Clearly vegan!

Clear apple and red grape juice thanks to plant-based fining agent for an unclouded drinking experience

| Clear Apple Juice | Clear Red Grape Juice | Fining Agent | Protein Composition | Vegan |

Juices are healthy, taste great, and with so many types of fruits and vegetables, there is a wide range to enjoy. Germans love juice – with a per capita consumption rate of more than 33 liters of fruit juice annually [1], they are global leaders in drinking juice. Apple juice is the market leader with 13 liters per person [2] followed in popularity by grape juice which includes healthy ingredients that reduce the risk of heart disease and cancer and support the immune system [3].

While some prefer naturally cloudy juices, others love beautifully clear fruit juice. Naturally cloudy juices are produced directly from pressing the fruits and can also contain the flesh of the fruit. When the naturally cloudy juice is fined and then filtered, clear juice is created. Clear juices that nevertheless end up with turbidity in the glass are generally unpopular with consumers. If the results of the fining of clear juices are unstable, particles can settle at the bottom. This is often taken to indicate lower quality or that the product has spoiled, and leads to consumer complaints. In order to avoid this, it is important to understand what gives rise to the turbidity and what specific processes can prevent it.

Turbidity in juice is caused by various substances. The most common causes are micro-organisms (yeasts, molds, bacteria), polysaccharides, proteins, tannins, anthocyanins, crystals and other foreign matter, such as filter fibers, filter aids or sand [4]. In apple juice, proteins are the most common culprit, causing roughly 40 percent of turbidity. To obtain a clear juice from a naturally cloudy juice, various fining agents are added, which act as adsorbents binding undesirable ingredients in the juice that are then removed by filtration [5].

The traditional fining agent is a combination of bentonite, silica sol and gelatin – an animal protein that is obtained from bone marrow [4]. The growing popularity of the vegan diet, where one avoids all animal-based food products, has led to a high demand for vegan fruit juices that are free of substances like gelatin.

Plant proteins, in particular pea and potato proteins, can provide an alternative to gelatin in order to produce clear and stable fruit juices for individuals following a vegan diet. As part of Sabine Schütz's Bachelor thesis (University of Applied Sciences, Frankfurt, Germany) naturally cloudy apple juice and red grape juice were fined with gelatin and plant proteins under standard conditions and the composition of the proteins in the fined juice was then examined. The aim of the study was to find out which fining agents were best suited to separating out the proteins that cause turbidity from the juice, and whether the plant proteins are similarly effective to gelatin for clarifying and stabilizing juice.

Test process

Naturally cloudy non-concentrated apple juice and red Dornfelder grape juice were used for the series of tests. The optimal dosages for fining were determined in the laboratory using a 100-ml scale. In every test, a combination of bentonite, gelatin and silica sol was used as a control in comparison with the combinations of bentonite, plant proteins and silica sol. At the same time, the dosages of the respective fining agents and the sequence of additions of the plant proteins varied (see Tables 1 and 2). In one case, for example, bentonite was added before the plant protein, and then reversed in the next series of tests. Silica sol was always added as the final fining agent. In conclusion, the tests were repeated with the optimal dosages on a larger scale of 5 liters each and samples were taken. To check the results, the turbidity values were determined using scattered light measurement (NTU = Nephelometric Turbidity Units) after fining and the heat and cold test. The protein analysis of the samples took place at the University of Applied Sciences in Frankfurt, Germany. In preparation, a dialysis with distilled water was carried out, to remove interfering substances such as salts and sugar. The samples were then concentrated by freeze-drying (lyophilization) and the resulting lyophilisate was used for the SDS PAGE protocol (sodium dodecylsulfate polyacrylamide gel electrophoresis). SDS PAGE is an analytical method that separates the proteins according to their molecular size. The silver staining makes the different protein bands visible.

Results

Tables 1 and 2 show the optimal dosages and the turbidity values after fining the 5-liter sample of both juices. The turbidity is measured in NTU. After fining, the samples were subjected to the heat and cold test to check their stability and consequent success of the fining. A juice is deemed stable if the difference in turbidity values before and after the heat and cold test is a maximum 1 NTU. For a clear juice, the total turbidity value after fining and subsequent filtration should not exceed 2 NTU.

The untreated apple juice had an initial turbidity of 553 NTU, the untreated red grape juice an initial turbidity of 47.1 NTU. The combination of bentonite-gelatin-silica sol achieved the highest reduction in turbidity with 1.15 NTU in apple juice and 0.87 NTU in grape juice. Fining with the plant proteins also successfully reduced the turbidity of both juices. Particularly noteworthy is the bentonite-pea protein-silica sol fining sequence. This achieved 1.55 NTU in apple juice and 1.24 NTU in grape juice. The potato protein-bentonite-silica sol combination achieved a good result with 1.99 NTU in apple juice. In comparison with apple juice, the reduction in turbidity in grape juice was slightly lower, which can be attributed among other things to different phenol and protein structures and pH levels.

The results from the subsequent heat and cold test confirmed the stability of all fined samples. In apple juice, the fining combination of bentonite-pea protein-silica sol achieved the best result with a difference of 0.19 NTU (1.74 minus 1.55 NTU) and a final turbidity value of 1.74 NTU. In grape juice, the bentonite-gelatin-silica sol combination achieved the highest reduction with a difference of 0.45 NTU and a final turbidity value of 1.32 NTU, closely followed by pea protein-bentonite-silica sol with a difference of -0.16 NTU and a final turbidity value of 1.49 NTU.

Protein composition

In order to study the protein composition, the samples were applied to an SDS gel. The associated scale of 10 to 250 kDa (kilodalton) means that the individual protein bands can be mapped according to their size. Figures 1 and 2 show the protein bands for both juices, made visible with the silver stain.

Table 1: Fining agent sequences and dosages in naturally cloudy non-concentrated apple juice including turbidity values and stability check (Sample R: checking non-fined juice: NTU 553)

	1st fining step		2nd fining step		3rd fining step		Turbidity	
	Fining agent	Dosage [g/hl]	Fining agent	Dosage [g/hl]	Fining agent	Protein: Silica sol ratio	After fining [NTU]	After heat and cold testing [NTU]
Sample G	Bentonite	200	Gelatin	30	Silica sol	1:3	1.15	2.13
Sample E1	Bentonite	150	Pea protein	30	Silica sol	1:3	1.55	1.74
Sample E2	Pea protein	10	Bentonite	200	Silica sol	1:3	2.53	2.95
Sample K1	Bentonite	150	Potato protein	10	Silica sol	1:3	2.34	3.60
Sample K2	Potato protein	15	Bentonite	100	Silica sol	1:3	1.99	2.65

Table 2: Fining agent sequences and dosages in red grape juice including turbidity values and stability check (Sample R: checking non-fined juice: NTU 47.1)

	1st fining step		2nd fining step		3rd fining step		Turbidity	
	Fining agent	Dosage [g/hl]	Fining agent	Dosage [g/hl]	Fining agent	Protein: Silica sol ratio	After fining [NTU]	After heat and cold testing [NTU]
Sample G	Bentonite	150	Gelatin	30	Silica sol	1:3	0.87	1.32
Sample E1	Bentonite	200	Pea protein	10	Silica sol	1:3	1.24	2.02
Sample E2	Pea protein	20	Bentonite	200	Silica sol	1:3	1.65	1.49
Sample K1	Bentonite	200	Potato protein	20	Silica sol	1:3	2.34	2.18
Sample K2	Potato protein	20	Bentonite	150	Silica sol	1:3	2.24	3.03

Proteins with a molecular weight between 15 and 31 kDa, i.e. Haze Active Proteins (HAPs), are discussed as relevant to turbidity [6, 7]. The SDS PAGE tests only identified a few proteins in this range in the untreated apple juice (Sample R). The combination of bentonite-gelatin-silica sol (Sample G) and bentonite-potato protein-silica sol (Sample K1) reduced the proteins relevant to turbidity the most. The comparison of the protein bands provides evidence that gelatin can be replaced with potato protein.

Potato protein performs well in suspension, forming large flakes. In combination with bentonite and silica sol, it bonds with proteins relevant to turbidity and draws them down during sedimentation, forming a compact sediment on the bottom and a clear supernatant. The sediment is then separated off in the next step of the process.

Far more proteins relevant to turbidity were found in red grape juice in the range of 15 to 31 kDa using SDS gel. The

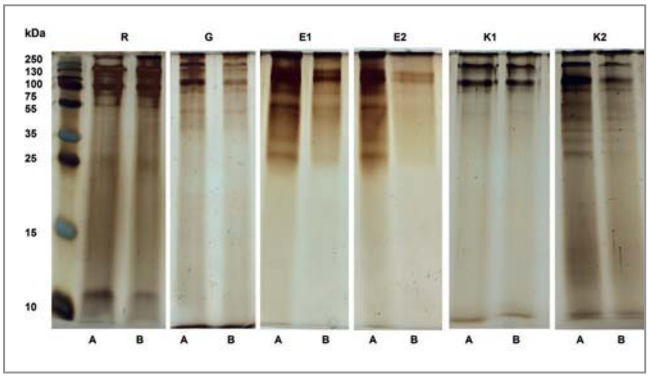

Figure 1: 15 % SDS gel: Protein composition of the apple juice

Note: A stock solution of 20 mg lyophilisate was dissolved in 1 ml H_2O_{dist} and the dilution was applied according to the pockets. Visualization took place using silver stain.
R: Reference (untreated juice);
G: Fining with bentonite-gelatin-silica sol;
E1: Fining with bentonite-pea protein-silica sol;
E2: Fining with pea protein-bentonite-silica sol;
K1: Fining with bentonite-potato protein-silica sol;
K2: Fining with potato protein-bentonite-silica sol;
A dilution 1:1 and B dilution 1:2

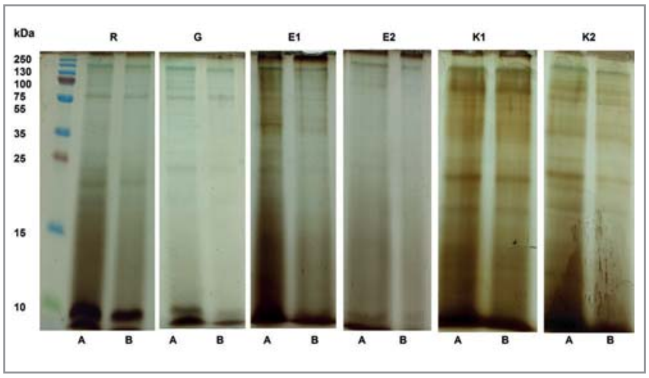

Figure 2: 15 % SDS gel: Protein composition of the red grape juice

Note: A stock solution of 20 mg lyophilisate was dissolved in 1 ml H_2O_{dist} and the dilution was applied according to the pockets. Visualization took place using silver stain.
R: Reference (untreated juice);
G: Fining with bentonite-gelatin-silica sol;
E1: Fining with bentonite-pea protein-silica sol;
E2: Fining with pea protein-bentonite-silica sol;
K1: Fining with bentonite-potato protein-silica sol;
K2: Fining with potato protein-bentonite-silica sol;
A dilution 1:1 and B dilution 1:2

best results were achieved with the combinations of bentonite-gelatin-silica sol (Sample G) and pea protein-bentonite-silica sol (Sample E2). Gelatin could also be replaced with a plant protein in this fining sequence. In red grape juice, the lower pH value and higher total phenolic content (red grape juice: 1957 mg/l, apple juice: 1181 mg/l) influenced the reactivity of the pea protein positively. The pea protein therefore achieved better results than the potato protein. The results from the SDS PAGE protocol were also supported by the NTU measurements (see Table 2).

Conclusion

Fining agents help remove undesirable turbidity from fruit juices, making them more visually and sensorily appealing to consumers and stable to produce. The growing demand for vegan fruit juices means that an alternative to the traditional fining agent of gelatin in combination with bentonite and silica sol must be found. The above results show that plant proteins can effectively separate out the proteins that cause turbidity and achieve similarly good juice clarification and stabilization as gelatin. Thus they qualify as a vegan alternative to gelatin.

The raw ingredients used influence the individual composition (degree of turbidity, pH value, total phenolic and protein content) of each juice. The vegan fining agents pea and potato protein differ in their suspension characteristics, flake formation and size as well as the reaction and sedimentation time. In addition, the juice temperature influences the reactivity of the fining agent. With regard to the subsequent filtration, the degree of turbidity of the juice as well as the flake size and sedimentation rate of the plant proteins must be considered as a whole. Given the many variables, small scale preliminary fining tests can help determine the dosage and sequence of the plant fining agents for the most effective result.

By using these plant fining agents, there is no longer any obstacle to enjoying juices as part of a vegan diet. The love of juice can thus be "unclouded" for everyone.

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Plant proteins

The plant proteins from Eaton, SIHA® pea protein and SIHA potato protein meet the requirements for vegan and kosher diets. They are also free of genetically modified organisms and allergens.

To improve clarification of fruit juices before fining, fruit juice producers can, for example, use the Panzym® YieldMASH XXL enzyme from Eaton. The enzymation positively influences filtration performance and simplifies the fining process by splitting off the high molecular structures of the pectins and glucans even before starting to prepare the fruit juice. The vegan range of fining products is supplemented with SIHA activated carbon, which is used to correct color, odor and flavor defects before bottling.

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