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Isolation of Peroxidase from Watermelon Rinds Using Three Phase Partitioning

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Abstract: Background and Objective: Peroxidase is an oxidoreductase that uses different compounds as substrate and thus can be utilized for different applications. The goal of this work is to isolate peroxidase from watermelon rinds using three phase portioning (TPP).
Methods: TPP was set by adding varying amounts of salts and alcohol and the enzyme activity recovery was measured for each variable. Different parameters were optimized successively in order to achieve the highest enzyme activity recovery including salt type, salt concentration, pH, alcohol/crude extract ratio and type of alcohol and then, combining all optimized conditions together.
Results: Salt that gave maximal recovery was sodium potassium tartarate, optimal salt concentration was 17%, optimal pH was 8, optimal alcohol/crude extract ratio was 2 and 1-butanol was preferred to t-butanol. Efficiently, upon combining all optimized factors, activity recovery of 116% was obtained.
Conclusion: Peroxidase can be isolated from watermelon rinds with high efficiency using TPP

Keywords: peroxidase, three phase partitioning, isolation, activity recovery, enzyme.

1. Introduction

Enzymes are highly efficient protein catalyst that speeds various chemical reactions up. Based on the reaction they catalyze, enzymes can be classified into six groups, namely oxidoreductase, transferases, hydrolases, lyases, isomerases and ligases. Under each class there are subclasses. Peroxidases enzyme belong to oxidoreductase [1]. Being oxidoreductases, peroxidases (E.C. 1.11.1.X) oxidize a wide spectrum of substrates by decomposing hydrogen peroxide. The substrates peroxidase can catalyze include aliphatic, aromatic, organic and inorganic. Peroxidase are distributed almost throughout all life kingdoms from bacteria to human but dominates in plants [2]. This type of enzymes differs in their primary as well as tertiary structure since the molecular weight ranges from 30 kDa to 150 kDa. Due to the identification, characterization and application of this enzyme from several sources for a plethora of purposes, a database was established to contain such information called PeroxiBase [3].

Peroxidase can be used for different purposes involving food industry such as milk sterilization, environmental remediation such as dyes and mycotoxin degradation, chemical syntheses like purpurogallin and as biosensor in the diagnostic kits of glucose and triglycerides, among others.

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Such wide application spectrum is attributed to the wide acceptability of the enzyme toward plenty of substrates whatever the source [4].

The first step prior to application is enzyme isolation. The most commercial, commonly used source of peroxidase isolation is horseradish [5]. Nevertheless, the commercial source is not usually feasible owing to economic cost issues. In contrast, new sources were employed for the isolation of peroxidase and demonstrated their replaceability to horseradish peroxidase [6]. Three phase portioning (TPP) is a non-chromatographic, rapid, easy-to-use method for the isolation and purification of various biomolecules including, bioactive phenolic compounds, polysaccharides, enzymes, proteins, and drugs. As its name implies, it is composed of three phases but with two components, i.e. upper alcohol phase (dominantly tert-butanol), middle phase (dominantly insoluble protein clumps) and lower aqueous phase (saturated with salt). The fractionation principle is based on the affinity of the fractioned molecules to each phase of the system. Of note, TPP has payed attention in the recent years due to high recovery yield of the isolated biomolecule and can be coupled with ultrasound and microwave [7].

Herein, we aimed to isolate peroxidase from watermelon rinds using TPP.

2. Materials and Methods

2.1. Preparation of watermelon rinds

Fresh watermelon was purchased from local market of Aleppo city, Syria. About 3 mm was the thickness of the excised rind that was cut from the washed watermelon which further washed twice with distilled water. Then, 10 g of the rinds together with 100 ml of cold phosphate buffer (pH 7) were homogenized using commercial blender. Afterwards, the obtained solution was filtered and centrifuged at 5500 rpm for 20 mins and the supernatant was stored at 4 °C for later use.

TPP setup

. To 15 ml test tube, 5 ml of the crude extract was saturated with different salt concentrations and mixed until completely dissolved. Then, various volumes of alcohol were added to the tube and vigorously mixed and settled for 3 h. After that, brief centrifugation step was done to facilitate the separation of the layers (3000 rpm for 5 mins). The enzymatic activity was measured for the bottom layer. However, many parameters were optimized to achieve maximum separation efficiency involving different salt types, salt concentrations, pH degrees, different alcohol types, and varying the alcohol/crude extract ratios [6].

2.2. Determination of peroxidase assay

We followed the methods described by [8]. In short, 2.4 ml of phosphate buffer (pH 7) was added to a test tube followed by the addition of 300 µl 5.3% pyrogallol, 200 µl 0.6% hydrogen peroxide and 100 µl of the corresponding enzyme source at 420 nm for 4 mins. The activity recovery was calculated by dividing the obtained activity by the activity of the crude extract multiplied by 100.

3. Results and Discussion

The measured activity of the crude extract was found to be 753 U/L which instigates us to further isolate the enzyme from such attainable fruit waste.

Effect of salt type

To test the influence of salt type on the separation of peroxidase into the bottom layer, various salts were examined. Among those, sodium potassium tartarate exhibited the highest relative activity (82%) in comparison with the crude extract as depicted in Fig 1. This was followed by magnesium sulfate and sodium acetate with activity recovery of 75% and 72% respectively.

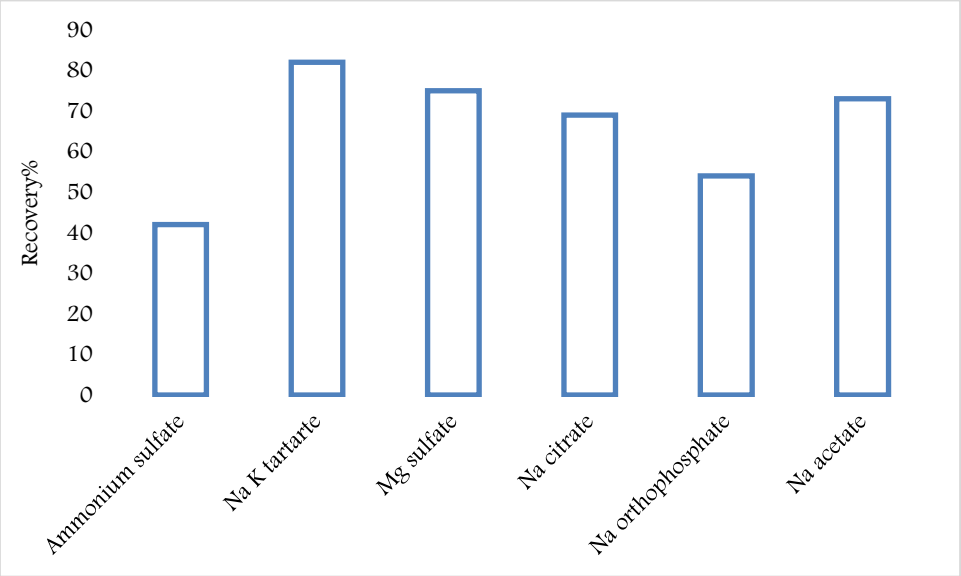

Figure 1. Effect of salt type used to saturate aqueous phase on enzyme recovery. Salt concentration was set at 20%, pH at 7, alcohol/crude extract ratio at 1, and alcohol used was t-butanol.

Effect of salt concentration

After selecting the best salt source, we optimized sodium potassium tartarate that gives maximum separation by varying the concentration range from 10% to 30%. We found that as the salt concentration increases, separation increases and enzyme activity recovery raises to give maximal activity (106%) at 17%. However, after that concentration, raising the salt concentration poses negative impact on the separation process (bell curve-like graph) as shown in Fig 2.

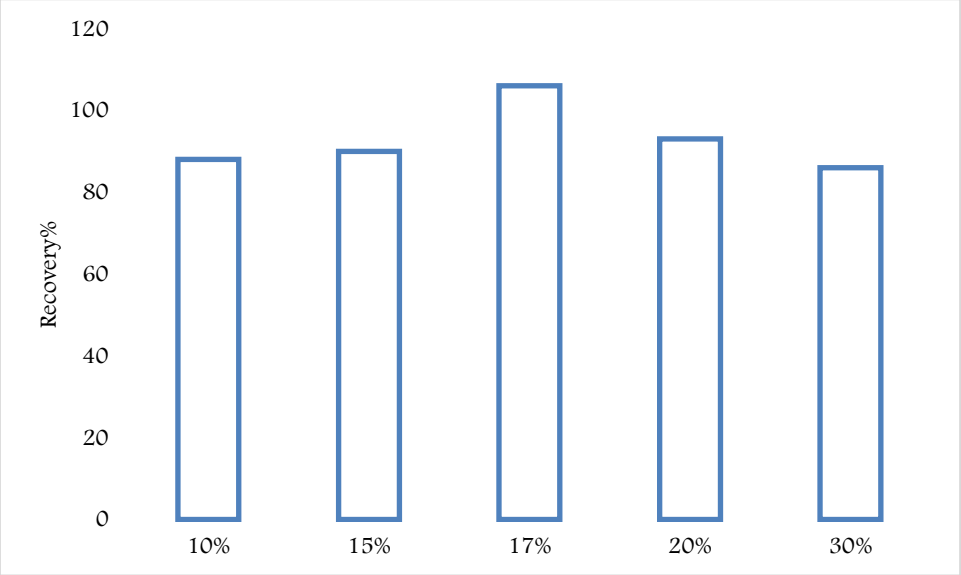

Figure 2. Effect of varying sodium potassium tartarate on peroxidase recovery. pH was set at 7, alcohol/crude extract ratio at 1, and alcohol used was t-butanol.

Effect of pH

Next, pH range from 6-9 was tested for its impact on the separation of TPP. As the pH get increased the activity recovery increased reaching optimal separation (107%) at pH 8 and afterwards, a decline in activity was observed (Fig 3).

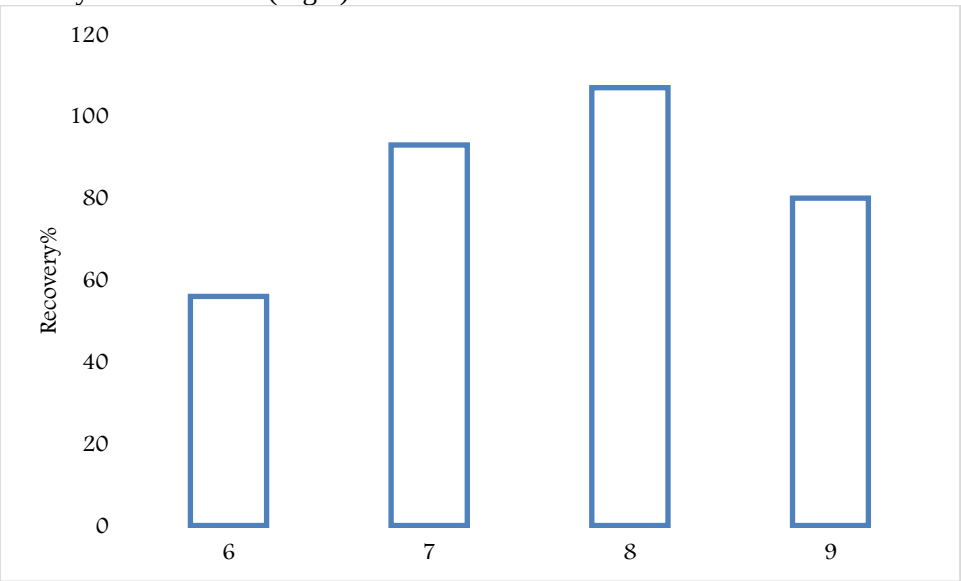

Figure 3. Influence of pH on peroxidase recovery. Salt concentration was set at 20%, salt used was sodium potassium tartarate, alcohol/crude extract ratio at 1, and alcohol used was t-butanol.

Effect of Alcohol type

We also tested 1-butanol versus the standard alcohol used in TPP, T-butanol. Surprisingly, 1-butanol gave better findings than T-butanol, i.e. 96% versus 86% of activity recovery, respectively as shown in Fig 4.

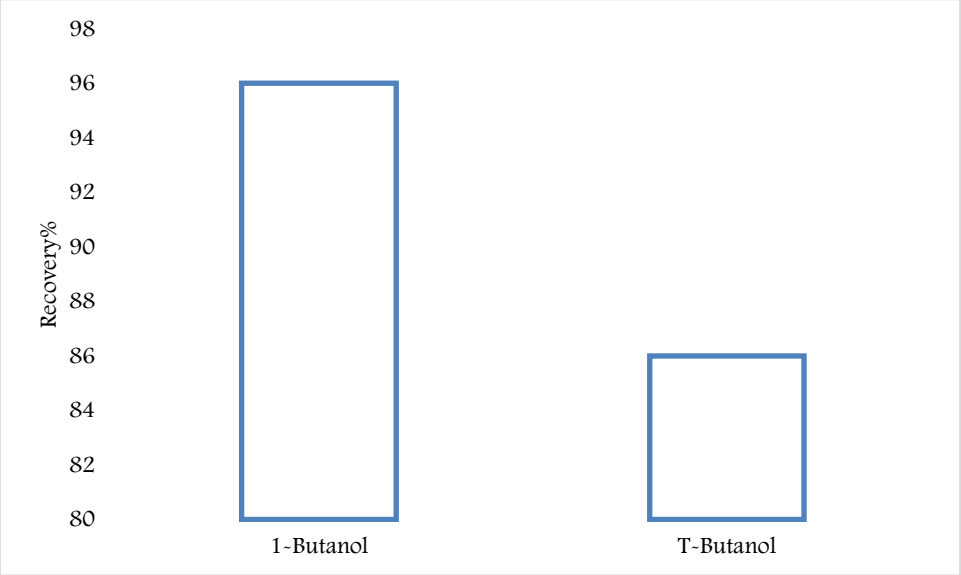

Figure 4. Influence of type of alcohol used on enzyme recovery. Salt used was sodium potassium tartarate, salt concentration was set at 20%, pH at 7, alcohol/crude extract ratio at 1.

Effect of alcohol/crude extract ratio

Finally, the last parameter to be optimized was the ration between alcohol and crude extract in terms of volumes on the fractionation. Sharp increase was observed in the ration 2, i.e. when the volume of alcohol was the double amount of crude extract, the fractionation was maximal (74%). However, below this ratio, less than half of activity recovery was observed in the bottom phase (Fig 5).

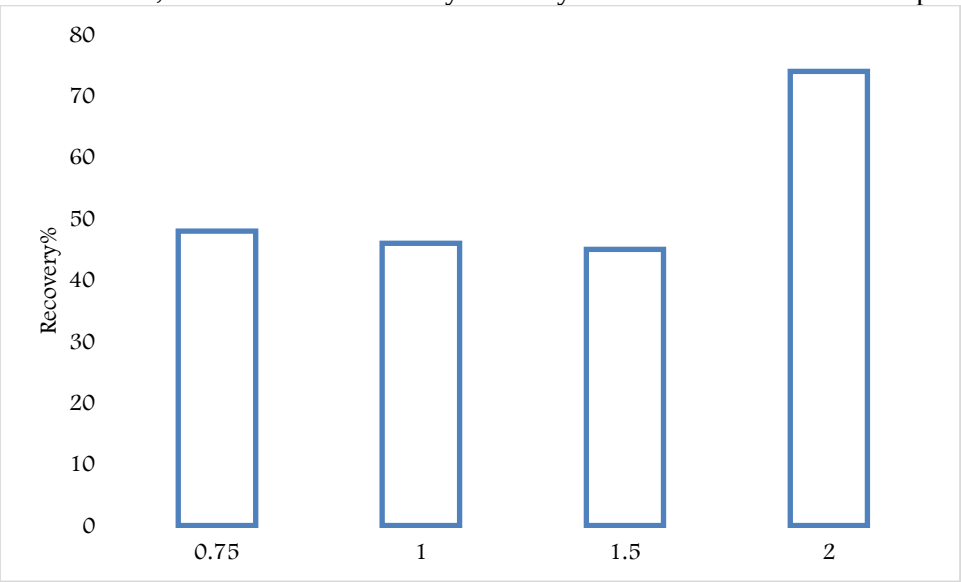

Figure 5. Effect of alcohol/crude extract ratio on peroxidase recovery. Salt used was sodium potassium tartarate, salt concentration was set at 20%, and pH at 7.

Upon combining the optimized parameters, namely, using sodium potassium tartarate as the salt source, with a concentration 17%, at pH 8, alcohol to crude extract ratio of 2 and 1-butanol as the alcohol source, approximately 116% of the separated enzyme activity was obtained in the bottom phase while other proteins and phenolic compounds were aggregated in the middle and upper layers respectively. This indicates that almost all of the enzyme was transferred into the lower layer

reflecting the high separation efficiency. In addition, the extracting salt (sodium potassium tartarate) had a slight activating action of peroxidase activity which account for the extra in the percentage over 100%. TPP was previously utilized to isolate and purify peroxidase from different sources. Vetal and Rathod [9] upon applying TPP for peroxidase isolation from orange peels, the activity recovery of the enzyme obtained was 93% after 180 mins. Karakus et al. [8] purified peroxidase enzyme from *Amsonia orientalis* plant using TPP. The activity recovery was after 30 mins 162%. Moreover, TPP can be coupled with ultrasonication to further reduce the time of fractionation. This approach was employed to purify peroxidase enzyme from orange peels with activity recovery 91% within 6 mins [10]. Nonetheless, this technique had a negative effect on the activity recovery of our study (diminished to 76%).

4. Conclusion

This study demonstrates the explitation of TPP to isolate peroxidase enzyme from a waste material (watermelon rinds). Successive optimizations were performed so as to harvest the highest activity recovery of peroxidase. Salt that gave maximal recovery was sodium potassium tartarate, optimal salt concentration was 17%, optimal pH was 8, optimal alcohol/crude extract ratio was 2 and 1-butanol was preferred to t-butanol. Indeed, upon combining all optimized factors, activity recovery of 116% was obtained reflecting the high yield of enzyme isolation as well as the feasibility and efficiency of the TPP as a method of isolation. We recommend to purify peroxidase from other waste materials using the same approach.

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