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Comparison of seventeen different fining agents used with wine

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Several fining trials with commonly used fining agents have been published before, but generally referring to quantities of fining agents suggested by the manfacturer. Usually, protein fractions of fining agents are the operative components, which agglomerate with suspended particles, tanning agents, anthocyanins and other phenolic compounds. This study comparatively investigates the efficiency of 17 fining agents with 20 wines made from seven grape varieties. Fifteen proteins including rather new proteins of vegetable origin as well as two non-proteins were applied. Their quantity was calculated based on their given protein content (for calculations non-proteins were treated as pure proteins). The following analytical parameters were measured: colour density, colour hue, brilliance, total phenolics and tannin content. Collected data were statistically evaluated by means of PCA and ANOVA. The strongest effect on the analysed parameters was obtained using fish proteins, KalCasin (casein) and ErbiGel (gelatine), whereas vegetable proteins had the slightest effect. Especially the corn protein showed no significant differences to the control sample. In conclusion, the efficiency of fining agents mainly depends on the given wine composition, thus preliminary tests are indispensable.
Keywords: fining agents, wine, colour, phenols, tannins

Vergleichender Versuch von siebzehn Schönungsmitteln, angewendet in Wein. *Viele Schönungsversuche mit derzeit verwendeten Schönungsmitteln wurden bereits veröffentlicht, jedoch wurde immer eine vom Hersteller vorgegebene Menge an Schönungsmittel angewendet. Wirksamer Bestandteil des Schönungsmittels sind üblicherweise die Proteine, die durch Agglomeration mit Weininhaltsstoffen Trubpartikel, Gerbstoffe, Anthocyane und andere phenolische Verbindungen entfernen. In dieser Studie wurden die Auswirkungen von 17 Produkten an insgesamt 20 Weinen sieben verschiedener Rebsorten untersucht. Fünfzehn Proteine, unter anderem neuartige pflanzliche Proteinprodukte, sowie auch zwei Nichtproteine wurden dabei verwendet. Die Anwendungsmenge wurde über den jeweiligen Proteingehalt berechnet, um eine Vergleichbarkeit der Effektivität zu ermöglichen (für die Berechnungen wurden Nichtproteine als reine Proteine angesehen). Untersuchte Parameter waren Farbdichte, Buntton, Brillanz, Gesamtphenol- und Tanningehalt. Zur statistischen Datenanalyse wurden PCA und ANOVA verwendet. Deutlichste Wirkungen, besonders in Bezug auf die Parameter Farbdichte, Tannin- und Gesamtphenolgehalt, wurden mit den Fischproteinen, dem Kaseinpräparat KalCasin sowie dem Gelatinepräparat ErbiGel erzielt. Die pflanzlichen Proteinpräparate zeigten durchwegs die schwächsten Wirkungen, besonders das Maisprotein konnte keine signifikanten Änderungen hervorrufen. Grundsätzlich wirkten die Schönungsmittel in jedem Wein anders, so dass Vorversuche vor einer Schönung unverzichtbar bleiben.*
Schlagwörter: Schönungsmittel, Wein, Farbe, Phenole, Tannine

Essai comparatif avec 17 produits de collage, appliqués au vin. *Beaucoup d'études sur ces produits de collage ont été publiées, dans lesquelles on a toujours utilisé un dosage recommandé par le producteur. Le composant actif dans le*

produit de collage est la protéine, qui forme des agglomérats avec des composants particuliers du vin à savoir la lie, les tannins, l'anthocyanes et autres composants phénoliques et les élimine. Dans cette étude nous avons analysé l'effet de 17 produits de collage sur 20 vins de 7 cépages. Au total, nous avons utilisée 15 protéines, entre autre des nouveaux produits à base de protéine d'origine végétale, ainsi que 2 non-protéines. La quantité du produit de collage était calculé également selon la teneur en protéine, pour garantir la comparabilité de l'effectivité (pour le calcul nous avons considéré les non-protéines comme protéines pures). Les paramètres analysés étaient: l'intensité de la couleur, la nuance de couleur, le brillant, la teneur en phénols totaux et tanins. Pour l'analyse statistique nous avons employé l'ACP et l'ANOVA. Les produits de poisson, la caséine KalCasin et le produit gélatine ErbiGel ont été les plus actifs. Les produits d'origine végétale ont été les moins réactifs, la protéine de maïs en particulier, qui n'avait pas du tout provoqué d'effet signifiant. En général, chaque produit de collage provoque un effet différent dans chaque vin de sorte qu'un test pilote pour un collage reste indispensable.
Mots clés: agent de collage, vin, couleur, phénol, tanin

The generally applied use of proteins as fining agents in wine has now advanced to the point that a large variety of products is commercially available. Gelatine and collagen of diverse origin, isinglass and various fish skin proteins, egg albumin, milk proteins (potassium caseinate and whey protein) and, recently, proteins of vegetable origin are commonly used, as are the non-proteins polyvinyl-polypyrrolidone (PVPP) or activated charcoal. The major reason for their application is the elimination of excessive astringency and bitterness, contributing to the improvement of organoleptic characteristics of wines (Bonerz et al., 2003; Donner et al., 1993; Gómez-Plaza et al., 2000; Maury et al., 2001; Sarni-Manchado et al., 1999; Sims et al., 1995).

Depending on the desired effect, a suitable fining agent and its adequate application quantity must be found for each wine. The use of PVPP, charcoal and gelatine eliminates polyphenols in general with the undesirable side effect of reducing colour density (Castillo-Sánchez et al., 2008; Lopéz et al., 2001), but with no specific effect in preventing browning (Barroso et al., 1995). Egg albumin and caseinates have only little effect on colour, mainly on shades of brown (colour hue), especially in white wines or sherry wines (Amati et al., 1979; Barroso et al., 1995; Fischerleitner et al., 2003; Giacomini, 1987; Jouve et al., 1989; Manfredini, 1989).

Table 1: Used fining agents (molecular weights analysed by N-Zyme BioTec, Darmstadt (Germany), protein contents analysed by [1)]Erbslöh Geisenheim AG, Geisenheim (Germany) or by [2)]N-Zyme BioTec, Darmstadt (Germany); MW = molecular weight, kDa = kilo-Dalton, n/a = not applicable)

Fining agent	Protein group	Specifications / origin	MW (kDa)	Condition	Protein content (%)
ErbiGel	Gelatine	Pig skin gelatine, 90-100 Bloom	>45.0	Powder	90[1)]
GelitaKlar		Dissolved pig skin gelatine	n/a	Fluid	20[1)]
Peptan NFC		Bovine skin collagen hydrolysate	n/a	Powder	85[1)]
VP 6935-1		Pig skin collagen paste	n/a	Fluid	1,9 [1)]
IsingClair	Fish protein	Isinglass (swim bladder) paste	n/a	Fluid	2,2[1)]
GelaFish		Fish skin gelatine, 130-170 Bloom	n/a	Powder	65[1)]
Drifine		Hydrolysed isinglass, lyophilised	45.0 >150.0	Powder	90[1)]
AlbuVin	Egg protein	Egg white albumen, pasteurised, spray-dried	50.0	Powder	78[1)]
Fresh egg white		Beaten fresh egg white	50.0	Fluid	11[2)]
KalCasin	Milk protein	Potassium caseinate, modified casein of water-insoluble milk protein fraction	21.0	Powder	75[1)]
Whey protein		Water-soluble milk protein	15.2	Powder	75[1)]
Lupine protein	Vegetable protein	Lupine protein isolate	>20.0	Powder	75[2)]
Gluten		Wheat protein fraction	40.0	Powder	79[2)]
Corn protein		Corn protein isolate	23.0	Powder	59[2)]
Hydrol. wheat protein		Enzyme-modified wheat protein	<20.0	Powder	75[2)]
Activated charcoal	Non-protein	Amorphous carbon, water-insoluble	-	Powder	-
PolyclarV (PVPP)		Polyvinylpolypyrrolidone, water-insoluble	-	Powder	-

To prevent browning reactions right from the beginning, fining agents, especially caseinates like potassium caseinate, are increasingly used prior fermentation (Puig-Deu et al., 1999).
Fish proteins and egg white are used to optimise the sensory characteristics of wines, concerning taste and especially rounding of aroma (Bonerz et al., 2003). The gentle effect of fish proteins on colour density and hue was shown earlier (Bonerz et al., 2003), comparing the finings with the use of PVPP, silica sol, casein, gelatine and activated charcoal. The fish protein isinglass caused the only significant difference on the aromatic potential of 'Gewürztraminer' in a trial with bentonite, casein and PVPP (Cabaroglu et al., 2003).
The desired effect, i.e. reduction of astringency, bitterness or browning, is mostly achieved by increasing the amount of the applied fining agent. According to quantity, the application always reduces characteristics of consumers' interest as well, like colour density, taste complexity or flavour.
Former studies compared fining agents and their effect on wines with different quantities following manufacturers' instructions (Cabaroglu et al., 2003; Castillo-Sánchez et al., 2006; Castillo-Sánchez et al., 2008; Cosme et al., 2007; Cosme et al., 2008). The aim of this study was to compare fining agents of different origin using the same protein amount, demonstrating effectiveness and significant differences to control samples. Fining agents were not used in combinations, neither with other proteins nor with clarification substances like bentonite. Only the effect of the fining agent protein should be presented.
A comparative fining trial was carried out to overview the effectiveness on parameters like colour density, colour hue, brilliance, total phenolics and tannin contents. Twenty wines (red and white) were treated with 17 fining agents with equal protein concentration.

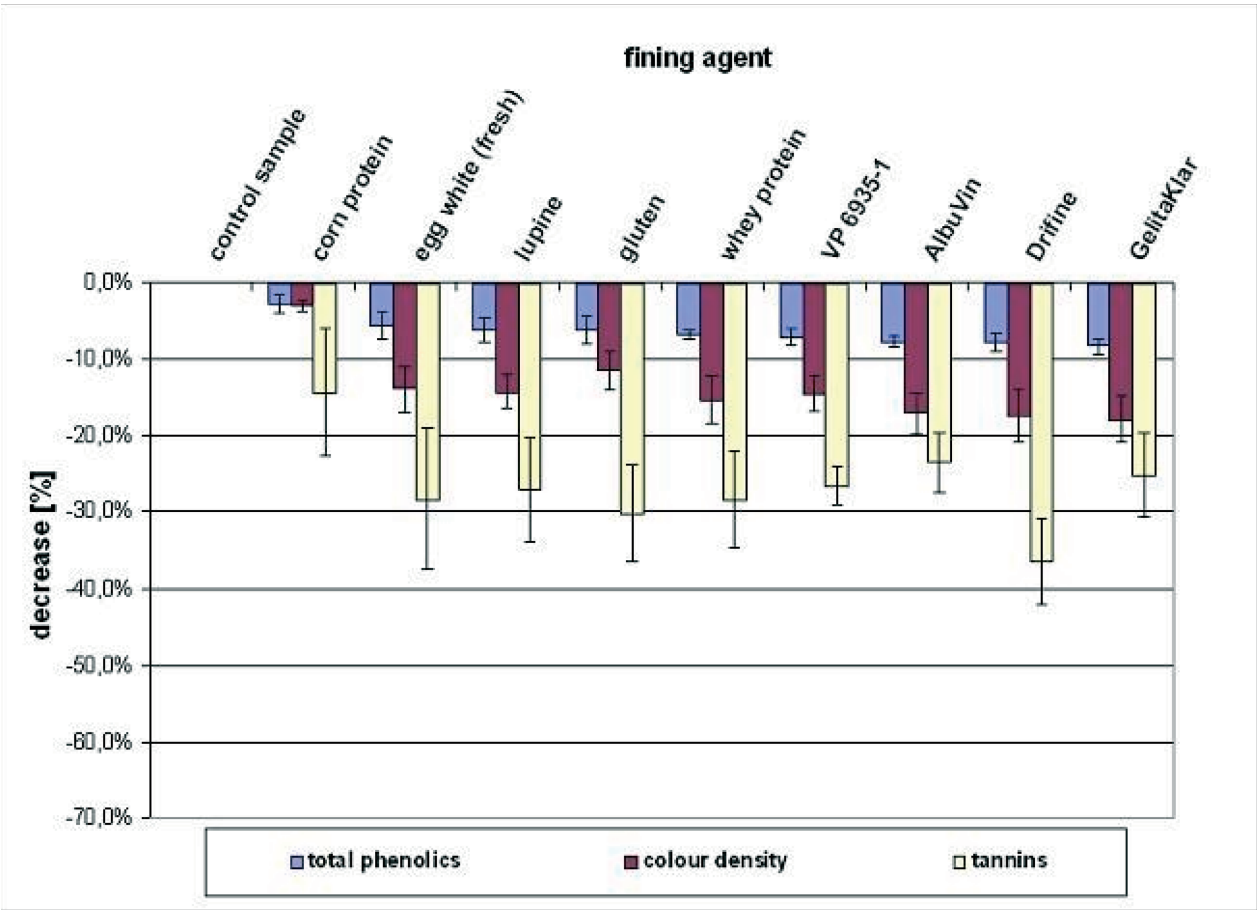

Fig. 1: Decrease of total phenolics, colour density and tannin content in fined red wines (in %), part 1; data averaged of 15 wines (all determinations performed in duplicate)

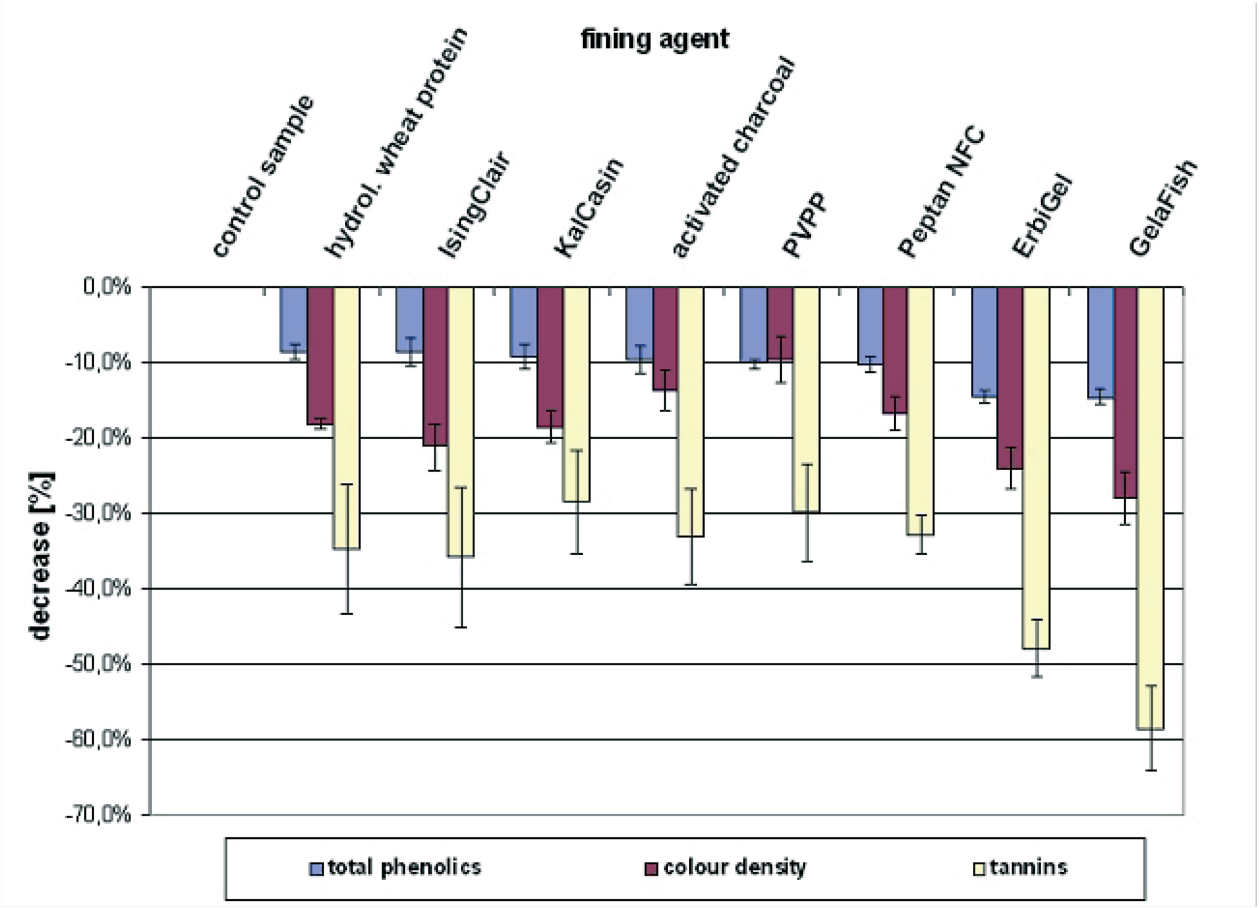

Fig. 2: Decrease of total phenolics, colour density and tannin content in fined red wines (in %), part 2; data averaged of 15 wines (all determinations performed in duplicate)

Material and methods

Fining agents and wines

Seventeen fining agents (Table 1) were collected: two egg-albumin preparations (AlbuVin, fresh egg white), three fish-based proteins (Drifine, IsingClair, GelaFish), four gelatines (ErbiGel, VP 6935-1, GelitaKlar, Peptan NFC), one potassium caseinate (KalCasin) and one of each whey protein, corn-based protein, gluten, hydrolysed wheat protein and lupine protein as well as two non-proteins (activated charcoal and PVPP PolyclarV). The fining agents were made available by Erbslöh Geisenheim AG, Geisenheim (Germany) and N-Zyme BioTec GmbH, Darmstadt (Germany), except fresh egg white. Fining agents were used with wines from the seven grape varieties 'Spätburgunder' ('Pinot Noir'), 'Lemberger', 'Trollinger', 'Samtrot', 'Schwarzriesling' ('Pinot Meunier'), 'Riesling' and 'Grauburgunder' ('Pinot Gris') with a total of 20 wines. Fourteen wines from vintages 2004 to 2006 were collected from the Staatsweingut Weinsberg (Germany), six from vintages 2005 and 2006 were made available by the WG Lehrensteinsfeld eG (Germany) and WG Flein-Talheim eG (Germany).

Fining

Protein contents of fining agents were provided by Erbslöh Geisenheim AG and N-Zyme BioTec GmbH and estimated according to Kjeldahl-method EN ISO 3188 (ISO, 1978). Considering the protein content of fining agents (Tab. 1) a 1 % (w/w) protein suspension of each fining agent was prepared in deionised water. In certain cases (gelatines, fish protein powders and corn protein) suspensions were carefully warmed up to a maximum of 50 °C to achieve better solubility. Using a 250 ml Erlenmeyer flask 5 ml of the fining agent suspension were added to 200 ml of wine. The concentra-

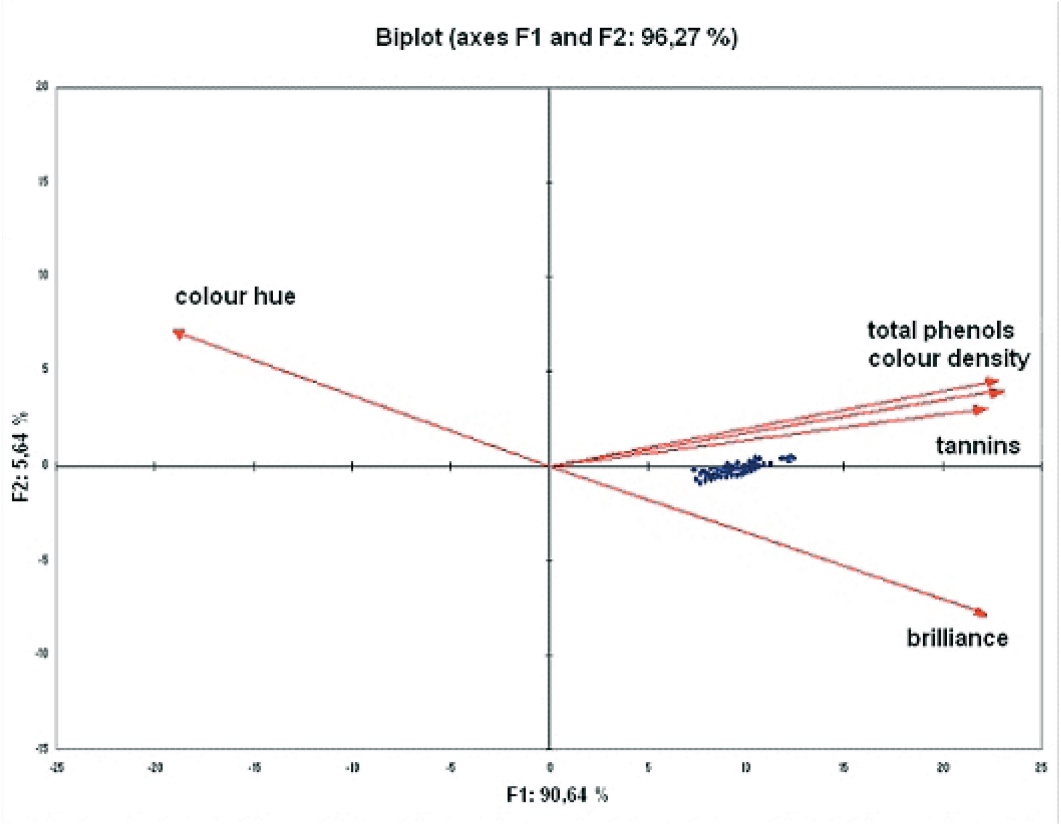

Fig. 3: Biplot of PCA with variables (red) and red wine data (blue, cf. Fig. 4)

tion, equivalent to 25 g pure protein per hectolitre wine, was calculated from commonly used concentrations of end-products for fining. The concentration of non-proteins was 1 %.
The prepared wines were filled into 250 ml measuring cylinders taped with parafilm and stored in the dark at 8 °C for 24 h. The wines were decanted through a folded filter (Schleicher & Schuell 616 1/6, Dassel, Germany) into 150 ml amber screw-cap bottles. Unprepared wines used as control were treated equally for all experimental sets. The experiments were performed in duplicate.

Wine analysis

The determinations were performed using a Cary 50 Bio UV spectrophotometer with a multicell holder (Varian Deutschland GmbH, Darmstadt, Germany).
The colour density was determined by measuring absorbance at 420, 520 and 620 nm in macro disposable cuvettes (ratiolab 10mm Q-VETTES macro, Dreieich, Germany) according to Glories (1984). Colour hue was quantified as the ratio of absorbance at 420 and 520 nm (Glories, 1984), and brilliance was calculated according to Riberéau-Gayon et al. (2000).
Total phenolics were determined by Folin method (Singleton and Rossi, 1965, modified by Ritter et al., 1994) at 720 nm using (+)-catechin as a standard. Tannins were quantified as (+)-catechin equivalents using the methyl cellulose precipitation (MCP)-assay (Sarneckis et al., 2006). Absorbance at 280 nm was determined in quartz cuvettes.

Data analysis

The data analysis was carried out using XLSTAT Version 2008.1.01 (Addinsoft Deutschland, Andernach, Germany).

Results and discussion

White wines

The dosage equivalent to 25 g protein/non-protein per hectolitre wine did not result in a significant improvement of both grape varieties ('Riesling' and

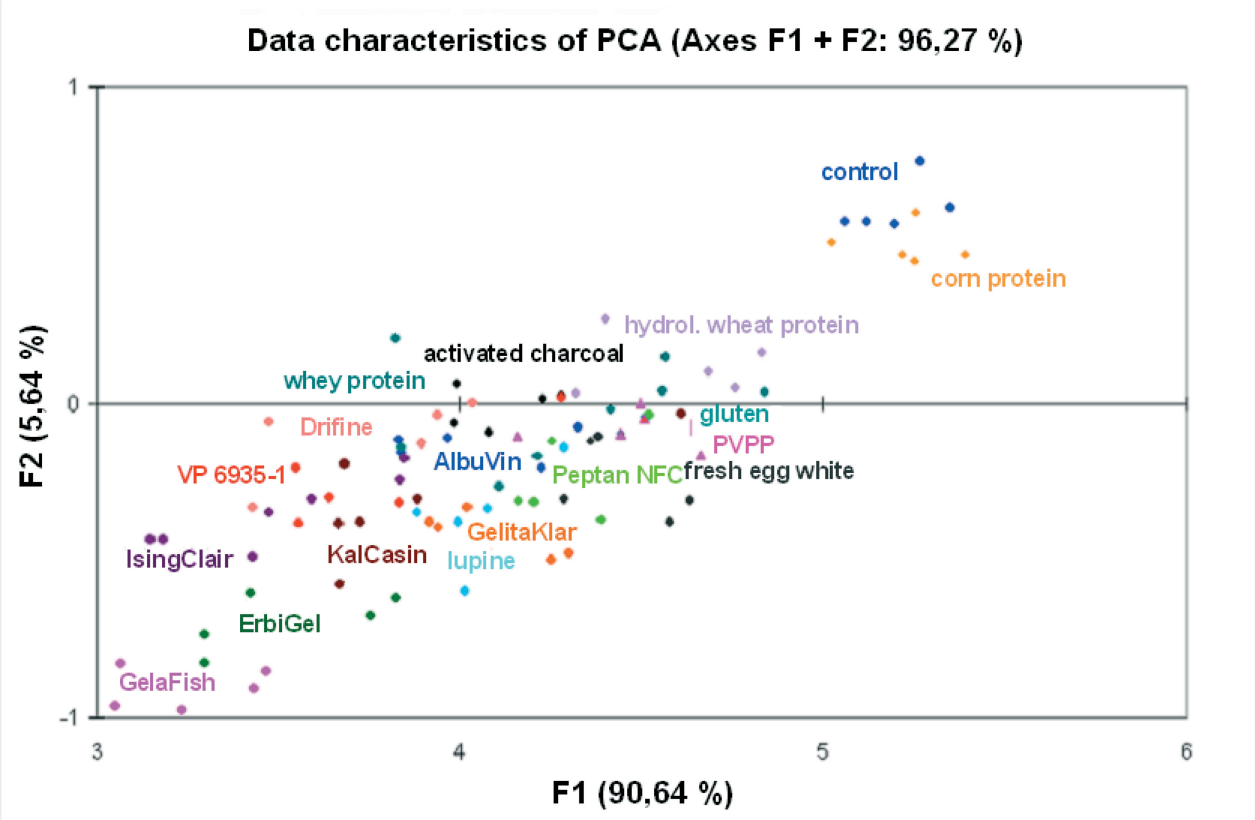

Fig. 4: PCA-data of 15 red wines, averaged per grape variety; corn protein causes slightest deviations from control, GelaFish, ErbiGel and IsingClair with highly prominent differences

'Grauburgunder') concerning browning and brilliance, except activated charcoal (brilliance) and KalCasin (browning). Significant loss in colour density was generally not observed. Phenols were reduced by 4.7 to 5.7 % (vegetable proteins), 6.2 to 6.8 % (fish proteins), 7.5 to 8.5 % (gelatines), 10.5 % (PVPP) and 13.5 % (activated charcoal), but the results were not significant (confidence interval CI = 95 %).

Tannin content of 'Grauburgunder' wines was below 10 mg/l and thus below detection limit. In 'Riesling' wines tannins were rather decreased by the addition of PVPP (up to 90 %), followed by charcoal (76 %) and fish proteins (61 to 74 %); vegetable proteins showed the slightest effect.

Red wines

The large range of values (caused by grape variety, e.g. colour density in 'Trollinger' and 'Lemberger') had a major impact on standard deviation (σ) and accordingly variance (σ^2) (SACHS, 1997). Comparing these remote data, no significant differences between control samples and treated wines could be observed. Therefore, analysis was performed using data averaged by grape variety. Contrary to former fining trials, the gelatine ErbiGel and the fish proteins GelaFish, IsingClair and Drifine showed the strongest effects on colour, tannin and phenolic content. The differences between GelaFish- and ErbiGel-treated red wines and control samples were significant at all times (CI = 95 %). Colour density was decreased by 26.8, 20.3 and 17.0 % by GelaFish, IsingClair and Drifine, respectively (Fig. 1 and 2). ErbiGel removed 23.6 % of colour density, so the effect was much stronger than applying the non-proteins charcoal (12.8 %) and PVPP (maximum 8.9 %). Red wines treated with corn-protein and PVPP did not show any significant difference at all.

Concerning total phenol content, again the most significant differences between control and treated wine could be shown with GelaFish (14.7 %) and ErbiGel (14.3 %), with a considerable difference to other fining agents. Within the red wine trial consisting of 15 wines the maximum decrease was 17.3 % with GelaFish.

Table 2: Analysis of variance (ANOVA) giving an overview of significant differences to control, differentiated by grape varieties (c = colour density, p = total phenolics, t = tannins, n.d. = not detectable, +/- = significant / no significant difference to control (confidence interval CI = 95%), P. = Pinot)

	Riesling (3)			P. Gris (2)			Lemberger (3)			P. Noir (3)			Trollinger (3)			P. Meunier (3)			Samtrot (3)			No. of signif. diff.
	c	p	t	c	p	t	c	p	t	c	p	t	c	p	t	c	p	t	c	p	t	
Corn protein	-	-	-	-	+	n.d.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Lupine	-	-	-	+	-	n.d.	-	-	-	+	-	-	-	+	+	-	-	+	+	-	+	7
Fresh egg white	+	-	+	+	-	n.d.	-	-	+	+	-	-	-	-	+	-	-	-	+	-	+	8
Gluten	-	-	-	+	-	n.d.	-	-	+	+	-	+	-	+	+	-	-	+	+	-	-	8
Whey protein	+	-	+	+	-	n.d.	-	-	+	+	+	-	-	-	+	+	-	-	+	-	+	10
PVPP	-	-	+	+	+	n.d.	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	11
VP 6935-1	-	-	+	-	+	n.d.	+	-	+	+	+	-	-	-	+	-	+	-	+	+	+	11
Activated charcoal	-	-	+	+	+	n.d.	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	13
AlbuVin	-	-	+	+	+	n.d.	-	-	+	+	+	-	+	+	+	+	-	-	+	+	+	13
GelitaKlar	-	-	+	-	+	n.d.	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+	13
Hydr. wheat protein	-	-	-	-	-	n.d.	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	13
Drifine	-	-	+	-	+	n.d.	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	14
Peptan NFC	-	-	+	+	-	n.d.	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	14
IsingClair	-	-	+	+	-	n.d.	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	15
Gelafish	-	-	+	-	-	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
KalCasin	-	-	+	+	+	n.d.	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	16
ErbiGel	-	-	+	-	+	n.d.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17

The reduction of phenol contents by vegetable proteins ranged from 2.7 % (corn protein) to 8.8 % (hydrolysed wheat protein). Differences between control samples and corn protein or egg white (5.7 %), respectively, were not significant with all grape varieties.

The fish proteins and ErbiGel achieved the highest reduction of tannins (GelaFish 58.4 %, Drifine 36.5 %, IsingClair 35.8 %, ErbiGel 47.9 %). Other fining agents removed 20 to 30 % of the tannins, except for hydrolysed wheat protein (34.7 %), activated charcoal 33.1 %) and PVPP (32.9 %). Merely corn protein (14.3 %) had no significant effect on tannin removal, regarding all five grape varieties studied.

Principal Component Analysis (PCA) was applied with the data collected of 15 red wines, referring to total phenolics, tannins, colour density, colour hue and brilliance. PCA was carried out to determine dependencies of considerable parameters and to visualize responsible parameters for deviation.

As described with individual parameters, corn protein had hardly any effect on treated wines, while GelaFish, ErbiGel and IsingClair indicated the highest differences to control samples (Fig. 1 and 2). Deviation was caused by the parameters colour density, total phenolics and tannin content, which pointed into the same direction (Fig. 3). Colour hue and brilliance had no effect on deviation, but were directly opposed. This means that their effect was contrary. With a higher removal of colour hue (shades of brown) the value of brilliance increased, as expected. Deviations from control samples are shown in Figure 4 (showing data points of Fig. 3 in detail), dependent on fining agents.

Analysis of variance (ANOVA, Tab. 2) gives an overview of significant differences to control samples of each grape variety (CI=95 %). The synoptical table is confined to the parameters responsible for deviation at PCA. The determined total number of significant differences shows that fish proteins, KalCasin and ErbiGel caused most effects while corn protein had hardly any effect on all parameters. Table 2 points up the fining agents and their different impact on analysed parameters in the wines.

Conclusion

The fining trial with a protein concentration, equivalent to 25 g per hl wine, showed some already described, but also some new aspects. Results making a statement about the effect of a fining agent on colour, total phenolics or tannins could not be applied to other wines. The efficiency of the fining agent firstly depended on grape variety and secondly on the individual wine matrix, which makes preliminary tests indispensable.

Important trends, however, could be observed. Strongest effects on all analysed parameters were achieved using fish proteins and gelatines. Fish proteins were ge-

nerally described as gentle fining agents (Bonerz et al., 2003) as a result of using these products in low concentrations, recommended by the producers. Using an equivalent protein concentration comparable to all other protein fining agents, a gentle fining result with these fish proteins for merely finishing touches could not be proved.

Activated charcoal had even less impact on colour than the fish proteins in general, which underlines the potential of fish proteins for removal of desired wine ingredients.

With the exception of hydrolysed wheat protein, wine structure was hardly influenced by the new products of vegetable origin. Especially corn protein had no effect on wine composition, probably due to its relatively high insolubility. Modified/hydrolysed products of these protein sources will possibly lead to more effective finings in future trials.

This trial focused on differences in general, but not on differences concerning specific wine components, such as individual polyphenols. Further research will reveal, whether similarities of the present study can be found in sensory tests. The focus will especially be on changes of bitterness and astringency in red wines, i.e. the major reason why fining agents are added. The reduction of tannin contents, for example, seems to be directly related to astringency removal (Kennedy et al., 2006). In addition, HPLC-analyses will be carried out, mainly referring to the analysis of specific polyphenols responsible for bitterness and astringency.

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