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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 microorganism 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.

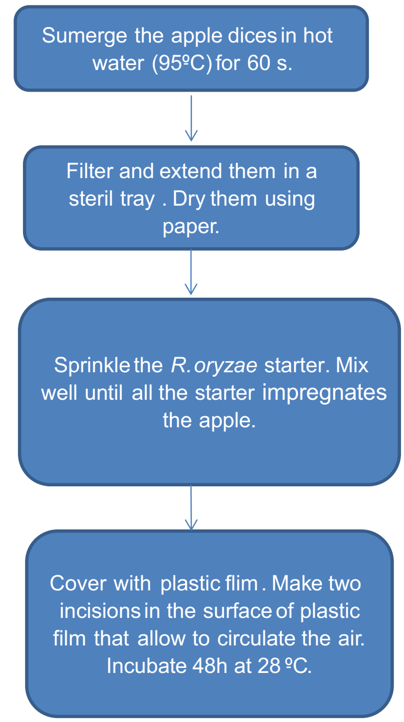

Fig. 1. Workflow diagram for apple-dice fermentation.

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 visual, gustative and flavor characteristics using an open record to annotate their impressions. Sensorial definitions obtained in 2 or 3 different sessions per products were collected, grouped according to their sensorial significance, summarized in consensus descriptors and recorded. Positive and negative descriptors were considered.

Results and discussion

Fruits

One of the best substrates for fungal growth is fruits. In this paper, we described the fermentation of apple dices with *R. oryzae*. Ingredients are described in Table 1 (prototype A) and the workflow is described in Fig. 1. Briefly, apples (Golden and Granny Smith were used separately in several tests), were diced in cubes of 2 cm, boiled for 60 s in tap water, cooled, dried and fermented for 48 h at 28 °C (measured in the incubator). Time, temperatures and distances between apple dices were the same for both types of apples. After the initial 24 h, fungal filaments could be observed in both apple varieties, and dices were completely covered with *R. oryzae* after 48 h. *Rhizopus* (as all fungus) is an aerobic organism that will colonize the surfaces exposed to air; we, therefore, recommend a minimal distance of 2 cm between apple dices to assure a good coverage. After several trials with both apple varieties, it is observed that the fungus develops faster in more acidic (sensorial) varieties, like the Granny Smith apple. *R. oryzae* would be favored in acidic environments because it counterbalances their own ammonia (and therefore alkalizing) production. It can be also speculated that these varieties favor the fungal growth due to the malic acid concentration differences (Nour et al., 2010), but further experiments are required to confirm this point. Only Granny Smith variety was used for sensorial characterization.

Among the evident visual alterations (seen in Fig. 10), several sensory changes were detected after fermentation comparing raw fruits with fermented. The texture changed from very raw, crunchy, gritty and somewhat fibrous apple dices to dices with a very ephemeral outer layer and succulent interior. Considering flavor, fermentation enhances more sweetness and acidity while eliminating some fruity notes from the reference.

Externally, products gain a new esthetic appearance with a white and filamentous cover in thereby introducing a new concept for western consumers that is both safe and surprising. The first eye-contact with the product would generate rejection from consumers, and, about 40% (2 of 5) of the panelists showed reservations about its safety. May be this is because Occidental consumer is not very usual this visual effect in food and it is joined with food in bad conditions to be consumed. This appreciation was observed in all products referred in this paper.

Table 1
List of ingredients for each prototype.

Prototype	Ingredient	Weight/volumen
A	Cooked apple dices of 2 cm	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
B	Cooked rice	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
C	Cooked burghul	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
D	Cooked barley	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
E	Cooked rye	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
F	Cooked nuts	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
G	Cooked peas	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
H	Cooked fava beans	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g
I	Cooked beans	200 g
	Water for cooking	1000 ml
	R. Oryzae starter	1.0 g

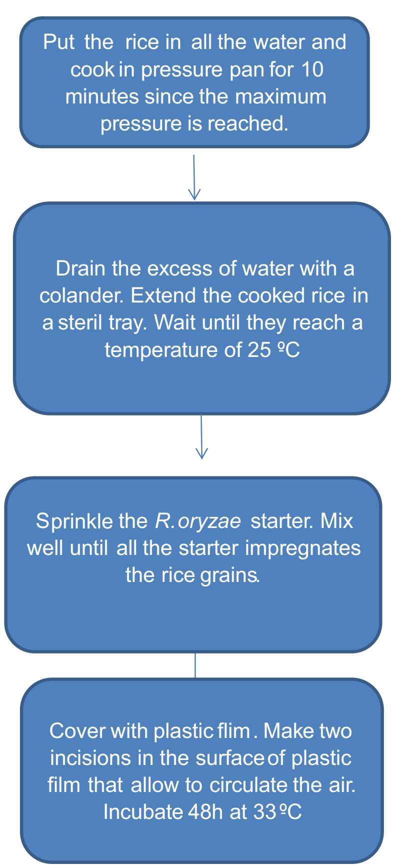

Fig. 2. Workflow diagram for rice fermentation.

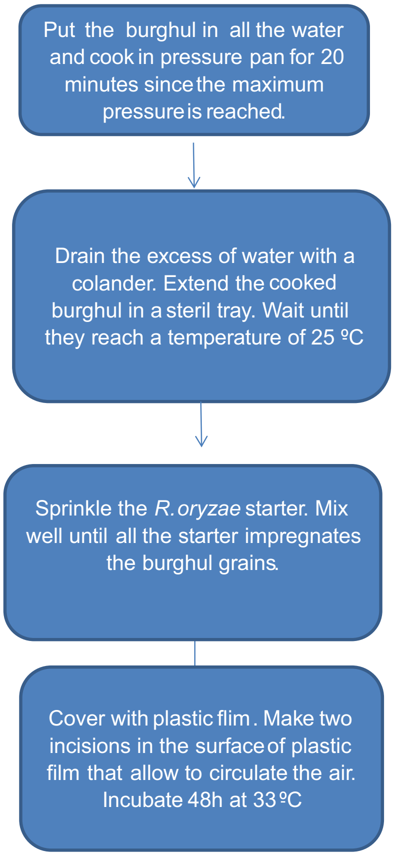

Fig. 3. Workflow diagram for burghul fermentation.

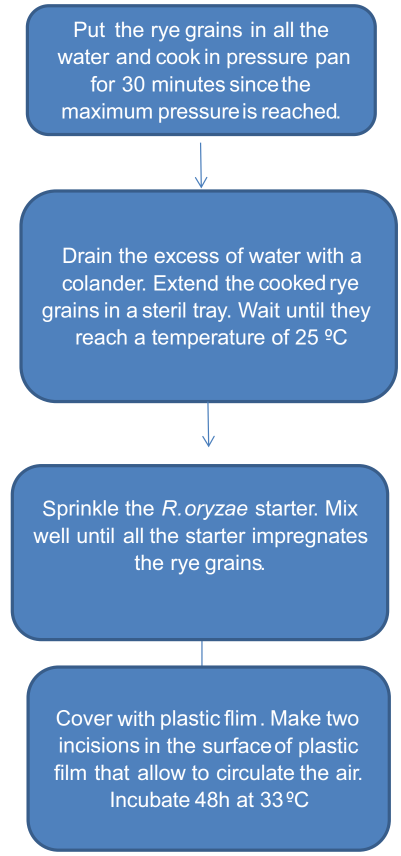

Fig. 5. Workflow diagram for rye fermentation.

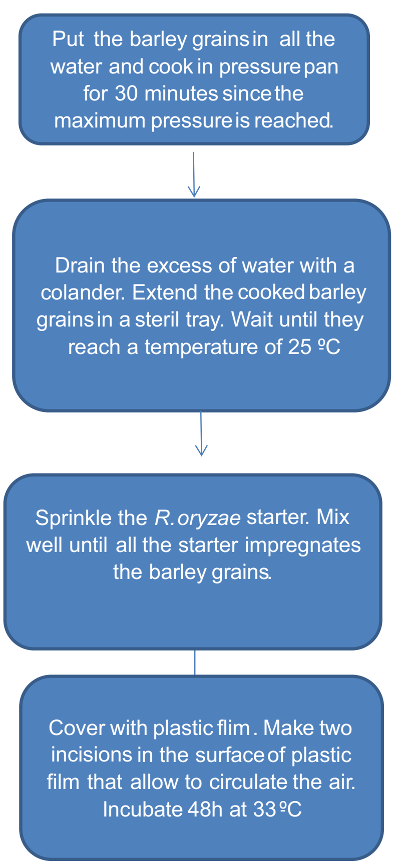

Fig. 4. Workflow diagram for barley fermentation.

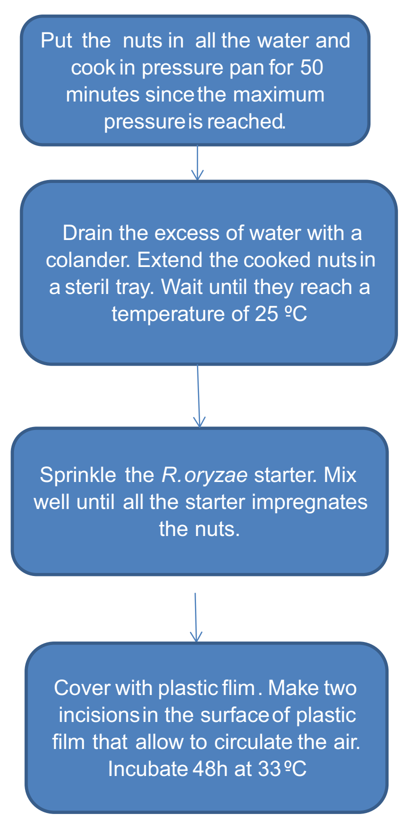

Fig. 6. Workflow diagram for nuts fermentation.

Grains

Traditionally, sake is obtained through rice fermentation with *Aspergillus oryzae* (Gauntner, 2002). The hereby research presents *R. oryzae* strains as an option to obtain alternative alcoholic products from cooked rice fermentation with particular sensorial characteristics. As occurs with Koji, *R. oryzae* grows best on partially cooked grains and free water should be avoided. This approach is not completely new, because *R. oryzae* is a part of the starters used to prepare other traditional rice wine (Hong Qu glutinous rice wine), (Lv et al., 2012).

R. oryzae was inoculated in different cooked cereals, including rice (*Oryza sativa, L*), burghul (mainly *Triticum durum, L*), barley (*Hordeum vulgare L*) and rye (*Secale cereal, L*). Ingredients are described in Table 1 (rice is prototype B, burghul is prototype C, barley is prototype D, rye is prototype E). The workflow is described in Fig. 2 for rice, Fig. 3 for burghul, Fig. 4 for barley and Fig. 5 for rye. Briefly, grains were boiled separately in a pressure pan until they were completely cooked, mixed with the fungus and then fermented for 48 h at 33 °C. After 48 h, mycelia covered the entire surface exposed to air (Fig. 6).

After the initial 24 h, all tested cereals showed acidity notes with acetic and alcoholic flavors. After another 24 h at refrigeration temperature, the flavor changed to sweet notes. Examples of visual effects with different cereals are shown in Fig. 4. Sensorial panel judged the preparation using rice as a fermentation ingredient as the most acceptable, specially comparing with other fermented cereals. Fermented rice appeared similarly to jelly, like a thick layer of rice-milk pudding, even overcooked rice, without visible mycelium. Initially, the smell was sweet and afterwards appears a sour smell, both pleasant for the sensorial panel. The texture was creamy and melted in the mouth. The flavor was intense, between acidic and sweet, and panelists described it as “rare but not disgusting”. The after-taste was less intense and more likeable than in trials with other legumes (results not shown) and grains. Several authors prevent the use of longer fermentation time due the ammonia produced by *Rhyzopus* in these substrates (Steinkraus, 1996).

Other authors have tested different tempeh-like fermentations with grains in order to obtain high-value products. Hachmeister and Fung (1993) fermented hard red winter wheat, triticale, yellow sorghum (milo), and red sorghum with *R. oligosporus*, obtaining only positive sensorial and technological results in the case of red sorghum. In our case, the sensory characteristics of rice after fermentation have completely changed. They were surprising having come from plain cooked rice.

Menu Plate: Cured lobster meat and fermented rice.

Nuts

Following the line of work done with seeds, fruits and vegetables, different tests have also been performed using nuts as fermentations substrates. (Fig. 11)

As a general consideration, the fungus required cooked nuts to increase its water activity and make fermentation possible.

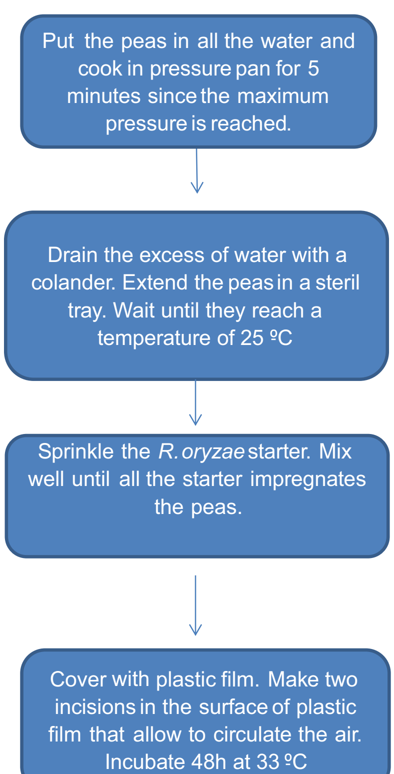

Fig. 7. Workflow diagram for peas fermentation.

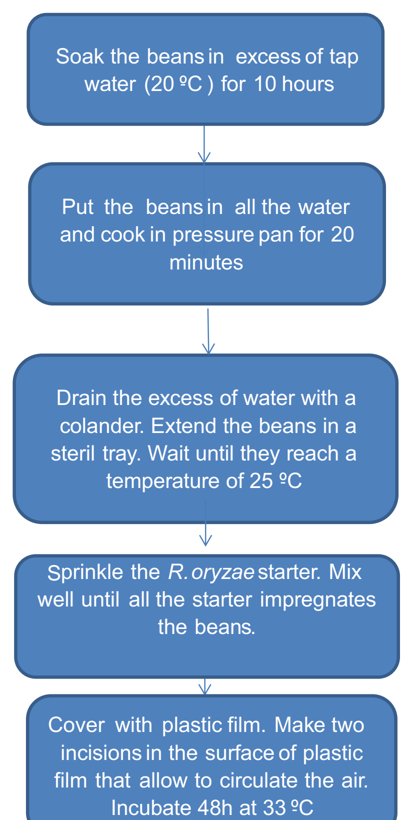

Fig. 8. Workflow diagram for beans fermentation.

For example, a preparation with hazelnuts is described in this paper. Ingredients are described in Table 1 (prototype F) and workflow is described in Fig. 5. Briefly, nuts were cooked in a pressure-cooker with water for; they were then drained, tempered, spread on a metal tray and inoculated with fungal starter. The time of incubation lasted 48 h at 33 °C. After this time, the development of mycelium was observed in between individual nuts, sticking them together in a continuous product and forming a structure similar to traditional tempeh (Fig. 12).

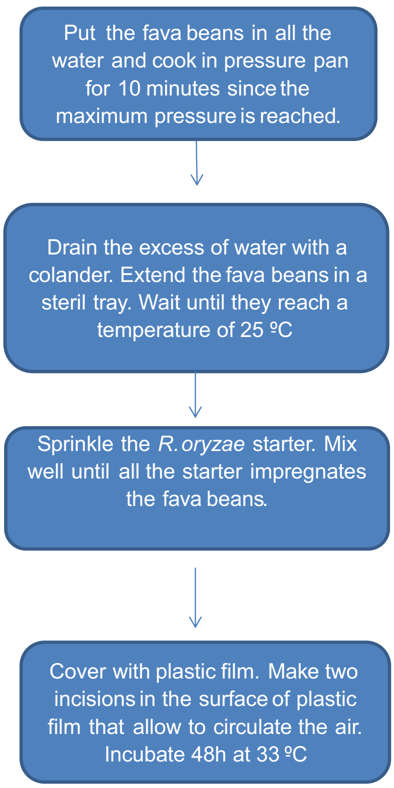

Fig. 9. Workflow diagram for fava beans fermentation.

The visual aspect of the product was similar to a nougat; with a combination of aromas of hazelnut and light mold. The flavor consisted of cooked hazelnuts, lightly sweetened with notes of honey. The texture was soft, creamy and lightly starchy.

Menu plate: Cooked nougat savory praline and peppercorn.

Legumes

Using the same concept as traditional tempeh (made from cooked soy seeds) *R. oryzae* can be inoculated in other cooked legumes. We tested cooked peas (*Pisum sativum, L*), beans (*Phaseolus vulgaris, L*) and fava beans (*Vacia faba, L*). Ingredients are described in Table 1 (peas are prototype G, fava beans are prototype H and beans are prototype I). Workflow is described in Fig. 7 for peas, in Fig. 8 for beans and in Fig. 9 for fava beans. Briefly, legumes were cooked separately in a pressure cooker with water for 5 min (peas) 10 min (fava beans) and 20 min (beans soaked) since the full pressure was reached (1 bar); they were then drained, tempered, and spread on a metal tray and inoculated with a fungal starter. As it happens in traditional tempeh making, there is an initial bacterial fermentation or acidifying step in legumes when they are soaked before cooked. The time of incubation lasted for 48 h at 33 °C. After this time, mycelium

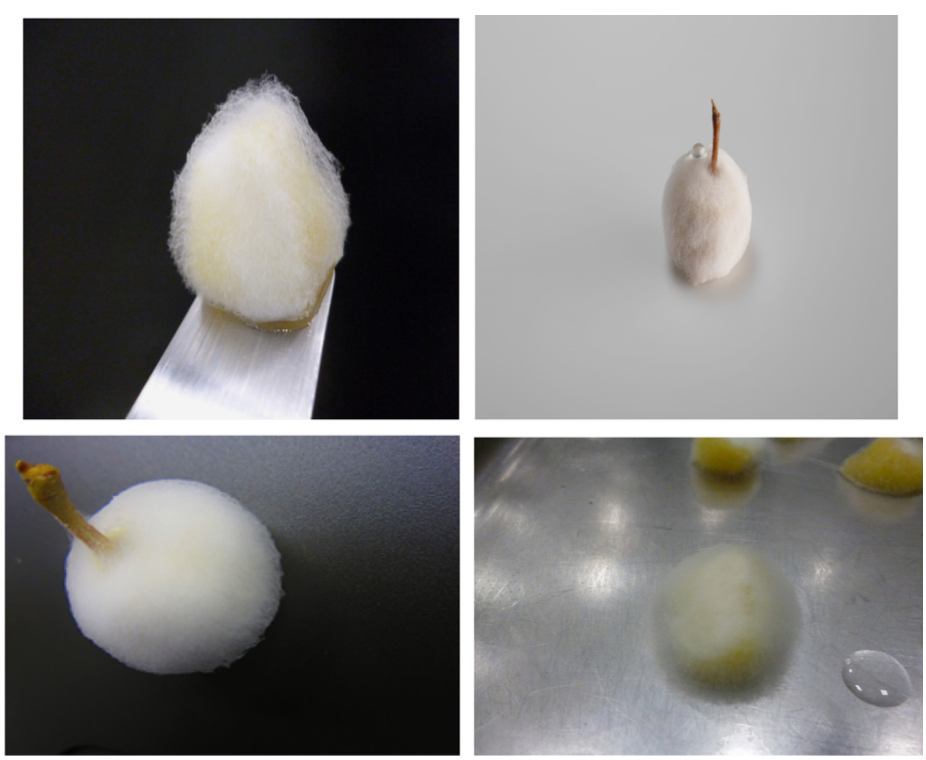
Fig. 10. Fruits colonized by *R. oryzae* (A) apple dice, (B) whole wild apples, (C) apple puree colonized with *R. oryzae*, and (D).

was observed between the legumes, sticking them together in a continuous product and forming a structure similar to traditional tempeh (Fig. 13).

In all cases, visual presence was similar to tempeh, forming a compact and stable block of legumes connected and fused by the fungal hyphae. Molds were visible in the legumes exterior. Flavor was similar to unleavened bread, fungal, with acid and sweet notes. Texture was soft, velvety and juicy. In the three cases, initial taste was acidic, bitter and slightly sweet. As fermentation continues, proteins and lipids hydrolysis promote stronger flavor, and, eventually, ammonia was released. Initial color was bright white, then grey and black because spores are produced by the mold. Similar effect on color was observed in tempeh (Handoyo and Morita, 2006).

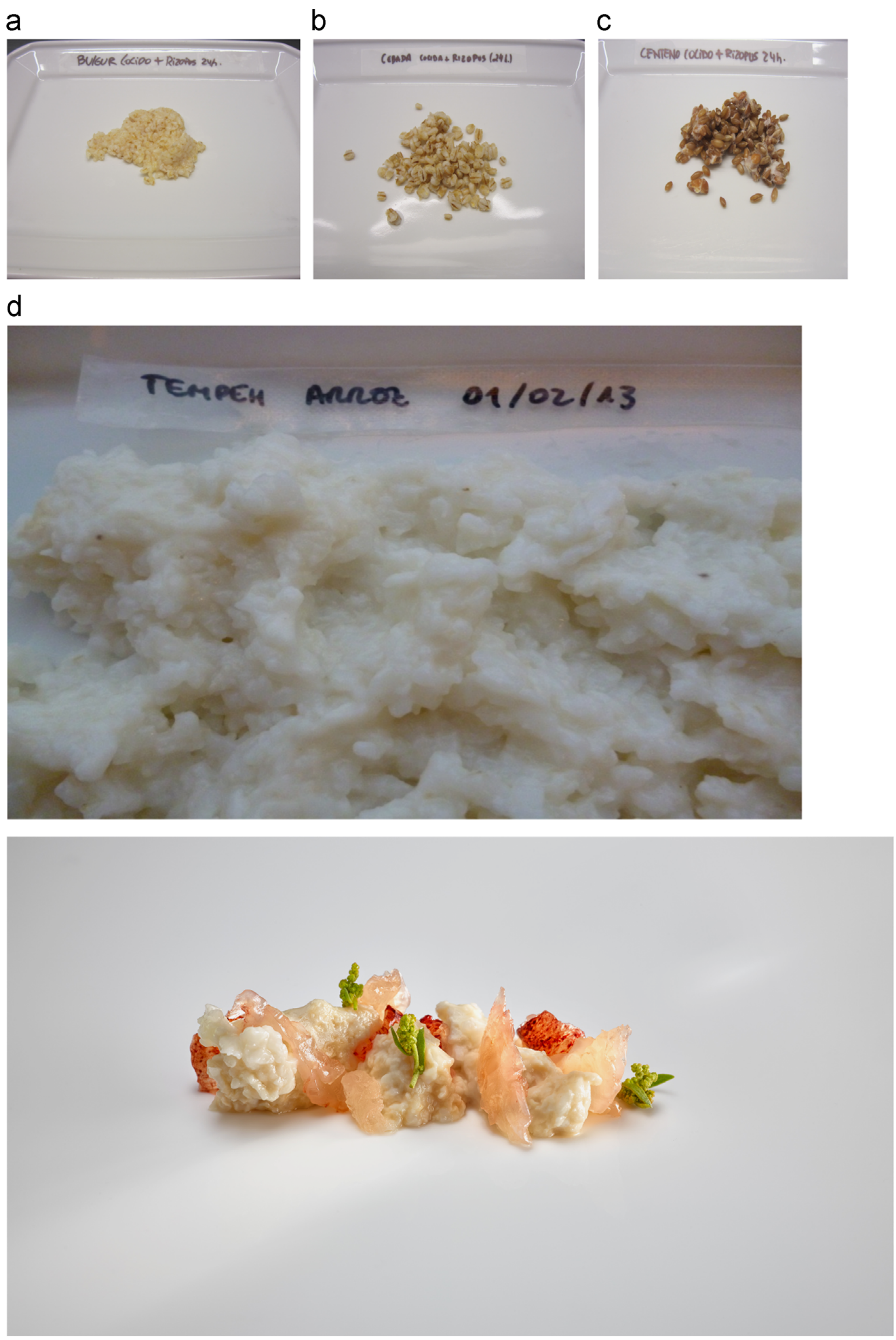

Fig. 11. Grains colonized by *R. oryzae* (A) rice colonized with *R. oryzae*, (B) burghul colonized with *R. oryzae*, (C) barley colonized with *R. oryzae*, and (D) rye colonized with *R. oryzae*.

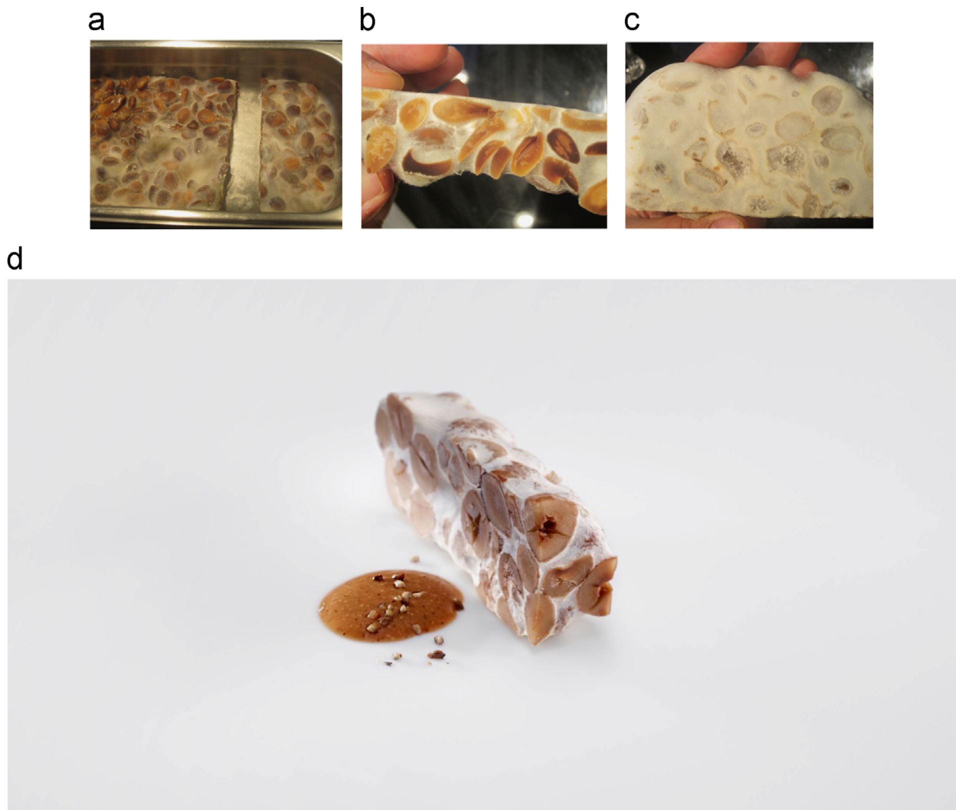

Fig. 12. (A, B and C) Helznuts colonized with *R. oryzae* and (D) Turrón.

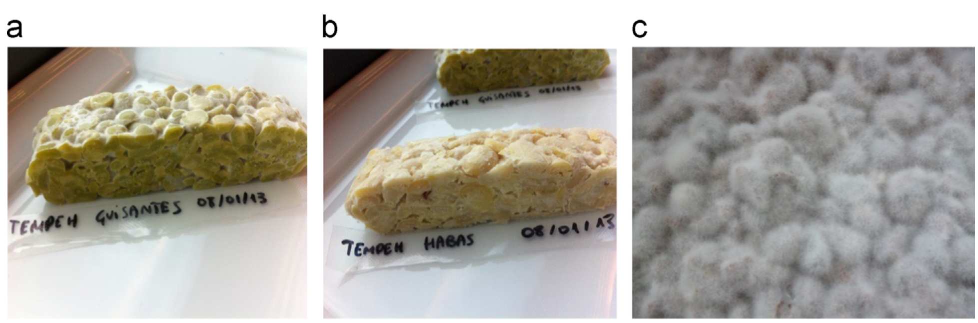

Fig. 13. Legumes colonized by *R. oryzae* (A) Peas, (B) Broad beans, and (C) beans.

Menu plate: Vegan “alubiada”: *Carpaccio* of fermented and toasted Tolosa beans.

In general, and beyond the initial prejudices, mainly because the unusual external appearance, consumers find a range of complex flavors outside of the conventional and expected. Fermented foods, already present in the menu of the restaurant, has served to be a real test with consumers by checking the degree of acceptability. Further studies would transfer them to the food industry in order to obtain new, healthy and delicious products. This transference would require strains, substrates and procedure adaptation, but would allow a completely new product repertoire.

Conclusions

Fruits, grains, nuts and legumes mold-fermentation with *R. oryzae* produces profound sensory changes in foods at different levels. New flavors including acidic, sweet and bitter appears in treated products in varying degrees, depending on the fermented food, and modify their sensory profile. The texture is also deeply modified, and, in all cases, visual aspects of fermented products are completely unusual.

Acknowledgments

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