Activity of Extracted Lipase and Phospholipase of (Perlette) Grape Cultivar Seed Meal

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Summary: The activity of lipase and phospholipase, extracted from the seeds meal of grape cultivar, was studied with the help of spectrophotometer at different pH, temperature and solvents. Both lipase and phospholipase presented an optimum activity at pH 6.0 and 45 °C. Their activity also maximized when n-hexane was used as solvent media.

Introduction

Grape belongs to the family Vitaceae cultivated originally in Asia. Minor varieties are grown in South Europe, in North Africa and Middle East [1]. The seeds of grape are dicotyledonous with two ovules in each cell, which develop into succulent pedicellate berry of spherical of ovoid form in which the cells are obliterated [2].

The fruit of grape contains grape sugar, gum, tannin, tartaric acid, citric acid, chloride of potassium and sodium. Grapes are demulcent, laxative, refrigerant, stomach, diuretic and cooling. Raisins are laxative demulcent and expectorant also considered as alternant. Supportive nutrition and blood purified juice of unripe grape are astringent [3]. Oil of grape seeds is used in sunburn lotions, hair products, body hygience cream, lip balm and hand cream [4]. Grape seed oil also has syntactic anticancer effect [5] and has proven helpful in Pakistan for candiomuxe [6]. Through the perusal of literature, it was seen that no work on the lipase and phospholipase of cultivation of Perlette grape had been reported previously. Grape seed oil has health promoting chemistry and has marvelous medicinal importance [7]. The activity of enzymes in the seed may cause hydrolyses of oil which results in the degradation of oil, which is essential as oil is the main source of energy in Pakistan. The degradation in the present study was an attempt to extract the enzyme from the seeds to determine their optimum activity using triglycerols of olive oil and egg lecithin as substrates under different conditions of pH, temperature and solvents.

The literature survey revealed that similar investigations have been carried out on castor bean [8], oat grains [9], wheat grains [10], corn [11] and Niwtina tobacum [12]. The objective of this work was to establish an optimum condition for the hydrolysis of sample triglycerols and phospholipase by grape seed meal for laboratory and industrial usage.

Results and Discussion

Enzymes play a vital role in *in vivo* syntheses and in the break down of a number of organic compounds in animals and plants. The present study was concerned with lipase and phospholipase enzymes of Perlette grape cultivars seed, which were involved in degradation of lipids. The enzymes hydrolyze triglycerides and phosphoglycerides, respectively, and the liberated fatty acids serve as an indicator of their activity.

Experimental

The seeds of cultivars of Perlette grape seed were ground to a fine powder and defatted by using Soxlet apparatus and diethyl ether as solvent. 100 gms of defatted seeds of each condition powder were suspended in 0.1M citrate buffer (citric acid 0.1M and disodium hydrogen phosphate 0.2M) of pH 6.0 and shaken for one hour at 45 °C.

The supernatants containing the enzyme [13] were obtained by centrifugation for 20 minutes at 12 rpm. The extract was then diluted to 200 ml water 0.1 M citrate buffer, and utilized to study the enzyme activities under different conditions. 0.1gm of pure triglycerol of olive oil was emulsified by blending

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Table- 1: Lipase Activity of Grapes Seed at Different pH

pH	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
3	90	0.72
4	110	0.88
5	240	1.92
6	460	3.68
7	310	2.48
8	280	2.24

a. Taken from standard curve.
b. Calculated by Guven's Method
c. Activated equals to μ equiv./ gm per hour.

Table- 2: Phospholipase Activity of Grape Seed at Different pH

pH	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
3	50	0.40
4	80	0.64
5	280	2.24
6	310	2.48
7	240	1.92
8	206	1.65

The activity of lipase and phospholipase at different is reported in Table 1 and 2. The tables show that maximum activity of lipase is 3.68 μU and phospholipase is 2.48μU at pH 6.0 respectively. This indicates that enzymes are more active inslightly acidic media.

with 10 % gum acacia solution in aqueous media to determine lipase activity [14], whereas 10 % egg lecithin emulsion [15] was used as a substrate for the determination of phospholipase activity. The hydrolysis of substrate by enzymes extracted from the seeds of grape under different parameters is described below.

Effect of pH

Different experiments were conducted at pH ranging between 3 and 9 to observe the effect of pH on hydrolysis of substrates. The pH of the enzyme extracted was adjusted either to acidic or alkaline with 0.1 M solution of citric or 0.2 M solution of

Table- 3: Lipase Activity of Grapes Seed at Different Temperature

Temp. (°C)	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
30	206	1.65
35	240	1.92
40	398	3.18
45	458	3.66
50	390	3.12
55	280	2.24
60	110	0.88
65	80	0.64
70	40	0.32

Determination of activities of the two enzymes at different temperature from 20 °C to 70 °C with an increase of 5 °C at pH 6.0 revealed maximum activities as 3.66 and 3.18μU respectively (Table 3 and 4) at
45 °C.

Table-4: Lipase Activity of Grapes Seed at Different Temperature

Temp. (°C)	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
30	125	1.00
35	180	1.44
40	280	2.24
45	398	3.18
50	325	2.60
55	240	1.92
60	206	1.65
65	95	0.76
70	30	0.24

The activities were affected by changing the temperature and it was noted that activity decreased either the temperature was decreased or increased from 45 °C. This was supported by the reported work of Rakhimov and Aizonom [18].

sodium hydrogen phosphate, respectively. Sodium carbonate 0.1 M and sodium bicarbonate 0.1 M were used to achieve 0.1 M. Lipase and phospholipase fractions (4 m/ L each) extracted at pH 6.6 were incubated at 45 °C for 1 hour in 6ml substrate citrate buffer (5 m/ L) of pH 6.0 and 0.1 M calcium chloride (1 m/ L) in 50 m/ L stoppered conical flasks. The fatty acid released after extraction with 5 m/ L hexane : chloroform 1 : 1 v/v was treated with 2.5 m/

Table- 5: Lipase Activity of Grapes Seed at Different pH

Solvents	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
n-Heptane	390	3.12
Cyclohexane	240	1.92
Di-isopropyl Ether	115	0.92

Solvent also affected on lipase and phospholipase activities of seeds of grape.

Table- 6: Lipase Activity of Grapes Seed at Different pH

Solvents	Cone. Of F. A (μ equiv./L)[1]	Activity (μU)[2]
n-Heptane	325	2.60
Cyclohexane	206	1.65
Di-isopropyl Ether	105	0.84

n-Heptane was found to be the best solvent for. maximum enzymatic activity (Table- 5 and 6) of both enzyme s compared to cyclohexane and di-isopropyle ether. The resent work shows that the lipase and phospholipase enzyme of defatted seeds of grape exhibit maximum activity at pH 6.0 in n-heptane solvent at 45 °C.

L of cu tea regent in a test tube, shaken for 5 minutes and then centrifuged. The upper layer (3 m/ L) was reacted with 0.5 m/ L of 0.1 % sodium diethyl dithiocarbonate to develop a golden yellow color whose absorbance was measured at 440 nm on a spectrophotometer (Beckmann) against a blank prepared by boiled enzyme powder extract. A standard curve was drawn between the concentration against absorbance at the same wavelength. The standard curve was used to calculate u equivalent of fatty acid released per gram per house using Guvens method [16]. The activity concentration of lipase and phospholipase was calculated as follows by the method of Guvens et al:

$$\frac{\text{Conc. of fatty acid}}{1000} \times 5.0$$

Effect of Temperature

Experiments were conducted to study the hydrolysis of substrate under different conditions of temperature. The temperature incubation was changed from 30 °C to 70 °C interval [17]. Incubation was carried out for 1 hr at 6.0 pH.

Effect of Solvent

Defatted seed powder of Perlette grape (1 g) was placed in a 50 m/ L stoppered conical flask containing 50 ul water and 5.0 m/ L of triglyceride solvent (1 : 9 w/v) to observe the effect of different organic solvents on lipase activity, and lecithin solvent (1 : 9 w/v) was used to study the effect of solvent on phospholipase activity. Both the above mixtures for the two enzymes were shaken separately for two hours at 40 °C [16] and cooled to room temperature. 3 m/ L of solvent was further added to the mixture and mixed thoroughly. The remaining experimental conditions were kept same as those maintained to the effect of pH experiment.

Reference

1. Chopra, I. C., Handa, K. L., Kaphur, L. D. *Indigenous drugs of India Second Edi Academic Publisher, Calcutta, New Delhi.* pp. 530. (1954).
2. Gesundh, Arb., *Amt. Berl. Chem. Zbl.*, **11**. 1085 (1929).
3. Nadkarni, A. K. *The Indian Medica.* Vol-1, Bombay Popular Prakashan 4th Ed. pp. 1284 (1957).
4. Sharma, G., TYAGI, A. R., Singh, R. P., Chan, D. C, Agarwal, R. *Brest Cancer Res. Treat.*, **85**. 1. (2004).
5. Wang. Yu. H., Zhaoc, Sc., Ghengyan, Xu. G. Wei, *Mutant Res.*, **523**. 524 (2003).
6. Joshi, S. S., Kuszunski, C. A., Bagchi. D. *Curr Pharm. Biotechonol.* **2**. 187 (2001).
7. Hifza, A., Hamid, S., Waheed, A and Bashir, R. *Jour. Chem. Soc. Pak.* 28: 93-100 (2006).
8. Ory, R. L. *Acid lipase of castor bean. Lipids.* 4. 177 (1969).
9. Berner, D. L., and Hammond, E. G, Specificity of Lipase from Several Seeds. *Lipids.*, **5**.572. (1972).
10. Ferrion, M., and Geddes, W. E. Distribution of lipase in the commercial mil powder from hard red spring wheat cereal. *Lipids.* **35.** 422 (1958).
11. Banu, C, and Serban, L. *Ind. Ailment.*, **21**, 367. (1970).
12. Waheed, A, Mahmood, S, and Saleem, M,

Natural Product Sciences., **7.** 17. (2001).
13. Rakhimov, M. M. Kh. Ataullaer, A. *Maslo. Zhir. Prom. St.*, **11.** 8. (1976).
14. Aizono, Y. Funatau, M. Fujiki, Y., and Watanabe, M. *Argic. Biol. Chem.*, **40** . 317. (1976).
15. Zaka, S. Akhtar, M. W. and Khan, S. A. *Pak. J. Sci. Ind. Res.*, **32.** 27. (1989).
16. Guven, K. C. Bergisadi, N. and Guler, E. 1989. *Fette. Seifen. Anstrichmittel.* **81**: 152-154 (1989).
17. Akhtar, H. W., Parveen, H. Kausar, S., and Chughtai, M.I.D. *Pak. J. Bio. Chem.*, **81.** 77. (1975).
18. Kausar, N., and Akhtar, W. M. *Pak. J. Bio. Chem.*, **12.** 58. (1979).