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Scientific Paper

Uses of *Rhizopus oryzae* in the kitchen

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Abstract

The use of fungal cultures in modern cuisine can provide a broad number of new textures, flavors and tastes from unexpected substrates; such as fruits, vegetables and nuts. The presented research describes how *Rhyzopus oryzae*, a fungi used in Asian culture to produce traditional recipes, results in fruits, vegetables and grains with unique sensorial properties. Throughout the paper, different examples of these novelty uses are presented showing different examples of prototypes those have been successfully incorporated into our menus, their production procedures and their sensorial evaluations.

Keywords: Rhyzopus oryzae; Mold fermentations; Innovative gastronomy

Introduction

Tempeh is a popular fermented product in Indonesia and Malaysia that includes, traditionally, fresh or cooked soybean and a mixture of fungus, yeast and bacteria that ferments the wet seed and produces a solid paste that can be fried, boiled or consumed raw (Shurtleff and Aoyagi, 2001). During fermentation, microorganisms use the seed as a substrate to feed themselves and subsequently obtain energy and organic material. It is a complex process where fungal metabolism plays a key role and results in significative changes in the texture, flavor and taste of the vegetal material. Although tempeh is not usually commercialized in Western countries, it is widely used in Asia and it has become an emergent food industry. Transformation from artisanal to modern food production requires pure, standardized and well-defined starter strains. They make it possible to expand the uses of these microorganisms in different food products and can yield more consistent results in controlled conditions and at production facilities.

In most cases, traditional products are fermented with a mixture of yeast, bacteria and fungi. Among others genus, *Rhizopus* is part of the fungal ecosystem responsible of these fermented products. Scientific classification of this fungus is Class, *Phycomycetes*; Order, *Mucorales*; Family, *Mucoraceae*; Genus, *Rhizopus.* This genus is composed of 10 species, including plant spoilage species (like *Rhizopus arrhizus* or *Rhizopus artocarti*) or food related species, like *Rhizopus oligosporus* and *Rhizopus oryzae*, those involved in tempeh production (Wiesel et al., 1997). Different characteristics make this genus interesting for massive application in food production. *Rhizopus* requires a simple ecosystem to survive and can grow vigorously between 25 and 45 °C. All these characteristics make them almost omnipresent in nature, and allow almost all vegetables material colonization. The main constraint to fungal growth is humidity, which should remain at an elevated percentage. During fermentation, *Rhizopus*' amylase, lipase and protease activity (Baumann and Bisping, 1995), increases the biodisponibility of the nutrients and their ability to use many compounds as energy and carbon source. Also,

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this fungus has a very rich secondary metabolism, it produces a high number of compounds with sensorial and nutritional interest (Denter et al., 1998).Finally, *Rhyzopus* can produce anti-fungal and anti-bacterial compounds, which have been reported by Dinesh Babu et al. (2009).

As with other microorganisms, safety concern is critical when human food application is considered. *R. oryzae* is considered GRAS (Generally Recognized As Safe) by the U.S Food and Drug Administration (FDA) and thus, can be used for human consumption within the U.S. However, filamentous fungi is not considered QPS (Qualified Presumption of Safety) by the EFSA (European Food Safety Agency), which raise safety concerns, in which mycotoxins presence therefore should be analyzed in individual products marketed in Europe. To our best of knowledge, no toxin production by *R. oryzae* has been reported in scientific literature. Actually, the *Rhizopus* species has been used on the one hand, as a detoxifying agents against food toxins, like Ochratoxin A (Varga et al., 2005), and on the other hand, to increase the digestibility of certain legumes (Azeke et al., 2007).

One of the main advantages of working with this micro-organism in the kitchen is that molds open a window in sensorial world in terms of new textures and flavors. In the case of *R. oryzae*, there is a special interest to obtain innovative results in matrix apart of traditional soy beans. Among of this, molds change nutritional characteristics during fermentation, those may improve nutritional profile in menus. Examples of this modifications are described in Baumann and Bisping (1995), in Denter et al. (1998) and in Dinesh Babu et al. (2009).

In Western gastronomy, mold-fermentations are not usual, except significant exceptions (i.e. Roquefort-like cheeses). In this paper we present examples of different products that can be obtained using *R. oryzae* as a fermentation agent, in an effort to communicate the amazing textures and flavor modifications that can be induced by fungal fermentation from an exclusively gastronomic point of view. This paper does not try to be an exhaustive relation of fermentable substrates and we encourage each cook to test with their own “magical recipe”.

Materials and methods

Used microorganisms, ingredients and cooking conditions

Commercial *R. oryzae* culture was provided by Top Cultures, (Belgium). The inoculum was stored at 4 °C. The specification sheet claims 8,000,000 spores per gram of starter when it was packaged. Starter was inoculated without previous rehydratation. In all the cases described in the paper, raw material was cooked in tap water and 200 g of cooked substrate was inoculated with 1 g of dry starter (see Fig. 1). Preliminary results (not shown) demonstrated that using smaller ratio of inoculum produce incomplete colonization and, visually, very heterogeneous results.

Used raw materials (Golden and Granny Smith apples, rice, seeds cereals and legumes) were provided by local markets. Cooking was done using habitual material present in professional installations, including induction cooking facilities and industrial ovens with air circulation.

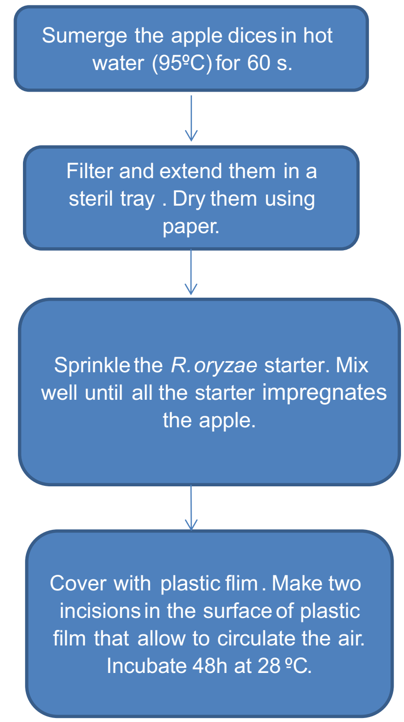

Fig. 1. Workflow diagram for apple-dice fermentation.

Inoculation and growth; safety considerations

Fungal growth requires long incubation periods (24–48 h) at 28 °C, which would make the food matrix a good substrate for possible pathogens. Incubation conditions as well as the absence of later thermal treatment of most of the products make food safety our first concern. Possible contamination with pathogens should take in mind and extremely hygienic practices should be observed, including some modification in the usual kitchen practices. Among others, all material in contact with the ingredients, including knives, bowls and trays should be cleaned with 70% ethanol before use. Gloves should be used and cleaned frequently with 70% ethanol. To inoculate efficiently, one recommends use tools that allow the starter to spread on different foods. In this case, the starter is a powder that we spread with the help of a thin colander cleaned with 70% ethanol. Incubation should be done as sterile as possible, in trays cleaned with 70% ethanol and covered with plastic film as soon as the sample is introduced. Small incisions should be performed for fungal respiration and to prevent condensation in the plastic cover.

Sensorial analysis

Sensorial analysis of the dishes was performed by the sensory panel of AZTI and Mugaritz according to the flavor profile methodology described by Keane (1992). In essence, 4–5 panelists with broad expertise in sensorial analysis evaluate