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Bioaerosol formation during grape stemming and crushing

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Abstract

Indoor formation of airborne particles during pre-fermentation grape processing was assessed by particle counting using laser particle sizers. Particle numbers of four different aerodynamic size classes (0.3 to 0.5 μm, 0.5 to 1 μm, 1 to 5 μm, and >5 μm) were determined during unloading of harvest containers and subsequent grape stemming and crushing. Regarding these size classes, composition before grape handling was determined as 87.9%, 10.4%, 1.7%, and 0.1%, respectively, whereas the composition changed during grape handling to 50.4%, 15.2%, 33.0%, and 1.5%, respectively. Airborne bacteria and fungi originating from grape processing were collected by impactor and liquid impinger samplers. Grape handling resulted in a sixfold increase in total (biological and non-biological) airborne particles. The generation of bacterial and fungal aerosols was associated mostly with particles of aerodynamic diameters >5 μm (mainly 7 to 11 μm) as determined by flow cytometry. This fraction was increased 150fold in relation to background levels before grape crushing. Maximum concentrations of culturable bacteria reached 485,000 colony forming units (cfu/m^3), whereas 146,000 cfu of fungi and yeasts were detected per cubic meter of air. Culturable Gram-negative bacteria occurred only in small numbers (180 cfu/m^3). In relation to the total number of airborne particles emitted, culturable microorganisms comprised 0.1% to 0.2%. As soon as grape crushing was stopped, particle concentrations decreased rapidly either due to passive settling or due to air currents in the occupational indoor environment reaching background levels.

Keywords: Wine; Grapes; Grape processing; Bioaerosol; Particle counting; Impaction; Impingement; Flow cytometry

1. Introduction

The first step of indoor pre-fermentation grape handling includes stemming and crushing (Jackson, 1994). Stems, leaves and other extraneous materials are removed and the grapes are crushed. The resulting pulp is transferred to a dejuicer unit where grape juice and flesh are separated. Generally, the surface of grapes harbors a rich microflora including yeasts (e.g. *Saccharomyces, Kloeckera, Pichia*), bacteria (e.g. *Lactobacillus*), and fungi (e.g. *Rhizopus*). Yeasts and bacteria are of major importance in alco-

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holic fermentation (alcohol formation, acid degradation) (Delfini and Formica, 2001). It has been estimated that 1000 to 100,000 of colony forming units (cfu) per gram are present on the grape surface (Fleet, 1999). Interestingly, *Saccharomyces* (a "key player" in alcoholic fermentation) is found only in low numbers. However, massive colonization of the grape surface can also occur by unwanted microorganisms such as the filamentous fungus *Botrytis cinerea* due to damage of the grape skin resulting from herbivory or from hail events which lead to increased microbial fouling (Fleet, 1999). Under these circumstances, population densities of 10^6 to 10^8 cfu might be found per gram of grape. Nevertheless, during stemming and crushing the grape-specific microflora can be aerosolized together with other plant parts and distributed within the occupational environment.

In general, processing of agricultural products often results in the formation of fine-grained dust and particles which are released into the atmosphere (Al-Dagal and Fung, 1990). A significant part of these airborne particles is of microbiological origin and is mainly composed of bacterial and fungal spores as well as vegetative cells (Lighthart, 1997). A series of reports describe the occurrence of bioaerosols in specific occupational environments. Regarding agricultural workplaces, fungal and bacterial aerosols have been determined in cow shelters (Adhikari et al., 2004) or swine barns (Duchaine et al., 2001). In relation to specific agricultural products, airborne microorganisms during processing (e.g. picking, sorting, cleaning, cutting, drying, grinding, or packing) of hop (Gora et al., 2004), potatoes (Dutkiewicz et al., 2002), herbs (Dutkiewicz et al., 2001), tobacco (Reiman and Uitti, 2000), or grain (Dacarro et al., 2005) have been characterized. As example, it has been reported that during hop processing (picking and sorting) 2000 to 130,000 cfu can be found per cubic meter of air (Gora et al., 2004). Composition of bioaerosols regarding different physiological microbial groups was variable ranging from 22% to 96% for Gram-positive bacteria, and 4% to 65% for fungi. In about half of all samples, Gram-negative as well as thermophilic actinomycetes were not detected, in others in concentrations of up to 24,000 and 7500 cfu/m^3, respectively. Herb processing produced airborne microorganisms in concentrations of up to 600,000 cfu per cubic meter of air (Dutkiewicz et al., 2001). During cigar manufacturing, 220,000 mesophilic bacteria and 2900 mesophilic fungi have been found per cubic meter of air resulting from tobacco handling.

Besides agricultural processes, industrial processes such as wastewater treatment (Prazmo et al., 2003), handling of municipal solid waste (Lavoie and Guertin, 2001), or paper recycling (Breum et al., 1999) have been investigated regarding the formation of bioaerosols. However, with a few exceptions, all publications do not include information on hourly, daily, or seasonal patterns, fluctuations, or impact loads in relation to time.

Here we report the formation of airborne microorganisms resulting from post-picking grape processing (stemming and crushing). The objectives of the work were (i) to determine quantitatively total particle emission during grape crushing; (ii) to determine quantitatively airborne culturable bacteria and fungi originating from grape crushing; and (iii) to correlate bioaerosol emission with specific particle size classes determined either by laser particle sizing or by flow cytometry after specific staining.

2. Material and methods

2.1. Description of the sampling site

Harvested grapes are transported from the vineyard in vats (containing approx. 500 kg of grapes) to an indoor processing facility (barn). The vats are emptied by the help of a crane and their content is transferred to a crushing unit which is usually operated for 5 to 10 min. During the process of emptying followed by stemming and crushing, particulate bioaerosols (microflora from the grape surface, plant parts) are generated and distributed within the working environment. Air samples were collected by impingement and impaction in a distance of approximately 3 m from the crushing unit.

2.2. Particle counting

Three laser particle sizers (MetOne "227B", purchased from SKAN AG, Allschwil, Switzerland) were used to determine particle numbers. In total, particles