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

Supporting document 1

Risk and technical assessment report – Application A1117

Extension of Use of L-cysteine as a Food Additive

Executive summary

Food Standards Australia New Zealand (FSANZ) received an Application from Link Trading (Qld) Pty Ltd seeking approval for extension of use of L-cysteine monohydrochloride (hereafter referred to as L-cysteine) as a food additive. The Applicant is seeking approval for the use of L-cysteine on peeled and/or cut avocado and banana at levels consistent with good manufacturing practice (GMP). L-Cysteine is intended for use in reducing enzymatic browning of fresh cut/peeled avocado and banana and so extend their shelf-life.

In the *Australia New Zealand Food Standards Code* (the Code), L-cysteine is currently permitted for use in accordance with GMP as a food additive to treat root and tuber vegetables that are peeled, cut, or both peeled and cut. In addition, L-cysteine is a permitted processing aid with the technological purpose of dough conditioner. L-Cysteine is also a required nutrient for infant formula and follow-on formula products and is permitted to be added to formulated supplementary sports foods.

Efficacy studies provided show that dipping peeled and cut avocado and banana pieces in solutions of L-cysteine reduces enzymatic browning to an extent that results in substantial increases in shelf-life. The proposed use of L-cysteine is therefore considered to be technologically justified.

L-Cysteine is an amino acid which occurs widely in dietary proteins. In a normal diet, amino acids are ingested as components of food proteins and not as free amino acids. Based on the amino acid composition of soy bean protein, an intake of 100 g protein per day is equivalent to an L-cysteine intake of 2.2 g/day. When given as a chronic nutritional supplement (in the form of *N*-acetylcysteine), typical doses range from 300 to 600 mg/day, with up to 2400 mg/day used in the treatment of certain conditions. No evidence of adverse effects has been reported at these levels of supplementation. Any additional dietary exposure to L-cysteine resulting from the requested extension of use is expected to be negligible in comparison to L-cysteine intake from the consumption of dietary protein.

It is concluded that evidence submitted in support of this Application provides adequate assurance that L-cysteine fulfils the stated technological function to reduce enzymatic browning of cut/peeled avocado and banana, and there are no identifiable public health and safety concerns associated with the proposed use.

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1 Introduction

Food Standards Australia New Zealand (FSANZ) received an Application from Link Trading (Qld) Pty Ltd seeking approval for extension of use of L-cysteine monohydrochloride (hereafter referred to as L-cysteine unless the monohydrochloride salt needs to be referred to) as a food additive. The Applicant is seeking approval for the use of L-cysteine on peeled and/or cut avocado and banana at levels consistent with good manufacturing practice (GMP). L-Cysteine is intended for use in reducing enzymatic browning of fresh cut/peeled avocado and banana and so extend their shelf-life.

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1.1 Objectives of the risk and technical assessment

The objectives of this risk and technical assessment are to assess whether the addition of L-cysteine to cut/peeled avocado and banana is technologically justified and whether addition of L-cysteine to cut/peeled avocado and banana presents any public health and safety concerns. The following key questions have been posed:

1. Does the use of L-cysteine achieve its stated technological function in the form and quantity proposed as a food additive to cut/peeled avocado and banana?

2. Are there any public health and safety concerns associated with the use of L-cysteine as a food additive to cut/peeled avocado and banana?

2 Food technology assessment

2.1 Current permissions

L-Cysteine monohydrochloride is currently permitted in the Code as a permitted food additive within the table to section S15—5 to treat root and tuber vegetables that are peeled, cut, or both peeled and cut at Good Manufacturing Practice (GMP) in food subcategory 4.1.3.2. It would appear the technological purpose of the food additive is as an antioxidant, to limit browning but the Code does not state what technological purpose individual food additives are performing when they are listed in the Code.

L-Cysteine is also a required L-amino acid nutrient for infant formula and follow-on formula products (section 2.9.1—10, which references the table to section S29—6) and a permitted amino acid that may be added to formulated supplementary sports foods (paragraph 2.9.4—3(1)(b) which references the table to section S29—18).

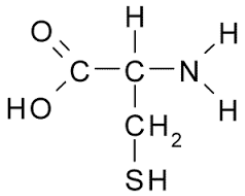
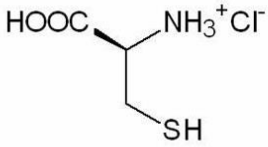
L-Cysteine (or the HCl salt) is also a permitted processing aid, with the technological purpose of dough conditioner being listed in section S18—9 (Permitted processing aids, various technological purposes).

2.2 Chemical and physical properties

The chemical structures of L-cysteine and L-cysteine monohydrochloride are provided in Table 1. L-Cysteine is commercially available as the monohydrochloride, which means the molecule also includes one molecule of hydrochloric acid (HCl). As well the substance can include one water molecule (H_2O) or be anhydrous (no water molecules).

The chemical and physical properties of the substance are summarised in Table 1.

Table 1 Chemical and physical properties of L-cysteine and L-cysteine monohydrochloride (Food Chemicals Codex 2014)

Property	**L-cysteine**	**L-cysteine monohydrochloride**
Alternative names	L-2-amino-3-mercaptopropanoic acid	L-2-amino-3-mercaptopropanoic acid monohydrochloride
IUPAC[1] name	(2R)-2-amino-3-sulfanylpropanoic acid	(2R)-2-amino-3-sulfanylpropanoic acid monohydrochloride
Molecular formula	$C_3H_7NO_2S$	$C_3H_7NO_2S.HCl$ (anhydrous) $C_3H_7NO_2S.HCl.H_2O$ (monohydrate)
Molecular structure		
Food additive number (INS)	920	920
Molecular weight g/mol	121.16	157.62 (anhydrous) 175.63 (monohydrate)
CAS registry number	52-90-4	52-89-1 (anhydrous) 7048-04-6 (monohydrate)
Appearance	White (colourless) crystals	White crystalline powder
Solubility	Soluble in water and alcohol, 16 g/100 ml at 20°C in water	Soluble in water and alcohol
Melting point	240°C, decomposes	175°C (anhydrous form melts with decomposition)

2.3 Analytical method of detection

L-Cysteine is an amino acid and the analysis of amino acids is well developed with well-established methods available to measure amino acids. It is also noted that L-cysteine is an already permitted food additive and nutrient in the Code.

2.4 Manufacturing process

There are a number of ways to commercially manufacture the food additive L-cysteine; some methods source the raw material from natural sources such as feathers or hair before further processing steps are undertaken. The Applicant confirmed that the L-cysteine it uses is

[1] IUPAC International Union of Pure and Applied Chemistry

synthetically produced and not from natural sources.

2.5 Specifications

Subsection 1.1.1—15(2) requires that a substance used as a food additive (paragraph 1.1.1—15(1)(a)) must comply with a relevant specification in Schedule 3 – Identity and purity. Food Chemicals Codex, which is a primary source of specifications, contains a specification for L-cysteine monohydrochloride (paragraph S3—2(1)(c) in Schedule 3). Therefore no specification is required to be included in Schedule 3. If this Application is successful then the commercial preparation of L-cysteine used to treat food products would need to comply with the identity and purity requirements of this specification.

2.6 Stability

The data in the Application, as well as in the technical literature, indicates that L-cysteine is stable and functional for the proposed purpose. That is to limit enzymatic browning when peeled and cut fruit, including avocados and bananas, are dipped into an aqueous solution containing it and the treated fruit pieces are stored at refrigeration temperature.

2.7 Technological purpose

The technological purpose of the substance as a food additive for the proposed use is similar to that for the already permitted purpose (limit browning and so act as an antioxidant). But it is different to that of its technological purpose as a permitted dough conditioner processing aid.

The purpose of the Application is to use L-cysteine as a food additive to control enzymatic browning on peeled and cut avocados and bananas and so extend their commercial shelf life (between 7−11 days when stored at 4°C, see Table 1). A limitation for commercial sale of peeled and cut fruit (or vegetables) is the browning of the exposed surfaces due to oxidation. Such browning of the surfaces is unacceptable to consumers and is a commercial limitation of the shelf life of such products. Consumers select fruit products, including peeled and cut pieces, on their physical appearance, including colour.

Enzymatic browning is discolouration of fruit and vegetables (most likely to occur when the food product has been peeled and cut to remove the protective outer skin and allow oxygen access to the now exposed surface). It is the outcome from the reaction of a group of enzymes, called polyphenol oxidases, which naturally occur in many fruits and vegetables. Enzymatic browning is a major cause of food deterioration; second only to microbiological contamination for food spoilage and wastage.

Polyphenol oxidases include the enzymes polyphenol oxidase and peroxidase. Polyphenol oxidase catalases the oxidation of phenols to diphenols, while peroxidase catalyses the oxidation to *o*-quinones. The produced *o*-quinones are reactive species that readily polymerise to form larger molecules termed melanins which are the cause of the brown discolouration. The phenols and diphenols also naturally occur in fruit and vegetable cells, and especially when the cells are damaged during processing (peeling and cutting) and are exposed to oxygen in the atmosphere.

The enzymatic browning reaction schematic is provided in the Application, taken from Laurila et al. (1998) and provided in Figure 1.

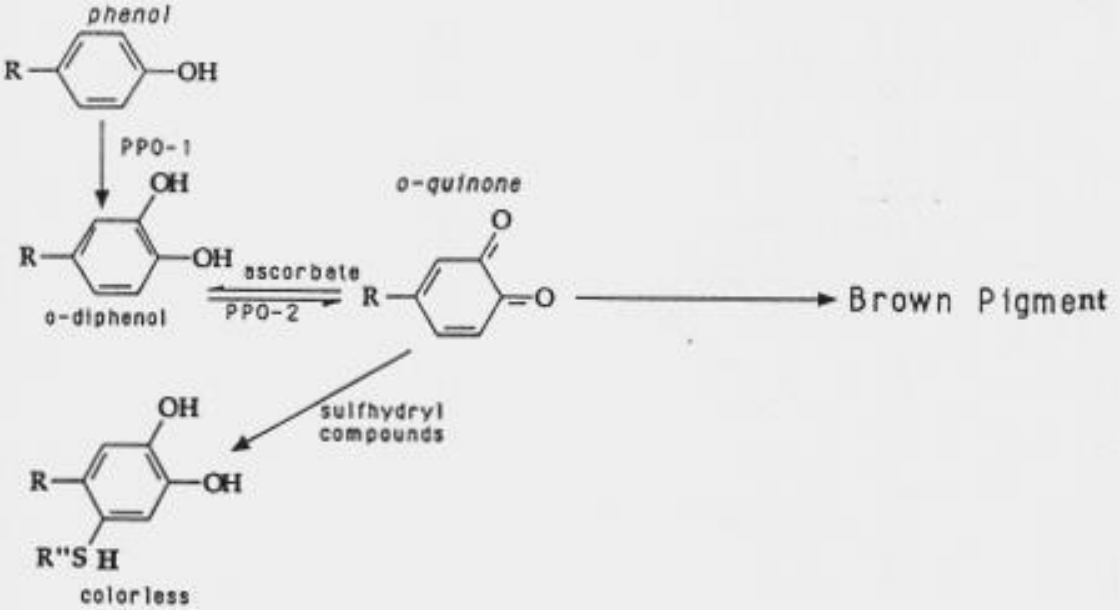


Figure 1: Reaction schematic of enzymatic browning (taken from the Application as amended from Laurila et al. 1998)

There are a limited number of permitted treatments (chemical and physical) that reduce the rate of enzymatic browning that occurs in peeled and cut fruits. The Application lists the advantages and disadvantages of possible treatments identified. These include physical treatment such as temperature adjustment (heating, or alternatively cooling), modification of storage gases (modified atmosphere packaging), high pressure processing and gamma radiation. There are a number of disadvantages of these techniques for treating peeled and cut avocados and bananas which include not being suitable to produce commercially acceptable product, being too expensive and not being permitted.

Chemical options include antioxidant food additives such as ascorbic acid, erythorbic acid (iso-ascorbic acid), citric acid and glutathione. Such food additives either do not provide the shelf life extension required or would need to be used in such high concentrations to produce unacceptable flavour impacts. Sulphur dioxide and various sulphites were also considered as they do limit enzymatic browning. However, there has been a worldwide regulatory approach to reduce the use of sulphites to treat food, noting also that a number of consumers have intolerance reactions to sulphites. Sensitive consumers could also perceive that the fruit has been treated with sulphites due to the strong odour sulphur dioxide (the active substance) has and this could likely produce negative consumer perceptions compared to fresh fruit.

As explained in the Application, the purpose of L-cysteine is to react with the *o*-quinones intermediates in the enzymatic browning process to form other non-coloured substances and not lead to the formation of the brown coloured melanins. This means the food additive is performing the technological purpose of an antioxidant (definition in section S14—2, technological purposes in Schedule 14 – Technological purposes performed by substances used as food additives; antioxidant, “retards or prevents the oxidative deterioration of a food”). The Application proposes using L-cysteine as an antioxidant and calcium chloride as a firming agent in a dipping solution (with or without ascorbic acid) to treat peeled and cut avocados and bananas to limit enzymatic browning.

Data provided in the Application and supporting references indicated that shelf life extensions can be obtained by the use of such dipping solutions.

2.7.1 Avocados

Such treatment provides shelf life extension of between three (diced product) to seven days (halves, skin on or off) at 4°C for peeled and cut avocados using a concentration of 2.5% L-cysteine or nine to eleven days using 5% solution.

The summary of trial results for avocados reported in the Application is provided in Table 2.

Table 2: Summary results for treatment of peeled and cut avocados using L-cysteine (and calcium chloride)

Product	Shelf life - control untreated (days)	Treatment (L-cysteine % w/v)	Shelf life - treated (days[1])	Shelf life extension (days[1])
Halves (skin off)	4	2.5%	11	7
Halves (skin on)	4	2.5%	11	7
Slices (5 mm)	4	2.5%	9	5
Diced (15 mm)	3	2.5%	6	3
Halves (skin off)	4	5.0%	13	9
Halves (skin on)	4	5.0%	13	9
Slices (5 mm)	4	5.0%	15	11
Diced (15 mm)	3	5.0%	14	11

1. Storage at refrigeration temperature (4°C)

2.7.2 Bananas

The results for peeled and cut bananas were reported in the Application to indicate that untreated peeled and cut bananas were unacceptable after 8 days storage while treated samples were acceptable though with some browning in the centre of the slices.

Dipping peeled and cut bananas in a solution containing L-cysteine (0.5% (w/v)), calcium chloride (1.0% w/v) and ascorbic acid (1.0% w/v) prevented enzymatic browning and softening up to 6 days stored at 5°C. This was increased to 7 days if the cysteine concentration was increased to between 0.5–1.0%. The shelf life of control, untreated samples was less than two days (Vilas-Boas and Kader 2006).

Further research work also using dipping solutions containing L-cysteine (0.75% w/v), calcium chloride (1.0% w/v) and ascorbic acid (0.5% w/v) on peeled and cut bananas indicated using this solution was the most effective treatment investigated in limiting enzymatic browning and retarding softening. The shelf life of such treated slices was five days at 5°C, compared to less than two days for control untreated product (Bico et al. 2010).

Both calcium chloride (INS 509) and ascorbic acid (INS 300) are additives permitted at GMP, being listed in the tables to section S16—2. The food category 4.1.3 (Fruits and vegetables that are peeled, cut, or peeled and cut) within the table to section S15—5 permits additives permitted at GMP, so both these two food additives are permitted for such food products.

2.8 Food technology conclusion

An analysis of the testing results performed by dipping peeled and cut avocado and banana pieces in a solution containing L-cysteine concludes that it reduces enzymatic browning and so extends the commercial shelf life of such products at refrigeration temperatures compared to untreated product. L-Cysteine may be used in solution with calcium chloride and ascorbic acid to limit enzymatic browning and maintain fruit firmness by limiting softening. The assessment

concludes that L-cysteine performs the technological purpose of an antioxidant for the proposed purpose.

3 Hazard Assessment

L-Cysteine is an amino acid which occurs widely in dietary proteins. In a normal diet, amino acids are ingested as components of food proteins and not as free amino acids. EFSA (2008) stated that an intake of 100 g protein per day is not an unusual intake for an adult European individual. Based on the amino acid composition of a typical protein such as soy bean protein, an intake of 100 g protein would amount to an intake of 2.2 g cysteine (EFSA 2008). When given as a chronic nutritional supplement (in the form of *N*-acetylcysteine), typical doses range from 300 to 600 mg/day, with up to 2400 mg/day used in the treatment of certain conditions (van der Poll 2006). No evidence of adverse effects has been reported that could be ascribed to *N*-acetylcysteine at these levels of supplementation. Oral ingestion of L-cysteine hydrochloride in aqueous solution (the form relevant to this Application) will result in systemic absorption of the free amino acid, as is the case for L-cysteine exposure resulting from ingestion of dietary protein or *N*-acetylcysteine as a nutritional supplement.

4 Dietary Exposure Assessment

A dietary exposure assessment was not conducted because any additional dietary exposure to L-cysteine resulting from the requested extension of use is expected to be negligible in comparison to L-cysteine intake from the consumption of dietary protein.

5 Risk Characterisation

Intake of L-cysteine from the normal consumption of dietary protein can exceed 2 g per day. Similar levels of intake resulting from nutritional supplementation have not been associated with adverse effects. Any additional dietary exposure to L-cysteine resulting from its use as a food additive on cut/peeled avocado and banana presents no identifiable public health and safety concerns.

6 Risk and technical assessment conclusions

This risk and technical assessment evaluated the technological suitability and safety of the proposed addition of L-cysteine to cut/peeled avocado and banana.

6.1 Reponses to risk and technical assessment questions

1. ***Does L-cysteine achieve its stated technological function in the form and quantity used as a food additive on cut/peeled avocado and banana?***

Section of report	***Summary response/conclusion***
Section 2	Evidence submitted in support of this Application provides adequate assurance that L-cysteine fulfils the stated technological function to reduce enzymatic browning of cut/peeled avocado and banana.

2. Are there any public health and safety concerns associated with the use of L-cysteine as a food additive on cut/peeled avocado and banana?

Section of report	*Summary response/conclusion*
Section 3, 4 and 5	There are no identifiable public health and safety concerns associated with the proposed use of L-cysteine as a food additive on cut/peeled avocado and banana.

7 References

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