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Study of the Lipidic and Proteic Composition of an Industrial Filmogenic Yeast with Applications as a Nutritional Supplement

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The lipid and protein contents of yeast strains that form "flor velum" during the aging of sherry wines have been studied during their fermentation and "velum" phases. The same analyses were carried out on two other strains that do not form velum (fermentative strains). The results show a high lipid content in velum yeast during its two phases. This strain changes its lipidic components while passing from the fermentative to the velum phase, with palmitic, palmitoleic, and stearic acid concentrations decreasing, while the oleic, behenic, and lignoceric acid concentrations increase. Furthermore, a higher proteic content can be seen during the filmogenic stage of velum yeast as compared to the fermentative stage of this strain. A well-balanced distribution of amino acids is observed, which includes all essential amino acids. The sulfurated amino acids are shown to be the most limited, and a high quantity of lysine has been detected. Finally, the values of PDCAAS (Protein Digestibility Corrected Amino Acid Score) and MEAA (Modified Index of Essential Amino Acids) of this strain make it recommendable for dietary uses.

KEYWORDS: "Flor" yeast; lipidic composition; protein content; nutritional value

1. INTRODUCTION

The production process of sherry wine is characterized by the action of certain yeasts, commonly denominated by what are known in winemaking vernacular as "flor velum" yeasts. Once the alcoholic fermentation of the must has finished, these yeasts develop spontaneously on the wine surface and form a film. This film prevents oxidation of the wine through oxidative metabolism and is the primary agent that produces the unique organoleptic characteristics of sherry wines. This microbiological phenomenon is denominated as the biological aging of wine and is the most important stage in the industrial production process. Flor yeasts belong to the *Saccharomyces cerevisiae* genus, although they are physiologically different from typical fermentative yeasts (*24, 35*), which are unable to grow aerobically in wine.

During aging, the metabolic activity of flor yeasts is greatly increased as a consequence of the shift from anaerobic to aerobic metabolism. This biochemical change results in partial consumption of many fermentation products, including ethanol, glycerol, acetic acid, and free amino acids, and the production of other components, such as acetaldehyde, 2,3-butanediol, and ethyl lactate (*16*).

To develop on the surface of the previously fermented medium, flor yeasts must reconvert their enzymatic characteristics and adapt to the new aerobic conditions to handle an atmosphere with a high alcohol content. It has been noted that ethanol not only has a role as a source of energy in these flor yeast processes but also can be incorporated into the microorganism as part of the cellular substance (*36*).

Genetic research on industrial *S. cerevisiae* yeast strains has yielded data indicating that these yeasts are capable of rapidly adapting to the special environmental conditions that are found in the industrial production process (*27*). Other studies have shown that the genetic background of the industrial flor strain is very similar to a sequenced *S. cerevisiae* strain that does not form velum. However, a large part of the flor yeast genome shows evidence of genomic rearrangements that are reflected in the DNA copy number changes observed (*22*). Such rearrangements produce amplified chromosomal segments that result in an increased expression of certain genes located within the amplicons, producing the physiological characteristics of the flor yeast. According to Benítez et al., the genes involved in tolerance to ethanol are different in different strains, so genes coding for membrane functions could be responsible for an increase in the tolerance to ethanol. In this sense, the mitochondrial genome is directly involved in the ethanol tolerance of flor yeast (*8*).

Several changes in cellular fatty acids in *S. cerevisiae* (capensis) during the fermentation and flor velum formation have been reported (*2*). Fatty acids having 16 carbon atoms are prevalent in the cells during the exponential growth phase in fermentation, with significant quantities of decanoic, lauric, and myristic acid present. These findings suggest a high capacity

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for biosynthesis in these yeasts. The ability to rise to the surface of the liquid and develop as a film is linked to cellular changes, such as an increase in size or changes in cellular density and hydrophobicity (*25*). This increase in hydrophobicity could result from an increase in the concentration of cellular lipid components (*17*). Furthermore, the adaptation of the yeasts to flor film formation in presence of oxygen also determines the increase in oleic content (*37*).

The use of nutritional supplements is becoming increasingly common and is growing at a rapid pace, and there are certain commercial nutritional supplements that include brewer's yeasts in their composition. For some time, flor yeast has been of interest to researchers; however, studies related to the chemical–nutritional characteristics of these strains have not been published. These characteristics could be very interesting from a human health and nutrition perspective and may also expand their utility in the oenological industry.

To assess the nutritional valuation of a food, it is only necessary initially to determine the protein and essential amino acid content of this food (*34*). Eight amino acids are generally regarded as essential for humans: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine (*41*). Essential amino acids are so-called not because they are more important to life than the others but because the body does not synthesize them, making it essential to include them in one's diet to obtain them.

The proteins act as a nitrogen source for the synthesis of the nonessential amino acids, which humans are able to synthesize. Moreover, a low sulfurated amino acid content usually limits the nutritional value of proteins. In general, the level of protein of nutritional quality is smaller when the amount of limiting amino acid is low. The synthesis of proteins stops when these amino acids are exhausted, and the rest of the amino acids are used in other metabolic pathways that involve deamination. Thus, these compounds are of biological importance since they determine the net use of proteins by the human organism.

A food that presents a well-balanced profile of limiting amino acids is a good candidate for inclusion in diets. For this reason, the concept of a chemical score was proposed to analyze the comparative quality of different proteins (*9*). Thus, the ratio of the essential amino acid that shows maximum deficit in a given protein to the quantity that is represented in a reference protein is denoted as the chemical score.

However, chemical analysis of essential amino acids is not an absolute measure of the nutritional quality of a protein (*34*). This is mainly due to the limitations imposed by the digestibility of the protein. It is sometimes the case that an existing amino acid is not liberated during physiologic digestion or it is not available in a usable form. Thus, there are several factors that affect the digestibility of a protein (*15*), such as its structural conformation, its interaction with other compounds that originate from the animal or vegetable source, and the type of processing that it is subjected to, including additives. In spite of the aforementioned inconveniences, recent observations indicate that the chemical score, when corrected for by digestibility values, presents good correlations with the results of human feeding biological experiments.

To evaluate the nutritional composition of velum yeast, the lipid and protein contents have been studied in both the fermentative and the velum phases. To make this study more complete, the digestibility, chemical score, PDCAAS, and MEAA index have also been calculated. For reference, the same analysis has been carried out in two other common fermentative yeasts.

2. MATERIALS AND METHODS

2.1. Yeast Strains. An autochthonous yeast strain capable of filmogenic growth was used in this study (velum yeast). This strain was isolated from the biological aging system of a winemaking company in the Sherry region (Jerez de la Frontera, Spain). It was later identified as *S. cerevisiae* (beticus), following the standard identification protocol for yeasts (*6*).

In addition, two other yeast strains that do not form velum were studied. One, an industrial fermentative strain (oenological use) that was also classified as *S. cerevisiae*, was provided by the laboratories of another winemaking company in the same region. The other, a commercial strain used for alimentary purposes (Santiveri, Spain), was also identified as *S. cerevisiae*.

2.2. Biomass Production. For biomass production of all strains (except for the commercial alimentary strain), multiple 2 L fermenters loaded with high-quality grape must were used and inoculated with small quantities of each strain from agar slopes. The must was always extracted from the Palomino Fino variety of mature grapes, which is the variety normally used in the yeast-producing industry. Successive fermentations were carried out every week, taking 100 mL of each previously fermented media and inoculating it into new fermenters with fresh media. All of the fermentations (three for each strain) were carried out at 22 °C and 100 rpm in rotary shakers.

At the end of each fermentation batch, the media from the oenological fermentative strain (which does not form velum) were collected and centrifuged (12000*g*, 5 min). The concentrate was dried in Petri dishes at 110 °C until it reached a constant weight, and the dry residue was frozen and stored at −22 °C until use.

In the case of the velum strain, at the end of each fermentation batch, the alcohol concentration of the medium was corrected from 12 to 15% (v/v) with ethanol. This modification of media was carried out to mimic the culture conditions usually employed in the flor velum industrial growth process. The modified media were distributed into 50 cm × 35 cm × 35 cm PVC receptacles suitable for velum formation. These receptacles were incubated at 22 °C without agitation and in total darkness for 21 days. During the incubation, the velum growths were observed daily.

In all cases, the velum development started around the eighth day of incubation, when some biomass was observed floating on the liquid surface. Later, after a period of approximately 12 days, the velum formed a thick layer covering the entire liquid surface. During the following days, this layer became thicker and more wrinkled. Finally, on the 21st day, one sample from each fermenter was collected for analytical determinations. If the velum remained under incubation without restoration with fresh medium, the Biofilm color changed progressively from white or clear yellow to ochre or brown, indicating the evolution of oxidation phenomena.

The composition of the biomass collected for analysis in each case at the end of the fermentations or cultivations was determined after centrifuging the resulting wine and washing the separated biomass three times with demineralized water. The centrifugations were carried out in all cases at 12000*g* for 5 min. In the case of the commercial alimentary strain, the sample was previously rehydrated. The moisture content of the obtained yeast samples was determined by drying 5 g of sample at 110 °C until a constant weight was reached, according to the AOAC method (*4*). The following analytical determinations were expressed in terms of the dry weight of yeast.

2.3. Lipids Determination. Studies carried out with flor velum yeast sediments demonstrated that the best lipid extraction was achieved with water-saturated butanol (*18*), as previously proposed (*30*). This method was therefore chosen for lipid extraction of the wine yeasts in this study. Because polar lipids such as glycolipids or phospholipids cannot be extracted with relatively nonpolar solvents like hexane, diethyl ether, or chloroform, it was necessary to use solvents with greater polarity.

The first extraction was carried out with water-saturated butanol, using 1 g of sample and 10 mL of solvent. This mixture was magnetically stirred for 30 min and then centrifuged at 12000*g* for 5 min. The extraction process was repeated twice more, and after the micture was centrifuged, the organic phases were combined in a

previously tared tube. The solvent was removed on a rotary evaporator, at a temperature below 40 °C, to turn the extracted lipids into a residue. The total lipid weight was determined by gravimetric methods.

2.4. Fatty Acids Analysis. The qualitative and quantitative determination of yeast fatty acids was carried out by determining the corresponding methyl esters using GC (gas chromatography); these derivatives had lower boiling points than the acids, thus allowing analysis by this technique.

Prior to identification and quantification, the lipidic fraction was treated with sulfuric acid in methanol solution to obtain the methyl esters (*5*). According to this method, the lipidic extract was deposited in a tube (25 mL capacity) with a threaded top (fitted with a Teflon membrane), and 10 mL of a sulfuric acid solution in 1% (v/v) methanol was added.

A nitrogen current was flushed through the tube for 5 min, and the sample tube was hermetically sealed and warmed at 50 °C for 10 h. The methyl ester solution was allowed to cool, concentrated on a rotary evaporator, and extracted by adding 3 mL of hexane and 1 mL of water. The upper layer was agitated and removed. The extraction was repeated by adding the same quantities of hexane and water, and the final hexane solution was evaporated to dryness under a current of nitrogen and was redissolved in 200 mL of benzene.

The separation and identification of fatty acid methyl ester were carried out by GC using a Hewlett-Packard 5890 gas chromatograph in conjunction with a model 3390A Integrator. The stationary phase was a Supelco Wax 10 M column (30 m × 0.53 μm i.d.), with hydrogen at a flow rate of 4.6 mL/min as the mobile phase, and a temperature control program of 100−240 °C at a rate of 4 °C per minute. The injector temperature was 200 °C, and the detector temperature was 250 °C, with a 1 μL injected sample. The standards were supplied by Sigma (37H8395 reference) assay 99%. Heptadecanoic or margaric acid (C17:0) was used as the internal standard. The quantitative determination was based on the principle that the quantity of each of the separated components in the mixture was proportional to its peak area in the chromatogram.

2.5. Amino Acids Determination. First, the total protein content of the samples was determined with an automatic protein analyzer (Electric Foss, model Macro-N Elemental) based on the oxygen combustion principle. Later, the samples were hydrolyzed in HCl (6 N) with phenol 1% (v/v), and a derivatization of the resultant amino acids was carried out.

For the derivatization of the standards and samples, we used the AccQ-fluorine reagents kit (Waters), following the method recommended by this supplier (AccQ-Tag Manual). The amino acid standards were supplied by Pierce (Amino Standard Acid H).

High-performance liquid chromatography (HPLC) equipment was used for the determination and quantification of amino acids, including a Waters 600 pumps, a fluorescence detector (Waters 474), and a reverse phase column (AccQ-Tag 3.9). The areas under the registered peaks were automatically integrated and compared with the areas obtained for the standards (*28*). The results are expressed in grams of amino acid/100 g protein.

2.6. Nutritional Analysis. The "in vitro" digestibility test was carried out by hydrolysis with the pepsin−pancreatin system (*1*). The total nondigested protein was determined by the automatic protein analyzer, and the digested protein was determined by the difference, with the results expressed as a digestibility percentage. The chemical score of proteins from each sample was also calculated (*9*). The PDCAAS was determined according to the method recommended by the FAO/WHO (*13*), and MEAA was determined using the procedure described by Oser (*29*) and modified by Mitchell (*26*).

The results obtained were statistically analyzed with Statgraphics 3.1 software (Statistical Graphics Corp., Rockville, MD), and the data were expressed as means ± standard deviation (SD) (statistical significance degree $\alpha < 0.05$).

3. RESULTS AND DISCUSSION

Figure 1 shows the results of the global lipidic contents of the yeast strains under investigation. It can be observed that the velum yeast in the fermentative phase has a higher lipidic content than the fermentative strains, increasing by 34.9 and 56.7%, respectively. Furthermore, velum yeast increases its lipid content by 10.3% when passing from the fermentative to the velum phase.

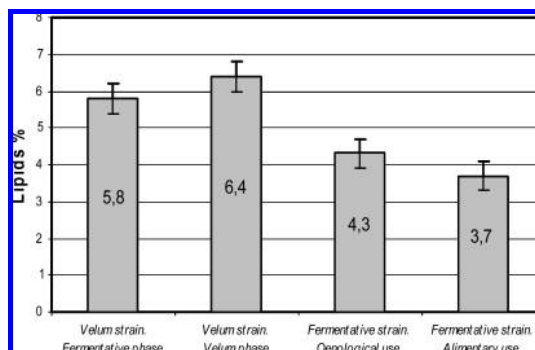

Figure 1. Global lipidic content of the different strains (%) in terms of yeast dry weight (means ± SD; $\alpha < 0.05$).

Ethanol is the principal stress factor for yeasts during the fermentation stage. Some authors have suggested a relationship between tolerance of the yeast to a high alcoholic grade and their internal reserves of lipids and carbohydrates (*20*). As a consequence, it seems reasonable to think that velum yeast had more lipidic content than the fermentative strains and that velum yeast increases its total lipidic content when passing from the fermentative phase to the velum phase to handle the high alcoholic grade of the filmogen medium (15% v/v vs 12% v/v).

The global lipidic contents for the nonvelum-forming strains are similar to those found in a *S. cerevisiae* strain used for alimentary purposes (3.15%) (*38*). Data concerning the lipidic content of velum yeasts, however, cannot be compared to previous data since no previous literature on this subject is available. The only comparable data concerning the lipidic content of velum yeasts comes from a study carried out on sherry lies (*18*). Lies are the remains of dried fermentative yeasts that are found in deposits along with other precipitates and fermentation residues. The lipidic content of these deposits is on the order of 5%. This value is slightly higher than that shown by the fermentative strains, although it is lower than the value detected in active velum cells at any stage.

The distributions of different fatty acids determined for the different strains are shown in **Table 1**. In this study, those fatty acids with an odd number of carbon atoms were not determined (e.g., C15:0, C15:1, C17:0, and C17:1), since it is known that although they can be found in other yeast species, they only appear in *S. cerevisiae* in trace concentrations (*18*).

In general, the majority of fatty acids in all of the strains studied were observed to be palmitic, palmitoleic, stearic, and oleic acids. Nevertheless, velum yeast in both phases (fermentative and filmogen) clearly has a greater oleic acid percentage than nonfermentative yeasts (both oenological and alimentary strains), approximately 30% in comparison to 10%. In velum yeast, on the other hand, a lower linoleic acid level than in the fermentative strains was found (1−2% in comparison to 3−4%) as well as a lower erucic acid level (0.5 vs 1.8%), although these differences are much less important.

Most significant differences in the fatty acid composition of the velum yeast when passing from the submerged culture (fermentative phase) to the filmogen culture (velum phase) are shown in **Figure 2**. The amounts of palmitic, palmitoleic, and stearic acids are observed to diminish when passing to the velum phase. However, the oleic, behenic, and lignoceric acid amounts increase during the same metabolic transformation. In particular,

Table 1. Relative Concentration of Fatty Acids (% w/w) in Terms of Yeast Dry Weight (Means ± SD; α < 0.05)

fatty acid (%)		velum strain		fermentative strain	
		fermentative phase	velum phase	oenological use	alimentary use
C8:0	caprylic acid	0.06 ± 0.01	0	0.07 ± 0.02	0.03 ± 0.01
C10:0	capric acid	0.13 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	1.06 ± 0.04
C12:0	lauric acid	1.31 ± 0.10	0.49 ± 0.04	5.76 ± 0.24	1.35 ± 0.08
C14:0	myristic acid	0.44 ± 0.08	0.99 ± 0.10	2.15 ± 0.12	0.88 ± 0.04
C16:0	palmitic acid	25.71 ± 0.21	20.39 ± 0.21	29.72 ± 0.21	34.45 ± 0.21
C16:1	palmitoleic	24.14 ± 0.23	11.67 ± 0.15	25.04 ± 0.26	31.08 ± 0.15
C18:0	stearic acid	11.25 ± 0.09	6.25 ± 0.34	13.65 ± 0.18	10.61 ± 0.31
C18:1	oleic acid	27.16 ± 0.13	34.30 ± 0.26	12.18 ± 0.18	9.57 ± 0.20
C18:2	linoleic acid	2.07 ± 0.02	0.51 ± 0.08	3.17 ± 0.12	4.47 ± 0.11
C18:3	linolenic acid	0.82 ± 0.04	0.09 ± 0.02	1.18 ± 0.09	0.58 ± 0.02
C20:0	arachidic acid	2.97 ± 0.03	0.85 ± 0.04	0.69 ± 0.08	0.18 ± 0.04
C22:0	behenic acid	0.65 ± 0.03	11.57 ± 0.11	1.43 ± 0.15	0.33 ± 0.09
C22:1	erucic acid	0.30 ± 0.01	0.68 ± 0.05	1.70 ± 0.05	1.82 ± 0.12
C24:0	lignoceric acid	3.01 ± 0.05	12.17 ± 0.14	3.21 ± 0.14	3.58 ± 0.14
saturated fatty acids (%)		45.5	52.7	56.7	52.5
unsaturated fatty acids (%)		54.5	47.3	43.3	47.5

the velum strain in the aerobic phase is the only one that presents high values of these last two acids, which have not previously been detected in such high quantities in any other strain. In this regard, the characteristic that best identifies the velum strain in the aerobic phase is the high amount of these long-chain fatty acids.

In general, the data obtained in this study agree well with those presented in previous studies (*3*), which found the predominant fatty acids to be the palmitic, palmitoleic, stearic, and oleic acids in studies of a *S. cerevisiae* fermentative strain. However, it has been determined that fatty acids having 16 carbon atoms prevail in the yeast cells during the fermentative phase (*2*). In that study, the main fatty acids in fermentative strains were found to be palmitic, stearic, and oleic acids. These authors also found the main fatty acids in a velum yeast strain during the fermentative phase to be oleic and stearic acids.

Palmitoleic acid is usually observed to be quantitatively the most important fatty acid in yeasts (*33*), regardless of the culture type in which the strains have grown. Ribes et al. (*33*) add that, in aerobic conditions, this fatty acid can end up representing more than 50% of the total fatty acids (both saturated and unsaturated). In general, the most abundant fatty acids in yeasts were observed to be the linear chain acids having 16 and 18 carbon atoms (saturated and unsaturated) (*32, 33*).

In relation to the lipidic composition of the *S. cerevisiae* yeast membrane, several authors have observed an increase in the yeast's saturation index (*3*) and a correlation between ethanol tolerance and the increased degree of fatty acid unsaturation of membrane lipids in *S. cerevisiae* (*40*). A decrease in the level of palmitic acid was detected in these cultures, and this was

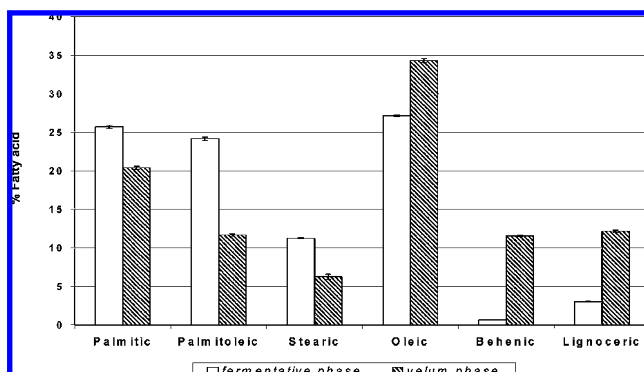

Figure 2. Main changes in fatty acid composition (%) during the two phases of velum yeast (means ± SD; α < 0.05).

accompanied by a proportional increase in the oleic acid concentration.

As described above, this study found that a decrease in the proportion of palmitic acid occurs in velum yeast when passing from the fermentative (12% v/v alcohol grade) to the aerobic phase (15% v/v). A corresponding increase is detected in the oleic acid proportion. Despite this increase, in this work, we have found a small increase in the percent of unsaturated fatty acids, principally due to the palmitoleic acid observed decrease.

It seems likely that the presence of ethanol activates these metabolic differences. In this sense, the cell modifies the membrane composition in fatty acids to minimize the effects of the membrane fluidity that the ethanol causes (*21*). This is a consequence of the replacement by ethanol of the water molecules associated with the polar groups. Other studies have also suggested that the adaptation of yeast to ethanol happens because of changes in the lipidic composition of the cell membranes (*10*). Another possible reason for this metabolic change could be the difference in available oxygen concentration when yeast passes from the fermentative to the aerobic phase.

The total protein content and the relative concentration of amino acids for the strains studied are presented in **Table 2**. As shown, an incremental difference in the protein content of the velum yeast is detected when passing from the fermentative phase to the velum phase. However, the fermentative yeasts present different values, with the protein content of the winemaking strain being lower than the content of the alimentary strain. The protein content of this last strain is slightly higher than those reported (*31, 38*), around 45% in several alimentary strains of *S. cerevisiae*. On the basis of these data, we can propose that the velum strain (during the velum phase of culture) is a reasonably good source of protein in comparison with other alimentary sources.

In general, the amino acid profiles found in the four samples of yeast studied show a very well-balanced distribution in comparison with the recommendation proposed by the FAO (*13*). This result is in agreement with the fact that many yeast proteins that carry out common or specific functions usually display similar amino acid patterns. Moreover, we have also found in our results that the sulfurated amino acids are the most limiting in the protein composition, something that is true in other microorganisms (*23*).

High values of lysine were also noticed in all of the strains studied, values that were greater than the FAO standard (*13*). This result is very interesting because, bearing in mind that this

Table 2. Total Protein Content (% w/w in Terms of Yeast Dry Weight) and Relative Concentration of Amino Acids (Grams of Amino Acid/100 Grams of Protein) of Strains (Means ± SD; α < 0.05)

	velum strain		fermentative strain		
	fermentative phase	velum phase	oenological use	alimentary use	FAO/WHO pattern[a]
total protein (%)	38.63 ± 0.08	47.03 ± 0.27	39.44 ± 0.32	48.00 ± 0.01	
ALA	5.90 ± 0.04	5.70 ± 0.27	6.07 ± 0.38	6.74 ± 0.04	
ARG	5.91 ± 0.32	5.45 ± 0.11	4.93 ± 0.13	5.66 ± 0.03	
ASP	10.35 ± 0.43	9.89 ± 0.58	10.01 ± 0.27	9.43 ± 0.02	
GLU	11.01 ± 0.54	11.69 ± 0.46	10.50 ± 0.34	12.07 ± 0.03	
GLY	5.20 ± 0.52	5.22 ± 0.38	5.11 ± 0.32	4.90 ± 0.01	
HIS	2.42 ± 0.15	2.32 ± 0.13	2.37 ± 0.05	2.57 ± 0.01	1.9
ILE[d]	4.84 ± 0.73	4.82 ± 0.38	4.74 ± 0.53	4.98 ± 0.04	2.8
LEU[d]	7.00 ± 0.18	6.68 ± 0.22	6.75 ± 0.09	6.66 ± 0.03	6.6
LYS[d]	8.75 ± 0.27	9.01 ± 0.37	8.71 ± 0.17	8.63 ± 0.06	5.8
MET + CYS	1.84 ± 0.69	1.88 ± 0.32	1.62 ± 0.22	1.76 ± 0.07	2.5
PHE[d]	4.47 ± 0.85	4.46 ± 0.41	4.96 ± 0.33	5.39 ± 0.08	6.3[b]
PRO	4.22 ± 0.11	4.93 ± 0.97	4.00 ± 0.13	4.77 ± 0.05	
SER	6.76 ± 0.25	7.18 ± 0.31	6.85 ± 0.61	5.72 ± 0.03	
THR[d]	6.44 ± 0.39	7.18 ± 0.09	6.39 ± 0.05	5.07 ± 0.09	3.4
TYR	2.61 ± 2.32	3.61 ± 0.97	3.66 ± 0.22	3.32 ± 0.06	
VAL[d]	8.81 ± 0.65	7.40 ± 0.25	8.96 ± 0.61	7.53 ± 0.09	3.5
other AAs (TRP + ASN + GLN)[d]	3.47 ± 8.5	2.58 ± 6.26	4.37 ± 4.54	4.8 ± 0.78	1.1[c]

[a] FAO/WHO pattern (*15*). [b] PHE + TYR. [c] TRP. [d] Essential amino acids (*41*).

Table 3. Values of the “in Vitro” Digestibility, Chemical Score, Protein Digestibility Corrected Amino Acid Score (PDCAAA) Index, and Modified Index of Essential Amino Acids (MEAA) Index for the Proteins of Each Strain (Means ± SD; α < 0.05)

strain	digestibility (%)	chemical score	PDCAAS index	MEAA index
velum strain, fermentative phase	82 ± 3	73.6	0.60	88 ± 3
velum strain, velum phase	83 ± 3	75.2	0.62	90 ± 3
fermentative strain, oenological use	82 ± 3	64.8	0.53	91 ± 3
fermentative strain, alimentary use	83 ± 2	70.4	0.59	87 ± 2

amino acid is restrictive in many cereals, the yeasts studied could be proposed as an appropriate complement to improve the quality of these kinds of alimentary sources.

Apart from having a good essential amino acid profile, an important characteristic for any protein used for alimentary purposes is good digestibility. Although the amino acid content is the main indicator of the quality of a protein, its true quality also depends on how useable these amino acids are for the human organism. Thus, the digestibility affects overall proteic quality.

The results obtained for the different studied strains with regard to digestibility factors are shown in **Table 3**. These values are similar to those found for whole cells of *S. cerevisiae*, in the order of 83 (*38*) and 79% (*39*). These values are higher than those for millet (79%) and lower than those for wheat (86%), corn (85%), or soya flour (86%) (*12*).

The chemical score values for the different yeast proteins studied (based on methionine and cysteine) are also presented in **Table 3**. The value obtained for the velum yeast is superior to that of the wheat, corn, or rice values (chemical score of 40, 43, and 59, respectively).

The PDCAAS index (Protein Digestibility Corrected Amino Acid Score) is also used to evaluate the quality of a protein. This index is based on a protein’s amino acid content, its digestibility, and its capacity to provide the essential amino acids in appropriate quantities for human needs. This index substitutes the protein efficiency ratio (PER), which has been the accepted method of evaluating the quality of the proteins since 1919 (*14*). The results obtained for the studied strains are shown in **Table 3**. As can be deduced, the deficit of sulfurated amino acids (methionine and cysteine) and the digestibility of the proteic source have strongly determined these values.

Finally, the modified index of essential amino acids (MEAA) is a procedure that offers values very close to those obtained in human feeding biological experiments. For example, an MEAA index of 85 has been determined for *Spirulina* microalgal (*7, 11*) and a value of 92 for casein (*19*). The resultant values of this index for all studied strains are available in **Table 3**.

In short, the total lipid content of the filmogenic strain, its proteic composition (with all of the essential amino acids), and the high in vitro digestibility obtained for the flor velum yeast provide support for this yeast being deemed very similar or slightly superior to the common alimentary yeast and, so, quite appropriate as a nutritional complement to other off-balanced vegetable sources. Finally, the general composition of the flor velum yeast and the different alimentary ratios measured show good possibilities for its use as a nutritional supplement.

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