Fungal Control Through the Hygiene Process

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Contamination caused by fungi stands out as a significant microbiological issue in the food industry, particularly leading to premature spoilage across various food segments, including the dry-fermented meat industry. The emergence of undesired fungi on product surfaces results in substantial economic losses. Once microorganisms infiltrate the food, contamination ensues, and their subsequent proliferation can adversely impact the product's appearance, odor, flavor, and texture. This, in turn, leads to consumer rejection and negatively affects the commercial brand. Additionally, concerns persist regarding the potential presence of mycotoxins in these products. Given the detrimental effects of spoilage fungi in the food industry, practices such as thorough cleaning and sanitization become crucial to prevent contamination and subsequent premature deterioration. These measures play a pivotal role in ensuring the quality and safety of food, while also extending the shelf life of products.

Keywords: sanitizers ; food industry ; hygiene process ; sodium hypochlorite ; peracetic acid ; benzalkonium chloride ; fungal

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1. Introduction

Dry-fermented meat products are widely consumed worldwide, and the occurrence of common molds on their surfaces is generally deemed normal. This can even be considered a quality indicator, as long as these molds do not synthesize mycotoxins or antibiotics [1]. Fungi play a pivotal role in the technological process by releasing enzymes that elevate the sensory characteristics of dry-fermented products, resulting in distinctive flavors [2]. However, a potential drawback exists, as undesirable species capable of producing mycotoxins may also develop on the product surface, posing a threat to consumer exposure to harmful compounds [1][3][4][5][6].

The capacity of fungi to thrive in acidic pH, along with their resilience to the low water activity (aw) and high salt concentration found in dry-fermented meat products, promotes the growth of filamentous fungi over other microbial groups [4][7]. The richness and diversity of species existing in raw materials and the production environment of dry-fermented meat products play a significant role in shaping the mycobiota of the end product [8][9][10].

Species that produce ochratoxin A are especially undesirable in dry-fermented meat products. In temperate climates, the most relevant are *Penicillium nordicum* and *Penicillium verrucosum* [4][5][7][8][9], while in warmer climates, *Aspergillus* from Section *Circumdati* (mainly *A. westerdijkiae* and *A. ochraceus*) stands out, both in South America and Mediterranean countries [4][7][8][10]. The occurrence of both fungi and mycotoxins in these products is linked to contamination from raw materials, particularly spices, and the air in the maturation chamber, where products of different ages often mature together [2][8][10][11][12]. A study conducted by Almeida [11] demonstrated a two-log increase in the fungal population when comparing cured sheep ham with spices to ham produced without this raw material.

Contamination by fungi, encompassing molds and yeasts, is a critical factor contributing to losses and waste resulting from premature fungal spoilage in food. Fungi, predominantly molds, pervade the entire food production chain and can originate from various stages, including seed contamination, cultivation, harvesting, post-harvest activities, food processing, transportation, and storage [3]. This pervasive presence poses a significant risk, leading to substantial economic losses and potential health hazards for consumers. Certain fungi have the capacity to produce toxic secondary metabolites, such as mycotoxins, with adverse effects on both human and animal health [1][4]. Furthermore, the proliferation of fungi in food is associated with adverse effects on the sensory attributes of products, such as appearance, texture, and flavor properties [3][5]. These consequences not only prompt consumer rejection but also contribute to economic losses for producers.

To guarantee the production of high-quality products, predominantly free from microbiological pathogens and spoilage agents, the food industry needs to establish measurable and monitorable limits. These limits should ensure the effectiveness of procedures and the attainment of predefined objectives [13]. Both the sanitizer and the hygiene process should facilitate the production of food with an extended shelf life while ensuring the safety of consumers' health [14][15].

For the process to be effective, it is crucial to choose sanitizers that contain active ingredients proven to be effective against the target microorganisms. The concentrations applied should be adequate for fungal inactivation without unnecessary waste, adhering to microbiological recommendations set with technical criteria for sanitized surfaces, processing environments, food handlers, and equipment [16][17].

A critical aspect of the sanitization phase within an effective hygiene process is the careful selection of the sanitizer. Several factors must be taken into account, including the spectrum of action, antimicrobial or antifungal activity [18], formation of toxic by-products [19] and whether the sanitizer complies with safety and legal requirements stipulated by relevant regulatory bodies [20].

A sanitizer can only be registered and authorized for use after demonstrating its antimicrobial efficacy for the intended purposes. This verification is typically conducted through efficacy testing on the finished product and in the dilutions specified for use by the manufacturer on the label. These analyses may follow the methodology of the Association of Official Analytical Chemists (AOAC) or methods endorsed by the European Committee for Standardization (CEN) for liquid sanitizers (European Standard n 13697) [21]. For smoke sanitizers, compliance with the French Standard (NF-T-72281) [22] is essential, as it outlines the methodology for evaluating the effectiveness of smoke-generating agents. **Figure 1** provides an overview of the sanitizing efficacy analysis scheme based on the modeling of efficacy tests as described by Bernardi et al. [18][23].

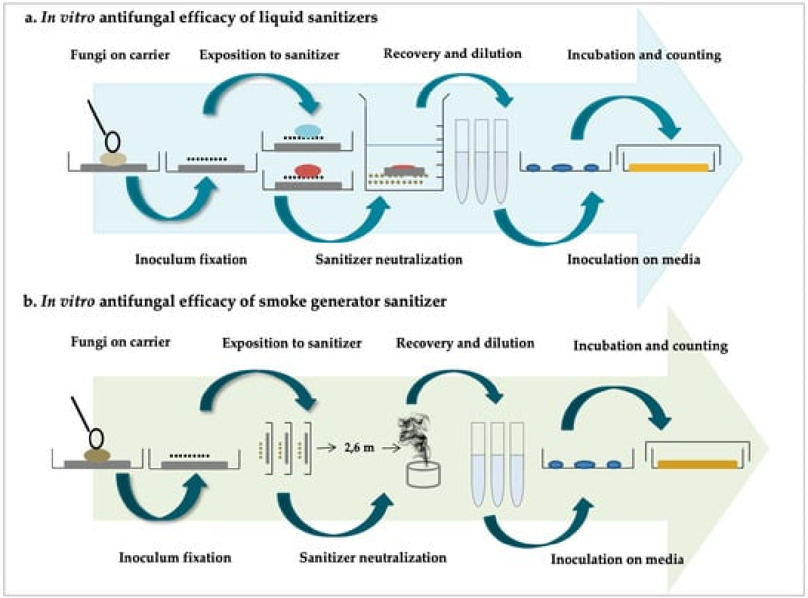

Figure 1. Schematic of in vitro efficacy testing for liquid (**a**) and smoke (**b**) sanitizers.

The selection of a sanitizer should take into consideration various factors, including the type of equipment surface and the specific location to be disinfected, the presence of residual organic load (soil), temperature, water quantity, contact time, the spectrum of action of the agent, and the residual efficacy of the product, among other considerations [20]. Unfortunately, it is a common occurrence for sanitizers to be employed in inappropriate concentrations or combined with multiple products, resulting in formulations that may compromise the antimicrobial activity of the product [17].

In the following section, some common sanitizers used for fungal control in the food industry for the disinfection of work surfaces, air, and production and processing environments will be discussed.

2. Sodium Hypochlorite

Sodium hypochlorite stands out as the most commonly used sanitizer, often serving as a reference in comparative analyses of sanitizers [19][21]. The mechanisms of action of sodium hypochlorite are grounded in its physicochemical properties [22]. Sodium hypochlorite (NaOCl) reacts with water to produce hypochlorous acid (HOCl) [24], also known as free chlorine, through hydrolysis ($NaOCl + H_2O \rightarrow HOCl + NaOH^-$). Subsequently, hypochlorous acid dissociates into the hypochlorite ion (ClO^-) and H^+ (proton) [25][26]. In solution, the hypochlorite ion (ClO^-), one of the active oxidizing forms, directly acts on microorganisms, rendering them inactive by inhibiting enzymatic reactions, denaturing proteins, and inactivating nucleic acids within the cells [27]. This agent serves as a chlorine source and is recognized as a potent oxidizing agent. It is extensively used for cleaning and sanitization in the food industry. Notable features of this compound include its broad spectrum of activities, highlighted by its whitening action [28] and cost-effectiveness [29][30][31], along with its minimal impact on the nutritional qualities of food [32].

The permissible maximum concentration of sodium hypochlorite for use on food contact surfaces typically ranges from 0.005 to 0.02% (50 to 200 parts per million) and 0.05 to 0.08% (500 to 800 parts per million) in non-food contact areas. However, efficacy tests on non-food contact surfaces have indicated that while sodium hypochlorite effectively combats bacteria and yeasts, it is unable to achieve a 4-log inactivation of fungal spores for most tested species [33][34][35][36], including dry-meat spoilage fungal species [37]. This specified concentration is outlined in legislation for assessing the efficacy of sanitizers permitted for use in the food industry. Considering these results, sodium hypochlorite may not be an adequate choice in industries aiming for fungal inactivation, as it exhibits limited effectiveness in controlling fungal contamination [35][36][38].

3. Peracetic Acid

Considered an environmentally friendly oxidant and disinfectant with low environmental impact [39][40], peracetic acid stands out as a key alternative to chlorinated compounds [41][42][43][44]. One of its notable advantages is its minimal reactivity with proteins, effectively preventing the formation of biofilms [45].

Commercially, peracetic acid is available as a mixture containing hydrogen peroxide (H_2O_2) (10–40%), acetic acid (3–40%) [19][29][46][47], and water [48]. This agent, known for its environmental compatibility, decomposes into harmless derivatives (acetic acid, water, and oxygen) [49], which are swiftly metabolized by microorganisms [50]. Possessing lipid solubility properties, as highlighted by Lazado et al. [51], peracetic acid acts directly and robustly on cell membranes through hydroxyl radicals [52]. Reactive oxygen species cause damage to DNA and lipids, disrupting membranes, and blocking enzymatic and transport systems [53].

Owing to its demonstrated fungicidal and sporicidal efficacy in various applications, the use of peracetic acid as a disinfectant in the food industry has gained increased attention in recent years [54]. It is considered highly effective and is applied in different environments, including food processing, beverages, water from cooling towers, and wastewater [41]. In the minimally processed industry, which seeks sustainable alternatives to chlorine [55], peracetic acid is also applied to food contact surfaces [56]. Furthermore, it finds use in cheese and meat facilities [23], bakeries [35], and is also effective for controlling mycotoxin-producing species [38]; usually reaching high fungal inactivation when used in intermediate to high concentrations.

4. Benzalkonium Chloride

Benzalkonium chloride belongs to the group of quaternary ammonium compounds (QACs) [57], specifically from the first generation [35]. Quaternary ammonium compounds typically have at least one long hydrophobic alkyl chain substituent at one end and a short alkyl chain (methyl, benzyl, or ethyl benzyl) at the other end of the quaternary ammonium cation [58]. The antimicrobial activity of these compounds depends on the length of the alkyl chain, with homologous C12 being effective against yeasts and molds, C14 acting well on Gram-positive bacteria, and C16 on Gram-negative bacteria [59]. These compounds can be formulated for specific target microorganisms [60][61][62] and are known for their low toxicity [63][64][65][66]. In lower concentrations (0.5 to 5 mg/liter), quaternary ammonium compounds, including benzalkonium chloride, also exhibit fungistatic properties [67].

The performance of benzalkonium chloride against food spoilage fungi has shown promising results. Evaluations of its efficacy at different concentrations (0.3%, 2.5%, and 5%) against spoilage fungal species from bakery products (such as *Penicillium roqueforti*, *Penicillium paneum*, *Hyphopichia burtonii*, and *Aspergillus pseudoglaucus*) revealed its effectiveness in inactivating strains of *P. roqueforti* [35]. When exposed to different concentrations of benzalkonium chloride, fungi associated with the spoilage of dairy and meat products, including *A. westerdijkiae*, *A. pseudoglaucus*, *Penicillium commune*, *P. roqueforti*, and *P. polonicum*, exhibited varying degrees of resistance, with meat product spoilers generally showing higher resistance to this sanitizer [33]. Studies have also reported its effectiveness against aflatoxigenic fungi, with benzalkonium chloride proving effective against multiple strains of *Aspergillus* spp. [38]. Additionally, it has demonstrated good antifungal action against heat-resistant strains of *Paecilomyces variotii, Paecilomyces niveus,* and *Aspergillus neoglaber* [36].

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