GELITA AUSTRALIA PTY LTD

Additional Submission for the use of L-Cysteine as a food additive

In response to application A1117

[redacted]

8/8/2016

In response to Application A1117 made by Link Trading (QLD) Pty Ltd, Gelita Australia calls to extend the use of L-Cysteine monohydrochloride (hereafter referred to as L-Cysteine) as a food additive in gels, gummies and liquids containing reducing sugars, protein and/or peptides.

INTRODUCTION

1.1 Organisation

Gelita Australia Pty Ltd- Gelatine Manufacturer

1.2 Authorised By

Josh Hemelaar- Vice President Sales China/APA

1.3 The Submission

In response to Application A1117 made by Link Trading (QLD) Pty Ltd, Gelita Australia Pty Ltd calls to extend the use of L-Cysteine monohydrochloride (hereafter referred to as L-Cysteine) as a food additive in gels, confectionery and liquids containing reducing sugars, protein and/or peptides. As indicated by the application A1117 L-Cysteine controls enzymatic browning but Gelita Australia Pty Ltd recommends that L-cysteine also inhibits non-enzymatic browning. The addition of L-Cysteine has been shown to inhibit the Maillard reaction in products containing reducing sugars, protein and/or peptides. For example, this application of L-cysteine could be used in confectionery items, gelatine containing gels and desserts. *See appendix 4 for an example of a product patent that would require L-Cysteine to be viable, currently the product is unfavourable due to discolouration caused by the Maillard reaction.*

1.4 The Current Standard

See Appendix 1 point 1.3. There are no permissions in the current standard to use L-cysteine monohydrochloride as a food additive in gels, gummies and liquids containing reducing sugars, protein and/or peptides.

1.5 International and National Standards

See Appendix 1 Section 1.3.1

2.1 Risk Assessment

See Appendix 1 Section 2.1. At this point in time there are no allowances made for the use of L-Cysteine for the aforementioned purposes (*See Appendix 1 Section 1.3*). Given the low dosage that would be required and the finding of the Risk and Technical Support document for application A1117 Section 5 showing that a normal dietary intake of L-Cysteine can exceed 2g

per day *(See Appendix 2)* Gelita Australia Pty Ltd proposes that there would be no risk to public health and safety.

2.2 Labelling Requirements

As indicated in *Appendix 1 Section 2.2.2 and described in section1.2.4-7 in Standard 1.2.4* of the Food Code.

2.2.3 Specifications

See Appendix 1 Section 2.2.3

2.2.4 Analytical Methods

See Appendix 1 Section 2.2.4

Discussion

Gelita Australia seeks permission to use L-cysteine as a food additive in products containing reducing sugars and proteins for confectionary items, desserts and gels- specifically products containing mixtures of gelatines, collagens and sugars.

It is required that the Maillard reaction (non-enzymatic browning) be controlled to ensure integrity and shelf stability of products created. This involves controlling/inhibiting the amount of Maillard reaction products (MRPS) Kwak et al (2003) formed. During the Maillard reaction MRPs are formed, including the dark-brown polymeric compound Melanoidin Wijewickreme (1997). By inhibiting the formation of these MRPs it is possible to control the discolouration in products containing protein and reducing sugars.

There have been some reports about the ability of sulphur-containing compounds, such as L-Cysteine to be a successful means of inhibition of browning Friedman et al (1990). As such initial testing was commenced by Gelita AG in Eberbach to discern if such application of L-Cysteine would be applicable for the aforementioned purposes. The addition of L-cysteine was successful in the prevention of discolouration *(see Appendix 3)*. At this time any results regarding prevention of crosslinking and inhibition of toxic compound formation is conjecture as analysis is still in its preliminary stages.

In addition to this, multiple publications are available that support the claim that L-Cysteine can successfully inhibit non-enzymatic browning including *Appendix 5 "Study of Maillard Reaction Inhibitors For The sugar Cane Industry"* (*Pacheco et al,2012*) and *Appendix 6 "Inhibition of Browning by Sulphur Amino Acids" (Friedman et al, 1990).*

Pacheco et al 2012 states that the Maillard reaction produces Melanoidins that create an unacceptable dark colour and as such looks at potential inhibitors including L-Cysteine. In this study glucose and amino acids were mixed together at a rate of 0.05%, 0.1% and 0.15%, pH was adjusted with NaHO and results analysed.

Figure 1 (Pacheco et al., 2012) indicates that the addition of L-Cysteine was successful in reducing the quantity of MRPs (The soluble matter is expressed °Bx and indicates the quantity of MRPs as macromolecules and particles). L-Cysteine showed greater effect in the formation of these particles than the other inhibitors.

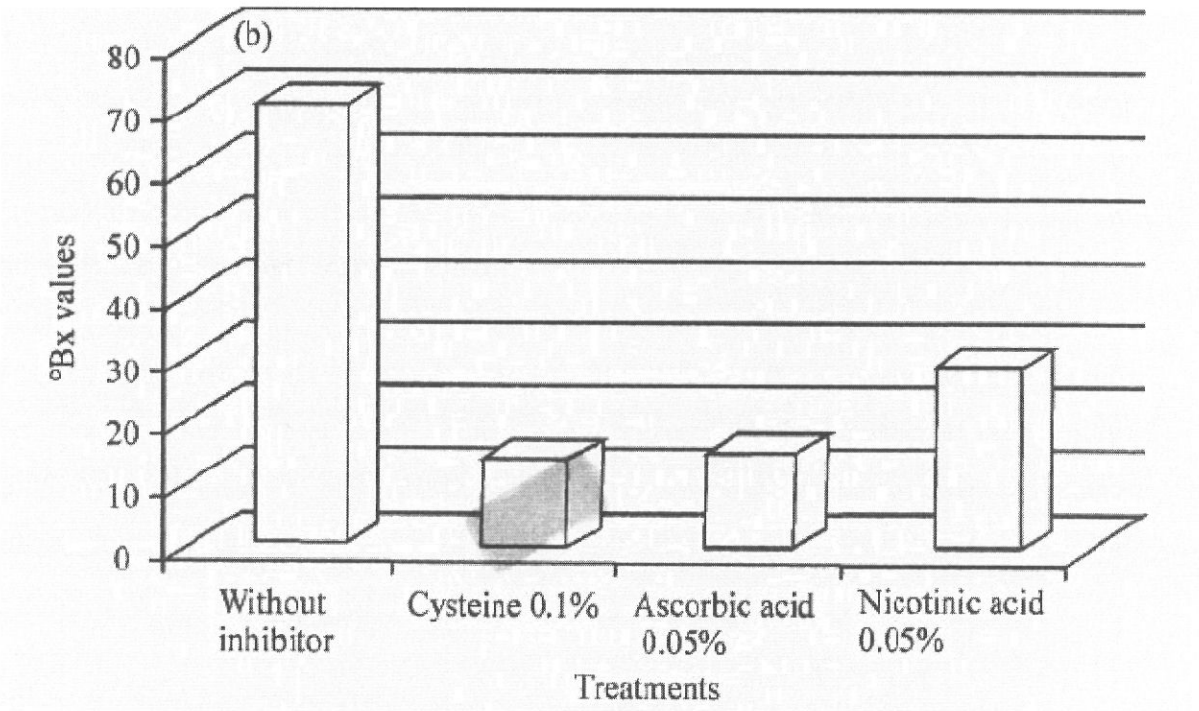

Figure 1 Inhibition of browning using different additives based on measurement of MRPs (Pacheco et al., 2012).

Figure 2 Demonstrates the effect the trialled inhibitors have on colour formation. L-Cysteine again was shown to have the greatest positive effect on colour formation from the Maillard reaction.

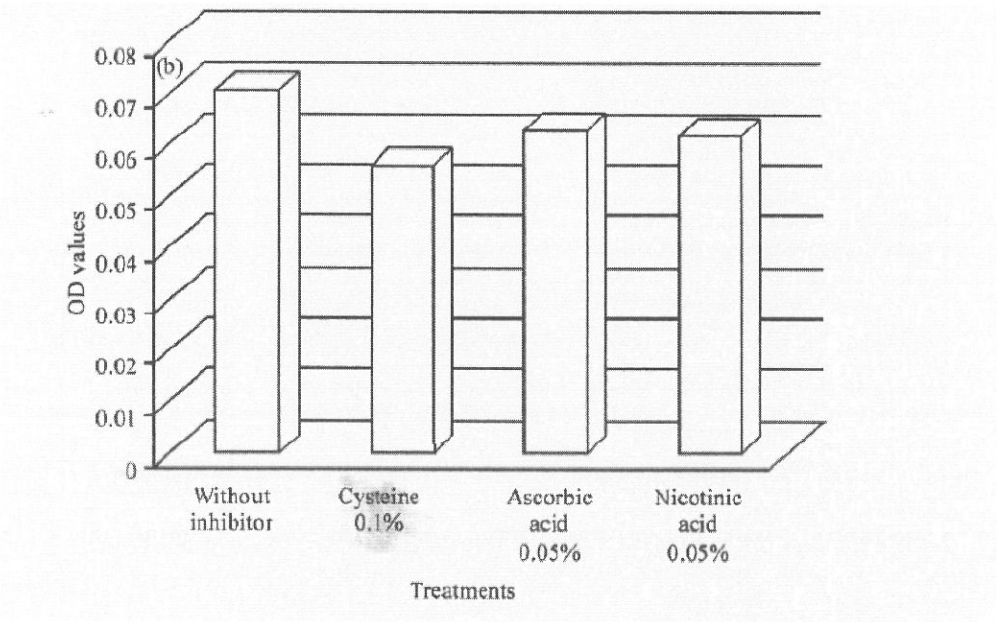

Figure 2 *Inhibitor effects on colour* (Pacheco et al.,2012)

This study, based on the above results concluded that L-Cysteine provided a high level of inhibition and was successful in reducing the amount of MRPs produced.

Friedman et al (1990) examined sulphur amino acids and sulphur rich proteins and their effect on inhibiting browning of amino-glucose system. One notable finding was the ability of these sulphur amino acids (including L-Cysteine) to prevent the formation of anti- nutritional and toxic browning products by trapping intermediates. Figure 5 represents the results from the study regarding the effectiveness of the inhibitors on browning reactions. It was noted that varying concentrations were required for each inhibitor.

Table III. Effectiveness of Inhibitors on Browning Reactions (I–IV) of D-Glucose with β-Alanine (I), N^{α}-Acetyl-L-lysine (II), a Mixture of Amino Acids (III), and Glycylglycine (IV)

reaction	inhibitors	IP,[a] %	stoichiometry of inhibition,[b] mol of inhibitor/mol of MP
I	*N*-acetyl-L-cysteine	70	0.2
II	*N*-acetyl-L-cysteine	83	0.4
III	*N*-acetyl-L-cysteine	91	2.0
IV	*N*-acetyl-L-cysteine	89	0.4
I	L-cysteine	79	0.05
II	glutathione	83	0.08
I	sodium bisulfite	79	0.02
II	sodium bisulfite	96	0.12
III	sodium bisulfite	74	0.16
IV	sodium bisulfite	91	0.05
I	urea	91	12
II	urea	88	25
III	urea	95	12
IV	urea	89	12

[a] Index of prevention (IP) = 100 – (molar absorptivity value of the amine compound + glucose + inhibitor) × 100/(molar absorptivity value of the amine compound + glucose). [b] Minimum mole ratios needed to achieve the corresponding IP value. MP, Maillard reaction product precursor.

Figure 5 Effectiveness of different inhibitors on browning reactions

Along with the aforementioned studies and evidence supplied by GELITA AG, it can be concluded that L-Cysteine is of practical value in preventing browning caused by the Maillard reaction in a variety of food products.

References

Wijewickreme AN, Kitts DD, Durance TD (1997) *"Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard products"*. Journal of Agricultural Food Chemistry, 45:4577-4583

Pacheco MD, Christian JI, Feng B (2012) *"Study of Maillard Inhibitors for the Sugar Cane Processing Industry"*. American Journal of Food technology, IISN 1557-4571:470- 478

Friedman M, Molnar-Perl I (1990) *"Inhibition of Browning by Sulphur Amino Acids. 1. Heated Amino Acid-Glucose Systems"*. American Chemical Society, 39:1642-1647

Kwak EJ, Lim SI (2003) *"The effect of sugar, amino acid, metal ion, and NaCl on Model Maillard reaction under pH control"*. Amino Acids, 27:85-90

Appendix 1

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

29 June 2016
[16–16]

Call for submissions – Application A1117

Extension of Use of L-Cysteine as a Food Additive

FSANZ has assessed an Application made by Link Trading (Qld) Pty Ltd to extend the use of the food additive, L-cysteine, to limit enzymatic browning of peeled and cut avocado and banana and so extend the shelf life and has prepared a draft food regulatory measure. Pursuant to section 31 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act), FSANZ now calls for submissions to assist consideration of the draft food regulatory measure.

For information about making a submission, visit the FSANZ website at information for submitters.

All submissions on applications and proposals will be published on our website. We will not publish material that is provided in-confidence, but will record that such information is held. In-confidence submissions may be subject to release under the provisions of the *Freedom of Information Act 1991*. Submissions will be published as soon as possible after the end of the public comment period. Where large numbers of documents are involved, FSANZ will make these available on CD, rather than on the website.

Under section 114 of the FSANZ Act, some information provided to FSANZ cannot be disclosed. More information about the disclosure of confidential commercial information is available on the FSANZ website at information for submitters.

Submissions should be made in writing; be marked clearly with the word 'Submission' and quote the correct project number and name. While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website via the link on documents for public comment. You can also email your submission directly to submissions@foodstandards.gov.au.

There is no need to send a hard copy of your submission if you have submitted it by email or via the FSANZ website. FSANZ endeavours to formally acknowledge receipt of submissions within 3 business days.

DEADLINE FOR SUBMISSIONS: 6pm (Canberra time) 10 August 2016

Submissions received after this date will not be considered unless an extension had been given before the closing date. Extensions will only be granted due to extraordinary circumstances during the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

Questions about making submissions or the application process can be sent to standards.management@foodstandards.gov.au.

Hard copy submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 5423
KINGSTON ACT 2604
AUSTRALIA
Tel +61 2 6271 2222

Food Standards Australia New Zealand
PO Box 10559
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Supporting document

The following document which informed the assessment of this Application is available on the FSANZ website at http://www.foodstandards.gov.au/code/applications/Pages/A1117-L-cysteineasaFA.aspx

SD1 Risk and Technical Assessment Report

Executive summary

Link Trading (Qld) Pty Ltd submitted an Application seeking to extend the permission for a currently permitted food additive, L-cysteine monohydrochloride, to treat peeled and/or cut avocados and bananas to control enzymatic browning and so extend their shelf life.

The table to section S15—5 in Schedule 15 – Substances that may be used as food additives in the *Australia New Zealand Food Standards Code* (the Code) contains permissions for food additives across different food categories.

L-Cysteine monohydrochloride is a permitted food additive for root and tuber vegetables (peeled, cut or both peeled and cut), but not for fruits.

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-acetyl cysteine), 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.

FSANZ's risk assessment concluded that there were no public health and safety concerns associated with the proposed extension of use of the food additive for the proposed purpose. The assessment also concluded that its use was technologically justified.

There is a primary source of specifications within Schedule 3 – Identity and Purity for L-cysteine monohydrochloride. The current labelling requirements in subsection 1.2.4—7 apply for ingredient labelling of products containing the food additive. L-cysteine is an amino acid and the analysis of amino acids is relatively well-developed, with well-established methods available.

FSANZ proposes the creation of a new sub subcategory of 4.1.3.3 (Avocados and bananas) to be added to the table to section S15—5 with a permission for L-cysteine monohydrochloride as a food additive to treat this food category at Good Manufacturing Practice (GMP).

1 Introduction

1.1 The Applicant

Link Trading (Qld) Pty Ltd is a supplier of raw materials to the food and beverage processing industry.

1.2 The Application

The Application seeks to extend the permissions for a currently permitted food additive, L-cysteine monohydrochloride (hereafter referred to as L-cysteine unless reference is required to monohydrochloride salt), to treat peeled and cut avocado and banana to control enzymatic browning and so extend their shelf life.

1.3 The current Standard

The table to section S15—5 in Schedule 15 – Substances that may be used as food additives) contains permissions for food additives across different food categories.

Food category 4 (Fruits and vegetables (including fungi, nuts, seeds, herbs and spices)) contains subcategory 4.1.3 (fruits and vegetables that are peeled, cut, or both peeled and cut). All the additives permitted at GMP (i.e. the food additives listed in the table to section S16—2), as well as sorbic acid and sodium, potassium and calcium sorbates, and ethyl lauroyl arginate are permitted to be added to these food products.

There are also further sub subcategories, being 4.1.3.1 (products for manufacturing purposes) which has permissions for the sulphur dioxide and various sulphites, but only to treat processed apples and potatoes and the sub subcategory of root and tuber vegetables, which also has permissions for sulphur dioxide and various sulphites and L-cysteine monohydrochloride.

The terms L-cysteine and L-cysteine monohydrochloride are used interchangeably throughout this report, as the monohydrochloride salt is the usual permitted form of L-cysteine.

Food additive permissions from the table to section S15—5 for subcategory 4.1.3 are:

4.1.3	**Fruits and vegetables that are peeled, cut, or both peeled and cut**		
	Additives permitted at GMP		
200 201 202 203	Sorbic acid and sodium, potassium and calcium sorbates	375	
243	Ethyl lauroyl arginate	200	
4.1.3.1	***Products for manufacturing purposes***		
220 221 222 223 224 225 228	Sulphur dioxide and sodium and potassium sulphites	200	Only apples and potatoes
4.1.3.2	***Root and tuber vegetables***		
220 221 222 223 224 225 228	Sulphur dioxide and sodium and potassium sulphites	50	
920	L-cysteine monohydrochloride	GMP	

There are no permissions to use L-cysteine monohydrochloride as a food additive for peeled, cut, or both peeled and cut avocado and banana since neither fruit is a root or tuber vegetable.

L-Cysteine (or the hydrochloride salt) is also a permitted processing aid used as a dough

conditioner up to a maximum level of 75 mg/kg. This permission is listed in the table to section S18—9 (Permitted processing aids – various technological purposes) of Schedule 18 – Processing Aids. The Code regulates the substance as a processing aid and not a food additive since it performs the technological purpose during the manufacture of the food. That is, it is used during the conditioning of the dough as part of the manufacturing process for bread and baked goods, and does not have a technological purpose in the final baked food.

This permission for dough conditioning is similar to that listed in Codex Alimentarius (see section 1.3.1 below), the United States of America (USA) (see section 1.3.1.1) and the European Union (EU) (section 1.3.1.3 below), except the other regulations permit the substance as a food additive not a processing aid. The Code has a specific processing aid Standard and processing aid permissions which differ from how Codex, the USA and Europe regulate processing aids.

1.3.1 International and National Standards

There are limited international and national permissions for the use of L-cysteine monohydrochloride.

L-Cysteine and its hydrochloride and sodium and potassium salts has the Codex Alimentarius International Number System (INS) of 920 and function class and technological purpose of flour treatment agent. This information is obtained from the Codex Standard CAC/GL 36-1989 (Class names and the international numbering system for food additives).

L-cysteine monohydrochloride has a specification in the Food Chemicals Codex (9th edition) but not in the Joint WHO/FAO Expert Committee for Food Additives (JECFA).

1.3.1.1 The USA

L-Cysteine as a nutrient amino acid is permitted to be added to foods in accordance with the conditions in section 172.320 of the Code of Federal Regulations (CFR), Title 21.

There is also permission in the CFR for the use of both L-cysteine (§184.1271) and L-cysteine monohydrochloride (§184.1272) as food additives with the technological purpose of dough strengthener in yeast-leavened baked goods and baking mixes. The permission is for 0.009 part of total L-cysteine per 100 parts of flour in dough (i.e. 90 mg/kg, parts per million (ppm)).

1.3.1.2 Canada

The Canadian Food and Drug Regulations Division 16, Table XI, Part IV permits the use of L-cysteine hydrochloride as a food additive sulphite replacement formulation for prepared fruits and vegetables consistent with Good Manufacturing Practice.

This use is similar to that proposed by the Application.

1.3.1.3 EU

L-Cysteine is permitted as a food additive in the EU for use in two types of food categories within the Commission Regulation (EU) No 1129/2011. They are:

- flours and other milled products and starches (category number 06.2.1) at level of *quantum satis* (comparable to GMP in the Code)

- processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC (food category 13.1.3). A maximum permit limit of

1000 mg/kg applies for biscuits for infants and young children.

1.3.1.4 Japan

L-Cysteine monohydrochloride is permitted as a food additive in Japan, as mentioned in Table 1 in Article 12 of the Food Sanitation Law Enforcement Regulations.

This listing does not detail how the food additive may be used.

1.3.1.5 Singapore

L-Cysteine is a permitted flavour enhancer under paragraph 23 – (2)(d) of the Food Regulations of the Agri-Food & Veterinary Authority of Singapore.

1.4 Reasons for accepting Application

The Application was accepted for assessment because:

- it complied with the procedural requirements under subsection 22(2) of the FSANZ Act
- it related to a matter that warranted the variation of a food regulatory measure.

There currently are permissions for the use of L-cysteine monohydrochloride as a food additive in the Code. This Application is seeking an extension of use of the food additive by requesting an amendment to the Code, being a variation of a food regulatory measure.

1.5 Procedure for assessment

The Application is being assessed under the General Procedure.

2 Summary of the assessment

2.1 Risk assessment

FSANZ conducted a risk assessment on the extension of use of L-cysteine which is provided as SD1. The conclusions of this assessment are provided below.

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.

The food technology assessment concluded that L-cysteine performs the technological purpose of an antioxidant for the proposed purpose of treating peeled and cut avocado and banana pieces by reducing enzymatic browning. The fruit pieces are dipped into an aqueous solution containing L-cysteine, which extends the shelf life of such products stored at refrigeration temperature compared to untreated product.

2.2 Risk management

The conclusion of the risk assessment (section 2.1 and SD1) is that the extension of use of

L-cysteine for the proposed purpose is both safe and technologically justified. There are, however, a number of risk management issues to consider; specifically how to add permissions into the Code and labelling and specification aspects of the Application.

2.2.1 Draft amendments to the Code

The Application has requested approval for L-cysteine monohydrochloride as a food additive to treat only two types of fruits that are peeled and/or cut, being avocados and bananas. As noted in section 1.3, there is a food subcategory 4.1.3 (Fruits and vegetables that are peeled, cut, or both peeled and cut) within section S15—5 which details food additive permissions for different food categories.

There are two further sub subcategories being 4.1.3.1 (products for manufacturing purposes) and 4.1.3.2 (Root and tuber vegetables) which are both not applicable for the requested products. The hierarchical nature of food additive permissions in Schedule 15 means that if permissions are provided for in subcategory 4.1.3 for these particular foods, even with a qualification statement, possible misinterpretations could be made that L-cysteine monohydrochloride is then also permitted to treat food in both sub subcategories 4.1.3.1 and 4.1.3.2. Therefore it was proposed to create a new sub subcategory called 4.1.3.3 (Avocados and bananas) and provide permission for the food additive at GMP.

2.2.2 Labelling requirements

Substances used as food additives are required to be declared in the list of ingredients on the label of most packaged foods. Section 1.2.4—7 in Standard 1.2.4 – Information requirements – statement of ingredients requires food additives to be declared by their class name followed by the prescribed name, or code number in brackets.

Schedule 7 – Food additive class names (for statement of ingredients) provides the list of food additive class names for labelling purposes, while Schedule 8 – Food additive names and code numbers (for statement of ingredients) provides the lists of food additive names and code numbers. For the purposes proposed for this Application, FSANZ is proposing the class name 'antioxidant' be used for L-cysteine monohydrochloride, with either the prescribed food additive name 'L-cysteine monohydrochloride' or the code number '920'.

There are some exemptions to these requirements that apply to food for sale that is not required to bear a label. These exemptions are set out in Standard 1.2.1 - Requirements to have labels or otherwise provide information. The exemptions include whole or cut fresh fruit and vegetables (other than seed sprouts or similar products) in a package that does not obscure the nature or quality of the food, and food made and packaged on the premises from which it is sold. This means that L-cysteine monohydrochloride would not need to be declared if an exemption applies. FSANZ considers this approach to be appropriate, given that the risk assessment (refer to SD1) has concluded there are no public health and safety concerns associated with the use of L-cysteine monohydrochloride. This approach aligns with the approach taken for other permitted food additives.

2.2.3 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 under paragraph S3—2(1)(c), contains a specification for L-cysteine monohydrochloride. Therefore, no additional specification is required to be included in Schedule 3.

2.2.4 Analytical methods

L-Cysteine is an amino acid and the analysis of amino acids is relatively well developed with well-established methods available to measure amino acids.

2.3 Risk communication

FSANZ has developed a basic communication strategy for this Application.

2.3.1 Consultation

Consultation is a key part of FSANZ's standards development process. The process by which FSANZ considers standard development matters is open, accountable, consultative and transparent. Public submissions are called for to obtain the views of interested parties on the Application and the impacts of the regulatory options. All calls for submissions are notified via the FSANZ Notification Circular, media release, FSANZ's social media tools and Food Standards News.

The Applicant, individuals and organisations that make submissions on this Application will be notified at each stage of the assessment. Subscribers and interested parties are also notified via email about the availability of reports for public comment.

Following consultation, the FSANZ Board will consider the proposed variation taking into account comments received through submissions. If the draft variation to the Code is approved by the FSANZ Board, that decision will be notified to the Australia and New Zealand Ministerial Forum on Food Regulation (Forum). If the decision is not subject to a request for a review, the Applicant and stakeholders, including the public, will be notified of the gazettal of the variation to the Code in the national press and on the FSANZ website.

2.3.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obliged to notify WTO members where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are not any relevant international standards and amending the Code to permit L-cysteine monohydrochloride to treat peeled and cut avocado and banana is unlikely to have a significant effect on international trade as this is a voluntary food additive permission which would liberalise trade. Therefore, a notification to the WTO under Australia's and New Zealand's obligations under the WTO Technical Barriers to Trade or Application of Sanitary and Phytosanitary Measures Agreement was not considered necessary.

2.4 FSANZ Act assessment requirements

When assessing this Application and the subsequent development of a food regulatory measure, FSANZ has had regard to the following matters in section 29 of the FSANZ Act:

2.4.1 Section 29

2.4.1.1 Cost benefit analysis

FSANZ is required to consider the impact of various regulatory and non-regulatory options on all sectors of the community, especially relevant stakeholders.

The benefits and costs associated with the proposed amendments to the Code have been

considered based on regulatory impact principles. The level of analysis is commensurate to the nature of the Application and significance of the impacts.

The Office of Best Practice Regulation, in a letter dated 24 November 2010 (reference 12065), provided a standing exemption from the need to determine whether a Regulation Impact Statement is required for applications relating to food additives, as they are machinery in nature and their use is voluntary.

However, FSANZ has undertaken a limited qualitative impact analysis.

Two regulatory options have been considered:

(1) prepare a draft variation to the revised Code to permit L-cysteine monohydrochloride to treat peeled and cut avocado and banana
(2) reject the Application.

The likely impacts of these options were considered but this is not intended to be an exhaustive, quantitative economic analysis. Rather, the qualitative effects of each option are described below, and are deliberately limited to broad areas such as trade and consumer choice.

Option 1 – prepare a draft variation to the Code

Sector	Costs or benefits
Consumers	There are direct benefits to consumers as they would have access to fresh peeled and cut avocados and bananas for a longer period due to the use of this technology. There are no expected costs to consumers, unless companies charged a premium for such treated products. Consumers would potentially have access to a greater range of products with an extended shelf life.
Industry	There are benefits to the fresh peeled and cut avocados and bananas industry and retailers of such products who can offer a longer shelf life for these products to consumers. The only cost to these companies will be setting up processes to treat their product with this food additive before packaging, and the cost of the food additive itself and the uptake of this technology is optional.
Governments	There should be minimal costs to government agencies, to check or enforce whether the food additive has been used on product. The additive is already permitted for use on a range of other fresh fruits and vegetables. Some packaged products will require the usual ingredient labelling which includes reference to food additives used.

Option 2 – reject the Application

Sector	Costs or benefits
Consumers	There are no benefits to consumers. However there are costs as the shelf life of peeled and cut avocados and bananas will not be as long as it could be and product will turn brown earlier and so be discarded.
Industry	There are no benefits to the food industry but there are costs. The peeled and cut fresh fruit industry will not have access to new technology that can maintain the fresh appearance of peeled and cut avocados and bananas longer. Companies and retailers will need to discard product that shows signs of browning which is unacceptable to consumers earlier than they would need.
Governments	There would be no direct impacts on government agencies.

FSANZ considered that Option 1 to permit L-cysteine monohydrochloride to treat peeled and cut avocado and banana was the preferred option and has prepared a draft variation to the Code.

The direct and indirect benefits that would arise from a food regulatory measure developed or varied as a result of the application outweigh the costs to the community, Government or industry that would arise from the development or variation of the food regulatory measure.

2.4.1.2 Other measures

There are no other measures (whether available to FSANZ or not) that would be more cost-effective than a food regulatory measure developed or varied as a result of the Application.

2.4.1.3 Any relevant New Zealand standards

There are no relevant New Zealand Standards. Schedule 15 applies to both Australia and New Zealand.

2.4.1.4 Any other relevant matters

Other relevant matters are considered below.

2.4.2 Subsection 18(1)

FSANZ has also considered the three objectives in subsection 18(1) of the FSANZ Act during the assessment.

2.4.2.1 Protection of public health and safety

FSANZ has undertaken a safety assessment (SD1) and concluded there are no public health and safety concerns with permitting L-cysteine monohydrochloride as a food additive to treat peeled and cut avocado and banana.

2.4.2.2 The provision of adequate information relating to food to enable consumers to make informed choices

In accordance with existing labelling provisions for substances used as food additives, L-cysteine monohydrochloride would be required to be declared in the list of ingredients on the label of most packaged foods, unless there is an exemption that applies for food for sale that is not required to bear a label.

2.4.2.3 The prevention of misleading or deceptive conduct

No issues were identified for this Application relevant to this objective.

2.4.3 Subsection 18(2) considerations

FSANZ has also had regard to:

- **the need for standards to be based on risk analysis using the best available scientific evidence**

FSANZ has used the best available scientific evidence to conduct the risk analysis which is provided in SD1. The Applicant submitted a dossier of scientific studies as part of their Application. Other technical information including scientific literature was also used in assessing the Application.

- **the promotion of consistency between domestic and international food standards**

Section 1.3.1 details the current permissions for L-cysteine monohydrochloride in different countries. Permitting this Application will ensure consistency between the Code and other international food standards.

- **the desirability of an efficient and internationally competitive food industry**

Permitting L-cysteine monohydrochloride as a food additive to treat peeled and cut avocado and banana to extend the shelf life of such treated food products will improve and make such products more competitive and useful for consumers and so providing opportunities for interested companies.

- **the promotion of fair trading in food**

No issues were identified for this Application relevant to this objective.

- **any written policy guidelines formulated by the Forum on Food Regulation**

The Policy Guideline 'Addition to Food of Substances other than Vitamins and Minerals'[1] includes specific order policy principles for substances added to achieve a solely technological function, such as food additives. These specific order policy principles state that permission should be granted where:

- the purpose for adding the substance can be articulated clearly by the manufacturer as achieving a solely technological function (i.e. the 'stated purpose')
- the addition of the substance to food is safe for human consumption
- the amounts added are consistent with achieving the technological function
- the substance is added in a quantity and a form which is consistent with delivering the stated purpose
- no nutrition, health or related claims are to be made in regard to the substance.

FSANZ has determined that permitting L-cysteine monohydrochloride to treat peeled and cut avocado and banana is consistent with these specific order policy principles.

3 Draft variation

The draft variation to the Code is at Attachment A and is intended to take effect on gazettal.

A draft explanatory statement is at Attachment B. An explanatory statement is required to accompany an instrument if it is lodged on the Federal Register of Legislation.

Attachments

A. Draft variation to the *Australia New Zealand Food Standards Code*
B. Draft Explanatory Statement

[1] http://www.foodstandards.gov.au/code/fofr/fofrpolicy/pages/default.aspx

Attachment A – Draft variation to the *Australia New Zealand Food Standards Code*

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

Food Standards (Application A1117 – Extension of Use of L-cysteine as a Food Additive) Variation

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The Board of Food Standards Australia New Zealand gives notice of the making of this variation under section 92 of the *Food Standards Australia New Zealand Act 1991*. The variation commences on the date specified in clause 3 of this variation.

Dated [To be completed by Standards Management Officer]

Standards Management Officer
Delegate of the Board of Food Standards Australia New Zealand

Note:

This variation will be published in the Commonwealth of Australia Gazette No. FSC XX on XX Month 20XX. This means that this date is the gazettal date for the purposes of clause 3 of the variation.

1 Name

This instrument is the *Food Standards (Application A1117 – Extension of Use of L-cysteine as a Food Additive) Variation*.

2 Variation to a standard in the *Australia New Zealand Food Standards Code*

The Schedule varies a Standard in the *Australia New Zealand Food Standards Code*.

3 Commencement

The variation commences on the date of gazettal.

Schedule

[1] Schedule 15 is varied by adding the following to subcategory 4.1.3 in the table to section S15—5, in numerical order

4.1.3.3	***Avocados and bananas***	
920	L-cysteine monohydrochloride	GMP

Attachment B – Draft Explanatory Statement

1. Authority

Section 13 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) provides that the functions of Food Standards Australia New Zealand (the Authority) include the development of standards and variations of standards for inclusion in the *Australia New Zealand Food Standards Code* (the Code).

Division 1 of Part 3 of the FSANZ Act specifies that the Authority may accept applications for the development or variation of food regulatory measures, including standards. This Division also stipulates the procedure for considering an application for the development or variation of food regulatory measures.

FSANZ accepted Application A1117 which seeks to extend the permission of the food additive, L-cysteine, so as to permit its use for limiting enzymatic browning of peeled and cut avocado and banana and so extend the shelf life. The Authority considered the Application in accordance with Division 1 of Part 3 and has prepared a draft variation.

2. Purpose

The Code does not currently permit the use of the food additive L-cysteine monohydrochloride to treat fruit. The purpose of this instrument is to amend the table of permissions for food additives to permit the use of L-cysteine monohydrochloride to treat peeled, cut, or both peeled and cut avocado and banana. The food additive is used to prevent enzymatic browning of the cut surfaces which is unacceptable to consumers and so extend the shelf life of the treated food products.

3. Documents incorporated by reference

This variation to a food regulatory measure does not incorporate any documents by reference.

4. Consultation

In accordance with the procedure in Division 1 of Part 3 of the FSANZ Act, the Authority's consideration of Application A1117 will include one round of public consultation following an assessment and the preparation of a draft Standard and associated report.

A Regulation Impact Statement was not required because the proposed variation to Schedule 15 is likely to have a minor impact on business and individuals.

5. Statement of compatibility with human rights

This instrument is exempt from the requirements for a statement of compatibility with human rights as it is a non-disallowable instrument under section 94 of the FSANZ Act.

6. Variation

The variation amends the table to section S15—5 in Schedule 15 of the Code by adding new food sub subcategory 4.1.3.3. The new food sub subcategory provides permission for the use of L-cysteine monohydrochloride (INS 920) in avocados and bananas subject to a maximum permitted level of GMP (Good Manufacturing Practice).

Appendix 2

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.

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.

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).

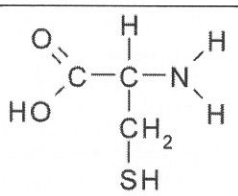
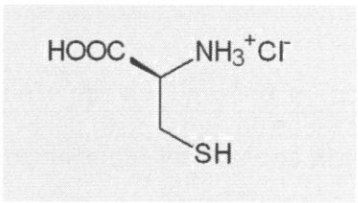
L-Cysteine (or the HCl salt) is also a permitted processing aid, with the technological purpose of dough conditioner being listed in section S18—9 (Permitted processing aids, various technological purposes).

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.

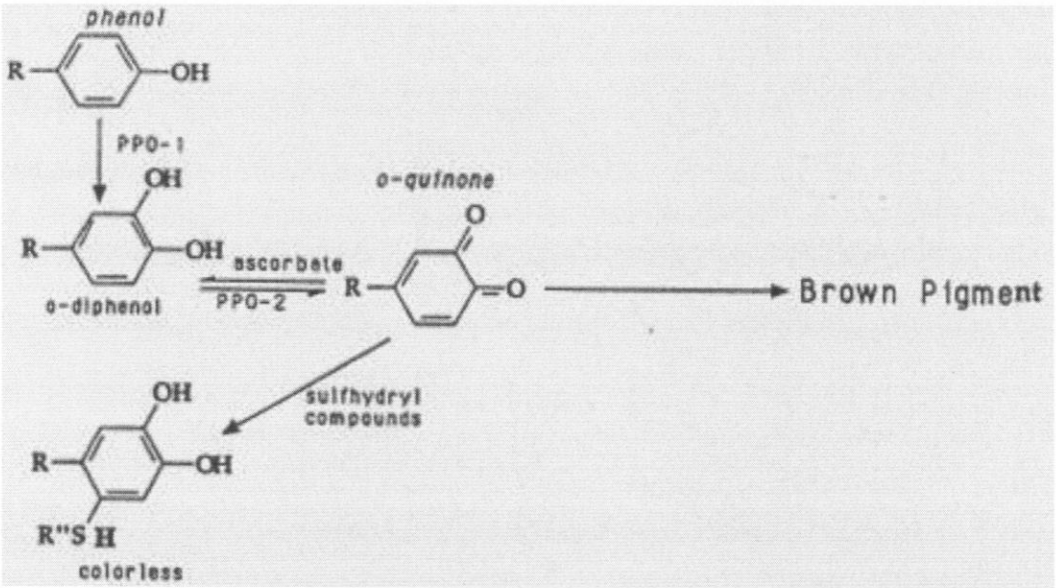


Figure 1: Reaction schematic of enzymatic browning (taken from the Application as amended from Laurila et al. 1998)

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

Bico SLS, de Jesus Raposo MF, de Morais RMSC, de Morais AMMB (2010) Chemical dips and edible coatings to retard softening and browning of fresh-cut banana. *Int J Postharvest Technology and Innovation*, **2**(1):13–24.

Codex (2015) Codex Alimentarius. CAC/GL 36-1989 Class names and the International numbering system for food additives. Amendment 2015
http://www.fao.org/input/download/standards/13341/CXG_036e_2015.pdf

EFSA (2008) Amino acids from chemical group 34. Flavouring Group Evaluation 26, Revision 1. Scientific opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food. *The EFSA Journal*, **790**:1–51.

Food Chemicals Codex (2014). L-cysteine monohydrochloride, 9th ed, United States Pharmacopeial Convention, Rockville, MD.
http://online.foodchemicalscodex.org/online/pub/index?fcc=9&s=3&oYr=2015&oMo=12&oDa=1
(accessed 11 April 2016)

Laurila E, Kervinen R, Ahvenainen R (1998) The inhibition of enzymatic browning in minimally processed vegetables and fruits. *Postharvest News and Information*, **9**(4):53N–66N.

van de Poll MC, Dejong CH, Soeters PB (2006) Adequate range for sulfur-containing amino acids and biomarkers for their excess: lessons from enteral and parenteral nutrition. *J Nutrition*, **136**(6 Suppl):1694S–1700S.

Vilas-Boas EV and Kader AA (2006) Effect of atmospheric modification, 1-MCP and chemicals on quality of fresh-cut banana. *Postharvest Biology and Technology*, **39**:155–162.

APPENDIX 3

TRIAL

Experiment conducted in Eberbach Germany commencing 25/05/16 [redacted]

LM-3416 Bräunungstest

Basic Recipe Verisol ST gummy candies:

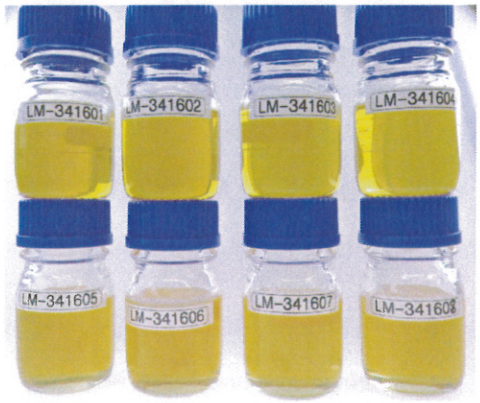
Ingredients	[%]
Sucrose	17.58%
Glukose syrup DE40	23.47%
Water	2.95%
Verisol ST	34.32%
Water	21.68%
	100.00%

Varieties of the basic recipe by the addition of:

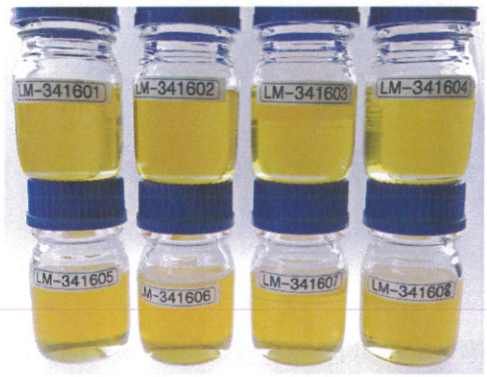
Batch nr.	Fructose [%]	Cystein [%]	Citric acid [50%] pH adjusted on 4.5
LM-341601	-	-	yes
LM-341602	2	-	yes
LM-341603	2	0.1	yes
LM-341604	-	0.5	yes
LM-341605	-	-	no
LM-341606	2	-	no
LM-341607	2	0.1	no
LM-341608	-	0.5	no

Browning conditions storage at 40°C in water vapour sealed Duran bottles:

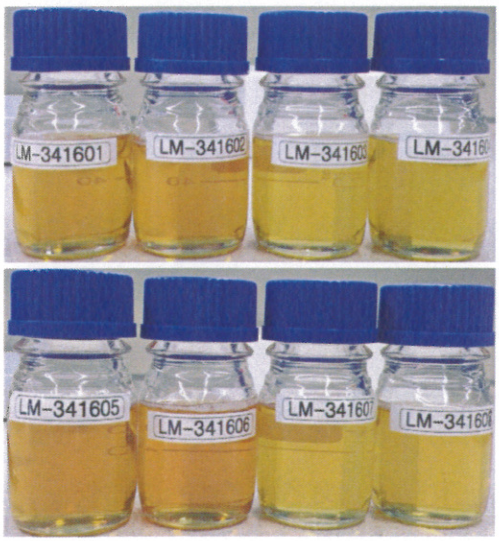
After 0 min of storage (25.05.16)



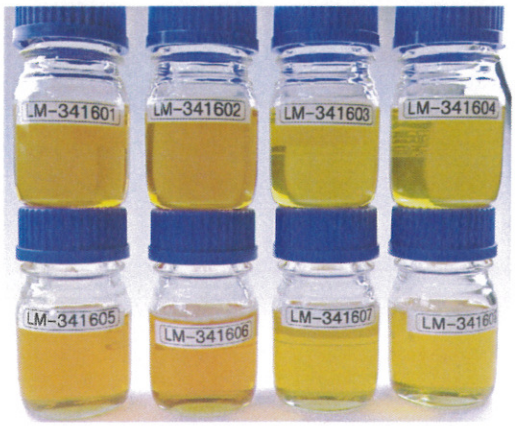
After 4h (25.05.16)



After 2 days (27.05.16)



After 5 days (30.05.16)



After 6 days (31.05.16)

After 7 days (01.06.16)

After 8 days (02.06.16)

After 13 days (07.06.16)

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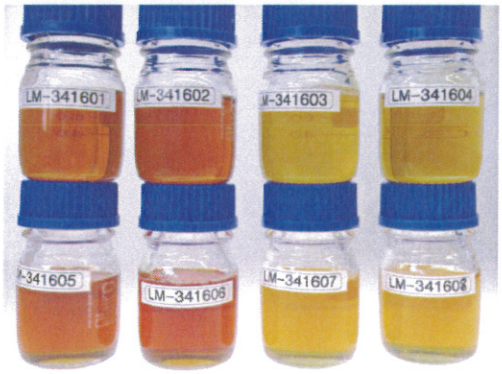

Appendix 4

Free-flowing gelatin composition

The invention relates to a novel free-flowing gelatin composition, in particular for use as a food precursor. The novel free-flowing gelatin composition retains its flowability in particular even at temperatures below 30°C, for example at 25°C.

The industrial processing of gelatin, particularly in the food industry, utilizes in particular the gel-forming properties of dissolved gelatin in the manufacture of food products. Traditionally, for this purpose gelatin in a dry state, in particular in powder form, is used as an initial product, which is dissolved while adding water and heating. A joint dissolving of gelatin with any further ingredients is as a rule hardly possible since, because of the competition for available water, the gelatin fraction can scarcely be dissolved. For this reason, first a solution of the gelatin fraction is produced and then the solution is mixed with the remaining components of the food product before then allowing this to gel as it cools.

In view of the fact that gelatin, especially in powder form, does not have a particularly highly pronounced wettability and does not dissolve in cold water, its mixing with water and conversion to liquid form are a laborious part of the manufacturing process. This is connected in particular also to the fact that the gelatin particles when stirred into liquids readily stick together and form lumps, thereby slowing down the uniform swelling of the gelatin particles into gelatin gel particles and their dissolving in the liquid.

Excessive agitation to prevent lump formation, on the other hand, may lead to intensive foaming, which has equally an extremely disruptive effect on the production process.

This so-called instant gelatin is admittedly cold soluble and may be processed directly mixed with all the ingredients without previously having to dissolve this special gelatin separately. However, with such gelatin products it is not possible to produce genuine gels but merely gel-like structures that, given identical dosing, possess very much lower gel strengths than a gel manufactured conventionally from a comparable powder gelatin.

A further restriction on the usability of instant gelatin is the many times greater risk of lump formation compared to powder gelatin, for which reason for many applications the use of instant gelatin as an alternative to powder gelatin is not possible.

A certain remedy is found by mixing sugar-containing carrier materials or gelatin hydrolysate with the instant gelatin, wherein the sugar-containing carrier materials and/or the gelatin hydrolysate is used to agglomerate the gelatin particles of the gelatin powder. When the agglomerated particles are stirred into liquids, the carrier materials and/or the gelatin hydrolysate dissolve faster than the gelatin particles themselves and then leave the latter behind, finely distributed in the liquid. The carrier materials and/or the gelatin hydrolysate moreover facilitate the initial wetting. The problem of the reduced gel strength however cannot be solved in this way, unless the mixture as a whole is heated beyond the melting point

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of the gelatin in order thereby to produce a genuine solution. Because of the laborious manufacturing process such instant gelatin products are more expensive than powder gelatin, and this, particularly in the case of cost-sensitive food products, has an adverse effect on the cost situation.

The present invention provides an economical gelatin product that is capable of simplified industrial further processing.

In one embodiment, the present invention provides a free-flowing gelatin composition, which comprises an aqueous liquid with gelatin particles dispersed therein having a mean particle size in the swollen state of about 0.01 to about 3mm and/or with gelatin hydrolysate dissolved therein, as well as a sugar component, wherein the sum of the contents of gelatin, gelatin hydrolysate and sugar component is selected such that the water activity (aw value) of the composition is less than or equal to 0.97, and wherein the composition has a water content from about 25% to about 58%.

In one embodiment, the composition described above is used as a food precursor. In another embodiment, the composition is used in the manufacture of capsules for the pharmaceutical industry.

The industrial-scale processing of gelatin that is obtained in a drying process from an aqueous solution generally entails, as a first processing step, renewed dissolving of these dry products. It would therefore be advantageous to avoid this renewed dissolving and supply gelatin or gelatin hydrolysates directly without drying to the users. However, apart from only very short interim storage periods, pure gelatin and gelatin hydrolysates are stable in storage only in the dry state. For this reason, at least according to the present state of the art these products directly after their manufacture, when they are still in liquid form, have to be dried immediately in order to render them stable in storage and transportable.

The reasons why storage in liquid form and/or transportation at temperatures below 30°C is impossible are on the one hand the low microbiological stability of such solutions and on the other hand, in the case of gelatin, the fact that gelatin at these temperatures takes the form of a solid gel. Higher storage temperatures, which both minimize the risk of spoilage and prevent the gelling of gelatin, do not offer a solution because under these conditions massive thermal damage occurs, which renders the product unsuitable for further use, with the result that this alternative also may be used at most for short-term interim storage.

A further alternative, at least for gelatin hydrolysates that are still liquid even at temperatures below 30°C, would naturally be the use of preservatives. The use of such substances is not without problems globally from the point of view of food regulations and is considered unacceptable by many users.

The free-flowing gelatin compositions according to the invention may be transported in tanker lorries without difficulty, often without these having to be heated, and surprisingly also present the required microbiological stability for transportation and storage, this being achieved in particular by selecting the sum of the contents of gelatin, gelatin hydrolysate and sugar component such that the water activity of the composition is less than or equal to 0.97.

A water activity less than or equal to 0.97 means that the water vapour partial pressure at the surface of the free-

flowing gelatin composition is less than or equal to 0.97 times the water vapour partial pressure that arises directly above the surface of pure water.

The microbiological stability of the free-flowing gelatin composition thereby achieved is adequate for manufacture, storage, transportation and stockpiling by the industrial user.

Because of the ability of gelatin to absorb water to a large extent, it is in practice impossible to achieve a water activity less than or equal to 0.97 by the dosing of the gelatin particles in the aqueous solution alone. In the present case, the addition of one or more sugar components is an ideal solution to the problem because sugar components are themselves often contained in food recipes and the content already supplied in the free-flowing gelatin composition may easily be taken into consideration by the industrial user during formulation in the course of further processing.

The novel free-flowing gelatin composition according to the invention may easily be further processed in the industrial process as the free-flowing composition need merely be heated in order to melt the gelatin particles dispersed therein completely and hence bring the gelatin into solution. During cooling solid gel structures, such as are customary from the processing of conventional powder gelatin, are then obtained.

In the case of the exclusive use of gelatin hydrolysate, heating is not necessary because it already forms a

molecularly disperse, homogeneous mixture with the other components of the composition according to the invention.

The gelatin hydrolysate content is not limited to a soluble fraction. Rather, within the scope of the invention it is possible also to cite compositions, in which dissolved and undissolved gelatin hydrolysate are present alongside one another. The undissolved fractions of gelatin hydrolysate are then present preferably dispersed in the composition according to the invention.

According to the invention, saccharides, in particular mono-, di- and oligosaccharides, in particular sucrose, glucose, fructose, glucose syrups, oligofructose syrups, dextrins and the like are suitable as sugar components.

Further suitable sugar components are sugar substitutes, in particular alditols, such as for example glycerine, threitol, mannitol, isomalt, lactitol, sorbitol, xylitol, erythritol, arabitol and maltitol, as well as polydextrose.

The previously cited sugar components may be used individually or in any combination in the composition according to the invention.

A further advantage of the use of the composition according to the invention is simplified handling because the relatively high outlay when manufacturing in particular highly concentrated gelatin solutions is eliminated.

Gelatin in the form of gelatin gel particles and gelatin hydrolysate may be used alongside one another in any combination in the free-flowing gelatin composition,

wherein the gelatin as well as the gelatin hydrolysate may be exclusively present in the composition.

The free-flowing gelatin composition according to the invention is particularly economical to manufacture because the operation of drying to a water content of 10 wt. % that is necessary when manufacturing gelatin powder or gelatin hydrolysate powder may be omitted. In particular, in the case of the gelatin component, it is possible to start from an intermediate product of gelatin manufacture, the so-called gelatin noodles, which contain ca. 30 wt.% dry gelatin substance and ca. 70 wt.% water. These gelatin noodles may easily be chopped by a cutting mechanism (cutter) under cooling conditions (temperature below 20°C) to produce sufficiently fine gelatin gel particles.

These gelatin particles may be mixed with the sugar component and optionally the desired gelatin hydrolysate fraction, thereby resulting in a free-flowing dispersion of the gelatin gel particles in a liquid matrix that is pumpable and hence may be used in an easily dosable manner in the industrial process.

At the same time, gelatin gel particles may also be mixed directly with the sugar component in solid form, wherein a pumpable dispersion arises already during the mixing operation since because of osmotic effects some of the water bound in the gelatin gel particles escapes and is available to dissolve the sugar component.

Given the water activity of less than or equal to 0.97 that is defined according to the invention, at temperatures of ca. 20°C a microbiological stability remains guaranteed for

at least 2 to 3 weeks, provided that the conventional hygienic conditions of gelatin manufacture are observed.

All the customer or processor has to do is add the dosed further recipe ingredients to the free-flowing gelatin composition and mix them with the free-flowing composition, wherein this mixture may then pass through an, in any case, necessary cooking system in order to obtain a gelling pouring solution for the food product for example, in particular for gumdrops or jelly babies.
In terms of manufacture, the free-flowing gelatin composition according to the invention therefore eliminates not only a drying step but also the interim storage of the product prior to the drying step for further laboratory tests and the grinding and mixing as well as the packing of the gelatin powder. The - compared to this - additional cost of chopping the gelatin noodles, mixing for example with sugar and glucose syrup and the extra cost of transportation are therefore easily justifiable.

For the processor, the complete step of swelling and dissolving the gelatin is eliminated, and the dosing and mixing of the free-flowing gelatin composition according to the invention with the remaining recipe ingredients is simplified. This offers the processor economies not only in terms of equipment but also in terms of personnel costs because, unlike gelatin in powder form, processing of the free-flowing gelatin composition according to the invention may take place fully automatically without any problems.

Furthermore, processors who process powder gelatin often in batch processes that require complicated supply- and weighing systems may use a continuously operating system,

which may easily be set up in existing installations by appropriate refitting.

A more extensive microbial stabilization may be achieved by lowering the pH value, for example to values lower than 5, in particular ca. 3 to 4.5, wherein for this purpose preferably edible acids may be used. The aw value remains substantially unaffected by this.

If an even longer microbiological stability of the gelatin composition according to the invention is required, it is recommended that the water activity of the composition be lowered to 0.93 or less. The formulation, i.e. the fractions of gelatin gel particles and/or dissolved gelatin hydrolysate, on the one hand, and sugar component, on the other hand, has to be adapted accordingly.

If gelatin gel particles are used as the sole gelatin ingredient in the free-flowing gelatin composition, the gelatin gel particle content (expressed as dry mass with a water content of ca. 10 wt.%) may vary within the range of 20 to 40 wt.%, in relation to the total weight of the composition.

Higher concentrations of gelatin or, in other words, water contents of 50 wt.% or less (in the case of gelatin hydrolysate-based compositions 35 wt% or less) are on the one hand technically realizable only with difficulty and moreover result in viscosities that extremely limit the pumpability.

Given predominant or exclusive use of gelatin hydrolysate as the gelatin ingredient in the liquid gelatin

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composition, its content may be varied without difficulty within the range of 20 to 60 wt.%.

The mean molecular weight of the gelatin hydrolysate is preferably selected within the range of ca. 1,000 to ca. 20,000 Da.

The gelatin gel particles in the swollen state (i.e. with a maximum water content) have a mean particle size of ca. 0.01 to 3 mm, in particular 0.1 to 1 mm.

Since, given the use of gelatin hydrolysate as the gelatin component in the composition according to the invention, higher whole protein contents are possible, the sugar component content in such compositions need not be set as high as is the case for compositions with gelatin gel particles as the main gelatin component. Here, sugar component contents of ca. 10 wt.% or more may already bring about adequate microbiological stabilization.

For the compositions according to the invention gelatin hydrolysates may be used in the form of solutions, such as are currently already being used in the manufacture of hydrolysate powder by the spray drying process.

The sugar component, optionally edible acids and, depending on the client's requirements, any further recipe ingredients are added to these solutions. The requisite mixing and dissolving steps may be carried out within a broad temperature range.

Typically representative of the sugar components of the gelatin composition that are used according to the

invention are - as already mentioned - saccharides, which in a recipe that is geared mainly to gelatin gel particles as the gelatin component are used preferably in a quantity of 30 wt.% or more, in relation to the total composition.

The saccharides are preferably selected from mono-, di- and/or oligosaccharides, wherein in particular mono- and disaccharides are used, in compositions that are intended as food precursors.

If the gelatin composition according to the invention is to be used in recipes that contain no saccharides, possible alternatives are the above-mentioned sugar substitutes, in particular alditols, such as for example glycerine or other sugar alcohols, oligofructose syrups, polydextrose and dextrins, in particular wheat dextrin.

Such compositions are suitable in particular for the manufacture of dietetic low-sugar products, in particular for the manufacture of low-sugar gumdrops.

As regards the pumpability of the free-flowing gelatin composition according to the invention, it is preferred if the composition has a viscosity of at most ca. 20,000 cP, more preferably at most 10,000 cP. However, even compositions having viscosities of ca. 100,000 cP are free-flowing and may be processed, pumped and proportioned using conventional food technology equipment.

These and further advantages of the invention are described in more detail below by way of the examples.

Where in the following examples swollen gelatin gel particles are used, these originate from an intermediate step of gelatin production, in which so-called gelatin noodles with a water content of ca. 70 wt.% arise. These noodles have been chopped, as described further above, in a so-called cutter to the particle sizes indicated in the individual examples.

Example 1: gelatin dispersion

Example A

61.4 wt.%	swollen gelatin gel particles (dry substance ca. 30 wt.%) mean particle size 0.4 mm; Bloom = 260; gelatin type A
28.0 wt.%	sucrose
10.60 wt/%	glucose syrup (78 wt.%)

The recipe ingredients may be mixed with one another without adding water and produce a free-flowing composition according to the invention with a water content of ca. 43.4 wt.%. The aw value is 0.97.

At 20°C this gelatin composition according to the invention has a viscosity of ca. 2500 cP.

Example B

60.00 wt.%	swollen gelatin gel particles (dry substance ca. 30 wt.%), mean particle size 0.4 mm; Bloom = 280; gelatin type A
40.00 wt.%	sucrose

The recipe ingredients may be mixed with one another without adding water and produce a free-flowing composition according to the invention with a water content of ca. 42 wt.%. The aw value is 0.963.

At 20°C this gelatin composition according to the invention has a viscosity of ca. 14000 cP.

It is suitable in particular as a food precursor for the manufacture of jelly babies or gumdrops.

The processor needs to add to the gelatin composition according to the invention only glucose syrup, sucrose and flavourings as well as optionally colourings and pass this mixture through a cooking system in order easily to obtain a finished pouring solution that may be poured into conventional moulds.

Example 2: gelatin dispersion sugar-free

50 wt.%	swollen gelatin gel particles (dry substance ca. 30 wt.%), mean particle size 0.3 mm, Bloom = 240; gelatin type A
25 wt.%	wheat dextrin (obtainable as Nutriose® from Roquette Frères, France)
25 wt.%	polydextrose

The water content of this recipe is ca. 36.5 wt/%, the aw value is 0.95.

At 20°C this gelatin composition according to the invention has a viscosity of ca. 6000 cP.

It is suitable in particular as a precursor for the manufacture of low-sugar and/or sugar-free confectionery.

Example 3: liquid hydrolysate

40 wt.% gelatin hydrolysate (dry substance), mean molecular weight = 3000 Da
16 wt.% sucrose
[1 wt.% citric acid for lowering the pH to ca. pH 4.5; optional]
the remainder water

The aw value is 0.942.

At 20°C this gelatin composition according to the invention has a viscosity of ca. 1120 cP.

This gelatin composition according to the invention is likewise usable as a food precursor, for example for the manufacture of protein-enriched gumdrops or the manufacture of edible bars.

It is self-evident that the recipes of Examples 1 to 3 may be modified in such a way that the gelatin content is formed partially by gelatin hydrolysate (Examples 1 and 2) and/or by gelatin gel particles (Example 3).

If in Example 1 gelatin hydrolysate is additionally used, the result is already a precursor for the manufacture of protein-enriched food, for example gumdrops or marshmallows.

The following Tables 1 and 2 demonstrate how with differing contents of the components of the composition according to the invention it is easily possible to adjust the required aw value.

Table 1

Wt.% gelatin (Bloom=220;type A)	Wt.% sugar (sucrose)	Wt.% DS mix	aw value
25.0 %	0.0 %	25.0 %	0.994
19.2 %	23.1 %	42.3 %	0.970
24.4 %	25.9 %	50.3 %	0.966
29.3 %	28.6 %	57.9 %	0.959
34.7 %	33.3 %	68.0 %	0.932

Table 2

Wt.% hydrolysate DS (MW = 3000 Da) starting product	Wt.% sugar 100 (sucrose)	Wt.% DS mix	aw value
51.5 %	0.0 %	51.5 %	0.972
42.9 %	18.0 %	60.9 %	0.935
34.3 %	34.1 %	68.4 %	0.883
28.6 %	43.3 %	71.9 %	0.848
25.7 %	49.7 %	75.4 %	0.808

From the Tables it is likewise evident that the gelatin composition (mix) according to the invention advantageously comprises very high fractions of dry substance (DS), wherein not only is the advantage of microbiological stability achieved but further processing into the finished product may also be effected in an advantageous manner in

terms of energy because only relatively low water contents have to be expelled in the product drying operation.

Example 4:

In order to even further improve the solubility of the gelatin gel particles during further processing, it is possible even during manufacture of the gelatin gel particles moreover to add to the gelatin solution arising in the initial stage a sugar component, for example sugar, which is then dissolved in the solution and distributed in a molecularly disperse manner. The gel particles manufactured from this are, like the gel particles comprising only gelatin and water, mixed with sugar, glucose syrup etc., in order to produce from this a stable dispersion.

Recipe example:

In a ca. 30 wt.% gelatin solution that arises as an intermediate product in gelatin production sugar is dissolved so that the solution has the following composition:

water	54 wt.%
gelatin (DS)	23 wt.%
sugar	23 wt.%

Then the solution is cooled and gelled (as in the normal processing of gelatin) and the gel particles produced therefrom are mixed with sugar and glucose syrup to produce a dispersion according to the invention that has for example the following composition:

gelatin (DS)	11.7 wt.%	
sugar	26.6 wt.%	
glucose syrup	24.0 wt.%	(78 % DS)
the remainder	water	

The sugar content of 26.6 wt.% is composed of 13.3 wt.% sugar contained in the gelatin gel particles and 13.3 wt.% sugar added as dry substance in pure form.

The viscosity of this composition according to the invention is 3,000 cP. The aw value achieved is 0.961.

Example 5: gelatin hydrolysate composition A

37.5 wt.%	gelatin hydrolysate (dry substance) mean molecular weight = 3000 Da
25.0 wt.%	Nutriose (95 wt.% dry substance)
the remainder	water

In such a composition a fraction of the gelatin hydrolysate is present in dissolved form and a further fraction in solid, dispersed form.

The resulting aw value is 0.935. At 20°C the viscosity is ca. 24,570 cP.

Example 6: gelatin hydrolysate composition B

To the composition of Example 5 further fractions of the gelatin hydrolysate were added in powder form, resulting in the following composition:

43.3 wt.%	gelatin hydrolysate (dry substance)

	mean molecular weight = 3000 Da
22.2 wt.%	Nutriose (95 wt.% dry substance)
the remainder	water

The resulting aw value is 0.920. At 20°C the viscosity is ca. 68,800 cP.

Although the composition according to the invention described in this example has a much higher viscosity than the one previously recommended as preferred, such compositions are free-flowing and may be pumped and dosed using conventional food technology equipment.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

The claims defining the invention are as follows:

1. Free-flowing gelatin composition, comprising an aqueous liquid, gelatin gel particles dispersed therein having a mean particle size in the swollen state of about 0.01 to about 3mm and/or gelatin hydrolysate dissolved therein, and one or more sugar components, wherein the sum of the contents of gelatin, gelatin hydrolysate and sugar component(s) is selected such that the water activity (aw value) of the composition is less than or equal to 0.97, and wherein the composition has a water content from about 25% to about 58%.

2. Composition according to claim 1, characterized in that the water activity (aw value) is 0.93 or less.

3. Composition according to claim 1 or 2, characterized in that the gelatin gel particle (dry mass) content is 20 to 40 wt.%.

4. Composition according to one of claims 1 to 3, characterized in that as a sugar component one or more saccharides are contained, wherein the saccharide content is 30 wt.% or more.

5. Composition according to one of claims 1 to 4, characterized in that the gelatin gel particles in the swollen state have a mean particle size of 0.1 to 1 mm.

6. Composition according to one of claims 1 to 5, characterized in that the gelatin hydrolysate content is 20 to 60 wt.%.

7. Composition according to claim 6, characterized in that the mean molecular weight (MW) of the gelatin hydrolysate is ca. 1,000 to ca. 20,000 Da.

8. Composition according to claim 6 or 7, characterized in that as a sugar component one or more saccharides are contained, wherein the saccharide content is 10 wt.% or more.

9. Composition according to one of claims 1 to 8, characterized in that the saccharide is selected from mono-, di- and/or oligosaccharides.

10. Composition according to one of claims 1 to 9, characterized in that as a sugar component one or more alditols are contained.

11. Composition according to one of claims 1 to 10, characterized in that the composition comprises a fraction of one or more edible acids.

12. Composition according to claim 11, characterized in that the pH value of the composition is lower than 5.

13. Composition according to claim 12, characterized in that the pH value of the composition is ca. 3 to ca. 4.5.

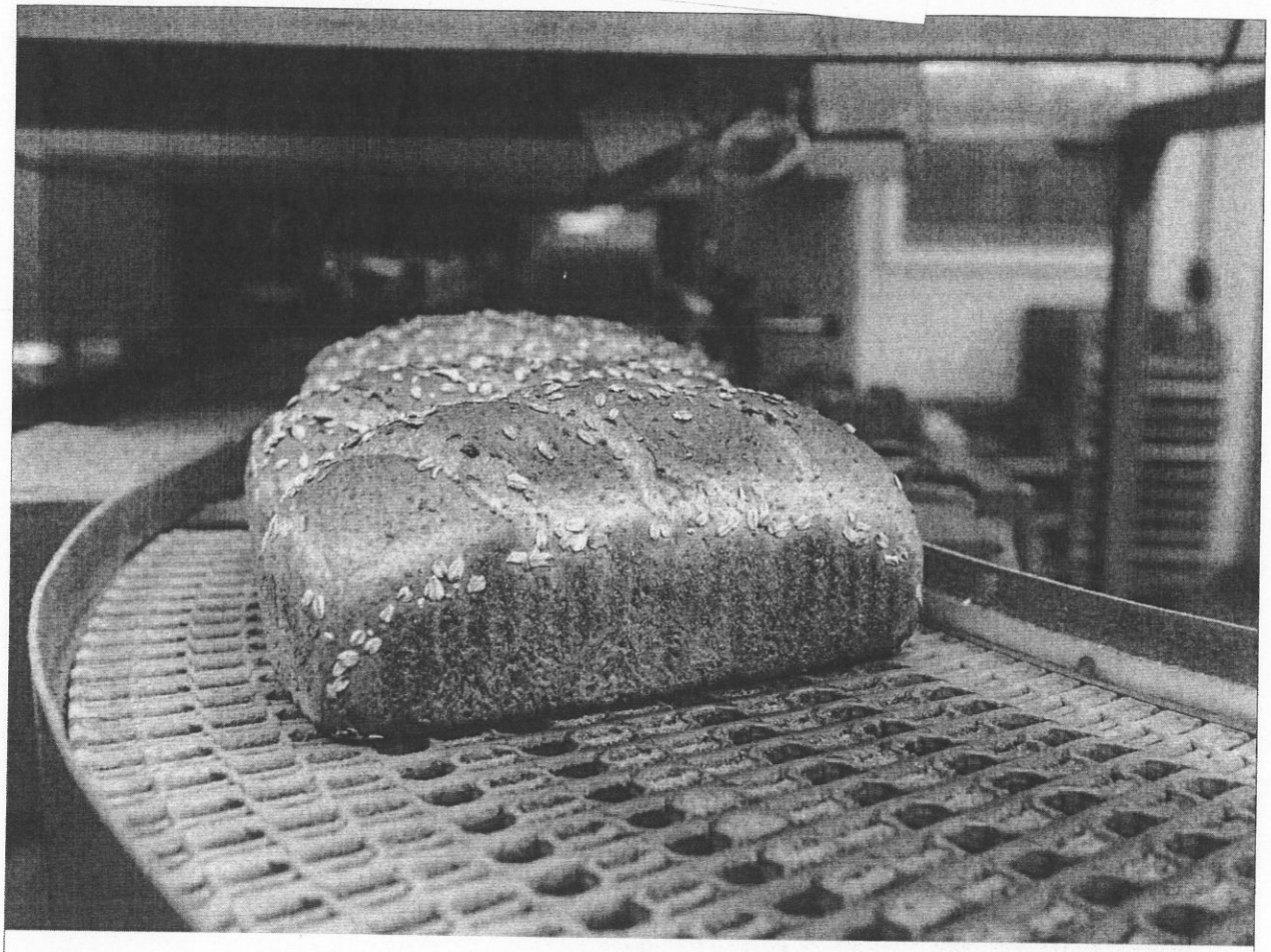
14. Composition according to one of claims 1 to 13, characterized in that the composition has a viscosity of at most 30,000 cP.

15. Use of a composition according to one of claims 1 to 14 as a food precursor.

16. Use of a composition according to one of claims 1 to 14 in the manufacture of capsules for the pharmaceutical industry.

17. Free- flowing gelatine composition, or use of the composition, substantially as herein described with reference to the Examples.

Appendix 5



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Study of Maillard Reaction Inhibitors for the Sugar Cane Processing

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ABSTRACT

Maillard Reaction (MR) is a complicated non-enzymatic browning which can happen in many kinds of food, processed products such as breweries, dairy products and sugar products. Studies have done in order to remove the Maillard reaction products, to decolorize colored compounds or to treat wastewater from sugar cane factory. For this reason, this research was performed to study and identify MR inhibitors during sugar cane processing using model systems with different concentrations of amino acids and glucose, 0.09 mol L^{-1} for asparagine (Asn); 0.08 mol L^{-1} for glutamine (Gln) and 0.2 mol L^{-1} for glucose (Glc), which were heated at 85-90°C for two hours, based on physicochemical analyses and assays. The influence of the physical factors such as temperature, pH or water activity ($a_{\square}$) were investigated as well as chemical agents such as Ascorbic Acid (AA), Cysteine (Cys), Nicotinic Acid (NA), NaCl and vitamin B6 at different concentrations (0.05, 0.1 and 0.15%), respectively. According to the results, AA 0.05%, NA 0.05% and Cys 0.1% have significantly reduced the browning effect, soluble matter (°Bx) and improved the pH compare with the untreated samples. These findings open new possibilities for strategies in the study and control of MR.

Key words: Maillard reaction, glucose, asparagine, glutamine, inhibitors

INTRODUCTION

During sugar cane processing many reactions and other factors affect quantity and quality of the final product (sucrose). These factors are related to the formation of non-sugars, mainly colored compounds and reducing sugars. The formation of these components during sugar cane processing is the result of physical variation such as pH, temperature, reaction time and autocatalytic effects (Bento and Sa, 1998). The thermal treatment of reducing sugars with amino acids or other proteins in an aqueous system with low water activity, colored compounds appeared in few minutes, browning increased with longer reaction times (Knerr *et al.*, 2001). In the past, many studies have been done on MR. More work has been concentrated on low molecular weight, fine particulates and colored compounds which could be the main constituents of Maillard reaction products. The principal components of the sugar cane are sugar and fiber (Honig, 1953). The average composition of sugar cane depends on the state of maturity of the plant (fiber 10-18%; water 70-77%; saccharose 12-16% and non-sugar compounds 2-5%). According to Roberts and Martin (1959), 100 mL of sugar cane juice contain about 15.5% of asparagine and glutamine which was the highest composition of amino acid.

Some developing countries where people are consuming non-refining sugar, the performance of sugar factories remains to be improved. The MR not only causes the loss of saccharose but also produces melanoidins (Coca *et al.*, 2004); acrylamides (Zyzak *et al.*, 2003; Knol *et al.*, 2005) and the persistence of the unaccepted dark brown color (Pant and Adholeya, 2007). Engineers should take action to slow down the MR.

So far, few studies have concentrated on preventing the MR during sugar cane processing and its effect on non-refining sugar quality. Previous knowledge supports that Maillard reaction can be controlled by the physical factors such as temperature, reaction time, pH and water activity. The present paper concerns on the study and identification of the chemical inhibitors which can reduce or stop the Maillard reaction and its intermediary to be formed during the sugar cane processing before the crystallization (sugar boiling) phase.

MATERIALS AND METHODS

Materials: D-glucose-monohydrate, L-asparagine-monohydrate, L-glutamine, L-ascorbic acid, L-Cysteine, nicotinic acid, pyridoxine-hydrochloride, sodium hydroxide, all chemicals were the best available analytical grade reagents and supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium chloride was bought from the local market.

Simulation of MR: The present study was conducted in the years 2011-2012 at School of Food Science and Technology, Jiangnan University, People's Republic of China. To investigate the influence of different inhibitors on the MR, a simulating solution was prepared with glucose and amino acids. 0.025 mol of glucose was mixed with 0.09 mol of Asn, 0.08 mol of Gln. To obtain a homogeneous mixture both reactants were carefully heated (IKA Werke GmbH and Co. KG, Branch Laboratory Instrument Co., Ltd. Guangzhou, China) at 85-90°C. During the heating process, the pH of the two mixtures was adjusted to 8.5 with NaOH (0.5%) to initiate MR in the systems. The pH was measured using the METTLER TOLEDO pH-meter FE 20 Five Easy pH (Shanghai Toledo Instrument, Co., Ltd., China). The color was measured by the UV-VIS spectrophotometer UV1600 (Shanghai MAPADA Instruments, Co., Ltd., China). The dry matter content was measured by portable refractometer FG-108 and expressed on Brix.

Inhibition of MR: Three different concentrations (0.05, 0.1 and 0.15%) of each inhibitor (AA, Cys and NA) previously prepared were used to investigate their effect on the Maillard reaction of the systems. Each concentration of the inhibitors prepared was separately added to both mixtures during the heating and the process allowed to proceed for a little over two h. After two hours, the mixture was cooled to room temperature. A control experiment was carried out without addition of inhibitors. The resultant products obtained after the heating process were then considered as Maillard reaction products. The resultant products were subjected to the further analysis in order to determine the effects of the inhibitors on the reaction. All experiments were prepared in triplicate.

Analysis of samples: The digital pH-meter (METTLER TOLEDO) was used to measure the pH of the systems. The systems (Asn+Glc and Gln+Glc) had the same water activities (0.95) measured at room temperature using the formula:

$$a_w = \frac{p}{p^0} = \frac{n_2}{n_1 + n_2}$$

where, n_1 is the number of moles of solute and n_2 is the number of moles of water. Titration assay was used to add the different inhibitors in the samples in the order to control or to stop the Maillard reaction. Samples color was measured (brown pigment formation) at 560 nm (Clement *et al.*, 2010). When necessary, samples were diluted with distilled water (1/5 v/v).

RESULTS AND DISCUSSION

Two model systems (Asn+Glc and Gln+Glc) were investigated for studying the effect of inhibitors on the Maillard reaction in the sugar cane process. Glucose is the typical reducing sugar in sugar cane and asparagine and glutamine have the highest percentage as amino acids in the sugar cane (Roberts and Martin, 1959). Chemical reagents were used to slow down the MR.

Effect of the inhibitors on the pH: The pH plays an important role in the reaction. Maillard reaction needs a particular pH (≤8.5) to initiate the reaction. At the beginning of the reaction, the pH of the systems was acidic (≤4.6) and at this pH the Maillard reaction cannot occur. To allow the reaction to run, sodium hydroxide 0.5% was added to raise the pH to the favorable value for Maillard reaction. The pH was recorded every ten minutes to see its variation and the results are shown in the Fig. 1a for the system Glc-Asn and Fig. 1b for the system Glc-Gln, which explained

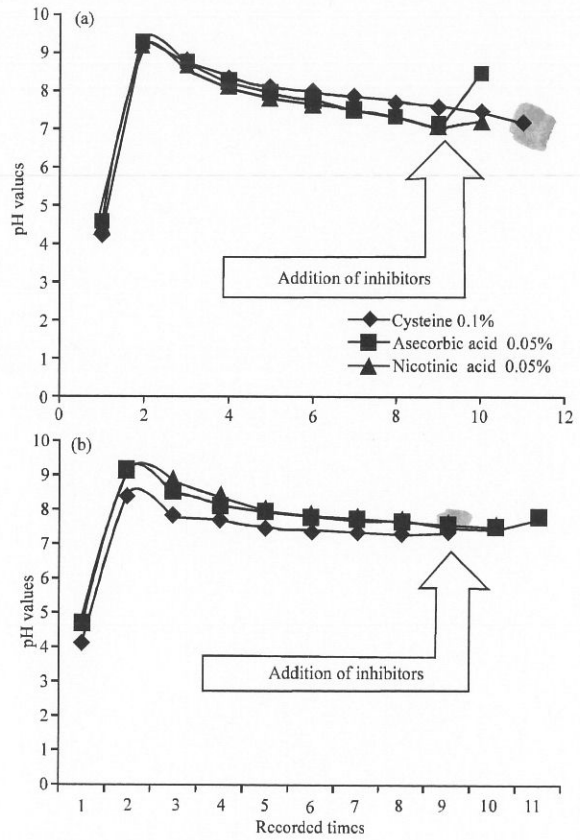

Fig. 1(a-b): Illustration of pH before, during and after the reaction according to time in the model systems (a) Gln+Glc and (b) Asn+Glc

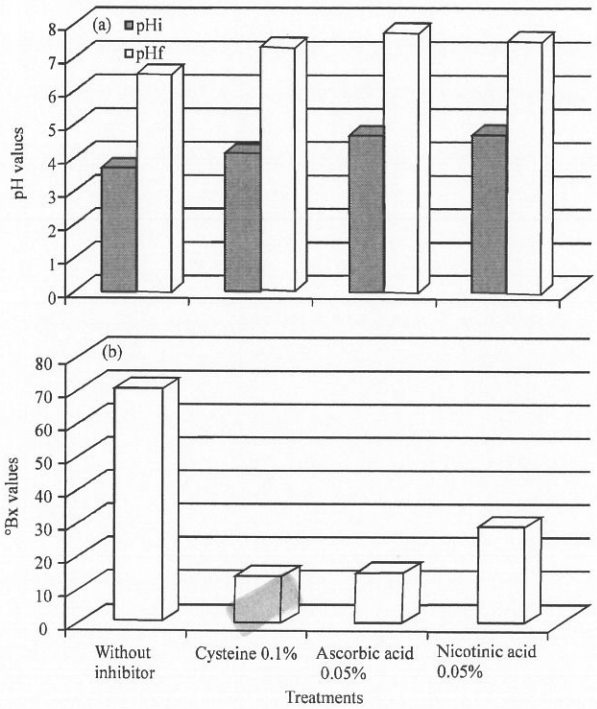

Fig. 2(a-b): Illustration of the difference between pHi and pHf in the model systems (a) Asn+Glc and (b) Gln/Glc to compare treated samples (with inhibitors) to untreated samples

that after the NaOH was added in the model systems, the pH started to decrease with the reaction time, temperature and water activity. This fall of pH was to promote the MR. The pH can be used as an indication for the degree to which the MR has occurred.

Contrary to temperature and water activity which favored the occurrence of MR, the effect of inhibitors on the pH increases or stabilizes this one. An increase in the final pH (pHf) was observed after the addition of chemical inhibitors. Figure 2a showed that in the Asn-Glc system AA (7.74) has the highest pHf, after NA (7.49) and the smallest is Cyst (7.3). Figure 2b showed in the Gln/Glc model that AA (8.47) has the highest pHf, after NA (7.24) and finally Cyst (7.19) has the smallest pHf. This augmentation of pH confirmed as well as the one of the important factors of Maillard reaction (Mao *et al.*, 2007).

Effect of the inhibitors on the soluble matter (°Bx): The soluble matter was expressed in °Bx. It increases when the temperature increases and the water activity decreases (Hedegaard *et al.*, 2007). Chou *et al.* (2002) have reported that the ultrafiltration reduced the concentration of macro-molecules and well dispersed fine particulates which are nothing other than Maillard reaction products. Figure 3a (Asn-Glc) showed that the untreated sample (70.5°Bx) has the highest concentration of soluble matter compare to the samples treated with inhibitors. Soluble matter has been affected more by Cyst (14°Bx) than AA (15°Bx) and NA (29°Bx). The Gln-Glc (Fig. 3b) model was treated in the same conditions and the result shown that AA (9°Bx) has more effect on the system more than NA (10°Bx) and Cys (11°Bx). However, this study has shown that the inhibitors

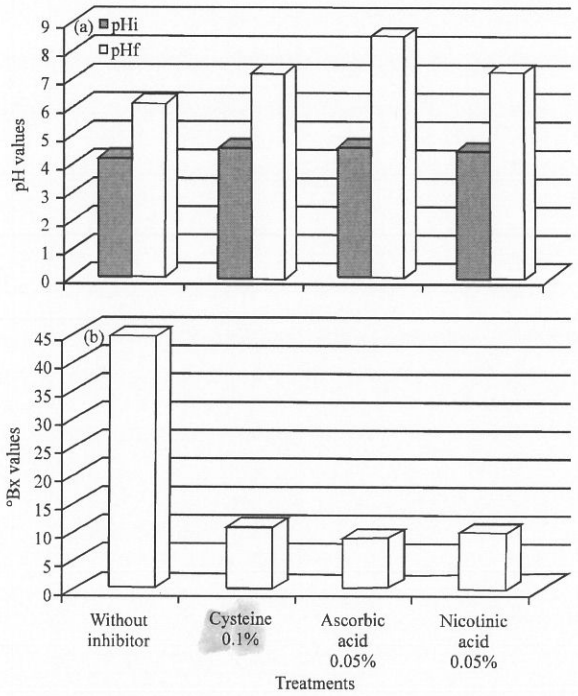

Fig. 3(a-b): Illustration of the inhibitors effects on the soluble solids (Brix) in the model systems (a) Asn/Glc and (b) Gln/Glc treated samples compared to untreated samples

strongly affected (reduced or stopped) the Maillard reaction products which happened during the experiments. Therefore, the percentage of soluble matter (°Bx) was reduced in both systems. This effect of the inhibitors on the soluble matter can facilitate the crystallization (sugar boiling) of sucrose and reduce the percentage of Maillard reaction products such as melanoidins, acrylamide, macro-molecules and other colored compounds.

The Maillard reaction produces many kinds of products such as organic acids (De Vleeschouwer *et al.*, 2010); acrylamides melanoidins (Ibarz *et al.*, 2009) and unknown ones which increased with the temperature and heighten the concentration of soluble matter in the samples which can affected the quality of the final product.

Effect of inhibitors on color: Browning is a classical feature of the extent of the Maillard reaction in its advanced and final stages and it has been directly related to the colored compounds formation in both model systems (Asn+Glc and Gln+Glc). Thus, the rates of Maillard reaction could be represented by the color formation and expressed directly by the optical density. The variation of color between the samples (Fig. 4a for Asn-Glc and Fig. 4b for Gln-Glc) was the effect of inhibitors on the reaction. Table 1 (Asn-Glc) showed that the untreated sample (without inhibitors)has the highest value (0.156 nm) compare to the samples which were treated with inhibitors. The addition of inhibitors reduced significantly the browning with NA (0.065 nm) afterward Cyst (0.077 nm) and then AA (0.078 nm). Table 2 (Gln-Glc) showed the same phenomena but the difference was with Cys (0.056 nm) which has more effect than NA (0.063 nm) and AA (0.062 nm).

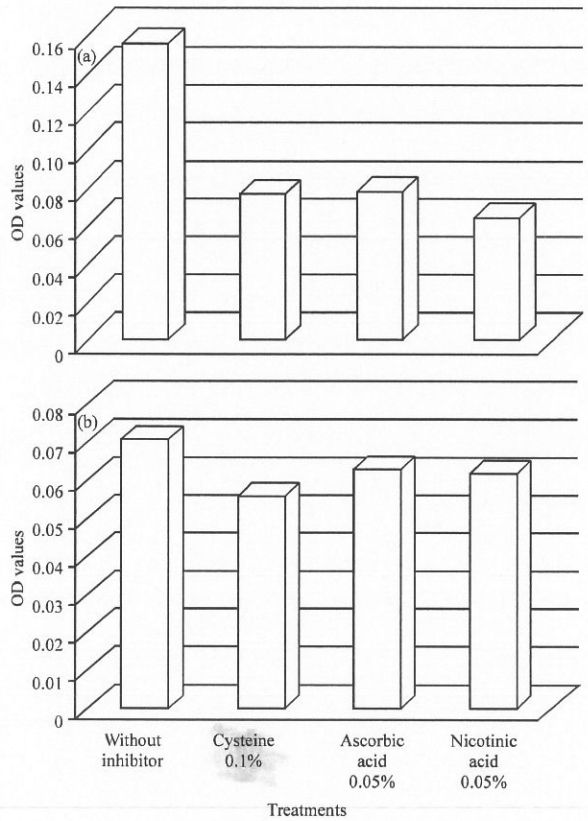

Fig. 4(a-b): Illustration of inhibitors effect on color (OD value) in the systems (a) Asn/Glc and (b) Gln/Glc to see the difference between treated samples (with inhibitors) and untreated samples

Table 1: Inhibitors effects on different parameters compare to the untreated samples in the model systems Asn/Glc

Asn/Glc*	Bx	pHi	pHf	OD
Without inhibitor	70.5±3.0	3.70±0.5	6.51±0.5	0.156±0.002
Cysteine 1%	14.0±3.0	4.15±0.5	7.30±0.5	0.077±0.002
Ascorbic acid 0.5%	15.0±3.0	4.67±0.5	7.74±0.5	0.078±0.002
Nicotinic acid 0.5%	29.0±3.0	4.71±0.5	7.49±0.5	0.065±0.002

*Parameter values (1, 0.5 and 0.5%) are transformed values

Table 2: Inhibitors effects on different parameters compare to the untreated samples in the model systems Gln/Glc

Glc/Glc*	Bx	pHi	pHf	OD
Without inhibitor	44.5±3.0	4.21±0.5	6.14±0.5	0.071±0.002
Cysteine 1%	11.0±3.0	4.58±0.5	7.19±0.5	0.056±0.002
Ascorbic acid 0.5%	9.0±3.0	4.60±0.5	8.47±0.5	0.063±0.002
Nicotinic acid 0.5%	10.0±3.0	4.45±0.5	7.24±0.5	0.062±0.002

*Parameter values (1, 0.5 and 0.5%) are transformed values

Sugar refining processes used the operations such as carbonation; filtration and ion exchange to remove colored substances before crystallization to produce white sugar. Any or combination of above operations selected should have performed the highest color removal with minimum

environmental problems. In the order to produce good quality of sugar, many studies have concentrated on removing of color during the sugar cane processing. Donovan and Williams (1992) have concluded that the color occlusion in sugar crystal which gave the higher molecular weight colorants can be separated by membrane. Chou *et al.* (2002) concluded that the SAT process is a direct replacement of the sulfitation, carbonation and blanco directo processes for plantation white sugar productions.

In the same way of thinking, chemical factors (inhibitors) were used in this study to reduce the browning substances generated by MR (Fig. 4a, 4b) showed the extent of browning in samples without inhibitors and samples with inhibitors. On the one hand, Asn/Glc system, browning increased rapidly with the increase of temperature and water activity to reach 0.156 nm (Fig. 4a). On the other hand, Gln/Glc system, browning increased with the raise of temperature, afterward became stable (Fig. 4b). At 85-90°C, the model Asn/Glc without inhibitors the absorbance reached the highest point 0.071 nm (Fig. 4a). However, for the Asn/Glc system, the color had fallen to 0.077 nm in presence of Cys 0.1 and 0.078% nm in the presence of AA 0.05 and 0.065% nm in the presence of NA 0.05%. For the Gln/Glc system treated in the same conditions, the results showed (Fig. 4b) that the samples without inhibitors, the browning was higher than the samples which were treated with inhibitors. However, the variation in the color between untreated samples and the treated samples was less than 0.015 nm for Cys 0.05%, less than 0.01 nm for AA 0.05% and NA 0.05%. These results have shown that the three inhibitors (AA, Cys and NA) strongly reduced the advanced and the final stages of the Maillard reaction and its intermediate compounds in the systems especially for Asn/Glc model.

The effects of different inhibitors on Maillard reaction in the systems (Asn/Glc and Gln/Glc) were investigated. For this purpose, three kinds of inhibitors such as ascorbic acid (Cortez-Vega *et al.*, 2008), cysteine (Tochi *et al.*, 2009) and nicotinic acid at different concentration (0.05, 0.1. 0.15%) were added into the model systems when the browning started between 85-90°C during two hours. Results are shown in Table 3 for Gln-Glc and Table 4 for Asn-Glc the percentage necessary of the inhibitors to inhibit the Maillard reaction compared to the samples without inhibitors. Both systems (Asn/Glc, Gln/Glc) have shown inhibition activities to some extent for all concentrations. However, the model systems have respondedpositively with AA, Cys and NA

Table 3: Results with all inhibitors and different concentrations in the model systems Gln/Glc

	Gln/Glc			
Inhibitors (%)	Bx	pHi	pHf	OD
Cysteine				
0.05	11	4.28	6.57	0.081
0.10	11	4.58	7.19	0.056
0.15	9	4.7	7.16	0.058
Ascorbic acid				
0.05	9	4.6	8.47	0.063
0.10	8	4.67	7.36	0.06
0.15	9	4.56	7.46	0.063
Nicotinic acid				
0.05	10	4.45	7.24	0.062
0.10	9	4.28	7.12	0.055
0.15	10.2	4.38	7.12	0.054

Table 4: Results with all inhibitors and different concentrations in the model systems Asn/Glc

	Asn/Glc			
Inhibitors (%)	Bx	pHi	pHf	OD
Cysteine				
0.05	20	4.1	7.28	0.124
0.10	14	4.15	7.3	0.077
0.15	19	4.17	6.9	0.083
Ascorbic acid				
0.05	15	4.67	7.74	0.078
0.10	18	4.35	7.45	0.075
0.15	21	4.71	7.7	0.077
Nicotinic acid				
0.05	29	4.71	7.49	0.065
0.10	25	4.43	7.43	0.069
0.15	20.5	4.5	7.23	0.058

(Table 1 for Asn-Glc and Table 2 for Gln-Glc) because inhibitors affected the different steps of Maillard reaction and stopped its intermediaries tobe formed (browning compounds). Among these inhibitors Cys provided the highest inhibition effects followed by AA and NA. This explains that Maillard reaction has been affected by these chemical factors (AA, Cys and NA) which greatly influenced the formation of browning compounds especially AA which is known to have antioxidant effect (this point will be more developed in the second part of our work).

CONCLUSION

The physico-chemical analyses andassays were used to identify the chemical inhibitors on Maillard reaction occurred during the sugar cane processing which may affected the final product. This study revealed that ascorbic acid, cysteine and nicotinic acid have inhibitory effects on the Maillard reaction. Despite all these, more investigations are required regarding the effect of the Maillard reaction in sugar cane processing before the crystallization (boiling sugar) of the sucrose. Future studies of MR inhibitors for the sugar cane processing should be targeted on the antioxidant effect of these three chemical inhibitors (AA, Cys and NA) and should be focused on their action in the different steps of MR.

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REFERENCES

Bento, L.S.M. and S. Sa, 1998. Study of high molecular weight compounds in sugar using gel permeation chromatography with an evaporative light scattering detector. Carbohydr. Polym., 37: 257-261.

Chou, C.C., K. Iqbal, Y.G. Min, D.W. Gao and E. Duffaut, 2002. White and refined sugar production from cane sugar factories. First Biennial World Conference On Recent Development in Sugar Technologies. May 16-17, 2002, Marriott Hotel, Delray Beach, Florida, USA,.

Clement, A., L. Lagace and B. Panneton, 2010. Assessment of maple syrup physico-chemistry and typicity by means of fluorescence spectroscopy. J. Food Eng., 97: 17-23.

Coca, M., M.T. Garcia, G. Gonzalez, M. Pena and J.A. Garcia, 2004. Study of colored components formed in sugar beet processing. Food Chem., 86: 421-433.

Cortez-Vega, W.R., B.P.A. Maria, S.J. Marques and F.G. Graciano, 2008. Effect of L-Ascorbic acid and sodium metabisulfite in the inhibition of the enzymatic browning of minimally processed apple. Int. J. Agric. Res., 3: 196-201.

De Vleeschouwer, K., I. Van der Plancken, A. Van Loey and M.E. Hendrickx, 2010. The effect of high pressure-high temperature processing conditions on acrylamide formation and other Maillard reaction compounds. J. Agric. Food Chem., 58: 11740-11748.

Donovan, M. and J.C. Williams, 1992. The factors influencing the transfer of color to sugar crystals. Proceedings of the Sugar Processing Research Conference, September 27-29, 1992, USA., pp: 31-48.

Hedegaard, R.V., H. Frandsen, K. Granby, A. Apostolopoulou and L.H. Skibsted, 2007. Model studies on acrylamide generation from glucose/asparagine in aqueous glycerol. J. Agric. Food Chem., 55: 486-492.

Honig, P., 1953. Principles of Sugar Technology. Vol. 1, Chapter 4, 7, 8 and 9, Elsevier Pub. Co., Netherlands, Pages: 767.

Ibarz, A., A. Garvin, S. Garza and J. Pagan, 2009. Toxic effect of melanoidins from glucose-asparagine on trypsin activity. Food Chem. Toxicol., 47: 2071-2075.

Knerr, T., H. Lerche, M. Pischetsrieder and T. Severin, 2001. Formation of a novel colored product during the Maillard reaction of d-glucose. J. Agric. Food Chem., 49: 1966-1970.

Knol, J.J., W.A.M. Loon, J.P.H. Linssen, A.L. Ruck and M.A.J.S. van Boekel, 2005. Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system. J. Agric. Food Chem., 53: 6133-6139.

Mao, L.C., Y.Q. Xu and F. Que, 2007. Maintaining the quality of sugarcane juice with blanching and ascorbic acid. Food Chem., 104: 740-745.

Pant, D. and A. Adholeya, 2007. Biological approaches for treatment of distillery wastewater: A review. Bioresour. Technol., 98: 2321-2323.

Roberts, E.J. and L.F. Martin, 1959. Progress in determining organic nonsugars of sugarcane juice that affect sugar refining. Proceedings of the 6th Technical Session on Bone Char, Montreal, Canada, (TSBC'59), Bone Char Research Project, Inc., pp: 67-99.

Tochi, B.N., Z. Wang, S.Y. Xu and W. Zhang, 2009. Effect of stem bromelain on the browning of apple juice. Am. J. Food Technol., 4: 146-153.

Zyzak, D.V., R.A. Sanders, M. Stojanovic, D.H. Tallmadge and B.L. Eberhart *et al.*, 2003. Acrylamide formation mechanism in heated foods. J. Agric. Food Chem., 51: 4782-4787.

J. Agric. Food Chem. 1990, 38, 1642–1647

Appendix 6

Inhibition of Browning by Sulfur Amino Acids. 1. Heated Amino Acid–Glucose Systems

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Amino acids interact with carbohydrates to form Maillard browning products. Such reactions reduce the nutritional value of foods containing amino acids and carbohydrates and may lead to the formation of compounds that are mutagenic and clastogenic or chromosome-damaging. A need therefore exists to inhibit these heat-induced interactions. To demonstrate whether SH-containing sulfur amino acids minimize nonenzymatic browning, β-alanine, N^{α}-acetyl-L-lysine, glycylglycine, and a mixture of amino acids were each heated with glucose in the absence and presence of the following potential inhibitors: *N*-acetyl-L-cysteine, L-cysteine, reduced glutathione, sodium bisulfite, and urea. Inhibition was measured as a function of temperature, time of heating, and concentration of reactants. The extent of browning was estimated by absorbance measurements at 420 nm. Inhibition was independent of the amino group containing reactant. The minimum concentrations for optimum inhibition, in moles of inhibitor per mole of D-glucose, were as follows: sodium bisulfite, 0.02; L-cysteine, 0.05; *N*-acetyl-L-cysteine, 0.2; reduced glutathione, 0.2; urea, 8. An "index of prevention" (IP) was used to calculate the inhibition at the optimum mole ratio range, where IP = 100 − [molar absorptivity value (MAV) of the amine compound + glucose + inhibitor] × 100/(MAV of the amine compound + glucose). The calculated values were about 90% in all cases. Possible mechanisms of browning prevention are discussed.

INTRODUCTION

Sulfur-containing amino acids such as cysteine, *N*-acetylcysteine, and the tripeptide glutathione actively participate in the detoxification of xenobiotics in vivo. These sulfur-containing compounds also inhibit the action of mutagens, carcinogens, and other toxic compounds by direct interaction. These antioxidant and antitoxic effects are due to several mechanisms including the ability to act as (a) reducing agents, (b) scavengers of reactive oxygen (free-radical species), (c) strong nucleophiles that can trap electrophilic compounds and intermediates, (d) precursors for intracellular reduced glutathione, and (e) inducers of cellular detoxification. For example, we (Friedman, 1984; Friedman et al., 1982a) have shown that cysteine and related thiols inactivate the mutagenicity of aflatoxin. Other examples include (a) the demonstration by De Flora (1989) that coadministration of *N*-acetylcysteine dramatically decreased urethane-induced tumor formation in mice, (b) the report by Troll (1986) that the sulfur-rich protein called the Bowman–Birk protease inhibitor suppresses nitrosamine-induced carcinogenicity in the digestive tract of rats, (c) the reported protection in sheep against bitterweed (*Hymenoxys odorata*) poisoning by dietary components that stimulate the formation of SH-containing compounds in vivo (Calhoun et al., 1989), (d) the reported reduction of mutagen formation in fried beef by adding cottonseed flour (Rhee et al., 1987) or soy protein concentrate (Wang et al., 1982), and (e) the observed inhibition of lysinoalanine formation by cysteine (Finley et al., 1978; Friedman, 1978).

For these reasons, fruitful results are expected from evaluation of the effectiveness of sulfur amino acids and sulfur-rich proteins in (a) preventing the formation of antinutritional and toxic browning products by trapping intermediates and (b) reducing the toxicity of browning products in animals by preventing transformation of such compounds to biologically active forms.

This study examines the relative potencies of three SH-containing amino acids, sodium bisulfite, and urea in inhibiting browning of amino acids or peptides heated with glucose. Since sulfites are reported to induce asthmatic crises in 4–8% of exposed asthmatics (Gifon et al., 1989), a need exists to develop sulfite substitutes that can inhibit food browning. In two companion papers we cover related studies on the inhibition of browning in apples, potatoes, fruit juices, and protein-rich foods (Molnar-Perl and Friedman, 1990a,b).

MATERIALS AND METHODS

Matrials. L-Cysteine (free base) was obtained from U.S. Biochemical Corp., Cleveland, OH. All other amino acids, reduced glutathione, and urea came from Sigma, St. Louis, MO. Sodium bisulfite came from Malinckrodt, St. Louis, MO.

Instruments. Browning of amino acids was assayed by absorbance measurements with a Beckman DB spectrophotometer. A Radiometer pHM 26 meter and a Beckman 39030 thin-probe combination electrode were used for pH measurements.

Browning of Amino Acids with Glucose. Stock solutions of D-glucose (2 M), β-alanine (2 M), N^{α}-acetyl-L-lysine (0.5 M), mixed amino acids (0.6 M), glycylglycine (0.8 M), *N*-acetyl-L-cysteine (0.5 M), L-cysteine (0.4 M), glutathione (0.4 M), sodium bisulfite, (0.1 M), and urea (5 M) were adjusted to pH 6.5 before use. The solution of mixed amino acids was made up as follows (moles per liter): α-alanine (0.02), L-arginine (0.04), glycine (0.04), L-histidine (0.04), L-isoleucine (0.04), L-ornithine (0.04), L-phenylalanine (0.04), L-proline (0.04), L-serine (0.04), L-threonine (0.04), and L-valine (0.04) or 0.06 mol/L mixed amino acids.

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Table I. Molar Absorptivity Values (Am/M·cm) Obtained from the Reactions of Alanines (AL) and N^{α}-Acetyl-L-lysine (*N*-ALL) with D-Glucose (D-Glu)[a]

reactants	Am/(M·cm) 420 nm	Am/(M·cm) 300 nm	Am_{300}/Am_{420}
DL-Al–D-Glu	0.28 ± 0.015	7.0	25
L-Al–D-Glu	0.28 ± 0.015	7.0	25
D-Al/D-Glu	0.46 ± 0.010	11.0	24
β-Al–D-Glu	3.50 ± 0.20	52.5	15
N-ALL–D-Glu	1.43 ± 0.20	41.0	29

[a] Conditions: β-AL/D-Glu = 0.4 M/0.2 M; N-ALL/D-Glu = 0.10 M/0.2 M; temperature, 100 °C; reaction time, 90 min; initial pH 6.1–6.2; final pH 5.7–5.8.

To the mixture of 0.5 mL of D-glucose and 1.0–2.0 mL of one of the amino group containing solutions were added different amounts of the inhibitors in matched vials with screw caps (Kimble, Division of Owens, IL, No. 60910-1). The final volume of the solutions was adjusted to 5.0 mL with distilled water. The vials were placed in a boiling water bath and heated at 100 °C for periods up to 120 min. At the end of the heating period the vial rack was placed in a cold water bath. The absorbances of the solutions at 420 nm against distilled water blanks were determined using matched 1-cm cells. The standard error in preliminary experiments was estimated to be ±5%.

RESULTS AND DISCUSSION

Electron spin resonance studies (ESR) and related studies on the browning reaction (Feather and Huang, 1986; Friedman, 1982; Friedman et al., 1990; MacGregor et al., 1989; Montgomery, 1983; Namiki and Hayashi, 1983; Yen and Lai, 1987) revealed that both α- and β-alanines react with D-glucose under the influence of heat and that β-alanine is a much more reactive precursor of Maillard products than is α-alanine. For this reason, we selected β-alanine as the major model compound to assess the effectiveness of several potential inhibitors of nonenzymatic browning reactions. Table I compares molar absorptivity values of D-glucose heated with L-alanine, D-α-alanine, DL-α-alanine, β-alanine, and N^{α}-acetyl-L-lysine. The results at 420 nm show that β-alanine browning was about 12 times greater than that of α-alanine and about twice that of N^{α}-acetyl-L-lysine, a model for proteins. The values at 300 nm roughly parallel those at 420 nm. Figure 1 illustrates the absorbance maxima of the heated alanine–glucose mixtures. The results suggest that Maillard products produced by the alanines are probably similar in structure and that the structure of the alanine influences the concentrations of specific products in the equilibrium mixture.

Table II lists the molar absorptivity values at 420 nm of the browning products derived from various concentrations of β-alanine–D-glucose and N^{α}-acetyl-L-lysine–D-glucose heated at 100 °C for various times. The results strikingly illustrate that browning increases progressively as a function of concentration and time.

Figure 2 shows the pH profile of the molar absorptivities of β-alanine–D-glucose with initial pH ranging from 4.4 to 10.0, heated at 100 °C for 90 min. The sigmoid-shaped curve suggests that browning is strongly catalyzed by pH. Although initial and final pH values of the reaction mixtures were similar up to about pH 7, the pH decreased significantly in the final values for initial values beginning at about 8. The cause of this decrease is not immediately apparent.

On the basis of these results, we chose the following conditions to assess the effectiveness of several potential browning inhibitors: temperature 100 °C, reaction time 90 min, and pH 5.8–6.1 at a constant level of inhibitor. The following mole ratios of reactants were generally used:

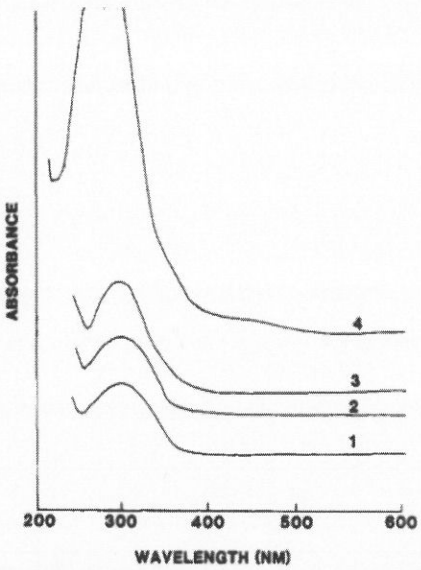

Figure 1. Absorbance spectra of the reaction products of alanines with D-glucose. Conditions: AL/D-Glu = 0.4 M/0.2 M; spectra taken with solutions diluted 1:10; 100 °C; 90 min; initial pH 6.1–6.2; final pH 5.7–5.8. Spectra: (1) L-AL–D-Glu; (2) D-AL–D-Glu; (3) DL-AL–D-Glu; (4) β-AL–D-Glu.

Table II. Molar Absorptivity Values (Am/M·cm) at 420 nm Obtained from the Browning Reactions at 100 °C of β-AL–D-Glu and N^{α}-Acetyl-L-lysine (*N*-ALL)–D-Glu as a Function of Concentrations and Mole Ratios of Reactants and Reaction Time[a]

β-AL:D-Glu mole ratio	β-AL:D-Glu concn, mol:mol	Am/(M·cm) at 420 nm 15 min	30 min	60 min	120 min
1:1	0.5:0.5	0.28	1.63	0.75	
1:2	0.5:1.0	0.47	1.90	2.25	
1:3	0.5:1.5	0.81	3.70	5.00	
2:1	1.5:0.5	1.02	3.60	5.00	
3:1	1.5:0.5	3.00	9.50	11.50	
3:1	0.75:0.25	0.88	3.40	5.00	22.80
	0.375:0.125	0.16	0.50	0.96	5.60
	0.150:0.050	0.10	0.16	0.24	0.90
	0.075:0.025	0.0	0.0	0.0	0.60
3:1	0.60:0.020			4.40	
6:1	0.60:0.10			3.40	
12:1	0.60:0.05			3.00	
24:1	0.60:0.025			2.92	

β-AL or *N*-ALL:D-Glu mole ratio	β-AL or *N*-ALL:D-Glu concn, mol:mol	β-AL:D-Glu (90 min)	*N*-ALL:D-Glu (90 min)
0.25:1	0.06:0.2	0.55 ± 0.05	1.08 ± 0.08
0.5:1	0.1:0.2	0.61 ± 0.04	1.43 ± 0.02
1:1	0.2:0.2	0.90 ± 0.03	3.25
1.5:1	0.3:0.2	2.07 ± 0.05	5.28 ± 0.20
2:1	0.4:0.2	3.50 ± 0.20	8.50
3:1	0.6:0.2	7.93 ± 0.35	
4:1	0.8:0.2	15.30 ± 0.40	
6:1	1.2:0.2	18.50 ± 0.10	

[a] Conditions: See Table I.

β-alanine/D-glucose, 0.4 M/0.2 M; N^{α}-acetyllysine/D-glucose, 0.1 M/0.2 M; glycylglycine/D-glucose, 0.16 M/0.2 M; and mixture of amino acids/D-glucose, 0.12 M/0.2 M.

Since oxygen may influence the extent of free-radical reactions as well as the extent of browning, mixtures of β-alanine–D-glucose with and without the inhibitor *N*-acetylcysteine were each saturated with nitrogen and oxygen, respectively, before heating. Since the extent of browning

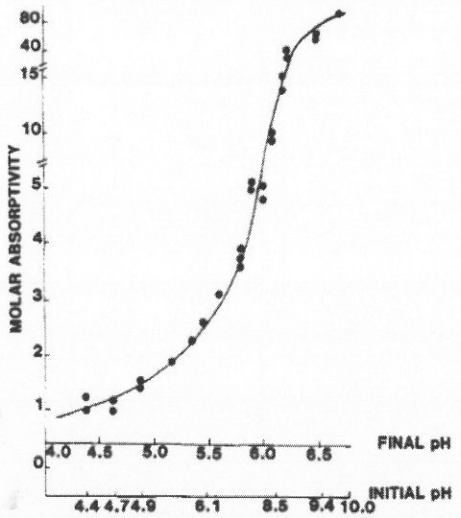

Figure 2. Molar absorptivity value–pH curve for the reaction of β-AL–D-Glu performed in solutions of various pH values. Conditions: β-AL/D-Glu = 0.4 M/0.2 M; 100 °C; 90 min. The initial (pH 4.40–10.0) and the final (pH 4.40–6.85) values are both indicated on the axis.

Table III. Effectiveness of Inhibitors on Browning Reactions (I–IV) of D-Glucose with β-Alanine (I), N^{α}-Acetyl-L-lysine (II), a Mixture of Amino Acids (III), and Glycylglycine (IV)

reaction	inhibitors	IP,[a] %	stoichiometry of inhibition,[b] mol of inhibitor/mol of MP
I	*N*-acetyl-L-cysteine	70	0.2
II	*N*-acetyl-L-cysteine	83	0.4
III	*N*-acetyl-L-cysteine	91	2.0
IV	*N*-acetyl-L-cysteine	89	0.4
I	L-cysteine	79	0.05
II	glutathione	83	0.08
I	sodium bisulfite	79	0.02
II	sodium bisulfite	96	0.12
III	sodium bisulfite	74	0.16
IV	sodium bisulfite	91	0.05
I	urea	91	12
II	urea	88	25
III	urea	95	12
IV	urea	89	12

[a] Index of prevention (IP) = 100 – (molar absorptivity value of the amine compound + glucose + inhibitor) × 100/(molar absorptivity value of the amine compound + glucose). [b] Minimum mole ratios needed to achieve the corresponding IP value. MP, Maillard reaction product precursor.

of the two solutions was the same, oxygen does not seem to influence the Maillard reactions.

Next, we used ultraviolet–visible and nuclear magnetic resonance spectroscopy (NMR) to systematically evaluate the relative suppression of browning by the following inhibitors: L-cysteine, *N*-acetyl-L-cysteine, reduced glutathione, sodium sulfite, and urea. The UV–visible spectra were plotted as molar absorptivities (Am) as a function of the molar ratios of the heated amino acid–D-glucose reaction mixtures in the absence and presence of each inhibitor (not shown). The tables and plots reveal the following information about browning and its prevention.

(1) Browning inhibition, as defined by an index of prevention (IP in Table III) varied between 70% and 96%. However, the amount of inhibitor needed to achieve this degree of inhibition varied. The minimum concentrations of each inhibitor needed for optimum inhibition per mole of D-glucose were as follows: sodium bisulfite, 0.02; L-cysteine, 0.05; *N*-acetylcysteine, 0.2; reduced glutathions, 0.2; urea, 8. For the first three, the concentration needed to inhibit browning was less than the theoretical amount needed to interact stoichiometrically with either partner in the browning reaction.

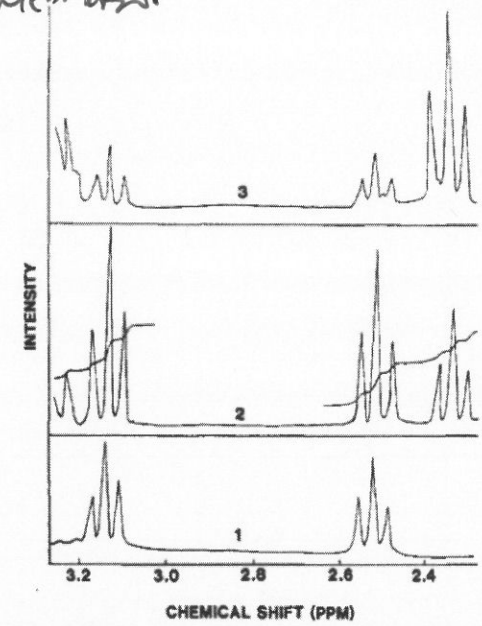

Figure 3. NMR spectra in the range 2.30–3.35 ppm of β-AL (0.4 M) (spectrum 1), β-AL (0.4 M) plus urea (3.0 M) (spectrum 2), and β-AL (0.4 M) plus urea (3.0 M) plus D-Glu (0.2 M) (spectrum 3).

(2) The molar absorptivity values for β-alanine–D-glucose and the inhibited Maillard reactions were surprisingly high. Mole ratios of 0.2, 0.5, and 0.6 of sulfur amino acids or sodium sulfite to Maillard product precursors resulted in absorptivity values that increased with inhibitor concentration. This suggests that autoxidation products of the inhibitors may contribute to the spectra.

(3) Preliminary NMR studies show that the addition of inhibitors to β-alanine–D-glucose caused no spectral changes. This result suggests that there are no apparent chemical interactions between cysteine, *N*-acetylcysteine, reduced glutathione, or sodium bisulfite and the Maillard product precursors. However, the NMR spectra do reveal possible interactions between urea and D-glucose. Thus, Figure 3, spectrum 1, shows two triplets associated with heated β-alanine. In the presence of urea, a third triplet appears at around 2.35 ppm, presumably arising from the interaction between urea and β-alanine (Figure 3, spectrum 2). The ratio of intensities of the two triplets centered at 2.70 and 2.35 ppm is 6:4. The spectrum of heated β-alanine plus D-glucose plus urea (Figure 3, spectrum 3) shows that D-glucose changes this ratio to about 1:4. The figure also shows that the initial triplets due to β-alanine at 2.53 and 3.14 ppm are further split as a result of reaction with urea, forming two additional triplets centered at 2.35 and 3.27 ppm. These observations suggest that urea inhibits browning by chemically interacting with β-alanine, thus preventing its reaction with glucose.

(4) Figures 4–7 show the absorption spectra in the region 200–600 nm for the Maillard product β-alanine–D-glucose (spectrum 1), for D-glucose plus inhibitors (spectrum 2), for β-alanine plus inhibitors (spectrum 3),

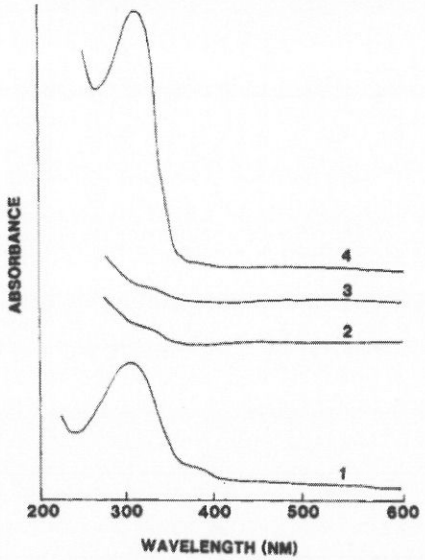

Figure 4. Absorbance spectra in the region 600–200 nm of solutions of β-AL/D-Glu = 0.4 M/0.2 M (spectrum 1), *N*-acetylcysteine (NAC)/D-Glu = 0.2 M/0.2 M (spectrum 2), NAC/β-AL = 0.2 M/0.4 M (spectrum 3), and β-AL/D-Glu/NAC = 0.4 M/0.2 M/0.2 M (spectrum 4), all four treated at optimum condition. Spectra have been taken at dilutions 1:50.

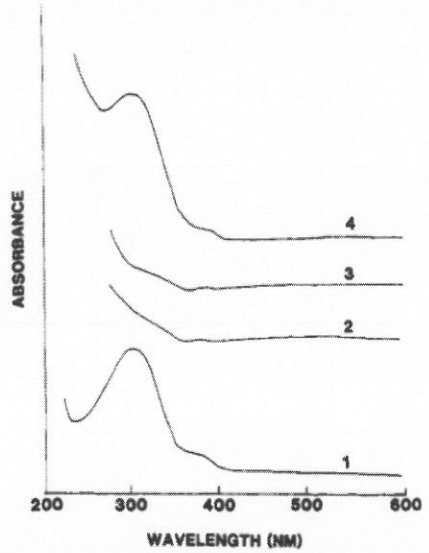

Figure 5. Absorbance spectra of solutions of β-AL/D-Glu = 0.4 M/0.2 M (spectrum 1), GSH/D-Glu = 0.05 M/0.2 M (spectrum 2), GSH/β-AL = 0.05 M/0.4 M (spectrum 3), and β-AL/D-Glu/GSH = 0.4 M/0.2 M/0.05 M (spectrum 4). Conditions as in Figure 1.

and for the inhibited Maillard products (spectrum 4). As with the NMR data, the electronic spectra show changes only for the urea-inhibited reaction (compare spectrum 1 to spectrum 4 in Figure 7), in which the characteristic absorption spectrum at 300 nm observed with Maillard product is decreased considerably. This difference may be due to the fact that any maximum absorption of the inhibited Maillard product is in a different part of the overlapping spectra. Evaluation of the spectra depicted in Figures 4–6 suggests the following: (a) the products of the inhibited reactions have absorption maxima at the same wavelength as the Maillard product precursors (Figure 4, spectra 1 and 4); and (b) the molar absorptivity values of the inhibited Maillard reaction are nearly the same as

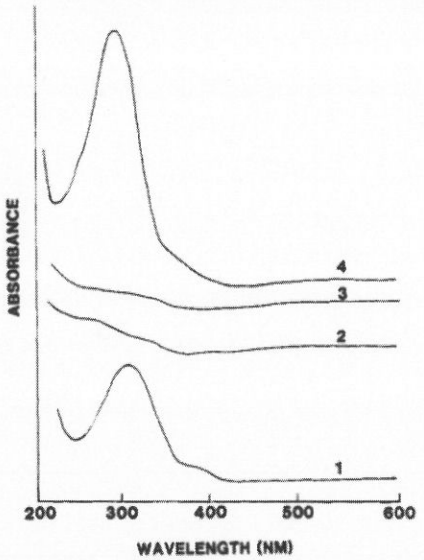

Figure 6. Absorbance spectra of solutions of β-AL/D-Glu = 0.4 M/0.2 M (spectrum 1), sodium bisulfite/D-Glu = 0.01 M/0.2 M (spectrum 2), sodium bisulfite/β-AL (spectrum 3), and β-AL/D-Glu/sodium bisulfite = 0.4 M/0.2 M/0.01 M (spectrum 4). Conditions as in Figure 1.

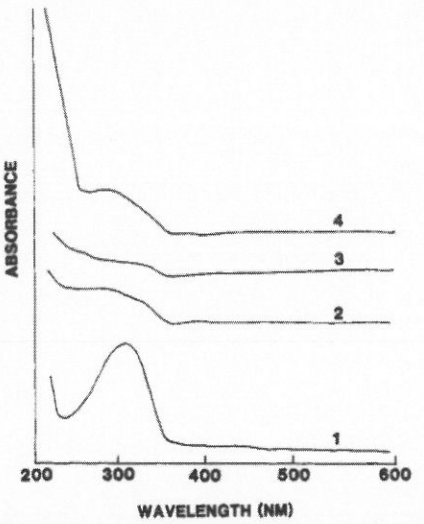

Figure 7. Absorbance spectra of solutions of β-AL/D-Glu = 0.4 M/0.2 M (spectrum 1), urea/D-Glu = 3.0 M/0.2 M (spectrum 2), urea/β-AL = 3.0 M/0.4 M (spectrum 3), and β-AL/D-Glu/urea = 0.4 M/0.2 M/3.0 M (spectrum 4). Conditions as in Figure 1.

(Figure 4 and 5) or somewhat higher than (Figure 6) that of the uninhibited Maillard reaction mixture. The spectral studies were repeated several times to validate the above conclusions.

Chemistry of Inhibition. Although the nature of the inhibition processes is not well understood, possibilities include (a) suppression of free-radical formation, whereby the formed radicals during heating are abstracted by and localized on the sulfur moiety of the thiol; and (b) interaction of the sulfhydryl compounds with intermediates formed during browning, thus trapping them and preventing them from forming the final browning product(s). Because of their strong nucleophilic reactivity and ability to dissipate free radicals, sulfur amino acids are especially capable of participating in the cited transformations on the basis of their extensively studied chemical properties. Thus, as previously noted (Fried-

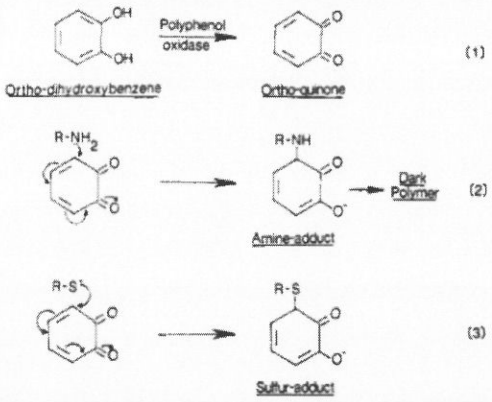

Figure 8. Postulated mechanism for the inhibition of polyphenol oxidase induced enzymatic browning by thiols: oxidation of a dihydroxybenzene to an *o*-quinone that can then participate in nucleophilic addition reactions with amino groups, leading to the formation of dark browning products, or competitively with mercaptide ions to form sulfur adducts, thus blocking polymer formation.

man, 1973), sulfhydryl groups in amino acids, peptides, and proteins participate in anionic, cationic, and free-radical reactions both in vitro and in vivo. The reactivity of the thiolate anion is much greater than would be expected from its basicity. Indeed, the thiolate anion appears to be one of the strongest nucleophiles known. This great reactivity presumably results from the polarizability of sulfur electrons and the availability of empty d orbitals, permitting d-orbital overlap. Another possibility is that the SH-containing compounds reduce carbonyl groups or react with carbonyl groups and double bonds in brown products to form colorless materials. These properties of the SH group can be taken advantage of to improve the quality and safety of our food supply.

Some of these concepts will be briefly illustrated with the aid of Figure 8, which schematically depicts the oxidation of a dihydroxy(poly)phenol by polyphenol oxidase to an *o*-quinone. This highly reactive intermediate then reacts with amino acids, peptides, and proteins to form dark polymers (Deshpande et al., 1984; Golan-Goldhirsh and Whitaker, 1984; Hurrell and Finot, 1984). In the presence of SH groups, which are known to react 200–300 times faster than amino groups in related nucleophilic addition reactions (Friedman et al., 1965), the quinone intermediate may be preferentially trapped as the sulfur adduct, thus preventing enzymatic browning.

Similar equations can be written for the preferential trapping of aldehyde or keto groups of reducing sugars to suppress nonenzymatic browning (Friedman, 1973). For example, in the pathway toward Maillard browning, it has been postulated that an aldehyde group of a reducing sugar interacts with an amino group to form a hemiketal adduct, which then dehydrates rapidly to form an aldimine (Schiff base). After an Amadori rearrangement, the latter is further transformed to dark browning products (eq 1). In the presence of a thiol, the aldehyde can com-

$$\underset{\text{carbohydrate}}{\text{R—CHO}} + \underset{\text{amine}}{\text{R'—NH}_2} \overset{H^+}{\rightleftharpoons} \underset{\text{hemiketal (carbinolamine)}}{\text{R—CH(OH)N}^+\text{H}_2\text{—R'}} \overset{-H_2O}{\rightarrow} \underset{\text{aldimine (Schiff base)}}{\text{RCH=NH—R'}} \rightarrow \text{dark products} \quad (1)$$

$$\text{R—CHO} + \underset{\text{thiol}}{\text{R'—SH}} \rightleftharpoons \underset{\text{thiohemiketal (carbinol sulfide)}}{\text{R—CH(OH)S—R'}} \quad (2)$$

$$\text{R—CH(OH)S—R'} + \text{R'SH} \overset{-H_2O}{\rightarrow} \underset{\text{thioketal}}{\text{R—CH—(SR')}_2} \quad (3)$$

petitively interact with one or two SH groups to form a thiohemiketal or thioketal, thus blocking Maillard browning (eqs 2 and 3).

Relative reactivities of aldehyde or ketone groups with SH or NH_2 groups in structurally different environments will dictate the extent of inhibition of Maillard browning by various thiols. More work is needed to define the exact chemistry of the browning inhibition, as was done for sulfur dioxide (Wedzicha, 1987).

CONCLUSIONS

Our results indicate that SH-containing amino acids such as cysteine, *N*-acetylcysteine, and reduced glutathione are nearly as effective as sodium bisulfite in preventing nonenzymatic browning of heated amino acid–glucose mixtures. Urea is less effective, but it may be of special value in feed products for animal consumption, where it can also serve as a nutritional source of nitrogen for ruminants such as cattle and sheep (Friedman et al., 1982b).

Conditions were defined to evaluate the extent of browning inhibition in terms of an index of prevention (IP). If widely adopted, this index could serve as a well-defined measure of the relative effectiveness of browning inhibitors. This would facilitate comparing results from different studies in terms of a single parameter of browning prevention. Our findings also suggest that SH-containing sulfur amino acids may be of practical value to prevent browning in various food products. This has been successfully demonstrated in two companion papers (Molnar-Perl and Friedman, 1990a,b).

Finally, a special need exists to prevent browning in solution used for parenteral nutrition, containing both amino acids and carbohydrates (Kies, 1989; Rassin, 1989; Neuhauser-Berthold, 1989). The described studies on inhibition of browning in heated amino acid–carbohydrate solutions may provide a basis for designing parenteral solutions containing both of these food ingredients, so that a single intravenous feeding could replace two separate feedings containing either amino acids or carbohydrates.

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LITERATURE CITED

Calhoun, M. C.; Baldwin, B. C.; Kuhlmann, S. W.; Kim, H. L. Experimental prevention of bitterweed (*Hymenoxys odorata*) poisoning in sheep. *Am. J. Vet. Res.* **1989**, *50*, 1642–1646.

De Flora, S.; Benicelli, C.; Serra, D.; Izzotti, A.; Cesarone, C. F. Role of glutathione and N-acetyl-cysteine as inhibitors of mutagenesis and carcinogenesis. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; Plenum: New York, 1989; Vol. 3, pp 19–53.

Deshpande, S. S.; Sathe, S. K.; Salunkhe, D. K. Chemistry and safety of plant polyphenols. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum Press: New York, 1984; pp 457–495.

Feather, M. S.; Huang, R. D. Some studies on a Maillard polymer derived from L-alanine and D-glucose. In *Amino-Carbonyl Reactions in Food and Biological Systems*: Fujimaki, M., Namiki, M., Kato, H., Eds.; Elsevier: Amsterdam, 1986; pp 183–192.

Finley, J. W.; Snow, J. T.; Johnston, P.; Friedman, M. Inhibition of lysinoalanine formation in food proteins. *J. Food Sci.* **1978**, *43*, 619–621.

Friedman, M. *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins*; Pergamon Press: Oxford, England, 1973; Chapters 1 and 10.

Friedman, M. Inhibition of lysinoalanine synthesis by protein acylation. *Adv. Exp. Med. Biol.* **1978**, *105*, 613–648.

Friedman, M. Chemically reactive and unreactive lysine as an index of browning. *Diabetes* **1982**, *31*, 5–14.

Friedman, M. Sulfhydryl groups and food safety. *Adv. Exp. Med. Biol.* **1984**, *177*, 31–63.

Friedman, M.; Wehr, C. M.; Schade, J. E.; MacGregor, J. T. Inactivation of aflatoxin B_1 mutagenicity by thiols. *Food Chem. Toxicol.* **1982a**, *20*, 887–892.

Friedman, M.; Diamond, M. J.; Broderick, G. L. Dimethylolurea as a tyrosine reagent and protein protectant against ruminal degradation. *J. Agric. Food Chem.* **1982b**, *30*, 72–77.

Friedman, M.; Wilson, R. E.; Ziderman, I. I. Effect of heating on mutagenicity of fruit juices in the Ames test. *J. Agric. Food Chem.* **1990**, *38*, 740–743.

Giffon, E.; Vervloet, D.; Charpin, J. Suspicion sur les sulfites. *Rev. Mal. Respir.* **1989**, *6*, 303–310.

Golan-Goldhirsh, A.; Whitaker, J. R. Relation between structure of polyphenol oxidase and prevention of browning. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum Press: New York, 1984; pp 437–456.

Hurrell, R. F.; Finot, P. A. Nutritional consequences of the reactions between proteins and oxidized polyphenolic compounds. In *Nutritional and Toxicological Aspects of Food Safety*: Friedman, M., Ed.; Plenum Press: New York, 1984; pp 432–435.

Kies, C. Comparative utilization from enteral formula diets by humans of intact proteins, polypeptides, peptides, and amino acids. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC: Boca Raton, FL, 1989; Vol. 2, pp 87–96.

MacGregor, J. T.; Tucker, J. D.; Ziderman, I. I.; Wehr, C. M.; Wilson, R. E.; Friedman, M. Non-clastogenicity in mouse bone marrow of fructose/lysine and other sugar/amino acid browning products with in vitro genotoxicity. *Food Chem. Toxicol.* **1989**, *27*, 715–721.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *J. Agric. Food Chem.* **1990a**, second of three papers in this issue.

Molnar-Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. *J. Agric. Food Chem.* **1990b**, third of three papers in this issue.

Montgomery, M. W. Cysteine as an inhibitor of browning in pear juice concentrate. *J. Food Sci.* **1983**, *48*, 961–962.

Namiki, M.; Hayashi, T. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. *ACS Symp. Ser.* **1983**, *215*, 21–46.

Neuhauser-Berthold, M. Amino acid derivatives as a source of amino acids in parenteral nutrition. In *Absorption and Utilization of Amino Acids*: Friedman, M., Ed.; CRC: Boca Raton, FL, 1989; Vol. 2, Chapter 7.

Rassin, D. K. Amino acid metabolism in total parenteral nutrition during development. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC: Boca Raton, FL, 1989; Vol. 2, pp 71–85.

Rhee, K. S.; Donnelly, K. C.; Ziprin, V. A. Reduction of mutagen formation in fried ground beef by glandless cottonseed flour. *J. Food Prot.* **1987**, *50*, 753–755.

Troll, W.; Frenkel, K.; Wiesner, R. Protease inhibitors: their role as modifiers of the carcinogenic process. *Adv. Exp. Med. Biol.* **1986**, *199*, 153–165.

Wang, Y. Y.; Vuolo, L. L.; Spingarn, L. E.; Weisburger, J. H. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. *Cancer Lett.* **1982**, *16*, 179–189.

Wedzicha, B. L. Review: chemistry of sulfur dioxide in vegetable dehydration. *Int. J. Food Sci.* **1987**, *22*, 433–450.

Yen, G. C.; Lai, Y. H. Influence of antioxidants on Maillard browning reaction in casein-glucose model system. *J. Food Sci.* **1987**, *52*, 1115–1116.

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Registry No. Glucose, 50-99-7; β-alanine, 107-95-9; *N*-α-acetyl-L-lysine, 1946-82-3; glycylglycine, 556-50-3; *N*-acetyl-L-cysteine, 616-91-1; L-cysteine, 52-90-4; glutathione, 70-18-8; sodium bisulfite, 7631-90-5; urea, 57-13-6.