EMERGING CONCEPTS FOR FOOD SAFETY

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Background : Food preservation implies putting microorganisms in a hostile environment, in order to inhibit their growth or shorten their survival or cause their death. The feasible responses of microorganisms to such a hostile environment determines whether they may grow or die. Related to these responses more basic research and its application are needed, because this might lead to new and better concepts for food preservation (Leistner, 1995; Leistner & Gorris, 1995).

Homeostatsis : A key phenomenon, which deserves more attention in food preservation is the interference by the food with 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, and this applies to higher organisms as well as to microorganisms. Much is already known about the homeostasis in higher organisms at the molecular, subcellular, cellular and systematic levels in the fields of molecular biology, biochemistry, physiology and medicine (Häussinger, 1988). This knowledge should now be transferred to microorganisms important for the poisoning or spoilage of foods. If the homeostasis of microorganisms, i.e. their internal equilibrium, is disturbed by preservative factors in foods (hurdles), they will not multiply, i.e. remain in the lag-phase or even die, before their homeostasis is re-established ("repaired"). Thus, food preservation is achieved by disturbing the homeostasis of microorganisms in food temporarily or permanently (Leistner, 1995).

The internal water activity (a_w) of living cells is another important feature of their homeostasis. Since food stability is often achieved by water activity reduction, the osmoregulation of microorganisms has been studied already extensively with respect to food preservation. Considerable knowledge is available about the osmoregulatory mechanisms of microorganisms, and the role of potassium as well as the accumulation of amino acids or polyols (as compatible solutes), in order to reverse plasmolysis and re-establish a metabolically suitable water content inside the cells living in an environment with low a_w. Gould et al. (1983) pointed out that cell osmoregulation mechanisms involve a considerable energy cost for the synthesis or accumulation of "compatible solutes". Thus, any restriction of energy supply will tend to be especially synergistic with lowered a_w, and such energy restriction is e.g. caused by anaerobic conditions (vacuum packaging).

The principles of other known "repair mechanisms" of the microbial cells under stress should be applied to food preservation too, i.e. could be related to the application of combined processes (hurdle technology).

Multi-target Preservation of Foods : For foods preserved by hurdle technology, it has been suspected for some time that different hurdles in a food could not just have an additive effect on stability, but act synergistically (Leistner, 1978). A synergistic effect could become true if the hurdles in a food hit, at the same time, 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 an intelligent combination of gentle hurdles.

In practical terms, this could e.g. 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, 1994a). This "multi-target preservation" of foods is a promising research area, because if small hurdles with different targets are selected, a minimal but most effective preservation of foods could be accomplished. It is anticipated that the targets in microorganisms of different preservative factors (or hurdles) for foods will be elucidated, and then the hurdles could be grouped into classes according to their targets (e.g. cell membrane, DNA, enzyme systems, pH, a_w, Eh) within microbial cells. A mild and effective preservation of foods, i.e. a synergistic effect of hurdles, is likely if the preservation measures are based on an intelligent selection and mix of multi-target hurdles taken from different "target classes". This approach seems not only valid for traditional food preservation procedures, but for modern processes (such as food irradiation, ultra high pressure, mano-thermo-sonication, etc.) too. An example for a multi-target novel process is the application of nisin (damages the cell membrane) in combination with lysozyme and citrate (which are then able to penetrate easily into the cell).

Food microbiologists could learn much in this respect from pharmacologists, because the mechanisms of action of biocides has been studied extensively in the medical field. At least 12 classes of biocides are distinguished which often have more than one target in the microbial cell, multiple lesions in microorganisms are known, often the cell membrane is the primary target, becomes leaky and unzips the organism, but biocides also impair the synthesis of enzymes, proteins, and DNA (Denyer, 1995).

It is of interest to note, that nitric oxide (NO) which is criticized in the area of meat curing, due to its possible contribution to nitrosamine formation, has some definite beneficial effects in the medical field, due to blood vessel relaxation and stimulation of the brain function. Furthermore, nitrite helps to kill undesirable bacteria in the stomach, because it is strongly bactericidal at the low pH of the stomach (Benjamin et al., 1994), and possibly this is a multi-target effect too.

Even mutations of the HIV virus, which are a major problem in fighting AIDS, could be overcome by a "multi-drug attack" early in the course of the disease (Ho et al., 1995). It is believed that only combinations of drugs have the potential to outgun the virus, because this tactic has worked against fast-mutating bacteria.

Stress Reactions of Microorganisms : Some bacteria become more resistant (e.g. toward heat) under stress (because the synthesis of protective stress shock proteins is induced by heat, a_w, pH, ethanol, etc.), or they become less resistant under stress (and this is caused by low pH or catalase inactivation in heat-injured cells). These responses certainly might influence the preservation of foods. Especially an increased resistance under stress could turn out to be problematic in the application of hurdle technology. However, multi-target preservation could provide in this respect an answer too. Because the switch on of genes for the synthesis of shock proteins, which help the microorganism to cope with a certain stress situation, should be more difficult if different stresses are received at the same time which would ask for the energy consuming synthesis of several or at least much more protective stress shock proteins. Nevertheless, further research in stress proteins, and the different mechanisms which switch them on, seems warranted also in relation to hurdle technology applied for food preservation.

Metabolic Exhaustion of Microorganisms : Another phenomenon of practical importance is the "autosterilization" of stable hurdle technology foods during storage. This was first observed by us and initially not believed, many years ago (Leistner and Karan-Djurdjic, 1970), with mild heated (95 °C core temperature) liver sausage adjusted to different water activities by the addition of salt and fat, and the product was inoculated with *Clostridium sporogenes* PA 3679 and stored at 37 °C. Clostridial spores which survived the heat treatment vanished in the product during storage, if the products were stable. Later this behaviour of clostridia as well as *Bacillus* spores was regularly observed during storage of SSP products (Leistner, 1994b), especially F-SSP. The most likely explanation is that bacterial spores which survive the heat treatment are able to germinate in these foods under conditions that are less favourable than those under which vegetative cells of bacilli and clostridia are able to multiply (Leistner, 1992). Therefore, during storage of these products some viable spores germinate, but the germinated spores or vegetative cells deriving from these spores die. Thus, the spore counts in stable SSP actually decrease during storage at ambient temperatures. Also during studies in our laboratory, with Chinese dried meat products, we observed a similar behaviour (Shin, 1984). If these meats were recontaminated after processing with staphylococci, salmonellae or yeasts, the counts of these microorganisms on stable products decreased fast during unrefrigerated storage, especially on meats with a water activity close to the threshold for microbial growth. Again the same phenomenon was observed by Latin American researchers (Sajur, 1985; Alzamora et al., 1993) in their studies with high moisture fruit products (HMFP), because the counts of a variety of bacteria, yeasts, and moulds which survived the mild heat treatment, decreased quite fast in the products during unrefrigerated storage, because the hurdles applied (pH, a_w, sorbate, sulfite) did not allow growth.

A general explanation for this behaviour might be that vegetative microorganisms which cannot grow will die, and they die more quickly if the stability is close to the threshold for growth, storage temperature is elevated, antimicrobial substances are present, and the organisms are heat-injured (Leistner, 1995). Apparently, the microorganisms in stable hurdle technology foods strain every possible repair mechanism to overcome the hostile environment, by doing this they completely use up their energy and die, if they become "metabolically exhausted", and this leads to autosterilization.

Thus, due to autosterilization the hurdle technology foods, which are microbiologically stable, become more safe during storage, especially at ambient temperatures. For example, salmonellae which survived the ripening process in fermented sausages, will vanish more quickly if the products are stored at ambient temperatures, and they survive longer in products stored under refrigeration (Leistner, 1995). Moreover, it has been convincingly demonstrated that *Salmonella enteritidis* survives in mayonnaise at refrigeration temperatures much better than at ambient temperature (Board, 1995).

Conclusions : Food preservation is achieved by disturbing the homeostasis of the microorganisms in a food temporarily or permanently. Furthermore, a gentle food preservation could be achieved by using an intelligent mix of hurdles, which secures safety and stability as well as the quality of a food.

If the homeostasis of microorganisms is better understood and a multi-target preservation of foods is applied which leads to synergistic effects of preservative factors (hurdles) and possibly to the control of stress shock proteins, then minimally processed foods could result which are safe, stable, and have fresh-like characteristics. Such foods, stabilzed by hurdle technology, could even undergo an autosterilization during storage due to metabolic exhaustion of the microorganisms present.

Therefore, a fresh look towards food preservation seems timely which could not only improve the quality of foods but their safety too. However, if hurdle technology foods are becoming now more advanced, they will require a thorough understanding of the principles involved as well as the back up of their production by guidelines based on good manufacturing practice (GMP), and if possible, by application of the HACCP concept (Leistner, 1994b, 1995).

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