EUROPEAN COMMISSION

FOOD PRESERVATION

BY

COMBINED PROCESSES

Final Report
FLAIR Concerted Action No. 7, Subgroup B
(Internet Word 6.0 Version- 1997)

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CONTENT

PREFACE

FLAIR (Food-Linked Agro-Industrial Research) was a programme launched by the Directorate General for Science, Research and Development (DG XII) of the Commission of the European Communities. Within this FLAIR programme, Shared-Cost Actions and Concerted Actions, in which European laboratories worked together on high rated research projects, were approved.

In the FLAIR Concerted Action No. 7, under the project leadership of Jeff G. Banks of the Campden Food & Drink Research Association, UK, two successful research proposals were merged, one on combined processes used for food preservation, and the other on the application of the HACCP concept in the European food industry. Therefore, this Concerted Action was carried out from autumn 1990 until spring 1994, in Subgroups A and B, under the heading "Food Safety and Quality based on the Application of Combined Processes and Hazard Analysis Critical Control Point (HACCP)".

To Subgroup B, working on "Combined Processes", contributed 14 scientists/laboratories (listed at the end of this Report) in 11 European countries (Belgium, Denmark, France, Germany, Ireland, Italy, The Netherlands, Slovenia, Spain, Sweden, United Kingdom). Since for Concerted Actions the Commission provides only funds for meetings and travel, the research done by the participating laboratories came out of their own funds, nevertheless, substantial progress has been achieved.

We are pleased to present our Final Report, which gives in its "General Part" an introduction to the application of combined processes (hurdle technology), and a description of the potential hurdles employed in food preservation. Thereafter, members of the Subgroup B describe in mini-overviews some of the "Emerging Hurdles", and outline "Examples of Hurdle Preserved Foods", demonstrated on some complex food systems. Finally, an "Index of Keywords" should assist the reader to locate data of special interest to him in our Final Report.

This report has been written with the intention to be a user friendly reference for the application of combined processes for food preservation in industrialized as well as in developing countries. In recent years the subject gained increasing attention, and it is felt that further developments are still in store. The members of Subgroup B of this FLAIR Concerted Action would be pleased if their Report would be of use to scientists, food processors, and students who are interested in the field of hurdle technology, applied in a gentle preservation of foods.

Spring 1994

Lothar Leistner
Subgroup B Project Leader

PART 1

GENERAL PART

FOOD PRESERVATION BY COMBINED PROCESSES

FINAL REPORT FLAIR Concerted Action No. 7, Subgroup B

1.1

INTRODUCTION TO HURDLE TECHNOLOGY

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ABSTRACT
An introduction to the concept of hurdle technology and its significance for the procurement of safe, stable, nutritious, tasty, and economical foods will be given; illustrated by some examples. Furthermore, the relation of hurdle technology to the homeostasis of microorganisms and to the total quality of foods will be outlined.

GENERAL ASPECTS OF HURDLE TECHNOLOGY
The microbial stability and safety of most foods is based on a combination of several factors (hurdles), which should not be overcome by the microorganisms present. This is illustrated by the so-called hurdle effect. This hurdle effect is of fundamental importance for the preservation of foods, since the hurdles in a stable product control microbial spoilage, food-poisoning, and the desired fermentation processes (Leistner, 1978; Leistner *et al.*, 1981). Furthermore, Leistner and co-workers acknowledged, that the hurdle concept illustrates only the well-known fact that complex interactions of temperature, water activity, pH, redox potential, *etc*. are significant for the microbial stability of foods.

From an understanding of the hurdle effect, hurdle technology has been derived, which allows improvements in the safety and the quality as well as the economic properties (*i.e*. how much water in a product is compatible with its stability) of foods, by an intelligent combination of hurdles (Leistner 1985; 1987; 1992; 1994). The application of this concept (synonymously called combined methods, combined processes, combination preservation, combination techniques, barrier technology, or hurdle technology) proved very successful, since an intelligent combination of hurdles secures the microbial stability and safety as well as the sensory, nutritive, and economic properties of a food.

EXAMPLES FOR THE HURDLE EFFECT
For each stable and safe food a certain set of hurdles is inherent, which differs in quality and intensity depending on the particular product, however, in any case the hurdles must keep the "normal" population of microorganisms in this food under control. The microorganisms present ("at the start") in a food product should not be able to overcome ("jump over") the hurdles present, otherwise the food will spoil or even cause food-poisoning. This concept is illustrated by Figure 1, which gives eight examples. Example 1 represents a food which contains six hurdles, and these are: high temperature during processing (F value), low temperature during storage (t value), water activity (a_w), acidity (pH), and the redox potential (Eh) of the product, as well as preservatives (pres.). The microorganisms present cannot overcome these hurdles, and thus the food is microbiologically stable and safe. However, Example 1 is only a theoretical case, because all hurdles are of the same height, *i.e*. have the same intensity, and this rarely occurs. A more likely situation is presented in Example 2, since the microbial stability of this product is based on hurdles of different intensity. In this particular product the main hurdles are

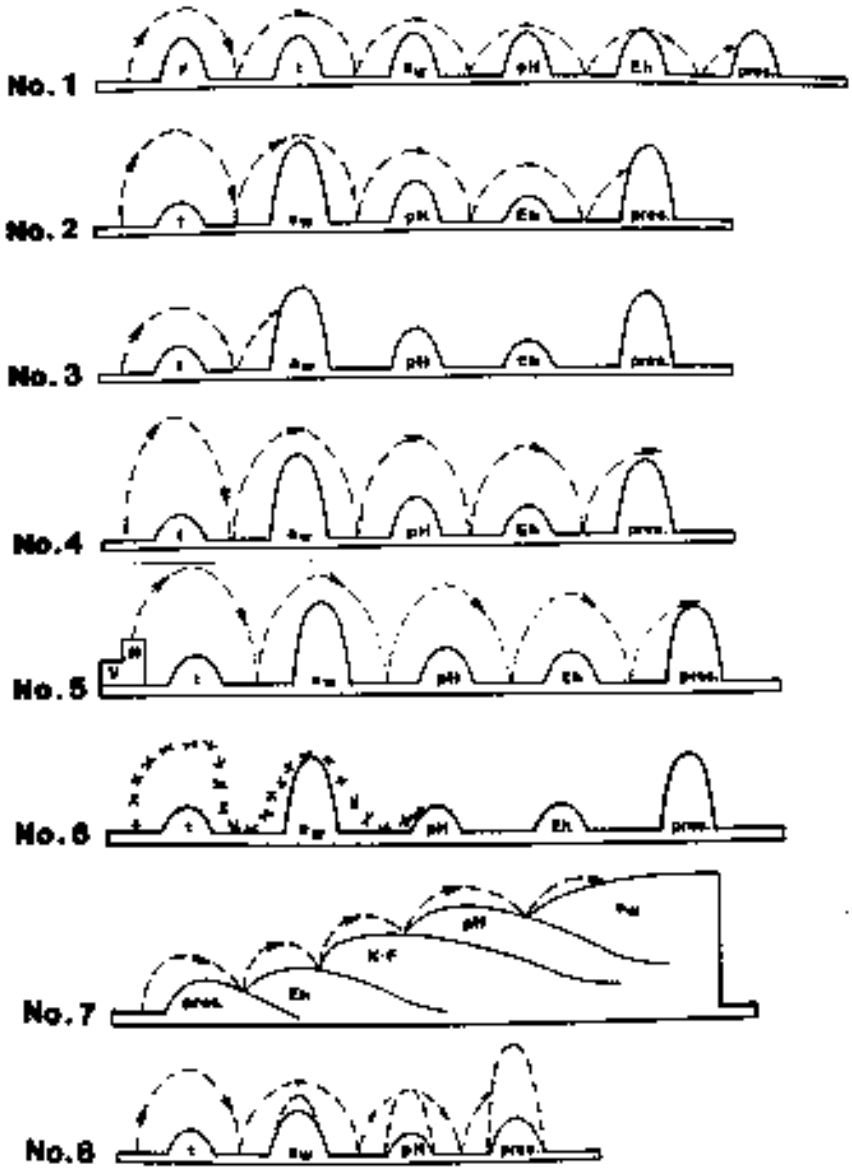

Figure 1. Illustration of the hurdle effect, using eight examples. Symbols have the following meaning: F, heating; t, chilling; a_W, water activity; pH, acidification; Eh, redox potential; pres., preservatives; K-F, competitive flora; V, vitamins; N, nutrients.

a_W and preservatives; other less important hurdles are storage temperature, pH and Eh. These five hurdles are sufficient to inhibit the usual types and numbers of microorganisms associated with such a product. If there are only a few microorganisms present at the start (Example 3), then a few or low hurdles are sufficient for the stability of the product. The aseptic packaging of perishable foods is based on this principle. On the other hand, as in Example 4, if due to bad hygienic conditions too many undesirable microorganisms are initially present, even the usual hurdles inherent in a product cannot prevent spoilage or food-poisoning. Example 5 is a food rich in nutrients and vitamins, which foster the growth of microorganisms ("trampoline effect"), and thus the hurdles in such a product must be enhanced, otherwise they will be overcome. Example 6 illustrates the behaviour of sublethally damaged organisms in foods. If, for instance, bacterial spores in meat products are damaged sublethally by heat, then the vegetative cells derived from such spores lack vitality, and therefore are already inhibited by fewer or lower hurdles. In some foods, such as fermented sausages and probably also ripened cheeses, the microbial stability is achieved during processing by a sequence of hurdles, which are important in different stages of the ripening process and lead to a stable final product. Example 7 illustrates the sequence of hurdles in fermented sausages. Important hurdles in the early stage of the ripening process of salami are salt and nitrite (pres.), which inhibit many of the bacteria present in the batter. Other bacteria multiply, use up oxygen and thus cause the redox potential of the product to decrease. This in turn enhances the Eh hurdle, which inhibits aerobic organisms and favours the selection of lactic acid bacteria. They are the competitive flora (K-F) and flourish, which causes acidification of the product, and thus an increase of the pH hurdle. In long-ripened salami the nitrite hurdle is depleted and the count of lactic acid bacteria decreases, whereas the Eh and pH increase again, *i.e.* all these hurdles become weak during a longer ripening of salami. Only the water activity hurdle (a_W) is strengthened with time, and it is mainly responsible for the stability of long-ripened raw sausage (Leistner, 1987). Probably also in other fermented foods, a sequence of hurdles will be important for the stability and quality of the product, and it should be challenging to elucidate them.

HOMEOSTASIS AND HURDLE TECHNOLOGY

An important phenomenon, which deserves attention in food preservation is the homeostasis of microorganisms (Gould, 1988). Homeostasis is the tendency to uniformity or stability in the normal status (internal environment) of the organisms. For instance, the maintenance of a defined pH within narrow limits is a prerequisite and feature of living organisms (Häussinger, 1988); this applies to higher organisms as well as to microorganisms. If the homeostasis of microorganisms, *i.e.* their internal equilibrium, is disturbed by preservative factors (hurdles) in foods, they will not multiply, *i.e.* remain in the lag-phase or even die, before their homeostasis is reestablished. Thus, food preservation is achieved by disturbing the homeostasis of microorganisms in foods temporarily or permanently.

In foods preserved by hurdle technology, the possibility exists that different hurdles in a food will not just have an additive effect on stability, but could act synergistically (Leistner, 1978). Example 8 in Figure 1 illustrates this. A synergistic effect could become true, if the hurdles in a food hit different targets (*e.g.* cell membrane, DNA, enzyme systems, pH, a_W, Eh) within the microbial cell, and thus disturb the homeostasis of the microorganisms present in several respects. Therefore, employing different hurdles in the preservation of a particular food should have advantages, because microbial stability could be achieved with a combination of gentle hurdles. In practical terms, this could mean, that it is more effective to use different preservatives in small amounts in a food than only one preservative in larger amounts, because different preservatives might hit different targets within the bacterial cell, and thus act

synergistically (Leistner, 1994). This "multi-target preservation of foods" could become a promising research area, because if small hurdles with different targets are selected, and the preservation of a food is made-up in this way, then a gentle but most effective preservation of foods could be accomplished. Certainly, the interrelationship of hurdle technology and homeostasis warrants further investigations (Leistner, 1994).

POTENTIAL SAFETY AND QUALITY HURDLES

The most important hurdles commonly used in food preservation, either applied as "process" or "additive" hurdles, are high temperature (F value), low temperature (t value), water activity (a_w), acidity (pH), redox potential (Eh), competitive microorganisms (*e.g.* lactic acid bacteria), and preservatives (*e.g.* nitrite, sorbate, sulphite). However, in addition, more than 40 hurdles of potential use for foods of animal or plant origin, which improve the stability and/or the quality of these products, have hitherto been identified (Leistner, 1994). These include oxygen tension (low or high), modified atmosphere (carbon dioxide, nitrogen, oxygen), pressure (high or low), radiation (UV, microwaves, irradiation), other physical processes (*e.g.* ohmic heating, pulsed electric fields, pulsed light processing, ultrasonication), new packaging (*e.g.* selective permeable films, advanced edible coatings), the microstructure of foods (*e.g.* solid-state-fermentations, emulsions), and various preservatives. In the following chapter of this report potential hurdles of significance for the safety and/or quality of foods are described in more detail. However, the list of possible hurdles for the preservation of foods is by no means closed, but not all of these hurdles will be commonly applied, and certainly not all of them for the same food product.

TOTAL QUALITY OF FOODS

Stanley (1991) indicated that the hurdle technology approach seems to be applicable to a wider concept of food preservation than just microbial stability, and he suggested that *e.g.* the oxidation of plant and animal membrane lipids is influenced by a number of positive and negative extrinsic and intrinsic factors. Undoubtedly, hurdle technology is not only applicable to the safety, but also to quality aspects of foods. Some hurdles (*e.g.* Maillard reaction products) influence the safety as well as the quality of foods, because they have antimicrobial properties and at the same time improve the flavour of the food. This also applies to nitrite used in the curing of meat. The possible hurdles in foods might influence the stability, sensory, nutritive, technological, and the economic properties of a product, and the hurdles present might be negative as well as positive for securing the desired total quality of a food (Figure 2). Moreover, the same hurdle could have a positive or a negative effect on foods, depending on its intensity. For instance, chilling to an unsuitable low temperature will be detrimental to fruit quality ("chilling injury"), whereas moderate chilling is beneficial. Another example is the pH of fermented sausages, which should be low enough to inhibit pathogenic bacteria, but not so low as to impair taste. In order to secure the total quality of a food, the safety and quality hurdles should be kept in the optimal range (Leistner, 1994), as illustrated with the example given in Figure 2.

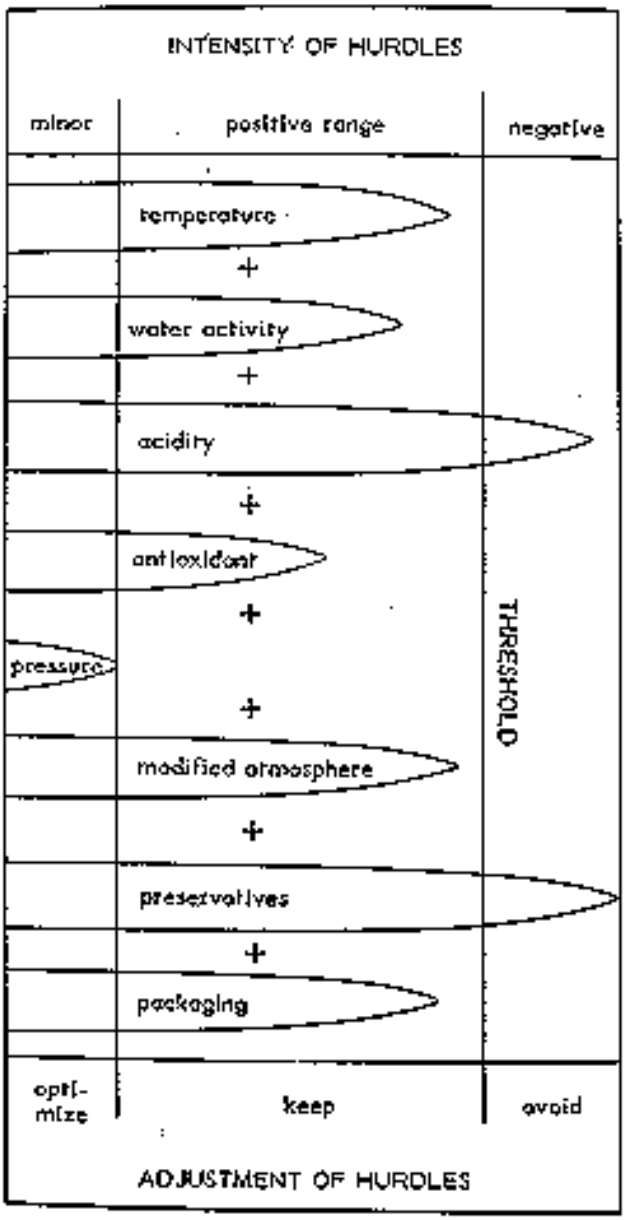

Figure 2. Examples of quality hurdles in a food, which could be, at the same time, safety hurdles and determine in summation the total quality of this product. If the intensity of a particular hurdle in a food is too small ("minor"), it should be strengthened ("optimized"). On the other hand, if it is detrimental ("negative") for the food quality, it should be lowered ("avoided"). By this adjustment, the hurdles in foods should be kept in the optimal ("positive") range, considering safety as well as quality.

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1.2

DISCRIPTION OF HURDLES

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INTRODUCTION.

The storage life, quality and safety of many foodstuffs is based on the combined effects of several factors (hurdles). These hurdles can have an additive or even synergistic effect.

Hurdles in foods are substances or processes inhibiting deteriorative processes. In many cases prevention of deteriorative processes and maintenance of quality are opposing actions. Therefore, to maintain optimal quality, the height of hurdles has to be kept as low as possible, *i.e.* by combination of various hurdles.

It should not be overlooked, that the food legislation is different in different countries, particularly regarding the permitted use of food additives. Therefore, it is not enough to determine the composition, manufacture *etc.* which leads to a wholesome foodstuff, it must also be checked that the manufacturing process, including composition, type and amount of additives *etc.* is in accordance with the legislation in the relevant countries.

This document gives a short description of the most important hurdles, especially the hurdles important for safety and stability, rather arbitrarily, a subdivision will be made between physical hurdles, physico-chemical hurdles, microbially derived hurdles and miscellaneous hurdles.

A. *PHYSICAL HURDLES.*

Most hurdles under this heading are processes used in food manufacture. When using processes intended to kill microorganisms, it is necessary to protect the food product against (microbial) recontamination after processing.

A 1. Heat processing.

Besides the purposes of pure cooking and changing food properties, the main purpose of heat processing of foods in food manufacture is to inactivate (kill, destroy) microorganisms and/or enzymes. When heat processing is used to kill microorganisms, it is necessary to protect the food product against recontamination by means of hermetically sealed packagings/containers.

Three types of heat processing will be mentioned here: sterilization, pasteurization and blanching. The heating method may be conventional heating (water and/or steam) but can also be ohmic heating, microwaves, *etc.*

A 1a. Sterilization.

The food is heated to a temperature at the coldest point (often the centre temperature) of 100°C or above. This is to obtain destruction of bacterial spores in order to make the product (canned foods) ambient shelf stable, *i.e.* stable for at least one year at ambient temperature.

The sterilization process is evaluated by means of the F value, defined as the period of exposure, in minutes, to 121.1°C (250°F), which will have a sterilizing effect equivalent to that of the process. The symbol F_0 is used when the sterilizing effect is calculated based on z= 10°C.

For low-acid foods, *i.e.* foods with pH > 4.5, F_0 should be at least 2.5 to secure a reduction of *Clostridium botulinum* spores by a factor of 10^{12} or more. A so-called Botulinum-cook, or 12D-cook, indicates that F_0 is 2.5 or above, often at least 3. When ingredients in the food have an inhibitory effect, such as salt (NaCl) and sodium nitrite ($NaNO_2$) in canned cured meat, lower F values will be sufficient. In such products, a sterilization process with F_0 around 2.5 would result in foods of an unacceptable quality. For foods with a pH of 4.5 or below, heat processing at temperatures below 100°C is often used, as *Cl. botulinum* is not able to grow at pH < 4.6.

Only hurdle: Canned foods must be in hermetically sealed containers. An F_0 value of at least 2.5 secures against *Cl. botulinum*, but lower F_0 values may be sufficient in canned cured meat products. Higher F_0 values may be necessary to ensure stability in low-acid canned foods.

A 1b. Pasteurization.

The food is heated to a centre temperature from around 60°C to around 85°C, depending on the food product. The normal temperature range is 65-75°C. At these temperatures all, or nearly all enzymes and vegetative microorganisms are inactivated, but generally, bacterial spores will survive.

The pasteurization process can be evaluated by means of the FP value, *i.e.* a pasteurization value determined and calculated according to the same principles as the F value, but using a reference temperature of 70°C and z= 10°C.

Only hurdle: Not applicable, as pasteurization is always combined with other preservation methods (hurdles), especially chilling. Also, packaging in hermetically sealed containers is necessary to protect against contamination after the pasteurization process.

A 1c. Blanching.

Blanching is heat processing at 70-100°C, mainly used for fruits and vegetables before further processing, *e.g.* before drying and freezing. The purpose is mainly to inactivate enzymes which could result in quality problems during subsequent storage. However, a substantial reduction in bacterial numbers will take place at the same time.

Only hurdle: Not applicable, as blanching is always combined with other preservation methods (hurdles), especially freezing and drying.

A 2. Storage temperature.

Two regimes of storage temperatures are relevant: chill temperature and freezer temperature. Storage at ambient temperature (or room temperature) is not a hurdle.

A 2a. Chill temperature.

Normally, chill storage means storage at temperatures between -1°C and +15°C. For perishable foods, *i.e.* meat, fish, dairy products, ready-to-eat dishes *etc.*, chill storage is from -1°C to maximally +7°C.

In some countries, the maximum temperature is +8°C. These temperature limits are related to minimum temperature for growth of pathogenic microorganisms, of which some are mentioned in the table below.

Microorganism	**Minimum temperature (°C)**
Salmonella spp.	5
Listeria monocytogenes	1
Yersinia enterocolitica	-1
Clostridium botulinum, type A and B	10
Clostridium botulinum, type E	3.3

For most fresh fruits and vegetables, storage at temperatures down to 0°C will increase storage life, and the above mentioned pathogenic microorganisms are normally irrelevant. However, for some fresh fruits and vegetables, mainly those from tropical origin, the so-called "Chilling injury" must be taken into consideration. This means that storage at a temperature below a minimum temperature, well above the freezing point, will result in severe quality degradation. For unripe bananas the minimum temperature is around 12-14°C, for unripe tomatoes it is around 8-10°C, for cucumbers around 8°C, for some oranges around 5°C, *etc.*

Only hurdle: For several foods, chilling is the only preservation method, the only hurdle, and normally the storage life is increased by lowering the storage temperature. "Chilling injury" should be taken into consideration. Chilling is very often used in combination with other hurdles, *e.g.* packaging, pasteurization, curing, *etc.*

A 2b. Freezer temperatures.

Freezing normally involves lowering the temperature to and storage at -18°C or below (deep freezing or quick freezing), although -10°C or -12°C are used in some countries for some foods. At temperatures below around -8°C there is in practice no growth of microorganisms. Quality degradation processes, often caused by enzymes, can take place down to -30°C or even colder.

Only hurdle: Freezing is often used as the only hurdle, but in most cases freezing is combined with other hurdles, *e.g.* blanching, packaging, *etc.*

A 3. Radiation.

Radiation processes using a frequency of 10^9 MHz or above, which have sufficient energy to excite or destroy organic molecules, will be described here. Electromagnetic radiation with a relatively low frequency, *i.e.* less than about 10^8 MHz is dealt with in section A 4.

A 3a. Ultraviolet radiation (UV).

The ultraviolet radiation region of the spectrum is from $8*10^8$ to $3*10^{11}$ MHz, at wavelengths below 450 nm. The most effective wavelength for destruction of microorganisms is 260 nm. The usual source of UV is a low-pressure mercury lamp, with about 80% of the UV emission at 254 nm.

Gram-negative bacteria are most easily killed by UV, while spores and moulds are much more resistant. UV is used for decontamination of air, and also of liquids (in thin layers, max 1 cm). UV can destroy microbes on a surface, but only on surfaces directly radiated, and only if the surface has been cleaned effectively beforehand. UV can be used to "sterilize" packages, *e.g.* food in thin plastic bags.

Only hurdle: Ultraviolet radiation cannot be used as the only hurdle. It is combined with, for example, packaging, chilling, heat processing or others.

A 3b. Ionizing radiation (irradiation).
Ionizing radiation is characterized by a very high energy content, and may be

- β Radiation: comprises highly charged electrons, mostly from linear accelerators.
- γ Radiation: emission from isotopes such as Co^{60}, at a frequency over $3*10^6$ MHz.

Ionizing radiation can kill microorganisms, depending on the dose. To reduce the number of viable microorganisms a million fold demands about 1-10 kGy (100 krad -1 Mrad) for vegetative bacteria and yeasts, and about 10-50 kGy for bacterial spores.

The advantage of this preservation method is that no (very little) heat is involved in destruction of microorganisms, meaning that the product has the characteristics of a "fresh" foodstuff after irradiation. The disadvantages are that off-flavour and off-taste occur in some irradiated foodstuffs, and in particular, that most consumers are very sceptical of the method. Consequently, ionizing radiation is subjected to severe restrictions, or even forbidden, in most countries.

Ionizing radiation with doses of about 5 kGy is sufficient to kill or inactivate most pathogenic and spoilage organisms, thus increasing the safety and stability of foods. Irradiation of spices, with doses about 10 kGy, can significantly reduce the bacterial number in spices, that often contain very high numbers of microorganisms (especially spores).

The irradiation of spices and a restricted number of other products is permitted in nearly all countries.

Only hurdle: As legislation presumably will prescribe a max dose of about 10 kGy, ionizing irradiation must be combined with other hurdles, *e.g.* chill storage, to increase safety and stability. Most irradiated products must be packaged to protect against recontamination.

A 4. Electromagnetic Energy (EME).
Electromagnetic energy (EME) results from high voltage electrical fields which reverse their polarity millions of times per second (frequency). Microwaves carrying the EME may be reflected, transmitted or absorbed. In the latter case, interaction with biological material is effected.

A 4a. Microwave energy.
Microwave energy alternates electrical fields at 500 to 1000 MHz. It is applied to heat food products fast by internal heating resulting from molecular friction between vibrating components (polar molecules, in foods often water) excited by the absorption of the microwave energy. In heterogeneous, multi-ingredient food products non-uniform heating often occurs resulting in hot and cold spots. Inactivation of microorganisms by microwave energy is due to its thermal effect and follows the same rules as conventional heating.

In the food industry, microwave energy is employed for pasteurization, drying, thawing and blanching, but normally not for sterilization processes. The main hazard associated with microwave heating is the non-uniform heat distribution throughout a food product, due to which localized survival of bacteria may occur. Resistance of microorganisms to microwave energy varies somewhat with species, but more so with the composition of the food (salt, fats, oils).

Only hurdle: Not applicable. Microwave pasteurization or blanching are used in combination with other preservation methods (hurdles), mainly with chilling, freezing and packaging.

A 4b. Radiofrequency energy.
Radiofrequency energy is characterized by frequencies of 1 to 500 MHz. It may be applied to heat food products, but especially at the very low frequencies specific non-thermal effects of radio-frequency waves on proteins and biological membranes have been reported. The effect may be a denaturation or an imbalancing of cell functionality. Although many of the effects are yet to be definitively confirmed, in potential this technique may be a useful mild preservation method, specifically inactivating spoilage microorganisms while minimally affecting product quality. Radiofrequency energy may be used in thawing.

Only hurdle: Radiofrequency energy cannot be used as a single hurdle.

A 4c. Oscillating magnetic field pulses.
One or more pulses of high intensity magnetic fields may be used at intensities of 2 to 5 Tesla and oscillating at a frequency of 5 to 500 MHz to destroy or inactivate bacteria and yeasts in in poorly electrically conductive food products. It is claimed that a single pulse may reduce the microbial load of a product by 99%, and up to 100 pulses do not cause an increase in product temperature by more than 5°C. Exposure times are extremely short, 0.025 to 10 milliseconds.

Application is advocated mainly for pasteurization processes, preferably in-pack. The mechanism of action on microorganisms is uncertain. It is suggested that the oscillating magnetic field may specifically affect large molecules such as DNA, rendering it unfunctional.

Only hurdle: This technique may not be used as a single hurdle.

A 4d. High electric field pulses.
This technique, also referred to as High Voltage pulse treatment, uses strong electric fields to inactivate microorganisms. An external electric field induces an electric potential over the membrane. When this electric potential equals or exceeds a critical value (for vegetative bacteria this is at an electric field strength of about 15 kV/cm) a reversible increase in membrane permeability is the result. Only when the critical electric field is greatly exceeded, irreversible pores are formed, membranes are destroyed and cells die. The field intensity required for cell disruption is inversely proportional to the cell diameter. Some processes employ 30 kV/cm for destruction of vegetative cells or higher for spores. At this intensity level, specific yeasts and bacteria could be reduced in number by 4-5 log cycles at an energy input of 100-200 kJ/l. Heat generation in the product is minimal, and especially heat-sensitive products would therefore benefit from application of this pasteurization technique.

Only hurdle: This technique may not be used as a single hurdle.

A 5. Photodynamic inactivation.
Photodynamic inactivation of microorganisms requires three basic components: light, molecular oxygen, and a photosensitizer. The photosensitizer (*e.g.* rose bengal, a xanthene dye) is a molecule which can absorb light of a specific wavelength (540 nm for rose bengal) which creates a store of chemical energy that can react with molecular oxygen to produce highly reactive free radicals. Singlet oxygen ($^1O_2$) is one of the main reactive species formed, and is a potent killing agent.

Photodynamic inactivation has chemical inhibitors called quenchers which can be natural, *e.g.* carotenoids, or artificial (*e.g.* several antioxidants). Photodynamic inactivation of bacteria can be achieved by means of incorporating photosensitizers into the packaging of the product.

Only hurdle: This technique may not be used as a single hurdle.

A 6. Ultrahigh pressure.
Foodstuffs treated under ultra high pressure, *i.e.* 3000 Bar and above, undergo physico-chemical changes, often leading to longer shelf life. The main factor for this is inactivation of enzymes and microorganisms. The chemical principle behind the effect of ultra high pressure is that non-covalent bonds, like hydrogen-, ion- and hydrophobic bonds in proteins, nucleic acids and carbohydrates undergo changes leading to different molecular structures.
The level of inactivation of microorganisms is dependent on various inherent properties, pH, a_W and temperature of the product. The killing mechanism is probably that high pressure destroys the cell membrane function, leading to cell leakage. Experimental data indicates a general trend, where Gram-negative bacteria are inactivated at 3 kBar, yeasts and moulds at 4 kBar, Gram-positive bacteria at 6 kBar. To kill the most resistant bacterial spores demands 12 kBar, or a combination of temperature and ultra high pressure.

Products suitable for ultra high pressure treatment recognized so far are fruit based products. Dry products such as cacao powder or spices are not suitable, as no inactivation of bacterial spores has been noticed in these products.

Only hurdle: Ultra high pressure must be combined with other hurdles such as pH and temperature, and especially packaging.

A 7. Ultrasonication.
Ultrasonics (ultrasounds) are vibrations similar to sound waves, but at a frequency too high (between 18 kHz and 500 MHz) to be heard by the human ear. In biological media these vibrations produce cycles of compression and expansion and the phenomenon of cavitation. The implosion of bubbles generates spots with very high pressures and temperatures that can disrupt cellular structures.

Ultrasounds have been used for cell disruption in the isolation of cytoplasmic components. A lethal effect on microorganisms has been reported. However, this effect is very low and in some cases (bacterial spores) negligible. Furthermore, the death rate kinetics of microorganisms by ultrasounds is not well known and the intensity of sonication for this effect to be obtained is very high. The use of ultrasounds as the only means of sterilization would probably be precluded by their harmful effect on physico-chemical and organoleptical properties of the food product. Ultrasounds, therefore, will probably have to be used in combined processes.

Recently, ultrasonication under pressure (Mano-Thermo-Sonication) was observed to increase drastically the lethality of pasteurization and sterilization processes of liquid menstrua (foods).

D values of some microorganisms and enzymes have been observed to decrease to 10 and 100 fold respectively, of those of heat treatments at the same temperature.

Only hurdle: Not applicable due to the intensity of the ultrasonication treatment required and its harmful effect on the characteristics of the product.

A 8. Packaging.

For most foods, packaging is necessary to preserve the quality and protect against damage during storage and distribution. Especially, packaging should act as a barrier, *i.e.* prevent entry of microorganisms, insects, dirt *etc.* Packaging can also act as a barrier against transfer/passage of water vapour, gases and aroma. Normally, packaging materials contain no or very few microorganisms at the time of packaging. The optimal packaging method and packaging materials depend on the food. In many cases, special packaging systems are used, *e.g.* vacuum packaging, "active" packaging, or MAP.

A 8a. Vacuum packaging.

The package is evacuated and closed, leaving a very low amount of air, especially oxygen (O_2), in contact with the food. In many cases, the CO_2 concentration in the package will increase considerably. This slows down many processes, and will also influence the type of microorganisms that are able to grow on/in the food, see B 7 and B 8. The package used must have a low or very low permeability to O_2 and other gases.

When using vacuum packaging of chilled foods, it must be remembered that *Cl. botulinum* (and some other pathogens too) grow well in the absence of O_2. Thus, a max temperature of 3.3°C is advisable for some vacuum packed foods, especially for pasteurized foods.

A 8b. Moderate vacuum packaging.

In moderate vacuum packaging/storage the product is stored under a pressure of around 400 mBar at chill temperature. This can take place in a rigid, airtight container or a plastic pouch. Thus, the amount of O_2 available to the food is about one third of the normal, slowing down the metabolism of respiring produce and the growth of spoilage microorganisms.

A 8c. Active packaging.

By means of special methods, the composition of the atmosphere in the package can be changed, *e.g.* the O_2 content may be reduced to less than 0.5% by placing sachets with oxygen scavengers in the package. Also, ethanol may be introduced into the package, see B 20. Normally, the package used must have a low permeability to O_2 and other gases.

A 8d. Edible coatings.

The use of edible coatings or films gives the food product a protective superficial layer. This has been used for many years, *e.g.* waxing of fruits. Currently, edible films and coatings which protect a food against microbial spoilage as well as loss of quality are developed on the basis of proteins, starches, waxes, lipids, *etc.* Also, edible films/coatings are developed which include food-grade antimicrobial (see D3) and antioxidant compounds. These will allow the use of greatly reduced amounts of additives because they are fixed at the product surface where the prime protection is required.

In MAP, see A9, such protective films/coatings should generate suitable modified atmosphere around respiring produce and should be compatible with high moisture foods in order to replace non-biodegradable plastic materials.

Only hurdle: Packaging is always used in combination with other hurdles. Only for fruits such as oranges can packaging be the only hurdle.

A 9. (Modified Atmosphere Packaging) (MAP).
MAP (Modified Atmosphere Packaging) means that an atmosphere with a gas composition different from that of atmospheric air is created in the package. The volume of product is about the same as the volume of air in the package. CO_2 and O_2 are the most important gases, see B7 and 8. The package must have low or very low permeability to O_2, CO_2 and other gases, except for fresh fruits and vegetables where a certain permeability is necessary to prevent anaerobic conditions in the package. Edible coatings, see A 8d, could be used in MAP.

In MAP of non-respiring ("dead") foods a high CO_2 content, *i.e.* > 20% is used, in most cases together with a low O_2 content, *i.e.* < 0.5%. For MAP with high CO_2 concentration, the storage temperature should be kept low (< 5°C), as the effect of CO_2 increases with lower temperature. In some cases, fresh meat (beef) and lean fish, more than 21% O_2 is recommended.

In MAP of respiring ("live") produce, *i.e.* fresh fruits and vegetables, once the atmosphere has been changed to the desired level, the respiration rate of the produce should equal the diffusion of gases across the packaging material in order to achieve an equilibrium atmosphere in the package. The O_2 concentration must be kept sufficiently high to preclude anaerobic respiration. Too high CO_2 concentrations may cause disorders in several commodities. Many plastic films do not have the proper O_2/CO_2 permeability ratio. Since respiration rate and gas permeability change with temperature, MAP for respiring produce is rather complicated.

Only hurdle: MAP is always used in combination with other hurdles, especially chilling.

A 10. Modified Atmosphere storage.
Modified Atmosphere storage (MA storage) means that produce is stored in airtight storage rooms with a modified atmosphere. This atmosphere is created by the respiration process of the produce. The O_2 level is decreased and the CO_2 level increased, the total of these two gases being around 20%. MA storage is only used in chilled storage for fruits and vegetables.

Only hurdle: MA storage is used in combination with other hurdles, especially chilling.

A 11. Controlled Atmosphere storage.
Controlled Atmosphere storage (CA storage) means that produce is stored in airtight chilled storage rooms, where a modified atmosphere is created and continuously controlled and regulated. CA storage can be maintained during transport, *e.g.* in containers. In practice, CA storage is still only used in chill storage of fruits and vegetables, especially of apples and pears. The composition of the atmosphere in the storage room is kept constant, *e.g.* about 3% O_2, and about 3% CO_2, as this slows down most quality degrading processes, see B 7 and B 8.

Only hurdle: CA storage is always used in combination with other hurdles, *i.e.* chilled storage.

A 12. Hypobaric storage.
In hypobaric (low pressure) storage, produce is stored at chill temperatures under a pressure of 10 to 100 mBar, and often with a constant circulation of fresh air at high RH (80-100%). The O_2 available to the produce is much lower than normal, see also A 8b, and the storage life of many horticultural and floricultural products may be increased considerably. Hypobaric storage can be maintained during transport, *e.g.* in containers. The system is not used as widely as MA or CA storage.

Only hurdle: Hypobaric storage is used in combination with other hurdles, especially chilling.

A 13. Aseptic packaging.
Aseptic packaging normally means that foods after heat processing are transferred to "sterile" and hermetically sealed containers under aseptic conditions, so that no re-infection takes place. The principle is well known for liquid products, *e.g.* (UHT) milk, fruit juices, *etc.*

The "clean room" technology is intended to drastically reduce the number of microorganisms in the areas where food products are produced, sliced or packaged, in order to increase safety and stability.

Only hurdle: Aseptic packaging is normally a combination of hurdles, *i.e.* heat processing and packaging. It may be combined with other hurdles, especially chilling.

A 14. Microstructure.
In certain foods the microorganisms present are not evenly distributed, their growth being restricted to definite areas in the product. In concentrated oil-in-water emulsions the bacteria form small colonies. In water-in-oil emulsions the bacterial growth is confined to the water droplets, which may loose their integrity due to coalescence. In fermented sausages or cheese the bacterial growth is immobilized in little cavities or nests, in which the bacteria are in keen competition with each other, and from which they influence (by metabolic products, *e.g.* acids, enzymes) the ripening process of the entire food as well as other bacterial nests which are located in distant locations of the food.

This impact of the microstructure on microbial growth, survival and death in foods has theoretical and practical implications. Certainly, under these circumstances predictive modelling is difficult. On the other hand, the number, size, and distance of the microbial nests in such foods, and thus the safety, stability and quality of the products, might be influenced by technological means.

Only hurdle: Not applicable.

B. *PHYSICO-CHEMICAL HURDLES.*

B 1. a_W.
Water activity (a_W) of a food is the ratio of the water vapour pressure of the food to that of pure water at the same temperature. a_W influences the growth, resistance and survival of microorganisms and the reaction rate of most quality degrading processes. In general, bacteria are less tolerant to a reduced a_W than yeasts and especially moulds. a_W may be decreased by dehydration, or by addition of solutes such as salt, sugar, *etc.* and by lowering of temperature.

Only hurdle: To make the food ambient shelf stable, a_W should be about 0.6 or lower, but very few microorganisms and no pathogens grow at a_W less than 0.7. a_W is very often combined with other hurdles, *e.g.* chilling. a_W may be the only hurdle in dry/dried foods. Normally, a packaging acting as a barrier against water vapour is necessary.

B 2. pH.

Most raw (unprocessed) foods have a pH between 5.6 and 6.6, although many fruits have a lower pH, and egg white a pH above 7. For thousands of years, the use of a pH decrease (or an increase of acidity) has enhanced microbiological stability. This has been (and is) done naturally by fermentation or artificially by the addition of acidulants. Hydrochloride and phosphoric acid are examples of strong acids used in food manufacture, *e.g.* in carbonated and non-carbonated drinks with a pH of 3.3 or below. However, in most cases weak organic acids are used, see B 10 below. Most microorganisms do not grow below a specified minimum pH, the most well known limit being 4.6 for *Cl. botulinum*.

Only hurdle: In very few cases, acceptable food quality can be combined with a pH so low that no microorganisms are able to grow. pH is often combined with packaging, and additives such as NaCl, organic acids (see B 10), and chilling or heating.

B 3. Redox potential (Eh).

Eh or redox potential (oxidation-reduction potential) indicates the oxidizing or reducing potential of a food system and is expressed in mV. In general, foodstuffs have an Eh value (corrected to pH 7.0) in the range of +300 to -200 mV, and they differ in intensity, but also in capacity of their Eh. Eh measurement is done with a potentiometer, a platinum electrode and a mercury chloride reference electrode; the electrodes are inserted into the food. Sufficient time after insertion must be allowed before the reading, *i.e.* until an equilibrium has been established or only small changes (2 mV within 30 minutes) still occur.

The Eh of a food is influenced by the removal of air (oxygen), the exclusion of light, the addition of reducing substances (ascorbic acid, sucrose, *etc.*), the growth of bacteria, the presence of nitrite, the temperature, and especially the pH. The redox potential determines whether aerobic (*e.g.* pseudomonads) or anaerobic (*e.g.* clostridia) microorganisms will grow in a foodstuff, and it influences considerably the colour and especially the flavour of the foodstuff.

Only hurdle: Eh cannot act as the only hurdle. It is used in combination with curing, chilling, packaging *etc.*

B 4. Salt (NaCl).

The addition of salt (NaCl) to foods has been known for centuries. The main effect of NaCl is the reduction in a_W, see above, but NaCl in itself seems to have some bacteriostatic effect. Today, most consumers prefer foods with a less salty taste, *i.e.* a lower salt content than before, meaning that salt must be combined with other hurdles. Salt-in-water (g NaCl per 100 g water) or salt-in-brine (g NaCl per 100 g water+g NaCl) are well known expressions of "effective" salt content. Curing is the process of the addition of NaCl and other curing ingredients such as nitrite.

Only hurdle: A stable product must contain at least 27g salt per 100g water ($a_W < 0.7$). To inhibit

growth and toxin formation of *Cl. botulinum* type E in fish products at 15°C, there must be at least 4.5g salt per 100g water. Curing is often combined with hurdles like packaging, chilling, smoking, *etc.*

B 5. Nitrite ($NaNO_2$) .
In curing of meat, salt is nearly always used in combination with nitrite (or nitrate). Nitrite, normally $NaNO_2$, at the level used commercially (and allowed in legislation) inhibits the growth of many organisms, depending on concentration, type of organism, *etc.* A very important aspect is that nitrite is rather effective against sporeforming bacteria, especially clostridia. The effect of nitrite is greater in systems where nitrite is heated together with meat, where a specific, more or less identified anti-botulinal compound (the Perigo-factor) seems to be formed. The anti-botulinal activity of nitrite is claimed to be due to its inhibition of nonheme, iron-sulphur enzymes. Nitrite is used to give cured meat products a pink colour, but it also improves flavour and can prevent or decrease off-flavours such as warmed over flavour (WOF).

Only hurdle: Nitrite is always used in combination with other hurdles, see B 4.

B 6. Nitrate ($NaNO_3$ or KNO_3) .
In cured products, nitrate is sometimes used. The effect of nitrate in itself is limited to a small reduction in a_W, but in many products, especially meat products, nitrate was used as a "reservoir" for nitrite, as bacteria may reduce nitrate to nitrite.

Only hurdle: Nitrate has a very limited effect, and is always used in combination with other hurdles, especially NaCl.

B 7. Carbon dioxide (CO_2) .
CO_2 is present in the atmosphere, at a concentration of about 0.03%. A higher CO_2 concentration slows down many quality degrading processes in foods, and at a concentration of above 20% the growth of most spoilage bacteria is reduced or inhibited. Thus, in MAP (see A 9) of most non-respiring foods, a CO_2 concentration of at least 20% is used. In respiring foods, an increased CO_2 concentration slows down the respiration, and thus increases shelf life. A too high CO_2 concentration results in quality disorders in most fruits and vegetables, but the critical limit (often 8-12%) is different for different commodities. The solubility of CO_2 increases dramatically with lower temperatures, down to the freezing point of the foodstuff. The effect of increased levels of CO_2 is utilized in other hurdles, see A8 - A12.

Only hurdle: An increased level of CO_2 is always used in combination with other hurdles, especially chilling, but often also packaging.

B 8. Oxygen (O_2) .
O_2 is present in the atmosphere, at a concentration of about 21%. Most organisms, including humans, prefer this concentration, and in practice only a lowering of the O_2 concentration can be considered as a hurdle. At low O_2 concentrations the growth of most, but not all (see A 8a) microorganisms is reduced or inhibited, the respiration rate of respiring produce is decreased, and many quality degrading processes (oxidation) are slowed down. Thus, the absence of O_2 should improve quality and safety.

However, this is not the case for respiring produce, and for displayed retail packed chilled meat (beef) oxygen is necessary for maintaining a bright red colour. For foodstuffs where *Cl. botulinum* may grow, some authorities consider anaerobic conditions a health hazard. The effect of reduced levels of O_2 is utilized in other hurdles, see A8 - A12.

Only hurdle: A reduced O_2 level is always used in combination with other hurdles, especially chilling, but often also packaging.

B 9. Ozone.

Ozone is a water-soluble gas with powerful oxidizing properties. Ozone will rapidly decompose to oxygen when exposed to water, and this limits the use of ozone. Ozone is also affected by temperature, pH and organic matter present. The lethal effect on microorganisms is due to the strong oxidizing activity, probably targetting on amino acids, RNA and DNA. Ozone treatment destroys Gram-negative bacteria particularly. Yeasts and moulds are more resistant than bacteria, and a very high concentration of ozone is required for the destruction of bacterial spores.

There are several applications of ozone in the food industry. Sterilization of spices would require 30-135 g/m^3, ozone treatment in order to reduce the microflora of poultry involves around 2-3 g/m^3, and for air-sanitizing in chill storage rooms for meat (beef) an ozone concentration of 0.3 g/m^3 is considered appropriate. Ozone should never be used for foods susceptible to rancidity and other quality deteriorating reactions caused by oxidation. In many countries there are legal limits for the max concentration of ozone in working areas.

Only hurdle: Ozone is never used as the only hurdle.

B 10. Organic acids.

Organic acids or their salts are used to aid the preservation of a wide variety of foods. In most countries, the amounts and types of organic acids are controlled by governmental agencies, and the amounts allowed are often small in comparison with the amounts occurring naturally in many fruits and fermented products. The short chain acids such as acetic, benzoic, citric, lactic, propionic and sorbic acids and their salts are most commonly used. It is mainly the undissociated molecule that is responsible for most of the antimicrobial activity. Generally, the organic acids are most effective in foods with pH lower than about 5.5, although the alkyl esters of parahydroxybenzoic acid (parabens) have an effect in foods with pH near 7, and propionic and sorbic acid have some effect in foods with pH 6 to 6.5. The organic acids differ in their effect against moulds, yeasts and bacteria. Many combinations of organic acids and other preservation systems (hurdles) are synergistic.

Only hurdle: In most cases, organic acids are used in combination with other hurdles, *e.g.* pH, NaCl, chilling, and packaging.

B 11a. Lactic acid, lactate.

Lactic acid is generally viewed as less effective than many of the other fatty acids used as preservatives. However, it has been shown experimentally that lactate is effective against several pathogenic bacteria, such as *Mycobacterium tuberculosis* or against *Listeria monocytogenes* together with salt, nitrite and an acidulant in a sliced, cooked meat product. Further, it has been demonstrated as an

excellent inhibitor of spore-forming bacteria at pH 5, and has been found to be inhibitory for growth of acid-tolerant yeasts. Finally, it has been found to inhibit formation of mycotoxins in some cases.

B 11b. Acetic acid, acetate.
Acetic acid is widely used as a preservative. Its mode of action is identical with the other fatty acids. Its inhibitory ability is generally considered to be better against bacteria than against yeasts and moulds. A special feature of acetic acid is its high pKa, which makes it very important to consider the pH of a given food, when evaluating the effect of adding acetic acid or acetate for preservation reasons. In meats, acetate is effective against *Listeria monocytogenes*, and other pathogens.

B 12. Ascorbic acid.
Ascorbic acid and isoascorbic (erythorbic) acid and their respective salts, referred to as ascorbates and isoascorbates, have several effects in food products. In some foods, ascorbates can act synergistically with nitrite to inhibit cell growth. In canned cured meats, for instance, ascorbates or isoascorbates may increase the anti-clostridial effect of nitrite. In cured meats, ascorbates may stabilize the colour and chelate pro-oxidants. In MA packaged fresh meat, ascorbate can act as a colour-stabilizing antioxidant. In other foods, ascorbates act as antioxidants, or as synergist in the presence of other antioxidants. Asorbic acid can also be used to lower the pH.

Only hurdle: Ascorbic acid and ascorbates are always used in combination with other hurdles.

B 13. Sulphite or SO_2.
The sources of SO_2 are dissolved salts, mainly sodium metabisulphite, or gaseous sulphur dioxide. Sulphite is a multifunctional food additive. 1) antioxidant: can prevent oxidation, minimize colour changes, and stabilize vitamin C. 2) enzyme inhibitor: can inhibit chemical and enzymic reactions, *e.g.* enzymic browning. 3) maillard reaction inhibitor: can prevent non-enzymatic browning. 4) reducing agent: modifies flour rheology. 5) antimicrobial agent: inhibits growth of yeasts and moulds in low pH and low a_W products, and inhibits Gram-negative bacteria in foods with higher pH and high a_W.

Most of the applications of sulphite are in fruit and vegetable products, and in alcoholic and non-alcoholic drinks. The reactivity of sulphite (SO_2) is very high, but during storage and processing (heating) very high losses often take place.

Only hurdle: Sulphite is normally used in combination with other hurdles, *e.g.* a_W (drying) and ethanol.

B 14. Smoking.
The smoking process was once an important component in the preservation of many cured meat and fish products. Today it is mainly used to give flavour and colour. However, smoking of raw sausages and hams is still an effective means of inhibiting undesirable mould growth. During the smoking process, depending on the temperature, a drying of the surface (decrease in a_W) and a reduction in bacterial numbers takes place. Equally important is that natural smoke contains a variety of organic compounds, especially phenolic compounds, with antimicrobial and/or anti-

oxidative effect. These compounds are absorbed on the surface of the product and contribute to preservation. The use of liquid smoke results in little or no preservative effect.

Only hurdle: Smoking is always combined with other hurdles, especially curing, chilling and packaging.

B 15. Phosphates.
In food manufacture, phosphates normally means polyphosphates, of which a number of polymeric phosphates are in commercial use. Some pyrophophates are also used. Phosphates are used as additives in several food products, mainly to improve water binding capacity. Phosphates may raise the pH. Some polyphosphates have an antimicrobial activity, and some have an antioxidative effect. Polyphosphates act as chelators (see B 18) of trace metals which are used as catalysts in the microbial metabolism.

Only hurdle: Polyphosphates are always used in combination with other hurdles, especially curing.

B 16. Glucono-δ-Lactone (GDL).

Glucono-δ-Lactone (GDL) is added to, for example, cured meat products. GDL slowly hydrolyses to gluconic acid, subsequently lowering the pH. This gives advantages during processing, and also contributes to safety and stability.

Only hurdle: GDL is always used in combination with other hurdles, especially curing.

B 17. Phenols.
The use of phenolic antioxidants is well known in order to prevent or reduce oxidative deterioration of food products. BHA (butylated hydroxytoluene), BHT (butylated hydroxyanisole) and TBHQ (2-tertiary butylhydroquinone) have some antimicrobial effect, especially in combination with other hurdles.

Only hurdle: Phenolic antioxidants can not act as the only antimicrobial hurdle. They are used in combination with other hurdles, *e.g.* packaging, to reduce quality deterioration.

B 18. Chelators.
Chelators are used in foods primarily for their antioxidative properties, mainly due to their ability to eliminate the pro-oxidative effects of metals. Some chelators occur naturally in foods, but the most commonly used are citrates, lactates, pyrophosphates and EDTA (ethylene diamine tetra-acetic acid). Chelators are not considered as antimicrobials, but they may potentiate other antimicrobial agents. Their effect is most important in overcoming the resistance of Gram-negative bacteria to chemical agents.

Only hurdle: Chelators are not used as the only hurdle.

B 19. Surface treatment agents.
This group includes substances which inhibit the growth of moulds, and which are authorized in most countries. Diphenyl: authorized for use on the peel of citrus fruits. o-Phenylphenol: authorized for use on the peel of citrus fruits. Thiabendazole: authorized for use on the peel of bananas and citrus fruits.

Only hurdle: These preservatives may be the only extrinsic hurdle for citrus fruits.

B 20. Ethanol.
Ethanol in foods can be present as 1) the product of fermentation, like alcoholic beverages (wine, beer, *etc.*) or fermented foods, 2) an ingredient (spirit or essence) in candies, confectionary products, sweets, *etc.*, 3) a residue after baking in yeast-fermented bakery products, 4) an additive (when permitted) in packed IMF (intermediate moisture foods), by spraying before packaging or by desorption from packaging films, microcapsules or cups.

In general, high molecular concentrations of ethanol are required to inhibit microbial growth, kill cells or block glycolysis and metabolism, but high ethanol concentration changes the physical nature of the aqueous environment. The effective concentration varies with the type of spoilage microorganisms and the conditions in the medium. Growth of bacteria and moulds is usually prevented at 8-11 vol% of ethanol; yeasts are more resistant, often requiring 15-18 vol%. The integrity of the cellular membrane is in many cases the primary site of ethanol damage, although ethanol clearly affects the properties of all biological molecules to some degree.

Ethanol affects properties of water, which generally is the major component of food; in fact, it is also known for its strong a_W lowering capacity: in microbial cells (*i.e.* yeasts) reduced values of a_W induces a series of metabolic changes resulting in a decrease of the cellular activities.

Only hurdle: Ethanol is the only hurdle in spirits and beverages with high contents of ethanol (liqueurs) and fruits preserved in liqueurs. In most cases, ethanol is used in combination with other hurdles such as sulphite.

B 21. Propylene glycol.
Propylene glycol is one of the humectants which can be used to lower the a_W in, for example, intermediate-moisture foods (IMFs). Propylene glycol may have some antimicrobial effect, and is used to inhibit moulds in IMFs.

Only hurdle: Propylene glycol is never the only hurdle. In addition, it is prohibited for use in foods in many countries.

B 22. Maillard reaction products (MRPs).
The Maillard reaction is a reaction between amino acids and reducing sugars. This reaction is very common during heating of foods, and the Maillard reaction products (MRPs) affect colour, flavour and physico-chemical properties of foods. Some MRPs have antioxidative properties and can slow down the formation of rancid flavour in foods, depending on the pH, heating time and temperature, the nature of reactants, *etc.* In some cases, MRPs have antimicrobial properties, but MRPs have been reported to act as stimulants in other cases. The formation of antibacterial compounds is influenced by the nature of reactants, pH, the heating temperature, and the

concentration of MRPs. MRPs often influence enzymatic activity. Depending on heating time and temperature, nature of reactants, *etc.*, MRPs may slow down or inhibit the activity of enzymes such as trypsin, lactase, polyphenol oxidase and peroxidase.

Only hurdle: MRPs are never the only hurdle.

B 23. Spices and herbs.

It is well known that numerous spices and herbs have antioxidative and/or antimicrobial properties which can contribute to the stability and safety of foodstuffs. The most active components of spices and herbs seem to be the phenolic compounds and essential oils, *e.g.* allicin in garlic, allyl isothiocyanate in mustard, *etc.* However, the concentration of spices or herbs in foodstuffs, necessary to result in significant antimicrobial activity is normally much higher than is organoleptically acceptable to most consumers. Several researchers investigate the possible use of spices such as rosemary as antioxidants, in combination with other components with antioxidative properties. Similarly, the use of spices and herbs as food antimicrobials is an interesting area.

Only hurdle: Until now, spices and herbs are not used as the only hurdle.

B 24. Lactoperoxidase.

A natural antimicrobial system is the lactoperoxidase system. Its bactericidal activity is due to the formation of short-lived hypothiocyanate and possibly other antimicrobial compounds by the oxidation of thiocyanate in the presence of H_2O_2. Lactoperoxidase in cow's milk can be activated by the addition of sodium carbonate peroxyhydrate and sodium thiocyanate. In countries which are short of refrigeration facilities, this could increase the supply of "fresh" milk.

Only hurdle: The lactoperoxidase system is not used as the only hurdle, but development in science and technology may change the situation.

B 25. Lysozyme.

One of the natural anti-microbial systems in animals is lysozyme, an acetylmuramidase. Lysozyme can retard microbial growth, and this is used commercially to control lactate fermentation in some cheeses. Egg-white lysozyme can kill or prevent growth of *Listeria monocytogenes*. In some foods, the addition of EDTA can increase the effect of lysozyme.

Only hurdle: Lysozyme is not used as the only hurdle, but research may change this.

C. *MICROBIALLY DERIVED HURDLES.*

C 1. Competitive flora.

Long before proper microbiological knowledge existed, the effect of competitive flora as a hurdle was known. The most striking example is fermentation, where "spontaneous" growth of distinct types of microorganisms may completely overgrow a food, and by their mere magnitude, supported by

extrinsic and intrinsic factors related to the food in question, arrest or inhibit growth of other microorganisms. Of course, the fermentation can also be initiated by the addition of microbial starter cultures (see C 2).

To a lesser degree, the same effect can be seen as antagonism in growth between two strains of microorganisms, such as competitive growth between different clostridial strains, or between *Salmonella* and a number of putrefactive, Gram-negative bacteria. In fresh meat, competitive flora inhibits the growth of *Listeria monocytogenes*.

Only hurdle: It is not possible to rely on a competitive flora as the only hurdle. The effect is only observed in connection with one or more intrinsic and/or extrinsic factors.

C 2. Starter cultures.

Traditional foods preserved with the aid of microorganisms include many dairy products (cheese, yoghurt *etc.*), vegetable products (sauerkraut, pickles, olives), fermented sausages, wine, beer, Asian-type products made from fish and cereals, *e.g.* Tempeh. There is an increasing interest in the use of starter cultures, where microbial cultures are added, also to non-fermented foods. Lactic acid bacteria are particularly suitable in food preservation, as they reduce pH, act as antagonistic microorganisms, or produce antimicrobial metabolites, *e.g.* bacteriocins (see C 3).

Only hurdle: Only in very few cases, the addition of starter cultures can act as the only hurdle. It is nearly always combined with other hurdles, especially chilling and curing.

C 3. Bacteriocins.

Bacteriocins are formed naturally by several types of microorganisms. Generally, the effect of bacteriocins is limited to closely related strains, although they are never toxic against the producing strain. Typically, bacteriocins are formed by various lactic acid bacteria. In addition, there are the colicins, formed by certain strains of *E. coli*, which have an effect against related coliform bacteria. Nisin is the best known bacteriocin from lactic acid bacteria. Nisin is a heat-resistant polypeptide (34 amino acids). Nisin is limited in its active antimicrobial effect, *e.g.* no effect against yeasts and moulds has been demonstrated, but its spectrum is much broader than is the case with most other bacteriocins. A very important aspect of the use of nisin is its ability to prevent the outgrowth of germinating bacterial spores. A number of bacteriocins are used experimentally together with other hurdles. The antimicrobial spectrum of bacteriocins may be widened by use of chelators such as EDTA.

Only hurdle: Today, the narrow effective antimicrobial spectrum makes it impossible to use bacteriocins as the only hurdle.

C 4. Antibiotics.

Generally, broad-spectrum antibiotics are prohibited for use in foods, although their use as a single (only) hurdle would be possible. A whole range of antibiotic-like substances have been tried, but only very few are permitted and used. Pimaricin (natamycin) is effective against yeasts and moulds in products undergoing ripening, *e.g.* cheese.

Only hurdle: Antibiotics are not allowed to be used as the only hurdle.

D. *MISCELLANEOUS HURDLES.*

Here are briefly described some hurdles which have not been used very much, and for which the effect is rather uncertain. None of these hurdles can be used as the only hurdle.

D 1. Monolaurin.

Monolaurin is a food-grade glycerol monoester of lauric acid. In addition to its properties as a food emulsifier, monolaurin has a rather broad antimicrobial spectrum against Gram-positive bacteria, yeasts and moulds. Monolaurin has the greatest overall antimicrobial activity among all fatty acids and their esters. In combination with organic acids such as acetic, benzoic or lactic acid, the effect of monolaurin may be increased. The effective concentration of monolaurin is in the order of 0.001%.

D 2. Free Fatty Acids (FFA).

Some fatty acids, depending on the degree of saturation and the chain length, have an inhibitory effect on bacteria. Free Fatty Acids (FFA), such a linoleic and arachidonic acids, are quite active against Gram-positive bacteria, including pathogens such as *Listeria monocytogenes*. Levels of FFA in foods of the order of 0.5-1% may result in detectable organoleptic changes, whereas slightly higher concentrations may be required for substantial growth inhibition.

D 3. Chitosan.

Chitosan is a high molecular weight polysaccharide shown to significantly inhibit the growth of a number of fungi directly or indirectly. Due to its polymeric nature, chitosan can form films which are permeable to gases and has the potential of being used as an edible coating (see A8d).

D 4. Chlorine.

It is not uncommon to treat food products with a chlorine wash, *e.g.* raw salad vegetables prior to MAP and retail distribution. The most widely used is probably a hypochlorite solution containing 50-100 ppm free chlorine in which the produce is dipped for a short time. A reduction in pH of the solution to about 5 may improve the disinfection effect. In several countries, raw produce are not allowed to come in contact with chlorine solutions. In others, residual chlorine after processing/packaging must be below a maximum level.

1.3

USER GUIDE TO FOOD DESIGN

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ABSTRACT
Hurdle technology as a concept has proved useful in the optimization of traditional foods as well as in the development of novel products. However, it should be combined, if possible, with the HACCP concept and predictive microbiology. A ten step procedure is suggested which could be quite suitable for an effective food design, since it combines hurdle technology, predictive microbiology and HACCP.

INTRODUCTION
Three related concepts for quality assurance of foods were investigated within several FLAIR projects of the European Communities: hurdle technology (used for food design), the HACCP concept (used for process control), and predictive microbiology (used for process refinement). By considering these different approaches, an overall strategy for securing stable, safe and tasty foods should now be accomplished. This strategy could be applied in an effective food design, for which a user guide is tentatively suggested.

HURDLE TECHNOLOGY
The principles and applications of hurdle technology have been dealt with in the previous chapter "Introduction to Hurdle Technology" of this Final Report, and thus are not repeated here.

HACCP CONCEPT
The principles and applications of HACCP have been already well established (*e.g.* by Pierson and Corlett, 1992). Subgroup A of FLAIR Concerted Action No. 7 has developed a user guide for HACCP (FLAIR C.A. No. 7, 1994) for the European food industry.

The Federal Centre for Meat Research, Kulmbach, demonstrated the efficiency of hurdle technology for food preservation in a study (supported by the Medical Corps of the German Army) on meat products with fresh product characteristics which nevertheless are storable without refrigeration. Eight categories of meat products were selected and optimized. Since these meats should be suitable for army provisions, even if produced by large or small size enterprises, the manufacturing processes must be standardised and reproducible. Therefore, for the first time, a linkage between hurdle technology and the HACCP concept was introduced (Hechelmann *et al.*, 1991; Leistner, 1993). In the manufacturing plant processing the recommended meats, no microbiological tests have to be carried out, however, other process parameters have to be strictly controlled. These are: time, temperature, pH, and a_W. These measurements should be done on-line, or at least close to the line. A new instrument became available (Rödel *et al*., 1989), which allows reliable a_W determinations of meats within 10 to 20 minutes. The army study mentioned could be used as a model for other instances, where hurdle technology and HACCP should be linked (Leistner and Hechelmann, 1994).

PREDICTIVE MICROBIOLOGY

The army project also raised the question, how food design should be done in general, by applying hurdle technology combined with HACCP, and possibly predictive microbiology too. The predictive microbiology (Gould, 1989; McClure *et al*., 1993; McMeekin *et al*., 1993) is a promising concept which allows computer-based and quantitative predictions of microbial growth, survival and death in foods. However, the predictive models constructed so far handle only up to four different factors (hurdles) simultaneously. Factors considered to date are temperature, pH, a_W (due to salt or humectants), preservatives (*e.g.* nitrite, lactic acid) and CO_2. As outlined in the preceding chapters, there are numerous other relevant hurdles to be considered, which are important for the stability, safety, and quality of foods. It is unlikely that all, or even a majority of these hurdles could be covered by a single predictive model.

Thus, predictive microbiology cannot be a quantitative approach to hurdle technology. However, it does allow quite reliable predictions of the fate of microorganisms in food systems, while considering few but the most important factors (hurdles). Because several hurdles are not taken into account, the predicted results are fortunately on the safe side, *i.e.* the limits indicated for growth of pathogens in foods by the models available are often more prudent ("fail-safe") than the limits in real foods (Leistner, 1994).

Predictive microbiology will be an important tool in future food design, because it can narrow down considerably the range over which challenge tests with relevant microorganisms need to be performed. Although it will never render challenge testing obsolete, it may greatly reduce both time and costs spent in product development. Thus, predictive microbiology should be an integral part of advanced food design.

RECOMMENDED STEPS FOR FOOD DESIGN

For the design of foods ten steps have been suggested (Leistner, 1993, unpublished data; Leistner and Hechelmann, 1994), which proved appropriate when solving real product development tasks in the food industry. These steps are listed in Table 1, but still should be considered as tentative, until further practical experiences with the application of this user guide have accumulated in the food industry.

In food design different disciplines, including technologists and microbiologists, must work together. The technologists should determine which processes or additives are proper for the enhancement of hurdles in a food, by taking the legal, technological, sensory, and nutritive limitations into account. The microbiologist should determine, which types and intensity of hurdles in a particular food are needed, for the desired safety and stability of the product. Because the engineering, economic, and marketing aspects have to be taken into consideration in food development too, food design is indeed a multidisciplinary endeavour.

Table 1. Recommended Steps For Food Design

1. First, for the modified or novel food product the desired sensory properties and the desired shelf-life must be defined.

2. Secondly, a tentative technology for the production of the food should be suggested.

3. The food is then manufactured according to this technology, and the resulting product is analyzed for pH, a_w, preservatives or other inhibitory factors, and the temperature for heating (if intended) and storage as well as the expected shelf-life are defined.

4. For preliminary stability testing of the suggested food product, predictive microbiology should be employed.

5. The product is now challenged with food-poisoning and spoilage microorganisms, using somewhat higher inocula and storage temperatures than "normal".

6. If necessary, the hurdles in the product are modified, taking the homeostasis of the microorganisms and the sensory quality of the food (*i.e.* "total quality") into consideration.

7. The modified product is again challenged with relevant microorganisms, and if necessary the hurdles are modified once more. Predictive microbiology for assessing the safety of the food might be helpful at this stage too.

8. Now the established hurdles of the modified or novel food are exactly defined, including tolerances. Then the methods for monitoring the process are defined (preferably physical methods should be used).

9. Thereafter, the designed food should be produced under industrial conditions, because the possibilities for a scale-up of the proposed process must be validated.

10. Finally, for the industrial process the critical control points (CCPs) and their monitoring have to be established, and thus the manufacturing process should be controlled by HACCP.

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PART 2

EMERGING HURDLES

FOOD PRESERVATION BY COMBINED PROCESSES

FINAL REPORT FLAIR Concerted Action No. 7, Subgroup B

2.1

FOOD PRESERVATION BY ULTRAHIGH PRESSURE

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ABSTRACT
Ultra high pressure (UHP) treatment can be used as a preservation technique. One of the key advantages of UHP is that is can inactivate certain microorganisms, while the product itself may not be affected as much as with other many preservation techniques. The combination of a mild heat treatment with UHP application has shown to be a quite effective technique for a range of food products.

However, only few studies have been devoted to elucidating the mechanism(s) through which high pressures influence the growth or functionality of microorganisms under various environmental conditions.

In our studies, some phenomena were observed with important consequences for application of UHP. Amongst others, the inactivation of microorganisms ny UHP treatment was dramatically reduced in foods with a water activity below 0.94. Also, the resistance of microorganisms to UHP remained constant over a relatively wide range of pH (3-8). To adequately inactivate bacterial spores, pressures over 8000 kg/cm^2 and elevated temperatures are required.

INTRODUCTION
From the literature it can be summarised that application of ultrahigh pressure (UHP) gives four important effects on food products: unique texture, preservation of natural flavour, inactivation of enzymes and inactivation of microorganisms.

The treatment of food by ultrahigh pressures have been shown to drastically reduce the original microbial population, leading to longer shelf-life. Although the discovery was made at the beginning of this century, the technique on food products, has not been explored until very recently. In particular the Japanese market have during the last years introduced high pressure treated food products like sources, dressings, yoghurt and fruit juices.

The development in Europe up to now have mainly being in research programs and have focused on safety issues like studying the effect of high pressure on the microbial population different food products or on a possible modification of enzyme activity. Data from research activities on UHP has also been in areas of product functionality (texture) and quality retention (flavour). In the future a successful industrialization of the UHP technology depends on the food and packaging industry and their success in developing large scale process equipment, although UHP has a great potential with certain advantages over more traditional food processing methods. Those advantages are:

* Lack of chemical additives
* Instantaneous effect of pressure throughout the food
* Operation at low or ambient temperature so natural taste and flavour can be preserved.

Altogether, food products acquire a higher quality when treated with high pressure technique. This overview deals with inactivation of microorganisms by UHP as a preservation technique. Several reviews are available for detailed information on UHP (Balny *et al*., 1992; Butz *et al*., 1986; Farr, 1990; Hayashi, 1989; Hoover *et al.*, 1989; Knorr, 1993; Lechowich, 1993).

STATE OF THE ART

The inactivation of the microorganisms when subjected to UHP is suggested to be that the cellmembrane function is destroyed, which leads to cell leakage. The reaction occurring under UHP involves mainly hydrogen bonds, electrostatic links and hydrophobic interactions. Factors affecting the killing rate are:

* sugar concentration
* salt concentration
* water content
* product concentration
* pH

Shelf life of foods is extended without using heat treatment or chemical additives. If the high pressure process can inactivate spores it could be used to sterilize acid and non-acid products.

UHP processing can be combined with other types of treatment parameters such as pH of products, partial pressure in the package, CO_2 or N_2 gassing, temperature and chemical additions to obtain sterilization, specifically elimination of spores.

The mechanism of killing microorganisms through pressurization is thought to be based on the destruction of the membrane and wall of the cells. The destruction is in part due to abrupt changes in the cell volume and also to denaturation of proteins. The effect of ultra high pressure on bacterial spores is mainly at the moment the pressure is released and the pressurized water moves with a high adiabatic expansion velocity. Based on this principle, cycles of pressurization and depressurization have been advocated for efficient spore inactivation rather than UHP treatments for prolonged holding times or at elevated temperatures.

Table 1. Inactivation of microorganisms on pork by ultra high pressure (25°C; 10 min).

Microorganisms (*genera*)		Inactivated at
Gram negative bacteria	*Campylobacter* *Pseudomonas* *Salmonella* *Yersinia*	3000 kg/cm^2
Yeast	*Candida* *Saccharomyces*	4000 kg/cm^2
Gram positive bacteria	*Micrococcus* *Staphyloccus* *Streptococcus*	6000 kg/cm^2
Bacterial spores	*Bacillus*	6000 kg/cm^2 only when temperature is increased to 60°C and time prolonged to 40 minutes
Bacterial toxins	*Clostridium botulinum*	Partly inactivated at 6000 kg/cm^2

Table 2. Use of high pressures (23°C; 10 min) for the sterilization of fruit juices: effects on yeast and moulds

Product	pH	Initial counts/ml	Counts/ml after 2000 kg/cm^2	Counts/ml after 3000 kg/cm^2
Oranges	3.4	5.2×10^3	1.2×10^2	0
Lemons	2.5	1.4×10^3	2	0
Mandarins	3.8	2.0×10^3	2.7×10^2	0

PRINCIPLE OF THE PROCESS
Food, pre-packed in containers made from conventional plastic materials, is charged into a high pressure vessel filled with normal tap water. The vessel is closed. The pressure therein is increased to the necessary value and then decompressed to ambient pressure again. The vessel is opened. The charge is lifted out and dried. The food package is ready for distribution.

OWN RESULTS
Food treated with UHP at 3 kBar (3000 kg/cm^2) and above inactivate microorganisms often leading to a longer shelf life. The level of inactivation of microorganisms are dependent on various inherent properties pH, water activity and temperature of the product. Experimental data from the literature indicate a general trend, where Gram-negative bacteria are inactivated at 3 kBar, yeasts and moulds at 4 kBar Gram-positive bacteria at 6 kBar (Table 1). A combined treatment UHP and heat treatment (6 kBar, 60°C for 40 min) kills *Bacillus* spores, whereas spores of *Clostridium botulinum* are unaffected. To kill the most resistant bacterial spores 12 kBar, or a combination of more elevated temperatures and UHP, is required.

Products suitable for UHP recognized so far are wet food systems like fruit based products. In these foods, a rather low UHP treatment of 2 kBar suffices to completely inactivate yeasts and moulds (Table 2). Their rather low pH adds to the effect of the presence of water. With dry products such as cacao powder, UHP treatment is not able to inactivate the bacterial spores, although the addition of water to the food product brings about complete inactivation (Table 3).

Table 3. Use of high pressure (40°C; 10 min) for the sterilization of cacao mass: effects on yeast and moulds

Process	Micro-organisms
8000 kg/cm$_2$	No inactivation
Addition of 30% water	All microorganisms inactivated

Table 4. Use of UHP (8 Kbar 23°C) to treat irradiated spices contaminated with *Bacillus subtilis* spores.

Product	Initial counts/g	Counts/g, 1 h after UHP treatment	Counts/g, 2 h after UHP treatment	Counts/g, 1 h after 20 min UHP pulse
Black pepper	6×10^7	4×10^7	5×10^7	-
Allspice	5×10^8	2×10^8	2×10^8	2×10^8
Thyme	8×10^8	1×10^8	3×10^8	1×10^8

Table 5. Bacterial spore inactivation by UHP in a wet food system (orange juice).

Microorganisms	Initial counts/ ml	Time (min) after processing (temperature)					
		1 min, 23°C	5 min, 23°C	1 min,	5 min, 40°C	60°C, 1 min	60°C, 5 min
Bacillus cereus	$2x10^6$	$6x10^5$	$4x10^5$	$4x10^5$	$3x10^5$	$2x10^3$	$1x10^3$
Bacillus stearothermophilus	$2x10^6$	$2x10^6$	$2x10^6$	$1x10^6$	$8x10^5$	$1x10^4$	$8x10^3$
Clostridium sporogenes	$3x10^6$	$5x10^6$	$2x10^6$	$2x10^6$	$2x10^6$	$2x10^6$	$1x10^6$

Using UHP up to 8 kBar, the survival of bacterial spores, which are considered to be one of the most resistant forms of life forms and thus a problem for food industries, was studied in more detail. The aim of the study was to acquire knowledge about the risks taken and the security required in respect of food reacted under high pressure. The results in Table 4 illustrate that there is no significant inactivation of bacterial spores in a dry food system such as spices.

In orange juice (pH 3.5) to which a number of bacterial spores were added and which was subjected to UHP at 8 Kbar at various temperature and times, *Bacillus* spores show a 3 log reduction, while *Clostridium* spores are unaffected.

CONCLUSION

The conclusion from our experiments on UHP treatment of bacterial spores in dry and wet food systems show that UHP treatments at 8 Kbar needs to be combined with temperature treatments in order to achieve sufficient inactivation of bacterial spores, even in wet and acidic foods.

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2.2

MANO-THERMO-SONICATION : A NEW METHOD OF FOOD PRESERVATION?

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ABSTRACT
A brief review on the attempts of using the effect of ultrasound for microbial inactivation is followed by a summary of the recent observations on the increase of lethality of heat treatments when combined with ultrasonication. A new combined process of microbial inactivation including heat and ultrasonication under pressure (Mano-Thermo-Sonication; MTS process) is described.

Lethality of this new procedure was observed to be much greater than that of heat treatments at the same temperature. The death rate of yeasts (*Saccharomyces cerevisiae*), some vegetative cells (*Aeromonas hydrophila*) and bacterial spores (*Bacillus subtilis*) was much higher (between 10 to 30 times approximately). Pressure was necessary to obtain MTS lethality at temperatures above the boiling point of the medium.

A discussion is included on the possible mechanisms of Mano-Thermo-Sonication.

INTRODUCTION
Different alternatives have been attempted to replace heat in the preservation of foods: one of them being ultrasound.

Ultrasound are vibrations similar to sound waves, but of a frequency too high (18 kHz - 500 MHz) to be heard by the human ear. In biological media these vibrations produce cycles of compression and expansion and a phenomenon of cavitation. The implosion of bubbles generates spots with very high pressures and temperatures that can disrupt cellular structures.

The lethal effect of ultrasound in some microorganisms has long been known. Although ultrasonication has been proposed as a means of sterilization of liquid foods (Jacobs and Thornley, 1954) the inactivation of extremely high resistant microbial forms such as bacterial spores would require such a drastic ultrasonication treatments that spoilage of the physico-chemical characteristics of the food would result. The combination of ultrasound with other preservation methods might help to overcome this difficulty.

In 1972 Burgos *et al.* reported that previous ultrasonic treatments decreased the heat resistance of *Bacillus cereus*. In a later work these authors demonstrated that a simultaneous combined heat-ultrasound treatment reduced heat resistance of *Staphylococcus aureus* by 63% (Ordoñez *et al*., 1987) and that of *B. subtilis* by 43% (Garcia *et al*., 1989), as compared to their heat resistance at the same temperatures. This effect decreased as the temperature of treatment approached the boiling point of water (Figure 1). This trend greatly hampered any eventual future utilization of this effect for developing a new preservation method. At sterilization temperatures it seemed to be ineffective and at pasteurization temperatures the ultrasonication treatment required would probably be too long.

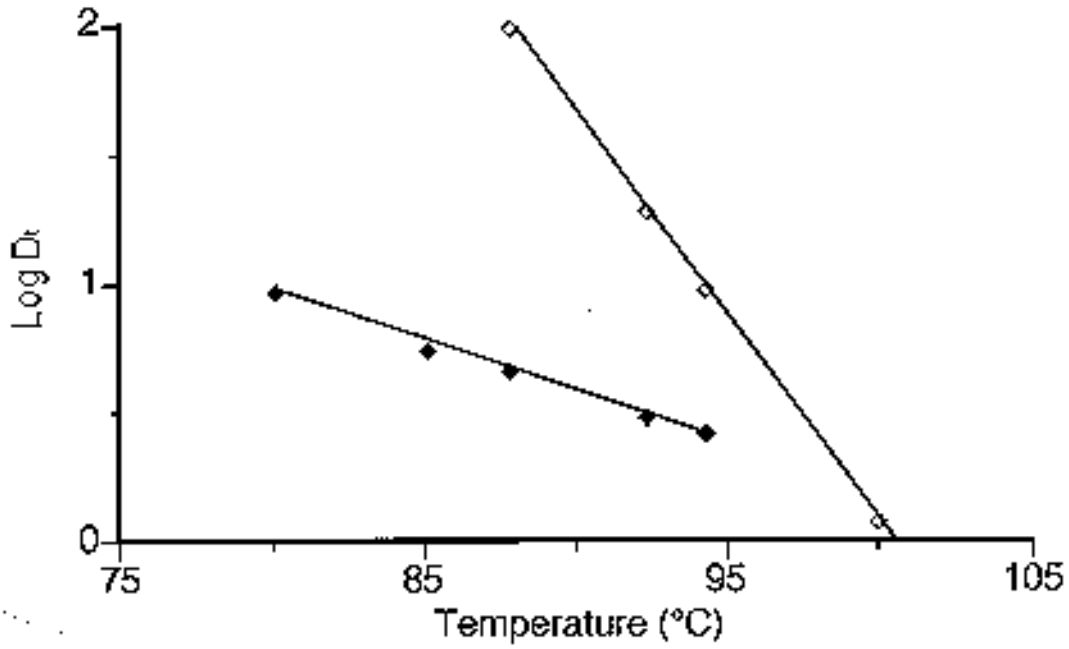

Figure 1. Resistance of *Bacillus subtilis* to heat (◊) and combined ultrasonic and heat treatment (t) at different temperatures (Garcia, 1985).

MANO-THERMO-SONICATION

The loss of lethality at temperatures close to the boiling point of the medium, observed by Garcia *et al.* (1989) was probably due to a loss of cavitation effect caused, amongst other possible factors, by the high water vapour tension at this temperature.

In 1990 Sala *et al.* (unpublished data) investigated with *B. subtilis* the effect of a simultaneous heat-ultrasonication treatment under pressure, designated as Mano-Thermo-Sonication (MTS). Pressure during heat-ultrasonication treatment retained the lethality at temperatures above the boiling point of the medium (Figure 2). The effect of MTS treatments was also investigated in other sporeforming bacteria (*Bacillus cereus, Bacillus coagulans* and *Bacillus stearothermophilus*), non sporeforming bacteria (*Aeromonas hydrophila*) and yeasts (*Saccharomyces cerevisiae*). In all cases, the lethality of MTS treatments was greater (5 to 30 times) than that of the corresponding heat treatment at the same temperature. The magnitude of this effect depended on the microorganism: yeasts were the most sensitive to MTS treatments, whereas spores were the most resistant. Also microenvironmental factors (pH and composition of the medium) and treatment parameters (amplitude of ultrasound and pressure) were observed to influence the increase in lethality.

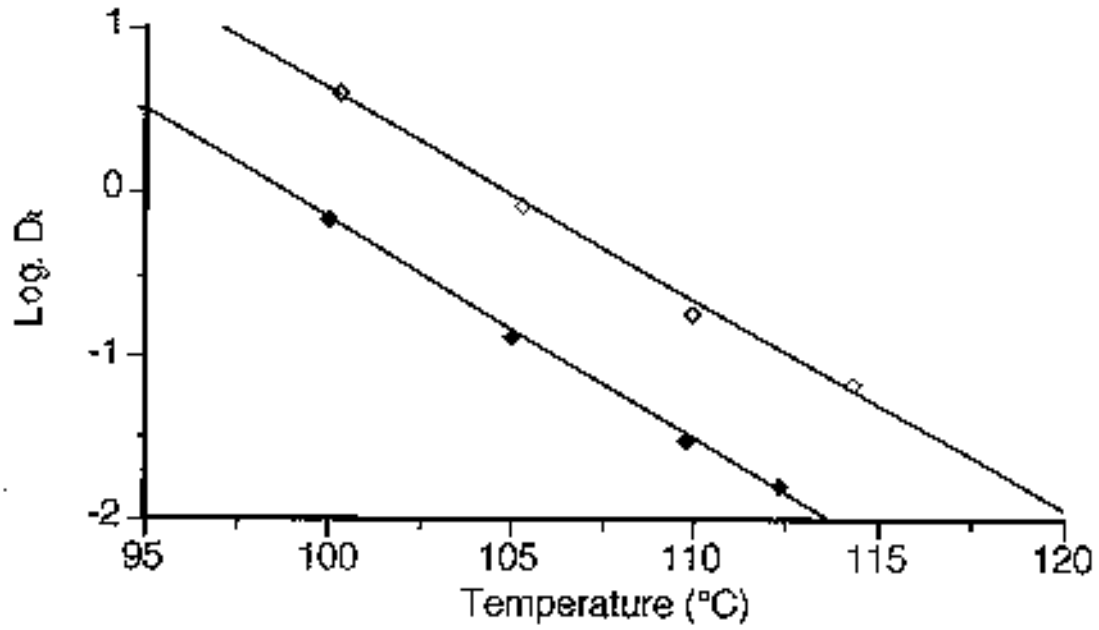

Figure 2. Resistance of *Bacillus subtilis* to heat (◊) and combined ultrasonic and heat treatment under pressure (Mano-Thermo-Sonication) (t) at different temperatures.

The effect of ultrasound during heat treatment is not an additive but a synergistic effect. As can be seen in Figure 3, the lethality of an ultrasonication treatment was negligible whereas the MTS treatment increased the lethality of heat treatment approximately 10 fold. Also, the kinetics of death rate of MTS treatments were the same as for heat treatments (first order reaction).

MECHANISM OF ACTION OF MTS

During MTS treatments cell structures would be submitted to very intense vibration and/or cavitation stresses which would cause a very strong shaking of molecules (even causing the breakage of bonds). In fact, Palacios *et al.* (1991) reported the liberation of dipicolinic acid and some low molecular weight polypeptides from the cortex of spores of some bacterial species. This cortex degradation would lead to rehydration of the protoplast which, as postulated by some authors (Gould and Dring, 1975), results in a loss of heat resistance.

According to Alderton and Snell (1969), the loss in heat resistance at acid pH of the medium is also caused by the rehydration of protoplast as a result of cortex degradation (protonization). The observation that the effect of MTS is slightly smaller at acidic pH would suggest that the mechanism of action of ultrasound and acidity is similar: through the degradation of the spore cortex.

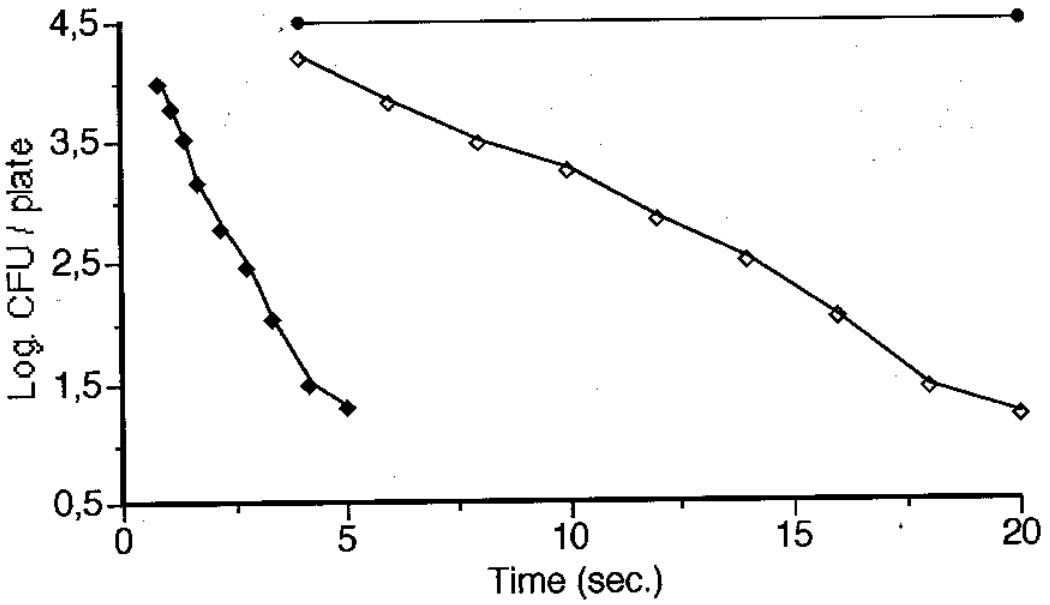

Figure 3. Survival curves of *Bacillus subtilis*: (l) Ultrasonication at 40°C and 117 µm; (◊) Heat treatment at 112°C; (t) Mano-thermo-sonication at 112°C, 20 kHz, 117 µm and 300 kPa.

The effect of MTS on vegetative cells could possibly be explained by a comparable mechanism affecting the integrity of the cell wall.

CONCLUSION

Data on microbial inactivation by ultrasound reported in the literature does not foresee its use in the future for microbial inactivation due to the very high resistance to ultrasound of bacterial spores. Although vegetative cells are sensitive to ultrasound their inactivation kinetics is still unknown and some authors (Jacobs and Thornley, 1954) question whether it follows a first order reaction pattern. This would hinder the establishment of adequate treatment parameters.

The combined use of heat and ultrasound under pressure increases very much the lethality of heat treatments allowing drastic reductions in time and/or temperature of heat processes. Microbial inactivation by MTS follows, as heat processes do, a first order reaction kinetics pattern, thus allowing prediction of the inactivation effect. As a consequence, if no undesirable side-effects in the processed food prevent its utilization, Mano-Thermo-Sonication could be an advantageous alternative to current heat processes.

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2.3

PHOTODYNAMIC INACTIVATION OF MICROORGANISMS

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ABSTRACT
A review of the principles and potential applications of natural and artificial photodynamic inactivation is presented. Factors necessary for photodynamic action, light, photosensitizer, and molecular oxygen are described in detail. The two main mechanisms by which a photodynamic process occurs are discussed and future applications and controlling factors are reviewed. It is
concluded that photodynamic inactivation offers many new opportunities to the food industry for preservation and hygiene; in this context areas for future research identified.

INTRODUCTION
Photodynamic action occurs when a photosensitizer absorbs light of a specific wavelength in the presence of oxygen. This causes oxidation processes which produce chemical and biological effects. The biological effect of photodynamic damage has been observed in virtually all classes of organisms; the effects include membrane damage, mutagenesis, interference with metabolism, reproduction and many other processes, and can be lethal. The products of the photodynamic reaction cause biochemical lesions in a variety of biological systems, and these have been extensively studied (Blum, 1964; Gallo, 1972; Spikes, 1970). Photooxidation of certain amino acids, nucleotides, lipids and other cell constituents appear to be the cause of lesions (Blum, 1964; Gallo, 1972). The first observations of photosensitization were reported by Oscar Raab, a medical student in Munich (Raab, 1900). He showed that acridine dyes sensitized paramecia to killing by visible light. The absorption of light in living organisms by endogenous or exogenous photosensitizers in the presence of oxygen, leads to many chemical and biological effects, most of which are detrimental to vital cell processes. Perhaps the most studied endogenous photosensitizer is chlorophyll which is contained in the chloroplasts of plant cells and green algae. Exogenous photosensitizers can create reactive species which if not quenched can cause damage and death to biological systems (Krinsky, 1968). The reactive species are thought to be produced by two main mechanisms called TYPE I and TYPE II reactions. TYPE I photooxidation mechanisms involve direct interaction of the excited sensitizer with the substrate resulting in radical formations and a subsequent reaction with oxygen. The TYPE II photooxidation pathway involves energy transfer to dioxygen, generating singlet molecular oxygen ($^1 0_2$) a powerful oxidant that mediates the photooxidation (Banks, 1985). Singlet oxygen was first detected in 1964 by its emission in microwave discharges (Bader, 1964).

As with any biological systems, photodynamic inactivation has chemical inhibitors referred to as quenchers which can be artificial or natural. The natural quenchers *e.g.* carotenoids are one of the group of chemicals that inhibit the photoinactivation properties of the endogenous photosensitizer chlorophyll in plants. Photodynamic reactions including the generation of singlet oxygen are promoted *in vivo* when the excited state of this pigment is not quenched. Carotenoid quenching is a mechanism capable of eliminating both singlet oxygen $^1O_2$ and (see *Mechanisms*

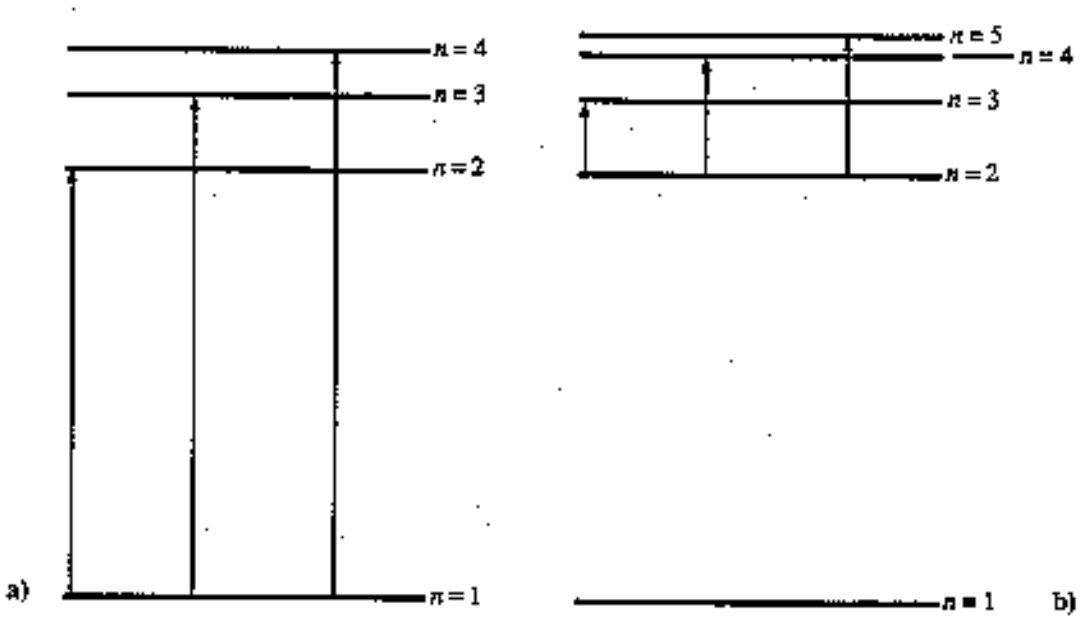

Figure 1. Energy level diagram for hydrogen showing a) absorption in the U.V. and b) absorption in the visible spectrum (Peet, 1978).

involved in photodynamic action) triplet chlorophyll (Krinsky, 1979; Dodge, 1983). Chemical quenchers work by 'mopping up' free radicals, like those produced by photodynamic action. Some quenchers which have been extensively studied include the carotenoids already mentioned, tryptophan, methionine (Dahl *et al*., 1988) L-histidine, crocetin (Carter, 1983) and propylgallate (Nitzan *et al.*, 1989), which is an antioxidant. Other antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may function as quenchers (Hudson, 1990).

COMPONENTS NECESSARY FOR PHOTODYNAMIC ACTION

A photosensitizer is a molecule which absorbs light to produce a chemical reaction which otherwise may not occur. Most photosensitizers in biological systems involve molecular oxygen present in photooxidation processes. Over 400 photosensitizing compounds have been reported (Gallo, 1972). The vast majority are triheterocyclic compounds (all are fluorescent), but fluorescence as such is not involved in photosensitization. The wavelength required to energise most photosensitized reactions is longer than 320nm but all photosensitizers have their own particular peak intensities. Rose bengal, a xanthene dye, for example has a peak absorbency at 540nm (Knox and Dodge, 1984), whereas the photosensitizer bacteriochlorophyll(a) absorbs optimally at 760nm (MacRobert and Philips, 1992). Molecular oxygen is needed in most photodynamic systems where the photosensitizer acts as a catalyst during the reaction. Photosensitizers which do not require the presence of oxygen do exist,

such as the furocoumarins which do not produce singlet oxygen, and are themselves consumed during their reaction. Photodynamic action is arbitrarily defined to require i) a sensitizer ii) light and iii) oxygen. Thus furocoumarins (such as the psoralens) are excluded because they do not require oxygen (Wacker, 1972).

MECHANISMS INVOLVED IN PHOTODYNAMIC ACTION

Photodynamic reactions involve electronically excited states of the sensitizer molecule. All elements have their own set of absorption energies. This is due to the fact all elements have different numbers of electrons, when these electrons are excited they are raised to higher levels or shells. The energy required to raise electrons to various excited levels has a value known as the "principle quantum number *n*". Each of the raised levels are given a quantum number from the lowest level *n*1 through to *n*5. For example all electrons raised from quantum level *n*1 to any of the quantum levels above it, represent absorption in the U.V. spectrum. Electrons raised from the *n*2 level correspond to absorption in the visible spectrum (Peet, 1978) (Figure 1).

In the dark, sensitizer molecules exist in the ground state **S**. On addition of light energy near U.V. or visible is absorbed by endogenous pigments, which can interact with substrates (firstly oxygen) yielding products (shown below) which are toxic/harmful to the cell. In order to act as efficient photosensitizers, these molecules have to undergo "intersystem crossing" (Figure 2) from their first excited singlet state to their first excited triplet state with a relatively high quantum yield. In fact, as the life time of the triplet state is considerably more than that of the singlet state (10^{-3} to 10^{-6}s as opposed to 10^{-9}s) the probability that a molecule in such a metastable state will interact with other molecules before decaying back to the ground state is significantly higher. Photodynamic processes can be classified as TYPE I and TYPE II reactions (Ghetti, 1992).

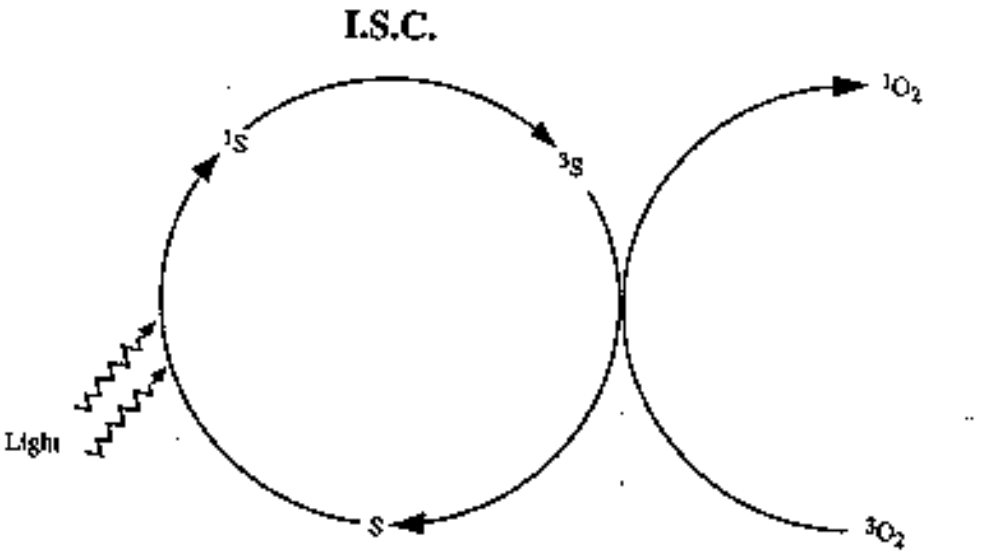

Figure 2. Photodynamic generation of $^1O_2$ by a sensitizer. S= ground-state sensitizer; ^1S= excited singlet state of sensitizer; ^3S= triplet state of sensitizer; I.S.C.= intersystem crossing; $^3O_2$= ground-state (triplet) molecular oxygen; $^1O_2$= singlet oxygen (Knox and Dodge, 1985).

In the TYPE I mechanism (electron transfer) the triplet sensitizer molecule ($^3\mathbf{S}$) can react directly with a reducing substrate "**A**" by electron transfer process to give a semireduced form of the sensitizer, $\mathbf{S}^{-\cdot}$, and a semioxidizied from of the substrate, $\mathbf{A}^{+\cdot}$

$$^3\mathbf{S} + \mathbf{A}(\text{substrate}) \rightarrow \mathbf{S}^{-\cdot}\ \mathbf{A}^{+\cdot}$$

The semioxidized substrate can react with molecular oxygen to give a fully oxidised product, **Aox**:

$$\mathbf{A}^{+\cdot} + {}^3\mathbf{O}_2 \rightarrow \mathbf{Aox}$$

The ground-state sensitizer can be regenerated from the semireduced form by reaction with oxygen:

$$\mathbf{S}^{-\cdot} + \mathbf{O}_2 \rightarrow \mathbf{S}_0 + \mathbf{O}_2^{-\cdot}$$

Which the oxygen superoxide, $\mathbf{O}_2^{-\cdot}$ can be produced in this process and in turn oxidise another molecule in the system:

$$\mathbf{O}_2^{-\cdot} + \mathbf{Aox}$$

The second and most common mechanism TYPE II (energy transfer) process is the interaction of the triplet sensitizer with ground-state oxygen, which is in the triplet state ($^3\mathbf{O}_2$).

$$^3\mathbf{S} + {}^3\mathbf{O}_2 \rightarrow \mathbf{S}_0 + {}^1\mathbf{0}_2$$

Singlet oxygen ($^1\mathbf{O}_2$) is the main species involved in photodynamic reactions. Singlet oxygen is more reactive than ground state oxygen, and can interact with a wide range of substrates to give a fully oxidised form of the substrate (Spikes, 1977). Sensitizers such as acridines, porphyrins, xanthenes (eosin Y, rose bengal) and thiazines (methylene blue, thionine) give good yields of singlet oxygen. Techniques available to determine whether a particular sensitizer produces photodynamic inactivation via the singlet oxygen mechanism have presented by (Nilsson, 1973; Foote, 1968; Higgins, 1968).

POSSIBLE APPLICATIONS FOR PHOTODYNAMIC ACTION

The main fields in science in which this technology is being exploited includes photodynamic therapy (P.D.T) for treatment of cancers (MacRobert and Philips, 1992), inactivation of bacteria (Lin-Q *et al.*, 1991); inactivation of viruses (Lenard *et al.*, 1993), and new selective media (Miyazawa *et al.*, 1974). Photodynamic therapy involves the use of specialised photosensitizers, which are administered intravenously to the patient. The criteria for a successful photosensitizer are very strict due to the nature of its function. Current clinical trails to eradicate tumours, or to alleviate the symptoms use haematoporphyrin derivative (HpD). A purified fraction of HpD is available commercially as a sensitizer called DHE, which has been one photosensitizer widely used on clinical trails (MacRobert and Philips, 1992).

The photodynamic inactivation of bacteria is of interest to the food microbiologist, and research in this field has focused on disinfection, and a preservation system that has a high safety and a low energy cost (Lin Q *et al*., 1991). These could be applied to surfaces of materials, and interfaces of solid-liquid, or solid-gaseous system. One system investigated, uses a

water soluble photosensitizer alpha-terthienyl (Alpha-T) immobilised in a film of polymethylmethacrylate (PMMA). Photodynamic inactivation of bacteria using U.V. light was observed. *Escherichia coli* strains exhibited multihit kinetics, whereas curves for *Pseudomonas aeruginosa* and *Staphylococcus aureus* exhibited single hit kinetics. Cell inactivation by irradiated Alpha-T film at an immobilised concentration of 20 $\mu g/cm^2$ was mainly caused by generated singlet oxygen. The activation rate depended on the surface density of Alpha-T in the film. A linear relationship between singlet oxygen production on the film surface and cell death was obtained (Lin *et al*., 1991).

Work has also been carried out on the inactivation of a range of microorganisms using the sensitizer rose bengal. The study involved finding the minimum concentration of rose bengal required to produce an effective kill, and also an investigation into the quenching compounds L-histidine and crocetin which protect bacteria from photodynamic inactivation (Banks, 1987). This application could be used to provide photodynamic preservation and increase the shelf-life of perishable products with photosensitizers incorporated into the packaging of the products sensitized by natural light. As mentioned earlier in this paper some common food preservatives/antioxidants may quench photodynamic action and therefore limit application. Photodynamic preservation in foods would need to be carefully assessed so that the foods contained no significant levels of natural or artificial quenchers. The commercial implication of successful photodynamic preservation systems are significant.

Studies of inactivation of *Staph. aureus* by the photosensitizer deuteroporphyrin and its inhibitors have been carried out. The use of deuteroporphyrin showed a 96% inactivation of *Staph. aureus* after 30 minutes. With the addition of propylgallate 42% inactivation of *Staph. aureus* was achieved. A range of reagents was tested including cysteine, histidine, and methionine (which provided 95.8%, 95.9% and 41% inactivation respectively) (Nitzan *et al.*, 1989).

New selective agar for the detection of coliforms in foods based on Rose Bengal Lactose (RBL) have been developed using photodynamic action as the selective agent against contaminating flora. Xanthine photosensitizers such as Rose Bengal show lethal activities against Gram positive bacteria. The RBL agar was found to be practical in the detection of coliforms from several types of food (Miyazawa *et al*., 1974).

The separation of the photosensitizer from the substrate on Gram-positive (*Streptococcus faecium*) and Gram-negative (*E. coli*) bacterial strains, has been studied using a thin air layer as the separating barrier. Under such conditions only singlet oxygen can reach and oxidize the substrate. The cell samples exposed to the singlet oxygen for various lengths of time, showed that *S. faecium* survival decreased rapidly. The Gram-positive *E. coli* became sensitive only when the integrity of the outer membrane was altered by treatment with calcium chloride or Tris-EDTA. Biochemical and ultrastructural analyses suggest that the cytoplasmic membrane and genetic material are the main sites damaged by singlet oxygen (Valduga *et al*., 1993).

The use of photodynamic action has also been investigated for the inactivation of infectivity of enveloped viruses, including the human immunodeficiency virus (Lenard *et al*., 1993). Rose Bengal and hypericin dyes were both shown to inactivate enveloped (but not unenveloped) viruses upon illumination by visible light. Human immunodeficiency virus was photodynamically inactivated by both photosensitizers at nanomolar concentrations. Both sensitizers used showed similar virucidal effects (Lenard *et al.*, 1993).

Photodynamic inactivation offers many new opportunities to food technologists and food scientists, particularly now that the basic mechanics of the process are understood. Photodynamic inactivation cannot become a universal food processing technique because of its intrinsic requirement for light, oxygen, and a photosensitizer in close proximity to target microbes; however, there are undoubtedly many niche areas where of this application should be considered.

AREAS FOR FUTURE RESEARCH

Future research needs to concentrate on the engineering aspects of delivering effective light doses and food grade photosensitizers to the intended site of application. Some benefit might also be gained from screening natural, food grade components for photosensitizing activity.

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2.4

MODIFIED ATMOSPHERE PACKAGING OF NON-RESPIRING FOODS

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ABSTRACT
The primary gases in MAP applications are CO_2, N_2 and O_2. For food safety carbon dioxide is the primary gas and has both bacteriostatic and fungistatic properties. The degree of growth inhibition offered by CO_2 differs widely depending on the species of microorganisms and proper-ties of the product, *i.e*. additional hurdles such as pH, water activity, salt and sugar. Applying MAP to food products, the producer needs to consider both external and internal factors. Among the most important factors to have knowledge about is the capacity of a product to take up added CO_2, *i.e*. the amount of CO_2 dissolved in the product. With MAP of non-respiring food products, the optimal gas mixture as well as the ratio of headspace to produce volume have both to be correctly dimensioned to ensure food safety.

INTRODUCTION
Modified atmosphere packaging (MAP) is a technology in which a food product enrobed in high barrier packaging material and in which the air in the headspace is replaced by a constructed gas mixture, a procedure that leads to an enhancement of the product shelf-life. The role of the gasmixture is to slow down the respiration rate of the packaged product, to reduce microbial growth and to retard enzymatic spoilage. For successful applications of MAP, knowledge of the most optimal gasatmosphere composition in the package is a prerequisite. Under optimal MAP conditions, high food quality may be retained for several days or weeks extra, without causing health hazards. Consumers today demand products of high quality, which means fresh, minimally processed foods which contain less preservatives and are safe to eat. MAP is very well suited to meet this demand.

However, MAP technology requires from the food producers that they take time to understand exactly what is involved in it. Ultimately this means that they have to expert knowledge on all three basic components, *i.e*. food, gas and packaging-material, and more so on the integration of these components for an optimal MAP system. Only the correct application of MAP-technology will result in better quality and safety of foods as compared with today's products.

PRODUCT SPOILAGE
Spoilage of food products is primarily due to chemical or biological reactions. What is first detected in spoilage or quality changes, depends mostly on the type of product, the composition of the gasmixture and the types of microorganisms present. The chemical reactions may be enzymatic oxidation of for example lipids, causing rancidity, or of myoglobin in red meat, resulting in brown discolouration. Organoleptic spoilage such as bad taste or odour may be due to the biological activity of moulds, yeasts and bacteria. Certain types of microorganisms may, under specific conditions, produce toxic compounds which pose a health hazard. Concerning the food safety issue, the major health risks in the past have been caused by anaerobic pathogens, more specifically the psychrotrophic (cold-tolerant), non-proteolytic clostridia. However, new hazards have been

signalled in the case of MAP preserved products. Because of their extended shelf-life at chill temperature, there is a potential risk that other psychrotrophic pathogenic bacteria, for instance species of the genera *Campylobacter*, *Listeria*, *Aeromonas* and *Yersinia*, may proliferate and reach dangerously high levels in products. During the 1990, a vast number of MAP products has become available to the consumer and it is of the utmost importance that their microbial safety is properly ensured.

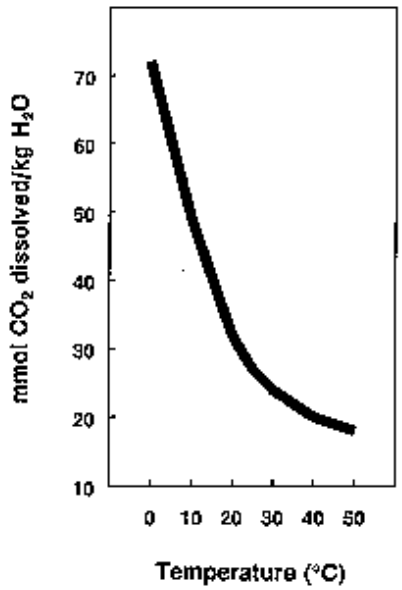
THE GASES

The main components to obtain an extended shelf-life with in MAP are the use of the optimal gases and the storage at chill temperature. The gases predominantly used in MAP storage are Carbon dioxide (CO_2), Nitrogen (N_2) and Oxygen (O_2). These gases are employed either alone or in mixtures. The gasproperties and the interaction of gases with the food-matrix, *e.g*. solubility in the foodstuff, should be taken into account when choosing the gascomposition.

Table 1. Solubility (L/kg at +20°C) in water at P_{GAS} = 100 KPa

Argon, Ar	0.033
Hydrogen, H_2	0.018
Carbon dioxide, CO_2	0.878
Nitrous oxide, N_2O	0.610
Nitrogen, N_2	0.015
Oxygen, O_2	0.030

Figure 1.Solubility of CO_2 at different temperatures



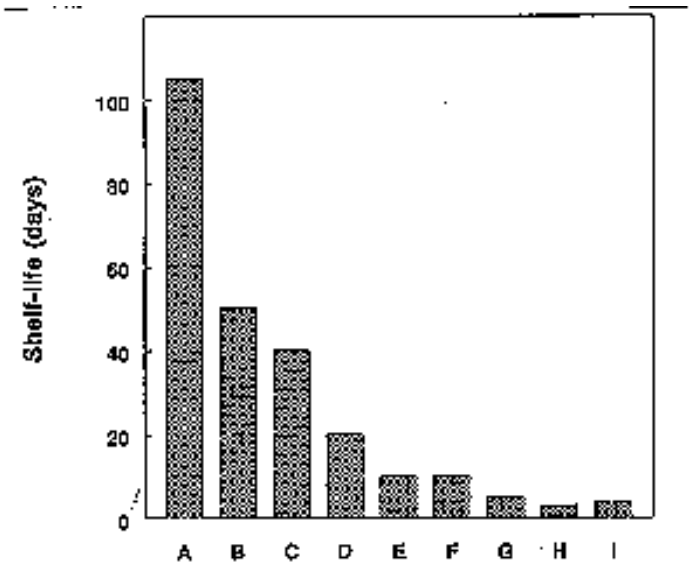
For example N_2 prevents oxidation, stops growth of obligatory aerobic microorganisms and can be used as a filler gas because it has only a low solubility in water. O_2 prevents growth of obligatory anaerobic microorganisms, many of which are toxigenic, and maintains the red or pink colours of meat. CO_2 finally has a bacteriostatic effects, generally inhibiting microbial growth at levels above 20 percent.

Table 1 gives some data on the solubility of CO_2 in water in comparison with other gases of interest for MAP. Figure 1 illustrates the dramatic increase in solubility of CO_2 in water at chilled temperatures.

THE PACKAGING MATERIAL

In order to keep the gas or gasmixture around the non-respiring products during the entire life time of the package, multi-layered plastic materials are commonly used. Headspace volume, gas and vapour permeabilities, shape and design are among the most determinative factors. The first two affect the time and extent to which a gas is present in sufficient amounts to be able to inhibit the microorganisms. Packages made of high-barrier materials and with a large headspace generally lead to the more extended shelf-lives. The shape of the packages should enable maximum contact between product and gases.

Designing MAP systems for products, producers need to consider many external and internal factors in order to maintain high quality and proper food safety throughout the period of product storage to human consumption. For example for food with low-fat and high-moisture contents, the prime aim of MAP is to inhibit the growth of microorganisms. For foods with high-fat and low-moisture contents, protection from oxidation is most important. In general, the initial numbers of microorganisms on the packaged product must be as low as possible. Well-controlled chilling and good hygiene during handling and packaging, are required to achieve an optimal shelf-life. Figure 2 summarizes literature data on approximate (microbiological) shelf-lives for beef products at different combinations of modified atmosphere and temperature. It adequately emphasizes the effectiveness of CO_2 as a hurdle.



A = 100% CO_2, 1°C
B = Vacuum, 1°C
C = 100% CO_2, 4-5°C
D = Vacuum, 4-5°C
E = 10-20% CO_2, 2-10% O_2, balance N_2, 4°C
F = 15% CO_2, 40% O_2, 45% N_2, 4°C
G = 50-60% CO_2, 40-50% O_2, 4°C
H = Air, 4°C
I = 100% O_2, 1-3°C

Figure 2. Shelf-lives for beef products as based on microbiological factors obtained with different combinations of gasatmosphere compositions and storage temperatures

Today we can see an increasing number of non-respiring products in modified atmosphere on the market. In table 2 has a number of different non-respiring products been collected where MAP-guidelines are given to the producers, for what is required for successfully retain high quality. The shelf-lives given should be seen as rough estimates, as they are determined by several factors such as initial flora of microorganisms and physical properties of the product. In reviewing the literature, sensory analysis measuring changes in odour and flavour has been used in combination with microbiological analysis as an criterion as whether a product is spoiled or not.

Table 2. Recommended MAP-conditions for a variety of non-respiring food products.

Product	Gas mixture	Gas volume (ml) per 100 g product	Shelf-lives (days)		Storage temp. (°C)
			in air	in MAP	
Red meat	80% O_2 + 20% CO_2	100-200	2-4	5-8	+2 to 3
Poultry meat	50-80% CO_2+ 20-50% N_2	100-200	7	16-21	+2 to 3
Sausages	20% CO_2 + 80% N_2	50-100	2-4	28-35	+4 to 6
Fatty fish	60-70% CO_2 + 30-40% N_2	200-300	3-5	5-9	+0 to 3
Cooked fish products	20% CO_2 + 80% N_2	50-100	2-4	28-35	+4 to 6
Hard cheeses	80-100% CO_2 + 0-20% N_2	50-100	14-21	28-70	+4 to 6
Rye wheat bread	20-40% CO_2 + 60-80% N_2	50-100	2-3	14	+20 to 25
Pizza	30-60% CO_2 + 40-70% N_2	50-100	7-14	14-35	+4 to 6

MEAT PRODUCTS

From Table 2 it may be concluded that for applications of MAP to meat products, levels of CO_2 commonly are around 20% CO_2 and will retard bacterial growth, particularly growth of the main spoilage organisms such as the obligatory aerobic species *Pseudomonas, Moraxella* and *Acinetobacter*, which present the greatest problem for fresh meat kept under normal air. Although most research is reported in terms of volume % of gas, the factors to be considered really are the amount of gas solubilized in the food and thus effectively available, expressed in terms of gas volume/product weight. 20% CO_2 is not inhibitory for gram-positive microorganisms such as lactobacilli which may turn out to be a spoilage flora on many types of MAP stored meat. CO_2 still is a beneficial gas to use, though, since lactobacilli produce byproducts of their metabolism more slowly as compared to typical spoilage organisms such as pseudomonads.

FISH PRODUCTS

Fresh fish has an extremely short shelf-life. By use of proper MAP systems, the shelf-life can be extended typically by 20-50% as compared to air storage. The sensitivity of fish and other seafood is caused by their high water activity, neutral pH and presence of enzymes. The safety of MAP stored fish products has been examined extensively over the past years and several reviews are available in the literature. The concern for health is growth and toxin production by non-proteolytic strains of *Clostridium botulinum*, that are able to grow down to 3.3°C. To assure safety, it is recommended to keep the products below 3°C at all times. For well cooked fish products it is assumed that all sporeformers are inactivated and 20% CO_2 is a sufficient hurdle.

DAIRY PRODUCTS

Cheeses are particularly susceptible to mould growth. Mould growth on hard cheeses is inhibited by levels of CO_2 of 80 to 100%. The very high CO_2 levels prevent moulds to grow, although lactobacilli which are naturally present in cheeses are slight affected this atmosphere too. However, in soft cheeses characterized by high water activities, CO_2 levels of 20-40% are commonly used to prevent bacterial growth and rancidity.

DRY FOODS AND BAKERY PRODUCTS

The main spoilage factors for bakery products are mould growth and chemical breakdown, resulting in staling. Products such as potato chips, peanuts, coffee and cocoa products are sensitive to oxidation reactions causing rancidity and water uptake leading to sogginess. The main biological hazard are moulds. MAP storage for the various products is favourable because of the low oxygen minimizes oxidation and suitable packaging materials may be adequate barriers to water vapour. Recommended CO_2 concentration are between 20-40%.

PREPARED FOOD

Prepared foods constitute a complex area. Products with several different ingredients are particularly suspectable to spoilage. A major difficulty associated with prepared foods is the introduction of microbial contamination during manufacturing process. Hygiene is therefore a very important factor. Also, only high quality raw materials can be used in successful MAP storage. Very-little or no O_2 is present in the headspace, whereas CO_2 levels are high (20-80%) in order to suppress development of pathogenic organisms. The most optimal CO_2 concentration strongly depends on the sensitivities of the different ingredients in the product towards the various spoilage factors. As a rule of thumb, the higher the water activity is, the higher CO_2 concentration should be applied in the package for optimal insurance of safety.

OWN RESULTS

Studies have been conducted on the sensitivity of different microorganisms to CO_2. The results obtained with a small number of spoilage and pathogenic bacteria are summarized in Table 3. However, there are many more pathogens to be considered in practice, like *Clostridium botulinum*, *Listeria monocytogenes*, *Aeromonas hydrophila* and *Vibrio parahaemolyticus*.

In general, CO_2 inhibits the growth of many bacteria and moulds but the extend to which this occurs is vary variable. From Table 3 it can be seen that *Bacillus cereus* is very sensitive but *Yersinia enterocolitica* is relatively resistant to CO_2. *Y. enterocolitica* may therefore may pose a considerable microbial risk if present in food to be stored in MAP, even when high levels of CO_2 are used. Carbon dioxide inhibits the microbial activity by dissolving into the liquid phase of the food, thereby reducing its pH. It it thought to penetrate the membrane of the microorganisms, thus causing changes in permeability and functionality. CO_2 may also affect cytoplasmic enzymes.

Research activities at SIK aiming at optimization of MAP applications and product safety have included both solubility and growth inhibition experiments.

SOLUBILITY EXPERIMENTS

The solubility of CO_2 in MAP model systems has been studied employing a coulometer. The aim was to see how solubility was affected by factors like pH, water activity, temperature, buffering capacity of product (media) and headspace to product volume (volume gas/media) inorder to accurately determine the level of CO_2 required for growth inhibition with, for

Table 3. Relative inhibition of various bacteria under 100% CO_2 as compared to under 95% N_2/5 CO_2

	0%	20%	40%	60%	80%
Bacillus cereus	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx				
Yersinia frederiksenii	xxxxxxxxxxxxxxxxxxxxxxxxxxxx				
Staphylococcus aureus	xxxxxxxxxxxxxxxxxxxxxxx				
Clostridium sporogenes	xxxxxxxxxxxxxxxxxxxx				
Citrobacter freundii	xxxxxxxxxxxxxxxxx				
Escherichia coli	xxxxxxxxxxxxxxx				
Streptococcus faecalis	xxxxxxxxxxxxxx				
Yersinia enterocolitica	xxxxxxxxxxx				
Brochotrix thermosphacta	xxxxxxxx				
Lactobacillus viridescens	xxxxx				
Lactobacillus plantarum	xxx				

example, CO_2 resistant microorganisms such as *Yersinia enterocolitica*. The results from the later two factors on the solubility of CO_2 are shown in Figure 3. From this it can be seen, that the CO_2 solubility increases with an increased buffering capacity of the medium (Figure 3A) and with an increased headspace to medium volume ratio (Figure 3B). In fact, this ratio and the buffering capacity of the food product are the two factors that influence the solubility of CO_2 most strongly. The cause of the differences in CO_2 solubility between the different types of product (medium) lies with the substantial differences in pH and content of salts and organic substances such as proteins. The increased solubility of CO_2 at increasing ratios of headspace to medium volume can be explained by the increase partial pressure of CO_2 under these conditions.

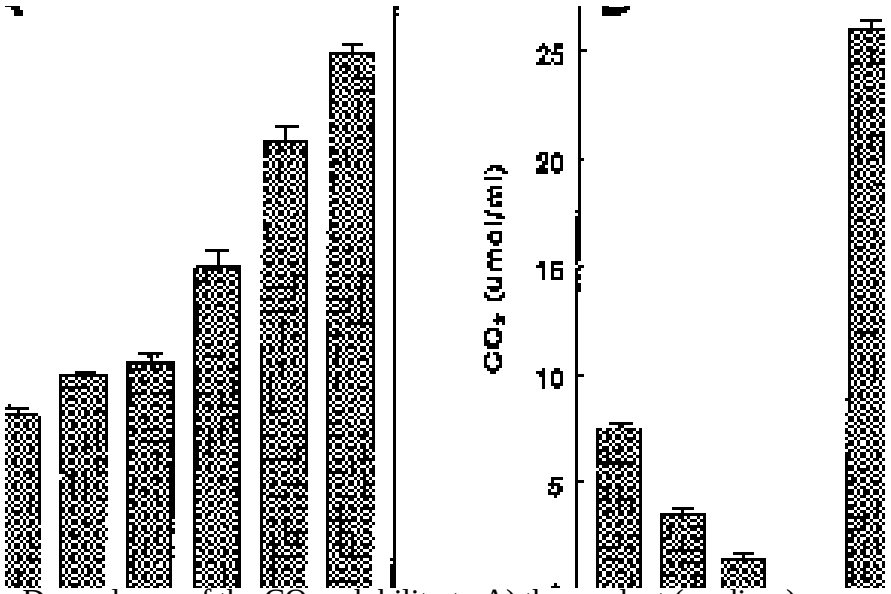


Figure 3. Dependency of the CO_2 solubility to A) the product (medium) composition and B) the ration of headspace volume to product (medium) volume.

INHIBITION EXPERIMENTS

Accurate data on the effect of CO_2 on pathogens are not readily available because earlier studies tended to calculate levels of CO_2 in terms of concentration in the headspace of the package rather than in the food, were its activity actually is. Using the coulometer method it is possible to get accurate estimation of levels of CO_2 solubilized in foods. In this way the effects of dissolved CO_2 present in different concentrations at chill temperatures on the activity of food related microorganisms can be effectively studied. In other words, it now is possible to determine precisely the real threshold values of CO_2 for either growth or no growth. Threshold values have been determined for *Y. enterocolitica* (Figure 4). It was found this pathogen had a great potential to grow in the presence of high CO_2 concentrations at low temperature. Inhibition was only observed at 79 µmol CO_2/ml medium at 4°C. To achieve this level of dissolved CO_2, a ratio of headspace to product (medium) volume of 11:1 and a concentration of 100% CO_2 was necessary. A smaller ratio of headspace to product volume does not give such a high concentration of CO_2 concentration in solution. This finding shows the importance of measuring dissolved CO_2 concentrations in MAP systems in order to be able to correctly dimension a MA package that properly prevents growth of pathogenic microorganisms.

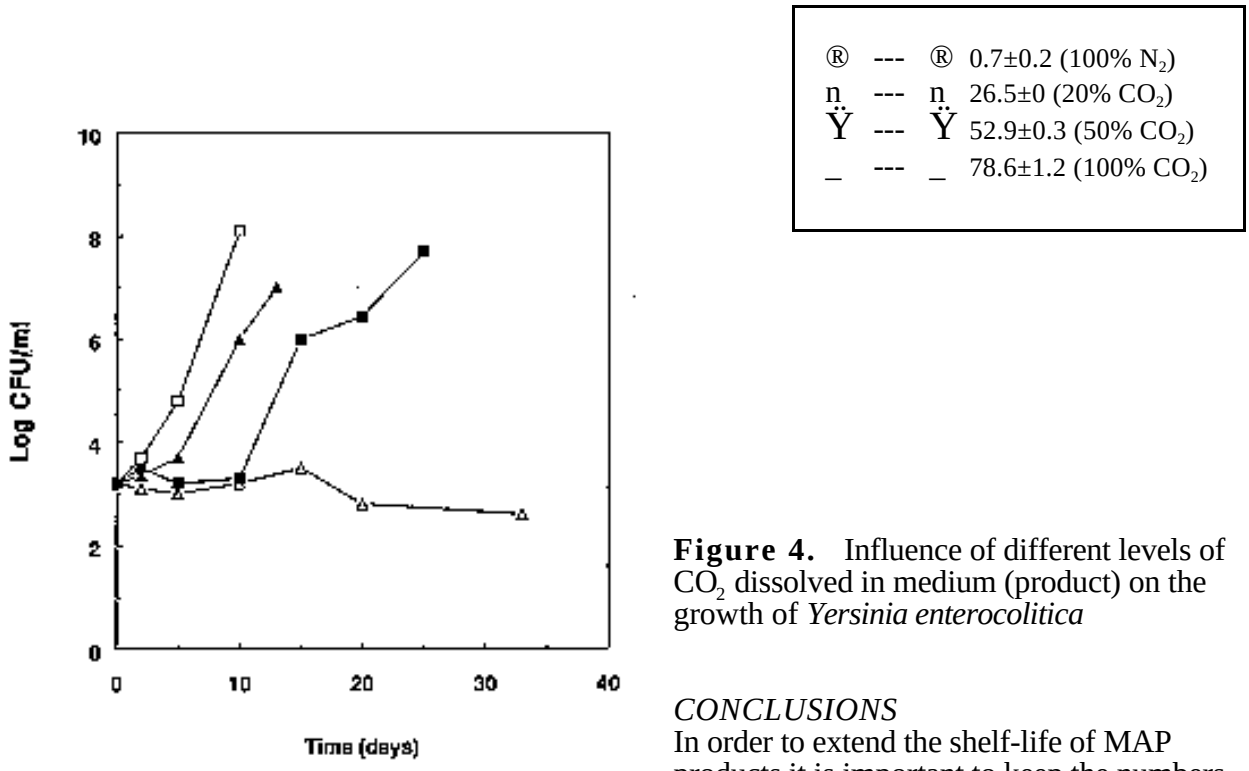


Figure 4. Influence of different levels of CO_2 dissolved in medium (product) on the growth of *Yersinia enterocolitica*

CONCLUSIONS

In order to extend the shelf-life of MAP products it is important to keep the numbers of spoilage and pathogenic microorganisms at very low levels. In doing so the primary hurdle is CO_2 gas. However it can not be the only hurdle. In order to build in safety, considering particularly the various, psychrotrophic pathogens, additional hurdles such as pH, water activity and temperature must be used on MAP products. Use of high quality raw materials only, hygienic manufacturing practices and educated system design are additional prerequisites for successful application of MAP technology for non-respiring products.

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2.5

MODIFIED ATMOSPHERE STORAGE OF RESPIRING PRODUCE

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ABSTRACT
Extension of the preservation period of respiring produce in bulk storage or in packages by means of Modified Atmosphere (MA) is dealt with in this chapter. Different techniques are used to create such a MA. A short overview is given on the modes of action of MA's, on the possibilities and limitations in their use. Specific problems encountered when optimizing Modified Atmosphere Packaging (MAP) of fresh produce are outlined and new developments in MAP applications indicated.

INTRODUCTION
Modified Atmosphere (MA) storage indicates the preservation technique whereby a perishable product is stored under an atmosphere that is different from the normal composition of air. This MA can be applied in rooms or containers for the storage and transport of bulk commodities (mainly fruits) or can be created within small packages for retail sale. When the concentrations of the different atmospheric components are kept within a narrow band around the desired optimal values through continuous control and regulation, the more specific term Controlled Atmosphere (CA) storage is applicable.

The modification of the atmosphere generally implies a reduction of the oxygen (O_2) content and/or an increase of the carbon dioxide (CO_2) concentration, but in some cases changing the level of carbon monoxide (CO), ethylene, ethanol or other compounds in the atmosphere can also contribute to the extension of the preservation period. A MA can be created passively by the respiration activity of the product inside the storage room or package and/or can be obtained in an active way with the aid of gas generators and scrubbers (MA or CA storage of bulk products), by evacuation of air (hypobaric storage, vacuum packaging), by introducing the desired gas mixture (gas packing) or chemical systems that absorb or generate gases or volatile compounds (active packaging) into packages.

This section will focus upon MA storage and more specifically upon Modified Atmosphere Packaging (MAP) of fruits and vegetables. MAP of fresh produce needs a different approach than MAP of other food commodities since fresh fruits and vegetables are living organisms. They are still respiring and as such contribute to the modification of the surrounding atmosphere. Moreover they are able to respond to these modifications. The problems encountered when optimizing MAP of fresh produce are therefore quite different from those related to MAP of non-respiring foods. Also the bases for the contribution of a MA to the extension of the shelf-life, and hence the possibilities and limitations in the use of a MA, are different for these respiring products.

HISTORICAL NOTE ON MA
MA has been used for ages for the storage of grains and root crops. The storage of these products in caves, underground pits and hermetically sealed containers is still in use today in developing countries, while recent innovations are added. Although there had been earlier

attempts at commercial use of MA techniques for the preservation of fruits, it was only after the basic, systematical studies on the effects of MA on physiological processes, initiated after World War I (Kidd and West, 1927), that risks with the use of this preservation technique were minimized and the commercial use of MA for long-term storage of bulk fruits became widespread.

The growing concern of consumers towards the use of potentially harmful chemical preservatives in and around foods stimulated the development of techniques for atmosphere control as well as biological research that lead to a broader application area for MA's. Although nowadays MAP is widely used, there is a still increasing interest in the development of CA and MAP techniques for the short term preservation of small amounts speciality crops and minimally processed fresh produce.

BIOLOGICAL BASIS OF MA PRESERVATION

Immediately after harvest the sensorial, nutritional and microbial quality of fresh produce will start to decline as a result of altered plant metabolism and microbial growth. These deteriorative processes include transpiration, senescence and ripening associated processes, wounding initiated reactions, development of postharvest disorders and microbial proliferation. Their relative importance in determining the end of the shelf-life will depend upon the product's characteristics as well as upon external factors. Low temperature and proper hygienic handling of the material are the prime factors that control these processes. A suitable modified atmosphere can further slow down or retard the onset of most of these undesired processes. A brief summary on the mode of action of MA's in preserving fresh produce will be presented here, but comprehensive reviews can among others be found in Kader (1986) and Weichmann (1986).

Lowering O_2 and increasing CO_2 concentrations in the first place will slow down respiration activity and decrease general metabolic activity. It will retard senescence, aging and ripening, and associated processes that may lead to quality loss, such as tissue softening and loss of green colour. Often the undesired reactions are mediated by specific enzymic activities or can be attributed to an increased oxidative state of the tissue as a result of loss of membrane integrity. Decreased oxygen levels may have a direct effect on these reactions.

Many of the ripening and senescence related processes are initiated or controlled by ethylene action. Part of the MA response can be attributed to its action upon ethylene production and sensitivity. Further improvement of storability of some commodities can therefore also be obtained by directly controlling the ethylene level.

MA's can also prolong postharvest life of some commodities by alleviating certain physiological disorders such as chilling injury of subtropical fruits or russet spotting in lettuce.

Modifying the atmosphere in general involves reducing the O_2 concentration from 20.9% in air to 1 to 5% and elevating CO_2 level from 0.03% in air to 2 to 15% or even higher. However these values are only indicative and the optimal concentrations required to improve quality retention greatly depend upon the commodity and the main processes involved in its deterioration, as well as upon external factors. Often the effects of O_2 and CO_2 levels are additive, sometimes even synergistic. In many cases products have to be kept in between very narrow borders of atmospheric composition for the MA to be beneficial and not detrimental.

Inappropriate MA's with concentrations for O_2 or CO_2 outside the limits of tolerance for a particular commodity may lead to new disorders or to irregular ripening. These tolerance limits may depend upon the physiological state of the product, as *e.g.* determined by growth conditions and maturity stage, but also upon temperature and rate and duration of atmosphere modification. Too low O_2 levels will increase anaerobic respiration and make the product unacceptable due to off-flavour development and membrane damage. Too high CO_2 concentrations will cause several

specific disorders *e.g.* development of brown stain in lettuce.

The constraints to MA's in view of the tolerance of the plant material imply that only in very rare cases does a MA directly act upon decay micro-organisms. If there is a MA benefit in retarding microbial deterioration of a produce, this is mostly the result of a better preservation of the integrity of the plant material due to retarded ripening and senescence processes.

CONTROLLED ATMOSPHERE STORAGE

Today, lists with optimal gas composition ranges for CA storage of many commodities are available. The International Controlled Atmosphere Research Conference, held every 4 years, includes in its Proceedings lists with up-to-date recommendations for CA storage. Similar information can be found in Calderon and Barkai-Golan (1990). Although a lot of data are already available, research is still going on to find optimal MA solutions for new products and to improve MA storing conditions for classical products. More basic research is needed on the combined effects of modified O_2 and CO_2 levels.

Care should be taken when trying to apply these recommended atmospheres to related products. Even cultivar, cultural and harvest related factors can greatly influence responses to a specific MA. A better understanding of the physiological basis of MA action and of product variability leading to different responses to MA, will aid the further development of CA storage.

New areas of attention in CA storage today are hypobaric storage, Ultra Low Oxygen (ULO) storage (1 to 2% O_2) and programmed CA storage, which only became possible with the new technological developments in automated systems that allow for very strict atmosphere control.

MODIFIED ATMOSPHERE PACKAGING

A modified atmosphere may be effected in a sealed package through counterbalancing the respiration activity of living produce inside with the diffusion of gases across the packaging film. Eventually at a given temperature the gases approach an Equilibrium Modified Atmosphere (EMA) when the rate of gas permeation through the film equals the respiration rate. This EMA should approximate as close as possible storage optima of the commodity inside.

The course of the atmosphere modification is determined by three interacting processes: respiration by the commodity, gas diffusion through the commodity and gas permeation through the film. Each of these processes in turn is strongly influenced by several commodity and environmental generated factors. Respiration of a certain commodity depends on its physiological stage and temperature, O_2 and CO_2 partial pressures, ethylene concentration, *etc.* Gas diffusion is affected by temperature, gas gradient across the limiting barrier and by the commodity's mass, volume, respiration rate, membrane permeability, gas diffusion path, *etc.* Some of these variables may vary with the maturity stage of the product. Some variables affecting gas permeation through the film are: temperature, gas gradient across the film and film structure, thickness and surface area. A change in product amount, free volume or any of the variables listed above will affect the EMA and/or the time in which the steady state conditions are established. Flushing the package with a gas only will influence the time needed to attain the EMA.

An extensive review on MAP of fruits and vegetables is given by Kader *et al.* (1989).

An average MAP has a quickly changing atmosphere. Small amounts of product show greater variability in mean respiration and sensitivity than large amounts. Strict temperature control in the distribution chain is wishful thinking. Temperature response of the permeabilities of most films is in general lower than temperature sensitivity of the respiration activity. Most of today's existing plastic films do not have the proper O_2/CO_2 permeability ratio to provide the ideal MA

for many commodities at a given temperature. In view of all these facts and knowing that any change within or around the package will alter the dynamic equilibrium between the product and its environment, it is obvious that MAP should provide amelioration of quality retention under real conditions and that looking for an optimal solution under particular conditions (especially temperature) makes little sense. Knowing the limits of tolerance of a certain commodity therefore is even more important for MAP than it is for CA storage.

MAP OF MINIMALLY PROCESSED FRUITS AND VEGETABLES

MAP has been proven to be especially useful for the extension of the shelf-life of minimally processed vegetables. The processing of the food will not contribute to its preservation but, on the contrary, will make it more deterioration sensitive. Chopping, cutting, slicing and peeling will all injure the plant material and lead to higher respiration activity and ethylene production and enhance senescence. Moreover these treatments will provide extra sites for pathogen entry and substrates for their growth. A third area of problems will arise from the mixing of enzymes and substrates due to disruption of cell membranes. An example of this is the browning of cut areas of lettuce. Oxidation reactions in general will be enhanced. All of these problems can, at least partly, be overcome with the use of a proper MA. Conflicting levels of O_2 en CO_2 however may be required for different aspects of the quality problem.

MA packages have a high relative humidity and a low O_2 level and thus provide an environment that promotes growth of pathogens that otherwise would be excluded by competitive flora. A low initial load and proper temperature control therefore are very important. The higher respiration activity of wounded material will make the danger of overmodification and very low O_2 levels more real. At the same time more extreme MA's will be tolerated by cut plant material and natural warning of the consumer will be retarded.

There is a strong feeling that risks associated with MAP of minimally processed material should be studied closer, especially for low acid vegetables missing an extra natural hurdle. How does a MA act upon growth of pathogenic microorganisms; how does the microbial profile change; do microorganisms adapt themselves to MA's; are all questions to be answered. Meanwhile high temperature or low oxygen indicators could be included in film packages as a safety precaution.

OPTIMIZATION OF MAP

A lot of interacting factors determine the atmosphere change in a MAP. This implies a lot of possibilities for optimizing a specific MAP and obtaining maximum benefit from the technique, but also means that optimization with trial-and-error experiments will be very slow. Alternatively predictive models could be used in MAP design for fresh produce.

Since temperature in the distribution chain often cannot be strictly controlled, it would be extremely useful to be able to evaluate the probable limits of atmospheric modification in order to determine whether they will remain within the limits of tolerance of the commodity, which itself is a variable. Other applications of models could be screening of commercially available films for a specific application and pointing out the potential limitations, or defining the requirements for new films in terms of their temperature sensitivity and CO_2/O_2 permeability ratio. Already some interesting models have been developed (*e.g.* Kader *et al.*, 1989; Zagory *et al.*, 1989), but the limiting factor in their use is the lack of detailed data and of insight into interacting processes and factors that cause variability.

The development of new films with more suitable gas permeability ratios could greatly improve quality retention under MAP. For products with high respiration activity the plastic film industry

is constantly in search for films with higher permeabilities. One way in achieving this is the use of microperforated films, although this solution means a loss of the selectivity of the film to the different atmospheric compounds. Another way is the use of labels that are put over holes in packages. Other solutions are being looked for in the area of laminated films and the so called "smart" films. These are temperature compensating films, whose "permeabilities" changes in response to temperature changes. This can mean that an irreversible change is induced by temperatures above a fixed safety level, to avoid the creation of anaerobic conditions. A more ideal solution would allow for a reversible reaction to changing external conditions.

Selection of suitable cultivars and tuning cultural practices in view of the product's processing is a practice that has been given little attention up until now, especially when it concerns vegetables. Today, raw material variability is a problem but when better understood, the selection of suitable homogenous material could contribute to and facilitate the optimization of the product-film-environment combination.

Selection of the most suitable material for a specific processing-preservation technique requires knowledge and understanding of the main processes involved in quality loss and deterioration of the processed product under optimal and suboptimal conditions. As outlined above different quality aspects may determine consumer acceptability and their relative importance may be determined by storage conditions and other factors. Multivariate analysis procedures can be a valuable tool in determining the relative importance of different quality aspects in the overall quality loss of the product and in revealing relations between sensory quality aspects that decline during storage and the physiological and microbiological parameters involved (Keteleer *et al.*, 1993). Once the underlying deteriorating mechanisms are known, process determining product properties (*e.g.* enzyme activities, respiration rate) can be identified. In this way "suitable material for minimal processing and/or MAP" can be defined in terms of product characteristics and can be selected for on an objective basis. Further research could then reveal to which degree these characteristics are genetically determined or can be influenced by preharvest factors.

CONCLUSIONS

MA does not increase product quality, neither can it replace proper product handling, but starting from high quality material and with strict hygienic and temperature control it can contribute to quality preservation of perishable products and often replace the use of chemicals on foods. However, producers should be well aware of the risks of irresponsible use of MA's and keep away from the dangerous attitude of pack and pray.

Modern consumer needs for convenient and fresh, wholesome produce together with the recent reorganization of its distribution chain will further stimulate the use and the broadening of the application area of MAP. Continued basic physiological and microbiological research on the action of MA's will minimize the risks of loss and safety associated with the use of MA's and will allow for faster MAP optimization by using models. Also new technological developments, especially in the area of more suitable packaging films, will contribute to the success of MA as a preservation technique.

The main limiting factors for further extension of MAP will probably arise out of environmental concern. A solution for the huge waste problem, in the long run, may be found in the use of edible and biodegradable films that are able to create a modified atmosphere.

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2.6

EDIBLE COATINGS AND OSMOTIC DEHYDRATATION

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ABSTRACT
Adjustment of hurdles can be obtained by "direct formulation" *i.e.* by immersion of food pieces in concentrated solutions of additives, acids, a_w lowering agents, *etc.* This technology initially refferred to as Osmotic Dehydratation has now been termed a Dewatering and Impregnation Soaking (DIS) process. Individual protection from external oxygen, external humidity or from superficial microbial development can be achieved by controlling the surface preservative concentration with an adequate Edible Protective Superficial Layer (EPSL) enriched with specific additives. Osmotic dehydration and edible coatings represent two ways to apply hurdle technology to solid foods without affecting their structural integrity.

INTRODUCTION
Physico-chemical and microbiological stabilisation of foodstuffs by combination of hurdles has generally been achieved by formulation of destructured food followed by a retexturation procedure. Two other alternatives can be used, which keep the original food texture and integrity. One is to achieve a direct formulation of foodstuffs by soaking them, whole or in pieces, in highly concentrated solutions. Various traditional food processes are obtained according to this technology. These are mainly salting (*e.g.* cheese-making, fish or meat curing processes), osmotic dehydration, candying and semi-candying and combination of acidification, salting and/or addition of sugar (*e.g.* pickles, olives, acidified vegetables, *etc.*). Such processes have recently been termed Dewatering and Impregnation Soaking (DIS) processes (Raoult-Wack *et al.*, 1992).

The other alternative, less used, consist of placing an edible protective superficial surface layer with a high concentration of a given preservative, *e.g.* antimycotic or antioxygen agent (Guilbert, 1988). Due to the ability to retain additives and to reduce water and gas (O_2, CO_2) transport between the food and its environment or between two compartments of a heterogeneous food, this layer allows control of the chemical hurdles but also acts as an additional hurdle for improving overall food quality and stability.

GENERAL ASPECTS OF DIS PROCESSES
When foods are soaked into concentrated solutions, three cross mass transfers are initiated, 1) an important water outflow, from product to solution, 2) a solute transfer, from solution to product and 3) a leaching of product's own solutes. Hence, this technique claims to achieve simultaneously dewatering and direct formulation of the product, *i.e.* through impregnation plus leaching (Raoult-Wack and Guilbert, 1990; Raoult-Wack *et al.*, 1992). All these transformations of the food are then reported on the soaking solution and its composition has to be adjusted continously by evaporation and/or by adding solute(s).

Mass transfer kinetics first of all depend on the structural properties of the product. In fresh biological systems, high water ouflow is accompanied by low solute impregnation. The

loss of water is usually attributed to osmosis phenomena through the semi-permeable cellmembranes (Ponting *et al*., 1966). Solute impregnation can be improved by pre-treating the food, in order to passivate tissue membranes, *e.g.* by freezing or by blanching. In this case, appropriate control of operating variables such as specific surface area of pieces, temperature, time/duration, concentration and composition of the solution (*i.e*. solute molecular weight and nature, presence of ions), and mode of phase contacting (solid/liquid phases) leed to a wide range of dewatering to impregnation ratios (Raoult-Wack *et al*., 1992).

A DIS process is generally implemented by using small food pieces (*e.g*. 1 cm^3) and highly concentrated (50 to 75 g of solute per 100 g of solution) of salts, sugars, organic acids, specific additives, *etc.* An increase in the concentration difference between product and solution has a very favourable effect on water loss, but generally a detrimental effect on solute gain (Lenart and Flink, 1984; Raoult-Wack *et al.*, 1991a). Using highly concentrated soaking solutions may also reduce losses of the product's own solutes, such as ascorbic acid in fruits (Vial *et al*., 1990), which is possibly due to the creation of a solute concentrated boundary layer preventing solute outflow (Raoult-Wack *et al*., 1991a). Impregnation is generally favoured by low molecular weight compounds but on the contrary, dewatering is greatly favoured by the maintainance of the concentration difference and from this point of view, the use of solutes with higher molecular weight is favourable (Lerici *et al.*, 1985; Raoult-Wack *et al*., 1991a).

Interactions between solutes are significant and may help the direct formulation effect. For instance, presence of sugar can decrease salt uptake in the product (Collignan *et al*., 1992). In some cases, a DIS process could be conducted in two parts: first, in a solution of high molecular weight solutes and then, in a solution of a low molecular weight solute, as suggested by Raoult-Wack *et al.* (1991a). This type of process effectively physically separates the stages of dehydration and the incorporation of the chosen solute and, therefore, can provide a better control on both.

Specific modelling of mass transfer phenomena during soaking were recently proposed by Le Maguer (1988), Raoult-Wack *et al.* (1991b, 1992) for the prediction of which modification of the product's composition is possible or not and for the industrial control of DIS processes.

GENERAL ASPECTS OF EPSL TECHNOLOGY

Edible films or coatings have long been used to improve food appearance or preservation (*e.g*. sugar, chocolate, wax or oil coatings for candies, fruits or various foods). Recently the use of coatings to maintain high concentrations of specifics additives at the surface of a food has been proposed (Torres *et al*., 1985; Guilbert *et al*., 1985; Guilbert, 1986). As an integral part of the food, edible films or coatings must have a composition which conforms to the regulations that apply to the food product concerned. Advantage, formation, types and properties of various films with examples have been comprehensively reviewed (Guilbert, 1986; Kester and Fennema, 1986; Guilbert and Biquet, 1989; Guilbert and Gontard, 1994).

The formulation of edible films and coatings must include at least one component that can form an adequately cohesive and continuous matrix. A wide range of compounds can be used: starch and derivatives, dextrins, alginates and other gums, cellulose derivatives, collagen, zein, gluten and other protein types, waxes and acetylated glycerides or other fatty materials. Hydrocolloids have generally good film-forming properties, but poor water resistance. Lipids seem to be the most effective edible barrier to water vapour movements but often cause application, mechanical, stability or organoleptic problems. Since the film structural integrity is necessary to maintain its barrier properties, edible films composed of polysaccharides or proteins (structural matrix) and lipids (moisture barrier) have been developed (Kamper and Fennema, 1984; Guilbert, 1986;

Kester and Fennema, 1989; Krochta *et al*., 1989; Gontard *et al*., 1994). The moisture barrier capacities of different films can be classified in decreasing order of efficiency as follows: waxes > solid lipids and fatty acids > lecithin, aceto-glycerides > liquid oils > insolubles proteins > other hydrocolloids. The addition of a plasticizing agent to edible films is required to overcome film brittleness. The most commonly used plasticizers are polyols, mono-, di- or oligo-saccharides, lipids and derivatives. Formation of a coating generally requires a liquid carrier or a solvent, limited to water, organic acids and/or ethanol for food products. For hydrocolloids based films, incorporation of reticulating or crosslinking agents such as divalent ions or organic acids, or exposure to denaturation conditions by heat or irradiation treatments may be carried out to improve mechanical or barrier properties (Guilbert, 1986). The application and distribution of the film coating material in a liquid form can be achieved by spreading, spraying, falling film, enrobing, dipping and subsequent dripping, distributing in a rotating pan (pan coating), fluidized bed or air brushing.

Certain specific agents (antimicrobial additives, antioxidants, organic acids, nutritional additives, flavours, colouring, *etc.*) can be incorporated into edible films to obtain functional effects localized on the surface. Using edible superficial layers of high antimicrobial or antioxygen concentrations allows the use of the additive without the destruction of food integrity and the use of smaller amounts of the additive relative to the total weight of the food (Guilbert *et al*., 1985; Guilbert, 1986). Therefore, the control of the protective effect, the diffusivity of the additive within the film and food is of particular importance (Guilbert *et al*., 1985; Giannakopoulos and Guilbert, 1986).

Films made of gelatin, casein, zein, stearic acid and waxes and also composite films (cellulose derivatives and fatty acid or zein and acetolglyceride) containing preservatives were investigated by various authors (Motycka and Nairn, 1978; Torres *et al*., 1985; Guilbert, 1988; Vojdani and Torres, 1990). These materials, placed in contact of model foods or real foods, significantly decreased the permeability to benzoic acid, sorbic acid and/or tocopherol.

The possibility of obtaining a permanent reduction of surface pH was explored by Torres and Karel (1985) by coating a food with a carrageenan-agarose gel matrix containing sorbic acid.

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2.7

ETHANOL

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ABSTRACT
Despite its widespread use as a germicidal agent, few studies have evaluated ethanol as a preservative for food products. Nevertheless, ethanol is present in a large number of foods for example wines, beer, spirits, confectionary products, bakery products, *etc.*
Ethanol is well known for its water activity (a_W) lowering capacity; this effect can induce a series of metabolic changes resulting in a decrease in the cellular activities of bacteria and yeasts.

Most of the studies on the growth inhibition or killing action of ethanol on microorganisms has been provided in alcoholic fermentation by yeast (particularly by *Saccharomyces cerevisiae*). However, it is unclear whether it is ethanol in the head space (vapour phase) or in the medium (liquid phase) that is mainly responsible for this action.

In recent papers and patents, ethanol has been proposed in modified atmosphere packaging, either adsorbed in the packaging or encapsulated as a vapour source. Ethanol has been shown to increase the shelf-life of bread, pizza, bakery filled products and filled pasta.

INTRODUCTION
The presence of ethanol in food is very common. It can be found in fermented food, as a consequence of fermentation processes; in formulated food, when ethanol is enclosed as a liqueur; in bakery products as a residue after baking; adsorbed in packed food when ethanol is released from a carrier inserted in the packaging or from packaging film. Examples are alcoholic beverages such as wine, beer, spirits, some milk derivatives, fillings in some sweets, chocolate liqueurs, confectionary and bakery products.

In the medical field, ethanol is widely used as a disinfectant, but only a few studies have been made on its use as a food preservative. Recently, the positive effects of the addition of low concentrations of ethanol as a means of prolonging the shelf-life of packaged foods, particularly from the microbiological point of view, has been recognized (Seiler and Russel, 1991).

ANTIMICROBIAL MODE OF ACTION
The antimicrobial mode of action of ethanol was reviewed by Ingram and Buttke (1984). The cell membrane is, in many cases, the primary site of ethanol damage, although ethanol clearly affects the properties of all biological molecules to some degree (Ingram, 1990). In general, high molar concentrations of ethanol are required to inhibit microbial growth, kill cells or block glycolysis and metabolism but high ethanol concentrations alter the physical nature of the aqueous environment. Ethanol affects the properties of water, which generally represents the major component of food.

From a chemical point of view, ethanol can be considered a water analogue. In mixtures of ethanol and water, the hydroxyls of ethanol compete with water during the formation of

hydrogen bonds. They also compete with molecules other than water, altering the macromolecular interactions, *e.g.* protein folding and the quaternary structure of macromolecules (Casey and Ingledew, 1986).

Ethanol is known to have a strong water activity (a_W) lowering capacity (Lerici *et al.*, 1983). In microbial cells (*i.e.* yeast), a reduced value of a_W may induce a series of metabolic changes resulting in a decrease in the cellular activities. The changes cause an overproduction of glycerol, which is the solute utilized for osmoregulation in yeasts and numerous other microorganisms.

ANTIMICROBIAL EFFECT

Most of the research on the growth inhibition or killing action of ethanol on microorganisms has been provided by the commercial interest in alcoholic fermentation by yeast. It was shown that it was the production of ethanol which was most responsible for limiting microbial growth and therefore the final alcohol content of the fermentation. Consequently, *Saccharomyces cerevisiae* has been the microorganism of choice for the majority of such studies (Seiler and Russel, 1991).

According to Ooraikul (1991) the consequences of ethanol presence on yeasts growth can be summarized as follows:

- the addition of ethanol to the medium generates a vapour into the headspace;
- ethanol competes for water causing the reduction of the water activity of the medium;
- ethanol can inhibit yeasts growth.

However it is unclear whether it can mainly carry out its inhibiting action in the vapour-phase, when it is present in the head space, or in liquid-phase, when it is present in the medium. Some studies showed that the ethanol concentration in the headspace of the package, required for inhibition, depends on the water activity of the system. The inhibitory effect was principally due to the ethanol vapour pressure rather than to the ethanol adsorbed in the medium, although the latter may affect the growth indirectly through the lowering of the water activity. In other words, the mode of action of ethanol on yeast growth can be divided into non-specific effects, which are characterized by reduced water activity, and specific effects, where the solvent works against the cell membrane and against particular enzymes (Jones and Greenfield, 1987).

For antimicrobial activity of ethanol, water is essential. At a concentration of 95 by volume (vol%) or greater, vegetative cells tend to be resistant, whereas at 60-70 vol% most microorganisms are destroyed in less than a minute. The concentration of ethanol necessary to prevent microbial growth varies with the type of spoilage microorganism and the nature and composition of the medium. With bacteria and moulds, growth is usually prevented within the range 8 to 11 vol% of ethanol; yeasts are more resistant and 15-18 vol% is often required. As the growth conditions become more adverse, the level of ethanol required for complete inhibition becomes less. It was even observed that the alcohol tolerance of yeasts increases with the concentration of sugar in the growth medium and with a lowering of the storage temperature (Seiler and Russel, 1992).

RECENT DEVELOPMENTS OF ETHANOL AS A PRESERVATIVE

The use of ethanol is proposed in a number of papers and patents for extending shelf-life of bread and bakery products, either by dipping or by spraying with water-ethanol solutions prior to packaging under vacuum. In modified atmosphere packaging, ethanol can be adsorbed in the packaging or encapsulated as a vapour source.

It has been shown that spraying alcohol directly onto the surface of a product prior to packaging and storage can increase the mould-free shelf life of bread and pizza (Ooraikul, 1991).

Recently Giavedoni (1994), used ethanol vapours as an environmental preservative in plastic bags containing a pasta-type product with a meat-based filling. Ethanol was found to be effective in inhibiting microbial growth (especially moulds, and *Micrococcaceae*). On the other hand, as the sensory characteristics of the product could be affected by ethanol presence, this should always be taken into consideration.

CONCLUSIONS

The inhibiting effect of ethanol on the growth of some moulds has already been proven, but very little information has been reported on its ability in retarding spoilage by yeasts and bacteria, in particular food-poisoning bacteria.

Further studies should be carried on to prove the effectiveness of ethanol as an environmental preservative, especially when it is present as vapour in the head space of modified atmosphere packaging.

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2.8

MAILLARD REACTION PRODUCTS

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ABSTRACT
The Maillard reaction can take place during heat treatment of foods rich in reducing sugars and amino acids, and it may result in a non-enzymatic browning of the product. The reaction occurs between reducing sugars and amino groups and causes changes in the flavour, colour, functional properties and nutritional value of food. Many processes of food technology are based on a controlled non-enzymatic browning as in caramel production, chocolate manufacture, bread baking, coffee roasting. In domestic cooking, the reaction is often desired to produce colour, flavour and aroma in heated and cooked foods.

Antioxidative properties of Maillard Reaction Products (MRPs) were described by several authors. In particular their ability in slowing down lipid oxidation has already been tested. Conflicting results are reported on the influence of MRPs on microorganisms. Inhibition has been demonstrated in some instances, while a stimulating effect has been described in others. Maillard reaction products can also inhibit enzymatic activity (*e.g.* proteolysis and enzymatic browning).

INTRODUCTION
The Maillard Reaction (MR), the most important "non-enzymatic browning" reaction, as it is well known, is a reaction between compounds containing amino groups (like free or protein-bound amino acids) and compounds containing reducing groups (like reducing sugars). MR is very common and occurs in all heated systems containing amino acids and sugars.

The initial stage of the reaction is the condensation of the amino- and the carbonyl-groups which can undergo the Amadori rearrangement to give amino-deoxy compounds; these compounds can react further depending on the different conditions. Intermediate products of the reaction can be water and carbon dioxide, while the end products are brown polymers, called melanoidins.

The products of the MR, the Maillard Reaction Products (MRPs), affect colour, flavour and chemical and physico-chemical properties of the food. So, the effects of the Maillard reaction are desired in some cases, such as in bakery products or in roasted coffee, whereas they are undesired in others, as in over-heated food such as sterilized milk or fruit and vegetable juices. In any case, a major negative consequence of the reaction is the loss of essential amino acids.

Pathways taken by the reaction are greatly influenced by the reaction conditions such as reactants nature, their concentration, and temperature, pH and water activity of the system. Contrasting results are reported about the toxicological and mutagenic effects of Maillard reaction products; in any case the problem should be taken into consideration.

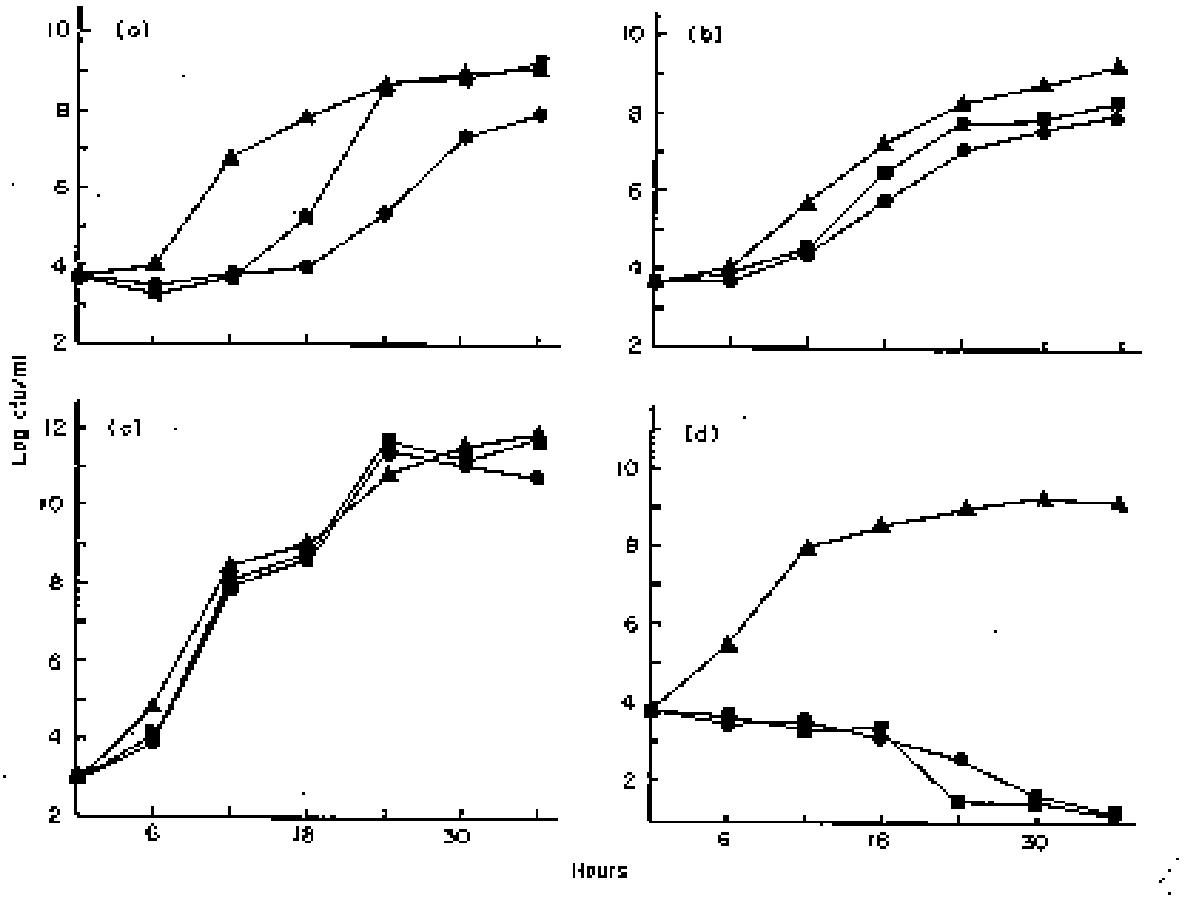

Figure 1. Effect of Maillard reaction products against (a) *Staphylococcus aureus*, (b) *Listeria monocytogenes*, (c) *Salmonella typhimurium* and (d) *Aeromonas hydrophila*. , control; , MRP-type A obtained from a mixture with initial pH of 6.0; , MRP-type B obtained from a mixture with initial pH of 8.8 (from Stecchini *et al*., 1991).

ANTIOXIDATIVE PROPERTIES OF MRPs

According to Lingnert and Eriksson (1980) some MRPs show an antioxidative effect on lipid oxidation and are able to slow the formation of rancid flavour in food. Reactants as well as reaction time and pH influence the development of antioxidative compounds (Eichner, 1981). The greatest antioxidative effect was found in mixtures having an initial pH of 9 or 7. Some authors observed that the higher the browning level of the MR, the more antioxidative effect resulted. Other studies showed that MRPs produced in the early stages of the reaction had a strong antioxidant activity. Elizalde *et al*. (1991) observed the antioxidative effect of Maillard Reaction Volatile Products (MRVP) developed by a heated glucose-glycine mixture.

ANTIMICROBIAL PROPERTIES OF MRPs

Despite various effects caused by MRPs in food, in the literature little data is available on the relationship between MRPs and microbial growth, even if Lewis in 1930 had already observed that inhibitory substances were formed in sterilised alkaline media of glucose, nitrogen compounds and phosphate (Einarsson, 1987).

Few reports are available on the influence of MRPs formed in foods, on microorganisms. Most investigations of the effect of MRPs on microorganisms have been carried out in microbial growth media. Inhibition has been demonstrated in some instances, while a stimulation has been described in others (Einarsson *et al.*, 1987). Stecchini *et al.* (1991), investigated the activity of MRPs against food poisoning microorganisms, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Aeromonas hydrophila* (Figure 1). The inhibitory effect of MRPs on food poisoning microorganisms is dependent on the type of MRP (formation of antibacterial compounds is influenced by the nature of reactants, by the pH and the temperature of reaction), their concentration and the type of bacteria tested (Einarsson *et al.*, 1987; Stecchini *et al.*, 1993). According to Stecchini *et al.* (1993), it is possible that the early MRPs promote the development of microorganisms, whereas late MRPs may have an inhibiting effect on their growth.

EFFECT OF MRPs ON ENZYMATIC ACTIVITY

Proteolysis can be inhibited by the presence of melanoidines: trypsin can be considered the enzyme most affected by the presence of water-soluble MRPs obtained from heated glucose-glycine mixtures, while pepsin and erepsin were only weakly inhibited. Oste *et al.* (1987) confirmed that the inhibiting effect on trypsin could be attributed to low molecular weight MRPs. Intestinal disaccharidase activities also are affected by MRPs. A large decrease in lactase, invertase and maltase activities caused by MRPs was observed in both *in vitro* and *in vivo* studies (Oste *et al.*, 1987). Nicoli *et al.* (1991) observed a strong inhibiting effect of MRPs on polyphenoloxidase (PPO) and peroxidase (POD). The inhibition became more evident with increasing heating times of a glucose/glycine mixture, reducing the activity of PPO and POD nearly to zero.

CONCLUSIONS

In heated food systems, Maillard Reaction Products were shown to have an antioxidant effect on lipid oxidation, and an inhibiting effect on microbial growth. These effects should be considered in food preservation and could be exploited to extend the shelf-life of packaged food. Whereas MRPs are already well known for their antioxidant properties, it was shown that they have a strong inhibiting effect on enzymatic browning too. Apart form these apparently advantageous properties, the possible toxicological and mutagenic aspects of application of MRPs should be taken into account.

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2.9

BACTERIOCINS: POTENTIAL APPLICATIONS IN FOOD PRESERVATION

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ABSTRACT
Bacteriocins are small proteins produced by many bacterial genera, including lactic acid producing bacteria. Most of the bacteriocins produced by lactic acid bacteria inhibit the growth of other lactic acid bacteria, but some are bactericidal to a number of food pathogens and food spoilage bacteria too. In all cases, these other bacteria are Gram-positive. Although about 30 different bacteriocins have currently been identified and their potential use as a food preservative is apparent, the only industrial application to date is of nisin, a bacteriocin produced by *Lactococcus lactis*. This limited application is mainly due to a) the rather small bactericidal range of most bacteriocins, b) the low efficiency of production, c) the limited stability in the food matrix and d) the disputed regulatory status. Nevertheless, the increasing doubt about the safety of the traditional chemical preservatives, such as nitrite and propionate, warrants a revival of interest in the introduction of natural preservative factors such as the bacteriocins.

INTRODUCTION
A bacteriocin is defined as a protein that has a bactericidal action against a limited range of organisms, which are mostly closely related. Bacteriocins have been discovered many decades ago. Bacteriocins produced by pathogenic bacteria such as *Escherichia coli* (colicin) or *Staphylococcus aureus* (epidermin) are not suitable for food application. A more interesting source of bacteriocins are the lactic acid bacteria (LABs). For centuries these have been used in food fermentation to produce stable food products, ranging from dairy (cheese), meat (sausages) and vegetable (sauerkraut), mainly through the preservative action of the lactic acid they produce. The fact that fermented products, which naturally contain these microorganisms and the bacteriocins they may produce, are consumed without a negative health effect, means that the LABs are 'generally regarded as safe' (GRAS) organisms.

Ever since the identification of the inhibitory activity of a strain of *Lactococcus lactis* subsp. *lactis* in 1928, LABs have been increasingly scrutinized for bacteriocin production. The inhibitory agent was later termed nisin, the first known and most extensively studied bacteriocin of LABs. Table 1 lists some of the potential applications of nisin. Today, about 30 different bacteriocins, produced by some 17 species of LABs, have been identified and a lot of information has been obtained on their biochemistry and range of bactericidal activity. For food preservation, advantageous features of several bacteriocins are their relatively high heat resistance and inhibition of Gram-positive foodborne pathogens and spoilage organisms. Much attention has been given to the inhibition of *Listeria monocytogenes*. This cold-tolerant bacterium may result in a high mortality rate and may occur in many different foods, causing problems specifically in dairy (soft cheeses) and meat (pate, sausages) products. Also the bactericidal impact of several bacteriocins on sporeforming bacteria, such as *Bacillus* and *Clostridium* species, has been subject of research for many decades and indicates the great potential these bacteriocins could have in food preservation.

In the following, a brief overview of the research on three interesting bacteriocins will be

presented. In addition, the potential application in food preservation and the regulatory status of bacteriocins in general will be discussed. Several excellent reviews are available giving more detail on food application of bacteriocins (Daeschel, 1990; Harris *et al*., 1992; Kim, 1993; Muriana, 1992; Schillinger, 1990).

NISIN

Nisin is a protein consisting of 34 amino acids, which is stable to autoclaving and effectively inhibits growth of important Gram-positive foodborne pathogens like *L. monocytogenes* and *Staphylococcus aureus*, and prevents outgrowth of spores of many species of *Clostridium* and *Bacillus*. It is especially active in acidic food matrixes. The bacteriocin is produced by some strains of *Lactococcus lactis* ssp. *lactis*, although different strains may produce structural variants slightly deviating in exact amino acid composition. Originally nisin was considered for use as an antibiotic, but because amongst others its range of inhibition is limited, it was not judged suitable for therapeutic use. However, because nisin is completely degraded in the alimentary tract and it therefore can be used safely as a food additive. Its potential use as a food preservative was firstly demonstrated through the successful employment of nisin-producing cultures in the manufacture of Swiss-type cheeses. Due to their inhibition of gas-producing clostridia, blowing of the cheeses is prevented. Although vegetative cells of these organisms are killed or reduced in number by normal processing conditions, the heat-resistant spores require an excessive "botulinum cook" or the use of chemical additives to prevent their outgrowth. Nisin may be used as a natural additive that inhibits spore outgrowth or reduces their heat-resistance.

Nisin has been used in conjunction with other preservative measures to enhance product safety or quality. In canned foods such as vegetables, soups and puddings, nisin has been applied in conjunction with heating to successfully counteract heat-resistant spores of flat-sour thermophilic bacteria. Normal heating and nisin may be combined for milk production in countries where pasteurization, refrigera-tion and transportation facilities are not adequate, and where it is difficult to assure the supply of good quality milk to the public. When nisin is used with acetic, lactic or citric acid, the effectiveness of blanching and pasteurization treatments may be better than with nisin or the organic acids alone. The use of nisin in combination with nitrite in meat systems has been reported frequently. Although the combined application may allow for less nitrite to exert an identical degree of inhibition of clostridia compared to nitrite alone, the meat systems seem to influence the effectiveness of nisin strongly. Inhibition of *L. monocytogenes* in raw meat, for instance, may remain after 2 weeks at 5°C but both the inhibitory effect and the nisin related activity diminish rapidly at room temperature. Comparable findings hold for clostridia suppression in bacon and sausages. Conceivably, binding of nisin to meat particles or high salt concentration may reduce the amount of nisin in solution where it may be active.

PEDIOCIN

Pediocin is the name given to bacteriocins produced by LABs of the genus *Pediococcus*. The first report on pediocin production dates back to 1975, when it was found that *Pediococcus pentosaceus* inhibited growth and acid production of *Lactobacillus plantarum*, an undesirable competitor in mixed brine cucumber fermentation. The active agent, designated as pediocin A, inhibited a broad range of LABs as well as several clostridia, *Staphylococcus aureus* and *Bacillus cereus*. The finding implied that pediocin production might be a favourable asset of starter cultures in the fermentation of sausages and vegetables, where reported staphylococci and naturally competing LABs are the major concern, respectively.

Table 1. Some foods and beverages in which the bacteriocin nisin has been used

FOOD PRODUCT	FUNCTION OR USE
Swiss-type cheese	Prevention of blowing faults caused by clostridia
Milk	Extension of shelf-life
Tomato juice	Allows lower heat processing requirements
Canned foods	Control of flat sour caused by thermophilic spoilage bacteria
Sauerkraut production	Optimizing starter function by improving competitiveness
Beer	Inhibition of spoilage by lactic acid bacteria
Wine	Control of spoilage lactic acid bacteria

Several applications of pediocins have been assessed with regard to food safety. Pediocin PA-1, produced by a strain of *Pediococcus acidilactici*, has been shown to inhibit growth of *L. monocytogenes* inoculated into cottage cheese, half-and-half cream and cheese sauce for 1 week at 4°C, whereas rapid growth to high cell densities was observed in the control samples (no bacteriocin added). The activity of pediocin PA-1 was not affected by fat or proteins present in the foods, while a synergistic action was noted between the effect of the bacteriocin and lactic acid. In bacon, a pediocin producing strain of *P. acidilactici* has been used in combination with reduced levels of nitrite to prevent toxin production by outgrowth of *C. botulinum* spores. Acting as what is known as a Protective Culture (Schutzkultur), the *P. acidilactici* culture would grow during conditions of temperature abuse, producing lactic acid and inhibitory pediocins. Strain *P. acidilactici* H, isolated from fermented sausage, exhibited a broader range of bactericidal activity than any other pediococcal bacteriocin due to the production of a bacteriocin termed pediocin AcH. Extensive tests have shown that this pediocin is non-toxic, non-immunogenic and is readily hydrolysed by gastric enzymes. The potent antilisterial activity and the effectiveness of pediocin AcH and other pediocins as biopreservatives has by now been well established experimentally in beef wieners, semi dry sausage, frankfurters and fresh meat.

SAKACINS

Sakacins, a group of bacteriocins produced by *Lactobacillus sake*, owe their discovery probably to the intensive search for natural antimicrobial compounds capable of increasing the shelflife of raw meat by inhibiting growth of meat spoilage microorganisms and of controling *L. monocytogenes*. Several different antimicrobials are known to be produced by strains of *Lb. sake* which normally reside on meat products. These strains are well adapted to the conditions in meats and conceivably are the best competitors in this food environment.

Lactocin S, produced by strain *Lb. sake* 45 isolated from naturally fermented sausage, is inhibitory against a range of LABs, including organisms from the same sausages. A similar bacteriocin is produced by a strain of *Lb. sake* isolated from Spanish dry sausages. However, the bactericidal range of this compound is much wider, including LABs and several Gram-positive

foodborne pathogens (*L. monocytogenes, Staph. aureus, Clostridium botulinum, Clostridium sporogenes*). *Lb. sake* strain Lb706, an isolate obtained from vacuum-packaged beef which produces a bacteriocin designated sakacin A, has been shown to inhibit *L. monocytogenes* in minced meat and comminuted cured raw pork filled into casing (German-type "Mettwurst"). The inhibitory effect of sakacin A was most pronounced in fresh Mettwurst (pH 5.5-5.8), resulting in over 90% reduction of *L. monocytogenes* in 2 days. In the presence of a sakacin A negative variant, strain *Lb. sake* Lb706-B, the pathogen was not hindered in its growth.

OTHER BACTERIOCINS

Most other bacteriocins identified are interesting mainly from a food quality point of view, since their bactericidal activity is directed towards closely related LABs only. The impact of this in the quality of starter cultures has been mentioned before. Bacteriocin producing strains of *Lactobacillus helveticus* (producing helveticins and lactocins), *Lactobacillus acidophilus* (lactacins, acidophilucin) and *Lactobacillus plantarum* (plantaricins, plantacin) have been most extensively studied in this respect. With regard to food safety, the reported inhibition of *Clostridium botulinum* and even the Gram-negative *Aeromonas hydrophila* by plantacin BN producing strains of *Lb. plantarum* is very noteworthy.

Several members of the genus *Carnobacterium*, a group of LABs which have been found in large numbers in chilled meat products, have been found to produce bacteriocins (carnocins) or bacteriocin-like compounds in relatively high amounts at chill temperatures, which would give them a favourable competitive edge over psychrotrophic foodborne pathogens and spoilage organisms. Again, the bactericidal range is restricted mainly to the closely related LABs, but inhibition of *L. monocytogenes* and *A. hydrophila* has been reported too.

MODES OF APPLICATION OF BACTERIOCINS AND BACTERIOCIN-PRODUCING LABs.

Bacteriocins can be applied in food systems by three basic methods:

- a pure culture of the viable bacteriocin-producing LAB.

  This offers an indirect way to incorporate bacteriocins in a food product. Its success depends on the ability of the LAB to grow and produce the bacteriocin to the desired extent in the food under the prevailing environmental conditions (temperature, pH, *etc.*)

- a (semi-)purified preparation of the bacteriocin.

  In this mode, dosage of the bacteriocin is most accurate and thus its effect most predictable. However, application is limited according to national regulations concerning food additives.

- a crude bacteriocin-preparation obtained by growing the bacteriocin-producing LAB on a complex, natural substrate (*e.g.* milk).

  This mode avoids the use of a purified compound and while still being able to use a preparation of known and constant activity.

This later method is now employed for the production of industrial scale nisin-preparation. A nisin-producing LAB is grown in milk whey at optimal temperature. During the course of incubation, nisin is expelled into the substrate. At a sufficiently high level, the substrate is pasteurized which kills the bacteria but does not affect the heat-stable nisin. The amount of active nisin in commercial preparations is about 1% of the total protein.

MISCELLANEOUS APPLICATION ASPECTS

Although Gram-negative bacteria, yeasts and moulds are not sensitive to the action of nisin, the presence of chelating agents (EDTA, Tween, Triton-X 100) or osmotic shock (high salt) may sensitize them. A combined preservation scheme would be advantageous here.

In discussions regarding wide scale application of bacteriocins in foods, resistance towards the bacteriocin is often a key issue. Obviously, the producing strains have a self-protection mechanism which renders them virtually immune. With respect to nisin, a natural variability in sensitivity has been noted and the identification of nisin-resistant strains has been reported for the genera *Streptococcus*, *Staphylococcus*, *Clostridium* and *Listeria*. The mechanism of resistance is unknown and may differ among strains. In any case, the resistance phenomenon, though relative infrequent yet, may hamper widespread application.

REGULATORY STATUS OF BACTERIOCINS AND BACTERIOCIN-PRODUCING ORGANISMS

In 1969, a joint FAO/WHO expert committee accepted nisin as a legal food additive, although it was not until 1988 that it was approved in the U.S.A. by the FDA for use in certain pasteurized cheese spreads. Presently nisin is permitted in at least 46 countries, for the inhibition of clostridia in cheese and canned foods. None of the other bacteriocins known to date has a fully approved legal status as a food additive, although also the application of a pediocin producing strain of *P. acidilactici* has been approved by the USDA for use in reduced nitrite bacon to aid in the prevention of botulinum toxin production by outgrowth of *C. botulinum* spores.

The regulation of bacteriocin preparations from LABs stands in sharp contrast to the common use of these organisms as starter cultures. Moreover, LABs are commonly consumed in high numbers in fermented or cultured products, and are often present as indigenous contaminants in many retail products. The general conception would be that the introduction of bacteriocins in foods at levels analogous to those capable of being produced by starter cultures, should be as safe as the consumption of the cultured products themselves.

CONCLUSIONS

Food preservation by natural means has developed to be the current challenge for food manufacturing industries of all sizes and is dictated by the changes in consumer attitude in recent years towards chemical, unnatural preservatives. All foods can be processed to extremes using physical methods that render them sterile and thus microbiologically safe. However, such foods would be unmarketable because consumers favour foods that are natural and "as-good-as-fresh". Current research trends in food microbiology and food technology focus on mild, physical preservation techniques and the use of natural antimicrobial compounds. The production of lactic acid and bacteriocins by LABs may well provide for a safe and natural means of (bio)preservation.

Despite their great potential, the application of bacteriocins and bacteriocin-producing LABs is negligible compared to chemical food additives. A further expansion of their application is limited for several reasons:

a) the antimicrobial activity range of most known bacteriocins is narrow and does not extend to the Gram-negative bacteria, which are often the prime cause of food poisoning and spoilage.

b) the amount of bacteriocin produced when a producing LAB is added to a food, in many instances, is quite low due to insufficient growth of the LAB and absorption of the

bacteriocin onto the food matrix
c) the genetic information allowing for bacteriocin-production in some cases is not stable
d) the GRAS status is needed before intentional application is permitted.

Looking at the various limitations above, the conclusion still seems to be warranted that biopreservation by bacteriocins or bacteriocin-producing LABs is a key natural preservation measure for the future, but no estimation can be made on its exact timescale. They potential of bacteriocins has been acknowledged by the Commission of the European Community, since they have funded several research & development projects in this area under the Programmes Bridge (BIOT CT910263), FLAIR (AGRF.0048) and AAIR (CT920125).

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PART 3

EXAMPLES OF HURDLE PRESERVED FOOD

FOOD PRESERVATION BY COMBINED PROCESSES

FINAL REPORT FLAIR Concerted Action No. 7, Subgroup B

3.1

PRESERVATION OF FRUIT JUICES

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ABSTRACT
In spite of opposition from some consumers, the application of preservatives is justified in avoiding toxin development, microbial spoilage, nutritional losses and aesthetic changes. Since some preservatives in high concentrations represent chemical hazards, a combination of chemical preservatives with other preservation methods is useful. Proper application of combined methods gives stable products, prevents the undesired side-effects of each individual treatment, saves energy and lowers the required concentration of added preservatives.

INTRODUCTION
Preservation of fruit juices and related products (pulps, concentrates, fruit nectars and soft drinks) by addition of even permitted and harmless preservatives represents a chemical method of food preservation that has been criticized as toxic. There is still a widespread aversion to preservatives among consumers. On the other hand it must be stressed, that their application involves less risk than their omission as they prevent poisoning by toxins formed by different microorganisms as well as microbial spoilage. The use of preservatives is a good preventive measure against forming mycotoxins by fungi. On the other hand fruit juices provide an especially good medium for growth of fungi. The use of preservatives is justified in processing of many fruit products since the high temperatures required to kill some spoilage microorganisms cause flavour changes, product discolorations and loss of nutrients. Refrigerating and freezing extend their shelf-life but do not stop the spoilage and aesthetic changes (David *et al*., 1986).

MICROBIAL SPOILAGE OF FRUIT PRODUCTS
The shelf-life of these products is primary determined by microbial growth. These products generally have a low pH (between pH 2.3 and 5), a high redox potential (Eh +100 to 400 mV) and mostly a high a_w and low content of vitamin B. All these factors represent a hurdle for the growth of most bacteria except those resistant to acidity like lactic, acetic and butyric acid bacteria. Moulds are few in number on the surface of fresh fruits, but after harvesting become important pathogens and spoilage factors. They are usually found in most fruit products as a result of primary contamination of the fruit and often result in rotting. Yeasts are usually responsible for the characteristic fermentations and off-flavour in crushed products, under-pasteurized juices and soft drinks. Fruit concentrates, pulps, jellies, jams and dried products are often spoiled by osmophilic yeasts and xerophilic moulds.

HURDLES USED TO INCREASE SHELF-LIFE
To prolong and maximize shelf-life various improved methods are applied. Such methods or hurdles used individually, except sterilization, do not completely destroy for spoilage microorganisms and cause quality losses as noted in the following table.

Table 1. HURDLES USED TO INCREASE SHELF-LIFE

Hurdle	Application	Shelf-life	Quality losses
initial selection; packaging; wax coatings	pretreatment; fresh refrigerated	a few weeks	market disorder; increased rot
refrigeration	fresh, MA[1] stored products; single strength juices	depending on perishability; > 1 year	chilling injuries; weight losses
freezing	fresh, blanched products; concentrates	> 1 year	nutritional, texture losses; discoloration, aesthetic change, enzymatic browning
CA[2]	fresh/refrigerated products	extended	softening; discoloration; retarded spoilage
pasteurization	for all products	stable, according to F-value	nutritional losses; sensory losses
canning (sterilization)	fruit juices/canned fruit	depending on initial contamination	nutritional losses; sensory losses
drying	fruits, juices	months, depending on moisture level	discoloration; flavour changes; mould growth
preservatives	juices, soft drinks, concentrates, pulp, nectars	rather short	consumer aversion, resistance

1 = Modified Atmosphere
2 = Controlled Atmosphere

PRESERVATIVES COMBINED WITH OTHER HURDLES

Compatible combination of preservatives with other hurdles ensure safe and stable fruit products, reduce the concentration of preservatives needed, diminish quality and aesthetic losses, lower energy costs and provide, because of gentle processing, the nutritional needs of the consumer (Leistner, 1992). It is not an easy decision to select a proper combination because of differing fruit products, legal requirements and economic aspects. Some examples are listed below.

1) Combinations of several preservatives with one another enhance their action against microorganisms and broaden the spectrum of their activity. The combined effect of two or more preservatives could be:
   - additive: arithmetic sum of the individual effects of each preservative,
   - synergistic: greater than the arithmetic sum of the individual effects,
   - antagonistic: smaller than the arithmetic sum of the individual effects or that of a sole preservative. (Davidson and Branen, 1993)

Since the most common spoilers in fruit products are yeasts and moulds, it is important to use mixtures having a specific synergistic effect against such spoilage combinations. A beneficial effect is obtained by combining salts of benzoic and sorbic acid with sodium sulphite. However in the case of contamination with osmophilic yeasts the combination of formic and propionic acid is more effective, since these yeasts can metabolize salts of sorbic and benzoic acid (Lück, 1980).

2) Combination of preservatives with pasteurization reduces F-values or lowers the temperature required to kill microorganisms. For fruit products the combination of Ultralow Pasteurization and addition of low doses of potassium sorbate or sodium benzoate (0.05%) guarantee stable products. Benefits of such combinations are evident in good nutritional and sensory quality, reduced energy costs and lower doses of preservatives.

3) Combination of preservation with refrigeration has a synergistic and destructive effect on yeasts and moulds spoiling fruit juices, concentrates pulps and pastes providing stable products. The combined effect of potassium sorbate and sodium benzoate with refrigeration, for instance, increases with the concentration of added preservative and inversely with the temperature reduction. So the same effect can be obtained with a lower and healthier concentration at 4°C as with a higher concentration at 0°C (Pokorn, 1990).

4) Combination of preservatives with drying is recommended in pretreatments. Light-coloured fruits are treated with sulphur dioxide combined with low concentrations (0.01% - 0.02%) of sorbic and benzoic acid to intensify the fungicidal action and prevent oxidative reactions, enzymatic and nonenzymatic browning, to improve colour and minimize vitamin losses. Intermediate moisture (IM) foods on fruit bases with a_W greater than 0.85 support the growth of moulds and yeasts. By adding potassium sorbate with concentrations of 0.02% or 0.05% of esters of polyhydroxy butyrate a reduction in their growth is obtained (Lück, 1980).

5) Combination of preservatives by carbonating is common by utilized in soft drinks production. CO_2 itself does not kill yeasts and moulds, at least not in short term. To extend the shelf-life of soft drinks and minimize their spoilage the combination of 0.1% of equal parts of potassium sorbate and sodium benzoate with 6.0g CO_2/l is recommended.

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3.2

HEAT PROCESSED CURED MEAT PRODUCTS

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ABSTRACT
The hurdles important for the safety and stability of three types of heat processed cured meat products will be briefly discussed: Shelf stable canned cured meat, "keep refrigerated" canned cured meat, and sliced cured meat products.
Besides, the temperature conditions in the different parts of the chill chain will be outlined.

INTRODUCTION
It has been known for many years that the safety and stability of perishable cured meat products depends on several factors (hurdles). Three categories of cured meat products are discussed:

a. Canned cured meat products (ambient shelf stable)
b. "Keep refrigerated" canned cured meat
c. Sliced cured meat products

An outline of the temperature conditions in the chill chain is also included.

CANNED CURED MEAT PRODUCTS
The most important products in this category are hams, shoulders, luncheon meat, chopped pork, *etc.*, containing around 2.5% NaCl, *i.e*. a salt/water ratio of at least 3.5. Leistner (1985) has included these products in the F-SSP group, meaning Shelf Stable Products where the heat treatment (the F-value) is the most important factor (hurdle) for safety and stability.

NaCl, nitrite ($NaNO_2$) and other curing ingredients (phosphates, and often ascorbate) are added during manufacturing. After packaging in hermetically sealed containers (normally metal cans), the product is heat processed to a F-value a little below 1. The reason for such a mild treatment (the maximum centre temperature will be around 104°C) is that the sensory properties of the product will deteriorate with more severe heat processing. Heat processing to a F-value below 1 is not sufficient to kill *Clostridium botulinum* spores, as it is well known that this would imply a F-value of at least 2.5. The safety of such products is demonstrated by the fact that millions of cans have been produced during the last 40-50 years, without food poisoning caused by *Cl. botulinum* surviving the heat process. The safety is based on a combination of three factors: Heat treatment, inhibition, low bacterial count.

The so-called preservation formula, developed by Mogens Jul, *c.f*. Bøgh-Sørensen (1993) indicates that Pr = Ds + In' - Ct.
Pr = Protection, the log of the smallest number of containers in which one toxic container will appear. Pr should be at least 10, and Pr = 12 was used in defining a "botulinum cook", a 12D heat treatment.

Ds (Destruction of spores due to heat processing) can be calculated when the F-value is known. Ct (Initial count) is the log of the number of bacterial spores present in each container. In' is the inhibition, which could be the log of smallest number of bacteria which would grow in

the product after the heat process used. This is difficult to measure, and only a few experiments have dealt with the question. However, there are some results, and calculations according to the formula seem to fit well with experience in the canned food industry.

As mentioned above, the heat treatment is rather mild, but a certain amount of bacterial spores (and all vegetative cells) will be killed by a F-value of 0.4-0.8.

Inhibition means that the conditions in the heat processed containers are such that surviving spores (of for example *Cl. botulinum*) are not able to grow and form toxin. The inhibition comes from NaCl (a_W and perhaps an effect of NaCl itself) and $NaNO_2$ (the max. input amount of $NaNO_2$ is around 125 ppm), but addition of ascorbate seems to increase the inhibitory effect. Other inhibitory factors are pH and the redox potential in the meat product.

A low initial count, *i.e.* a low number of, especially, *Cl. botulinum* spores is absolutely necessary in order to ensure the safety and stability of these products. The theory behind heat killing of bacterial spores clearly shows that the risk of surviving spores is reduced with a lower initial count.

"KEEP REFRIGERATED" CANNED CURED MEAT

The manufacturing process is similar to that used for shelf stable canned cured meat, but keep refrigerated products are pasteurized, often at 72-75°C, in order to achieve a centre temperature of 70°C or above. The heat process kills most vegetative bacteria, but does not kill spores. Therefore, these products must be kept refrigerated, e.g. at 5°C or below.

Traditionally, these products were packed in metal cans, but now several producers use plastic packaging, e.g. vacuum packaging in laminates of PA (nylon) and PE (polyethylene). Products such as hams and shoulders are often sold to slicing companies, where slicing and retail packaging takes place. The packages can be very large, containing up to 32 lbs. (± 15 kg).

The safety of this category of products depends on the storage temperature and the heat treatment (and a hermetically sealed container). The pasteurization kills vegetative pathogenic bacteria, and storage at temperatures less than 10°C means that spores of *Cl. botulinum* type A and B do not grow. Some heat stable bacteria can survive the pasteurization process and grow in the product, leading to reduced quality and, perhaps, to spoilage. The inhibitory effect of NaCl, $NaNO_2$, pH, *etc*., means that only few heat stable vegetative bacteria are able to grow in the product.

The calculation of the killing effect of pasteurization follows the same principle as for sterilization. A pasteurization value (a FP-value) should be based on a reference temperature of 70°C and z= 10°C, where an F-value is based on 121.1°C and z= 10°C. It seems that the FP-value should be about 40 or above to attain an adequate shelf life of these products. For some large products (10 kg and above), a heating time of 6-7 hours is used in order to achieve a centre temperature of 76°C. This results in a FP-value around 500.

SLICED CURED MEAT PRODUCTS

In several countries, sliced cured meat products are sold from chill cabinets in supermarkets. In some countries, the legislation prescribes storage at max. 5°C for such products.

This category of products includes ham, bologna type sausages, *etc.* The products to be sliced could be the products mentioned above (keep refrigerated cured meat), *i.e.* where normally, vegetative bacteria are not present before slicing. The colour of heat processed cured meats will fade if oxygen is present. Therefore, these products are often vacuum-packed, *e.g.* in

PA/PE laminates, or MAP is used, e.g. with 50% CO_2 and 50% N_2. The safety of such products is due to the storage temperature, as re-infection often occurs during the slicing process. As pathogenic bacteria, e.g. *Salmonella* and *Listeria monocytogenes*, could be introduced during slicing, and as some of them can grow at temperatures down to about 1°C, it is important for ensuring safety and stability that other hurdles are included. NaCl (and $NaNO_2$) act as inhibitors, and several experiments indicate that the use of lactate or acetate as curing ingredients increase the inhibitory effect of the meat system.

Also, a lowering of pH, caused by the addition of GDL (glucono-δ-lactone) or a starter culture, is beneficial for the safety and stability of these products.

Thus, a large number of hurdles are involved:

- heat treatment (pasteurization)
- good hygiene during slicing and packaging
- vacuum packaging, or MAP
- a_W (NaCl)
- $NaNO_2$
- pH
- Chill storage
- Smoking is used for some meat products

THE CHILL CHAIN

It should not be overlooked that the safety and stability of chilled foods, including the sliced cured meat products mentioned above, depend on the chill chain, *i.e.* the various links from the producers to the consumer. Surveys in several countries (Bøgh-Sørensen and Olsson, 1990), have shown that the intended temperatures are often exceeded.

- *Chill storage rooms* at the manufacturer, the grocer, terminals, *etc.*, are normally run at reasonably low temperatures, but especially near the doors, which often are frequently opened during the day, dramatic temperature increases may occur.

- *Transport by means of trucks etc.* should not cause increases in product temperatures. Sometimes, the cooling system breaks down or functions incorrectly, the thermostat setting may not be correct, the product temperature is too high during loading, *etc.*, leading to high product temperatures at arrival to a secondary cold store.

- *Local distribution.* During local distribution, the trucks often go to several shops (supermarkets), meaning frequent door openings during a voyage and frequent increases in air temperatures around the products. However, the transport time for local distribution is normally rather short.

  Product temperatures up to 16°C at arrival to supermarkets have been reported.

- *Loading/unloading.* A weak link in the chill chain is loading and/or unloading. These operations are not always carried out as they should, meaning that the products are left at room temperature (or exposed to sun and rain) for several hours. Of course, this may lead to very high product temperatures, and to reduced safety and stability.

- *Display cabinets.* It is difficult to maintain correct product temperatures in chill cabinets. The air temperature must not be so low that freezing occurs (freezing would be very slow and could result in severe quality deterioration), and it is necessary to run frequent checks of the cooling system. Especially packages in the outer (upper) layers can be warmer than 5°C, and in several countries measurements have shown that at least 50% of the packages are over 5°C; temperatures up to 14-16°C have been reported.

- *Home transport.* It is not uncommon that consumers place chilled meat products in the car and that the home transport may last several hours. Especially in a warm climate, this may lead to high product temperatures.

- *Home refrigerators.* Although most modern refrigerators may be set to run at reasonable temperatures, it has been shown that the temperatures are higher than necessary, sometimes due to the fact that someone in the household wants particular commodities to be not too cold. Also, there are rather large temperature variations in most refrigerators, meaning that many chilled products are stored at 8-10°C in the refrigerator. This fact does not improve the safety and stability of chilled foods.

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3.3

APPLICATIONS OF HURDLE TECHNOLOGY IN DEVELOPING COUNTRIES

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ABSTRACT
Foods based on hurdle technology are prevalent in industrialized as well as in developing countries. In the past and often still today hurdle technology was applied empirically without knowing the governing principles. But now with a better understanding of these principles and improved monitoring devices the intentional use increases. Recent examples of the intelligent application of hurdle technology in developing countries are the preservation of different fruits in Latin America, a dairy product in India, and a meat product in China.

INTRODUCTION
For developing countries, foods storable without refrigeration are of special interest, because refrigeration (energy) is costly and not continuously available. Furthermore, in less developed countries food preservation procedures should be inexpensive and simple, but reliable. Over centuries a treasure of knowledge of food preservation methods which fulfil these requirements has been accumulated in different parts of the world.

Most of the foods, which remain stable, safe and tasty during prolonged storage without refrigeration, even under the difficult climatic conditions prevalent in many developing countries, are intermediate moisture foods (IMF). The water activity (a_w) of IMF ranges from 0.90 to 0.60, and in addition to a_w, in general, other hurdles are inherent in traditional and novel IMF (Leistner and Rödel, 1976). However, information on the principles of preservation applied to IMF of developing countries was scarce and, in particular, quantitative data were lacking. For Ibero America this situation was overcome, due to an extensive study which was launched by Spain, and to which from 1986 to 1991 several countries of the region (Argentina, Brazil, Chile, Costa Rica, Cuba, Mexico, Nicaragua, Puerto Rico, Uruguay, and Venezuela) contributed (Aguilera *et al*., 1993). In the course of this study (CYTED-D, AHI) the researchers identified 246 traditional food items, representing fruits, vegetables as well as foods derived from milk, fish, cereals and meat, which are stable and safe without refrigeration. The majority of these items were intermediate moisture foods. However, many had a much higher water activity, in some instances as high as 0.97, and their stability and safety was based on empirically applied hurdle technology. The Latin American researchers compiled detailed descriptions (with photographs), the physico-chemical characteristics (including a_w, pH, chemical composition), technological information, and consumption patterns of these 246 foods in an "Inventario" (Aguilera *et al*., 1990). This was a major contribution to food science of Latin America, and in pursuance of this study many advances were made in the preservation of foods of the region, including fruits and vegetables as well as dairy, marine, and meat products by the use of combined methods, *i.e*. hurdle technology. Especially, the use of hurdle technology for the preservation of tropical fruits in small and bulk containers appears to have great potentials for

less developed countries (Aguilera *et al.*, 1993). In the opinion of these authors, the successful application of hurdle technology in Latin America deserves a closer look, particularly by developing countries where refrigeration is scarce. The approach of the Latin American researchers, *i.e.* to compile first an inventory of the traditional foods stable without refrigeration, then to characterize these products, and finally to improve them by application of hurdle technology, should become a model for other regions of the world.

In order to illustrate which improvements in traditional foods have already been achieved by using hurdle technology in different developing countries, a few examples are given below.

FRUITS OF LATIN AMERICA

Recently processes have been developed for the preservation of high moisture fruit products (HMFP: a_w higher than 0.92) in seven Latin American countries, and have been applied to peach halves, pineapple slices, mango slices and puree, papaya slices, banana puree, and chicozapote slices. The new technologies were based on the combination of a mild heat treatment (blanching for 1-2 min in saturated steam), slight reduction in a_w (0.97-0.92, with glucose or sucrose), lowering of pH (to 4.3-3.0, with citric or phosphoric acid), and the addition of antimicrobials (potassium sorbate or sodium benzoate, and sodium sulphite or sodium bisulphite). Thus, hurdle technology was applied for these novel processes (Alzamora *et al.*, 1993).

These minimal processes proved energy efficient and very simple to carry out, the resulting fresh-like products still scored high with consumer panels after three months of storage at 35°C for taste, flavour, colour, and especially for texture, which is often problematic for canned fruits. According to these authors, the combined methods applied allow storage of fruits, without losses between seasonal harvest peaks, for domestic consumption and further processing to confectionery, bakery and dairy products, or for preservatives, jams, and jellies. Fruit pieces can also be utilized as ingredients in salads, barbecues, pizza, and fruit drink formulations. Moreover, these novel HMFP open new possibilities for export markets. In general, they provide a better utilization of Latin American indigenous tropical and subtropical fruits, many of them having exotic and quite distinctive flavours, texture, and appearance. The high moisture fruit products, stabilized by hurdle technology, proved shelf-stable during at least 4 to 8 months storage at 25 to 35°C. Due to the blanching process the initial microbial counts were substantially reduced, and during the storage of the stabilized HMFP the numbers of bacteria, yeasts and moulds further decreased, sometimes below the detection limit (Alzamora *et al.*, 1993). The authors are of the opinion, that these technologies will attract much attention in many developing countries, because they are easy to implement and will improve considerably the quality of stored fruits.

DAIRY PRODUCT OF INDIA

Paneer is a traditional, cottage cheese type product in fried cubes, with tomato sauce, onions, and spices, frequently consumed and much liked in India, because of its nutritive value and characteristic taste. However, Paneer generally spoils at room temperature (in India often at 35°C) within two days, and this is an immense drawback for the industrial production. Sterilized Paneer in cans has severe sensory limitations with regard to flavour, texture and colour. Thus, together with a visiting scientist from India, Dr. K. Jayaraj Rao, the Federal Centre for Meat Research, Kulmbach, developed a mildly heated Paneer in cans, with the desired flavour (like prepared fresh), colour (no browning) and texture (not too hard). This product was stabilized by hurdle technology, and thus is stable and safe for several weeks without refrigeration. The following combinations of hurdles proved effective with this product: $a_w = 0.97$, heating to an F value of 0.8, pH= 5.0 or alternatively $a_w = 0.96$, F= 0.4, pH= 5.0 (Rao *et al.*, 1992).

After his return to India, Dr. Rao continued his work with the application of hurdle technology to fried Paneer in cubes made from buffalo milk. The product with gravy was packed either in tins or retort pouches, and a set of hurdles, *i.e.* F= 0.8, a_w= 0.95, pH= 5.0, and 0.1% potassium sorbate, was chosen, which had maximum lethal and inhibitory effects on microorganisms and minimum effects on textural and chemical characteristics (Rao and Patil, 1993; Rao, 1993). The water activity of Paneer and gravy was lowered by using humectants, such as dahi, skim milk powder, salt and glycerol. The pH was adjusted by changing the dahi to skim milk powder ratio. The resultant product had a keeping quality of one month at 30°C and over three months at 15°C. The product was compared with fresh samples from restaurants and was found to be equally acceptable. In the opinion of the authors, this method of preservation has a large scope for alterations in product formulations, depending on regional taste preferences, without affecting the keeping quality (Rao and Patil, 1993). Via paneer, hurdle technology was introduced into food science of India, and its application to a variety of indigenous foods is now anticipated.

MEAT PRODUCT OF CHINA

About 15% of the meat supply of the Peoples Republic of China, which in total uses more meat than any country in the world, are processed into meat products, and dried meats are most popular. There are 3 technologies in use for Chinese dried meat production, but one variety (Rou Gan) constitutes more than 95% of the dried meats of China. The technology used for Rou Gan has not changed for hundreds of years, but improvements are possible and desirable.

Chinese consumers prefer now products with a softer texture, lighter colour and less sugar addition. Shafu is an improved Rou Gan which fulfils these expectations, and therefore has been already widely accepted in the market. Whereas traditional Rou Gan has a water activity below 0.70, the novel product Shafu, with much superior sensory properties, has an a_w of about 0.79, and nevertheless is storable without refrigeration (Wang and Leistner, 1993). Compared with Rou Gan the moisture content of Shafu is higher, whereas salt and sugar contents are lower; to Rou Gan nitrate is added, but Shafu is produced with nitrite curing salt and the finished product is vacuum packaged. Both products have low residual levels of nitrite and nitrate, contain few microorganisms and in general no pathogenic or toxigenic bacteria, and may be stored for several months without refrigeration.

The superior sensory properties of Shafu, compared with the traditional Rou Gan, are due to an intelligent application of hurdle technology, and it may be expected that similar modifications in the future will be used for other Chinese foods.

CONCLUSIONS

The intentional application of hurdle technology has only recently been introduced into developing countries, but probably will be increasingly used for the improvement of traditional products as well as in the development of novel foods. Whereas the HACCP concept and the predictive microbiology still have difficulties in being widely accepted in the food industry of developing countries, because of the prevailing infrastructure, hurdle technology is easily understood and applied. It may be expected that hurdle technology foods with a relatively high water activity will partially replace intermediate moisture foods, because lower amounts of humectants (such as salt or sugar) and less drying are required, and this would be desirable from the sensory and nutritive point of view. However, hurdle technology should be applied without sacrificing the microbial stability and safety of the foods in developing countries, especially when stored without refrigeration.

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3.3

COMBINED PROCESSES AND TOTAL QUALITY MANAGEMENT

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ABSTRACT
The concept of using combined processes or 'hurdle technology' as a tool in total quality management is discussed and its potential assessed. It is emphasised that although desirable, this concept has not yet been converted into a usable form for the food industry. It is probable that the initial applications will be HACCP (critical control point analysis) procedures converted from safety targets to other quality targets, targets that need not have any safety implications.

INTRODUCTION
To date, the concept of combined processes has been oriented towards 'hurdle technology' and the provision of barriers to food spoilage processes. Obviously, the combination of barriers increases the probability of a safer product. Consequently, most barriers are considered as barriers to microbial growth and spoilage. However, food quality is a much broader field than microbiological safety and encompasses a wide range of physical, biological and chemical attributes. Some researchers feel that the tools being proposed elsewhere in this publication are so valuable that they can be applied with equal success to the consideration of barriers to other forms of quality deterioration and also to quality enhancement. Indeed, while not using the same terminology, researchers are starting to use combined processes towards such an end (Kormendy, 1993). The objective of this short paper is to emphasise the concept of combined processes working towards total quality rather than the narrow but important concept of bacterial quality and safety.

PROBLEMS IN IMPLEMENTING THE TECHNIQUE
One of the major difficulties that must be overcome before the combined processes philosophy is applied to food quality (and in particular, to physical quality) is the determination of interaction mechanism of physical attributes generated by different processes in the production chain. For example, one can readily compute how composition can be effected by mixing of two process streams of differing composition and flow rate. It is more difficult, however, to predict what the resulting colour of the mixture will be and more difficult again to predict what the resulting rheological attributes will be. Will the mixing of two Newtonian liquids result in a non-Newtonian behaviour. The only answer that currently be given is 'quite possibly'.

It is even more difficult to predict combined behaviour for properties that are in themselves complex combinations of other variables. For example, in my own laboratory, working on the 'instant' rehydration of dried products, the property in question depends on internal structure, diffusivities in various media, damage induced in various stages of the process and, of course, the rehydration process used. Also within the FLAIR programme, a group is developing predictive software for the shelf-life of foods using not only on predictive microbiology but also

texture change predictions based on both compositional and process variables and interactions (Verlinden and De Baerdemaeker, 1993). It is not necessary to say that the prediction of such quality properties from combined process concepts is currently no more than a pious aspiration. However, it should eventually be a reality and meanwhile, many of the tools associated with microbiological aspects of combined processes can usefully be applied.

Foremost amongst these is HACCP, a sister concept within this FLAIR Concerted Action #7. In applying this useful tool, most processors again consider 'hazard' as 'microbiological hazard' and identify suitable critical control points. Of course, the same concept can equally be applied to the hazard of deterioration of other properties of a physical or chemical nature or to the inhibition of development of a desirable physical attribute.

CONCLUSION

To conclude, one should reiterate that the application of combined processes philosophy to physical product quality is a future development. Certainly, the current emphasis on microbial safety and shelf life enhancement will quite correctly continue to be the dominant interest. The purpose of this short paper has been to encourage researchers to appreciate the wider power of the concept and to urge the food industry to initially use the tools of combined processes for as many quality enhancements as is possible.

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W

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