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# ENZYME ASSISTED AQUEOUS EXTRACTION AND PHENOLIC ANTIOXIDANTS OF ONION OIL

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**Abstract:** Enzyme-assisted aqueous extraction is a safe and efficient oil extraction process. In this study, onion oil was extracted with different types of enzymes (amylase, cellulase and amylase+cellulase), extraction time (8, 16 and 24 hours) and temperature (35$^{o}$C, 50$^{o}$C and 65$^{o}$C). The highest oil recovery (18.38%) was obtained by sample extracted with amylase+cellulase after 16 hours at 65˚C. The oil extracts along with the control sample were further analyzed to determine their total phenolic content by Folin-Ciocalteau method giving the highest value of 5.90 mg/mL by sample extracted with cellulase at 35$^{o}$C, after 16 hours. It was interesting to note that different types of enzymes, temperature and extraction time exhibited significant oil recovery and total phenolic content.

**Keywords:** enzyme-assisted extraction, oil recovery, total phenolic content.

## INTRODUCTION

Onions (*Allium cepa*) have been used for centuries in several societies against parasitic, fungal, bacterial and viral infections. Recent chemical characterisation of sulphur compounds in onions has allowed stating that they are the main active antimicrobial agents (Rose *et al*., 2005). However, some proteins, saponins and phenolic compounds can also contribute to this activity (Griffiths *et al*., 2002). Due to the great antimicrobial activity that onion posses, it could be used as natural preservatives, to control the microbial growth (Pszczola, 2002).

Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oil industry since it offers many advantages compared to conventional extraction. For instance, it eliminates solvent consumption which reportedly may also lower investment costs and energy requirements (Barrios *et al*., 1990). It has been reported that the low extraction yields of aqueous processes can be overcome by using enzymes that hydrolyse the structural polysaccharides forming the cell wall of oilseeds, or that hydrolyse the proteins which form the cell and lipid body membranes (Sosulski *et al*., 1988).

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Enzymatic treatment of vegetable samples can be a suitable alternative to increase polyphenols recovery. Enzymes commonly used in the food industry catalyze a variety of hydrolytic reactions and a high percentage act on cell wall polymers improving extraction yield of juices, oils and sugars. The main limitation for the application of those enzymes in industrial processes has been their high cost, but with current biotechnology advances it is already possible to obtain enzymatic formulations with lower costs and better quality (Zúñiga *et al.*, 2003). The aim of this study was, therefore, to measure the oil recovery from onion by using different types of enzymes at different temperature during the extraction process. Other than that, total phenolic content was also analysed in the onion oil extracted.

## MATERIALS AND METHODS

### Aqueous enzymatic oil extraction

Aqueous extraction method for onion oil was done according to Latif (2009). Fresh onion (*Allium cepa* L.) was purchased from Giant Superstore Tampin, Taman Sri Intan, Tampin, Negeri Sembilan, Malaysia. The raw onions were cut into smaller pieces, weighed and added to distilled water with the solid:water ratio of 1.6:1. Then, the mixture was boiled and stirred on the hot plate for 10 minutes, and allowed to cool down to room temperature. The pH was adjusted to pH 7 by using 0.5M NaOH and 0.5M HCl. The mixtures with different types of enzymes; 2% (v/v) amylase (Science Technics, Malaysia), 2% (v/v) cellulase (Novozymes Malaysia), 1% (v/v) amylase+1% (v/v) cellulase) and without any enzyme added (control) were prepared. The samples were extracted at different periods of time (8, 16 and 24 hours) and temperature (35˚C, 50˚C and 65˚C) with constant shaking (100 rpm). After that, the samples were centrifuged (10,000 rpm, 30˚C) for 10 minutes by using Bench Top Centrifuge (Sigma 3K15). The supernatants were collected and filtered into a beaker. Finally, the extracts were dried in a drying oven at 60˚C. The oil recovery of the oil samples was calculated based from Guyer *et al.,* (2003) by calculating the percentage of weight of onion oil extracted with the weight of onion used in the extraction.

### Determination of total phenolic content

Total phenolic content of onion oil extracted from different parameters done previously was determined according to the method described by Siti Fairuz *et al.,* (2011) where 1500 µL of Folin-Ciocalteu reagent and 1.2 mL of 7.5% (w/v) sodium carbonate were added to 0.3 mL of sample extract. The sample was covered with parafilm and allowed to stand for 2 hours in the dark. The absorption of the sample was measured at 765nm using UV-Visible

Spectrophotometer against blank. The total phenolic contents were expressed in gallic acid equivalents (GAE).

## RESULT AND DISCUSSION

### Effect of different enzymes, temperature and time on oil extraction

The effect of different types of enzymes, temperature level and extraction time on onion oil recovery are shown in Table 1. It is clear by comparing the control from the experiments carried out with the two enzymes (amylase, cellulase) and mixture of both enzymes, it shows the extraction yields obtained with enzyme assisted were much higher than those obtained with the non enzyme assisted. It is also possible to see that the increases that happened due to the action of the enzymes were in general very significant in comparison to the control. Aqueous extraction in the absence of added enzyme did not give high oil recovery. The highest oil recovery (18.38%) was obtained by amylase+cellulase, at 65$^{o}$C, after 16 hours of extraction.

**Table 1:** Percentage of Onion Oil Recovery

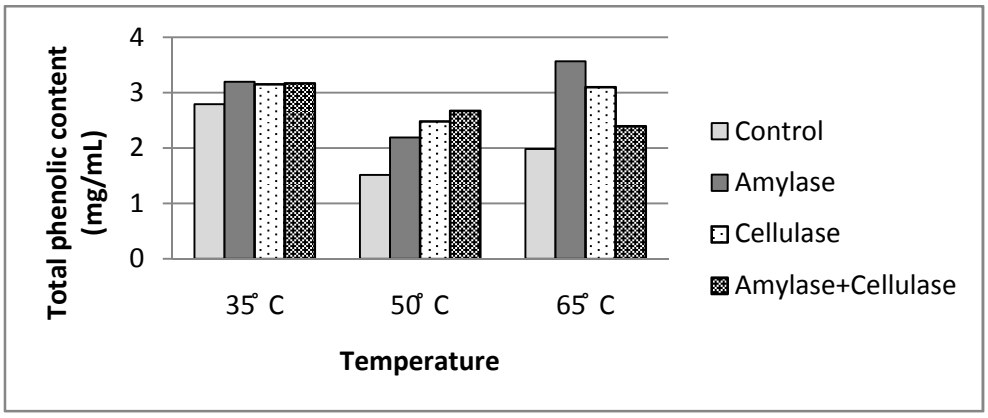
| Time | Enzyme | 35$^{o}$C | 50$^{o}$C | 65$^{o}$C |
|---|---|---|---|---|
| 8 hours | Nil (control) | 2.46 | 3.24 | 2.65 |
| | Amylase | 3.76 | 4.35 | 3.17 |
| | Cellulase | 3.54 | 4.13 | 3.91 |
| | Amylase+Cellulase | 4.05 | 4.73 | 3.53 |
| 16 hours | Nil (control) | 3.04 | 3.51 | 3.77 |
| | Amylase | 5.25 | 10.75 | 13.40 |
| | Cellulase | 5.02 | 14.67 | 13.11 |
| | Amylase+Cellulase | 4.81 | 16.73 | 18.38 |
| 24 hours | Nil (control) | 2.14 | 3.22 | 3.29 |
| | Amylase | 7.80 | 12.55 | 9.84 |
| | Cellulase | 7.12 | 11.68 | 10.33 |
| | Amylase+Cellulase | 11.98 | 14.84 | 9.94 |

The addition of specific enzymes during extraction enhances the oil recovery by breaking the cell wall and lipid bodies (Rosenthal *et al*., 1996; Singh *et al*., 1999). Enzyme mixtures with

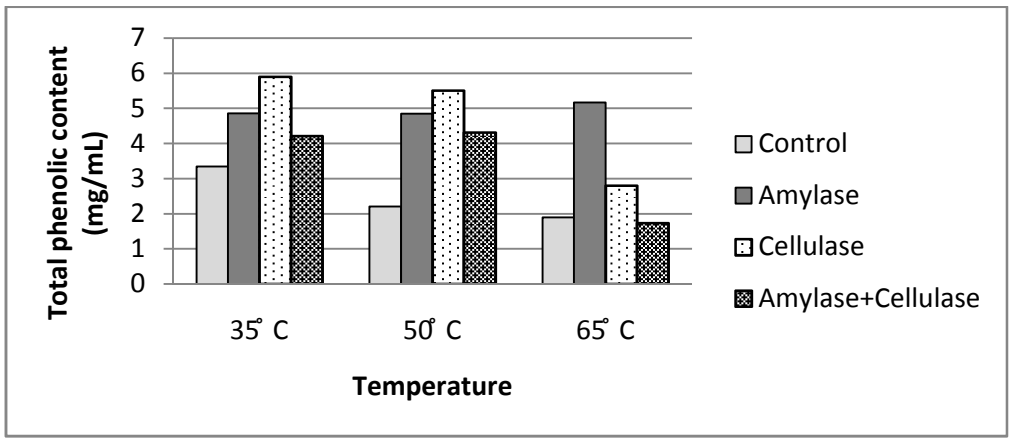
combined activity give, in general, better results than individual enzymes (Buenrostro and Lopez-Munguia, 1986).

**Effect of different enzymes, temperature and time on total phenolic content**

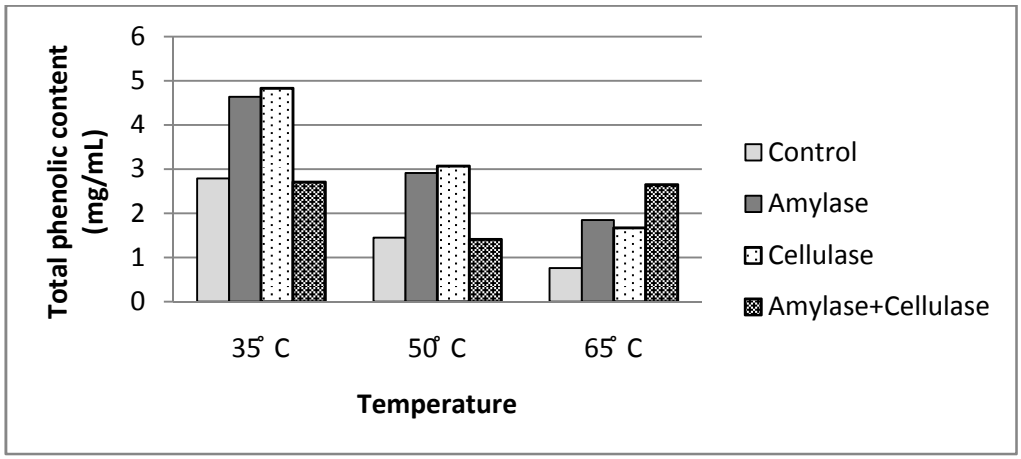
Figure 1 (a), (b) and (c) show the effect of different types of enzymes at different temperature (35°C, 50°C and 65°C) after 8, 16 and 24 hours of extraction on total phenolic content. It was observed that the highest total phenolic content (5.90 mg/mL) was obtained by sample extracted with cellulase at 35°C after 16 hours of extraction as shown in Figure 1 (b). The second highest total phenolic content (5.50 mg/mL) was also obtained by sample extracted with cellulase but at a higher temperature of 50°C, after 16 hours of extraction as shown in Figure 1 (b). The total phenolic content decreased after 24 hours for samples extracted at 50°C and 65°C. These results show that time and temperature during the extraction process also played a role in achieving higher total phenolic content as well as types of enzymes.




(a)




(b)




(c)

Fig. 1(a): Effect of different enzymes at different temperature after 8 hours of extraction. Fig. 1(b): Effect of different enzymes at different temperature after 16 hours of extraction. Fig. 1(c): Effect of different enzymes at different temperature after 24 hours of extraction.

Compared to control, adding enzymes to assist in the oil extraction process significantly increased the recovery of total phenolic content. This is probably due to enzymatic mediated extraction of phenolic antioxidants from the vegetable matrix may occur via hydrolytic degradation of the cell wall polysaccharides, which can retain phenolics in the polysaccharide lignin network by hydrogen or hydrophobic bonding. Another mechanism may be the direct enzyme catalyzed breakage of the ether and/or ester linkages between the phenols and the plant cell wall polymers, as it is mentioned by Pinelo *et al*. (2008).

The recovery of phenolic compounds from plant materials is also influenced by the extraction time and temperature, which reflects the conflicting actions of solubilization and analyte degradation by oxidation (Robards, 2003). An increase in the extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate. In addition, the viscosity and the surface tension of the solvents are decreased at higher temperature, which helps the solvents to reach the sample matrices, improving the extraction rate. However, many phenolic compounds are easily hydrolyzed and oxidized. Long extraction times and high temperature increase the chance of oxidation of phenolics which decrease the yield of phenolics in the extracts (Dai and Mumper, 2010).

Similar results have been referenced previously by García *et al*. (2001) where enzyme incorporation in oil extraction processes produce a high content of antioxidant compounds in olive oil), in borage oil and its defatted meal (Soto *et al*. 2008). Meyer *et al*. (1998) and

Maier *et al*. (2008) also showed an increase of phenolic compounds extracted from grape pomace by means of enzyme incorporation.

## CONCLUSION

The extraction process for edible oils based on aqueous extraction media with or without enzymes contributes to environmental, safety, and economic aspects. However, further studies need to be carried out in terms of finding more specific and effective enzymes to be used for extraction. The stability of phenolic compounds and other beneficial content of the extracted oil should also be considered. Prior investigation of the systematic process engineering and economic evaluation need to be done before scaling up the enzyme assisted aqueous extraction system.

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