Spectrophotometric Determination of Carbaryl Residues in Vegetables Using Crude Peroxidase from Wheat Bran

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Abstract

A spectrophotometric method for the determination of carbaryl based on the catalytic reaction of peroxidase enzyme is presented. The method involves the alkaline hydrolysis of carbaryl into 1-naphthol followed by enzymatic reaction with peroxidase in the presence of 4-aminoantipyrine and hydrogen peroxide to yield a red-coloured product which is measured at 502 nm. Wheat bran was exploited as a source of peroxidase in this study and the enzyme was extracted by a simple method without any purification. Parameters influencing the enzyme assay activity such as concentrations of NaOH, hydrogen peroxide, 4-aminoantipyrine, pH and incubation time were investigated and optimized. Under optimized conditions, absorbance of the system was found to be proportional to carbaryl concentration in the range of 0.5– 2.5 mg L^{-1} *with* R^2 *value of 0.987. Limit of detection (LOD) and limit of quantitation (LOQ) for the system were 0.22 mg* L^{-1} *and 0.67 mg* L^{-1} *respectively. Application of the method to the determination of carbaryl residues in spiked vegetable samples revealed good recoveries ranging from 86 - 118 %. Relative standard deviation (RSD) for intraday measurements (n=7) were 4.25 % and 2.72 % for 0.5 and 1 mg/L concentration respectively. Overall, the investigated method showed good sensitivity and selectivity for the quantitative analysis of residual carbaryl.*

Keywords: *Carbaryl, Peroxidase, 1-naphtol, Enzyme, Spectrphotometry*

1.0 INTRODUCTION

Carbaryl (1-naphthyl methylcarbamate) is a broad spectrum insecticide widely used for the eradication of agricultural pests. The insecticide is highly effective against numerous insect pests that affect fruits, vegetables and many other crops. Large scale and improper use of this pesticide leads to residual concentrations in food products and consequently ingested by humans. Carbaryl and its major hydrolysis metabolite (1-Naphtol) are known to be toxic to human health causing nausea, bronchoconstriction, blurred vision, excessive salivation, muscle twitching, cyanosis, convulsion, coma, respiratory failure, and cancers (Vichapong *et al.*, 2011). The toxic effects of carbaryl are mostly linked to inhibition of the key enzyme acetylcholinesterase (AChE) which controls neurotransmitters. Carbaryl is also known to degrade readily to 1-naphthol, which is several times more toxic than its precursor.

Various analytical techniques have been reported for the determination of carbaryl and its metabolites in different sample types. The major methods used are chromatography (Zhan *et al.,* 2003), spectrophotometry (Kumar *et al.,* 2005; Portela *et al.,* 2003), Immunoassay (Marco *et al.,* 1995; Wang *et al.,* 2005), fluorescence spectroscopy (Jia *et al.,* 2007), titrimetry (Sharma *et al.,* 2003) colorimetry (Lee *et al.,* 2018) and biosensors (Mashuni *et al.,* 2022; Liu et al., 2016). Most of the reported techniques require expensive instrumentation, complex sample preparation, purification procedures and skilled personnel. Simple methods are desirable for routine analysis of a large number of samples. In this respect, enzyme catalytic methods are gaining great attention as they offer rapid determination of a wide range of substances. Enzymatic methods have considerable advantages over the traditional analytical procedures because of their general low cost and rapidity, and because they do not require a preliminary purification of the substrates to be analyzed. The high specificity of enzymes enables the analysis of substrates in complex sample matrixes without complicated sample preparation techniques. This makes enzymatic analysis a highly valuable tool because it saves time, reduces costs and gives reliable results independent of the sample matrix. Additionally, enzymatic methods use non-hazardous reagents and thus are environmentally friendly.

Analysis of carbaryl have been reported using different enzymes such as acetylcholinesterase (Caetano and Machado, 2008, Liu *et al.,* 2016), choline oxidase (Pires *et al.,* 2007) peroxidase (Supharoek *et al.,* 2019, Didpinrum *et al.,* 2019) among others. The reported procedures involve either the catalytic action of the enzymes on certain reactions with carbaryl or indirect methods which involve measuring the inhibitory action of carbaryl on certain enzymes. The methods were shown to be highly efficient for the determination of carbaryl with many advantages in terms of high sensitivity, medium to high selectivity, high precision and high accuracy.

In the present work, crude peroxidase enzyme from wheat bran is being exploited for the determination of carbaryl. Wheat bran has been previously shown to be a rich source of peroxidase enzyme (Hamid *et al.,* 2015). The advantage of utilizing non-purified enzyme is that the natural environment for the enzymes prevents conformational changes in the protein structure that would lead to loss of activity in non-conventional medium. Also, the use of crude enzymes reduces analysis time through the minimum process involved in obtaining the enzyme. Studies employing peroxidase from rubber tree bark and jack fruit latex for the determination of carbaryl have been reported (Supharoek *et al.,* 2019; Didpinrum *et al.,* 2019). The enzyme is used for catalytic reaction of 1-naphthol (hydrolysis product of carbaryl) with 4-aminoantipyrine in the presence of hydrogen peroxide. The reaction gives a product which is monitored spectrophotometrically and related to concentration of the analyte (carbaryl). This is the first report utilizing peroxidase enzyme from wheat for the determination of cabaryl residues.

2.0 Materials and Methods

2.1 Materials

All chemicals and reagents used were of analytical grade. Working carbaryl standard solution was prepared by diluting the 1000 mg L^{-1} carbaryl (Sigma Aldrich) with deionized water. Working solutions of hydrogen peroxide were prepared by diluting 30% hydrogen peroxide (Flinn Scientific) in deionized water. Stock Solution of sodium hydroxide (0.1 mol L^{-1}) was prepared by dissolving 0.2 g NaOH (Sigma Aldrich) in 50 mL of deionized water and working

solutions were prepared by serial dilution of the 0.1 mol L^{-1} stock solution. Stock solution of 4-aminoantipyrine (1000 mg L^{-1}) was prepared by dissolving 0.1 g of 4-aminoantipyrine (Sigma-Aldrich) in 100 mL of deionized water and working solutions were prepared by diluting the stock in appropriate volumes of deionized water. Phosphate buffer (50 mmol L^{-1}, pH 6.0) was prepared from monosodium dihydrogen phosphate and disodium hydrogen phosphate (both from BDH chemicals). Wheat grains were purchased from a local market in Wukari, Nigeria and dehulled to obtain the bran. Vegetable samples (Spinach, cabbage and lettuce) were also purchased from local markets.

2.2 Extraction of Peroxidase Enzyme from Wheat Bran

Peroxidase was extracted from wheat bran according to the following procedure; Ten (10) grams of pulverized wheat bran was added into 50 ml of phosphate buffer (50 mmol L^{-1} pH 6.0) and homogenized thoroughly. The homogenate was filtered through four layers of cheese cloth and then centrifuged at 4000 rpm for 30 minutes using Centurion centrifuge (C2041). The clear supernatant was collected into amber-colored bottles (as crude peroxidase extract) and stored at 4 °C until required.

2.3 Determination of Peroxidase Enzyme Activity

Peroxidase activity was evaluated according to the method described by Magomya *et al.*, (2021) with slight modification. The assay was based on the quantification of tetraguaiacol formation in a 5 -mL reaction mixture containing 2.5 ml of 50 mM phosphate buffer (pH of 7.0) 1 ml of 0.5 mM 2-methoxyphenol (Guaiacol), 1mL of 2 mM H_2O_2 and 0.5 ml of enzyme extract. The absorbance of the assay mixture was taken against a blank at 5 minutes on a Uv-Vis spectrophotometer (T60) at 470nm. One unit (U) of enzyme activity is the amount of that causes the oxidation of 1 µmol of guaiacol per minute under the stated experimental conditions.

2.4 Peroxidase Assay for Determination of Carbaryl

For the measurement of carbaryl via peroxidase enzymatic reaction, the method of Didpinrum *et al.,* (2019) was used with slight modification; the reaction was initiated by mixing 0.5 mL carbaryl standard or sample with 5 mmol L^{-1} NaOH for 5 min to hydrolyse carbaryl to 1-naphthol. Then, 100 mg L^{-1} 4- aminoantipyrine and 0.2 mmol L^{-1} hydrogen peroxide were added followed by 0.5 mL of crude peroxidase extract from wheat bran as a catalyst. Phosphate buffer (50 mmol L^{-1}, pH 6.0) was used to adjust the volume to 10 mL and the solution was left at ambient temperature (approximately 35 °C) until a red-coloured product was obtained. Absorbance was taken at a wavelength of 502 nm on a UV-Vis spectrophotometer (T60 U)

2.5 Optimization of Experimental conditions for Carbaryl Detection

Effect of various influencing parameters such as pH, concentration of sodium hydroxide, concentration of H_2O_2, concentration of 4-aminoantipyrine and incubation time were investigated and optimized using a univariate experimental method. The effect of pH was optimized between 4 and 7. Concentrations of sodium hydroxide for carbaryl hydrolysis were investigated in the range of 1–20 mmol L^{-1}. Effect H_2O_2 concentrations in the range of 0.05–0.50 mmol L^{-1} was studied. Concentrations of 4-aminoantipyrine from 50–500 mg L^{-1} were examined and reaction time of peroxidase was evaluated between 1 – 30 min.

2.6 Calibration curve for Determination of Carbaryl

Working standard solutions of carbaryl (0.5 – 5.0 mg/L) were prepared by diluting the 1000 mg L^{-1} stock solution with deionized water. The standards were subjected to enzymatic assay as described in 2.4. A calibration graph of absorbance against concentration of standards was plotted for the estimation of carbaryl concentration.

2.7 Determination of Carbaryl in Vegetable Samples

Three different vegetable samples were analysed for carbaryl using the proposed method. Preparation of the vegetable samples was done by means of a modified QuEChERS method. The samples were cut into small pieces and then mixed homogeneously using a cooking blender. A 10 g portion of sample was weighed and transferred into a 50 mL centrifuge tube, 10 mL of acetonitrile was added, and sample and solvent were homogenized for 10 min. Magnesium sulfate (4g) and sodium chloride (1g) were added and the sample was further homogenized and then centrifuged at 4000 rpm for 5 min. The supernatant was filtered through Whatman No.1 filter paper and the solution was adjusted to 10 mL with acetonitrile. Subsequently, 3 mL of the solution was pipetted into a centrifuge tube with 20 mg of charcoal activated powder and vortexed for 1 min followed by centrifugation to remove the charcoal. The sample solution was then filtered through a 0.45 μm syringe filter.

The sample extracts were subjected to peroxidase enzymatic reaction as described in 2.4. Standard addition method was employed in the anlaysis and concentration of carbaryl was estimated from the standard calibration curve.

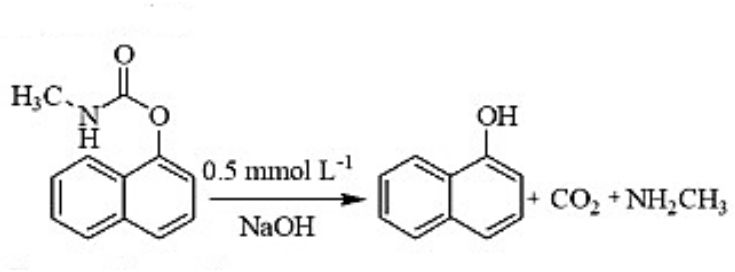
2.8 Method Validation

The method was validated in accordance with ICH guidelines (ICH Harmonised Tripartite Guideline 2005). The parameters validated were linearity, sensitivity (LOQ and LOD), accuracy and precision. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method. Limit of detection (LOD and limit of quantitation (LOQ) were determined by analysing different solutions of carbaryl and measuring the signal-to-noise ratio; LOD = 3.3σ / S, and LOQ = 10σ / S. where σ is the standard deviation of the response and S is the slope of the calibration curve. The accuracy of the method was determined by recovery studies at two concentration levels (0.5 and 1 mg/L). The precision (repeatability) of the method was determined by seven measurements of a sample on the same day.

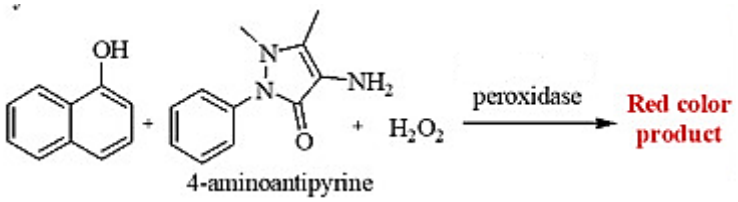
3.0 Results and Discussion

The spectrophotometric method for determination of carbaryl presented in this study is based on the peroxidase catalysed reaction of 1-naphthol as hydrolysis product of carbaryl. Carbaryl is first hydrolysed to 1-Naphtol by NaOH and then reacted with 4-aminoantipyrine in the presence of hydrogen peroxide and peroxidase according to the following equations:

Step I: Hydrolysis of carbaryl to 1-Naphtol



Step II: Enzymatic Reaction



The reaction leads to a red coloured product which absorbs maximally at a wavelength of 502 nm. Without peroxidase enzyme or any other catalyst, the reaction usually takes a very long time or does not occur at all; the presence of peroxidase accelerates the rate of the reaction by reducing the activation energy (Robinson, 2015). A linear relationship was established between absorbance and carbaryl concentration and this forms the basis for the use of the reaction in quantitative estimation of carbaryl.

The peroxidase employed in this study was extracted from wheat bran and its characterization revealed an enzyme activity of 57 ± 1.79 U/mL. Comparison of this activity with peroxidases from other plant sources revealed that peroxidase from *Ipomea batata* (sweet potato) had higher activity (130 U/mL) than our current source (Magomya *et al.,* 2021). The value recorded in this study was however higher than the peroxidase activities reported for *Beta vulgaris L.* (17.69±4.34 U/mL), *Cucumis sativus L.* (50.90±3.29 U/mL) and *Raphanus sativus L.* (17.17±4.20 U/mL) (Ulfat *et al.,* 2012). Lower peroxidase activity (13.178 U /mL) than our current study was also reported for crude peroxidase from Broccoli (Ahmad *et al.,* 2019).

Experimental conditions for the analytical reaction employed in this study were investigated and optimized. NaOH plays the role of hydrolysing carbaryl to 1-naphthol which is subsequently reacted under peroxidase catalysis to yield a measureable product. Effect of NaOH concentration was studied within 1 – 20 mmol L^{-1} concentration range. Our findings (presented in Fig. 1) showed increase in assay absorbance as NaOH concentration was increased from 1 to 5 mmol L^{-1}. Increase in concentration from 5 to 10 mmol L^{-1} did not cause any significant change in absorbance. However, higher concentrations of 15 and 20 mmol L^{-1} lead to decreased analytical signal. The decreased signal at higher NaOH concentration could be due to conformational changes or enzyme denaturation.

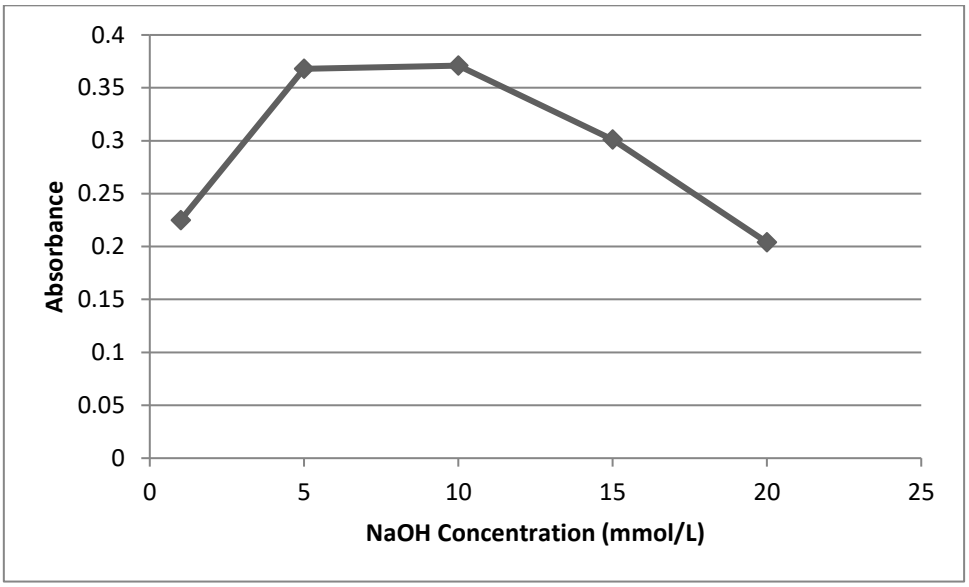


Figure 1: Optimization of NaOH concentration

The effect of pH on the peroxidase-catalysed reaction was studied in the range 4.0 – 7.0. Our findings (Figure 2) Indicate that maximum assay activity was obtained at a pH of 5.5 and it remained relatively stable up to a pH of 6. After this a steady decline in signal was observed with increase in pH. Enzymes are proteins therefore; they are sensitive to changes in the hydrogen ion concentration. They may be denatured by extreme levels of hydrogen ions (whether high or low) as this alters the degree of ionization of an enzyme's acidic and basic side groups as well as the substrate components. The active sites of enzymes must be in the proper ionic form in order to maintain the conformation for substrate binding and reaction catalysis. In general, optimum pHs for activity of most peroxidases usually range between 4 and 7 (Nicolas *et al.*, 2003). The value we recorded in this study falls within this range.

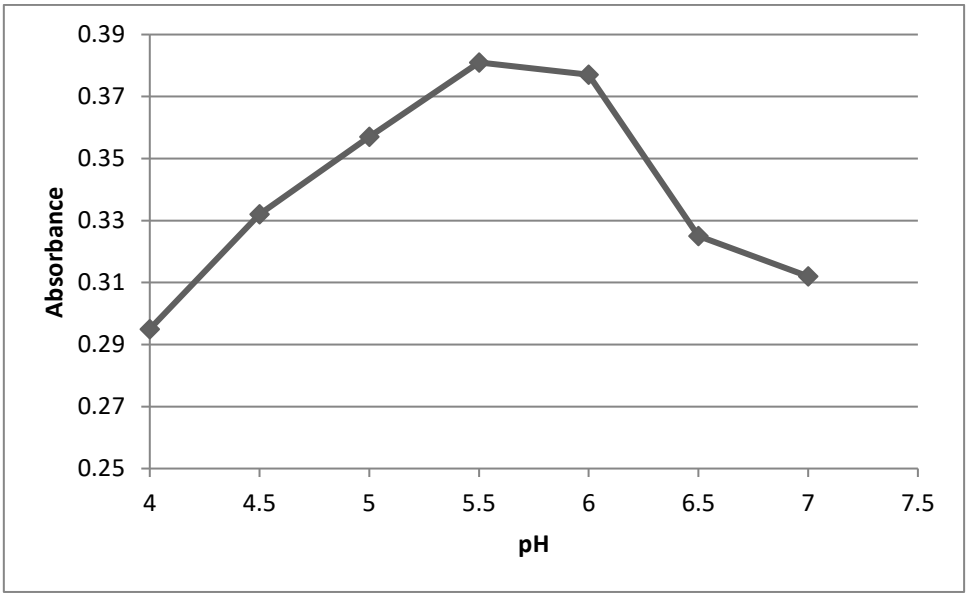


Figure 2: Optimization of pH

Figure 3 presents the effect of H_2O_2 on the reaction assay which was studied within 0.1 – 0.5 mmol/L concentration range. A sharp increase in analytical signal was observed from 0.1-0.2 mmol/L and optimum signal was recorded at 0.3 mmol/L. After this concentration, no significant change in activity was observed. Our finding here is in agreement with the mechanisms for enzyme-substrate reactions. Many enzymes are known to exhibit a course of reaction rate that represents a rectangular hyperbolic curve; in the presence of a given amount of enzyme, the rate of an enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached. At this point, further increase in the substrate concentration produces no significant change in the reaction rate because all enzyme molecules are saturated with substrate hence the excess substrate molecules will cause no further effect.

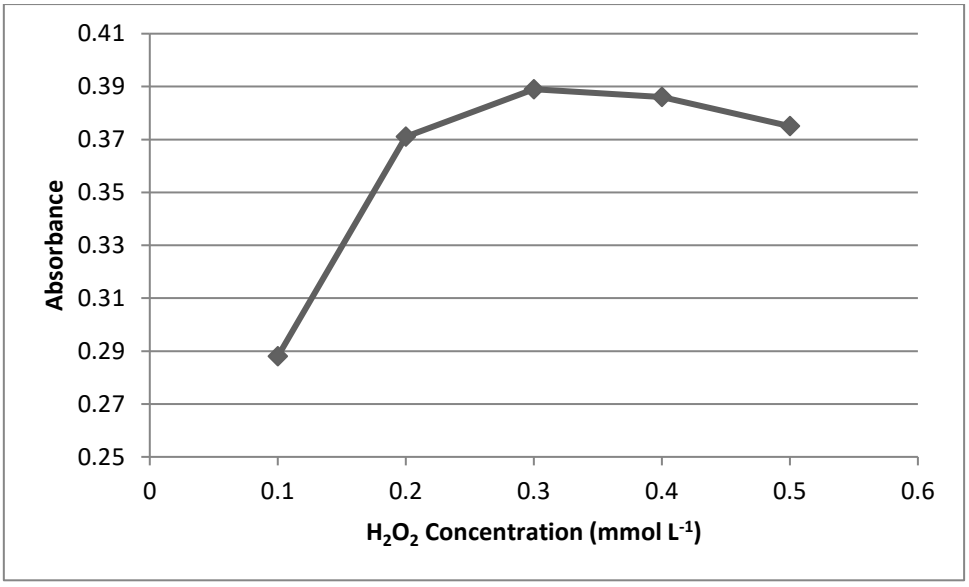


Figure 3: Optimization of H_2O_2 concentration

The aromatic substrate 4-aminoantipyrine acts as the hydrogen donor in the peroxidase enzymatic reaction for determination of carbaryl. In this study, the effect of 4-aminoantipyrine concentration was tested in the range of 25–200 mg L^{-1} with a view to establishing the optimal concentration for the analytical system. Results obtained (Figure 4) showed that there was steady increase in absorbance with increase in 4-aminoantipyrine concentration up to 100 mg L^{-1}. Thereafter, the system absorbance remained relatively constant. Our finding here agrees with previous reports by Didpinrum *et al.* (2019) and Supharoek *et al.* (2019); both studies established 100 mg L^{-1} as optimum concentration of 4-aminoantipyrine for the analytical reaction under consideration.

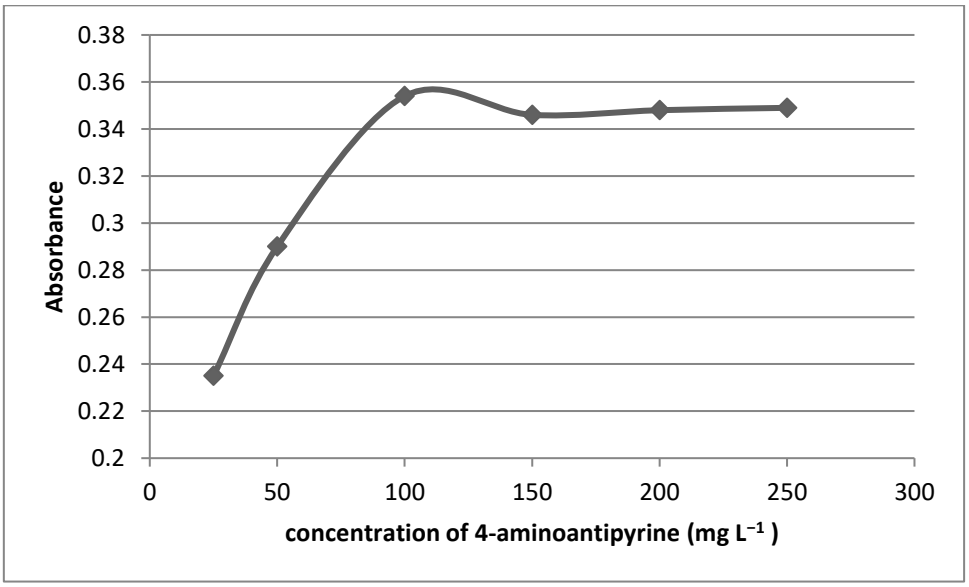


Figure 4: Optimization of 4-aminoantipyrine concentration

The influence of time on the rate of reaction was studied at two minute intervals from 0 to 20 minutes. Our results showed that increasing reaction time resulted in increased absorbance from 1 – 12 minutes. With longer incubation time the assay absorbance remained stable up to 16 minutes and thereafter it began to decline. The longer an enzyme is incubated with its substrate, the greater the amount of product that will be formed. However, the rate of formation of product is not a simple linear function of the time of incubation. All proteins suffer denaturation, and hence loss of catalytic activity, with time. Some enzymes, especially in partially purified preparations, may be noticeably unstable, losing a significant amount of activity over the period of incubation. This explains the observed trend in the current study presented in Figure 5. A general rule in selecting incubation time is that it should be long enough to permit a moderate amount of product to be formed, but not so long that there is detectable levelling off of the curve. Incubation time of 10 minutes was selected for this study.

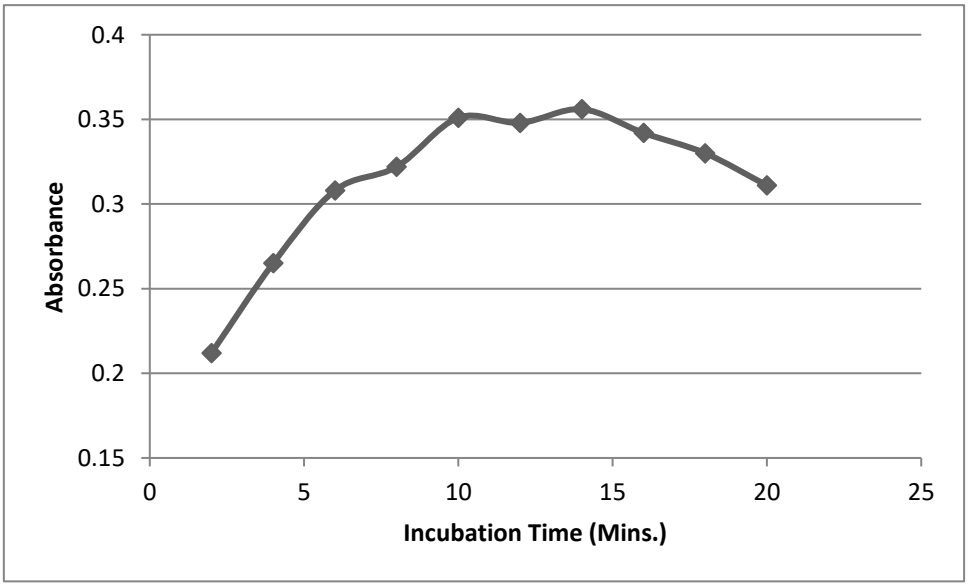


Figure 5: Optimization of Incubation Time

Using optimized conditions, calibration curve for determination of carbaryl was constructed by plotting absorbance against concentration of carbaryl standards. Linear relationship was observed in the range 0.5–2.5 mg L^{-1} according to the linear equation y= 0.0810x + 0.2733 (R^2 0.987). Limit of detection was determined to be 0.22 mg/L while limit of quantification was 0.67 mg/L. In order to evaluate the applicability and feasibility of the proposed method, determinations of carbaryl residues were carried out in real vegetable samples. The samples (cabbage, spinach and lettuce) were prepared by means of a modified QuEChERS method and spiked at two levels (0.5 and 1.0 mg/L). The results of the analysis revealed percentage recovery in the range of 86 to 118% with RSD values of 1.76 – 4.67 %. The values obtained here indicate the presence of carbaryl residues in cabbage and lettuce but no residues detected in spinach. Intraday repeatability (precision) which was evaluated in terms of relative standard deviations (RSDs) for carbaryl concentration of 0.5 and 1 mg L^{-1} (n=7) were 4.25 % and 2.72 %. The findings here show good method accuracy and precision.

Table 1: Determination of Carbaryl in Vegetable Samples

Vegetable Samples	Added (mg L^{-1})	Recovered (mg L^{-1})	Recovery (%)	%RSD (n =3)
Cabbage	0.5	0.56	112	3.34
	1.0	1.18	120	2.05
Spinach	0.5	0.46	92	1.76
	1.0	0.86	86	4.67
Lettuce	0.5	0.54	108	3.48
	1.0	1.05	105	2.08

4.0 Conclusion

This study has shown the feasibility of an enzyme-based method for determination of carbaryl pesticide using peroxidase extract from wheat bran. Our findings showed that the enzyme-based system is simple and could rapidly determine the analyte with good linear detection range and reproducibility. The use of a crude enzyme from plant source and a simple spectrophotometry equipment is a low-cost approach hence the method can be easily applied by low resource laboratories. The approach is also a green concept which minimizes the use of reagents. Overall, the presented method appears to be a reliable alternative for the determination of carbaryl in vegetable samples and can be extended to environmental samples such as water and soil.

REFERENCES

Ahmad, Q., Mehmood, A., Saeed, Z., & Fayyaz, M., (2019). Purification and Characterization of Peroxidase from Broccoli (*Brassica oleracea l. Var. Italica*) Stems. *Electronic J Biol,* 15 (1):38-45

Caetano,J., Sergio A.S. & Machado, (2008). Determination of carbaryl in tomato “in natura” using an amperometric biosensor based on the inhibition of acetylcholinesterase activity, *Sensors and Actuators B: Chemical,* 129(1): 40-46

Didpinrum, P., Ponhong, K., Siriangkhawut, W., Supharoek, S. & Grudpan, K. (2019). A cost-effective spectrophotometric method based on enzymatic analysis of jackfruit

latex peroxidase for the determination of carbaryl and its metabolite 1-napthol residues in organic and chemical-free vegetables. *Food Analytical Methods*, 13(2)

Hamid, M., Majeed, H. & Al-Assie, A.H (2015). Purification and characterization of peroxidase extracted from wheat bran. *World Journal of Pharmaceutical Research* 5(01): 77-86.

ICH Harmonised Tripartite Guideline (2005). Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, 1-13

Jia, G., Li, L., Qiu, J., Wang, X., Zhu, W., Sun, Y. & Zhou, Z. (2007). Determination of carbaryl and its metabolite 1-naphthol in water samples by fluorescence spectrophotometer after anionic surfactant micelle-mediated extraction with sodium dodecylsulfate. *Spectrochim. Acta Part A* 67: 460–464

Kumar, K.S., K. Suvardhan, K. & Chiranjeevi, P. (2005). Preparation of reagents for the sensitive spectrophotometric determination of carbaryl in environmental samples," *Analytical Letters* 38(4): 697–709

Lee, M., Patil,V., Na, Y., Lee, D.S., Sung H. Lim, S.H., Gi-Ra Yi, G.R (2018). Highly stable, rapid colorimetric detection of carbaryl pesticides by azo coupling reaction with chemical pre-treatment, *Sensors and Actuators B: Chemical*, 261: 489-496,

Liu, Y., Liu, K., Hongming, D., Zhang, L., Deng, Yan., Ma, Chao. & Wang, Z., (2016). Acetylcholinesterase biosensor for carbaryl detection based on nano-porous pseudo carbon paste electrode. *Nanoscience and Nanotechnology Letters*, 8(9):785-790

Magomya, A.M., Okpaegbe, U.C., Yebpella, G.G., Hikon, B.N & Y. Joshua, Y. (2021). Development of a Peroxidase-based assay for assessment of heavy metals in aqueous samples *Elixir Applied Chemistry*, 160 :55724-55729

Marco, M.P., Chiron, S., Gascón, J., Hammock, B.D. & Barceló, D. (1995). Validation of two immunoassay methods for environmental monitoring of carbaryl and 1-naphthol in ground water samples, *Analytica Chimica Acta*, 311 (3): 319–329

Mashuni,M., Ritonga, H.,. Jahiding, M., Rubak, B., & Fitri Handayani Hamid, F.H (2022). *Chemosensors*, 10 (4): 138

Nicolas,J., Billaud, C., Philippon, J. & Rouet-Mayer, M.A. (2003). BROWNING | Enzymatic – Biochemical Aspects, Benjamin Caballero, Encyclopedia of Food Sciences and Nutrition (Second Edition), Academic Press, 678-686,

Zhan, Gu¨ L O¨., Topuz, S & Alpertunga, B. (2003). A Simple Method for the Determination of Carbaryl and 1-Naphthol in Fruit Juices by High-Performance Liquid Chromatography–Diode-Array spectrophotometry. *Journal of Food Protection 66 (8):* 1510–1513

Pires, C.K., Morales-RubioII, A., Miguel de la GuardiaII., Limall, José L. F. C. & Elias A. G., ZagattoI, E.A.G, ReisI, B.F (2007). A chemiluminescence flow-based procedure for determination of carbaryl in natural waters exploiting multicommutation and enzymatic reaction. *J. Braz. Chem. Soc.* 18 (3): 519 - 525

Portela, D.C., Pereira, I.M.F., Paíga, P., Delerue-Matos, C. & Vaz, M. C. V. F. (2003). Amperometric and spectrophotometric determination of carbaryl in natural waters and commercial formulations," *Analytical and Bioanalytical Chemistry*, 377 (2): 356–361

Robinson, P.K. (2015). Enzymes: principles and biotechnological applications. *Essays Biochem.* 59:1-41.

Sharma, D.K., Kumar, R., Kumar, S. & Verma, B.C. (2003). A simple titrimetric method for the determination of carbaryl, carbofuran and propoxur in insecticide formulations and in admixtures. *National Academy Science Letters* 26(3-4):85-89

Supharoek, S.A, Ponhong, K., Siriangkhawut, W. & Grudpan, K. (2019). A new method for spectrophotometric determination of carbaryl based on rubber tree bark peroxidase enzymatic reaction. *Microchem J.* 144: 56–63.

Ulfat, M., Fazal, S., Javed, M.M & Khan, Z.U (2012). Evaluation Of Peroxidase Activity In *Beta Vulgaris L.*, *Cucumis Sativus L.* and *Raphanus Sativus L. Pak. J. Bot.*, 44: 91-96

Vichapong, J., Burakham, R., Srijaranai, S. & Grudpan, K. (2011). Room temperature imidazolium ionic liquid: A solvent for extraction of carbamates prior to liquid chromatographic analysis, *Talanta* (5):1253-1258

Wang, S., Chundi Yu, C. & Wang, J. (2005). Enzyme immunoassay for the determination of carbaryl residues in agricultural products, *Food Additives & Contaminants,* 22(8): 735-742