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Stable Hurdle Technology Foods and Packaging -Worldwide

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The stability and safety of most foods is based on a combination of preservative factors (hurdles) which the microorganisms present cannot overcome. This is true for traditional foods with inherent empiric hurdles, as well as for novel products for which hurdles are intelligently selected and intentionally applied (Leistner, 1995a). The most important hurdles in foods are temperature (high or low), water activity, acidity, redox potential, some preservatives, and competitive microorganisms. However, in a recent European research project more than 50potential hurdles which influence the preservation or quality of foods have been identified and described (Borg-Sorensen, 1994),and the list of possible hurdles for food preservation is by no means closed. At present especially non-thermal processes are of interest, since they may be used in combination with other hurdles for minimally processed, fresh-like food products with little induced degradation of nutritional and sensory properties (Barbosa-Canovas et al.,1995). Packaging (e.g. vacuum or modified atmosphere packaging, active packaging, scavengers, absorbers, antimicrobial packaging material, edible food coatings and films, aseptic packaging)is important for most hurdle technology foods, however, again it has to be used in combination with other hurdles.

The hurdle technology approach is applicable not only to safety, but also to quality aspects of foods. In order to secure an optimal total quality of a food the preservation and quality hurdles must be adjusted to an optimal range (Leistner, 1994a). 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 flavor of the product. Moreover, the same hurdle could have a positive as well as 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 (Leistner, 1995a).

The hurdle effect was introduced be Leistner (1978), and from an understanding of the hurdle effect the hurdle technology has been derived (Leistner, 1985). More recently out of the comprehension of the hurdle technology new concepts for food safety have emerged (Leistncr, 1995b). The latter focus on homeostasis, metabolic exhaustion and stress reactions of microorganisms and the multi-target preservation of foods.

Homeostasis is the tendency to uniformity and stability in the normal status (internal environment) within organisms. For instance, the maintenance of a defined pH in narrow limits is prerequisite to all living cells. If the homeostasis of microorganisms, i.e. their internal equilibrium, has been disturbed by hurdles in a food, they will not multiply, i.e. they remain in the lag-phase or even die, before their homeostasis is re-established ("repaired"). Therefore, food preservation is achieved by disturbing the homeostasis of microorganisms in foods temporarily or permanently (Gould, 1988, 1995; Leistner, 1995a). Repair of a disturbed homeostasis, e.g. the osmoregulation of bacterial cells in foods of low water activity, demands much energy of the microorganisms, and therefore restriction of the energy supply inhibits repair mechanisms of the microbial cells and leads to a synergistic effect of hurdles. Energy restrictions for the present microorganisms are e.g. caused by anaerobic conditions, such as in modified atmosphere or in vacuum packaging of foods.

In general microorganisms which cannot grow will die, and they die more quickly if the stability of a food is close to the threshold for microbial growth, the storage temperature is elevated, antimicrobial substances are present, and the organisms are sublethally injured (e.g. by heat). Apparently, 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 metabolicly exhausted. This leads to an autosterilization of such foods (Leistner, 1995a; Alzamora et al., 1993, 1995). Due to autosterilization 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 temperature, and they will survive longer in products stored under refrigeration (Leistner, 1995a).

Some bacteria become more resistant (e.g. toward heat) or even more virulent under stress, since they generate their "safety net" (Booth, 1996). Synthesis of protective stress shock proteins is induced by heat, pH, aw, ethanol, etc. as well as by starvation. These responses of microorganisms under stress could turn out to be problematic for the application of hurdle technology. However, the switch on of genes for the synthesis of stress shock proteins should become more difficult if different stresses arc received at the same time, because to counter different stresses will ask for the energy consuming synthesis of several or at least much more protective stress proteins, which the microorganisms cannot deliver since they become metabolicly exhausted (Leistner, 1995b).

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, aw, Eh) within the microbial cell, because then the repair of the homeostasis of the microorganisms should be more difficult. In practical terms this could mean that it would be more effective to use different preservatives in small amounts in a food than only one preservative in larger amounts, because different hurdles might hit different targets within the microbial cell, and thus act synergistically (Leistner, 1994a, 1995a). This multi-target preservation of foods might become a promising research area. It is now anticipated that the targets in microorganisms of different preservative factors (hurdles) for foods will be elucidated, and then the hurdles could be grouped in classes according to their targets within the microbial cells. A mild and effective preservation of foods, i.e. a syngergistic effect of hurdles, is likely if the preservation measures are based on an intelligent selection and mix of hurdles taken from different "target classes" (Leistner, 1996).

Over the years the insight into the hurdle effect has been broadened and the application of the hurdle technology extended. In industrialized countries hurdle technology is of particular interest for minimally processed foods, whereas in developing countries now high-moisture foods storable without refrigeration, due to stabilization by hurdle technology, are of paramount importance. The application of hurdle technology advances worldwide, even though this concept is synonymously called combined methods, combination preservation or Hurden-Technologie in German, Technologic des Barrieres in French, Tecnologia degli Ostacoli in Italian, Tecnologia de Obstaculos in Spanish, and Zanglangishu in Chinese. In numerous publications traditional and novel hurdle technology foods have been described, e.g. meat products of Germany (Leistner, 1994b), Taiwan (Kuo et al., 1994), and China (Wang and Leistner, 1993, 1994, 1995), dairy products of India (Rao, 1993; Hossain, 1994), as well as high-moisture fruit products from Latin America (Alzamora et al., 1995; Argaiz et al., 1995). The application of hurdle technology in Europe was fostered by a three-year project of the European Commission on "Food preservation by combined processes", to which scientists of 11 European countries have contributed. More than 2000 copies of the final report (Leistner and Gorris, 1994) were requested by scientists and industrialists. Furthermore, hurdle technology was featured in Trends in Food Science & Technology (Leistner and Gorris, 1995), and indeed this concept is now worldwide in the trend.

Little research has been done towards the packaging of hurdle technology foods as a group. Because for the various types of hurdle technology foods known, the appropriate packaging materials and procedures must be selected and applied individually. However, for some types of these foods already much information is available e.g. on the suitable packaging of fresh-like and minimally processed foods, which all are based on hurdle technology (Zagory, 1995; Swanson et al., 1995; Yang, 1995). Such foods (e.g. MAP, CAP, vacuum packaged or sous-vide products) need rigorous refrigeration as the main hurdle and therefore they are prevalent primarily in industrialized countries. Furthermore, already extensive information on the appropriate packaging of hurdle technology foods which are mildly heated or dried or fermented is available (Leistner, 1994b). In these instances packaging procedures which are in general use are suitable for such hurdle technology foods too, if they are intelligently selected for packaging of the various food items. The stable hurdle technology foods have an improved microbial stability, nevertheless their microbial recontamination after processing must be excluded, however a complete integrity of the seal is not essential, i.e. clipped casings are sufficient. For the packaging of hurdle technology foods casings and pouches are more suitable than cans, since condensation of water vapor inside of the package (i.e. formation of water droplets in the headspace of cans) might unbalance the adjusted water activity, and this will be detrimental to the product stability (Hechelmann et al., 1985). For hurdle technology foods in the intermediate-moisture rage, which are prevalent in developing countries, reabsorption of water is a problem and must be avoided, and this is achieved by simple packaging devices (e.g. metal cans, glass jars). For stable high-moisture foods, based on hurdle technology, which are of current interest in developing countries (e.g. high moisture fruit products in Latin America) appropriate packaging procedures have not yet developed. Generally, there is a lack of knowledge related to the packaging of hurdle technology foods in developing countries, and only sporadic information (e.g. Argaiz et al. 1995) has been published on this subject. However, further exploration into the application of easy to use, cheap and efficient packaging for hurdle technology foods of developing countries, which are stored in small consumer packages or large containers in bulk, should be a challenging and even lucrative approach.

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Application of Immobilized Enzymes in Active Packaging

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Ever since Appert discovered that food could be preserved by heating in sealed bottles, improvement in food packaging has been a major research goal. The traditional role of packaging in food preservation has been to withstand thermal processing and to act as a barrier to contamination. Modem food packaging influences the nutritional, quality, and convenience attributes of foods, insures availability year round, and is an important tool for marketing products.

The major advancements in food packaging technology have been the development of new materials, combinations of materials, and containers with specific technical and economic benefits. However, these new technologies are passive in that they act primarily as inactive barriers separating the food from the environment. The next frontier is to develop packaging which actively contributes to the preservation, quality, and safety of foods. Such packaging has been called "interactive" or "active" packaging.

Active packaging applies the advances in chemistry, biotechnology, materials science, and/or micro-electronics to food packaging. Examples of active packaging include antimicrobial films which prevent growth of spoilage and/or disease causing microorganisms, shelf life and temperature abuse indicators which warn of potential hazards, atmosphere modifiers (humidity and gas composition) which help preserve foods, flavor enhancers which maintain desirable flavor in products, off-odor absorbers which remove undesirable flavors, systems which intercept oxygen, and biosensors which detect microorganisms and toxins. These technologies promise to improve the safety and quality of many foods including non-sterile refrigerated foods such as milk, cheeses, and minimally processed fruits and vegetables. These foods represent one of the most rapidly growing segment of the food market in parts of the world.

The concept of using packaging in an active rather than passive sense is relatively new and largely undeveloped. One of the earliest active packaging concepts incorporated vapor or gas absorbers and emitters into packages after closure. This might be as simple as a desiccant (e.g. ,sodium or calcium chloride) which controls relative humidity, or more complex substances which absorb ethylene (to inhibit ripening), absorb undesirable odors, or emit ethanol to control molds in baked products. Some commercial absorbers remove both residual and ingress oxygen after the package is scaled and have been termed an "oxygen interceptors". These interceptors have been used commercially to remove trace amounts of oxygen from the headspace of bottled beer, for example.

Active packaging may improve safety by indicating the condition or history of a product. One available technology is the time-temperature indicator. These devices integrate the time and temperature history of a product and give a visual indication if the combination has exceeded some standard or desirable amount. Such technology would be especially useful when combined with other shelf life technologies modified atmosphere packaging and sub-sterilization ionizing radiation.

In the future it may be possible to directly detect the presence of specific toxins or microorganisms in packaged foods using biosensors. Immunologically-based sensors coupled to packaging could find applications in food safety, food processing, and detection of adulteration. Such sensors may, for example, detect the presence of bacterial toxins in packaged foods. They could also be used to determine if a food had been properly pasteurized, contained enzyme activity, or undesirable pesticides. Biosensors which combine electronics with biological specificity and sensitivity may find use in packaging as a monitors of safety and quality. Reportedly, methods to quantify the presence of microorganisms on fresh meats are near commercialization. Such systems could eventually be incorporated directly into food packaging.

ANTIMICROBIAL PACKAGING

Microbial contamination is the major factor in food spoilage and responsible for most food-borne disease outbreaks. Two approaches, heat sterilization and direct addition of antimicrobial additives, have been used to control microbial growth. In conventional thermal processing, foods are sealed in a package and the combined product-package thermally processed. This is the basis of the canning industry. More recently, the package and the product have been sterilized separately then filled and sealed aseptically. Foods can also be otherwise processed (e.g., dried) to reduce microbial growth.

The addition of antimicrobial additives directly to foods usually does not inhibit all growth but is selective. The use of these additives is regulated and their use, in most cases, must be stated on the label. The incorporation of an antimicrobial additive into the package, rather than into the food may have advantages in that less additive may be needed and the effect may be greater because most spoilage occurs at the food surface. If the additive remains in the packaging material and does not migrate, it is not considered a food additive and need not be labeled.

In solid or semi-solid foods, microbial growth occurs primarily at the surface. Surface treatment with antimicrobial agents for products such as cheeses, fruits, and vegetables has been practiced for decades. Anti-mycotic agents have been incorporated into waxes and other edible coatings used for produce items. More recently, the idea of incorporating anti-microbial agents directly into polymeric packaging films which would contact the surface of the food has been discussed.

Anti-microbial films can be divided into two types: those containing an ntimicrobial agent which migrates to the food and those that are effective against surface growth without migration to the food. A few commercial antimicrobial films have been introduced. One widely discussed commercial product is a synthetic zeolite which has had a portion of it's sodium ions replaced with silver ions. Silver is anti-microbial under certain situations. These zeolites are incorporated directly into a food contact film to allow for a slow release of silver ions to the food. This material is not approved for use in the U.S. but is reported to be in commercial use in Japan.

Other synthetic and naturally occurring compounds have been proposed and/or tested for antimicrobial activity in packaging. The anti-fungal agent, imazalil, is effective when incorporated into waxes used to coat fruits and vegetables. We have demonstrated that the same compound is effective at preventing mold growth on cheese surfaces when incorporated into LDPE films. The growth of several species of molds were inhibited when imazalil was incorporated into LDPE at levels of 500 to 2, 000 mg/kg. Although imazalil is not approved for cheese, this work established that anti-mycotic films could effectively control surface molds in foods.

Reports have appeared which demonstrated the effectiveness of adding food-grade anti-mycotic agents to cellulose-based edible films. Unfortunately, cellulose-based films are not heat sealable or good barriers in high humidity situations.

We overcame the incompatibility of simple anti-mycotic organic acids such as propionic, benzoic, and sorbic acids with polymers such as LDPE by forming the anhydride of the acid which removes the ionized acid function and decreases polarity. Anhydrides are stable when dry yet hydrolyze in aqueous environments such as foods. Hydrolysis leads to formation of the free acid which in turn leads to migration from the surface of the polymer to the food where they can be effective anti-mycotics. This is an example of "switched on" packaging; the active ingredient remains in the film until the film contacts a food at which point it is "switched on." The activity is initiated by the moisture in the food. Others have proposed the incorporation of naturally occurring microbial inhibitors into packaging materials. Using naturally occurring antimicrobial agents would have regulatory advantages in parts of the world were natural compounds receive less scrutiny than synthetic.

PEPTIDES, PROTEINS, AND ENZYMES AS ACTIVE PACKAGING AGENTS

The major drawback to all of these technologies is the antimicrobial agent must migrate from the packaging material to the food in order to be effective. The next generation of active packaging films may use biologically-derived materials that may not need to migrate to the food to be effective. For example, bacteriocins are proteins derived from microorganisms much in the same way penicillin is derived from mold. Bacteriocins are effective against organisms such as Clostridium botulism and one such compound, nisin, has been approved in the U.S. for food use. Several other anti-microbial short chain peptides have been isolated from natural sources.

For example, defensins, cecropins, and magainins have been isolated from mammalian phagocytes, frog skin, and insects, respectively. Each is a potent anti-bacterial agent of little or no mammalian toxicity. The low toxicity is due to the breakdown of the protein in the mammalian stomach. These peptides could theoretically, be attached to the surface of food contact films rendering them antimicrobial. A search of the world patent literature suggests that the use of anti-microbial peptides in polymer-based human implants in being investigated and perhaps used.

Enzymes have been immobilized on solid polymers including nylon, EVOH and cellulose acetate. Anti-microbial enzymes might be effective even if bound to the inner surface of food contact films. These enzymes would produce microbial toxins or act directly on microorganisms. Glucose oxidase which forms hydrogen peroxide is an example of one such enzyme. Lysozyme is a common enzyme which attack the cell wall in many microorganisms. Preliminary work in our lab has shown the feasibility of immobilizing lysozyme to an approved food packaging polymer. Immobilized lysozyme is effective at inhibiting microbial growth in defined media when incorporated into the film. Immobilization of the lysozyme prevents migration to the media while maintaining anti-bacterial activity.

FLAVOR ENHANCING ENZYMES

Flavor deterioration during processing and/or storage is likewise, a problem for most foods and often the limiting factor in shelf life. Bitterness can develop in citrus juices due to the formation of compounds such as naringin. A fungal enzyme (naringinase) is available which hydrolyzes the compound narigin and removes bitterness. Preliminary work in our lab has shown that this enzyme can be immobilized to a food approved polymer substrate and that the bitterness of citrus can be actively removed during storage. We have been successful at increasing the activity of the immobilized enzyme ten-fold over that reported in the literature. This raises the possibility that naringinase could be immobilized to the product contact surface of juice packaging and thus improve the product during storage. This technology would be especially applicable to aseptically filled juices.

CONCLUSIONS

Our preliminary experiments have convinced us that the use of immobilized peptides and enzymes on food-grade polymer substrates as interactive packaging materials bears more detailed investigation. While our initial work has focused on lysozyme and naringinase, other enzymes and peptides might be even more interesting. Naringin in grapefruit juice is decreased by approximately 80% in 4 days when stored at AC when in contact with cellulose acetate containing naringinase. Likewise, microbial suspensions of 10e4 CFU are rendered sterile in 24 hours when in contact with cellulose acetate containing lysozymc. These preliminary results indicate that the concept of interactive packaging using immobilized enzymes may be feasible.

What little literature that exists suggests that over the next few years, active packaging materials which arc capable of direct interaction with foods could be developed. Advances in biotechnology, micro-electronics, and materials science need to be applied to food packaging technologies. Our general approach will be to seek out recent scientific developments in the fields of materials science, biotechnology, and micro-electronics and to apply them to the development of active packaging systems. It is our intent to be active in their development.