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A new gentle fining agent for 'Pinot noir'

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Colour is a major quality component in red wines. In cool climate regions, such as New Zealand, colour problems occur due to a lack of anthocyanins and other phenolic compounds and/or the necessity to use colour-reducing fining agents. Thus, choosing the right fining agent in order to preserve as much genuine colour as possible is a critical decision. Therefore a preliminary comparative fining trial with a new proteinaceous fining agent, Cfine® (fish collagen), and six other protein-derived fining agents was conducted at laboratory scale to analyse influences on colour, phenolic compounds and antioxidative capacity in 'Pinot noir' wine. Furthermore, a fining trial with increasing Cfine® concentrations was carried out to analyse its impact on these parameters, on polyphenols causing bitterness and astringency as well as getting detailed information regarding the lees (phenolic-protein interactions) and possible over-fining related problems. Our results show that even when added at high concentrations Cfine® seems to be very gentle with colour, total polyphenol content, and monomeric phenols. HPLC analysis revealed that Cfine® mainly removes polymeric flavan-3-ols explaining the described reported effects of Cfine® on taste, such as leading to less bitterness and astringency.

Keywords: Wine, 'Pinot noir', fining, polyphenols, colour, antioxidative capacity, procyanidine, SDS-electrophoresis

Ein neues schonendes Schönungsmittel für 'Blauer Burgunder'. *Farbe ist eines der qualitätsbestimmenden Merkmale bei Rotwein und vor allem in kühleren Weinbauregionen, wie z.B. Neuseeland, sind Farbprobleme auf Grund geringerer Anthocyan-Gehalte und/oder durch die Notwendigkeit der Anwendung von Schönungsmitteln zu beobachten. Deswegen ist es wichtig, ein schonendes, farberhaltendes Schönungsmittel zu verwenden. Um den jeweiligen Einfluss auf Farbe, Phenole und antioxidative Kapazität zu analysieren, wurde Wein der Sorte 'Blauer Spätburgunder' im Rahmen von Vorversuchen mit sechs gängigen Schönungsmitteln sowie Cfine® (einem neuartigen Fisch-Kollagen) im Labormaßstab geschönt. Eine weitere Schönung mit Cfine® in ansteigenden Konzentrationen wurde zur Bestimmung der oben genannten Parameter und des möglichen Risikos einer Überschönung sowie des Einflusses auf die bitteren und adstringierenden Polyphenole durchgeführt. Der Schönungstrub wurde zur genaueren Bestimmung der Phenol-Protein-Wechselwirkungen getrennt analysiert. Auch bei höheren Anwendungsmengen bewirkte Cfine® eine geringe Abnahme der Farbe sowie der Gehalte an Gesamtphenolen und monomeren Phenolen. Mittels HPLC-Analyse wurde nachgewiesen, dass mit Cfine® hauptsächlich polymere Flavan-3-ole entfernt werden, wodurch die von der Praxis beschriebene Geschmacksverbesserung durch Abnahme an Bitterkeit und Adstringenz erklärt werden kann.*

Schlagwörter: Wein, 'Pinot noir', Schönung, Farbe, Polyphenole, Antioxidantien, Procyanidine, SDS-Elektrophorese

Un nouvel agent de collage fonctionnant en douceur pour 'Pinot noir'. *La couleur est l'une des caractéristiques déterminantes de la qualité du vin rouge. Dans des régions de viticulture plutôt fraîches, telles que la Nouvelle-Zélande, on peut constater des problèmes de couleur dues aux faibles teneurs en anthocyanes et/ou à la nécessité d'utiliser des agents de collage. Pour cette raison, il est nécessaire d'utiliser un agent de collage fonctionnant en douceur, conservant la couleur. Afin d'analyser l'influence de chacun des agents de collage sur la couleur, les phénols et la capacité antioxydante, le vin du cépage 'Blauer Spätburgunder' a été collé à six agents de collage courants ainsi qu'au Cfine® (un nouveau collagène de poisson) à l'échelle du laboratoire. Un autre collage au Cfine® avec des concentrations croissantes a été effectué dans le but de déterminer les paramètres mentionnés ci-dessus et le risque possible d'un surcollage ainsi que l'influence sur les polyphénols amers et astringents. Les troubles de collage ont été analysés séparément afin de déterminer plus exactement les interactions entre les phénols et les protéines. Même des quantités plus élevées de Cfine® ont provoqué une faible diminution de la couleur ainsi que des teneurs en phénols totaux et en phénols monomères. Il a été prouvé au moyen d'une analyse HPLC que le Cfine® a enlevé principalement les flavane-3-ols polymères, ce qui peut expliquer l'amélioration du goût décrite dans la pratique, due à la réduction de l'amertume et de l'astringence.*

Mots clés : vin, 'Pinot noir', collage, couleur, polyphénols, antioxydants, procyanidines, électrophorèse SDS

'Pinot noir', a premium grape cultivar, originates from the Burgundy and Champagne regions in France. In New Zealand, 'Pinot noir' production is said to expand rapidly within the next few years. In 2002, the production area of 'Pinot noir' grapes in New Zealand was 2209 ha and it is predicted to expand up to 3282 ha by 2005, an increase of 48 % within three years (http://www.nzwine.com/statistics/). Therefore, 'Pinot noir' will become more and more important to domestic and export markets and, following 'Sauvignon Blanc' and 'Chardonnay', it is already the largest export wine of New Zealand.

One major leading quality requirement for red wine is colour. In cool climate regions, such as New Zealand, especially 'Pinot noir' wines are known for their sensitive colour. Generally, colour in red wine depends on several factors, such as polyphenol (anthocyanin) content, grape cultivar, pH-value, winemaking technique, wine stabilisation (fining), and aging. With regard to the expectations consumers have of red wine - the trend is to prefer dark coloured red wines - colour loss during winemaking needs to be avoided as much as possible. Yet, especially in cool climate winegrowing areas winemakers often encounter great colour losses during vinification and maturation of the wine. A possible explanation could be a lack of anthocyanin biosynthesis and/or copigmentation factors, such as flavan-3-ols and hydroxycinnamic acids, evoked by cooler climate. Copigmentation is a phenomenon in which pigments and other noncoloured organic compounds form molecular associations or complexes resulting in enhanced absorbance (colour) and in some cases it occurs with a shift in the wavelength of the maximum absorbance of the pigment. Thus, copigmentation can account up to 50 % of colour in young wines (Boulton, 2001). Another important quality requirement is clarity. Visual satisfaction of the consumer is of great importance, and so, the presence of haze or sediment is considered a fault. Therefore, fining is a commonly used method to delay or avoid protein-polyphenol haze formation, helping to stabilise the wine and to accelerate the clarification during winemaking (Jackson, 2000). Furthermore, fining can be used to eliminate certain off-odours and to reduce astringency and bitterness (improving the 'mouthfeel'). Nowadays, a wide range of fining agents is used, such as activated carbon, silica sol, casein, gelatine, polyvinylpolypyrrolidone (PVPP), etc. These fining agents bind to or adsorb particular material and form new aggregates that, generally, are large enough to precipitate quickly (Macheix et al., 1990).

Yet, since the outbreak of BSE (bovine spongiform enzephalopathy) disease, there have been concerns about the possible hazard of animal proteins to humans.

Therefore the use of alternative fining agents derived from other possibly non-infectious sources (such as plant or fish derived fining agents) becomes more and more important (Fischerleitner et al., 2002). Several plant-derived proteinaceous fining agents, such as hydrolyzed wheat glutens and white lupin *(Lupinus alba)* showed relatively good results compared to fining trials conducted with gelatine (Lefebvre et al., 2000; Lefebvre et al., 2002). Before adapting other proteins for fining, it is necessary to study their efficiency in clarifying and the impact on phenolic compounds, especially bitter and astringent tasting tannins. Latest results showed the capability of plant proteins to precipitate

polymerised and condensed tannins without largely affecting the desirable "soft-tasting" monomeric phenolics in wine. Especially highly polymerised tannins, leading to bitter and astringent taste properties, bound to plant proteins (SDS analyses). With regard to gelatine, plant proteins removed less tannins but showed a comparable impact on highly galloylated tannins. Obviously, molecular weights of protein fractions play an important role on fining properties: lower molecular weights lead to a preferred precipitation of high mDP tannins (CHEYNIER et al., 2003). Plant proteins have also been successfully used for the clarification of must. Compared to gelatine, plant proteins showed similar clarifying effects during sedimentation and flotation (SCHMIDT et al., 2003). However, not only plant derived proteins might be useful for effective fining. Collagen extracted out of fish (e.g. isinglass) provides another opportunity to be used as fining agent and, for this reason, was tested in several fining trials (SIEBERT, 1999).

Cfine® is a relatively new proteinaceous fining agent extracted as a by-product from the skins of the deep-sea fish 'Hoki'/'Blue Grenadier' (*Macruronus novaezelandiae*) and has already been used to strip yeast and some proteins from beer during the brewing process (SIEBERT, 1999). Thus, Cfine® is reported to work very well with 'Pinot noir'. Unfortunately, to date these reported effects have not been backed up by scientific studies.

Therefore, a fining trial was conducted at laboratory scale to compare the effect of Cfine® versus six other commonly used proteinaceous fining agents, such as gelatine, isinglass, casein, egg albumen and PVPP on colour, antioxidative capacity and polyphenols in 'Pinot noir' wine. And, since removing bitter and/or astringent tasting compounds without largely affecting colour is desireable for every winemaker, a fining agent to obtain just 'soft-tasting' phenols is of great interest. Therefore the main focus of this work was to investigate, which polyphenols mainly react with the Cfine® protein and which protein fractions are responsible for the fining effect. Furthermore, as phenols are being made responsible for the health-benefits of moderate wine-consumption, it is worth knowing the influence of a fining agent on polyphenol content and hitherto, antioxidative capacity to assess the impact of fining on bioactive compounds, such as polyphenols.

Methods and Materials

Wine samples

'Pinot noir' wine was produced at Lincoln University Winery from grapes of New Zealand, Marlborough region, vintage 2001, clone 10/5. Grapes were picked at 21.7 °Brix, total acidity of 8.3 g/l (calc. as tartaric acid) and a mash fermentation with a total skin contact time of seven days was performed. The wine had no oak treatment, fining trials were carried out nine months after vintage. The pH-value of the wine was 3.15.

Fining agents

In all cases deionised nanopure water was applied.

1) Cfine® (Sealord Group Ltd., PO Box 11, Nelson, New Zealand) was supplied as a gel containing a total protein concentration of 35.8 g/l. For a better handling the gel was diluted 4:1 with water before use (8.95 mg/ml).
2) Laffort Gelatine Extra N°1 (Carter & Associates, Auckland, New Zealand), was dissolved in warm water (35 to 40 °C) to produce a 50 g/l stock solution.
3) Erbslöh Geisenheim's Hausengranulat Dryfine (Columbit N.Z. Ltd., Auckland) is also an isinglass and was dissolved in water at ambient temperature (20 °C) as according to manufacturer's instructions to produce a 6 g/l stock solution.
4) Polyvinylpolypyrrolidine (PVPP) (Sigma, Australia) was added directly to the wine.
5) Laffort soluble Casein (Carter & Associates, Auckland, New Zealand) was dissolved slowly in water (20 °C) and allowed to soak for 2 hours to produce a 50 g/l stock solution.
6) Laffort Egg Albumin (Carter & Associates, Auckland, New Zealand) was dissolved in water (20 °C) containing a few crystals of sodium carbonate to produce a 100 g/l stock solution.
7) Vickers Dryfine (Carter & Associates, Auckland, New Zealand) is an isinglass and was dissolved in water (20 °C) to produce a 1 g/l stock solution.

Preparation of fining trials

Above 12 °C Cfine® will denature and so, the trial was carried out in triplicate at 10 °C, nine different amounts of Cfine® (+ control) were added (concentration range: 0 to 200 mg/l). The trials were conducted on a labora-

tory scale in 250 ml measuring cylinders. They were filled with 200 ml of chilled wine and fining agent was added.
After 48 hours the wine was filtered through a GF/A filter paper (Schleicher & Schuell GmbH, Dassel, Germany) by means of a vacuum pump. To get comparable results all fining agents were used at the same protein concentration.

Colour

After filtering the fined wine through a 0.45 µm syringe filter (Schleicher & Schuell GmbH) sample colour of each replicate was measured at 420 nm, 520 nm and 620 nm and colour intensity (A420+A520+A620) as well as colour hue (A40/A520) were calculated.

TEAC

(Trolox Equivalent Antioxidative Capacity)
The antioxidative capacity was estimated using the method of MILLER et al. (1993) in a slightly modified form (POUR NIKFARDJAM, 2001). Briefly, 500 ml of a 500 mmol/l solution of ABTS (2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)) in phosphate buffer (pH = 7.3) were pipetted into a 1.5 ml half-micro cuvette and 100 ml of diluted sample solution were added. The reaction was induced by addition of 200 ml 10 mmol/l potassium persulfate. After 6 min the absorption was measured by means of a spectrophotometer at a wavelength of 734nm and compared to a standard curve, which had been prepared using Trolox® (6-Hydroxy-2,5,7,8-tetramehtylchromane-2-carbonic acid) as standard in a concentration range between 0.025 and 0.45 mmol/l in phosphate buffer.

Total Polyphenols

Total phenolics were determined with Folin-Ciocalteu reagent using gallic acid as a standard. Results were expressed as gallic acid equivalents (GAE) per liter (RITTER, 1994).

Monomeric Phenols (RP-HPLC)

For all analyses, the samples were filtered through 0.45 µm filters (Schleicher & Schuell GmbH, Dassel, Germany) and directly injected into a HPLC (Waters 600-MS) equipped with an autosampler (Waters™ 717 Autosampler) and a diode array detector (Waters™ 996 Photodiode Array Detector). Chromatogramms were recorded at 280 nm, 310 nm, 320 nm, 355 nm and 525 nm. The system was equipped with an RP-column (LiChrospher RP-18 Endcapped, 100A, 5 µm, 250 mm x 4.6 mm) maintained at 30 °C with a column heater (Spectra-Physics (SP) 8792 controller). The injection volume was 20 µl. For all measurements and calculations, the Millenium Chromatography Manager (Version 2.10) software was used. For identification of retention times and UV/VIS-spectra the resulting peaks were compared to different phenolic standard substances. For quantification calibration curves of different standards were recorded. Some of the compounds were calculated as unconjugated phenol: caftaric acid, p-coutaric acid, fertaric acid, and GRP (RITTER, 1994). All anthocyanins were calculated as malvidin-3-glucosid.

Mean Degree of Polymerisation (mDP)

This method was used as described elsewhere (RIGAUD et al., 1991). In brief, the "lower" terminal units of flavan-3-ols are split off by acid catalysed hydrolysis and released as corresponding flavonols while the "upper" and intermediate extention units react with phenylmethanethiol forming stable benzylthioethers. After thiolysis the samples were measured by means of RP-HPLC.

Analyses on the sediment

In order to get further information about the reactions between the various protein fractions of Cfine® and phenolic compounds the sediment of the fining trial was analysed. Therefore, the lees were filtered off and freeze-dried. Samples were measured by the Institute of Nutritional Science, University of Potsdam, Germany with the following methods:
SDS-PAGE: 15 mg of freeze-dried lees + 150 µl buffer solution (containing mercaptoethanol and SDS for better solubility) were shaken, heated and after adding TCA (trichloracetic acid) (flocking out of proteins) were centrifuged. The flock was washed with acetone, buffer solution and urea were added and the protein fractions were measured by SDS-PAGE.
UV-VIS-spectroscopy: 15 mg of freeze dried lees + 150 µl buffer solution (containing mercaptoethanol and SDS for better solubility) were shaken, heated and after adding TCA (trichloracetic acid) (flocking out of proteins) were centrifuged. The flock was washed with ace-

tone and dissolved in 1 ml of SDS-solution and then the UV-VIS spectra were recorded.

Results

Comparative fining trial

The aim of the fining trial was to investigate the effect of various proteinaceous fining agents on colour, polyphenol content and antioxidative capacity in 'Pinot noir' wines. Results are shown in Figure 1 and Figure 2, respectively. In total, Cfine® showed the most "gentle" effect on colour, polyphenols and antioxidative capacity as estimated by TEAC method. Dryfine from Erbslöh and Vickers Dryfine also showed good results but lead to higher reduction in colour, hue and polyphenol content. All fining agents except gelatine and PVPP had only little effect on the antioxidative capacity of 'Pinot noir' wines (Fig. 3). Thus, a fining trial with increasing concentrations of Cfine® was also conducted to assess the effect of high amounts of Cfine® on colour, polyphenol content and antioxidative capacity and also the possibility of potential over-fining reactions.

Cfine® fining trial

Colour

Even at extremely high amounts of Cfine® added to the wine, the colour of 'Pinot noir' seems to be stable. Cfine® showed only a minimal effect on the colour intensity in a concentration range between 0 and 200 mg/l (Fig. 4). Colour hue was also not strongly affected; the fined wine showed a desireable tendency to the redder part of the spectrum (Fig. 5).

Total Polyphenols (Folin-Ciocalteu Method)

In concentrations between 0 and 200 mg/l Cfine® showed a maximum reduction in the total polyphenolic content of about 8.5 %. Variations between these values could be due to the inhomogeneous Cfine® gel added to the wine (Fig. 6).

Trolox Equivalent Antioxidative Capacity (TEAC method)

As shown in Figure 7, Cfine® seems to be a good choice for removing polyphenols without having a large impact on the antioxidative capacity. The decrease is recognisable, but compared to other fining agents Cfine® showed good results. Gelatine and PVPP had the strongest effect on antioxidative capacity (Fig. 3) compared to other commonly used fining agents, however, concentration of PVPP was with 60 g/hl quite high.

Monomeric phenols (RP-HPLC)

As shown in Table1 Cfine® does not seem to have a large effect on monomeric phenols either. Only resveratrol (-29.7 %) (data not shown) and petunidin-3-glucoside (-21.5 %) are affected to a greater extent by the highest Cfine® concentration used, but resveratrol is generally known to be quite sensitive against fining (VRHOVSEK et al., 1997). Epicatechin (-11.5 %) and malvidin-3-glucoside (-5.8 %) decreased as well, but not as much as resveratrol. Within the concentration range used monomeric phenol concentration decreased less than 10%.

Mean Degree of Polymerisation (mDP-method)

The mDP-method gives more information about the influence of Cfine® on polyphenolic compounds, particularly flavan-3-ols and their polymers, the proanthocyanidins. Proanthocyanidins with a shorter chain length possess bitter tasting properties and are colourless whereas those with a longer chain length are astringent and tawny. As shown in Figure 8 mainly proanthocyanidins with a short chain length between 2 and 4 were removed by Cfine®. This would explain the effects of Cfine® fining on taste: with increasing Cfine® concentrations mainly bitter tannins are being removed leading to softer mouthfeel and better structure.

Sediment - UV/VIS-Spectra

With increasing Cfine® concentrations, the spectra of the polyphenols shift more and more towards those of flavan-3-ols and their derivatives such as catechin, epicatechin and proanthocyanidines with, according to the mDP-results, a chain length between 2 and 4 (Fig. 9 to 11).

Sediment - SDS-PAGE

Figure 12 shows the genuine protein fractions of Cfine®. Analysis of the lees (fining sediment) shows new protein fractions after treatment (table not shown) with a molecular weight of about 30,000 and between 50,000 and 57,000 Dalton. The genuine 70,000 Dalton band (No. 7 in Fig. 12) seems to split into these new fractions, which could be due to derived (changed) protein fractions. Presumably, and according to the mDP-

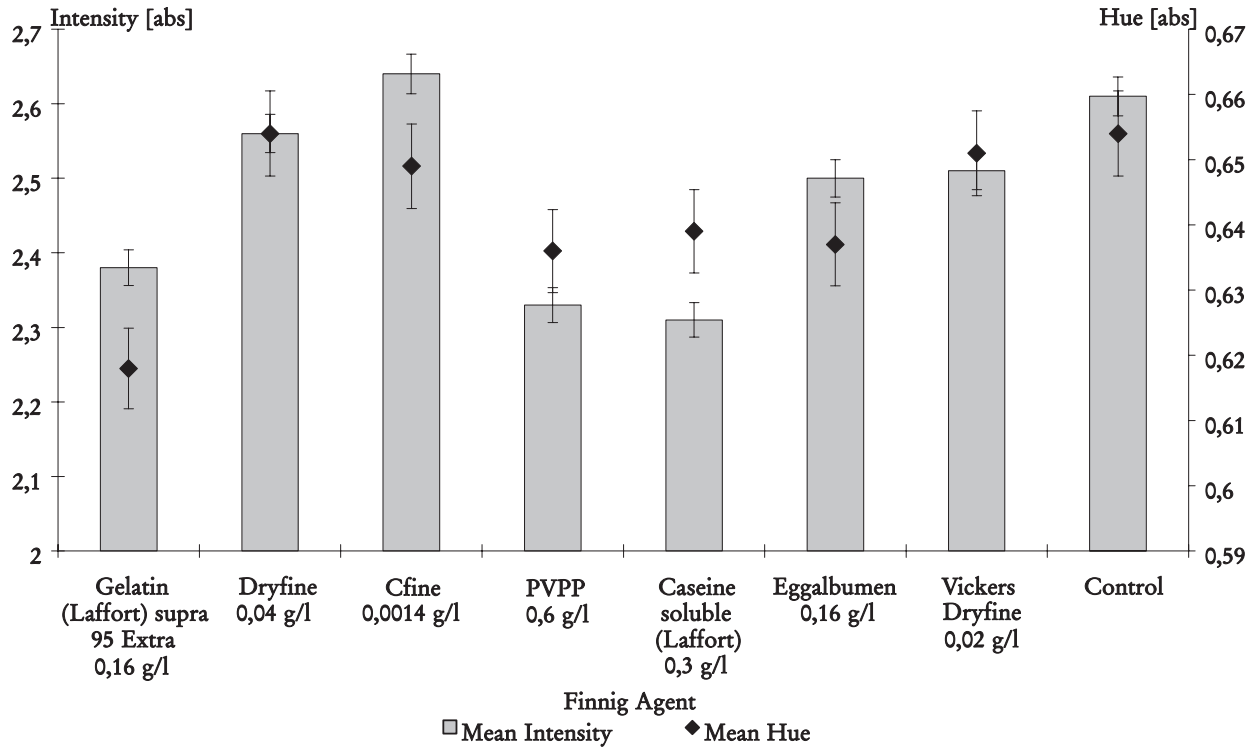


Figure 1: Influence of different fining agents on colour in 'Pinot noir' wines (mean values, n = 3)

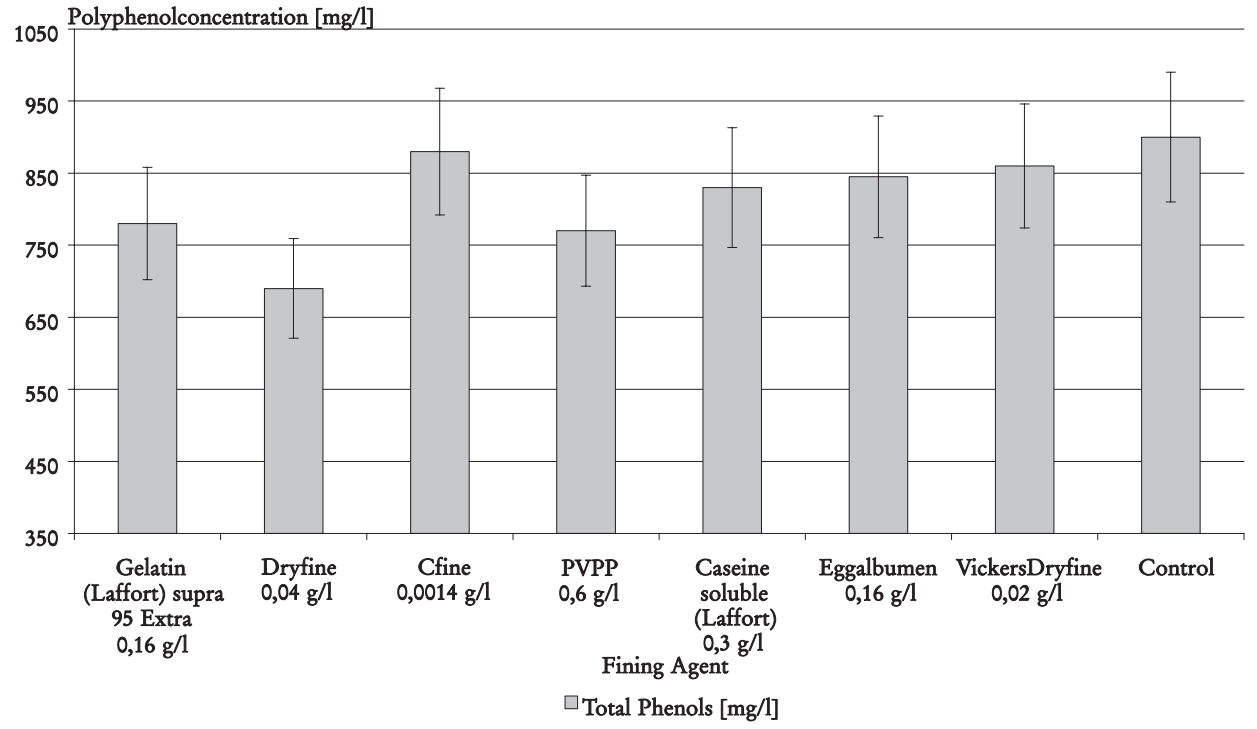


Figure 2: Influence of different fining agents on polyphenol content in 'Pinot noir' wines (mean values, n = 3)

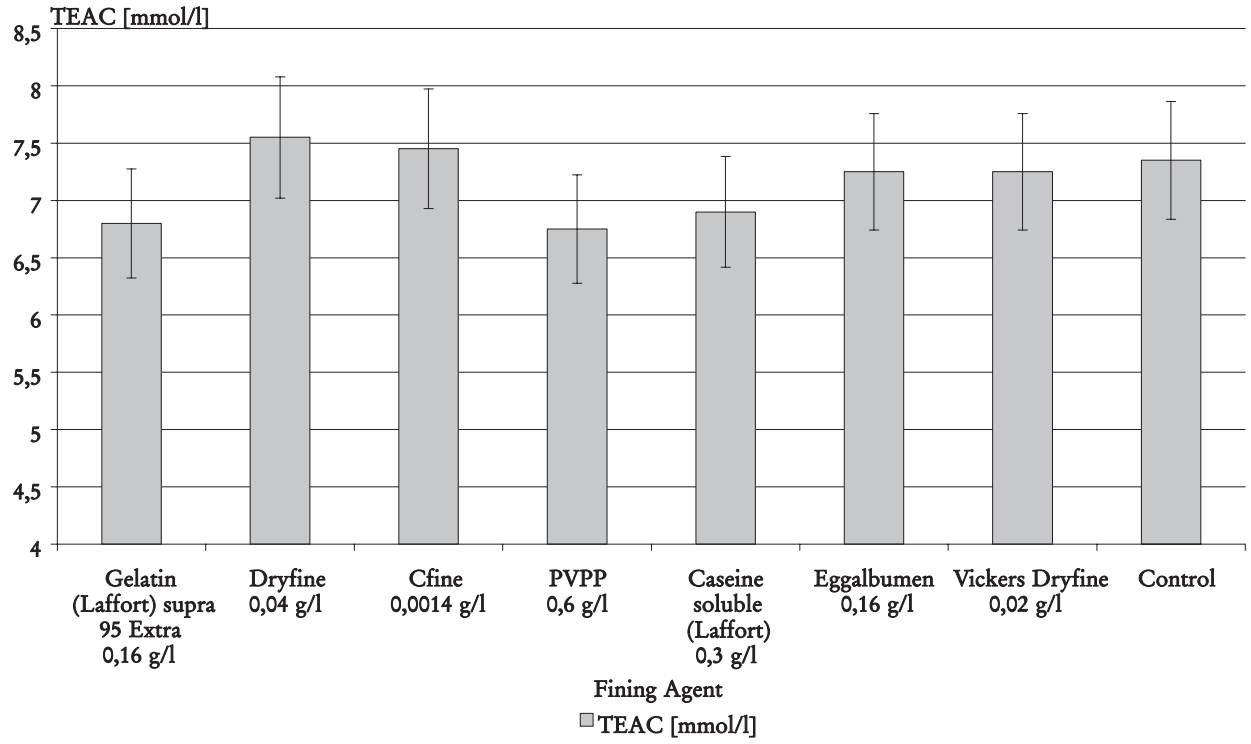


Figure 3: Influence of different proteinaceous fining agents on antioxidative capacity (TEAC test) in 'Pinot noir' wines (mean values, n = 3)

and UV/VIS-sprectroscopy results, flavan-3-ols and their polymers are bound in these new protein-fractions explaining the reported effects of Cfine® on sensory properties (better taste and less astringency and bitterness) in red wine. The amino acid composition of Cfine® mainly contains glycine, alanine, proline, gluta-

Table 1:
Concentration of monomeric phenols by HPLC analysis (mg/l)

Polyphenols		Cfine additions (mg/l)									
		0	5	10	20	40	60	80	100	150	200
Gallic acid	colourless phenols	27,9	25,0	26,8	28,0	26,2	30,2	30,2	29,2	29,6	30,1
Catechin		53,4	53,8	56,8	58,7	57,8	61,0	61,1	59,3	58,6	59,3
Epicatechin		40,7	37,8	39,0	40,1	39,5	35,9	36,0	35,1	34,7	36,0
Resveratrol		0,27	0,25	0,31	0,33	0,35	0,26	0,25	0,26	0,21	0,19
Caftaric acid		2,5	2,5	2,5	2,6	2,6	2,7	2,7	2,7	2,6	2,6
GRP		4,1	4,1	4,0	4,0	4,2	4,0	4,1	4,0	3,8	4,0
Coutaric acid		1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2
Caffeic acid		22,8	20,7	21,3	22,2	22,2	21,2	21,4	20,6	20,8	21,4
p-Coumaric acid		7,6	6,9	7,2	7,5	7,5	7,1	7,2	6,8	7,0	7,1
Ferulic acid		3,0	2,9	2,9	2,9	2,9	3,2	3,2	3,2	2,8	2,9
Malvidin-3-glc	Antho-cyanidins	164,1	153,7	156,9	161,7	161,2	156,3	154,5	152,3	153,0	154,6
Delphinidin-3-glc		6,6	6,2	6,4	6,6	6,6	6,1	6,0	6,0	6,0	6,1
Cyanidin-3-glc		13,5	12,7	13,0	13,5	13,5	12,8	12,5	12,3	12,3	12,5
Petunidin-3-glc		0,79	0,69	0,73	0,78	0,79	0,71	0,68	0,67	0,64	0,62
Peonidin-3-glc		9,1	8,5	8,7	8,7	8,7	9,2	9,2	9,0	9,1	9,2

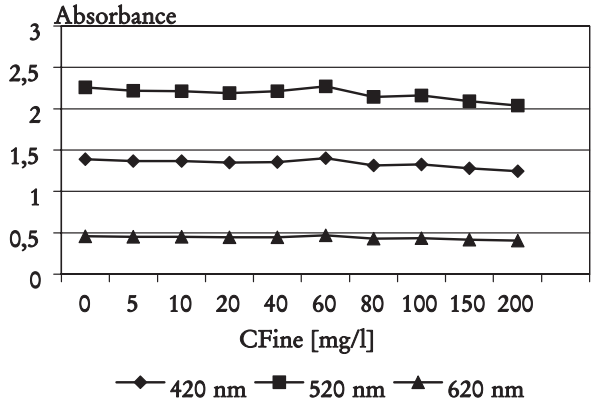


Figure 4: Influence of increased concentrations of Cfine® on colour of 'Pinot noir' wines at different wavelengths (n = 3)

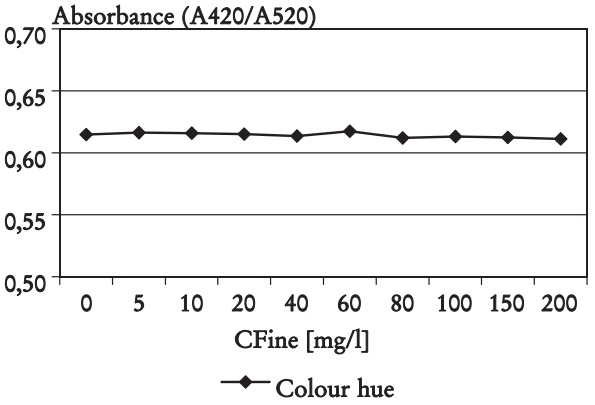


Figure 5: Influence of increased concentrations of Cfine® on colour hue of 'Pinot noir' wines (n = 3)

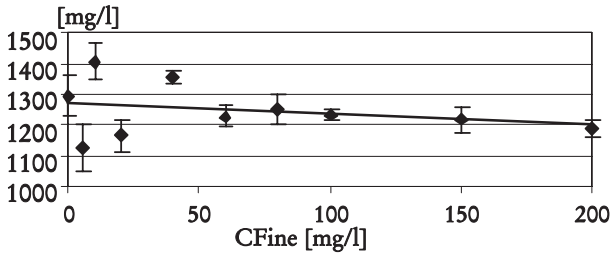


Figure 6: Total phenolic concentrations in 'Pinot noir' wine at different Cfine® concentrations (n = 3)

mic acid, serine, hydroxyproline and arginine in declining concentrations, gelatine (gelisol) is mainly composed of glycine, alanine, proline and hydroxyproline.

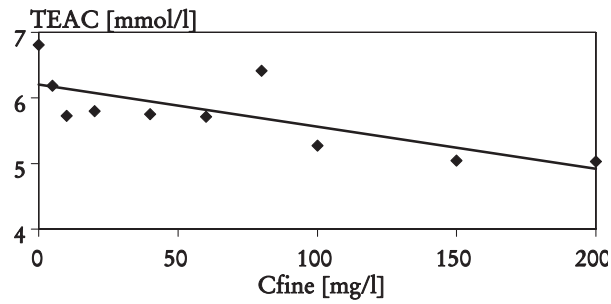


Figure 7: TEAC concentrations in 'Pinot noir' wines treated with different Cfine® concentrations (n = 3)

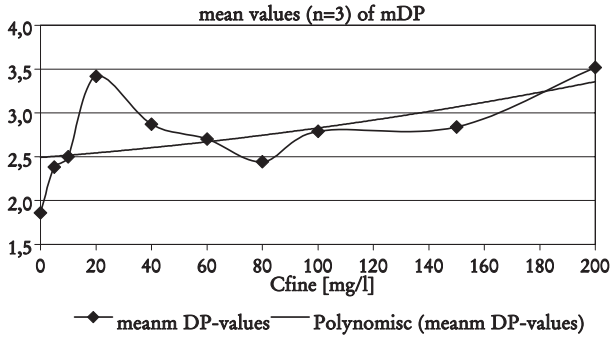


Figure 8: Mean degree of polymerisation (mDP values) of 'Pinot noir' wines treated with different Cfine® concentrations (n = 3)

Compared to Cfine® and gelisol, wheat protein preparations are mainly composed of glutamic acid/glutamine and proline, those of lupin of glutamic acid/glutamine and aspartic acid/asparagine (CHEYNIER et al., 2003). Compared to the main molecular weights of wheat gluten and lupin, those of Cfine® (as shown in Fig. 12) are relatively high.

Discussion

Cfine® seems to be a gentle fining agent regarding colour, antioxidative capacity and 'soft-tasting' monomeric phenols. It produced well related lees layers (depth) when added to 'Pinot noir' wine (data not shown). Cfine® can be used in small concentrations as a final "polish" to achieve an effective fining with regard to removing bitter tasting polyphenols and preserving 'soft-tasting' and colouring monomeric phenols, as well as preserving and enhancing colour itself. Compared to several plant proteins, such as wheat gluten and lupin,

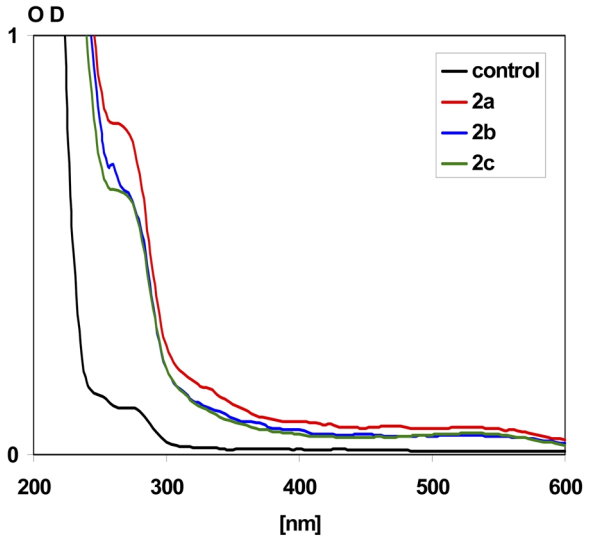


Figure 9: UV/VIS spectrum of the fining trial lees, Cfine® concentration 5 mg/l

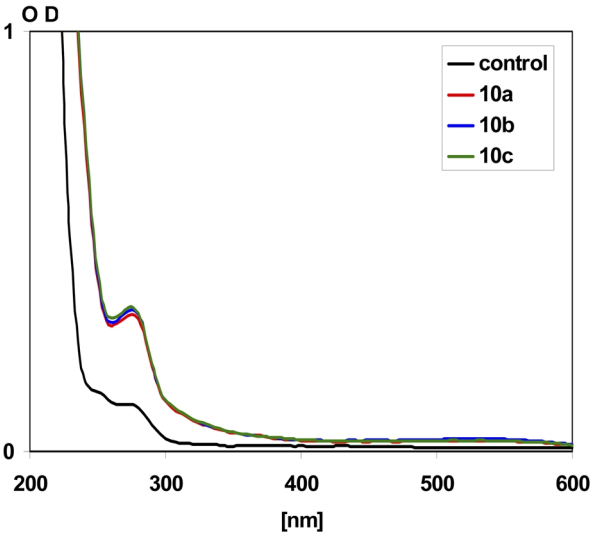


Figure 10: UV/VIS spectrum of the fining trial lees, Cfine® concentration 200 mg/l

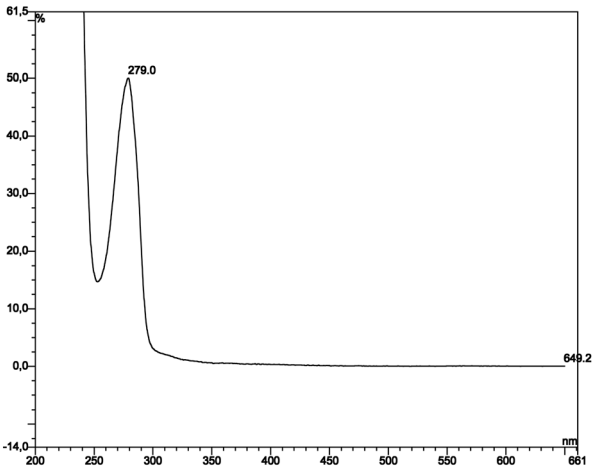
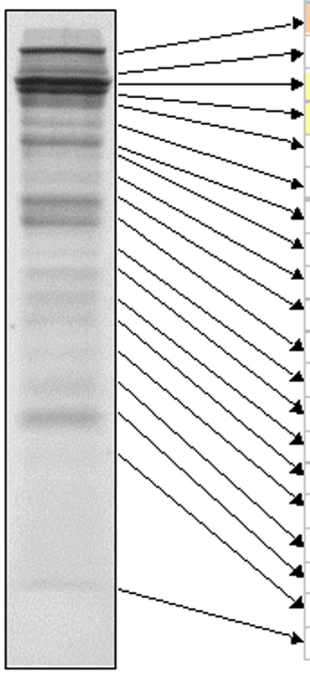


Figure 11: UV/VIS spectrum of catechin

Cfine® mainly removed lower 'mDP tannins' (lower degree of polymerisation) which might be due to higher molecular weights of the Cfine® protein fractions.
However, this preliminary study has not yet considered the sensory impact of the fining treatment and such study would be of great importance to prove the results of the fining trial with regard to taste (higher mDP values and increased mean tannin chain length after fining). Compared to gelatine, fining with certain plant proteins and wheat proteins, respectively, leads to less astringency, a lower flock height, good flotation properties, and, in some cases to spicy flavours as well as more dryness. Plant proteins seem to be a good choice as replacement for gelatine and other animal derived fining agents (LEFEBVRE et al., 2000 and 2002; FISCHER-LEITNER et al., 2002; SCHMIDT et al., 2003).
Further research might also investigate the effect of Cfine® on polysaccharide concentration and/or individual aroma compounds, which would give further knowledge on the effect and impact on wine composition after fining.
Finally, using *Cfine*® in Australia and New Zealand is more than a pure winemaking decision, because its use has legally to be labelled on the wine bottle using the words "this product may contain fish or fish-related products" according to "Australia New Zealand Food Standards Code" since 20 December 2002 (http://www.nzfsa.govt.nz/policy-law/legislation/food-standards/). Such labelling is under current discussion in the European Union as well and might determine its further use during winemaking due to consumer acceptance reasons of the final (labelled) product.
Although no allergic reactions are known so far, research on the allergenic potentials of Cfine® as a proteinaceous fining agent still needs to be done. It will not be avoidable to detect small amounts of fining agents remaining in the fined wine.
To sum it up, Cfine® mainly removes tannins with a shorter chain length, whereas hydrolysed wheat glutens



No.	Molmasse (Da)	[%]
1	102984	12.4
2	95316	6.6
3	90885	16.8
4	88040	12.7
5	85851	7.2
6	77883	3.5
7	71961	6.9
8	68844	2.3
9	62164	2.9
10	56658	6.8
11	52187	5.8
12	46125	1.1
13	42424	2.2
14	38262	2.2
15	35184	1.5
16	30586	1.1
17	26713	2.1
18	23541	3.7
19	20125	0.7
20	11967	1.4

Figure 12: Protein fractions of the fining agent Cfine® (bands making up the three highest amounts shaded)

as well as lupin mainly removed highly galloylated tannins. Compared to other common fining agents (Fig. 1 to 3) Cfine® showed pretty good results regarding the preservation of colour, total phenols and the antioxidative capacity. The latter is closely linked to the French paradox and health benefits, respectively. The impact of Cfine® as well as the plant proteins on monomeric phenols seems to be negligible. Therefore, Cfine®, as well as several plant proteins could be used for effective fining instead of the possibly hazardous fining agent gelatine (BSE). However, labelling of the usage of Cfine® or other fish-derived fining agent is under current discussion in the European Union and it already needs to be labelled in New Zealand and Australia.

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