SCIENTIFIC REVIEW OF THE MICROBIOLOGICAL RISKS ASSOCIATED WITH REDUCTIONS IN FAT AND ADDED SUGAR IN FOODS

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EXECUTIVE SUMMARY

There is a range of food products that have high fat and/or sugar levels. The development of low-fat/low-sugar recipes could have an impact on levels of energy consumption and would help achieve the Governments target to reduce saturated fat intakes and tackle obesity. Manufactured foods rely on their formulation for their sensory and textural properties as well as the microbiological safety and stability. Any changes to product composition could have an impact on one or more of all these essential product characteristics.

One of the main effects of reducing the sugar and/or fat content of foods will be to alter the water activity. Generally, the changes will cause an increase in water activity. This has two potential microbiological risks. It could increase the growth potential of any foodborne pathogens that may be present in the product and thus increase the risk of food poisoning. Or it could increase the growth of spoilage organisms and thus shorten the shelf-life of the product that would have financial implications for food manufacturers. The microbiological risks will depend on the original water activity before re-formulation. Foods which are close to the minimum water activity for growth of foodborne microorganisms may only need minor recipe changes to take them into the growth region.

Some products rely mainly on sugar as a preservative, e.g. jams, pickles, candies and the levels of fat are negligible. Changes to this type of product are more likely to result in quality issues or microbiological spoilage. Other products such as chilled savoury products, sausages and pastry products have a high level of fat. Changes to these products may be sufficient to cause microbiological spoilage and could allow growth of food poisoning organisms. Other products such as cakes and muffins rely on both fat and sugar for product composition and microbiological stability. Changes in both these factors could result in quality loss, microbiological spoilage and growth of food poisoning organisms.

For some products, e.g. candies and cookies, there does not appear to be any microbiological risks as the changes in water activity are still below the minimum levels required for microbial growth. It is recommended that any changes to product formulation be assessed using the principals of HACCP. Where necessary, any loss of preservation caused by changes in fat and sugar level should be balanced with addition of other suitable factors which may include, preservatives, reduced pH, reduced storage temperature, increased heat process or modified atmosphere storage conditions.

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1. INTRODUCTION

It is recognised that levels of obesity are rising in the population. In simple terms, obesity occurs when energy intake exceeds energy expenditure. The biggest contributors to energy in foods are fats (9 calories/g or 37.7 kJ/g) and sugars (4 calories/g or 16.7 kJ/g). There are a number of factors that contribute to increasing obesity levels. People are generally leading more sedentary lives thus lowering energy expenditure and there is a wide availability of manufactured foods for consumption outside the home. As one of the ways to improve consumer health, the Foods Standards Agency is working towards a target of reducing the average saturated fat intake for adults to below 11% from the current level of 13.4%.

There are a number of ways in which this can be done. Consumer education about the choice of food and amount of food consumed in a single portion may be a way forward. Take for example, consumption of hard cheddar-style cheese containing 500 calories per 100g and 34.9% fat. If a person consumed 80g instead of 100g then they would consume 400 calories (1674 kJ) instead of 500 calories (2092 kJ) and would consume 27.92g of fat and not 34.9g, thus reducing their fat intake by 20%. Another way to reduce the levels of energy intake is to lower the levels of total fats and sugars in manufactured foods. This may involve modifications of existing product formulations or the development of new recipes. As with any change to product recipe, the implications of any reduction in fat or sugar levels need to be considered following the principles of the HACCP system in order to maintain product safety.

Manufactured food products need to meet three main consumer drivers:

(i) They need to be of a desirable quality to the consumer.

(ii) They should provide a positive contribution to a balanced diet of the consumer.

(ii) Above all, they must be safe for consumption. One of the key targets is to achieve product safety with respect to the risks from foodborne pathogens.

Achieving a balance between these three aspects is not easy. Many components of the food such as salt, sugars and fats will give a desirable taste and texture but may not achieve the desired characteristics of a 'healthy' product if they are present in large amounts. Any reduction in the levels of these components to improve the nutritional aspects of the product has the potential to unbalance the preservation system of the food and may allow growth of pathogens and spoilage organisms that were previously inhibited within the product.

In order to understand the likely effects of reductions in fats and/or sugars, it is worth considering their contribution to food preservation. There are four main factors that can be used to control the growth of microorganisms in foods:

(i) Temperature: either to inactivate microorganisms during heat processing or to minimise their growth during reduced temperature storage.
(ii) pH: use of vinegars, lemon juice or other organic/mineral acids to increase the acidity of the product and thus reduce/prevent microbial growth.
(iii) Preservatives: chemical additives such as sodium benzoate that are added to foods to prevent growth of target groups of organisms.
(iv) Water activity: the use of reduced water availability to reduce/prevent microbial growth.

There are many ways in which the water activity of a product can be changed. Water can be evaporated from a product, or ingredients such as proteins, fats, sugars and salt can be added to a product to bind the water. Any change in the level of these ingredients will alter the water activity of the product. In products preserved by a combination of factors, a change in the level of any of the factors could potentially unbalance the system and needs to be balanced by changes in other factors to maintain physical characteristics and microbiological stability (Figure 1, Appendix). Another issue to consider is the drive to remove any kind of additives and E-numbers from foods. Concurrent plans to reduce salt, sugars, fats and additives yet maintain product safety and shelf-life present a large challenge to food manufacturers.

The scope of this report is to evaluate the microbiological risks from reductions in added fat and sugars in foods. The risks perceived are two-fold. Firstly, the increased risk of food poisoning and secondly, the increased risk of microbiological food spoilage. Food poisoning risks may arise when changes to product composition allow an enhanced growth or survival of food pathogens such that they or their toxins are present in sufficient numbers to cause illness. Microbiological food spoilage risks may arise when enhanced growth of spoilage microorganisms render a food unacceptable for consumption more quickly in a product of altered composition compared to the original. The report will look at the types of changes likely to be used to produce low-fat/low-sugar foods, their effect on product preservation factors and the subsequent potential for increased microbial growth or survival.

Information used in this review has predominantly been taken from peer-reviewed scientific literature. The main databases used were Food Science and Technology Abstract 1990 – 2006and 1969 to 1989. On-line searches were done through Foodline 1972 – 2006. Several hundreds of articles were highlighted in the searches and the most relevant papers chosen to obtain in full i.e. those articles that contained

information relevant to answer the questions asked in the project brief. Another source of information was discussions with members of the food industry that shall remain confidential due to reasons of confidentiality. Much of the current information on recipe formulation and technological advances is confidential to industry companies and is not available for general dissemination.

2. TYPES OF COMPOSITIONAL CHANGES LIKELY TO BE USED FOR LOW FAT/LOW SUGAR FOODS

There are two main ways in which the levels of fat and sugar can be reduced. They can be removed from a product and not replaced with anything. This may be feasible where the level of reduction is low. Where the reduction in levels of fat and sugar is large enough to impact on the functionality of the food, then a fat or sugar replacer will be needed. There is a wide range of different ingredients that can be used. Some may be metabolised differently to the ingredient they are replacing or might have different effects on the structure and properties of the food. Careful consideration needs to be given to the choice of fat and sugar replacer to maintain product safety and stability.

2.1 Alternatives to Fats and Sugars

2.1.1 Fats*:* many of the alternatives to fats are based on an aqueous suspension of fibres (Aronson *et al*, 2005) and other hydrocolloids (Lobato-Calleros *et al*, 2006). There are many patents describing the composition and potential uses of such compounds (e.g. Buttini *et al*, 2006, Shukla *et al*, 2005, Best *et al*, 2005). A good fat replacer needs to have three essential qualities: a thickening agent, a bulking agent and a microparticulate component to give the smoothness of fat (Khouryeh *et al*, 2005).

Whilst use of fat replacers may be able to achieve a product with lower fat and energy content which have acceptable organoleptic qualities, it may also have the potential to increase the moisture content of the products. This will have two main effects. Firstly, the water activity may be increased which may allow or enhance the growth of a range of microorganisms including food pathogens. Secondly, the antimicrobial effects of any salt or preservatives that were in the water phase of the product may be reduced, as their concentration will decrease due to the increasing level of water. For example, if a product with a moisture content of 70% containing 1000ppm potassium sorbate was reformulated so that the moisture content was 80% then the level of potassium sorbate would only be 875ppm unless more sorbate was added to address the change in concentration. The effect on the water activity of the product will depend on which product category under consideration and which fat replacer has been used as the water binding properties will be different for each type.

In meat products there are many ways of producing a low fat version. Either the fat is removed and replaced by iced-water, or a fat replacer such as carageenan and pectin gel (Candogan and Kolsarici, 2003) or konjac gel (Osburn and Keeton, 2004) is added, in addition to the fat removal, to stabilise the physical properties of the products. Reducing the fat content of the product may have little effect on the final water activity, particularly, if the water activity is already quite high and additives with good humectant properties, such as lactates are used. Unfortunately, not all refereed publications give crucial details on water activity and it is not possible to calculate these values from moisture content alone.

Katsaras and Dressel (1994) looked at the effect of soy protein on the water activity of sausages and inclusion of up to 10% soy protein made little difference to the a_w of 0.96 present in the control. Low fat sausages (1.45% fat), made with fat replacer (konjac flour/carageenan and soy protein) had a similar a_w (0.936) to high fat (21.5% fat) sausages (Choi *et al*, 2003).

Frankfurters which had 40% reduced fat levels had a higher moisture content (68%) compared with the controls (56% moisture) (Caceres *et al,* 2006). No specific a_w values are given for each recipe but it states that the a_w ranged from 0.95 to 0.99. If, as seems likely, the higher moisture/low fat frankfurters were those with the highest a_w values, then this could allow a greater microbial growth in the low fat version. There would be a marked difference in lag time and growth rate of various microorganisms, including pathogens, over a 10 day shelf life at 8ºC (Table 1, Appendix).

Other studies (Bloukas *et al*, 1997) showed that low fat frankfurters (10% fat) had a higher moisture content than high fat controls (24.6 % fat). As a result, the shelf-life at 4°C was only 3 weeks for the low fat version compared with 4 weeks for the control. The decrease in shelf-life was attributed to increased microbial growth in the low fat product.

Studies on the use of fat replacers in low fat pepperonis (Vickery and Rogers, 2002) showed that low fat product (6.5% fat) made with starch, rice flour and beet flour yielded products that were satisfactory from a sensory perspective, i.e. colour and texture. No data is given on a_w but the moisture content of low fat recipe (47%) was higher than the control (33%) which may impact on the microbiological shelf-life.

In cheeses, the formulation of products with reduced fat levels affected the water activity of the products (Kreisman and Labuza, 1978). Different intermediate moisture cheese products were made with varying amounts of water to give cheeses with the following values for fat and water activity; 21.7% fat/a_w 0.81; 21% fat/a_w

0.86; 19.1% fat/a_w 0.90; 17.8% fat/a_w 0.91; 16.8% fat/a_w 0.94. The cheeses were generally acceptable from a sensory point of view with the estimated shelf-life ranging from 6 months to 2 years. However, the increased water activity seen in the lowest fat cheeses allowed microbial growth to occur. The cheeses were inoculated at the beginning of life with moulds, *Salmonella* and *Staphylococcus aureus* and stored at room temperature for 6 months. *Salmonella* was not able to grow in any of the cheeses, even though its minimum a_w for growth is 0.92. This demonstrates the effect of substrate and food matrix on the minimum a_w for growth. *S. aureus* grew in the highest a_w cheese (0.94) and the moulds grew in the three highest a_w cheeses (0.91-0.94). This demonstrates the effect of fat reduction on product a_w and the subsequent increase in growth potential of spoilage organisms or food pathogens.

In any food product, there is a complex physico-chemical interaction of the various food constituents to produce a product that is balanced in terms of required pH, salt levels, fat levels etc. Once any part of the system is altered then all the other attributes may be affected. It has already been demonstrated that a decrease in fat level can increase the moisture content. In some products, the increase in moisture content may affect other sensory characteristics of the food and make them undesirable. This can be seen in a low fat cream cheese product. The normal fat level (19.4%) was reduced to 11.5% without including a fat replacer, and to 7.6% using a fat replacer (Dairy-Lo®). No a_w values are given but the moisture content was highest in the cheese with fat replacer (67%) than either the fat reduced (64%) cheese or the control (55%). As a consequence of the change in moisture level, other essential characteristics changed. The product pH fell with increasing moisture content and whilst this may be desirable with respect to microbiological inhibition, it was not acceptable with respect to sensory attributes. The pH was 5.3, 5.2 and 4.9 for the three cheeses with the pH decreasing with fat levels. The fat reduced cheese at pH 5.2 was acceptable to a sensory panel but the lowest fat recipe at pH 4.9 was not (Zalazar *et al*, 2002).

The inter-relationship between fat levels and other factors is demonstrated in a further study on reduced fat cheeses. Pasteurised processed cheese products were made using full fat (32.8% fat), reduced fat (19.6% fat) or skimmed milk cheeses (0.9% fat). The a_w values for the 3 cheeses were 0.95, 0.967 and 0.97 respectively. The study looked at the effects of various additives (e.g. sodium lactate, monolaurin and fat replacers) on the safety of these products with respect to *Cl. botulinum*. Toxin production appeared to be delayed in the low fat cheeses. Although the moisture content was higher in these low fat products, the fat levels were substantially lower. The antimicrobial factors used are fat soluble and in the higher fat cheeses were absorbed into the fat portion of the cheese thus reducing their antimicrobial properties (Glass and Johnson, 2004).

Another area where reduced fat recipes are being developed is mayonnaises and dressings. There has been an increasing development in new types of mayonnaise, e.g. low fat, no fat, flavoured mayonnaise (Anon, 2003). The fat replacer used is important with respect to functional properties of the low fat products. One potential replacer is β-Glucan (G) from spent brewers' yeast (Worrasinchai *et al*, 2006). Full fat product (84.8% fat) and reduced fat recipes made with 25% G (65% fat), 50% G (43% fat) and 75% G (24% fat) were evaluated for sensory properties over a 70 day storage period. There were large differences in water activity between the recipes although the pH values were consistently low for all fat levels. These are shown below (Table 2).

Table 2
pH and a_w of mayonnaise at different fat levels

Mayonnaise	a_w day1	a_w day 64	pH day1	pH day 64
Full fat	0.958	0.943	3.84	3.91
25% G	0.989	0.984	3.80	3.52
50% G	0.995	0.994	3.84	3.44
75% G	0.998	0.999	3.85	3.33

The microbiological levels (total plate count) were similar for all products and remained low during storage. Although the a_w was higher in the low fat products, the pH was sufficiently low to prevent growth of bacteria and allow only growth of yeasts and moulds. Based on sensory assessment, 25%G and 50%G mayonnaise was acceptable. The appearance and odour was poor for the 75%G mayonnaise, being too dense and pale.

Changing the type of fat from a saturated to a poly- or mono-unsaturated fat may be preferable with respect to potential microbial risks than reducing fat levels completely, as it would maintain the overall fat level and thus the water activity of the product. However, there are still some potential risks that may arise from the effects of changing fat on the structural properties of the food. Changing the fat type may affect the type of emulsion formed, e.g. it may change from a water-in-oil emulsion to an oil-in-water emulsion, or it could change the size and structure of the oil droplets. Studies have shown that the type and size of droplets within the emulsion can affect microbial growth (Brocklehurst *et al*, 1995; Parker *et al*, 1995). In addition, it is known that fat can protect microorganisms during heat processing. If the fat was reduced or the fat type changed then this may change the thermal death characteristics of some microorganisms (see 3.1.7).

For some products such as pastry, the type and level of fat present is an integral part of the structural properties of the final product. Puff and flaky pastries are made from equal parts flour and fat and this is important to achieve the raised and layered effect. Short crust and hot water pastries contains two parts flour to one part fat and are naturally lower in fat, whilst filo pastry contains flour and egg and minimal added fat. It is not possible to give recommendations on the level of fat reduction which is achievable whilst maintaining the essential product characteristics as the data is not available.

Conclusions: effects of reducing fat levels

C1. Reduction in fat levels can lead to an increase in moisture content. In some cases this may lead to a rise in water activity. This is dependent on the properties of the fat replacer and other additives used.

C2. Where the changes in increased water activity are balanced by other factors, such as low pH, the risks from growth of food pathogens are low and tend to be primarily due to spoilage organisms such as yeasts and mould.

C3. Where changes in water activity are not balanced by other preservative factors then the risks of microbial growth are increased. If the water activity is increased above 0.90-0.92 then there may be increased risk of growth of food pathogens if present.

C4. Often, the reduced fat products are acceptable with respect to microbiological quality but the sensory properties, e.g. appearance, odour, texture are not acceptable and limit the amount of fat reduction achievable.

C5. It is not possible to give recommendations of minimum achievable levels of saturated fat, total and/or added sugar levels in different products, which would still maintain the physical characteristics of the product, such as texture. Levels will vary between product type and such data is not available in the literature.

2.1.2 Sugars: with respect to reducing the amount of sugar in a product there are natural alternatives such as fructose which are sweeter than sucrose and can be used in smaller quantities to give the same sweet taste. Alternatively, there are artificial sweeteners such as aspartame and acesulfame-K, which are intensely sweet. Sugar alcohols or polyols constitute the major portion of sugar replacers, they are not completely metabolised and have fewer calories than sucrose (Anon, 2004). As the amount of sugar is reduced then bulking agents need to be used to maintain the correct ratios of other ingredients in the product and to achieve the same functionality. Careful consideration needs to be given to the humectant properties of the ingredients used when changing recipes to accommodate a reduction in sugar as the water binding

properties vary considerably. The relative humectant values of cake making ingredients are shown below (Table 3) where sucrose has a reference value of 1 (Mathlouthi, 2001). If the value is less than 1 then the ingredient is able to bind less water than sucrose. If it is higher then it has a greater water-binding capacity and thus will reduce the water activity more if present in the same weight.

Table 3
Relative humectancy of some cake ingredients

Ingredient	Humectancy
Sucrose	1
Flour	0.2
Glycerol	4
Egg	0
Skim milk	1.2
Salt	11
Glucose	1.4
Sorbitol	2

Reducing the total amount of sugar in a product may increase the water activity of the product that could increase the risk of microbial growth.

In addition, it could have further effects on the sensory properties of the foods. Sugars are often used to balance the effects of other preservatives such as salts or acids. If the intensity or nature of the sweetness was changed then levels of other preservative factors may also need to be altered to maintain organoleptic acceptability.

One of the largest areas where sugar reduction is being explored is with baked goods, cookies, cakes, pastries and muffins. Often the reduction in sugar is done in combination with a reduction with fat levels so both factors will be considered together for these goods.

The effect of the change in product composition will depend on the product under consideration. For cookies, the effects on water activity are unlikely to be sufficient to cause any increased risk from microbial spoilage or safety. The a_w of cookies has to be low to produce a product of acceptable sensory properties. Cookies were manufactured with four different fat replacers and 3 different sugar levels (Zoulias *et al*, 2002). The water activity of all combinations (0.11-0.22) was higher than the control (0.08) but sufficiently low to maintain microbiological stability with respect to bacteria, yeasts or moulds (an a_w level of <0.60 is sufficient to prevent any microbial

growth, Table 4). The sensory evaluation of the cookies showed that 35% fat replacement was acceptable whilst 50% was not.

Table 4
Water activity regions for growth of microorganisms

Aw region	Typical foods	Microorganisms inhibited at lower end of range	
		Spoilage organisms	Pathogens
1.0-0.95	Perishable fresh foods meats, vegetables.	*Pseudomonas*, *Proteus*, *Escherichia*	*Clostridium perfringens, Clostridium botulinum* (psychrotrophic). *Bacillus cereus, Escherichia coli*
0.95-0.91	Some cheeses, cured meats.	*Serratia, Lactobacillus, Pediococcus*	*Clostridium botulinum (proteolytic), Listeria monocytogenes, Salmonella*
0.87-0.91	Fermented meats, sponge cake, hard cheese, margarine	Many yeasts (Candida, Hansenula), Micrococcus	*Staphylococcus aureus*
0.87-0.80	Fruit concentrates, syrups, fruit cakes	Moulds, other yeasts species (S*accharomyces*)	*Staphylococcus aureus*
0.80-0.60	Jams, sugar, some dried fruits, honey, toffee, fudge	Xerophilic moulds, osmophilic yeasts	None in this range
<0.60	Pastas, spices, cookies, dried soups and milk powders, cereals, chocolate	No growth in this range	No growth in this range

An increase in water activity was shown for oatmeal and chocolate chip cookies (Perry *et al*, 2003) made with 50% reduction in either fat or both fat and sugar. Again, the increase in a_w was not sufficient to increase the risk from microbial growth. Oatmeal cookies (a_w 0.32) increased in a_w to 0.39 for fat reduced recipe and to a_w 0.41 for fat and sugar reduced recipe. Results were similar for the chocolate chip cookies going from a_w 0.33 to 0.38 and 0.42. The changes to recipe were acceptable with respect to sensory attributes and had a greater effect on texture than flavour.

With low sugar muffins or cakes, the initial water activity is higher and therefore any modification in recipe may be sufficient to take the water activity into regions in

which microorganisms can begin to grow. Four cake mixes were made with different levels of encapsulated aspartame and stored alongside a full sugar control (Wetzel *et al*, 1997). The water activity of the no-sugar cakes was 0.922 (32% moisture), rising to 0.934 (34% moisture) during 3 days storage at room temperature. The control cake started at a_w 0.854 (25.5% moisture) and rose to a_w 0.862 (25.7% moisture). The lower a_w of the full sugar cake was due to the humectant behaviour of sucrose which was not replaced by the bulking agents used in the no-sugar recipes. The difference in a_w between the full sugar and no sugar cakes would be sufficient to allow the growth of pathogens if present (see Section 3.1). Whilst most bacterial pathogens would not grow cakes at a_w <0.90, any changes in water activity caused by reductions in fats and sugars may be sufficient to allow increased growth of *S. aureus* and any spoilage organisms present, particularly moulds. This would impact on the shelf-life of the product, and whilst there may not be any immediate health risks to the consumer there may be economic risks to the manufacturers due to increased production costs or loss of spoiled product. In some cases, whilst there may be no bacterial risks, there is the potential for the production of mould aflotoxins in products stored for prolonged periods. However, it is likely that the food would be visibly spoiled before aflotoxin production became an issue.

An increase in water activity is a consistent finding in low fat and low sugar cakes. No-fat muffins made with a protein-based fat replacer were compared to a full fat (10% fat) control. No data is given on a_w but the moisture content of the control (39.5% moisture) was lower than the reduced fat product (45% moisture). The constituents of the fat replacer, e.g. xantham gum and monoglycerides have been shown to trap moisture during baking, thus leading to the increased moisture content (Conforti, 1997).

Similar results were found with low sugar muffins (Khouryieh *et al*, 2005). They were made with sucralose which is 600 times as sweet as sugar. Due to the small amount needed to achieve the correct sweetness, bulking agents such as maltodextrins were added to provide the functional properties of sucrose and maintain product weight. The a_w of the control was 0.882 (28.4% moisture) whilst the no-sugar recipes had an a_w of 0.91 to 0.92 (32-38% moisture). The muffins were acceptable over a 4 day period but again the reduced sugar muffins were in the range of a_w values with the potential to allow growth of pathogens.

The change in a_w caused by reductions in sugar level depends on cake recipe. Sponge cakes made with dextrin and sucralose showed no difference in a_w between the control and low sugar recipe at an a_w of approximately 0.91. The total plate counts were similar for the different recipes, ranging from 10^1 to 10^2 per gram and no coliforms were detected in any samples tested. Cakes with 0, 40 and 50% sucrose replacement

were sensorially acceptable whilst 60 and 80% were not (Lin, S-D and Lee, C-C, 2005).

One of the main issues with low-fat/low-sugar recipes is the structural change that occurs in the final baked product. Whilst a_w may not be affected directly after baking it may increase during storage. Recent studies (Roca *et al*, 2006) have looked at migration of moisture in a sponge cake with different fat levels and initial porosities. Cakes were made with fat levels of 0, 0.11 and 0.30 g/g dry basis. The fat free sponge was beaten for 0, 25 or 50 minutes to vary the amount of entrapped air and thus the density (porosity) of the cake. The cakes with added fat were beaten for 50 minutes only.

The water activities were between 0.86 and 0.88 after baking but during storage for 7 days the moisture uptake and thus the time taken to reach a critical a_w value of 0.92 varied between fat levels and density. Sponge with no fat and no beating time reached an a_w of 0.92 within 4.5 days. Similar absorption rates were present for cakes with 0.3g/g fat and a beating time of 50 minutes. Sponge with a reduced fat level (0.11g/g dry basis) beaten for 50 minutes had time to a_w 0.92 of 2 days, less than half that of the full fat cake. The reduction in fat may lead to a shorter shelf-life if manufactured in the same way as the full fat version. Beating times and fat levels need to be balanced to find the optimum density for the desired moisture absorption characteristics.

The levels of sugars and other preservatives are carefully balanced in food products. If the sugars are to be reduced then careful consideration needs to be given to the choice of replacement. Often, high sugar products are preserved with potassium sorbate as this prevents the growth of yeasts and moulds which are the dominant spoilage organisms in these types of product. Studies have shown that the use of potassium sorbate and aspartame in an aqueous sugar system produced non-enzymatic browning which limited the shelf-life due to colour formation (Gliemmo *et al*, 2001). The authors concluded that in the manufacture of low-calorie products, the interactions of ingredients and additives must be carefully considered.

The effect of water activity on confectionery, particularly candy type products is predominantly one of sensory quality. A low a_w is essential to maintain the crisp texture of hard boiled sweets or the correct chewyness of a toffee. Confectionery products cover a wide range of a_w values from 0.2 to 0.85 (Fontana, 2005) but are generally below the level required to allow bacterial growth (Table 5).

In addition to the microbiological issues, changing the a_w of the product may affect the other attributes of the product such as lipid oxidation, non-enzymic browning, chlorophyll degradation and vitamin loss. Browning is a complex series of reactions

between reducing sugars and free amino acids. The rate of browning is maximal at an a_w of 0.6 to 0.8 so any changes in composition which bring confectionery products into this range may increase non-enzymic browning issues. All these reactions are minimal below a_w 0.3 but increase rapidly above this level (Esse and Saari, 2004). Similarly, the higher water content in low fat/low sugar products may create additional difficulties during commercial food manufacturing operations.

Table 5
Typical a_w values of confectionery products

Product	a_w	% sugars
Boiled sweets	0.25-0.4	35-60
Chocolate	0.30-0.40	30-50
Caramel/toffee/fudge	0.45-0.6	40-70
Jams	0.80-0.85	0-70
Jellies	0.50-0.75	30-75
Chewing gum	0.4-0.65	20-35
Marshmallow	0.6-0.75	40-65
Fondants	0.65-0.8	15-30

Conclusions: effects of reducing sugar levels

C6. Reduction in sugar levels can lead to an increase in moisture content. In some cases this may lead to a rise in water activity. This is dependent on the properties of the sugar replacer used.

C7. Whilst there may not always be an immediate change to water activity, the structural properties of the food may be changed such that their absorption properties allow a greater uptake of moisture throughout storage in reduced sugar recipes.

C8. Where an increase in water activity is observed this is may be sufficient to raise the a_w of the product into the zone of potential growth of pathogens. The increase in microbial risk will also depend on the likelihood of presence of the pathogens in these products.

C9. A reduction in the sugar level of some foods might necessitate changes in the composition of to ensure consumer acceptability is retained. Some of these changes might be in factors relating to the preservation of foods.

3. IMPLICATIONS OF CHANGES IN PRODUCT COMPOSITION ON MICROORGANISMS

The growth of foodborne microoganisms is affected by many properties of the product formulation, packaging and storage conditions. Levels of pH, water activity and preservatives will have been chosen to minimise the potential for growth of target groups of organisms. Any changes to the product composition will have an impact on the preservative factors and may allow growth of a group of organisms that was previously unable to grow, or it may allow more rapid growth of organisms that were already able to grow. It has been shown that reducing levels of fats and sugars may allow an increase in water activity values. The impact of water activity on important food pathogens is discussed below. This choice of pathogens was made to represent enteric pathogen, sporeforming pathogens, toxigenic and infectious pathogens as well as those, such as *Staphylococcus aureus*, with particular resistance to low water activity environments. These pathogens also represent the majority of literature available on the survival and growth characteristics of pathogens in relevant food types.

3.1 Product safety

3.1.1 *Staphylococcus aureus*

Staphylococcus aureus is the bacterial pathogen with greatest tolerance to low water activity in foods. This is the organism most likely to be able to grow if modifications to product recipes due to reduced fats or sugars resulted in a slight increase in water activity. The minimum water activity for growth of this organism is dependent on product type and generally the limiting a_w for toxin production is higher than that for growth. In microbiological media, growth of *S. aureus* occurred down to a level of 0.86 and when the inoculum level was high $>10^6$ per ml, toxin was also produced (Ewald and Notermans, 1988).

In an intermediate moisture food made with peanuts, raisons and honey, *S. aureus* was able to grow at a_w values near to 0.9 (Boylan, 1976). Growth also occurred at a_w 0.90 in salamis when the pH was 5.5, but *S. aureus* could only grow at an a_w of 0.925 when the pH was decreased to 5.0 (Martinez). This demonstrates the interactions between preservative factors in food systems to prevent growth of pathogens.

In another low a_w product, ground bread crumb, the minimum a_w for growth was even lower. The a_w of the bread was adjusted to between 0.793 and 0.909 using glycerol. Despite the range of a_w values the moisture content was consistent at approximately 26% and the pH was 5.2 – 5.5 (Feeherry, 2003). Growth of *S. aureus* occurred down to an a_w of 0.84. Growth was not able to occur in processed cheese products at an a_w

of 0.851 or 0.870 although it could grow in a range of imitation cheeses at a_w values of 0.942-0.972 and pH 5.37-6.34 (Bennett, 1983).

3.1.2 *Salmonella*

Unlike pathogens such as *S. aureus*, *Salmonella* does not need to be able to grow to cause illness as it has a low infective dose. There is a risk to food safety if there are viable cells remaining in the product at the point of consumption. The effect of water activity on survival of *Salmonella* in foods as well as growth is therefore important, especially for intermediate moisture foods which may be stored for extended periods. Studies looking at survival in salad dressings inoculated levels of $\log_{10}$ 5.7 or 2.4 into a range of six ranch dressings (3 low-fat) and 4 blue cheese dressings (2 low-fat). The fat levels ranged from 5.98 to 56.8 % fat and the pH values were 3.06-3.69. *Salmonella* was not able to survive in any of the dressings irrespective of fat level and no viable cells were detected after 24hrs storage. Due to the high acidity of these products, the reduction in fat was not a significant factor on the survival of *Salmonella* (Beuchat, 2006).

The effect of fat levels on the survival of *Salmonella* during manufacture of cheesecake were studied (Hao *et al*, 1998). Cheesecake made with standard fat levels (19.4%) and reduced (10.8%) fat levels were inoculated with low (10) and high (10^6) levels of *Salmonella* per gram. The pH was 4.95 for both recipes and although the moisture levels were higher in the reduced fat cheesecake the a_w was the same for both fat levels. The cheesecakes were baked in an oven at for 232ºC for 10 minutes then the oven temperature was reduced. The centre of the cakes did not rise above 68ºC. There were no survivors from the low inoculation levels but some survivors were found in the cheesecakes with high levels of *Salmonella*. There were no differences between the low and high fat recipes.

Survival of *Salmonella* was tested in low fat and standard fat peanut butters and spreads. Seven different recipes were evaluated with different levels of salts, sugars and fat levels. The a_w values ranged from 0.2 to 0.33. Following inoculation, the samples were stored at 5 or 21ºC for up to 24 weeks. There was a reduction in numbers by week 1. Less reduction was seen in the low fat products during the first 2 weeks of storage. This difference had gone by weeks 6 and 24 storage. The death rate was quicker at 21 than 5ºC but *Salmonella* was able to survive in all products over a 24week period (Burnett *et al*, 2000).

Salmonella is known to be able to survive in low a_w products such as chocolate and outbreaks of salmonellosis have been linked to consumption of chocolate in a number of cases during 1973 to 2002 in USA and Canada, England and Wales, Norway,

Finland and Germany (Baylis *et al*, 2004). An important feature of these outbreaks was the low level of contamination, typically 1-10 cells per gram.

No indication is given on the effect of variations in fat levels on the length of survival of *Salmonella*, although in a study (Baylis *et al*, 2004) on other enteric pathogens, it was found that survival of verocytotoxigenic *E.coli* (VTECs) was greatest in confectionery products with the lowest water activity (see 3.1.3).

3.1.3 *Escherichia coli*

Verocytotoxigenic strains of *E. coli* are similar to *Salmonella* in that they have a low infective dose and their survival characteristics in reduced fat and sugar foods are as important as their potential for growth. In the study with low fat dressings described above (Beuchat, 2006) *E. coli* O157:H7 was detected in most samples after 24 hrs and in some samples after 3 days but no viable cells were detected after 6 days.

L. monocytogenes showed similar survival characteristics to *E. coli* O157:H7, with some cells detected after 1 and 3 days but not 6 days. The authors concluded that shelf-stable pourable dressings with high or low fat levels did not support the growth or survival of *Salmonella*, *E. coli* or *Listeria* and are not considered a safety hazard.

Another area in which survival of *E. coli* and *Salmonella* has received some attention is in fermented and cured meats. Clacero and Beuchat (1996) demonstrated that *E. coli* O157:H7 was able to survive for 32 days in salami at a_w 0.95. In samples at a_w 0.90, cells survived during 16 days storage but not 32. Lethal effects of reduced a_w may not be evident during initial storage periods but may be more evident after prolonged storage. Further studies (Hewett *et al*, 2006), on chorizos, looked at survival of *E. coli*, *Salmonella* and *Listeria* in product at a_w 0.85, 0.9, 0.93, 0.95, 0.97. The fat levels ranged from 9.0% to 23.7%, although there was no correlation between the fat levels and the a_w of the products. Numbers of the three pathogens declined during 7 days storage although not at a rate sufficiently rapid to ensure safety of the product within the shelf-life. Death rates of all three pathogens showed interesting relationship with a_w. The maximum death rate for *E. coli* was seen at a_w 0.876 and at 0.873 for *Salmonella*. For *L. monocytogenes* it was 0.824. It is often the case that the death of microorganisms is greatest at values that are just sub-optimal for growth.

In this chorizo study the maximum death rate occurred for *L. monocytogenes* at a lower a_w than the Gram negative pathogens. The minimum growth activity for *L. monocytogenes* is also lower (0.90-0.92) than for *Salmonella* and *E. coli* (0.92-0.95).

The survival of *E.coli* O157:H7 and other VTECs was studied in chocolate and other confectionery products (Baylis *et al*, 2004). Several VTEC strains were inoculated into milk chocolate (aw 0.4), biscuit cream (a_w 0.75) and mallow (a_w 0.7) and stored at 10, 22 and 38ºC for up to a year. Death of the organisms was more rapid at higher temperatures. For example, in chocolate, *E.coli* was detected up to 43 days at 38ºC, 85days at 22ºC and >365 days at 10ºC. Similar temperature effects were seen for biscuit cream and mallow, although the length of survival time varied between products, with survival being longest in the lower a_w products.

3.1.4 *Listeria monocytogenes*

L. monocytogenes is fairly resistant to low water activity and can therefore tolerate higher levels of salt and sugar than most other pathogens, with the exception of *S. aureus*.

Petran and Zottola (1989) demonstrated that this organism was able to grow in 39.4% sucrose solutions (a_w 0.92) within 24 hours at 30ºC but not in 50% sucrose solutions (a_w 0.88). Similar results were obtained for growth of *L. monocytogenes* and *L. innocua* in solutions of sucrose, salt or glycerol (Nolan *et al*, 1992; Farber, 1992) where minimum a_w values found were of 0.92 in sucrose and salt solutions and 0.90 in glycerol.

As for most pathogens, the minimum growth a_w is often higher in food products than liquid substrates due to the presence of other antimicrobial compounds. Ingham (2005) looked at the inactivation of *L. monocytogenes*, *Salmonella* and *E. coli* during manufacture of a spiced fermented beef product. Three batches were prepared and inoculated with 10^6 of the three pathogens per gram. Initial a_w values were trial 1: 0.87, trial 2: 0.95 and trial 3: 0.84. No detectable *E. coli* or *Salmonella* were detected from finished product in trial 1 or 3. Low levels were detected on finished product in trial 2 possibly due to the higher initial a_w. This is in agreement with the data previously described by Hewett (2006) where pathogens were able to survive on higher a_w products and were inactivated on lower a_w recipes.

3.1.5 *Bacillus cereus*

The minimum a_w for growth of this organism is higher than some of the other pathogens discussed but due to the presence of heat resistant spores, this organism may survive baking or cooking and grow in finished product if the a_w is increased due to recipe modifications. Studies (Jaaskelain, 2003) looked at the potential for *B. cereus* to produce an emetic toxin in a range of bakery products obtained from different bakers. There was a wide range of a_w and pH for the different products. Growth and toxin were found in the higher a_w products (>0.953) stored at 21ºC, i.e.

meat pastry (filling and dough) and rye bread. No growth was observed at 8ºC for these products or at 8 or 21ºC for the lower a_w products such as jam rolls and muffins.

There is a wide variation in a_w present in the same type of product from the different bakers. Where such a variation exists naturally, it is possible to envisage how small changes to recipe may push the a_w into growth regions for pathogenic organisms.

3.1.6 *Clostridium botulinum*

The minimum a_w for growth of *Cl. botulinum* is well documented to be 0.94 for mesophilic (proteolytic) strains and 0.97 for psychrotrophic (non-proteolytic) strains. As with all pathogens, the presence of other factors will interact with the water activity to alter these minimum levels. Daifas (2003) showed growth of a proteolytic strain of *Cl. botulinum* at a_w values of 0.953 to 0.997 in broth systems. Time to growth was 3 days with 0 and 2% added ethanol. This was delayed significantly (49 days) in the presence of 4% ethanol and did not occur after 365 days in 6% ethanol. These studies were done in broth systems although the authors concluded that the data had application to foods either as an ingredient or as incorporation into the packaging as an ethanol sachet.

The growth and toxin production of proteolytic *Cl. botulinum* was evaluated in peanut spreads at a different a_w values. Growth occurred at a_w 0.96 and 0.98 but not 0.94 or 0.92 (Clavero, 2000).

3.1.7 Effect of water activity on heat resistance of pathogens

In general terms, increasing the water activity of a system will decrease the heat resistance characteristics provided the matrix and heat transfer properties of the food remain the same.

Alvarez (2006) looked at the heat resistance of *Salmonella* Senftenberg in different a_w broths. At 60ºC the Decimal reduction (D) value (i.e. the time to reduce the population by 1-log order) was 1.27min at an a_w of 0.99. This increased to 10.5 min at an a_w of 0.96 and to 15.5 min at an a_w of 0.93. Further studies (Mattick *et al*, 2000) showed increased heat tolerance of *Salmonella* in low a_w environments which was increased further if the cells were habituated to these conditions prior to heat treatment. They concluded that exposure of *Salmonella* to low a_w environments could reduce the effect of any subsequent heat treatment. The converse is also likely to be true that increasing the water activity of a product is likely to make the heat treatment more effective.

If the reduction of fat and sugar in a product is achieved by replacement with another ingredient then this may have some effects on thermal resistance characteristics dependent on the replacer used.

Summer *et al* (1991) looked at *S.* Typhimurium in sucrose solutions and chocolate syrup. The D value at 65.6ºC was 0.29min at a_w of 0.98, 4.8mins at an a_w of 0.89 and 40.2 min at an a_w of 0.83. However, when tested in four chocolate syrups, at an a_w of 0.75 to 0.84 the heat resistance was much lower than in sucrose solutions ranging from 1.2 to 3.2 min. It was concluded that the *Salmonella* was less resistant in the chocolate syrups than sucrose solutions at a similar a_w because, the sweeteners present may not have had such a protective effect on thermal injury as sucrose.

Kenney (2004), looked at the effects of different fat levels on the heat resistance of *L. monocytogenes*. The following values were obtained at 60ºC (Table 6).

Table 6
Effect of fat level and product type on heat resistance of *L. monocytogenes*

Product	Fat level(%)	D_{60} (min)
Peanut beverage	3.1	3.2
Whole fat milk	3.5	3.3
Whole fat choc milk	4	4.5
Reduced fat choc milk	1	5.9
Choc peanut spread	39	37.5
Peanut spread	53	26

The heat resistance of *Salmonella* in the low fat chocolate milk was higher than the other beverage products although the fat level was lowest. No data are given on the a_w values of these products but the low fat chocolate milk was the only one to contain corn syrup. The authors concluded that this may have reduced the a_w or affected the heat resistance characteristics of the cells, but without further information, this conclusion is hard to substantiate.

Spore forming organisms are also affected by the a_w of the heating medium. *B. cereus* spores were heated at 100ºC in a range of broths where the a_w was adjusted by sucrose. The D value was 8.71 min at a_w 0.98 and 19.10 min at a_w 0.92. This time, when the heat resistance was tested in a food product (cream chocolate), the heat resistance was higher than expected and was attributed to other aspects of the food product such as structure and texture (Leguerinel *et al*, 2005).

In general terms reducing the water activity of any particular product should increase the heat resistance of organisms. Extrapolation from sucrose or laboratory medium to

different foods may not be reliable as the heat resistance may either be higher or lower in the food matrix than anticipated due to the exact product composition. The effect of heat treatments should be verified for each product under consideration.

Conclusions on product safety due to compositional changes:
C10. If changes to food composition due to reduced fats or sugars result in an increased water activity then foodborne pathogens may be able to grow.

C11. Critical level of water activity for most bacterial pathogens is a_w 0.92. Staphylococcus aureus is more resistant and in products where these organisms may be present, the minimum a_w to prevent growth is 0.83.

C12. If changes to product composition result in an increased water activity, other preservative factors will need to be used to maintain product safety or the shelf-life may need to be reduced. Whilst there is some data available on the implications of fat/sugar reduction on the shelf-life of foods, it is insufficient to allow general recommendations to be made on suitable reductions in shelf-life to compensate for recipe changes. It is necessary for individual manufacturers to assess the impact of recipe changes on their own products on a product by product basis.

C13. The water activity of a product affects the heat resistance characteristics of microorganisms. Generally, as the water activity of a food increases, the heat resistance of an organism will decrease. There is generally no increased risk from survival of microorganisms in heat treated products with reduced levels of fats and sugar.

3.2 Product stability

Changes in food composition may increase the risk from growth of spoilage organisms. Table 7 (Appendix) shows the minimum water activity values for growth of these groups. Yeasts and moulds are of particular interest to low a_w foods as they are able to grow in much lower a_w environments than bacteria. For example, Gock *et al* (2002), showed that xerophilic fungi could grow at a_w values as low as 0.70. At these a_w values the growth rate was higher at pH 4.5 than 7.5. Unlike bacteria which are adversely affected by low pH, many yeasts and moulds are able to grow down to pH values as low as 1.5 and many prefer to grow in an acidic environment. Changes in a food to inhibit one microbial population, may enhance the growth conditions for another group of organisms.

One area where changes to product recipe may reduce the shelf-life due to increased microbiological growth is baked goods, particularly with respect to potential for

growth of moulds. The microbiological implications of changes in bakery goods are discussed below.

3.2.1 Fat and sugar reduction in bakery products – microbiological implications

Fat is a major component of many bakery products. It influences the eating properties, acts as a release agent, ensures good flavour release, influences batter and baking properties, gives cohesiveness and consistency to the dough, enables aeration, facilitates colour development, enhances the appearance, carries fat soluble compounds, influences shelf life and reduces the rate of drying out.

Sugar, in the form of sucrose, also fulfils several functions in bakery goods. Although a prime function is to add sweetness to a product, it is also important as a bulking agent, in structure and texture formation, in colour development and in extending product shelf life.

Within the diverse range of bakery products, a reduction in the levels of fat and/or sugar will have most impact on the microbiological stability of cakes and sponges, where water activities typically range from 0.8 to 0.9. Bread contains little or no fat and sugar. Biscuits, although containing significant amounts of these ingredients, are baked to low moisture levels where microbiological stability is assured. Baked pastry has a low water activity, but it is often found in combination with another component of higher water activity where this constituent will have a greater impact on the microbiological stability rather than the pastry itself.

A practical exercise was done in order to evaluate the likely effect of recipe changes on generic cake recipes. Six formulations were selected to represent common cake and sponge types and the influence of reducing the fat and/or sucrose content on the products equilibrium relative humidity (ERH) was determined, using ERH CALC™ and BALANCE software programmes. ERC CALC™ can be used to calculate water activity and ERH values and to predict mould-free shelf life. ERH is another measure of the water activity of a product and an ERH of 85% would equate to an a_w of 0.85, whilst one of 95% would equate to an a_w of 0.95.

BALANCE is typically used to develop new products by checking and adjusting ingredient quantities against the rules of cake making to see if the ingredients are within acceptable ranges. In this study it was used to help define the upper and lower limits of ingredient addition that might be used to produce a product that would still be recognisable.

The formulations selected were:

- High-ratio cake (plain)

- High-ratio cake (chocolate)
- Low-ratio cake (plain)
- Basic sponge (plain)
- Swiss roll (chocolate)
- Enriched sponge (plain)

The ERH of the original formulation was calculated using ERH Calc™. The formulation was then entered into the Balance programme and was modified to take the sugar content to the minimum level within the limits of the ingredient range built into the software. On occasion the programme prompted changes to the levels of other ingredients in order to balance the total ingredient mix within the product. Returning to the initial formulation, the minimum fat formulation was similarly determined. As egg has a significant fat content, to reduce the fat content further the egg level was adjusted to the mid-point of the acceptable range for egg. Finally a balanced formulation was derived which contained the minimum levels of fat and sugar in each product type[1]. Using ERH Calc™, the ERH of all the revised formulations were calculated. The fat content of all the formulations was calculated (using shortening 100% fat, egg 12.1% fat, cocoa powder 21.7% fat and emulsifier 100% fat). The results are summarised in Table 8. On some occasions, the changes that were achievable resulted in only small reductions in sugar or fat. In addition, an initial reduction in either the fat or the sugar levels could result in the requirement for an increase in the other ingredient to balance the recipe.

The impact on the minimum mould-free shelf life of the products was quantified by using ERH Calc™ to determine the mould-free shelf life of both the original formulations and the minimum sucrose and/or fat formulations at 21 and 27°C and calculating the difference in mould-free shelf life that the formulation change had made. The results are included in Table 9 and show the decrease in shelf-life obtained for the re-formulated cake compared to the standard mix.

[1] Using Balance software to the limits of the ingredients' ranges is permissible as a theoretical exercise to evaluate the effect of fat and sugar reduction on product ERH, however in practice the resultant formulations may have taken the fat and sugar reduction to extremes where product characteristics are compromised.

Table 8
Summary of fat and sucrose contents and calculated ERH values for cake and sponge formulations (in-going amounts)

Formulation	Fat content of batter (% of total)	Sucrose content of batter (% of total)	ERH (%) by calculation
High ratio cake (plain)			
Original formulation	13.3	26.3	83.1
Min. sucrose formulation	17.1	22.2	87.7
Min. fat formulation	8.0	29.7	85.6
Min. sucrose and fat formulation	8.6	24.9	88.1
High-ratio cake (chocolate)			
Original formulation	17.6	24.9	84.5
Min. sucrose formulation	17.8	23.0	85.8
Min. fat formulation	8.7	28.0	85.2
Min. sucrose and fat formulation	8.9	26.2	86.2
Low-ratio cake (plain)			
Original formulation	28.4	25.0	81.8
Min. sucrose formulation	12.2	24.9	83.8
Min. fat formulation	7.0	32.5	85.8
Min. sucrose and fat formulation	12.2	24.9	83.8
Basic sponge (plain)			
Original formulation	5.1	30.8	88.8
Min. sucrose formulation[2]	3.0	32.0	88.5
Min. fat formulation	n/a	n/a	n/a
Min. sucrose and fat formulation	n/a	n/a	n/a
Swiss roll (chocolate)			
Original formulation	5.4	30.0	89.3
Min. sucrose formulation	2.6	28.2	92.8
Min. fat formulation	n/a	n/a	n/a
Min. sucrose and fat formulation	n/a	n/a	n/a

[2] When lowering the sucrose content, modifications to the other ingredients to balance the recipe resulted in a lower total quantity of ingredients and hence the percentage of sucrose in the mix actually increased relative to the amount in the original formulation. The fat content is however lower due to a decreased egg level in the formulation.

Formulation	Fat content of batter (% of total)	Sucrose content of batter (% of total)	ERH (%) by calculation
Enriched sponge (plain)			
Original formulation	9.8	24.7	90.7
Min. sucrose formulation	9.8	24.7	90.7
Min. fat formulation	5.1	28.5	87.6
Min. sucrose and fat formulation	5.1	28.5	87.6

Table 9
Difference in mould-free shelf life with minimal sucrose and/or fat formulations

Formulation	Difference in mould-free shelf life at 21°C compared to original formulation	Difference in mould-free shelf life at 27°C compared to original formulation
High ratio cake (plain)		
Min. sucrose formulation	-9 days	-5 days
Min. fat formulation	-6 days	-4 days
Min. sucrose and fat formulation	-10 days	-6 days
High-ratio cake (chocolate)		
Min. sucrose formulation	-3 days	-2 days
Min. fat formulation	-2 days	-1 days
Min. sucrose and fat formulation	-4 days	-2 days
Low-ratio cake (plain)		
Min. sucrose formulation	-6 days	-3 days
Min. fat formulation	-10 days	-6 days
Min. sucrose and fat formulation	-6 days	-3 days
Basic sponge (plain)		
Min. sucrose formulation	0 days	0 days
Swiss roll (chocolate)		
Min. sucrose formulation	-2 days	-1 days
Enriched sponge (plain)		
Min. sucrose formulation	0 days	0 days
Min. fat formulation	+3 days	+2 days
Min. sucrose and fat formulation	+3 days	+2 days

The results show the trend is for an increase in ERH value with a reduction in sugar and fat in cakes and some sponges, with a corresponding decrease in mould-free shelf life. In the enriched sponges a reduction in fat content, with a corresponding increase in sucrose content, leads to a decrease in ERH value and hence an increase in mould-free shelf life. The loss in mould-free shelf life varied for the different formulations but in general, within these model systems, the loss ranged from 15-60% for the high-ratio cakes, 25-50% for the low ratio cakes and 25-40% for chocolate swiss rolls stored at both 21 and 27°C. There was no loss in mould-free shelf life with the recipe modifications to the basic and enriched sponges as the sucrose levels were unchanged or even increased to ensure a balanced recipe.

4. RISKS WITH DIFFERENT FOOD GROUPS

Taking into consideration the likely changes to product composition and thus microbiological stability of the foods, it is possible to identify which product groups are likely to increase the risk of microbiological spoilage or food poisoning. A brief statement is given below for some food categories.

4.1 Cakes

This is one of the categories of products where changes in product composition could increase the risks from microbiological spoilage or poisoning. These products rely heavily on fat and sugar in their formulation. It has been shown how recipe changes may lead to an increased water activity. If this is not balanced by other preservative factors then the shelf-life may need to be reduced to ensure the product remains microbiologically safe and stable.

4.2 Confectionery

Hard boiled sweets, candies and jellies rely on sugars for their functionality and microbiological safety. Any reduction in sugar is unlikely to lead to an increased microbiological risk with these products as any increases in the a_w would not take it into the growth region of food poisoning or food spoilage bacteria.

Toffees and fudges rely on sugars and fats to provide the correct product functionality. Again, any increase in a_w seen as a result of changing formulation are unlikely to increase the microbiological risk as maintenance of low a_w is crucial for the sensory and physical properties of the product.

Chocolate has a low water activity (a_w 0.30 to 0.40) and any increase in a_w as a result of reducing fat or sugar levels is unlikely to increase microbiological risks as such changes are unlikely to take the products into the region of microbiological growth.

4.3 Dairy products

Butters and spreads

These products contain high level of fats but not sugars. Changes to the fat levels of a spread may change the types of structure. Spreads with more than 15 to 20% fat are generally water-in-oil emulsions whilst spreads with lower fat levels are oil-in-water emulsions. Droplet size is important with respect to microbial growth. Finer emulsions with a droplet size smaller that 10 microns are not conducive to microbial Those with larger sizes are better able to support microbial growth. In low fat products the water droplets are larger and provide more growth potential. The ability to grow will depend on the pH, and humectant level within the water droplets. In a study of 5 different yellow fat spreads (ranging from 30 to 80% fat), there was no growth of the inoculated pathogens, *Salmonella*, *E.coli* O157:H7 or *L.monocytogenes* (Holliday and Beuchat, 2003). In this study, reducing the fat level did not affect the survival of the pathogens.

Milk and cream

The heat treatment given to pasteurised or UHT treated milk and cream is designed to achieve a reduction in target pathogens ad enzymes which could affect the quality of the milk. The efficacy of the pasteurisation treatment is not affected by the level of fat present.

4.4 Savoury chilled foods

For meat products and pastry products such as sausage rolls, reduction of fat levels may affect the product water activity. The water activity of these products is fairly high before recipe modification so small changes has the potential to increase the microbiological risk from these products. These products contain minimal levels of sugar.

Pizza consists of a dough base and an assortment of toppings including cheese and meat products. For chilled pizzas it is likely that changes to one or more of these components would increase the growth potential of pathogens or spoilage organisms present. It is possible that the shelf-life may need to be reduced to compensate for recipe changes.

4.5 Ambient stable products

Jams and pickles contain large amounts of sugar which reduces the a_w below the growth region of bacterial pathogens. Reductions in sugar levels may be sufficient to allow the enhanced growth of yeasts and mould or spoilage bacteria. These products are not solely reliant on sugar but also heat treatments, pH and preservatives. These factors will need to be increased to minimise the microbiological risks.

4.6 Chips

The majority of manufactured chips eaten by consumers will be stored frozen. Any changes in fat levels on such products are unlikely to have any microbiological implications as growth of microorganisms is inhibited during frozen storage. Any organisms present are likely to be destroyed by subsequent heating.

4.7 Soft drinks

The stability of soft is based on low pH (2.0 –3.5), heat and presence of preservatives. Many drinks contain high levels of sugar which may reduce the water activity of the product sufficient to prevent growth of spoilage yeasts or moulds. The pH is low enough to prevent growth of any pathogenic bacteria. If changes to the sugar levels increase the water activity then it is possible that spoilage yeasts and moulds could grow. It should be ensured that the other antimicrobial factors present are sufficient to manage microbial spoilage.

4.8 Savoury snacks

This group consists of ambient stable snack products e.g. extruded flavoured maize products, crisps, cheese biscuits. The a_w of these products is critical to aspects of product quality such as texture. Products are often packaged in modified atmospheres such as nitrogen to prevent oxidative rancidity and staling. Changes in fat levels are unlikely to increase the microbiological risks from these products as they would not be sufficient to take the products into the region of microbiological growth.

Conclusions on product at greatest risk

C14. Any product group has a potential microbiological risk if the reduction in fat and/or sugar levels increase the water activity sufficiently high to increase microbiological growth.

C15. Some products rely exclusively on sugar for microbiological stability. Such products may be subject to microbial spoilage, e.g. jams. Such products are likely to be subject to microbiological spoilage if sugar levels are reduced. Other products

rely on fat levels in their formulation, e.g. sausages. Such products may be a potential risk from growth of food spoilage or food poisoning organisms if the composition is changed. Products which rely on both fats and sugars in their composition, e.g. cakes, may also be at potential risks from both food spoilage or food poisoning organisms.

C16. Any change to product composition should be assessed using the principles of HACCP. The shelf-life should be reduced accordingly or alternative preservation factors used.

5. ALTERNATIVE PRESERVATION STRATEGIES

Preservation techniques which are designed to prevent microbial growth in foods include low temperature storage, reduction of water activity (a_w), reduction of pH, modification of oxidation/reduction (E_h), addition of competitive microorganisms and addition of preservatives (Marechal *et al*, 1999). In addition, physical treatments such as heat, ultrasound, broad spectrum and ultraviolet light and high pressure could be used. It is unusual to use only one of these factors to achieve product stability. Combinations of preservatives can be more effective than just using one and the use of combinations is referred to as the hurdle effect (Leistner, 1978, 1995; Leistner and Gorris, 1995). By using a number of different means of inhibition, it is possible to apply each individual hurdle in a reduced intensity and result in food products that are safe, have adequate shelf life and may be more acceptable to consumers (Leistner, 1992, 1995). The combined hurdles may have an additive or even a synergistic effect, allowing combinations that achieve microbial stability and safety to be chosen.

One of the preservatives most commonly used in manufactured foods is salt. However, it is not recommended that the salt levels of foods are increased as a means of counteracting reductions in levels of fats or sugars as this is not in line with the Food Standard Agency's salt reduction strategy. Similarly, an increase in levels of chemical preservatives may not be desirable for many food manufacturers and retailers as there is a move towards, preservative free foods.

Where there are no easy alternative preservation systems that can be used, then it may be necessary to reduce the shelf-life of foods to maintain the microbiological quality of the product. No rules can be given on the amount of reduction that will be required as this will vary between products and will need to be assessed by practical studies on a product by product basis.

Changing fats and sugars has its primary effect on the water activity of a product. The amount of each factor needed to change the water activity is very different.

About ten times (w/w) as much sucrose is needed as salt to reach the same activity value (Table 10, Appendix). This is interesting with respect to product formulation as a reduction in sucrose of 10g per 100g would have the same effect on water activity as reducing 1g salt per 100g.

When developing new recipes or modifying old recipes the microbiological risks must be assessed. This can be done using microbiological models or laboratory studies such as shelf-life trials and challenge tests. No modifications to recipes should be done without assessing the impact of those changes. In addition, there may be non-microbiological consideration to take into account when modifying recipes. Such changes are outside of the scope of this review, but, for example, increasing the glycerol level in heat treated foods may well increase the level of undesirable chloropropanols in the food such as 3-MCPD. All changes to product safety should be considered by food manufacturers when changing recipes or developing new recipes.

An overview of different hurdles and how they impact on product shelf-life is given by Betts (2006). A summary of some of these strategies is given below:

Natural antimicrobials

Many natural food ingredients which are traditionally added to achieve a desired flavour, also have the potential to control microbial growth. This is known to be true for vegetable extracts, mustard, onion, garlic, horseradish and a range of other herb and spice ingredients including extracts and essential oils from the plants, which have been shown to inhibit the growth of a range of microorganisms. Natural antimicrobial compounds and their possible modes of action have been reviewed extensively (Nychas, 1995; CAST, 1998), but because they contain a variety of compounds from different chemical classes, it is not possible to identify a single mechanism by which all of these compounds act on microorganisms.

Essential oils are natural mixtures of aromatic compounds present in plants that are extracted by steam or solvent distillation, in yields of between 0.01% and 2.0% (calculated on the weight of fresh plant distilled). These compounds have been shown to have both fungistatic and bactericidal activity to suppress infection by plant pathogenic microorganisms. The flavour and fragrance industry and natural product industries use these compounds routinely. Such compounds that are approved for use in foods and combine antimicrobial activity with low toxicity have great potential as natural food preservatives.

The active components found in herbs and spices (e.g. thymol from thyme and oregano, cinnamaldehyde from cinnamon, and eugenol from clove) have been shown to have a wide spectrum of antimicrobial activity (Martini *et al*, 1996; Friedman *et al,* 2000; Lambert *et al*, 2001). There is the potential to add such products to savoury

pastry and meat dishes but the antimicrobial effects would need to be studied by challenge test studies on a product by product basis to demonstrate their efficacy.

Chemical Preservatives

There are a number of chemical preservatives than can be used in food products to inhibit growth of microorganisms.

For example, sodium benzoate and potassium sorbate are used for fruit products, pickles, mayonnaises and dried fruit. The active ingredient of many of these preservatives is the organic acid e.g. benzoic or sorbic acid. Preservatives are generally used for lower pH foods because they work better in acidic environments at or near their P_ka values.

Sorbic acid and potassium sorbate are widely used throughout the food industry for the preservation of cheese, in bakery products, vegetable based products (pickles, olives, fresh salads), fruit based products (dried fruits, fruit juices), beverages and some other products such as smoked fish, margarine and mayonnaise.

Sorbate is more inhibitory to yeasts and moulds than bacteria. They are more effective against catalase positive organisms that catalase negative, and aerobes rather than anaerobes (Sofos and Busta, 1983) which means that they are extremely useful in preservation of fermented foods.

Sodium benzoate is commonly used in products where pH is low, for example mayonnaise, pickled vegetables, fruit products and drinks. It is commonly combined with potassium sorbate in mayonnaise type products. This is because the mixture of the two preservatives is more effective than either of them individually and also sorbate is tasteless (Lueck, 1980). Benzoate exerts its primary antimicrobial action upon yeasts and moulds.

Propionates are used in cheese production to prevent mould growth on the cheese surface. The main forms used are sodium and calcium propionate It is also used widely with bakery products, and like sorbate and benzoate its action is pH dependent. However, it is able to work at higher pH values which makes it suitable for bakery use. Its mode of action is also similar to that of sorbate and benzoate, because it accumulates within the cell and acts upon enzymes (Leuck, 1980).

Physical treatments

There are a number of physical treatments that can be used to achieve inactivation of microorganisms instead of heat treatments.

High pressure

A wide range of pathogens and spoilage organisms are inactivated by high pressure and it also appears that there is a link between cell shape, size and cell wall structure, and the effectiveness of pressure treatment (Hoover *et al* 1989). For example, yeast cells are more sensitive that bacteria and inactivation begins at 200MPa. Gram negative rods are the next sensitive, where greater than 350MPa may be required to cause injury or death (e.g. *Escherichia coli* and *Pseudomonas aeruginosa*). The Gram positive bacteria are more resistant to pressure than Gram negative bacteria, requiring greater than 400MPa (e.g. *Listeria monocytogenes*) and Gram positive cocci such as *Staphylococcus aureus* may require pressures greater than 450MPa to cause inactivation. Finally vegetative cells are more susceptible than spores which require greater than 600MPa, usually in cycles (6 cycles of 600MPa at 70ºC), to achieve spore inactivation (Smelt 1998). A suggested explanation for the lower pressure resistance of Gram-negative microorganisms is that the more complex cell membrane has greater susceptibility to environmental changes brought about by pressurisation.

High pressure is used for liquid and fruit products, jams and pâtés and ethnic products like guacamole. It is used for heat sensitive products where a pasteurisation or sterilisation effect is required but where the application of heat may damage the product.

Ultrasound

Ultrasonication is known to disrupt biological structures and has been shown to be effective in destroying a range of different spoilage bacteria and pathogens. This technique relies on application of sound waves through a liquid medium and is useful for sterilisation of surface of vegetable or protein products such as chicken.

The research done to date involving ultrasound has important implications for the food industry. Present sterilisation and pasteurisation methods involve high temperature processing which can give rise to undesirable flavours and textures in some foods. It is envisaged that the use of ultrasound treatments could lead to a decrease in process times and/or temperatures and a subsequent improvement in product quality and convenience to the consumer.

Pulsed light

Light in the form of ultraviolet (UV) light has been used for many years to sterilise hospital equipment, water and other liquids. It has also been used to sterilise food packaging and the surface of food products such as meats or baked goods.

More recently, a different type of light energy has been investigated for use i.e. pulsed light technologies. The main difference between the germicidal UV lamps and pulsed

light technology is that the latter uses intense pulses of broad spectrum light that include all the wavelengths of natural sunlight.

Hard crusted white bread rolls inoculated with mould spores were exposed to 2 pulses of light (0.5 ms duration time, 16 J/cm^2 per flash and 1Hz). Pulsed light was able to eliminate mould spores without burning the food product. Baked cake surfaces inoculated with mould spores were packed in clear plastic containers. After treatment with 3 pulses of 16 J/cm^2 per pulse at 5 second intervals, they were stored at room temperature and checked for mould growth. Untreated caked exhibited mould growth within 3 days, whilst the average number of days before visible mould growth appeared on the treated cakes was 10. This technology has the application to surface treatment of bakery products.

6. NON-MICROBIOLOGICAL RISKS

The main issues with reducing the levels of saturated fat and sugar in foods is if they have to be replaced with something else. Often, this will be a low calorie substitute which may be metabolised differently than the original ingredient.

With regard to fats in foods, there is a requirement to reduce the total level of fats in order to reduce the energy in-take. As well as this, there is a requirement to reduce the type of fat in foods as they have different effects on consumer health. Fats exist in a number of forms. In simple terms they are saturated fats e.g. lard which are solid at room temperature, or unsaturated fats e.g. vegetable oils which are liquid at room temperature. Saturated fats are known to increase levels of undesirable types of cholesterol in the blood and have been linked to a range of diseases such as coronary heart disease, hypertension and Type 2 diabetes.

The basic structure of fat consists a backbone of carbon atoms, each of which can link to four other atoms. In saturated fats, all the bonds for each carbon atom are used by other carbon or hydrogen atoms. Thus a long straight chain is formed containing only single bonds. In unsaturated fats, not all the bonds for each carbon group are used by hydrogen atoms and so a double bond can be formed between two carbon atoms. Kinks can occur in the chain at the point of a double bond. Either both ends of the chain can pull towards each other (*cis* fatty acid) or they can pull away from each other as in a *trans* fatty acid (TFA). The type of rotation around the double bond in a TFA means it has the structure and function of a saturated fat and thus has the same implications for consumer health. TFAs are naturally present in some foods, e.g. milks, cheeses and meats but they are formed during hydrogenation of oils which is used to transform a liquid unsaturated fat into a solid fat with more desirable functional properties

With regards to sugar levels, the main non-microbiological risk involve the metabolic degradation of sugar replacers. Low digestible carbohydrates such as mannitol, xylitol, sorbitol, are only partially digested in the intestines. Excessive consumption may have a laxative effect and products containing these sugar replacers should be clearly labelled to this effect.

Conclusions on non-microbiological risks

C17. When reducing the levels of saturated fats in foods, the levels of Trans Fatty Acids present should be minimised and certainly not allowed to increase as a direct result of changes to recipe/processing conditions.

7. USE OF PREDICTIVE MODELS

Predictive microbiological models involve the use of mathematical equations to estimate the likely growth of bacteria under different conditions. They are developed using laboratory data from one set of experimental conditions and can be used to predict the likely responses under new sets of conditions not previously tested. Modifications of new or existing recipes can be evaluated on the computer using a predictive model very quickly and easily, before embarking on expensive laboratory experiments or pilot scale production runs.

With respect to product safety, there are a number of models which can be used to assess the likely growth, survival or death of foodborne pathogens under a variety of environmental conditions. Until recently, there were two systems that could be used for predicting growth of food pathogens; the US pathogen modelling program (PMP) http://www.arserrc.gov/mfs/pathogen.htm (PMP) and the UK Growth predictor system http://www.ifr.ac.uk/safety/GrowthPredictor/GPsetup.zip .

Recently, these systems have been merged to give COMBASE predictor. This system now allows fluctuating temperature profiles to be modelled. This system is free on the internet http://www.combase.cc/predictor.html. The system includes models for *Salmonella*, *Listeria* and *Escherichia coli* O157, *Bacillus cereus*, *Clostridium perfringens* and *Clostridium botulinum* (Table 11, Appendix).

It is important to be able to predict the growth of food spoilage organisms when considering the likely stability and shelf life of food products. The *FORECAST* system developed at CCFRA, contains models for specific spoilage groups, for example; *Pseudomonas* species, Enterobacteriaceae, lactic acid bacteria or for a mixture of spoilage organisms relevant to food commodities, fish products and meat products. Full range of models is shown in Table 12 (Appendix). In addition, a range of models were produced for acetic acid preserved foods which allow predictions to

be made on the likely grow of acid tolerant organisms as affected by levels of preservatives, acidity and sugars/salt (Table 13, Appendix).

These models are powerful tools to evaluate the effect of reducing the levels of fats and sugars in a product. It is worth noting that most models do not allow a direct input of water activity but rather, they rely on conversion of water activity into a level of solute, usually salt. Model predictions to demonstrate the effect of product changes on microbial growth will therefore be based on the water activity of the original and reformulated recipes converted to a solute level for input into the relevant models.

In order to demonstrate the effect of changing composition, a series of practical exercises were done. Table 1 shows the difference in lag time and time to reach a level of 10^6 cfu/g for a range of food spoilage and food poisoning organisms in two frankfurter recipes. Dependent on organism the lag time could be as much as thirty times shorter in the higher a_w product. Table 14 (Appendix) shows how the acid club models can be used to assess the effect of reducing sugar levels in a ambient stable product. At a pH of 3.5 and potassium sorbate level of 400ppm, there was no likely to be no microbial growth during a 93 day shelf-life for product with 40% and 30% sugar. When the sugar was reduced to 20% and 10%, growth was predicted to occur and required the sorbate levels to be increased to 800ppm and 1600ppm respectively to prevent growth.

Similar data can be seen for spoilage in broths containing various levels of sugar and preservatives (500ppm sorbate) and a low level of alcohol (2%v/v) (Table 16, Appendix). At a constant pH of 5.0, the time to visible growth was 194 days at 60% sucrose to as little as 12 days at 20% sucrose. By decreasing the pH level of the product, it was possible to extend the time to growth, thus demonstrating how changes in sugar level may necessitate changes in other factors.

A final example is shown in Figure 2, where growth of meat spoilage organisms have been predicted at different a_w values. It can be seen that growth rate increases as the a_w increases. If the shelf-life of this product was limited by a level of 10^6 organism then the shelf-life would be 5days, 7days, 10 days, or 15 days as the a_w decreased.

Conclusions on use of predictive models

C18. Any change in product recipe should be evaluated for effect on microbial growth. Predictive models are useful tools to enable predictions to be made of the likely change in product composition on food spoilage organisms and pathogens.

8. CONCLUSIONS

The scope of this review was to evaluate the microbiological risks associated with a reduction in fats and added sugars in foods. Consideration has been given to the likely changes in food composition that may arise as a consequence of changing levels of fats and sugars, and how these changes might impact on foodborne microorganisms.

Conclusions relevant to each section (C1 – C18) have been given in the body of the review. Some general conclusions are given below.

(i) Microbiological safety and structural properties of a food are both achieved by a careful balance of ingredients, manufacturing conditions and preservative factors. Any change in a single component of a food may affect its microbiological safety and stability.

(ii) Changing the levels of fats and sugars in a food (by their removal or replacement) is likely to result in a change of water activity. For many foods, the maintenance of a low water activity is essential to its safety and stability. Any increase in the water activity could increase the risks of microbiological spoilage or food poisoning.

(iii) In order to maintain the safety of reduced fat/reduced sugar foods, there may be a requirement to increase the levels of other preservative factors, e.g. the acidity or level of chemical preservatives. Where such a change is not desired, then the shelf-life may need to be reduced to maintain product safety.

(iv) An increase in the levels of water activity may impact on other aspects of the food preservative factors. This may have either a negative or a positive impact on the product safety. It is not possible to predict what these changes will be as they will vary from product to product. Any change to product recipe should be evaluated in accordance with the principles of HACCP.

(v) For some food products, e.g. candies, toffees and cookies, it is unlikely that the reduction in fat and sugar levels would increase the microbiological risks from these products as the changes in water activity would not be sufficient. In other products, e.g. cakes, muffins, savoury pastries, frankfurters and cheese spreads, it is likely that the reduction in levels of fats and sugars would be sufficient to increase the risks of microbiological growth either by food spoilage organisms or food pathogens.

(vi) This review has highlighted various gaps in the literature with respect to the effect of reduction in fats and sugars on growth of microbial pathogens. Much of the data available has looked at the effects of different levels of water activity on microbial growth in laboratory media. More research is needed in real foods. There is little or no information on the level of fat or sugar reduction that is technologically feasible in order to maintain the essential characteristics of a product. There is also limited information on the level of shelf-life reduction that may be required to balance the effects of reducing levels of fats and sugars in foods.

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APPENDICES

Appendix I: Industry Statements

The food industry are working closely with the Government to try to address healthy eating issues. A number of manufacturers and retailers were approached for comment on the reduction in fat and sugar in foods. Due to the time constraints of the project, it was only possible to obtain a few responses.

These are written anonymously below:

'Our overall position is, as ever, to offer consumer choice. In the first instance, that is about making the nutritional content of foods clear.

From the point of view of the fat/sugar issue, we backed the "gradual reduction" approach on salt and are of the view something similar may arise with fat/sugar. That being said, the scope for minimisation is probably far more limited, as the fats and sugars have a structural-function part to play in many foods, to a far greater extent than sodium. To take the analogy of Shortbread: Shortbread comprises flour, sugar, butter and a pinch of salt. There is little scope for change before the product simply stops being recognisable or acceptable as Shortbread. The same fundamentals apply to many types of foods.

So our development strategy is minimisation, but we believe scope is often limited. It is difficult to make, say, a Pork Pie without pork and lard or a Christmas Pudding without sugar.

We are mindful of microbiological risks and always take these in to account. We have very little reduced fat/sugar variants; this reflects the type of products we make. We believe the answer is clear labelling, clear choice'.

'We have significant work going on into reducing salt, sugar and fat in food aligned to our nutrition signposting work on front of pack.

Every modification in an own brand product has to be re-assessed for safety and shelf life prior to sale. Changes affecting water activity will generally result in the allocation of reduced shelf life for safety reasons unless of course the existing shelf life is already reduced due to quality reasons, i.e. the maximum safe shelf life is often not used as the product quality deteriorates much sooner.

This work is coinciding with a move to cleaner ingredient lists and hence the removal of preservatives and it would be very unusual to compensate for reduced salt or fat by adding a preservative - it is much more likely that we would reduce life or try to justify the same life with proper microbiological challenge tests/shelf life trials'.

Appendix II: Tables

Table 1

Use of predictive models to demonstrate the effect of frankfurter a_w on the growth of spoilage organisms and pathogens. Data assumes a constant pH of 6.0, and a constant temperature of 8ºC

Organisms	A_w 0.95		A_w 0.99	
	Lag time (hr)	Time to reach 10^6/g [a] (hr)	Lag time (hr)	Time to reach 10^6/g [a] (hr)
Enterobacteriaceae	1050	Not reached	30	92
Pseudomonas	55	132[b]	33	77
Meat spoilage organisms	84	328[c]	16	104
Lactic acid bacteria	1400	Not reached	44	107
Salmonella	100	710[d]	42	340
L. monocytogenes	100	500	30	180
B. cereus	300	1000	70	200
Cl. botulinum (psychrotrophic)	600	1400[e]	130	310
E. coli (10ºC) [f]	130	1000[g]	25	200
S. aureus	100	700	50	390

[a] initial level 10^2/g

[f] minimum temperature in the model

minimum a_w in model = [b] 0.977, [c] 0.964, [d] 0.973, [e] 0.974, [g] 0.961

Table 7
Minimum growth conditions for microorganisms which may be associated with chilled food

The table lists various species and indicates approximate growth and survival limits with the various factors acting alone. INTERACTIONS BETWEEN FACTORS ARE LIKELY TO CONSIDERABLY ALTER THESE VALUES.

Type of Microorganism	Minimum pH for Growth	Minimum A_w for Growth	Minimum Growth Temp □C
Salmonella	3.8 (1)	0.92 - 0.95 (2)	5.2 (3) 4 (3a)
Staphylococcus aureus	4.0 (1)	0.83 (4)	7 (4)
Bacillus cereus (spores/heat resistant)	4.9 (1)	0.93 – 0.95 (2)	4 (9)
Clostridium botulinum			
proteolytic A,B,F	4.6 (5)	0.94 (2)	10 (10)
non-proteolytic B, E,	4.7 (1) 5.0 (5)	0.97 (2)	3.3 (10)
Listeria monocytogenes	4.3 (1)	0.92 (6)	-0.4 (12)
Escherichia coli	4.4 (1)	0.935 (6)	Ca. 7-8 (15)
Psychrotrophic spoilage			
(*Pseudomonas*)	5.0 (5)	0.97 (27)	0 (36)
(*Enterobacter aerogenes*)			
(Lactic acid bacteria)	3.5 (7)	0.90 (18)	4 (35)
Micrococci			
Yeasts	1.5 (8)	0.62 (19)	Pink yeast –34 (37)

Type of Microorganism	Minimum pH for Growth	Minimum A_w for Growth	Minimum Growth Temp □C
Moulds	1.5 (8)	0.61 (17)	Unspecified moulds -12 (37)
Clostridium perfringens	4.5 (7)	0.93 – 0.95 (2)	
Vibrio parahaemolyticus	4.9 (1)	0.94 (2)	5 (13)
Yersinia enterocolitica	4.4 (1)	0.96 (6)	-1.3 (14))
Aeromonas hydrophila	<4.5** (11)	0.97 (6)	-0.1 (16)

Adapted from Betts *et al* 2004

Table 10
A_w values of different salt and sugar solutions –to be completed

% NaCl (w/w)	a_w	% Sucrose (w/w)	a_w
1.7	0.990	15.5	0.990
3.4	0.980	26.1	0.980
5	0.971	-	-
6.6	0.960	39.7	0.960
9.4	0.940	48.2	0.940
10	0.934	50	0.935
10.5	0.929	51	0.932
11	0.924	52	0.928
11.5	0.921	53	0.925
12	0.917	54	0.920
12.5	0.913	55	0.915
14.2	0.900	58.5	0.900
16.3	0.880	62.8	0.880

Table 11
Models available in Combase Predictor

Growth Predictor					
Model	**Temperature (°C) ***	**NaCl (% aq)**	**a_w**	**pH**	**Other Conditions (only one extra condition permitted per prediction)**
Aeromonas hydrophila	2.0-25.0	0.0-4.5	(0.974-1.000)	4.6-7.5	-
Bacillus cereus	5.0-34.0	0.0–9.4	(0.940-1.000)	4.9-7.4	CO_2 0-60%
Bacillus licheniformis	13.0–34.0	0.0–13.5	(0.907-1.000)	4.0-7.6	-
Bacillus subtilis	10.0–34.0	0.0–10.3	(0.933-1.000)	4.3-7.8	-
Brochothrix thermosphacta	0.0-30.0	0.0-8.0	(0.950-1.000)	5.5–7.0	-
Clostridium botulinum (non-proteolytic)	4.0-30.0	0.0-4.5	(0.974-1.000)	5.1-7.5	-
Clostridium botulinum - proteolytic)	14.0–30.0	0.0–7.5	(0.954–1.00)	4.7-7.2	-
Clostridium perfringens	15.0–52.0	0.0–5.0	(0.971-1.000)	5.0-8.0	-
E. coli	10.0-30.0	0.0–6.5	(0.961–1.00)	4.5-7.0	0-100% CO_2
Listeria monocytogenes/innocua	1.0-35.0	0.0–11.4	(0.924-1.000)	4.4-7.5	0 - 200 ppm $NaNO_2$ 0-100% CO_2 0-20,000ppm lactic acid 0-10,000ppm acetic acid
Salmonella	7.0-30.0	0.0–4.6	(0.973-1.000)	3.9-7.3	0 – 100% CO_2 0-200ppm nitrite
Staphylococcus aureus	7.5-30.0	0.0–13.5	(0.907-1.000)	4.3-7.1	-
Yersinia enterocolitica	0.0-30.0	0.0–7.0	(0.957-1.00)	4.4-7.1	0-10,000 ppm lactic acid 0 – 80% CO_2

* fluctuating temperature profiles can be used.

Table 12
Growth models available within the FORECAST system for growth of spoilage organisms

Model	Temperature (°C)	NaCl (% aq)	a_w	pH	Other Conditions
Pseudomonas	0-15	0.0-4.0	(0.970-1.000)	5.5-7.0	Fluctuating temperature
Bacillus spp.	5-25	0.5-10	(0.946-1.000)	4.0–7.0	Fluctuating temperature
Enterobacteriaceae	0-30	0.5-10	(0.943-1.000)	4.0-7.0	-
Yeasts (chilled foods)	0-22	0.5–10	0.870-1.000	2.6–6.0	Fluctuating temperature
Yeasts (fruit/drinks)	0-22	-	(0.946-1.000)	2.0–7.0	0 - 60 % Sucrose (w/v) 0 – 20% Ethanol (v/v) Potassium sorbate 0 – 1000 (ppm)
Lactic acid bacteria	2-30	0.5-10	(0.870-0.989)	3.0–6.0	Fluctuating temperature
Meat Spoilage	2-22	0–6		4.6–7.0	0 – 240 KNO_2 (ppm) Fluctuating temperature
Fish Spoilage	2-22	0-6	(0.943-1.000)	4.5– 8.0	Fluctuating temperature

Table 13
Acid preservation club models and ranges of conditions over which predictions can be made (at a constant temperature of 25°C)

Organisms	Phase	Prediction categories	pH	a_w	Salt %w/v	Preservative ppm
Cold fill spoilage	1	1 =G in 14d 2 =G in 15-30d 3 =G in 31-60d 4 =G in 61-182d 5 =NG in 182d	2.8 – 5.0	0.85 – 1.00	0.5 – 18	Benzoate Sorbate 0–2000 (in total)
Cold fill pathogens	1	1 =G in 120d 2 =NG in 120d	3.9 – 5.0	0.87 – 1.00	0.5 – 16	Benzoate Sorbate 0–2000 (in total)
Hot fill spoilage	1	1 =G in 14d 2 =G in 15-30d 3 =G in 31-60d 4 =G in 61-182d 5 =NG in 182d	3.7 – 5.2	0.86 – 1.00	0.5 – 18	Benzoate Sorbate 0–2000 (in total)

Cold fill spoilage = CIMSCEE culture, acid adapted yeast, moulds and lactic acid bacteria
Cold fill pathogens = *E. coli*, *S. aureus* and *Salmonella*
Hot fill spoilage = Sporeformers e.g. *B. coagulans*, *C. pastuerianum*

Table 14

Use of acid club models to demonstrate the effect of sucrose level on likely growth of acid tolerant spoilage organisms

Sucrose (%w/v)	40	30	20		10	
PH	3.5	3.5	3.5		3.5	
Sorbate (ppm)	400	400	400	800	400	1600
Growth	NG	NG	G	NG	G	NG

Table 15

Use of yeast model to demonstrate the effect of sucrose level and pH on time to growth (d) of spoilage yeasts (other factors fixed at 10ºC, 500ppm sorbate, 2% alcohol

	Sucrose level (% w/v)				
	60	50	40	30	20
pH 5.0	194	58	24	14	12
pH 4.5	297	88	37	22	18
pH 4	489	146	61	36	30
pH 3.5	869	260	109	64	53
pH 3	1664	498	209	123	102

Figure 2

Predicted growth of meat spoilage organisms as affected by variations in water activity (other conditions held constant at 8ºC, 100ppm nitrite and pH 6.0)

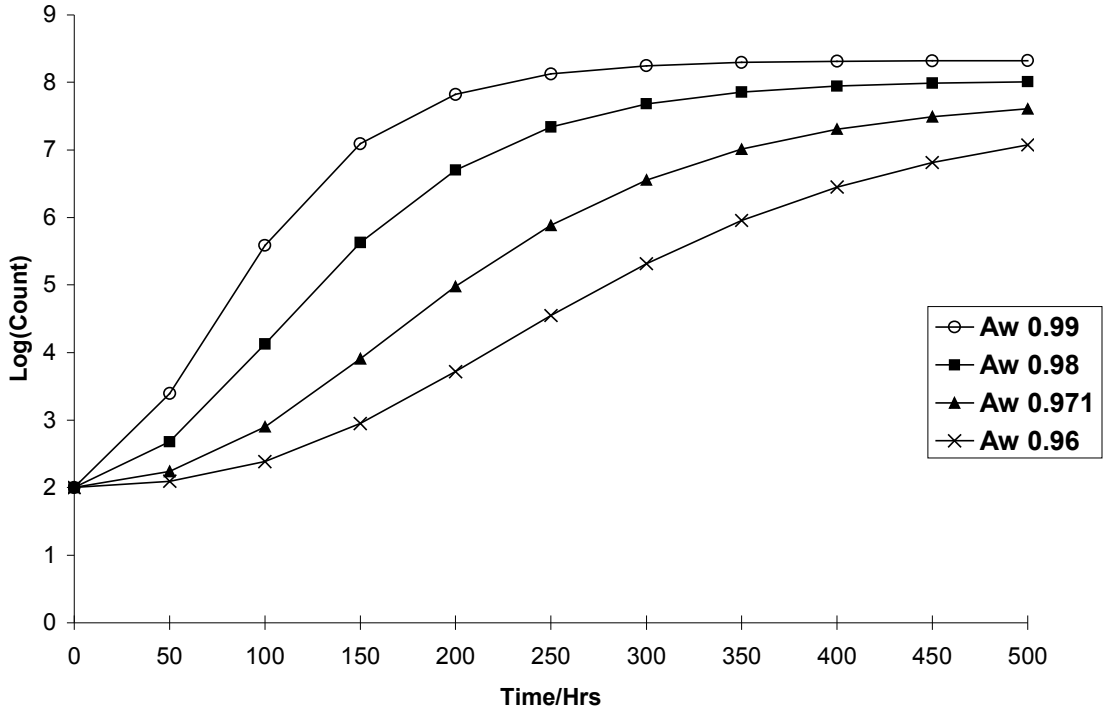

Figure 1
Interaction of product composition, manufacturing considerations and storage conditions on product quality and safety

A = product which is both microbiologically safe and stable and has the desired sensory properties. In this situation, all aspects of the product are perfectly balanced
B= product which has the desired sensory properties but might not be microbiologically safe or stable. In this situation, the sensory properties are acceptable but changes to the levels of intrinsic characteristics e.g. sugar may increase the microbiological risk.
C= product which is microbiologically safe and stable but may not have the desired sensory properties. In this situation, the product safety is maintained but changes to the intrinsic or extrinsic factors may affect the sensory attributes of the product.