October 2014

PROTEOTEST - THE TEST PERFORMED UNDER ENOLOGICAL CONDITIONS

THE EVALUATION OF PROTEIN STABILITY – A Background

In determining the optimal dose of bentonite to achieve protein stability in wine, several variables must be considered including the protein profile of the wine, the type of Bentonite to be used, rehydration procedures and clarification contact time.

It is of upmost importance to perform preliminary laboratory tests to ensure effective bentonite treatment on the commercial scale. The most common tests are considered 'orientation tests' which indiscriminately cause a change in the colloidal component resulting in an increase in turbidity.

The general procedure for determining protein stability is to prepare samples with increased doses of Bentonite (eg 10, 20, 30g/hL Bentonite), clarify the samples through filtration or centrifugation and then measure the level of turbidity.

The increase in turbidity is generally measured by a turbidity meter, in units of NTU. Where such equipment is not available clarity can be observed by eye in comparison with treated and untreated samples. When there is not visible turbidity the wine can be considered protein stable.

The two most common methods of determining protein stability are the Bentotest and Heat testing. Both of these treatments serve the winemaker well, however these tests are limited in either accuracy or time required to determine the result.

Bentotest has proven to be somewhat variable in its results being over vigorous in its estimation of bentonite required. This is due to Bentotest incorrectly accounting for polysaccharides and mannoproteins as unstable colloids. While the Bentotest serves a role as a quick estimation of protein stability, it is critical that the winemaker interpret the results not as an absolute result but as an indicator.

The Heat test, while more accurate, relies on heat to accelerate the speed of the reaction, thus altering the structure of the proteins involved, and of the colloidal system in general. This method is long, laborious and can be variable in its results due to inconsistencies in:

- Test temperature.
- Time for which it is kept at that temperature.
- The type of tannin that is used.

It is thus apparent that an ideal alternative for determining protein stability would incorporate:

- NO heat alteration of the colloidal nature of the wine.
- ONLY measures unstable colloidal material.
- Speed
- Simplicity
- Accuracy

The PROTEOTEST embodies all of these attributes, the first protein stability test to accurately determine bentonite fining rates quickly in enological conditions.

BSG™ Wine Division | Napa

474 Technology Way, Napa CA 94558
707.252.2550 (800) 585.5562 www.bsgwine.com

COMPARISON ON WHITE WINE BETWEEN BENTOTEST AND HEAT TEST WITH TANNIN (Δ NTU)

The Proteotest utilizes the reaction between the protein in the wine and a phenolic based material to induce turbidity in the presence of unstable proteins. The reactivity of the phenolic material is both strong and rapid resulting in a quick, accurate and reliable determination of protein stability.

During the design of Proteotest comparative tests were run, aimed at learning more about the reactivity of the tannins. Among the myriad of tannins analyzed, the two most reactive were selected and mixed in adequate proportions to create Proteotest.

The reactivity of the tannins has proven to accurately indicate stability at rates far below what is indicated by the Bentotest.

Table 1.1 below compares the Bentotest with the heat test using tannin where ΔNTU < 10, indicating protein stability, is achieved at 50g/hL as tested by heat test using Tannin, compared with 100g/hL bentonite addition rate as indicated by the Bentotest.

Table 1.1. COMPARISON ON WHITE WINE BETWEEN BENTOTEST AND HEAT TEST WITH TANNIN (where stability indicated at ΔNTU < 10.)

	BENTOTEST	HEAT TEST WITH TANNIN
UNTREATED	270	17
+25 g/hL bentonite	111	14
+50 g/hL bentonite	33	►7◄
+100 g/hL bentonite	►9◄	5
+200 g/hL bentonite	1	1
+250 g/hL bentonite	1	1

Using the Proteotest mixture at cold temperatures allows the execution of a practical and immediate test as with Bentotest, but conforming more to the reactions that happen in the wine. In fact, Proteotest is performed at the actual pH and temperature conditions of the wine. The turbidity limit is set at 15 NTU.

Table 1.2 below shows the results of a comparison of the reactions of the protein stability evaluation methods for different treatments with increasing dosages of bentonite.

Table 1.2 TESTS WITH BENTONITE ON UNSTABLE WINE (where ΔNTU <15 indicates stability)

	BENTOTEST	HEAT TEST WITH TANNIN	PROTEOTEST
UNTREATED	65	50	70
+30 g/hL bentonite	28	►5◄	►12◄
+60 g/hL bentonite	►8◄	5	8
+80 g/hL bentonite	6	4	8
+100 g/hL bentonite	4	3	6

On this unstable white wine, the heat test with the tannin indicates the product is stable, the Bentotest indicates 50 to 60 g/hL of bentonite is necessary, and with the Proteotest, the required amount of bentonite is consistent with the first test: less than 30 g/hL.

When the three treatments are compared on multiple wines (See table 1.3) the Proteotest can be seen to concur with the results indicated by the heat test with tannin. The Bentotest indicates a false instability thus suggesting the winemaker use higher bentonite addition rates.

Table 1.3: COMPARISON BETWEEN TESTS ON DIFFERENT WINES.

SAMPLE	WINE TYPE	BENTOTEST	PROTEOTEST	HEAT TEST	COMMENTS
169A	Red	13	22	30	Unstable - tests agree
169B	Rose	1	6	6	Stable - tests agree
162	White	0.28	10	0.75	Stable - tests agree
V1	Red	33	32	27	Unstable - tests agree
145A	Red	43	38	58	Unstable - tests agree
145B	Red	38	38	103	Unstable - tests agree
BTB	White	0.17	2	4	Stable - tests agree
150	Red	60	3	3	False positive with Bentotest, Proteotest agrees with heat test.
147	White	0.89	7	4	Stable - tests agree
T5LB	White	16	4	9	False positive with Bentotest, Proteotest agrees with heat test
B3LB	White	4	2	6	Stable - tests agree
R	Red	1	0.87	0.50	Stable - tests agree
RS	Rose	0.20	8	5	Stable - tests agree
163	Red	5	0.28	0.65	Stable - tests agree
183	Red	42	2	1	False postive with Bentotest, Proteotest agrees with heat test
138B	Red	13	18	72	Unstable - tests agree
138C	Red	1	5	3	Stable - tests agree
146B	Red	48	1	2	False postive with Bentotest, Proteotest agrees with heat test
146C	Red	22	2	2	False postive with Bentotest, Proteotest agrees with heat test

PROTEOTEST: THE TEST PERFORMED UNDER ENOLOGICAL CONDITIONS

During testing with PROTEOTEST precipitations can occur; this phenomenon can even be considered an integral part of the test. Before taking a reading with a turbidity meter, the sample should be agitated to bring the sediment into suspension.

With PROTEOTEST, the reactions that take place are those that manifest themselves in the wine, at the actual pH and temperature conditions.

The PROTEOTEST represents an indication that more closely conforms to enological conditions, and is therefore a more reliable test.