

Development and evaluation of biodegradable packaging of aryl from the *Hymenaea stigonocarpa* fruit

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ABSTRACT

The fruits of *Hymenaea stigonocarpa* have a sweet, starchy fibrous structure used as food by wild animals and humans. The present study investigated the physicochemical, optical, microstructural, and antibacterial properties of the fruit aril film. The starchy solution was obtained from aryl. The biodegradable film was prepared according to the "Casting" technique. The physicochemical characteristics of the thickness (mm), humidity (%), water solubility (%), biodegradability time, and transmittance (T%) were evaluated. Morphology was evaluated by optical and scanning electron micrography, 3D mathematical modeling, and mechanical parameters of resistance and biological properties on antibacterial activity were assessed. The results obtained for the biodegradable film were a yellowish-brown color, aroma, homogeneity, thickness of 0.27 mm, 12.45% humidity, water-solubility of 57.48%, 100% biodegradable, maximum transmittance of 82.25 (T%), small imperfections, and small cracks. 3D mathematical modeling showed surprising results in aiding imaging. The mechanical characteristics were maximum tension of 3.17 N, rupture tension of 1.34 MPa, elongation of 2.99%, and elasticity of 90.07 MPa. The bioactive film of *Hymenaea stigonocarpa* aryl showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis*.

Keywords: Biodegradable film, *Hymenaea Genus*, Food packaging, Natural packaging.

Desenvolvimento e avaliação de embalagem biodegradável a partir do arilo do fruto de *Hymenaea stigonocarpa*

RESUMO

Os frutos de *Hymenaea stigonocarpa* possuem estrutura fibrosa doce e amilácea usada como alimento entre os animais selvagens e o homem no campo. No presente estudo, foram investigadas as propriedades físico-químicas, ópticas, microestruturais e antibacterianas do filme de arilo do fruto. A solução amilácea foi obtida a partir do arilo. O filme biodegradável foi preparado de acordo com a técnica de "Casting". Foram avaliadas as características físico-químicas de espessura (mm), umidade (%), solubilidade em água (%), tempo de biodegradabilidade e transmitância (T%). Foi avaliada a morfologia por micrografia óptica e eletrônica de varredura, modelagem matemática em 3D, e para os parâmetros mecânicos de resistência e propriedades biológicas sobre a atividade antibacteriana. Os resultados obtidos para o filme biodegradável foram coloração marrom-amarelada, aroma e homogeneidade, espessura 0,27 mm, umidade 12,45%, solubilidade em água 57,48%, biodegradabilidade 100%, transmitância máxima 82,25 (T%), pequenas imperfeições e pequenas trincas. A modelagem matemática 3D exibiu resultado surpreendente no auxílio do imageamento. As características mecânicas para tensão máxima 3,17 N, tensão de ruptura 1,34 MPa, alongamento 2,99% e elasticidade com 90,07 MPa. O filme bioativo do arilo de *Hymenaea stigonocarpa* apresentou atividade antibacteriana contra *Staphylococcus aureus*, *Escherichia coli* e *Enterococcus faecalis*.

Palavras-chave: Filme biodegradável, Gênero *Hymenaea*, Embalagens para alimentos, Embalagens naturais.

1. Introduction

Hymenaea stigonocarpa Mart. ex Hayne, which belongs to the genus *Hymenaea*, popularly known as “jatobá-do-cerrado”, has a homogeneous distribution, occurring in tropical regions, mainly in areas of the Brazilian Cerrado domain (Paiva and Machado, 2006).

This species of the Fabaceae family: Caesalpinioidae, is an annual plant with large fruits in the form of dry, indehiscent, monospermic, or polyspermic vegetables, elongated, rounded, and slightly rectified apex, the rounded base, and the entire margin slightly wavy, with a diameter between 8.7-20.0 cm long, with a light brown to dark brown color, inside there is the yellow, aromatic, fibrous-floury, and sweet aryl, rich in starch that covers the seeds (Botelho et al., 2000; Menezes Filho et al., 2019).

Due to this characteristic, the aryl, being constituted by a certain percentage of starch, becomes a possible candidate for the development of biodegradable packaging. Currently, diverse natural polymer matrices are known, mainly based on starch, chitosan, gelatin, fish myofibrillar, corn zein, wheat gluten, collagen, polysaccharides, lipids, and proteins, where, in several studies, they incorporate essential oils, oil-resin, fixed oils, among other compounds such as plant extracts, and active substances such as lycopene, β -carotene, among others, promoting this product, antioxidant, and antifungal characteristics, glycerol is also incorporated, which aims to promote in the polymer matrix an organized arrangement acting as plasticizers (Kechichian et al., 2010; Assis et al., 2017; Caetano et al., 2018; Malherbi et al., 2019; Fuente et al., 2019; Marasca et al., 2020).

Biodegradable starch films are inexpensive, readily available, and have good physicochemical, morph structure, and mechanical characteristics (barrier to external and internal elements), guaranteeing their use in various activities, mainly for maintenance, avoiding contamination, and preserving characteristics of foods that you want to preserve for longer shelf life (Henrique et al., 2008; López et al., 2011; Ezeoha and Ezenwanne, 2013; Lucena et al., 2017; Silva et al., 2019). These can be extracted directly from vegetables, obtained by hydrolysis processes, known as modified starches (Asiri et al., 2019; Ulbrich et al., 2019; Oliveira Filho et al., 2020).

In addition, natural packaging contributes to environmental preservation and decreases the inappropriate use of synthetic petroleum-based packaging, which presents a long period of decomposition in the environment, being a severe problem in the conservation of aquatic species where they offer a greater risk (Nor Adilah et al., 2018; Bernardi; Hermes; Boff, 2018; Menezes Filho et al., 2020). The objective of this study was to develop and

evaluate a biodegradable packaging based on the starch solution obtained from the aryl of the fruit the *Hymenaea stigonocarpa* by the Casting process.

2. Material and Methods

H. stigonocarpa fruits were collected in February 2021, when the Cerrado region, located in Goiás State (GO), undergoes the rainy season. The collection was carried in Montividiu, GO (17°27'28.0''S, and 51°10'11.9''W). Biologist Antonio Carlos Pereira de Menezes Filho identified plant material and deposited in the Herbarium that belongs to the Instituto Federal Goiano, Rio Verde, Goiás, Brasil. Voucher (n° 11077).

The aryl was dried in an oven with forced air circulation at 60 °C (Nova Ética, Mod. 402-D) until a constant weight was reached, ground in a Wiley mill, and sieved to obtain a product with uniform granulometry. The obtained flour was stored in plastic packaging. The material was kept refrigerated at -12 °C until analysis.

The film was obtained by a casting technique, using the methodology proposed by Issa et al. (2017) with adaptations. Aryl (10 g) was dissolved in 250 mL of deionized water to produce the film. The solution was then moderately agitated at room temperature (25 °C) for 30 minutes. The solution was filtered under manual pressure on a thin nylon cloth, and the supernatant was collected.

Afterward, it was heated at 70 °C, with constant agitation for 30 minutes. After starch solution gelatinization, glycerol (Dinâmica, PA. – ACS, purity of 99,5%) was added as a plasticizer (35% p/p); this dispersion was agitated (BiomiXer, Mod. 78HW-1) for five more minutes. Filmogenic solution was poured on polystyrene (20 cm) (Kasvi, Mod. CRAL) slabs and dried in an air circulation (Ethik, Mod. 40 L) oven at 35 °C for about 36 hours.

Film thickness was measured with a digital caliper (Matrix, Mod. 150 mm Mtx) whose precision was 0.01 mm. Measurements were conducted in 15 spots on every film, and the thickness mean was calculated (Santos et al., 2020).

The film was weighed and then dried at 105 °C (Thoth, Mod. Th-510-480) for four hours, according to the methodology described by Rambabu et al. (2019). In order to measure the water solubility, the film (2 x 2 cm) was dried in an oven at 105 °C for 4 hours and then weighed to determine the initial mass (Mi). They were immersed in 60 mL distilled water and kept under constant agitation at 25 °C for 24 hours. Afterward, solutions with the films were filtered through filter paper (Unifil, Mod. C42) which had been previously weighed. Sheets of filter papers with films were dried at 105 °C for 24 hours and weighed so that the final mass

(M_f) could be found, in agreement with the methodology described by Jahed et al. (2017), adapted. The film solubility (%) was calculated by equation (1).

$$Wsol(\%) = (M_i - M_f)/M_i * 100 \quad \text{Eq. [1]}$$

Where: M_i = initial mass, and M_f = final mass.

The analysis was conducted by the methodology described by Martucci and Ruseckaite (2009), with adaptations. Film samples (2 x 2 cm) were dried up to constant weight to determine the initial mass (M_i). Samples were then placed in open polyethylene packages (high-density polyethylene - HDPE) to enable microorganisms and moisture to gain access to them.

After that, they were buried in organic soil, which had been previously prepared, at constant moisture, and room temperature. Twenty days after the experiment installment, the packages with the samples were removed from the soil, washed with distilled water, and dried up to constant weight (M_f). Biodegradability (%) and moisture (%) were calculated by equation (2).

$$Bio(\%) = (M_f - M_i)/M_i * 100 \quad \text{Eq. [2]}$$

Where: M_f = final mass and, M_i = initial mass.

Ultraviolet (UV) visible light transmittance of the film was conducted by UV-Vis spectrophotometer (Belphtonics, Mod. M-51). Film samples were cut and placed in cuvettes to measure transmittance over a wavelength between 250 and 850 nm (Hosseini et al., 2015). The film was fixed on a microscope slide. Then, it was observed under an optical microscope (Nikon, Mod. ED200) with magnifications of 4, 10, and 40x. Micrographs were taken using a camera attached to the microscope (Nikon, Mod. DS-Ri2). The film was fixed on brass sample holders. Images were conducted by a JEOL (JSM, Mod. 6610) (EDS, Thermo Scientific NSS Spectral Imaging) scanning electron microscope (SEM) in high-vacuum mode to detect secondary electrons at an electron accelerating voltage of 3 kV.

Mathematical modeling was performed using ImageJ software (free version, 1.8.0_172). The traction resistance was determined using a universal machine (Instron, Mod. 3360). Film strips were cut with a diameter of (15 x 120 mm) as described by Menezes, Souza, and Castro (2019). The system was adjusted with 100 mm hook spacing. The tensile strength was carried out with a speed of 0.21 mm/s in a maximum load cell of 650 N. The results were obtained for maximum tension, breaking stress, elongation, and modulus of elasticity.

Antibacterial activity was evaluated in vitro against three bacteria *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Enterococcus faecalis* (LB 29212) (Oliveira Filho et al., 2020). Briefly, 100 μL of bacteria culture, adjusted to 1×10^4 CFU mL^{-1} , were grown on plates with Tryptone Agar

Medium (Himedia – TAM) and 200 μL of spore suspension adjusted to 1×10^5 spores mL^{-1} on plates with Plant Count Agar (Kasvi – PCA). The film (10 mm diameter) was then placed on the surface of the agar and incubated (Nova Instruments, Mod. NI 1523i) at 37 °C for 36 h. The diameter of the zone of inhibition was measured with a digital caliper (Digimess, Mod. 100.174BL), resolution of 0.01 mm/0.0005.

Analyses were carried out in triplicate, and a standard deviation (\pm) was calculated. Means were obtained using the PAST 3 software program (free version, 2019).

3. Results and Discussion

The biodegradable film showed a brownish-yellow visual color, with a slight natural aroma of the fruit aryl. Menezes Filho et al. (2019) also obtained biodegradable yellow-colored film from the watermelon peel. According to Rocha et al. (2014), the color of biodegradable films can affect their acceptance in both edible and non-edible applications (packaging). The image of the biodegradable film obtained from the aryl of the Jatobá fruit (*H. stigonocarpa*) is shown in Figure 1.

The film had a good aspect, easy to handle, and was visibly homogeneous, with no presence of insoluble particles or brittle areas. Similar results were obtained by Malherbi et al. (2019) with gelatin-based corn starch biodegradable films incorporated with guabiroba pulp. The results of the physicochemical and mechanical parameters obtained by the biodegradable aryl film of the *H. stigonocarpa* fruit are shown in (Table 1).



Figure 1. The biodegradable film of the aryl from the fruit of *Hymenaea stigonocarpa*. Source: Authors, 2021.

Table 1. Physicochemical and mechanical parameters of the biodegradable film of the Jatobá (*Hymenaea stigonocarpa*) fruit.

Parameters	Results*
Thickness (mm)	0.27 ± 0.11
Moisture content (%)	12.45 ± 0.92
Water solubility (%)	57.84 ± 4.11
Biodegradability (%)	100 ± 0.00

*Average of the triplicate followed by (\pm) standard deviation.

The thickness obtained in the biodegradable film is within the range observed in other studies from alternative starch materials (Tab. 1). In the study by Assis et al. (2017), the researchers found thickness between 0.11-0.15 mm in biodegradable films of cassava starch incorporated with lycopene nanocapsules. Souza, Silva, and Druzian (2012) found a thickness between 0.12-0.14 mm in different formulations of biofilms of cassava starch incorporated with mango and acerola pulp and 0.12 mm in the control treatment. According to Hosseini et al. (2015), Adilah et al. (2018), and Santos et al. (2020), film thickness depends on factors related to drying and the method of preparation. The thickness should be measured because it affects the mechanical properties of films, such as water vapor permeability.

Possibly the aryl of the jatobá fruit has a high amylose content, and further studies are needed to determine this natural polymer. This polymer induces high sensitivity to moisture, which may also affect the mechanical properties of films (Thakur et al., 2019). The moisture content of the film presented similarity to other biodegradable starch-based packagings. Assis et al. (2017) reported a moisture content between 10.68 and 13.46% in biodegradable films of cassava starch incorporated with lycopene nanocapsules.

The biodegradable film of the Jatobá fruit aryl showed a moderate percentage of solubility (Tab. 1), which is, restricted use in environments with low humidity saturation. The lower the percentage of solubility, the better the quality and durability of the biodegradable packaging, being able to stay in an environment with a certain level of relative humidity. Solubility can also be influenced by the pH, and glycerol effects, which can interact with the structural conformation of the polymer (Rocha et al., 2014). Solubility is also an important parameter that should be analyzed in starch packaging. The ideal level of film solubility depends on their final use, for example, food, transport of liquids, and solids.

Starch is a hydrophilic material; thus, when a starch film is exposed to water its polymeric molecules form hydrogen bonds with water and lead to film dissolution (Bertuzzi et al., 2007; Kim et al., 2015). After the 20-days analysis, the polyethylene packages that contained film of the aryl of *H. stigonocarpa* fruit were removed from the soil. Since the film had been thoroughly degraded, the material could not be weighed to quantify biodegradability. This is a good result because it may be considered a promising material for biodegradable and eco-friendly packaging.

The same was observed in Santos et al. (2020) study, where they used different filmogenic solutions from arrowroot starch. The researchers at the time of 30-days observed the packages were completely degraded, being

impossible to determine the biodegradability. According to Martucci and Ruseckaite (2009) and Assis et al. (2017), biodegradable analysis of the film in natural soil seeks to play a degradation process in the natural environment where the soil is generally comprised of microflora consisting of bacteria, fungi, and protozoa which act in synergy in the biodegradability process in less time.

Similar results were observed by Medina Jaramillo et al. (2016) in cassava starch films with yerba mate extract and Seligra et al. (2016) in biodegradable films of starch with rapid biodegradability of the material in 12 and 15 days, respectively, where showed a significant degradation in 30-days, demonstrating the importance of development biodegradable packaging, and substitution packaging obtained from non-biodegradable polymers (petroleum-based synthetic).

According to Maran et al. (2014), Seligra et al. (2016), and Assis et al. (2017), biodegradable films obtained from native starch, and glycerol, which compounds exhibit hydrophilic character, can present a high loss of mass during the biodegradation due to increased water absorption by starch. The increased water absorption of polymers promotes the growth of microorganisms naturally present in the soil that act on the rich source of carbohydrates and results in greater and faster degradation of these compounds.

In Figure 2, the transmittance rate of the biodegradable film of the aryl from *H. stigonocarpa* fruit. The film showed similar behavior to other studies when subjected to UV analysis and visible light transmission at different wavelengths, from 250 nm to 850 nm. The maximum transmission was 82.25%, and the minimum was 1.32% (Fig. 2). A similar result was observed in the study of Assis et al. (2017) in cassava starch films with lycopene nanocapsules with maximum transmittance from 86.56% (800 nm) to 5.19% (200 nm).

Color is an important parameter to be evaluated since it influences the product acceptance of consumers (Lynch et al., 1986; Magnier and Schoormans, 2017). According to Romani et al. (2018), Rambabu et al. (2019), and Santos et al. (2020), films used as traditional packaging are usually transparent so that the product can be seen more easily. Transparent packaging is more attractive from the consumer's point of view; however, colorful and opaque biodegradable films help protect food exposed to visible and UV light, mainly in food with high-fat content, preventing them from undergoing oxidative degradation, which influences the quality negatively.

In Figure 3, we can observe the images by optical micrographic, electronic scanning, and 3D mathematical modeling of the surface area of the biodegradable aryl film from *H. stigonocarpa* fruit.

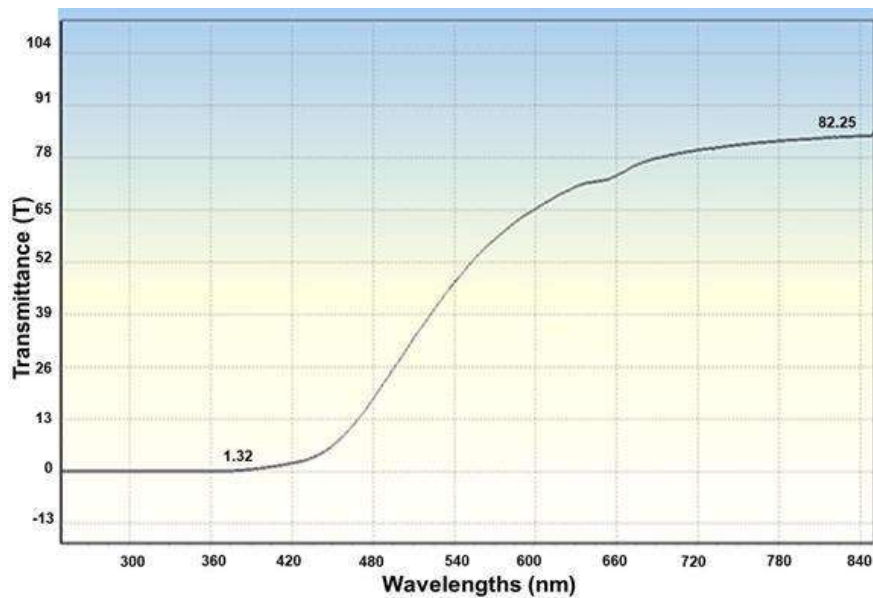


Figure 2. UV-Vis light transmittance rate in the biodegradable film of aryl from the Jatobá (*Hymenaea stigonocarpa*) fruit. Source: Authors, 2021.

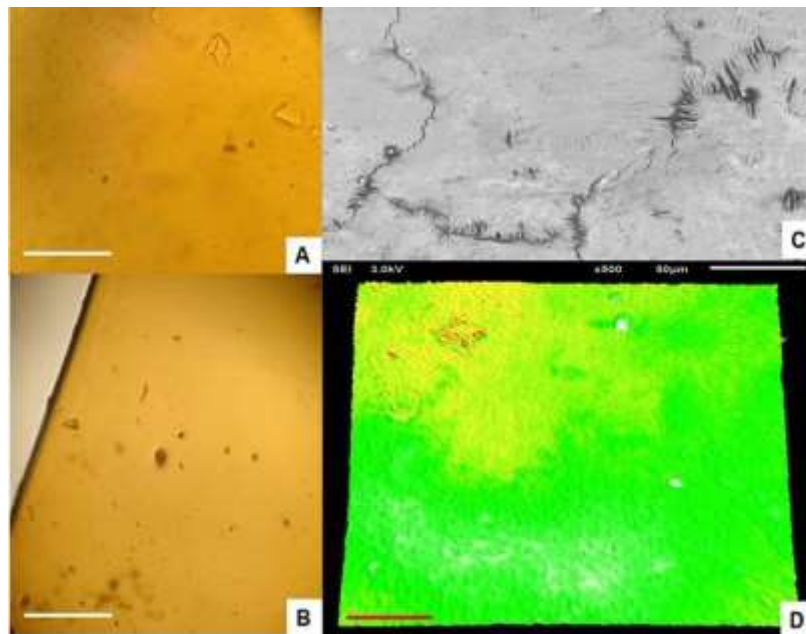


Figure 3. Micrographs of a superficial area of the starch film of aryl solution from *Hymenaea stigonocarpa* fruit. Bars: (A) 65 μm , (B) 80 μm , (C) 50 μm , (D) 90 μm . Source: Authors, 2021.

The biodegradable aryl film from Jatobá presents good malleability, appearance, and aroma. Brito et al. (2019) evaluated biodegradable films of flours and pectin from the residues of orange, passion fruit, and watermelon, where they found similar results to this study (Fig. 3A, 3B, and 3D), without roughness, with a shiny appearance, good malleability, and fruity aroma. In the scanning electron micrograph, it is possible to verify a surface area with cracks, probably related to the drying process and small starch granules (Fig. 3C).

In the 3D mathematical model, it is possible to observe the moderately uniform topography of an area of the surface on both faces (Fig. 3D). In green,

homogeneous surface area, a yellow area with small elevations, and in red, substantial elevations. The white dots represent the starch granules. The following results were found, maximum stress (N) = 3.17 ± 0.12 , rupture stress (MPa) = 1.34 ± 0.66 , elongation (%) = 2.99 ± 0.76 , elasticity (MPa) = 90.07 ± 14.21 .

They are similar to those observed by Menezes Filho et al. (2019) for biodegradable watermelon film, with maximum tension of 1.38 MPa, elongation of 2.26%, elasticity module of 91.09 MPa, and breaking strength of 3.01 N. Malherbi et al. (2019) found results between 10.95-79.04 MPa for tensile strength and 1.45-6.19% rupture elongation for biodegradable corn

starch films incorporated with guabiroba pulp. Already Assis et al. (2017) obtained tensile strength results between 2.43 to 18.13 MPa, and elongation at the break from 61.27 to 399.92% for biodegradable films of cassava starch incorporated with lycopene.

The aryl film of (*H. stigonocarpa*) presented low antibacterial activity, 7 mm *S. aureus*, 5 mm *E. coli*, and 8 mm *E. faecalis*. The natural polymer based on the aryl from *H. stigonocarpa* fruit showed potential bactericidal action. Jain et al. (2014) and Oliveira Filho et al. (2020) discuss the use of antimicrobial polymers are materials capable of killing or inhibiting the growth of microbes on a surface or surrounding environment. They have an inherent capacity to display antimicrobial activity such as chitosan, compounds with quaternary nitrogen groups, halamines, essential oil, fixed oil, resin oil, and poly- ϵ -lysine (ϵ -PL), or they can be a polymer acting as a backbone to incorporate small biocides and antibiotics to develop their activity. Complementary studies should be carried out in the phytochemical characterization of this aryl polymer by high-performance liquid chromatography (HPLC)

4. Conclusions

The development of biodegradable film from renewable sources, such as starch in the aryl from the *Hymenaea stigonocarpa* fruit, may be an alternative to non-biodegradable packaging with high biodegradability in a short period. The biodegradable film of the aryl from the jatobá fruit presented interesting results aiming at the easiness of obtaining the raw material and production.

The results of the physicochemical, morphological, and mechanical parameters and antimicrobial activity qualify this new product for the packaging production market as a possible substitute for synthetic packaging and provide a new way to improve microbial safety used of foods shelf life. This is the first of several studies on developing new biodegradable packaging that seeks to combine the preservation of an endemic species in the Cerrado with the production of green line packaging for sustainable products.

Authors' Contribution

Antonio Carlos Pereira de Menezes Filho contributed to the execution of the experiment, data collection, analysis and interpretation of results, writing of the manuscript, and final correction of the manuscript. Aparecida Sofia Taques contributed to the execution of the experiment and data collection. Ivan Alves contributed to the analysis, data collection, and

translation. Matheus Vinicius Abadia Ventura contributed to the analysis and data collection. Hellen

Regina Fernandes Batista-Ventura contributed to the analysis and data collection. Wendel Cruvinel de Sousa contributed to the final article writing and translation. Carlos Frederico de Souza Castro contributed to data analysis, funds, and final manuscript correction. Marconi Batista Teixeira contributed to data analysis and funds. Frederico Antônio Loureiro Soares contributed to the analysis, funds, and final correction of the manuscript

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