Efficiency of Enzymatic and Other Alternative Clarification and Fining Treatments on Turbidity and Haze in Cherry Juice

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Several alternative strategies were examined for improving conventional juice fining procedures for cherry juice clarification and fining in laboratory-scale experiments: Centrifugation of freshly pressed juice from 1000*g* to 35000*g* induced decreased turbidity according to a steep, negative power function. Individual and interactive effects on turbidity and haze formation in precentrifuged and uncentrifuged cherry juice of treatments with pectinase, acid protease, bromelain, gallic acid, and gelatin−silica sol were investigated in a factorial experimental design with 32 different parameter combinations. Gelatin−silica sol consistently had the best effect on juice clarity. Centrifugation of cherry juice (10000*g* for 15 min) prior to clarification treatment significantly improved juice clarity and diminished the rate of haze formation during cold storage of juice. Both treatment of precentrifuged cherry juice with Novozym 89L protease and co-addition of pectinase and gallic acid improved cherry juice clarity and diminished haze levels. None of the alternative treatments produced the unwieldy colloids notorious to gelatin−silica sol treatment. The data suggest that several alternative clarification strategies deserve further consideration in large-scale cherry juice processing. Precentrifugation of juice before clarification and fining is immediately recommended.

Keywords: *Clarification; fining; turbidity; haze; cherry juice; protease; pectinase; gelatin; silica sol*

INTRODUCTION

Industrial production of cherry juice includes a number of steps intended to clarify the pressed juice. Clarification treatment of cherry juice typically involves addition of pectinases and fining agents such as bentonite or gelatin−silica sol to remove cloud, sediments, and haze-active components (*1*). Usually, such juice clarification also includes a slow settling of the colloid flocs resulting from the action of added pectinase and fining agents and subsequent separation and recovery of juice from the flocculated material. In Europe, industrial production of cherry juice and cherry juice concentrates is largely based on local varieties of sour (or tart) cherries (*Prunus cerasus* L.) (*2*).

The main purpose of the clarification treatment employed in industrial cherry and other fruit juice processing is to take away constituents responsible for the immediate turbidity and cloudiness in freshly produced juice. The other important purpose is to remove substances that may cause haze and sediment formation during storage, at reconstitution of the concentrate, or after bottling of the juice.

Only very little is known about the identity of the substances responsible for cloud, turbidity, and haze development in cherry juice. However, in accordance with the knowledge on cloudiness and turbidity in apple juice (*3*, *4*), the current practical experiences from use of pectinases and fining agents in the clarification of cherry juice (*5*) indicate that the primary cloud in freshly pressed cherry juice is mainly due to the presence of suspended proteinaceous pectin particles. Fractions of other fruit cell wall material may also be responsible for some coarse turbidity as is the case in other types of fruit juices (*6*, *7*). The sediments and postclarification haze may result mainly from proteins, polyphenols, oxidized phenolics, insoluble tannins, and their interactions as demonstrated in apple juice, grape juice, wine, and haze model solutions (*4*, *8*−*10*). There exists no specific terminology to discriminate turbidity, cloud, sediments, and postclarification haze formation in fruit juices according to their chemical origins or times of emergence in the juice process. In this paper, haze will be used to describe the turbidity that eventually forms after clarification during cold storage of cherry juice.

Enzymatic depectinization in fruit juice clarification is assumed to work by pectinase-catalyzed electrostatic destabilization of suspended, cloud-causing pectin particles (*3*, *11*). Clarification of fruit juices by fining treatment with bentonite or gelatin and silica sol rests on unspecific binding of haze-active polyphenols and proteins and capture of sediments and cloud substances during subsequent sedimentation of the resulting colloidal structure (*6*, *12*). The fining treatment may be performed after, or concurrent with, pectinase clarification treatment. The generally used criterion for cherry juice clarity is that the turbidity of the final, freshly produced juice is <5 FNU (formazan nephelometric units at 3.0 °Brix) as evaluated by nephelometry (*13*).

In large-scale fruit juice processing, the gelatin−silica sol clarification step is slow, as it may last for a minimum of 6−18 h to accomplish the necessary sedimentation of the colloidal particles. Incomplete sedimentation of the colloidal material results in prolonged processing times and significant juice losses. The clari-

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fied supernatant juice fraction remaining after settling of flocs may be centrifuged prior to subsequent filtration treatments, but the voluminous, viscous precipitate requires the use of sludge frame filters with kieselguhr as a filtering aid or high-vacuum rotary filtration systems with diatomaceous earth as a filtering aid to recover some of the juice captured in the colloid sediment slurry (*6*). The filtering aids kieselguhr and diatomaceous earth both contain silica dust and are considered to be harmful hazardous materials. Environmental regulations therefore require special handling and disposal of these materials and the wastes from filtration operations. Altogether, this creates an incentive for the development of alternative clarification and fining strategies in the cherry juice and other fruit juice industries.

One option could be to remove the densest turbidity-causing substances directly by high-speed centrifugation of the freshly pressed juice. In particular, it would be relevant to explore if such centrifugation could enhance clarification efficiency if introduced prior to addition of clarification and fining agents. A direct alternative approach to gelatin−silica sol or bentonite fining treatment would be a more targetted removal of haze-active proteins via enzyme catalysis using proteases. Another approach could be to chemically block haze-active protein binding sites and thus prevent protein molecules from entering into multimolecule protein−polyphenol cross-binding (*10*). Addition of gallic acid and methyl gallate, which are not able to cross-link with other haze-active proteins, was thus recently shown to retard haze formation in beer model systems (*14*).

The purpose of this study was to explore, in laboratory-scale experiments, the effects of a range of alternative strategies for clarification and fining to decrease turbidity and haze in cherry juice. First, the impact of centrifugation on cloud removal was investigated. Then, individual and interactive effects on immediate juice turbidity and haze formation in cold-stored cherry juice of treatments with a pectinase, two different proteases, and gallic acid were examined coordinately on precentrifuged and noncentrifuged juice versus gelatin−silica sol treatment. A central composite experimental design consisting of 35 experimental points was used to study the effects of different combinations of treatments.

MATERIALS AND METHODS

Cherry Juice. Freshly pressed juice produced from sour cherries (*Prunus cerasus* L.) cv. Stevnsbær was sampled directly after pressing and pasteurization, but prior to clarification, from an industrial cherry juice process line (Vallø Saft A/S, Vallø, Denmark). The juice was divided into suitable small portions and kept frozen (−30 °C) until use. Juice samples were gently thawed overnight at refrigeration temperature (5 °C) prior to the day of use in clarification experiments.

Chemicals and Enzymes. Gelatin (100 AB 30 acid bone gelatin) was obtained from SKW Biosystems (Boulogne Billancourt Cedex, France), and silica sol (Klar-Sol Super) was from Erbslöh Getränke Technologie (Geisenheim, Germany). Macer8 FJ pectinase, a liquid preparation from an *Aspergillus* sp. with a declared activity of 1500 polygalacturonase units/g, was from Biocatalysts Ltd. (Pontypridd, U.K.). Novozym 89L acid protease produced by *Mucor miehei*, a liquid preparation with a declared activity of 0.18 Anson units/g, was obtained from Novo Nordisk A/S (Bagsværd, Denmark). Bromelain, a lyophilized preparation from pineapple stem with a declared activity of 1 gelatin digestion unit/mg of dry weight, was obtained from CalBiochem (La Jolla, CA). Carbazole, gallic acid, and Folin−Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO). Borax and galacturonic acid were from Merck (Darmstadt, Germany).

Centrifugation Experiments. The impact of centrifugation on primary turbidity was tested by spinning unclarified cherry juice samples (100 mL) for 10 min at 1000−35000*g* and for extended time periods at 35000*g* using a Sorvall, floor-model superspeed centrifuge (Buch and Holm, Herlev, Denmark). The efficiency of high-speed centrifugation prior to gelatin−silica sol treatment was evaluated on juice samples that were centrifuged in the Sorvall centrifuge at 20000*g* for 20 min.

Clarification Treatments. Individual and combined effects on immediate cherry juice turbidity and on turbidity development during storage (21 days, 2 °C) of the addition of Macer8 FJ pectinase (0.5 g/L), Novozym 89L acid protease (0.5 g/L), bromelain (0.05 g/L), gallic acid (0.05 g/L), and gelatin−silica sol (0.063 g of gelatin/L, 0.625 g of silica sol/L) were evaluated on uncentrifuged and centrifuged (15 min at 10000*g*) cherry juice samples. All treatments were at 50 °C for 4 h. Gelatin and silica sol were added to cherry juice samples according to the manufacturers' instructions at levels used in industrial berry juice processing: gelatin was thus preswelled and dissolved by heating (50 °C) in doubly distilled water (12.5 g/L) prior to addition to the juice, and silica sol was subsequently added directly to the juice. A randomized, fractional 2^6 factorial design comprising 32 different combinations of parameters and 3 replicated centerpoints (*15*) was used to examine individual and interactive effects of parameters on immediate juice turbidity and on haze formation during cold storage (2 °C) of the juice. Responses were evaluated by multiple linear regression. Spoilage of cherry juice during cold storage was prevented by adding potassium sorbate and sodium benzoate immediately after experimental treatments to a final, total concentration in juice of 1 ppm (0.5 ppm of each). During cold storage, haze formation was assessed by evaluating turbidity in samples at days 0, 2, 6, 15, and 21 after treatment.

Determination of Total Phenol, Protein, and Pectin Levels. The initial concentration of total phenols in cherry juice samples was determined by using the Folin−Ciocalteu procedure with total phenols expressed as milligrams per liter of gallic acid equivalents (GAE) (*16*). Protein content in cherry juice was assessed from analysis of nitrogen according to the Dumas method using a Nitrogen Analyzer Macro N instrument (Elementar Analysensysteme GmbH, Foss Electric, Hillerod, Denmark). The results are expressed as protein by using a multiplication factor of 6.25 from analyzed nitrogen levels. Pectin was determined after sulfuric acid hydrolysis of cherry juice samples as the amount of uronic acids reacting with carbazole, determined spectrophotometrically at 530 nm (*17*). The uronic acid results were calibrated against a standard curve of galacturonic acid and corrected for mutual interference effects as recommended by Hudson and Bailey (*18*).

Juice Turbidity Measurements. Turbidity in formazan nephelometric units (FNU) was measured by nephelometry at 90° light scattering, 860 nm, with a Nephla reader calibrated against hexamethylene tetramine formazin according to the instrument manufacturer's instructions (Dr. Lange, Düsseldorf, Germany). One FNU equals 2.5 mg/L SiO_2. Prior to measurement all cherry juice samples were diluted to 3.0 °Brix. °Brix values were measured with a manual refractometer (Carl Zeiss Gmbh, Vienna, Austria). To ensure equal comparison of treatments in the factorially designed experiment, the same dilution factor of 6 was used for all samples to obtain 3 °Brix.

Statistics. Differences in turbidity after different centrifugations were determined by one-way analysis of variances, where 95% confidence intervals, that is, $P \leq 0.05$ significance level, were calculated from pooled standard deviations using Minitab statistical software (Addison-Wesley, Reading, MA). The computer program Modde (Umetri AB, Umeå, Sweden) was used as an aid in the statistical design of the factorial experiment and to fit and analyze the data by multiple linear regression. Significance of the results was established at $P \leq 0.05$.

Table 1. Characteristics of Industrially Pressed Unclarified, Pasteurized Sour Cherry Juice (*P. cerasus* L. Stevnsbær)

juice property	
pH	3.3
sugar content (°Brix)	18
turbidity (FNU[a])	>1300
pectin (g/L)	1.84[b]
protein (g/L)	6.86[c]
phenols (mg of GAE[d]/L)	4947

[a] Formazan nephelometric units measured at 3 °Brix. [b] Measured as uronic acid. [c] Calculated from measured nitrogen (6.25 × N). [d] Gallic acid equivalents.

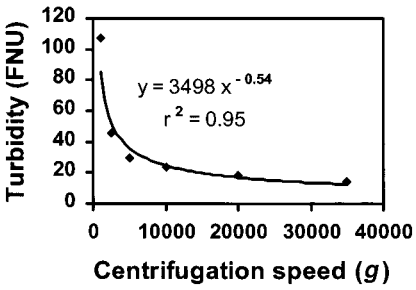

Figure 1. Effect of increased centrifugation speed on turbidity in unclarified sour cherry juice. Centrifugation time = 10 min.

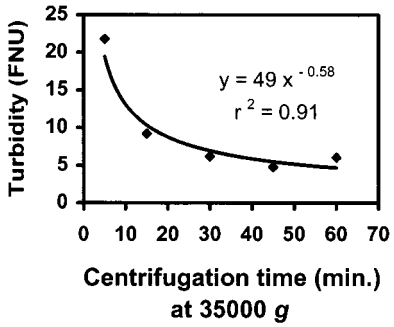

Figure 2. Effect on juice turbidity of extended centrifugation of unclarified sour cherry juice at 35000*g*.

RESULTS

Initial Cherry Juice Characteristics. A summary of the properties of the industrially produced unclarified, raw cherry juice that was used as a starting material for experimental clarifications is shown in Table 1. The pH of this juice was 3.3, and the sugar content was equivalent to ~18 °Brix. The raw sour cherry juice contained visible particles and cloud, and its turbidity was beyond the measurable range (>1300 FNU) (Table 1).

Effect of Centrifugation. Direct centrifugation of freshly pressed cherry juice samples induced significantly decreased turbidity with increasing centrifugation speed (*g*): The decrease in FNU levels obtained by centrifugation for 10 min at 1000−35000*g* was most profound from 1000*g* to 5000*g* ($P < 0.05$). The overall effect of centrifugation speed on juice turbidity could be described by a steep negative power function: $Y = 3498X^{-0.54}$ ($r^2 = 0.95$), where Y is turbidity in FNU and X is centrifugation power in *g* (Figure 1). Likewise, the turbidity decrease obtained by extended high-speed centrifugation of cherry juice at 35000*g* fitted a power function: $Y = 49X^{-0.58}$ ($r^2 = 0.91$) (Figure 2). By centrifugation for 10 min at 20000−35000*g* the lowest FNU levels obtained were from 14 to 18 FNU (Figure 1), but by extended centrifugation at 35000*g* turbidity levels as low as 5−6 FNU were achieved (Figure 2). Amounts of total phenols as well as protein content also decreased with increased centrifugation power, but, as expected, the observed decreases did not immediately correlate to the decrease in FNU or were strongly related to the centrifugation power applied to the cherry juice (Table 2).

Influence of Centrifugation prior to Gelatin—Silica Sol Treatment. High-speed centrifugation of raw cherry juice at 20000*g* for 20 min resulted in the same turbidity in the supernatant juice of 18 FNU as conventional fining treatment of raw cherry juice with gelatin−silica sol for 25 h at 25 °C (Table 3). As indicated in Table 3, fining treatments with gelatin−silica sol were followed by centrifugation, which was done to accelerate separation of colloid sludge. If cherry juice was centrifuged prior to addition of gelatin−silica sol fining agents, clarification efficiency improved significantly and a clear juice with an FNU level below 5 was obtained (Table 3).

Turbidity after Experimental Clarification Treatments. Treatments of precentrifuged and raw cherry juices with different combinations of a pectinolytic enzyme preparation (Macer8 FJ), two proteases (Novozym 89L, bromelain), gallic acid, and gelatin−silica sol resulted in very different juice turbidity responses immediately after treatment ranging from 3.5 to 35 FNU in the different experiments (day 0 column, Table 4). The 35 different treatments comprised a 2^6 factorial experimental design with 32 different experimental combinations and 3 center points: Nine of the experimental treatments resulted in FNU levels <10 immediately after treatment (day 0), and five treatments immediately gave a satisfactorily clear cherry juice with FNU ≤5 under the test conditions (experiments 5, 14, 15, 23, and 32, day 0, Table 4). As expected, precentrifuged juice samples (10000*g* for 15 min) were generally less turbid after different treatments having the lowest FNU levels, with several in the range of 3.5−6 FNU at day 0 (experiments 5, 14, 15, 22, 23, 29, and 32, Table 4). However, with some precentrifuged samples, the turbidity levels after experimental clarification treatments remained much higher and ranged from 29 to 31 FNU at day 0 (experiments 2, 9, 17, 20, 26, and 27, Table 4). It was beyond the scope of the present work to find a mechanistic explanation for this phenomenon. Nevertheless, precentrifugation of samples resulted in a strongly significant clarifying effect on cherry juice ($P < 0.001$), that is, with a negative correlation coefficient of −3.7 on turbidity at day 0 when evaluated by multiple linear regression (Table 5). Gelatin−silica sol treatment also consistently had a significantly positive impact on juice clarity by decreasing initial turbidity independent of sample precentrifugation ($P < 0.001$, Table 5). As expected, treatments with gelatin−silica sol produced an exaggerated colloid precipitate, which was not seen with samples not subjected to gelatin−silica sol. Individually, neither the pectinase, the two proteases Novozym 89L and bromelain, nor gallic acid addition influenced cherry juice turbidity (all P values >0.05, Table 5). However, treatment of cherry juice with Macer8 FJ pectinase in combination with gallic acid addition resulted in a significant clarifying effect immediately after treatment ($P < 0.05$, Table 5). Likewise, even though the turbidity after treatment of centrifuged cherry juice with Novozym 89L was rather high (experiment 3, day 0, Table 4), treatment of precentrifuged

Table 2. Summary of Effects of Increased Centrifugation Speed (10 min Centrifugation) and Extended Centrifugation Time at 35000*g* on Levels of Total Phenols and Protein in the Resulting Supernatant Sour Cherry Juice

centrifugation treatment	phenols range (mg of GAE/L)	descriptive function and data correlation[a]	protein range (g/L)	descriptive function and data correlation[a]
centrifugation for 10 min: speed increase from 1000*g* to 35000*g*	4147−3970	$y = 4118X^{-0.03}$ $r^2 = 0.64$	4.96−4.79	$y = -0.023X + 4.9$ $r^2 = 0.56$
centrifugation at 35000*g*: time extension from 5 to 60 min	3860−4007	no correlation	4.80−4.75	$y = -0.005X + 4.8$ $r^2 = 0.03$

[a] Functions calculated versus the increased centrifugation treatment.

Table 3. Effects of High-Speed Centrifugation of Industrially Pressed Unclarified, Pasteurized Sour Cherry Juice prior to Gelatin−Silica Sol Fining Treatment

juice treatment	turbidity (FNU[c])
none	>1300[c]
centrifugation[a]	17.9[b]
gelatin−silica sol[b] + centrifugation[a]	18.1[b]
centrifugation[a] + gelatin−silica sol[b] + centrifugation[a]	3.5[a]

[a] 20000*g* for 20 min. [b] 0.063 g of gelatin/L, 0.625 g of silica sol/L, contact time = 25 h at 25 °C (as indicated in the table, fining treatments were always followed by centrifugation to accelerate separation of colloid sludge). [c] Formazan nephelometric units measured at 3 °Brix. The FNU results having different roman superscript letters are significantly different at $P < 0.05$. Pooled SD = 0.7 FNU.

juice samples with Novozym 89L exerted an interactive clarifying effect that penetrated as a significant effect by multiple linear regression analysis ($P < 0.05$, Table 5).

Effects of Experimental Clarification Treatments on Haze Formation during Cold Storage. During cold storage of cherry juice haze progressively increased with time in all of the experimentally clarified juice samples as evaluated from FNU levels. However, the rates of increase differed considerably among differently treated samples, and the FNU levels recorded after 3 weeks of cold storage varied widely in the different cherry juice samples and ranged from 5 to 48 FNU (turbidity day 21, Table 4). The combined treatment comprising Novozym 89L, gelatin−silica sol, and gallic acid addition to precentrifuged juice resulted in the lowest turbidity of only 4.7 FNU after 3 weeks of cold storage at 2 °C and was thus the best treatment in preventing haze formation (experiment 15, Table 4). The treatment with Novozym 89L, gelatin−silica sol, and bromelain as well as addition of all the tested parameters combined also maintained low haze levels of ∼5−6 FNU during cold storage (experiments 23 and 32, Table 4). Evaluation of the effect of the tested parameters on haze levels after 21 days of cold storage affirmed that gelatin−silica sol as well as precentrifugation had significantly positive effects as single factors to retard haze development ($P < 0.001$, Table 5), that is, significances similar to their influence on the turbidity levels immediately after treatment. The significantly positive interaction on juice clarity of Novozym 89L and precentrifugation was maintained after 21 days in cold storage ($P = 0.01$, Table 5), but the clarifying interaction between pectinase and gallic acid had weakened ($P = 0.07$, Table 5). A comparison of the differences among samples in rate of haze development during cold storage revealed that, in general, the samples having the lowest initial turbidity levels after clarification treatment also had lower rates of haze formation during cold storage (haze rate column, Table 4). Thus, precentrifugation and gelatin−silica sol treatment retarded the rate of haze formation most efficiently ($P < 0.001$ for both parameters, Table 6). In contrast, pectinase plus gallic acid treatment unexpectedly had no effect on haze formation rate (Table 6), and neither did the positive interaction on turbidity levels of precentrifugation and Novozym 89L treatment penetrate to affect the rate of haze formation with a statistically strong significance ($P = 0.09$, Table 6). Novozym 89L in combination with gallic acid surprisingly produced an increase in the haze formation rate ($P = 0.05$, Table 6), but the coefficient was small (0.03, Table 6).

DISCUSSION AND CONCLUSIONS

This study showed that several alternative procedures show promise as novel approaches to clarification and fining treatments of cherry juice. In particular, direct removal of turbidity-causing material by high-speed centrifugation proved to be efficient in improving juice clarity. Furthermore, precentrifugation of juice significantly enhanced the fining efficiency of gelatin−silica sol (Table 3) and penetrated as a single factor as well as in interaction with Novozym 89L protease treatment to significantly improve juice clarity and haze in experimental clarification treatments (Tables 4 and 5). The power functions derived from our data describing the effect of centrifugation on cherry juice turbidity proved that extended high-speed centrifugation of unclarified juice at 35000*g* can clarify cherry juice and that centrifugation at 5000−10000*g* for 10 min have a significant impact on juice turbidity. Removal of fruit pulp by centrifugation at ∼360−15000*g* for 10 min has been used in research on orange juice and guave puree clouding substances (*19*, *20*), but quantitative data on the removal of turbid substances in freshly pressed cherry juice by centrifugation are scarce. High-speed centrifugation at ∼10000*g* is a procedure already used in large-scale cherry juice processing: According to flow sheets on cherry juice production available in the literature (*1*) industrial cherry juice processing may include a centrifugation step immediately after juice pressing, but many large-scale cherry juice processors in Europe use centrifugation only to separate particles in the supernatant cherry juice after settling out of the colloid gelatinous precipitate resulting from gelatineous fining treatment. In the fruit juice industry disk−bowl type centrifuges appear to be the most widely employed centrifuge types to allow for semicontinuous operation during processing of large fruit juice batches. With these centrifuges, the juice is usually centrifuged at a maximum force of 10000*g* for a few seconds only. Obviously, centrifugation is an energy-expensive procedure, and at present, extended high-speed centrifugation beyond a few seconds may be difficult to achieve with the centrifuge equipment currently available in many large-scale fruit juice processing plants. However, on the basis of the data obtained in this work, we recommend that the

Table 4. Influence of Pectinase, Proteases, Gelatin−Silica Sol, Gallic Acid, and Precentrifugation Treatment on Cherry Juice Turbidity and Rate of Haze Formation during Cold Storage

expt	Macer8 FJ pectinase (g/L)	Novozym89 protease (g/L)	gelatin + silica sol (g/L)	gallic acid (g/L)	bromelain protease (g/L)	precentrifugation[b]	turbidity (FNU)[c] day 0	turbidity (FNU)[c] day 21	haze rate[d] days 0−21 (ΔFNU/day)
1	0	0	0	0	0	0	31.9	39.6	0.33
2	0.5	0	0	0	0	centrifuged	30.6	32.4	0.08
3	0	0.5	0	0	0	centrifuged	22.5	26.6	0.15
4	0.5	0.5	0	0	0	0	36.3	43.0	0.29
5	0	0	0.06 + 0.6	0	0	centrifuged	5.0	6.5	0.06
6	0.5	0	0.06 + 0.6	0	0	0	15.1	20.9	0.21
7	0	0.5	0.06 + 0.6	0	0	0	11.3	12.7	0.06
8	0.5	0.5	0.06 + 0.6	0	0	centrifuged	6.8	8.2	0.06
9	0	0	0	0.05	0	centrifuged	29.2	32.6	0.13
10	0.5	0	0	0.05	0	0	33.8	37.5	0.15
11	0	0.5	0	0.05	0	0	35.4	46.2	0.37
12	0.5	0.5	0	0.05	0	centrifuged	26.7	29.3	0.10
13	0	0	0.06 + 0.6	0.05	0	0	18.9	21.5	0.11
14	0.5	0	0.06 + 0.6	0.05	0	centrifuged	4.8	6.2	0.07
15	0	0.5	0.06 + 0.6	0.05	0	centrifuged	3.4	4.7	0.05
16	0.5	0.5	0.06 + 0.6	0.05	0	0	14.7	21.8	0.26
17	0	0	0	0	0.05	centrifuged	30.4	33.1	0.10
18	0.5	0	0	0	0.05	0	33.4	42.0	0.39
19	0	0.5	0	0	0.05	0	35.4	44.7	0.34
20	0.5	0.5	0	0	0.05	centrifuged	29.4	31.7	0.10
21	0	0	0.06 + 0.6	0	0.05	0	6.6	7.5	0.03
22	0.5	0	0.06 + 0.6	0	0.05	centrifuged	5.7	7.1	0.07
23	0	0.5	0.06 + 0.6	0	0.05	centrifuged	4.3	5.8	0.06
24	0.5	0.5	0.06 + 0.6	0	0.05	0	17.3	19.6	0.09
25	0	0	0	0.05	0.05	0	34.5	36.3	0.05
26	0.5	0	0	0.05	0.05	centrifuged	31.3	33.7	0.11
27	0	0.5	0	0.05	0.05	centrifuged	29.1	31.7	0.09
28	0.5	0.5	0	0.05	0.05	0	37.5	48.2	0.40
29	0	0	0.06 + 0.6	0.05	0.05	centrifuged	6.1	8.4	0.09
30	0.5	0	0.06 + 0.6	0.05	0.05	0	13.6	15.1	0.07
31	0	0.5	0.06 + 0.6	0.05	0.05	0	12.1	20.6	0.29
32	0.5	0.5	0.06 + 0.6	0.05	0.05	centrifuged	3.5	5.1	0.04
33	0.25	0.25	0.03 + 0.3	0.025	0.025	50:50 mix	26.9	30.4	0.17
34	0.25	0.25	0.03 + 0.3	0.025	0.025	50:50 mix	22.2	28.3	0.27
35	0.25	0.25	0.03 + 0.3	0.025	0.025	50:50 mix	22.0	23.5	0.07

[a] All treatments were at 50 °C for 4 h; cold storage at 2 °C. [b] Precentrifugation: 15 min at 10000*g*. [c] Formazan nephelometric units measured at 3 °Brix. [d] Values for rate of haze formation are given as slopes of linear regression lines fitted from the turbidity data (from days 0, 2, 6, 15, and 21) vs time.

Table 5. Multiple Linear Regression Coefficients Describing the Influence of Different Treatments on Immediate Cherry Juice Turbidity and on Haze after 21 Days of Cold Storage[a]

term	immediate turbidity: regression coefficient	immediate turbidity: P^b	haze: regression coefficient	haze: P^c
constant	20.79	5.04×10^{-27}	24.64	1.12×10^{-26}
Macer8 FJ pectinase	0.76	0.10	0.74	0.19
Novozym89 protease	−0.16	0.73	0.61	0.28
gelatin + silica sol	−11.20	1.07×10^{-3}	−12.40	1.25×10^{-18}
gallic acid	0.40	0.38	0.55	0.33
bromelain	0.12	0.79	0.03	0.96
precentrifugation	−3.72	8.97×10^{-9}	−5.44	2.50×10^{-10}
Macer × gallic acid	−0.94	0.05	−1.05	0.07
Novozym × precentrifugation	−0.93	0.05	−1.67	0.01

[a] As estimated from turbidity data obtained at days 0 and 21 after treatment, respectively (Table 4). $P \leq 0.05$ indicates significance at a 95% level. [b] The 95% confidence limit on each regression coefficient was ±0.92 (±0.88 for the constant). [c] The 95% confidence limit on each regression coefficient was ±1.13 (±1.08 for the constant).

economic and technological feasibilities of introducing precentrifugation treatments with longer centrifugation times at 5000−10000*g* receive careful consideration in the design of novel approaches to fruit juice clarification in large-scale, commercial plant operations.

At present, detailed knowledge on the components, precursors, and mechanisms responsible for turbidity and haze in cherry juice is scarce: The findings in this work that pectinase treatment with Macer8 FJ in combination with gallic acid decreased cherry juice FNU levels and that the acidic protease Novozym 89L produced a positive interactive clarifying effect with precentrifugation provide support for the assumption that both pectin and protein−phenol interactions are involved in cherry juice turbidity. Moreover, the result that Novozym 89L and precentrifugation also interacted to give significantly lowered haze readings in cherry juice after 21 days of cold storage (Tables 4 and 5) also implies that protein is implicated in cherry juice haze development during cold storage. These assumptions are in line with the available knowledge on cloudiness, turbidity, and haze formation in apple juice, grape juice, wine, and various model solutions (*3*, *4*, *8*−*11*). In beer and apple juice there appears to be a preferential

Table 6. Multiple Linear Regression Coefficients Describing the Influence of Different Treatments on Rate of Haze Formation during Cold Storage of Cherry Juice[a]

term	regression coefficient	P^b
constant	0.154	5.18×10^{-11}
Macer8 FJ pectinase	0.005	0.72
Novozym89 protease	0.021	0.14
gelatin + silica sol	−0.049	2.06×10^{-3}
gallic acid	-3.17×10^{-4}	0.98
bromelain	-4.76×10^{-3}	0.74
precentrifugation	−0.066	1.03×10^{-4}
Macer8 × gallic acid	-4.42×10^{-3}	0.76
Novozym × precentrifugation	−0.025	0.09
Novozym × gallic acid	0.029	0.05

[a] As estimated from data in Table 4. [b] $P \leq 0.05$ indicates significance at a 95% level. The 95% confidence limit was ±0.03.

involvement of proline-rich proteins and particular dihydroxy polyphenols, notably procyanidins and catechins, in postclarification haze development (*8*−*10*). Although the proximate chemical and nutrient composition of sour cherry juice can be found in the literature, the amino acids composition is not yet available. In selecting proteases to be screened for clarification treatment and haze prevention in cherry juice in this work, we primarily included bromelain for its recognized efficiency in chill-haze prevention of beer but deliberately selected Novozym 89L because of its acid stability rather than for its proteolytic action pattern. According to the enzyme manufacturer Novozym 89L protease was originally developed as a rennet substitute for milk clotting in cheese-making.

The measured levels of protein of ∼5−7 g/L found in the cherry juice used as a starting material in this study (Tables 1 and 2) are in relatively good agreement with the available compositional data for cherry juice which indicate that the protein content in fully processed cherry juice typically averages 3 g/L (*1*). The discrepancy between our measured values and the literature data may have root in the fact that we analyzed the unclarified juice and that table values in the literature relate to ready to drink cherry juice. Second, compositional data on cherry fruit juices, as on other juices, may be influenced by the fruit variety, maturity stage, geographical origin, etc. Because the declared activities for the proteases used in this study had been produced with assay substrates unrelated to cherry protein (see activity declarations under Materials and Methods), we dosed the proteases in the experimental clarification treatments by weight in relation to the measured cherry juice protein contents, a dosing strategy we have used previously with other plant substrates of unknown composition (*21*): Thus, with the liquid protease preparation Novozym 89L we aimed for a ∼10% w/w enzyme/substrate (E/S) ratio and for the lyophilized bromelain powder 10 times less, that is, an addition level of ∼1% w/w in relation to the level of cherry juice protein.

The finding that a positive effect on cherry juice clarification was obtained with a protease (Novozym 89L) provides proof that protease catalysis may be a workable principle in cherry juice clarification to replace or minimize the use of traditional fining agents. The fact that the protease used was not specifically developed for application in fruit juice processing provides a promising basis to test if other acid proteases may in fact be even more suitable for fruit juice clarification and fining. Better knowledge on the composition, solubility, and turbidity characteristics of proteins in cherry and other fruit juices and testing of other acid proteases under various reaction conditions are needed to evaluate fully the applicability of protease clarification treatment in cherry juice and other fruit juice processing.

The rate of haze formation during cold storage of differently treated cherry juice samples was evaluated from the slopes of linear regression lines fitted from the turbidity data recorded during the storage period (Table 4). However, linear regression did not model equally well the progressive haze development in different experimentally clarified samples (data not shown). The differences in fit to a linear model of haze formation during cold storage may explain partly why the statistically significant interactions on turbidity established for Macer8 pectinase × gallic acid and for precentrifugation × Novozym 89L treatment (Table 5) did not penetrate as strongly as expected in multiple linear regression analysis of haze rates (Table 6).

The juice in this study was produced from Stevnsbær cherries. This cherry variety contains high levels of polyphenolic flavan-3-ols, that is, catechins, with reported values for catechin and epicatechin of 15 and 152 mg/kg of fresh weight (*22*). These levels are equivalent to 0.05 and 0.5 mmol/kg of fresh weight, respectively. Because the level of flavan-3-ols is associated with sediment formation and degree of haze development in various beverages (*9*), especially epicatechin, which is present at high levels, may be an important contributor to turbidity and haze development in sour cherry juice produced from Stevnsbær cherries. Like sweet cherries, sour cherries also contain relatively high amounts of simpler phenolic compounds, notably different conjugated hydroxycinnamic acids: The reported total contents of hydroxycinnamates in Stevnsbær cherries amount to ∼480 mg/kg of fresh weight, of which 3-*p*-coumaric acid constitutes almost half, ∼226 mg/kg of fresh weight (*22*). This level of 3-*p*-coumaric acid is equivalent to a concentration of ∼1.4 mmol/kg of fresh weight. On this basis it may seem surprising that addition of a comparatively small amount of 0.3 mM (=0.05 g/L) of another simple phenol, gallic acid, could produce a positive clarifying action—albeit in interaction with pectinase—in sour Stevnsbær cherry juice (Tables 4 and 5). Reactivity of phenols with proteins is known to increase with the number of hydroxyl groups, and the reactivity notably increases when the OH groups are adjacent on the phenolic ring (*23*). The fact that gallic acid possesses three ortho-positioned hydroxyl groups, whereas epicatechin only has two *ortho*-dihydroxy groups and 3-*p*-coumaric acid has only one hydroxyl, may explain why gallic acid could be more reactive to retard turbidity than the phenols native to the sour cherry juice. At present, there is a severe lack of knowledge on possible molecular interactions among different cherry juice constituents in relation to turbidity and haze formation. Any data on this would provide a significantly improved foundation to tailor more chemically targetted clarification and fining treatments to particularly substitute current unspecific fining procedures.

The data obtained in this explorative study have demonstrated that several alternative measures to gelatin-based clarification treatments show promise. None of the alternative clarification treatments produced an exaggerated, unwieldy colloid precipitate. Consequently, further developments of these novel clarification strate-

gies may allow for other fruit juice processing improvements. It is particularly pertinent to replace the use of kieselguhr filter aids downstream from the clarification step to ensure a safe and more environmentally friendly juice production. Hopefully, the results of the present work will enable rational selection and optimization of novel clarification and fining treatments in more research studies as well as in large-scale cherry juice processing development.

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