Expectations and Applications of Natural Antimicrobials to Foods:
A Guidance Document for Users, Suppliers, Research and Development, and Regulatory Agencies

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ABSTRACT
Efficient use of natural antimicrobials in food is predicated on the proper implementation of hurdle technology. These substances are meant to increase the robustness of existing food safety or quality assurance programs, not to correct or mask poor practices. The objective of this paper is to outline the important aspects of application of natural antimicrobials to foods, including selection of antimicrobial, determination of target microorganisms, efficacy testing against target microorganisms in vitro and in foods, and issues that must be addressed in the commercial application of the antimicrobial. Because natural antimicrobials are secondary hurdles, expectations of them must be realistic, and considerations should include other aspects, such as effect on sensory and quality attributes of the food, cost (and cost-in-use) of the antimicrobial, and regulatory and labeling considerations, in addition to efficacy against target microorganisms in the food matrix. The “idea-to-launch” business framework and governance is recommended for pairing of a potential antimicrobial with a complex food matrix, along with clearly defined objectives, inputs, outputs, and technical success criteria and business decision criteria. To help quantify the benefits of hurdles, including antimicrobials, we propose use of the “Food Protection Objective” (FPO), which is defined as the acceptable level of microbiological quality and/or safety at the moment of consumption or at the end of shelf life of a food.

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INTRODUCTION

Although food preservation methods have been used for millennia, interest in the use of natural antimicrobials has increased as more are discovered and made available to the food industry. Additionally, consumer demand for minimally processed foods and “clean” labels has become a strong driving force. While choices in antimicrobials have increased, much confusion exists regarding the proper application of these materials to foods. The purpose of this paper is to attempt to generate a uniform understanding of the potential for use of antimicrobials derived from natural sources (animal, plant, microbial) in foods (10). The document is not meant to be a comprehensive review of antimicrobials used in food, but rather a set of recommended guidelines based on the “idea-to-launch” business framework for proper application of natural antimicrobials based on experiences of the authors. The guidelines are designed for end users of natural antimicrobials as well as those who study and potentially commercialize natural antimicrobials.

Antimicrobials may be used to improve the safety of a food product by inhibiting or inactivating pathogenic microorganisms or to improve the shelf life of food by inhibiting or inactivating spoilage microorganisms. Selecting the appropriate natural compound would be a simple process if the only things one had to be concerned with were the antimicrobial and the target microorganism(s). However, to set realistic expectations for antimicrobials, one must consider many other factors, including efficacy against target microorganisms in the food matrix, effect of the compound(s) on sensory properties of food, effect of processing method on the antimicrobial (e.g., compound degradation or activity enhancement), cost of the antimicrobial and cost in use, regulatory aspects, and labeling considerations. This can be a rather complex exercise.

At the outset, it is vital to remember that the use of antimicrobials in food is meant to increase the robustness of existing food safety and quality assurance programs, not to correct or mask poor practices. In fact, existing antimicrobials are not efficacious enough to overcome marginal or poor microbiological quality of a food. Thus, effective use of antimicrobials in food begins with the presence of sound prerequisite programs, such as Good Manufacturing Practices (GMPs) and sanitation. It may be argued that prerequisite programs and, in fact, any measures used to enhance the safety and quality of foods can be viewed as hurdles and be included in a hurdle concept plan. Although this is not the classical view of hurdle technology, it may be practical to think in this way when setting up food protection programs in the manufacturing environment.

HURDLE TECHNOLOGY AND ANTIMICROBIAL USE

As interest in the use of “natural” antimicrobials in food products has increased, so have the sometimes unrealistic expectations of their capabilities in solving food safety and spoilage problems. Thus, the hurdle concept and hurdle technology (15) are central to successful utilization of antimicrobials in food. While use of antimicrobial ingredients to inhibit or reduce populations of spoilage or pathogenic microorganisms in food is a well-known practice, care must be taken not to rely on these substances alone to give the level of safety or quality desired in food products. They are best utilized in the context of hurdle technology, as part of the framework of total microbial control in a food manufacturing facility and/or in food products. Hurdles can be applied externally or internally. Many external hurdles (e.g., thermal treatments, non-thermal treatments, sanitation) are designed to inactivate and reduce microbial numbers (cidal effect). Internal hurdles are often designed to inhibit or retard growth of unwanted microorganisms (stasis) by manipulating intrinsic factors such as pH, water activity, or redox potential.

Hurdle technology encompasses the use of interventions to create products with the desired level of safety and quality. The hurdle concept can be applied to the entire production chain, from farm to fork. The beauty of this technology in the creation of food products is that, by understanding the role of each hurdle, the producer can optimize each so that the resultant product is safe, has a long shelf life, and is of the highest possible sensory quality. Use of certain antimicrobial ingredients may make it possible to raise pH or moisture levels or reduce thermal processing times or temperatures and still obtain safe products with superior sensory qualities. Ideally, hurdle systems have components both to kill unwanted microorganisms and to prevent growth of survivors. For example, pasteurization of milk is designed to eliminate pathogenic microorganisms as well as the majority of spoilage microorganisms; subsequent refrigeration is used as an additional hurdle to slow the growth of remaining microorganisms. Extending this example, if the pasteurized milk were to be used as an ingredient in another food, addition of a natural antimicrobial to that food might further retard the growth of the remaining microorganisms in the milk and extend shelf life of the product.

Figure 1 is a diagrammatic representation of the hurdle concept with regard to growth of microorganisms. As the population of undesirable microorganisms encounters more hurdles, each hurdle adds additional stress on microbial growth, resulting in lengthening of the lag phase (the time needed for the microbial population to adapt to the environment and begin to grow). In general, when the population begins to increase in the logarithmic phase, the rate of growth is unchanged regardless of the number of hurdles introduced. However, the use of multiple hurdles may lead to an increase in time to reach the stationary phase by extending the lag phase, resulting in increased food protection (which may be manifested as increased shelf life).

RESEARCH & DEVELOPMENT PERSPECTIVES

Many companies have an idea of what microorganism(s) should be the target for antimicrobial control but not what type of antimicrobial compound may be useful against the target microorganism(s). Thus, the first step in selecting an antimicrobial is to determine its efficacy. Although many studies on the antimicrobial activity of natural antimicrobials have been published, it may be necessary to establish efficacy de novo. Because there are no standard methods for determining efficacy, researchers have generally used methods used by clinical microbiologists, such as agar diffusion assays, microbroth dilution assays, agar dilution assays and “time-kill” curves (9). Because many food antimicrobials are partially hydrophobic, the commonly used agar diffusion assay, which relies on consistent and rapid diffusion of compounds in the polar agar gel, may yield
inaccurate results. Dilution assays are more appropriate for testing food antimicrobials. Reports in the literature on efficacy of compounds tested with agar diffusion method might be considered suspect unless these compounds have been highly standardized, such as with nisin.

In the evaluation of natural antimicrobials for potential use in foods, the suggested steps include in vitro testing to determine endpoints and dynamic inhibition, followed by application to foods and challenge studies. The endpoint assays, generally broth or agar dilution assays, involve adding the compound to a microbiological media, adding the test microorganism and incubating for a specific time. This type of assay generates a “minimum inhibitory concentration (MIC),” or the concentration that prevents growth of the microorganism, as measured by a lack of turbidity (in broth) or colony formation (on agar). A “minimum lethal concentration (MLC)” may be determined in the broth dilution assay by transferring media from tubes or wells where no growth occurred to fresh media. If no growth occurs in the fresh media, the assumption is that the microorganism was inactivated and thus that the concentration was lethal to the population. An alternate definition of MLC is the concentration that results in a 99.9% (3 logs) reduction in microbial numbers. Obviously, both an MIC and MLC depend highly upon environmental growth conditions (e.g., pH) and initial number of microorganisms.

Following an endpoint assay to determine appropriate concentrations, one can determine the influence of the compound on dynamic growth by incubating the target microorganism in a microbiological broth medium and taking repeated samples over time to determine number of survivors. The plot of survivors over time is sometimes referred to a “time-kill” curve, a term that is used in clinical microbiology. From this type of assay, it can be determined what type of inhibition the test compound causes over time. The type may manifest itself in a number of different ways (Fig. 2). Compared with the control, concentrations of an antimicrobial that are at the MIC may reduce the final cell number (enough to depress the absorbance or turbidity), delay the lag phase, inactivate and then allow recovery, or inactivate to undetectable levels. A success criterion for further evaluation of an antimicrobial in such a test would likely be an increase in lag phase or some type of inactivation. One point to remember in these types of assays is that an antimicrobial neutralizer should be used in the medium being used for enumeration of survivors so as to avoid obtaining any false positive results.

Before investing in elaborate and expensive challenge studies in actual food matrices, it is customary to assess efficacy of promising antimicrobials in simple food systems. These studies may be done in culture tubes, using commercially sterile shelf-stable apple juice or UHT-sterilized shelf-stable 2% fat milk. These simple food models can
be used to evaluate the effect of the food, including pH and binding of the antimicrobial by fat or protein. Obviously, antimicrobials that are bound or inactivated during processing are not available to act against target microorganisms. Generally, one can expect the effect of juice to be similar to that of microbiological media because of the lack of protein and fat. In milk, there generally will be a dramatic drop in activity because of the high pH and binding by protein and fat. The purpose of these tests is to get an idea of what concentrations might be efficacious in the food product of interest. The next logical progression is to evaluate the antimicrobial in the actual food matrix of interest, simulating production, processing and packaging conditions present at the manufacturing plant.

Combination studies

As mentioned throughout this document, natural antimicrobials are generally not effective enough or have too negative an effect on food properties to be used alone. Thus, it is often desirable to use them in combination with other natural antimicrobial or with physical preservation processes, such as heat. When combinations of antimicrobials are elevated, three outcomes are possible. A combination may be “additive,” i.e., the effect of the combined treatments is equivalent to the sum of the effects of the treatments acting independently. The two components can be “antagonistic” toward one another, actually resulting in a reduced efficacy of the combined treatments compared with their use independently. This might result, for example, from a chemical reaction between components to form a new, non-inhibitory, compound. The most desirable outcome is termed “synergistic,” in which the activity of the combination is enhanced compared with the sum of individual treatments. Measuring synergism in vitro is most easily done with a microtiter “checkerboard” assay and by calculating a fractional inhibitory concentration (FIC), defined as the concentration of each antimicrobial in combination which produces inhibition of growth expressed as a fraction of the concentration that inhibits growth when the antimicrobial is used alone (3, 9), or a fractional lethal concentration (FLC), defined as the concentration of each antimicrobial in the combination that produces lethality, expressed as a fraction of the concentration that is lethal when the
regulatory adoption. Recommendations for the use of standard methods of inhibition or inactivation of microorganisms. While this may not be as antimicrobials, with the exception of nisin and lysozyme. Thus, most commercially available antimicrobial food preservatives used as antimicrobials. In fact, there are no governmental standards concerning the efficacy of most commercially available antimicrobial food preservatives used as antimicrobials, with the exception of nisin and lysozyme. Thus, many commercial antimicrobial food preservatives, such as sorbate or benzoate, have not been evaluated for their intended purpose, i.e., inhibition or inactivation of microorganisms. While this may not be a large problem if one is attempting to extend shelf life, it certainly is important if the compound is being used to control pathogenic microorganisms. Recommendations for the use of standard methods were called for over 20 years ago (9) but, to date, there has been no regulatory adoption.

**CONSIDERATIONS FOR COMMERCIAL APPLICATION OF ANTIMICROBIALS IN FOOD**

Attempts at pairing a specific food matrix in need of a secondary barrier for food protection with a potential antimicrobial is very rarely a linear or straightforward exercise. In the food industry, several competing factors need to be reviewed and co-optimized to meet predetermined technical success criteria and business decision criteria, as illustrated in Fig. 3. Key factors that must be considered include (a) efficacy against target microorganisms in the food matrix during processing, (b) business case and justification, (c) cost-in-use, (d) sensory effects, (e) storage, (f) end use by consumers, (g) regulatory and labeling considerations, and (h) sustainable supply (7). To achieve the goal of successful application of a natural antimicrobial, certain “technical success criteria” must be established up front for managing business expectations, cost structure and implementation at the manufacturing plant.

*Figure 3* is a modified Stage Gate™ business process based on the “idea-to-launch” framework for product innovation and reducing time-to-market (5). The proposed framework is for systematic pairing of a potential antimicrobial system with a food matrix, with clearly defined objectives, inputs, outputs and success criteria for each of the three phases: Phase 1 – Discovery (Proof of Concept), Phase 2 – Technology Development, Phase 3 – Technology Transfer (Scale-up and Commercialization).

**Phase 1 – Discovery or proof of concept**

This phase consists of high throughput screening of promising antimicrobials against target microorganisms via appropriate assays to determine MIC and MLC. Antimicrobials differ in their ability to inhibit or inactivate vegetative cells and spores of Gram-positive bacteria, Gram-negative bacteria and yeasts and molds. As previously stated, the first step in choosing an antimicrobial is to correctly identify and characterize target spoilage and/or pathogenic microorganisms from food. In addition, one should have a good understanding of factory microbial ecology, including vectors, incoming bio-burden load in ingredients, and data trends from environmental monitoring program. No single antimicrobial can control all types of bacteria, yeasts and molds in all food matrices. Lower dose concentrations for MIC and MLC are indicative of higher efficacy. Also, an order of magnitude of reduction in microbial numbers relative to initial inoculum level at time zero can be approximated. Thus, for example, successful candidate antimicrobials causing a 4 to 5-log reduction would be moved to the next phase of technology development. It is customary to review those antimicrobials with a score of 1 — 3 log reduction for other good traits as well, such as polarity, pKa, sensory, effects, GRAS status, etc. Even though most antimicrobials come with vendor-generated technical information and MIC and log reduction values, it is prudent for the user to re-check the MIC and MLC under desired environmental conditions of pH and temperature and against microorganisms isolated from product recall or spoiled product or the factory-specific environmental microbiome.

A quick test for antimicrobial impact on odor and taste of the target product is essential. Usually, 3 levels of antimicrobials (MIC, below MIC, above MIC) are mixed with finished product to assess concentration of the subject antimicrobial. Because finished product is the basis for this quick test, it does not account for the impact of processing conditions on final product sensory characteristics or efficacy of the antimicrobial. Combination systems with other antimicrobials or other intrinsic or extrinsic hurdles may also help lower the use and dosage of individual antimicrobials for minimizing negative sensory impact and optimizing cost-in-use.

**Phase 2 – Technology development**

This step is where the bulk of the investment (resources and cross-functional teams), testing and assessment work are staged and completed to facilitate making the “go/no-go” business decision. Often, natural antimicrobials are more expensive than traditional chemical preservatives, and cost can be higher by a factor of ten or more. Vendor-provided cost per pound price for Phase 1 successful antimicrobials helps one to assess whether the product in question can absorb upcharge per case of finished product, and thus to make a reasonable business case. The rule of thumb is that cost of antimicrobials should be less than or equal to $0.01 per pound of finished packaged product.
**Antimicrobial Efficacy Against Target Spoilage or Pathogenic Microorganisms**

**Business Decision Criteria**
- Screen Antimicrobials Efficacy in Microbiological Media (MIC, MLC, FIC, FLC)
- Antimicrobial Efficacy in Simple Food Models (Time-kill assay, Cidal and/or Stasis Effects)
- Antimicrobial Efficacy in Complex Food Matrix (Challenge Study)

**Patent Landscape & Intellectual Property Review:**
- Technology & Ingredient Patents
- Ingredient Lock Out or Ingredient Use Patents

**Microbiological Media**
- <1 log reduction - Failure
- 1-3 log reduction - review other good traits: polarity, pKa, sensory, GRAS, etc.
- 4-5 log reduction - Pursue

**Simple Food Models**
- Juices similar to media
- Milk: 2-4 log reduction
- Lag phase increase – Inhibition 1 to 2 times the targeted shelf life

**Complex Food Matrix**
- Challenge Study
- 1-2 log reduction
- Inhibition 1.5 to 2 times the targeted shelf life (stasis)

**Delivery System**
- 10-1,000X efficacy compared to control

**Combination:** Additive, Synergistic, Antagonistic

**Phase 1: DISCOVERY & PROOF OF CONCEPT**
- Sign or Combination of Antimicrobials
- Body & Taste of Food
- Antimicrobial Mixed With Food

**Phase 2: TECHNOLOGY DEVELOPMENT**
- Sensory Product Formulation
- Business Case & Cost Justification
- Commercially Available
- Regulatory Assessment

**Phase 3: TECHNOLOGY TRANSFER (SCALE-UP & COMMERCIALIZATION)**
- Formal Sensory & Shelf Life Tests
- Sustained Supply

**Early Failure = Success (Cost Less)**

**FIGURE 3.** The “Idea-to-launch” framework, governance and decision criteria for pairing a food product with a potential antimicrobial system for requisite food protection at an acceptable product cost structure.
Higher costs of $0.015 – 0.02 per pound of finished product can be justified for antimicrobials meeting or exceeding many or all of the success criteria shown in Fig. 3.

Natural antimicrobials may leave a residual odor or taste that can potentially impact finished product sensory and quality attributes. It is important to do a formal cutting of food product with antimicrobial concentrations equal to and less than MIC values established from Phase 1 work, to quickly evaluate any negative sensory impact. A more formal sensory cutting is required during Phase 3 prototyping and scale-up work streams. It may be important to develop a “tool box” of antimicrobials to appropriately pair with different food matrices, e.g., savory, dessert (sweet) and neutral. For example, sweet essential oils may be appropriate for use in puddings and pies, whereas essential oils from coriander, lemon grass or mustards may be more compatible with savory sauces, meats and vegetable entrees. Combinations of antimicrobials need to be developed that may be more efficacious (additive or synergistic) and cause less impact on the sensory properties of the food.

Patent landscape and intellectual property (IP) review are in order at this stage to ensure that there is room for creating unique and compelling consumer solution and innovation, and to create substantial competitive advantage via IP and patents. It is important to ensure that application of an antimicrobial or combination of antimicrobials in target categories of product are not protected by either technology or ingredient patents, or by ingredient lock out or by ingredient use patents. For example, there may be restrictions on use of a lactate-diacetate system in certain types of fresh meat products or restrictions on use of nisin plus natamycin for control of spoilage due to lactic acid bacteria in salad dressings. Due diligence is the key so as not to infringe on any domestic or international published patents or patent applications.

Regulatory assessment is also done at this stage to ensure that there are no “red flags” with regard to use, human safety, and toxicology of both parent antimicrobial and breakdown chemical compounds in buffers or food models. In addition, one needs to be cognizant of constraints due to regulatory limits set for most additives and GRAS antimicrobials (11, 22). At this phase, an assessment should be made of FDA and USDA boundaries for labeling of antimicrobials in any subject food.

**Phase 3—Technology transfer (scale-up and commercialization)**

A determination of efficacy against target microorganisms in the food matrix of choice is a prerequisite for further investment of resources. A desired success criterion needs to be formulated for these efficacy tests, as shown in Fig. 3.

Efficacy of natural antimicrobials in foods is most easily determined and interpreted in carbohydrate-based beverages, followed by bakery products, fruits, vegetables and produce, dairy products, and meat, poultry and seafood products (8). Carbohydrate-based beverages such as fruit juices and soft drinks present the fewest challenges associated with incorporating natural antimicrobials. They are not dissimilar to microbiological media; these systems have relatively low pH and very low to non-existent protein and lipid contents, and they provide a homogenous system for the dispersion of antimicrobials. The only hurdle for application in this type of food would be interaction with simple and complex carbohydrates and minerals (e.g., the chelator sodium hexametaphosphate works only in unfortified fruit juice beverages, and not in the calcium fortified version). Bakery products may also be suitable for use of natural antimicrobials to inhibit growth of spoilage organisms and increase shelf life. These products have relatively low protein content, and surface application would inhibit spoilage fungi.

Negative aspects of bakery products as to antimicrobial application are a neutral pH and non-homogeneity of the food system. Application of natural antimicrobials to minimally processed fruits should be similar to application to fruit juices. Fruits generally have low lipid and protein content, which decrease the opportunity for interactions. They generally also have lower pHs and lower aw than vegetables, which may stress the target microorganisms. The only negatives to application on fruits are the non-homogeneity as to composition and contamination sites of fruits; those with higher pH (e.g., cantaloupe) may show decreased efficacy of natural antimicrobials, since low pH would not be working in concert with the antimicrobials to inhibit target organisms. Vegetables and fresh produce generally have low lipid and protein content but have high pH and water activity and are highly non-homogenous as to composition and site of contamination. Dairy products are very difficult systems in which to achieve activity of natural antimicrobials because these products have high pH, high aw and high levels of protein, fat and divalent cations as interfering compounds, all of which can interact to reduce activity of natural antimicrobials. As a result, natural antimicrobials have been applied to dairy products with varying success. Meat and poultry products may be the commodities with the most inherent properties that will limit effective application of natural antimicrobials. These include a non-homogenous substrate, neutral pH and high concentrations of lipids and proteins. In addition to commodity effects, products formulated with gums, phosphates, titanium dioxide or other additives may tend to bind or inactivate antimicrobials, leading to diminished efficacy and much higher cost-in-use. Developing appropriate delivery systems, such as encapsulation or emulsions or micelles for natural antimicrobials, can reduce their interactions with food components and increase interaction with the target microorganisms and allow for controlled release of the antimicrobial during the shelf life of the food (8).

Challenge studies are done in a food matrix with the antimicrobials at the pre-process, in-process or post-process stages (discussed under FPO) at storage and abuse conditions, and in packaging over the 1x and 1.5x the normal shelf life of the product. The National Advisory Committee on Microbiological Criteria for Foods (1) has developed guidelines for conducting challenge studies with regard to pathogen inhibition and inactivation that should be adapted when developing and executing challenge studies.

Cost-in-use is one of the business critical and sensitive constraints that should be evaluated early on for determination of increased cost per unit of finished packaged product. The cost-in-use analysis should also include cost of inert carrier (e.g., maltodextrin, vegetable oil, glycerol, NaCl, etc.), enablers (i.e., emulsifiers, surfactants, and wetting agents) and delivery systems for carrying the antimicrobial to target microorganisms in the food matrix. It is important to understand and develop specifications for incoming microbial loads (including bacterial spores) for the above ingredients. Capital expendi-
ture may also need to be factored in, such as a need for installation of special mixing and dosing units at batching or at filler operations. For example, special mixing and dosing units are needed for application of natamycin and dimethyl dicarbonate (DMDC). Precise delivery of approved antimicrobial into food is critical for compliance with rules on regulatory agency approved dose at the point of application. The regulatory maximum limit is based on concentration of antimicrobial at the point of application and not on residual activity in downstream finished product during or at the end of shelf life.

In conclusion, unacceptable cost-in-use, negative impact on the quality and sensory attributes of the final product and inadequate efficacy in the food matrix are the primary reasons for failure when attempts are made to couple a food with an antimicrobial system. From a business point of view, early technical failure may be considered a business success, as it helps in reallocation of valuable resources and in re-prioritizing opportunity costs.

**ESTABLISHING FOOD PROTECTION OBJECTIVES FOR ANTIMICROBIAL USE IN FOODS**

Antimicrobials serve potentially important roles in protecting quality and safety of foods by inactivating or inhibiting spoilage and/or pathogenic microorganisms. The authors would like to propose the term “Food Protection Objective,” or “FPO,” to define this secondary barrier effect against spoilage and/or pathogenic microorganisms. This is similar to the concept of Food Safety Objective (FSO) originally described by the International Commission for the Microbiological Specifications for Food (12, 13). The full definition for FPO is the acceptable level of microbiological quality (determined by a specified level of spoilage microorganisms) and/or microbiological safety (determined by absence or acceptable levels of pathogens or opportunistic pathogens of public health significance) at the moment of consumption or at the end of shelf life of a food. It is interesting to note that the hurdle concept is a qualitative or descriptive tool to showcase benefits of preventive controls (Fig. 1). The concept of FSO proposed by ICMSF is probably the only tool that attempts to quantify, at least partially, the benefits of hurdles, including antimicrobials.

This modified concept can be expressed in the following equation:

\[ H_0 \leq \sum R + \sum I \leq PO \]  

where \( H_0 \) is the level of the microbial quality or microbial safety hazard(s) in the raw incoming material, \( \sum R \) is the total (cumulative) effect of processes in that step that reduce hazard levels, \( \sum I \) is the total (cumulative) effect of processes in that step that increase hazard levels in-process or post-process recontamination and/or growth or regrowth due to survivors (14), and PO is the Performance Objective for that step. If the step considered is the final step in the food supply chain, i.e., just prior to consumption, then the PO is the FPO. All variables \( H_0, R, I, \) and PO and FPO in Equations 1, 2, 3 and 4 are expressed in log units.

**Order of addition of antimicrobial**

Two equations are proposed to describe the order of addition of an antimicrobial to a food, pre-process (during formulation, batching) or post-process (but prior to primary packaging). The pre-process addition is preferred by food processors as it is much simpler, i.e., it does not involve use of any special dosing or spraying unit and may also enhance the antimicrobial system in combination with other intrinsic and extrinsic factors that may contribute to increased efficacy (additive or synergistic). For example, low pH and heat may exert combination effects with an antimicrobial system in some instances. It must be noted that sometimes food or extrinsic factors may also exert an antagonistic effect due to binding or inactivation or degradation of the antimicrobial due to excessive heat, resulting in decreased efficacy of added antimicrobial. Therefore, any known potential loss of antimicrobial caused by processing (temperature, shear, pressure, etc.) and binding should be compensated for by adding enough antimicrobial upstream in formulation batch for adequate efficacy (FPO) in downstream finished product.

The equation for pre-process or in-process addition of an antimicrobial is:

\[ H_{PO(1)} - \sum R_{(1)} + \sum I_{(1)} \leq PO_{(1)} (= FPO) \]  

where \( \sum R_{(1)} \) is the total (cumulative) effects of all intervention processes in that step, including secondary barrier effects due to an antimicrobial added at pre- or in-process stages.

Post-process addition of an antimicrobial is the preferred order of addition for control of sporadic adventitious re-contamination from the environment during filling or cross-contamination from food contact surfaces during packaging. Special dosing or spraying equipment will be needed for this unit operation. Some examples are: (1) spraying of natamycin at the exit of line on baked breads and pastries for control of surface molds, and (2) post-lethality spraying of lauric arginate on sliced refrigerated RTE meats prior to packaging, for control of pathogens.

The equation for post-process addition of an antimicrobial is:

\[ H_{PO(2)} - \sum R_{(2)} + \sum I_{(2)} \leq PO_{(2)} (= FPO) \]  

The equation can be sequentially applied so that PO for one step becomes \( H_0 \) for a subsequent step. Equation 3a is similar to 1 and represents all other preceding pre-process and in-process intervention steps (chemical and physical, including thermal and non-thermal) to reduce hazard levels. The antimicrobial effect is shown as a component of \( \sum R_{(2)} \) in Equation 3b. Also, some synergy is possible in this case because of the combined effect of antimicrobial and residual heat in capped containers and native pH, as in the case of hot-fill-hold processed acid juice or acidified beverages.

It is worth noting that by using the above equations it is possible to describe both the initial microbial reduction and residual inhibitory effect of antimicrobial action (Fig. 2). The term “- \( \sum R \)” is a
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