JULY–AUGUST 2013

FOOD PROTECTION TRENDS

FPT

food protection trends

SCIENCE AND NEWS
from the International Association for Food Protection

Vol. 33, No. 4 | ISSN: 1541-9576 | Periodicals
6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2864, USA

Expectations and Applications of Natural Antimicrobials to Foods:

A Guidance Document for Users, Suppliers, Research and Development, and Regulatory Agencies

JAIRUS R.D. DAVID,[1]* LARRY R. STEENSON[2] AND P. MICHAEL DAVIDSON[3]

[1]ConAgra Foods, Inc., Six Conagra Drive, Mail Stop 6-330, Omaha, NE 68102–5903, USA

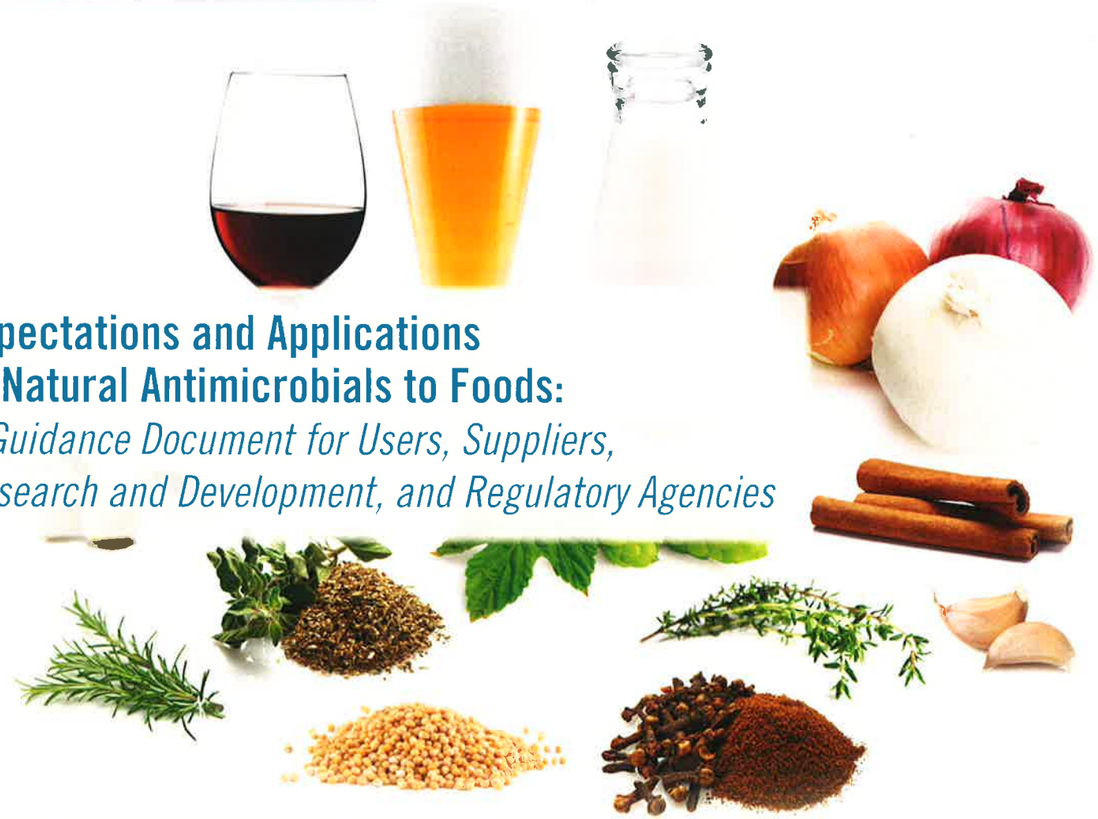
[2]DuPont Nutrition and Health, Four New Century Pkwy., New Century, KS 66031–1144, USA

[3]Dept. of Food Science and Technology, University of Tennessee, 2605 River Drive, Knoxville, TN 37996–4591, USA

PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 33, No. 4, p. 241–250

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864



Expectations and Applications of Natural Antimicrobials to Foods:

A Guidance Document for Users, Suppliers, Research and Development, and Regulatory Agencies

JAIRUS R.D. DAVID,[1]* LARRY R. STEENSON[2] AND P. MICHAEL DAVIDSON[3]

[1]ConAgra Foods, Inc., Six Conagra Drive, Mail Stop 6-330, Omaha, NE 68102–5903, USA

[2]DuPont Nutrition and Health, Four New Century Pkwy., New Century, KS 66031–1144, USA

[3]Dept. of Food Science and Technology, University of Tennessee, 2605 River Drive, Knoxville, TN 37996–4591, USA

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.

*Author for correspondence: Phone: +1 402.240.6808; E-mail: Jairus.David@conagrafoods.com

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

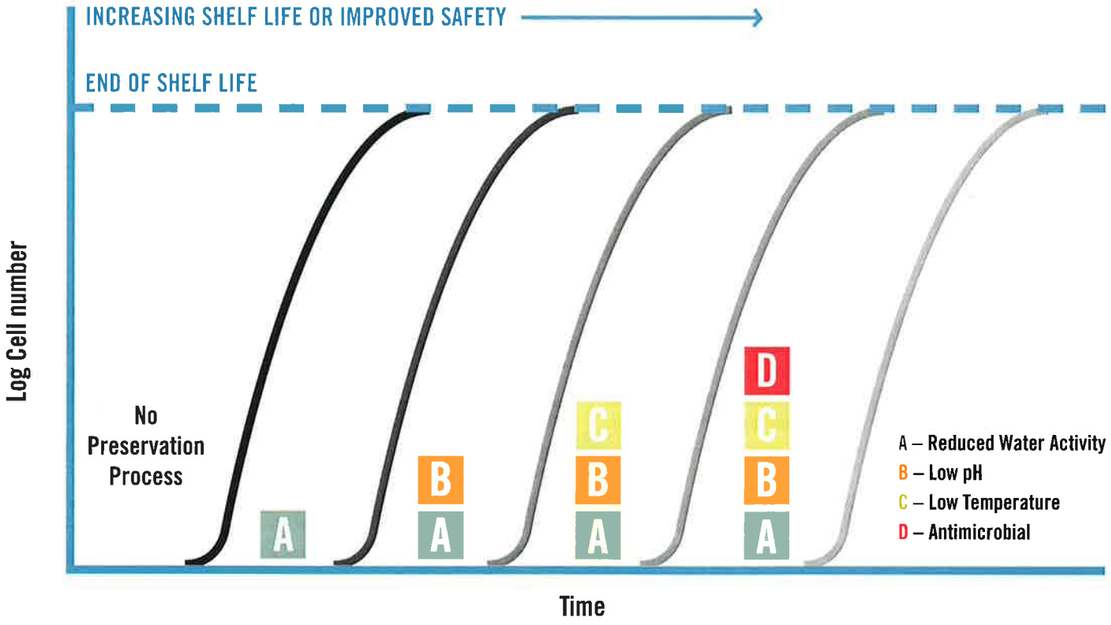


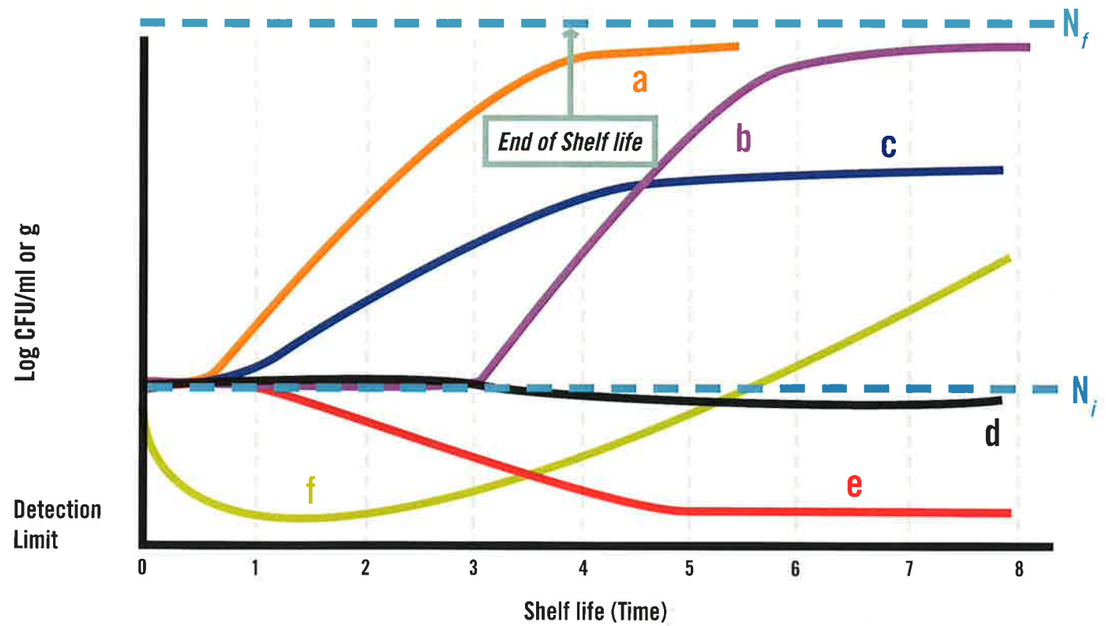
FIGURE 1. A diagrammatic representation of the role of antimicrobials as a secondary barrier against the growth of microorganisms in the context of Hurdle Technology. The diagram is not meant to imply that hurdles are applied in a linear fashion or have equivalent antimicrobial effects, but only to demonstrate the overall effects of multiple hurdles. By themselves, antimicrobials are normally not adequate to inhibit growth of microorganisms in food to the extent often desired, but when combined with primary hurdles, they can be very powerful interventions. Their power is in the way they interact with other hurdles, and the ability to target specific microorganisms. This allows flexibility in their usage

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



a – Control (no antimicrobial)
b – Extended Lag Phase
c – Reduced Growth Rate & Final Level
N_i – Initial Number of Target Microorganism
d – Static (Inhibition)
e – Cidal (Inactivation)
f – Initial Cidal Effect Followed by Regrowth
N_f – Final Number of Target Microorganism

FIGURE 2. Diagramatic representation of the impact of antimicrobials use on antimicrobials on microorganism growth: (a) control (b) extended lag phase (c) reduced growth rate and final level (d) static (inhibition) (e) cidal or inactivation (f) Initial cidal effect followed by regrowth

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

antimicrobial is used alone. In foods, it is more difficult to determine synergistic activity, although a modified checkerboard assay is possible.

Natural antimicrobials may be used with physical preservation processes to enhance the effectiveness of the process or as a safeguard for post-process contamination. Corbo et al. *(6)* described potential process interactions as (a) partial inactivation of the microorganism by the preservation process, followed by continued inhibition or inactivation by the antimicrobial during storage, (b) enhancement of the process inactivation of the microorganism by the antimicrobial or vice versa, or (c) totally independent effects. Such physical processes might include heat (pasteurization) or non-thermal processes, such as high hydrostatic pressure, high-pressure homogenization, or pulsed electric fields. Several studies have demonstrated that natural antimicrobials enhance the effectiveness of physical preservation processes in inactivating target microorganisms *(2, 4, 16–21)*.

Standardization of efficacy determination

One of the major needs in the arena of food antimicrobials, both natural and traditional, is the adoption of standard methods for determination of efficacy. While medically important antimicrobials (i.e., antibiotics) and sanitizers have regulatory guidelines on efficacy evaluation, no such guidelines exists for food 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.

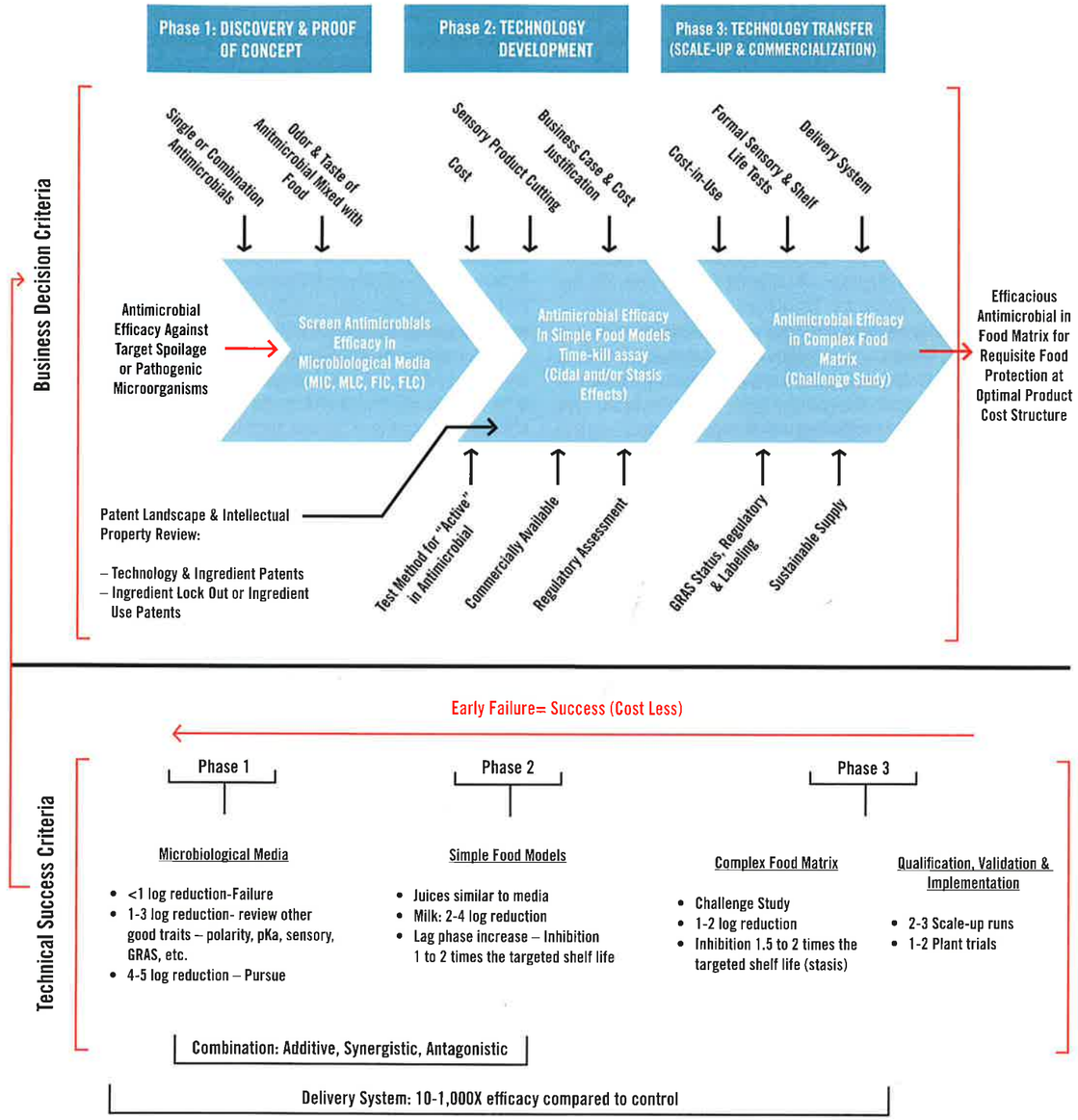


FIGURE 3. The "Idea-to-launch" framework, governance and decision criteria for pairing a food product with a potential antimicrobial system for requisite food protection at an acceptable product cost structure

combined parameter incorporating all risk reduction steps, including instantaneous microbial reduction due to antimicrobial. Similarly, the terms PO and/or FPO can be viewed as combined parameters to describe the inhibitory effect of an antimicrobial. As shown in *Fig. 2*, the extended lag phase of the target microorganism caused by the antimicrobial should be longer than the target shelf life of the product. Most of the antimicrobials have predominantly stasis effect or in some instances a combination of stasis and cidal effects. The desired effect is determined by target shelf life (FPO) and agreed-upon business objectives. Equations (1) and (3b) can be approximated and recast to describe cidal and stasis effect, shown below.

$$H_0 - \sum R + \sum I \leq PO \rightarrow H_0 - \sum R + 0 \leq PO \rightarrow H_0 - \sum R \leq PO\ (=FPO) \quad (4a)$$

(for cidal effect without re-growth during the shelf life of the product)

$$H_0 - \sum R + \sum I < PO \rightarrow H_0 - 0 + 0 \leq PO \rightarrow H_0 = PO\ (= FPO) \quad (4b)$$

(for stasis effect with no re-growth or no additional reduction in numbers)

Even though the concept of FSO is the only tool that attempts to quantify benefits of hurdles, the equation is not mathematically correct. While this concept and equation works for exponential processes such as microbial growth or microbial inactivation, it is not logically correct when arithmetic processes, such as cross contamination or post-process recontamination, are considered, as shown in Equation 3b. In spite of this limitation, the concept of FSO (FPO) has been useful for design and management of food safety (food protection) of the product.

SUMMARY

The utilization of natural antimicrobials as food protection interventions has gained new popularity with their increasing availability and the advent of the "natural" food movement. Many of these antimicrobials are derived from materials readily available in nature, or from fermentation processes utilizing food grade microorganisms. With more natural antimicrobials becoming readily available to the food industry, there is confusion as to what they can and cannot do for the food manufacturer. The importance of the food matrix and its interactions with antimicrobial compounds cannot be minimized, as these interactions are often directly responsible for success or failure. Because target microorganisms may partition differently from the antimicrobial, there may be no interaction between the two and thus no inhibition. The use of targeted delivery systems may need to become part of the future application of natural antimicrobials *(10)*. In addition, blends of antimicrobials, especially those with synergistic components, potentially can be powerful tools for microbial inhibition or inactivation. Combination systems with other antimicrobials or other hurdles, as well as use of targeted delivery systems, should help lower the use and dosage of individual antimicrobials to minimize impact on finished product sensory and quality attributes and for optimizing cost-in-use.

ACKNOWLEDGMENTS

The authors acknowledge John Wyatt, Ware Flora, Ricky Singh, Athula Ekanayake, Richard McArdle and Brennan David for their valuable insights to *Fig. 3* on business process diagram and success criteria for pairing a food product with a potential antimicrobial system for requisite food protection at an acceptable product cost structure and thank George Weber for his contribution on hurdle technology.

REFERENCES

1. Anonymous. 2009. Parameters for inoculated pack/challenge study protocols. National Committee on the Microbiological Specifications for Foods. http://www.fsis.usda.gov/About_FSIS/NACMCF_Subcommittee_Inoculated_Pack/index.asp. Accessed 28 March 2013.
2. Black, E. P., A. L. Kelly, and G. F. Fitzgerald. 2005. The combined effect of high pressure and nisin on inactivation of microorganisms in milk. *Innov. Food Sci. & Emerg. Technol.* 6:286–292.
3. Branen, J. K., and P. M. Davidson. 2004. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *Intl. J. Food Microbiol.* 90:63–74.
4. Calderon-Miranda, M. L., G. V. Barbosa-Canovas, and B. G. Swanson. 1999. Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *Intl. J. Food Microbiol.* 51:7–17.
5. Cooper, R. G., and S. J. Edgett. 2012. Best practices in the idea-to-launch process and its governance. *Res. Technol. Management.* 55(2):43–54.
6. Corbo, M. R., A. Bevilacqua, D. Campaniello, D. D'Amato, B. Speranza, and M. Sinigaglia. 2009. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches – A review. *Intl. J. Food Sci. & Technol.* 44:223–241.
7. David, J. R. D., A. Ekanayake, I. Singh, B. Farina, and M. Meyer. 2013. Effect of white mustard essential oil on inoculated *Salmonella* sp. in a sauce with particulates. *J. Food Prot.* 76:580–587.
8. Davidson, P. M., F. J. Critzer, and T. M. Taylor. 2013. Naturally-occurring antimicrobials for minimally processed foods. *Ann. Rev. Food Sci. Technol.* 4:163–190.
9. Davidson, P. M., and M. E. Parish. 1989. Methods for testing the efficacy of food antimicrobials. *Food Technol.* 43(1):148–155.
10. Davidson, P. M., T. M. Taylor, and S. E. Schmidt. 2013. Chemical preservatives and natural antimicrobial compounds, p. 765–801. *In* M. P. Doyle and R. L. Buchanan, (ed.). Food microbiology: Fundamentals and frontiers, 4th ed., ASM Press: Washington, D.C.
11. FDA. 2012. Code of Federal Regulations – Title 21. U.S. Government Printing Office, Washington D.C.
12. ICMSF. 2002. Microorganisms in foods 7. Microbiological testing in food safety management. Kluwer Academic/Plenum Publishers, New York, NY.
13. ILSI. 2004. Food safety objectives – role in microbiological food safety management. Summary report of a workshop held in April 2003 in Marseille, France.
14. ILSI. 2005. Recontamination as a source of pathogens in processed foods – A literature review. ILSI Europe Risk Analysis in Microbiology Task Force.
15. Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *Intl. J. Food Microbiol.* 55:181–186.
16. Liang, Z. W., G. S. Mittal, and M. W. Griffiths. 2002. Inactivation of *Salmonella* Typhimurium in orange juice containing antimicrobial agents by pulsed electric field. *J. Food Prot.* 65:1081–1087.
17. Mosqueda-Melgar, J., R. M. Raybaudi-Massilia, and O. Martin-Belloso. 2012. Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. *Food Bioprod. Proc.* 90:205–214.
18. Nakimbugwe, D., B. Masschalck, G. Anim, and C.W. Michiels. 2006. Inactivation of gram-negative bacteria in milk and banana juice by hen egg white and lambda lysozyme under high hydrostatic pressure. Intl. *J. Food Microbiol.* 112:19–25.
19. Nguyen, P., and G. S. Mittal. 2007. Inactivation of naturally occurring microorganisms in tomato juice using pulsed electric field (PEF) with and without antimicrobials. *Chem. Engr. Proc.* 46:360–365.
20. Pathanibul, P., T. M. Taylor, P. M. Davidson, and F. Harte. 2009. Inactivation of *Escherichia coli* and *Listeria innocua* in apple and carrot juices using high pressure homogenization and nisin. *Intl. J. Food Microbiol.* 129:316–320.
21. Pol, I. E., H. C. Mastwijk, R. A. Slump, M. E. Popa, and E. J. Smid. 2001. Influence of food matrix on inactivation of *Bacillus cereus* by combinations of nisin, pulsed electric field treatment, and carvacrol. *J. Food Prot.* 64:1012–1018.
22. USDA-FSIS. 2012. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Food Safety and Inspection Service, United States Department of Agriculture, Washington, D.C.