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# Potential of lees from wine, beer and cider manufacturing as a source of economic nutrients: An overview

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ABSTRACT

Lees are the wastes generated during the fermentation and aging processes of different industrial activities concerning alcoholic drinks such as wine, cider and beer. They must be conveniently treated to avoid uncontrolled dumping which causes environmental problems due to their high content of phenols, pesticides, heavy metals, and considerable concentrations of nitrogen, phosphate and potassium as well as high organic content. The companies involved must seek alternative environmental and economic physicochemical and biological treatments for their revalorization consisting in the recovery or transformation of the components of the lees into high value-added compounds. After describing the composition of lees and market of wine, beer and cider industries in Spain, this work aims to review the recent applications of wine, beer and cider lees reported in literature, with special attention to the use of lees as an endless sustainable source of nutrients and the production of yeast extract by autolysis or cell disruption. Lees and/or yeast extract can be used as nutritional supplements with potential exploitation in the biotechnological industry for the production of natural compounds such as xylitol, organic acids, and biosurfactants, among others.

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## 1. Definition, composition and importance of lees

Lees are the wastes generated during the fermentation and aging processes of different industrial activities involving alcoholic drinks such as wine, cider and beer (Pérez-Bibbins et al., 2014). They are composed by solid and liquid fractions, whose composition depends on their regions of origin and their agronomic and edaphoclimatic characteristics.

The solid fraction contains all the deposits precipitated at the bottom of the tanks, which primarily consist on microbial biomass (yeasts and bacteria), insoluble carbohydrates (cellulosic or hemicellulosic materials), phenolic compounds that contribute to color and flavor, lignin, proteins, metals, inorganic salts, organic acid salts (mainly tartrates in the case of wine lees) and other materials such as pips, fruit skins, grains and seeds. The type of phenolic compounds depends on the origin of grape, apple and barley variety as well as the climate. Phenolic compounds contribute to color, flavor, astringency, bitterness, enzymatic or nonenzymatic browning, haze formation and aging behavior (Ziegler, 1990; Bustos et al., 2004a).

The liquid phase mainly consists in the exhausted fermentation broth, namely wine, cider and beer. In consequence, the liquid phase is rich in ethanol and organic acids. Lactic acid from the malolactic fermentation, where malic acid is dicarboxylated into lactic acid by bacteria metabolism (*Oenocuccus oeni*) (Moreno-Arribas and Lonvaud-Funel, 2000), and acetic acid, which is the product of the bacterial metabolism of *Gluconobacter oxydans*, *Acetobacter pasteurianus* and *Acetobacter aceti* present in wine mature stages (Joyeux et al., 1984; Bustos et al., 2004a), can be also present in significant amounts in liquid lees.

## 2. Composition of lees and market of wine, beer and cider industries in Spain

### 2.1. Wine and vinification lees

Spain is the third wine producing country in the world, occupying the 12.1% of the worldwide production in 2012 (30.4 millions

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of hl) after France (16.4%) and Italy (15.9%), with 41.4 and 40.1 millions of hl respectively. The Iberian Peninsula is an ideal place for producing different kinds of wines. The International Organisation of Vine and Wine (OIV) reported 1.018 millions of hectares for vine growing in Spain, remaining as the largest area of vineyards of the European Union (OIV-OEMV (2013), www.winesfromspain.com). Spain has 89 production areas of quality wines with 69 Designation of Protected Origin (DOP), which 67 are DOP, 2 with Designation of Qualified Origin, 6 are Heading Quality Wines with Geographical Indication and 14 are Wine of "Pago". The first authorized designations were approved in 1932 and are: Xères-Jerez-Sherry, Manzanilla de Sanlúcar de Barrameda, Málaga, Montilla-Moriles, Rioja, Tarragona, Priorato, Alella, Utiel Requena, Valencia, Alicante, Ribeiro, Cariñena, Penedés, Condado de Huelva, Valdepeñas, La Mancha, Navarra and Rueda. The most common grape varieties in Spain are Airén (23.5%), Tempranillo (20.9%), Bobal (7.5%), Garnacha Tinta, Monastrell, Pardina, Macabeo and Palomino, where Tempranillo, Bobal, Garnacha Tinta and Monastrell are red varieties and the rest are white varieties.

Vinification, the process involving all the steps for wine elaboration, is a seasonal activity that accounts approximately 3 months preferably during the autumn (Torrijos and Moletta, 2003). This activity is considered one of the most important agricultural activities in Spain, representing up to 10% of the total agricultural production (Rivas et al., 2006), and producing large volumes of wastes that can be estimated in $18 \times 10^6$ $m^3$/year, a value 6 times higher than the wine wastewater produced in France or Italy.

As a result of the winemaking process in wineries, one ton of grapes approximately generates 0.13 t marc, 0.06 t lees, 0.03 t of stalks and 1.65 $m^3$ of wastewater (Oliveira and Duarte, 2014). According to the European Council Regulation No. 79/337, wine lees are defined as "the residue that forms at the bottom of recipients containing wine, after fermentation, during storage, after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product" (Pérez-Serradilla and Luque de Castro, 2008). Meanwhile, vinasses are the liquid fraction waste from the distillation process of the wine lees, which is carried out to recover ethanol and elaborate distilled beverages. Their effluents show a high organic matter content, which is responsible for their high Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) (de Bustamante and Temiño, 1994; Beltrán et al., 1999), as well as a acidic and reducing properties (de Bustamante, 1990). These acid characteristics provoke that their disposal into soils lead to the karstification of the calcareous component of the terrain and the formation of $CH_4$ and $CO_2$ in anaerobic environments, which, if the environment is closed, can produce explosions (de Bustamante and Temiño, 1994). On the other hand, the free spill of vinasses and/or wine lees in water environments results in local fish kill and damage in the aquatic biota (Niranjan and Shilton, 1994). In spite of this, particularly some small wine-producers do not obey the laws in force, making illegal waste discharge to water body, sewage or uncontrolled deposition, for which fines can reach up to 60,000 € (Oliveira and Duarte, 2014).

The composition of wine lees depends on the winemaking technology (see Table 1) although, according to de Bustamante and Temiño, 1994 the main characteristics are a pH between 3 and 6, a COD that can be higher than 30,000 mg/L, an organic matter content between 900 and 35,000 mg/L, potassium concentrations that can be higher than 2500 mg/L, phenolic components in quantities up to 1000 mg/L, and discharge temperatures of 90 °C.

Red wine is made by fermenting the grape must in contact with skins and seeds, thus releasing high levels of soluble phenolic compounds (Bustos et al., 2004a), which can polymerize and form complexes with proteins and polysaccharides during winemaking and precipitate again. Therefore, red wine lees also contain skins, pips and polymeric phenolic compounds.

**Table 1**
Composition of cider, beer, white and red wine (for 100 g of edible portion).

| Components | Beer | Cider | White wine | Red wine |
|---|---|---|---|---|
| *General composition* | | | | |
| Alcohol (ethanol) (g) | 3.96 | 3.7 | 8.58 | 9.82 |
| Total energy | 176 KJ (42 Kcal) | 209 KJ (50 Kcal) | 252 KJ (61 Kcal) | 294 KJ (71 Kcal) |
| Total fat (total lipids) (g) | 0 | 0 | 0 | 0 |
| Total protein (g) | 0.5 | Trace | 0.1 | 0.23 |
| Water (humidity) (g) | 92.4 | 90.3 | 91.2 | 89.7 |
| *Carbohydrates* | | | | |
| Sugars (g) | 3.12 | 6 | 0.1 | 0.3 |
| Fiber (total dietary) (g) | 0 | 0 | 0 | 0 |
| *Fat* | | | | |
| Fatty acids, total monounsaturated (g) | 0 | 0 | 0 | 0 |
| Fatty acids, total polyunsaturated (g) | 0 | 0 | 0 | 0 |
| Total saturated fatty acids (g) | 0 | 0 | 0 | 0 |
| Cholesterol (mg) | 0 | 0 | 0 | 0 |
| *Vitamins* | | | | |
| Vitamin A[a] (μg) | – | Trace | – | – |
| Vitamin D (μg) | 0 | 0 | 0 | 0 |
| Vitamin E[b] (mg) | 0 | Trace | 0 | 0 |
| Niacin total equivalents (Vitamin B3) (mg) | 0.43 | 0.01 | 0.08 | 0.09 |
| Riboflavin (Vitamin B2) (mg) | 0.03 | 0.01 | 0.05 | 0.02 |
| Thiamine (Vitamin B1) (mg) | 0 | 0.01 | 0.01 | 0.01 |
| Vitamin B12 (μg) | 0.15 | 0 | 0 | 0.01 |
| Vitamin B6 (mg) | 0.06 | 0.01 | 0.02 | 0.02 |
| Vitamin C (ascorbic acid) (mg) | 0 | 0 | 0 | 0 |
| *Minerals* | | | | |
| Calcium (mg) | 8 | 8 | 9 | 7.6 |
| Total iron (mg) | 0.01 | 0.49 | 0.6 | 0.9 |
| Potassium (mg) | 37 | 72 | 82 | 93 |
| Magnesium (mg) | 9.6 | 3 | 10 | 11 |
| Sodium (mg) | 4.4 | 7 | 2 | 4 |
| Phosphorous (mg) | 55 | 3 | 15 | 14 |
| Iodide (μg) | 8 | 0 | 0 | 0 |
| Total selenium (μg) | 1.2 | Trace | 0.3 | 0.2 |
| Zinc (Cinc) (mg) | 0.01 | Trace | 0.07 | 0.05 |

BEDCA. Base de Datos Española de Composición de Alimentos [computer file]. <http://www.bedca.net>.
Ortega et al. (2004).
[a] Equivalents of retinol as retinos and carotenoids activities.
[b] Alpha tocopherol equivalents from E vitamers activities.

In white wine making technology the must is extracted from the unfermented grapes and separated from the seeds and skins before fermentation. In consequence, white lees do not contain the phenolic compounds from pips and skins. These lees are rested in tanks or barrels to precipitate the solid particles to the bottom.

Additionally, white and red wine lees composition differs depending not only on the kind of wine but also on the number of decanting steps performed. Total solids, ashes, nitrogen and carbon contents are generally higher in the first decanting step in white/red wine lees because they have more concentration of dead yeast and solids in suspension (such as other microorganisms and organic matter) than in the second one. Carbon and nitrogen contents are usually higher in white non-distilled lees (C = 10.9 g/100 g dry basis, N = 0.7 g/100 g dry basis) in comparison with the values observed after distillation (C = 6.4 g/100 g dry basis, N = 0.4 g/100 g dry basis). Conversely, the behavior observed with red lees was opposite, with the higher contents quantified after distillation (C = 11.7 g/100 g dry basis; N = 1.2 g/100 g dry basis).

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Higher concentrations of metals (Cu, Mg, Fe, Mn, Ca, Al and Zn) were found for red wine lees in comparison with white wine due to the contact of the fermenting must with the grape skins (Bustos et al., 2004b).

### 2.2. Beer lees

Beer is defined by the Spanish Food Code as a beverage resulting from the fermentation with a selected yeast of the wort made from barley malt, alone or mixed with other starches converted into sugars by enzymatic digestion, cooking and flavored with hops flowers. Beer lees are the main by-products of the brewing industry generated during the fermenting step, occupying 80% of all wastes in breweries (Shibata et al., 2009).

The term "beer lees" is sometimes used to refer to the wastes generated during beer elaboration in the different steps of milling, malting, mashing, worting, fermentation and clarification (Kao and Wu, 2013). In these cases beer lees are mainly composed of malt husk, which contains 16.8–25.4% cellulose, 21.8–28.4% hemicellulose, 11.9–27.8% lignin, 3.4–4.6% ash and 15.2–24.2% proteins (Bando et al., 2013). But strictly, "beer lees" must be considered as the solid reside accumulating at the bottom of the wort fermentation tank, which is composed mainly by dead yeasts. Beer lees can also include protein–polyphenol complexes, lipidic compounds and insoluble hop components that precipitate during worth boiling, cooling and fermentation, especially if trub (the particulate matter generated during wort boiling) is not removed (Priest and Stewart, 2006).

The Spanish beer market in 2013 recorded a production of 32.7 million of hectoliters, which places Spain as the fourth European Union country of beer volume production (Van de Walle, 2014).

### 2.3. Cider lees

Cider lees are the main by-products of cider elaboration, a fermented alcoholic beverage made from the unfiltered juice of apples. The composition of cider includes (Table 1) alcohol, fermentable sugars and variable amounts of glycerol, mannitol, organic acids such as malic, lactic, acetic, quinic, succinic, pyruvic and citric acids, and nonvolatile polyphenols responsible for bitterness and astringency of musts (0.5–3.5 g/L) (BEDCA: http://www.bedca.net; Ortega et al., 2004). It also contains acetaldehyde, ethyl acetate, acetoin and acrolein, which can produce changes on sensorial properties. Higher alcohols (propanol, isobutanol and amyl alcohols) contribute to the flavor of the cider (Le Quéré et al., 2006).

The production of cider and sparkling cider is an important economic resource in Europe and a popular drink, with consumption rates over 14 million hectolitres a year (Association of the Cider and Fruit Wine Industry of the European Union, 2010). The affiliated members represent over 180 cider and fruit wine manufacturing companies in the EU. Their largest producers are in England, France, Spain, Germany, Ireland, and Belgium (http://www.aicv.org).

## 3. Applications of lees

The activity of beer, cider and wine industries generates organic residues, mainly trimmings (from farming), lees (post-fermentation) and bagasse. They must be conveniently treated to avoid uncontrolled dumping that causes environmental problems. Therefore, it is important for the producing companies to consider alternative strategies to disposal or simple removal of lees for making the process sustainable in both, environmental and economic senses. It is necessary to seek physicochemical and biological treatments to remove or reduce contamination in the waste lees due to phenols, pesticides, heavy metals, considerable concentrations of nitrogen, phosphate and potassium as well as high organic content (Mena et al., 1997). For the depuration treatment of the liquid fraction ("vinasses"), the suspended organic material can be removed by sedimentation, filtration or flotation in order to reduce the BOD of these liquid wastes. Nevertheless, the valorization treatments consisting in the recovery or transformation of the components of the lees into high value-added compounds are more environmental and economic attractive alternatives.

### 3.1. Applications of wine lees

Wine lees can be used to recover ethanol by distillation in wineries (>55 g/L) and polyphenolic compounds used for the production of aromatic flavor liquors (Bustos et al., 2004a). Wine lees could also be used for the recovery of salts, although the evaporation process could be too expensive because of the high-energy demanded to implement the process to a high scale.

Among salts, tartrate species are especially interesting because of their abundance in grapes and, consequently, in lees and bagasse. Tartaric acid has a large range of potential applications for many food industries as a natural acid, being a popular alternative to the widely used citric and phosphoric acids in the food and beverage industries (Salgado et al., 2010a) for the elaboration of candies, bakery goods like cookies, and beverages like sodas, since the relative microbial stability of tartaric acid makes these products more stable with less need for chemical or thermal preservation (Boulton et al., 1995). Tartaric acid can be obtained from potassium hydrogen tartrate and calcium tartrate recovered in wineries and distilleries of wine lees. Thus, Nurgel and Canbas (1998) proposed the production of tartaric acid from pomace of some Anatolian grape cultivars, meanwhile Versari et al. (2001) reported the recovery of tartaric acid from three types of industrial enological wastes, namely cream of tartar, lees, and wastewater from the regeneration of anion exchange resins used in the production of grape liquid sugar. More recently, Rivas et al. (2006) have optimized an integral process for valorization of wine lees, further improved by Salgado et al. (2010a), which includes the extraction of tartaric acid with a high degree of purity and reducing the costs of evaporation.

Liu et al. (2010) used vinasses as C source for L-lactic acid production by enzymatic hydrolysis of cellulose and hemicellulose and fermentation with *Lactobacillus casei*. The key issue was to destroy the join of cellulose-lignin -hemicellulose, reduce the crystallinity of cellulose and remove lignin to improve the accessibility of enzymes to substrates. To achieve high value-added utilization, the best pretreatment was the combination of 8% alkali concentration and 700 W microwave power, reaching an utilization rate of cellulose and hemicellulose of 23.8% and 71%, respectively, and making vinasses an ideal source for producing lactic acid.

It has been also studied the use of wine lees as soil fertilizers, but their residual phenolic compounds could have an adverse environmental impact for soil microbiota (Bustamante et al., 2008). Thus, Moldes et al. (2008) submitted vinification lees to biological tests employing cress, spring barley and ryegrass seeds, and observed that although they were rich in essential nutrients for plants like P (2520 mg/Kg), K (36,738 mg/Kg) and Mg (462 mg/Kg), they have low pH (3.9) and high C/N ratio. Consequently, when vinification lees were submitted to biological tests, no germination was observed for garden cress and ryegrass seeds and almost no germination for spring barley seeds, showing the negative effect of discharging lees on crop fields. Lignin also induces inhibition of germination (Morthup et al., 1998) and of nitrogen immobilization in soil (Bustamante et al., 2008).

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Recently, wine lees have also found application for the production of biogas and high quality digestate through anaerobic co-digestion of winery waste (wine lees) and waste activated sludge under mesophilic or thermophilic conditions. Both conditions showed similar yields (0.40 $m^3$/kg COD fed) but different biological process stability (Da Ros et al., 2014).

### 3.2. Applications of beer lees

Beer lees can be employed as livestock feed. Some researchers are studying the production of activated carbon from beer lees with high specific surface area by chemical activation with KOH (Hayashi et al., 2000) or biological treatments such as methane fermentation to recycle wastes discharged from the brewing process (Mahamat et al., 1989). Additionally, one attractive application of beer lees is the production of hydrogen as an excellent alternative energy source by fermentation with anaerobic mixed bacteria optimizing the influence of environmental factors such as pH, HCl pretreatment and phosphorous concentration (Cui et al., 2009; Fan et al., 2006). More recently, Bando et al., 2013 examined 33 environmental microflorae for hydrogen-fermentation from beer lees. The maximum hydrogen yield, 29.3 mL $H_2$/g total solid, was 9-times greater than that of previous studies.

### 3.3. Applications of cider lees

Conversely, scarce information can be found about the final destination of cider lees. As it happens with beer, cider lees can be also used for animal feed. A recent work also describes the use of subcritical water mediated hydrolysis for recovering intracellular yeast contents, phenolic compounds adsorbed on the cell walls and yeast cell polymers (Bahari, 2012).

## 4. Potential of lees as economic nutrients for biotechnological processes

Another potential application for wine, beer and cider lees is based on their content in interesting compounds that could be used as nutritional supplements for industrial fermentation processes (Pérez-Bibbins et al., 2014). The residual content of lees in carbohydrates and nitrogen compounds, but especially the presence of the metabolites and components from the yeasts which include essential vitamins for other microorganisms as lactic acid bacteria, makes lees a promising supplement similar to yeast extract or corn steep liquor for formulating culture media (Salgado et al., 2009).

Fermentation technologies must be competitive with chemical synthesis to carry out the biotechnological processes at industrial scale. Yeast extract and peptone are the most common N sources used in fermentation processes. Yeast extract is especially useful due to its additional high content of purine and pyrimidine and B vitamin. But peptone and yeast extract are very expensive, accounting for almost 30–40% of the total cost of the process (Miller and Churchill, 1986). For that reason, the search for alternative economically competitive nutrient sources is particularly interesting.

Different commercial media such as cheese whey permeate, molasses, casamino acids, soybean hydrolyzate, corn steep liquor, or ram horn protein can be formulated from industrial or agricultural byproducts in order to reduce the production costs of industrial fermentation processes. Among them, corn steep liquor is the most effective and inexpensive nutrient source used in some biotechnological processes including the production of xylitol by *Debaryomyces hansenii* (Carvalheiro et al., 2007).

In this sense, lees could be attractive as a potential economic nutrient source for subsequent larger-scale bioproductions to obtain high-added natural food additives as xylitol, lactic and citric acids, or biosurfactants with applications in food, medical and biotechnological industries (Salgado et al., 2009).

### 4.1. Formulation of economic culture media for lactic acid bacteria development

Vinification lees have been used as economic nutrients for lactic acid production from synthetic glucose by *L. rhamnosus* (Bustos et al., 2004a; Salgado et al., 2009), *Lactobacillus plantarum*, *Lactobacillus pentosus*, *L. casei* and *Lactobacillus coryniformis* subsp *torquens* (Bustos et al., 2004b) achieving high fermentative parameters, as it can be seen summarized in Table 2. Among these microorganisms, and using red or white lees from different decanting steps, *L. rhamnosus* produced the highest concentration of product (103.4 g lactic acid/L) corresponding to a product yield of 0.91 g/g when 20 g/L of white wine lees from the second decanting step without being submitted to distillation, were used as a economic nutrients. These values overcame the threshold of $P = 104.3$ g/L and $Q_P = 2.251$ g/L h, obtained with the costly Man–Rogosa–Sharpe medium (MRS) (which contains per L 10 g peptone, 8 g beef extract, 4 g yeast extract, 20 g D-glucose, 2 g $K_2HPO_4$, 2 g di-ammonium hydrogen citrate, 5 g $CH_3COONa$, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.05 g $MnSO_4{\cdot}2H_2O$ and 1 g Tween-80) proposed by Mercier et al. (1992).

Rivas et al. (2006) improved the profitability of the lees valorization process recovering tartaric acid from distilled vinification lees (coming from the white and red winemaking technology) in a first step, and further use of the residual lees as economic nutrients for lactic acid production by *L. pentosus* using hemicellulosic vine shoot hydrolyzates as carbon source. In this case, distilled lees after tartaric acid extraction used as nutrients provided values of lactic acid concentrations (18.4–18.9 g/L), global volumetric productivities (0.82–0.84 g/L·h), and product yields (0.69–0.70 g/g) similar to those achieved when using the general medium for *Lactobacilli* (18.6 g/L, 1.11 g/L h, and 0.62 g/g, respectively) or lees without tartaric acid extraction (16.4–17.2 g/L, 0.96–1.21 g/L h, and 0.61–0.66 g/g, respectively).

In a same way, Rodríguez et al. (2010) showed not only the potential of *Lactococcus lactis* as a lactic acid and biosurfactant producer but also that the economical cost of *L. lactis* cultures could be effectively reduced by replacing the MRS by the use of two waste materials: trimming vine shoots as C source, and 20 g/L distilled wine lees (vinasses) as N, P and micronutrient sources (see Table 2). Using the solid and liquid fractions obtained after tartaric acid recovery from a total concentration of 20 g/L vinasses, 19.6 g lactic acid/L were obtained corresponding to a global volumetric productivity ($Q_P$) of 0.633 g/L h and a product yield ($Y_{P/S}$) of 0.88 g/g.

Lees were also evaluated in continuous fermentation of hemicellulosic hydrolyzates using *L. pentosus* (Bustos et al., 2007) for lactic acid as well as biosurfactants production. Using 10 g/L of corn steep liquor and 10 g/L of yeast extract as nutrients, 22.1 g lactic acid/L were attained ($Q_P = 1.276$ g/L h and $Y_{P/S} = 0.73$ g/g) for a dilution rate of 0.02 $h^{-1}$. When nutrients were replaced by 20 g/L of distilled lees from white wine production, similar results were obtained ($P = 21.8$ g/L, $Q_P = 1.258$ g/L h and $Y_{P/S} = 0.70$ g/g). Furthermore, it was observed that lees did not interfere in the lactic acid recovery from the fermentation medium, and that extracted biosurfactants reduced the superficial tension in 23.5 mN/m, similar to the 25.5 mN/m observed in the culture broth formulated with corn steep liquid plus yeast extract.

Bustos et al. (2005) assayed *L. rhamnosus* for simultaneous saccharification of the cellulosic solid residue obtained from prehydrolysis of trimming wastes of vineshoots, and fermentation

**Table 2**
Fermentative parameters reported in bibliography in culture broths formulated with lees as economic nutrient.

| Nutrients | Microorg | *P* | $P_{max}$ (g/L) | $Q_P$ (g/L h) | $Y_{P/S}$ (g/g) | References |
|---|---|---|---|---|---|---|
| Control (MRS broth) | *L. casei* | LA | 104.8 | | 0.97 | Bustos et al. (2004a) |
| Control (MRS broth) | *L. coryniformis* | LA | 74.6 | | 0.72 | Bustos et al. (2004a) |
| Control (MRS broth) | *L. pentosus* | LA | 84.7 | | 0.92 | Bustos et al. (2004a) |
| Control (MRS broth) | *L. plantarum* | LA | 84.7 | | 0.88 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the white wine making technology | *L. casei* | LA | 65.8 | | 0.82 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the white wine making technology | *L. casei* | LA | 92.1 | | 1.05 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the white wine making technology | *L. coryniformis* | LA | 25.6 | | 0.69 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the white wine making technology | *L. coryniformis* | LA | 29.3 | | 0.74 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the white wine making technology | *L. pentosus* | LA | 50.1 | | 1.02 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the white wine making technology | *L. pentosus* | LA | 59.2 | | 0.89 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the white wine making technology | *L. plantarum* | LA | 49.3 | | 0.86 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the white wine making technology | *L. plantarum* | LA | 67.4 | | 0.89 | Bustos et al. (2004a) |
| 20 g/L white lees extracted with organic solvents | *L. pentosus* | LA | 84.1 | | 0.87 | Bustos et al. (2004a) |
| 20 g/L red lees extracted with organic solvents | *L. pentosus* | LA | 73.3 | | 0.88 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the red wine making technology | *L. casei* | LA | 66.2 | | 1.13 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the red wine making technology | *L. casei* | LA | 72.7 | | 0.92 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the red wine making technology | *L. coryniformis* | LA | 8.9 | | 0.69 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the red wine making technology | *L. coryniformis* | LA | 11.6 | | 0.58 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the red wine making technology | *L. pentosus* | LA | 30.6 | | 0.98 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the red wine making technology | *L. pentosus* | LA | 64.2 | | 0.85 | Bustos et al. (2004a) |
| 20 g/L no distilled lees from the red wine making technology | *L. plantarum* | LA | 30.2 | | 0.62 | Bustos et al. (2004a) |
| 20 g/L distilled lees from the red wine making technology | *L. plantarum* | LA | 64.8 | | 0.87 | Bustos et al. (2004a) |
| Control (MRS broth) | *L. rhamnosus* | LA | 103.0 | | 0.97 | Bustos et al. (2004b) |
| Control (10 g/LCSL) | *L. rhamnosus* | LA | 58.0 | | 0.78 | Bustos et al. (2004b) |
| 10 g/L of wine lees (from pressed bagasse without distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 92.8 | | 0.89 | Bustos et al. (2004b) |
| 10 g/L of white wine lees (1st decanting step, no distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 92.0 | | 0.92 | Bustos et al. (2004b) |
| 10 g/L of white wine lees (2nd decanting step, no distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 93.3 | | 0.89 | Bustos et al. (2004b) |
| 10 g/L of white wine lees (after distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 94.7 | | 0.92 | Bustos et al. (2004b) |
| 10 g/L of red wine lees (1st decanting step, no distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 93.1 | | 0.88 | Bustos et al. (2004b) |
| 10 g/L of red wine lees (2nd decanting step, no distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 96.6 | | 0.92 | Bustos et al. (2004b) |
| 10 g/L of red wine lees (after distillation) and 10 g/L CSL | *L. rhamnosus* | LA | 103.2 | | 0.97 | Bustos et al. (2004b) |
| 20 g/L of wine lees (from pressed bagasse without distillation) | *L. rhamnosus* | LA | 97.4 | | 0.86 | Bustos et al. (2004b) |
| 20 g/L of white wine lees (1st decanting step, no distillation) | *L. rhamnosus* | LA | 95.2 | | 0.88 | Bustos et al. (2004b) |
| 20 g/L of white wine lees (2nd decanting step, no distillation) | *L. rhamnosus* | LA | 103.4 | | 0.91 | Bustos et al. (2004b) |
| 20 g/L of white wine lees (after distillation) | *L. rhamnosus* | LA | 96.8 | | 0.93 | Bustos et al. (2004b) |
| 20 g/L of red wine lees (1st decanting step, no distillation) | *L. rhamnosus* | LA | 83.3 | | 0.82 | Bustos et al. (2004b) |
| 20 g/L of red wine lees (2nd decanting step, no distillation) | *L. rhamnosus* | LA | 90.2 | | 0.81 | Bustos et al. (2004b) |
| 20 g/L of red wine lees (after distillation) | *L. rhamnosus* | LA | 75.2 | | 0.82 | Bustos et al. (2004b) |
| 20 g/L vinification lees (simultaneous saccharification and fermentation) | *L. rhamnosus* | LA | 21.8 | 0.36 | | Bustos et al. (2005) |
| Control (MRS broth) | *L. pentosus* | LA | 18.6 | 1.11 | 1.11 | Rivas et al. (2006) |
| WDL after TA extraction | *L. pentosus* | LA | 18.4 | 0.84 | 0.84 | Rivas et al. (2006) |
| RDL after TA extraction | *L. pentosus* | LA | 18.9 | 0.82 | 0.82 | Rivas et al. (2006) |
| WDL before TA extraction | *L. pentosus* | LA | 17.2 | 1.21 | 1.21 | Rivas et al. (2006) |
| RDL before TA extraction | *L. pentosus* | LA | 16.4 | 0.96 | 0.96 | Rivas et al. (2006) |
| 20 g/L vinification lees (continuous fermentation) | *L. pentosus* | LA | 21.8 | 1.258 | 0.70 | Bustos et al. (2007) |
| 20 g/L of distilled vinification lees | *L. pentosus* | LA | | 0.89 | 0.58 | Moldes et al. (2007) |
| 30 g/L corn steep liquor | *L. rhamnosus* | LA | 27.6 | 0.726 | 0.90 | Salgado et al. (2009) |
| 30 g/L corn steep liquor | *D. hansenii* | Xylitol | 25.9 | 0.326 | 0.50 | Salgado et al. (2009) |
| 30 g/L corn steep liquor | *A. niger* | CA | 13.4 | 0.141 | 0.61 | Salgado et al. (2009) |
| 20 g/L vinasses after TA extraction + stream B (15.3 mL) | *L. rhamnosus* | LA | 28.8 | 0.800 | 0.95 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction + stream B (29.0 mL) | *L. rhamnosus* | LA | 22.3 | 0.925 | 0.80 | Salgado et al. (2009) |
| 40 g/L vinasses after TA extraction + stream B (38.8 mL) | *L. rhamnosus* | LA | 22.6 | 0.924 | 0.75 | Salgado et al. (2009) |
| 20 g/L vinasses after TA extraction | *L. rhamnosus* | LA | 22.2 | 0.594 | 0.90 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction | *L. rhamnosus* | LA | 22.2 | 0.681 | 0.81 | Salgado et al. (2009) |
| 40 g/L vinasses after TA extraction | *L. rhamnosus* | LA | 21.5 | 0.910 | 0.77 | Salgado et al. (2009) |
| 20 g/L vinasses before TA extraction | *L. rhamnosus* | LA | 26.9 | 0.528 | 0.84 | Salgado et al. (2009) |
| 30 g/L vinasses before TA extraction | *L. rhamnosus* | LA | 21.1 | 0.575 | 0.74 | Salgado et al. (2009) |
| 40 g/L vinasses before TA extraction | *L. rhamnosus* | LA | 20.7 | 0.619 | 0.73 | Salgado et al. (2009) |
| 20 g/L vinasses after TA extraction + stream B (15.3 mL) | *D. hansenii* | Xylitol | 5.5 | 0.037 | 0.20 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction + stream B (29.0 mL) | *D. hansenii* | Xylitol | 22.7 | 0.149 | 0.46 | Salgado et al. (2009) |
| 40 g/L vinasses after TA extraction + stream B (38.8 mL) | *D. hansenii* | Xylitol | 22.6 | 0.149 | 0.40 | Salgado et al. (2009) |
| 20 g/L vinasses after TA extraction | *D. hansenii* | Xylitol | 3.8 | 0.122 | 0.13 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction | *D. hansenii* | Xylitol | 13.0 | 0.110 | 0.32 | Salgado et al. (2009) |
| 40 g/L vinasses after TA extraction | *D. hansenii* | Xylitol | 11.4 | 0.096 | 0.26 | Salgado et al. (2009) |
| 20 g/L vinasses before TA extraction | *D. hansenii* | Xylitol | 17.0 | 0.116 | 0.44 | Salgado et al. (2009) |
| 30 g/L vinasses before TA extraction | *D. hansenii* | Xylitol | 35.3 | 0.380 | 0.69 | Salgado et al. (2009) |
| 40 g/L vinasses before TA extraction | *D. hansenii* | Xylitol | 32.9 | 0.354 | 0.61 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction + Stream B (29.0 mL) | *A. niger* | CA | 13.3 | 0.106 | 0.48 | Salgado et al. (2009) |
| 30 g/L vinasses after TA extraction | *A. niger* | CA | 8.3 | 0.088 | 0.32 | Salgado et al. (2009) |
| 30 g/L vinasses before TA extraction | *A. niger* | CA | 13.9 | 0.146 | 0.63 | Salgado et al. (2009) |
| MRS broth | *L. rhamnosus* | LA | 27.2 | 0.760 | 0.87 | Salgado et al. (2009) |

(*continued on next page*)

**Table 2** (*continued*)

| Nutrients | Microorg | P | $P_{max}$ (g/L) | $Q_P$ (g/L h) | $Y_{P/S}$ (g/g) | References |
|---|---|---|---|---|---|---|
| Control (3 g/L yeast extract + 3 g/L malt extract + 5 g/L Peptone) | *D. hansenii* | Xylitol | 26.8 | 0.258 | 0.52 | Salgado et al. (2009) |
| $NH_4NO_3$ (25 g/L), $MgSO_4{\cdot}7H_2O$ (2.5 g/L), $CuSO_4$ 0.04 (g/L) | *A. niger* | CA | 14.1 | 0.181 | 0.45 | Salgado et al. (2009) |
| 20 g/L distilled wine lees (vinasses) without TA recovery | *L. lactis* | LA | 8.9 | 0.275 | 0.93 | Rodríguez et al. (2010) |
| 20 g/L vinasses using the solid obtained after TA recovery | *L. lactis* | LA | 9.2 | 0.312 | 0.91 | Rodríguez et al. (2010) |
| 20 g/L vinasses using the solid and liquid fractions obtained after TA recovery | *L. lactis* | LA | 19.6 | 0.633 | 0.88 | Rodríguez et al. (2010) |
| Control (3 g/L yeast extract + 3 g/L malt extract + 5 g/L Peptone) | *D. hansenii* | Xylitol | | 0.193 | 0.55 | Salgado et al. (2010a) |
| Vinasses after TA recovery | *D. hansenii* | Xylitol | | 0.232 | 0.57 | Salgado et al. (2010a) |
| 30 g vinasses/L from CBO 1 (solid + liquid fractions) | *D. hansenii* | Xylitol | 13.7 | | 0.28 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 2 (solid + liquid fractions) | *D. hansenii* | Xylitol | 21.9 | | 0.43 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 3 (solid + liquid fractions) | *D. hansenii* | Xylitol | 24.6 | | 0.45 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 4 (solid + liquid fractions) | *D. hansenii* | Xylitol | 40.4 | | 0.58 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 5 (solid + liquid fractions) | *D. hansenii* | Xylitol | 24.9 | | 0.46 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 1 (liquid fraction) | *D. hansenii* | Xylitol | 34.2 | | 0.53 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 2 (liquid fraction) | *D. hansenii* | Xylitol | 30.7 | | 0.62 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 3 (liquid fraction) | *D. hansenii* | Xylitol | 29.8 | | 0.48 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 4 (liquid fraction) | *D. hansenii* | Xylitol | 37.1 | | 0.62 | Salgado et al. (2010b) |
| 30 g vinasses/L from CBO 5 (liquid fraction) | *D. hansenii* | Xylitol | 32.0 | | 0.52 | Salgado et al. (2010b) |
| 30 g/L vinasses after TA extraction (sequential fermentation) | *L. pentosus* | LA | 21.4 | 0.703 | 0.69 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 7.9 | 0.0258 | 0.23 | |
| 30 g/L vinasses after TA extraction + stream B (sequential fermentation) | *L. pentosus* | LA | 24.2 | 0.0853 | 0.28 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 14.3 | 0.0415 | 0.27 | |
| 30 g/L vinasses without TA extraction (sequential fermentation) | *L. pentosus* | LA | 24.2 | 0.0620 | 0.31 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 17.1 | 0.118 | 0.44 | |
| 40 g/L vinasses after TA extraction (sequential fermentation) | *L. pentosus* | LA | 23.4 | 0.0678 | 0.28 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 12.6 | 0.0872 | 0.36 | |
| 40 g/L vinasses after TA extraction + stream B (sequential fermentation) | *L. pentosus* | LA | 22.6 | 0.924 | 0.65 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 18.1 | 0.0800 | 0.38 | |
| 40 g/L vinasses without TA extraction (sequential fermentation) | *L. pentosus* | LA | 23.0 | 0.126 | 0.41 | Salgado et al. (2011) |
| | *D. hansenii* | Xylitol | 8.6 | 0.0516 | 0.38 | |
| Control (3 g/L yeast extract, 3 g/L malt extract and 5 g/L peptone) | *D. hansenii* | Xylitol | 12.6 | 0.26 | 0.50 | Pérez-Bibbins et al. (2013) |
| Vinasses 25% | *D. hansenii* | Xylitol | 12.3 | 0.26 | 0.51 | Pérez-Bibbins et al. (2013) |
| Vinasses 50% | *D. hansenii* | Xylitol | 13.7 | 0.29 | 0.56 | Pérez-Bibbins et al. (2013) |
| Vinasses 75% | *D. hansenii* | Xylitol | 12.2 | 0.25 | 0.53 | Pérez-Bibbins et al. (2013) |
| Control (3 g/L yeast extract, 3 g/L malt extract and 5 g/L peptone) | *D. hansenii* | Xylitol | 39.9 | 0.478 | 0.78 | Pérez-Bibbins et al. (2014) |
| Beer lees (liquid fraction) | *D. hansenii* | Xylitol | 25.4 | 0.239 | 0.60 | Pérez-Bibbins et al. (2014) |
| Cider lees (liquid fraction) | *D. hansenii* | Xylitol | 0.9 | 0.011 | 0.43 | Pérez-Bibbins et al. (2014) |
| Wine lees (liquid fraction) | *D. hansenii* | Xylitol | 31.9 | 0.301 | 0.63 | Pérez-Bibbins et al. (2014) |
| Control (3 g/L yeast extract, 3 g/L malt extract and 5 g/L peptone) | *D. hansenii* | Xylitol | 42.7 | 0.445 | 0.80 | Pérez-Bibbins et al. (2014) |
| Beer (100%) (*whole* lees) | *D. hansenii* | Xylitol | 0.5 | 0.003 | 0.13 | Pérez-Bibbins et al. (2014) |
| Beer (75%) (*whole* lees) | *D. hansenii* | Xylitol | 0.0 | 0.000 | 0.00 | Pérez-Bibbins et al. (2014) |
| Beer (50%) (*whole* lees) | *D. hansenii* | Xylitol | 24.2 | 0.144 | 0.94 | Pérez-Bibbins et al. (2014) |
| Beer (25%) (*whole* lees) | *D. hansenii* | Xylitol | 27.2 | 0.162 | 0.60 | Pérez-Bibbins et al. (2014) |
| Cider (100%) (*whole* lees) | *D. hansenii* | Xylitol | 2.5 | 0.015 | 0.58 | Pérez-Bibbins et al. (2014) |
| Cider (75%) (*whole* lees) | *D. hansenii* | Xylitol | 13.2 | 0.079 | 0.32 | Pérez-Bibbins et al. (2014) |
| Cider (50%) (*whole* lees) | *D. hansenii* | Xylitol | 20.7 | 0.173 | 0.44 | Pérez-Bibbins et al. (2014) |
| Cider (25%) (*whole* lees) | *D. hansenii* | Xylitol | 25.7 | 0.214 | 0.55 | Pérez-Bibbins et al. (2014) |
| Wine (100%) (*whole* lees) | *D. hansenii* | Xylitol | 0.4 | 0.002 | 0.40 | Pérez-Bibbins et al. (2014) |
| Wine (75%) (*whole* lees) | *D. hansenii* | Xylitol | 0.0 | 0.000 | 0.00 | Pérez-Bibbins et al. (2014) |
| Wine (50%) (*whole* lees) | *D. hansenii* | Xylitol | 22.5 | 0.134 | 0.70 | Pérez-Bibbins et al. (2014) |
| Wine (25%) (*whole* lees) | *D. hansenii* | Xylitol | 17.8 | 0.106 | 0.48 | Pérez-Bibbins et al. (2014) |

LA = lactic acid concentration; $P_{max}$ = maximum concentration of product (g/L); $Q_P$ = global volumetric productivity (g/L h); $Y_{P/S}$ = product yield (g/g); TA = tartaric acid; CSL = corn steep liquor; CBO = certified brands of origin (CBO1: Valdeorras, CBO2: Ribeiro, CBO3: Rías Baixas, CBO4: Ribeira Sacra, and CBO5: Monterrei).

using 20 g/L of residual vinification lees coming from the white wine making industry. In this case they observed a partial delay in glucose and cellobiose production in comparison with the use of the expensive and complex MRS broth, probably due to the presence of phenolics and other organic compounds in lees that did not affect significantly the fermentation stage but produced inhibitory effects on the enzymatic hydrolysis, thus decreasing the lactic acid productivity from 0.69 to 0.36 g/L h, although the final lactic acid concentrations were similar in both cases, 23.8 g/L using the MRS broth and 21.8 g/L using lees. Using detoxified hydrolyzates from auto-posthydrolysis of trimming vine shoots as C source for *L. pentosus*, Moldes et al. (2007) obtained lower fermentative parameters adding vinification lees as nutrients ($Q_P$ = 0.89 g/L h; $Y_{P/S}$ = 0.58 g/g) in comparison to those obtained with Mercier medium ($Q_P$ = 1.14 g/L h; $Y_{P/S}$ = 0.70 g/g), which they attributed to a negative synergic effect on lactic acid fermentation of some compounds in the lees or to an insufficient amount of nitrogen compounds in this case.

### 4.2. Formulation of economic culture media to produce xylitol

Vinification lees have been assayed for the formulation of economic culture media for xylitol production under different conditions. For instance, Salgado et al. (2010a) recovered the tartaric acid and calcium tartrate from vinasses, and the residual streams were successfully employed as economic nutrients for the xylose to xylitol bioconversion by *D. hansenii*, achieving higher global volumetric productivities ($Q_P$ = 0.232 g/L h) and product yields ($Y_{P/S}$ = 0.57 g/g) than fermentations carried out using commercial nutrients ($Q_P$ = 0.193 g/L h and $Y_{P/S}$ = 0.55 g/g respectively).

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On the other hand, Pérez-Bibbins et al. (2013) assayed the use of corn cob as carbon source and vinasses diluted in different proportions as economic nutrients, finding that in the series supplemented with twofold diluted vinasses, xylitol concentration increased up to 13.7 g/L ($Q_P$ = 0.29 g/L h; $Y_{P/S}$ = 0.56 g/g) regarding the series supplemented with synthetic medium ($P$ = 12.6 g/L; $Y_{P/S}$ = 0.50 g/g; $Q_P$ = 0.26 g/L h), which indicates that wine vinasses provide the necessary nutrients for *D. hansenii* to compensate the deficit in corncob hydrolyzates without showing remarkable inhibitory effects.

The best results obtained with this yeast can be explained considering that *Lactobacilli* species comprise fastidious-growing bacteria with numerous requirements for growth (Brusch Brinques et al., 2010). Therefore, *Lactobacilli* need rich media containing expensive compounds such as amino acids, peptides, vitamins, and nucleic acids (Axelsson, 2004), meanwhile *D. hansenii* shows less nutritional requirements.

Salgado et al. (2009) showed a strong dependence of *D. hansenii* with the initial amount of $Mg^{2+}$ and calculated an economic efficiency parameter that identified vinasses as a lower cost and more effective nutrient source in comparison to corn steep liquor (see Table 2). On the other hand, evaluating vinasses from five certified brands of origin, Salgado et al. (2010b) did not observe significant differences among them. These authors assayed the use of the whole lees (solid and liquid fractions) and the liquid lees, obtaining the best results using only the liquid part of the vinasses as source of nutritional factors (30.7–37.1 g xylitol/L), probably because of an excess amount of nutrients when using both solid and liquid fractions.

Furthermore, Salgado et al. (2011) assayed vinasses to transform sequentially concentrated synthetic media containing glucose and xylose as carbon sources, into lactic acid and xylitol by *L. rhamnosus* and *D. hansenii*, respectively. Thus, using 30 g/L of vinasses without tartaric acid extraction, 24.2 g lactic acid/L and 17.1 g xylitol/L were efficiently produced (see Table 2), showing that these nutrients can represent an interesting alternative to minimize the final cost of the products achieved.

Finally, residual lees from the beer and cider industries were also evaluated, along with wine lees, as a source of nitrogen and micronutrients for xylitol production by *D. hansenii* by Pérez-Bibbins et al. (2014). The liquid fraction of wine or beer lees supplemented with 60 g/L xylose can be employed as suitable culture media for xylitol production with acceptable xylitol productivities and yields (see Table 2), although the liquid fraction of cider lees did not allow the development of the yeast, probably due to a negative synergism between phenolic compounds and metals in this case. The lyophilization of wine lees did not improve the results. On the other hand, the use of complete lees (solid and liquid fractions) resulted inhibitory for *D. hansenii*, but they allowed acceptable xylitol productions when they were progressively diluted, even in the case of cider lees. The best results ($P$ = 27.2 g/L; $Q_P$ = 0.162 g/L h; $Y_{P/S}$ = 0.60 g/g) were achieved when fermentation media were prepared using beer lees 4-fold diluted. Finally, the use of the solid fraction of wine, cider and beer lees did not allow the development of *D. hansenii* cultures, being necessary to search for alternative methods, as explained in Section 5.

### 4.3. Formulation of economic culture media for Aspergilli species

Other applications where lees or vinasses have been assayed included the use of *Aspergilli* species. For instance, wastes from olive oil and wine industries (including exhausted grape marc, vineshoot trimmings, two-phase olive mill waste, vinasses, and olive mill wastewater) were evaluated for lignocellulolytic enzyme production (as endocellulases, endoxylanases, and feruloyl esterases) by solid-state fermentation (SSF) with *Aspergillus niger*, *Aspergillus ibericus*, and *Aspergillus uvarum* in order to reduce the production costs of lipases (Salgado et al., 2014a, 2014b). Alternatively, wine vinasses, before or after tartaric acid extraction, where assayed by Salgado et al. (2009) for the formulation of economic culture media during the citric acid production by *A. niger* (see Table 2). Using 30 g/L vinasses before tartaric acid extraction, the fermentative parameters ($P$ = 13.9 g/L; $Q_P$ = 0.146 g/L h; $Y_{P/S}$ = 0.63 g/g) slightly overcame those results achieved with synthetic medium ($P$ = 13.4 g/L; $Q_P$ = 0.141 g/L h; $Y_{P/S}$ = 0.61 g/g). The results indicated that the amino acid profile, protein concentration and metals in vinasses played an important role during the bioconversion.

## 5. Preparation of yeast extract from lees or spent cells

The preparation of yeast extract from lees is a promising alternative for the valorization of this residue, when lees cannot be directly applied as nutrient, as it happened with the use of the solid fraction of wine, cider and beer lees. Yeast extract consists primarily of amino acids, peptides, nucleotides and other soluble components of yeast cells. Up to now, the major raw material used in Europe for yeast extract is high protein yeast of *Saccharomyces cerevisiae* grown on molasses-based media. Meanwhile in other countries, such as the United States, yeast extract is manufactured from debittered brewers yeasts of *S. cerevisiae* or *Saccharomyces uvarum*. Alternative, other raw sources includes the use of *Kluyveromyces fragilis* fermented on whey or *Candida utilis* grown on high carbohydrate waste-products.

Yeast extract is manufactured by the breaking down of cells. Manufacturing processes are mainly divided into autolysis and cellular disruption.

### 5.1. Autolysis

Autolysis is the hydrolysis of cellular components by hydrolytic yeast enzymes that breakdown cell membranes liberating intracellular constituents. Autolysis consists on the degradation of the cell structures and the hydrolysis of the intracellular polymeric compounds by the action of endogenous enzymes. Autolysis occurs naturally in yeasts when they complete the cell growth cycle and enter the death phase. Autolysis process has some disadvantages such as low extraction yield, difficulty in solid-liquid separation, poor taste characteristics as a flavor enhancer and risk of deterioration due to microbial contamination. In a modified autolysis process referred to as plasmolysis inorganic salts such as sodium chloride or non-polar organic solvents are often used to accelerate autolysis (Kollar et al., 1991; Nagodawithana, 1992; Belousova et al., 1995). Despite its simplicity, the yeast extract manufactured by plasmolysis may have limited use since there is a growing demand for processed foods containing low salt content (Nagodawithana, 1992).

Yeast extracts are mainly used in the fermentation industry as substrates and in the food industry as flavor improvers in soup, sauces, gravies, stews, snack food and canned food. Other applications include vitamin supplements in health foods (York and Ingram, 1996).

Although a variety of nitrogen sources have been tested for lactic acid production, not one gave product concentrations as high as those obtained with yeast extract (Nancib et al., 2001). However, the addition of commercial yeast extract powder for large-scale fermentation is probably unrealistic due to the extra cost (Selmer-Olsen and Sørhaug, 1998). Consequently, an alternative option could be the use of extracts from autolyzed biomass such as brewery yeast. Some examples of this utilization can be found in literature. For instance, autolyzed spent yeast cells have been

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**Table 3**
Fermentative parameters reported in bibliography in culture broths formulated with spent yeasts or yeast extract obtained from cell disruption as economic nutrient.

| Nutrients | Microorg | P | $P_{max}$ (g/L) | $Q_P$ (g/L h) | $Y_{P/S}$ (g/g) | References |
|---|---|---|---|---|---|---|
| Yeast extract (5 g/L), peptone (10 g/L), sodium acetate (5 g/L), sodium citrate (2 g/L), $K_2HPO_4$ (2 g/L), $MgSO_4 \cdot 7H_2O$ (0.58 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 116.1 | | 0.96 | Rivas et al. (2004) |
| Peptone (10 g/L), sodium acetate (5 g/L), sodium citrate (2 g/L), $K_2HPO_4$ (2 g/L), $MgSO_4 \cdot 7H_2O$ (0.58 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 91.63 | | 0.84 | Rivas et al. (2004) |
| Biomass grown in synthetic xylose medium (5 g/L), peptone (10 g/L), sodium acetate (5 g/L), sodium citrate (2 g/L), $K_2HPO_4$ (2 g/L), $MgSO_4 \cdot 7H_2O$ (0.58 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 92.65 | | 0.79 | Rivas et al. (2004) |
| Biomass grown in hydrolyzate medium (5 g/L), peptone (10 g/L), sodium acetate (5 g/L), sodium citrate (2 g/L), $K_2HPO_4$ (2 g/L), $MgSO_4 \cdot 7H_2O$ (0.58 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 95.48 | | 0.89 | Rivas et al. (2004) |
| Biomass grown in synthetic xylose medium (10 g/L) | *L. rhamnosus* | LA | 36.99 | | 0.84 | Rivas et al. (2004) |
| Biomass grown in synthetic xylose medium (10 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 29.54 | | 0.85 | Rivas et al. (2004) |
| Corn steep liquor (10 g/L) (from Sigma Chemical CO., St. Louis, USA) | *L. rhamnosus* | LA | 58.63 | | 0.91 | Rivas et al. (2004) |
| Corn steep liquor (10 g/L), biomass grown in synthetic xylose medium (10 g/L) | *L. rhamnosus* | LA | 90.22 | | 0.82 | Rivas et al. (2004) |
| Corn steep liquor (10 g/L), biomass grown in synthetic xylose medium (10 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 92.05 | | 0.85 | Rivas et al. (2004) |
| Biomass grown in hydrolyzate medium (10 g/L) | *L. rhamnosus* | LA | 30.92 | | 0.75 | Rivas et al. (2004) |
| Biomass grown in hydrolyzate medium (10 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 21.95 | | 0.73 | Rivas et al. (2004) |
| Corn steep liquor (10 g/L), biomass grown in hydrolyzate medium (10 g/L) | *L. rhamnosus* | LA | 108.91 | | 0.90 | Rivas et al. (2004) |
| Corn steep liquor (10 g/L), biomass grown in hydrolyzate medium (10 g/L), $MnSO_4 \cdot H_2O$ (0.12 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L) | *L. rhamnosus* | LA | 110.87 | | 0.90 | Rivas et al. (2004) |
| Control (3 g/L yeast extract, 3 g/L malt extract and 5 g/L peptone) | *D. hansenii* | Xylitol | 39.3 | 0.409 | 0.80 | Pérez-Bibbins et al. (2014) |
| Yeast extract control (3 g/L yeast extract) | *D. hansenii* | Xylitol | 40.2 | 0.559 | 0.80 | Pérez-Bibbins et al. (2014) |
| Beer lees after cell disruption using a high-pressure homogenizer | *D. hansenii* | Xylitol | 0 | 0 | 0 | Pérez-Bibbins et al. (2014) |
| Cider lees after cell disruption using a high-pressure homogenizer | *D. hansenii* | Xylitol | 0 | 0 | 0 | Pérez-Bibbins et al. (2014) |
| Wine lees after cell disruption using a high-pressure homogenizer | *D. hansenii* | Xylitol | 0.1 | 0.001 | 0 | Pérez-Bibbins et al. (2014) |
| Beer (whole lees after autolysis) | *D. hansenii* | Xylitol | 15.6 | 0.106 | 0.44 | Pérez-Bibbins et al. (2014) |
| Cider (whole lees after autolysis) | *D. hansenii* | Xylitol | 0 | 0 | 0 | Pérez-Bibbins et al. (2014) |
| Wine (whole lees after autolysis) | *D. hansenii* | Xylitol | 0.1 | 0.001 | 0.03 | Pérez-Bibbins et al. (2014) |
| Beer (solid lees after autolysis) | *D. hansenii* | Xylitol | 34.8 | 0.363 | 0.67 | Pérez-Bibbins et al. (2014) |
| Cider (solid lees after autolysis) | *D. hansenii* | Xylitol | 0.3 | 0 | 0.12 | Pérez-Bibbins et al. (2014) |
| Wine (solid lees after autolysis) | *D. hansenii* | Xylitol | 0.1 | 0 | 0.03 | Pérez-Bibbins et al. (2014) |

LA = lactic acid concentration; $P_{max}$ = maximum concentration of product (g/L); $Q_P$ = global volumetric productivity (g/L h); $Y_{P/S}$ = product yield (g/g).

assayed as a source of extra free amino nitrogen in wheat fermentations, resulting in significantly accelerated rates of sugar utilization and ethanol production comparable to those obtained in urea-supplemented fermentations (Jones and Ingledew, 1994). Meanwhile, crude yeast autolyzates (10 g/L, containing 5.8 g solubles/L) supplemented with minerals and vitamins served as a nutrient supplement to produce 46 ± 1.7 g ethanol/L by the recombinant bacterium *Escherichia coli* KO11, which were comparable to the 44–48 g ethanol/L obtained using 5–15 g/L of purified, soluble, commercial protein hydrolyzates (York and Ingram, 1996). On the other hand, Selmer-Olsen and Sørhaug (1998) observed almost equal growth of *L. plantarum* and lactic acid production in media based on whey supplemented with either commercial yeast extract or home-made autolyzate from brewery yeast biomass, thus confirming that autolyzate of yeast cell biomass is a growth stimulant when added to whey, both for growth of lactic acid bacteria and for their production of organic acids and other compounds.

However, the suitability of using 2.5% (w/v) glucose and 1% (w/v) brewer's yeast extract (BYE) prepared by autolysis of complete beer slurry, for growth and sporulation of *Bacillus thuringiensis* subsp. *kurstaki* resulted in a low biomass production with considerable byproduct formation, including organic acids and a concomitant low medium pH, incomplete glucose utilization and marginal sporulation in comparison with the use of a commercial laboratory-grade yeast extract (Difco) (Saksinchai et al., 2001). In this particular fermentation iron was identified as the limiting factor in BYE since the addition of 3 mg iron sulphate/L doubled the formation of biomass, hardly producing byproducts and occurring complete sporulation. Finally, spent yeast cells coming from the fermentation of xylose to xylitol by *D. hansenii* have also been assayed for lactic acid fermentation by *L. rhamnosus* (Rivas et al., 2004). In this case, under selected conditions, media containing glucose, biomass of *D. hansenii* and corn steep liquor as unique components led to product yields similar to those obtained in a fully supplemented medium. The results attained using different formulations are summarized in Table 3.

Amrane and Prigent (1994) demonstrated the relevance of yeast extract, obtained as the water-soluble portion of autolyzed yeast cells, as supplement of whey hydrolyzate for the growth of *Lactobacillus helveticus* assuming that the main contribution of yeast extract was probably due to purines and pyrimidines and vitamins of the B group. On the other hand, as lactic acid bacteria have a limited capacity to synthesize B-vitamins and amino acids (Hofvendahl and Hahn-Hagerdal, 2000), yeast extract is often used to supply all of these factors in bacterial cultures (Difco, 1984).

### 5.2. Cell disruption

There are several methods of cellular disruption: mechanical, permeation, acid hydrolysis, enzymatic hydrolysis, thermolysis, plasmolysis and permeabilization. Mechanical disruption is performed by high pressure processing with temperature control to avoid degradation of thermolabile compounds. The permeabilization is carried out with organic solvents such as ethyl acetate and ethanol at high concentrations and temperatures for disrupting proteins, the cell membrane and the cell wall, thus favoring the release of intracellular enzymes that contribute to lysis. Acid hydrolysis is

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carried out by hydrochloric acid. Despite a high production yield, acid hydrolysis is less attractive to the manufacturers because or relatively high capital investment cost, high salt content and high probability of generating carcinogenic compounds such as monochloropropanol and dichloropropanol (Nagodawithana, 1992). Enzymatic hydrolysis is carried out by combinations of enzymes, including proteolytic enzymes (Knorr et al., 1979), cell wall lysis enzymes (Rayan and Ward, 1988), β-1,3 glucanases or the culture broth of *Streptomyces* sp. (Lim, 1997), and produces yeast extract low in salt content. Thermolysis or plasmolysis is performed by yeast autoclaving at high salt concentrations to produce a saline shock. Permeabilization is accomplished by freezing and thawing to activate the intracellular activity of α-glucosidase and make the cell wall permeable to exogenous enzymes.

Although the elaboration of yeast extract needs the disruption of the cell structures (namely, membrane and wall) for solubilization of the intracellular components, different yeast extract qualities can be obtained depending on the degree of hydrolysis of these compounds, mainly proteins and nucleic acids. Both the use of yeast extract as nutritional supplement for microbial cultures and as food grade flavoring agent require the hydrolysis of the proteins for being assimilable for non proteolytic microorganisms, and the hydrolysis of the nucleic acids for generating the flavoring nucleotides 5′-guanosine monophosphate and 5′-inosine monophosphate. In consequence, the cell disruption methods only consisting in the disintegration of the cellular structure must be combined with a hydrolytic process developed by exogenous enzymes.

Recently, Pérez-Bibbins et al. (2014) observed that the use of the solid fraction of wine, cider and beer lees did not allow the development of *D. hansenii* cultures. In consequence, two methods of cell disruption were assessed: one mechanical using a high-pressure homogenizer, and one non-mechanical, consisting in the autolysis of cells. Only autolysis of beer lees proved to be successful (see Table 3) for producing xylitol using whole lees or the solid fraction. Therefore, using the solid fraction of beer lees after autolysis, the fermentative parameters ($P$ = 34.8 g/L, $Q_P$ = 0.363 g/L h and $Y_{P/S}$ = 0.67 g/g) were close to those achieved using synthetic media ($P$ = 39.3 g/L, $Q_P$ = 0.409 g/L h and $Y_{P/S}$ = 0.80 g/g) or yeast extract as the only nutrient ($P$ = 40.2 g/L, $Q_P$ = 0.559 g/L h and $Y_{P/S}$ = 0.80 g/g), meaning that the solid fraction of beer lees could be employed as economic nutrient after an autolysis treatment. These authors conclude that these results could be attributed to the amount of available nitrogen after the treatment.

## 6. Conclusion and future perspectives

Wine, beer and cider lees are produced in high quantities around the world in the respective industrial sectors, but faraway from being a potential valuable resource, they are considered a waste to be managed in order to avoid the disposal into soils or free spill in water environments.

Although different applications have been assayed, including the recovery of ethanol by distillation, polyphenolic compounds or salts, as fertilizers, as raw material for L-lactic acid production, or even for the production of biogas, waste lees appear as an undervalued byproduct up to now.

Recent studies aim to evaluate lees as nutritional supplements for industrial fermentation processes considering that the residual content of lees in carbohydrates, nitrogen compounds, or essential vitamins makes this residue a promising supplement similar to yeast extract or corn steep liquor for formulating culture media, although, in some particular cases, lees must be submitted to autolysis to break down the cells and make available the yeast extract.

The results compiled in this review suggest that lees could be attractive as potential economic nutrient sources for subsequent larger-scale bioproduction to obtain high added value natural food additives as xylitol, lactic and citric acids. Some studies seem to corroborate this since economic efficiency parameters calculated identify lees as a lower cost and more effective nutrient source in comparison to corn steep liquor.

However, more studies with other microorganisms, substrates and fermentation technologies must be developed to confirm these promising results. In particular, more work has to be done in order to clarify the reasons for the worse productions observed in some cases with lees, especially when they are applied in high concentrations or the solid fractions are used. Deepening in the study of metals and phenolic compounds and the synergisms between them could be critical for designing lees treatments that allow avoiding their potential negative effects and extent the use of these materials for different bioproductions.

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