Chapter 14

New Trends in *Schizosaccharomyces* Use for Winemaking

Ángel Benito, Fernando Calderón and Santiago Benito

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64807

Abstract

Several researchers are studying the winemaking potential of non-*Saccharomyces* yeast strains in order to improve wine quality. For that purpose, yeast species such as *Torulaspora delbrueckii, Lachancea thermotolerans, Metschnikowia pulcherrima, Candida zemplinina, Kloeckera apiculata, Hansenula anomala* and *Pichia guilliermondii* were studied in the past. Yeasts from the genus *Schizosaccharomyces* have been traditionally studied from a winemaking point of view due to its rapid malic acid deacidification, by converting malic acid to ethanol and CO_2. Nevertheless, during the last 5 years, it has been discovered that *Schizosaccharomyces pombe* possesses several remarkable metabolic properties different from its traditional malic acid deacidification that may be useful in modern quality winemaking, including a malic dehydrogenase activity, high autolytic polysaccharides release, ability of gluconic acid reduction, urease activity in order to avoid ethyl carbamate formation, elevated production of pyruvic acid related to colour improvement, and low production of biogenic amines.

Keywords: *Schizosaccharomyces*, malic acid, pyruvic acid, ethyl carbamate, biogenic amines

1. Introduction

In modern traditional winemaking, *Saccharomyces cerevisiae* has been considered as the main species used in the production of quality wines. The incidences of non-selected *Saccharomyces* or non-*Saccharomyces* opportunistic yeasts during fermentations were usually related to off-flavours such as high levels of acetic acid, ethyl phenols and great levels of higher alcohols. On the other hand, at present, scientists and winemakers have started to believe in the helpful effect of some non-*Saccharomyces* in winemaking in matters such as aroma complexity [1–6].

The main problem about using non-*Saccharomyces* in oenology is their inefficiency to finish alcoholic fermentation in a proper way. So, most of the time, it is required the combined use of *Saccharomyces cerevisiae* strains during alcoholic fermentations in order to ensure a proper fermentation end without any residual sugar at industrial levels. The production of remarkable metabolites by non-*Saccharomyces* in higher amounts than *S. cerevisiae* such as glycerol, pyruvic acid and mannoproteins has awakened especial interest during the last few years [3, 7]. The better performance of enzymatic activities by non-*Saccharomyces* such as the type glycosidase or β-lyase is a relatively new issue in modern oenology. The use on non-*Saccharomyces* also looks to be the only microbiology way to get wines with lower alcohol content in warm areas.

Some studies have analysed the use and influence of different non-*Saccharomyces* species in wine quality. Some of this yeast species are *Kloeckera apiculata, Hanseniaspora uvarum, Hanseniaspora vineae, Torulospora delbrueckii, Metschnikowia pulcherrima, Starmerella bacillaris, Zygosaccharomyces bailii, Pichia guilliermondii, Schizosaccharomyces pombe, Lachancea thermotolerans* and *Hansenula anomala* [1]. **Table 1** summarizes the main quality improvements about using these different yeast species in winemaking.

Yeast species	**Oenological interest**
Kloeckera apiculata	Aroma complexity
Hanseniaspora vineae	Aroma complexity, high 2-phenyl-ethyl acetate production ,biogenic amines reduction
Torulospora delbrueckii	Aroma complexity, acetic acid reduction.
Metschnikowia pulcherrima	Aroma complexity, increase in esters, terpenes and thiols.
Starmerella bacillaris	
Zygosaccharomyces bailii	Polysaccharides increase
Pichia guilliermondii	Formation of high stability colour compounds
Pichia kluyveri	Aroma complexity, increase in varietal thiols and esters.
Lachancea thermotolerans	Acidification, L-lactic acid production
Hansenula anomala	Decreased of C6 alcohols
Schizosaccharomyces pombe	Deacidification, L-Malic acid consumption
Candida stellata	High glycerol production

Table 1. Summary of oenological interest of some non-*Saccharomyces* species.

The chance to modify the flavour and elegance of fermented beverages through different fermentation methodologies is increasing the awareness in researching most imaginable blends of non-*Saccharomyces* and *Saccharomyces* [8]. Regarding this matter, most scientific trials performed fermentations with non-*Saccharomyces* strains by their own, with mixed fermentations (synchronized) and sequential inoculation, comparing them against an alcoholic fermentation performed by *S. cerevisiae* by itself. Most studies testimony sequential inoculation as the finest option in winemaking.

http://dx.doi.org/10.5772/64807

Among non-*Saccharomyces* yeast genera, *Schizosaccharomyces* has been traditionally used to reduce acidity in wines presenting high levels of malic acid. This fact is related to its unique ability to transform L-malic acid into ethanol [9–11]. Nevertheless, novel uses of these *Schizosaccharomyces* species related to different abilities not so well studied until the last few years have been developed to increase wine quality and food safety [12–14]. **Figure 1** summarizes these new uses. One of this novel uses is the performance of specific *Schizosaccharomyces* mutants to decrease the original content of gluconic acid from rotten grape juices [15]. Other modern use is its application in ageing over lees, thanks to their superior polysaccharide release [16]. *S. pombe* metabolism also offers a method of increasing the pyranoanthocyanin content in red wines [12]. *Schizosaccharomyces* is also of great interest in food safety. The urease activity of *Schizosaccharomyces* reduces high ethyl carbamate content in wine by reducing the concentration of urea (main precursor of ethyl carbamate) [13, 14, 17]. *Schizosaccharomyces* can also reduce biogenic amines contents avoiding the classical malolactic fermentation performed by lactic bacteria.

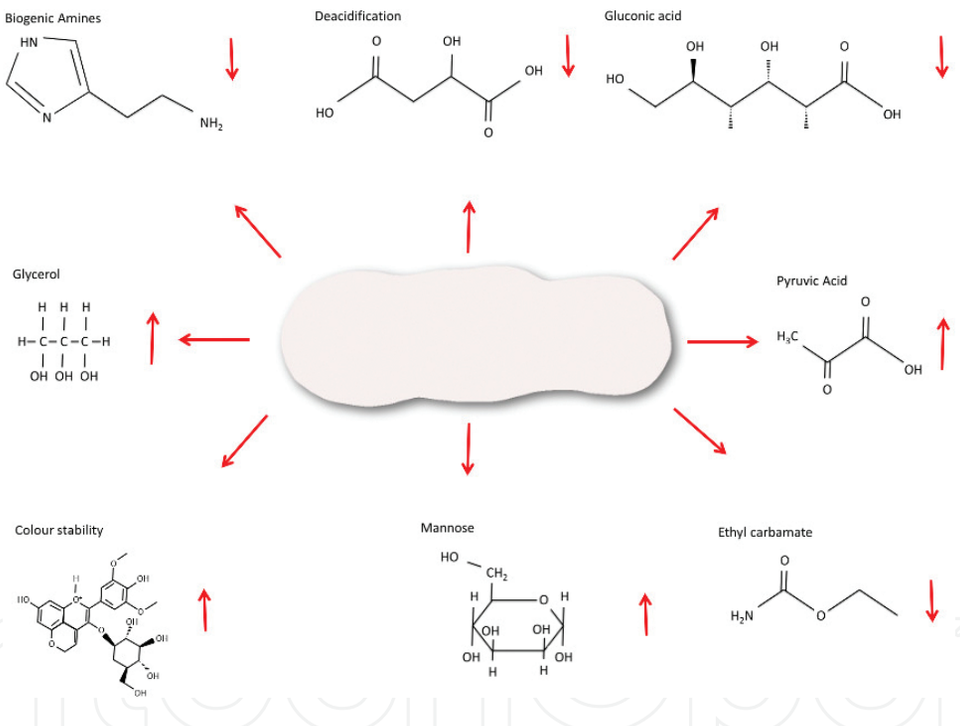

Figure 1. Summary of the new uses of *Schizosaccharomyces*.

The use of the genus *Schizosaccharomyces* in winemaking was approved by the International Organisation of Vine and Wine (Resolution OENO/MICRO/97/75/phase 7). However, *Schizosaccharomyces* was not commonly used due to specific off-flavours associated with the metabolism of non-selected wild strains of this genus [12]. Indeed, *Schizosaccharomyces* has been described to be isolated from wines showing strong organoleptic and chemical deviations such as high levels of acetic acid, sulfidric acid, acetaldehyde, acetoin and ethyl acetate [12]. Due to the enormous variability in the genetic composition of any species such as *S. pombe* [18], recent selection processes have been performed in order to obtain proper strains for winemaking

purposes [19, 20]. The last studies regarding *Schizosaccharomyces* genus have demonstrated that it is possible to produce quality wines through the combination of wild *Schizosaccharomyces* strains with selected *Saccharomyces* strains or more recently through the use of selected *Schizosaccharomyces* strains that are able to perform by themselves a complete proper fermentation process, especially under very acidic conditions. This study aims to show the main new potential of this genus in modern winemaking.

2. Physiology, morphology and taxonomy of *Schizosaccharomyces* genus

In the past, Lodder and Kreger van Rij documented four species belonging to *Schizosaccharomyces*: *Schizosaccharomyces pombe* Lindner (1883), *Schizosaccharomyces octosporus Beijerinck* (1894), *Schizosaccharomyces japonicus* var. versatilis Wickerhan and Duprat (1945) and *Schizosaccharomyces malidevorans* Rankine and Fornachon (1964) [12]. Nowadays, it is believed that the genus *Schizosaccharomyces* is a compound of three species: *S. pombe*, *S. octosporus* and *S. japonicus*. They have been mainly classified according to the principle that involves the number of spores per ascus and their ability to ferment Sucrose and Raffinose [12] (**Table 2**). Most of the time, their presence is related to hot climate regions.

	Fermentation		**Assimilation**	
	Sucrose	**Raffinose**	**Sucrose**	**Raffinose**
S. pombe	+	+	+	+
S. japonicus	+	+	+	+
S. octosporus	+	+	+	+

Table 2. Summary of fermentation and assimilation properties of species from *Schizosaccharomyces* genus.

The species *S. pombe* is usually long rectangular cells of about 2–4 × 5–18 μm (**Figure 2**). They commonly appear as single cells or in pair groups. *S. pombe* is a sporulating species. It can reproduce asexually by binary fission (**Figure 2**) when it forms a septum in the midpoint of the cell. *S. pombe* is not able to assimilate nitrates, it does not have α-glucosidase activity and it cannot breaking down arbutin by enzymatic activity. The species possesses urease positive activity. It reacts with diazonium blue that makes it possible to distinguish it from other basidiomycetous (**Figure 3**). It possesses a high fermentative power, producing 11°–13° of alcohol in anaerobiosis and 14–15.5° with slight aeration.

S. pombe can metabolize malic acid and to convert it into ethanol and CO_2. In the past, a strain of *S. Pombe* was denominated—*Schizosaccharomyces acidovorans* (acidodevoratus)—by Chalenko (1941) [12] due to its special ability to eliminate most of the malic acid from growing media, nowadays this ability is highly strain-dependent [19, 20].

http://dx.doi.org/10.5772/64807

Schizosaccharomyces genus owns a cell structure known as *Schizosaccharomyces*-type that is very particular among ascomycetes. It is richer in polysaccharides and α-galactomannose than any other known yeast species [16, 21].

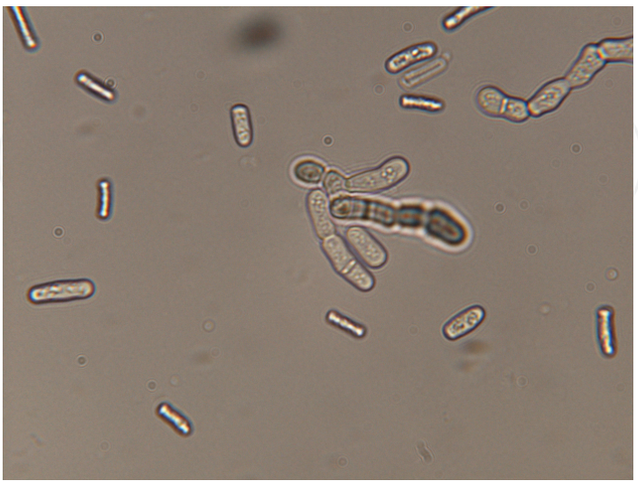

Figure 2. Details of *Schizosaccharomyces pombe*.

Figure 3. Details of *Schizosaccharomyces pombe* reaction with diazonium blue that makes it possible to distinguish it from other yeast specie.

3. Physiological and biochemical properties of *Schizosaccharomyces* genus

Due to its peculiar fission cell division (**Figure 2**), *Schizosaccharomyces* genus is considered as a model organism to study this phenomenon for molecular and genetic microorganism cycle studies [18]. On the other hand, until the last decade, just little information had been published

related to factors that influence its growth, survival and biochemical activities of these microorganisms in industry processes. Most of the few studies available are focus on *S. pombe*. During the last few years, several studies have been performed, especially for wine-making industry, but further studies are needed.

This genus is facultative anaerobic and able to metabolize hexose sugars such as glucose and fructose or disaccharides such as sucrose. They mainly ferment by means of glycolytic pathway producing ethanol and carbon dioxide as main products; several secondary metabolites are also produced. *Schizosaccharomyces* was occasionally described as a higher producer of hydrogen sulphide when it was compared to *S. cerevisiae* [12]. This genus has also been described as a high glycerol producer [22].

Schizosaccharomyces genus is notable known among yeast genera due to its high capacity to metabolize malic acid into ethanol during anaerobic fermentation processes. A NAD-dependent malic enzyme decarboxylates malate to pyruvate. Later, pyruvate is decarboxylated to ethanol that is finally reduced to ethanol (**Figure 4**). A proton-dicarboxylate symport was proved for the transportation of L-malic acid into *S. pombe*, and the presence of glucose is required for malic acid metabolism.

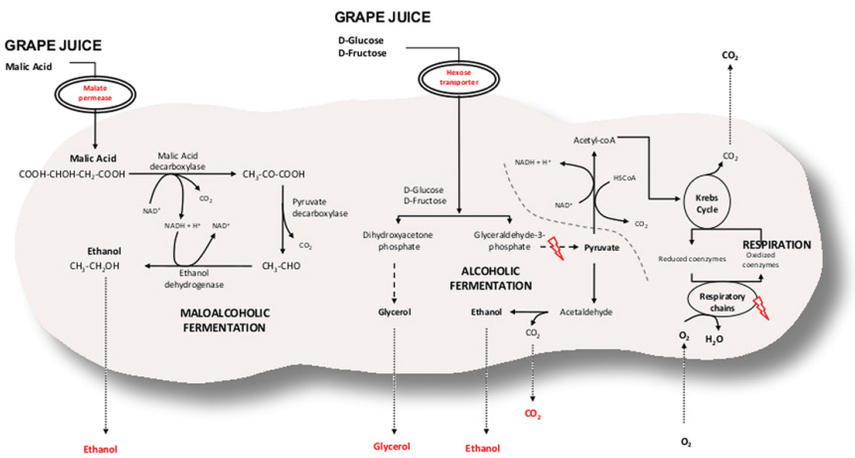

Figure 4. Summary of main metabolic routes performed by *Schizosaccharomyces pombe*.

During the last few years, it was suggested that extracellular amylases, pectolytic enzymes and proteases were not produced by *Schizosaccharomyces* spp., but some new studies start to show that those activities could be strain-dependent. Nevertheless, *S. pombe* has been used to degrade starch with plasmids carrying the glucoamylase gene of *Sacch. diastaticus*. *Schizosaccharomyces octosporus* produces an extracellular lipase that can hydrolyse lard to produce significant quantities of stearic acid, but the lipid degrading ability of other species of *Schizosaccharomyces* is not known.

Schizosaccharomyces pombe has been reported as being able to develop at higher temperatures than *S. cerevisiae*, up to 35°C, data from other *Schizosaccharomyces* species do not have been reported [12]. Other data indicate that *S. japonicus* is skilful of growing at 37°C, but earlier literature reports the growth of *S. pombe* and *S. octosporus* at this temperature. Nevertheless,

the optimum fermentation temperatures are reported to be between 24 and 30°C [23], further studies are needed to determinate the minimum and maximum performing temperatures of this genus. The pH influence is not very well known. It grows properly in pH close to 7, but it is usually isolated from grape juices with pH 2.9–3.1, probably due to the inhibition of other competitor microorganisms that cannot develop as so low pH such as *S. cerevisiae*.

The *Schizosaccharomyces* genus shows a special ability to develop in food media of high sugar content and osmotic pressure [24]. Some authors have reported it as osmotolerant yeasts, capable of growth in the presence of 50% glucose (and possibly 60% glucose) at water activity (aw) values as low as 0.78 [24]. This ability has been described as species dependent for *S. pombe* and *S. octosporus*, minimum a_w values of 0.89–0.90 with glycerol, glucose and fructose are described as stressing levels; in the case of *S. japonicus*, those levels are higher up to 0.92–0.94. Conversely, the genus is less resistant to high salt concentrations [19] and does not develop at a_w levels less than 0.95 of this solute. Most *S. pombe* strains are incapable of growing in the presence of 3% NaCl, pH 5.5. Growth in low a_w environments is accompanied by the production of intracellular glycerol as a compatible solute.

Schizosaccharomyces genus has been widely described to be higher tolerant to several stabilizers than other microorganisms such as *S. cerevisiae* or *Dekkera bruxellensis* [24]. Some of those preservatives are acetic acid, sulphur dioxide, benzoate or sorbate that are normally used during food processing (**Table 3**). This genus looks to be notably higher tolerant to these preservers than *S. cerevisiae*. Opposition to inactivation by heat treating was studied for *S. pombe*. About 99% of the population suspended in phosphate buffer, pH 6.5, containing 48% sucrose (a_w 0.95), was destroyed at 65°C in 3 min (D65 1.99 min). Quicker death proportions were achieved when sucrose was absent from the buffer. Nevertheless, higher thermotolerance is achieved by yeast pre-exposition to minor heat (40°C). This phenomenon makes the production of intracellular trehalose that is used as a thermoprotectant agent.

S. pombe	**Temperature**	**37ºC**
	Acetic acid	1% v/v
	SO_2	120 mg/kg
	Benzoate	>600 mg/L
	Sorbate	>600 mg/L
	Actidione	>100 mg/L

Table 3. Summary of several resistance factors of *S. pombe*.

4. *Schizosaccharomyces* strain isolation

Schizosaccharomyces genus strains have been occasionally isolated from fermented drinks and similar products such as wine, must, grapes and beer. However, most of the isolates related to genus *Schizosaccharomyces* have been reported in foods containing high sugar levels, such

as dried fruit, sweets, molasses and honey [20, 25]. Indeed, no yeast species belonging to *Schizosaccharomyces* genus are included in the 20 Food-Borne Yeasts most frequently described [24, 25] (**Figure 5**). This lack of yeast strains from this genus in nature has avoided the obtaining of commercial strains with proper industrial abilities and free of collateral effects. On the other hand, there is an especial interest from oenological industry yeast manufacturers to get strains able to perform properly at industry level, as a result of the OIV's approval of "Deacidification by Schizosaccharomyces" as an authorized/recommended practice (Resolution OENO/MICRO/97/75/Stage 7). However, at this moment, there are no any commercial strains selected after performing an appropriate selection process, due to their low presence in grape juices and the absence of an adequate method for isolating strains from this complicate genus.

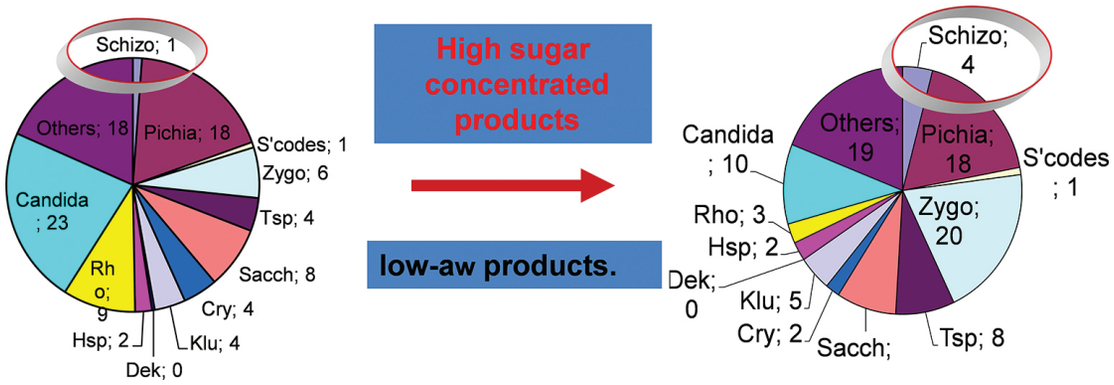

Figure 5. Estimated frequencies (%) of yeast species in fruit and beverages and in high sugar concentrated products. Estimated from [18].

In the past, some authors suggested the use of culture media rich in tryptone glucose yeast extract agar combined with some antibacterial antibiotics, such as streptomycin gentamicin, oxytetracycline and chloramphenicol. It was also suggested the use of sugar and acetic acid in high concentrations as selective agents. Lysine as a selective source of nitrogen was suggested as a way to inhibit the growth of the main yeast species competitor *S. cerevisiae*. Our personal experience showed us that, in spite of using these culture media, there are too many false positives produced by competitor microorganisms; this fact makes impossible to isolate an elevated number of *Schizosaccharomyces* strains. So, in spite of the fact that most important yeasts posse an specific selective-differential method, no specific culture medium has been described until the last few years for isolating yeasts of the genus *Schizosaccharomyces* [24], this media has appeared as a consequence to the great interest an demand that these species have awakened in oenological industry.

A novel and specific isolation method for *Schizosaccharomyces* is nowadays in process of patenting. It has been developed and optimized during the last few years in order to isolate and to select strains of *S. pombe* (**Figure 6**). This new method uses a differential selective medium that contains selective factors such as actidione antibiotic. This antibiotic has been described before in most differential selective media regarding the genera *Brettanomyces/Dekkera*. Among the reported false positives for those media, the genus *Schizosaccharomyces* appeared on some occasions.

http://dx.doi.org/10.5772/64807

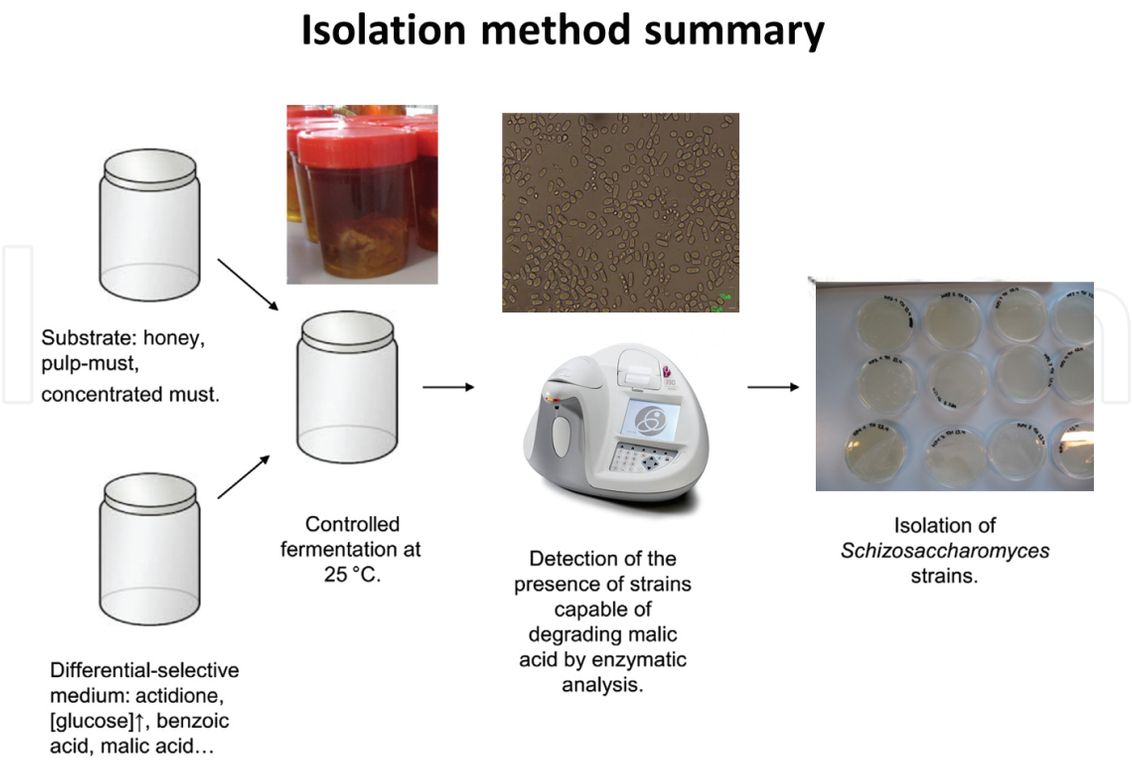

Figure 6. Summary of the specific isolation method developed to isolate *Schizosaccharomyces* strains.

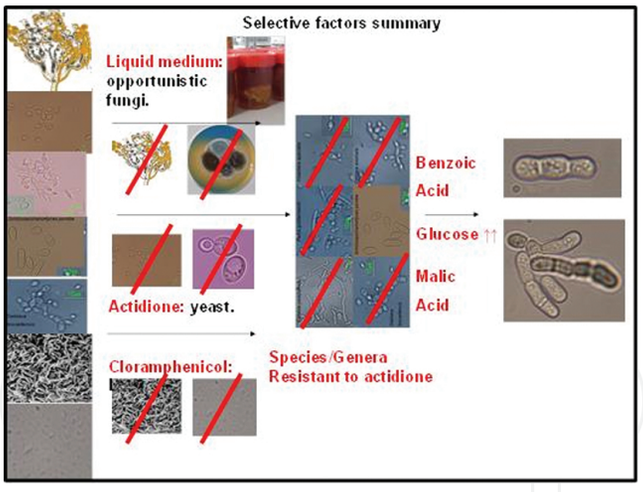

Figure 7. Summary of the main points regarding *Schizosaccharomyces pombe* selective-differential media.

Even though other false positive remain in the described method due to their resistance to actidione (**Figure 7**). Those can also be avoided throw the use of other inhibitor agents such as benzoic acid, acetic acid or high sugar concentration (**Table 3**). To improve the differentiation process, malic acid is commonly used as it makes possible to identify the presence of micro-organism able to degrade it throw pH control or enzymatic analyse. This methodology (**Figure 6**) has allowed generating *Schizosaccharomyces* collections of hundreds of different strains [12].

5. *Schizosaccharomyces* selections for winemaking

The isolation methodology explained above has allowed obtaining representative universes of *Schizosaccharomyces* genus that made it possible to perform basic selection processes to get strains with especial aptitude for winemaking [20]. It has been observed that just a small percentage up to 5% of strains could perform a proper alcoholic fermentation process without collateral effects.

Some of the basic parameters studied in this initial selection processes have been correct sugar consumption, moderate acetic acid production, complete malic acid degradation, glycerol production and the correct sensory profile of the wines produced with these strains.

5.1. Sugar consumption

Most recent studies report that *S.pombe* ferments great quantities of sugars during alcoholic fermentation up to 240 g/L. However, most studies report a slower kinetic metabolism than that described for *S. cerevisiae* [12]. Some authors have reported differences up to 2–4 days to complete an alcoholic fermentation process when duration was compared against a *S. cerevisiae* control [9, 19, 22]. Nevertheless, we must consider that the second fermentation performed by lactic bacteria in red wines is not needed in the case of wines made by *Schizosaccharomyces* yeasts [7]. This process usually takes long time than the alcoholic fermentation by yeasts and the risk of deviations is higher.

5.2. Malic acid consumption

Malic acid consumption has been reported in most studies regarding *S. pombe* to be completed in most cases. Nevertheless, great differences regarding different kinetics depending on the strains have been reported [19, 20]. On some occasion, especially in very acidic musts with malic acids contents over 6 g/L [9] from northern regions, the deacidified wines were preferred by the testing panels due to the excessive acidity described for the controls performed by regular *S. cerevisiae* without malate dehydrogenase activity. Increments of about 0.4 in pH were produced after malic acid consumption by *S. pombe* [7, 9].

5.3. Acetic acid production

Acetic acid is the factor that has showed the biggest variety among the studied strains in most studies [19, 20]. On some occasions, values over 1 g/L have been reported [9]. These values are not compatible with quality wines. Nevertheless, in other studies, moderate levels have been reported. According to the last studies, we can report that it is possible to select *S. pombe* strains that produce wines with as low content of acetic acid as regular wines performed by *S. cerevisiae* if they are properly selected [19, 20]. Another option to reduce this possible collateral effect was to combine the use of *S. pombe* with other yeast species that produce lower levels of acetic acid such as *L. thermotolerans* [7].

5.4. Urease activity/ethyl carbamate reduction

Schizosaccharomyces genus has been described among the few yeast species that can develop urease activity [17, 19, 25]. This enzymatic activity was observed in several fermentation trials [4, 10, 12–14] were *Schizosaccharomyces* fermentations always reported final urea values after fermentation of about 0 mg/L. However, controls regarding *S. cerevisiae* reported notably higher values up to 3 mg/L. The enzymatic activity in winemaking could reduce the initial level of the main precursor (urea) for ethyl carbamate (one of the most toxic compounds reported in wine) formation. Nowadays, ethyl carbamate is a main problem for human health as it is considered a powerful carcinogen with an especial incidence in fermented beverages [19, 27]. It is also a very important problem regarding to wine exportations as several countries have already set specific limits for this toxicological compound that varied from 10 to 30 μm.

5.5. Pyruvic acid

Fermentations performed by *Schizosaccharomyces* always were reported as higher producers of pyruvic acid than *S. cerevisiae*. Nevertheless, important differences have been observed depending on the *Schizosaccharomyces* strains [12, 13]. Maximum values during the first days of alcoholic fermentation up to about 0.5 g/L have been achieved by *Schizosaccharomyces* fermentations while maximum values up to about 0.1 g/L have been reported for specific *S. cerevisiae* strains selected according to this criterion [6]. The oenological meaning about producing high levels of pyruvic acid is related to the strong correlation between the amount of pyruvic acid released during alcoholic fermentation by yeasts and the formation of vitisin A [15, 16]. Vitisin A is known as a very stable coloured pigment that directly influences wine colour quality and stability. This parameter is considered nowadays an important criterion in red wine yeast selection processes. Until now, *Schizosaccharomyces* genus is the highest producer yeast of pyruvic acid in winemaking.

5.6. Glycerol

One of the first experiments involving *Schizosaccharomyces* [7] indicated that *Schizosaccharomyces pombe* possesses a highly developed glyceropyruvic pathway compared to other yeast species. This fact explains also the greater production of pyruvic acid explained before. Modern trials have reported glycerol productions up to 10 g/L [13] and values higher than 1 g/L when they were compared to *S. cerevisiae* controls [4, 6]. Increased glycerol content is described as one of the main contributions of some non-*Saccharomyces* strains in winemaking [5] because it directly influences positively to the mouth-feel. Even though other yeast species such as *Candida stellata* have been described as higher producer of glycerol [5], the use of *Schizosaccharomyces* could be interesting in order to improve this quality parameter.

5.7. Ethanol

Many winegrowing areas observe an increase in the alcohol content of their wines, on some occasions to more than 14% by volume. This phenomenon may become increasingly common due to the effects of climate change. Several practices are proposed to decrease the ethanol

levels in fermented beverages either completely or partially, for instance the use of great temperatures to drive off the ethanol, chemical extraction, cryoconcentration, filtration using semipermeable membranes and supercritical fluids extraction [26]. Some authors have reported that some non-*Saccharomyces* types of yeast produce lower ethanol levels than *Saccharomyces*. *Schizosaccharomyces* has been described in some occasions as lower producer of ethanol than *S. cerevisiae* in amounts of about 0.4-0.2% vol [2, 3, 7, 9]. However, other authors have reported no significant differences when compared to *S. cerevisise* strains selected for its high-developed glyceropyruvic pathway [19]. The sugar metabolism can be used to produce different compounds other than ethanol, such as glycerol or pyruvic acid, or to increase the yeast biomass [12]. Other authors observed lower final ethanol levels using other non-*Saccharomyces* species under very specific settings related to high aeration conditions [2]. In those cases, reductions higher than 1 or 2% vol in ethanol can be achieved. This reduction is higher and more efficient than those described for *Schizosaccharomyces*, so *S.pombe* could be interesting when the needed reduction in ethanol is about 0.5% vol in ethanol.

5.8. Biogenic amines

Biogenic amines are other toxicological compounds that can appear in wine. Several authors have described harmful effects in human beings produced by biogenic amines [7, 17]. For these reasons, this topic is considered a serious matter in food safety that must be considered. Several countries have established legal limits. A histamine value of 2 mg/L is considered the most restrictive level in some countries. Several trials performed by *Schizosaccharomyces* show that *S. pombe* does not produce higher levels of biogenic amines than *S. cerevisiae* [7, 17]. Reduction in biogenic amines that come from spoilage grapes such as cadaverine have been reported to decrease for *Schizosaccharomyces* in quantities up to a few mg/L. Similar processes have been described before for other yeast species [7]. However, the main use of *Schizosaccharomyces* about reducing/avoiding biogenic amine formation is based on the fact that most biogenic amines are produced during wine ageing and especially during malolactic fermentation [7, 17], as they are compounds produced primarily by lactic acid bacteria. Thus,wines fermented by *Schizosaccharomyces* do not need malic acid consumption by lactic bacteria any more.This fact notably decreases the risk of biogenic amines formation [17].

5.9. Volatile aroma

Schizosaccharomyces strains were not used in the past because of specific off-flavours commonly associated with the metabolism of non-selected strains. In the past, *Schizosaccharomyces* was commonly isolated from wines suffering from organoleptic and chemical faults through the appearance of sulfidric acid, acetic acid, acetaldehyde, acetoin and ethyl acetate [12]. First, control fermentations performed by mixed non-selected *Schizosaccharomyces* strains combined with *S. cerevisiae* fermentations reported higher concentrations of acetaldehyde, propanol and 2,3-butanediol up to several mg/L [9]. Nevertheless, the last fermentations performed by selected *Schizosaccharomyces* strains show lower levels in higher alcohols than the non-selected *Schizosaccharomyces* and the *S. cerevisiae* controls [7, 18]. In those studies, the tested *S. pombe* strains produced also less esters than the *S. cerevisiae* strains. Similar effects have been reported

for other non-*Saccharomyces* [1]. This finding could be of interest in facilitating the making of wines with varietal character for specific grape varieties or to increase wine complexity avoiding the influence of higher alcohols or esters. No differences between selected *S. pombe* and *S. cerevisiae* strains have been observed with respect to compounds considered negative, such as ethyl acetate and diacetyl when selected strains have been employed [19, 20]. Nevertheless, compounds such as acetaldehyde and ethyl acetate show significant differences depending on the different *S. pombe* strains [20].

5.10. Gluconic acid

Rotten grape musts contain high concentrations of gluconic acid formed by fungal attacks and acetic bacteria from rotten grapes. This fact drastically reduces the quality of wines made from those grapes. The sensory properties of wines are considerably altered by the presence of gluconic acid, which decreases the wine's microbiological stability and raises long-term storage problems that can be solved only by reducing its concentration in the wine. Specific *S. pombe* strains have been used to remove gluconic acid in 50% rates from wines up to 2.5 g/L obtaining in the end better final wines. This fact produced a good influence in volatile compounds spoilage by gluconic acid influence [15].

5.11. Polysaccharides

The methodology of ageing over lees is nowadays considered important in the making of red wines because it has been probed that it produces high quality wines with peculiar identity. It allows winemakers to produce new different wines in a market that shows great homogeneity. This methodology, however, demands appreciable investment in resources and is not free of problems. Many research groups are now working on how to minimize these difficulties, and on how to obtain balanced products quicker and simpler.

Schizosaccharomyces pombe is yeast species whose cell wall has a particular structure and composition owed to the presence of polysaccharides and sugar derivatives that are unusual within the family Saccharomycetaceae. The main difference between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* is the possession of a-galactomannose rather than mannose, along with the presence of α-(1=>3) glucan. The use of *Schizosaccharomyces pombe* in over-lees ageing has reported faster release kinetics and increases of about 100 mg/L in polysaccharides pullulans than *S. cerevisiae* in 142 days [16].

5.12. Sensory impact

Wines developed by *Schizosaccharomyces* usually show big differences in the sensory perception of acidity when they are compared to *S. cerevisiae* controls (**Figure 8**) [7, 9, 19]. This fact is related with the malic acid consumption and pH increased explained above. In some occasions, wines fermented by *Schizosaccharomyces* have been described as sweeter than those fermented by *S. cerevisiae* in spite of the fact that all wines did not contain any residual sugar [9, 19]. This phenomenon is explained by the new balance generated between acidity, sweetness, bitterness and salty perception when acidity is highly reduced. Severe faults have been

reported in trials involving non-selected *S. pombe* strains regarding to high acetic acid, reduction and sulfidric acid characters [19, 22]. Nevertheless, in modern fermentation trials, some selected *S. pombe* have received the best scores when compared against non-selected *Schizosaccharomyces* strains and *S. cerevisiae* strains when fermentations took place in high acidic musts [19, 22]. The preference commonly has been related to excessive high acidity for the tasters due to high levels of malic acid up to 6 g/L and in the case of the non-selected *Schizosaccharomyces* strains due to their several collateral effects.

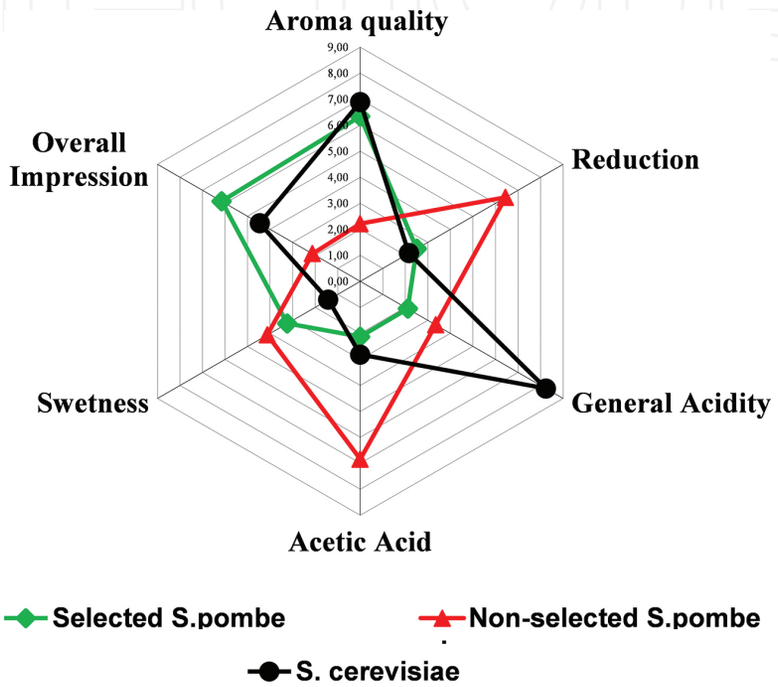

Figure 8. Summary of sensory profiles performed by Selected *S. pombe*, non-selected *S. pombe* and selected *S. cerevisiae* strains from very acidic grape musts.

5.13. Combination with other yeast species

New trends involving mixed fermentations between *S. pombe* and *L. thermotolerans* have been recently performed in warm viticulture regions with few malic acid content and high pH of about 4. The objective of this combination is to avoid biogenic amines formation during malolactic fermentation at high pH. In that case, *L. thermotolerans* is used in order to avoid an excessive deacidification through lactic acid formation. These wines showed lower final levels of biogenic amines up to almost 2 mg/L than the controls that underwent malolactic fermentation [7]. The pH was also reduced in 0.25 instead of increasing.

6. Conclusion

There are many new uses related to *Schizosaccharomyces* genus that can be applied in modern enology different from the classic malic acid deacidification. These new applications are not

only related to improve wine quality. They can also improve food safety parameters doing the act of drinking wine a healthier habit. Last few studies have demonstrated that it is possible to make quality wine by using *Schizosaccharomyces* genus when selected strains are used. These strains can be used to produce wines with low levels of malic acid, acetic acid, gluconic acid, ethyl carbamate and biogenic amines, and with an appropriate volatile aroma profile.

Author details

Ángel Benito, Fernando Calderón and Santiago Benito*

*Address all correspondence to: santiago.benito@upm.es

Department of Chemistry and Food Technology, Polytechnic University of Madrid, University Campus S/N, Madrid, Spain

References

[1] Benito S, Hofmann T, Laier M, Lochbühler BC, Schüttler A, Ebert K, Fritsch S, Röcker J, Rauhut D. Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-Saccharomyces and *Saccharomyces cerevisiae*. European Food Research and Technology. 2015; 241: 707–717. DOI: 10.1007/s00217-015-2497-8

[2] Belda I, Navascués E, Marquina D, Santos A, Calderón F, Benito S. Dynamic analysis of physiological properties of *Torulasporadelbrueckii* in wine fermentations and its incidence on wine quality. Applied Microbiology and Biotechnology. 2015; 99: 1911–1922. DOI: 10.1007/s00253-014-6197-2

[3] Benito A, Calderón F, Palomero F, Benito S. Quality and composition of airen wines fermented by sequential inoculation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae*. Food Technology and Biotechnology. 2016; 54(2) 135–144. DOI: 10.17113/ftb.54.02.16.4220

[4] Rojas V, Gil JV, Piñaga F, Manzanares P. Studies on acetate ester production by non-*Saccharomyces* wine yeasts. International Journal of Food Microbiology. 2001; 70(3): 283–289. DOI: 10.1016/S0168-1605(01)00552-9

[5] Ciani M, Comitini F, Mannazzu I, Domizio P. Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Research. 2010; 10(2): 123–133. DOI: 10.1111/j.1567-1364.2009.00579.x

[6] Comitini F, Gobbi M, Domizio P, Romani C, Lencioni L, Mannazzu I, Ciani M. Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with *Saccha-*

romyces cerevisiae. Food Microbiology. 2011; 28(5): 873–882. DOI: 10.1016/j.fm.2010.12.001

[7] Benito A, Calderón F, Palomero F, Benito S. Combine use of selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* yeast strains as an alternative to the traditional malolactic fermentation in red wine production. Molecules. 2015; 20: 9510–9523. DOI: doi:10.3390/molecules20069510

[8] Jolly NP, Varela C, Pretorius IS. Not your ordinary yeast: non-Saccharomyces yeasts in wine production uncovered. FEMS Yeast Research. 2014; 14: 215–237. DOI: 10.1111/1567-1364.12111

[9] Benito S, Palomero F, Morata A, Calderón F,Palmero D, Suárez-Lepe JA. Physiological features of *Schizosaccharomyces pombe* of interest in making of white wines. European Food Research and Technology. 2013; 236: 29–36. DOI: 10.1007/s00217-012-1836-2

[10] Maconi E, Manachini PL, Aragozzini F, Gennari C, Ricca GS. A study of the maloalcoholic fermentation pathway in *Schizosaccharomyces pombe*. Biochemical Journal. 1984; 217(2): 585–588. DOI: 10.1042/bj2170585

[11] Taillandier P, Strehaiano P. The role of malic acid in the metabolism of *Schizosaccharomyces pombe*: substrate consumption and cell growth. Applied Microbiology and Biotechnology. 1991; 35(4): 541–543. DOI: 10.1007/BF00169765

[12] Benito S, Palomero F, Calderón F, Palmero D, Suárez-Lepe JA. Schizosaccharomyces. In: Batt CA, Tortorelo ML, editors. Encyclopedia of Food Microbiology. 2nd ed. Amsterdam: Elsevier; 2014. p. 365–370.

[13] Lubbers MW, Rodriguez SB, Honey NK, Thornton RJ. Purification and characterization of urease from *Schizosaccharomyces pombe*. Canadian Journal of Microbiology. 1996; 42(2): 132–140. DOI: 10.1139/m96-021

[14] Bacanamwo M, Witte CP, Lubbers M, Polacco J. Activation of the urease of *Schizosaccharomyces pombe* by the UreF accessory protein from soybean. Molecular Genetics and Genomics. 2002; 268(4): 525–534. DOI: 10.1007/s00438-002-0769-z

[15] Peinado RA, Moreno JJ, Maestre O, Mauricio JC. Removing gluconic acid by using different treatments with a *Schizosaccharomyces pombe* mutant: effect on fermentation by products. Food Chemistry. 2007; 104: 457–465. DOI: 10.1016/j.foodchem.2006.11.070

[16] Palomero F, Morata A, Benito S, Calderón F, Suárez-Lepe JA. New genera of yeasts for over-lees aging of red wine. Food Chemistry. 2009; 112: 432–441. DOI: 10.1016/j.foodchem.2008.05.098

[17] Benito S. The use of *Schizosaccharomyces* yeasts in order to reduce the content of biogenic amines and ethylcarbamate in wines. Journal of Food Processing & Technology. 2015; 6: 9. DOI: 10.4172/2157-7110.S1.026

[18] Jeffares DC, Rallis C, Rieux A, Speed A, Převorovský M, Mourier T, et al. The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. Nature Genetics. 2015; 47: 235–241. DOI: 10.1038/ng.3215.

[19] Benito A, Jeffares D, Palomero F, Calderón F, Jurg J, Bai Y, Benito S. Selected *Schizosaccharomyces pombe* strains have characteristics that are beneficial for winemaking.2016; 11(3) e0151102. DOI: 10.1371/journal.pone.0151102 Accepted.

[20] Benito S, Palomero F, Calderón F, Palmero D, Suárez-Lepe JA. Selection of appropriate Schizosaccharomyces strains for winemaking. Food Microbiology. 2014; 42: 218–224. DOI: 10.1016/j.fm.2014.03.014

[21] Belda I, Navascués E, Marquina D, Santos A, Calderón F, Benito S. Outlining the influence of non-conventional yeasts in wine ageing over-lees. Yeast. 2016. Accepted.

[22] Benito S, Palomero F, Gálvez L, Morata A, Calderón F, Palmero D, Suárez-Lepe JA. Quality and composition of red wine fermented with *Schizosaccharomyces pombe* as sole fermentative yeast, and in mixed and sequential fermentations with *Saccharomyces cerevisiae*. Food Technology and Biotechnology. 2014; 52(3): 376–382.

[23] Benito S, Palomero F, Morata A, Calderón F, Suarez-Lepe JA. New applications for *Schizosaccharomyces pombe* in the alcoholic fermentation of red wines. International Journal of Food Science and Technology. 2012; 47: 2101–2108. DOI: 10.1111/j.1365-2621.2012.03076.x

[24] Benito S, Gálvez L, Palomero F, Calderón F, Morata A, Palmero D, Suárez-Lepe JA. *Schizosaccharomyces* selective differential media. African Journal of Microbiology Research. 2013; 7(24): 3026–3036. DOI: 10.5897/AJMR2013.5684

[25] Deák T. Handbook of Food Spoilage Yeasts. 2nd ed. CRC Press, Boca Raton, Florida.

[26] Loira I, Morata A, González C, Suárez-Lepe JA. Selection of glycolytically inefficient yeasts for reducing the alcohol content of wines from hot regions. Food and Bioprocess Technology. 2012; 5(7): 2787–2796. DOI: 10.1007/s11947-011-0604-9

[27] Xia C, Hong M, YunFeng Z, Ning WY. Research progress on toxicity and contamination of ethyl carbamate in fermented foods. Journal of Food Safety and Quality. 2014; 5(9): 2617–2262. DOI: 10.5897/AJMR2013.5684

IntechOpen