ISSN 1330-9862
(FTB-2997)

minireview

In taberna quando sumus: A Drunkard's Cakewalk Through Wine Proteomics

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Received: January 13, 2012
Accepted: February 28, 2012

Summary

Analysis of white and red wine trace proteomes *via* capture with combinatorial peptide ligand libraries (CPLL) is reported here. Most of the alcoholic beverages tested (all of Italian origin) were found to contain only traces of casein (on average from 20 to 60 μg/L, with a detectability of as low as 1 μg/L) and not any grape protein any longer, as they had been fined with bovine casein (surprisingly also red wines for which the typical fining agent is egg albumin). However, analysis of untreated white wine (Recioto, from Garganega grapes in the Veneto region) *via* CPLL capture indeed permitted to detect close to 100 unique gene products from the grapes, suggesting the possibility of proteotyping *grand crus*, *i.e.* those aged, high quality wines that should not be treated with fining agents. Thus the CPLL technique could become a formidable tool for traceability of beverages in particular and of foodstuff in general. For trace protein analysis, a new, most powerful CPLL methodology emerges: capture at pH=2.2 in 0.1 % trifluoroacetic acid (TFA) under the conditions mimicking reversed-phase mechanisms of adsorption.

Key words: alcoholic beverages, trace proteome, combinatorial peptide ligand libraries, food traceability

Introduction

Since Noah's colossal drunkenness wines have been one of the preferred beverages among all peoples and all nations. In particular, in Italy, wine production had become almost a religious event already in Roman times, to the point at which Italy was dubbed *Enotria tellus*, *i.e.* the wine-producing territory par excellence. Italian wines have been 'celebre' since antiquity, starting perhaps with the highly famous Falernum, a most costly wine appreciated by Emperors and patricians in ancient Rome (Julius Caesar spent a fortune on this wine in the year 47 BC to celebrate his victories described in *De Bello Gallico*). They were so fond of wines that they even tried to take them along in the nether world. A smart tourist visiting Rome with a well-annotated Baedeker might shun from the Vatican Museums or the Coliseum, but will surely notice, upon visiting San Lorenzo Fuori le Mura, on the left side of the entrance portico, a superbly sculptured sarcophagus, dubbed *Sarcofago degli Amorini Vendemmianti* (of the grape-picking putti; Fig. 1). It is a feast for the eye: some putti are seen climbing the vines and competing with gluttonous peacocks for the same grapes they are harvesting and depositing in wicker baskets. This love for wines continued well into the Middle Ages. There are rumours that in the year 1111 AD the German bishop Johannes Defuk (Johann Fugger in German) made a pilgrimage to Rome, taking the well-known (in those days) Via Francigena. He had a friar scouting the territory to mark the best tavern in each town or village, in which, in the evening, they could rest drinking wine. The tavern would be marked by the friar with the secret symbol '*est*' (the verb 'to be' in Latin, meaning here it is). At one point of the pilgrimage the tavern had been marked by a 'triple *est*'. If you visit the town of Montefiascone (close to Rome) you will find a

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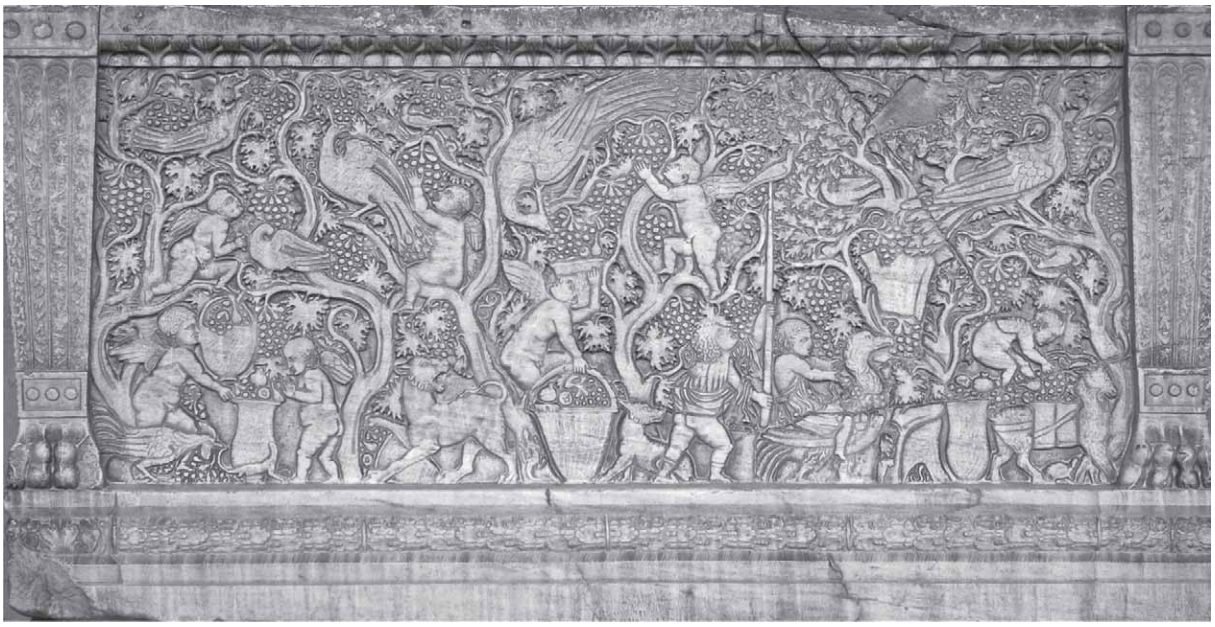

Fig. 1. *Sarcophago degli Amorini Vendemmianti* (of the grape-picking putti) in the portico of San Lorenzo Fuori le Mura in Rome (photo courtesy by P.G. Righetti, personal collection)

white wine with the label *Est!*, *Est!!*, *Est!!!*, in memory of this event. Even throughout the Renaissance Popes and Cardinals appreciated a lot these wines, as nicely told in the detective and gastronomic novels by Barrière (*1*).

In present times, the commerce of alcoholic beverages represents a very large proportion of the global food market and it is in continuous expansion. Just as an example, Table 1 gives a 2009 survey of the global world-wide wine production. It can be appreciated that Italy has taken the lead over France, with 50 million hectolitres produced. Moreover, the first three countries (Italy, France and Spain) produce just about 50 % of the world global and 80 % of the total European productions. Additionally, Table 2 gives a survey (from 2003 to 2007) of the wine consumption (litres per head per annum) in several countries. Here too it is seen that France and Italy have an equal share of approx. 50 L *per capita*, closely followed by Portugal with approx. 45 L (although not reported in this table, it is almost embarrassing to us to note that the Vatican City State in 2009 was a leader with 70 L *per capita*). The most curious data are those of China: in 2005 the average wine intake for Chinese people at large was barely 0.7 L per year. It would be highly desirable, for producers and customers alike, to have an easy method for controlling the origin of these wines and to assess whether, in case of *grand crus* (*e.g.* Brunello di Montalcino, one of the wines that suffers from a great deal of counterfeited products released onto the market), they are truly from the origin stated on the label or have been counterfeited and are thus a fraud. The loss for both the producers and customers could be huge in the absence of proper controls. This problem is acutely felt, as recently addressed by Hamburg in an editorial in *Science* (2): 'Ensuring the safety and quality of food products has never been more complicated. Societies around the world face increasingly complex challenges that re-

Table 1. Wine statistics 2009

Country	Millions of hectolitres
Italy	50
France	46
Spain	35
USA	22
Australia	12
Argentina	12
Chile	10
Germany	9
all Europe	163
world globally	270

Table 2. Wine consumption (litres *per capita per annum*) from 2003 to 2007

Country	2003	2004	2005	2006	2007	Variation from 2003/%
France	56.6	54.8	55.0	53.8	52.1	–8.0
Italy	50.4	49.4	48.1	48.1	48.0	–4.1
Portugal	51.1	46.9	46.5	45.3	42.5	–16.8
Greece	27.8	29.8	32.3	28.8	29.7	+6.8
Spain	32.8	32.5	31.5	30.8	29.7	–9.5
Argentina	32.5	29.0	28.3	28.4	28.3	–12.9
Germany	25.2	26.4	26.8	27.7	27.5	+9.1
Uruguay	22.7	25.5	26.1	25.9	25.9	+14.1
Australia	21.1	21.7	22.3	22.3	22.9	+8.5
USA	7.3	7.5	7.7	7.8	8.0	+10.0
Japan	1.8	1.8	1.9	2.0	2.2	+18.0
Brazil	1.5	1.6	1.6	1.7	1.8	+15.0
China	0.6	0.6	0.7	0.8	0.9	+30.0

quire harnessing the best available science and technology on behalf of consumers... We must also develop new science to protect the safety of our food supply'. And the problem of food traceability (and of certifying its genuineness) is becoming more and more a serious challenge.

To complicate the matters further, modern wines might be quite different from those drunk by our ancestors. One of the main reasons is that the residual grape proteins, which survived the fermentation process, slowly aggregate leading to amorphous sediments or flocculates, causing turbidity. A haze or deposit in bottled wine indicates that the product is unstable, has a low commercial value and is therefore unacceptable for sale. For these reasons, it has become customary, especially in white wine production, to remove the residual proteins remaining in the finished product, so as to prevent haze formation and sediment in the bottled wines available for sales. Among the fining agents, one of the most popular is casein derived from bovine milk. However, caseins are also known as major food allergens and therefore, according to the Directive 2007/68/EC of the European Community (EC), 'any substance used in production of a foodstuff and still present in the finished product' must be declared on the label, especially if it originates from allergenic material. Due to the fact that caseins are nearly insoluble at the pH of white wines and that they form insoluble complexes with phenolic compounds, they are considered to be almost completely coagulated and thus eliminated by precipitation after treatment, so no wine maker has reported the presence of caseins in their fined product (although this mandatory labelling has first been postponed to the end of December 2010 and then extended until the end of June 2012, likely due to protest from wine producers). Yet, classical chemistry laws suggest that traces of caseins should remain even after their massive co-precipitation with residual grape proteins. Unfortunately, the official ELISA test of the EC has a sensitivity limit of 200 μg of casein per litre (*3*), in other words not enough to detect traces of it.

A technique that might help out of this impasse is the combinatorial peptide ligand library (CPLL) that, *via* its unique performance in enhancing and thus detecting the low-abundance proteome, might enable harvesting sufficient amounts of either trace additives or original grape proteins (for untreated wines) (for recent reviews see *4–9*). Its methodological aspects have also been described *in extenso* (*10*). We have in fact applied CPLLs to the analysis of white (*11*) and red (*12*) wines, as well as beers (*13*), in a number of articles that might go down to history as 'the drunkard's trilogy', second perhaps only to the famous Dante's trilogy. The present review will critically summarize these data (but not the ones of beer, this would be anathema in *Enotria tellus*!) and offer also some recent, unpublished findings.

Analysis of White Wines

In the first of our investigations, starting with white wines, as we suspected that the residual amounts of potential fining agents added (casein) could be rather minute, we decided to treat the entire content of each bottle of white wine (750 mL), a rather large volume to be processed by an analytical laboratory. Moreover, in order to recover the proteins from the CPLL beads in the smallest possible volume, barely 200 μL of ProteoMiner beads (the trade name of CPLLs, as sold by Bio-Rad, Hercules, CA, USA) were added to such large sample volume and protein adsorption was implemented *via* gentle shaking for 3 h at room temperature. The beads were then collected by filtration and washed twice with their respective buffer to eliminate the unbound protein excess. The captured proteins from each peptide library sample were then desorbed using a solution composed of 4 % sodium dodecyl sulphate (SDS) and 25 mM dithiothreitol (DTT) for 5 min under boiling conditions, as per Candiano *et al.* (*14*). The efficiency of the capturing process was tested at three different pH values, as recommended by Fasoli *et al.* (*15*): pH=3.3 (the actual pH value of most white wines); pH=7.2 (by adding 40 mM phosphate buffer) and pH=9.3 (*via* the addition of 40 mM Tris). In the final experiments, however, a modified, highly performing library, produced in our lab, was adopted and the best capture was accomplished at the most acidic pH investigated (pH=3.3). The results were well above our expectations: as shown in Fig. 2, as little as 1 μg of casein could be assessed in some analyzed wines. For the first time, we could demonstrate that our CPLL technique could: (*i*) harvest even minute quantities of proteins with quite high efficiency (in the case of our home-made CPLLs as high as 80 %); (*ii*) handle large sample volumes (up to 1 L) in a user-friendly manner (*e.g.* no need for on-column operations, which would require long periods of time to filter the entire liquid volume through, but a simple batch-adsorption protocol); (*iii*) allow signal amplification factors by more than 6000-fold (considering the ratio between the sample and CPLL bead

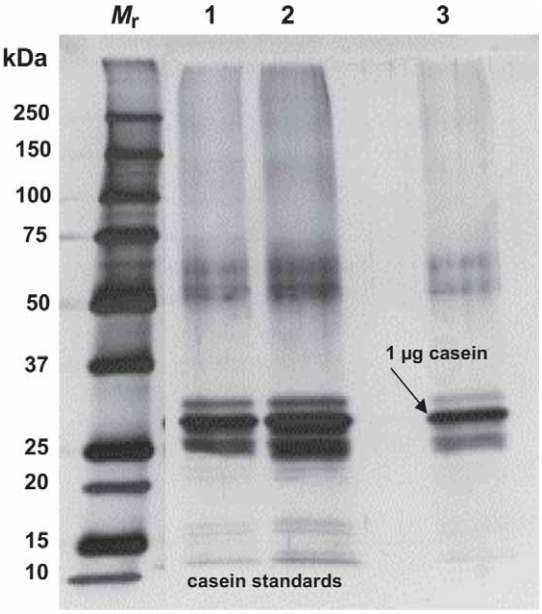

Fig. 2. SDS-PAGE of casein standards and a casein eluate with home-made CPLL beads in a wine-like mixture consisting of 12.5 % ethanol in water at pH=3.3 (with acetic acid) with the addition of 1 μg/L of casein. M_r: molecular mass standards; lanes 1 and 2: 2 and 4 μg of casein standards treated in conventional Laemmli buffer; lane 3: casein recovered from the wine-like mixture. Detection by silver staining (from Cereda *et al.* (*11*), with permission)

volumes, and taking into account the efficiency of the process, as well as the small elution volume, 100 μL), *i.e.* somewhere in between 3 to 4 orders of magnitude (*11*).

White Wines: The Debate Continues

The major result of the above investigation was that we were able to prove that the CPLL technique had a sensitivity 200 times higher than the current ELISA test, a non-negligible accomplishment. But there was more to it. Just as our paper (*11*) appeared at the web site of *Journal of Proteomics*, another one was posted at the web site of *Journal of Chromatography A* (*16*): in this latter paper, the authors stated 'when fined wine samples were considered, the lowest added concentration for which the peptide marker could be detected was 50 μg/mL' (the peptide marker referring to casein digests, as identified by mass spectrometry). Now, if we are not mistaken, this means that our CPLL treatment for harvesting and detecting minute traces of caseins in white wines (as well in red wines, as discussed below) has a sensitivity 50 000 times better than the mass spectrometry (MS) method of Monaci *et al*. (*16*) (it goes without saying that we too identified the captured caseins *via* MS). These authors, actually, continued in their search of traces of caseins in white wines and reported yet another method for tracing residual milk allergens, this time based on the use of a single-stage LTQ Orbitrap (Thermo Fisher Scientific, Prague, Czech Republic) MS instrument (*17*). Yet, the improvement in detectability was not spectacular, in their own words: 'minimum detectable added caseinate concentrations, *i.e.* those corresponding to response with signal to noise ratio *S/N*=3, were estimated between 39 and 51 μg/mL'. What is also disturbing, in their data, is that they can only find caseins which they add to wines, whereas we go shopping in supermarkets and analyse wines as we take them from the shelves, *i.e.* as commercialized by wine makers, without any prior knowledge on how such wines had been treated. There was a further evolution on this topic. Recently, Palmisano *et al*. (*18*) published an extensive investigation on grape proteins present in white Chardonnay. They adopted a multiplexed glycopeptide enrichment strategy in combination with tandem mass spectrometry in order to analyze the glycoproteome of this brand of white wine, thus identifying a total of 28 glycoproteins and 44 glycosylation sites. The identified glycoproteins were of grape and yeast origin. In particular, several glycoproteins derived from the grape, like invertase and pathogenesis-related (PR) proteins, and from the yeast were found after the vinification process. Bioinformatic analysis revealed sequence similarity between the identified grape glycoproteins and known plant allergens. This paper made headlines in the lay press, which placed a strong accent on the well known fact that about 7 % of the world wine drinkers lamented disturbances after sipping white wine, no doubt, concluding, thanks to these newly-found glycoprotein allergens. We were surprised in a way by this report, since, in our hands, all the white wines that we had investigated contained only traces of bovine casein, due to the well-known fining protocols adopted in the vast majority of vineyards around the world (although also other protocols are adopted, such as adsorption of grape proteins *via* bentonite treatment). After reading their manuscript carefully, we found out that this Chardonnay had been produced in the village of Turi (Puglia, Italy) by a local wine maker who had had strict orders not to treat the produced wine with any fining agent. In fact, all analyses were carried out within one month from the wine production to avoid any protein loss. It just so happened that one of the co-authors has a small wine production farm in the Veneto Region (outside Verona), so we set apart a few bottles of Recioto wine (a dessert wine made from partly dehydrated grapes left for longer periods on the plants) and of Garganega wine, a white table wine produced from the same grapes picked at ripening. Both types of wine were not treated with any fining agent and were also left to age in the bottle for just one month. The Recioto bottles were subjected to the same CPLL treatments illustrated in section Analysis of White Wines, with an additional capture at very acidic pH values (pH=2.2) in the presence of 0.1 % TFA (trifluoroacetic acid) as an ion-pairing agent, so as to mimic reversed-phase (RP) adsorption (here, of course, we used a special home-made library acting on the RP principle). The results are shown in Fig. 3: *via* CPLL treatment we can capture a large body of proteins at all pH values, spanning from approx. 10 up to 75 kDa, all of them in equilibrium with the sediment, which had been collected by centrifugation and dissolved directly in boiling Laemmli buffer. Additionally, it can be appreciated that the capture at pH=2.2 under RP conditions harvested a much higher quantity of proteins (at least three times as much as assessed by densitometry of the Coomassie-stained bands) and enriched particularly two bands at around 25–30 kDa, seen only as faint zones in all other SDS-PAGE tracks. All lanes were sectioned into 10 segments (from cathode to anode), protein digested and subjected to LTQ-XL linear ion trap mass spectrometry analysis. We could identify more than 100 grape proteins, a bountiful harvest never before reported in any investigation, once more proving the power of the CPLL technology (*19*). This might suggest that if one could obtain bottles of untreated wine, one could type them (especially the *grand crus*, *i.e.* prestigious, expensive wines) in order to protect them from fraudulent imitations. However, this 'proteomic signature' (proteotyping) might not be quite realistic, considering the following Italian scenario: (*i*) 350 DOC (denominazione di origine controllata, *i.e.* controlled designation of origin) wines, >35 DOCGs (denominazione di origine controllata e garantita, *i.e.* controlled designation of origin guaranteed), >120 IGTs (indicazione geografica tipica, *i.e.* typical geographical indication); (*ii*) there are in excess of hundred grape varieties in regular use and hundreds more in limited use; (*iii*) ampelographers talk about undiscovered treasures (biodiversity) that lurk in historic Italian vineyards.

Analysis of Red Wines

The next logical step was to extend our investigation to red wines, here too either to detect traces of fining agents or the entire grape-proteomic asset in non-treated samples. Knowing the Italian market, in reality we expected to find only traces of fining agents, which, in the case of red wines, should have been ovalbumin or

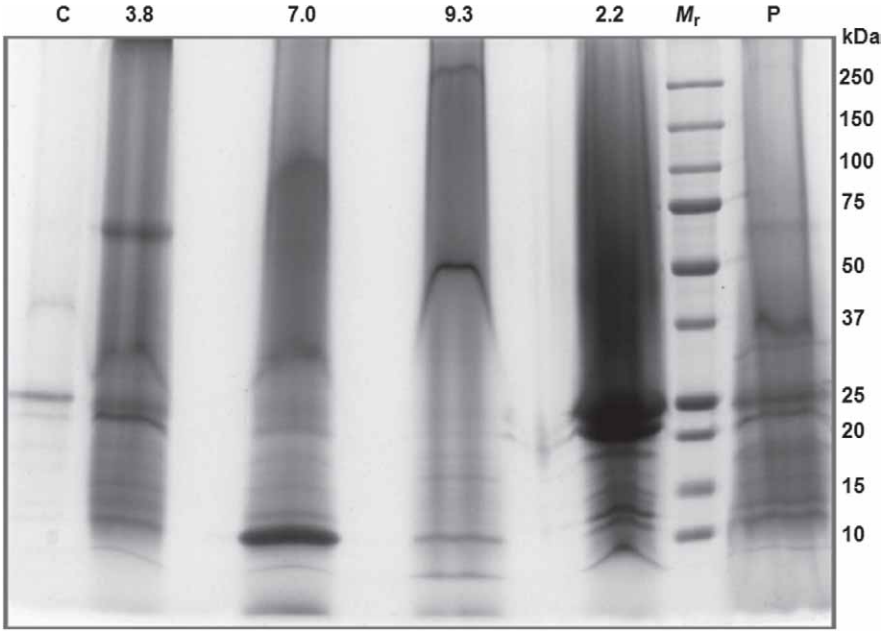

Fig. 3. SDS-PAGE profiling of Recioto wine (Garganega grapes). C: control, untreated wine; 3.8, 7.0 and 9.3: eluates of sequential captures of 750 mL of Recioto at pH=3.8, 7.0 and 9.3, respectively; 2.2: eluate of a special capture performed with homemade beads in 0.1 % TFA under reversed-phase adsorption conditions; P: precipitate dissolved directly in Laemmli buffer; M_r: molecular mass markers. Staining with micellar Coomassie Brilliant Blue (Fasoli *et al.*, unpublished)

entire egg white, since this was the customary treatment officially reported. We analyzed mostly Valpolicella from different producers around Chiari, in province of Brescia, and in Verona (all of them 2009 vintage), as well as aged (2006 vintage) Chianti from Tuscany. Here we had the first big surprise: all the red wines we analyzed did not contain traces of egg albumins but again of bovine caseins (*12*), especially α- and κ-caseins, with essentially no residual grape proteins, except for traces of thaumatin fragments. And then came the second big surprise: our data point out that adverse events must have happened in Northern Italy during the 2009 grape harvest, since most of the analyzed bottles of Valpolicella contained (albeit in traces) abnormal amounts of proteins originating from fungal infection, such as from *Botryotinia fuckeliana*, *Sclerotinia sclerotiorum* or *Aspergillus aculeatus*. This seems to be quite unusual and has not been reported, to our knowledge, by any other paper on wine proteomics (*3*,*20*), except by Cilindre *et al.* (*21*), with the proviso, though, that they infected their grapes on purpose with *B. fuckeliana*, just to see how the infection would affect the residual wine proteins. There is more on the analysis of red wines: recently, Tolin *et al.* (*22*) reported the finding of egg white proteins in red wines treated with this fining agent. According to these authors their gel-free MS method 'detects residual egg white proteins in red wines with high sensitivity'. However, it is not at all clear what this 'high sensitivity' is, since they state 'this allowed the detection of egg proteins in red wines fined down to 5 g/hL of commercial egg white preparation and also in the commercial red wine'. Now, if this amount were the residual amount left in red wine after treatment, this would mean a detection sensitivity of only 50 μg/mL, which cannot be taken seriously (in fact not any better than that reported by Monaci *et al.* (*16*,*17*) in detecting caseins in white wines)! Had we been able to find commercial red wine preparations fined with egg white, we have no doubt that, just as we reached a lower detection limit of a few micrograms per litre (*i.e.* ng/mL, in the region of low- to very-low abundance proteins sorely missed in the analysis of sera in search of disease biomarkers) for caseins, we would have had a similar sensitivity also in the case of egg white proteins.

A Possible Mechanism of Protein Survival in Beverages

What could be the possible mechanism for protein survival in alcoholic and nonalcoholic beverages? Perhaps a general survival mechanism begins to emerge, namely the fact that most likely the proteins that remain in a solution after the industrial processing are either small-size species or high-molecular-mass components, which have been degraded to smaller fragments possibly by proteases acting during the fermentation/industrial manipulations. A case in point is represented by our recent findings on the proteome of coconut milk (*23*), in which one of the major components detected is the 7S globulin. This macromolecule is a storage protein present in essentially all seeds. It has a theoretical M_r of 67 008 Da, yet we found it as one of the three major bands centred at 15–25 kDa in the SDS-PAGE profile of Fig. 3 (*23*). This means that this protein is not recovered intact but, either during homogenization of the coconut flesh or during the industrial processing, due to protease activation, it is cut into fairly large fragments. This seems to occur also for the other high-abundance proteins found in coconut milk, namely glutelin, which has a theoretical M_r of 42 811 Da but is also found in the same 15–25 kDa region. Interestingly, this could be a major event occur-

ring in general in the preparation of these beverages. Another example is represented by prunin, which is the major storage globulin of almonds. It is 551 amino acids in length, corresponding to a molecular mass of 63 321 Da. It is the major band we find in almond milk, yet it is not found in the SDS-PAGE in a zone corresponding to the intact M_r, but rather in a zone of approx. 20–25 kDa, suggesting that it is also broken down into large fragments (*24*). In a third instance, another high-abundance protein detected in the Recioto wine, the 'whole genome shotgun sequence of line PN40024, scaffold_22.assembly-12x', with a theoretical M_r of 60 746 Da, has also been found in the SDS-PAGE gel at around 20 kDa, here too suggesting a degradation product (*19*). An additional interesting observation comes from the Recioto wine proteome (*19*): out of 106 unique gene products found, the vast majority are low-molecular-mass proteins, ranging from 10 to 35 kDa. It would thus appear that, in addition to the presence of fragments of high-molecular--mass proteins, the proteins that survive the treatment of these beverages (fermentation, *etc*.) are mostly low--molecular-mass species, the high-molecular-mass ones being either present as fragments or simply precipitated out of the solution in the industrial processing (however, it must be stated that, in the case of 7S globulin and glutelin, small amounts were also detected in the SDS--PAGE gel at the correct M_r, suggesting a coexistence among degraded and undegraded forms, although the undegraded species represented <5 % of the total) (*23*). The same seems to apply to the proteome of beer: out of 22 unique gene products that we detected, 19 had M_r values ranging from 10 to 33 kDa and only three proteins (two serpins and barwin) had M_r of 43 000 Da (*13*). This degradation phenomenon is even more apparent when running two-dimensional (2D) maps. Again, in the case of the beer proteome, Imure *et al*. (*25*) found no less than 31 spots of lipid transfer protein 1, ranging in size from 10 to 16 kDa and 16 spots of Z-type serpin in the M_r interval 30 to 43 kDa. In the case of the champagne proteome, Cilindre *et al*. (*21*), here too *via* 2D mapping, found no less than 16 spots of vacuolar invertase-1 ranging in size from 20 up to 75 kDa (the latter being the native M_r), the native, undegraded form representing <5 % (see Fig. 4). These data were obtained without the use of CPLLs. When using such libraries, in a recombinant human albumin preparation, Fortis *et al*. (*26*) detected 21 albumin spots, ranging in size from 8 to 71 kDa (the latter representing the M_r of the native form), in addition, of course, to host (*Pichia pastoris*) proteins not declared by the producers, who had also failed to report the presence of albumin degradation products!

Discussion

Some important data emerge from our results of wine analyses: to start with, it is now clear that trace proteins can be detected, even down to a very low limit of barely 1 μg/L of protein (something that at present seems to materialize only *via* CPLL capture). A second, most important aspect of our research is the introduction of a fourth pH value for capturing proteomes with CPLLs: namely pH=2.2. This *per se* would not be of interest, since it is clear that, in addition to modulating the capture by selecting three pH values (acidic, neutral and basic) one could envision a series of captures at 1-pH unit increments to further modulate it. This however would not make much sense, since it would greatly add to the experimental burden without most probably bringing any discovery of new species. But the fourth pH value here adopted (pH=2.2) has a special valence in

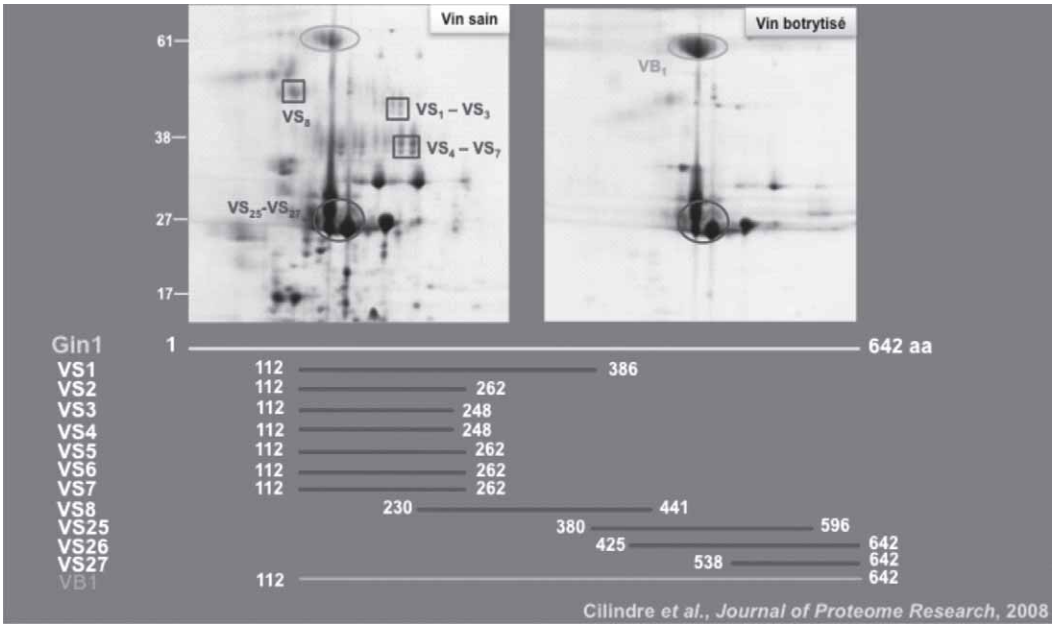

Fig. 4. Two-dimensional maps of control (left) and treated (with *Botryotinia fuckeliana,* right panel) champagne. The squares and circles identify the major spots of invertase-1 (GIN1), while the lines underneath (delimited by numbers) give the amino acid sequences of the fragments of detected invertase. It can be seen that only a small percentage is found at the native M_r value (line and circles marked with VB1), whereas the vast majority is seen as degraded fragments (courtesy of Dr C. Cilindre, University of Reims, France, modified from *21*)

that it is introduced in order to force a capture *via* reversed-phase mechanisms, *i.e.* maximizing hydrophobic interactions on a strongly hydrophobic surface. According to the data presented here (Fig. 3), it appears that such a capture might be the best to harvest trace proteins with unique efficiency (we have applied it too to nonalcoholic beverages and we can confirm that this is a general mechanism). The above data strongly suggest that a new, powerful technique is emerging, namely the CPLL methodology, which has found now wide applications especially in biomarker discovery in biological fluids. The results we obtained when exploring the human red blood cell proteome (*27*) and cerebrospinal fluid (*28*) were simply spectacular. And this notwithstanding the three-pronged attack that came recently from three German scientists against this methodology. Basically, one of them stated that CPLLs could not discover any low-abundance proteins in sera, but only medium to high-abundance ones (*29*); the other put forward the notion that CPLLs (which act on bioaffinity recognition) are not any different from C_{18} resins (acting on the principle of affinity for…petrol!) (*30*); the third group, finally, stated that modern MS instrumentation is powerful enough to allow discovery of low-abundance species even in the overwhelming presence of very high-abundance ones, negating any need for CPLL treatments (*31*). Without entering into the merits of such extravaganzas, we refer the readers to recent publications dismantling such absurdities and setting the record straight (*32*,*33*).

Conclusions

Protein traces are clearly present in most if not all wines. They are derived not only from grape, but also from the fermentation process involving yeasts and bacteria. To improve the stability of wines over time, the use of additives is becoming a general rule even if there is the obligation to remove traces of additives at the end of the process (see Table 3; *3*,*11*,*12*,*16*–*22*,*34*). Unfortunately, among additives there are proteins from egg white and from milk, both sources of potential allergens. Most of the wines, analyzed by using very sensitive approaches and especially associated with enrichment tools such as CPLL, showed the presence of foreign proteins, encouraging further investigations of the low-abundance proteome.

For the good or the bad a lot remains to be discovered in wine proteomes. This will not be limited to the scientific knowledge, but also to the wine composition, age and possible 'adulteration'. The presence or absence of additives and the presence of pathological components from microorganisms generating for instance potential allergic reactions is another feature that cannot be neglected. The contribution of our laboratories with the help of tools capable to enhance the low-abundance protein species present is an important step in that direction. In spite of being probably one of the oldest prepared beverages, it is still dependent on a sort of more or less secret art of making while considered also one of the most fascinating drinks.

It seems that our papers on wines are one way or another intimately connected to *Carmina Burana:*

Quid agatur in taberna, ubi nummus est pincerna….

Primo pro nummata vini, ex hac bibunt libertini….

Quarter pro Chistianis cunctis, quinquies pro fidelibus defunctis

Sexies pro sosoribus vanis, septies pro militibus silvanis

Octies pro fratibus perversis, nonies pro monachis dispersis….

Tam pro papa quam pro rege, bibunt omnes sine lege….

To minimize your suffering with the above (pseudo) Latin verses, this time we will give you below the translation:

What happens in a tavern, where money buys a drink….

The first toast is for money to keep buying drinks, after that it is for libertines….

The fourth toast is for all Christians, the fifth one for all dead souls

The sixth toast is for all sinning nuns, the seventh for bandits in the forests

The eighth one for libertine friars, the ninth toast for friars lost (in sins)

Be it for Popes or for Kings, all of us drink incommensurably….

Table 3. Main published investigations on proteins present in wines

Type of wine	Topic covered	Proteins found/analyzed	Year	Ref.
white	quantitative immunostaining determination of allergens in wine	casein, lysozyme	2009	(*3*,*34*)
white	low-abundance proteome detection in wine	casein	2010	(*11*)
red	low-abundance proteome detection	casein, thaumatin, few fungal proteins	2010	(*12*)
white	detection of allergenic milk markers by LC-ESI-MS/MS	α and β casein	2010	(*16*,*17*)
white	glycoprotein profile from grape, additive and yeast allergens	various glycoproteins from grape and yeast origin	2010	(*18*)
white	proteome of untreated Recinoto wine	more than 100 gene products from grape and a dozen from yeast	2011	(*19*)
red and white	comparison of protein content by LC-MS/MS	12 grape and 6 yeast proteins, LTP	2009	(*20*)
white	comparison of proteomes of Champagne from healthy and botrytized grapes	pectinolytic enzymes, invertase, PR proteins	2008	(*21*)
red and white	search of egg white proteins by immunochemical methods and LC-MS/MS	ovalbumin, ovotransferrin, lysozyme, ovomucin and few others	2012	(*22*)

Acknowledgements

Supported in part by a grant from MIUR (PRIN2008, Rome). We thank Dr C. Cilindre, University of Reims, France, for the kind gift of Fig. 4.

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