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FOOD TECHNOLOGY

Araucaria angustifolia and the pinhão seed: Starch, bioactive compounds and functional activity – a bibliometric review

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ABSTRACT: *Araucaria angustifolia* characterizes mixed Ombrophilous Forests. This Paraná pine tree has been of great economic, cultural and social importance for southern Brazil. Its cutting is restricted, as it is threatened with extinction and the use of its seed has been encouraged. This study highlights scientific research on this conifer by bibliometric analysis and reviews trends in new research on its seed and some of its food applications. The Web of Science© database revealed 620 scientific articles and the bibliometric analysis through VOSviewer showed the worldwide interest in growing. The increase in research in the areas of silviculture, phytoscience and ecology reflects the concern with the preservation of "Matas das Araucárias". Concurrently, research in food science and technology has increased, as pine nut seed can produce starch-rich food flour with low glycemic response and source of dietary fiber and some minerals. Also, along with its husk, provide bioactive compounds with potential application in the special food, active/smart and reinforced packaging and even pharmacological industries.
Key words: resistant starch, gluten-free food, functional food, phenolics compounds, trace elements, cosmetic.

Araucaria angustifolia e a semente de pinhão: Amido, compostos bioativos, e atividade funcional – uma revisão bibliométrica

RESUMO: A *Araucaria angustifolia* caracteriza as Florestas Ombrófilas mistas. Este pinheiro do Paraná tem tido grande importância econômica, cultural e social para o Sul do Brasil. Seu corte está restrito, pois está ameaçada de extinção e o uso de sua semente tem sido incentivado. Este estudo destaca as pesquisas científicas sobre esta conífera por análise bibliométrica e revisa as tendências de novas pesquisas sobre sua semente e algumas de suas aplicações alimentícias. A base de dados Web of Science© revelou 620 artigos científicos e a análise bibliométrica por meio do VOSviewer demonstrou o interesse mundial em ascendência. O aumento das pesquisas nas áreas de silvicultura, fitociência e ecologia refletem à preocupação com a preservação das Matas das Araucárias. Concomitantemente, pesquisas em ciência e tecnologia de alimentos têm aumentado, pois a semente de pinhão pode produzir uma farinha alimentar rica em amido com baixa resposta glicêmica e fonte de fibra dietética e de alguns minerais. Ainda, junto com sua casca, disponibilizar compostos bioativos com potencial de aplicação nas indústrias de alimento especial, de embalagem ativa/inteligente e reforçada e, até mesmo, farmacológica.
Palavras-chave: amido resistente, alimentos sem glúten, alimentos funcionais, compostos fenólicos, oligoelementos, cosméticos.

INTRODUCTION

Araucaria angustifolia (Bertoloni) Otto Kuntze (Gymnosperm, Araucariaceae Family) is the predominant species in the Mixed Ombrophilous Forest located in the southwestern and southern regions of Brazil (IBGE, 2012) and northeastern Argentina (ZONNEVELD, 2012). The pine nut seed of the conifers have been consumed by the natives for a long time (CONFORTI & LUPANO, 2008). This Brazilian pine (Figure 1a) is economically important for the South of Brazil, where it is popularly known as the "pinheiro do Paraná" (FIGUEIREDO FILHO et al., 2011). The cutting of *A. angustifolia* trees (Figure 1a) has been restricted by law, and the use of its seed (Figure 1d) has been fomented and popularized (MEDINA-MACEDO et al., 2016). As for agricultural importance, a total of 9,342 tons of pinhão were produced in 2019, being that 2,055 of which were composed of pine nut seed. Most of it was produced in the southern region of Brazil, with the state of Paraná producing 3,290 ton, Santa

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Catarina producing 3,120 ton and Rio Grande do Sul producing 819 ton (IBGE, 2020).

One of the original ways to consume the pine nut seed at home is the "sapecada". The seed is thrown into a fire made with the pine leaves themselves ("grimpas"). The most common way to eat the pine nut seed is to wash the pinhão seed and cook them in salted water for 40 min in a pressure cooker or about 75 min in a normal pot (Figure 1h). It must be peeled while still hot to facilitate the operation. They are later taken out and served. More recently, the cooked pine nut seed has been used to compose typical dishes, such as "entrevero", pinhão soup, "farofa", etc. (EMBRAPA, 2013).

The mature feminine strobilus or cone (Figure 1b,c) consists of seeds (full pinhão, Figure 1d), unfertilized pinhão ("chocho") and bracts (flaws, Figure 1c,e), which are undeveloped seeds that are usually discarded in the environment (SOUZA et al., 2014; ZANETTE et al., 2017). The pinhão seed comprises a husk or shell (integument, Figure 1f,i,j), an endosperm and an embryo (pine nut seed, Figure 1f,i) (BRASIL, 2009), and the innermost part (endotesta; Figure 1g) of the shell usually adheres to the starchy part of the seed of raw pinhão nuts (Figure 1g). The seed is a seasonal product that is available between the months of April and August. Population consume it and is sold in stands at the side of the road, supermarkets and regional festivals in the South of Brazil (CLADERA-OLIVEIRA et al., 2012; ZORTÉA-GUIDOLIN et al., 2017a). The husks are a residue with low added value (PERALTA et al., 2016), but which is a source of phenolic compounds with antioxidant and minerals (SANTOS et al., 2018), and that can be used to promote and improve human health (BELWAL et al., 2017; PERALTA et al., 2016). Like bark, bracts contain high levels of phenolic compounds, including high molecular weight condensed tannins, which have a higher antioxidant capacity than common phenolic compounds (KOEHNLEIN et al., 2012; SOUZA et al., 2014). Condensed tannins also aid in the curing of

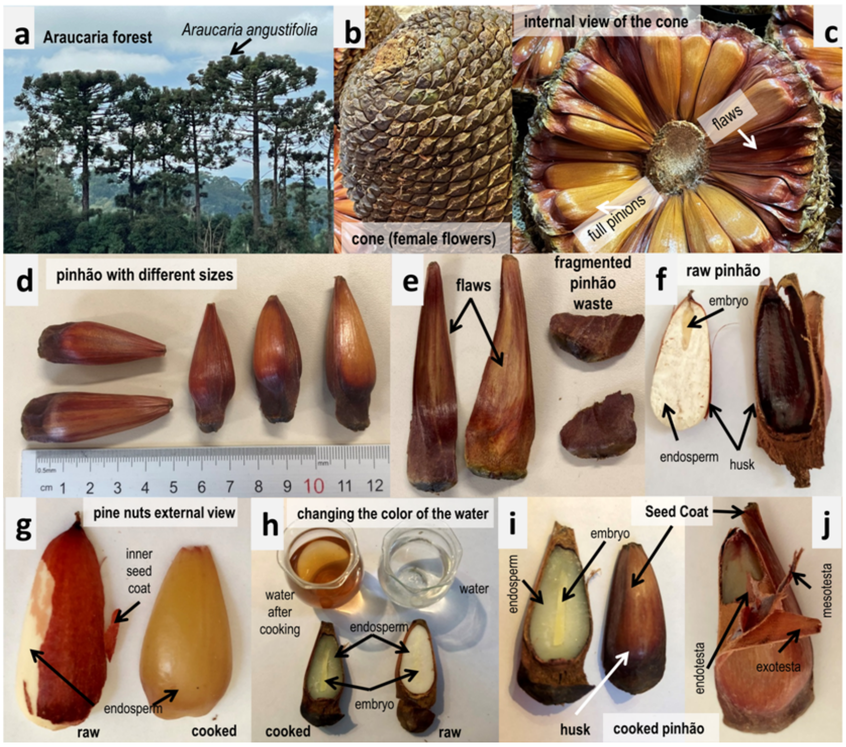


Figure 1 - Araucaria Forest, *Araucaria angustifolia*, cone and seeds cooked or not, as well as their parts.
a) Araucaria forest; b) External view of the cone; c) Internal view of the cone; d) Pinhão with different sizes, e) Flaws and pinhão waste, f) Internal view of raw pinhão. h) External views of raw and cooked pinhão; i) internal view of cooked pinhão and its husk; j) Seed coat and its three layers.

wounds, reduce pain from pancreatitis, reduce insulin resistance in diabetics and help protect from the toxicity of medications. Within this context, the use of condensed tannins has a high potential for use in alternative therapies for several associated oxidative and inflammatory diseases (ABU ZARIN et al., 2016) and demands complementary studies *in vivo*.

The high carbohydrate content (DA SILVA et al., 2016) in the pinhão seed, the presence of essential fatty acids (BARBOSA et al., 2019) and macro and microminerals (BARBOSA et al., 2019; CONFORTI & LUPANO, 2008) of pinhão stimulate its direct consumption in the cooked form and in the hundreds of dishes based on cooked pinhão (EMBRAPA, 2013). Additively, the raw pine nut flour for the production of "alfajores" is already a reality of its industrial application (CONFORTI & LUPANO, 2008). Furthermore, this flour can potentially also be used in the production of gluten-free cake (IKEDA et al., 2018), gluten-free bread (POLET et al., 2019) and extruded foods (ZORTÉA-GUIDOLIN et al., 2017b). More sophisticatedly, the pine nut starch, whether natural (DAUDT et al., 2014) or modified (GONÇALVES et al., 2014), opens up other application opportunities, such as films for use in food (GONÇALVES et al., 2014; DAUDT et al., 2017) and even pharmacological excipients (DAUDT et al., 2014). Finally; although, the husk is not considered an edible portion, it contains components that can be used as a food additive (TIMM et al., 2020), an antimicrobial agent with potential application in the food industry (TROJAIKE et al., 2019), composites for improvement of food packaging films (ENGEL et al., 2020), etc. The husk can still be used for health promotion due to the presence of bioactive compounds in foods and as a control of lipid levels in the blood (OLIVEIRA et al., 2015, LIMA et al., 2020).

Within this context, a bibliometric study, which provides a conceptual profile of the scientific development of specific areas and fields through qualitative and quantitative analyzes (DI STEFANO et al., 2010), can identify new trends and perspectives of scientific studies on the theme. Thus, the present study followed the previously applied methodology (RAAN, 2009; ARAÚJO, 2006; RAASCH et al., 2018) to describe and analyze the timeline, authors and co-authors, institutional links and the geographical distribution of scientific articles linked to *Araucaria* and Pinhão.

This study provided an overview of the consolidated scientific research on *Araucaria angustifolia* in terms of areas of knowledge, journals, institutions, research networks and countries involved, as well as to present some data on its seed to stimulate new research on food science and technology.

METHODOLOGY

Bibliometric and literary review methodology

This bibliographic review is composed of a bibliometric analysis, followed by a review of the literature (ARAÚJO et al., 2020) based on a quantitative research approach using the technique of bibliometric, which measure the production and propagation indices of scientific knowledge (ARAÚJO, 2006). Data collection was conducted during the month of January of 2021 using the Web of Science© (WOS) database, since it is a consolidated database with an index of more than 15,000 periodicals (FORLIANO et al., 2021) which is practical for data mining using its filters (MERIGÓ et al., 2015). The data was filtered using the *topic* item, which includes the title, abstract and keywords with the following descriptors: "*Araucaria angustifolia*" OR "Brazilian pine seed" OR "pinhão seed" OR "pinhão coat". Thus, ensuring international publications. The study period was between the years 2000 and 2020, due to the growing number of studies regarding the fields included in the selected keywords.

A total of 652 scientific publications were identified, which were also composed of publications in proceedings, reviews, abstracts of meetings, corrections and early accesses. Then, a filter was applied to analyze the 620 records of the research article type in relation to the distribution in relation to the year of publication, language and region of records. The quantitative analyzes of the bibliometric indexes were compiled in an electronic spreadsheet using the Microsoft Office Excel® 2010 software. Finally, VOSviewer was used to set up cooperation networks between countries and organizations.

DISCUSSION

Bibliometric analysis

Year, language, region of publication, organizations, cooperation between countries and journals.

Six hundred and twenty scientific articles on *Araucaria angustifolia* were published between 2000 and 2020, of which 485 were published in English, 125 in English and 10 in Spanish. Remembering that Portuguese is the official language in Brazil and Spanish is the official language in Argentina and Paraguay, which are the countries where *Araucaria angustifolia* occurs (ZONNEVELD, 2012).

The average number of publications between 2000 and 2006 was 9 scientific papers (standard deviation = ±3) (Figure 2), but it tripled (34±4) between 2007 and 2014 and practically quintupled between 2015 and 2020 (48±8). The year 2020 revealed the highest number (56 articles) of publications, which surpassed the 55 articles of 2016. This reveals the increase in interest on the topic (highlighting 108 articles between 2019 and 2020), and the need for investment for studies on this topic and topics related to its application.

Scientific articles about *Araucaria angustifolia* were published in 77 fields of knowledge. Each of the first 10 fields of knowledge with the largest number of publications has at least 23 publications. The remaining fields range from 20 to at least one publication, including fields such as biology, engineering, chemistry, horticulture, entomology, cellular biology, organic chemistry, propagation and vegetable physiology. The largest concentration of studies involving *Araucaria angustifolia* was in the area of forestry, followed by plant sciences and ecology, which may be attributed to the preoccupation to preserve the Araucaria Forest (“Mata das Araucárias”) (ZANETTE et al., 2017). The field of Food Science and Technology is in fourth place, with 50 publications.

The representativeness of authors and co-authors is achieved by means of the organizations. Among the 77 fields of knowledge, 369 organizations published scientific articles related to *Araucaria angustifolia*, of which the 9 principal organizations are Brazilian (Figure 3) and located in southern and southwestern Brazil, the region where *Araucaria angustifolia* occurs (WREGE et al., 2017; ZONNEVELD, 2012). Each organization published at least 20 articles involving *Araucaria angustifolia*, of which the Federal University of Paraná held the record with 104 published articles.

The *Araucaria angustifolia* is a native Brazilian species (WREGE et al., 2017), but small spots also occur in Argentina and in Paraguay (ZONNEVELD, 2012). This explains articles from Argentina (Figure 4), but also from cooperation with institutions from other countries (Figure 3), such as Eberhard Karls University of Tuebingen (Germany), University of California (USA) System and Polytechnic Institute of Bragança (Portugal), among other institutions.

In this sense, Brazil is the country with the largest number of publications (549, Figure 4) involving *Araucaria angustifolia*, who is native to of southern and southeastern Brazil and northeastern Argentina (CORDENUNSI et al., 2004). Even so, 28 other countries have made contributions to the production of data involving this specie. Brazilian institutions interact nationally and internationally in scientific production (Figure 3), thus revealing relationships between Brazil and other countries such as Germany, USA, Canada, Australia and others. Italy and Chile are the only countries that have made publications indirectly with Brazil (CORDENUNSI et al., 2004), being that *Araucaria araucana* occurs in Chile, as well as in Argentina (CONFORTI & LUPANO; 2008; ZONNEVELD, 2012), which may justify the interest in the subject in a more. These publications are related to environmental issues involving Araucaria forest and address the influence

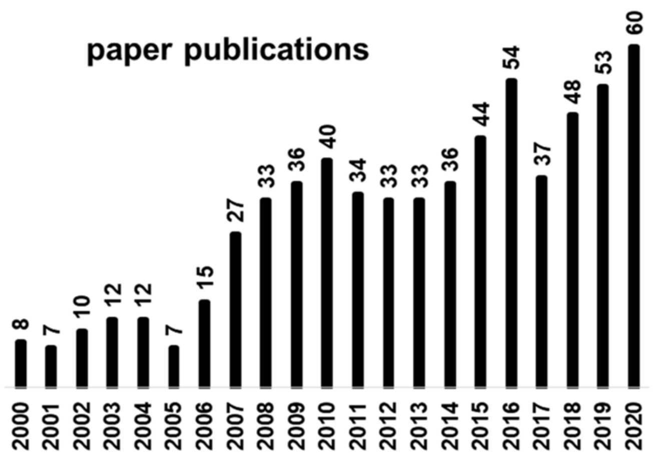
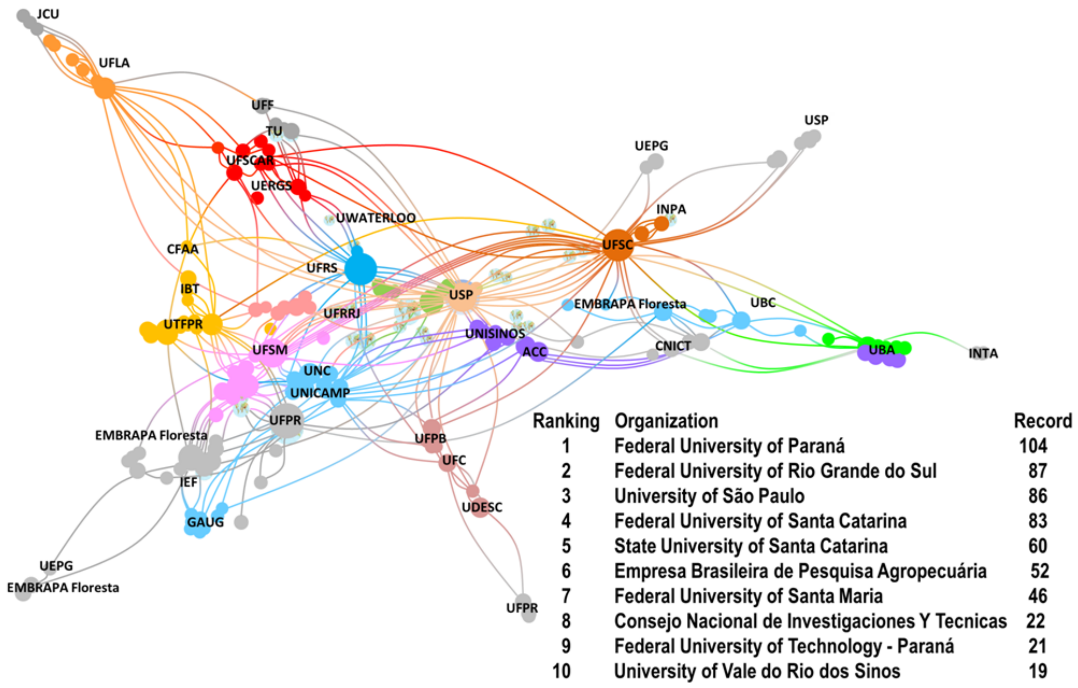


Figure 2 - Scientific articles related to *Araucaria angustifolia* over the last 20 years.



Ranking	Organization	Record
1	Federal University of Paraná	104
2	Federal University of Rio Grande do Sul	87
3	University of São Paulo	86
4	Federal University of Santa Catarina	83
5	State University of Santa Catarina	60
6	Empresa Brasileira de Pesquisa Agropecuária	52
7	Federal University of Santa Maria	46
8	Consejo Nacional de Investigaciones Y Tecnicas	22
9	Federal University of Technology - Paraná	21
10	University of Vale do Rio dos Sinos	19

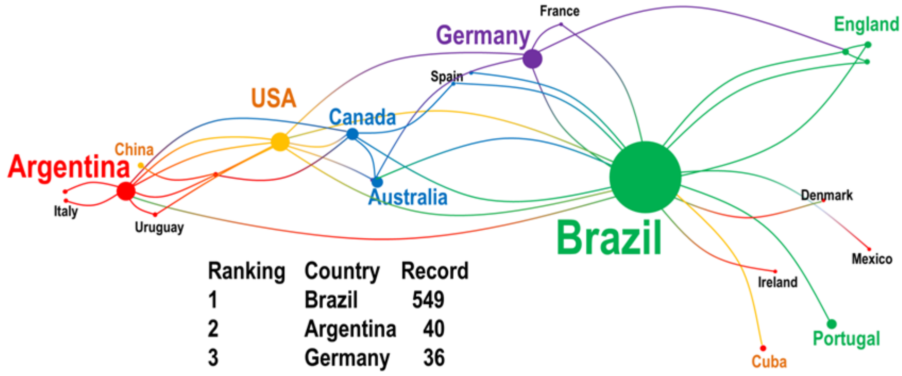
Figure 3 - Cooperation network among organizations according to co-authorship. (Adapted from VOSviewer).
ACC = Academia de Ciencias de Cuba; CFAA = Casa da Floresta Assessoria Ambiental; CNICT = Consejo Nacional de Investigaciones Y Tecnicas; EMBRAPA Floresta = Empresa Brasileira de Pesquisa Agropecuária – PR; GAUG = Georg-August-Universität Göttingen; IBT = Instituto Politécnico de Bragança; IEF = Instituto Estadual de Florestas; INPA = Instituto Nacional de Pesquisas da Amazônia; INTA = Instituto Nacional de Tecnología Agropecuaria (Famaillá); JCU = James Cook University; TU = Universität Tübingen; UBA = Universidad de Buenos Aires; UBC = University of British Columbia; UDESC = Universidade do Estado de Santa Catarina; UEPG = Universidade Estadual de Ponta Grossa; UERGS = Universidade Estadual do Rio Grande do Sul; UFC = Universidade Federal do Ceará; UFF = Universidade Federal Fluminense; UFLA = Universidade Federal de Lavras; UFPB = Universidade Federal da Paraíba; UFPR = Universidade Federal do Paraná; UFRS = Universidade Federal do Rio Grande do Sul; UFSC = Universidade Federal de Santa Catarina; UFRRJ = Universidade Federal Rural do Rio de Janeiro; UFSM = Universidade Federal de Santa Maria; UFSCAR = Universidade Federal de São Carlos; UNISINOS = Universidade do Vale do Rio dos Sinos; UNC = Universidade do Contestado; UNICAMP = Universidade Estadual de Campinas; USP = Universidade de São Paulo; UTFPR = Universidade Tecnológica Federal do Paraná; UWATERLOO = University of Waterloo.

of fauna, solar activity and genetic variability of species and so on, thus revealing a global interest in preserving this species that is important to the South of Brazil (WREGE et al., 2017).

Analysis of journals and keywords

The publication knowledge field was limited by using a filter to include only scientific paper in the area of Food Science and Technology. From this subset, the journals with the highest number of publications, the most cited and the highest Impact Factor were identified (Table 1). Thus, it was to be expected that journals were essentially related to the themes. The four journals with the largest number of publications were cited a total of 350 times and have an impact factor greater than 2.2 (year 2020), but there is no correlation between them, which is confirmed by the relationship between citation numbers and scientific articles. Starch Starke alone has 9 publications, corresponding to 18% of all publication and that is expected to be a seed with edible fraction (CONFORTI & LUPANO, 2008; CORDENUNSI et al., 2004; IKEDA et al., 2018; POLET et al., 2019; ZORTÉA-GUIDOLIN et al., 2017b). The journal Food Hydrocolloids has 84 citations with only 4 publications, which makes it occupy the second place in this aspect. The ability to form hydrocolloids with starch is of great industrial interest (DAUDT et al., 2014; GONÇALVES et al. 2014).

To identify and verify search trends, a network of words was generated based on keywords (Figure 5) in which the size of the circle represents



Ranking	Country	Record
1	Brazil	549
2	Argentina	40
3	Germany	36
4	USA	35
5	Canada	16
6	Australia	15
7	England	8
8	Portugal	8
9	Cuba	5
10	China	5

Figure 4 - Cooperation network among countries according to co-authorship. (Adapted from VOSviewer).

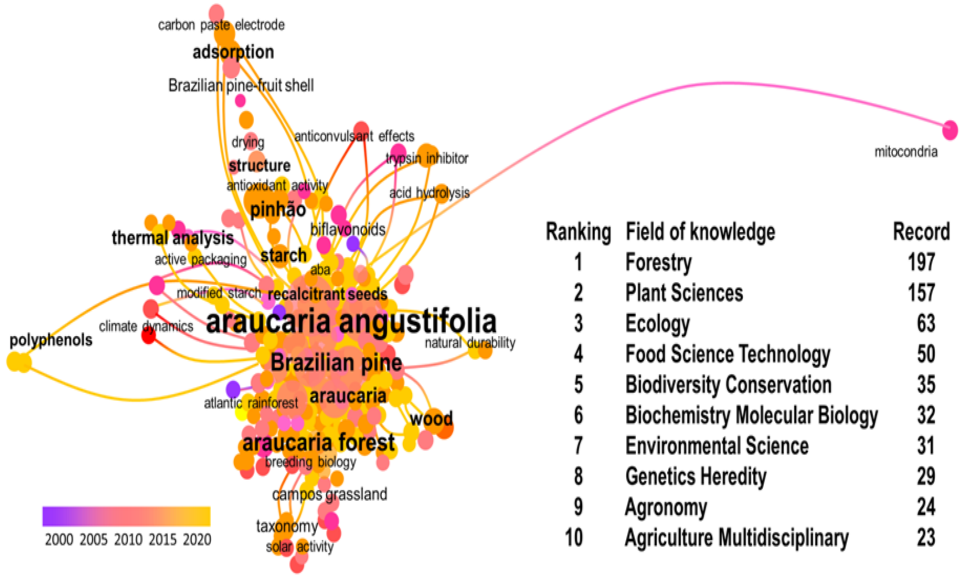
the number of occurrences of keywords and the color represents the year of publication of the article. Research trends involving the pinhão seed, food portion, and its shell involve starch, polyphenols, antioxidant activity, active packaging, and recalcitrant seed, among other topics. Information on these topics was scarce until a few decades ago, but has been intensified recently (Figure 2). This bibliometric analysis revealed that there is a growing generation of knowledge in these areas, which reveals the need for a literary review on the pinhão.

Literature review

Araucaria angustifolia and pinhão seed

Araucaria angustifolia, also known as the Brazilian pine or the Paraná pine, is one of the most important tree species of the Brazilian flora, being the most economically important of the native Brazilian flora (ZANDAVALLI et al., 2004). The intensive exploitation of this species began in the 1930s, always being associated with the acquisition of wood for lumber and for supplying the paper industry. This activity resulted in an 88% reduction of the total forest area (from approximately 253,000 km^2 to only 32,000 km^2) (RIBEIRO et al., 2009). Therefore, the species is on the list of Brazilian species under threat of extinction due to uncontrolled exploitation (PERALTA et al., 2016), only 31% (981 km²) of the total area is being protected (FIGUEIREDO FILHO et al., 2011). Global climate changes are potentially additional threats to *Araucaria angustifolia* due to the increase in temperature and changes in the water regime, which reduces its potential for survival and reestablishment in new planting areas (WREGE et al., 2017). Natural forests and plantations are mainly distributed among the Brazilian states of Paraná, Santa Catarina and Rio Grande do Sul. The seed of A. angustifolia, known as pinhão and can be acquired between April and September (ANSELMINI & ZANETTE, 2008).

The *A. angustifolia* is a tree with a height ranging from 30 to 50 m and a chalice-shaped crown and a straight trunk with an approximate diameter of 50 cm. Its optimal development occurs at an approximate age of 30 years, and its lifespan ranges from 200 to 300 years (BRDE, 2005). It is a dioecious species, having reproductive structures that are organized into masculine and feminine strobili (pine cones) (CARVALHO, 2002). Pollination is accomplished by wind, occurring between the months of August and December. The reproduction (maturation) of the cone (Figure 1b) occurs two years after pollination and the feminine tree is capable of producing an average of 80 cones per year, each of which weighs between 0.61 kg e 4.1 kg and produces approximately 90 pinhões (BRDE, 2005). The weight of the pinhão seed varies between 7 and 9 g, and its husk accounts for 22% of the entire mass of the structure (LIMA et al., 2007). Nutritionally, the pine nut seed exhibits significant nutritional values, containing 36% starch, 3% protein



Ranking	Field of knowledge	Record
1	Forestry	197
2	Plant Sciences	157
3	Ecology	63
4	Food Science Technology	50
5	Biodiversity Conservation	35
6	Biochemistry Molecular Biology	32
7	Environmental Science	31
8	Genetics Heredity	29
9	Agronomy	24
10	Agriculture Multidisciplinary	23

Figure 5 - Co-occurrence of keywords in published scientific articles related to *Araucaria angustifolia* (Adapted from VOSviewer).

and 1% lipids, in addition to calcium, iron and phenolic compounds (CORDENUNSI et al., 2004).

Physico-chemical composition of the pinhão seed

The moisture, ash, crude protein, total lipid, fiber and other carbohydrate contents in the endosperm of the (*in natura*) pine nut seed are 43.70%, 1.50%, 3.42%, 1.67%, 1.29% and 48.42%, respectively (DA SILVA et al., 2016). The dry matter of pine nut seed represents 3.43 g of seed^{-1}. The elemental composition of the seed has C (383 g kg^{-1}) as the main macroelement , followed by K (11.8), N (10.0), P (4.18), Mg (0.78) and Ca (0.29). The microelements (mg kg^{-1}) reported are Fe (25.83), Zn (18.86), Mn (9.11), Cu (7.23), Ni (1.30), Mo, (0.93), Ba (0.93), Co (0.45), Cr (0.65) and Cd (0.19). Contributions according to the recommended diet indices (RDA, %) for the intake of 100 g of Araucaria angustifolia seeds (45% moisture) are N-Protein (6.2), K (18.5), P (32.8), Mg (11.7) and Ca (1.6), Fe (9.7), Zn (11.4), Mn (25.0), Cu (43.6) and Mo (115). The tolerable upper intake (TUI, %) was also compared for Mo (2.6), Ni (7.2), Ba (4.0), Co (1.8) and Cd (16.3). Thus, pine nut seed can be a source of beneficial nutrients (K, P, Mn, Cu, Mo, and Cr), while values for Ba and Cd does not indicate health risks (BARBOSA et al., 2019). In this sense, the pinhão nut seed was also considered a source of starch, dietary fiber, Mg and Cu (CORDENUNSI et al., 2004).

The accumulation of nutrients occurs during the dehydration of the seeds in the final maturation stages, generally during the months of April and May, when the protein content increases (PERALTA et al., 2016). Low molar mass sugars in largest quantities is glucose (0.56 g 100g^{-1}), followed by saccharose (0.05 g 100g^{-1}) and fructose (0.03 g 100g^{-1}). However, phenolic compounds migrate from the husk to the pine nut seed (Figure 1h) when they are cooked and enrich this edible (CORDENUNSI et al., 2004), which reinforces the low glycemic response by eventual inhibition of amylase (SILVA et al., 2014). Furthermore, the insoluble dietary fiber content is greater in cooked seeds, a fact that may be related to the non-negligible quantities of resistant starch. The high amylose content in the pinhão starch may contribute to the formation of resistant starch after the cooked seeds have cooled (CORDENUNSI et al., 2004).

The main components of the lipid fraction are linoleic acid (18:2n-6), oleic acid (18:1n-9) and palmitic acid (16:0). The first two are considered essential fatty acids, as they are not synthesized by the body. Also, the omega-6 series (400.76) was more abundant than the omega-3 series (27.54). In a cleaner technology, high concentrations of essential fatty acids, total tocopherol and total phytosterol were obtained in the extracts of oil from seeds endosperms by subcritical fluid extraction with n-propanol at 40 ºC and 8 MPa (DA SILVA et al., 2016).

Table 1 - Published paper number in journal in the field of Food Science and Technology, number of citations, JCR (2020) and citation and publication relationship.

Journal	Record#	NC&	JCR*	NC/Record
Starch Starke	9	158	2.226	17.6
International Journal of Food Science and Technology	4	85	2.773	21.3
Food Hydrocolloids	4	84	7.053	21.0
Food and Bioprocess Technology	3	23	3.356	7.7
Journal of Food Processing and Preservation	3	1	1.405	0.3
Journal of Food Engineering	2	74	4.499	37.0
LWT Food Science and Technology	2	66	4.006	33.0
Food Chemistry	2	52	6.306	26.0
Food Research International	2	50	4.972	25.0
Food Function	2	6	4.171	3.0
Journal of Food Science	2	5	2.479	2.5
Journal of Agricultural and Food Chemistry	1	89	4.192	89.0
Food Science and Technology	1	64	0.49	64.0
Food Packaging and Shelf Life	1	14	4.244	14.0
Journal of Food Process Engineering	1	25	1.703	25.0
Journal of the Science of Food and Agriculture	1	10	2.614	10.0
Boletim do Centro de Pesquisa de Processamento de Alimentos	1	9	nf	9.0
Food Biophysics	1	6	2.387	6.0
International Journal of Food Properties	1	6	1.808	6.0
Food and Chemical Toxicology	1	3	4.679	3.0
Journal of Food Science and Technology Mysore	1	3	1.946	3.0
Journal of the Institute of Brewing	1	1	1.504	1.0
Ukrainian Food Journal	1	0	0.453	0.0

Record = paper number registered in the journal; & NC: number of citations; *Impact factor during the year 2020; nf = not found.

The seed coat of *Araucaria angustifolia* (Figure 1j) is composed of 46% of α-cellulose, 9% hemicellulose, 34% of lignin, 7% of extracts and 1.6% of ash (SAMPAIO et al., 2019). As for the extractive of the bark, obtaining with the use of a soxhlet extractor is enhanced with the use of organic solvents of increasing polarity (cyclohexane, ethyl acetate and methanol) for an uninterrupted period of 24 h for each solvent. The total extracted for the three layers of the bark revealed the highest yield for the endotesta (27.53%), followed by the mesotesta (17.44%) and exotesta (11.91%). Still, methanol showed the highest extractive capacity (BARROS et al., 2021).

Characterization of pinhão starch

The starch content on pine nut seed is 36.28 ± 0.11 g 100g^{-1} *in natura* and 34.48 ± 0.72 g 100g^{-1} in cooked pinhão, that is, about 70% on a dry basis (CORDENUNSI et al., 2004). Pinhão starches exhibited C-type crystallinity and had amylopectin with larger proportions of medium-short branch-chains (DP 13-24) and average branched chain-length of 19.7-21.4 anhydrous glucose units (AGU) (ZORTÉA-GUIDOLIN et al., 2017a). Using x-rays diffraction, the *in natura* pinhão starch is seen to be a semi-crystalline solid, whereas the cooked pinhão starch is an amorphous solid like any other pre-gelled starch (DAUDT et al., 2014). Pinhão starch presented considerable levels of slowly digestible starch (SDS) and resistant starch (RS). The amylopectin of these starches presented weight-average molecular mass (Mw) of 3.0-3.9 x 10^8 g mol^{-1}, z-average radius of gyration (Rz) of 270-283 nm, which allows promising use in healthy food/nutraceuticals; like shakes and nutritional supplements (ZORTÉA-GUIDOLIN et al., 2017a). The pine nut seed starch can be isolated with a yield of approximately 70% (BELLO-PÉREZ et al., 2006). The average size of the starch granules is approximately 12 μm (CONFORTI & LUPANO, 2007), and they exhibit oval, hemispherical or truncated ellipsoid shapes and smooth surface (ZORTÉA-GUIDOLIN et al., 2017b), and also exhibit a white color and partial crystallinity (DAUDT et al., 2014). They are insoluble in cold water but may form gel at low temperatures (50 ºC and 60 ºC), and a quantitative estimation of the relative crystallinity of the pinhão starch classifies it as type C (ZORTÉA-GUIDOLIN et al., 2017a).

Some factors present in the starchy foods influence the rate at which the starch is hydrolyzed and absorbed *in vivo*. The high concentration of amylose is not the only fact involved with the starch retrogradation and the formation of resistant starch. Thus, the contents of total starch, resistant starch, digestible starch, amylose and dietary fiber is recommended, and the formation of RS in foods does not follow an easily correlated behavior (ROSIN et al., 2002). CORDENUNSI et al. (2004) proposed that the high amylose content (almost 30%) in pinhão starch can contribute to the formation of resistant starch after cooling the cooked seeds and that the resistant starch value (3.27%) reported are similar to legumes, such as beans, chickpeas, lentils and peas. Resistant starch is not digested as quickly as regular starch and may resist enzymatic digestion in the upper parts of the gastrointestinal tract, but it can be fermented by microorganisms residing in the large intestine. This also contributes to the low glycemic response related to SILVA et al. (2014). It has some unique functions, in addition to some biological benefits, such as traditional fiber smoothing postprandial blood glucose, preventing colon cancer (REGASSA & NYACHOTI, 2018).

Phenolic compounds and antioxidant activity

Phenolic compounds are secondary metabolites that may exist in plants in high concentrations (HUANG et al., 2019). Their health benefits are attributed to properties that protect against cardiovascular and neurodegenerative diseases (DEL RIO et al., 2013). The phenolic content in *in natura* pine nut seed is very low in comparison to that of the interior lining of the seeds. However, when the pinhão with shell are cooked, phenolic compounds migrate from the husk to the pine nut seed and enrich this part of the seed (CORDENUNSI et al., 2004), being also responsible for changing the color of the cooking water (Figure 1h). The three main phenolic compounds identified in both *in natura* and cooked seeds were quercetin, catechin and gallic acid. The gallic acid and quercetin contents in the cooked seeds were at least two and ten times higher to those of the *in natura* seeds, respectively (KOEHNLEIN et al., 2012).The main isomer of the tocopherol composition is α-tocopherol. Regarding phytosterols, stigmasterol and β-sitosterol represent 96% of total phytosterols in the lipid fraction (DA SILVA et al., 2016). Thirteen phenolic compounds were identified in the hydro-alcoholic extract of the pinhão seed, among which were nine proanthocyanidins (derived from catechin and epicatechin), two phenolic acids (derived from protocatechuic and ferulic acids), one flavonol (quercetin-3-O glycoside) and one flavanone (eriodictyol-O-hexoside) (DE FREITAS et al., 2017; SANTOS et al., 2018).

The seeds contain several polyphenols belonging to the flavonoid class, including catechin and epicatechin (subclass flavan-3-ol); rutin, quercetin (subclass of flavonol); and apigenin (subclass of flavone) (BRANCO et al., 2015a). The main flavonoids that have been isolated belong to the class of biflavonoids: amentoflavone, monomethyl amentoflavone, di-O-methyl amentoflavone, ginkgetin, tri-O-methyl amentoflavone, tetra-O-methyl amentoflavone, which differ according to the number and position of the methoxyl group in relation to amentoflavone molecule (MOTA et al., 2014). The biflavonoids reported in the pinhão seeds act as free radical sequestration agents and exhibit efficient protection against damage from oxidation. They are excellent option for use as antioxidants and photoprotection agents (YAMAGUCHI et al., 2005; MICHELON et al., 2012).

Functional properties of the pinhão husk and nut seed

Phenolic and polyphenol compounds are frequently detected in larger quantities in the husks than in the edible portions, a fact which accounts for the characteristic darker color of the pinhão husk. Their occurrence in the outer portion of the seed is related to their defensive role in plants (MOTA et al., 2014). The female strobilus consists of seeds (the edible part of A. angustifolia) and bracts (non-developed seeds). These bracts, which represent approximately 80% of the female strobilus, have usually no use. Catechin, epicatechin and rutin were the main phenolic compounds reported in the extract. Its extract has antioxidant activities according to *in vitro* and *in vivo* assays and extracts dilutes were non-mutagenic and avoided DNA damage induced by hydrogen peroxide in yeast cells. Highlighting that dietary intake of antioxidants could be a useful strategy to reduce the incidence of diseases associated with oxidative stress, such as cancer, atherosclerosis and neurodegenerative disorders (MICHELON et al., 2012). The extract of ground and dried bracts at 37 °C in distilled water (5%, w/v) under reflux at 100 °C for 15 min showed total phenolic content of 1586 ± 14.53 mg gallic acid equivalents 100 g^{-1}. The main phenolic compounds found were catechin (140.6 ± 2.86 mg 100 g^{-1} of bracts), epicatechin (41.3 ± 2.73 mg 100 g^{-1} of bracts), quercetin (23.2 ± 0.06 mg 100 g^{-1} of bracts) and apigenin (0.6 ± 0.06 mg 100 g^{-1} of bracts) (SOUZA et al., 2014). This stratum scavenged DPPH radicals and exhibited potent action on superoxide dismutase and catalase activities, including significantly protecting MRC5 cells against H_2O_2-induced mortality and oxidative damage to lipids, proteins and DNA.

The tannin content is responsible for the color of the pinhão husk and possesses activity that inhibits the human pancreatic and salivary α-amylase. The inhibition of the α-amylase results in a delay in the digestion of carbohydrates and absorption of glucose, with a lessening of postprandial hyperglycemic excursions (SILVA et al., 2014), as is the case with acarbose therapy, which is first-line treatment for newly diagnosed patients with type 2 diabetes (LAUBE, 2002). This suggested that the tannin of the husk may be used to eliminate postprandial hyperglycemia in patients with diabetes (SILVA et al., 2014). Regarding pancreatic lipase, the inhibition by the husk extract was achieved by means of a non-competitive parabolic mechanism. The levels of triglycerides in the plasma of rats were reduced after the administration of husk extract (OLIVEIRA et al., 2015). This report was reinforced by the study that reported that a diet of a suspension formulation of husk nanofibril is able to reduce cholesterol and triglyceride levels in rats (LIMA et al., 2020).

Use and potential industrial application of the pine nut seed and its husk

The direct commercialization of seed is essentially associated with a low level of industrialization (ZORTÉA-GUIDOLIN et al., 2017b). However, more studies can reverse this phenomenon. The flour of pine nut seed of the *Araucaria angustifolia* and *Araucaria araucana* are used to prepare traditional dishes and sweets such as "alfajores" (Table 2), which proves that its industrial use is already a reality. *Araucaria angustifolia* has a higher starch content and lower fiber content than *Araucaria araucana,* with the first species showing greater digestibility (CONFORTI & LUPANO, 2008). Pinhão flour can replace up to 50% of the rice flour used to produce gluten-free cakes, essential for people with celiac disease. This reduces cake firmness, but formulations with substitution between 25 and 37.5% produce a specialty product with greater global acceptance (IKEDA et al., 2018). Gluten-free breads can also be produced with floury mixtures of pinhão associated with: a. potato and buckwheat starch, b. potato starch, or c. potato starch and rice flour. Compared with the traditionally wheat flour product, sensory analysis of gluten-free breads revealed that the last two formulations (b and c) are

Table 2 - Some potential industrial use of different parts of the structure of the pinhão of the Paraná pine recently reported in relevant scientific journals.

Application	Material	Reference	Journal
Flour used in commercial product	*Pinhão* seed	CONFORTI & LUPANO, 2008	Starch-Starke
Gluten-free cakes	*Pinhão* seed	IKEDA et al., 2018	Ciência Rural
Gluten-free breads	*Pinhão* seed	POLET et al., 2019	Journal of Culinary science & Technology
Extruded foods	Pinhão seed	ZORTÉA-GUIDOLIN et al., 2017b	Journal of Food Science
Starch extraction and pharmaceutical excipient	*Pinhão* seed	DAUDT et al., 2014	Industrial Crops and Products
Modified starch nanoparticles	*Pinhão* seed	GONÇALVES et al., 2014	LWT - Food Science and Technology
Cereal bar using 20% of pine nut seed	*Pinhão* seed	CONTO et al., 2015	Chemical Engineering Transactions
Edible films of seed flour reinforced or not with husk powder	*Pinhão* seed and husk	DAUDT et al., 2017	Food Hydrocolloids
Nanosuspension applied to cereal bars	*Pinhão* husk	TIMM et al., 2020	Journal of Food Processing and Preservation
Broad spectrum of antimicrobial activity	*Pinhão* husk	TROJAIKE et al., 2019	Food and Bioprocess Technology
Composite for food packaging	*Pinhão* husk	ENGEL et al., 2020	Journal of Polymers and the Environment
Nanosuspension for therapeutic feeding	*Pinhão* husk	LIMA et al., 2020	Food & Function
Extract encapsulated in electrospun starch fibers	*Pinhão* husk	FONSECA et al., 2020	Food Biophysics

promising alternatives to replace traditional gluten-free bread (POLET et al., 2019). Pinhão flour can be extruded allowing the production of extruded foods with good expansion, texture properties and sensory acceptance. Extrusion depends on moisture content, screw speed and heating temperature to obtain a marketable product. The resistant starch contents is almost reduced to zero after extrusion cooking while the slowly digestible starch content is increased (ZORTÉA et al., 2017b). Noting that extrusion reduced the levels of trypsin, chymotrypsin, α-amylase inhibitors and hemagglutinating activity without modifying the protein content of fever and beans. In this sense, it is expected that this heat treatment will improve the digestion of protein and starch in the pine nut seed as well (ALONSO, R., et al., 2000).

The pine nut seed and husk of the pinhão or its components can be applied directly to create new foods and other consumer goods (Table 2). Sensory tests (appearance, aroma, flavor, texture and overall acceptance, and purchase intent) of cereal bars with crystal sugar/glucose syrup, rice flakes and oat bran, with or without replacement of the latter by up to 20% of dehydrated pine nut seed showed good scores. Also, oat bran replacements up to 10% and the proportion of crystal sugar up to 50% perform better. The food rich in fiber and which contributes to the preservation of *Araucaria angustifolia* can be a successful commercial appeal (CONTO et al., 2015). Active packages can be made incorporating functional ingredients into edible films and coatings. The aqueous solution of pinhão flour (5%) and glycerol (1.5%) can be used to produce edible films when dried at room temperature. The addition of pinhão husk flour (0.5, 1.0, 1.5, 2.0 and 2.5%) increases the thickness, apparent porosity, roughness, hydrophilicity, permeability to water vapor , the content of total soluble phenols, the antioxidant capacity, the Young's modulus and the content of dietary fiber (mainly insoluble fiber) and leaves or intensifies the reddish-yellowish color. Conversely, addition decreases clarity and elongation at break. Thus, a wide variety of films can be produced and applied to specific situations (DAUDT et al., 2017).

Starch is the main component of pinhão nut seed (about 72% on a dry basis) and can be easily isolated by water treatment under mild conditions (CORDENUNSI et al., 2004). The starch extraction yield in raw pinhão (94.53%) is higher than if it is cooked (73.84%) (Table 2). Crude pinhão starch granules are more homogeneous in size and have a narrow size distribution. They are also more rounded, have a lower gelatinization temperature, more neutral pH and lower moisture content than cornstarch. Cooked pinhão starch is irregular, more variable in size, brownish in color, presence of phenolic compounds, amorphous, flowable and poorly soluble. The physicochemical and morphological characteristics explored preliminarily in the present study showed the applicability of crude pinhão starch as a pharmaceutical excipient (DAUDT et al., 2014). Native starch (NA) from pine nut seed can be produced with aqueous extraction and spray-drier dried. Starch is a homopolysaccharide of D-glucose units composed by amylose (glycosidic α-1,4 bonds) and amylopectin (glycosidic α-1,4 and α-1,6 bonds) chains. The treatment of NA particles (15.34 µm) by ultrasound (UA) produces nanoparticles of 453 nm, while the treatment by acid hydrolysis (AA) produces much smaller particles (22 nm). Furthermore, AA has a lower amylose fraction, is more soluble, is more hygroscopic and forms a more clarified paste than the other two types. These kinds of nanoparticles can be useful for development of novel composites with special properties to be employed as coating materials or films (GONÇALVES et al., 2014).

The pinhão shell can be applied in the production of consecrated foods, although the raw shell has an astringent taste and can make it difficult to accept (Table 2). CONTO et al. (2015) developed a cereal bar using pine nut seed and the results indicated that the formation with 40% pine nut seed was the best formulation according to sensory analysis. The high acceptance rate obtained reflects a great purchase potential of this product, in addition to the nutritional appeal because it contains high amounts of fiber. Despite this, cereal bars were developed with its nanosuspension to minimize astringency and as a potential binding agent. This bar has greater strength, despite the low contribution of nanosuspension as a binder. Conversely, this additive contributes positively to uniformity, texture, crispness, color and shine, but without harming the flavor of the product without this additive. Therefore, the addition of this nanosuspension is recommended for the production of functional cereal bars due to the high content of fiber, protein and phenolic compounds (TIMM et al., 2020). The pinhão husk extract can be encapsulated (62 to 100%) in electrospun starch fibers, producing an interaction of the components that leaves the fibers with better morphology, makes the extract components more stable, and provides high levels of total phenolic compounds (225.32 $\mu g \cdot g^{-1}$) and catechin/epicatechin and catechin dimer. Still, the starch fibers with added antioxidant activity, and *in vitro* release were revealed to be dependent on the content used. Thus, this biodegradable nanomaterial can be applicable as an antioxidant agent in the food industry (FONSECA et al., 2020). Additively, the aqueous extract of pinhão shell presents antibacterial activity against important bacteria of food origin and its combination with thermal processing can be an interesting tool to be used in food preservation. For example, the concentration of 10 kg m^{-3} has antimicrobial activity against a broad spectrum of bacteria and fungi, and its synergism with heat treatment against Listeria monocytogenes at temperatures between 55 and 70 °C (TROJAIKE et al., 2019).

The pinhão shell can be used to produce a nanosuspension after bleaching treatment or not (Table 2). The nanoformulation added to the rat's daily diet reduced cholesterol and triglyceride levels, as well as causing body weight gain, but without showing toxicity effects at a histopathological level. Nanofibrils have antioxidant activity and high levels of phenols and sterols, but these are removed by bleaching. Also; although, the nanoformulation incorporates the polyphenols from the tegument and beneficial effects have been reported, such as antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (HAN et al., 2017), its incorporation into the developed food source has not been proven. Therefore, the positive impact was attributed to the dietary fibers provided (LIMA et al., 2020). The pinhão shell can also be used in the production of composites based on cassava starch through a thermocompression process for the manufacture of environmentally sustainable single-use (unidirectional) packaging. For example, this composite can be used in active packaging of food products with low moisture content in some specific situations, such as the transport of chips to avoid mechanical impacts (ENGEL et al., 2020).

The pinhão seed can be used in other industries, such as pharmaceuticals (Table 2). For example, starch has properties suitable for use as a pharmaceutical excipient (DAUDT et al., 2014). The pinhão husks contain condensed tannins that function as antioxidants and exhibit also antimutagenic and antigenotoxic functions against hydrogen peroxide (BRANCO et al., 2015b; MICHELON et al., 2012), as well as anticarcinogenic and antimicrobial properties (SOUZA et al., 2014). Regarding the use of pinhão in the pharmaceutical industry, starch and pinhão bark extract can also be used as raw materials for cosmetic gel and emulgel formulations (DAUDT et al., 2015). There is rheological stability for pH between 6.17 and 6.37, that is, within the demand range (pH 4.5 to 7.5), and at storage temperatures (CASTELI et al., 2008; LEONARDI et al., 2002). Finally, the formulation with pinhão starch showed greater spreadability on the skin, lower viscosity, better sensation and less perception on the skin 5 min after application (DAUDT et al., 2015). Bark, bracts and the skin of the cooked pine nut also have components with potential application as raw material in the pharmaceutical and cosmetic industries (PERALTA et al., 2016)

Additionally, the aqueous solution of the pinhão husk may be used as an alternative for promoting adsorption of metallic ions and colorants in the treatment of industrial effluents from both regular and metallurgy industries (LIMA et al., 2008; CALVETE et al., 2010). The action occurs due to the presence of tannins, the compounds that are mainly responsible for the adsorption of metallic ions (LIMA et al., 2007).

CONCLUSION

The Brazilian pine tree, or *Araucaria angustifolia* or "Pinheiro do Paraná", also occurs in Argentina and Paraguay. Araucaria is the name of its forest and it was intensively used, leaving it in a state of extinction risk. The bibliographic study revealed that the scientific research of this Brazilian pine and its seed has been intensified and extended by an international scientific network. Regarding the area of food science and technology, the use of its seed for the production of consumer goods can value it as food and pharmaceutical inputs and also as a component of effluent treatment. The pine nut seed from its seed is rich in starch with a low glycemic response. This part of the seed can be used as flour for the production of gluten-free foods, such as cookies, cake, bread, extruded foods, among others. Its starch can be easily extracted from the raw seed, which is difficult if the seed is cooked, and it can be modified to open up new opportunities for food use and as a pharmaceutical ingredient. The cooked seed is consumed directly or in the preparation of various traditional dishes by the population of the region. Cooking causes the formation of resistant starch and the migration of compounds from the husk to the seed, which is beneficial for human health. Thus, the incorporation of husk flour in food, such as a cereal bar, provides bioactive compounds. The fibers or bark extract can also be applied in smart packaging with potential antimicrobial capacity, which reveals the potential for innovative, disruptive and environmentally sustainable applications. It even values when this residue is generated in the production of pinhão starch flour. In addition, unformed pine nut seed (bracts), parts of the cone filling and seed husk residues can have the same application, as well as compose an effluent treatment input to remove cations. Thus, it is noteworthy that the seed is a source of functional compounds, such as resistant starch and substances that reduce the efficiency of α-amylase that minimize the glycemic peak, as well as containing substances with antioxidant capacity and, reducing the level of cholesterol, controlling glycaemia, stimulates body mass gain and with a photoprotection agent. Finally, it is expected that these new possibilities of demand for seeds will contribute to the economic development of the population involved with their use and consequently to the sustainable perpetuation of the species.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript and critically revised the manuscript approving the final version.

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