YEAST HULLS: EFFECT ON SPONTANEOUS FERMENTATION IN DIFFERENT VINIFICATION CONDITIONS

ÉCORCES DE LEVURE : EFFET DES FERMENTATIONS SPONTANÉES DANS DIFFÉRENTES CONDITIONS DE VINIFICATION

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Abstract : The effect of the addition of yeast hulls in vinification was investigated during three consecutive years. The study was carried out in the experimental winery of C.I.D.A in La Rioja (Spain) with free running white grape juice of the Viura variety. Four different vinifications were studied. In two of these vinifications, stuck fermentations were detected. In all the studies, the addition of yeast hulls (yeast ghosts) improved the fermentation kinetics, increasing the number of viable yeasts at the end of the exponential stage and decreasing the final content of reducing sugars. This work revealed a new effect of yeast hull addition which had not been identified previously; their selection effect on the wild yeast strain in spontaneous fermentation.

Résumé : On a étudié pendant trois années consécutives l'effet de l'addition des écorces de levure dans le développement de la fermentation alcoolique du moût de raisin. Nous avons réalisé quatre vinifications dans la cave expérimentale du CIDA dans La Rioja (Espagne). Dans deux des quatre fermentations on a détecté des arrêts de fermentation. Dans tous les essais, le traitement aux écorces de levure améliore la vitesse de fermentation, accroît la population vivante des levures pendant la phase exponentiel et fait diminuer les sucres résiduels dans les vins. Ce travail a mis en évidence un nouveau effet de l'addition d'écorces que l'on n'avait pas encore identifié. Il s'agit de l'effet d'écorces sur la sélection des souches de levure qui conduisent une fermentation alcoolique spontanée. Ce mécanisme pourrait expliquer l'efficacité de l'addition d'écorces pour l'amélioration de la fermentation alcoolique.

Key words : yeast hulls, yeasts, stuck fermentation

Mots-clés : écorces de levure, levures, arrêts de fermentation

INTRODUCTION

Fermentation difficulties are one of the major problems in current vinification. Stuck and sluggish fermentations are a common phenomenon. Two very problematic vintages (1995 and 1997) have occurred in the Rioja region (Spain) in the last ten years.

Different resources can be applied to prevent the risk of stuck fermentations in white wine vinification. These include the addition of Assimilable Nitrogen (FAN) (CASTINO and DI STEFANO, 1990; LORENZINI, 1996), survival factors (CHARPENTIER, 1993; DELFINI *et al.*, 1993; SABLAYROLLES *et al.*, 1996), substances with a support effect (LARUE *et al.*, 1985; LAVIGNE and DARRIET, 1991) and yeast hulls (LAFON-LAFOURCADE *et al.*, 1984; RIBÉREAU-GAYON, 1985; INGLEDEW, 1995). Another option is the use of controlled aeration in the musts (ALEXANDRE and CHARPENTIER, 1995; RIBÉREAU-GAYON, 1999).

Several studies have reported on the improvement given by addition of yeast hulls to the fermentation. Their action mechanism has been postulated to be due to physical adsorption of toxic fatty acids present in the must (GENEIX *et al.*, 1983; LAFON-LAFOURCADE *et al.*, 1984). Yeast hulls also release sterols and long chain fatty acids, compounds considered as oxygen substitutes (MUNOZ and INGLEDEW, 1989; BERNATH and BERTRAND, 1992). These effects enhance the survival rate of non-proliferant yeasts.

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Saccharomyces cerevisiae is the dominant yeast specie in alcoholic fermentation. In recent years, several genetic methods have been developed for yeast strain identification and these have demonstrated that there is a wide genetic diversity among the enological strains of *Saccharomyces cerevisiae* driving the fermentation (MOLINA *et al.*, 1992; QUEROL *et al.*, 1992; QUESADA and CENIS, 1995). However, only a few strains actually control the fermentation (FLEURENT *et al.*, 1993; VERSAVAUD *et al.*, 1993), and so they determine the final wine composition.

The influence of yeast hulls on the presence and distribution of *Saccharomyces cerevisiae* strains in spontaneous fermentations has not been widely evaluated. This paper describes the effect of the addition of yeast hulls in different spontaneous fermentations carried out over three consecutive years. Information is provided to show that the physical adsorption of medium chain length fatty acids, and the supplementation of sterols and unsaturated fatty acids, are not the only possible explanations for the effect of yeast hulls in preventing stuck or sluggish fermentations.

MATERIALS AND METHODS

Fermentations: These were carried out during the 1996, 1997 and 1998 vintages, in a experimental winery using free-running white grape juice of the Viura variety from La Rioja (Spain). The musts had been strongly clarified by settling, and sulphur dioxide (6 g/hl) was added before the fermentation started. The pure yeast hulls for winemaking used are produced by Fould-Springer (F 94701 Maisons-Alfort). They were added at the recommended level (20 g/hl) during the exponential stage of fermentation (when the ºBrix had decreased by 3-4 units). In all cases, control vinifications (without yeast hull addition) were made.

In 1996, the tests were carried out in 5 l glass tanks at 18ºC and 30ºC. In 1997 and 1998, the fermentations were carried out at 18ºC in stainless steel tanks of 25 l and 250 l respectively. Fermentations were conducted in triplicate and ºBrix measured once daily with a digital ATAGOdbx-30 refractometer.

Analysis methods: The yeast viable counts were determined daily from all tanks by plating out onto chloramphenicol glucose agar (Biokar Diagnostics) at serial decimal dilutions and incubation at 28ºC for 48 hours. Plates containing between 30 and 300 colonies were examined. In all plates from samples taken at the end of the exponential stage (density 1025), ten colonies were randomly selected and their mitochondrial DNA was analyzed (QUEROL *et al.*, 1992). This analysis is able to differentiate between *Saccharomyces* and non-*Saccharomyces* yeasts and also between strains of *Saccharomyces cerevisiae* (GUILLAMON, 1996). The different strains of *Saccharomyces cerevisiae* were designated with capital letters in 1996, roman numerals in 1997 and lower case letters in 1998.

The physico-chemical analysis of the musts and wines were determined according to Official EU Methods (Diario Oficial de la CEE, 1990). Assimilable nitrogen was analyzed using the Formol Titration method (AERNY, 1996).

Fatty acids were extracted with a mixture of chloroform and ethanol (at 2:1 v/v), three times in succession. After evaporating the organic phase to dryness in a rotating evaporator, the dry remnant was submitted to saponification with $NaOH/CH_3OH$ and to methylation with $F3B/CH_3OH$. The methyl esters formed were extracted three times with hexane, finally concentrating the extract to a volume of 0.4 ml (SANTAMARIA *et al.*, 1995). The extracts were analysed by gas phase chromatography (Hewlett-Packard 5890, Series II with FID detection and FFAP capillary column of 0.22 mm x 30 m). The analysis was carried out on 2 µl of the methylated extract with split/splitless injection. The injector and detector temperatures were 250ºC; the oven temperature was programmed to rise from 50ºC to 220ºC at 10ºC/min, remaining at 220ºC for 15 minutes.

RESULTS AND DISCUSSION

The composition of musts from the three years are given in table I. The 1996 must had the most balanced composition, in spite of its high sugar level. The 1997 must was low in long chain fatty acids (activators) and high in medium chain fatty acids (inhibitors). The 1998 must contained the lowest amount of assimilable nitrogen, just on the minimum limit recommended (HENSCHKE and JIRANEK, 1993).

Yeast hulls had stimulatory effects on all the fermentations when compared with the control (figure 1). In all of the fermentations tested, yeast hull addition gave more cell growth and decreased the final reducing sugar and volatile acidity content as compared with the control fermentations (table II). These results were more noticeable when the amount of long chain fatty acids in the must was limited (1997), and when the fermentation were carried out under unfavourable conditions of 30 ºC (1996).

It has been postulated that the action of yeast hulls is due to the physical adsorption of medium chain fatty acids, compounds with an inhibitory action on the fermentation. (LAFON-LAFOURCADE, 1984). In our experiment, it is not clear that the yeast hulls act via this physical adsorption (figure 2). It is true that the

treatment caused decreases in the inhibitor fatty acids content of samples carried out in 1997 and 1998. However, in 1996 this action did not appear: in fermentations carried out at 18 ºC, no significant differences were detected, whereas at the 30 ºC fermentation temperature, the content of these compounds increased when the yeast hulls were added, with respect to the control fermentations. In these laster cases, the detoxification theory cannot be the hull action mechanism. Therefore, the improvement produced in the fermentations carried out at 30 ºC was not due to the adsorption of the inhibitor fatty acids. Furthermore, the quantity of inhibitory fatty acids generated was significantly lower in the problematic fermentations (1996-30ºC and 1997) than in the normal ones (1996-18ºC and 1998). This data questions the role of these compounds in some stuck fermentations, contrary to the conclusions reached until now (LARUE *et al.*, 1984).

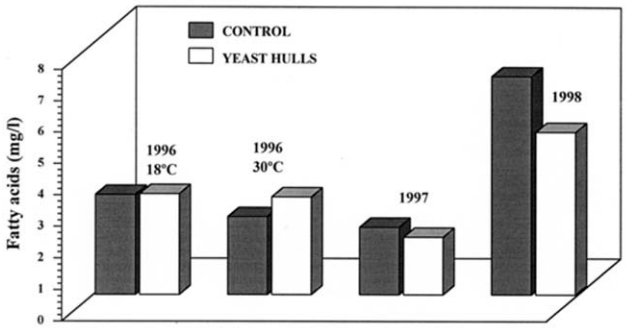

Fig. 2.- Effect of the addition of yeast hulls on the medium chain fatty acids (C6+C8+C10+C12) in the vigorous stage of fermentation

Fig. 2.- Effect de l'addition des écorces de levure sur les teneurs en acides gras à moyenne chaîne (C6+C8+C10+C12) pendant la phase stationnaire de la fermentation.

TABLE I
Composition of the musts in the 1996, 1997 and 1998 vintages.

Tableau I – Composition des moûts (Vendanges de 1996, 1997 et 1998)

Parameters	Must		
	1996	1997	1998
Sugar (g/l)	243	191	201
Turbidity (NTU)	10	9	24
FAN (mg/l)	244	250	139
Activator fatty acids (C14-C22) (mg/l)*	2,001	0,855	3,331
Inhibitor fatty acids (C6-C12) (mg/l)**	0,125	0,366	0,143

* Myristic acid (C14) + Myristoleic acid (C14:1) + Palmitic acid (C16) + Palmitoleic acid (C16:1) + Stearic acid (C18) + Oleic acid (C18:1) + Linoleic acid (C18:2) + Linolenic acid (C18:3) + Arachidic acid (C20) + Behenic acid (C22)
** Caproic acid (C6) + Caprylic acid (C8) + Capric acid (C10) + Lauric acid (C12)

Microbiological studies of the yeasts present in the vigorous stage of fermentation revealed that yeast hull addition influenced the distribution and frequency of the yeast strains. Table III shows that in nearly all cases, (with one exception in 1997), the addition of yeast hulls decreased the number of different strains present at this fermentative stage. In the tests carried out during the 1996 vintage, mtDNA restriction patterns of the isolates revealed the presence of 10 different strains (designated with letters from A to J). The fermentations carried out at 18 ºC were completed in both the control and hull addition fermentations. Four *S. cerevisiae* strains were present at frequencies of more than 10 p. cent, in the control. Pattern A was clearly dominant. Hull addition increased the A strain from 60 to 90 p. cent and only two different strains were detected. All the vinifications carried out at 30 ºC were affected by stuck fermentations, but the wines produced in the presence of hulls had a lower residual sugar content. Pattern A was again dominant in this test (30 p. cent) among the 7 different strains found in the control fermentations; its percentage increased to 70 p. cent in the treated fermentations, while the number of different strains appearing during the fermentations fell to half (4 different strains). The temperature was the only different parameter between the tests car-

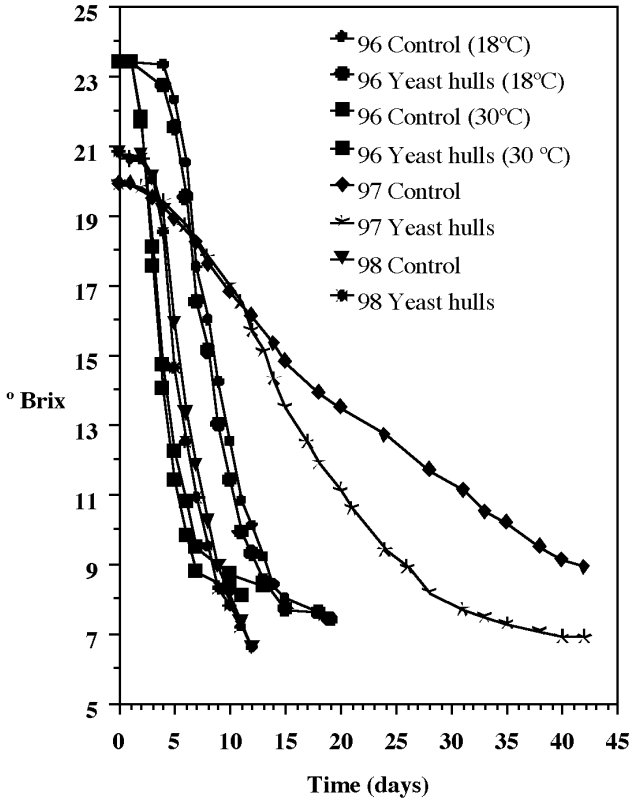

Fig. 1.- Effect of the addition of yeast hulls on the fermentation rate.

Fig. 1.- Effet de l'addition des écorces de levure dans la vitesse de fermentation.

TABLE II
Indicative parameters of the fermentations kinetic.

Tableau II – Paramètres indicatifs de la vitesse des fermentations.

Vintage	Tª Ferm.	Tests	Yeast formed* (cfu/ml x 10^6)	Ethanol (% vol/vol)	Volatile acidity (g/l)	Residual sugar (g/l)
1996	18º	Control	147	14,6	0,62	2,39
		Yeast hulls	172	14,4	0,60	2,23
	30º	Control	85	12,9	0,69	22,8
		Yeast hulls	89	13,4	0,61	16,8
1997	18º	Control	14	9,33	3.88	48,8
		Yeast hulls	19	11,4	2.33	9,90
1998	18º	Control	122	12,5	0,55	2,20
		Yeast hulls	126	12,4	0,46	2,13

Data are the average of three replications
* Maximun yeast viable counts produced during fermentation (millions colony forming units per ml)

TABLE III
Frequency of different strains of yeast identified in the vigorous stage of fermentation in the four vintages.

Tableau III - Fréquence d'apparition des différents souches de levure identifiées dans la phase stationnaire de la fermentation dans les quatre vinifications.

Clones	1996 (18ºC)		1996 (30ºC)		1997 (18ºC)			1998 (18ºC)		
	Control	Yeast hulls	Control	Yeast hulls	Clones	Control	Yeast hulls	Clones	Control	Yeast hulls
A	60	90	30	70	I	10	50	a	50	50
B	10		10		II	10		b	10	30
C	10	10			III*	70	10	c	20	
D	20				IV*	10		d	20	
E			10		V		20	e		20
F			10		VI		10			
G			20	10	VII		10			
H			10	10						
I			10							
J				10						

*Non-*Saccharomyces* strains

species at the end of the exponential stage was detected with frequencies of 80 p. cent (clones III and IV). The yeast hull addition caused an important change in the distribution of wild yeasts: the non-*Saccharomyces* strains practically disappeared, decreasing from 80 p. cent to 10 p. cent, whileclone I (*S. cerevisiae*), increased from 10 to 50 p. cent. This change to the growth dynamic could explain the improvements in fermentation when the hulls were added. The data from 1997 shows that the reason for the stuck fermentation that year was a microbial imbalance, meaning that the normal succession of yeast species did not happen, in agreement with BISSON (1999). For 1998, the effect of yeast hull addition on the clonal distribution of *Saccharomyces cerevisiae* can also be seen. Strain « b » increased from 10 to 30 p. cent, other strains did not appear and the Clone « e » was present.

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