Introduction

While acidified foods enjoy an enviable history of microbiological safety, some risks do exist for high-acid products as evidenced by several food-borne illness outbreaks in recent years (1, 11, and 5). Furthermore, acidified foods fall under a stringent set of FDA regulations under the requirements of Title 21 of the Code of Federal Regulations, Sections 108.25 and 114. Compliance with these requirements has been a point of emphasis by FDA inspectors and has resulted in numerous citations and warnings. Particular attention has been given to the classification of a given food product as an “acidified food.” Upon FDA inspection, many products previously judged as exempted from 21CFR108/114 by the food processor and/or its processing authority have been reclassified and subjected to the requirements of 21CFR108/114. Many of these products are filled at ambient temperature and rely on formulation control to provide protection against microbiological stability and/or safety risks. The efficacy of the formulation control approach must be scientifically demonstrated in order to be acceptable to the FDA.

A draft guidance document intended to assist the food industry in gaining understanding of these requirements was drafted and published by the FDA in September of 2010. Unfortunately, many statements within this draft have been widely questioned by the industry and many of its provisions remain uncertain as of this date.

This white paper aims to provide the reader with a basic understanding of the acidified food regulations as well as providing strategies to assess and improve the microbiological stability and safety of products in this category.

Current FDA Regulations and Guidance Document Governing Acidified Foods

The FDA regulations governing acidified foods were published in the Federal Register on March 16, 1979 (Vol. 44 No.53) under the Code of Federal Regulations, Title 21, Part 108.25 and Part 114 – Acidified Foods. These regulations apply to low-acid foods to which acid or acid food(s) are added. These shelf-stable products are packaged in hermetically sealed containers, have a water activity greater than 0.85, and a finished equilibrium pH of 4.6 or below. In brief, the regulations require the filing of a scheduled process consisting of both proper acidification and thermal processing with the FDA. The scheduled process must be judged to be adequate by a recognized processing authority, must be applied by personnel working under the supervision of an individual having attended an FDA-recognized school, and records of the process must be maintained.

While the regulations are fairly prescriptive as to the requirements for processing and packaging these food products, it is evident that the authors recognized the diversity of intrinsic parameters, acidity levels, microbiological risks, and risk mitigation strategies associated with acidified foods. Therefore, it is the opinion of this author that some aspects of the regulation were intentionally left open or generically addressed to allow flexibility and scientific judgment by a qualified authority. Unfortunately, over the years, some of these points resulted in different interpretations between the industry and the regulatory agency. An attempt by the agency to provide clarification to these points of contention resulted in the issuance of a draft guidance document for acidified foods published in September, 2010 (7).
Finally, acidified foods are also under the purview of the Food Safety Modernization Act and the proposed rule, Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food (proposed 21CFR117). Among the most important requirements imposed by the Preventive Controls Rule are those involving validation and verification for control measures involving not only biological hazards but also chemical, physical and radiological hazards.

Microbiological Safety of Acidified Foods

For decades, the prevailing scientific opinion on naturally acidic and acidified foods was that the acidity of these products provided sufficient protection against contamination by bacterial pathogens. In fact, the risk of potential survival and subsequent toxin production by heat tolerant spores of *Clostridium botulinum* is effectively addressed by the acidic environment present in these products. However, a number of outbreaks involving minimally processed and/or fresh juices (1, 5) and high-acid dressings (11) demonstrated that pH alone was an insufficient barrier to protect public health in the event of contamination by other bacterial pathogens. Outbreaks related to high-acid juices prompted the promulgation of the Juice HACCP Regulations - 21CFR120 - in 2001 (6). While these incidents were limited to naturally acidic products with minimal or no thermal process, it became evident that the safety of high acid products, including acidified foods, could not be assured based solely on their acidity level or pH.

The acidity of these products (pH ≤4.6) precludes the growth and, in most cases, the extended survival of pathogenic bacteria. However, some pathogens such as *Salmonella* and *Escherichia coli* 0157:H7 possess relatively high resistance to acidic environments and are able to survive for periods of time ranging from a few hours to several weeks at pH values at or below 4.6. While these organisms are unable to replicate/grow in the acidic environment, their very low infectious dose (10 – 100 cells) presents a public health concern even in the absence of growth (8).

In spite of the outbreaks cited above for naturally acidic products, acidified foods, as regulated under 21CFR114, have not been associated with significant food-borne illness outbreaks in the USA. This is likely the effect of the multi-barrier protection inherent in most of these foods. These protective barriers include:

1. The application of a thermal process sufficient to render the product shelf-stable.
2. The application of a thermal process, via hot-fill or other means, sufficient to bring the container and its closure to a condition of commercial sterility.
3. Regulatory requirements for careful acidification and confirmation of adequate pH.
4. The antimicrobial properties of organic acids often used in the formulation of these products.
5. The addition of preservatives such as sodium benzoate or potassium sorbate.
6. Regulatory requirements for adequate training for persons in charge of the process.
7. Regulatory requirements for oversight by a competent processing authority.

In contrast to the historical or conventional acidified foods, a new generation of products is entering the market that are processed and formulated in ways that remove some of the barriers listed above. For example, many high-acid and acidified products in the market today are preservative-free, ambient-filled, use packaging materials that do not tolerate hot-filling, and/or use inorganic acids as acidifying agents. These new products may exhibit a lesser margin of safety relative to microbiological risks as compared to the traditional acidified products.
Again, the majority of the risk associated with bacterial pathogens in acidified foods is not related to the ability of microorganisms to proliferate within properly acidified foods, but rather to their ability to potentially survive in these products for extended periods of time. The length of potential survival of these pathogens in high-acid foods is product dependent and influenced by pH, type of acid, storage temperature, and the presence of preservatives. It has been reported that achieving a 5-log reduction of E. coli O157:H7 may take as long as two weeks in cucumber juice adjusted to a pH of 3.8 and stored at 10°C (4). Extensive work conducted by Breidt et al. clearly shows that, as expected, survival is enhanced at higher pH values (3, 4). However, these authors also reported enhanced survival at lower storage temperatures (i.e., 10 versus 25°C) in acidic environments.

While vegetative pathogens such as E. coli O157:H7, Salmonella, and Listeria monocytogenes represent a potential food safety risk to high-acid products, these pathogens are readily inactivated by thermal treatments normally applied to acidified foods for the purpose of rendering the product shelf-stable. However, careful attention must be paid to the risk presented by the acid tolerance of these organisms when a thermal process is excluded or ambient filling procedures are followed. In such cases, it is important to assess via challenge study the ability of the formula to deliver a bactericidal effect equivalent to a 5-log reduction of these pathogens within a reasonable exposure time (i.e., 5-log reduction occurs prior to product leaving the control of the processing facility).

An additional, albeit unlikely, microbiological risk noted by the FDA (7) is the possibility of metabiotic effects resulting from the potential outgrowth of aciduric spore-forming bacteria (i.e., Bacillus subtilis, B. licheniformis, etc.) which could germinate in the product and metabolize acids in a manner that increases the pH of product above 4.6. Such a situation could allow for the outgrowth of pathogenic organisms, including C. botulinum, in the product. This microbiologically-induced rise in pH has been reported to occur at the upper range of the pH limits permissible for acidified foods, pH ≥4.4 (10). While this situation has been observed experimentally, it has not been documented in a commercial food product. Nonetheless, it is recommended to formulate acidified foods so that the maximum pH is ≤4.2, or alternatively, to evaluate via challenge study the ability of these aciduric spore-formers to grow and elevate the pH in the given product formula.

Is My High-Acid Product Exempted from the Requirements of 21CFR108/114?

Acidified foods are defined in the FDA regulations (21CFR114) as: “...low-acid foods to which acid(s) or acid food(s) are added... They have a water activity greater than 0.85 and have a finished equilibrium pH of 4.6 or below.” A controversial and confusing aspect of the regulation is the interpretation of a statement granting an exemption from the rule to certain product categories. Some products are explicitly exempted (i.e., alcoholic beverages, refrigerated foods, carbonated beverages, jams, jellies and naturally acidic foods). The point of controversy surrounds the statement granting exemption to foods that: “...contain small amounts of low-acid food(s) and have a resultant pH that does not significantly differ from that of the predominant acid or acid food...” The regulation does not elaborate as to what constitutes a “small amount of low-acid food” or a “significant change in pH.” Numerous food manufacturers have concluded that some of their high-acid products fall under this exemption and opted not to file their processes under the requirements of the acidified food regulations. Products such as light dressings, light syrups, isotonic beverages, and nutraceuticals have been among the most frequently judged to be exempted under this clause. However, many of these same products have been later judged, upon plant inspections by FDA personnel, to be in
violation of the requirements set forth by 21CFR108/114. This situation is further complicated by the fact that frequently these products are not thermally processed and/or are ambient filled without the benefit of an aseptic fill or alternative treatment. In the majority of these cases the microbiological safety and stability of the product is achieved by a combination of intrinsic parameters (i.e., pH, salt content, acidity, water activity, lack of essential nutrients for microbial growth, natural antimicrobial properties, etc.) and the addition of preservatives such as sodium benzoate and potassium sorbate. Many of these products cannot tolerate a conventional thermal process due to loss of sensorial, physical, or nutritional attributes; or are produced in processing lines that do not support a thermal treatment for the product and/or package. Since the regulation explicitly requires a thermal process addressing both the product and the container, the decision as to whether the products are exempted from the regulation has a tremendous impact on the feasibility of manufacturing these products. Whether or not these products fall under the requirements of 21CFR114/108, it is strongly recommended that the microbiological safety of the process (or lack thereof) be scientifically demonstrated, preferably with the assistance of a qualified processing authority. The confusion related to the interpretation of the “small amounts of low-acid foods” clause was a major catalyst in the issuance of the FDA’s Draft Guidance Document on Acidified Foods on September, 2010 (7). Unfortunately, while a major thrust of this document was to provide clarity around this issue, there is still a great amount of confusion and more than four years later the guidance document still has not been finalized. It has become quite apparent based on informal discussions with FDA personnel and industry representatives that some of the provisions of this guidance document will be revised or will not be included in a final version of this document. However, FDA inspectors and local regulatory agencies have adopted this draft document and are exercising judgment on many products upon plant inspections based on its provisions. The major provisions of the guidance document as it relates to the classification of a product as an acidified food are highlighted below:

Draft Guidance Document on Acidified Foods: The guidance document addresses the question related to what constitutes a “small amount” of low-acid foods by recommending that such amount should not exceed 10% (by weight) of the finished product (excluding water and oil). It also declares that in the case of “water-based liquids” that water be considered a low-acid ingredient, hence recommending that all of these products (i.e., sports beverages) be considered acidified foods. The document also addresses the question of a significant shift on pH by providing a table detailing what constitutes a significant change in pH upon addition of low-acid foods (Table 1). It is clear that the net result of following these recommendations would be the reclassification of a very large number of products that have been traditionally considered exempt by the food industry. It is important to note that the draft guidance does not represent a regulatory mandate but rather recommendations by the agency to the industry.

Table 1. Determining if resultant equilibrium pH does not significantly differ from that of the predominant acid or acid food

<table>
<thead>
<tr>
<th>Equilibrium pH of acid food</th>
<th>Shift in pH is significant if:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;4.2</td>
<td>Any shift in pH</td>
</tr>
<tr>
<td>4.2</td>
<td>Shift in pH &gt; 0.2</td>
</tr>
<tr>
<td>&gt;3.8 and &lt;4.2</td>
<td>Shift in pH &gt;0.3</td>
</tr>
<tr>
<td>&lt;3.8</td>
<td>Shift in pH &gt;0.4</td>
</tr>
</tbody>
</table>
As previously stated, the above guidance document remains in draft status and some of its recommendations are likely to be modified or excluded. In the meantime The National Food Laboratory, acting as a processing authority, is recommending filing the process if your product falls into this gray area. However, our emphasis is on evaluating the microbiological safety and stability of the product regardless of its regulatory status. If a thermal process is applied, we focus on the adequacy of the process in terms of providing sufficient microbial lethality to both product and its container/closure. If a thermal process is not applied, then our focus is on identifying the critical factors (i.e., formulation, intrinsic parameters, etc.) that provide microbial stability and safety and scientifically providing evidence that the critical factors provide the necessary protection (validation) as discussed later in this document.

Can My Acidified Food be Filed with the FDA Without a Full Thermal Process?

As previously stated in this document, a thermal process that addresses the microbiological safety and stability of acidified foods and their package is a requirement of the FDA regulations (21CFR114). However, it is possible to file an acidified process without the benefit of a thermal process for the product and/or the package if scientific evidence is provided demonstrating that the formula’s intrinsic parameters provide the necessary microbial lethality. The agency’s expectation in this case is that evidence be provided demonstrating a 5-log reduction of pertinent pathogens in the product within a period of time not to exceed the time that product remains under the control of the processing facility. This evidence may be provided by citing studies from the peer-reviewed scientific literature or by conducting scientifically sound challenge studies. When this approach is utilized, we at The National Food Laboratory classify this type of filing as a “formulation control filing.” Formulation control is defined as “a food where the growth of microorganisms is controlled through multiple physical and/or physiochemical hurdles.” These “hurdles” are identified, validated, and filed with FDA as critical factors capable of controlling the microbiological risk associated with the product.

The work published by Breidt et al. (3, 4) has been used by The National Food Laboratory to scientifically justify filings of ambient-filled acidified products with pH 3.8 or lower that are acidified with acetic acid. However, this approach may only be used when there is reasonable assurance that the product formula would behave similarly to the test matrices used in the studies published in the literature. Alternatively, The National Food Laboratory recommends the execution of a scientifically designed microbiological challenge study that at minimum demonstrates a 5-log reduction of pertinent vegetative pathogens, following the recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) on the design and execution of challenge studies (9). For testing, the bacterial pathogens are introduced into the food matrix to be tested and incubated at appropriate temperatures.

Important considerations in the execution of a challenge study using pathogenic organisms include the following:

1. Identify the formulation parameters to be validated as critical factors (i.e., pH, titratable acidity, salt content, preservative levels, etc.). Only sorbic acid and benzoic acid (or their respective salts – potassium sorbate and sodium benzoate) are allowed by the FDA in assessing the suitability of a formulation to provide a 5-log reduction against pathogenic organisms.
2. Adjust the test formulation to the minimal values to be filed (i.e., critical limits).
3. Assure that inoculation procedures do not affect the intrinsic parameters of the food product.

4. Select pertinent organisms consistent with the product. Typically, various strains of each of the three most important vegetative pathogens are used (E. coli O157:H7, Salmonella, and L. monocytogenes).

5. Strains from the same organisms can be combined into a single cocktail. Different organisms must be tested separately.

6. The test organisms must be adapted to acidic conditions prior to use.

7. Incubation temperatures must include lowest expected during the holding period of the product prior to release. For shelf-stable products, testing is often conducted at 10 and/or 25°C. Survival of vegetative pathogens is longest at lowest incubation temperatures.

8. Adjust inoculation levels to allow for the determination of a 5-log reduction. The background microflora of the product must be considered and monitored during the study as it may have an impact on the 5-log reduction determination.

9. Sufficient sampling intervals should be used to determine the reduction of viable test organisms during the product incubation.

10. Bacteriological media used for determining the number of surviving organisms at various sampling times over the course of the study must be suitable for the recovery of injured organisms. The use of selective media is discouraged as such media may be inadequate for recovery of acid-injured bacterial cells.

It is important to point out that only some preservatives (as noted in #1 above) are allowed in challenge study testing to demonstrate the log reduction of bacterial pathogens in formulation controlled products. The acidified food regulations (21CFR114) state the following: “Permitted preservatives may be used to inhibit the reproduction of microorganisms of non-health significance (in lieu of thermal process)”. In the past, this clause was cited by representatives of the agency to disallow the use of any preservatives to demonstrate log reductions against pathogens. However, more recently, the agency has permitted the use of potassium sorbate and sodium benzoate up to maximum allowable concentrations (i.e., 0.1%) in challenge studies for pathogenic organisms.

A properly designed challenge study allows for the determination of the exposure time necessary to achieve a 5-log reduction of pertinent pathogens in a given acidified food. This time frame may vary from a few hours to several weeks depending on the formula’s antimicrobial properties. Once the time period is determined, it becomes a critical factor in the FDA filing of the product along with the minimum (not maximum) temperature that the product must be held during this period.

**Pasteurization Processes for Acidified Foods**

Acidified foods are thermally processed as required by the regulations to (1) provide microbiological safety by inactivating the vegetative cells of bacterial pathogens capable of surviving in acidic environments and causing illness even if present at low concentrations and (2) render the product shelf-stable by inactivating the cells of spoilage organisms capable of reproducing in the product under normal conditions of storage, distribution, and retail. The most common pasteurization approach used for acidified foods is the hot-fill and hold process. This process normally involves heating the product to temperatures at or above 190°F and holding it at that temperature for several minutes followed by hot filling at temperatures at or above 180°F.
While hot-fill processes have a long history of safe application by the food industry, it is imperative that the process be adequately validated as per the requirements of FSMA. Particular attention must be paid to sterilization of the container headspace and the interior of the closure as these surfaces may not be adequately heated by simply filling the container with hot product and holding it in an upright orientation. Sterilization of these surfaces is normally accomplished by inclination or inversion of the container to expose all surfaces of the container to the hot product. The efficacy of these procedures should be a point of emphasis during the validation efforts. Alternatives to the common hot-fill-hold process include aseptic processing/package, tunnel pasteurization (in-container process), or atmospheric heating in a retort-type vessel.

The National Food Laboratory Can Help

Questions on exemptions and interpretations by industry and regulatory agencies on acidified foods exist. FSMA guidelines and proposed rules have heightened attention to this area, thus continuing to provide challenges for manufacturers seeking to meet their business needs while simultaneously adhering to compliance requirements. While some aspects of regulatory compliance remain a “gray area,” the design and execution of proactive and appropriate process validation studies is a prudent and recommended brand protection strategy for this class of products. At The National Food Laboratory we can help processors of high-acid foods (naturally acidic or acidified) in all aspects of the regulatory compliance and thermal process selection and validation. Our services in this area are listed in chronological order:

1. Categorize the product as per the regulatory requirements as high-acid or acidified.
2. If categorized as an acidified food, The NFL will assist with the required filing of the product/process with the US FDA.
3. Recommend a thermal process for the specific product based on standard NFL processes or scientific literature.
4. If a standard process is not available for the specific product or if process optimization is desired, The NFL will conduct the necessary microbial thermal death time studies and process validations to select the most efficient process to achieve food safety and quality objectives and fulfill regulatory requirements.
5. If an ambient fill is desired, The NFL will conduct non-thermal challenge studies to verify that the product is suitable for this type of approach and to generate the necessary microbial challenge data required by the FDA.

About The NFL

The National Food Laboratory is a food and beverage consulting and testing firm providing creative, practical and science-based solutions for the following areas: Food Safety and Quality; Product and Process Development; and Sensory and Consumer Research. We create value for our clients by enabling them to develop commercially safe, high quality and great tasting foods and beverages. For more information about The National Food Laboratory, please visit us at www.TheNFL.com.

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References:


