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A generic model for spoilage of acidic emulsified foods: Combining physicochemical data, diversity and levels of specific spoilage organisms

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ABSTRACT

The spoilage pattern of three emulsified, vegetable-based spreads of low pH (3.90–4.15) adjusted with acetic acid was characterized by correlating the growth of spoilage flora with the organoleptic and physicochemical changes, as well as the changes in the species composition of the dominant microflora during storage under isothermal conditions. In a further step, a generic (hereafter called 'unified') model was developed to describe the maximum specific growth rate of the specific spoilage organisms (SSOs) in all acetic acid acidified products, including literature data and additional in-house data from similar products, as a function of the storage temperature, pH (3.61–4.25) and initial concentration of the undissociated acetic acid in each product. The predictions of the unified model were compared with those of product-specific models, with temperature as the sole predictor variable. Two independent batches of commercially prepared pepper- (*PS*), fava beans- (*FS*) and eggplant-based (*ES*) spreads were stored at 4, 7, 10, 12, 15, 18, 20 and 25 °C. The growth of lactic acid bacteria (SSOs; LAB) was correlated with changes in pH, titratable acidity and organic acids concentration, as well as sensory characteristics, in order to define the shelf-life of the products. Isolates from each spread and storage temperature were grouped with SDS-PAGE and were identified with 16S rRNA, determining the association between spoilage and species diversity. Product-specific models were developed using the square root model, while a polynomial and the Ratkowsky model were used for the development of the unified model. Products with lower pH and/or higher acetic acid content showed higher microbial stability. *Lactobacillus plantarum* or *Lactobacillus brevis* dominated the LAB association in all three spreads, although their relative percentage at the beginning of storage varied significantly. These facultative or obligate hetero-fermentative bacteria increased lactic acid and, sporadically, acetic acid levels in the spreads. The developed models were validated under real chill chain conditions and showed very good agreement with the observed data in *PS* and *FS*. The spoilage perception patterns of the different products were similar and thus, the proposed unified model may provide accurate predictions for the spoilage of a wide variety of acetic acid-acidified spreads, regardless of differences in the formulation (e.g., raw materials) and the manufacturing procedure.

1. Introduction

Acidified sauces, salad dressings, spreads and dips are emulsified foods of animal or plant origin, which may be served as complementary dishes of most meals. Mayonnaise and mayonnaise-based dressings (e.g., ranch, blue cheese, Thousand Island, Caesar dressing), ketchup, barbeque sauce, cheddar cheese dip etc., are some well-known products of such nature. The microbial stability of these foods relies on the low pH achieved through acidulants and weak acid preservatives, such as salts of sorbic or benzoic acid, which also offer buffering capacity to the system (Vermeulen et al., 2007). These intrinsic factors contribute to a shelf-life of several months, even if the products are stored under ambient temperatures. Currently most food industries use the code of the Committee of the Industries of Mayonnaises and Table Sauces of the European Economic Community (CIMSCEE, Comité des Industries des Mayonnaises et Sauces Condimentaires de Communauté Economique Européenne, 1992) in order to determine the probability of microbial growth in any acetic acid ambient-stable sauce. This probability (growth/no growth) model calculates a numerical value (Σ), based on the products content (% w/w) in undissociated acetic acid, NaCl, hexose (glucose and fructose) and disaccharides (Eq. (1)).

$$\Sigma = 15.75 \times [\mathrm{UAC}] + 3.08 \times [\mathrm{NaCl}] + \mathrm{hexose} + 0.5 \times [\mathrm{disaccharides}] \quad (1)$$

If Σ value is above 63 the product is considered shelf-stable and thus, no refrigeration is required for a long-term shelf-life (i.e., ketchup and ranch). Indeed, this formula has been widely used to formulate mayonnaise-based and/or ambient-stable products, like dressings. However, in products such as guacamole, Σ value may not exceed the stability threshold and therefore, chilled storage should be implemented as additional hurdle for maintenance of product quality. The guacamole-style

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category also includes a variety of well-known spreads and dips, namely traditional Greek salads (i.e., tzatziki, pepper spread, feta-cheese spread, Russian salad), which are produced based on vegetables, cheese, yogurt and/or mayonnaise, with the addition of acetic acid as acidulant. An extensive review of this food category was recently presented by Panagou et al. (2013). As long as proper chill-chain conditions are applied, the spoilage of these products is more likely related to slow physicochemical changes, such as dissociation of the emulsion, oxidation, hydrolysis and discoloration (Waite et al., 2009). However, chill chain conditions are often not properly maintained (Nunes et al., 2009; Willocx et al., 1994), and products may experience temperature abuse, especially in retail and home refrigerators (Manios et al., 2009). These conditions may favor the growth of undesirable bacteria, capable of compromising the quality of the products. The acidic environment of these spreads usually favors the proliferation of aciduric bacteria (i.e., lactic acid bacteria) and inhibits other non-acid tolerant microorganisms such as pseudomonads or enterobacteria. The growth of these microorganisms commonly causes an increase of the acidity due to the release of acidic metabolites, swelling of the package due to excessive production of gasses, or slime formation (Vermeulen et al., 2007; Huis in't Veld, 1996). However, changes of the environmental conditions may result in significant diversity of the specific spoilage microorganisms (SSOs) and subsequently affect the profile of the produced metabolites that render the product organoleptically unacceptable (Ercolini et al., 2011). Considering that these products are multi-ingredient ecosystems and that they are usually stored under fluctuating temperatures, the quantification of the microbial metabolites and their correlation with the growth of the SSOs and changes in sensory characteristics may contribute to a more accurate determination of their shelf-life (Dainty, 1996).

Predictive models may be used as means for rapid and inexpensive determination of the “best-before” date, or the remaining shelf-life of products already exposed to chill chain conditions. The available mathematical models for the prediction of the growth of spoilage microorganisms are quite limited (Bruckner et al., 2013; Mataragas et al., 2006, 2011; Manios et al., 2009). This is mainly due to the fact that the development of a shelf-life model requires identification of SSOs and study of their growth potential under a range of environmental conditions (Bruckner et al., 2013). Another drawback of spoilage models is that their predictions account for a specific food and thus, their validity for other, even similar products could be limited and require extensive validation. However, the spoilage of closely related products is primarily affected by the same extrinsic and/or intrinsic parameters (e.g., temperature, pH, a_w, packaging gas composition), since other parameters such as diversity of the SSOs and structure may not differentiate significantly. Models which account for a group of products rather than a single product are termed “unified” (Bruckner et al., 2013; Wilson et al., 2002). Although this “global” approach may overlook significant factors for the microbial growth, it is assumed that their overall effect is represented by the impact of the variables which are taken into account. By these means, a carefully designed and validated model, may describe the microbial behavior in a whole food category with limited data and therefore, could assist in Decision Support Systems (DSS) for the management of such products in the chill-chain.

The objectives of the present study were: (i) to simultaneously monitor microbial growth, SSO species diversity and physicochemical changes during storage of three commercial vegetable-based spreads under isothermal conditions, (ii) to develop product-specific predictive models for the growth of the SSOs of these products in response to temperature, and (iii) to evaluate the performance of a unified mathematical model based on temperature, initial undissociated acetic acid concentration and pH of products under real chill-chain (dynamic) conditions.

2. Materials and methods

2.1. Product characteristics

Three commercial vegetable-based emulsified spreads, which are prepared using traditional Greek recipes, were kindly provided by a multinational company in commercial packages of 250 g. In particular, a pepper-based (*PS*), an eggplant-based (*ES*) and a fava beans-based spread (*FS*) were used. The ingredients and the physicochemical characteristics of each product are presented in Table 1. The manufacturing process includes the preparation of fresh vegetable pulps without any thermal treatment and further mixing of the ingredients. The addition of stabilizers avoids the disruption of the emulsion. All samples were freshly produced and were stored at 4 °C for less than 24 h until use.

2.2. Microbiological analysis

In order to evaluate the batch variability, two different batches of products were obtained and tested; the first batch was stored at 4, 10, 15 and 20 °C (or 25 °C for fava beans spread) and the second at 7, 12 and 18 °C. In total, 24 packages of each spread were stored at each temperature in high-precision incubators. Periodically, portions (10 g) of each sample were diluted in 90 ml of Maximum Recovery Diluent (MRD; ref. No 401691, Biolife Italiana Srl, Milan, Italy) in a sterile stomacher bag and macerated for 1 min in a stomacher (Lab Blender 400, Seward Medical, London). After sampling, the containers were discarded and independent, sealed packages were used for next sampling, in order to avoid contamination during sampling, or the ‘disruption’ of the ecosystem defined by the food and the headspace of packaging. Serial 10-fold dilutions were made and aliquots of 1 ml or 0.1 ml of the appropriate dilution were poured or surface plated, respectively, on selective or non-selective media. Total viable counts were enumerated on tryptic glucose yeast agar (PCA; ref. No 402145, Biolife) and incubated at 30 °C for 48 h. Lactic acid bacteria (LAB) were enumerated on DeMan Rogosa Sharpe agar with pH adjusted to 5.8 (MRS; ref. No 401728, Biolife) following incubation at 30 °C for 48 to 72 h, while yeasts and molds were enumerated on chloramphenicol glucose yeast extract agar (YGC; ref. No 401289, Biolife) at 25 °C for 72 to 120 h.

2.3. Physicochemical analysis

Following microbiological analysis, the pH of each homogenized (10-fold) sample was measured by a digital pH meter (pH526 WTW)

Table 1
Ingredients and suggested shelf-life of the vegetable spreads examined in the present study, as indicated on the package label.

Type of product	Suggested shelf-life	Ingredients
Pepper-spread (*PS*)	90 days under refrigeration	80% fine chopped sweet pepper, vinegar, extra virgin olive oil, vegetable oil, sugar, eggplant pulp, wheat fibers, salt, garlic, spices and seasonings, 0.1% potassium sorbate (preservative)
Fava beans-spread (*FS*)	90 days under refrigeration	65% fava beans, vegetable oil, extra virgin olive oil, onion, modified starches, salt, spices and seasonings, vinegar, 0.1% potassium sorbate (preservative)
Eggplant-spread (*ES*)	90 days under refrigeration	80% eggplant pulp, sweet pepper, extra virgin olive oil, vegetable oil, vinegar, wheat fibers, salt, garlic, spices and seasonings, 0.1% potassium sorbate (preservative)