

Supporting Document 1 (Approval)

Risk and Technical Assessment Report – Application A1068

Hydrogen Peroxide as a Processing Aid

Executive Summary

Hydrogen peroxide is currently permitted for use as a processing aid in the Australia New Zealand Food Standards Code (the Code). Current permitted uses are as a bleaching, washing and peeling agent for use in all foods; an inhibiting agent for dried vine fruits, fruit and vegetable juices, sugar, vinegar and yeast autolysate; for removal of glucose from egg products; and removal of sulphur dioxide. Hydrogen peroxide is also permitted for use in the manufacture of packaged water and in water used as an ingredient in other foods. For each of these uses, FSANZ has set a maximum permitted level (MPL) of residual hydrogen peroxide of 5 mg/kg in the final product.

This Application seeks approval to use hydrogen peroxide as a processing aid to control the population of lactic acid producing microorganisms, and in so doing, stabilise the pH during the manufacture of dairy products made using lactic acid producing microorganisms. The Applicant has proposed an MPL of residual hydrogen peroxide of 5 mg/kg for dairy products manufactured using this substance as a processing aid. Alternative methods of pH control such as refrigeration and heat treatment are costly, less effective and may result in poorer quality products.

The Applicant has clearly articulated the technological function of hydrogen peroxide when used as proposed. Hydrogen peroxide fulfils the stated technological function at the proposed level of use, i.e. it is effective for maintaining a stable pH in the production of dairy products manufactured using lactic acid producing microorganisms. Furthermore, hydrogen peroxide has a long history of use as a food processing aid and it is technically feasible to attain hydrogen peroxide residues below the MPL in the finished food. There are suitable analytical methods for verifying whether products manufactured as proposed comply with the proposed MPL.

Hydrogen peroxide is a product of normal mammalian metabolism produced in gram quantities by the body daily. It also occurs naturally in food. Estimates of dietary exposure to hydrogen peroxide indicate negligible exposure relative to that produced by the body. Toxicity following oral exposure is evident only with concentrations of hydrogen peroxide that are sufficiently high to produce local corrosive/irritant effects or result in oxygen embolism due to degradation of the substance. Such concentrations are at least 1000-fold greater than the MPL. An Acceptable Daily Intake (ADI) has not been established by FSANZ or any other regulatory body and is not considered necessary. The presence of hydrogen peroxide in food at levels at or below the MPL presents no health and safety concerns.

The overall conclusion of this risk and technical assessment is that the use of hydrogen peroxide as a processing aid for fermented dairy ingredients and products is technologically justified and raises no public health and safety issues for consumers.

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1. Introduction

1.1 Background

On 18 October 2011, Food Standards Australia New Zealand (FSANZ) received an Application from Fonterra Co-operative Group Limited, New Zealand, seeking an amendment to the Table to clause 14 of Standard 1.3.3 – Processing Aids in the *Australia New Zealand Food Standards Code* (the Code) to permit the use of hydrogen peroxide to control the population of lactic acid producing microorganisms, and in so doing, stabilise the pH during the manufacture of dairy products made using lactic acid producing microorganisms.

The Code currently includes permissions for use of hydrogen peroxide as a bleaching, washing and peeling agent for use in all foods; an inhibiting agent for dried vine fruits, fruit and vegetable juices, sugar, vinegar and yeast autolysate; for use in the removal of glucose from egg products; and for removal of sulphur dioxide. Hydrogen peroxide is also permitted for use in the course of manufacture of packaged water and in water used as an ingredient in other foods. A maximum permitted level (MPL) for hydrogen peroxide of 5 mg/kg applies to food/water resulting from each of these permitted uses.

The Applicant's proposed MPL is also 5 mg/kg for the use of hydrogen peroxide as a processing aid for dairy products made using lactic acid producing microorganisms.

1.2 Risk Assessment Questions & Scope

The following questions are addressed in this Risk and Technical Assessment Report:

- Is the use of hydrogen peroxide as a processing aid for dairy products made using lactic acid producing microorganisms technologically justified?
- Are dairy products made using hydrogen peroxide as a processing aid safe for consumption?

This Risk and Technical Assessment Report addresses the above questions in order and comprises the following components:

- (1) Food Technology Assessment, which describes the chemical properties of the compound and considers whether the use of hydrogen peroxide as a processing aid is technologically justified.
- (2) Risk Assessment, which evaluates the intrinsic toxicity of hydrogen peroxide and the potential risk to consumers from residual hydrogen peroxide in food produced through its use.

2. Food Technology Assessment

2.1 Hydrogen peroxide Characteristics

The following information regarding the identity and chemical and physical properties of the processing aid hydrogen peroxide has been taken from the Application and various references.

2.1.1 Identity

Common name:	hydrogen peroxide
Chemical name (IUPAC):	hydrogen peroxide
Other names:	dihydrogen dioxide, hydroperoxide, dioxidane
C.A.S. registry number:	7722-84-1
Molecular formula:	H ₂ O ₂
Structural formula:	H-O-O-H
Molecular weight:	34.0147 g/mol
Marketing names:	Albone, Hioxyl

2.1.2 Chemical and physical properties

Hydrogen peroxide is a colourless to very light blue liquid at ambient temperature and pressure. It is thermally stable under an inert gas atmosphere at less than 400⁰C and is stable in neutral and dilute acidic and alkaline solutions. Table 1 summarises other pertinent physicochemical properties of hydrogen peroxide.

Table 1: Physical and chemical properties of hydrogen peroxide

Melting point	-0.43°C at 1 atmosphere
Boiling point	150.2°C at 1 atmosphere
Density	1.4425 g/cm ³ at 25°C
Vapour pressure	3 hPa at 25°C
Solubility in water	Miscible

Hydrogen peroxide is a strong oxidising agent (United States National Library of Medicine, 2012). It is weakly acidic in aqueous solution, and undergoes a highly exothermic decomposition in the presence of small amounts of catalysts such as ions, oxides or hydroxides of metals e.g. iron, copper, manganese (Goor, 1989).

2.1.3 Production

The predominant method for industrial production of hydrogen peroxide employs the organic autoxidation process, primarily that of 2-alkylanthrahydroquinone (Goor *et al.*, 1989; Hess, 1995; European Chemicals Bureau, 2003).

In this method, a 2-alkylanthraquinone is hydrogenated to the corresponding 2-alkylanthrahydroquinone using a palladium or nickel catalyst. The alkylanthrahydroquinone solution is then separated from the catalyst and aerated with an oxygen-containing gas e.g. compressed air, to give hydrogen peroxide and the original alkylanthraquinone. The hydrogen peroxide is extracted using demineralised water, then purified and concentrated by fractionation (Hess, 1995).

Other methods of manufacturing hydrogen peroxide include electrolysis of aqueous ammonium sulphate or sulphuric acid solution in water (European Chemicals Bureau, 2003; Goor *et al.*, 1989). However, these methods have been superseded by the autoxidation process and are now of limited industrial significance (Goor *et al.*, 1989).

2.1.4 Specifications

The Applicant proposes to use the specification for hydrogen peroxide in the Food Chemicals Codex (2010). The Food Chemicals Codex is already listed as a primary source in clause 2 of Standard 1.3.4 – Identity and Purity, so no separate specifications for hydrogen peroxide need to be written.

2.1.5 Methods of analysis in foods

The Applicant has cited the International Dairy Federation (IDF) *Provisional Standard 74A:1991: Anhydrous Milk fat – Determination of Peroxide Value* as a suitable method for detecting and quantifying the presence of hydrogen peroxide in milk and anhydrous milk fat. FSANZ notes that the International Organisation for Standardisation (ISO) and the IDF have since issued a joint international standard “*ISO 3976 | IDF 74 Milk fat – Determination of peroxide value*” which cancels and replaces *IDF 74A:1991: Anhydrous Milk fat Determination of Peroxide Value* (ISO and IDF, 2006).

FSANZ notes that the ISO 3976 | IDF 74 method is not specific for hydrogen peroxide. It will also detect the products of lipid peroxidation in milk, so that the peroxide value obtained may overestimate the amount of hydrogen peroxide in the sample. This has been discussed with the Applicant, who acknowledged the potential for this to occur and indicated they are able to meet an MPL of 5 mg/kg.

The interference by the products of lipid peroxidation is likely to be minimal at the point that the peroxide is used during processing, and the Applicant has stated that other methods of analysis are available including AOAC Official Method 957.08 *Hydrogen Peroxide in Milk* as well as test strips. The Applicant has advised that hydrogen peroxide content can be quickly screened during processing using widely available semi-quantitative test strips which can measure from 0.5 to 25 mg hydrogen peroxide per litre (e.g. the Merck Millipore 110011 Peroxide Test¹) and that in countries where hydrogen peroxide is used as proposed in this Application this is routine practice. The Application also lists several other alternative methods for detecting the presence of hydrogen peroxide in dairy products (see page 19 of the Application).

Therefore, FSANZ concludes that there are suitable analytical methods for verifying whether products manufactured as proposed comply with the proposed MPL.

2.2 Technological function

This Application seeks approval to use hydrogen peroxide as a processing aid to control the population of lactic acid producing microorganisms, and in so doing, stabilise the pH during the manufacture of dairy products made using lactic acid producing microorganisms.

Fermented dairy products are typically made from pasteurised milk, whey, or related dairy materials that are inoculated with suitable dairy starter microorganisms. The Applicant states that for each fermented dairy product, optimum functionality² and quality occur at a specific pH. The starter microorganisms inoculated into dairy material produce lactic acid which lowers the pH of the material. If the organisms are allowed to continue producing acid after the optimal pH has been reached for a specific product, the functionality and quality will be compromised, hence the need to maintain the pH at a specific value.

¹ see http://www.merckmillipore.com/new-zealand/peroxide-test/MDA_CHEM-110011/p_29.b.s1LRy4AAAEWI.IfVhTI?attachments=PI

² According to the Applicant “functionality” describes the performance in the product, or the ability to create an emulsion, a foam, a gel etc., thereby contributing to the body and texture of the finished product.

The Applicant is proposing to use hydrogen peroxide to stabilise the pH of dairy material at the optimal value. The Applicant reports that adding sub-lethal concentrations of hydrogen peroxide to the dairy material inhibits growth of the starter population, which curtails lactic acid production and stabilises the pH. However, after approximately 12 hours, the population of starter bacteria will have recovered to a point where the amount of lactic acid being produced begins to lower the pH again.

The Applicant proposes an MPL of 5 mg/kg in foods produced using this process, which is the same as for other uses of hydrogen peroxide already permitted in the Code. To bring the level of residual hydrogen peroxide to below the MPL, the Applicant proposes to use the enzyme catalase, which breaks down hydrogen peroxide to water and oxygen (International Union of Biochemistry and Molecular Biology, 1999). FSANZ notes that the Code permits the use of catalase EC 1.11.1.6 sourced from *Aspergillus niger* and *Micrococcus luteus* as a food processing aid.

Other methods that could be used to control pH include heat treatment and refrigeration. However, the Applicant considers both methods unsuitable for various reasons. Refrigeration incurs additional energy costs during both cooling and reheating. Moreover, the microorganisms continue to produce acid during the cooling and heating cycle, making it an inefficient process for maintaining a stable pH.

According to the Applicant, heat treatment is not suitable for manufacturing the particular products of interest because:

- it kills the starter organisms
- it denatures whey proteins resulting in poor product functionality
- it alters product flavour and colour
- it reduces the nutritional value of the proteins by destroying lysine
- it promotes caking of packed product
- it creates cooked flavours.

2.3 Uses Excluded from this Application

The technological function of hydrogen peroxide proposed in this Application is limited to stabilising pH by controlling the population of lactic acid producing microorganisms during the manufacture of:

- fermented milk
- fermented milk products
- cheese made using lactic acid producing microorganisms
- cheese products made using lactic acid producing microorganisms.

These products are collectively referred to as 'dairy products made using lactic acid producing microorganisms' in the rest of this document.

This Application excludes use of hydrogen peroxide as a sanitiser, i.e. hydrogen peroxide is not proposed to be used as an alternative to good hygiene practices. The Application also excludes the use of hydrogen peroxide to stabilise deteriorating milk.

2.4 Food Technology Conclusion

The Applicant has clearly articulated the technological function of hydrogen peroxide when used as a processing aid in the manufacture of dairy products made using lactic acid producing microorganisms.

Hydrogen peroxide fulfils the stated technological function at the proposed level of use i.e. it is effective for maintaining a stable pH in the manufacture of these products. Furthermore, hydrogen peroxide has a long history of use as a food processing aid and it is technically feasible to attain hydrogen peroxide residues below the MPL in the finished food. There are suitable methods for detecting and quantifying the presence of hydrogen peroxide in dairy products.

3. Risk Assessment

3.1 Introduction

Hydrogen peroxide occurs naturally in food and is also a product of normal mammalian metabolism. The toxicological properties of hydrogen peroxide via the oral route of exposure have been addressed in several monographs and reviews (FASEB 1979, ECETOC 1993, Desesso et al 2000, ECB 2003). No new data on hydrogen peroxide relevant to hazard assessment were submitted as part of this Application. FSANZ considers the information submitted was adequate for purposes of assessing the safety of hydrogen peroxide as a processing aid.

3.2 History of Use

Hydrogen peroxide has a long history of safe use in the food industry. Experimental studies on its use as a milk preservative were first conducted in the late 19th century (references cited in Lück 1962). It has been subsequently used in a variety of food applications. Standard 1.3.3 includes the following uses for hydrogen peroxide: bleaching agent, washing and peeling agent for use in all foods; inhibiting agent for dried vine fruits, fruit and vegetable juices, sugar, vinegar and yeast autolysate; removal of glucose from egg products; removal of sulphur dioxide. Hydrogen peroxide is also permitted for use in the manufacture of packaged water and in water used as an ingredient in other foods. A maximum permitted hydrogen peroxide level of 5 mg/kg applies to food/water resulting from each of these uses.

3.3 Overseas Approvals

In the USA, hydrogen peroxide is approved for use in a variety of applications including the preservation of milk intended for cheese production (antimicrobial agent), and the preparation of whey (antimicrobial agent), dried egg products (oxidising agent, GMP³), starch, and corn syrup (to reduce sulphur dioxide levels). Residual hydrogen peroxide in the final foods is to be removed by appropriate physical or chemical means (USCFR 2003).

In Canada, hydrogen peroxide is approved for use in the manufacture of beer (clarification aid); processing of liquid whey destined for the manufacture of dried whey products (to decolourise and maintain pH); oat hulls used in the manufacture of oat hull fibre (bleaching agent; GMP), starch modifying agent (GMP); and liquid whole egg, egg yolk, or egg white destined for drying (CFDR 2012).

In Japan, the Food Sanitation Act allows for hydrogen peroxide to be used as a sterilising agent with the condition that it must be decomposed or removed prior to preparation of the final food (JETRO 2011).

³ Good Manufacturing Practice

3.4 Absorption, Distribution, Metabolism and Excretion

Hydrogen peroxide is a naturally occurring product of mammalian metabolism. It is produced by the body in gram quantities per day and eliminated as oxygen and water by intracellular and extracellular protective mechanisms that maintain concentrations in the range of 10^{-8} to 10^{-3} molar (ECETOC 1993). The daily amount of hydrogen peroxide produced by the human liver has been estimated to be approximately 7 g for an adult male (Boveris et al 1972). The steady-state concentration of hydrogen peroxide within cells, estimated at 10^{-8} molar (Chance et al 1979), is a function of its production by mitochondria and its enzymatic degradation by catalase and various peroxidases. Data on oral absorption of hydrogen peroxide are lacking because of the difficulty in distinguishing administered from endogenous hydrogen peroxide.

Ingested hydrogen peroxide presumably undergoes rapid degradation in the gastrointestinal (GI) tract leaving little of the intact compound available for absorption (FASEB 1979).

3.5 Toxicity

3.5.1 Acute toxicity

Oral LD₅₀ values in rats have been reported to be 800 mg/kg bodyweight (bw) for 70% hydrogen peroxide to more than 5000 mg/kg bw for 10% hydrogen peroxide. With 70% hydrogen peroxide, compound-related gross changes included ulcerative necrosis of the tongue, oesophagus, stomach and duodenum, and adhesions in the peritoneal cavity in rats found dead. In a rat study with 10% hydrogen peroxide, one female died on day 1 and the only necropsy findings noted were blood filled stomach and intestines and reddened lungs (ECB 2003).

There are a number of reported incidents of human poisoning by oral ingestion of aqueous hydrogen peroxide solutions, but few reports include dose information. Ingestion of 3% hydrogen peroxide usually results in only mild gastritis while ingestion of concentrated solutions (e.g. 35%) can result in severe erosion in the gastro-intestinal tract (GIT), ulceration and perforation (Pritchett et al 2007). Systemic toxicity can arise from gas embolism due to the liberation of oxygen from hydrogen peroxide decomposition *in vivo* (French et al 2010). Oral lethal doses in children range from approximately 600 to 3800 mg/kg bw. Fatalities in adults are less common and doses resulting in death could not be located in the published literature.

3.5.2 Sub-chronic toxicity

Sub-chronic repeat dose toxicity studies in rodents have been conducted using gavage dosing and administration in drinking water. In studies using higher concentrations of hydrogen peroxide (1% and greater) direct corrosive effects on the oral cavity and stomach were observed. Repeated exposure to hydrogen peroxide via drinking water has been shown to be lethal to mice and rats at concentrations above 1%.

In studies using lower, non-corrosive concentrations, reduced food and water intake and reduced body weight gain are the only consistently observed findings with no observed adverse effect levels (NOAELs) in the range of 30 to 60 mg/kg bw/day for both mice and rats (FASEB 1979, ECETOC 1993, ECB 2003).

3.5.3 Chronic toxicity and carcinogenicity

Hydrogen peroxide reacts with DNA in selected *in vitro* systems and has tested positive in various *in vitro* genotoxicity assays (see **Genotoxicity** below).

Its carcinogenic potential has therefore been extensively studied in chronic rodent studies. The results of these studies indicate that chronic oral exposure to hydrogen peroxide solutions sufficiently dilute to be non-corrosive/non-irritant does not present a risk for carcinogenicity (Desesso et al 2000, ECB 2003).

3.5.4 Genotoxicity

As indicated above, hydrogen peroxide can react with DNA and has frequently tested positive in a variety of *in vitro* genotoxicity assays. In bacterial tests, mutagenicity assays and DNA damage and repair assays have yielded positive results. With mammalian cells, positive results have been observed in assays investigating mutagenicity, DNA damage and repair, and chromosomal aberrations. However, *in vivo* genotoxicity studies in mice, rats and hamsters consistently showed a lack of genotoxicity associated with oral exposure to hydrogen peroxide (Desesso et al 2000, ECCB 2003).

3.5.5 Reproductive and developmental toxicity

No appropriate animal studies have been located for the evaluation of reproductive and developmental toxicity. However, hydrogen peroxide is thought to be rapidly degraded in the GIT, so that very little of the intact substance is available for systemic absorption. Therefore, it is unlikely that exogenously administered hydrogen peroxide could be associated with any effects on reproduction or development.

3.6 Residual Levels in Food

In the current Application, use of the permitted enzyme catalase is proposed for reducing the residual levels of hydrogen peroxide in dairy products, however no data were provided on the resulting levels of hydrogen peroxide. The proposed MPL of 5 mg/kg is equivalent to the MPL for the existing permissions for hydrogen peroxide in the Code.

3.7 Dietary Exposure

It has been estimated that the maximum dietary exposure to hydrogen peroxide occurring naturally in food is 1 mg/day. Additional exposure arising from the use of hydrogen peroxide in food production was also estimated to be 1 mg/day at most (ECB 2003). For an adult of bodyweight 60 kg, the resulting total dose of 2 mg/day results in a maximum estimated exposure of 0.033 mg/kg bw/day, whereas for a 15 kg child maximum estimated exposure is 0.13 mg/kg bw/day.

FSANZ considers that the additional dietary exposure to hydrogen peroxide resulting from its proposed use as a processing aid will be negligible. A dietary exposure assessment was not considered necessary.

3.8 Discussion

Hydrogen peroxide is a product of normal mammalian metabolism, occurs naturally in food, and has a long history of safe use as a food processing aid. Estimates of dietary exposure to hydrogen peroxide indicate negligible exposure relative to that produced normally by the body. Toxicity following oral exposure is evident only with concentrations of hydrogen peroxide that are sufficiently high to produce local corrosive/irritant effects or result in oxygen embolism due to degradation of the substance. Such concentrations are at least 1000-fold greater than the MPL. An Acceptable Daily Intake (ADI) has not been established by FSANZ or any other regulatory body and is not considered necessary. The presence of hydrogen peroxide in food at levels at or below the proposed MPL presents no health and safety

concerns for consumers.

3.9 Risk Assessment Conclusion

The use of hydrogen peroxide as a processing aid for dairy products as proposed in this Application with a maximum permitted level of 5 mg/kg raises no public health and safety concerns.

4. References

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