RESOLUTION OIV-OENO 363-2012

DETERMINATION OF PECTIN METHYLESTERASE ACTIVITY IN ENZYMATIC PREPARATIONS (COMPLEMENT TO RESOLUTION 9-2008)

THE GENERAL ASSEMBLY

IN VIEW of article 2, paragraph 2 IV of the Agreement of 3 April 2001, by which the International Organisation of Vine and Wine was founded,

CONSIDERING the works of the group of experts Specifications of Oenological Products,

CONSIDERING the resolution Oeno 9/2008 adopted in 2008 concerning the pectinemethyl esterase

HAS HEREBY DECIDED to modify the title of the resolution 9/2008 by "Determination of pectin methylesterase activity in enzyme preparations" and to complete the monograph on the determination of Pectine Methyl Esterase activity (Oeno 9/2008) published in the international Oenological Codex by the following modification:

DECIDE to add a title to the existing method indicating "Determination of Pectine methylesterase activity using methanol dosage" and to renumber the relevant point

DETERMINATION OF PECTIN METHYLESTERASE ACTIVITY IN ENZYMATIC PREPARATIONS

General specifications

These enzymes are usually present within an complex enzymatic preparation. Unless otherwise stipulated, the specifications must comply with the Oeno resolution Oeno 365-2009 concerning the general specifications for enzymatic preparations included in the International Oenological Codex.

1. Origin

Reference is made to paragraph 5 "Source of enzyme and fermentation environment" of the general monography on Enzymatic preparation

The enzyme preparations containing such activity are produced by directed fermentations such as Aspergillus niger, Aspergillus oryzae, Aspergillus sojae, Aspergillus Tubigensis, Aspergillus Awamori, Rhizopus oryzae and Trichoderma

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longibrachiatum (T.reesei)

2. Scope /Applications

Reference is made to the International Code of Oenological Practices, OENO 11/04; 12/04; 13/04; 14/04 and 15/04.

These enzyme activities are used to support grape maceration and grape juice extraction as well as to help the clarification of musts and wines and finally to improve their filterability.

Determination of Pectinmethylesterase activity using acid based titration

1. Principle

The demethylation activity of the pectinmethylesterase results in the appearance of free carboxylic groups at the level of the galacturonic acids forming the chains. To determine the activity of pectinmethylesterase, the carboxyl groups can be titrated during the enzymatic hydrolysis with sodium hydroxide solution at constant temperature and constant pH-value.

2. Equipment and materials

- Titration equipment (burette)
- Temperature controlled heat plate and magnetic stirrer/magnetic stir bar
- pH meter
- Glass cup, filled with water
- Chronometer
- Graduated flasks (different volume)
- Beakers (preferably 50 mL)
- Precision pipettes (different volume)

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3. Chemicals and reagents

- Pectin; highly esterified; p.a. quality (Sigma P9135-100G); CAS 9000-69-5
- 0,01 M NaOH solution (Titrisol) p.a. quality; CAS 1310-73-2
- NaOH pellets p.a. quality ; CAS 1310-73-2

4. Preparation of soultions

4.1. 1 M NaOH

Dissolve 4 g NaOH in 100 mL H_2O

4.2. Substrate solution

As substrate solution 1 % Pectin in H_2O, is used by solving 2.0 g Pectin very slowly in 150 ml H_2O. Subsequently the pH value is adjusted at pH 4.0 and at 40 °C with 1 M NaOH. The solution must be filled up to 200 mL exactly. Just before measuring, the pH-value should be controlled and adjusted again at pH 4,0, if necessary

4.3. Enzymatic solution

The enzymatic solution consists of approximately 30 to 50 mg/L commercial enzyme preparation diluted in cold water. This solution should be prepared directly before using.

4.4. 0.01M NaOH

This precast solution should be diluted according to the description of the producer.

5. Performance of enzyme activity determination

20 ml of substrate solution are put in a beaker (magnetic stirrer is added) on the temperature controlled heat plate in a glass cup, which is filled with water heated up to 40 °C. The pH electrode is put in substrate solution. It is necessary to have a control and maybe a new setting up of the pH-value at 40 °C before starting the analysis. Then 0.1 ml of the enzymatic solution is added. Exactly at this time the chronometer is started. During the analysis the pH value must be measured and the

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sample has to be titrated up to pH 4.0 with 0.01 M NaOH for 10 minutes at 40 °C. After 10 min the analysis is stopped and the consumption of 0.01 M NaOH is read off.

The consumption of 0,01 M NaOH should amount to values between 3,5 mL and 8,5 mL. Otherwise it is recommended to dilute or concentrate the enzymatic solution.

6. Calculation of the enzymatic activity

Enzymatic activity is calculated by using following formula:

Activity (U/mg) = n / (t*v*c)

Activity (nkat/g) = (Activity (U/mg) * 1000/60) * 1000

- n = consumption of 0.01 M NaOH in µmol
- t = time in min (in this case 10 min)
- v = quantity of enzymatic solution introduced in ml (=0.1 ml)
- c = concentration of the enzymatic solution in g/L

Validation of the acid based titration to determine the activity of Pectin methylesterase

The mean value of the standard deviation was determined of 8 different enzymes.

Each enzyme was analysed 6 times.

Mean value of the standard deviations of the different enzymes = 3.91 %

	Enzyme 1 40 mg/ml	Enzyme 2 40 mg/ml	Enzyme 3 40 mg/ml	Enzyme 4 40 mg/ml	Enzyme 5 40 mg/ml	Enzyme 6 40 mg/ml	Enzyme 7 30 mg/ml	Enzyme 8 50 mg/ml	Enzyme 8 30 mg/ml
Mean Value (nkat/g)	14527.7	19291.7	12756.8	9534.7	9444.5	18577.8	31591.7	10888.9	9446.5
Standard Deviation (nkat/g)	282.3	449.5	366.4	227.4	272.3	145.6	540.9	944.4	1096.1
Standard Deviation %	**1.9**	**2.3**	**2.9**	**2.4**	**2.9**	**0.8**	**1.7**	**8.7**	**11.6**
s2(r)	66410	168402	111863	43097	61786	17654	243773	743210	1001244
s (r)	257.7	410.4	334.5	207.6	248.6	132.9	493.7	862.1	1000.6

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Repeatability r (nkat/g)	729.3	1161.3	946.5	581.5	703.4	376.0	1397.3	2439.7	2831.8

Validation of the acid based titration to determine the activity of PME

Enzyme	Concentration	U/mg	nkat/g
Enzyme 1	40 mg/ml	0.89	14833
Enzyme 1	40 mg/ml	0.89	14750
Enzyme 1	40 mg/ml	0.88	14667
Enzyme 1	40 mg/ml	0.85	14083
Enzyme 1	40 mg/ml	0.87	14500
Enzyme 1	40 mg/ml	0.86	14333

Enzyme 1 40 mg/ml			(X-MW)^2
mean value (nkat/g)	14527.7		93228.4
standard deviation (nkat/g)	282.30		49432.1
standard deviation %	1.9		19413.8
variance	3.8		197728.4
$s^2(r)$	66410.6		765.4
s(r)	257.7		37895.1
r (nkat/g) repeatability	729.3	sum	398463.3

Enzyme	Concentration	U/mg	nkat/g
Enzyme 2	40 mg/ml	1.185	19750
Enzyme 2	40 mg/ml	1.155	19250

Enzyme 2 40 mg/ml		(X-MW)^2
mean value (nkat/g)	19291.7	210069.4
standard deviation (nkat/g)	449.54	1736.1

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Enzyme 2	40 mg/ml	1.130	18833	standard deviation %	2.3		210069.4
Enzyme 2	40 mg/ml	1.125	18750	$s^2(r)$	168402.8		293402.8
Enzyme 2	40 mg/ml	1.190	19833	s(r)	410.4		293402.8
Enzyme 2	40 mg/ml	1.160	19333	r (nkat/g) repeatability	1161.3		1736.1
						sum	1010416.7

Enzyme	**Concentration**	**U/mg**	**nkat/g**	Enzyme 3 40 mg/ml			(X-MW)^2
Enzyme 3	40 mg/ml	0.78	13042	mean value (nkat/g)	12756.8		81320.0
Enzyme 3	40 mg/ml	0.79	13208	standard deviation (nkat/g)	366.38		203551.4
Enzyme 3	40 mg/ml	0.76	12708	standard deviation %	2.9		2384.7
Enzyme 3	40 mg/ml	0.76	12583	$s^2(r)$	111863.1		30218.0
Enzyme 3	40 mg/ml	0.77	12833	s(r)	334.5		5801.4
Enzyme 3	40 mg/ml	0.73	12167	r (nkat/g) repeatability	946.5		347903.4
						sum	671178.8

Enzyme	**Concentration**	**U/mg**	**nkat/g**	Enzyme 4 40 mg/ml			(X-MW)^2
Enzyme 4	40 mg/ml	0.57	9500	mean value (nkat/g)	9534.67		1201.8

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Enzyme 4	40 mg/ml	0.59	9875	standard deviation (nkat/g)	227.41		115826.8
Enzyme 4	40 mg/ml	0.56	9333	standard deviation %	2.4		40669.4
Enzyme 4	40 mg/ml	0.56	9250	$s^2(r)$	43096.9		81035.1
Enzyme 4	40 mg/ml	0.58	9583	s(r)	207.6		2336.1
Enzyme 4	40 mg/ml	0.58	9667	r (nkat/g) repeatability	587.5		17512.1
						sum	258581.3

Enzyme	**Concentration**	**U/mg**	**nkat/g**	Enzyme 5 40 mg/ml			(X-MW)^2
Enzyme 5	40 mg/ml	0.55	9167	mean value (nkat/g)	9444.5		77006.3
Enzyme 5	40 mg/ml	0.59	9792	standard deviation (nkat/g)	272.29		120756.3
Enzyme 5	40 mg/ml	0.55	9083	standard deviation %	2.9		130682.3
Enzyme 5	40 mg/ml	0.57	9458	$s^2(r)$	61785.6		182.3
Enzyme 5	40 mg/ml	0.57	9542	s(r)	248.6		9506.3
Enzyme 5	40 mg/ml	0.58	9625	r (nkat/g) repeatability	703.4		32580.3
						sum	370713.5

Enzyme	**Concentration**	**U/mg**	**nkat/g**	Enzyme 6 40 mg/ml		(X-MW)^2

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Enzyme 6	40 mg/ml	1.105	18417	mean value (nkat/g)	18577.8		25956.8
Enzyme 6	40 mg/ml	1.118	18633	standard deviation (nkat/g)	145.55		3086.4
Enzyme 6	40 mg/ml	1.125	18750	standard deviation %	0.8		29660.5
Enzyme 6	40 mg/ml	1.105	18417	$s^2(r)$	17654.3		25956.8
Enzyme 6	40 mg/ml	1.112	18533	s(r)	132.9		1975.3
Enzyme 6	40 mg/ml	1.123	18717	r (nkat/g) repeatability	376.0		19290.1
						sum	105925.9

Enzyme	**Concentration**	**U/mg**	**nkat/g**	Enzyme 7 30 mg/ml			(X-MW)^2
Enzyme 7	30 mg/ml	1.920	32000	mean value (nkat/g)	31591.7		166736.1
Enzyme 7	30 mg/ml	1.947	32450	standard deviation (nkat/g)	540.86		736736.1
Enzyme 7	30 mg/ml	1.873	31217	standard deviation %	1.7		140625.0
Enzyme 7	30 mg/ml	1.860	31000	$s^2(r)$	243773.1		350069.4
Enzyme 7	30 mg/ml	1.893	31550	s(r)	493.7		1736.1
Enzyme 7	30 mg/ml	1.880	31333	r (nkat/g) repeatability	1397.3		66736.1
						sum	1462638.9

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Enzyme	Concentration	U/mg	nkat/g
Enzyme 8	50 mg/ml	0.578	9633
Enzyme 8	50 mg/ml	0.682	11367
Enzyme 8	50 mg/ml	0.706	11767
Enzyme 8	50 mg/ml	0.712	11867
Enzyme 8	50 mg/ml	0.596	9933
Enzyme 8	50 mg/ml	0.646	10767

Enzyme 8 50 mg/ml			(X-MW)^2
mean value (nkat/g)	10888.9		1576419.8
standard deviation (nkat/g)	944.38		228271.6
standard deviation %	8.7		770493.8
$s^2(r)$	743209.9		956049.4
s(r)	862.1		913086.4
r (nkat/g) repeatability	2439.7		14938.3
		sum	4459259.3

Enzyme	Concentration	U/mg	nkat/g
Enzyme 8	30 mg/ml	0.69	11444
Enzyme 8	30 mg/ml	0.067	8667
Enzyme 8	30 mg/ml	0.063	8889
Enzyme 8	30 mg/ml	0.065	8429
Enzyme 8	30 mg/ml	0.07	9625

Enzyme 8 30 mg/ml			(X-MW)^2
mean value (nkat/g)	9446.5		3990006.3
standard deviation (nkat/g)	1096.13		607620.3
standard deviation %	11.6		310806.3
$s^2(r)$	1001243.9		1035306.3
s(r)	1000.6		31862.3

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Enzyme 8	30 mg/ml	0.067	9625

r (nkat/g) repeatability	2831.8		31862.3
		sum	6007463.5

mean value of the standard deviations %	**3.91**

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