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Molecular monitoring of spoilage yeasts during the production of candied fruit nougats to determine food contamination sources

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Abstract

In the present work, we have analysed the yeast microbiota present in a manufacturing plant of candied fruits and nougats. Four yeasts species (*Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, *Sporobolomyces roseus*, and *Debaryomyces hansenii*) and a filamentous fungi (*Nectria mauriiticola*) were identified according to restriction analysis of 5.8S-ITS rDNA. These identifications were subsequently confirmed by sequencing the D1/D2 domain of the 26S rRNA gene. *Z. rouxii* and *Z. bailii* were isolated at high frequency along the whole manufacturing process. Since food alteration by *Z. bailii* and *Z. rouxii* is the cause of important economic losses for the food industry, there is a need for differentiating yeasts at the strain level as an essential part of quality control programs in this industry. For this purpose, we have tested the performance of three molecular techniques (RFLP mtDNA, RAPD-PCR, and microsatellite with $(GAC)_5$ and $(GTG)_5$ primers) to differentiate strains belonging to these two *Zygosaccharomyces* species. Those techniques with the best discriminatory power were applied to differentiate *Zygosaccharomyces* species isolates. The results of this analysis indicate that one strain of *Z. bailii* and two strains of *Z. rouxii* were involved in the spoilage of candied fruits. Moreover, the *Z. bailii* strain was also present in the spoiled nougat, hence being responsible of this alteration.

Keywords: *Zygosaccharomyces bailii*; *Zygosaccharomyces rouxii*; Candied fruits; Molecular techniques; Spoilage

1. Introduction

Yeasts have been exploited for their commercial benefits for hundreds of years in the production of diverse foods and alcoholic beverages. However, yeasts have also been shown to be involved in the spoilage of an extensive range of foods according to

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their metabolic and physiological capabilities. These capabilities allow them to overcome the environmental conditions and preservative measures applied to control yeast spoilage (Fleet, 1992; Loureiro and Querol, 1999). Extensive measures have been taken by the food industry to control the threat of yeast spoilage. However, recent demands by consumers for high-quality, preservative-free, and safe foods, which have then undergone milder processing while maintaining their extended shelf-life, impose new challenges to the food industry (Van der Vossen and Hofstra, 1996). Moreover, the number of organisms capable of tolerating the environmental conditions present in the food processing, including the ability to resist and overcome preservative measures used to control spoilage, is dramatically increasing (Brul and Coote, 1999).

The stability of high-sugar products depends on a_w, pH, the presence of preservatives, and temperature. At a_w values between 0.61 and 0.75, the growth rate of spoilage yeasts is very slow. Spoilage may then become apparent only after many months. If the high-sugar products are stored in an atmosphere of high relative humidity, the a_w increases due to hygroscopy, and these conditions allow a significantly faster yeast growth (Deak and Beuchat, 1996).

Preservative resistant organisms include a wide range of yeasts species of the genera *Candida*, *Debaryomyces*, *Dekkera*, *Issatchenkia*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, and *Zygosaccharomyces* (Pitt and Hocking, 1985). They are responsible for the spoilage in foods that have been processed and packaged according to normal standards of good manufacturing practice. However, the yeasts most frequently isolated from products with high sugar content are species of the genus *Zygosaccharomyces*. The species *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* are of particular importance to the food industry as major spoilage organisms of fruit juices, sauces, carbonated soft drinks, salad dressings, and ketchup. *Z. bailii* exhibits a high tolerance to acid preservatives and SO_2, together with a tolerance for low-pH and low-a_w foods. *Z. rouxii* is characterised by its ability to tolerate a_w environments with low a_w (being one of the most xerophilic organisms known) and to grow at an a_w of 0.62 in fructose solutions. Moreover, the ability to ferment hexose sugars makes both species causative agents of fermentative food spoilage (James and Stratford, 2003).

The rapid identification of spoilage yeasts is of great importance to the food industry. Several molecular-based methodologies have also been used to identify these yeasts (for a review, see Loureiro and Querol, 1999). The restriction analysis of the 5.8S-ITS rDNA region has proved to be the most suitable methodology for a rapid and accurate identification and differentiation of *Zygosaccharomyces* species, as proposed by Esteve-Zarzoso et al. (1999).

Another important problem associated with the identification of foodborne yeast consists in the specific detection and differentiation of strains at the within-species level. Discrimination at subspecies or strain level and the analysis of within-species genetic polymorphisms and population variability have been shown as very helpful to determine contamination sources during food processing (Loureiro and Querol, 1999). Some of the molecular techniques reviewed in Loureiro and Querol (1999) generate low reproducible or non-comparable results, while others have proved to be useful only for the differentiation of *Zygosaccharomyces* species. Preliminary results, obtained from the restriction analysis of mtDNA from strains of the species *Z. bailii*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces fermentati*, *Zygosaccharomyces mellis*, and *Z. rouxii* (Guillamón et al., 1997) and from electrophoretic karyotyping of strains from the species *Z. bailii*, *Z. bisporus*, *Z. fermentati*, and *Z. rouxii* (Török et al., 1993) have demonstrated the utility of both techniques for strain characterization and differentiation within the genus *Zygosaccharomyces*. Moreover, the resolution power of RFLP analysis of mtDNA for strains of this genus allowed the characterization of strains from the same species, even when they were isolated from the same source (Esteve-Zarzoso et al., 2003). This study demonstrated that the resolution of electrophoretic chromosome analysis is better in confirming the differences between species of this genus rather than characterizing them at the strain level.

As mentioned before, species of *Zygosaccharomyces* have been involved in the spoilage of candied fruits due to their exceptional tolerance to preservatives and high sugar contents (Thomas and Davenport, 1985). In the present study, we applied different molecular techniques of identification and character-