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Research Article

Optimization for Ultrasound-microwave Assisted Extraction of Pectin Methylesterase from Jujube Using with Orthogonal Design Methodology

[1]Yanan Xia, [2]Yuan Liu, [1]Yanli Ma, [1]Lijuan Hou and [1]Jie Wang
[1]College of Food Science and Technology, Agricultural University of Hebei, Baoding 071000, China
[2]Department of Food Science, Hebei North University, Zhangjiakou 075000, China

Abstract: Optimization of conditions for Jujube pectin methylesterase extraction was investigated using orthogonal design methodology. Extraction parameters which are employed in this study are Liquid-Solid Ratio (LSR) (1:5-20), NaCl content (0-2.0 N), ultrasonic time (0-80 min) and they were optimized using a three-factor orthogonal Design coupled with range and variance analysis. The results showed that, NaCl content and solid-liquid ratio have significant effect on the activity of pectin methylesterase. The satisfactory conditions for Jujube pectin methylesterase extraction were obtained as follows: 1:10 of LSR, 1 N of NaCl content and 40 min of ultrasonic time. Among the studied factors, NaCl content had the greatest influence on yield. Under these conditions, the experimental activity of Jujube pectin methylesterase reach the highest, was 0.6841 U/g.

Keywords: Exaction, jujube, pectin methylesterase, ultrasound-microwave

INTRODUCTION

Pectin is abundant in jujube and mainly high methoxyl pectin. Pectin is widely distributed in plants, constituting the main component of plant cell walls. Pectin is chain polymer with alpha-D-galacturonic acid as structure unit, connected with alpha-1, 4-glycosidic bond (Lacroix *et al*., 2005). Before pectin secreted into the cell wall, galacturonic acid in the main chain would esterification with methanol. Pectin methylesterase could catalyze pectin demethyl esterification to take off free carboxyl groups, methanol and hydrogen ions (Polydera *et al*., 2004). This will change the pH value around cell wall, which affects other pectic enzymes, such as polygalacturonase and the activity of pectin lyase, etc. Pectin methylesterase play an important role in plant cell wall metabolism process (Micheli *et al*., 1998).

At present, enzyme exaction and purification method include crude enzyme preparation, salting out with ammonium sulfate, organic solvent precipitation, chromatography, polyacrylamide gel electrophoresis, Sephadex gel filtration, isoelectric focusing, aqueous two-phase extraction (Kobayashi *et al*., 1999). Determination methods include viscosity reduction, titration, AJDA, ultraviolet absorption and so on. Given to obtain the larger pectin methylesterase activity and ease operation, crude enzyme preparation and sodium iodate titration are chosen as exaction and determination method (Jaramillo *et al*., 2013).

Humidicool storage inhibited Lipoxygenase (LOX) and Peroxidase (POD) activities of Winter-jujube and delayed decreasing of superoxide dismutase activity, increasing Malondialdehyde (MDA)content, which are beneficial to keep integrality of cell membrane (Xiaojun, 2004; Liu *et al*., 1996; Cyong and Hanabusa, 1980). Pectin methylesterase contribute to obtain apple quality could be tested in structure, esterification, water soluble pectin content (Di *et al*., 2005; Kinter III and van Buren, 1982) also happened in tomato (Lin *et al*., 2013; Christiaens *et al*., 2012; Anthon and Barrett, 2012). Research on pectin methylesterase inhibitor have also been improved in recent years (Mei *et al*., 2008; Micheli, 2001; Johansson *et al*., 2002; Schmohl *et al*., 2000; Camardella *et al*., 2000).

However, extraction technology of pectin methylesterase in jujube has not reported clearly until now. In this study, pectin methylesterase preparation technology is optimized in Liquid-Solid Ratio (LSR), NaCl content, ultrasonic time using a three- factor orthogonal design methodology, so as to provide advice for pectin methylesterase function on improving fruit and vegetable quality and its activity regulation research.

MATERIALS AND METHODS

Jujube: samples are dried ziziphus jujube (Hebei, Cangzhou):

Corresponding Author: Jie Wang, College of Food Science and Technology, Agricultural University of Hebei, Baoding 071000, China, Tel.: +86-13131262819

Table 1: Single factor experiment design of pectin methylesterase extraction

Parameter	1	2	3	4	5
Liquid solid ratio	1:5	1:8	1:10	1:15	1:20
NaCl content (N)	0	0.5	1.0	1.5	2.0
Ultrasonic time (min)	1:5	1:8	1:10	1:15	1:20

Table 2: Factors and levels for $L_9(3^4)$ orthogonal array design of the preparation of crude enzyme solution

Parameter	Liquid solid ratio (A)	NaCl content (B)	Ultrasonic time (C)
1	1:8	1N	40min
2	1:10	1.5N	60min
3	1:15	2.0N	80min

Pectin methylesterase preparation process:

- **Sampling:** Sampling was performed by quartering. Choose proper liquid ratio.
- NaCl added (Choose proper content), incubate 2 h at 4°C in order to pectin methylesterase fully dissolved. Na+ can be regarded as enzyme activator, accelerate the enzymatic reaction and inhibit microbes growth.
- Ultrasonic processing by intermittent 5 min with ice water in order to protect the enzyme protein. Choose proper ultrasonic time.
- Filter, rude pectin methylesterase sealed and preserved in 4°C.

Choose of extraction parameters (liquid ratio, NaCl content, ultrasonic time):

Liquid solid ratio: Solid-to-liquid ratio of extraction designed from 1:5, 1:8, 1:10, 1:15, 1:20, 1 N NaCl added, ultrasonic processing 60 min to choose proper liquid solid ratio by enzyme activity. Table 1 showed single factor experiment design of pectin methylesterase extraction.

NaCl content: Solid-to-liquid ratio of 1:20, NaCl content designed from 0N, 0.5N, 1N, 1.5N and 2.0N, ultrasonic processing 60 min to choose proper NaCl content by enzyme activity.

Ultrasonic time: Solid-to-liquid ratio of 1:20, 1 N NaCl added, ultrasonic time designed from 0, 20, 40, 60, 80 min to choose proper ultrasonic time enzyme activity.

Orthogonal design: Based on the results of single factor experiment on liquid ratio, NaCl content and ultrasonic time, L_9 (3^4) orthogonal test designed as Table 2 to choose proper extraction parameters of pectin methylesterase.

Determination method of pectin methylesterase:

- Sampling 50 mL crude pectin methylesterase, incubate 10 min at 30°C water bath.
- Sampling 50 mL 1% pectin solution adjust pH value to 7.5 with 0.01 N NaOH solution, incubate 10 min at 30°C under magnetic stirring.
- Add crude pectin methylesterase to pectin solution, adjust pH value to 7.8 quickly, timing start when pH value decrease to 7.5.
- Titrate with 0.01 N NaOH to obtain pH value stay in 7.5, record the consumption volume of NaOH after 30 min.

Pectin methylesterase activity calculation formula is:

$$\text{PME activity (U/g)} = (V_{NaOH} * c_{NaOH} * 10000)/(V_{PME} * t_{PME})$$

RESULTS AND DISCUSSION

The influence of liquid ratio on pectin methylesterase activity: It can be seen from Fig. 1 that pectin methylesterase activity increased first, reach the peak of 0.67U/g at liquid ratio of 1:10, significantly higher than other treatment ($p<0.05$), then decreased gradually with liquid ratio adding. So it is advisable to choose liquid ratio of 1:10.

The influence of NaCl content on pectin methylesterase activity: Pectin methylesterase activity presented a increase-stable-decrease trend with NaCl content adding, peak at NaCl content of 1.0 and 1.5 N, reach maximum pectin methylesterase activity of 0.66 U/g (Fig. 2). So it is advisable to choose NaCl content of 1.0 N.

The influence of ultrasonic time on pectin methylesterase activity: From Fig. 3, pectin methylesterase activity rose first, reach the peak of 0.68U/g at 60 min, significantly higher than other treatment ($p<0.05$), then reduced with ultrasonic time. That is because ultrasonic wave has the effect of splitting cells, certain ultrasonic treatment is beneficial for jujube cell to release pectin methylesterase, so enzyme activity showed a trend of increase at first. However, protein would be fractured after a long time of ultrasonic treatment, so as to enzyme activity decrease later. So it is advisable to choose ultrasonic time of 60 min.

Orthogonal test result of extraction of pectin methylesterase: The orthogonal test result is shown in

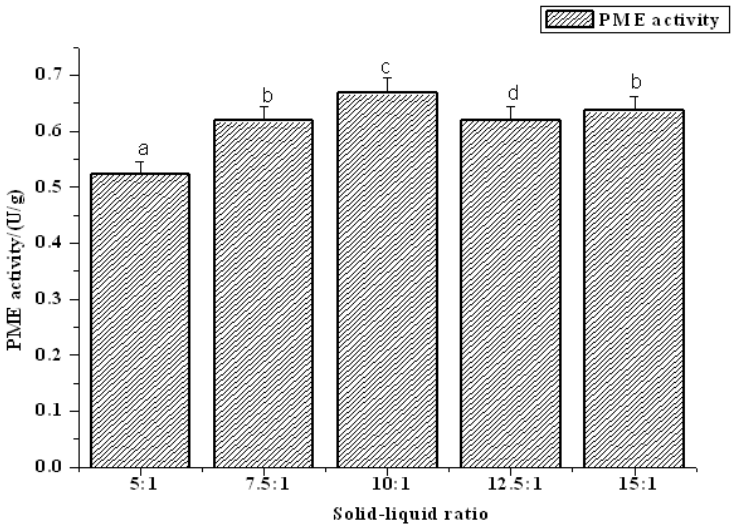

Fig. 1: Effect of solid-liquid ratio on the activity of PME

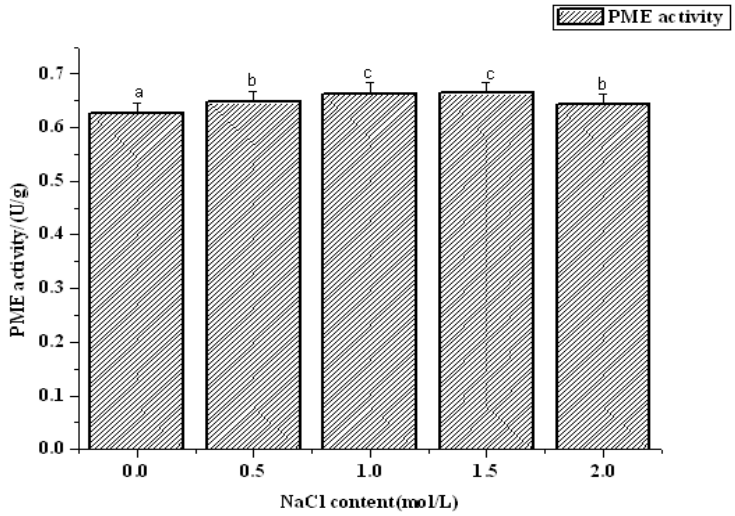

Fig. 2: Effect of NaCl content on the activity of PME

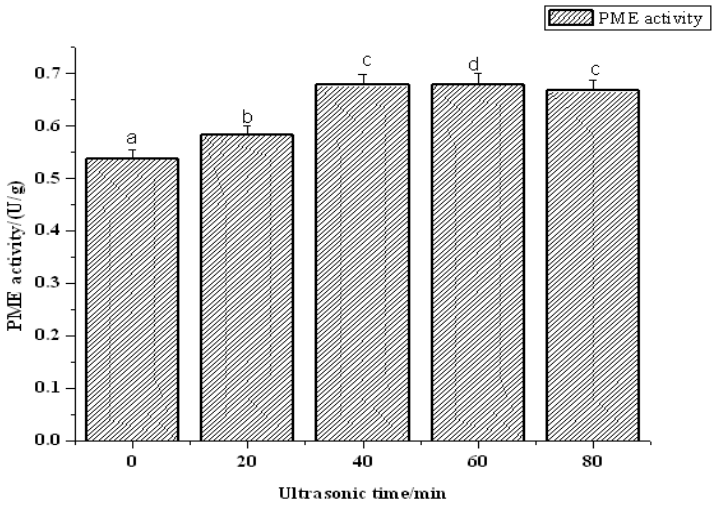

Fig. 3: Effect of ultrasonic time on the activity of PME

Table 3: Factors and levels for L_9 (3^4) orthogonal array design and results of the preparation of crude enzyme solution

No.	A	B	C	Enzyme activity (U/g)
1	3	3	1	0.5438
2	1	2	3	0.6017
3	3	1	3	0.5932
4	1	3	2	0.6253
5	2	3	3	0.5694
6	3	2	2	0.6169
7	2	2	1	0.6123
8	2	1	2	0.6839
9	1	1	1	0.6221
K1	0.6164	0.6331	0.5927	
K2	0.6219	0.6103	0.6420	
K3	0.5846	0.5795	0.5881	
R	0.0372	0.0228	0.0539	

Table 3. Range analysis results show the importance of extraction parameter on enzyme activity: NaCl content (B)> olid-liquid ratio (A)>ultrasonic time (C); Variance analysis show that NaCl content and solid-liquid ratio affect enzyme activity significantly ($p<0.05$), ultrasonic time does not show significant effect on enzyme activity. In order to get highest pectin methylesterase activity, according to the principle of significant factor choose the highest level, non-significant factor choose the most economic level, appropriate combination of extraction parameter is A2B1C1, namely liquid ratio of 1:10, NaCl content of 1 N, ultrasonic time of 40 min.

Verification test: Three repeat tests performed according to A2B1C1 conditions, pectin methylesterase activity reach a mean of 0.6841 U/g, higher than the 9 test results in Table 3, so the A2B1C1 is appropriate extraction parameter of pectin methylesterase.

Compared with liquid-liquid extraction of pectinase produced by Aspergillus oryzae using aqueous two-phase micellar system, pectinase activity varied from 0.233 to 0.601 U mL1. The highest activity was obtained in the top phase at conditions 25 C, 8% Triton X-114 (wt/wt) and 20% concentrated crude extract (wt/wt) (Jaramillo *et al*., 2013). If processed for long time period, a gradual further inactivation following different, slower kinetics will be achieved. This has also been observed by other researchers (Basak and Ramaswamy, 1996; Goodner *et al*., 1998).

CONCLUSION

Single factor test result show proper extraction parameter is Liquid-Solid Ratio (LSR) (1:10), NaCl content (1.0N), ultrasonic time (60 min). After a three-factor orthogonal Design coupled with range and variance analysis. The results showed that, NaCl content and solid-liquid ratio have significant effect on the activity of pectin methylesterase. The satisfactory conditions for Jujube pectin methylesterase extraction were obtained as follows: 1:10 of LSR, 1 N of NaCl content and 40 min of ultrasonic time. Among the studied factors, NaCl content had the greatest influence on enzyme activity. Under these conditions, the experimental activity of Jujube pectin methylesterase reach the highest, was 0.6841 U/g.

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