

Juice

Production of clear, stable juices and concentrates

Guidelines

Guidelines on processing and quality control
Customer Solutions
Novozymes Switzerland AG

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Introduction

Although the method for producing clear and stable fruit juices has been well known for years, problems with turbidity in juices and concentrates occasionally occur.

As a leading producer of microbial enzymes (pectinases, cellulases, amylases, proteases, etc.), we want to pass on information about the production technology for apple and pear juice and show you ways to prevent turbidity.

The production of juices from other fruits (e.g. stone fruits, berries, and tropical fruits) or vegetables is often very similar and does not therefore require significant changes in production control.

Section 1: Covers process control. It deals with the critical production phases, clarification and process hygiene. In many factories, these controls are daily routines which ensure smooth production and optimum, consistent quality of end-products.

Section 2: Deals with troubleshooting. If there is turbidity (haze) in your juice/concentrate, this information will help to evaluate the haze and provide guidance on correcting it.

Section 3: Provides a survey of methods and tests for process control and troubleshooting.

Section 4: (Checklists) and **Section 6** (Literature) provide support and information on process control and troubleshooting.

We hope this guideline, provided by the CUSTOMER SOLUTIONS DEPARTMENT at Novozymes Switzerland AG, will help you to produce clear, stable juices and concentrates.

1 Process control

The quality of the raw material is critical. Only high-quality raw material ensures the value and purity of the final product. Processing plants should insist that the raw material is delivered in clean, dry and undamaged condition. Dirty fruits are an ideal medium for microorganisms which can lead to the production of lactic acid or poisonous substances such as patulin, etc.

Traditionally, apples are stored in silos or bins. Transportation of apples to the washer/grinder is mostly accomplished through a fluid channel with circulating water. The transportation and/or washwater should be frequently changed.

Preparation of the mash, grinding and particle size depend on the condition of the fruits (fresh, stored, ripe/unripe) and on the pressing equipment. Fresh, hard apples need to be broken into smaller pieces than soft apples.

Whether a press or a decanter is used, enzymatic mash treatment (e.g. with PECTINEX® YIELDMASH, PECTINEX SMASH XXL, PECTINEX ULTRA MASH, PECTINEX ULTRA SP-L) gives increased yield and higher capacity in the separation system with no adverse effect on juice quality. Mash treatment reduces costs and is therefore economically significant.

Clarification, including juice enzymation, fining and filtration is the most sensitive part of the process.

1.1 Enzyme treatment

1.1.1 Pectin and starch

The importance of polysaccharides in juice turbidity (haze) and sediment formation is well established. The use of commercial enzymes (e.g.

PECTINEX ULTRA CLEAR, PECTINEX XXL, AMYLASE™ AG 300 L, AMYLASE AG XXL) and fining agents (bentonite, gelatin and silica sol) in achieving satisfactory juice clarification and stability is state of the art. To avoid potential haze or sediment formation in clarified single-strength juice or concentrate, the following guidelines are important.

1. Enzymatic hydrolysis of pectin in the juice should proceed until a negative pectin test (ethanol test) is obtained. See "Pectin test", page 13.

2. The juice must be heat-treated (e.g. aroma recovery) to gelatinize the starch, allowing enzymatic degradation with amylase (e.g. AMYLASE™ AG 300 L, AMYLASE AG XXL) until a negative starch test (iodine test) is obtained. Retrogradation of incompletely degraded starch must be avoided! See "Starch test", page 14.

Polysaccharides such as pectins and starch not only prevent clarification, but also tend to block filters. Pectin and starch degradation must be completed before the addition of fining agents!

1.1.2 Arabinans

When production technologies for higher yields are applied (high mechanical forces, mash enzymation, post extraction), araban is released from the apple mash and can cause secondary haze in the concentrate. The risk of arabinan haze cannot be countered during processing or prior to concentration because no quick process control exists. The application of a pectinase with sufficient arabinanase activity (e.g. PECTINEX ULTRA CLEAR, PECTINEX XXL) completely avoids the risk of araban haze.

1.2 Fining treatment

1.2.1 Protein and phenolic compounds

Depending on the type of juice (e.g. apple juice, pear juice and grape juice), proteins and phenolic compounds, independently or by association, can both be important in turbidity and post- clarification haze and sediment formation [HEATHERBELL et al., 1984]. See "Heat/cold stability test (phenolic/protein compounds)", page 20.

How to avoid sediment formation:

1. Remove unstable phenolic compounds by correct gelatin fining treatment (temperature, time, stirring).
2. Use only high-quality gelatin (approx. 100 bloom) and avoid overfining by using standardized laboratory clarification steps. See "Determination of gelatin demand for

fining", page 17.

3. Remove unstable proteins by bentonite fining.
4. A good policy is to combine bentonite, gelatin and silica sol fining as a routine procedure. The inclusion of silica sol has the added advantage of rapid settling and compacting of cloudy substances.

1.2.2 Metal ions

Several polyvalent cations are known to exert an effect on haze and sediment formation. These cations readily form stable complexes with phenolics and can combine with proteins, pectins and starch. Copper ions destroy ascorbic acid. The presence of metallic cations should be avoided by using only stainless steel or non-metallic processing equipment.

1.3 Filtration treatment

As the final step in the juice treatment process, filtration gives the pre-treated juice its crystal clarity [WEISS, 1995]. The degree of clarity is governed by the given filtration system. Prior to 1980, pre-coated and sheet filters were used exclusively for such filtration. Within the last 10 years, ultrafiltration has been introduced as a successful alternative. Enzyme preparations (e.g. PECTINEX UF) reduce membrane fouling during filtration, thereby increasing flux rates [STUTZ, 1993].

Various technologies (partial fining, enzymatic treatment with poly-phenoloxidase, PVPP or adsorber resin treatment, nanofiltration, etc.), divided into pre-treatment and final treatment processes, are available to avoid the risk of post-haze of ultrafiltered juices and concentrates [MAIER et al., 1994].

2 Troubleshooting

2.1 Most frequent causes of turbidity (haze)

Every day the CUSTOMER SOLUTIONS DEPARTMENT at NOVOZYMES Switzerland AG receives samples of turbid juices and concentrates from all over the world for analysis. In most cases, the turbidity (haze) is of organic origin, caused by improper clarification, fining and filtration or contamination with different microorganisms. In rare cases, the problems are caused by insufficient or incorrect enzymatic treatment. The samples examined in our laboratory typically show the following causes of turbidity:

- Phenolic compounds
- Proteins
- Protein/phenolic complexes
- Microbial infections (bacteria, yeasts and moulds)
- Gelatin
- Starch
- Pectin
- Arabinan
- Filter aids
- Metal ions

Turbidity is often caused by more than one substance, which makes identification more difficult. The most common causes of turbidity are polyphenol/protein complexes and microbial infections (yeasts and bacteria).

Higher concentrations of metal ions such as iron, copper or calcium can also cause post-hazing. The hazes are usually mixed organic/inorganic complexes. The partners of the metal ions are often phenolic compounds, pectin fragments or protein components.

The quality of the water used for redilution is therefore extremely important. Purely inorganic turbidities (diatomaceous earth, crystals, salts, etc.) also occur in the beverage industry, although they are rare.

See "Checklist for haze factors in clear juices and concentrates", page 23, for more information on avoiding and removing haze.

2.2 Troubleshooting strategy

If the guidelines given in Chapter 1 are observed, juices and concentrates normally remain clear and stable. However, if conditions change (raw material, temperature, etc.), haze may occur during or after production.

To determine the origin of turbidity (haze) in an unstable juice or concentrate, the following procedure is recommended:

Step 1: Optical and sensory judgment on origin of turbidity - Analytical data (pH, color, turbidity)

An optical and sensory judgment of color, turbidity, sediment, aroma, etc., makes a first overview of the origin of turbidity possible. Some analytical data such as °Brix, pH, turbidity (TE/F, NTU, %Transmission at 620 nm) and color measurement (%Transmission at 440 nm) also facilitate quick evaluation of the origin of turbidity.

See Section 3 for a survey of methods and stability tests.

Step 2: Observations under the microscope

It is important to examine the sample under the microscope. Concentrates can be examined without dilution. The juice should be centrifuged prior to analyzing the sediment under the microscope. In cases of uncertainty about the determination of microorganisms, arabans, filter aids, etc., please contact NOVOZYMES Switzerland AG for advice or a copy of relevant literature such as LUETHI and VETSCH [1981]. See "Observations under the microscope", page 10.

Step 3: - Stability tests

Stability tests allow the origin of turbidity in a juice or concentrate to be detected. Several causes of haze can often be identified by means of these tests:

Pectin residues	Ectin test (alcohol test)
Starch residues	Starch test (iodine test)
Gelatin residues	Gelatin demand, overfining test
Phenolic compounds	Underfining test, folin test
Proteins	Overfining test, bento test [JAKOB, 1995]
Phenolics/proteins	Heat/cold stability test
Arabinan	Microscopic evaluation

Table 1. Stability tests

3 Survey of methods and stability tests in the standard industrial laboratory

The initial analysis of haze is very important. If there is an off-flavour or gas build-up in a hazy juice/concentrate, an infection with microorganisms is probable. This can be detected under a microscope or through determination of the alcohol content in the sample. Further information can be obtained from analytical data, color and turbidity measurements described in TANNER and BRUNNER [1987].

3.1 Observations under the microscope

3.1.1 Microorganisms (bacteria, yeasts and moulds)

Microorganisms such as yeast cells viewed under the microscope (magnification times 100-400) can be identified as round, often constricted particles. Their size ranges around 10 µm. Bacteria are similar, but their size ranges around 1 µm. The problem is often that turbidity (haze) has different causes. In cases of uncertainty about the determination of microorganisms, filter aids and arabinan haze, the relevant literature should be consulted [LUETHI and VETSCH, 1981].

Prevention/correction:

Contamination with microorganisms can be avoided through proper process control. If contamination does occur, the juice or concentrate can be clarified by means of sterile or aseptic filtration (0.45 µm).

3.1.2 Filter aids (diatomaceous earth, cellulose fibres, perlites, etc.)

If the pre-coated or sheet filter is faulty, diatomaceous earth or cellulose fibres may pass into the juice. This can be avoided through the use of a sheet or membrane filter. Observation under a microscope can confirm the presence of filter aids.

Prevention/correction:

Routine checks of the filter and filter systems will help to prevent this problem if contamination occurs, additional filtration is required.

3.1.3 Arabinans

Arabinans are fruit polysaccharides (especially in apples and pears). They are soluble in the juice and pass through fining and filtration treatments. Arabinan turbidity only appears in concentrates weeks or even months after storage.

The concentrate becomes clear when heated above 80 °C (176 °F).

Under the microscope (magnification times 600), arabinan particles can be identified as round, often constricted particles. Their size ranges between that of yeast cells and that of starch granules (10 to 100 µm). After the sample is heated, the arabinan particles are no longer visible under a microscope.

Prevention/correction:

Prevention:

Arabinan haze can be avoided by depectinization of the juice using pectinases with sufficient arabanase activity (e.g. Pectinex Ultra CLEAR, Pectinex XXL). Arabinan is broken down during depectinization.

Correction:

Cloudy concentrates containing arabinan haze can be treated as follows:

The concentrates are heated to 40-45 °C (104 - 113 °F) without redilution (at 70-72 °Brix) and filtered through pre-coated filters.

Heating is only carried out to reduce viscosity. Concentrates should not be heated above 45 °C (113 °F) or the arabinans will resolubilize and pass through the filter. If heating takes place within the given temperature range, arabinans will remain in the diatomaceous earth.

3.1.4 Botrytis β-Glucan

The extremely uncertain nature of Botrytis cinerea in agriculture is well indicated by the contrasting descriptive terms in which it is known. Under ideal circumstances, this parasitic fungus with usual involvement of other spoilage microorganisms is able of shrivelling and rotting the ripened fruit.

Botrytis activity occurs within the intact fruit, but splitting of the ripe fruit, as from rain or prolonged humidity, leads to a rapidly or even uncontrolled infection by Botrytis and other spoilage organisms. Though very large compositional changes may occur during parasitisation of fruits by Botrytis cinerea, the overall influence on juice composition depends of course on the degree of infection in the crop. As one of the negative consequences, the highly molecular polysaccharide Glucan is produced. Unless there has been special care in the pressing procedure, juices may have sufficient Glucan content (Glucan level > 5 mg/l) to be troublesome during filtration and concentration.

Prevention/correction:

Prevention:

Glucan can be prevented by using only high quality raw material for processing.

Correction:

Processing of infected raw material (*Botrytis cinerea* fungus) needs a strictly controlled adaption of the production parameters, e.g. gentle pressing (liquid-solid separation), quick clarification (enzymatic pectin degradation), improved fining to ensure a Glucan level in the juice before concentration as low as possible. Very often the use and application of a Glucan degrading enzyme in case of grape juices Novozymes Glucanex® or Novozymes VinoTaste® Pro can be a helpful tool to resolve the Botrytis β -Glucan problem.

The complete enzymatic degradation of Glucan, even at high temperature (45 – 50 °C) needs much more time (3 – 10 days) as usual under industrial processing conditions (e.g. complete Pectin- and Starch degradation in 1 to 2 hours). Please contact our Customer Solutions Staff if assistance is needed!

Important note: Glucan in juices even at low quantities leads to a quick gelification during the evaporation (concentration) step.

3.2 Stability tests

3.2.1 Pectin test (ethanol test)

This test detects pectins in juices. Pectins have to be degraded until a negative test is obtained in order to:

- enable fining and filtration treatment
- ensure that the juice will not gelify during and after concentration
- stabilize the clear juice or concentrate

For this purpose, we recommend the following test procedure: Add two volumes (10 ml) of acidified ethanol (add 1 ml of 37% hydrochloric acid to 100 ml of 96% ethanol) to one volume (5 ml) of filtered juice (cleared of insoluble particles by filtering through a paper filter). Gently tip the test tube upside down 2 or 3 times, but avoid shaking! Allow to stand for 15 minutes before making a judgement!

Judgement:

If no gel or flocculation appears, the pectin is degraded! Pectin is present if poured air bubbles do not move to the top and gelatinous flocculation is observed. In the case of a high pectin content, a gel clot may even be observed. Low pectin content can be seen as gritty particles at the glass wall of the test tube.

A homogenous milky turbidity without any gelatinous flocculation or gritty particles does not indicate pectin.

Acidification of the ethanol ensures that only pectin will cause a precipitation. If the ethanol is not acidified, other soluble polysaccharides (celluloses, hemicelluloses) will cause precipitation [WILL et al. 1993].



Fig. 1:
Pectin test (Alcohol test)
Left: positive (gelification, flocculation)
Right: negative (Pectin degraded)

Prevention/correction:

Problems with pectins can be avoided by means of proper depectinization. If a problem occurs, adjust the enzyme dosage (e.g. PECTINEX ULTRA CLEAR, PECTINEX XXL), the temperature and the reaction time until a negative pectin test is obtained. Concentrated juice must be diluted with demineralized water to 20 – 30 °Brix and again treated with enzyme (e.g. PECTINEX ULTRA CLEAR, PECTINEX XXL).

3.2.2 Starch test (iodine test)

3.2.2.1 Soluble starch

An iodine test is used for rapid detection of starch (carbohydrate reserve) in fruit juices. The starch content varies throughout the season. Starch causes problems in clarification and filtration treatments and may cause haze in the final product. If starch is present and a clear juice or concentrate has to be produced, the starch must be degraded before the juice or concentrate is stored. Starch, which is mostly crystalline in the fruit, will be solubilized (gelatinized) during heat treatment (temperatures above 55 – 60 °C [131 – 140 °F], e.g. aroma recovery, pasteurization, concentration step). Soluble starch can be enzymatically degraded to glucose (e.g. using AMYLASE AG 300 L, AMYLASE AG XXL).

The detection and degradation of the starch should be followed by an iodine test:

Heat 10 ml of juice to above 80 °C/176 °F (not necessary if the juice is sampled directly after heat-treatment, e.g. aroma recovery). Allow the juice to cool to room temperature. Add around 1 ml of iodine solution gently to the top of the juice without mixing and observe the color at the interface (reaction zone).

(Iodine solution: solution of 1 g of iodine and 10 g of potassium iodine in 1 litre of water).

Judgement:

Color	Starch
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No color change of the iodine solution (reddish brown)	No starch
Color change from reddish brown	Not completely degraded starch
Brown, blue, dark-blue or black color	Starch

Table 2. Judgement

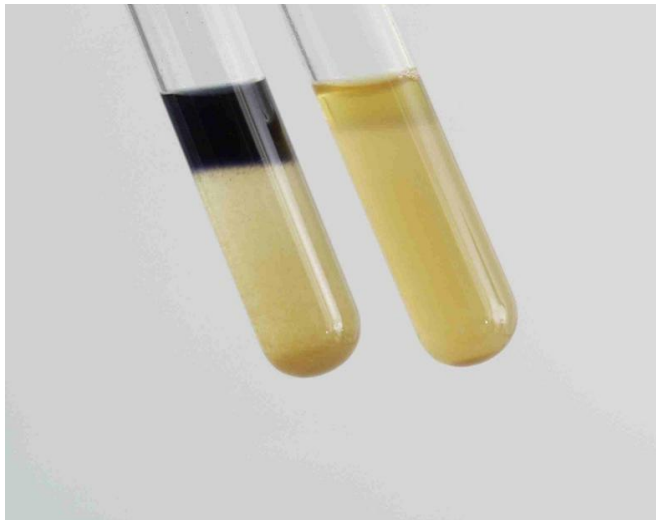


Fig. 2.
Starch test (Iodine test)
Left: Positive (blue color)
Right: negative (yellow color, Starch degraded)

3.2.2.2 Retrograded starch

If the gelatinized starch is not degraded, it will recrystallize ("retrograde"), causing haze in the final product. In this form, starch is very difficult to eliminate.

Characteristics of retrograded starch:

- Solubilization only at temperatures above 120 °C (248 °F) or above pH 10
- Poorly degradable using enzymatic treatment

It is therefore necessary to ensure that the starch is enzymatically degraded (e.g. using AMYLASE AG 300 L, AMYLASE AG XXL) in the juice in a solubilized form. Retrograded starch is not detectable using the normal iodine test.

The following test is recommended for retrograded starch:

1. Add 1 ml of 1N NaOH to 2 ml of sample material in a test tube and shake thoroughly (this pH-treatment will resolubilize the retrograded starch).
2. Wait 5 minutes, add 1 ml of 1N HCl and shake.
3. Check that the pH is slightly acidic (pH 5 – 6).
4. Put 2 ml on a glass plate. Place the plate on a white background.
5. Add 3 drops of iodine solution from the side (not the top) and observe the color reaction at the interface between the solutions (see Fig. 3).

Prevention/correction:

Starch problems can be avoided with proper enzyme treatment (e.g. using AMYLASE AG 300

L, AMLASE AG XXL) followed by a routine starch test.

If a bluish color appears with the iodine solution, the enzyme dosage (e.g. AMYLASE AG 300 L, AMYLASE AG XXL), the temperature and the reaction and holding time need to be adapted according to the starch test.

If a problem with retrograded starch occurs, please do not hesitate to contact the CUSTOMER SOLUTIONS DEPARTMENT at NOVOZYMES Switzerland AG for further help.

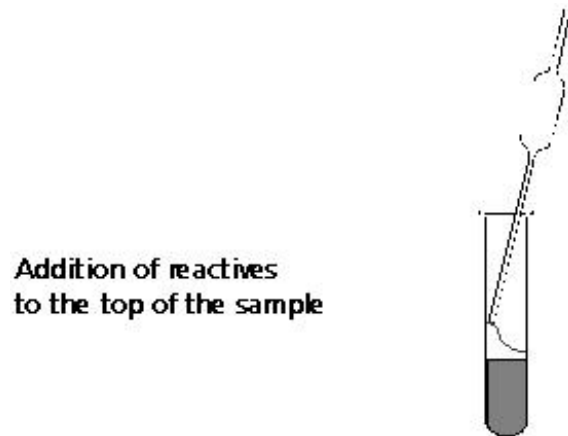


Fig. 3

3.2.3 Determination of gelatin demand for fining

This test determines the optimum gelatin dosage. It should only be carried out when both pectin and starch are completely degraded! As the correct implementation of this preliminary laboratory test is a decisive step towards optimum clarification, it is described in detail below:

Materials

- 1% fresh gelatin solution (the same quality as used in the factory)
- 10% silica sol solution (e.g. Baykisol 30%: add 2 parts water to 1 part silica sol 30%)
- 1 litre juice from fining tank or sample with turbidity
- 100 ml graduated glass cylinders (8)
- Paper filters (8)
- Glass test tubes (24)

Procedure for determining gelatin demand

Step 1:

Add 100 ml of juice to 8 glass cylinders respectively. Add gelatin in increasing dosages. The following serves as an example:

100 ml cylinders	1% gelatin solution	g/100 l juice	oz/100 gal
1	0.5 ml	5	0.67
2	1.0 ml	10	1.34
3	1.5 ml	15	2.00
4	2.0 ml	20	2.68
5	2.5 ml	25	2.34
6	3.0 ml	30	4.01
7	4.0 ml	40	5.34
8	5.0 ml	50	6.68

Table 3. Dosage

Mix well and allow 30-60 minutes for flocculation to take place.

Step 2:

Sample 10-15 ml of juice from each cylinder and filter it through a paper filter.

Step 3:

Divide the clear juice into 2 test tubes. Arrange the tubes in two rows. Add 2 drops of gelatin solution to the top of the 5 ml of juice in the first series (underfining test for additional gelatin demand).

Add 2 drops of silica sol solution to the top of the 5 ml of juice in the second series (overfining test shows if gelatin was overdosed). Do not mix!

Allow the test tubes to stand for 5 – 10 minutes.

Step 4: Judgement

Hold the tube against a dark background and examine the turbidity at the interface between the solutions in each test tube.

The test tube which does not show turbidity in the underfining test with gelatin or in the overfining test with silica sol indicates the dosage of gelatin/silica sol necessary for the juice in question (see Fig. 4, p. 19).

Notes

1. The above test is only an example. The quantities of gelatin to be added must be adjusted accordingly. In many cases it is advisable to carry out two series of tests, the first to establish the dosage range and the second series to determine the exact amount.
2. The optimum gelatin dosage determined in the test can be combined with bentonite and silica sol.
3. The sequence for adding the different fining agents (e.g. bentonite, followed by gelatin and silica sol) can sometimes be of importance in obtaining a better sedimentation.
4. Both overfining and underfining with gelatin will lead to haze in the final product. The overfining test and the underfining test therefore ensure the right fining in the final product. These combinations should produce the following results:

- Faster sedimentation
- More compact sediment
- Clearer supernatant
- Easier filtration
- Crystal-clear and stable juices or concentrates

A recommendation for the dosage of gelatin, silica sol and bentonite for a juice with around 12 °Brix is given below (phenolics: 300 – 500 mg/l, acidity: 5 – 6 g/l, pH 3.5 – 4):

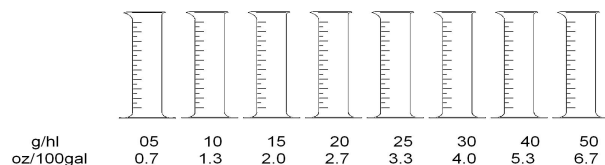
Gelatin: 10 – 15 g/hl (1.3 - 2.0 oz/100 gal.)

Silica sol: 3 – 10 times the amount of gelatin

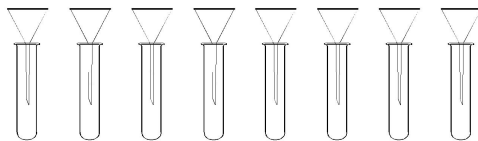
Bentonite: 50 – 100 g/hl (6.5 - 13.0 oz/100 gal.)

Fig. 4: Scheme for the Determination of Gelatin Demand

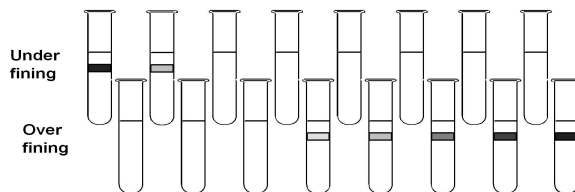
Step 1: Add 100ml juice respectively into glass cylinders
Add gelatine in increasing amounts



Step 2: Sample 10-15 ml juice from each cylinder
Filter it through paper filter



Step 3: Divide clear juice into two test tubes
Arrange the tubes in 2 rows
Underfining test: add 2 drops of gelatin solution
Overfining test: add 2 drops of silica solution
Do not mix!



Step 4: Judgement
Example: in this case the gelatine demand = 15g/hl = 2oz/100gal

3.2.4 Heat/cold stability test (phenolic/protein compounds)

This test determines whether an enzyme-treated, fined and clear-filtered juice runs the risk of turning turbid after concentration (e.g. after redilution and bottling). This test is suitable for checking a clarification formula developed in the laboratory. However, it also represents a good method for the production process.

The following is recommended for the heat/cold stability test:

- Measure the turbidity (TE/F or NTU) of the clear-filtered juice sample

- Heat the sample to 80 – 100 °C (176 – 212 °F)
- Freeze the sample (minus 18 °C) (0 °F)
- Thaw the sample to room temperature and measure the turbidity (TE/F or NTU)

Normally the turbidity is less than 2 TE/F (NTU) units before heating. If the turbidity difference after the heat/cold stability test is less than 1 TE/F (NTU) unit and/or the juice remains clear, the juice (and hence the concentrate) is stable vis-à-vis phenolic/protein compounds for several months.

If the juice becomes hazy, there is a risk of subsequent turbidity development. This turbidity (haze) may have several causes.

Prevention/correction:

Cause	Solution
The pectin is not completely degraded (check using the pectin test)	Control the depectinization temperature, increase the enzyme dosage (e.g. PECTINEX ULTRA CLEAR, PECTINEX XXL) or change the enzyme reaction time
The starch is not completely degraded (check using the starch test)	Control the enzymation temperature, increase the enzyme dosage (e.g. AMYLASE AG 300 L, AMLASE AG XXL) or adjust the reaction time
The juice is either underfined or overfined (check using the fining test)	Solution: Increase/reduce the gelatin dosage
The juice contains protein (check using the overfining test or bento test)	Increase the bentonite or silica sol dosage during fining
The juice or concentrate is contaminated with microorganisms	Check plant equipment. Additional sterile or aseptic filtration

Table 3. Prevention/correction

3.2.5 Detection of Botrytis β -Glucan

The detection of glucan is a qualitative test in Juice beverages (berries-, peach- and grape juices) and wine. Filtration experiments have shown that 5 mg of glucan/l can be sufficient to provoke filtration problems.

Only beta-glucan (polymer from Glucose >1 Mio. Dalton) produced by Botrytis cinerea strain could be detected. Yeast Glucan (around 0.2 Mio. Dalton) cannot be detected.

Botrytis-glucan in 30% alcohol is not soluble. This behaviour is specific for botrytis-glucan.

Depending of the glucan concentration in the sample, the following detection techniques should be applied:

1. For glucan levels higher than 15 mg/l (simple Glucan test) 10 ml of the sample to be checked are poured into a test tube and mixed slowly with 5 ml 96% alcohol. The occurrence of a filament-like precipitation indicates the presence of Glucan.
2. For glucan levels lower than 15 mg/l (modified glucan test) This test includes a pre-concentration of glucan. This can be done as follows: 5 ml of the sample are mixed with 5 ml 96% alcohol. The mixture is left at room temperature for 15-30 min. and then centrifuged at 3000 rpm (700 – 800 g) for 20 min. The supernatant is carefully

separated from the sediment. The sediment (colloids) is first dissolved in 1 ml water and then 0.5 ml 96% alcohol is added. The occurrence of a filament-like precipitation indicates the presence of glucan.



Fig. 5.
Glucan test (Alcohol test)
Detection of Botrytis β -Glucan on a raspberry juice (from concentrate)
Left: Test without acidified alcohol
Right: Test with acidified alcohol

4.1 Checklist for successful application of enzyme preparations

Parameter	Optimum influences
Fruit	- Ripeness, origin, season, etc., have a major influence on enzyme dosage; e.g. early season = high starch content, late season = high pectin content
pH	A pH Δ = 0.3 up to 50% difference in enzyme activity; e.g. Pectinex ULTRA CLEAR , Pectinex XXL pH 3.2 = 60% pH 3.5 = 100% pH 3.8 = 150%
Temperature	Temperature law: increase of +10 °C (18 °F) = around double enzyme activity; 50 °C (122 °F) = optimum for juice clarification $\geq$ 55 °C (131 °F), enzyme inactivation starts
Enzyme treatment time (E.T.)	Time concentration rule/law: Doubling the enzyme concentration reduces the reaction time by half; Enzyme time = filling + holding
Enzyme dosage	Optimum dosage: pre-trials in lab e.g. Pectinex ULTRA CLEAR , Pectinex XXL 2 – 4 ml/hl (2.5 - 5.1 fluid oz/1,000 gal.) for 12 ° Brix, with 2 h at 50 °C (122 °F) or 8 h at 20 °C (68

	°F)
Enzyme addition	Homogenous mixing important Dosing pump or direct to tank prior to filling
Enzyme solution	~10% (1 l Enzyme in 10 l [40 lit/100 gal] water at ambient temperature) Use enzyme dilutions for max. 10 hours of preparation
Enzyme storage	0 – 10 °C (32 – 50 °F), avoid storage temperature above 20 °C (68 °F)
Enzyme inhibitors	Bentonite! (during enzyme treatment) Polyphenols, SO ₂ over 500 mg/l Ethanol over 20% v/v Heavy metals

4.2 Checklist for haze factors in clear juices and concentrates

Haze factor	Detection	How to avoid or repair
Phenolic compounds	Gelatin demand Underfining test Microscope	- Stability tests - Correct treatment with gelatin, PVPP, casein, etc. - Repeat fining treatment
Proteins	Heat/cold test Overfining test, Bento test	- Stability tests - Correct bentonite or silica sol dosage - Repeat fining treatment
Yeasts, bacteria, moulds	Microscope	- Control cleaning of all equipment: tank, filter, pump, evaporator, etc. - Additional aseptic or sterile filtration
Gelatin	Overfining test	- Stability tests - Control fining preparation - Repeat fining treatment - Correct bentonite or silica sol dosage
Pectin	Pectin test (Ethanol test)	- Pectin test negative!! before adding fining agents - Reprocess the juice/concentrate
Starch	Starch test (Iodine test)	- Iodine test negative!! - Enzyme treatment (e.g. AMYLASE AG 300 L, AMYLASE AG XXL) - Difficult to reprocess - Contact our CUSTOMER SOLUTIONS DEPARTMENT
Arabinan	Microscope Heating test	- Pectinase preparation insufficient - Use high arabinanase activity enzyme (e.g. PECTINEX ULTRA CLEAR, PECTINEX XXL,) - Pre-coat filtering at 40 – 45 °C (104 – 113 °F)
Filter aids (diatomaceous earth, cellulose fibres, perlites)	Microscope	- Control filter set up - Additional aseptic or sterile filtration
Other haze factors	Microscope Stability test	- Water quality for dilution - Contact our CUSTOMER SOLUTIONS DEPARTMENT

Table 4. Haze factors in juice and concentrates

4.3 Customer Solutions report for troubleshooting

Here is an example of our internal CUSTOMER SOLUTIONS REPORT. In some instances not all analyses are required.

Customer Solutions report

General indications

Reference:

Customer:

Indication marks:

Problem:

Date of entry:

Internal code number:

Person in charge:

Distributor:

Date:

Optical / sensory judgement
(Aroma, color, haze, sediment)

Judgement and recommendations

Date: Visa:

Analytical report (results and judgement)

°Brix concentrate:

°Brix ref. rediluted:

°Oechsle/density:

pH:

Alcohol (vol. %):

Turbidity (TE/F; %Transmission at 620 nm):

Color (%Transmission at 440 nm):

Observations under the microscope

Microbiological status (sediment after centrifuging):

Other origin:

Results and judgement of the stability tests

Stability test: Heat/cold test (protein/-phenolics)

Folin test (phenolics)

Protein test:

Bento test

Bio-Rad

Gelatin demand: Overfining

Underfining

(Overfining: reaction with silica sol; Underfining: reaction with gelatin)

Pectin test (1 part juice:2 parts ethanol):

without HCl

with HCl

(Positive: no degradation; negative: degradation)

Starch test:

Iodine test

Araban test:

a) Heating test

b) Microscope

Glucan test (berries):

a) Normal test b) Modified test

Prevention and correction

(Results→ of enzyme test and fining test with customer sample)

Products Dosage/Time/Temperature Results Judgement

5 Summary

Based on laboratory and industry experiences, the CUSTOMER SOLUTIONS DEPARTMENT at NOVOZYMES Switzerland AG feels confident in saying that nearly all turbidity (haze) problems are attributable to the causes discussed above and that these causes can be identified using the proper testing. However, should you not achieve satisfactory results in your laboratory, do not hesitate to ask our CUSTOMER SOLUTIONS DEPARTMENT for assistance. To carry out our examination, we need at least 1 litre of juice or 300 ml of concentrate.

6 Literature

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7 Appendix

7.1 Shipping instructions

If possible, avoid placing samples in glassware!

Use proper plastic containers with safety stoppers.

Samples should be stabilized against microbial spoilage (either/or: concentrated, pasteurized, frozen, preserved e.g. with 0.1 - 0.2% sodium benzoate).

Ensure that all the necessary information is attached (customer, plant, kind of complaint, information about production process, etc.).

We recommend that you send samples by recorded/registered mail or by courier.

Company address:

Novozymes Switzerland AG

Attn.: Customer Solutions Juice & Wine

Neumattweg 16

CH - 4243 Dittingen / Switzerland

Time frame for results:

In general, you can expect a response within 3 – 5 working days after receipt of the sample.

If a response is needed more urgently, please let us know.

7.2 Contact persons for questions about the use of enzyme preparations in Juice & Wine

Contact Persons / Direct Phone / Internet

Tel.: + 4161 765 61 11

Fax: + 4161 765 63 33

Website: www.novozymes.com

Customer Center: www.mynovozymes.com

Email address: beverage@novozymes.com

Customer Solutions Manager:

Dr. Frank Rittig email: frri@novozymes.com

Tel.: + 4161 765 63 13 Fax: + 41 61 765 63 33

Customer Solutions:

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Mobile : +41794356020

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Yan Zhe email: zhyx@novozymes.com

Tel.: +86 138 105 728 46 Fax: +41 61 765 63 33

Other contact persons

Global Marketing Manager (Beverage Juice & Wine):

Elmar Janser email: elja@novozymes.com

Tel.: + 4161 765 63 20 Fax: + 41 61 765 63 33

Instructions for obtaining copies of technical literature cited:

Please contact our Marketing Office Juice & Wine

Marketing Assistant Beverage

Eveline Jeger email: evje@novozymes.com

Tel.: + 4161 765 64 20 Fax: + 41 61 765 63 33

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4 Checklists