ABSTRACT

Sweet brioche-like products (pH 5.8-6.2, aw 0.83) are considered to be intermediate moisture foods, which are significantly susceptible to fungal spoilage and sensory deterioration. Purpose of the study was the molecular identification and determination of in vitro growth potential of fungi isolated from filled sweet bread products and their raw materials. Freshly produced praline-, biscuit- or strawberry-filled brioche or raw materials (flour and fillings) were daily supplied by the manufacturer and analyzed immediately or during their shelf-life (20-37°C, 50-60 days) for fungal presence. A total of 102 fungal isolates were molecularly identified by sequencing the internal transcribed spacer (ITS) region. In vitro fungal growth of selected isolates (42 out of 102), representing the major fungal genera identified, was assessed with spot inoculation on Malt Extract Agar of pH 6.2 (adjusted using 5 N NaOH) and aw 0.99 (without glycerol) and 0.83 (adjusted with 38% v/v glycerol). The low aw was studied to imitate the respective values of sweet brioche-like products as well as fillings. Fungal growth was monitored at 25°C (recommended temperature by the industry) and 37°C (as temperature abuse) by measuring radial diameters for max. 60 days. More than 65% of fungal isolates was identified as Penicillium sp., followed by 20% of Cladosporium sp. and 7% of Aspergillus sp. In vitro growth assessment revealed that high aw permitted the growth of all isolates (n=42) at 25°C, whereas only 33% had a growth potential at 37°C, most of which belonged to genus of Penicillium. On sweet brioche-like media (aw 0.83), no growth was observed at all isolates except for three Aspergillus sp. isolates at 37°C. Contrary to temperature abuse conditions, more than 80% of the isolates, which included all the identified genera, were able to grow at 25°C and aw 0.83, highlighting the potential visual deterioration of brioche products during their shelf-life. Results of the present study could contribute to the identification and minimization of the risk of fungal spoilage in sweet brioche-like products.

OBJECTIVE

Molecular identification and determination of in vitro and in situ growth potential of fungi isolated from filled sweet brioche-like products and their raw materials.

MATERIALS AND METHODS

A. Isolation & Characterization of Fungi

- Samples:
  - Freshly produced products filled with cream (praline, biscuit, strawberry), were analyzed on receipt (fresh products),
  - upon receipt (fresh products),
  - storage samples, raw materials).
- Fungal isolation:
  - Collection of fungal isolates from final product (using 5 N NaOH) and aw 0.99 (without glycerol) and aw 0.83 (adjusted with 38% v/v glycerol).
- Characterization of isolates:
  - Fungal growth was monitored at 25°C and 37°C for up to 60 days (corresponding to shelf life of the product).
- Fungal growth was monitored on the ral surface of the product.

B. In vitro assessment of growth potential

- Microorganism:
  - 42 fungal isolates representing the main genera identified.
- Nutrient substrate:
  - Malt Extract Agar of pH 6.2 (adjusted using NaOH) and aw 0.99.
- Temperature:
  - 25°C for optimum fungal growth and
  - 37°C for growth potential at 37°C (aw 0.99). The low aw was studied to imitate the respective values of sweet brioche-like products as well as fillings.
  - Fungal growth was monitored at 25°C (recommended temperature by the industry) and 37°C (as temperature abuse) by measuring radial diameter for max. 60 days.
  - More than 65% of fungal isolates was identified as Penicillium sp., followed by 20% of Cladosporium sp. and 7% of Aspergillus sp. In vitro growth assessment revealed that high aw permitted the growth of all isolates (n=42) at 25°C, whereas only 33% had a growth potential at 37°C, most of which belonged to genus of Penicillium. On sweet brioche-like media (aw 0.83), no growth was observed at all isolates except for three Aspergillus sp. isolates at 37°C. Contrary to temperature abuse conditions, more than 80% of the isolates, which included all the identified genera, were able to grow at 25°C and aw 0.83, highlighting the potential visual deterioration of brioche products during their shelf-life. Results of the present study could contribute to the identification and minimization of the risk of fungal spoilage in sweet brioche-like products.

C. In situ assessment of growth potential

- Microorganism:
  - Mix of (5) Penicillium sp. isolates (that demonstrated the highest growth potential in product imitating aw) (Fig. VII).
- Growth assessment:
  - High aw permitted the growth of the majority of fungi isolates at optimum aw and T conditions.
- The highest heterogeneity in growth ability was observed at optimum aw and conditions.
- The majority (89%) of fungi was isolated from final products (Fig. I), while visible fungal growth was observed in discrete sites of the final product and mainly at the end of storage (Fig. II).
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- Penicillium (64%), Cladosporium (24%) and Aspergillus (7%) were the main genera identified (Fig. III) and were similarly distributed across different origin of isolation (Fig. IV).
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RESULTS

A. Isolation & Characterization of Fungi

- Percentage of fungal isolates (n=102) per origin (fresh products, storage samples, raw materials).

B. In vitro assessment of growth potential

- Percentage of fungal isolates demonstrating growth/ no growth under different combinations of temperature and aw.

C. In situ assessment of growth potential

- Penicillium sp. isolates at aw 0.82 and 25°C. Arrows indicate the isolates used in subsequent in situ experiments.

CONCLUSIONS

- The majority of fungal isolates could potentially spoil sweet brioche-like products.
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- Potential abuse temperature could favor the outgrowth of some Aspergillus isolates.
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- Combination of hurdles (i.e., preservative, MAP) could successfully control fungal spoilage in sweet brioche-like products.
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