

Sodium Metabisulphite Alternatives

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Seafish Technology Implementation

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Sodium Metabisulphite Alternatives

Summary:

Sodium metabisulphite has been used in the UK fishing industry for many years to control the effects of melanotic blackening in prawns (*Nephrops norvegicus*). Recent requirements for allergen labelling and court cases resulting from high levels of sulphite residue have prompted interest to secure alternative treatments to control melanosis. Further motivation has come from the highly corrosive nature of sodium metabisulphite. This causes extensive damage to vessels and processing equipment and has serious operational health and safety issues.

A number of treatments were trialled in a commercial environment to determine a viable alternative to sodium metabisulphite. Prawns were treated at sea and then assessed for shelf life and sulphite residue by processors in NE Scotland.

5 treatments were competitive with sodium metabisulphite, these include sulphite based and non sulphite based treatments. As such, vessels and factories are provided with a variety of treatment options depending on their own product requirements. Alternative sulphite based treatments exhibit reduced operational hazards and lower sulphite residues in the tail meat while selected non sulphite based treatments are classified as 'processing aids' and are exempt from any form of food additive labelling.

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1. Introduction

Black spots (melanosis) form on prawns and other shellfish within a few hours after harvest. This was once thought to be microbial but is now considered to be an enzymatic process (Lerner, 1953). The reaction involves the oxidation of phenols to quinines by the enzyme polyphenol oxidase (PPO). Following this, a non-enzymatic polymerisation of quinines occurs. This leads to the formation of dark, high molecular weight melanins. In shellfish, PPO is involved in shellfish cuticle hardening and wound repair and indirectly in the immune system (Bartolo, 1995). After capture and death, these enzymes remain active and promote the development of black pigments on the shell. The same series of reactions leads to the characteristic browning of cut fruit and vegetables (Labuza & Schmidl, 1986). Refrigeration alone does not prevent but only slows this process. Blackspot formation begins on the head of the animal and proceeds to the tail and legs with time. The pigments themselves do not pose a risk for human consumption, but affected crustaceans are rejected by consumers due to an unsightly appearance. The current practice adopted aboard fishing vessels and by shellfish processors to avoid or at least delay melanosis, is to treat prawns with sulphite based chemicals, most commonly with a dipping solution of sodium metabisulphite. Relatively high concentrations of metabisulphite are frequently used and it is common for treatment to take place at sea and subsequently onshore. As a result there is currently very little control over application practices, dosage or total residual content in edible tail meat.

1.1. Sodium metabisulphite as a food additive

Sodium metabisulphite is currently listed in Annex III Part B of Directive 95/2/EC as an authorised food additive. As such food labelling is required where sulphite residues exceed 10 mg/kg (ppm). This is labelled E223 with maximum permitted residues in crustacean products set at 150 mg/kg. However it is now accepted that sulphites, when present in foodstuffs, can cause allergic reactions in vulnerable persons and can constitute a danger to health (Collins-Williams, 1983). Metabisulphite is regarded as one causative agent of asthma attacks (Gunnison & Jacobsen, 1987). In accordance with this, EU Directive 2003/89/EC will make allergen labelling a requirement for all food stuffs produced in the European Community, which have been treated with sodium metabisulphite. This directive came into force on 25th November 2005 and will be implemented by The Food Labelling (Amendment) (No.2) (England) Regulations 2004, with parallel legislation in Scotland, Wales and Northern Ireland. This will be enforced using powers and penalties given in The Food Safety Act 1990. For the purpose of allergen labelling the additive class and 'E' number, as used for general food labelling, will not be considered sufficient detail. The presence of the allergen, when not clear from the name of the food product, should be identified clearly using a name which is easily understood by the consumer. Prawns treated with sodium metabisulphite should therefore be labelled as such or by using the term 'sulphur dioxide' or 'sulphite'. The effect of additional allergen labelling will firstly promote buyers (most prominently supermarket chains wishing to push an additive free product) to source non sulphite treated prawns and secondly will prompt heightened consumer awareness of prawn additives.

Operational health and safety is an issue of serious concern surrounding the use of sodium metabisulphite within industry. Metabisulphite, when used to treat prawns, is dissolved in water to form a dipping bath. Prawns are dipped in solution before either storage aboard a vessel or packaging ashore. On contact with acids, metabisulphite liberates gaseous sulphur dioxide (SO₂). This can cause severe irritation and respiratory disorders. Sodium metabisulphite is a severe irritant through topical and ocular contact, through ingestion and depletes the oxygen content of water courses. At the time of writing a legal case is being pursued by a fisherman who is claiming respiratory trouble and asthma like symptoms through exposure to SO₂ while working with metabisulphite. This is undoubtedly not the only case within the seafood industry where operators have suffered the detrimental effects of this treatment. Occupational exposure standards for sulphur dioxide, prior to being withdrawn in April 2003, were 2ppm (8hr ave.) and 5ppm (15 mins. exposure) (www.hse.gov.uk). These standards were withdrawn due to the concern they may not be adequate to protect the health of workers. At present an employer's legal obligation under COSHH regulations remains to achieve adequate control and limit exposure as far as is reasonably practicable, if possible to below 1ppm for both short and long term exposure. To give some sort of idea of the ambient SO₂ levels which may be reached through using metabisulphite, the Dept. of Environmental and Occupational Medicine at Aberdeen University re-created the exposure levels in a test chamber. 35ppm SO₂ was deemed to be a fair representation of the concentrations experienced by the fisherman onboard. Despite this the legal case is ongoing and the results from the exposure trials are, as yet unpublished.

There is a requirement within the fishing and seafood processing industry for an alternative treatment to be found which is not subject to allergen labelling, is not handled with the same level of health risk, and yet provides comparable benefits in terms of maintaining prawn appearance and extending shelf life.

1.2. Alternative treatments

The simplest mechanism for control of melanosis is through application of good handling practices to minimise mechanical damage to the prawns and rapid sorting, washing and packing to ensure chilling as soon as possible after capture. It is possible that novel packaging techniques may be of benefit in slowing melanotic blackening such as vacuum or modified atmosphere packaging (Greve, 1995). The oxidation reaction catalysed by PPO would potentially be inhibited with the removal of oxygen. However, this is beyond the scope of this project although would certainly be of potential interest for future work. Only alternative chemical treatments are covered in this report.

Many alternative treatments are available, sold to prevent melanotic blackening in prawns. These fall into two categories, firstly sulphite based treatments which contain sodium metabisulphite (E223) or sodium hydrogen sulphite (E222). These are subject to allergen labelling although are far less hazardous than the raw chemical in operation. Typically these treatments constitute ~10% sulphite which is combined with organic acids and stabilisers. The technological function of the

organic acids is to act as colour enhancers. These counter the natural bleaching effect of the sulphite. Secondly, non sulphite based treatments are not subject to allergen labelling although are still classed as additives and will require inclusion on the packaging ingredients list. Selected non sulphite treatments however, hold special derogation from all forms of food labelling. These are categorised as 'processing aids' under Directive 89/107/EEC. Processing aids are classified as treatments which do not perform a function in the final product and leave no residues that present a health risk.

9 treatments were obtained for trial and were tested against a control.

Control

Untreated prawns were used to provide a benchmark from which to compare the effectiveness of each chemical treatment. Untreated prawns can be kept on ice for 8-11 days after which they are considered inedible (Stroud *et al.* 1982). Melanosis develops within 6-8 days (Greve, 1995). The mechanism of melanosis forms part of the animals immune and injury repair system. The PPO enzyme is activated by the onset of trauma and mechanical damage, which is an inevitable consequence of capture and handling. It is widely regarded that prawns caught in trawls develop melanosis earlier during storage than those caught by creel. This is a direct result of differing stress levels experienced through different capture methods. It is therefore beneficial for fishermen, whatever the fishing method, to follow guidelines for good shellfish handling and storage procedures (Seafood Scotland report, July 2004). Significant quality can be retained through correct handling.

Sodium metabisulphite

Beyond careful handling and efficient icing procedures, melanosis inhibition and shelf life extension is commonly pursued with the use of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$). Sulphites are strong reducing agents which absorb oxygen in competition with PPO and irreversibly inhibit the formation of coloured melanins (Ferrer *et al.*, 1986). Sulphite in this form is widely attainable for use by vessels and processors. Application is by dissolving in seawater to the desired concentration. Baskets of prawns are then dipped in the metabisulphite solution prior to icing and storage. Guidelines for correct dipping procedure include a 2 minute dip at 1.25% by the Danish Ministry of Fisheries (Bartolo, 1995) and a 3 minute dip at 2.5% (BIM and Seafood Scotland). Despite this, many vessels routinely dip for 10 minutes at 5% highlighting the lack of continuity throughout industry. Dipping in an aqueous bath is the most common method for treating large numbers of prawns at a given concentration. It has been suggested that some onboard practices involve the application of metabisulphite 'dry' to the top layer of a full, iced box of prawns. This leads to inconsistent treatment of prawns throughout the box and is likely to result in erratic sulphite residues on the product.

Sodium metabisulphite is an off white crystalline powder which emits harmful sulphurous odour. In solid form metabisulphite is harmful to the skin, digestive system and in particular the lungs. All exposure should be limited or preferably removed through correct handling procedures. Protective equipment for handling and operation should comprise oilskins, boots, rubber gloves and respirator. All handling should be carried out in a well ventilated area. Exposure may cause an allergic reaction and it is recommended that workers with respiratory disorders

should not be employed to work with this chemical. Of particular relevance on fishing vessels is the potential for a build up of sulphur dioxide (SO₂) in the fish hold from metabisulphite reacting with water and ice. Metabisulphite is highly corrosive and should not be allowed to accumulate in the bilges or discharged via seacocks.

Pluscolour

Pluscolour is an Argentinean treatment specifically formulated to maintain the freshness characteristics of shrimp and prawns. The main selling point is the claim to inhibit melanosis whilst also maintaining a good, bright colour in the product. Pluscolour comprises a suite of ingredients, sodium metabisulphite, citric acid, sodium ascorbate and saccharose stabiliser. As a sulphite based product its usage falls under the same legislative constraints as sodium metabisulphite. Application and handling are similar to metabisulphite with a recommended concentration of 1.3% and a 1 minute dip time.

Hasenosa

Hasenosa is a similar product to Pluscolour. It is intended for external use on either fresh or frozen crustaceans to reduce the enzymatic process of melanosis. The ingredients are sodium metabisulphite, citric acid, ascorbic acid, EDTA acid and sodium bicarbonate. PPO is dependent on copper ions for activity. EDTA inhibits enzyme activity by binding to the copper ions. Prawns treated with Hasenosa will be subject to allergen labelling. Despite sodium metabisulphite content, Hasenosa is claimed to maintain the percentage of SO₂ residue in the edible part of the crustacean to well below the maximum limits imposed by European legislation. Application is at a concentration of 2% for 5 minutes.

Melacide SC20

Melacide is also a sulphite based product although contains sodium hydrogen sulphite (E222) as the active compound. This is mixed in the product with sodium ascorbate, potassium citrate and citric acid. The use of sodium hydrogen sulphite will be subject to allergen labelling the same as sodium metabisulphite. The advantages of sulphite in the form of E222 are reduced fumes during operation and safer handling characteristics. Application is by immersion at 1-2% for 5 - 20 minutes depending on the size of prawns. At the correct concentration between 20-40ppm SO₂ residue is claimed to remain on the muscular tissue.

Xyrex - Prawnfresh

Prawnfresh is a product made from organic acids and salts, plant extracts and inorganic stabilising buffers. It is non sulphite based and is not subject to allergen labelling. Furthermore, all Xyrex products for use in fish and shellfish processing have been classified by the Food Standards Agency as processing aids. Prawns treated with Prawnfresh will require no food additive labelling of any kind. Application of the liquid treatment is dilution with sea water, ratio 1:1000 with a dipping time of 15 minutes. There are no operational hazards associated with the use of Prawnfresh, which is biodegradable and non bioaccumulatory.

Everfresh

Everfresh is a product of Sun Opta Ingredients, USA and has been formulated for use on shrimps. The active ingredient is 4-hexylresorcinol (C₁₂H₁₈O₂). This is combined with sodium chloride as a carrier agent. The mechanism of PPO inhibition

by 4-hexylresorcinol is by inactivation of the enzyme by binding or hydrolysis at specific sites necessary for activity (Taoukis *et al*, 1990). 4-hexylresorcinol is used in medicines and is classified as GRAS (generally recognised as safe) in America although at the time of writing has not been approved in the E.U. as a permitted additive for use on crustaceans. France obtained temporary derogation under Article 5 Directive 89/107/EEC which classifies the use of 4-hexylresorcinol on crustaceans as a 'processing aid'. The European parliament has recently voted in favour of selected amendments to Directive 95/2/EC. This includes an entry permitting 4-hexylresorcinol (E586) to be used in fresh, frozen and deep-frozen crustaceans at 2 mg/kg as residues in edible meat. The new amending Directive is expected to be formally adopted early 2006. Everfresh is packaged in 200g sachets ready for use by dilution in 125 litres of sea or fresh water. Dip time is recommended for 2 minutes. Acute exposure to mucous membranes may cause irritation, treatment is by flushing with water.

Aquabon 14W

Aquabon is a liquid treatment with a broad range biocidal activity and strong antioxidant properties. It is intended for use in fish storage and processing and a wide range of crustaceans. It is a mixture of bioflavonoid extracts blended with citric acid, ascorbic acid and glycerine. All ingredients are GRAS listed, the product non-toxic for humans or animals. The recommended application concentration is 2% for 5 minutes.

Soft acid 'aqua e'

Soft acid is a liquid product consisting of organic acids and modified lignosulphonic acids. These inhibit bacterial growth and delay the degradation of fish.

Citric acid and Ascorbic acid

Citric and ascorbic acid are natural antioxidants and in solution ~ pH 2-2.5. PPO activity may be reduced at low pH levels. However, natural acids become oxidised during inhibition, and therefore may have only a temporary effect. Their products of oxidation can give rise to off-flavours, odour and discolouration. Ascorbic acid forms undesirable yellow oxidation products in shellfish (Otwell & Marshall, 1986). Derivatives of ascorbic acid that are more resistant to oxidation were used in tests with fruit with promising results (Sapers *et al*, 1989). Past studies have trialled citric and ascorbic acid at 0.5-5%. For this trial ascorbic acid was trialled at 1% and citric acid at 3%, dipped for 10 minutes.

A summary table of treatment information is given in Appendix 1.

2. Method

During August / October 2005 Seafish conducted sea trials aboard MFV Virtuous and MFV Fair Morn to determine the effectiveness of different chemical treatments for inhibiting melanosis in prawns (*Nephrops norvegicus*). Commercial assessment was carried out in conjunction with processors in NE Scotland and was designed to look at the practicalities of using each treatment as well as shelf life benefits. The partner processors were Macrae Foods and Iceberg of Fraserburgh and Laeso Fish of Peterhead.

Onboard, all treatments were diluted to the manufacturers specifications. A 20 litre drum was used for all tests and only good quality prawns were selected for trial. After dipping each sample was placed into a prelabelled bag stating treatment, date and analysis details. This was either sensory, sulphite residue or microbial. Samples were stored on polystyrene boxes and kept on ice for the duration of the sea trial. All samples were delivered to the processor as soon as possible after landing.

Dipping at sea was typically carried out on day 2,4,6,8 over a 10 day period. Two trips were carried out in total and 9 treatments selected for trial. Metabisulphite was trialled at 2.5%, dipping for 3 minutes and 5% for 10 minutes. Untreated prawns were used for reference and labelled 'control'. The more effective treatments from the first trial were selected for repeat testing during the second trial.

Onshore, sensory analysis was conducted over a one week period with sampling days structured to observe prawns over the full period of shelf life. A numerical scoring system was developed for the purpose of this trial to allow a quantitative measure of quality. Results were collected describing appearance, odour, texture and taste, as well as for microbial and sulphur dioxide residues. Visual scale was allocated 5 levels of quality because this spoilage characteristic was considered most directly attributable to melanosis. Odour, Texture and Taste were allocated a 3 or 4 point scale in line with the sensory descriptions received from the processors. Table 2.1 shows the full scoring system used.

		Score
Visual	Good / Orange	1
	Occasional black / grey	2
	Med / some blackening	3
	Mostly blackened	4
	All / very black	5
Odour	Good / Fresh	1
	OK / slight ammonia	2
	Med ammonia	3
	Strong / pungent	4
Texture	Firm	1
	Med / OK	2
	Soft / mushy / v. slimy	3
Taste	Sweet	1
	Slightly sweet	2
	Bland / neutral flavour	3
	Bad flavour / not eaten	4

Table 2.1 Sensory scoring system

3. Results

3.1. Sensory analysis

Sensory results are average scores for each day over the 14 day trial and include results from all processors.

Visual	Days on ice										
	4	5	6	7	8	9	10	11	12	13	14
Control	3	2.7	3.3	3.8	3.5	2.9	3.1	4	4	4	4.5
Meta 2.5%		2	1.7	2.3	3	2.7	4	4	4	3	
Meta 5%		1	2.5	2	1.8	1.7	2.5	2.5	2	3	2
Pluscolour	1	1.7	1.8	1.6	2.1	2.3	1.9	2.6	2	3.5	3
Hasenosa		1.3	1.2	1.8	2	1.2	2.2	3	1.5	2.5	2.5
Melacide SC20		1	1.3	2	1	1.5	1.8	2.5	1.5	2.5	2
Xyrex Prawnfresh		1.5	1	1.5	1.6	2	1.8	2	2	2.5	2
Everfresh		1.5	1.5		1.5	1.7	1.8	3	1	3	2
Aquabon		2	2.5		3.5	3	2.3	3	2	3.5	4
Soft acid	3	2.8	2.6	3.6	3.6	4	4	4.7			
Citric acid				5	4	4		5			
Ascorbic acid			5		4.5	4	5	5			

Table 3.1 Average visual scores by treatment

Visual appearance in untreated prawns declined consistently with initial blackening evident after only 4 days on ice. By day 14 blackening was evident in all prawns. Metabisulphite at 5% is more effective than 2.5% for maintaining prawn appearance. There is comparable visual performance between the three sulphite based treatments; all show good melanosis prevention to day 7 / 8. Xyrex was the most effective non sulphite based treatment for maintaining appearance. Only limited blackening was observed and these samples were frequently the best looking of all treatments throughout the trial. Everfresh treated samples showed good appearance to day 10, with overall melanosis prevention comparable to sulphite based treatments. Some melanosis prevention was evident in samples treated with Aquabon. Prawns showed better appearance than untreated samples although had significantly reduced shelf life to preceding treatments. Soft acid, citric acid and ascorbic acid did not show any control of melanosis. Despite the limited sample size it is apparent that citric and ascorbic acid had a negative effect on freshness. Total blackening was evident after 10 days with both treatments.

Odour	Days on ice										
	4	5	6	7	8	9	10	11	12	13	14
Control	3	2.8	3.3	3.5	3.4	2.4	2.6	3.3	2.5	2	3
Meta 2.5%		3	2.7	2.6	2.8	2.7	3	3.7	3	3	
Meta 5%		3	2.5	3	2.7	2.3	2.8	3.5	3	2	2
Pluscolour	1	2	2.1	2.5	2.5	2.1	2.1	2.2	2	3	2
Hasenosa		2.3	2.2	2.8	2.4	1.8	2.2	2.6	2.5	2.5	2.5
Melacide SC20		1.3	1.7	2.5	2	1.3	1.6	2.5	2	2	1.5
Xyrex Prawnfresh		2.5	2.2	2.8	2.1	1.6	1.6	2	2.5	2	1.5
Everfresh		1.5	1		1.5	1	1.5	2	1	2.5	2
Aquabon		1.5	1.5		1.5	1.3	1.8	2	1	2.5	2.5
Soft acid	3	3.3	3.4	3.6	3.7	3.8	3.7	4			
Citric acid				4	3	4		4			
Ascorbic acid			4		4	4	4	4			

Table 3.2 Average odour scores by treatment

Overall, odour showed least variation over the 10 day trial period. This was consistent for all treatments with ammonia generally evident in all samples from day 5. Melacide, Everfresh and Aquabon performed most favourably while citric and ascorbic acid treated samples were consistently poor. Metabisulphite has little benefit on odour development over untreated samples.

Texture	Days on ice										
	4	5	6	7	8	9	10	11	12	13	14
Control		3	2.5	2.3	1.7	1.8	2	2	2	2.5	2
Meta 2.5%		3	2.7	1.8	3	1.7	2	3	3	2	
Meta 5%		2.7	3	1.8	2.2	2.3	3.3	3	2	3	3
Pluscolour		2.3	2.2	1.3	1.9	2.2	1.8	1.8	1.5	3	2
Hasenosa		2	2.2	2	2.4	1.8	2.5	2.6	2	2.5	2
Melacide SC20		3	2.3	2.5	1.8	1	2.8	3	1.5	2	3
Xyrex Prawnfresh		2.8	2.2	1.8	2.1	2.6	2.2	2.4	2	2	2.5
Everfresh		2.5	2		2	3	1.8	3	1	2	3
Aquabon		2.5	3		3	2	2.5	2.5	1	2.5	2.5
Soft acid		3	2.5	3	2	3	3	3			
Citric acid				3	3	2		3			
Ascorbic acid			3		3	3	3	3			

Table 3.3 Average texture scores by treatment

Taste	Days on ice										
	4	5	6	7	8	9	10	11	12	13	14
Control		2.5	2.8	2.8	2.6	2.2	2.7	3.3	2.5	3	3
Meta 2.5%		3.5	3.3	2.5	3.3	3	4	4	4	3	
Meta 5%		2	3	2.5	2.2	2.3	3.3	3	2	3	3
Pluscolour		2	1.8	2.3	2.4	2.6	2.7	2.7	2.5	2.5	3
Hasenosa		1.8	2.2	2.8	2.3	2.2	3	3	3	3	3
Melacide SC20		2	1.7	1.5	2	1.3	2.4	2.8	2	3.5	2.5
Xyrex Prawnfresh		2	1.6	2.3	2.1	2.6	2.2	2.8	2	3	3
Everfresh		2.5	2		3.5	1.7	2.5	2.5	1	3	2
Aquabon		2.5	1.5		3	2	2.8	3	2	3	3.5
Soft acid		3	3	3.5	3.3	3	3.5	3.7			
Citric acid				4	3	3		3			
Ascorbic acid			4		3.5	3	4	3			

Table 3.4 Average taste scores by treatment

Texture and taste showed little change from day 4 to 14. Initial sweetness appears to be lost within the first 4 days storage with treated samples showing little difference to the control. Of all sulphite based treatments, Hasenosa treated samples were of consistently poor flavour compared to competitors.

Treatment	Days on ice									
	5	6	7	8	9	10	11	12	13	14
Ascorbic acid	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
Citric acid	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
Soft acid	Yellow	Red								
Control	Yellow	Yellow	Red							
Meta 2.5%	Green	Yellow	Yellow	Yellow	Red	Red	Red	Red	Red	Red
Aquabon	Green	Yellow	Yellow	Yellow	Yellow	Red	Red	Red	Red	Red
Meta 5%	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Red
Melacide	Green	Green	Green	Yellow						
Everfresh	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Red
Hasenosa	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Pluscolour	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Xyrex Prawnfresh	Green	Green	Green	Green	Green	Yellow	Yellow	Yellow	Yellow	Yellow

Red	Blackening on all prawns. Unacceptable quality
Yellow	Occasional / slight darkening of head (<3.5)
Green	Good, bright appearance (<2)

Table 3.5 Table showing the performance of all products on shelf life

Table 3.5 summarises all sensory scores and indicates overall freshness. Untreated prawns start to develop melanotic blackening within 5 days of capture and reach an unacceptable quality after 6 days. Treatment with ascorbic, citric or soft acid appeared to increase overall spoilage and prawns reached an unacceptable condition in 5-6 days. Metabisulphite 5% was found to extend prawn shelf life and prevent blackspot formation for up to 7 days. This was found to decrease when used at lower concentrations. Other sulphite based treatments showed similar performance to 5% metabisulphite. The two most effective non-sulphite treatments were Everfresh and Xyrex Prawnfresh. The latter was found to have the greatest effect on maintaining quality and extended blackspot prevention to 10 days.

3.2. Sulphite residues

Treatment	Sulphur dioxide residue (ppm)										
	Iceberg		Laeso				Macrae				
	Trial 1	Trial 2	Trial 1		Trial 2		Trial 1		Trial 2		
Control	21	20	<10	23	<10	10	<10	<10	<10	<10	<10
Metabisulphite 2.5%			46	41			61	37			
Metabisulphite 5%			144	171	557		36	43	49	755	
Pluscolour	25	<10	<10	60	<10		<10	54	<10	<10	
Hasenosa			58	59	10	153	48	96	<10	10	
Melacide SC20					<10	10	34	48	<10	26	
Xyrex Prawnfresh		24	<10	<10	<10	<10	<10	19	<10	61	
Everfresh		0			<10				<10	<10	
Aquabon		0			<10				<10	27	
Soft acid	28		<10	73			<10	28			
Citric acid			<10				20	31			
Ascorbic acid			<10				<10	<10			

Table 3.6 Sulphur dioxide residues by treatment

Metabisulphite 2.5% gave SO₂ residues of between 37 to 61 ppm, however at 5% these increased to a maximum of 171 ppm (it is likely that the values of 557 and 755 have been erroneously recorded or were the result of sample contamination). Most sulphite based treatments yielded residues within the legal maximum with Pluscolour reaching a maximum of 60 ppm and Melacide, 48 ppm. Hasenosa was the only formulated treatment to record sulphite residues above 150 ppm. All non sulphite based treatments returned low sulphite residues of <10 ppm. Any sulphite recorded was likely due to contamination between treatment sample batches.

3.3. Micro analysis

Microbial analysis was carried out on all samples. Full results are given in Appendix 3. Results for TVC varied greatly within each treatment and did not appear to change with storage time. No meaningful results were observed and no variation evident between treatments.

4. Discussion

The sensory results of untreated prawns confirm that blackspot appears in *Nephrops* within the first four days after capture and increases gradually throughout storage. Changes in odour, texture and flavour however did not decline alongside visual appearance. Initial sweetness was lost and ammonia smell present within the first four days of capture. Little change was detected from day 4-14. Treated prawns which showed little blackspot development retained better flavour and texture than untreated or ineffectively treated prawns. Melanosis appears to occur independently of and prior to spoilage of the tail meat and as such is not a direct indicator of poor quality prawns. No prawns were found to be of poor flavour despite retaining a favourable appearance.

There is no doubt that metabisulphite, applied in solution, delays blackspot formation. This has greater effect at high concentrations (trialled to 5%) although natural shell pigmentation is reduced through bleaching. Bleaching did not take place with formulated treatments such as Hasenosa, Pluscolour and Melacide because of reduced sulphite concentration. Furthermore, it is likely that sulphite bleaching is countered in these treatments through incorporation of ingredients such as citric and ascorbic acid, which act as colour enhancers. When these acids were trialled independently, colour enhancement was masked by rapid blackspot development. Citric and ascorbic acid do not have any effect in preventing melanosis. From Fig 3.5 it appears that citric and ascorbic acids have a negative effect on prawn appearance and promote melanosis. The reason for this is unclear as both are natural chemical antioxidants and acidulents, which would be expected to have an inhibitory effect on enzyme activity. Sensory tests for odour and taste show that all samples were in poor condition by day 4. Due to limited sample quantities each acid was only trialled at one concentration. It is possible that citric and ascorbic acid would have had more effect at higher concentrations. Further trials would be of value given the widespread use of these acids throughout the food industry and effective treatment of oxidative browning in cut fruit and vegetables.

Laboratory results indicate that prawns treated with 5% metabisulphite contained the highest sulphite residues, often above the maximum permitted level of 150 ppm. Prawns with high sulphite residue were found to have the least favourable flavour. This was evident with metabisulphite 5% and Hasenosa treated prawns.

Sensory analysis indicates that non sulphite based treatments can be effective at preventing melanosis and are a viable alternative to the use of sulphites on prawns. While blackspot inhibition is similar to that of effective sulphite based treatments, Xyrex treated prawns often showed the best colouration, both before and after cooking. No poor taste qualities were experienced as was often the case with sulphite based treatments.

Everfresh was found to be an effective treatment of melanosis although sensory scores show far greater variation to other treatments. This treatment was only tested in one set of trials due to availability and only limited results were obtained. Everfresh is manufactured in America for use on shrimps at a recommended concentration of 0.2% and a 2 minute dip time. This may be sufficient when treating

shrimps which have a comparatively soft shell but it is likely that effective treatment through toughened *Nephrops* cuticles would require higher concentrations or increased dipping time. Neither of these possibilities were pursued during the scope of this trial although would be of value for further investigation with legislation to permit the use of 4-hexylresorcinol on crustaceans in the UK due to be passed in the near future.

Throughout the trials all samples were stored in plastic food bags which may have contributed to a build up of ammonia during day 1-3. This effectively masked any further increase in odour through day 4-14. Trials were carried out during late summer when sea temperatures were at their highest (11.5°C was measured during fishing operations for the second trial) which may have accounted for the rapid development of ammonia. Despite this, all sensory assessment is highly subjective and although shore based analysis was carried out by experienced factory staff, only crude changes in quality were reported.

5. Conclusions

No chemical treatment can prevent melanotic blackening. This is an inevitable part of the spoiling process with blackspots appearing in time with all treatments. At best, melanotic blackening can be delayed.

Due to the scale of the trial it was not possible to replicate within sample batches leaving results of limited statistical robustness. However this trial is of use as an initial appraisal of treatments and indicates a number of viable competitors to sodium metabisulphite for extending effective shelf life of prawns.

Treatments which contain a sulphite ingredient will be subject to allergen labelling although alternatives exhibited reduced operational hazards in comparison to sodium metabisulphite in chemical form. Pluscolour, Hasenosa and Melacide all gave comparable shelf life qualities. Of these Melacide and Pluscolour returned more favourable sulphite residues.

Selected non sulphite based treatments are an effective option for suppliers wishing to extend shelf life and market an allergen free product. Xyrex Prawnfresh was the most effective non sulphite based treatment.

It is intended that fishermen and processors use this information to decide the best direction for their particular business but it is stressed that chemical dipping does not substitute GMP. High standards of hygiene and handling throughout the chill chain are fundamental for operations looking to improve product shelf life.

6. References

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Appendix 1 - Product specification sheet

Product	Constituents	Legislation	Subject to allergen labelling ?	Price	Cost to treat 100kg prawns	Dilution	Dip time (mins)	Product treated with 50L dip tank	Application	Performance
Sodium metabisulphite	E223	Permitted food additive in UK. Annex III Part B Directive 95/2/EC.	Yes	£12.93 for 25kg (52p per kg)	52p	5%	10	200kg	Offwhite powder	Prevention of blackspot to 7 days. Visually some bleaching effect. Taste often tainted.
Hasenosa HA-5502 Freskor (Spanish)	Sodium metabisulphite (E223) Citric acid (E330) Ascorbic acid (E300) EDTA acid (E385) Sodium bicarbonate (E500ii)	Permitted in UK Directive 95/2/EC	Yes	£29 for 25kg bag (£1.16 per kg)	£2.32	2%	5	50kg	Offwhite powder	Good visual qualities to 8 days. Effective melanosis control.
Pluscolor Fish 3425	Sodium metabisulphite (E223) Citric acid (E330) Sacarose (E451(i)) Sodium ascorbate (E301)	Permitted in UK Directive 95/2/EC	Yes	£1.20 per kg	£1.60	1.3%	1	50kg	Offwhite powder	Prawns good bright appearance, blackspot prevention to 8 days. Effective treatment.
Melacide SC-20	Sodium hydrogen sulphite (E222) Sodium ascorbate (E301) Potassium citrate (E332) Citric acid (E330)	Permitted in UK Directive 95/2/EC	Yes	£1.75 per kg	73p	3%	10	250kg	Offwhite powder	Good visual qualities to 8 days. Effective melanosis control.
Xyrex Prawnfresh	Inorganic stabilising buffers, organic acids.	Processing aid as defined by Directive 89/107/EEC	No	0.5L @ £30. 5L @ £250	£2.50-£3	1:1000	15	100kg	Liquid	Good blackspot prevention and best eating qualities. Max. blackspot prevention to 9 days.
Everfresh	4-Hexylresorcinol (E586) Sodium chloride	Included in amendments package for Directive 95/2/EC. Expected formal adoption early 2006	No	\$6 USD (£3.45) per 200g pouch.	£1.38	200g : 125L	2	100 kg	Powder contained in sachets	Overall performance good - comparable to sulphite based treatments.
Aquabon SD / 14W	Citrus bioflavonoids Citric acid (E330) Ascorbic acid (E300) Glycerine (USP)	GRAS additives listed in Annex II of Directive 95/2/EC	No			2%	5		Liquid	Some blackspot prevention to 5 days. Good flavour.
Soft acid Aqua E	Organic + lignosulphonic acids		Yes	1/3L : 100L water (0.3% trialed)			10	Liquid antibacterial treatment for fish		No control of blackspot
Citric acid	E330	Permitted in UK Directive 95/2/EC	No	£163 for 25kg		3%			Powder	Poor prevention of blackspot, performance worse than no treatment
Ascorbic acid	E300	Permitted in UK Directive 95/2/EC	No	£273 for 25kg		1%			Powder	

Appendix 2 - Sensory results

Appendix 2 (Sensory results)

Iceberg (trial 1)

Treatment	Catch date	Sensory test		
		Date (days on ice)	Visual	Smell
Control	22-Aug	29-Aug (7)	3	3
		30-Aug (8)	4	4
		31-Aug (9)	4	4
		01-Sept (10)	5	4
	23-Aug	29-Aug (6)	3	3
		30-Aug (7)	4	4
		31-Aug (8)	4	4
		01-Sept (9)	5	4
	24-Aug	29-Aug (5)	3	3
		30-Aug (6)	4	4
		31-Aug (7)	4	4
		01-Sept (8)	5	4
25-Aug	29-Aug (4)	3	3	
	30-Aug (5)	4	4	
	31-Aug (6)	4	4	
	01-Sept (7)	5	4	
Soft acid	22-Aug	29-Aug (7)	4	4
		30-Aug (8)	4	4
		31-Aug (9)	4	4
		01-Sept (10)	4	4
	23-Aug	29-Aug (6)	3	3
		30-Aug (7)	3	3
		31-Aug (8)	4	4
		01-Sept (9)	4	4
	24-Aug	29-Aug (5)	3	3
		30-Aug (6)	3	3
		31-Aug (7)	4	4
		01-Sept (8)	4	4
25-Aug	29-Aug (4)	3	3	
	30-Aug (5)	3	3	
	31-Aug (6)	4	4	
	01-Sept (7)	4	4	
Pluscolour	22-Aug	29-Aug (7)	1	1
		30-Aug (8)	2	2
		31-Aug (9)	2	3
		01-Sept (10)	2	4
	23-Aug	29-Aug (6)	1	1
		30-Aug (7)	2	2
		31-Aug (8)	2	3
		01-Sept (9)	2	4
	24-Aug	29-Aug (5)	1	1
		30-Aug (6)	2	2
		31-Aug (7)	2	3
		01-Sept (8)	2	4

	25-Aug	29-Aug (4)	1	1
		30-Aug (5)	2	2
		31-Aug (6)	2	3
		01-Sept (7)	2	4
Frescan	22-Aug	29-Aug (7)	3	2
		30-Aug (8)	3	3
		31-Aug (9)	4	4
		01-Sept (10)	4	4
	23-Aug	29-Aug (6)	3	2
		30-Aug (7)	3	3
		31-Aug (8)	4	4
		01-Sept (9)	4	4
	24-Aug	29-Aug (5)	3	2
		30-Aug (6)	3	3
		31-Aug (7)	4	4
		01-Sept (8)	4	4
	25-Aug	29-Aug (4)	3	2
		30-Aug (5)	3	3
		31-Aug (6)	4	4
		01-Sept (7)	4	4

Iceberg (trial 2)

Treatment	Catch date	Sensory test		
		Visual	Odour	Texture
Cidox	09/10/2005	3	2	3
	11/10/2005	4	3	2
	13/10/2005	4	3	3
	15/10/2005	4	4	3
Everfresh	09/10/2005	1	1	1
	11/10/2005	1	1	1
	13/10/2005	1	2	2
	15/10/2005	2	3	3
Pluscolour	09/10/2005	1	1	1
	11/10/2005	2	1	1
	13/10/2005	3	2	2
	15/10/2005	3	3	3
Xyrex	09/10/2005	1	1	1
	11/10/2005	1	1	1
	13/10/2005	1	2	2
	15/10/2005	1	2	3
Aquabon	09/10/2005	3	2	2
	11/10/2005	4	2	2
	13/10/2005	4	2	3
	15/10/2005	4	2	3

Control	09/10/2005	3	2	2
	11/10/2005	4	3	3
	13/10/2005	4	3	3
	15/10/2005	4	4	3

Laeso (trial 1)

Treatment	Catch date	Test date (days old)	Sensory test			
			Visual	Smell	Texture	Taste
Control	22/8/05	29-Aug (7)	3	Med	Med	Med
		31-Aug (9)	3	Slight ammonia	Med	Bland
		02-Sept (11)	5	Strong rotten	Bad	Bad
	23/8/05	29-Aug (6)	2	Med	Soft / Med	Med
		31-Aug (8)	2	Med	Firm	Sweet
	24/8/05	02-Sept (10)	5	Strong ammonia	Very soft	Just edible
		30-Aug (6)	3	Strong ammonia	Very soft, easily broken	Med
		01-Sept (8)	5	Strong ammonia	Disintegrated	Very bland
	25/8/05	30-Aug (5)	3	Strong ammonia	Soft	Med
		01-Sept (7)	5	Ammonia	Disintegrated	Very bland
	Meta 2.5%	22/8/05	29-Aug (7)	2	Med	Med
31-Aug (9)			2	Med	Watery	Bland
02-Sept (11)			5	Ammonia	Very soft	Bitter
23/8/05		29-Aug (6)	1	Med	Med	Med
		31-Aug (8)	2	Med	Watery	Meta flavour evident
24/8/05		02-Sept (10)	4	Ammonia	Med	Meta flavour
		30-Aug (6)	1	Med	Very soft	Very bland
		01-Sept (8)	3	Slight ammonia	Gluey texture	Strong acid taste
25/8/05		30-Aug (5)	1	Med	Very soft	Very bland
		01-Sept (7)	2	Med	Med	Med
Meta 5%		22/8/05	29-Aug (7)	4	Med	Soft, crumbly
	31-Aug (9)		2	Med	Firm texture	Meta flavour
	02-Sept (11)		5	Strong ammonia	Soft, crumbly	Strong meta flavour
	23/8/05	29-Aug (6)	4	Med	Soft, crumbly	Strong meta flavour
		31-Aug (8)	2	Med	Firm texture	Meta flavour
	24/8/05	02-Sept (10)	4	Strong ammonia	Slightly soft	Bitter
		30-Aug (6)	1	Med	Soft	Med

		01-Sept (8)	2	Med	Crumbly	Bland
	25/8/05	30-Aug (5)	1	Med	Med	Sweet
		01-Sept (7)	2	Med	Med	Bland
Soft acid	22/8/05	29-Aug (7)	2	Med	Soft	Med
		31-Aug (9)	4	Ammonia	Watery texture	Med
		02-Sept (11)	5	Bad	Bad	Bad
	23/8/05	29-Aug (6)	1	Med	Med	Med
		31-Aug (8)	3	Ammonia	Slightly soft	Med
		02-Sept (10)	5	Ammonia	Very soft	Sour
	24/8/05	30-Aug (6)	2	Sour	Watery	Bland
		01-Sept (8)	4	Sour	Gluey texture	Bitter / Sour
	25/8/05	30-Aug (5)	2	Sour	Watery	Bland
		01-Sept (7)	4	Sour	Gluey texture	Bitter / Sour
Ascorbic acid	23/8/05	29-Aug (6)	5	Strong ammonia	Poor	Ammonia
		31-Aug (8)	5	Ammonia	Soft and watery	Revoltng
		02-Sept (10)	5	Bad	Bad	Bad
Hasenosa	22/8/05	29-Aug (7)	1	Med	Firm	Sweet, very good
		31-Aug (9)	1	Med	Soft texture	Slight sour
		02-Sept (11)	4	Slight ammonia	Gluey	Strong acid
	23/8/05	29-Aug (6)	1	Med	Firm	Sweet, very good
		31-Aug (8)	2	Med	Spongy	Sweet
		02-Sept (10)	4	Slight ammonia	Soft	Bitter
	24/8/05	30-Aug (6)	1	Med	Very soft, watery	Bland
		01-Sept (8)	3	Slight ammonia	Very soft, watery	Bitter
	25/8/05	30-Aug (5)	1	Med	Firm	Sweet
		01-Sept (7)	3	Slight ammonia	Soft	Bitter
Citric acid	22/8/05	29-Aug (7)	5	Strong ammonia	Very poor	Ammonia
Frescan	22/8/05	29-Aug (7)	2	Med	Soft	Med
		31-Aug (9)	5	Ammonia	Disintegrated	Very bland
		02-Sept (11)	5	Bad	Bad	Bad
	23/8/05	29-Aug (6)	1	Med	Firm	Med
		31-Aug (8)	4	Ammonia	Very soft	Med
		02-Sept (10)	5	Ammonia	Soft	Bitter
	25/8/05	30-Aug (5)	4	Ammonia	Very soft	Sour

		01-Sept (7)	4	Ammonia	Soft	Bland
Pluscolour	22/8/05	29-Aug (7)	1	Med	Firm	Sweet
		31-Aug (9)	3	Slight ammonia	Soft	Bitter
		02-Sept (11)	2	Slight ammonia	Firm	Med
	23/8/05	29-Aug (6)	1	Med	Firm	Sweet
		31-Aug (8)	2	Med	Firm	Good flavour
		02-Sept (10)	3	Slight ammonia	Med	Med
	24/8/05	30-Aug (6)	1	Med	Med	Sweet
		01-Sept (8)	3	Med	Mushy	Bland
	25/8/05	30-Aug (5)	1	Med	Med	Sweet
		01-Sept (7)	2	Med	Med	Med
Xyrex	22/8/05	29-Aug (7)	1	Med	Very good	Sweet
		31-Aug (9)	2	Slight ammonia	Very soft, gluey	Med
		02-Sept (11)	5	Slight ammonia	Soft	Med
	23/8/05	29-Aug (6)	1	Med	Very good	Sweet
		31-Aug (8)	2	Med	Firm texture	Sweet
		02-Sept (10)	4	Med	Soft	Med
	24/8/05	30-Aug (6)	1	Med	Soft	Sweet
		01-Sept (8)	2	Med	Med	Med
	25/8/05	30-Aug (5)	1	Med	Soft	Sweet
		01-Sept (7)	2	Med	Firm	Sweet

Laeso (trial 2)

Treatment	Catch Day	Test day	Days on ice	Visual	Odour	Texture	Taste
Control	1	19/10/05	10	2	1	1	1
	1	21/10/05	11	3	2	2	2
	1	26/10/05	16	5	3	3	4
	2	20/10/05	9	1	2	2	2
	2	25/10/05	14	5	4	2	4
	3	19/10/05	6	2	1	1	1
	3	21/10/05	8	3	2	2	2
	3	26/10/05	13	5	3	3	4
	4	20/10/05	5	2	2	3	3
4	25/10/05	10	3	3	3	4	
Meta 5%	1	19/10/05	10	1	1	3	2
	1	21/10/05	11	1	2	3	3
	1	26/10/05	16	2	2	2	3

	2	20/10/05	9	1	2	3	2
	2	25/10/05	14	2	2	2	3
	3	19/10/05	6	3	2	3	3
	3	21/10/05	8	1	2	3	3
	3	26/10/05	13	3	2	3	3
	4	20/10/05	5	1	2	3	2
	4	25/10/05	10	3	2	3	3
Everfresh	1	19/10/05	10	2	1	2	2
	1	21/10/05	11	2	2	3	3
	1	26/10/05	16	2	2	2	3
	2	20/10/05	9	1	1	3	1
	2	25/10/05	14	2	2	3	3
	3	19/10/05	6	2	1	2	2
	3	21/10/05	8	2	2	3	4
	3	26/10/05	13	4	4	3	3
	4	20/10/05	5	2	2	3	3
	4	25/10/05	10	2	2	3	2
Xyrex	1	19/10/05	10	1	1	2	1
	1	21/10/05	11	1	1	2	2
	1	26/10/05	16	2	2	3	3
	2	20/10/05	9	3	2	3	3
	2	25/10/05	14	2	2	3	3
	3	19/10/05	6	1	1	1	1
	3	21/10/05	8	1	1	2	2
	3	26/10/05	13	3	3	3	3
	4	20/10/05	5	2	2	3	3
	4	25/10/05	10	2	2	3	3
Hasenosa	1	19/10/05	10	1	1	3	2
	1	21/10/05	11	1	1	1	2
	1	26/10/05	16	3	2	3	2
	2	20/10/05	9	1	1	2	3
	2	25/10/05	14	3	3	3	3
	3	19/10/05	6	1	1	2	2
	3	21/10/05	8	1	1	2	2
	3	26/10/05	13	3	3	3	3
	4	20/10/05	5	2	2	3	3
	4	25/10/05	10	3	3	3	3
Pluscolour	1	19/10/05	10	1	1	1	1
	1	21/10/05	11	2	2	2	2
	1	26/10/05	16	2	2	3	2
	2	20/10/05	9	1	1	3	2
	2	25/10/05	14	3	3	3	3
	3	19/10/05	6	1	1	3	2
	3	21/10/05	8	1	2	2	2
	3	26/10/05	13	3	4	3	2
	4	20/10/05	5	1	1	1	2
	4	25/10/05	10	2	2	3	2

Melacide	1	19/10/05	10	1	1	3	2
	1	21/10/05	11	2	2	3	3
	1	26/10/05	16	3	2	3	2
	2	20/10/05	9	1	1	1	1
	2	25/10/05	14	2	2	3	2
	3	19/10/05	6	1	1	3	2
	3	21/10/05	8	1	2	2	2
	3	26/10/05	13	3	3	3	4
	4	20/10/05	5	1	1	3	2
	4	25/10/05	10	2	2	3	3
Aquabon	1	19/10/05	10	2	2	3	3
	1	21/10/05	11	3	2	3	3
	1	26/10/05	16	5	4	3	4
	2	20/10/05	9	3	2	1	2
	2	25/10/05	14	3	2	3	3
	3	19/10/05	6	2	2	3	2
	3	21/10/05	8	4	2	3	3
	3	26/10/05	13	3	4	3	4
	4	20/10/05	5	2	2	3	2
	4	25/10/05	10	3	2	3	2
Cidox	1	19/10/05	10	4	4	3	4
	1	21/10/05	11	5	3	3	4
	1	26/10/05	16	5	4	3	4
	2	20/10/05	9	4	3	3	4
	2	25/10/05	14	5	4	3	4
	3	19/10/05	6	5	4	3	4
	3	21/10/05	8	5	3	3	4
	3	26/10/05	13	4	3	2	3
	4	20/10/05	5	5	3	3	4
	4	25/10/05	10	5	4	3	4

Macrae (trial 1)

Treatment	Catch Date	Test date (days old)	Sensory test			
			Visual	Smell	Texture	Taste
Control	22/08/05	30-Aug (8)	4	4	1	4
		31-Aug (9)	4	3	1	2
		2-Sept (11)	4	4	1	3
	23/08/05	30-Aug (7)	2	3	1	1
		31-Aug (8)	2	4	1	2
		2-Sept (10)	3	4	2	4
	24/08/05	30-Aug (6)	5	4	3	4
		1-Sept (8)	4	4	2	3
		5-Sept (12)	5	4	3	4
25/08/05	30-Aug (5)	2	3	3	2	
	1-Sept (7)	4	4	3	4	
	5-Sept (11)	4	4	3	4	
Meta 2.5%	22/08/05	30-Aug (8)	3	3	3	1

		31-Aug (9)	3	3	1	3
		2-Sept (11)	3	4	3	4
	23/08/05	30-Aug (7)	2	1	1	1
		1-Sept (9)	3	2	1	3
		5-Sept (13)	3	3	2	3
	24/08/05	30-Aug (6)	3	2	3	4
		1-Sept (8)	4	3	3	4
		5-Sept (12)	4	3	3	4
	25/08/05	30-Aug (5)	3	3	3	4
		1-Sept (7)	3	3	2	3
		5-Sept (11)	4	4	3	4
Meta 5%	22/08/05	30-Aug (8)	2	3	3	1
		31-Aug (9)	2	2	3	1
		2-Sept (11)	2	4	1	2
	23/08/05	30-Aug (7)	1	2	1	1
		31-Aug (8)	2	2	1	1
		2-Sept (10)	2	4	2	4
	24/08/05	30-Aug (6)	2	2	3	2
		1-Sept (8)	2	3	2	2
		5-Sept (12)	2	3	2	2
	25/08/05	30-Aug (5)	1	4	3	3
		1-Sept (7)	1	4	1	2
		5-Sept (11)	2	4	2	3
Frescan	22/08/05	30-Aug (8)	3	4	3	4
		31-Aug (9)	3	3	1	1
		2-Sept (11)	5	4	3	4
	23/08/05	30-Aug (7)	2	2	1	2
		31-Aug (8)	3	3	1	2
		2-Sept (10)	4	2	1	3
	24/08/05	30-Aug (6)	3	3	2	2
		1-Sept (8)	3	3	2	2
		5-Sept (12)	4	4	3	4
	25/08/05	30-Aug (5)	4	4	3	3
		1-Sept (7)	5	4	3	4
		5-Sept (11)	4	4	3	4
Xyrex	22/08/05	30-Aug (8)	1	2	3	2
		31-Aug (9)	1	2	3	4
		2-Sept (11)	1	2	1	3
	23/08/05	30-Aug (7)	1	1	3	2
		31-Aug (8)	1	1	3	2
		2-Sept (10)	1	2	1	2
	24/08/05	30-Aug (6)	1	3	3	2
		1-Sept (8)	3	4	3	3
		5-Sept (12)	3	4	3	3
	25/08/05	30-Aug (5)	2	4	3	2
		1-Sept (7)	2	4	2	3
		5-Sept (11)	2	4	3	3
Citric acid	22/08/05	30-Aug (8)	4	3	3	3

		31-Aug (9)	4	4	2	3
		2-Sept (11)	5	4	3	3
Pluscolour	22/08/05	30-Aug (8)	2	2	3	4
		31-Aug (9)	1	2	1	2
		2-Sept (11)	1	2	3	3
	23/08/05	30-Aug (7)	1	1	1	2
		31-Aug (8)	1	2	1	1
		2-Sept (10)	2	3	1	4
	24/08/05	30-Aug (6)	5	3	3	3
		1-Sept (8)	2	3	2	3
		5-Sept (12)	3	3	2	3
	25/08/05	30-Aug (5)	3	4	3	3
		1-Sept (7)	2	3	1	3
		5-Sept (11)	2	3	1	2
Ascorbic acid	22/08/05	30-Aug (8)	4	4	3	3
		31-Aug (9)	4	4	3	3
		2-Sept (11)	5	4	3	3
Soft acid	22/08/05	30-Aug (8)	3	3	2	3
		31-Aug (9)	4	4	3	3
		2-Sept (11)	4	4	3	3
	23/08/05	30-Aug (7)	3	3	3	3
		31-Aug (8)	3	4	1	3
		2-Sept (10)	3	4	3	3
	25/08/05	30-Aug (5)	3	3	3	3
		1-Sept (7)	5	4	3	4
		5-Sept (11)	5	4	3	4
Hasenosa	22/08/05	30-Aug (8)	4	4	3	2
		31-Aug (9)	1	3	1	1
		2-Sept (11)	4	4	3	4
	23/08/05	30-Aug (7)	1	2	1	3
		31-Aug (8)	1	2	1	2
		2-Sept (10)	2	3	2	3
	24/08/05	30-Aug (6)	2	3	3	3
		1-Sept (8)	2	4	3	2
		5-Sept (12)	2	4	3	3
	25/08/05	30-Aug (5)	1	3	3	2
		1-Sept (7)	2	4	3	3
		5-Sept (11)	2	4	3	3
Melacide	22/08/05	30-Aug (8)	1	2	1	1
		31-Aug (9)	2	2	1	1
		2-Sept (11)	1	2	2	3
	23/08/05	30-Aug (7)	2	2	2	1
		31-Aug (8)	1	2	3	3
		2-Sept (10)	2	2	3	2
	24/08/05	30-Aug (6)	2	3	2	2
		1-Sept (8)	1	3	2	3

		5-Sept (12)	2	3	2	3
	25/08/05	30-Aug (5)	1	2	3	1
		1-Sept (7)	2	3	3	2
		5-Sept (11)	3	4	3	3

Macrae (trial 2)

Treatment	Catch Day	Test day	Days on ice	Visual	Odour	Texture	Taste
Control	09/10/05	19/10/05	10	1	1	1	2
	11/10/05	19/10/05	6	1	1	1	2
	13/10/05	20/10/05	9	4	1	1	1
	15/10/05	20/10/05	5	2	1	3	2
	09/10/05	21/10/05	12	3	1	1	1
	11/10/05	21/10/05	8	2	1	2	3
	13/10/05	24/10/05	15	4	1	2	2
	15/10/05	24/10/05	13	3	1	2	2
	09/10/05	24/10/05	11	4	3	2	4
	11/10/05	24/10/05	9	2	1	3	2
	13/10/05	25/10/05	14	4	2	2	2
	15/10/05	25/10/05	10	3	1	2	2
Cidox	09/10/05	19/10/05	10	2	1	4	4
	11/10/05	19/10/05	6	2	1	4	4
	13/10/05	20/10/05	9	4	1	4	4
	15/10/05	20/10/05	5	4	1	4	4
	09/10/05	21/10/05	12	2	2	4	4
	11/10/05	21/10/05	8	2	1	4	4
	13/10/05	24/10/05	15	4	1	1	2
	15/10/05	24/10/05	13	4	1	1	2
	09/10/05	24/10/05	11	3	1	1	2
	11/10/05	24/10/05	9	4	1	1	1
	13/10/05	25/10/05	14	5	1	1	2
	15/10/05	25/10/05	10	2	1	1	2
Aquabon	09/10/05	19/10/05	10	2	1	2	2
	11/10/05	19/10/05	6	3	1	3	1
	13/10/05	20/10/05	9	4	1	2	2
	15/10/05	20/10/05	5	2	1	2	3
	09/10/05	21/10/05	12	2	1	1	2
	11/10/05	21/10/05	8	3	1	3	3
	13/10/05	24/10/05	15	4	1	1	2
	15/10/05	24/10/05	13	4	1	2	2
	09/10/05	24/10/05	11	3	2	2	3
	11/10/05	24/10/05	9	2	1	3	2
	13/10/05	25/10/05	14	5	3	2	4
	15/10/05	25/10/05	10	2	2	2	4
Xyrex	09/10/05	19/10/05	10	1	1	2	2
	11/10/05	19/10/05	6	1	1	3	3

	13/10/05	20/10/05	9	2	1	2	2
	15/10/05	20/10/05	5	1	1	2	2
	09/10/05	21/10/05	12	1	1	1	1
	11/10/05	21/10/05	8	1	1	1	2
	13/10/05	24/10/05	15	2	1	1	1
	15/10/05	24/10/05	13	2	1	1	3
	09/10/05	24/10/05	11	1	1	3	3
	11/10/05	24/10/05	9	2	1	3	1
	13/10/05	25/10/05	14	2	1	2	3
Melacide	09/10/05	19/10/05	10	1	1	2	2
	11/10/05	19/10/05	6	1	1	2	1
	13/10/05	20/10/05	9	1	1	1	1
	15/10/05	20/10/05	5	1	1	3	3
	09/10/05	21/10/05	12	1	1	1	1
	11/10/05	21/10/05	8	1	1	1	1
	13/10/05	24/10/05	15	4	2	1	3
	15/10/05	24/10/05	13	2	1	1	3
	09/10/05	24/10/05	11	4	2	3	2
	11/10/05	24/10/05	9	2	1	1	2
	13/10/05	25/10/05	14	2	1	3	3
	15/10/05	25/10/05	10	3	2	3	3
Hasenosa	09/10/05	19/10/05	10	1	1	2	2
	11/10/05	19/10/05	6	1	1	2	2
	13/10/05	20/10/05	9	1	1	1	1
	15/10/05	20/10/05	5	1	1	1	1
	09/10/05	21/10/05	12	1	1	1	3
	11/10/05	21/10/05	8	1	1	2	3
	13/10/05	24/10/05	15	2	1	1	2
	15/10/05	24/10/05	13	2	2	2	3
	09/10/05	24/10/05	11	4	2	3	2
	11/10/05	24/10/05	9	2	1	2	3
	13/10/05	25/10/05	14	2	2	1	3
	15/10/05	25/10/05	10	2	3	2	4
Everfresh	09/10/05	19/10/05	10	1	1	1	3
	11/10/05	19/10/05	6	1	1	2	2
	13/10/05	20/10/05	9	2	1	3	1
	15/10/05	20/10/05	5	1	1	2	2
	09/10/05	21/10/05	12	1	1	1	1
	11/10/05	21/10/05	8	1	1	1	3
	13/10/05	24/10/05	15	2	1	2	3
	15/10/05	24/10/05	13	2	1	1	3
	09/10/05	24/10/05	11	4	2	3	2
	11/10/05	24/10/05	9	2	1	3	3
	13/10/05	25/10/05	14	2	2	3	1
	15/10/05	25/10/05	10	2	2	1	3
Pluscolour	09/10/05	19/10/05	10	1	1	1	2
	11/10/05	19/10/05	6	1	1	1	2
	13/10/05	20/10/05	9	3	1	1	2

	15/10/05	20/10/05	5	2	1	3	2
	09/10/05	21/10/05	12	1	1	1	2
	11/10/05	21/10/05	8	4	1	1	3
	13/10/05	24/10/05	15	2	1	2	3
	15/10/05	24/10/05	13	4	2	3	3
	09/10/05	24/10/05	11	4	2	2	3
	11/10/05	24/10/05	9	4	2	3	3
	13/10/05	25/10/05	14	3	1	1	3
	15/10/05	25/10/05	10	2	2	2	4

Appendix 3 - Micro results

Appendix 3 (Micro results)

Iceberg (Trial 1)

Treatment	Day	Catch date	Date tested	TVC (cfu/g)	Enteros	Listeria	Salmonella	Lactic acid
Soft acid	1	22/08/2005	31/08/2005	1400	<10	ABSENT	ABSENT	N/D
	2	23/08/2005	31/08/2005	7400	<10	ABSENT	ABSENT	N/D
	3	24/08/2005	01/09/2005	6400	5	ABSENT	ABSENT	N/D
	4	25/08/2005	01/09/2005	370000	<10	ABSENT	ABSENT	N/D
Pluscolour	1	22/08/2005	31/08/2005	2300	<10	ABSENT	ABSENT	N/D
	2	23/08/2005	31/08/2005	1500	<10	ABSENT	ABSENT	N/D
	3	24/08/2005	01/09/2005	1700	<10	ABSENT	ABSENT	N/D
	4	25/08/2005	01/09/2005	38000	<10	ABSENT	ABSENT	N/D
Frescan	1	22/08/2005	31/08/2005	2100	<10	ABSENT	ABSENT	N/D
	2	23/08/2005	31/08/2005	6600	<10	ABSENT	ABSENT	N/D
	3	24/08/2005	01/09/2005	8800	<10	ABSENT	ABSENT	N/D
	4	25/08/2005	01/09/2005	23000	<10	ABSENT	ABSENT	N/D
Control	1	22/08/2005	31/08/2005	3000	<10	ABSENT	ABSENT	N/D
	2	23/08/2005	31/08/2005	2900	<10	ABSENT	ABSENT	N/D
	3	24/08/2005	01/09/2005	15000	<10	ABSENT	ABSENT	N/D
	4	25/08/2005	01/09/2005	13000	<10	ABSENT	ABSENT	N/D

Iceberg (Trial 2)

Treatment	acidity (as lactate) %	TVC (cfu/g)	Enterococcus (cfu/g)	Salmonella	Listeria
Cidox	2.529	3500	<20	absent	absent
Everfresh	0.027	1000	<20	absent	absent
Pluscolour	3.35	3200	<20	absent	absent
Xyrex	0.057	8600	<20	absent	absent
Aquabon	0.053	2000	<20	absent	absent
Control	1.018	5800	<20	absent	absent

Laeso (Trial 1)

	Day	Catch date	SO2 mg/kg	Aerobic colony count	Coliforms	E.coli	Staphylococcus	Salmonella
Control	1	22/08/2005	<10	22000	120	<10	<10	0
	2	23/08/2005		11000	<10	<10	<10	0
	3	24/08/2005		13000	<10	<10	<10	0
	4	25/08/2005	23					
Meta 5%	1	22/08/2005	171	6700	<10	<10	<10	0
	2	23/08/2005		23000	<10	<10	<10	0
	3	24/08/2005		1800	<10	<10	<10	0
	4	25/08/2005	144					
Meta 2.5%	1	22/08/2005	46	9900	150	<10	<10	0
	2	23/08/2005		24000	300	<10	<10	0
	3	24/08/2005		6800	<10	<10	<10	0
	4	25/08/2005	41					
Soft acid	1	22/08/2005	<10	320	<10	<10	<10	0
	2	23/08/2005		24000	<10	<10	<10	0
	3	24/08/2005		1200	<10	<10	<10	0
	4	25/08/2005	73					
Xyrex	1	22/08/2005	<10	1100	<10	<10	<10	0
	2	23/08/2005		32000	<10	<10	<10	0
	3	24/08/2005		2400	<10	<10	<10	0
	4	25/08/2005	<10					
Pluscolor	1	22/08/2005	60	1400	<10	<10	<10	0
	2	23/08/2005		17000	<10	<10	<10	0
	3	24/08/2005		9000	<10	<10	<10	0
	4	25/08/2005	<10					
Citric acid	1	22/08/2005	<10	<10	<10	<10	<10	0
	2	23/08/2005						

	3	24/08/2005						
	4	25/08/2005						
Hasenos a	1	22/08/2005	59	10000	<10	<10	<10	0
	2	23/08/2005		16000	<10	<10	<10	0
	3	24/08/2005		6900	<10	<10	<10	0
	4	25/08/2005	58					
Ascorbic acid	1	22/08/2005	<10	6400	10	<10	<10	0
	2	23/08/2005						
	3	24/08/2005						
	4	25/08/2005						
Frescan	1	22/08/2005	56	2600	<10	<10	<10	0
	2	23/08/2005		22000	<10	<10	<10	0
	3	24/08/2005		16000	<10	<10	<10	0
	4	25/08/2005	27					

Laeso (Trial 2)

	Day	Catch date	SO2 mg/kg	Aerobic colony count	Coliforms	E.coli	Staphylococcus	Salmonella
Control	1	09/10/2005	<10					
	2	11/05/2005		280	<10	<10	<10	0
	3	13/05/2005	10					
	4	15/05/2005		320	<10	<10	<10	0
Meta 5%	1	09/10/2005	557					
	2	11/05/2005		240	<10	<10	<10	0
	3	13/05/2005						
	4	15/05/2005		22000	<10	<10	<10	0
Everfresh	1	09/10/2005	<10					
	2	11/05/2005		480	<10	<10	<10	0
	3	13/05/2005						
	4	15/05/2005		480	<10	<10	<10	0

Xyrex	1	09/10/2005	<10					
	2	11/05/2005		280	<10	<10	<10	0
	3	13/05/2005	<10					
	4	15/05/2005		6400	<10	<10	<10	0
Hasenosa	1	09/10/2005	153					
	2	11/05/2005		5400	<10	<10	<10	0
	3	13/05/2005	10					
	4	15/05/2005		19000	<10	<10	<10	0
Aquabon	1	09/10/2005	<10					
	2	11/05/2005		200	<10	<10	<10	0
	3	13/05/2005						
	4	15/05/2005		25000	<10	<10	<10	0
Cidox	1	09/10/2005	<10					
	2	11/05/2005		800	<10	<10	<10	0
	3	13/05/2005	10					
	4	15/05/2005		2400	<10	<10	<10	0
Malacide	1	09/10/2005	<10					
	2	11/05/2005		240	<10	<10	<10	0
	3	13/05/2005	10					
	4	15/05/2005		100	<10	<10	<10	0
Pluscolour	1	09/10/2005	<10					
	2	11/05/2005		200	<10	<10	<10	0
	3	13/05/2005						
	4	15/05/2005		9000	<10	<10	<10	0

Macrae (Trial 1)

Sample Date	Rep Date	Catch date	Treatment	Aerobic colony count	Salmonella	Listeria
01/09	05/09/05	Day 2 23/08	Citric Acid	2.4x10 ³	< 10	< 10
01/09	05/09/05	Day 2 23/08	Soft Acid	4.8x10 ³	< 10	10
01/09	05/09/05	Day 2 23/08	Meta 2.5%	1.2x10 ⁴	< 10	< 10
01/09	05/09/05	Day 2 23/08	Meta 5%	8.9x10 ³	< 10	< 10
01/09	05/09/05	Day 2 23/08	Control	1.4x10 ⁴	< 10	< 10
01/09	05/09/05	Day 2 23/08	Melacide	1.0x10 ⁴	< 10	110
01/09	05/09/05	Day 2 23/08	Ascorbic Acid	900	< 10	< 10
01/09	05/09/05	Day 2 23/08	Xyrex	1.3x10 ³	< 10	< 10
01/09	05/09/05	Day 2 23/08	Pluscolour	6.0x10 ³	< 10	< 10
01/09	05/09/05	Day 2 23/08	Hasenosa	1.9x10 ⁴	< 10	< 10
01/09	05/09/05	Day 2 23/08	Frescan	1.2x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	5% Meta	2.1x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	2.5% Meta	1.4x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	Pluscolour	1.2x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	Malacide	8.6x10 ³	< 10	< 10
06/09	09/09/05	Day 3 24/08	Hasenosa	1.1x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	Soft Acid	2.5x10 ⁴	< 10	< 10
06/09	09/09/05	Day 3 24/08	Frescan	8.5x10 ³	< 10	< 10
06/09	09/09/05	Day 3 24/08	Xyrex	9.4x10 ³	< 10	< 10
06/09	09/09/05	Day 3 24/08	Melacide	9.6x10 ³	< 10	< 10