Research, Society and Development, v. 9, n. 10, e6059108996, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8996

N$^{\alpha}$-lauroyl-l-arginine ethyl ester monohydrochloride, an antimicrobial agent and its use: a review

N$^{\alpha}$-lauril-l-arginina etil éster monoclorohidrato, um agente antimicrobiano e seu uso: uma revisão

N$^{\alpha}$-lauroyl-l-arginina etilo ester monohidrocloruro, un agente antimicrobiano y su utilizacíon: una revisión

Received: 10/02/2020 | Reviewed: 10/05/2020 | Accept: 10/08/2020 | Published: 10/10/2020

Joyce Fagundes Gomes Motta
ORCID: https://orcid.org/0000-0001-8591-7472
Universidade Federal Rural do Rio de Janeiro, Brasil
E-mail: joycefgmotta@hotmail.com
Regiane Ribeiro-Santos
ORCID: https://orcid.org/0000-0002-2424-9447
Instituto Federal de Pernambuco, Brasil
E-mail: regiane.ribeiro@vitoria.ifpe.edu.br
Maria Clara Guimarães
ORCID: https://orcid.org/0000-0002-5512-1163
Universidade Federal Rural do Rio de Janeiro, Brasil
E-mail: mcguimaraes@id.uff.br
Lívia de Aquino Garcia Moura
ORCID: https://orcid.org/0000-0001-9905-1059
Universidade Federal Rural do Rio de Janeiro, Brasil
Universidade Federal Fluminense, Brasil
E-mail: livia.lag@gmail.com
Letícia Vitorazi
ORCID: https://orcid.org/0000-0002-6626-9016
Universidade Federal Fluminense, Brasil
E-mail: leticiavitorazi@id.uff.br
Nathália Ramos de Melo
ORCID: https://orcid.org/0000-0002-9533-506X
Universidade Federal Fluminense, Brasil
Universidade Federal Rural do Rio de Janeiro, Brasil

E-mail: nathaliarm@id.uff.br

Abstract

Growing demand for safe foods coupled with the intent to reduce food waste, seeing as much of it is lost through contamination by spoilage microorganisms, leads to research on antimicrobial agents such as LAE (N^{α}-lauroyl-L-arginine ethyl ester monohydrochloride). This compound has great antimicrobial potential against a range of microorganisms and, therefore, its use may be of extreme importance for the food industry in the search for antimicrobial agents with a broad spectrum of action. Thus, the objective of this article is to review the research involving LAE, when studied in vitro, in vivo and in the incorporation in different packaging in order to be released in a controlled manner for food products. In conclusion, despite the fact that it has a strong antimicrobial activity, it is still little known and is not accepted in all countries, including Brazil. With greater insight into this antimicrobial agent, more countries could use it, supporting worldwide in food preservation.

Keywords: LAE; Food safety; Cationic surfactant; Broad spectrum of action; GRAS.

Resumo

A crescente demanda por alimentos seguros atrelado com o intuito de reduzir o desperdício de alimentos, tendo em vista que grande parte é perdida através da contaminação por microrganismos deteriorantes, faz com que pesquisas sejam realizadas em torno de agentes antimicrobianos, como o LAE (N^{a}-lauril-l-arginina etil éster monoclorohidrato). Este composto apresenta grande potencial antimicrobiano frente à uma gama de microrganismos e, deste modo, sua utilização pode ser de suma importância para a indústria alimentícia na busca de agentes antimicrobianos com amplo espectro de ação. Assim, o objetivo deste artigo é fazer uma revisão sobre as pesquisas envolvendo o LAE, quando estudado *in vitro*, *in vivo* e na incorporação em diferentes embalagens com o intuito de ser liberado controladamente para os produtos alimentícios. Concluindo que o mesmo apesar de apresentar forte atividade antimicrobiana, ainda é pouco conhecido além de não ser aceito em todos os países, inclusive Brasil. Tendo maior discernimento deste agente antimicrobiano, mais países poderiam utilizá-lo, auxiliando mundialmente na preservação dos alimentos.

Palavras-chave: LAE; Alimentos seguros; Surfactante catiônico; Amplo espectro de ação; GRAS.

Resumen

La creciente demanda por alimentos seguros junto al incentivo de reducir el desperdicio de alimentos, teniendo en cuenta que grande parte es perdida a través de la contaminación por microorganismos deterioradores, hace con que las investigaciones sean realizadas alrededor de agentes antimicrobianos, como LAE (N^{α}-lauroyl-L-arginina etilo ester monohidrocloruro). Este compuesto presenta grande potencial antimicrobiano contra una variedad de microorganismos y de esta manera su utilización puede ser de suma importancia para la industria alimentaria, en la búsqueda de agentes antimicrobianos con amplio espectro de acción. Así, el objetivo de este artículo es revisar la investigación que involucra a LAE, cuando se estudia in vitro, in vivo y en la incorporación en diferentes envases para su liberación de forma controlada para productos alimenticios. En conclusión, a pesar de que tiene una fuerte actividad antimicrobiana, todavía es poco conocido y no es aceptado en todos los países, incluido Brasil. Con una mayor comprensión de este agente antimicrobiano, más países podrían usarlo, ayudando en todo el mundo a la conservación de alimentos.

Palabras clave: LAE; Inocuidad de alimentos; Tensioactivo catiónico; Amplio espectro de acción; GRAS.

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), 1.3 billion tons of food is wasted annually in Brazil along production chains. This represents 30% of the total volume of all food produced on the planet in a year. Losses and wastes have a negative impact on the environment by being linked to the sustainability of food systems, reducing local and global food availability, generating fewer resources for producers, and increasing end-product prices for consumers (FAO, 2017).

FAO also mentions that food waste is largely related to contamination by spoilage microbial agents, toxins, chemical, and/or physical compounds. In contrast, foodborne diseases (FBD) caused by pathogenic microorganisms pose a great risk to public health. In addition to the health problems of affected individuals, it causes economic losses to society due to the expenses involved with treatment, hospitalization, and lost working days (FAO/WHO, 2003).

FBD represents one of the leading causes of illness, hospitalization, and death (Broner, Torner, Dominguez, Martínez, & Godoy, 2010). According to the Brazilian Ministry of Health, data from the Brazil Information System for Notifiable Diseases (SINAN) show that, on average, there are 700 outbreaks of FBDs in the country involving 13,000 patients and can lead to 10 deaths annually (BRASIL, 2019).

To reduce waste and ensure food safety, consequently minimizing FBD, the food industry investigates the use of food additives to increase commercial validity and/or improve the physicochemical, sensory, and microbiological properties of processed foods (Damodaran & Parkin, 2017).

For all approved food additives, legislation should indicate the food, the conditions of use, and determine the permissible limit based on a level that ensures consumer safety. To this end, industries and regulatory agencies carry out strict quality control in identifying the class of food additives and the level of quantification they are employing, ensuring that any illegal additives are avoided and that the maximum permitted quantities are respected (Martins, Sentanin, & Souza, 2019).

Among food additives that act as antimicrobial agents, there is LAE (N^{α}-lauroyl-L-arginine ethyl ester monohydrochloride). It has the characteristic of being a cationic surfactant, seen as a new and one of the most powerful antimicrobial compounds on the market, but little known (Moreno et al. 2018; Muriel-Galet, Carballo, Hernández-Muñoz, & Gavara, 2016).

Therefore, this review aims to discuss the characteristics of the LAE, as well as its advantages and disadvantages, and its use, which may occur either by direct addition to food or when it is used as a component for active packaging formulation.

2. Methodology

In this study, a qualitative methodology was used, containing the interpretation by the researchers and their opinions about the phenomenon under study, in this case the LAE (Pereira, Shitsuka, Parreira, & Shitsuka, 2018). To this end, research related to the LAE antimicrobial compound was carried out using the Scopus, Web of Science and Google Scholar databases. The articles, book chapters and websites cited in this review were in Portuguese or English.

3. N^{α}-lauroyl-l-arginine ethyl ester monohydrochloride (LAE)

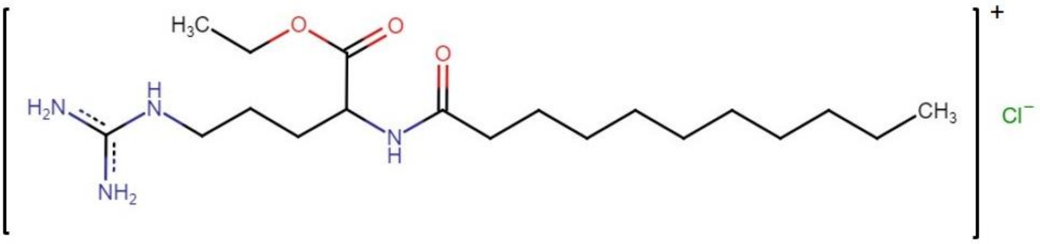
LAE is a cationic surfactant whose antimicrobial activity is attributed to disturbances that this compound causes in membrane potential, as well as changes in structure and loss of cell viability without cell lysis (Rodríguez, Seguer, Rocabayera, & Manresa, 2004). To Muriel-Galet et al. (2016), its antimicrobial effect is a result of its chemical structure, as it is electrostatically absorbed on the cell surface of microorganisms and has as its main target the plasma membrane.

Thus, this antimicrobial agent acts on gram-negative bacteria in altering the outer and cytoplasmic membrane, and on gram-positive bacteria in the cell and cytoplasmic membrane (Rodríguez et al., 2004). Concerning filamentous fungi, there are not many studies explaining the action of LAE against them. However, Xu et al. (2018) found an antimicrobial effect of this surfactant on *Penicillium digitatum* hyphae and spores and observed in transmission electron microscopy images that the LAE action behavior for this microorganism was similar to that for the bacterium *Pectobacterium carotovorum*. LAE caused a rough surface, irregular cell organelles, protoplast shrinkage, intracytoplasmic coagulation, and empty cavities in the hyphae and spores of *Penicillium digitatum* and in the bacterium cell *Pectobacterium carotovorum*.

Miret S.A. (Lamirsa) laboratory in Spain holds the patent for the compound, which has a white powder aspect. However, when used for food, the same compound is found in the market in the liquid form as Minerat®. Each Minerat is specific for a food group and is composed of LAE and one or more components. For example, Minerat-G consists of 90% glycerine and 10% LAE and is suitable for a range of food products, while Minerat-S consists of a mixture of special extracts, aroma, propylene glycol, glycerin, water, and LAE, and also has a wide application in food products. Additionally, LAE is used in cosmetics under the name Aminat® as a preservative in products such as lotions, hair conditioners and shampoos, and deodorants (VEDEQSA, 2018).

LAE (Figure 1) comes from compounds commonly found in human food, such as lauric acid found in coconut oil (Dayrit, 2015), arginine, amino acid found in many food products like walnuts (Martínez, Labuckas, Lamarque, & Maestri, 2010), and ethanol, being easily hydrolyzed by the human body (Asker, Weiss, & McClements, 2009).

Figure 1. LAE structure.



Source: Own authors.

Figure 1 represents the chemical structure of a CHOCl-shaped surfactant whose boiling temperature is from 107 °C and the melting temperature is between 50.5 - 58.5 °C. It has a hydrophobic part and a hydrophilic part characterizing the nature of a surfactant, in addition to having solubility in water at 20 °C greater than 247 g / kg and critical micellar concentration (CMC) between 2100 and 2500 ppm. The behavior of this compound can be influenced by the pH variation, so it is not indicated that LAE is used in applications that combine very low pH (for example, <2 or 3) with high temperatures (for example, 50ºC) for long periods of time. time (more than 10 to 20 days) (EFSA, 2007; VEDEQSA, 2015). The chemical structure of LAE according to FT-IR analyzes shows characteristic bands of surfactant in 1526 -1176 cm^{-1} and 2927 cm^{-1}, beyond the band in 1734 cm^{-1} attributed to the stretch C=O (Motta, 2020).

Metabolic studies by Ruckman et al. (2004) found that LAE is rapidly hydrolyzed to the amino acid arginine by hydrolysis of the ethyl ester and lauryl amide functions. Then arginine is metabolized to ornithine and urea, and possibly CO_2, by normal biochemical pathways. Lauric acid enters the normal metabolism of fatty acids, instead.

Therefore, LAE shows low toxicity, and its high antimicrobial activity makes it a powerful antimicrobial agent to control or inhibit microbial growth in food (Asker et al., 2009). To be used as such, the Food and Drug Administration (FDA) published in 2005 a no objection letter for this compound to be generally recognized as safe (GRAS), and used at levels up to 200 ppm. In 2007, the European Food Safety Authority (EFSA) considered LAE a new food preservative for use in various food matrices (EFSA, 2007).

Although it is a GRAS and has been considered by EFSA as a food preservative, each country has different legislation for this antimicrobial agent. MERCOSUL member countries such as Brazil, Argentina, Paraguay, Uruguay, and Venezuela have been awaiting approval since 2009. Other countries such as the United States, Mexico, Colombia, Chile, Canada, and Israel allow use as long as it does not exceed the 200 ppm limit. Additionally, some places establish their own concentration of use. European countries, in this case, establish a limit of 160 ppm in heat-treated meat products. As for Australia and New Zealand, they determine the use limit of 200 ppm for most food products, except for fish products, which may go up to 400 ppm (VEDEQSA, 2015).

The worldwide non-standardized legislation for this compound makes LAE still little known, despite having many advantages over other antimicrobials.

4. Pros and cons of using LAE

LAE has high thermal stability, allowing it to be used in products that require high temperatures during processing, as well as in the incorporation of active packaging materials. This compound also remains stable over a wide pH range (3 to 7), allowing it to be used in a wide range of food matrices (Becerril, Manso, Nerin, & Gómez-Lus, 2013).

The sensory characteristics of the products are preserved, which is an important property as other antimicrobial compounds can modify the flavor of the products. An example is essential oils (EOs), which must be compatible with the taste of the final product for it to be used in food. On the other hand, LAE can be incorporated into any food matrix (Becerril et al., 2013).

The antimicrobial potential against a range of microorganisms including gram-positive and gram-negative bacteria, filamentous fungi, and yeast, demonstrates an advantage of LAE over nisin and pediocin, for example. The latter, being bacteriocins, only have antimicrobial effect against gram-positive bacteria (Hernandez, Cardell, & Zarate, 2005; Nerin et al., 2016).

As mentioned before, LAE is derived from compounds present in the human diet and is rapidly hydrolyzed. Therefore, it can replace other preservatives, such as nitrate, whose use is limited due to the possible conversion of nitrate to nitrite by bacteria, which may react with primary and secondary amines, producing carcinogenic nitrosamines, which are harmful to consumer health (Jansen, Dera, Ruth, Syofyasti, & Yosy, 2018).

On the other hand, some disadvantages have to be mentioned, such as the possibility of reducing the antimicrobial potential by reacting with anionic components present in foods; and the perception of bitterness when in high concentration, due to reaction with anionic biopolymers present in the mouth. These circumstances can lead to transformations in compound functions, motivated by interactions with other molecules present in the system (Asker, Weiss, & McClements, 2011).

5. Studies on the antimicrobial effect of LAE

5.1. In vitro antimicrobial activity of LAE

Several studies have demonstrated LAE's broad spectrum of antimicrobial activity on pathogenic and spoilage microorganisms for food preservation (Al-Nemr, Mohamed, Barbabosa, & Salem, 2016; Apicella et al., 2018; Coronel-León et al., 2016; Higueras, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013; Soni, Shen, & Nannapaneni, 2014).

Al-Nemr et al. (2016) studied the antimicrobial potential of LAE, individually and in combination with nisin, against *Escherichia coli* bacteria and spore-forming bacteria *Bacillus subtilis* and *Clostridium sporogenes*. While *E. coli* was completely inhibited at 600 ppm LAE, sporulated bacteria had their action reduced with higher antimicrobial value (1000 ppm). In this study, it was also observed the synergistic effect of nisin with LAE, in a 1:3 w/w combination, potentiating the antimicrobial action against the studied microorganisms.

In a similar study, Becerril et al. (2013) studied the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of LAE against the microorganisms: *Escherichia coli*, *Listeria innocua*, *Salmonella enterica*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and found MIC values at 25 ppm for *Escherichia coli*, *Listeria innocua*, and *Salmonella enterica*; 12.5 ppm for *Staphylococcus aureus*, and 100 ppm for *Pseudomonas aeruginosa*. In addition to this inhibitory effect, the authors found that LAE exhibited equal inhibitory bactericidal concentration to all microorganisms studied, except for *Staphylococcus aureus*, whose MBC was 50 ppm.

Other microorganisms commonly found in foods, *Yersinia enterocolitica* and *Lactobacillus plantarum*, had a 100% reduction in cell viability after 60 minutes in contact with LAE in MIC at 8 and 32 ppm, respectively (Coronel-León et al., 2016).

Higueras et al., 2013, found that the LAE MIC was 8 ppm for gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*, and 16 ppm for gram-negative bacteria *Escherichia coli*, *Pseudomonas putida*, and *Salmonella enterica*, regardless of medium, solid or liquid, in which the tests were performed. A variation in LAE MIC of 4 to 16 ppm and 32 to 104 ppm was observed in liquid and solid assays, respectively, for the yeasts: *Candida utilis*, *Saccharomyces cerevisiae*, and *Torulopsis pinus*. For filamentous fungi, an antimicrobial variation of 24 to 120 ppm was observed in solid medium.

Growth inhibition of *Listeria monocytogenes* was studied by Soni, Shen, and Nannapaneni (2014), using three antimicrobials considered GRAS in different concentrations in liquid medium: LAE (200, 2000, and 20000 ppm), bacteriophage P100 (10^9 PFU/mL), and nisin (500, 5000, and 50000 ppm). The results showed a remarkable reduction in microbial growth for all antimicrobials tested. Higher antimicrobial concentrations and temperature (30°C) resulted in greater inactivation. It can be noted that LAE was the only one that exhibited a total reduction of *Listeria* after 1h (at 2000 and 20,000 ppm concentration) and 24h (at 200 ppm concentration) incubation at both 4°C and 30°C.

The efficacy of LAE was also verified against *Campylobacter jejuni* with 50, 100, and 200 ppm LAE solutions at 4°C for 2h. The authors noted that LAE was highly effective against the tested organism, where no survivors were detected (Nair, Nannapaneni, Kiess, Mahmoud, & Sharma, 2014).

5.2. Antimicrobial effect of LAE when applied to food

Since LAE has shown satisfactory in vitro antimicrobial results, studies have also proven its effectiveness when inserted directly into food.

LAE in combination with nisin (1:3, w/w) showed a positive synergistic effect inhibiting the growth of the food pathogens: *Bacillus subtilis*, *Clostridium sporogenes*, and *Escherichia coli* in feta cheese and spread cheese after storage for 7 and 30 days, respectively (Al-Nemr et al., 2016).

Cold smoked salmon is a ready-to-eat food and is considered a possible vehicle for contamination by pathogenic microorganisms such as *Listeria monocytogenes*. Soni, Shen, and Nannapaneni (2014) studied the use of LAE (200 ppm) associated with other antimicrobials, bacteriophage P100 (10^8 UFP/cm) and nisin (500 ppm), to inhibit the growth of this microorganism previously inoculated in food. Samples treated with LAE alone for 24 h at 4°C reduced 2-3 log CFU/mL and were shown to be more effective than P100 and nisin treatments. As for the combinations, nisin plus LAE exhibited the greatest effect against this microorganism, also treated at 4°C for 24h.

Kozak et al. (2018) evaluated the listericidal activity of LAE in whole milk purposely contaminated with *Listeria monocytogenes* and found that there was no antimicrobial efficiency at 100, 200, and 400 ppm for 21 days at 7ºC. However, at 800 ppm, LAE was able to exhibit a significant reduction in *L. monocytogenes* levels. In addition to LAE, other antimicrobial agents were tested, namely: caprylic acid, sodium caprylate, and ε-polylysine, which had an effect on *Listeria* at concentrations of 3200 ppm, 3200 ppm, and 100-400 ppm, respectively. The only synergistic effect observed was the combination of sodium caprylate plus ε-polylysine.

Moore et al. (2017) evaluated the efficacy of antimicrobials (0.1% peracetic acid, 0.6% cetylpyridinium chloride, 0.005% sodium hypochlorite, 1.5% acidified lactic acid, 0.3% propionic acid, and 0.1% LAE) in reducing *Salmonella heidelberg* and *Campylobacter jejuni* in chicken meat previously inoculated with the above microorganisms and stored at 4°C for 24h. Peracetic acid and LAE showed the largest reduction in Salmonella count. All treatments had the same effect on *Campylobacter jejuni* after 24h, except cetylpyridinium chloride and sodium hypochlorite, which had no effect when compared to the control.

Additionally, Nübling et al. (2017) used LAE to reduce the count of pathogenic bacteria *Escherichia coli* O157:H7 and *Listeria monocytogenes* in lettuce, and it was found that LAE was able to decrease the count of these bacteria in this vegetable.

Although the antimicrobial effect of LAE has been observed when applied directly to food, the compound can interact with other compounds present in the food matrix and consequently have its effect reduced or inhibited, which can also be caused by the processing under which the food is submitted. However, studies in this scope have not been found (Yildirim et al., 2018).

Thus, systems encompassing active packaging become alternatives to carry compounds and mitigate the risks caused by their direct insertion into the products.

5.3. Active packaging incorporated with LAE

Active packaging is considered a trend for the food industry as it interacts with the product. By incorporating additives into the polymeric matrix such as antimicrobial agents, they can be released into foods, acting as preservatives or even acting only by contact (Sharma & Ghoshal, 2018).

Different biopolymers have been studied for food packaging, such as pectin, polylactide, and whey proteins. These materials have been incorporated with different food additives, with the purpose of preserving packaged foods (Apicella et al., 2018; Asker et al., 2009; Aznar, Gómez-Estaca, Vélez, Devesa, & Nerín, 2013; Gamarra-Montes, Missagia, Morató, & Muñoz-Guerra, 2017). When applied to food packaging, LAE has also shown a positive effect due to its migration from packaging to product (Apicella et al., 2018; Aznar et al., 2013).

Apicella et al. (2018) produced BoPET (Biaxially-Oriented Polyethylene Terephthalate) films with a coating layer based on PLA (Polylactic acid) and LAE powder at different concentrations (0, 5, 10 and 20% w/w). In vitro antimicrobial analyses of films against *Escherichia coli* bacteria were evaluated, and the authors concluded that films containing over 5% LAE significantly decreased gram-negative bacterial counts, while films containing 20% LAE inhibited growth completely.

In another study by Gaikwad et al. (2017), LAE in powder (0, 1, 3, 5 and 10% w/w) was incorporated in low-density polyethylene (LDPE) package aiming at the elaboration of an antimicrobial package for fresh strawberries. The packaging was tested against spoilage fungi

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8996

and all concentrations incorporated into LDPE exhibited antifungal activity against *Botrytis Cinerea*, with emphasis on the highest concentrations. While the efficiency toward mycelium growth of the fungus *Rhizopus stolonifer* was only exhibited at concentrations of 5 and 10% (w/w).

Packaging of LAE-inserted γ-polyglutamic acid-based at a ratio of 1:1 and 1:0.5, respectively, was evaluated by Gamarra-Montes et al. (2017) against gram-negative bacteria *Salmonella enterica* and *Escherichia coli*, and gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus* by the liquid medium method as a function of time. It was noted by the authors that there was a reduction in growth after 24 hours of testing for gram-positive bacteria. Gram-negative bacteria also decreased growth, but at a longer time than gram-positive bacteria, proving a greater resistance of gram-negative bacteria to the antimicrobial effect of LAE.

However, Haghighi et al. (2019) found lower resistance of gram-negative bacteria to investigate and produce chitosan, gelatin, blend, and lamination films of these polymers containing 0.1% LAE (v/v). The antimicrobial analysis was performed in vitro with these films with the bacteria *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, and *Campylobacter jejuni*. LAE-containing films were found to have antimicrobial activity and were more efficient against gram-negative *Campylobacter jejuni* bacteria.

In turn, Ma; Zhang; & Zhong (2016) incorporated LAE alone and in combination with cinnamon essential oil and EDTA in chitosan films. Films incorporated with 0.1% LAE and 0.25% EDTA showed greater inhibition for the bacteria: *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* O157:H7, after 24 hours of incubation. The combination with EDTA exhibited a synergistic effect. The increase in LAE concentration (0.2%) in pure film or combined with 0.25% EDTA showed no significant effect against gram-negative bacteria (*S. enterica* and *E. coli* O157:H7). This may be due to the insignificant action of EDTA, because of the high volume of LAE, or due to the ineffective action of EDTA against gram-negative bacteria. Despite the incorporation of cinnamon oil, no significant increase in the inhibition zone was observed, which may have been caused by the low diffusion of the hydrophilic LAE and EDTA in the matrix after the addition of a hydrophobic component, the oil.

Finally, starch gelatin-based active film (1:1) was incorporated with LAE (10%) and used as an active layer in contact with vacuum-packed chicken breast fillet in polyamide and polyethylene pouches, and stored at 4°C for 19 days. The active layers were thermoprocessed or fused over the plastic film. In general, the total bacterial count (psychrotrophic, lactic acid, total bacteria, anaerobic, and *E.coli*) was significantly lower and the thermoprocessor films were oxidation resistant, therefore serving as antimicrobial packaging material to increase commercial validity of chicken breast fillets (Moreno, Cárdenas, Atarés, & Chiralt, 2017).

6. LAE publication history

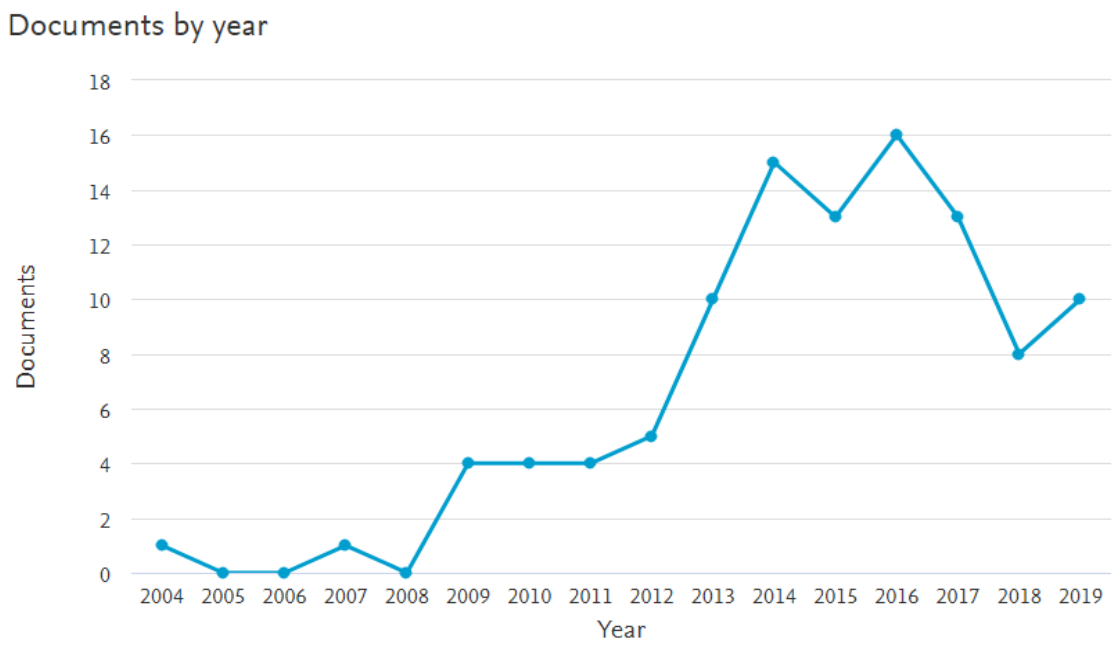
A search was conducted around publications about LAE to observe how much the compound has been studied. For this, SCOPUS was chosen since it is considered a large database with a broader spectrum of journals when compared to others (Bartol, Budimir, Dekleva-Smrekar, Pusnik, & Juznic, 2014). A search query was constructed and employed to retrieve data that contained the term "LAE" and the search was limited to the document title, summary, and keywords. These three sections - title, abstract, and keywords - always capture the essence of a publication reflecting the content of the main text (Mack, 2012).

The combination of the following words was used to retrieve LAE data from the SCOPUS database: “LAE” and “antimicrobial”.

The results showed that studies on this surfactant began in 2004, when Rodríguez et al. (2004) studied the efficacy of LAE against bacterial cells of Staphylococcus aureus and Salmonella thyphimurium using the minimum inhibitory concentrations for both microorganisms: 8 and 32 ppm, respectively.

From then on, 104 documents were published regarding the keywords used in the research until 2019 (Figure 2).

Figure 2. Historical series of published documents referring to the keywords: "LAE" and "antimicrobial".



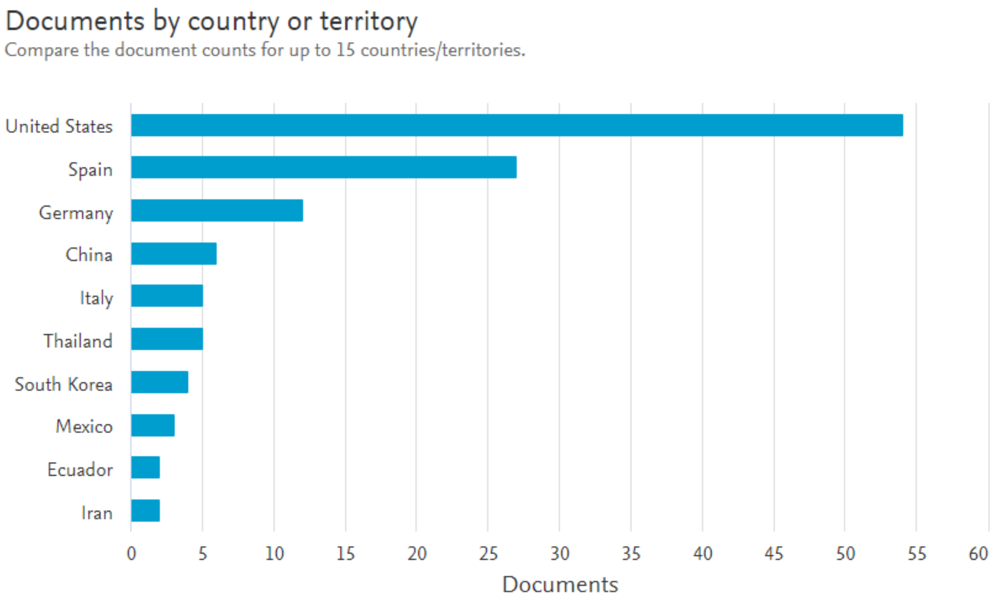
Source: Scopus (2019a).

According to Figure 2, it can be seen that between 2004 and 2008, the number of publications on the LAE was very low, between 0 and 1. As of 2009, this number remained constant in 4 publications until 2012 , where LAE publications skyrocketed, reaching a peak in 2016 with 16 publications. Subsequently, as of 2016, publications have decreased, however, when compared to the beginning, this number can still be considered high, reaching a total of 104 publications so far.

This number may be considered small compared to other searches for other antimicrobial compounds commonly used in foods, such as essential oils. In the case of essential oils, for better comparison purposes, we searched for documents between 2004 and 2019 using the keywords: "essential oils" and "antimicrobials", with a total of 8,670 publications in this period (Scopus, 2019b).

A reason why LAE is still a poorly known and studied antimicrobial agent might be the fact that there is no worldwide standardized legislation on the specific limits of use of this compound. This justifies why research around this cationic surfactant is concentrated in less than 15 countries (Figure 3).

Figure 3. Number of documents published using "lae" and "antimicrobial" keywords in each location



Source: Scopus (2019b).

It can be seen in Figure 3 that the United States has the top ranking of publications on LAE. This result is not unexpected since the North Americans are considered the largest investors in R&D in the world, followed by China (WEFORUM, 2018), which appears in 4th in the number of publications of LAE. Spain in 2nd place also does not show an unexpected result, since LAMIRSA owns the LAE manufacturing patent and is located in Spain. In the case of South American countries, only Ecuador appears in the ranking of LAE publications, although it is part of the MERCOSUR countries and therefore its use is still awaiting approval in this country.

7. Conclusions and perspectives

LAE, a GRAS-considered FDA compound and food preservative by EFSA, has a broad spectrum of action at low concentrations and is considered a potent antimicrobial compound for use by the food and food packaging industries (provided it does not exceed the maximum limit permitted by current legislation), thus aiming to ensure food safety and reduce food waste.

Research on LAE antimicrobial activity has been intensified in recent years and results have already proven its effect both *in vitro* and *in vivo*, and when incorporated into different polymeric matrices for active packaging production.

Additionally, as it has characteristics such as being odorless, tasteless, obtaining good thermal stability, and at different pH values, it increases its potential use in a variety of food matrices, and can extend the commercial validity of these products without changing their sensory characteristics.

However, for its use to be more effective today, it is necessary to have a dissemination of its application, emphasizing that its use must be released by more countries, large food producers.

Acknowledgements

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq), Federal University of Rio de Janeiro (UFRRJ), Fluminense Federal University (UFF) and Dynatech Indústrias Químicas LTDA.

References

Al-Nemr, T. M., Mohamed, S. E., Barbabosa, A., & Salem, A. Z. M. (2016). Influence of nisin and lauryl arginine ester against some foodborne pathogens in recombined feta and processed spread cheese. *Journal of Food Safety*, *36*(2), 172–179. https://doi.org/10.1111/jfs.12224.

Apicella, A., Scarfato, P., D'Arienzo, L., Garofalo, E., Di Maio, L., & Incarnato, L. (2018). Antimicrobial biodegradable coatings based on LAE for food packaging applications. *AIP Conference Proceedings 1981*, 2018, 020010 https://doi.org/10.1063/1.5045872.

Asker, D., Weiss, J., & McClements, D. J. (2009). Analysis of the interactions of a cationic surfactant (Lauric arginate) with an anionic biopolymer (Pectin): Isothermal titration calorimetry, light scattering, and microelectrophoresis. *Langmuir*, *25*(1), 116–122. https://doi.org/10.1021/la803038w.

Asker, D., Weiss, J., & McClements, D. J. (2011). Formation and stabilization of antimicrobial delivery systems based on electrostatic complexes of cationic-non-ionic mixed micelles and anionic polysaccharides. *Journal of Agricultural and Food Chemistry*, *59*(3), 1041–1049. https://doi.org/10.1021/jf103073w.

Aznar, M., Gómez-Estaca, J., Vélez, D., Devesa, V., & Nerín, C. (2013). Migrants determination and bioaccessibility study of ethyl lauroyl arginate (LAE) from a LAE based antimicrobial food packaging material. *Food and Chemical Toxicology*, *56*, 363–370. https://doi.org/10.1016/j.fct.2013.02.018.

Bartol, T., Budimir, G., Dekleva-Smrekar, D., Pusnik, M., & Juznic, P. (2014). Assessment of research fields in Scopus and Web of Science in the view of national research evaluation in Slovenia. *Scientometrics*, *98*(2), 1491–1504. https://doi.org/10.1007/s11192-013-1148-8.

Becerril, R., Manso, S., Nerin, C., & Gómez-Lus, R. (2013). Antimicrobial activity of Lauroyl Arginate Ethyl (LAE), against selected food-borne bacteria. *Food Control*, *32*(2), 404–408. https://doi.org/10.1016/j.foodcont.2013.01.003.

Brasil - Ministério da Saúde. Doenças transmitidas por alimentos: causas, sintomas, tratamento e prevenção. (2019). Retrieved from http://www.saude.gov.br/saude-de-a-z/doencas-transmitidas-por-alimentos/

Broner, S., Torner, N., Dominguez, A., Martínez, A., & Godoy, P. (2010). Sociodemographic inequalities and outbreaks of foodborne diseases: An ecologic study. *Food control*, *21*(6), 947–951. https://doi.org/10.1016/j.foodcont.2009.12.002.

Coronel-León, J., López, A., Espuny, M. J., Beltran, M. T., Molinos-Gómez, A., Rocabayera, X., & Manresa, A. (2016). Assessment of antimicrobial activity of Nα -lauroyl arginate ethylester (LAE®) against Yersinia enterocolitica and Lactobacillus plantarum by flow cytometry and transmission electron microscopy. *Food Control*, *63*, 1–10. https://doi.org/10.1016/j.foodcont.2015.10.050.

Damodaran, S., & Parkin, K. L. (2017). *Fennema's food chemistry*. (5th ed.). Boca Raton: CRC press.

Dayrit, F. M. (2015). The properties of lauric acid and their significance in coconut oil. *Journal of the American Oil Chemists' Society*, *92*(1), 1–15. https://doi.org/10.1007/s11746-014-2562-7.

EFSA. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to an application on the use of ethyl lauroyl arginate as a food additive question number EFSA-Q-2006-035. (2007). Retrieved from https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2007.511

FAO. FAO: 30% de toda a comida produzida no mundo vai parar no lixo. (2017). Retrieved from https://nacoesunidas.org/fao-30-de-toda-a-comida-produzida-no-mundo-vai-parar-no-lixo/

FAO/WHO. The codex alimentarius commission and the fao/who food standards programme. (2003). Retrieved from http://www.fao.org/3/y5307e/y5307e01.htm/

Gaikwad, K. K., Lee, S. M., Lee, J. S., & Lee, Y. S. (2017). Development of antimicrobial polyolefin films containing lauroyl arginate and their use in the packaging of strawberries. *Journal of Food Measurement and Characterization*, *11*(4), 1706–1716. https://doi.org/10.1007/s11694-017-9551-0.

Gamarra-Montes, A., Missagia, B., Morató, J., & Muñoz-Guerra, S. (2017). Antibacterial films made of ionic complexes of Poly(γ-glutamic acid) and ethyl lauroyl arginate. *Polymers*, *10*(1), 1-14 . https://doi.org/10.3390/polym10010021.

Haghighi, H., De Leo, R., Bedin, E., Pfeifer, F., Siesler, H. W., & Pulvirenti, A. (2019). Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packaging and Shelf Life*, *19*, 31–39. https://doi.org/10.1016/j.fpsl.2018.11.015.

Hernandez, D., Cardell, E., & Zarate, V. (2005). Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by Lactobacillus plantarum TF711. *Journal of applied microbiology*, *99*(1), 77–84. https://10.1111/j.1365-2672.2005.02576.x.

Higueras, L., López-Carballo, G., Hernández-Muñoz, P., Gavara, R., & Rollini, M. (2013). Development of a novel antimicrobial film based on chitosan with LAE (ethyl-Nα-dodecanoyll-arginate) and its application to fresh chicken. *International Journal of Food Microbiology*, *165*(3), 339–345. https://doi.org/10.1016/j.ijfoodmicro.2013.06.003.

Jansen, S., Dera, R. T. S., Ruth, M. S. M., Syofyasti, M. N., & Yosy, C. E. S. (2018). Analysis of nitrite and nitrate in the corned beef and smoked beef by Using Visible Spectrophotometry method. *IOP Conference Series: Earth and Environmental Science*, *205*(1), 1-6. 10.1088/1755-1315/205/1/012039.

Kozak, S. M., Brown, S. R. B., Bobak, Y., & D'Amico, D. J. (2018). Control of Listeria monocytogenes in whole milk using antimicrobials applied individually and in combination. *Journal of Dairy Science*, *101*(3), 1889–1900. https://doi.org/10.3168/jds.2017-13648

Ma, Q., Zhang, Y., & Zhong, Q. (2016). Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil, and ethylenediaminetetraacetate. *LWT - Food Science and Technology*, *65*, 173–179. https://doi.org/10.1016/j.lwt.2015.08.012.

Mack, C. A. (2012). How to write a good scientific paper: title, abstract, and keywords. *Journal of Micro/Nanolithography, MEMS, and MOEMS*, *11*(2), 20101. https://doi.org/10.1117/1.JMM.11.2.020101.

Martínez, M. L., Labuckas, D. O., Lamarque, A. L., & Maestri, D. M. (2010). Walnut (Juglans regia L.): genetic resources, chemistry, by-products. *Journal of the Science of Food and Agriculture*, *90*(12), 1959–1967. https://doi.org/10.1002/jsfa.4059.

Martins, F. C. O. L., Sentanin, M. A., & De Souza, D. (2019). Analytical methods in food additives determination: Compounds with functional applications. *Food Chemistry*, *272*, 732–750. https://doi.org/10.1016/j.foodchem.2018.08.060.

Moore, A., Nannapaneni, R., Kiess, A., & Sharma, C. S. (2017). Evaluation of USDA approved antimicrobials on the reduction of Salmonella and Campylobacter in ground chicken frames and their effect on meat quality. *Poultry science*, *96*(7), 2385–2392. https://doi.org/10.3382/ps/pew497.

Moreno, O., Atarés, L., Chiralt, A., Cruz-Romero, M. C., & Kerry, J. (2018). Starch-gelatin antimicrobial packaging materials to extend the shelf life of chicken breast fillets. *Lwt*, *97*, 483–490. https://doi.org/10.1016/j.lwt.2018.07.005

Moreno, O., Cárdenas, J., Atarés, L., & Chiralt, A. (2017). Influence of starch oxidation on the functionality of starch-gelatin based active films. *Carbohydrate Polymers*, *178*, 147–158. https://doi.org/10.1016/j.carbpol.2017.08.128.

Motta, J. F. G. (2020). Desenvolvimento de filmes ativos antimicrobianos a base de amidos não iônico, catiônico e aniônicos incorporados com o surfactante catiônico LAE (N^a-Lauril-L-arginina-etil-éster monoclorohidrato). Dissertação de mestrado, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil.

Muriel-Galet, V., Carballo, G. L., Hernández-Muñoz, P., & Gavara, R. (2016). Ethyl Lauroyl Arginate (LAE): Usage and Potential in Antimicrobial Packaging. In J. Barros-Velázquez (Eds.), *Antimicrobial Food Packaging* (pp. 313-318). London: Elsevier science.

Nair, D. V. T., Nannapaneni, R., Kiess, A., Mahmoud, B., & Sharma, C. S. (2014). Antimicrobial efficacy of lauric arginate against Campylobacter jejuni and spoilage organisms on chicken breast fillets. *Poultry Science*, *93*(10), 2636–2640. https://doi.org/10.3382/ps.2013-03858.

Nübling, S., Wohlt, D., Saile, N., Weiss, A., & Schmidt, H. (2017). Antimicrobial effect of lauroyl arginate ethyl on Escherichia coli O157:H7 and Listeria monocytogenes on red oak leaf lettuce. *European Food Research and Technology*, *243*(5), 1–9. https://doi.org/10.1007/s00217-016-2802-1.

Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. Metodologia da pesquisa científica. (1st ed). Santa Maria: Núcleo de Tecnologia Educacional da Universidade Federal de Santa Maria.

Rodríguez, E., Seguer, J., Rocabayera, X., & Manresa, A. (2004). Cellular effects of monohydrochloride of L-arginine, Nα- lauroyl ethylester (LAE) on exposure to Salmonella typhimurium and Staphylococcus aureus. *Journal of Applied Microbiology*, *96*(5), 903–912. https://doi.org/10.1111/j.1365-2672.2004.02207.x.

Ruckman, S. A., Rocabayera, X., Borzelleca, J. F., & Sandusky, C. B. (2004). Toxicological and metabolic investigations of the safety of N-α-Lauroyl-l-arginine ethyl ester monohydrochloride (LAE). *Food and Chemical Toxicology*, *42*(2), 245–259. https://doi.org/https://doi.org/10.1016/j.fct.2003.08.022.

Scopus. Analyze search results - antimicrobial and lae. (2019a). Retrivied from https://www-scopus.ez26.periodicos.capes.gov.br/term/analyzer.uri?sid=4d09ecee50cf9ca603c0aad4f6e52fac&origin=resultslist&src=s&s=%28TITLE-ABS-KEY%28lae%29+AND+TITLE-ABS-KEY%28antimicrobial%29%29&sort=plf-f&sdt=cl&sot=b&sl=53&count=104&analyzeResults=Analyze+results&cluster=scopubyr%2c%222020%22%2cf&txGid=51d09f83417f4bf4bb0b5956145fdfef

Scopus. Analyse search results - antimicrobial and lae. (2019b). Retrivied from https://www-scopus.ez26.periodicos.capes.gov.br/term/analyzer.uri?sid=4d09ecee50cf9ca603c0aad4f6e52fac&origin=resultslist&src=s&s=%28TITLE-ABS-KEY%28lae%29+AND+TITLE-ABS-KEY%28antimicrobial%29%29&sort=plf-f&sdt=cl&sot=b&sl=53&count=104&analyzeResults=Analyze+results&cluster=scopubyr%2c%222020%22%2cf&txGid=51d09f83417f4bf4bb0b5956145fdfef

Sharma, R., & Ghoshal, G. (2018). Emerging trends in food packaging. *Nutrition and Food Science*, *48*(5), 764–779. https://doi.org/10.1108/NFS-02-2018-0051.

Soni, K. A., Shen, Q., & Nannapaneni, R. (2014). Reduction of Listeria monocytogenes in cold-smoked salmon by bacteriophage P100, nisin and lauric arginate, singly or in combinations. *International Journal of Food Science and Technology*, *49*(8), 1918–1924. https://doi.org/10.1111/ijfs.12581.

VEDEQSA. Etil lauroil arginato E-243 (LAE®): Um nuervo conservante para la industria alimentaria. (2015). Retrivied from http://media.firabcn.es/content/S051015/docs/presentaciones_IMP/VEDEQSA-MIRENAT.pdf/

VEDEQSA. Specialties for the food industry. (2018). Retrieved from https://www.lamirsa.com/catalogo/ved2018eng/mobile/html5forpc.html

WEFORUM. Innovators wanted: these countries spend the most on R&D. (2018). Retrieved from https://www.weforum.org/agenda/2018/12/how-much-countries-spend-on-r-d/

Xu, X.-H., Jiang, Z.-L., Feng, F.-Q., & Lu, R.-R. (2018). Mechanisms of N α-lauroyl arginate ethyl ester against Penicillium digitatum and Pectobacterium carotovorum subsp. carotovorum. *Journal of food science and technology*, *55*(9), 3675–3682. https://doi.org/10.1007/s13197-018-3296-6.

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., … Coma, V. (2018). Active Packaging Applications for Food. *Comprehensive Reviews in Food Science and Food Safety*, *17*(1), 165–199. https://doi.org/10.1111/1541-4337.12322.

Percentage of contribution of each author in the manuscript

Joyce Fagundes Gomes Motta – 30%

Regiane Ribeiro-Santos – 15%

Maria Clara Guimarães – 10%

Lívia de Aquino Garcia Moura – 10%

Letícia Vitorazi – 15%

Nathália Ramos de Melo – 20%