Acid (OA), in turn, has been studied as an enhancer in the penetration of active ingredients into the skin, through its action mainly in the stratum corneum. The lipids of the layer are found in the intercellular spaces, organized in lamellar bilayers, constituting the primary permeability barrier of the skin. When applied to the skin, OA acts by disorganizing these lamellar layers and thus increases percutaneous permeation (JIANG; ZHOU, 2003).

In addition to these bioactive compounds present in the BO, for the elaboration of the cosmetic formulation, active ingredients were incorporated to enhance these beneficial effects on the skin. In this sense, vitamin E and glycerin were added as adjuvants for the development of the rejuvenating serum. In the cosmetic industry, vitamin E is widely used due to its antioxidant and protective effects against UV radiation, which act in photoprotection and consequently delaying skin aging (ZAFFARIN et al, 2020).

A study conducted by Makpol et al. (2013) in the stress-induced premature senescence model of human diploid fibroblasts, to determine collagen expression at genetic and protein levels, demonstrated that vitamin E exhibited an anti-aging effect through its ability to increase collagen synthesis and prevent collagen degradation.

Additionally, the use of glycerin is highly used in the development of cosmetics, due to its humectant power. The use of effective actives in retaining skin moisture in these products can help against skin aging, since the loss of hydration results in dryness, which will lead to wrinkles and sagging skin. In this sense, several studies have sought humectants that have high efficacy in retaining water in the human stratum corneum (CHEN et al, 2022).

COSMETIC QUALITY, STABILITY AND DEGRADATION TESTS

Contamination with microorganisms affects the vast majority of cosmetic products. Therefore, the microbiological quality control of non-sterile products admits the limited presence of microbial load and has as its main objective to prove the absence of pathogenic microorganisms and determine the number of viable microbial cells (SILVA, 2019).

Therefore, according to the results of the microbiological control available, the specific plates for the growth of both bacteria and fungi, containing the serum, did not show growth of microorganisms outside the specified standards, as governed by the Pharmacopoeia (BRIASIL, 2019).

In accordance with what is recommended by this Brazilian legal registration, in its VI edition (2019), the maximum limit allowed for mesophilic aerobic bacteria is 2 x 10^2 CFU/g and also establishes that the maximum limit for fungi is 2 x 10^1 CFU/G. Therefore, although the sample showed microbial and fungicidal growth, the number of CFU is below the maximum established limit, with an average of 1.2 x 10 ± 1 CFU for bacteria and 1.5 x 101 ± 2.08 CFU for fungi.

In addition to the microbiological parameters in the quality control of cosmetics, it is essential to excel in a physicochemical understanding of it. Therefore, the *Shelf-Life* test allows the evaluation of the shelf potential of the product, observing patterns related to its stability and degradation. Table 6 shows the results of this test, and in view of this, it is clear that in the spatio-temporal curve evaluated, the product followed profiles consistent with the current legal guidelines (BRASIL, 2004).

Table 06: Shelf-Life *Assay*

	ENS	**AIO *SHELF-LIF***	***And***	
PARAMETER	**AUGUST**	**SEPTEMBER**	**OCTOBER**	**NOVEMBER**
ph	5.54 ± 0.016	5,48 ± 0.021	5,51 ± 0.012	5,49 ± 0.008
CONDUCTIVITY	50,1 ± 3.193 mV	53,7 ± 1.248 mV	51,4 ± 2.169 mV	52,336 ± 1.274 mV
TITRATABLE ACIDITY	0,212 ± 0.024 KOH/g	0,215 ± 0.037 KOH/g	0,219 ± 0.017 KOH/g	0,222 ± 0.041 KOH/g
PEROXIDES	7,21 ± 0.741 mEq	7,274 ± 0.211 mEq	7,721 ± 0.424 mEq	7,728 ± 0.424 mEq
DENSITY	1 ± 0,022 g/mL	1 ± 0,018 g/mL	1 ± 0,013 g/mL	1 ± 0,017 g/mL

Source: AUTHORS, 2025

In addition, the freeze-thaw cycle test was also conducted, which is included in the parameters of forced degradation studies, allowing to denote physicochemical aspects after an intense stress of thermal variations. And with this evaluation, it was possible to perceive that the variations are established within the standards established by ANVISA (2004).

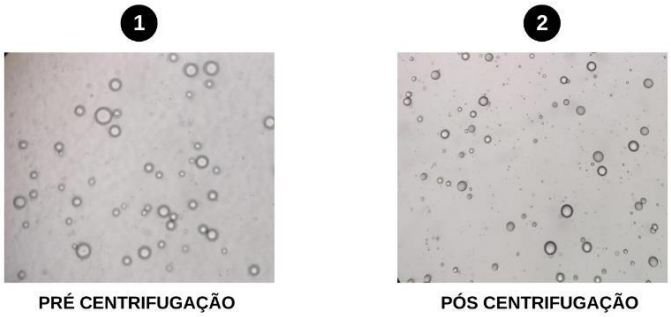
Table 07: Freeze-defrost cycle

ENSAI	THE ICE CYCLE-	THAW
PARAMETER	**PRE-REHEARSAL**	**POST TEST**
pH	5,54 ± 0.016	5,17 ± 0.012
CONDUCTIVITY	50,1 ± 3.193 mV	69,1 ± 1.257 mV
TITRATABLE ACIDITY	0,212 ± 0.024 KOH/g	0.427 ± 0.068 KOH/g
PEROXIDES	7,21 ± 0.741 mEq	9,071 ± 0.327 mEq
DENSITY	1 ± 0.022 g/mL	0.98 ± 0.015 g/mL

Source: AUTHORS, 2025

In addition, with the centrifugation test, it was possible to evidence the stability of the formulation, with little physical damage to the emulsion capsules, as can be seen in figure 01. According to Gonçalves et al. (2020), this finding highlights the structural quality of the formula, confirming the quality of the formulation, in its physical criteria.

Figure 01: Centrifugation test



Source: AUTHORS, 2025

PERSPECTIVES AND DIRECTIONS OF RESEARCH

According to Santos and partner authors (2019), the continuity of scientific studies, despite sometimes manifesting itself as a challenge, is essential for the progress of society. In view of this, it is visible the need for progress in investigations about the developed

product, primarily regarding its toxicity, and later its cosmeceutical effectiveness, in the effect proposed in the work.

In addition, evidence exposed in secondary data allows the formulation of hypotheses of biological activity aimed at aesthetic purposes, such as trichological purposes focused on the therapy of androgenetic alopecia, through the ability to inhibit the enzyme 5-alpha-reductase of certain components of the BO, as Katzer et al (2019) have already highlighted in a study.

In addition to hair products, the antioxidant and anti-inflammatory effects create a research plot for the therapeutics of acne and melasma, with the possibility of developing various cosmetic forms. Because, as reported by Miot et al. (2009) and Costa et al. (2008), the pathophysiology of such conditions requires protocols with active ingredients that exert such biological effects, highlighting the gaps and fields of possible applications of the BO.

In addition, there is the possibility of incorporating other active ingredients into the formulation, associating it with oral supplement therapies, aiming to provide functional and nutritional matrices so that biochemical systems can perform an accentuated response in skin structuring. Alves et al (2020) and Dini and Laneri (2019), emphasize the fundamentalness of the association of topical and internal protocols, for better therapeutic effectiveness, with regard to aesthetic maintenance with health.

CONCLUSION

In conclusion, the biotechnological development of the antioxidant rejuvenating serum, containing the lipid active ingredient from *Rana catesbeiana Shaw*, represents a promising innovation in skin care research. The developed formulation, supported by comprehensive physicochemical and microbiological assays, not only evidences a potential rejuvenating effect of the product, but also ensures its safety and stability. The synergy between biotechnology and natural actives, by exploiting bioactive compounds from sources such as *Rana catesbeiana Shaw*, highlights an emerging trend in the search for sustainable and effective ingredients in the cosmetics industry. This study not only offers an innovative option for the consumer in terms of skincare, but also contributes to the evolution of scientific knowledge, driving innovation and integrity in the production of cosmetics.

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