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Innovative formulation containing tocotrienol: A proposal for a food supplement

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ABSTRACT
The global population aging trend has been studied for more than 30 years. Data indicate that the Brazilian population of elderly will be larger than that of young people by the year 2050. In the United States this phenomenon has also been observed. The human biological system most affected with aging is the central nervous system, resulting in the decrease of neurons and loss of nerve conduction velocity, limitations in motor areas and incidence of pathological events related to advancing age. In this context, science has pointed out the effectiveness of the consumption of Vitamin E and its isoforms (tocotrienols) in neuroprotection, being effective in the prevention and treatment of senile diseases. Thus, the present work aims to propose an innovative formulation containing tocotrienol of annatto (Bixa orellana) to be consumed as a food supplement. Through a bibliographic survey and online market research, the supplements containing tocotrienol available to the consumer were evaluated. These supplements, for the most part, are presented for consumption in the form of soft gel. From this study, it was possible to define a proposal for a theoretical formulation in the form of an orodispersible tablet, which was tested in the pharmacotechnical laboratories of the Biopark, enabling the elaboration of a prototype of an innovative product. As a result, it was possible to obtain samples of the product with the possibility of being marketed in the American market, where dietary supplements are sold freely in supermarkets and pharmacies.

Keywords: Pharmaceutical Technology, Research, Pharmacy, Pharmaceutical Research.

1 INTRODUCTION

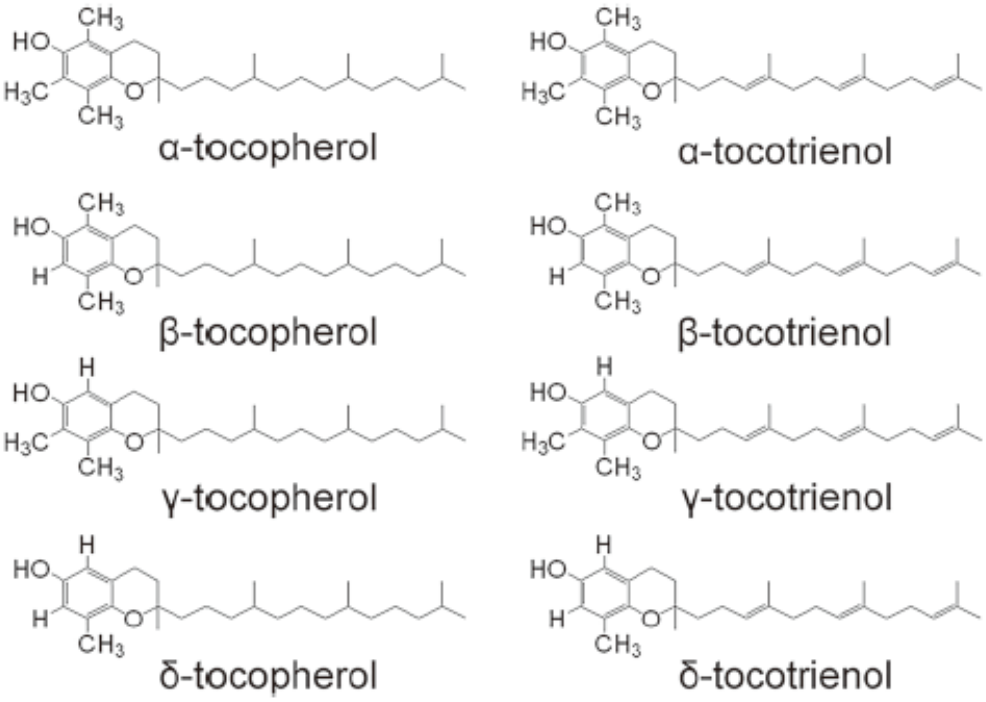
Scholars for nearly 30 years have been observing the aging population as a demographic revolution that has manifested itself at the global level. Especially in emerging countries, a reduction in fertility and mortality rates is perceived, resulting in older population groups and with very advanced individuals (KALACHE, VERAS, RAMOS, 1987). For example, the Brazilian Institute of Geography and Statistics (IBGE) (2018) conducted a study between 2012 and 2017 that demonstrates the population increase of the elderly group, which reached an approximate number of 30.2 million people. Estimates indicate that in 2050 the group of young people will be lower than that of the elderly in Brazil.

As a characteristic of the human species, the body begins the aging process naturally after reproductive age. The human body is affected gradually, presenting cumulative effects that vary in their severity according to biological factors and the influences of the individual's lifestyle. Thus, the elderly person becomes prone to present diseases of a physical and mental nature (CHAGAS, ROCHA, 2012). As the body ages, changes in the nervous system interfere with the individual, resulting in reduced brain function, changes in the activities of neurotransmitters and nerve cells, accumulation of toxins, manifestations arising from genetic changes, decreased blood supply and reduction of nerve cells (MAIESE, 2021).

Therefore, authorities and scientists have been concerned about the health of the elderly population, seeking an increase in life expectancy, and envisioning how this population will reach the end of life (IBGE, 2018). Thus, the scientific community has been studying the theme of aging to obtain treatments for the ills involving the central nervous system (CNS) and cognitive improvement (FUKUI, 2019). In this context, foods can contribute to the state of disposition, behavior and health of the population thanks to the functional properties they present. The neuroprotective activity of some substances has shown promising results in recent scientific research, demonstrating actions that directly impact on metabolic changes that are, or maybe, responsible for the onset of diseases of the central nervous system (GUEDES, MATIAS, 2021).

Scientifically highlighted, the medicinal substances discovered in the seed of annatto (*Bixa orellana*) can be mentioned, which have been studied and may contribute to the increase of the culture of this plant in Brazil. In the seeds of annatto are found high dosages of tocotrienols (CARVALHO, 2020). This group of compounds is part of the vitamin E family, as can be seen in Figure 1, and is relevant for its ability to prevent or treat several diseases associated with aging (MALAVOLTA et al., 2018; PLA, 2018). Thus, authors, such as Fukui (2019), Gopalan et al., (2014) and Mbachiantim (2021), studied tocotrienol and highlighted it as a potent neuroprotective agent, with chemical structures very similar to those of tocopherols.

FIGURE 1 - Chemical structure of tocopherols and tocotrienols



Source: Fukui, 2019.

Tocotrienols manifest themselves differently in the body, acting as a neuroprotector and inducer of the elimination of damaged cells of the nervous system, which represents advantages compared to tocopherols (FUKUI, 2019). In this same line of reasoning, Plá (2018) presents a relationship between the consumption of tocotrienols and the mechanisms described as neuroprotective in already published works.

The first studies proved the neuroprotective action of tocotrienol from cellular and animal models. However, Gopalan et al. (2014) published a study considering the experience conducted with 121 volunteers aged 35 years or older who had white matter lesions which were confirmed by magnetic resonance imaging. Those studied were randomly assigned to receive 200 mg of mixed tocotrienol twice a day, and the result of the study showed that patients who received the product showed attenuation in white matter lesions.

Following this aspect, it is possible to verify, through an online search, that there are already products being marketed in the North American market that contain tocotrienols as a bioactive substance. Most of the form presented is in soft gelatin capsules or *softgels*.

The supplement market is interesting if we consider that the consumption of these products increased by 48% in Brazil in 2021, covering 59% of the country's households (BAIMA, 2022). In addition, another study demonstrated that the consumption of plants and herbal medicines increased by 49% during the period of the COVID-19 pandemic in Brazil (BRAGA, DA SILVA, 2021). On the other hand, in the United States this consumption reaches 80% of the adult population habitually. Even

so, forecasts point to a 50% growth in consumption in five years. This trend is also reflected globally with estimated sales of 252 billion dollars in 2025 (BAIMA, 2022).

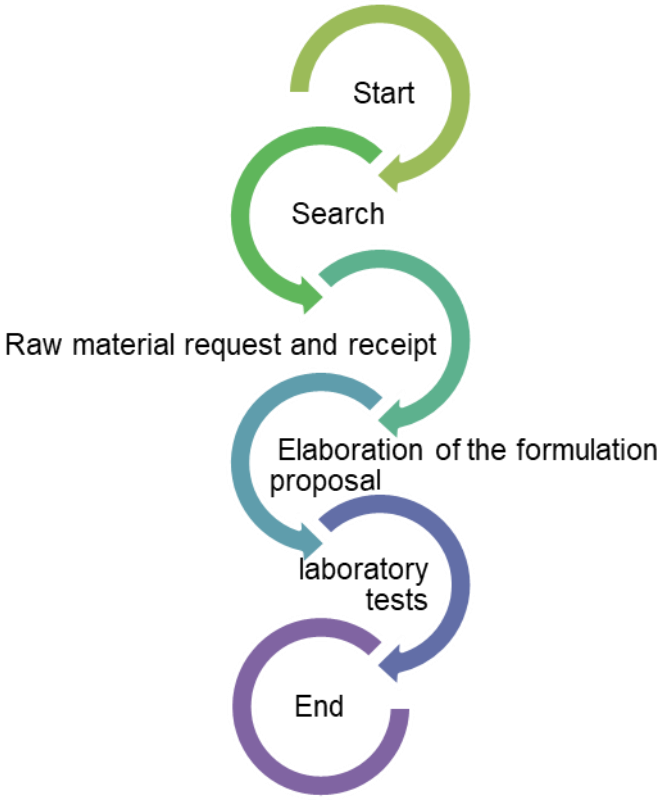
It is worth mentioning that dietary supplements follow specific regulations in each country. In Brazil, normative instruction 28 of July 27, 2018 presents the list of constituents and their respective limits of use, permitted claims and information on complementary labeling for dietary supplements (MINISTRY OF HEALTH, 2018). However, in this list of permitted bioactive substances, tocotrienol is not included. It is important to note that the company New Max Industrial LTDA has been working to insert this input in this list since 2021 (FERREIRA, CARVALHO, CARVALHO, 2021). When it comes to the U.S. market, which is regulated by the U.S. Food and Drug Administration (FDA), tocotrienol is already considered a foodstuff. The FDA considers safe the use of tocotrienol derived from annatto (*Bixa orellana*) by humans (FERREIRA, CARVALHO, CARVALHO, 2021). About the final product, according to the guidelines of this agency, the producer is responsible for the information described in the labeling, the ingredients of the formulation, safety and accuracy of the facts and does not require that chemical analyses be performed on the product, since the supplements do not have the purpose of diagnosing, treating, curing or preventing diseases. All product information must be on the label (BAIMA, 2022).

Thus, the present work aims to develop a proposal for an innovative formulation of a food supplement with tocotrienol from annatto (*Bixa orellana*) and is justified by the care to the elderly population that tends to increase, as cited by statistics. The tested form will be an orodispersive tablet aiming at the ease of swallowing the product and the use of tocotrienol whose studies have shown high benefits in the treatment and protection of the CNS.

2 MATERIALS AND METHODS

The activities planned for the elaboration of the proposal for the formulation of a food supplement containing tocotrienol are described in Figure 2.

Figure 2 - Flowchart of activities related to the proposed formulation of the food supplement



Source: Own elaboration, 2023.

The studies used to obtain data related to research on tocotrienol, concentration, studies on neuroprotective effects, toxicological data, safety and efficacy were selected from a bibliographic research with literature review, and the first stage of the methodological referral is shown in Figure 2. Books, scientific publications and technical materials available on the *web* pages Google Scholar, Scientific Electronic Library Online (SciELO), Pubmed and website of the company sponsoring tocotrienol, New Max Industrial LTDA., were searched. The descriptors used were "tocotrienol", "neuroprotective," and "*neuroprotective*," being performed between March 2022 and March 2023. The approximate results were 200 thousand articles and papers, which were submitted to the exclusion and inclusion criteria. As exclusion criteria, only studies published after 2010 that presented research containing tocotrienol as a neuroprotective agent were maintained. As inclusion criteria, the works that presented clinical research data and bibliographic reviews in Portuguese, English, and Spanish were maintained. The selection of articles was performed by evaluating the title and abstract and resulted in the full reading and use of eight works that were used in the theoretical basis for the theme of tocotrienol.

From this selection and research, we advanced to the next stage, as shown in Figure 2. A sample of the product Naturalmax Tocotrienol do Urucum (Powder) 30% and the additional inputs were requested to start the formulation tests at the level of academic bench in the laboratories of Biopark Education to obtain a prototype of the food supplement. The company New Max Industrial LTDA.,

headquartered in Americana – S.P. (Brazil) sponsored the bioactive compound Naturalmax. The other ingredients were provided by the university.

Regarding the regulatory basis, the research and reading of the norms established by the FDA, Anvisa and other bodies recognized by global regulatory agencies were carried out. Even so, the present study was carried out with a main focus on American regulations since, as already presented in the introduction chapter of the present study, the American standard allows the researcher to have the freedom to explore his technical knowledge, the consumption habits of dietary supplements by the North American population and the fact that the use of tocotrienol is already authorized as a food supplement by the regulations of that country. Therefore, it is possible to expect that the proposed formulation can be commercialized in the United States after its development and transposition of scale for industrial purposes.

3 RESULTS AND DISCUSSION

The theoretical research carried out served as an important source of information for the beginning of the development of this project, bringing truthful data on the amount of the bioactive compound to be used, determination of the limits of safety and efficacy of the consumption of tocotrienol as an option in the treatment of diseases related to brain degeneration. The main work reporting the study in humans, dated 2014 and written by Golapan et al, brings conclusions about the daily use of 200 mg of tocotrienols mix for two years, proving to be safe and effective in the treatment of brain injury. In addition, Plá (2018) contributes to these data by presenting his literature review highlighting data from five in *vitro and in* vivo *studies with tocotrienols demonstrating safety and efficacy in neuroprotection and considering testing at high concentrations*. Other authors such as Xia, Mo (2015) and Malavolta et al., (2018) have also studied the neuroprotective actions of tocotrienol, all of which concluded that the compound shows evidence of efficacy. Finally, it can be noted that dietary supplements containing tocotrienol are freely marketed in the North American market. The concentrations of these products vary between 50 and 125 mg of tocotrienol, being below the range tested by the authors. Thus, the present work presents the formulation test considering the use of 125 mg of Naturalmax Tocotrienol do Urucum (Powder) 30% as the concentration used in the tests performed in the laboratory.

From the definition of the amount of the bioactive compound to be used, a sample of the product Naturalmax Tocotrienol do Urucum (Powder) 30% was requested. The sponsoring company New Max Industrial Ltd. sent the sample in February 2023, which was subsequently used.

Since the objective of the present work was to develop a proposal for an innovative formulation, the excipients that make up dispersive tablets and that are marketed in Brazil were researched and analyzed. The study of these ingredients was carried out having as main guide the *Handbook of*

Pharmaceutical Excipients, 6th Edition *(2009), and the* parameters evaluated were the physical, chemical, and physicochemical properties, functional category, applications of the input in pharmaceutical and food formulations, description, incompatibility, safety, precautions and comments. Thus, the use of the inputs described in Table 1 was defined.

TABLE 1 - Inputs, their functions and recommended amounts for use in pharmaceutical or food formulations, according to the *Handbook of Pharmaceutical Excipients, 6th Edition,* 2009

INGREDIENT	FUNCTION	% RECOMMENDED FOR USE
Mannitol	Diluent	10 - 90
Crospovidone	Dismemberment	2 - 5
Microcrystalline cellulose	Diluent/disintegrating	5 - 90
Sorbitol	Diluent/sweetening agent	25 - 90
Sucralose	Sweetening agent	0,03 - 0,24
Magnesium stearate	Sliding/lubricant	0,25 - 5
Vanilla aroma	Flavoring	Not defined
Aroma of solid orange	Flavoring	Not Defined

Source: Own elaboration, 2023.

All ingredients were tested on the bench of the pharmaceutical technology laboratory of Biopark, considering that the formulation proposal should present reproducibility conditions on an industrial scale. Therefore, between November 2022 and March 2023, five cycles of bench tests were carried out, in which the parameters of mixture, humidity, average weight of the tablets, hardness, thickness, diameter, friability and disintegration were evaluated.

The parameters and the determination of specifications for the tablets were determined from the evaluation of guides and official compendia made available by the regulatory agencies whose application covers the areas of dietary supplements and medicines, also serving to meet the in-process control evaluations. The selected tests are presented in Chart 1.

TABLE 1 - List of parameters and specifications selected for laboratory bench tests using tocotrienol

TEST	SPECIFICATION	REFERENCE	PURPOSE
Appearance	Circular, non-grooved, light yellow tablet	NSF; DL	CQ
Middleweight	300 mg ±5%	FB	CP
Hardness	≥ 3 N	NSF; FB	CQ
Diameter	10 mm ± 5%	DL	CP
Thickness	4,10 mm ± 5%	DL	CP
Friabilidade	Loss ≤ 1.5% of your weight	NSF; FB	CQ
Disintegration	< 3 minutes	NSF; FB	CQ

Source: Own elaboration, 2023.
Note: DL - Local Development; F.B. - Brazilian Pharmacopoeia; C.P. - Control in Process; C.Q. - Quality Control.

Another guide consulted was the *Stability Testing Guideline for Dietary Supplements* (2011), which suggests a list of trials that differ according to each type of pharmaceutical form. From this guide, the appearance, hardness, friability and disintegration tests for tablets were selected. Other

parameters that define the specifications of the bench tests performed followed the Brazilian Pharmacopoeia, 6th edition, volume 1 (2012). On the other hand, the researchers were responsible for defining the specification for the appearance test, considering that this test should describe the characteristics of the product elaborated and tooling used. Thus, the specifications for the diameter and thickness evaluations were also defined, which are performed only as in-process control, since they are related to the tooling used in the powder compression equipment.

To know the characteristics of the inputs, the first three bench tests were performed. These tests also served to define the quantities of each of the inputs and understand the process of mixing and compressing the powders. The set of punctures chosen for these tests was the one that uses the 10mm matrix. The average weight of the tablets was defined at 300 mg, considering the use of the amount of 60 mg of Naturalmax Tocotrienol do Urucum (Powder) 30%. The mean of the results obtained in each test can be seen in Table 2.

TABLE 2 - Comparison of the mean of the results obtained in the formulation tests using the compression matrix of size 10mm

COMPARISON OF RESULTS						
TEST	**MIDDLEWEIGHT**	**HARDNESS**	**THICKNESS**	**DIAMETER**	**FRITABILITY**	**DISINTEGRATION**
Test 1	301 mg	1.60 N	4,16 mm	10,11 mm	0,01%	47”
Test 2	302 mg	3.10 N	4,20 mm	10,10 mm	0,01%	32’’
Test 3	312 mg	1.35 N	4,30 mm	10,13 mm	0,01%	1'10"

Source: Own elaboration, 2023.

To achieve the stipulated goal of producing a food supplement with 125 mg of the bioactive compound Naturalmax Tocotrienol do Urucum (Powder) 30% and improve the formulation, two more bench tests were performed. The compression matrix was modified to 12 mm size, increasing the size and weight of the tablets to 500 mg. In this context, the parameters defined for in-process control were reconsidered: average weight, thickness and diameter, as shown in Chart 2.

TABLE 2 – New parameters and specifications defined, due to the test of increase in the concentration of the bioactive compound

TEST	**SPECIFICATION**	**REFERENCE**	**PURPOSE**
Middleweight	500 mg ± 5%	FB	CP
Diameter	12 mm ± 5%	DL	CP
Thickness	4.50 mm ± 5%	DL	CP

Source: Own elaboration, 2023.
Note: DL - Local Development; F.B. - Brazilian Pharmacopoeia; C.P. - Control in Process.

The mean of the results obtained after evaluation of the tablets elaborated in tests 4 and 5 are presented in Table 3.

TABLE 3 - Comparison of the mean of the results obtained in the concentration increase tests, using the compression matrix of size 12mm

COMPARISON OF RESULTS						
TEST	**MIDDLEWEIGHT**	**HARDNESS**	**THICKNESS**	**DIAMETER**	**FRITABILITY**	**DISINTEGRATION**
Test 4	498 mg	1.60 N	4,76 mm	12,15 mm	0,17%	2’04”
Test 5	497 mg	3.9 N	4,88 mm	12,11 mm	0,01%	1'00"

Source: Own elaboration, 2023.

The tablets produced in test number 5 met the new specifications determined for the prototype of the proposed food supplement. After compiling the results of the process control and quality control tests, the tablets were approved and can be seen in Figure 3.

FIGURE 3 - Tablets obtained in test 5

Source: Own elaboration, 2023.

Thus, the tests were finalized and the composition of ingredients used in the bench test number 5 was defined as the best combination for the tablet composed of tocotrienol.

4 CONCLUSION

Health experts have shown great concern regarding the care to be taken with the elderly population in the face of global trends of population aging and consequent increase in senile diseases, which generates the need to develop proposals focused on health treatments in the chemical and natural spheres. Given this scenario, the present project met the objective of elaborating a proposal for the formulation of a food supplement with the compound tocotrienol of annatto (*Bixa orellana*). This study resulted in the development of a prototype of a pleasant-tasting orodispersive tablet that can be used as a form of neuroprotection. The product followed the guides and compendia available in the year 2023 and, therefore, can only be marketed in the North American market at this first moment. However, after being regularized by Anvisa it can be sold in Brazil.

It is important to highlight that, if there is interest of the industry in commercializing the product, the proposal described here will need to be retested and receive the necessary adjustments aimed at industrial production, including performing stability studies to meet the requirements of the regulatory agency of the country intended for commercialization, in addition to developing the analytical method to attest to the final quality of the finished product and the realization of the financial feasibility study for the composition of the selling price.

The inclusion of a product composed of annatto (*Bixa orellana*) in the form of an orodispersive tablet represents an innovative product, probably capable of generating an excellent financial return for the producing industry since market research has shown that the products currently marketed are presented in the soft gel pharmaceutical form.

REFERENCES

Agência Nacional de Vigilância Sanitária - ANVISA. Farmacopeia Brasileira, 6ª edição. Volume I. Brasília. 2012.

BAIMA, C. A complicada ética dos suplementos alimentares. Revista questão de ciência. Maio de 2022. Disponível em: https://www.revistaquestaodeciencia.com.br/artigo/2022/05/16/complicada-etica-dos-suplementos-alimentares. Acesso em: 22 ago. 2022.

BRAGA, J. C. B., & DA SILVA, L. R. (2021). Consumo de plantas medicinais e fitoterápicos no Brasil: perfil de consumidores e sua relação com a pandemia de COVID-19 / Consumption of medicinal plants and herbal medicines in Brazil: consumer profile and its relationship with the COVID-19 pandemic. Brazilian Journal of Health Review, *4*(1). https://doi.org/10.34119/bjhrv4n1-303

CARVALHO, P. R. N. Urucum: uma semente com a história do Brasil. 1. ed. Campinas, SP: Evidência.B.R., 2020.

CHAGAS, A. M.; ROCHA, E. D. Aspectos fisiológicos do envelhecimento e contribuição da Odontologia na saúde do idoso. Rev. Bras. Odontol. Vol.69, no.1. Rio de Janeiro. Jan./Jun. 2012.

FERREIRA, T. S.; CARVALHO, P. R. N.; CARVALHO, A. L. Risk assessment of tocotrienols from anatto (Bixa orellana L.): regulatory contributions. Pharmaceut Reg Affairs, Volume 10:7, 2021.

FUKUI, K. Neuroprotective and anti-obesity effects of tocotrienols. Journal of Science Vitaminol, 65, S185 – S187, 2019.

GOPALAN, Y.; SHUAIB, I. L.; MAGOSSO, E.; ANSARI, M. A.; BAKAR, M. R. A.; WONG, J. W.; KHAN, N. A. K.; LIONG, W. C.; SUNDRAM, K.; HONG, N. G. B.; KARUTHAN, C.; YUEN, K. H. Clinical investigation of the protective effects of palm vitamin E tocotrienols on brain white matter. Stroke Journal of the American Heart Association. 2014;45:1422-1428. CONS CALIFORNIA DIG LIB. DOI: 10.1161/STROKEAHA.113.004449.

GUEDES, M. O.; MATIAS, A. G. C. Neuronutrition in the perspective of the prevention and treatment of depressive disorder / Neuronutrição na perspectiva da prevenção e tratamento da desordem depressiva. Brazilian Journal of Health Review. Curitiba, v.4, n.3, p. 12434-12450 may./jun. 2021. DOI:10.34119/bjhrv4n3-217.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA ESTATÍSTICA. Pesquisa Nacional por Amostra de Domicílios Contínua. Rio de Janeiro: IBGE, 2018. Disponível em: https://agenciadenoticias.ibge.gov.br/agencia-noticias/2012-agencia-de-noticias/noticias/20980-numero-de-idosos-cresce-18-em-5-anos-e-ultrapassa-30-milhoes-em-2017. Acesso em: 22 jul. 2022.

KALACHE, A.; VERAS, R. P.; RAMOS, L. R. O envelhecimento da população mundial: um novo desafio. Revista de Saúde Pública. 21(3) 200-10. 1987. Disponível em: https://www.scielo.br/j/rsp/a/RRbSJj3PsLtCXyLPqzTJh6Q/?lang=pt. Acesso em: 25/07/2022.

MAIESE, K. Efeitos do envelhecimento sobre o sistema nervoso. Manual DSM Versão Saúde para a Família. 2021. Disponível em: https://www.msdmanuals.com/pt-br/casa/dist%C3%BArbios-cerebrais,-da-medula-espinal-e-dos-nervos/biologia-do-sistema-nervoso/efeitos-do-envelhecimento-sobre-o-sistema-nervoso. Acesso em 29 ago. 2022.

MALAVOLTA, M; PIERPAOLI, E.; GIACCONI, R.; BASSO, A.; CARDELLI, M.; PIACENZA, F.; PROVIANCIALI, M. Anti-inflamatory activity of tocotrienols in age-related pathologies: a SASPected involvement of cellular senescence. Biologicals Procedures Online (2018) 20:22.

MBACHIANTIM, J. M.; JOHNSON, N. C.; OGBAMGBA, V. M. Vitamin E: the “magic bullet” for neuro-protection. European Journal of Science, Innovation and Technology. Vol. 1. No. 6. P. 78. 2021.

Ministério da Saúde. Agência Nacional de Vigilância Sanitária - ANVISA. INSTRUÇÃO NORMATIVA - IN N° 28, de 26 de julho de 2018. Estabelece as listas de constituintes, de limites de uso, de alegações e de rotulagem complementar dos suplementos alimentares.

NSF Stability Testing Working Group. Stability Testing Guideline for Dietary Supplements Final Draft – January 2011.

PLÁ, A. C. P. Mecanismos de neuroproteção dos tocotrienóis: revisão de literatura. Universidade de Brasília, Faculdade de Ciências da Saúde, Graduação em Nutrição. Brasília. 2018.

ROWE, R.C.; SHESKEY, P. J.; QUINN, M. E. Handbook of pharmaceutical excipients. APhA. Pharmaceutical Press. London - U.K. 6th Edition, 2009.

Xia, W.; Mo, H. Potential of tocotrienols in the prevention and therapy of alzheimer’s disease. The Journal of Nutritional Biochemistry (2015). Doi: 10.1016/j.jnutbio.2015.10.011.