FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting document 3

Food Technology Report – Application A1015

Ethyl Lauroyl Arginate as a Food Additive

Executive Summary

Ethyl lauroyl arginate is a synthetically produced chemical compound. Its active component is a cationic surfactant, ethyl-N^{α}-lauroyl-L-arginate·HCl, which has a broad spectrum of activity against bacteria, yeasts and moulds.

The Application requested ethyl lauroyl arginate as a preservative in a wide range of food groups as listed below:

- food additive preparations
- cheeses – soft, cream, processed, mozzarella, hard and semi hard
- peeled and/or cut fruit and vegetables – rehydrated legumes
- cereal products – cooked rice, noodles and pasta
- semi processed fish and fish products – salted fish and roe
- processed meat, poultry and meat products in whole or cut pieces or comminuted products
- non-alcoholic beverages - fruit and vegetable juices and juice products, water based flavoured drinks and high energy drinks and soft drinks
- savoury toppings or fillings, dairy based desserts, dips and snacks

Within these foods, the Applicant proposed ethyl lauroyl arginate, expressed as the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl to be used in levels ranging between 50 mg/kg (e.g. beverages) and 400 mg/kg (in protein based foods, e.g. cheese and fish products).

The Applicant provided a number of experimental studies to support their claims that ethyl lauroyl arginate effectively suppresses a broad spectrum of micro-organisms in a wide range of food matrices. The Applicant provided information to demonstrate ethyl lauroyl arginate may be a potential alternative for some of the currently approved preservatives such as sulphites, benzoates and sorbates, which have some inherent limitations.

The data provided by the Applicant supplemented with published scientific reports indicate that ethyl lauroyl arginate is an effective food preservative to extend shelf life of foods in the food groups proposed above and that it also reduces the levels of certain pathogenic bacteria. This new antimicrobial agent is stable in storage and processing of a range of food groups.

Use of ethyl lauroyl arginate as a preservative in the specified food types up to the maximum requested level is technologically justified and along with good manufacturing practice could be a useful component of food preservation systems.

1. Introduction

An Application was received from Laboratarios Miret SA on 28 August 2008. The Applicant seeks the listing of a new food additive, ethyl lauroyl arginate, in Schedule 1 of Standard 1.3.1 (Food Additives) of the *Australia New Zealand Food Standards Code* (the Code).

Ethyl lauroyl arginate is used as a chemical food preservative to protect food against growth of micro-organisms including food spoilage and to improve the storage capabilities of food products. Its active component, ethyl-N^{α}-lauroyl-L-arginate·HCl, is a cationic surfactant which has a wide spectrum of activity against Gram positive and negative bacteria, yeasts and moulds. It is therefore proposed to be used in a wide range of foods.

2. Ethyl lauroyl arginate

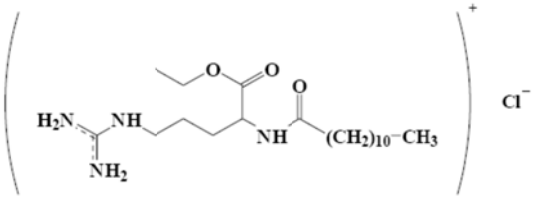
Chemistry

The active ingredient of ethyl lauroyl arginate is the hydrochloride salt of ethyl-N^{α}-lauroyl-L-arginate (ethyl-N^{α}-lauroyl-L-arginate·HCl, CAS number 60372-77-2). Ethyl lauroyl arginate contains between 85-95% of this active ingredient and it is a white powder.

The other names for ethyl lauroyl arginate are:

Lauric arginate ethyl ester
Lauramide ethyl ester
LAE
INS No. 243
Lauric arginate (Trade name)

The active ingredient is described as follows:

Chemical name:	ethyl-N^{α}-lauroyl-L-arginate·HCl
IUPAC name:	ethyl-N^{α}-dodecanoyl-L-arginate·HCl
C.A.S. number:	60372-77-2
Chemical formula:	$C_{20}H_{41}N_4O_3Cl$
Structural formula:	
Formula weight:	421.02 g/mol
Assay:	Not less than 85% and not more than 95%

Physical properties

Physical appearance:	White powder
Solubility:	Freely soluble in water (at 20°C, solubility greater than 247 g/kg) Soluble up to 20% in propylene glycol, glycerine and ethanol
pH (1% aqueous solution):	Between 3 and 5
Melting temperature:	50.5 – 58.0°C
Boiling temperature:	Decomposes from 107°C
Stability:	Over 2 years when the solid form is stored in a closed container

Methods of analysis in foods

The amount of ethyl-N^{α}-lauroyl-L-arginate·HCl (the active) in food matrices can be measured by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Different sample preparation techniques are required, which depend on the nature of the food matrix to be analysed (i.e. solid, semi-solid or liquid foods).

Stability

Ethyl lauroyl arginate as manufactured is a powdered substance and was shown to have a shelf life of more than two years when kept in a closed container at room temperature. However, the product is to be sold in a solution form with ethyl lauroyl arginate dissolved in appropriate carriers such as water, propylene glycol, glycerine or ethanol.

The stability of ethyl-N^{α}-lauroyl-L-arginate·HCl in aqueous solution has been evaluated under different pH conditions at 25°C. Results showed the active is most stable at pH 4, with a half life of greater than 1 year. Its half life decreased drastically at higher pH at that same temperature; that is, 57 days at pH 7 and 34 hours at pH 9.

The Applicant tested the stability of ethyl-N^{α}-lauroyl-L-arginate·HCl dissolved in propylene glycol at pH of 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 and temperatures of 4°C, 25°C and 50°C. The tests included different food acids such as phosphoric, citric, tartaric, malic and fumaric acids.

Results showed that high temperature (i.e. 50°C) combined with very low pH (less than 3) causes the hydrolysis of ethyl-N^{α}-lauroyl-L-arginate·HCl to N^{α}-lauroyl-L-arginine (LAS) as the main product. Further hydrolysis of LAS produced arginine and lauric acid. However, the active compound was relatively stable at room temperature even at low pH.

It was concluded that ethyl lauroyl arginate should not be used in a food application that combines high temperatures (e.g. 50°C) and low pH (<3) for a period of time in excess of 10 days. The Applicant pointed out that the conditions studied above are unlikely conditions to be experienced in the proposed used of ethyl lauroyl arginate and therefore its stability would not be an issue under these storage conditions.

Stability of ethyl lauroyl arginate combined with other components

The Applicant evaluated the potential interaction of the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl, with other components in foods such as hydrocolloids, food preservatives and antioxidants, enzymes, colour additives and proteins or protein extracts. Out of a total of 33 samples, nine showed interaction between the active and the compounds that constituted the sample (EFSA 2007).

In four of these samples, ethyl-N^{α}-lauroyl-L-arginate·HCl was shown to decrease over time due to its hydrolysis to LAS (the main hydrolysis product). The remaining five samples showed interaction with other components including meat, soya proteins, ovo-albumin and lacto-albumin, resulting in degradation of ethyl-N^{α}-lauroyl-L-arginate·HCl to ethanol, arginine and lauric acid. Interaction between ethyl-N^{α}-lauroyl-L-arginate·HCl and nitrite was observed but the applicant indicated that no nitrosamines were detected.

Stability of ethyl lauroyl arginate within different food matrices

A further stability study was conducted on eight different food matrices, three uncooked foods and five processed foods. Ethyl-N^{α}-lauroyl-L-arginate·HCl was found to be stable through the duration of shelf life of all processed foods but a decrease was seen in the uncooked foods. This is because ethyl-N^{α}-lauroyl-L-arginate·HCl is subjected to enzymatic action and undergoes hydrolysis by the presence of inherent enzymes found in chickpeas, marinated meats, dried and salted cod and potentially other foods. As a result, a higher level of ethyl lauroyl arginate was used in these foods during the study to achieve the required shelf life.

3. Manufacturing

The manufacturing process

A Spanish patent application (ES-A-512643) (Beltran and Bonaventura 2001) and European Patent No. 1294678 describe how ethyl lauroyl arginate is produced (Kawamura and Whitehouse, 2008).

The manufacturing process involves esterification of the carboxyl group of L-arginate·HCl with ethyl alcohol, utilising thionyl chloride as the esterification agent, with ethyl arginate 2HCl as a resulting product in this step. The next step involves condensation of lauroyl chloride with the α-amino group of ethyl arginate·2HCl in an aqueous medium. The final production step of ethyl lauroyl arginate is the filtration of the reaction mixture through a press filter. After the filtration process, a white solid is obtained with the active ingredient content of between 71% and 81% and a water content of 12 to 19%. This final mixture can be further dried to produce a product with an active content of between 85 and 95%. The possible impurities are residual materials and by-products of the reactions and they are listed in the Product Specification below (Table 5.1).

Specifications

Table 5.1: Specification for ethyl lauroyl arginate

Compound	Purity
Ethyl-N^{α}-lauroyl-L-arginate·HCl	Between 85% and 95%
N^{α}-lauroyl-L-arginine	Not more than 3%
Lauric acid	Not more than 5%
Ethyl laurate	Not more than 3%
L-arginine·HCl	Not more than 1%
Ethyl arginate·2HCl	Not more than 1%
Ash	Not more than 2%

Compound	Purity
Water	Not more than 5%
Ethanol	Not more than 0.2%
pH of 1% solution	Not less than 3 and not more than 5
Arsenic	Not more than 3 mg/kg
Cadmium	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg
Mercury	Not more than 1 mg/kg

The commercial product of this Application, ethyl lauroyl arginate complies with a relevant monograph published in the FAO Combined Compendium of Food Additive Specifications (Monograph 5) (JECFA, 2008). Monograph 5 is not yet a primary source of product specification, as required in clause 2 of Standard 1.3.4 – Identity and Purity. FSANZ proposes to update clause 2 to include reference to Monograph 5.

The specification for ethyl lauroyl arginate was revised at JECFA's 71st meeting in July 2009. There is no change in the main product specification. The revision is in the analysis of two impurities (L-arginine·HCl and Ethyl arginate·2HCl) where quantification procedures were modified.

Allergenicity

No allergenic materials (as listed in Table to clause 4, Standard 1.2.3) are likely to be present in the manufacture of this food additive.

Commercial preparations

Commercial products are formulations comprising of 20-25% solutions of ethyl lauroyl arginate in appropriate food grade solvents e.g. water, ethanol, propylene glycol, isopropyl alcohol, other glycols or mixtures of these. Examples of commercial product names are: Mirenat-N, Mirenat-NA, Mirenat-TT, Mirenat-LA and Mirenat-G.

4. Antimicrobial activity

Mode of Action

Ethyl lauroyl arginate is a cationic surfactant. The antimicrobial properties of ethyl lauroyl arginate include the reduction of surface tension and the formation of ionic aggregates leading to changes in the conductivity and solubility of cell membranes (Rodriguez *et al*., 2004). The disruption of proteins in the cellular membrane can lead to leaking of ions and other cellular constituents resulting in permanent alterations in cell permeability and subsequent inhibition of growth, or inactivation, of the microorganism. Ethyl lauroyl arginate is reported to have a broad spectrum of activity against Gram-negative and Gram-positive bacteria, yeasts and moulds (Bakal and Diaz, 2005).

The level of action of cationic surfactants against specific microorganisms is influenced by cell structure and physiology. Sakagami *et al*. (1989) reported that an increased quantity of phospholipids, fatty acids and neutral lipids in cell membranes inhibits the penetration of cationic surfactants. Another mechanism that has been associated with reduced sensitivity includes the increased activity of efflux pumps which act by reducing intracellular surfactant concentrations (Ishikawa *et al*., 2002).

Rodriguez *et al.* (2004) studied the structural alterations of cell membranes and subsequent changes in membrane potential following exposure of *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 6538) to ethyl lauroyl arginate. Cell membrane damage was analysed by staining cells with fluorescent nucleic acid dyes: SYTO-13 which penetrates all cellular membranes and propidium iodide (PI) which only penetrates damaged membranes (non-viable cells).

Following exposure of *S. typhimurium* to ethyl lauroyl arginate at the minimum inhibitory concentration (MIC) of 32 µg ml^{-1} for 30 min, up to 94% of the population were stained with PI. For *S. aureus* treatment with 8 µg ml^{-1} ethyl lauroyl arginate (MIC) for 30 min resulted in 43% of the population being stained with PI, however there was also a subpopulation of 21% that was double stained, indicating partially damaged membranes. Loss of viability was confirmed using conventional culture techniques.

Changes in membrane potential were determined by measuring the proton flux across the cell membrane and leakage of potassium ions. The flow of protons in cells treated with ethyl lauroyl arginate was slightly less than that for untreated samples however this was not statistically significant. Leakage of potassium ions was rapid following exposure to ethyl lauroyl arginate. Despite these observed structural changes in membrane integrity, it was reported that cells remained intact when viewed by electron transmitting microscopy.

5. Technological justification

The Applicant proposed that ethyl lauroyl arginate be permitted as a preservative in a range of foods and claimed that it may be used alone or in conjunction with other food preservatives such as sorbates, benzoates, sulphites and nitrates/nitrates. The function of a preservative is to retard or prevent the deterioration of a food by microorganisms. The use of preservatives is not a substitute for good hygienic practices, particularly in relation to control of microbial pathogens.

Results from an independent laboratory study provided by the Applicant demonstrated the activity of ethyl lauroyl arginate against a broad range of Gram-negative and Gram-positive bacteria, yeasts and moulds, with minimal inhibitory concentrations of 4-128 µg/mL depending on the microorganism (summary provided in Bakal and Diaz, 2005).

There has been limited peer-reviewed published data describing the use of ethyl lauroyl arginate in food products because of the novelty and originality of the food preservative.

The Applicant submitted additional information to support their claims of the efficacy of ethyl lauroyl arginate in preserving cheese, meats, vegetables, beverages and other foods. The data was gathered from internal laboratory studies of specific food products using standardised techniques. Many of these internal studies were done in collaboration of LAMIRSA's potential customers and therefore there were partial oversight from these potential users. These studies contain confidential commercial information and hence full details cannot be disclosed in this report. But the data have been evaluated fully as part of this assessment.

Milk and dairy products

The Applicant has requested that ethyl lauroyl arginate be permitted in milk and dairy products at levels ranging from 225 to 450 mg/kg. The Applicant has provided laboratory data to demonstrate that ethyl lauroyl arginate inhibited the growth of microorganisms in the following products:

- Studies conducted with hard cheese show that ethyl lauroyl arginate is an effective preservative by inhibiting microbial growth (especially moulds) on cheeses when used as a surface treatment. It was applied by dipping the cheese in an aqueous solution. Ethyl lauroyl arginate was found to migrate about 1 mm into hard cheeses (e.g. parmesan and Granda Padano). In these studies, no hydrolysis of ethyl lauroyl arginate was found to have taken place.

- Studies conducted with the soft cheese mascarpone showed that at the higher moisture content of soft cheese, it is more likely for ethyl lauroyl arginate to interact with milk protein and become inactivated. However, growth of mesophilic aerobes was inhibited by increasing the concentration of the preservative in the cheese to 500 mg/kg.

- Studies conducted with mozzarella cheese (with ethyl lauroyl arginate added to the brine in which mozzarella is stored) showed that when ethyl lauroyl arginate was added at levels between 400 and 800 mg/kg to the brine, microbial growth (aerobic mesophiles, coliforms and yeasts) was inhibited in both the brine and the cheese for at least 21 days of the 28-day study period. Upon analysis of the aerobic bacteria population, it was found that thermophilic bacteria were largely unaffected by ethyl lauroyl arginate. The Applicant has requested a maximum concentration of 225 mg/kg of ethyl lauroyl arginate for mozzarella, which results from soaking the cheese in brine with a concentration of 400 mg/kg of ethyl lauroyl arginate.

- Studies conducted with ricotta cheese inoculated with *Listeria monocytogenes* showed that treatment with ethyl lauroyl arginate inhibited growth (compared to controls) over the study period of seven days at 4ºC.

- Studies were conducted on gorgonzola cheese by the Applicant to illustrate the need to maintain control of microbial populations throughout the production process. Applying ethyl lauroyl arginate to fresh and mature cheese was shown to inhibit growth of both mesophilic aerobes and *Listeria* spp.

The use of ethyl lauroyl arginate in liquid dairy products may be limited by its reaction with the protein, casein, which can lead to the formation of a precipitate and consequent loss of activity. The Applicant has recommended that ethyl lauroyl arginate is not to be used in liquid milk products.

Vegetables

Studies have been conducted on various vegetables showing that application of ethyl lauroyl arginate inhibited microbial growth in rehydrated chickpeas, carrots and prepared salads.

- Chickpeas soaked in a water bath containing 100 mg/kg ethyl lauroyl arginate showed inhibition of fermentation and reduction of the total viable microbiological counts in the chickpeas and the soaking bath water compared with an untreated control over the 18 hour study period at 19ºC.

- Sliced carrot dipped in a bath containing ethyl lauroyl arginate solution (resulting in 200 mg/kg on the finished product) showed that the treated carrots had inhibited growth of aerobic mesophiles over the study period of nine days at 4ºC compared to controls.

- A prepared ready-to-eat salad including washed, sliced, chopped or shredded vegetables combined with a dressing was treated with a final concentration of 200 mg/kg ethyl lauroyl arginate. Inhibition of microbial growth was compared with an untreated control salad and a sodium benzoate plus potassium sorbate treated salad stored at 4ºC.

The results indicated that ethyl lauroyl arginate inhibited growth of aerobic microorganisms and enteric bacteria in the ready-to-eat salad at a level similar to that of sodium benzoate plus potassium sorbates for up to 30 days duration.

Meat and meat products

The Applicant provided data that demonstrated ethyl lauroyl arginate inhibited microbial growth in ham, stewed veal, marinated meat, smoked turkey slices, roast turkey slices and bratwurst sausages.

- The Applicant provided a published research paper that investigated the activity of ethyl lauroyl arginate when applied to commercially-prepared hams using an innovative delivery method (the 'Sprayed Lethality In Container' – SLIC). In the presence of 8 mL of a 5% ethyl lauroyl arginate solution, growth of L*. monocytogenes* was inhibited over the study period of 60 days at 4ºC (Luchansky *et al.,* 2005).

- Stewed veal vacuum sealed in a plastic bag inoculated with a mixture of bacteria (*E. coli, En. aerogenes, S. aureus, B. cereus*) and yeasts (*C. albicans, Saccharomyces bailii*) was evaluated and showed that addition of ethyl lauroyl arginate at 100 mg/kg had greater inhibition of microbial growth compared to nitrite treated or control (no treatment) veal over the study period of 35 days at 8-10ºC.

- Growth of microorganisms in marinated non-cooked cured meat treated with 180 mg/kg ethyl lauroyl arginate was compared with marinated meat treated with an alternative preservative, sodium nitrite ($NaNO_2$), at 150 mg/kg. Results indicated that ethyl lauroyl arginate suppressed the growth of mesophilic aerobes in marinated meat more effectively than sodium nitrate over the two week trial at 4-7ºC.

- Independent laboratory studies of sliced smoked turkey topically treated with ethyl lauroyl arginate solutions at 180 mg/kg showed a reduction of *L. monocytogenes* population over the six week study period compared to untreated samples stored at 4.4ºC and 15ºC. Ethyl lauroyl arginate was also found to inhibit the growth of aerobic spoilage bacteria and thus has the potential to extend the shelf life of refrigerated sliced smoked turkey. Similar reductions in *L. monocytogenes* were also obtained from a study conducted on sliced roast turkey; however, the effect of ethyl lauroyl arginate on inhibiting growth of *L. monocytogenes* was only observed in the first two weeks of the eight week study period.

- Ethyl lauroyl arginate added at 100 mg/kg to bratwurst sausages inhibited the growth of total aerobic bacteria over the 90 day study when stored at 5ºC. Aerobic bacteria counts remained the same inside both ethyl lauroyl arginate treated sausages and non-treated control sausages, but the growth of *Clostridium* sp. was inhibited in the treated samples.

Fish

The Applicant provided data that demonstrated ethyl lauroyl arginate inhibited microbial growth in fish roe and during rehydration of dried and salted cod.

- In fish roe, growth of *S. aureus, E. coli* and total bacteria was inhibited by ethyl lauroyl arginate at 200 mg/kg (similar level of inhibition to that observed for benzoic acid at 200 mg/kg) compared to the untreated control stored at 30ºC over the nine day study period.

- Adding 80-160 mg/kg ethyl lauroyl arginate to a desalting bath during rehydration of salted cod inhibited microbial growth and development of unpleasant odours compared to untreated controls over the eleven day study period at 4ºC.

Processed foods

The Applicant provided data to demonstrate that ethyl lauroyl arginate inhibits microbial growth in a broad range of prepared foods, including:

- Refrigerated soup (chicken soup with vegetables and rice), in which ethyl lauroyl arginate was added at 200 mg/kg, had inhibited growth of aerobic mesophiles compared to controls over the study period of 31 days at 4ºC.

- Sauces with added ethyl lauroyl arginate showed inhibited microbial growth including; guacamole (350 mg/kg) over a 12 day study period at 4ºC; and fresh tomato sauce (222 mg/kg) over a 12 day study period at 5ºC with periodic storage at 25ºC for 8 hours to simulate potential temperature abuse.

- Addition of ethyl lauroyl arginate to pizza topping at levels of 200 mg/kg inhibited growth of total viable bacteria over the study period of 14 days at 10ºC compared to controls. A similar level of inhibition was observed in pizza topping treated with sorbic acid (potassium sorbate) at 1000 mg/kg.

- Cooked pasta treated with 100 mg/kg ethyl lauroyl arginate showed inhibited growth of mesophilic bacteria over the 19-day study period at 4ºC compared to controls, and similar inhibition to that observed for cooked pasta treated with 1000 mg/kg potassium sorbate.

- Cooked rice treated with 100-300 mg/kg ethyl lauroyl arginate had reduced growth of mesophilic bacteria compared to both potassium sorbate treated and untreated samples over the study period of 11 days at 4ºC and 20ºC.

Beverages

The Applicant provided studies on the use of ethyl lauroyl arginate as a preservative in the following beverages:

- A carbonated orange juice drink where ethyl lauroyl arginate was used at 50 mg/kg had inhibited growth of *S. bailii, Zygosaccharomyces bailii* (a yeast resistant to sorbates and benzoates) and mesophilic aerobes compared to controls over the nine week study period at 25ºC.

- Fruit based concentrates with 100 mg/kg ethyl lauroyl arginate had inhibited growth of yeast compared to controls over the study period of seven days at 20ºC.

- Sport drinks (citrus flavour) and flavoured tea based drinks treated with 50 mg/kg ethyl lauroyl arginate had inhibited growth of yeast (including those resistant to sorbate and benzoate, mould and mesophile bacteria) over the study period of 28 days at 20ºC. The Applicant claims that ethyl lauroyl arginate has the potential to replace benzoate and sorbate in these drink categories.

As for any preservative, the extent of inhibition will vary depending on the physical and chemical nature of the food, type of microorganism, and the conditions of application, including the environment (e.g. temperature of storage).

While the data submitted by the Applicant demonstrate the inhibition of specific microorganisms in wide variety of food types, empirical laboratory data would need to be gathered to confirm efficacy in specific food products and under different environmental conditions.

6. Regulatory status

Please see comments under Section 9.1.2.

7. Conclusion

Based on an assessment of the data provided to FSANZ, ethyl lauroyl arginate fulfils the technological purpose of a food preservative in a variety of foods.

The data provided by the Applicant supplemented with published peer reviewed information indicate that ethyl lauroyl arginate is an effective food preservative at the proposed usage levels in the following food categories cheeses, meats, semi preserved fish and fish products, vegetable products, salads, some prepared foods and beverages. This preservative is effective against a broad range of microorganisms and is relatively stable in a variety of food matrices. Table 5.2 (below) specifies the proposed food groups and proposed maximum allowable levels of ethyl lauroyl arginate.

Table 5.2: Intended uses of ethyl lauroyl arginate

Food types*		Ethyl lauroyl arginate** (mg/kg; maximum)
0.1	Preparations of food additives	200
1.6	Cheese - soft/cream/processed and mozzarella	400 except for mozzarella at 200
1.6	Cheese – Hard/Semi-hard	1 mg/cm^2 of surface area of cheese (taken to a depth of 3 mm and not more than 5 mm)
4.1.3	Peeled and/or cut fruits and vegetables	200
4.3.8	Processed fruits and vegetables—rehydrated legumes only	200
6.3	Processed cereal and meal products-cooked rice only	200
6.4	Flour products (including noodles and pasta) – cooked pasta and noodles only	200
8.2	Processed meat, poultry and meat products in whole cuts or pieces	200
8.3	Processed comminuted meat and poultry products	315
9.3	Semi preserved fish and fish products	400
14.1.2	Fruit and vegetable juices and fruit and vegetable juice products***	50
14.1.3	Water based flavoured drinks	50
20.2	Savoury toppings or fillings - essentially sauces such as tomato paste used in ready to eat pizzas, etc.	200
20.2	Dairy and fat based desserts, dips and snacks	400

* The code number and food types are as listed in the Code, Standard 1.3.1, Schedule 1.
** Ethyl lauroyl arginate shall be calculated as ethyl-N$^{\alpha}$-lauroyl-L-arginate·HCl.
******* (NOT apple juice)" has been removed following comments received on the Assessment Report (see Section 9.1 for explanation)

References

Bakal, G. and Diaz, A. (2005) The lowdown on lauric arginate. *Food Quality:* 12, 54-61.

European Food Safety Authority. (2007) 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. *The EFSA Journal* 511:1-29.

Ishikawa, S., Matsumura, Y., Yoshizako, F. and Tsuchido, T. (2002) Characterization of a cationic surfactant-resistant mutant isolated spontaneously from *Escherichia coli*. *J Appl Microbiol* 92(2):261-268.

JECFA (2008) *Ethyl Lauroyl Arginate*. http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-506-m5.pdf . Accessed on 16 December 2008.

Kawamura, Y. and Whitehouse, B. (2008) *Ethyl Lauroyl Arginate - Chemical and Technical Assessment*. http://www.fao.org/ag/agn/agns/jecfa/cta/69/Ethyl_Lauroyl_arginate_CTA_69.pdf. Accessed on 10 December 2008.

Luchansky, J.B., Call, J.E., Hristova, B., Rumery, L., Yoder, L. and Oser, A. (2005) Viability of *Listeria monocytogenes* on commercially-prepared hams surface treated with acidic calcium sulfate and lauric arginate and stored at 4 degrees C. *Meat Science* 71(1):92-99.

Rodriguez, E., Seguer, J., Rocabayera, X. and Manresa, A. (2004) Cellular effects of monohydrochloride of L-arginine, N-lauroyl ethyl ester (ethyl lauroyl arginate) on exposure to Salmonella *typhimurium* and *Staphylococcus aureus*. *J Appl Microbiol* 96(5):903-912.

Urgell Beltran, J.B. and Seguer Bonaventura, J. (2001) Use of Cationic Preservatives in Food Products. PCT/EP01/12358(WO 03/034842 A1): International.

Appendix 1

US FDA proposed list of intended uses of ethyl lauroyl arginate.

Up to 200 ppm ethyl-N$^{\alpha}$-lauroyl-L-arginate hydrochloride in the following food categories in 21 CFR 170.3(n)
(3) Beverages and beverage bases, not including dairy products, soft drinks, or alcoholic beverages.
(3) Carbonated beverages*.
(5) Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip, and miscellaneous cheeses.
(6) Chewing gums, including all forms**
(7) Tea, including regular, decaffeinated, and instant types.
(8) Condiments and relishes, including plain seasoning sauces and spreads, olives, pickles, and relishes, but not spices or herbs.
(11) Egg dishes including egg roll, egg foo young, egg salad, and frozen multicourse egg meals, but not fresh eggs.
(12) Margarine and margarine-like table spreads, mayonnaise, and spoonable and pourable dressings for salads.
(13) Fish products, including all prepared main dishes, salads, appetizers, frozen multicourse meals, and spreads containing fish, shellfish, and other aquatic animals, but not fresh fish.
(15) Fresh fish, including only fresh and frozen fish, shellfish, and other aquatic animals.
(17) Fresh meats, including only fresh or home-frozen beef or veal, pork, lamb or mutton and home-prepared fresh meat-containing dishes, salads, appetizers, or sandwich spreads made therefrom.
(18) Fresh poultry, including only fresh or home-frozen poultry and game birds and home-prepared fresh poultry-containing dishes, salads, appetizers, or sandwich spreads made therefrom.
(22) Pie fillings.
(24) Gravies and sauces, including all meat sauces and gravies, and tomato, milk, buttery, and specialty sauces.
(29) Meat products, including all meats and meat-containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.
(34) Poultry products, including all poultry and poultry-containing dishes, salads, appetizers, frozen multicourse poultry meals, and sandwich ingredients prepared by commercial processing or using commercially processed poultry with home preparation.
(35) Processed fruits and fruit juices excluding apple juice, including all commercially processed juices and juice punches, concentrates, dilutions, ades, and drink substitutes made therefrom, and dried fruits; also including strained fruits and fruit juices excluding apple juice as baby or toddler foods.
(36) Processed vegetables and vegetable juices, including potato salads, raw vegetables, vegetable juices and blends, and tomato sauces
(40) Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes.

* The maximum concentration of ethyl-N$^{\alpha}$-lauroyl-L-arginate hydrochloride is 100 ppm.

** The maximum concentration of ethyl-N$^{\alpha}$-lauroyl-L-arginate hydrochloride is 5000 ppm.

Appendix 2

JECFA's list of proposed use levels for ethyl lauroyl arginate in food

Food category	Level of use as ethyl-N^{α}-lauroyl-L-arginate HCl (mg/kg)
Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip and miscellaneous cheeses	Up to 200
Tea, including regular, decaffeinated and instant types	Up to 200
Condiments and relishes, including plain seasoning sauces and spreads, olives, pickles and relishes, but not spices or herbs	Up to 200
Egg dishes, including egg roll, egg foo yung, egg salad and frozen multicourse egg meals, but not fresh eggs	Up to 200
Margarine and margarine-like table spreads, mayonnaise and spoonable and pourable dressings for salads	Up to 200
Fish products, including all prepared main dishes, salads, appetizers, frozen multicourse meals and spreads containing fish, shellfish and other aquatic animals, but not fresh fish	Up to 200
Fresh fish, including only fresh and frozen fish, shellfish and other aquatic animals	Up to 200
Fresh meats, including only fresh or home-frozen beef or veal, pork, lamb or mutton and home-prepared fresh meat–containing dishes, salads, appetizers or sandwich spreads made therefrom	Up to 200
Fresh poultry, including only fresh or home-frozen poultry and game birds and home-prepared fresh poultry–containing dishes, salads, appetizers or sandwich spreads made therefrom	Up to 200
Pie fillings	Up to 200
Gravies and sauces, including all meat sauces and gravies and tomato, milk, buttery and specialty sauces	Up to 200
Meat products, including all meats and meat-containing dishes, salads, appetizers, frozen multicourse meat meals and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation	Up to 200
Poultry products, including all poultry and poultry-containing dishes, salads, appetizers, frozen multicourse poultry meals and sandwich ingredients prepared by commercial processing or using commercially processed poultry with home preparation	Up to 200
Processed fruits and fruit juices, excluding apple juice, including all commercially processed juices and juice punches, concentrates, dilutions, ades and drink substitutes made therefrom, and dried fruits; also including strained fruits and fruit juices, excluding apple juice, as baby or toddler foods	Up to 200

Table 6. (contd)

Food category	Level of use as ethyl-N^{α}-lauroyl-L-arginate HCl (mg/kg)
Processed vegetables and vegetable juices, including potato salads, raw vegetables, vegetable juices and blends, and tomato sauces	Up to 200
Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable and combination soups and soup mixes	Up to 200
Beverages and beverage bases, not including dairy products, soft drinks or alcoholic beverages	Up to 200
Carbonated beverages	Up to 100

Appendix 3

EFSA's list of uses of ethyl lauroyl arginate.

Food categories	Ethyl lauroyl arginate (ppm)
Flavourings for flavoured water	250 in the flavourings with a maximum limit of 5 ppm in the finished product
High energy drink	55
Dried salted fish of the "Gadidae" species/salted, dried fish	225
Meat products: heat treated, marinated or dried meat products	225
Savoury toppings or fillings	225
Rehydrated legumes	225
Fish roe products	225
Prepared salads	225
Processed cheese, prepacked and sliced cheese, unripened cheese, blue cheese, surface ripened cheese	115
Surface treatment of hard cheese, semi-hard and semi-soft cheese	1 mg/cm^2
Fruit preparations used in fruit containing dessert	225, with a maximum limit of 22.5 ppm in the finished product