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Yeast diversity and native vigor for flavor phenotypes

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***Saccharomyces cerevisiae*, the yeast used widely for beer, bread, cider, and wine production, is the most resourceful eukaryotic model used for genetic engineering. A typical concern about using engineered yeasts for food production might be negative consumer perception of genetically modified organisms. However, we believe the true pitfall of using genetically modified yeasts is their limited capacity to either refine or improve the sensory properties of fermented foods under real production conditions. Alternatively, yeast diversity screening to improve the aroma and flavors could offer groundbreaking opportunities in food biotechnology. We propose a 'Yeast Flavor Diversity Screening' strategy which integrates knowledge from sensory analysis and natural whole-genome evolution with information about flavor metabolic networks and their regulation.**

Food fermentation and consumer preference

Fermented foods were originally developed by our ancestors as a biological way to preserve different fresh agricultural products such as fruit juices, milk, or meat. The challenge in those times was to extend shelf-life, freshness, flavor, and edibility of food after harvest. The objective was to conserve freshness without adding preservatives such as salt or vinegar, which can dramatically affect sensory characteristics. After many centuries of accumulating practical knowledge, mastery of fermentation enabled the management of what chemists of the 19th century called 'enzyme activities.' This involved decreasing pH or generating compounds such as ethyl alcohol in fermented food to prevent microbial food spoilage. Interestingly, the discovery of the biological basis of yeast fermentation was first accomplished in industrial brewing settings [1,2]. The role of yeasts in the fermentation of sugars to alcohol and carbon dioxide has been known for almost two centuries. However, well over a half of the 19th century elapsed before the role of yeast strains in the production of different wines was published by Pasteur in 1866 [3].

Today, food fermentation is all about increasing the sensory quality for the consumer, and obtaining unique signature flavors that help to distinguish a product from others on the market [4]. Consumer flavor sensations, not other benefits such as increased shelf-life or nutritional value, may in fact be the key factor that defines a successful food product. The increasing preference for designer wine, beer, or cheese among consumers is an excellent illustration of the power of flavor. This increased interest in specialty foods will continue to grow among affluent consumers (see Glossary) as they refine, improve, and discover their tasting abilities all around the world. These discerning consumers select products based on taste, preferring to pay more for a refined sensation, rather than less for quantity [5].

Gene manipulation and flavors

The aroma profile of fermented foods and beverages comprises hundreds of compounds, many of which have

Glossary

Affluent consumers: in marketing and financial services, consumers whose wealth or income is above the average. Luxury consumers look for products of outstanding quality and high performance that are well worth the price. In food products these consumers are considered as 'enthusiastic' or more knowledgeable for enjoying flavors and premium foods.
Ecotilling: the mutation detection technology used in 'Tilling' (targeting induced local lesions in genomes) was adapted to the discovery of polymorphisms in natural populations. This technology could help to find native proteins with improved functional designs. The technology is applicable to any organism including those that are heterozygous and polyploid, as is the case for many native types of yeast.
Evolutionary engineering: continuous evolution procedures based on the application of an artificial selection pressure to obtain a desired phenotype.
Flavor complexity: multisensory experience through which the human beings perceive a complex mixture of volatile and non-volatile molecules in foods.
Globalizing quality: the term is employed in the context of assuring a basic uniform quality of fermented beverages using a pure and standard ferment inoculum. This lack of differentiation between the final products does not favor consumer attention in a huge competitive market.
Natural biodiversity: the degree of variation of life in terms of genetic variation, ecosystem variation, or species variation (number of species) within a given area.
Polyphenolic maturity: during red grape maturation in the vineyard, sugars increase concentration and the phenolic compounds, mainly present in seeds and skin, are usually considered mature once sugar maturity is reached. After fermentation, higher alcohols are obtained, and mature phenolics are softer and not astringent as they can be if the grapes are still green.
Sensory analysis: scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch, and hearing) for the purpose of evaluating consumer products.
Yeast native vigor: genetic variation present in particular native yeast strains that can outperform laboratory strains for specific traits such as speed of fermentation, temperature-tolerance, or the production of compounds that may influence the aroma of the fermented food product. These strains are expected to be better adapted to industrial conditions in non-sterile fermentation media.

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sensory thresholds corresponding to very low concentrations (μg/L). An aroma profile interacts with hundreds of olfactory receptors triggering the transduction and integration of diverse and complex signals within the human brain [6,7]. Current yeast genetic engineering (GE) approaches designed to improve fermented beverage flavor take one or very few aroma compounds as the subject of study and manipulation. Examples of these approaches are scarce [8–10] and, to the best of our knowledge, subsequent formal sensory analysis and evaluation of the final product is exceptional [10]. Besides negative consumer perception of genetically modified organisms, the current limitation to the use of these strains under real winemaking conditions is the lack of stability or cell vigor of these strains in industrial settings [11]. Other breeding strategies or generation of interspecific wine yeast hybrids have successfully improved wine flavor by reducing off-flavor production and enhancing volatile thiol release in *Saccharomyces* [12,13].

From a different perspective, genetic manipulation to improve other traits can affect flavor-compound levels indirectly as was seen in several attempts to decrease ethanol yield in wines. In many wine regions the optimal polyphenolic maturity of grapes is obtained with excess sugar content, but this results in the undesired byproduct of high ethanol-containing wines [14]. The strategy to resolve this issue was to decrease ethanol yield, thus increasing glycerol formation from sugars during wine fermentation [15–20]. Overexpression of the *GPD1* gene, which encodes glycerol-3-phosphate dehydrogenase, in combination with the deletion of *ALD6*, encoding acetaldehyde dehydrogenase, yields the desired changes in metabolite accumulation (Box 1). These two alterations resulted in increased glycerol production and reduced acetate formation [17,18]. Yeast strains were successfully engineered, yielding the desired levels of glycerol, ethanol, and acetate, but the wines presented unacceptable aroma characteristics. This undesired outcome was the result of significant changes in the aroma flavor profiles (higher alcohols, acids, esters, etc.) due to redox balance-dependent compensatory regulation of NAD^+/NADH pools in the modified strains (Box 1).

Primary metabolism is resilient to changes in flux distributions that are optimal for cell growth [21]. Genetic alterations that target primary metabolism (affecting compound concentrations by g/L) generate unpredicted redistributions of micrometabolic fluxes, profoundly affecting the concentration of compounds that are within the μg/L range [21,22]. These changes are mainly due to compensatory regulation of redox balance within the cell, which can affect many flavor pathways [23]. Recently it was demonstrated in cheese, yogurt, and wine that slight changes of a few mV in

Box 1. Carbon metabolism and intracellular redox balance significantly affect wine flavor phenotype

In a scheme (Figure I) based on experiments reported by Eglinton *et al.* and Cambon *et al.* [17,18], the aim was to construct strains that produce wine with increased glycerol and decreased ethanol contents. Manipulation of primary metabolism alters NAD^+/NADH balance, and concomitant reinstallation of redox homeostasis largely impacts on small-scale (μg/L) byproduct yields. For example, the formation of higher alcohols increases NAD^+, and biosynthesis of isoacids increases NADH. Both families of compounds could be considered as undesired aromas. Flavor metabolic pathways had a significant susceptibility to genetic modifications that affect redox and also ATP/ADP balances during fermentation.

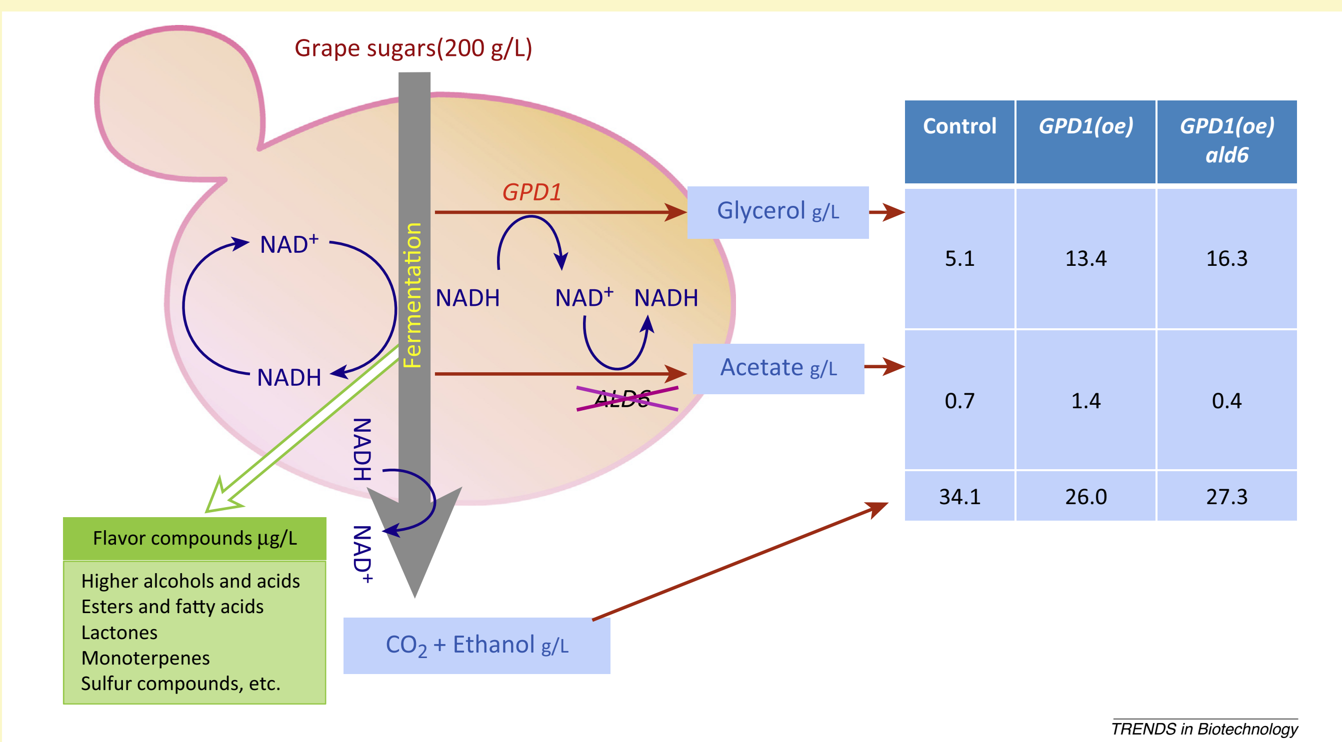

Figure I. Carbon phenotypes and wine flavor. Abbreviations: ALD6, aldehyde dehydrogenase; GPD1, glycerol-3-phosphate dehydrogenase; oe, overexpression.