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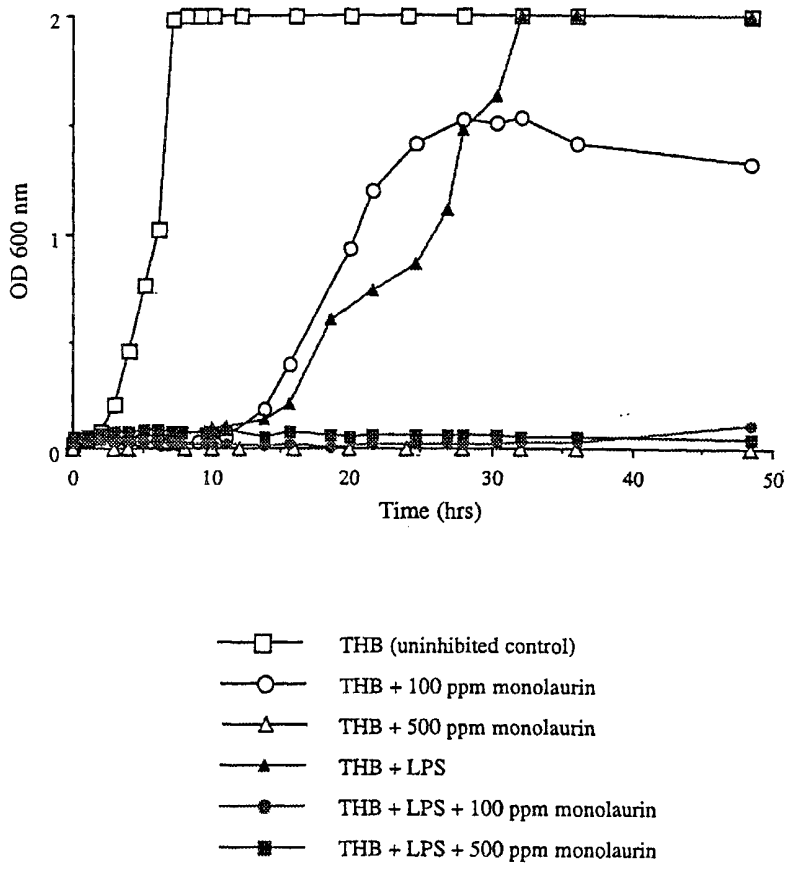
(57) Abstract

Peroxidase systems such as the Lactoperoxidase system (LPS) are combined with antimicrobial fatty acids or fatty acid derivatives to produce a synergistic antimicrobial composition. These compositions are disclosed as being useful for rendering surfaces, and also products such as food or cosmetics, resistant to microbes. A preferred form of the composition contains lactoperoxidase, monolaurin or sodium dodecyl sulphate, a source of peroxide, and thiocyanate ions.



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MICROBIALLY RESISTANT COMPOSITIONS

This invention relates to microbially resistant compositions and methods for their preparation. It further relates to methods of use of such compositions and to products which are made microbially resistant due to the addition or application of such compositions.

BACKGROUND

Lactoperoxidase (LP) is an enzyme that is part of the natural non-immune defence systems in milk and mucous secretions (such as saliva, tears and intestinal secretions). LP, together with various naturally occurring cofactors, forms the Lactoperoxidase system (LPS) that has pronounced anti-microbial activity.

The LPS incorporates LP (extracted from bovine milk), a source of peroxide and a cofactor (generally thiocyanate). In many situations, glucose oxidase (from a microbial source) and glucose are incorporated to provide a source of hydrogen peroxide. The use of an enzyme system is often preferred as this ensures that the delivery of peroxide is sustained. However this requires aerobic conditions so that in anaerobic conditions a direct source of peroxide may be required.

Reactions catalysed by LP and where the cofactor is thiocyanate yield short-lived intermediary oxidation products of thiocyanate that show the anti-microbial activity. LP utilises peroxide to catalyse the oxidation of the thiocyanate ion in the presence of water to generate the hypothiocyanite ion (OSCN-). The hypothiocyanite then is believed to react with the sulphydryl groups on the bacterial membranes with catastrophic effects on the bacteria. Much of the thiocyanate is regenerated in the process.

The LPS is regarded as being bactericidal against Gram-negatives (eg. *E. coli, Yersinia entercolitica, Pseudomonas spp., Salmonella spp, Campylobacter spp*) and bacteriostatic against Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Streptococcus spp*). The LPS is also suggested to have anti-viral activity in some situations.

While the focus of the LPS is on the use of lactoperoxidase, it will however be appreciated that, in some circumstances, other peroxidases can be used, particularly

those of GRAS status. Such systems are generically called peroxidase systems (PS), with the LPS being but one example.

The anti-microbial effects of a number of fatty acids are also well-documented. The most active are the medium chain fatty acids lauric (dodecanoic) acid and myristic (tetradecanoic) acid. The fatty acids are regarded as especially effective against the Gram-positive bacteria and the fatty acid derivative monolaurin (1-monododecanyol-rac-glycerol) is generally regarded as the most active. In addition, anti-viral activity (against the enveloped viruses) has been claimed for the fatty acid derivatives, including sodium dodecyl sulphate.

Monolaurin has GRAS status as an emulsifying agent and is used mostly in vegetable shortenings and to some extent in ice creams and baked goods.

Monolaurin is marketed as Lauricidin Æ for use as an anti-microbial in food systems. However, it has not found wide acceptance as an anti-microbial because of the concentrations required and the resultant effects on organoleptic quality of the treated food products.

The applicants have now found that PS (such as the LPS) and fatty acids/fatty acid derivatives such as monolaurin can be used in combination and that, when combined, the anti-microbial effect exceeds that which could have been predicted based upon the known properties of the components. It is therefore broadly upon this unexpected finding of enhanced anti-microbial effect or synergistic interaction which the present invention is based.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect, the present invention provides a method of preparing a microbially resistant composition which comprises forming a mixture of the following components:

(a) a peroxidase system (PS) comprising:
  (i) a peroxidase;
  (ii) a source of peroxide; and

(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and

(b) at least one fatty acid or a derivative of a fatty acid,

wherein said fatty acid or derivative is present in an amount effective to interact with said PS to produce an enhanced anti-microbial effect.

The term "enhanced anti-microbial effect" means an anti-microbial effect which is more microbiocidal against at least one type of microorganism than would be predicted from the known properties of the individual components.

As used herein, the term "microorganism" means microbial pathogens, ineffective particles and spoilage organisms, including those of bacterial, viral, fungal or protozoal origin.

Preferably, said fatty acid is an anti-microbial fatty acid, or an anti-microbial derivative of a fatty acid.

Preferably, the peroxidase is a lactoperoxidase.

Conveniently, the peroxide is hydrogen peroxide.

Preferably, the cofactor is selected from thiocyanate or iodide, and is most preferably thiocyanate.

Preferably, component (b) is or includes an anti-microbial fatty acid selected from C_8, C_{10}, C_{12}, C_{14} and C_{16} fatty acids or their derivatives.

More preferably, component (b) is or includes an anti-microbial ester of a fatty acid or a salt thereof.

Still more preferably, component (b) is or includes monolaurin (1-monododecanoyl-rac-glycerol) or its salt, sodium dodecyl sulphate.

In the currently most preferred embodiment, component (b) is monolaurin.

Conveniently, the anti-microbial fatty acid or derivative of a fatty acid is present in an amount which is at least 5% by weight of the total lipid present in the composition.

Preferably, said composition is formed by the addition of one or more of the components to a pre-formed mixture which already contains the remaining component(s).

Conveniently, the pre-formed mixture is a food, cosmetic or healthcare product.

The food product may be a dietary supplement or nutraceutical. It may also be a dairy product, meat product or fish product.

The food product may also be an animal feed, which in its simplest form may be water.

Alternatively, said composition, when formed, consists of said components in admixture.

In a further aspect, the invention provides a preparative composition suitable for use in preparing a microbially resistant composition which comprises at least two components selected from:

(i) a peroxidase;
(ii) a source of peroxide;
(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and
(iv) at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid thereof,

wherein the peroxidase and peroxide source, if both present, are kept separate.

Preferably, the peroxidase is a lactoperoxidase.

In yet a further aspect, the invention provides a preparative pack suitable for use in preparing a microbially resistant composition which comprises, in separate containers, a peroxidase and at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid.

Preferably, the peroxidase is a lactoperoxidase.

Preferably, said pack further includes a source of peroxide and/or a cofactor which is capable of yielding anti-microbial oxidation products. Where provided, the source of peroxide and/or said cofactor are in separate containers.

In yet a further embodiment, the invention provides an anti-microbial composition which comprises a peroxidase, a cofactor which is capable of yielding anti-microbial oxidation products and at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid, wherein said fatty acid or derivative thereof is present in an amount effective to synergistically interact with said peroxidase and said cofactor, in the presence of peroxide, to produce an enhanced anti-microbial effect.

Preferably, the peroxidase is a lactoperoxidase.

Preferably, said anti-microbial composition further includes a source of peroxide.

The composition may also include a further anti-microbial agent or a chelating agent.

Preferably, the further anti-microbial agent is selected from phenols, organic acids, bacteriocins, derivatives of these and mixtures of these, or anti-microbial components or mixtures of components extracted from or found in milk, such as lactoferrin.

Preferably, the chelating agent is EDTA.

In yet a further embodiment, the invention provides a microbially resistant product which is prepared by a method as defined above.

In still a further aspect, the invention provides a product which includes the following components:

(i) a peroxidase;
(ii) a source of peroxide;
(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and
(iv) at least one anti-microbial fatty acid or anti-microbial derivative thereof, which product is resistant to the growth of microorganisms.

Preferably, the peroxidase is a lactoperoxidase.

Preferably, said product is resistant to the growth of both Gram positive and Gram negative bacteria.

In one form, said product is a food product, such as a dietary supplement, nutraceutical dairy product, meat product, fish product or animal feed.

In another form, said product is a cosmetic product.

In still a further form, said product is a healthcare product.

In yet a further aspect, the invention provides a method of treating a surface which comprises the step of applying to said surface an effective amount of an anti-microbial composition as defined above.

In one embodiment, the surface is a surface which is used in the preparation and/or handling of food products.

In a final aspect, the invention provides a method of treating a product for the purpose of rendering that product microbially resistant which comprises the step of adding to said product an effective amount of an anti-microbial composition as defined above.

DESCRIPTION OF THE DRAWINGS

While the invention is broadly as defined above, it will also be appreciated that it includes embodiments of which the following description provides examples. Furthermore, a better understanding of the invention will be gained through reference to the accompanying drawings in which:

Figure 1 is a graph showing the effect of monolaurin concentration on the growth of bacteria in a broth culture inoculated with *S. aureus* at a rate of 8 x 10^6 cfu per ml (equivalent to a 1% inoculum of the specially prepared stock culture). The Optical Density (OD) measured at 600 nm was used as an index of bacterial cell numbers.

Figure 2 is a graph showing the inhibition of the growth of bacteria by various compositions in a broth culture inoculated with *E. coli* at a rate of 1.5 x 10^6 cfu per ml (equivalent to a 0.05% inoculum of the specially prepared stock culture).

Figure 3 is a graph showing the inhibition of the growth of bacteria by various compositions in a broth culture inoculated with *S. aureus* at a rate of 4 x 10^5 cfu per ml (equivalent to a 0.05% inoculum of the specially prepared stock culture).

Figure 4 is a graph showing the inhibition of the growth of bacteria by various compositions in a broth culture inoculated with *S. aureus* at a rate of 8 x 10^6 cfu per ml (equivalent to a 1% inoculum of the specially prepared stock culture).

Figure 5 is a graph showing the inhibition of the growth of bacteria by various compositions in a broth culture inoculated with *L. monocytogenes* at a rate of 1.5 x 10^6 cfu per ml (equivalent to a 0.05% inoculum of the specially prepared stock culture).

Figure 6 is a graph showing the inhibition of the growth of bacteria by various compositions in a broth culture inoculated with *L. monocytogenes* at a rate of 3 x 10^7 cfu per ml (equivalent to a 1% inoculum of the specially prepared stock culture).

DESCRIPTION OF THE INVENTION

As broadly defined above, the primary focus of the present invention is on microbially resistant compositions. Such compositions exert an anti-microbial effect through the synergistic combination of the PS and an anti-microbial fatty acid or fatty acid derivative component.

The surprising finding made by the applicants is that the anti-microbial efficacy of the PS can be supplemented markedly by addition of an anti-microbial fatty acid component. The improvement in anti-microbial efficacy is enhanced or synergistic in character. This synergism is particularly evident against Gram-negative microorganisms such as *E. coli*.

The PS requires three components. These are in turn a peroxidase, a source of peroxide and a cofactor. The cofactor is one which yields intermediary anti-microbial oxidation

products. Examples of suitable cofactors include thiocyanate and halides (particularly iodide).

The peroxidase itself can be any of those which are commercially available. GRAS status peroxidases are preferred, with a lactoperoxidase being particularly preferred.

The peroxide can be directly added (for example as hydrogen peroxide) or can be the product of enzymic digestion of an appropriate substrate. For example, a combination of glucose oxidase and glucose can provide the source of hydrogen peroxide. Sodium percarbonate-based systems can also be used.

The components of the PS will be provided in art standard amounts. For example, where the peroxidase is lactoperoxidase, the peroxide source is glucose/glucose oxidase and the cofactor is thiocyanate, the components can be included in a liquid medium in the following amounts (mg per litre):

LP	6.85
Glucose oxidase	3.17
Glucose	31.7
SCN^-	29.0

For a solid substrate, the same components can be included, for example, in the following amounts (mg per kg):

LP	205
Glucose oxidase	9.5
Glucose	31.7
SCN^-	200.

The fatty acid or fatty acid derivative component will generally be a C_8-C_{16} fatty acid or derivative. However, C_{10}, C_{12} and C_{14} fatty acids are generally regarded as the most anti-microbial and therefore these are preferred if the fatty acid component is to be added in the form of a fatty acid *per se*.

The use of anti-microbial derivatives includes the use of esters for fatty acids or their salts. One particular ester which the applicants found to be useful is monolaurin

(Lauricidin Æ). The sodium dodecyl sulphate salt can also be used as a suitable derivative.

It is also to be emphasised that the fatty acid component need not be in pure form. The useful fatty acid/ fatty acid derivative can be included in a mixture such as an extract of bovine milk fat or coconut oil in which the lipid has been treated to ensure that the requisite proportion of anti-microbial components are present.

In order for the fatty acid component to induce synergism with the PS, the applicants have found that a synergistically effective amount of fatty acid/fatty acid derivative must be present. This amount is greater than the levels at which anti-microbial fatty acids are present in standard bovine milk (in which the lipid is predominantly in the form of triglycerides), and reflects the applicants finding that when the PS plus fatty acid component is applied to milk, significantly enhanced and effective anti-microbial result is achieved.

Specifically, the applicants have determined that where the synergistic anti-microbial effect is to be produced in a composition which contains lipid, the anti-microbial fatty acid/fatty acid derivative must be present in an amount which is at least 5% by weight of the total lipid in the composition.

The invention will now be illustrated with reference to the following non-limiting experimental section.

EXPERIMENTAL

SECTION A

Materials

Bacterial strains, media and chemicals

L. monocytogenes strain L45 and *S. aureus* strain R37 were obtained from Dr Roger Cook, the Meat Industries Research Institute of New Zealand strain culture collection and *E. coli* O157:57 strain NCTC 12900 was obtained from Dr Heather Brooks, the Department of Microbiology, University of Otago strain culture collection. Stock cultures of all strains were stored in skim milk at -70°C and when required were

subcultured onto Plate Count Agar (PCA) (Difco Laboratories, Detroit, Michigan, USA) or blood agar (BA) (Columbia Agar Base (GIBCO BRL, Life Tech Ltd, Paisly UK) supplemented with 5% whole human blood (Dunedin Public Hospital, Dunedin, NZ)). Strains in regular use were maintained as plate cultures and subcultured every two weeks. All commercial media were prepared according to the manufacturer's specifications. Monolaurin (1-monolauroyl-rac-glycerol, Sigma Chemical Co., St Louis, MO, USA) was prepared by dissolving 1g in 10 mL ethanol, dispensing in 1 mL volumes and storing at -20°C until required. All LPS components were filter sterilized. Glucose (Sigma) was prepared by dissolving 18.016 g glucose (Sigma) in 100 ml of MilliQ water dispensing in 3 mL volumes and stored at room temperature (RT) until required. Glucose oxidase (GOX) and glucose oxidase were sourced from Sigma; lactoperoxidase was sourced from Tatua Biologics, Morrinsville, NZ; and the sodium or potassium thiocyanate was sourced from Bio Serae SA Limited, Montolieu, France.

Methods

Test system

Growth experiments were conducted in 100 x 15 mm screw capped glass test tubes containing 8 mL Todd-Hewitt broth (THB). As required, monolaurin was added to each tube prior to autoclaving of tubes at 121°C for 15 min. As required, components were added to autoclaved tubes in the following order, cell inoculum, 16 LPX stock, glucose stock, thiocyanate stock and glucose oxidase stock. In all cases tubes were inoculated with 0.05% and 1.0% (v/v) of an overnight THB culture of the appropriate bacterial strain. The tubes were incubated at 37°C for 48 hours and their OD_{600nm} read by use of a spectrophotometer (Spectronic 20D+, Milton Roy Company, USA) at intervals as appropriate. Viable counts of each bacterial strain were determined by dilution in saline of five overnight cultures of each strain and plating of each dilution onto PCA by use of a spiral plating machine (Spiral Systems, Cincinnati, USA).

The concentration (mg/litre) of the components of the LPS in the culture solution was as follows: 6.85 for lactoperoxidase, 3.17 for glucose oxidase, 31.7 for glucose and 29.0 for thiocyanate.

Results

The test system

The viable count of overnight broths for each test strain used in the experiments was 3 x 10^9, 8 x 10^8 and 3 x 10^9 cfu per ml for *E coli* 0157:57 strain NCTC 12900 ("*E. coli*"), *S*

aureus strain R37 ("*S. aureus*") and *L monocytogenes* strain L45 ("*L. monocytogenes*") respectively.

The degree of inhibition of monolaurin against both Gram-positive strains appeared to be proportional to the concentration of monolaurin used, as is illustrated by the results shown for a 1% inoculum of *S. aureus* grown in the presence of 50 – 150 ppm monolaurin (Figure 1). For both monolaurin and LPS, the degree of inhibition observed was inversely proportional to the bacterial load imposed on the system; that is, the greater the starting inoculum the lesser the degree of inhibition observed. For both monolaurin and LPS, *L. monocytogenes* was the strain most sensitive to inhibition and *E. coli* the strain least sensitive to inhibition.

Effectiveness against E. coli

Monolaurin did not inhibit the growth of *E. coli* when tubes were inoculated at either 0.05% or 1.0%. The LPS slightly inhibited the growth of *E. coli* when tubes were inoculated at 0.05% but not when inoculated at 1.0%. Growth of the *E. coli* was strongly inhibited by combinations of the monolaurin and LPS (Figure 2).

Effectiveness against S. aureus

Growth of *S. aureus* inoculated at 0.05% (Figure 3) was completely inhibited by monolaurin at concentrations of 100 and 500 ppm. These cultures were also strongly inhibited by LPS, with the time of culture stationary phase being extended for approximately 20 hours beyond cultures containing no LPS. No growth was observed in tubes containing both monolaurin and LPS. Growth of *S. aureus* inoculated at 1.0% (Figure 4) was completely inhibited by monolaurin at a concentration of 500 ppm but was only partially inhibited by monolaurin at a concentration of 100 ppm. In the case of the 1% inoculum with 100 ppm monolaurin cultures, the stationary phase was extended for approximately 10 hours beyond that of the control tubes and these cultures never reached densities comparable to those of the control tubes. These cultures were strongly inhibited by LPS, with the stationary phase being extended for approximately 13 hours beyond that of the tubes containing no LPS. Significantly, the only growth seen in tubes containing both monolaurin and LPS was in the 100 ppm monolaurin + LPS system where the last time point at 48 hours showed a slight but statistically significant increase in turbidity.

Effectiveness against L. monocytogenes

L. monocytogenes was completely inhibited at all inoculum densities by all systems containing monolaurin at either 100 or 500 ppm (Figures 5 & 6). In the case of the 1% inoculum with LPS cultures, the stationary phase was extended for approximately 22 hours beyond that of the uninhibited control tubes. In the case of the 0.05% inoculum with LPS cultures, stationary phase was extended for approximately 27 hours beyond that of the uninhibited control tubes and these cultures never reached densities comparable to those of the control tubes.

Discussion

The three strains of bacteria used in this study were chosen because of their status as food-borne pathogens and the range of their reported sensitivities to monolaurin. It was not surprising that growth of the *E. coli* O157:H7 strain used in this study was not affected by monolaurin at concentrations of up to 500 ppm, as Kabara *et al* reported growth of *E. coli* to be unaffected by monolaurin concentrations of greater than 1000 ppm (Kabara *et al*, 1977). By contrast, the growth of *L. monocytogenes* has been reported to be inhibited by relatively modest concentrations of monolaurin, with concentrations as low as 5 ppm having been reported to delay lag phase growth in broth cultures by 8 hours (Wang *et al*, 1977). Although growth of the strain of *L. monocytogenes* used in this study was not affected by monolaurin at a concentration of 10 ppm, it was completely inhibited by monolaurin at concentrations of 100 and 500 ppm. *S. aureus* is a bacterium with an intermediate sensitivity to inhibition of growth by monolaurin as it has been reported to be sensitive to inhibition at concentrations of about 200 ppm (Kabara *et al*, 1977), a result which compares well with the sensitivity of the strain used in this study.

The LPS has been reported to inhibit the growth of a wide range of bacteria including *E. coli, S. aureus* and *L. monocytogenes* (Wolfson *et al*, 1993). Unfortunately, the wide range of component (glucose, H_2O_2, GOX, LPX & SCN^-) concentrations, incubation media, and incubation temperatures used in these studies, make direct comparisons with the present study difficult. Generally though, it appears that the degree of relative sensitivity of the strains to LPS alone seen in this study is consistent with that reported by others (Gaya *et al*, 1991; Kamau *et al*, 1990; Bjork *et al*, 1975; Siragu *et al*, 1989). Despite the bactericidal nature of the action of LPS against *E. coli*, the degree of inhibition of growth of the O157:H7 strain used in this study was considerably less than that seen against the Gram-positive species. It was also considerably less than

that seen against *E. coli* DH5α, a common laboratory strain (Simmonds & Kennedy, unpublished data). Large variations in the sensitivity of *E. coli* strains to LPS has been noted by others, with Grieve *et al* reporting 6 hour reductions in viable count for different enterotoxigenic strains ranging between 3.6 and 7.3 log units (Grieve *et al*, 1992).

The results reported above provide strong evidence that the combination of monolaurin and LPS, has great potential for use as a preservation system. There was no inhibition of *E. coli* O157:H7 by monolaurin used alone and we are not aware of any reported degree of sensitivity of any *E. coli* strain to monolaurin alone used at any concentration. Thus, the synergistic effect of the monolaurin + LPS combinations against *E. coli* O157:H7 (inhibition far in excess of that expected from LPS alone) was unexpected. By contrast, the inhibitory effect of the monolaurin + LPS combinations against *S. aureus* was not as surprising on the basis of its significant inhibition by each agent used alone. One unexpected result however, was the degree of inhibition of *S. aureus* obtained in the combined systems. One problem with the use of monolaurin as a food preservative has always been that the quantity of monolaurin required to obtain the desired level of inhibition is such that it may make the process uneconomic, or result in the development of undesirable organoleptic properties (texture, flavour) in the food. The results reported above indicate that monolaurin + LPS combinations will be effective at concentrations of monolaurin much lower than those required to achieve an equivalent inhibitory effect by use of monolaurin alone.

SECTION B

This section further illustrates aspects of the invention.

A broth culture system (Todd-Hewitt Broth, THB) inoculated with either *S. aureus* R37 or *E. coli* O157:H7 (-vt) was used as a screening system to evaluate different lipid components. The strains were cultured in THB overnight at 37 °C and dispensed to tubes. The initial loadings were 1 x 10^5 per ml for both the *S. aureus* and the *E. coli*.

The components of the lactoperoxidase system, LPS, were present at concentrations of: 20 mg/litre of lactoperoxidase (c.3000 Units activity/litre), glucose oxidase (c.300 Units activity/l), 290 mg/l of thiocyanate ion (496 mg/l of NaSCN) and 12000 mg/l of glucose. The growth of the organisms was then followed by measuring the increase in

absorbance (at 600nm) of the broth regularly over 48 hours using a spectrophotometer (Spectronic 20D+) and attached data logger.

A number of lipid components (fatty acids and monoesters) exhibit anti-microbial effects when combined with the lactoperoxidase system as evidenced in the experiments summarised in Table 1. Monolaurin was the most effective lipid component against *S. aureus* and in this simple system, it was just as effective whether or not the LPS was included. The Table 1 data indicate that synergy is apparent for both the Gram positive *S. aureus* and the Gram negative *E. coli* but the effect is greater with the *S. aureus*. The effect of monopalmitoleate was very similar to that of monolaurin against *E. coli* alone or in the presence of the LPS. However sodium lauryl sulphate was very effective against *E. coli*, whether or not the LPS was present.

Table 1: Inhibition of bacterial growth in broth culture by a combination of a lipid component and the lactoperoxidase system. The data are expressed as the concentration of the lipid component when bacterial growth is inhibited by about 50% or 100% after 48 hours; eg. 100/250 means that growth is about 50% inhibited at 100ppm and completely inhibited at 250ppm of the lipid component; the * indicates that the organism was not 100% inhibited at any concentration up to 1000ppm.

Lipid component	*S. aureus* R37		*E. coli* O157:H7	
	Lipid only	***Lipid + LPS***	***Lipid only***	***Lipid + LPS***
Monolaurin	**<50/50**	**<50/50**	100/*	**50/***
Lauric acid	250/500	50/100	500/*	50/1000
Sodium lauryl sulphate	500/*	250/*	**100/250**	**100/250**
Caprylic acid (C8:0)	500/*	100/1000	500/*	500/1000
Palmitoleic acid (C16:1)	50/250	**<50/50**	250/*	**50/***
Monopalmitoleate	500/*	**<50/50**	**50/***	**50/***

Tables 2 and 3 present the results of experiments in which milk and mince were inoculated with *S. aureus* R37. The synergistic effects of the LPS/monolaurin combination are apparent.

Table 2: Evaluation of the monolaurin (1000ppm) + lactoperoxidase (LPS with 20mg per litre of lactoperoxidase) system in milk (*S. aureus* R37 at 37°C; cfu per ml).

Milk with S. aureus R37		*Experiment 1*		*Experiment 2*		*Treatment effect compared with Control*
	0 hours	**5 h**	**24 h**	**5 h**	**24 h**	
Control	7×10^4	1×10^8	1×10^8	5×10^7	1×10^8	Control
Monolaurin	7×10^4	2×10^5	9×10^8	1×10^4	9×10^8	3 log @ 5h; nil @ 24h
LPS	7×10^4	5×10^2	nil	nil	2×10^4	4 log to complete kill @ 5h & 24h
Monolaurin + LPS	7×10^4	Nil	nil	nil	nil	Complete kill @ 5h & 24h

Table 3: Evaluation of the monolaurin (1000ppm) + lactoperoxidase (LPS with 200mg per kg of lactoperoxidase) system in mince (*S. aureus* R37 at 37°C; cfu per g).

Mince with S. aureus R37	*Experiment*			*Treatment effect compared with Control*
	0 hours	**5 hours**	**24 hours**	
Control	2×10^5	3×10^8	7×10^8	Control
Monolaurin	8×10^4	2×10^8	9×10^8	Nil @ 5h & 24h
LPS	9×10^4	3×10^5	1×10^3	3 log @ 5h & >5 log @ 24h
Monolaurin + LPS	9×10^4	4×10^3	2×10^2	5 log @ 5h & >6 log @ 24h

Tables 4 and 5 present the results of experiments in which milk was inoculated with *E. coli* O157:H7 (a Gram negative organism) or *S. aureus* R37 (a Gram positive organism).

Table 4: Evaluation of the monolaurin + lactoperoxidase system in milk against a Gram negative organism (*E. coli* O157:H7, at 12°C; cfu per ml). The ratio of LPX to GOX (Units of enzyme activity) was 9:1, with the thiocyanate ion and glucose each present at 12mg/l.

Milk with E. coli O157:H7	**0**	**1 day**	**2 days**	**3 days**	*Treatment effect compared with Control*
Control	2×10^5	1×10^7	1×10^9	1×10^9	Control
Monolaurin (500 ppm)	2×10^5	2×10^6	3×10^8	2×10^9	1 log @ 1 & 2d; nil @ 3d
LPS (50 mg LPX/litre)	2×10^5	4×10^4	7×10^7	7×10^7	2-3 log @ 1d; 1-2 log @ 2 & 3d
Monolaurin (500)+ LPS (50)	2×10^5	3×10^4	2×10^4	3×10^6	2-3 log @ 1d; 5 log @ 2d; 2-3 log @ 3d

Table 5: Evaluation of the monolaurin + lactoperoxidase system in milk against a Gram positive organism (*S. aureus* R37 at 37°C; cfu per ml; component concentrations as for Table 4).

Milk with S. aureus R37	**0 hours**	**5 hours**	**24 hours**	***Treatment effect compared with Control***
Control	9×10^4	1×10^8	3×10^9	Control
Monolaurin (1000 ppm)	1×10^5	4×10^6	2×10^9	1-2 log @ 5h; nil @ 24h
LPS (5 mg LPX)	1×10^5	6×10^7	2×10^9	nil @ 5 & 24h
LPS (50 mg LPX)	1×10^5	1×10^8	1×10^9	nil @ 5 & 24h
Monolaurin (1000)+ LPS (5)	9×10^4	4×10^4	1×10^9	3-4 log @ 5h; nil @ 24h
Monolaurin (1000)+ LPS (50)	9×10^4	9×10^4	2×10^9	3 log @ 5h; nil @ 24h

Again, the efficacy of the LPS/monolaurin combination is apparent.

Table 6 presents the results of two experiments that show in certain circumstances, the presence of the milk itself actually has an inhibitory effect on the efficacy of the monolaurin treatment. That is, the anti-microbial effect of the monolaurin is adversely affected by the normal levels of lipid (generally in the form of triglycerides) naturally present in milk. Similarly, the efficacy of the combination of monolaurin plus the LPS is also affected by the concentration of milk (and hence the lipid) present in the culture medium (Table 7). However the effect of the (competing) lipid content is less with the combination of monolaurin + LPS than with monolaurin alone; in other words, the synergistic effect is greater.

Table 6: Comparison of different levels of milk (ie lipid) on the efficacy of the monolaurin system against S. *aureus* R37 (cfu per ml) in a Todd-Hewitt Broth (THB)/milk mixture at 37°C.

Treatment	**Microbial count**		**Effect of monolaurin treatment on *S. aureus***
	0 hours	**6 hours**	
100% THB [Control, 10ml THB only)	1×10^5	3×10^7	Control
100% THB + 500ppm Monolaurin	1×10^5	nil	7 log @ 6h
25% milk/75% THB	1×10^5	5×10^7	Control
25% milk/75% THB + Monolaurin	1×10^5	5×10^3	4 log @ 6h

Treatment	Microbial count		Effect of monolaurin treatment on *S. aureus*
	0 hours	6 hours	
50% milk/50% THB	1 x 10^5	7 x 10^7	Control
50% milk/50% THB + Monolaurin	1 x 10^5	2 x 10^3	4-5 log @ 6h
75% milk/25% THB	1 x 10^5	8 x 10^7	Control
75% milk/25% THB + Monolaurin	1 x 10^5	3 x 10^6	1-2 log @ 6h
100% milk	1 x 10^5	4 x 10^7	Control
100% milk + Monolaurin	1 x 10^5	1 x 10^7	nil @ 6h

Table 7: Comparison of different levels of milk (ie lipid) on the efficacy of the monolaurin + lactoperoxidase system (with 5 ppm of LPX) against S.*aureus* R37 (cfu per ml) in a THB/milk mixture at 37°C.

5

Treatment	Microbial count		Effect of monolaurin + LPS treatment
	0 hours	6 hours	
100% THB (Control, 10ml THB only)	1 x 10^5	2 x 10^7	Control
100% THB + 500ppm Monolaurin + LPS	1 x 10^5	nil	7 log @ 6h
25% milk/75% THB	1 x 10^5	3 x 10^7	Control
25% milk/75% THB + Monolaurin + LPS	1 x 10^5	nil	7 log @ 6h
50% milk/50% THB	1 x 10^5	5 x 10^7	Control
50% milk/50% THB + Monolaurin + LPS	1 x 10^5	3 x 10^3	4 log @ 6h
75% milk/25% THB	1 x 10^5	8 x 10^7	Control
75% milk/25% THB + Monolaurin + LPS	1 x 10^5	8 x 10^3	4 log @ 6h
100% milk	1 x 10^5	6 x 10^7	Control
100% milk + Monolaurin + LPS	1 x 10^5	4 x 10^4	3 log @ 6h

The experimental results reported in Tables 8 and 9 further demonstrate the synergistic efficacy of the LPS/monolaurin combination.

10 **Table 8:** The synergistic effect of the components through comparison of the effect of the efficacy of the LPS or monolaurin systems alone and the monolaurin + LPS against the Gram-positive bacteria, *S. aureus* R37 in milk at 37°C.

Treatment Monolaurin @ 1000 ppm & LPS (with LPX @ + 5 or 50 ppm)	Effect of treatment @ 5h (reduction in microbial count, cfu per ml)				
	LPS alone	Monolaurin alone	Monolaurin + the LPS	Synergistic effect of the combination compared with	
				LPS alone	Monolaurin alone
Expt 67 (from Table 5)					
1. 100% milk + 5 LPX	Nil	1-2 log	3-4 log	3-4 log	2 log
1. 100% milk + 50 LPX	Nil	1-2 log	3 log	3 log	1-2 log

Table 9: The synergistic effect of the components through comparison of the effect of different levels of milk (ie fat) on the efficacy of the monolaurin system alone and the monolaurin + LPS against the Gram-positive bacteria, *S. aureus* R37 at 37°C.

Treatment Monolaurin @ 500 ppm (& LPX @ + 5 ppm)	Effect of treatment @ 6h (reduction in microbial count, cfu per ml)		
	Monolaurin alone	Monolaurin + the LPS	Synergistic effect compared with monolaurin alone
Expt 72 & 77 (from Tables 6 & 7)			
2. 100% THB (Control, 10ml THB only)	Control	Control	NA
3. 100% THB + Monolaurin (+LPS)	7 log	7 log	Nil
4. 25% milk/75% THB + Monolaurin (+ LPS)	4 log	7 log	3 log
5. 50% milk/50% THB + Monolaurin (+ LPS)	4 log	4 log	Nil
6. 75% milk/25% THB + Monolaurin (+ LPS)	1-2 log	4 log	2-3 log
7. 100% milk + Monolaurin (+ LPS)	Nil	3 log	3 log

As summarised in Tables 8 and 9, LPS alone had no inhibitory effect on against S.*aureus* R37. Monolaurin alone at 1000 ppm had a small effect (1-2 log) as reported in Table 8 but had no effect at the lower level of 500 ppm as reported in Table 9. The synergistic effect of the combination of monolaurin and the LPS (compared with

monolaurin alone or the LPS alone) however is clearly evident. The extent of the synergy is affected by both the presence of and by the level of lipid. In the presence of lipid, the anti-microbial efficacy of the combined composition is much enhanced compared with the monolaurin alone. However, the efficacy of the anti-microbial composition is influenced by the proportion of lipid present.

Table 10 presents the results of an experiment in which the effect of the concentration of monolaurin alone on *S. aureus* in THB was evaluated. In this experiment, a concentration of only 25 or 50ppm was required to have a significant effect on the population of *S. aureus*. The efficacy of the 50ppm monolaurin treatment was equivalent to that achieved with 500ppm in Table 6. As the experiment reported in Table 10 was conducted in a fat-free medium, the comparison provides further evidence of the compromising effect of the presence of other lipids on the efficacy of the monolaurin.

Table 10: Comparison of different levels of monolaurin on the population of *S. aureus* R37 in THB (cfu per ml) at 37°C.

Treatment	0 hours	6 hours	24 hours	Effect of monolaurin treatment
8. Control THB only	3×10^4	4×10^7	2×10^9	Control
9. THB + 1ppm Monolaurin	3×10^4	2×10^7	2×10^9	Nil @ 6 & 24h
10. THB + 5ppm Monolaurin	3×10^4	1×10^7	2×10^9	Nil @ 6 & 24h
11. THB + 10ppm Monolaurin	3×10^4	8×10^6	1×10^9	Nil @ 6 & 24h
12. THB + 25ppm Monolaurin	3×10^4	5×10^1	2×10^6	6 log @ 6h & 3 log@ 24h
13. THB + 50ppm Monolaurin	3×10^4	nil	1×10^6	Complete kill @ 6h & 3 log@ 24h

With reference to Tables 6 to 10, a solution of 100% milk contains around 3% milk lipid. Monolaurin at a concentration of 500 ppm represents 0.05 grams per 100ml (0.05%). From the above it is clear that a certain threshold concentration of selected anti-microbial lipid components (expressed as a percentage of the total lipid) must be exceeded in order to ensure a significant anti-microbial effect of the total composition.

Table 11 provides a summary of the relevant data classified according to the level of added anti-microbial lipid (in this case monolaurin) both in the actual amount present and as a proportion of the total lipid. The data in Table 11 indicate that 3.2% monolaurin was marginal in that the effect was variable and ranged from a 1 log to a 4 log reduction in microbial count at 5 or 6 hours for monolaurin alone and a 3 log to 8 log reduction for monolaurin + LPS.

Table 11, Summary of data from Tables 2, 8, 9 & 10: Evidence for the effectiveness of, and the synergistic effect of, the components of the anti-microbial composition as affected by the quantity of monolaurin present and the proportion of the total lipid present as monolaurin (reduction in population of *S. aureus* after 5 or 6 hours at 37°C).

Treatment No ex Table	**Monolaurin as a proportion and as a percentage of total lipid**	**Effect of monolaurin**	**Effect of monolaurin + LPS**	**Synergistic effect**
11, Table 10	0.001/0.001 100% of total lipid	Nil	Not done (ND)	ND
13, Table 10	0.005/0.005 100%	7 log	ND	ND
3, Table 9	0.05/0.05 100%	7 log	7 log	Nil
4, Table 9	0.05/0.8 6.3%	4 log	7 log	3 log
ex Table 2	0.10/3.1 3.2%	3 log	8 log	5 log
1, Table 8	0.10/3.1 3.2%	>1 log	3 log	2 log
5, Table 9	0.05/1.6 3.2%	4 log	4 log	Nil
6, Table 9	0.05/2.3 2.2%	>1 log	4 log	3 log

It is therefore the applicants view that the concentration of the selected anti-microbial lipid components must be equal to or greater than 5% of the total lipid present in order to include the synergistic anti-microbial effect.

In the case of bovine milk the free fatty acids with anti-microbial properties (or their derivatives) must therefore constitute more than 5% of the total lipids present for the milk to be transformed into anti-microbial composition to achieve a substantial and consistent anti-microbial effect. Such a composition will be effective against both Gram positive (as exemplified by *S. aureus*) and Gram negative organisms (as exemplified by *E. coli,* see Table 5).

Such an amount of anti-microbial lipid can only be achieved by addition of the selected anti-microbial lipid or derivative in accordance with the invention.

The importance of the presence of all components of the system in ensuring an effective anti-microbial composition was tested in the experiment summarised in Table 12.

A simple medium of the following composition was prepared:

Phosphate Buffered Saline (pH 7) with bactotryptone and yeast extract (PBS/T/Y): 195 ml of 0.2 M NaH_2PO_4, 305 ml of 0.2 M Na_2HPO_4 and 8.994 g NaCl, 10 g bactotryptone, 5 g yeast extract made up to 1 litre with MilliQ water and autoclaved in screw-capped tubes at 121 °C for 15 min. The tubes were held at incubation temperature (37 °C) until used.

Two strains *S. aureus* R37 or *E. coli* O157:H7 (-vt) were used in the experiments (as for the experiments in Table 1), with the following base combinations of monolaurin and the LPS selected for the two strains based on titrations of the organisms against monolaurin + LPS to define the sensitivity: *S. aureus* (100mg LPX (lactoperoxidase) per litre in the LPS and 20mg/litre of monolaurin) and *E. coli* (100mg LPX per litre in the LPS and 50mg/litre of monolaurin). The LPX to glucose oxidase (GOX) ratio was 9:1 and the thiocyanate and glucose were incorporated at 12mg/litre.

Table 12: The importance of the individual components in ensuring the efficacy of the anti-microbial system. The degree of inhibition is defined by the time (hours of incubation 37 °C) at initiation of the logarithmic growth phase of the organism and the time at plateau (maximum absorbance).

***E. coli* O157:H7**

No inhibition (0 hours and 12 hours)	**Partial inhibition (4 hours and 16 hours)**	**Maximal inhibition (12 hours and 20 or >20 hours)**
Monolaurin (ML) alone	ML + LPX + GOX	ML + LPX + GOX + SCN
ML + LPX	ML + GOX + glucose	ML + the complete LPS
ML + glucose	ML + GOX + SCN	
ML + thiocyanate (SCN)	ML + GOX + glucose + SCN	

No inhibition (0 hours and 12 hours)	**Partial inhibition (4 hours and 16 hours)**	**Maximal inhibition (12 hours and 20 or >20 hours)**
ML + LPX + glucose	ML + LPX + GOX + glucose	The above may be interpreted as the effects of monolaurin plus the complete LPS as some glucose would have been present in the medium.
ML + LPX + thiocyanate	All of the above may be interpreted as the effects of monolaurin plus peroxide as the GOX would generate peroxide with glucose as the substrate.	
ML + glucose + thiocyanate		
ML + LPX + glucose + SCN		

***S. aureus* R37**

No inhibition (8-12 hours and 16-20 hours)	**Partial inhibition (12-16 hours and 24 hours)**	**Maximal inhibition (16 hours and 24-28 hours)**
ML + glucose	Monolaurin (ML) alone	ML + LPX + GOX + SCN
ML + thiocyanate (SCN)	ML + LPX	ML + the complete LPS
ML + LPX + GOX	ML + LPX + glucose	
ML + LPX + thiocyanate	ML + SCN + glucose	
ML + LPX + glucose + SCN		
	ML + GOX	The above may be interpreted as the effects of monolaurin plus the complete LPS as some glucose would have been present in the medium.
	ML + GOX + glucose	
	ML + GOX + SCN	
	ML + GOX + glucose + SCN	
	ML + LPX + GOX + glucose	
	The above group of 5 treatments may be interpreted as the effects of monolaurin plus peroxide as the GOX would generate peroxide with glucose as the substrate.	

Again, the efficacy of the applicants approach is demonstrated.

INDUSTRIAL APPLICATION

The applicants findings in respect of synergism between the LPS and monolaurin (which exemplify the interaction between PS and anti-microbial fatty acid/fatty acid derivatives) has a number of applications. Principal amongst these is that this synergistic anti-microbial effect can be reproduced in products which are prone to contamination or spoilage. Such products include food products, cosmetic products and healthcare products.

In food applications, the present invention has particular benefit where the fatty acid component is monolaurin. This reflects the fact that monolaurin has GRAS status and is already included in some food products as a emulsifying agent.

Food products in which the components can be included are any foodstuffs subject to spoilage as well as dietary supplements and nutraceuticals. The invention has particular application to dairy products (such as yoghurts), fish products (particularly shellfish) and meat products (including both ground meats and carcasses), as well as to animal feeds. It should however be appreciated that "feed" is intended in its most general sense, and can include water which is fed to farmed animals including but not limited to bovines, ovines, pigs, caprines, equines and avians (such as poultry).

To such products, the individual components can be added together or independently. In some instances, where a product may inherently contain one or more of the components in appropriate amounts, the remaining components only need be added.

The components can also be added to form a mixture as part of the product (such as a dairy product), or can be applied to at least partially coat the products (such as shellfish).

Where the ingredients are to be added individually, a preparative pack can be provided with at least the peroxidase and fatty acid/fatty acid derivatives in different containers.

The invention can also be applied in the formation of an anti-microbial composition for general use. Such a composition will include all four components in appropriate amounts. The composition can then be used to treat surfaces (for example, surfaces used in the preparation or handling of foodstuffs or in healthcare) to ensure that the microbial population is at least reduced if not eliminated.

The anti-microbial compositions of the invention can also be used to supplement the action of other agents. In such circumstances, the additional agent can be used separately or, more usually, as part of a mixture with the present components.

Other optional components which can be included where desirable include further anti-microbial agents, chelating agents, enzymes and nutritional nutraceutical components. Specifically, the composition may also include any of the following:

- a chelating agent, such as EDTA;
- a phenol, such as the esters if *para*-hydroxybenzoic acid (the parabens) including the methyl, ethyl, propyl, butyl or heptyl esters of *tert*-butyl hydroxyanisole (BHA);
- an organic acid, (which is recognised as a preservative), such as formic acid, acetic acid, propionic acid, lactic acid, sorbic acid, benzoic acid, citric acid or derivatives of any of these acids;
- a bacteriocin, such as nisin;
- lyzozyme;
- extracts of milk.

It will be appreciated by those persons skilled in the art that the above description is provided by way of example only and that modifications and/or variations thereto can be made without departing from the scope of the invention, which is limited only by the lawful scope of the appended claims.

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CLAIMS

1. A method of preparing a microbially resistant composition which comprises forming a mixture of the following components:

(a) a peroxidase system (PS) comprising:

(i) a peroxidase;

(ii) a source of peroxide; and

(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and

(b) at least one fatty acid or a derivative of a fatty acid,

wherein said fatty acid or derivative is present in an amount effective to interact with said PS to produce an enhanced anti-microbial effect.

2. A method according to claim 1 wherein the peroxidase is a lactoperoxidase.

3. A method according to claim 1 or claim 2 wherein the peroxide is hydrogen peroxide.

4. A method according to any one of claims 1 to 3 wherein the cofactor is selected from thiocyanate or iodide.

5. A method according to claim 4 wherein said cofactor is thiocyanate.

6. A method according to any one of claims 1 to 5 wherein said fatty acid is an anti-microbial fatty acid, or an anti-microbial derivative of a fatty acid.

7. A method according to claim 6 wherein said anti-microbial fatty acid or anti-microbial derivative of a fatty acid is present in an amount which is at least 5% by weight of the total lipid present in said composition.

8. A method according to any one of claims 1 to 7 wherein component (b) is or includes an anti-microbial fatty acid selected from C_8, C_{10}, C_{12}, C_{14} and C_{16} fatty acids or their derivatives, or mixtures thereof.

9. A method accordingly to any one of claims 1 to 8 wherein component (b) is or includes an anti-microbial ester of a fatty acid or a salt thereof.

10. A method according to any one of claims 1 to 5 wherein component (b) is or includes monolaurin (1-monododecanoyl-rac-glycerol) or its salt, sodium dodecyl sulphate.

11. A method according to claim 10 wherein component (b) is monolaurin.

12. A method according to any preceding claim wherein said composition is formed by the addition of one or more of the components to a pre-formed mixture which already contains the remaining component(s).

13. A method according to claim 12 wherein the pre-formed mixture is a food, cosmetic or healthcare product.

14. A method according to claim 13 wherein said food product is a dietary supplement, nutraceutical, dairy product, meat product or fish product.

15. A method according to claim 13 wherein said food product is an animal feed.

16. A method according to any one of claims 1 to 11 wherein said composition, when formed, consists of said components in admixture.

17. A microbially resistant composition which is prepared by a method as defined in any one of claims 1 to 16.

18. A preparative composition suitable for use in preparing a microbially resistant composition which comprises at least two components selected from:

(i) a peroxidase;
(ii) a source of peroxide;
(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and
(iv) at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid thereof,

wherein the peroxidase and peroxide source, if both present, are kept separate.

19. A preparative composition according to claim 18 wherein said peroxidase is a lactoperoxidase.

20. A preparative pack suitable for use in preparing a microbially resistant composition which comprises, in separate containers, a peroxidase and at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid.

21. A preparative pack according to claim 20 wherein said peroxidase is a lactoperoxidase.

22. A preparative pack according to claim 20 or claim 21 wherein said pack further includes a source of peroxide and/or a cofactor which is capable of yielding anti-microbial oxidation products.

23. An anti-microbial composition which comprises a peroxidase, a cofactor which is capable of yielding anti-microbial oxidation products and at least one anti-microbial fatty acid or anti-microbial derivative of a fatty acid, wherein said fatty acid or derivative thereof is present in an amount effective to synergistically interact with said peroxidase and said cofactor, in the presence of peroxide, to produce an enhanced anti-microbial effect.

24. An anti-microbial composition according to claim 23 which further includes a source of peroxide.

25. An anti-microbial composition according to claim 23 or claim 24 wherein said peroxidase is a lactoperoxidase.

26. An anti-microbial composition according to any one of claims 23 to 25 which include(s) a further anti-microbial agent or a chelating agent, or both.

27. An anti-microbial composition according to claim 26 wherein the further anti-microbial agent is selected from phenols, organic acids, bacteriocins, derivatives of

these and mixtures of these, or anti-microbial components or mixtures of components extracted from or found in milk.

28. An anti-microbial composition according to claim 26 wherein the chelating agent is EDTA.

29. An anti-microbial composition according to any one of claims 23 to 28 wherein said anti-microbial fatty acid or derivative is selected from C_8, C_{10}, C_{12}, C_{14} and C_{16} fatty acids or their derivatives, or mixtures thereof.

30. An anti-microbial composition according to any one of claims 23 to 28 wherein said anti-microbial fatty acid or derivative is an anti-microbial ester of a fatty acid, or a salt thereof.

31. An anti-microbial composition according to any one of claims 23 to 28 wherein said anti-microbial fatty acid or derivative is or includes monolaurin (l-monododecanoyl-rac-glycerol) or a salt thereof.

32. An anti-microbial composition according to claim 31 wherein said anti-microbial fatty acid or derivative is or includes sodium dodecyl sulphate as a salt of monolaurin.

33. An anti-microbial composition according to any one of claims 23 to 32 wherein said anti-microbial fatty acid or anti-microbial derivative of a fatty acid is present in an amount which is at least 5% by weight of the total lipid present in said composition.

34. A product which includes the following components:

(i) a peroxidase;
(ii) a source of peroxide;
(iii) a cofactor which is capable of yielding anti-microbial oxidation products; and
(iv) at least one anti-microbial fatty acid or anti-microbial derivative thereof, which product is resistant to the growth of microorganisms.

35. A product according to claim 34 wherein said peroxidase is a lactoperoxidase.

36. A product according to claim 34 or claim 35 which is resistant to the growth of both Gram positive and Gram negative bacteria.

37. A product according to any one of claims 34 to 36 in which said anti-microbial fatty acid or anti-microbial derivative of a fatty acid is present in an amount which is at least 5% by weight of the total lipid present in said composition.

38. A product according to any one of claims 34 to 37 which is a food product.

39. A food product according to claim 38 which is a dietary supplement, nutraceutical dairy product, meat product, fish product or animal feed.

40. A product according to any one of claims 34 to 37 which is a cosmetic product.

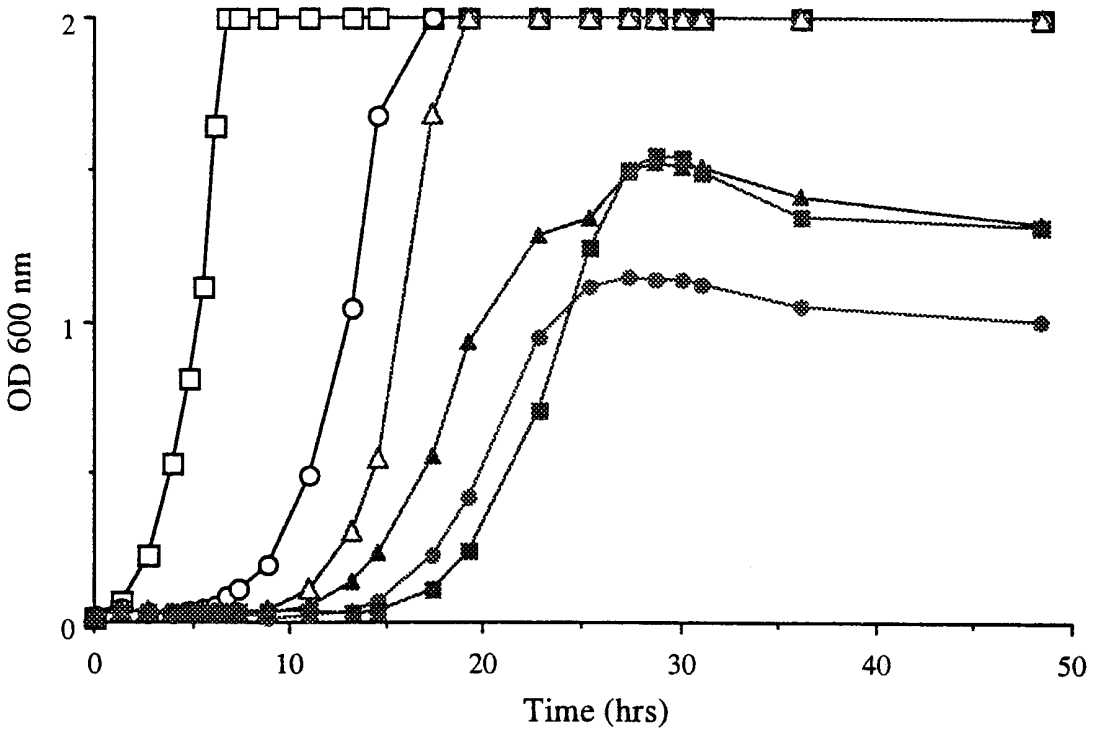
41. A product according to any one of claims 34 to 37 which is a healthcare product.

42. A method of treating a surface which comprises the step of applying to said surface an effective amount of an anti-microbial composition as defined in any one of claims 23 to 33.

43. A method according to claim 42 wherein said surface is a surface which is used in the preparation and/or handling of food products.

44. A method of treating a product for the purpose of rendering that product microbially resistant which comprises the step of adding to said product an effective amount of anti-microbial composition as defined in any one of claims 23 to 33.

Figure 1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50
Time (hrs)

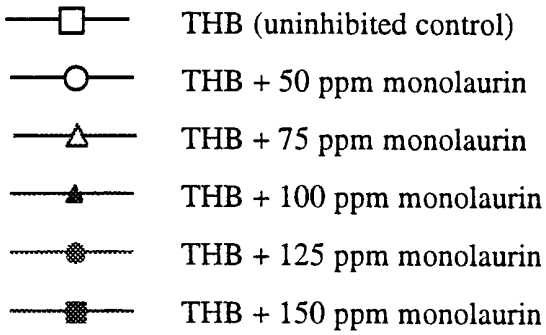

THB (uninhibited control)
THB + 50 ppm monolaurin
THB + 75 ppm monolaurin
THB + 100 ppm monolaurin
THB + 125 ppm monolaurin
THB + 150 ppm monolaurin

Figure 2

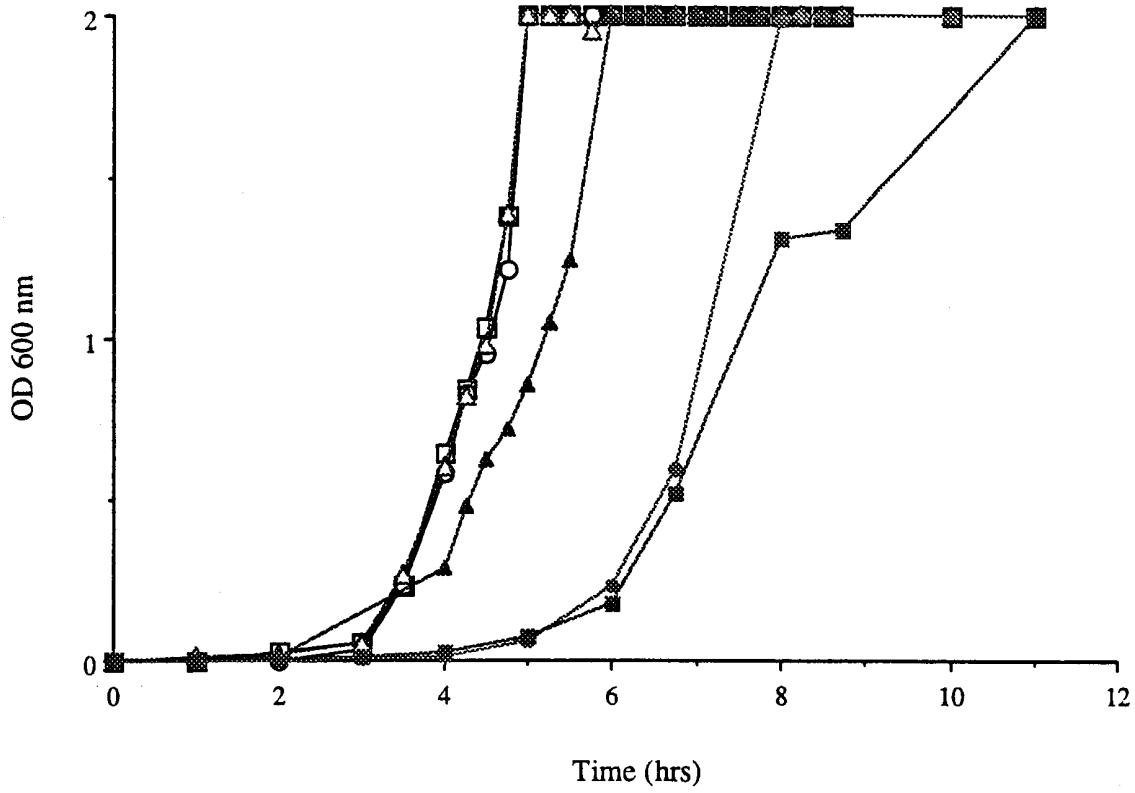

OD 600 nm
2
1
0
0
2
4
6
8
10
12
Time (hrs)
THB (uninhibited control)
THB + 100 ppm monolaurin
THB + 500 ppm monolaurin
THB + LPS
THB + LPS + 100 ppm monolaurin
THB + LPS + 500 ppm monolaurin

Figure 3

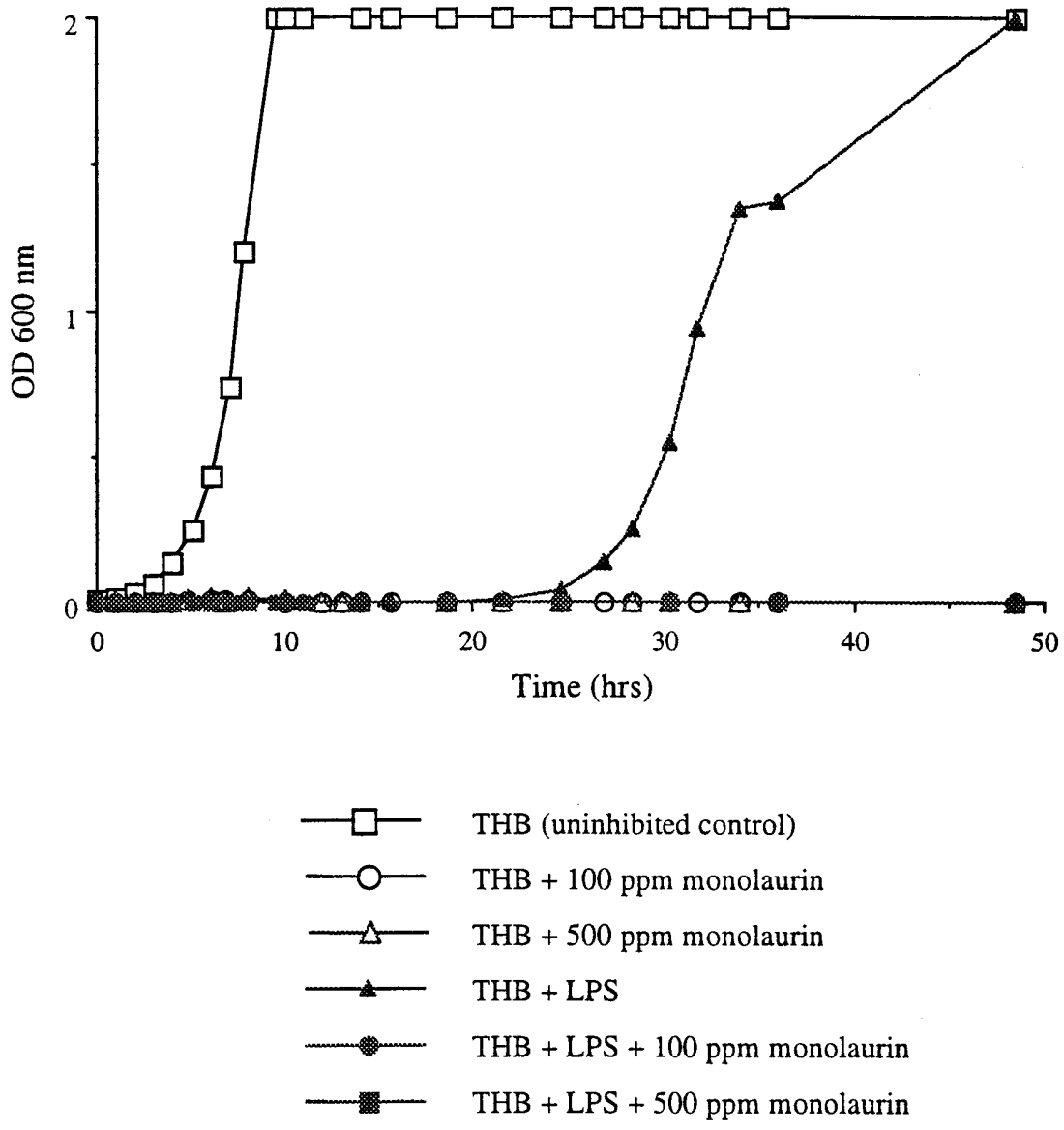

OD 600 nm
2
1
0
0
10
20
30
40
50
Time (hrs)
THB (uninhibited control)
THB + 100 ppm monolaurin
THB + 500 ppm monolaurin
THB + LPS
THB + LPS + 100 ppm monolaurin
THB + LPS + 500 ppm monolaurin

Figure 4

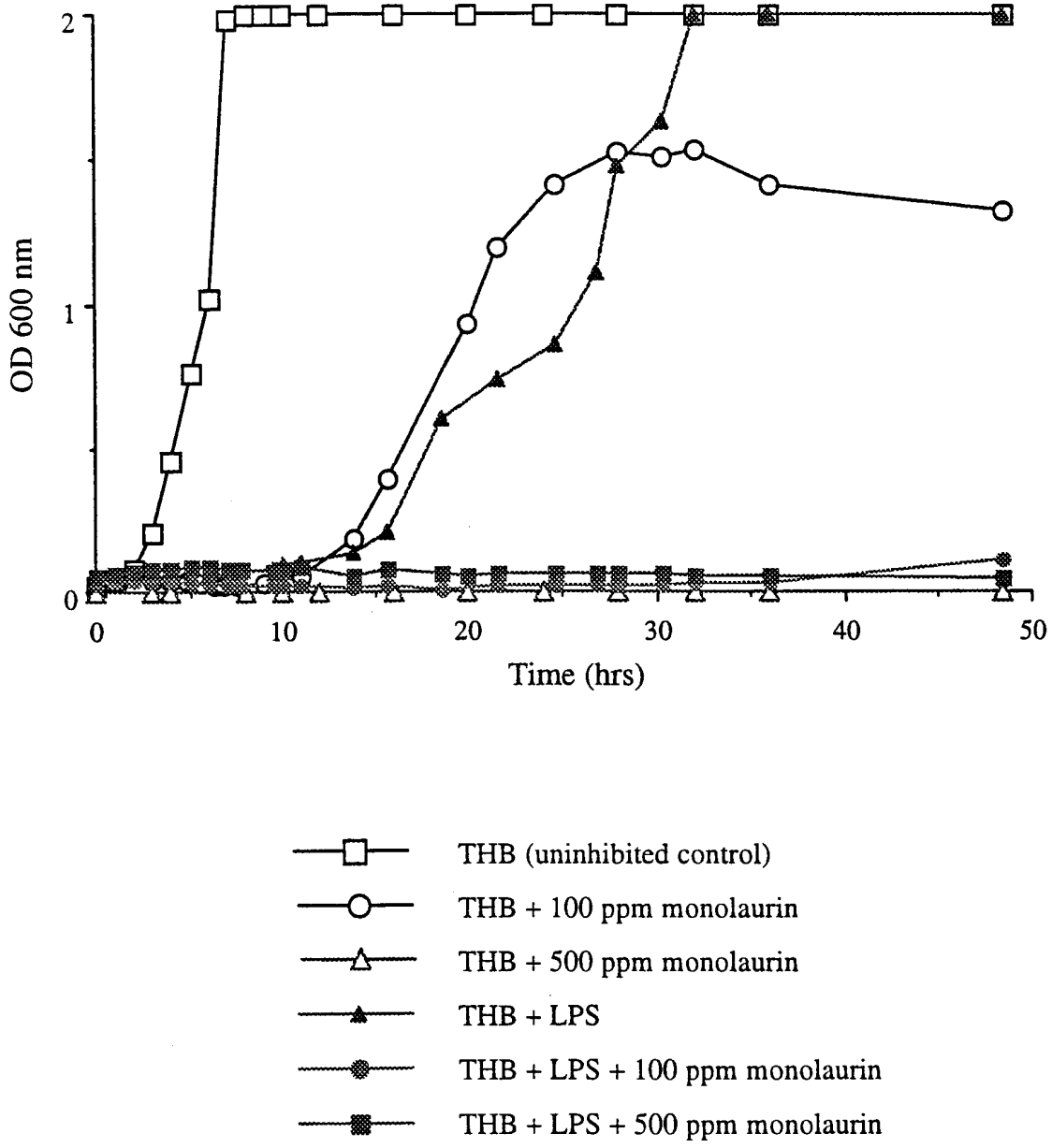

OD 600 nm
2
1
0
0
10
20
30
40
50
Time (hrs)
THB (uninhibited control)
THB + 100 ppm monolaurin
THB + 500 ppm monolaurin
THB + LPS
THB + LPS + 100 ppm monolaurin
THB + LPS + 500 ppm monolaurin

Figure 5

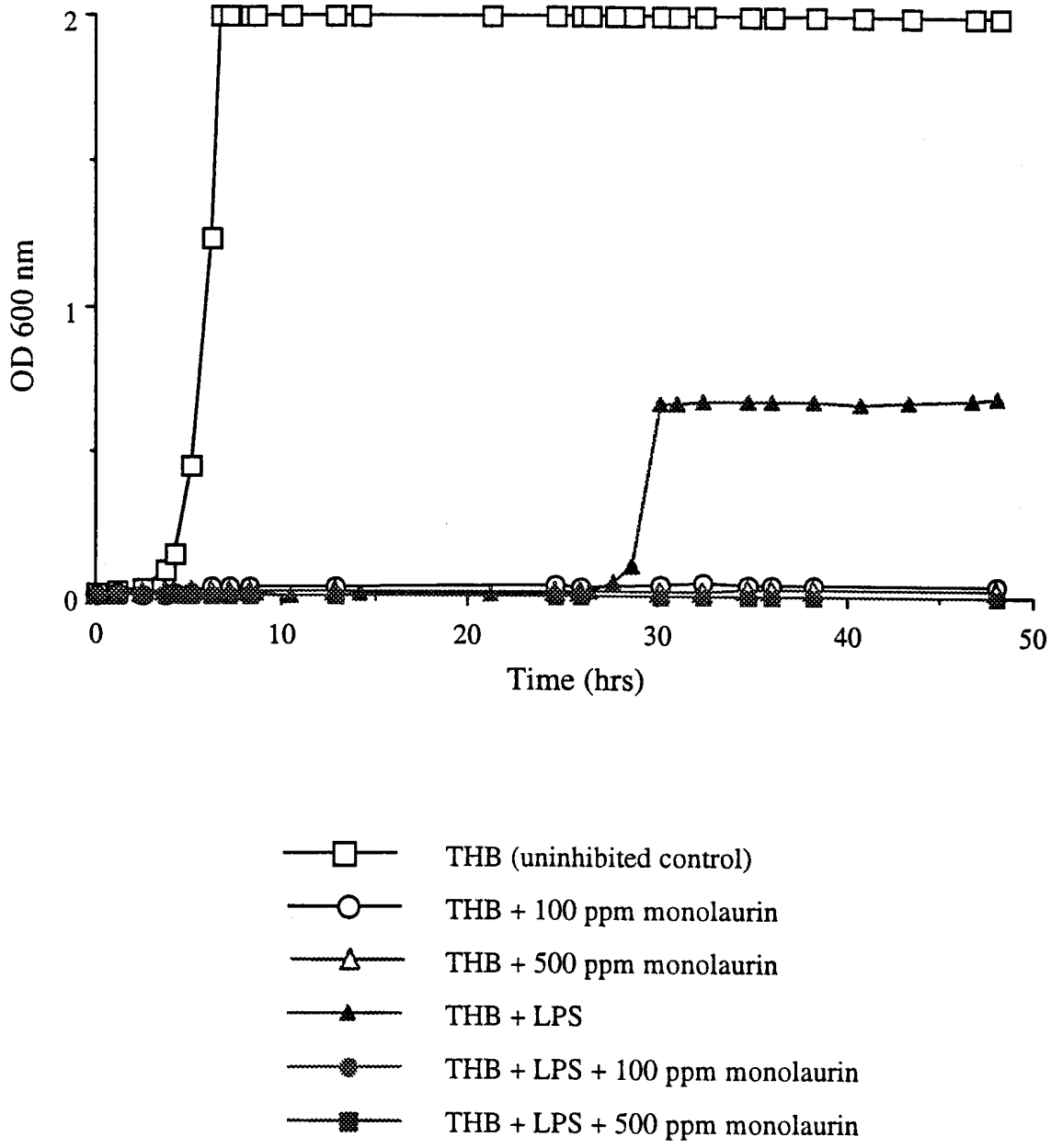
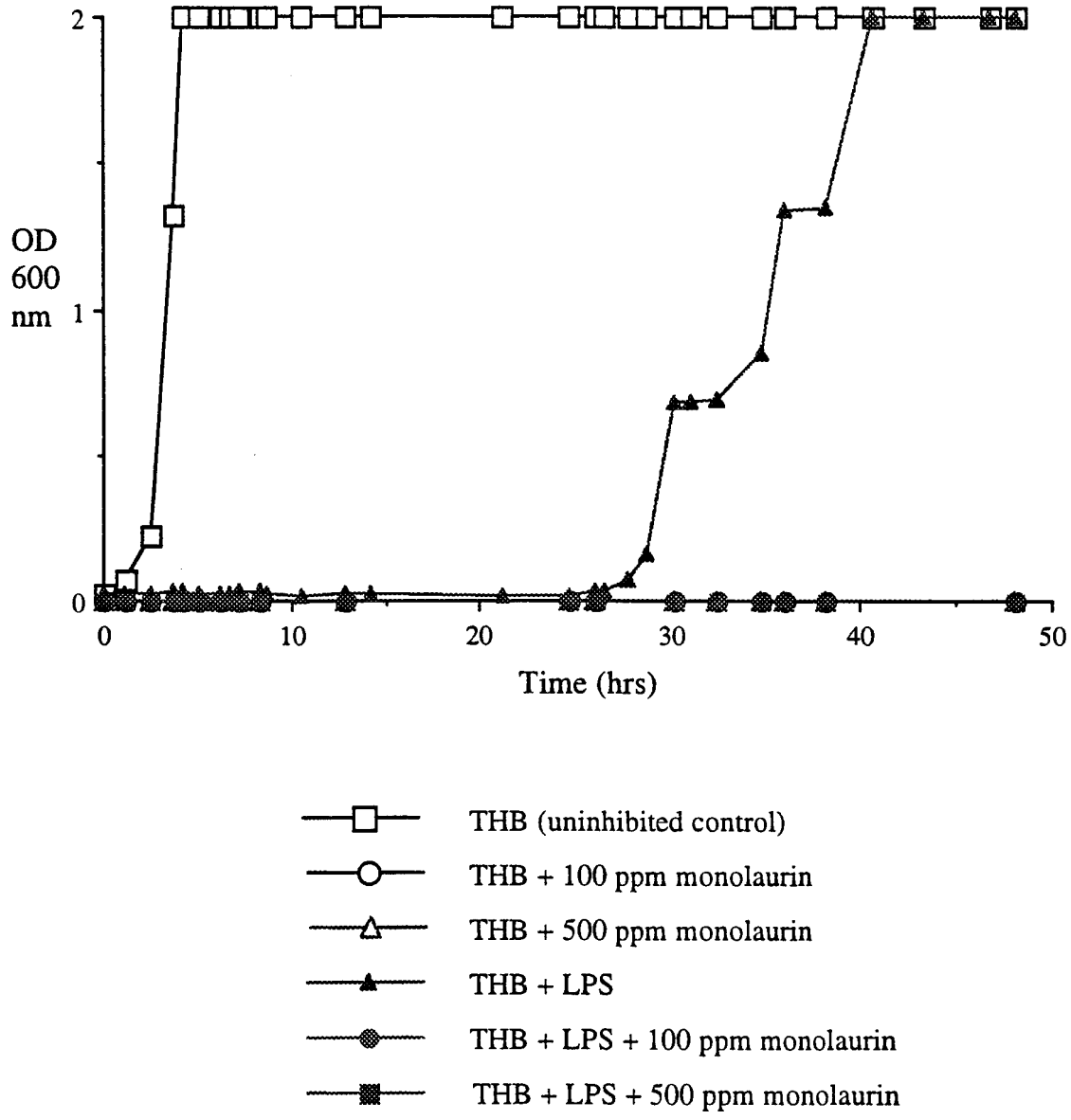

OD 600 nm
2
1
0
0
10
20
30
40
50
Time (hrs)
THB (uninhibited control)
THB + 100 ppm monolaurin
THB + 500 ppm monolaurin
THB + LPS
THB + LPS + 100 ppm monolaurin
THB + LPS + 500 ppm monolaurin

Figure 6


OD
600
nm
2
1
0
0
10
20
30
40
50
Time (hrs)
THB (uninhibited control)
THB + 100 ppm monolaurin
THB + 500 ppm monolaurin
THB + LPS
THB + LPS + 100 ppm monolaurin
THB + LPS + 500 ppm monolaurin

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00074

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A01N 63/00, 63/02, A23K 3/00, A23L 3/3571

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, JAPIO, FSTA, CA, AGRICOLA, KEYWORDS: LAURICIDIN, LAURIC, MYRISTIC, FATTY ACID, PEROXIDE, LACTOPEROXIDE, BIOCIDE, PRESERVATIVE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5227161 A (KESSLER) 13 July 1993 column 3 line 45 - column 4 line 46, column 6 lines 11-16 and 40-61, examples.	1-44
X/Y	US 5250299 A (GOOD) 5 October 1993 whole document	1-44
X/Y	US 5085873 A (DEGRE) 4 February 1992 whole document	1-44

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2000	29 AUG 2000

Name and mailing address of the ISA/AU	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00074

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	GIESE, J, "Antimicrobials: Assuring Food safety" FOOD TECHNOLOGY Vol 48 (6) (1994) pp 102-110.	
Y	pp 102-105	1-44
	US 4557935 A (EKENSTAM) 10 December 1985	
X	column 3 lines 19-27	18-19
Y		1-44
	US 5607681 A (GALLEY) 4 March 1997	
X	column 6, examples, claims.	18-23, 25-30 42, 44
	US 5206156 A (SAMAIN) 27 April 1993	
X	whole document	18, 19
A	US 4372982 A (HAASL) 8 February 1983	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ00/00074

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5227161 A	US	5370815 A	EP	404198 A		
US	5250299 A						
US	5085873 A						
US	4557935 A	CA	1182043 A	EP	73709 A	WO	3173/82 A
US	5607681 A	AU	72101/91 A	BG	96716 A	BR	9105930 A
		EP	514417 A	FI	923252 A	IL	97112 A
		NO	923033 A	NZ	236990 A	WO	91/11105 A
		ZA	9100763 A	CA	2073768 A		
US	4372982						
US	5206156 A	CA	2014847 A	EP	397227 A	FR	2646777 A
		JP	3193708 A				

END OF ANNEX