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Standards Management Officer
Food Standards Australia New Zealand
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Canberra 2610
ACT, Australia

October 31, 2011

Dear Sir/Madam

RE: Public Comment - APPLICATION A1045
BACTERIOPHAGE PREPARATION P100 AS APROCESSING AID 1st ASSESSMENT REPORT

I am writing in regards Application A1045 seeking the approval from EBI Food Safety Ltd to amend the Australian and New Zealand Food Standards Code to permit the use of bacteriophage (phage) preparation Listex P100 (Designated P100 preparation) as a processing aid to reduce numbers of Listeria monocytogenes in non- liquid ready-to-eat foods.

FreshBins would like to bring to the attention of Food Standards Australia that there is no need to use biochemical products such as P100 to remove the presence of Listeria monocytogenes in foods when Ozone can be used to achieve the same outcome, without the need for any form of additive. Ozone is increasingly being recognised world-wide for its ability to destroy many pathogens and bacteria, including listeria. The only bi-product from the Ozone sanitation process is the generation of Oxygen gas, a bi-product of the breakdown of ozone.

The objective of this document is to comment on the A1045 submission based on the use of Ozone instead of using the P100 approach as we believe it would be far more effective to use Ozone to destroy the Listeria viruses rather than adding another additive to the food to achieve the same outcome.

I look forward to the opportunity to provide you further information as required to satisfy any questions you may have in the application of Ozone in the food treatment process..

Yours sincerely,

David Nelson
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FreshBins Pty Ltd

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CONTENTS; ASSESSMENT REPORT is in BLUE.
SUBMISSION REPORT is in BLACK.

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20 September 2011 [17-11] APPLICATION A1045 BACTERIOPHAGE PREPARATION P100 AS A PROCESSING AID 1st ASSESSMENT REPORT

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The following material, which was used in the preparation of this Assessment Report, is available on the FSANZ website at
http://www.foodstandards.gov.au/foodstandards/applications/applicationa1045bact4797.cfm SD1 Risk Assessment Report

Executive Summary

Purpose

FSANZ received an Application from EBI Food Safety Ltd to amend the *Australia New Zealand Food Standards Code* (the Code) to permit the use of a bacteriophage (phage) preparation Listex P100 (designated P100 preparation) as a processing aid to reduce numbers of *Listeria monocytogenes* in non-liquid ready-to-eat foods. It is proposed that the bacteriophage preparation is applied to food as a spray or dip immediately prior to packaging.

Background

Bacteriophages infect, and destroy bacteria. They are highly specific and do not infect bacteria other than the species they infect. They are unable to infect plant, animal or human cells and are the most abundant biological entities on earth – being present wherever bacteria exist.

The Applicant proposes the use of P100 as a technology to be used in combination with other listericidal techniques currently applied in food processing. It is designed to complement good hygienic practices (GHP) used in food manufacturing. It is not meant for use as a surfactant, disinfectant or a general bactericide intended for other purposes within the processing facility. Phages used to treat food should be both lytic1 and non-transducing2 to ensure food safety.

Ready-to-eat foods are defined as any foods which are normally eaten in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form which is normally eaten without further preparation. Ready-to-eat non-liquid products may be treated with this preparation. FSANZ has confirmed with the Applicant that liquid foods are excluded from the scope of this Application.

1 Bacteriophages that undergo replication within the bacterial hosts to release phage particles by rupturing the host cells without integrating into the bacterial chromosome.

2 Transduction is the mechanism whereby bacterial genetic material is transferred between bacteria through a bacteriophage vector.

Ii FSANZ has assessed the safety and the proposed technological function of the P100 preparation.

In doing so, the efficacy and the continuity of the technological function under proposed use has been assessed. FSANZ has concluded that the P100 preparation is safe, effective and has no ongoing technological function when used under commercial conditions in non-liquid ready-to-eat foods
The Application is being assessed under the Major procedure and will include two rounds of public consultation.

Risk Assessment

FSANZ has assessed the scientific evidence submitted by the Applicant and other peer reviewed scientific information. FSANZ has concluded that the bacteriophage preparation poses no risk to public health and safety for Australian or New Zealand consumers.

The stated purpose for this bacteriophage preparation is to reduce or eliminate *L. monocytogenes* in a range of ready-to-eat foods. The evidence presented to support this use provides adequate assurance that the bacteriophage preparation, in the form and amounts added is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

Freshbins technology will eliminate *L. monocytogenes* in a range of ready-to-eat foods.

Furthermore, the weight of evidence, coupled with the restricted functionality of the bacteriophage in commercial conditions and in non-liquid food matrices, supports the conclusion that P100 has no ongoing technological function in non-liquid ready-to-eat food according to the use and levels proposed by the Applicant.

Freshbins technology has no ongoing technological function in non-liquid ready-to-eat food.

FSANZ reviewed evidence examining potential toxicity associated with the P100 preparation. There were no hazards identified which would preclude permitting the use of the P100 preparation to treat food for the stated purpose.

In assessing the allergenicity and toxicity of the P100 preparation, a comparison of the genomic sequences of P100 proteins and known allergens and toxins was carried out. No biologically significant similarity was found between the genes coding for the P100 proteins and any known allergens or toxins.

FSANZ reviewed the information on the possibility of emergence of bacteriophage resistant mutants of *Listeria monocytogenes.* FSANZ concluded after considering the scientific evidence, backed by views of experts in the field, that resistance development to phage treatment is minimal in food processing environments when appropriate user instructions are provided and adhered to. FSANZ further concluded that there would be no negative impact on humans caused by the ingestion or contact with this bacteriophage preparation.

There will no resistance development in Freshbins ozonated water systems.

The key risk assessment findings are detailed in Supporting Document 1.

Risk Management

P100 functions as a processing aid for the stated purpose when treating non-liquid foods so it is proposed to include permission for P100 within Standard 1.3.3 – Processing Aids. This would most likely be within the

Table to clause 14 – Permitted processing aids with miscellaneous function.

Because there are currently no specifications for P100 in the Code a new specification would need to be written into the Schedule of Standard 1.3.4 – Identity and Purity. Processing aids permitted under Standard 1.3.3 are exempt from labelling under subclause 3(d) of Standard 1.2.4 – Labelling of Ingredients.

Iii Assessing the Application

In assessing the Application, FSANZ has had regard to the following matters as prescribed in section 29 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act): whether costs that would arise from a food regulatory measure developed or varied as a result of the Application outweigh the direct and indirect benefits to the community,

Government or industry to permit P100 as a processing aid in non-liquid foods whether other measures would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.

Freshbins technology will be would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.

 Any relevant New Zealand standards
 Any other relevant matters.

Preferred Approach

Proceed to development of a food regulatory measure to vary Standard 1.3.3 –
Processing Aids to add P100 as an approved processing aid for the surface treatment of non-liquid ready-to-eat foods.

Reasons for Preferred Approach

The development of an amendment to the Code to give approval to use P100 as a processing aid in Australia and New Zealand is proposed on the basis of the available scientific evidence, for the following reasons:
 the safety assessment did not identify any public health and safety concerns

No health concerns with Freshbins technology, only by product is oxygen and water.

 the assessment concluded that for the purpose proposed by the Applicant, P100 has a technological function as a processing aid in non-liquid ready-to-eat foods. It has no ongoing technological function in these foods.

Freshbins technology also has no ongoing technological function in these foods.

 approval for use of P100 as a processing aid is consistent with Ministerial Council policy guidance on the *Addition to Food of Substances other than Vitamins and Minerals*

Freshbins technology falls within these guidelines.

 there are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.

Freshbins would be more cost-effective than a variation to Standard 1.3.3 and would achieve the same end.

Consultation

Public submissions are now invited, in particular on:

 scientific aspects of the Application, in particular any information relevant to the safety and technological function assessment

 the appropriate requirements that should be contained in a specification for P100

 parties that might be affected by having this Application approved or rejected.

Iv Invitation for Submissions

FSANZ invites public comment on this Report based on regulation impact principles for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in further considering this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information, separate it from your submission and provide justification for treating it as confidential commercial material. Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked 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 using the Changing the Code tab and then through Documents for Public Comment.

Alternatively, you may email your submission directly to the Standards Management Officer at submissions@foodstandards.gov.au. There is no need to send a hard copy of your submission if you have submitted it by email or the FSANZ website. FSANZ endeavours to formally acknowledge receipt of submissions within 3 business days.

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 1 November 2011 SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED

Submissions received after this date will only be considered if agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

Questions relating to making submissions or the application process can be directed to the Standards Management Officer at standards.management@foodstandards.gov.au.

If you are unable to submit your submission electronically, hard copy submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand Food Standards Australia New Zealand
PO Box 7186 PO Box 10559
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INTRODUCTION

FSANZ received an Application from EBI Food Safety Ltd to amend the *Australia New Zealand Food Standards Code* (the Code) to permit the use of a bacteriophage preparation Listex P100 (called P100 preparation for the rest of the report) as a processing aid in ready to- eat foods (RTE). FSANZ confirmed with the Applicant that the request was specifically for non-liquid ready-to-eat foods. The bacteriophage preparation was proposed for use to reduce numbers of *Listeria monocytogenes* in foods. The Applicant claims P100 acts as a processing aid in ready-to-eat foods, and so requested that Standard 1.3.3 Processing

THE FOOD INDUSTRY IS CURRENTLY IN NEED OF Innovative processing technologies in order to meet consumers' demand of fresher and safer ready-to-eat products.

High pressure processing, pulsed electric field, and high intensity pulsed light are some of these emerging technologies.

Attention is now focused on ozone as a powerful sanitizer that may meet expectations of the industry, approval of the regulatory agencies, and acceptance of the consumer. Regulatory agencies in the United States have been hesitant in the past to approve the use of ozone for treatment of drinking water and direct food applications. Currently, there are more than 3000 ozone-based water treatment installations all over the world and more than 300 potable water treatment plants in the United States (Rice and others 2000). This widespread application is a clear indication of the efficacy and usefulness of ozone. A petition submitted in August 2000 to the Food and Drug Administration (FDA) for approval of ozone as a direct food additive for the treatment, storage, and processing of foods in gas and aqueous phases has been recently accepted (Federal Register 2001).

Major advantages of ozone made it one of a few top candidate technologies attracting the attention of the food industry.

Ozone is one of the most potent sanitizers known. Excess ozone auto-decomposes rapidly to produce oxygen, and thus it leaves no residues in food. The sanitizer is active against all forms of microorganisms at relatively low concentrations.

The rapid developments in this field—appearance of a new body of knowledge and potential approval of ozone as a direct food additive by the U.S. government—justify the present review of various aspects of ozone-microorganisms interactions. Food processors who are introducing ozone in their facility and researchers who are exploring the feasibility of ozone use in food processing are in need of relevant and concise information about this sanitizer. This review article should address these needs.

Physicochemical properties of ozone

Ozone (O3) results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electric discharge. The product is a bluish gas with pungent odor and strong oxidizing properties (Horvath and others 1985). Physicochemical properties of ozone are closely related to its efficacy, and thus these properties will be discussed.

Solubility of ozone in water

The gas does not appreciably react with water; therefore it forms a true physical solution (Horvath and others 1985).
Dissolution of gasses that are partially soluble in water (for example, ozone) follows Henry's law which states that the amount of gas in solution, at a given temperature, is linearly proportional to the partial pressure of the gas. Consequently, saturation concentration (Cs) of a dissolved ozone in water under thermodynamic ideal conditions follows this equation (Bablon and others 1991a).

$$Cs = bM \times Pg$$

Where Cs: kg O3/m3 water; b (absorption coefficient): volume of ozone (expressed at NTP) dissolved per unit volume of water (at a given temperature) in the presence of equilibrating ozone at 1-atm pressure; M: mass volume of ozone, kg/m3, at NTP (2.14 kg/m3); Pg: partial pressure of ozone in the gas phase

Solubility of gasses can be compared if their â values are known. Solubility in water is greater for ozone than for nitrogen and oxygen; â values are 0.64, 0.0235, and 0.049, respectively.

Ozone, however, is less soluble in water than are carbon dioxide (b = 1.71) and chlorine (b = 4.54). Dissolution of ozone in water also can be expressed in a more practical term, the solubility ratio (Sr).

$$Sr = \frac{\text{mg/L O3 in water}}{\text{mg/L O3 in the gas phase}}$$

Solubility ratio for ozone increases as the temperature of water decreases (Bablon and others, 1991a). These authors showed a negative logarithmic relationship between Sr and water temperature in the range of 0.5 °C to 43 °C.

In addition to pressure and temperature, which directly affect the solubility, other parameters practically influence the dissolution of ozone in water. When a solution is prepared by bubbling ozone in water, smaller bubble sizes result in larger surface area of contact which increases the solubility (Katzenelson and others 1974). According to these authors, an optimum dissolution of ozone in water occurs when bubbles are 1 to 3 mm in dia. The flow rate of ozone and contact time affect the transfer of the gas to water. Appropriate mixing or turbulence increases bubble contact and solubilization in water (Katzenelson and others 1974). Design of ozone-water contractors, in general, greatly affects the rate of solubilization (Schulz and Bellamy 2000).

Purity and pH of water greatly affect the rate of ozone solubilization. J-G Kim (1998) bubbled gaseous ozone (1 mM) into double distilled, deionized or tap (from two sources) water. Ozone gas dissolved faster in deionized and distilled water than in tap water. Higher maximum ozone concentration was also obtained in the water from the former two sources. The pH values, measured before ozonation, were 5.6 and 5.9 for deionized and distilled water, respectively, and 8.23 and 8.39 for tap water from the two sources. The high

pH of tap water may have destabilized ozone, and thus the apparent rate of solubilization decreased. In addition, tap water may contain organic matter that consumes ozone.

Presence of minerals in water may also catalyse ozone decomposition (Hoigné and Bader 1985). Therefore, solubility of ozone increases when purity of water increases.

Stability of ozone

Ozone is relatively unstable in aqueous solutions. It decomposes continuously, but slowly, to oxygen according to a pseudo first-order reaction (Tomiyasu and others 1985). The half-life of ozone in distilled water at 20 °C is generally considered to be 20 to 30 min. However, Wynn and others (1973) found that ozone has a half-life of 165 min in distilled water at 20 °C and Wickramanayake (1984) reported a shorter half-life (2 to 4 min) in aqueous solution at pH 7.0 and 25 °C.

Wickramanayake (1984) attributed this short half-life to the mechanical stirring that kept the reactor's contents completely mixed.

The pH greatly affects the stability of ozone in aqueous solutions. J-G Kim (1998) added ozonated water, having different concentrations, into phosphate buffers (0.01 M) with pH 5.0 to 9.0, mixed for 15 s and measured the concentration of ozone using the indigo method. Stability of ozone in solution was the greatest when pH was 5.0. Ozone stability decreased as pH increased, and no ozone was detected in buffers with pH 9.0.
Decomposition of ozone follows first-order kinetics with respect to both ozone molecule and hydroxide ion.

$$-d[O3]/dt = k[O3][OH-]$$

According to Staehelin and Hoigné (1985), decomposition of ozone includes initiation, promotion, and inhibition reactions

(Figure 1).

(1) Initiation is the rate-limiting step which leads to formation of free radicals; these are the superoxide radical ion (~O2 –) and its hydrogenated form, the hydroperoxide radical (HO2~).

$$O_3 + OH^- \xrightarrow{k = 70\ mole^{-1}\ sec^{-1}} HO\sim_2 + \sim O_2$$

$$\sim O_2^- + H^+ \quad [pK_a = 4.8]$$

Formation of these radicals will lead to the generation of the highly reactive hydroxyl radical (~OH) and consumption of an ozone molecule (Figure 1). The ozonide radical ion (~O3-) is formed as an intermediate reaction product. Factors that enhance this stage of ozone decomposition (initiators) include hydroxyl ions,

some cations such as Fe2+, organic compounds such as glyoxylic acids, and ultraviolet radiation (UV) at 253.7 nm.

(2) Promotion reactions regenerate the hydroperoxide and superoxide radicals. Promotors include formic acid, glyoxylic acids, primary alcohols, and aryl groups.
(3) Inhibition refers to reactions leading to consumption of hydroxyl radical without regenerating the superoxide radical ion

$$k = 4.2 \times 108 \text{ mole}^{-1} \text{ sec}^{-1}$$

$$\sim OH + HCO3^- \longrightarrow OH^- + HCO\sim 3$$

Inhibitors include bicarbonate, carbonate, tertiary alcohols, and alkyl groups.

In practical terms, stability of ozone in aqueous solutions depends on the source of water. Water used in food processing or drinking usually contains readily oxidizable organic and inorganic substances. These substances may react rapidly with ozone, considerably decreasing its half-life. J-G Kim (1998) bubbled ozone in distilled, deionized, HPLC-grade and tap water from two sources, and phosphate buffer (0.5M, pH 7) to attain 0.10 to 0.15 absorbance at 258 nm (A258). Ozone decomposition rate was monitored during storage at 25 °C for 8 min. Concentration of ozone decreased during storage, but rates of decrease were greater in buffer and tap water than in distilled, deionized, and HPLC-grade water. These data indicate that ozone degrades faster in buffer and tap water than in purer water. It is apparent that high pH and presence of ozone-demand materials enhance decomposition of ozone.

Reactivity of ozone

The ozone molecule acts as dipole with electrophilic and nucleophilic properties. Organic and inorganic compounds in aqueous solutions react with ozone in one of two pathways (Staehelin and Hoigné 1985):

(a) Direct reaction of organic compound (M) with molecular ozone.

$$O3 + M \longrightarrow Mox$$

(b) Decomposition of ozone in water into a radical (for example, OH) which reacts with the compound (M).

$$O3 \xrightarrow{OH^-} OH \xrightarrow{M} Mox$$

Molecular ozone reactions are selective and limited to unsaturated aromatic and aliphatic compounds.

Ozone oxidizes these compounds through cycle-addition to double bonds (Bablon and others 1991a). Oxidation of sulfhydryl groups, which are abundant in microbial enzymes, may explain rapid inactivation of microorganisms and bacterial spores by ozone.

Ozone reacts with polysaccharides slowly, leading to breakage of glycosidic bonds and formation of aliphatic acids and aldehydes (Bablon and others 1991a). Reaction of ozone with primary and secondary aliphatic alcohols may lead to formation of hydroxy-hydroperoxides, precursors to hydroxyl radicals, which in turn react strongly with the hydrocarbons

(Anbar and Neta 1967). Perez and others (1995) showed that N-acetyl glucosamine, a compound present in

the peptidoglycan of bacterial cell walls and in viral capsids, was resistant to the action of ozone in aqueous solution at pH 3 to 7. Glucosamine reacted relatively fast with ozone, but glucose was relatively resistant to degradation. This observation may explain, at least in part, the higher resistance of gram-positive bacteria compared to gram negative ones; the former contains greater amounts of peptidoglycan in their cell walls. The action of ozone on amino acids and peptides is significant especially at neutral and basic pH. Ozone attacks the nitrogen atom or the R group or both.

Ozone reacts slowly with saturated fatty acids. Unsaturated fatty acids are readily oxidized with ozone and cycle-addition products are formed. Ozone reacts quickly with nucleobases, especially thymine, guanine, and uracil. Reaction of ozone with the nucleotides releases the carbohydrate and phosphate ions (Ishizaki and others 1981).

Factors altering reactivity and antimicrobial efficacy.

A factor such as treatment temperature affects solubility, stability, and reactivity of ozone differently. Consequently, it is difficult to predict the influence of this factor on the efficacy of ozone in real applications. Factors that affect these interrelated parameters simultaneously will be discussed.

Temperature.

The rate of destruction of microorganisms by a disinfectant generally increases with increasing temperature. According to the van't Hoff-Arrhenius theory (Fair and others 1968), temperature partly determines the rate at which the disinfectant diffuses through the surfaces of microorganisms and its rate of reaction with the substrate. At constant reagent concentration, increasing the temperature by 10 °C increases the reaction rate with the substrate by a factor of 2 or 3. In the case of ozone, however, as temperature increases ozone becomes less soluble and less stable, but the ozone reaction rate with the substrate increases. As the temperature increased from 0 °C to 30 °C, the rate of inactivating *Giardia* cysts increased (Wickramanayake and others 1984). However, Kinman (1975) reported that when bacteria were treated with ozone at 0 °C to 30 °C, treatment temperature had virtually no effect on the disinfection rate.

The researcher related this observation to the decrease in solubility and increase in the decomposition and reactivity of ozone as temperature increases. Achen and Yousef (2001) treated *Escherichia coli*-contaminated apples with ozone at 4, 22, and 45 °C, and observed that counts of the bacterium on the surface decreased 3.3, 3.7, and 3.4 log10-units, respectively.

Statistical analysis showed no significant differences among the three treatments ($P > 0.05$). The residual ozone concentration was greatest at the lowest temperature (4 °C) and decreased with increasing temperature. It appears that when treatment temperature increased, the increase in ozone reactivity compensated for the decrease in its stability, and thus no appreciable change in efficacy was observed. On the contrary, J-G Kim (1998) observed that ozone reduced more microbial contaminants when it was applied at higher than the refrigeration temperatures.

pH value.

Under constant residual ozone concentrations, the degree of microbial inactivation remained virtually unchanged for pH's in the range of 5.7 to 10.1 (Farooq and others 1977). However, efficacy of ozone seems to decrease at alkaline pH for rotaviruses (Vaughn and others 1987) and poliovirus type 1 (Harakeh and Butler 1985). Ozone is more stable at low than at high pH values, as indicated earlier.

Inactivation of microorganisms is mostly through reaction with molecular ozone when the pH is low. Ozone decomposes at high pH values and the resulting radicals contribute to its efficacy.

The relative importance of these two inactivation mechanisms may vary with the microorganism and treatment conditions (for example, presence of ozone-demanding contaminants).

Ozone-consuming compounds.

Presence of organic substances with high ozone demand may compete with microorganisms for ozone. Viruses and bacteria associated with cells, cell debris, or feces are resistant to ozone, but purified viruses are readily inactivated with the sanitizer (Emerson and others 1982). Similar results have been found in our laboratory for ozone inactivation of rotavirus in suspension comparative to 1-h adsorbed virus to the MA 104 cell monolayers (Khadre and Yousef 2001c). Hence, the presence of organic matter in water intended for use in ozone-associated food processing is highly undesirable. Furthermore, unwanted by-products from ozone action on organic compounds may shorten the shelf-life, change the organoleptic quality, or jeopardize the safety of the final product.

Determination of ozone concentrations

Physical, physicochemical, and chemical methods have been used for determination of ozone. Physical methods measure direct absorption in the UV, visible, or infrared region of the spectrum. Physicochemical methods are dependent upon effects such as heat or chemiluminescence caused by the reaction. Chemical methods quantitate the products released when ozone reacts with a chemical reagent such as potassium iodide.

The iodometric method has been approved by the International Ozone Association (Gordon and Grunwell, 1983).

Ozone oxidizes iodide ion, releasing iodine; the latter is then titrated with sodium thiosulfate to a starch endpoint. This method measures not only ozone, but also all other oxidizing species resulting from ozone decomposition in solutions; for example, ~O3 –, HO2~, and ~O2 –. Hence, measurement of residual ozone cannot be accurately done by the iodometric method.

The commonly used indigo method (Bader and Hoigné 1981) is precise, fast, and sensitive (lowest detection level is 0.005 mg/mL). The indigo reagent reacts additively with the carbon-carbon double bond of sulfonated indigo dye causing its decolorization and the resulting change in color is determined spectrophotometrically. Ozone measurement by the indigo method is not compromised by the presence of

hydrogen peroxide, organic peroxides, manganous ions, and oxidized species in drinking water. Compared to the iodometric method, the indigo method is more suitable for measuring residual ozone.

Several manufacturers produce instruments that measure ozone by determining the amount of UV light absorbed.
Gaseous ozone absorbs short-UV wavelengths with a maximum absorption at 253.7 nm and the gas-phase absorption coefficient of 3000 6 30 mole–1 cm–1 at 273 °K and 1 atm (Gordon and Grunwell 1983). Calorimetric methods of ozone measurement depend on the decomposition of ozone in the presence of a catalyst producing heat. Instruments using amperometric methods to measure the oxidation-reduction potential of ozone are available commercially.

Kinetics of microbial inactivation by ozone

Ozone is a strong, broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores. There is a little agreement, however, among researchers regarding the relative sensitivity of different microorganisms to ozone. Additionally, reported sensitivity of a single microorganism varies among studies. Strain of the microorganism, age of the culture, density of the treated population, presence of ozone-demanding medium components, method of applying ozone (that is, gas bubbles, or uniform aqueous solution), accuracy of ozone measuring procedures and devices, and method of measuring antimicrobial efficacy (for example, single point determinations in contrast to systematic kinetic studies) are some of the confounding factors that make comparison among different studies unfeasible. Based on our experience, sensitivity of bacteria to aqueous ozone ideally is tested as follows;

(1) grow cells to late exponential or early stationary phases,

(2) separate and wash cells from the growth medium,

(3) suspend washed cells uniformly in ozone demand-free medium, for example, pure water, to attain 107 to 108 CFU/mL,

(4) apply a dose of ozone that kills a significant portion of the population (~2 to 3 log10-units) but without leaving residual ozone in the treatment mixture,

(5) measure cell viability at the end of the treatment, and

(6) correlate the population inactivated with the ozone dose. If this procedure is carefully executed, results may be used to estimate the number of ozone molecules sufficient to inactivate a single bacterial cell (nz). Relative sensitivity of different microorganisms or the same microorganism under different cultural, physiological, or experimental conditions may be reliably determined by comparing their nz values. Using a similar approach, Kim and Yousef (2000) estimated nz for *Leuconostoc mesenteroides* at 109. In an earlier study, Finch and others (1988) found that 3 × 108 molecules of ozone were used to inactivate each cell of *E. coli*.

The procedure just described can be modified to estimate inactivation rate {D (log10 CFU/mL)/ (D time)} in response to a given ozone concentration. The modification replaces steps (v) and (vi) as follows: (v) at suitable treatment time intervals, quench the reaction and measure cell viability, and

(vi) construct the survivor's plot. The rate of microbial inactivation {D(log10 CFU/mL)/(D time)} is calculated using the linear plot or the steepest slope on the survivor curve. The negative reciprocal of this inactivation rate, known as decimal reduction time or D-value, is a useful term in comparing resistance to ozone of different microorganisms or of the same microorganism under different conditions. Microbial inactivation by ozone does not seem to produce linear survivor plots (Figure 2). Finch and others (1988), Kim and Yousef (2000) and many other researchers observed a tailing in these plots. Tailing of heat inactivation survivor plots are normally attributed to poorly designed experiments or to inaccuracies in measurements, but these causes do not necessarily explain the tailing in ozone survivor plots. While heat and other physical factors are applied constantly during the course of the treatment, ozone is commonly applied as a single dose at the beginning of the treatment; therefore, it may be reasonable to predict the nonlinearity in the latter case.

These nonlinear plots, nevertheless, may be used to measure initial inactivation rates and calculate the corresponding D values.

Kim and Yousef (2000) applied ozone to bacterial cell suspensions in a continuous, rather than a batch, mode and obtained survivor plots that are linear for 5 to 20 s of the treatment. This study proved that ozone reacts with microorganisms rapidly, and a nonlethal threshold concentration is reached quickly in a batch treatment. Continuous treatments, coupled with rapid sampling techniques, allow a relatively accurate determination of D-values.

Determination of microbial inactivation kinetics in a continuous treatment system may be simplified by measuring ozone dose as C.T value. Based on this concept, C.T is a measure of disinfectant concentration (C) multiplied by the time (T) required to achieve a given inactivation level of a microorganism.

It was originally introduced by Watson (1908) as a solution for the occasional absence of a straight line in disinfectant log plots. However, Watson emphasized the importance of a constant disinfectant concentration during the time of contact. Although the C.T concept provides an excellent measure of ozone doses, accurate determination of C.T value is difficult in the case of ozone due to its instability and short half-life. To overcome this problem, some authors used residual ozone concentration at the end of the contact period as an estimate of "C" in the "C.T" term; this approach obviously results in inaccurate dose measurement. Gyurek and others (1997) questioned the validity of the C.T concept.

They stipulated that extrapolation of a C.T product at a high concentration for chlorine to low concentration conditions is inappropriate because of the modelling discontinuity that may exist between high and low concentrations.

Mechanism of microbicidal action of ozone

Inactivation of bacteria by ozone is a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes,

peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids. Some authors concluded that molecular ozone is the main inactivator of microorganisms, while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition such as ~OH, ~O2 –, and HO~3 (Chang 1971; Harakeh and Butler 1985; Glaze and Kang 1989; Bablon and others 1991b; Hunt and Marinas 1997).

Cell envelopes.

Ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids leading to leakage of cell contents and eventually causing lysis (Scott and Lesher 1963; Murray and others 1965). When the double bonds of unsaturated lipids and the sulfhydryl groups of enzymes are oxidized by ozone, disruption of normal cellular activity including cell permeability and rapid death ensues. In our laboratory, Dave (1999) found that treatment of *Salmonella enteritidis* with aqueous ozone disrupted the cell membranes as seen in transmission electron micrographs (Figure 3). However, Komanapalli and Lau (1996) found that short-term exposures of *E. coli* K-12 to ozone gas compromised the membrane permeability but did not affect viability, which progressively decreased with longer exposure.

Bacterial spore coats.

Foegeding (1985) found that *Bacillus cereus* spores with coat proteins removed were rapidly inactivated by ozone, compared to intact spores. The researcher concluded that the spore coat is a primary protective barrier against ozone. Recently, Khadre and Yousef (2001b) found that spores of *Bacillus subtilis* treated with aqueous ozone showed heavily disrupted outer spore coats

(Figure 3).

Enzymes.

Several authors referred to enzyme inactivation as an important mechanism by which ozone kills cells. Sykes (1965) reported that chlorine selectively destroyed certain enzymes, whereas ozone acted as a general protoplasmic oxidant.
Ingram and Haines (1949), in view of their finding general destruction of the dehydrogenating enzyme systems in the cell, proposed that ozone kills *E. coli* by interfering with the respiratory system. Takamoto and others (1992) observed that ozone decreased enzyme activity in *E. coli* at a greater degree in case of cytoplasmic â-galactosidase than in case of the periplasmic alkaline phosphatase. Inactivation of enzymes by ozone is probably due to oxidation of sulfhydryl groups in Cysteine residues (Chang 1971).

Nucleic material.

Reaction of aqueous ozone with nucleic acids *in vitro* supports the notion that it may damage nucleic material inside the cell. Ozone modified nucleic acids *in vitro* , with thymine being more sensitive than cytosine and uracil (Scott 1975; Ishizaki and others 1981). In another study, ozone opened the circular plasmid DNA and reduced its transforming ability, produced single- and double-strand breaks in plasmid

DNA (Hamelin 1985), and decreased transcription activity (Mura and Chung 1990). Studying *E. coli,* l'Herault and Chung (1984) found that ozone may induce mutations. However, other investigators did not detect any mutagenic effect of ozone on *Salmonella* spp. (Victorin and Stahlberg 1988). Compared to other known mutagens, ozone was found to be a weak mutagen on *Saccharomyces cerevisi- ae* (Dubeau and Chung 1982). The effect of ozone on viral nucleic acids is discussed in a later section.

Viruses.

Sproul and Kim (1980) and CK Kim and others (1980) found that aqueous ozone inactivated both f2 and T4 bacteriophages by attacking capsid protein, with liberation and inactivation of the nucleic acid. The RNA from f2 bacteriophage was partially inactivated prior to release from the capsid. They suggested that ozone breaks the protein capsid into subunits liberating RNA and disrupting virus adsorption to the host pili, and that the RNA may be secondarily inactivated.

The DNA released from T4 bacteriophage was rapidly inactivated by ozone at about the same rate as that in the intact phage. CK Kim and others (1984) confirmed the results of Sproul and Kim (1980) about bacteriophage T4; they found that ozone randomly destroyed the head, collar, contractile sheath, end plate, and tail fibers and liberated the DNA from the head.

Yoshizaki and others (1988) found that aqueous ozone caused the coat proteins subunits of tobacco mosaic virus (TMV) to aggregate with each other and cross-link with the viral RNA. Despite their observation of a good correlation between loss of infectivity and decrease of recovery of viral RNA, Yoshizaki and others (1988) and Shriniki and others (1988) concluded that the major cause of TMV inactivation by ozone was the inability of the treated virus to uncoat. Roy and others (1981) found that ozone altered two of the four polypeptide chains in the poliovirus protein coat. They, however, attributed the inactivation of the virus to the damage in its RNA by ozone. The observation by Herbold and others (1989) that 0.38 mg/mL aqueous ozone was needed for complete inactivation of hepatitis A virus (HAV) and only 0.13 mg/mL for complete inactivation of poliovirus may support the hypothesis that damage to viral envelopes is the main cause of inactivation of viruses by ozone. Enveloped viruses such as HAV are expected to be much more resistant to ozone compared to non enveloped viruses such as poliomyelitis.

Efficacy of ozone

Efficacy of ozone is demonstrated more readily when targeted microorganisms are suspended and treated in pure water or simple buffers (with low ozone demand) than in complex systems such as food. The simplicity of low-ozone demand aqueous environment makes it possible to compare ozone efficacy against microorganisms within the same study, and occasionally among different studies. Ozone also may be compared with other sanitizers when experiments are done in the simple treatment environments just indicated, but differences in experimental designs, treatment conditions, and microbial strains tested should be considered.

Therefore, in the following discussion we will compare efficacies with consideration to the factors just indicated. This discussion will be limited to bacteria and viruses since they were more extensively investigated than other groups of microorganisms.

Inactivation spectrum

Bacteria. Studies summarized in Table1 show that 0.12 to 3.8 mg/mL aqueous ozone inactivated gram-positive bacteria by 1 to 7 log10 CFU/mL. When gram-negative bacteria were treated with 0.004 to 6.5 mg/mL aqueous ozone, their populations decreased 0.5 to 6.5 log10 CFU/mL (Table 2). It may not be possible to compare ozone sensitivity of gram-positive and gram-negative bacteria using summaries of data in Tables 1 and 2; therefore, studies that directly compare these two categories will be presented. Sobsey (1989) reviewed studies to inactivate health-related microorganisms in water by several disinfectants and concluded that gram-positive bacteria, including *S. aureus* and *Bacillus* spp., and the *Mycobacteria* were more resistant than were gram-negatives.

Lee and Deniniger (2000) observed the dominance of gram positive bacteria among the surviving microorganisms in ozonated drinking water. When gram-positive and gram-negative bacteria were compared in side-by-side experiments, however, variable results were obtained. Restaino and others (1995) studying a group of food-related microorganisms, observed that gram-negative bacteria were substantially more sensitive to ozone in pure water than were the gram-positive ones including *L. monocytogenes*. Kim and Yousef (2000) and J-G Kim and others (1999b) treated foodborne spoilage and pathogenic bacteria with ozone under identical conditions and found results inconsistent with the previous conclusion.

Resistance of bacteria tested in this study followed this descending order: *Escherichia coli* O157:H7, *Pseudomonas fluorescens*, *Leuconostoc mesenteroides*, and *Listeria monocytogenes*.

Ozone is generally more effective against vegetative bacterial cells than bacterial and fungal spores. In our laboratory, J-G Kim and others (2001) studied inactivation kinetics of different microorganisms that commonly spoil fruit juices

(Figure 2). Results of this study show that *Alicyclobacillus acidocaldarius* vegetative cells and *Zygosaccharomyces bailii* ascospores were inactivated rapidly with aqueous ozone.

Spores of *A. acidocaldarius* were the most resistant to ozone, and survivor's plot exhibited both a shoulder and a tail. Mold spores (*Neosartorya fischeri*) were intermediate in resistance to ozone, and tailing of survivor plots was apparent. Khadre and Yousef (2001b) measured ozone efficacy against spores of 8 *Bacillus* spp. *B. stearothermophilus,* which is known for high resistance to heat, also possessed the highest resistance to ozone among the species tested.

Viruses.

A limited number of studies on inactivation of viruses with ozone have been published. Researchers (Table3) tested ozone concentrations in the range of 0.1 to 15.9 mg/ mL against 8 different viruses; the treatment caused destruction of 0 to 7 log10-units. This may indicate that viruses are comparable to bacteria in sensitivity to ozone. Sobsey (1989), however, concluded that viruses are generally more resistant than vegetative bacteria and that bacteriophages are the most sensitive to ozone among the viruses tested. Other researchers (CK Kim and others 1980; Hall and Sobsey 1993) also reported the sensitivity of the bacteriophages MS2, and f2 to ozone. Based on the limited studies in Table3, it may be

concluded that bacteriophages are the least resistant to ozone, followed by polioviruses, whereas human rotavirus was the most resistant to the sanitizer. This conclusion is in agreement with those reports by Herbold and others 1989) and Hall and Sobsey (1993).

Combination treatments for increased efficacy

Advanced oxidation processes.

Advanced oxidation processes (AOPs) are processes designed to generate highly reactive intermediates, particularly the hydroxyl radical (~OH), for treatment of recalcitrant organic compounds in water.

Among the AOPs are ozonation at high pH, H2O2/O3 processes and UV photolysis of H2O2 (Arselan and others 1999).
Hydrogen peroxide in aqueous solutions partially dissociates to hydroperoxide anion (HO2 -) which is highly reactive with ozone (Taube and Bray 1940).

H2O2 + H2OÖHO2
– + H3O+
The hydroperoxide ions consumed by ozone are quickly replaced by shifting the equilibrium in the above reaction to the right. Hence, very small concentrations of H2O2 should be effective in initiating ozone decomposition.

Different AOPs vary in efficacy.

Arselan and others (1999) found that ozone at pH 11.5 was more effective than a combination of H2O2/O3 at pH 7.5 for decreasing color in dye house wastewater and removing dissolved organic compounds.
Cortes and others (2000) found that O3/catalyst (Fe2+, Fe3+ and Mn2+) combination was more effective than O3/high pH for the elimination of chlorobenzenes, which are stable non biodegradable and toxic substances, in industrial wastewater. Other researchers disputed the efficacy of AOPs. Rajala-Mustonen and Heinoen-Tanski (1995) reported that ozone alone in tap water was much more effective in inactivation of coliphages than were AOPs using UV light with hydrogen peroxide. Harakeh and Butler (1985) found that 0.2 ppm ozone at pH 4 gave significantly higher reduction of poliovirus than at pH 7.2 or 9. In the presence of 0.5 M sodium bicarbonate, an ozone decomposition inhibitor, viral sensitivity to ozone increased about 10-fold at each pH value tested.

Hence, enhancing ozone efficacy through generation of AOPs seems theoretically feasible but still lacks sufficient proof for practical application in foods.

Ozone-Chlorine.

Ozone seems to possess an activity that is lacking in chlorine; it alters membrane permeability. This is evident from the work of Gyurek and others (1996), who found that free chlorine is relatively ineffective against *Cryptosporidium parvum* oocysts unless it is preceded by a small dose of ozone. They assumed that

preozonation alters the permeability of the oocyst membranes, thus allowing free chlorine to penetrate and cause a significant inactivation of the oocysts.

Ozone-pulsed electric field.

Unal and others (2001) studied inactivation of *E. coli* O157:H7, *Listeria monocytogenes*, and *Lactobacillus leichmannii* by combinations of ozone and pulsed electric field (PEF). Cells were treated with 0.25 to 1.00 mg ozone/mL cell suspension, PEF at 10 to 30 kV/cm, or selected combinations of ozone and PEF. Treatment of *L. Leichmannii* with PEF (20 kV/cm), after exposure to 0.75 and 1.00 mg/mL ozone, inactivated 7.1, and 7.2 log10 CFU/mL, respectively; however, ozone at 0.75 and 1.00 mg/mL and PEF at 20 kV/cm inactivated 2.2, 3.6, and 1.3 log10 CFU/mL, respectively.

When *E. coli* O157:H7 and *L. monocytogenes* were treated with ozone and PEF, less pronounced synergistic bactericidal effects were observed. Ohshima and others (1997) also reported a synergistic effect of the simultaneous application of ozone and PEF on *E. coli*. Inspecting the data of Ohshima and others (1997), however, we found that ozone and PEF combinations, as tested in this study, had an additive rather than a synergistic action.

Ozone application in food processing

Ozone is one of the most effective sanitizers known, yet it leaves no hazardous residues on food or food-contact surfaces.
The precursors for industrial production of ozone (that is, O2 or H2O) are abundant and inexhaustible.

Ozone treatment requires no heat and hence saves energy. Ozone must be produced on-site; this leads to considerable savings in the costs of transporting and storing sanitizers. The initial cost of ozone generators may be of concern to small processors; however, long-term application may justify these costs.

The economics of ozone application is beyond the scope of this review; but the fact that ozone has been and is still being used in Europe and some places in the United States suggests that it is reasonably economical.

Products tested

Several investigators demonstrated the microbicidal effects of ozone gas injection or sparging in reconditioning poultry chiller water (Waldroup and others 1993; Diaz and Law 1999). Effective prefiltration of chiller water prior to ozone treatment is recommended for optimum reduction of microbiological levels and efficient use of ozone (Sheldon 1986). Aqueous ozone also was used to decontaminate beef and beef brisket fat (Gorman and others 1997), poultry meat (Dave 1999), salmon (Goche and Cox 1999), apples (Achen and Yousef 2001; McLoughlin 2000), strawberries (Lyons- Magnus 1999), lettuce (J-G Kim and others 1999a) and broccoflower (Hampson and Fiori 1997). Microbial studies typically show 2-logs

reduction of total counts and significant reduction of spoilage and potentially pathogenic species most commonly associated with fruit and vegetable products.

Some researchers treated raw ingredients with ozone before processing of food. M-J Kim and others (1993) treated various spices, used to prepare kimchi, with gaseous ozone and improved the fermentation of the final products. In our laboratory, K-G Kim and others (2001) used gaseous ozone injection to decontaminate the ingredients of fruit juices such as high-fructose corn syrup. The researchers speculated that ozone treatment of ingredients rather than final juice products can reduce ozone usage and minimize the damage to the sensory quality of the final product. Naitoh and others (1989) reported that the treatment of wheat flour with gaseous ozone inhibited microbial growth in namamen products and increased their storage life.

Gaseous ozone can be used during storage of foods.

Ozone was tested to prevent the growth of surface contaminants on meat (Greer and Jones 1989), grapes (Sarig and others 1996), and broccoli florets (Zhuang and others 1996). Low concentration (< 1 ppm) and long contact time (several days) were needed to inhibit microbial growth during storage.

Aqueous ozone was also used to treat packaging and food contact materials (Khadre and Yousef 2001a). Combinations of ozone with other oxidants such as hydrogen peroxide were also used to sanitize packaging films (Gardner and Sharma 1998), a confectionery plant (Naitoh 1989), and hatchery equipments (Whistler and Sheldon 1989). Ozone decreased surface flora by ~ 3 log10-units when tested in wineries for barrel cleaning, tank sanitation, and clean-in place processes (Hampson 2000).

In spite of its efficacy against microorganisms both in the vegetative and spore forms, ozone is unlikely to be used directly in foods containing high-ozone-demand materials, such as meat products. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of these products. Additionally, microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to the sanitizer. Application of aqueous ozone on products having smooth intact surfaces with low ozone demand (for example, fruits and vegetables) produced promising results (Achen and Yousef, 2001; Kim and others 1999a). Application methods, however, must assure direct contact of ozone with the target microbial cells. A variety of methods have been used to accomplish this, including stirring, pumping, flumming, bubbling, sonication, abrasion, and pressure washing.

Microorganisms for measuring ozone efficacy

The efficacy of a sanitizer in food processing is ideally tested by inoculating targeted microorganisms (spoilage or pathogenic) on the surface of food, equipment, or food-contact surface, and treating these surfaces with the sanitizer at conditions that simulate normal processing. Alternatively, an indicator (surrogate) microorganism with resistance to the sanitizer that is similar or greater than that of the targeted microorganism may be used. The indicator is ideally similar biologically to the targeted microorganism, but it should not be pathogenic if the study is carried out in the processing facilities.

Since sanitization commonly targets a variety of microorganisms, an indicator with the greatest resistance to the sanitizer is preferable in these challenge studies.

Clostridium sporogenes PA 3679 has been effectively used as a surrogate to *C. botulinum* in heat inactivation studies, but *Bacillus stearothermophilus* is also used (Russell 1982). *L. innocua* has been used to study treatments that target *L. monocytogenes* (Gervilla and others 1997). Selected *B. Subtilis* strains are used in determining the efficacy of H2O2 and heat in aseptic fillers (Anonymous 1995 and 1999). Very little research has been done in the quest for the ideal microorganism to use in measuring ozone efficacy.

In a comparative study, Khadre and Yousef (2001b) found that resistance of *Bacillus* spp. spores to ozone was highest for *B. stearothermophilus* and lowest for *B. cereus*. Spores of *B. subtilis* var niger ATCC 9372 are used as indicators in dry heat and ethylene oxide sterilizations (Anonymous 1995 and 1999), but in our study these spores were sensitive to ozone.

Hence, we suggest using *B. stearothermophilus* spores in testing the efficiency of sanitization by ozone.

Residual ozone and process efficacy.

During treatment of food, ozone may desolubilize, decompose, or react with food constituents and targeted microorganisms.

The rapid reaction and degradation of ozone diminish the residuals of this sanitizer during processing. The lack of residuals may limit the processor's ability for in-line testing of efficacy; this is an often-cited disadvantage of using ozone as a disinfectant. Stalder and Klosterkoetter (1976) clearly illustrated this problem—they observed that 1.5 mg/ mL ozone treatment kept water sterile for greater than 1 mo with no detectable residuals. However, passage of this water through a 12 m-long pipeline led to recontamination and considerable growth of microorganisms. Lack of residual ozone in the water led to this recontamination problem.

Food is packaged after processing; therefore, product recontamination is less likely in food than in drinking water. Lack of residues, however, minimizes a processor's ability to monitor ozone level in wash water as an important critical control point within a hazard analysis and critical control point (HACCP) plan.

Validation

Process validation is a practice that accompanies introduction of a new processing technology or unit operation.

Results of validating ozone use in drinking water at the Neuilly- sur-Marne plant in France has been published (Bablon and others 1991b). This plant produces 600,000 m3/d of water from the Marne River near Paris. The filtered water is disinfected with ozone at an average dose of 1.5 mg/mL for an average contact time of 12 min. Ozone is diffused through porous plates to contact chambers. The residual ozone concentration at the end of the contactors is 0.4 mg/mL. A post disinfection dose of chlorine is added to give a residual chlorine concentration of 0.4 mg/mL in the water leaving the plant and entering the distribution system. The bacteriological results of samples taken during 1988 to monitor bacterial levels before and after ozonation indicate a substantial reduction in microbial population. Fecal streptococci were not detected in water samples (100 mL), total and fecal coliform bacteria decreased > 4 and 3 log10-units, respectively, and heterotrophic plate count bacteria were reduced 2 to 3 log10-units.

Sheldon and others (1985) tested the effects of ozone on the microbiological characteristics of spent poultry prechiller water (95 L obtained from a poultry plant and tested in a pilot plant-size ozone contactor). Ozone was generated at a rate of 292 ppm per min for 60 min. After ozonation, the total aerobic population decreased ~7 log10-units, the coliform count decreased > 3 log10-units, and the fecal coliforms, *E. coli* and *Salmonella*, were not detected. These authors concluded that ozone qualifies for recycling poultry chiller water under the USDA's guidelines. In 1993, Waldroup and others reported their evaluation of a prototype water recycling ozonation system installed in a commercial turkey poultry processing facility over a 4-mo period. They found similar results like those of Sheldon and others (1985) and were able to obtain USDA approval for this system for recycling poultry chiller water in 1991. Tests for validation of the use of ozone for red meat processing (Greer and Jones 1989; Gorman and others 1997) have given modest results, and more research is needed in this area probably involving ozone in combination with other factors such as hot water or hydrogen peroxide.

Monitoring ozone in work environment and safety

Ozone toxicity. Low concentrations of ozone (~0.1 mg/L) cause irritation to the nose, throat, and eyes (Witheridge and Yaglou 1939). Thorp (1950) indicated that an hour exposure to ozone concentrations of 2, 4, 15, and 95 mg/L induces symptomatic, irritant, toxic, and irreversible lethal effects, respectively, in humans. The human lung is the primary target of ozone gas. Initially, there is pulmonary edema accompanied by capillary hemorrhage and inflammation of the respiratory tract. On prolonged exposure, ozone may cross the alveoli, causing damage to blood cells and serum proteins (Buckley and others 1975).

Ozone appears to react with substances in the water supply, such as humic acids, to form numerous disinfection by-products which cause minor toxicological reactions, if any (Bablon and others 1991a).

Personnel safety. Safety-of-use is of prime importance for the practical application of ozone in food processing. Systems for ozone detection and destruction in addition to respirators are essential for the safety of workers in food processing facilities. An ultraviolet analyzer equipped with a large measuring cell adapted to a range of 0.01 to 100 ppm by volume (0.02 to 200 mg/m3 NTP) must be installed in ozonation rooms at intervals covering the ozone gas distribution pipes, contactor access galleries, and at the ozone destruction point. The analyzer must trigger both a displayed and acoustic warning signal as soon as the ozone content in the ambient air exceeds 0.1 ppm (0.2 mg/m3 NTP) (Damez and others 1991).

The off-gas from the plant must pass through a thermal or catalytic ozone destructor. A continuous ozone analyzer that functions within a range of the standard of 0.1 ppm by volume (0.2 mg/m3 NTP) must be fitted to the air line leaving the destructor. Any overshooting of this value will trigger a general alarm (Damez and others 1991). The reason for ozone destruction is to protect personnel, equipment, structural components, and the general environment from exposure to high levels of ozone.

According to U.S. regulations (CFR 1997), an individual must not be exposed to a concentration of ozone higher than: (a) 0.1 ppm by volume (0.2 mg/m3 NTP), on an 8-h/d basis, of a 40-h work wk; and (b) 0.2 ppm by volume (0.4 mg/ m3 NTP), as a limit for an exposure time of 10 min. Furthermore, protective canister-type respirators must be kept available. There should be plans for remedial action in case of accidents, and

response procedures for accidental ozone inhalation and training of personnel covering the nature and dangers of ozone, precautions, and first aid for ozone inhalation.

Conclusion

OZONE IS A POTENT SANITIZER WITH PROMISING Applications in the modern food industry. The sanitizer is effective against a wide spectrum of microorganisms, and it can be used in an environment-friendly manner. Stability and efficacy of ozone at chilling temperatures constitute attractive savings to the industry which is already burdened by rising energy costs.

Chlorine and hydrogen peroxide are probably the most commonly used sanitizers in the food industry. These sanitizers have been used successfully to decontaminate processing environment, equipment surfaces, and occasionally the surfaces of solid foods. Their drawbacks, however, have prompted the quest for more effective and economical sanitizers.

Currently, ozone is the most likely alternative to chlorine and hydrogen peroxide in food applications. Transition from traditional sanitizers to ozone requires a great understanding of its benefits and limitations and realistic expectations from the alternative sanitizer. Further research is still needed to explore new applications for ozone and to best utilize the unique features of this sanitizer.

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Aids be amended.

L. monocytogenes is a well-known foodborne pathogen, and can be a contaminant of raw and RTE food products such as poultry, seafood and dairy products. Currently, the Code permits no tolerance of *L. monocytogenes* in several ready-to-eat foods. During the past 10 years, 48% of food recalls carried out in Australia for microbial contamination has been due to *L. monocytogenes* (FSANZ 2011).

Bacteriophages are specific to the strains of bacteria they infect and are not pathogenic to plants, animals or humans. They are the most abundant biological entities on earth and occur everywhere in the environment.

It is important to ensure food safety that phages used to treat food are both lytic3 and nontransducing4.

This is to ensure there is no transfer of genes (or DNA) between host bacteria. Phage-related research and application began in Europe and USA in the 1880s, but soon declined with the advent of antibiotics.

However, clinical use and research were maintained in Eastern European countries. The application of bacteriophages for various uses has recently become increasingly important due to concerns about antimicrobial resistance development in pathogenic microorganisms. Greater accessibility to Eastern

European research has resulted in an increase in bacteriophage-related knowledge development during the last decade and much more is now known about their biology.

Bacteriophage-based products are being produced and used in the Netherlands, US and Georgia for a range of applications. Food-related use has been fairly recent and more products are being researched and developed. The use of the P100 preparation and others to treat food has been approved by US, Canada and The Netherlands.

1. The Issue / Problem

The Applicant has requested that the P100 preparation be approved as a processing aid to reduce levels of *L. monocytogenes* in ready-to-eat food. A pre-market assessment is required before any new processing aid is permitted to be used to process food sold in Australia and New Zealand.

There is currently no permission for the use of bacteriophages preparations as processing aids in the Code.

A safety assessment of the use of P100 as a processing aid is required and must be undertaken before any permission may be granted. This assessment includes the safety of the P100 preparation and of using it to treat food, as well as an assessment of the technological function of P100 for its proposed use.

2. Current Standard

2.1 Background

All new processing aids must undergo a pre-market assessment before they can be permitted to treat food. The following definitions in the Code have been used for this assessment.

The use of processing aids is regulated by Standard 1.3.3. The purpose of this Standard includes a definition for 'processing aids' which is as follows:

Processing aid means a substance listed in clauses 3 to 19, where –

(a) the substance is used in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food; and

(b) the substance is used in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.

Clause 14 (permitted processing aids with miscellaneous functions) is the most applicable clause.

The use of food additives is regulated by Standard 1.3.1 – Food Additives. The purpose of this Standard includes a definition for food additives:

A food additive is any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions specified in Schedule 5.

2.2 Overseas approvals

The European Food Safety Authority (EFSA) issued a scientific opinion on the use of bacteriophages in food products and concluded that each phage/food application should be considered on a case-by-case basis taking into consideration the biology and safety aspects of each bacteriophage and the food matrix to which it is applied (EFSA 2009).

On 14 July 2009, the Dutch Ministry of Public Health permitted the use of P100 as a processing aid for use on all foods in The Netherlands.

FreshBins Pty. Ltd. Will be working with these agencies in the future to discuss our technology advances.

P100 was granted generally recognised as safe (GRAS) status by the FDA in 2006 for use as a processing aid in cheese and in 2007, extended its use to all food products susceptible to *Listeria monocytogenes*. Ingredient labelling requirements were initially specified for bacteriophage treated meat and poultry products by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture

(USDA). However, in 2011, USDA permitted its use as a processing aid on the surface of ready-to-eat meat and poultry products to achieve a level of 107 to 109 plaque forming units (pfu) per gram, without the need for labelling. The letter of permission requires that the treatment is integrated into the HACCP programs of the industry.

3 Bacteriophages that undergo replication within the bacterial hosts to release phage particles by rupturing the host cells without integrating with the bacterial chromosome

3 The assessment also considers whether it functions as a processing aid (no extended technological function in the final food) or as a food additive (having a technological function in the final food).

3. Objectives

The objective of this assessment was to determine whether it is appropriate to amend the Code to permit the use of P100 bacteriophage as a processing aid to reduce *L. Monocytogenes* in foods. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

 the protection of public health and safety; and
Freshbins process protect public health and safety

 the provision of adequate information relating to food to enable consumers to make informed choices; and
Freshbins will leave no chemical residue on products for enhanced public safety.

 the prevention of misleading or deceptive conduct.
Freshbins adheres to this.

In developing and varying standards, FSANZ must also have regard to:
 the need for standards to be based on risk analysis using the best available scientific evidence;
Freshbins applications have withstood the most rigorous scrutiny in all aspects. Evidence is provided.

 the promotion of consistency between domestic and international food standards;
Freshbins technology will provide consistency between domestic and international food standards;

 the desirability of an efficient and internationally competitive food industry;
Freshbins will enable Australia to be a more efficient and internationally competitive food industry;

 the promotion of fair trading in food; and
Freshbins will enable fairer trading in food because of the reduced costs to the industry, and less food spoilage and higher yields.

 any written policy guidelines formulated by the Ministerial Council.
The Ministerial Council Policy Guideline, Addition to Food of Substances other than Vitamins and Minerals, includes specific order policy principles for substances added to achieve a solely technological function,

such as processing aids. These specific order policy principles state that permission should be permitted 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'); and
Freshbins meets these requirements.

 the addition of the substance to food is safe for human consumption; and
Freshbins ozonated water treatment will guarantee that the addition of the substance to food is safe for human consumption;

 the amounts added are consistent with achieving the technological function; and
Freshbins systems are monitored to achieve consistent and constant requirements to achieve each technical function required.

 the substance is added in a quantity and a form which is consistent with delivering the stated purpose; and
Freshbins can guarantee 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. The main objective which applies to this assessment is the primary objective of protection of public health and safety.
Freshbins agrees with this statement, the primary objective of protection of public health and safety.

4 Transduction is the mechanism whereby bacterial genetic material is transferred between bacteria through a bacteriophage vector.

4 On 3 September 2010, Health Canada issued a 'letter of no objection' for the use of the P100 preparation as a processing aid in several foods, 'mainly deli meat and poultry products (e.g. wieners, sliced ham), cold-smoked fish, vegetable prepared dishes, soft cheeses and/or other dairy foods'. A recommendation was made to provide clear instructions on the conditions of application to potential users. A proposed level of use within the range of 107 to 109 pfu/g was also specified.

This objective has been considered by conducting a risk assessment. This risk assessment has also investigated the technological function and justification for using the phage P100 preparation, to address the Ministerial Council Policy Guideline: *Addition to Food of Substances other than Vitamins and Minerals.*

4. Questions to be answered

For the purpose of the Application, FSANZ has considered the following risk assessment questions:

 Is the P100 bacteriophage preparation suitably well characterised?
Freshbins believes that our Ozonation system is far superior and safer.

 Does the P100 preparation achieve its stated technological purpose?

Freshbins systems achieve a kill and clean with no chemical residue, which exceeds the values required.

 Has the technological need been articulated clearly?
There is now no need for P100 even to exist in the market.

 Is the preparation added in a quantity and form which is consistent with delivering the stated purpose?
Yes, Freshbins can deliver the preparation added in a quantity and form which is consistent with delivering the stated purpose.

 Can development of resistance render the P100 preparation ineffectual?
No resistance can ever develop using Freshbins technology.

 Does the P100 preparation present any food safety issues?
There are always risks associated P100. Freshbins however, there are no risks known for the applications that are required for this application.

 Are there potential allergens present in the P100 preparation?
There are no potential allergens present in the preparation of ozonated water.

 Are there toxicological safety issues?
No.

RISK ASSESSMENT

In addition to information supplied by the Applicant, other available resource material including published scientific literature and general technical information was used in this assessment.

The summary and conclusions from the risk assessment, provided in Supporting Document 1 (SD1), are presented below.

5. Risk Assessment Summary

5.1 Characterisation

5.1.1 Is the P100 bacteriophage preparation suitably well characterised?

The Applicant has provided information detailing the identity of the P100 bacteriophage as belonging to the Order *Caudovirales,* Family *Myoviridae* and Group SPO-1. The host (production) organism is a non-pathogenic type strain of *Listeria innocua* (ATCC 33090,

DSM 20649, NCTC 11288, SLCC 3379). The bacteriophage P100 and production organism are completely characterised.

5.2 Technological function

5.2.1 Does the P100 preparation achieve its stated technological purpose?
FSANZ has made an assessment of the efficacy and the possibility of an ongoing technological function when the P100 preparation is used for the stated purpose. The P100 is effective in reducing numbers of *L. monocytogenes* in treated foods.
The overall weight of evidence, noting the restricted functionality of the bacteriophage in commercial conditions and in non-liquid food matrices, supports the conclusion that P100 has no ongoing technological function in non-liquid ready-to-eat food according to the use and levels proposed by the Applicant.

It is important to note that P100 cannot be assumed to be a complete single treatment that will destroy and eliminate all *L. monocytogenes* from treated food. It should be considered only as additional technology food manufacturers can use along with their current processes to control *L. monocytogenes.*

Food manufacturers will need to determine appropriate process optimisation and SOP's (Standard Operating Practices) to establish efficacy on a case-by-case basis for different foods and different production plants and to monitor efficacy consistently.

The risk assessment reviewed the information on the possibility of emergence of bacteriophage-resistant strains of *L. monocytogenes.* The conclusion from the scientific evidence, supported by experts in the field and international regulators, is that when using bacteriophages to treat food, is that development of resistance in food processing environments is minimal, provided adequate information on the use, application and disposal of unsold product is provided to food manufacturers, and that manufacturers have regard to that information. This is no different to resistance developed by bacteria as a stress response to other bactericidal treatments applied during food processing. Treated products are not expected to re-enter the processing facility.

Adherence to GHP ensures phage-treated product that is not appropriate to be processed for commercial sale needs to be removed from the production facility on a regular basis, along with appropriate cleaning regimes to ensure there is no build-up of bacteriophage reservoirs in the facility. Continuous screening and monitoring of host susceptibility and phage resistance development in food premises using the P100 preparation, is being maintained by the Applicant. The P100 bacteriophage species could be updated as necessary, to maintain efficacy, while conforming to the specification.

5.3 Safety Assessment

5.3.1 Does the P100 preparation present any food safety issues*?*

No food safety issues were identified from the available toxicity data. This conclusion is supported by the absence of biologically significant homology between the P100 proteins and any known allergens or toxins.

P100 bacteriophage is only effective against bacteria of the genus *Listeria.* It cannot infect plant, animal or human cells. Ingestion or contact with P100 does not present a public health risk.

5.4 Conclusion

The findings of the risk assessment for Application A1045 show that the use of the P100 preparation is completely characterised and it is technologically justified and safe for use in non-liquid ready-to-eat foods as proposed by the Applicant. There is no ongoing technological function performed by the P100 preparation in non-liquid ready-to-eat foods.

RISK MANAGEMENT

6 Risk Management Issues

The risk assessment conclusions from Section 5.4 are that the use of P100 is technologically justified and is safe for use in non-liquid ready-to-eat foods. FSANZ has a number of regulatory risk management matters to address these risk assessment conclusions. These matters are considered in the following sections.

6.1 Technological function: processing aid or food additive?

An important regulatory issue relates to the technological function performed by the Applicant's phage preparation. The purpose statement in the Application is to: eradicate or decrease *L. monocytogenes* on various ready-to-eat food products for human consumption.

FSANZ assessed how the phage preparations performed their technological function; i.e. during processing only (therefore as a processing aid) or in the final food (food additive) (see Section 2.1). Section 5.2.3 of SD1 concludes that P100 performs its technological function during the processing and manufacture of food and has no ongoing technological function in non-liquid final foods. It was further concluded that phages that may remain on the surfaces of treated food do not have any active technological function to further reduce *L. Monocytogenes* after the initial reduction or possible recontamination.

There is an important distinction between being able to isolate so called 'active' phages from treated food surfaces, even after several days' storage and these phages having a functionality to seek, locate and destroy bacteria. It is concluded from the studies that the phages are 'bound' to the food surfaces and have limited mobility in non-liquid foods to locate and destroy remaining *L. monocytogenes* and therefore have no ongoing functionality.

The situation is different for liquid foods (which are not being assessed in this Application). This is explained by the hypothesis that phages have greater diffusion in liquid media and so a greater likelihood to locate and destroy bacteria than when bound or less mobile on solid media.

FSANZ concludes that the P100 preparation acts as a processing aid in non-liquid ready-to eat food products for the purpose of reducing levels of *L. monocytogenes* in these foods.

6.2 Proposed Regulatory Permissions

Based on the conclusions in Sections 5.4 and 6.1 of this Assessment Report, FSANZ concludes that it is appropriate to permit P100 as a processing aid to treat non-liquid ready to- eat food and it therefore proposes to amend Standard 1.3.3.

FSANZ has not concluded at this stage what the permission should encompass. This will be considered further following public comment on this Report (in particular FSANZ seeks comments on the questions noted in Section 10 – Consultation). It is possible that P100 could be used under conditions of Good Manufacturing Practices (GMP) in appropriate processed foods and during the processing of these foods.

That is, the substance could be added to the Table to clause 14 – Permitted processing aids with miscellaneous function in Standard 1.3.3. P100 could be permitted as a processing aid for non-liquid ready-to-eat foods.

Permitting P100 as a processing aid means that food manufacturers could use it as a technology in the concentrations and method recommended by the manufacturer.

Food manufacturers may also need to use other technologies that are available to control *L. monocytogenes* in foods and food processing (e.g. as part of their HACCP program). As with all new processes or technologies, manufacturers will need to consider their specific products and process requirements and conduct trials before use of the P100 preparation. In particular, P100 concentrations and contact time required to reduce bacterial levels should be determined.

The Applicant has advised that the manufacturers will be provided with clear instructions on the use of the P100 preparation as part of an ongoing assurance program to limit phage resistance developing in food production facilities.

The Applicant has also advised that they will continuously work with users to monitor phage resistance development and to update the P100 preparation as required to maintain efficacy.

The specification will provide for ensuring the ongoing efficacy of the P100 preparation.

6.3 Labelling implications

The Applicant sought approval for the use of P100 as a processing aid. Under paragraph 3(d) of Standard 1.2.4 – Labelling of Ingredients, processing aids are exempt from ingredient labelling. Based on the evidence submitted by the Applicant, as well as information from other scientific information, FSANZ concludes that the P100 preparation achieves the technological function (control of *L. monocytogenes*) as a processing aid in non-liquid foods.

Ingredient listing for P100 is therefore not required.

6.4 Analytical methods for determining presence of P100 in food

The Applicant has provided analytical methods for determining the presence of P100 in food which are summarised below.

A standard agar overlay method can be employed. A dilution or suspension of the bacteriophage treated food sample is mixed in a small volume of molten agar containing host bacteria (e.g. *L. innocua*) and poured onto the surface of a nutrient agar plate.

Following overnight incubation, the host bacterial cells grow uniformly throughout the top agar layer (forming a bacterial 'lawn'). The bacteriophage infects the bacteria causing lysis of the bacterial cells, thereby forming clear areas on the bacterial lawn (plaques). Plaques are enumerated resulting in the bacteriophage titre which is determined by this plaque assay.

The Application contains information relating to a Polymerase Chain Reaction (PCR) method applicable for determining the presence of P100 bacteriophage on treated food. To confirm the presence of P100, the following primers are used: Forward: 5′ -ccttcacgcatctttgttacag (binds P100 genome bp: 108867-108888); reverse: 5′ -cagggttgtatttaggtactc (binds P100 genome bp: 109957-109937). This analytical method is available and could be used by analytical laboratories for enforcement purposes if required.

6.5 Specification for P100 bacteriophage preparation

There are currently no specifications for bacteriophages, or more specifically P100, in any of the primary or secondary references for specifications or in the Schedule for Standard 1.3.4

– Identity and Purity. Therefore, a P100 specification is required in the Schedule for Standard 1.3.4. A draft is provided below. Specifications for lead and arsenic are addressed by the additional requirements of clause 4 of Standard 1.3.4. The Applicant has demonstrated that the P100 preparation is manufactured according to GMP.

This specification would permit P100 bacteriophage preparations though similar, but non identical phage preparations such as A511 would not be permitted. The specification would permit phage manufacturers to modify the P100 preparation to ensure efficacy as *L. monocytogenes* may adapt and alter with time.

Biological classification and microbiological properties for the P100 preparation are listed in

FSANZ seeks assistance from relevant stakeholders as to what other requirements should be incorporated into a P100 specific specification to ensure food safety.

6.6 Addressing the FSANZ objectives

The legislative objectives that FSANZ is required to meet when developing or varying a food standard are detailed in Section 3. FSANZ considers the main objective which applies to this Application is the primary objective of protection of public health and safety. The other two primary objectives are considered of less direct importance. How FSANZ has addressed these objectives during the consideration of this Application is noted below.

6.6.1 Risk to public health and safety

FSANZ's risk assessment concludes that approving the use of the P100 bacteriophage preparation to treat non-liquid ready-to-eat foods does not present any public health and safety risks.

6.6.2 Providing adequate information for consumers to make informed choices

For this Application the P100 preparation has been determined to perform its technological function to treat non-liquid ready-to-eat food as a processing aid. Processing aids are exempted from labelling requirements on package foods due to subclause 3(d) of Standard 1.2.4. FSANZ does not believe there are any appropriate reasons to exclude the labelling exemption for the P100 preparation, especially since there are unlikely to be any phage preparation remaining on treated food.

6.6.3 Prevention of misleading and deceptive conduct

FSANZ has considered this objective and concludes there are no misleading or deceptive conduct aspects to this assessment.

6.7 Consistency with Policy Guidelines

As noted in Section 3, FSANZ is required to have regard to the Ministerial Council Policy Guidelines relevant to the Application, in this case being the Policy Guideline: *Addition to Food of Substances other than Vitamins and Minerals*. Since the purpose for use of the P100 preparation is as a processing aid, consideration falls under 'Technological Function'.

FSANZ has therefore considered the Application under the five specific policy principles noted in Section 3.

The Application has provided a clear stated purpose, being the technological function that P100 performs when it is used as proposed to treat non-liquid ready-to eat food. The risk assessment has concluded that use of P100 to treat food is safe for human consumption and that the amounts added in the proposed quantity and forms are consistent with delivering the stated purpose. The Applicant does not wish to make any nutrition, health or related claims related to the use of P100 to treat food.

7. Options

Two options are available for consideration by FSANZ at the next stage of the assessment of this Application. These are:
Option 1 Reject the Application
Option 2 Prepare a draft food regulatory measure

8. Impact Analysis

FSANZ is required to consider the impact of various regulatory and non-regulatory options on all sectors of the community, especially relevant stakeholders who may be affected by this Application. The benefits and costs associated with the proposed amendments to the Code have been analysed using 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 (OBPR) in a letter dated 24 November 2010 (reference 12065) provided a standing exemption from the need to assess if a Regulation Impact Statement is required for applications relating to processing aids as they are machinery in nature.

8.1 Affected Parties

The affected parties for this Application may include:
 Sectors of the food manufacturing industry may wish to use P100 to reduce incidence of *L. monocytogenes* in the foods they process. These manufacturers will be able to take advantage of a new technology which will permit them to market products with increased confidence and to broaden their product range.
Freshbins technology is the answer to their problems.

An initial cost will be incurred in performing validation trials, advertising and marketing.
This will not necessarily have to happen.

Manufacturers may need to manage consumer response to this new technology.
That is old technology. Manufacturers need to embrace the new chemical free applications of Freshbins revolutionary technology.

 Consumers may have access to a wider choice of ready-to-eat products which may be available for consumption.
Agree.
 Food enforcement agencies responsible for ensuring compliance with the Code may require the development of skills relating to the verification and inspection applicable to a new technology.
Not necessary, Freshbins associated technicians service the equipment on a quarterly basis to ensure correct use and procedures are being followed.

 Laboratories may require training on aspects of testing associated with bacteriophage technology.
Not necessary.

8.2 Benefit Cost Analysis

8.2.1 Option 1 – Reject the Application

This option would disadvantage those members of the food industry who wish to use the P100 preparation as an additional process step to reduce the concentrations of *L. monocytogenes* on ready-to-eat food.
Disagree. Freshbins technology will reduce the concentrations of L. monocytogenes on ready-to-eat foodstuffs, and eliminate the need to use P100 preparation.

There are no benefits to relevant stakeholders of this option.
Disagree. Benefits include chemical free food safety. Staff and customer safety. Improvement in OH&S standards for the food industry. Improvement in shelf life and transportation of vegetables. Higher yields to market, less spoilage of stock. More money in the supplier's pocket.

8.2.2 Option 2 – Accept the Application and prepare a variation to Standard 1.3.3.

FSANZ notes that the permission of P100 as a processing aid is for application as an additional technology, not as a sole alternative to currently used procedures, to control levels of *L. monocytogenes*. *L. monocytogenes* is a major food safety concern for ready-to-eat food as confirmed by FSANZ's most recent recall information. Recalls due to *L. monocytogenes* alone has amounted to 48% of the total number of recalls due to microbiological contamination. This is despite the application of currently available technologies by food manufacturers.

Not true, the technology we have developed in the past 18 months and patent pending worldwide ,has enabled us to hold ozone in cold water, units designed can simply be put on existing infrastructure machinery. The use of our system will eliminate recalls. The elimination of these toxic chemicals from the food chain is now available, after L.monocytogenes is eliminated during wash treatment the only by product is oxygen and water. A far safer by-product for OH&S standards and staffing considerations.

FSANZ's risk assessment concludes that the P100 preparation is technologically justified and safe for use in non-liquid ready-to-eat foods as proposed by the Applicant to reduce the levels of *L. monocytogenes.* Therefore, its use as an additional new technology by food manufacturers has been considered safe and appropriate for use. The proposed use is a benefit to both producers and consumers of processed food.

Producers will benefit from our new technology in a lot of ways. The production of ozone on site and therefore on demand to the producers requirements, it can be produced at the demand concentrations that are required for each of their individual applications, it requires only 240Volts and cold water, not heating of water is required in our process. Additional comments below.

Ozone is now approved by the FDA for use in meat and vegetable food packaging / processing plants (and other foods) for more thorough cleansing of fruits, vegetables, other fresh produce, and meats.

Preservation, storage and transportation of fruit and vegetables

Ozone has a favourable effect on many refrigerated goods and is especially suitable for use in food packaging lines, ships and trucks. Ozone:

- Deodorizes and sterilizes refrigerating spaces and stores.
- Prevents transmission of odours.
- Controls the growth of mildew.
- Retards the ripening of fruit.

More information on the specifics of preservation and storage, effect on metabolism, disinfection, removal of odours, prevention of odor transmission, combating fungus, the effects of ozone on various refrigerated foods and operating costs is available on request.

The use of P100 for the proposed purpose is voluntary. Food manufacturers will use a range of factors to determine which techniques best suit their purpose. Such factors will include cost, suitability for the desired purpose, any consumer issues and the net benefit of using the processing aid in food preparation.

Approving a new processing aid may impose a modest added cost to government enforcement agencies, to widen the scope of their activities. Jurisdictions may require familiarisation and integration of this relatively new technology into their existing food regulatory framework.

Chemical free & environmentally friendly material Ozone

A material from the past.....with futuristic applications

Discovered in the 19th century Ozone, an allotropic form of activated oxygen generally produced during lightning storms and continuously occurring in the stratosphere due to action of ultraviolet (UV) rays, is being rediscovered in the 21st century. This naturally occurring compound may be artificially produced by the action of high voltage discharges in air or oxygen as follows,

$$O_2 + O \text{ -------energy ----------> } O_3$$

This unstable form of oxygen breaks down to oxygen molecules and oxygen atoms which have a high oxidation potential. If we examine the oxidation power of Ozone by measuring its REDOX potential we find that O3 is about 5 times more oxidising than oxygen and about twice as much as chlorine.

This high potential increases its reactivity with other elements and compounds. Ozone is about 20 to 50 times more reactive than chlorine and permanganates as is well documented by its high kill rate of micro-organisms (Funguses, Bacteria & Viruses).This high kill rate equates to smaller retention times and storage tanks for the same level of disinfection as other oxidants. In other words the capital cost for building these tanks and treatment plants is considerably reduced.

Ozone is a God given gift to all developing nations, most of which lack adequate chemical handling, storage , transportation infrastructure and production facilities. Ozone requires only electricity which is readily available from hydro, solar, wind or fuel electric generators. For the developed countries O3 will allow decentralisation of services which will provide greater flexibility and better cost management.

Here is non exhaustive list of Ozone applications where data & references are available:

Ozone chemical free treatments and applications			
Waste water effluents	**Industrial /Agriculture**	**Food Industry**	**Others**
Domestic/Municipal	Cooling towers treatment	Drinking & water bottling	Smoke & odour treatment
Pulp & paper	Boiler water treatment	Grain silo disinfecting	Semiconductor wafers clean
Mining (Cyanide, Arsenic)	Chilled water treatment	Fruit & vegetable storage	Laundry water recycling
Pharmaceutical (Phenol)	Cutting fluids recycling	Meat storage	Med. instrument sterilisation
Textile	Barn disinfecting (air/water)	Slaughter house disinfecting	Hospital air sterilisation
Leather	Hydroponics	Fruits & vegetable wash	Aqua-culture
Petroleum/Petrochemicals	Animal waste treatment	Food containers sterilisation	Paper pulp bleach
Electroplating	Water dripping treatment	Wine/Beer SO2 replacement	Sour gas desulfurisation
Heavy metal precipitation	Animal drinking water	Chicken egg wash	Zebra mussels treatment
Landfill leachates	Irrigation water disinfecting	Ozonated meat grinders	Rubber recycling ...etc.

This begs the question: if O3 is so good why is it not widely spread? The answer is simple. Up until recent times mid-range Ozone generators were very expensive costing an average $ 7000 US/Lb/day.

Today, with advent of new materials, power supplies, high frequency generator prices are starting to go down to around $ 4000 US/Lb/day and are expected to decline further still to $ 2000/Lb/day or even lower in the near future. At this price level Ozone will compete with the other oxidising alternatives such as Chlorine, Hydrogen peroxides...etc.

The time has come when this chemical free technology is an affordable reality.

8.3 Comparison of Options

Given that the acceptance of this Application imposes no significant financial burden (noting possible government costs) on any sector of the community; the use of this preparation raises no public health and safety issues, option 2 is the preferred option.

Communication and Consultation Strategy

9 Communications

As this is the first application FSANZ has assessed for the use of a bacteriophage preparation as processing aid to control a foodborne pathogen (*L. monocytogenes*) in food, an enhanced communication strategy will be employed.

Communication will include:

 a website fact sheet at the start of consultation
 a media release at the start of consultation.
Interested parties will also be notified about the availability of the assessment reports for public comment.

FSANZ considers standard matters in an open, accountable, consultative and transparent way. Public submissions are invited to obtain the views of interested parties on the issues raised by the Application and the impacts of regulatory options. The issues raised in the public submissions are evaluated and addressed in FSANZ assessment reports.

The Applicant, individuals, and organisations making submissions on this Application will be notified at each stage of the Application. If the Board approves a variation to the Code, that decision will be notified to the Ministerial Council. If a request to review the decision is not made by the Ministerial Council, the variation will be gazetted and registered as a legislative instrument. Stakeholders (including the Applicant) and submitters will be advised of the notification and gazettal in the national press and on the FSANZ website.

10. Consultation

FSANZ is seeking comment from the public and other interested stakeholders to assist in the further consideration of this Application. As the Application is being assessed under the Major procedure, two rounds of public consultation will be held. FSANZ seeks comments about the scientific aspects of the Application as well as the proposed approach to vary the Code. In particular FSANZ is seeking submitters' views on the following questions:
 Is there additional information relevant to the safety assessment of the use of P100 as a processing aid in manufacture of non-liquid ready-to-eat foods?

Yes.

 Do submitters agree with FSANZ's conclusion that P100 functions as a processing aid for the Applicant's stated purpose to reduce concentrations of *L. monocytogenes* on non-liquid ready-to-eat foods?

No.

 Do submitters agree with the proposed FSANZ specification for P100 provided in Section 6.5 and are there any additional processing and microbiological requirements needed to be added to the specification to ensure food safety?

Yes.

Following the consultation on this 1st Assessment Report, if FSANZ prepares a draft variation to the Code, a second round of public comment on the draft variation will be held.

The FSANZ Board will then consider the draft variation for approval.

10.1 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), either Australia or New Zealand are obligated to notify WTO member nations when 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 no relevant international standards directly applicable to the use of the P100 preparation in food. Amending the Code to allow the P100 preparation as a processing aid to control *L. monocytogenes* in non-liquid foods is unlikely to have a significant effect on international trade as it is proposed for use as a technology, thereby providing for a choice for use by food manufacturers.

This matter will be considered at the next stage of the assessment and, if necessary, notification will be recommended to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Technical Barriers to Trade (TBT) or Sanitary and Phytosanitary Measures (SPS) Agreements. This will enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

CONCLUSION

11. Conclusion and Preferred Approach

This Application has been assessed against the requirements of section 29 of the FSANZ Act.

FSANZ concludes that the P100 preparation is technologically justified as a processing aid for the purpose of reducing *Listeria monocytogenes* levels in non-liquid ready-to-eat foods.

There is no ongoing technological function performed by the P100 preparation in treated non-liquid ready-to-eat foods. The use of the P100 preparation for this purpose does not pose any public health and safety risks.

The Ministerial Council Policy Guidelines relevant for this Application have been addressed in this assessment. The technological function (the stated purpose) of using P100 as a processing aid has been articulated and has been assessed as being met. The assessment has concluded that use of P100 as proposed by the Applicant is both safe and suitable.

Preferred Approach

Proceed to development of a food regulatory measure to vary Standard 1.3.3 –

Processing Aids to add P100 as an approved processing aid for the surface treatment of non-liquid ready-to-eat foods.

Reasons for Preferred Approach

The development of an amendment to the Code to give approval to use P100 as a processing aid in Australia and New Zealand is proposed on the basis of the available scientific evidence, for the following reasons:

 the safety assessment did not identify any public health and safety concerns

 the assessment concluded that for the purpose proposed by the Applicant, P100 has a technological function as a processing aid in non-liquid ready-to-eat foods. It has no ongoing technological function in these foods.
Freshbins achieves the same results without the use of harsh chemicals.

 approval for use of P100 as a processing aid is consistent with Ministerial Council policy guidance on the

Addition to Food of Substances other than Vitamins and Minerals

 there are no other measures that would be more cost-effective than a variation to Standard 1.3.3 that could achieve the same end.
Freshbins can achieve safer and better results, being more cost effective and safer for the environment and humanity.

References

Codex (2007) Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods CAC/GL 61.

EFSA (2009) The use and mode of action of bacteriophages in food production. The EFSA Journal (2009) 1076:1–26

FSANZ (2001) Recall guidelines for packaged ready-to-eat foods found to contain *Listeria monocytogenes* at point of sale. FSANZ, Canberra, Australia. http://www.foodstandards.gov.au/consumerinformation/listeria/listeriarecallguidel1321.cfm. Accessed 15 August 2011

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