Effects of pH and Water Activity on Microbiological Stability of Salad Dressing

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(Received for publication December 8, 1988)

ABSTRACT

The water activity and pH of an experimental starch-based salad dressing were varied to evaluate inhibitory effects on microorganisms selected from groups known to be principal dressing spoilage agents. Dressing samples were inoculated with Lactobacillus fructivorans, Zygosaccharomyces bailii, or a yeast isolated from a spoiled commercial salad dressing. Both yeast and L. fructivorans displayed a minimum growth pH of approximately 3.55 to 3.60. The minimum aw observed was 0.89 for yeast growth and 0.91 for L. fructivorans. Combinations of aw and pH which imparted microbial stability without use of preservatives are described.

Commercially-prepared salad dressings are bactericidal to vegetative cells of pathogenic microorganisms (11). The pH of such dressings ranges from approximately 2.9 to 4.4 due to incorporation of acetic acid at a concentration of approximately 0.5% to 2.0%. Although these conditions are generally bactericidal, a limited group of aciduric microorganisms is capable of fermentative growth under these conditions which is initially evident by generation of carbon dioxide or off flavors.

The earliest studies of salad dressing spoilage were reported over 60 years ago (4,5,6). Early studies implicated members of the genus Bacillus whose origin was traced to contaminated spices. In 1934, Charlton et al. (2) isolated a microorganism from spoiled salad dressing which was designated Lactobacillus fructivorans due to enhanced growth in the presence of fructose. Kurtzman et al. (7) later analyzed 17 samples of spoiled dressing and found that one-third contained L. fructivorans. Smittle and Flowers also confirmed that this species was one of the most common spoilage agents in shelf-stable acidified dressings (12). It is possible that L. fructivorans went undetected in early studies. Even in a medium selective for lactobacilli with added fructose and an enriched carbon dioxide atmosphere, 104 cfu are required for development of colonies. In keeping with such slow growth, spoilage of commercial dressing is typified by a lag time of weeks or months. Gradual inversion of sucrose to component glucose and fructose molecules under acidic conditions could make a salad dressing containing a small number of L. fructivorans cells progressively more supportive of proliferation by these fructose fermenting bacteria. Similarly, recent trends to substitute high fructose corn syrups for sucrose in salad dressing might make them more prone to support growth of L. fructivorans.

Certain yeasts are also capable of growth in salad dressings. Torulopsis sp., Pichia sp., Rhodotorula sp., and Debaromyces sp. have been isolated from dressings (9,12). However, Zygosaccharomyces (Saccharomyces) bailii is by far the most commonly isolated species in salad dressings (1,3,8,16). Not only is this species tolerant of low pH and high sodium chloride concentrations, it is able to actively transport weak acids to the exterior of the cell, hence reducing their effectiveness as preservatives (15).

In recent years, consumer demand has created pressure to reduce concentrations of salt, oil, and preservatives in salad dressings. While these alterations are perceived to create more healthful products, they may affect the microbiological stability of the product. The purpose of this research was to determine combinations of pH and water activity which prevent the growth of L. fructivorans and Z. bailii.

MATERIALS AND METHODS

Lactobacillus fructivorans (NRRL 4000) and Zygosaccharomyces (Saccharomyces) bailii (Y2547) were obtained from the USDA Northern Regional Research Center, Peoria, Illinois. In addition, a yeast culture was isolated from a spoiled starch-based salad dressing and termed VT Yeast. An inoculum of L. fructivorans was prepared by incubating an LBS broth (BBL, Cockeysville, Maryland) culture anaerobically at 27°C for 10 d. Yeast inocula were prepared by incubating YM Broth (Difco, Detroit, Michigan) at 27°C for 24 h. Microorganisms were pipetted into salad dressing in sterilized glass containers, mixed manually and incubated for two weeks at 27°C. Enumeration involved introduction of 11 g inoculated salad dressing into 99 ml sterile phosphate buffer, mixing for 1 min in a Stomacher Model 6021 laboratory blender (Tekmar, Inc., Cincinnati, Ohio) and serially diluting (10). Pour plates were prepared using acidified PDA (Difco) for yeast and LBS agar for L. fructivorans followed by incubation for one week.

A spoonable starch-based salad dressing was used for inoculation studies. The formulation contained 458 ml soybean oil, 40 ml...
water, 67 ml salted egg yolk, and approximately 652 ml starch paste. Starch paste contained 364 ml water, 54 g cornstarch, 35 ml vinegar, 19.5 g sodium chloride, 130 g corn syrup, and 27.3 g isomerose. Starch was mixed with cold water and heated while mixing until thickened. Sugar, salt, corn syrup, and isomerose were then added to complete the starch paste. Egg yolk and water were then mixed together and added to one-half of the starch paste. Soybean oil was then added while mixing. Vinegar was then added to the remaining starch paste and this mixture was added to the dressing while mixing for 2-3 min. The pH of experimental salad dressing batches was altered by varying vinegar, compensating with water for consequent changes in total volume of liquid, and measuring pH with a Cole Parmer Model 5983 pH meter (Chicago, Illinois).

Water activity was adjusted by varying the quantity of sucrose and salt in the starch paste according to the empirical formula: \[ a_w = 0.97 - 2.91 \times 10^{-3} \times (\% \text{ sucrose}) - 0.01 \times (\% \text{ NaCl}) \], where \% sucrose and \% NaCl are the total weight percent of each component in the final dressing. Water activity was measured with a thermocouple psychrometer Model SC-10A equipped with a Model NT-3 nanovoltmeter thermometer (Decagon Devices, Inc., Pullman, Washington).

RESULTS AND DISCUSSION

The effects of pH and \( a_w \) on microbial growth in the experimental salad dressing are indicated in Figures 1 and 2. The apparent growth limit for yeast was approximately pH 3.6 and \( a_w 0.89 \) (Fig. 1). \textit{L. fructivorans} was able to grow in the dressing at pH values as low as 3.5, but only when \( a_w \) was above 0.95 (Fig. 2). In the experience of the authors, the spoilage agent of commercial starch-based salad dressing is most commonly yeast. Similarly, Kurtzman et al. (7) reported that yeast were the dominant spoilage organisms in 10 of 11 spoiled mayonnaise-like salad dressings. This correlates with the data in Fig. 1 and 2, since most salad dressings have a pH of 3.2 to 3.9 and water activity of 0.93, conditions apparently inhibitory to \textit{L. fructivorans} (12,14).

A composite of Fig. 1 and 2 revealed conditions under which the selected aciduric spoilage microorganisms are incapable of growth (Fig. 3). The polygon ABCDE enclosed combinations of pH and \( a_w \) which should ensure shelf stability of salad dressing while meeting palatability and economic constraints (Fig. 4). If the pH of a dressing is below approximately 3.2, the flavor of the product is undesirably acidic and must be ameliorated, typically by inclusion of sugars. This may be undesirable in the manufacture of low calorie dressings. Reduction of \( a_w \) to a value below that represented by the polygon requires that a salad dressing contain a relatively high concentration of solutes or vegetable oil (13). This may impose economic constraints and may also be undesirable in the manufacture of low calorie dressings. The region within the polygon represents a balance between microbial stability, balanced flavor, and economic considerations. Figure 4 also indicates the relationship of \( a_w \) and pH to several food categories in addition to salad dressings. The pH of some foods, such as pickles or soda pop, is sufficiently low that stability is ensured even at high \( a_w \). Conversely, the water activity of beef jerky or other low moisture foods is low enough that pH is not a crucial
Microbial Stability of Salad Dressing


Figure 3.

Acknowledgment

We gratefully acknowledge the technical assistance of Jann Wakefield Renggli.

References