

New spectrophotometric assay for assessments of catalase activity in biological samples

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ARTICLE INFO

Keywords:

H₂O₂
Catalase activity
Ammonium metavanadate
Sulfuric acid
Dichromate method

ABSTRACT

A novel, simple, and accurate colorimetric assay was established for assessments of catalase activity in biological fluids and tissues. H₂O₂ dissociation rates are directly proportional to catalase activity, and the principle of the present assay is based on reactions of ammonium metavanadate with H₂O₂ under acidic conditions. The resulting reduction of vanadium (V) to vanadium (III) produces a red–orange peroxovanadium complex with absorbance maxima at 452 nm. Biological samples containing catalase were incubated with 50-mM phosphate buffer solution containing 10-mM H₂O₂ as a substrate for two min. Subsequently, ammonium metavanadate in sulfuric acid was used as an indicator reagent and was added to reaction mixtures to determine remaining H₂O₂ concentrations.

The precision of the present novel assay was indicated by coefficients of variation of 4.09% within runs and 2.56% between runs. Moreover, in experiments with homogenized red blood cell solutions, peroxovanadate and dichromate assays of catalase activities were highly correlated ($r = 0.993$). In further experiments, we demonstrated application of the peroxovanadate method to assessments of catalase activity in bacterial and liver homogenates. The present method is accurate, simple, rapid, and inexpensive and can be used for routine clinical measurements and scientific investigations.

Introduction

Glutathione peroxidase and catalase are key antioxidant enzymes that mitigate reactive oxygen species and free radicals, and protect lipids, DNA, and proteins from oxidative modification [1]. Although these enzymes compete as scavengers of H₂O₂, their relative contributions to H₂O₂ detoxification remain unclear [2]. Catalase is ubiquitously expressed and degrades H₂O₂ into oxygen and water [3], and protects against H₂O₂ that is produced by host immune cells to attack pathogens [4].

Numerous methods have been devised to assess catalase activity [5–20], and the most popular of these involves UV spectrophotometric determinations of H₂O₂ at 240 nm. However, because various proteins and DNA absorb UV light, this spectrophotometric method is not appropriate for assessments of catalase activity in protein containing biological solutions [8]. In addition, release of molecular oxygen gas from catalase reactions hampers spectrophotometric measurements, leading to low H₂O₂ sensitivity and failure to assess physiological levels of H₂O₂ (below 1.0 mM). Conversely, physiologically high substrate levels can inhibit catalase, further hampering assessments of activity

[8,9]. Other methods assess changes in intact H₂O₂ concentrations or oxygen release from the decay of substrate. Oxygen production can be measured accurately using low-flow gas meters [10] or oxygen electrodes [11]. Other applicable methods employ iodometry [12], titrimetry [13], chemiluminescence [14,15], polarimetry [16], and spectrophotometry [17,18]. Among these, recent assessments of catalase activity were performed using an iso-nicotinic acid hydrazide-pyrocatechol system to monitor catalytic consumption of H₂O₂ [19] according to the formation of a chromogenic complex with an absorption maxima at 490 nm. Another modern application was developed using a flow injection unit with an amperometric sensor that monitors unreacted H₂O₂ [20], but this method requires specific laboratory instruments.

Herein, we report a novel optimized spectrophotometric method for determining catalase activities in biological samples. To this end, we used a novel reagent to estimate catalase activities, and show the absence of interference from fats, amino acids, proteins, and sugars in biological samples.

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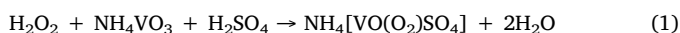
Materials and methods

Chemicals

All chemical reagents were purchased from commercial sources and were of analytical grade.

Principle

The principle of the method involves the reaction of ammonium metavanadate with H_2O_2 under acidic conditions, and depends on the reduction of vanadium (V) to vanadium (III) by H_2O_2 . Although H_2O_2 is considered a strong oxidant, it can act as a reductant under certain redox conditions. Accordingly, reduction of vanadium (V) leads to the formation of a red–orange peroxovanadium complex, which has a maximum absorbance at 452 nm [21,22]. The reaction between vanadium and H_2O_2 is shown in the following equation (1) [23]:



Catalase enzyme activity was determined by monitoring absorption of the red–orange peroxovanadium complex at 452 nm.

Reagents

Sulfuric acid solution (0.5 M) was prepared by appropriate dilution of concentrated sulfuric acid in 200 ml of distilled water. Ammonium metavanadate solution (0.01 M) contained 0.2925 g of ammonium metavanadate in 200 ml of 0.5-M sulfuric acid. Phosphate buffer (50 mM; pH 7.0) was prepared by mixing solutions a and b at a ratio of 1:1.5. Solution (a) was prepared by dissolving of 6.81 g of KH_2PO_4 in one liter of distilled water, and solution (b) was prepared by dissolving a 8.90 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in one liter of distilled water. Fresh H_2O_2 (10 mM) solutions were prepared by mixing 0.1134 ml of 30% H_2O_2 with 100 ml of phosphate buffer, and the solution was adjusted to 10-mM using the molar extinction coefficient of H_2O_2 at 240 nm ($43.6 \text{ M}^{-1} \text{ cm}^{-1}$). Catalase standard solution was prepared by dissolving 20 mg of catalase enzyme powder (HiMedia; Product code: TC037, New Delhi, India) in 100 ml of 50-mM phosphate buffer solution (pH 7.0). Catalase was diluted to 5 U mL^{-1} and final catalase activity was adjusted using the dichromate method as described by Sinha [20] and modified by Hadwan [26].

Blood samples

Erythrocyte lysates were prepared using 4-ml aliquots of whole blood from a researcher at the Advanced Biochemistry Laboratory (Chemistry Dept., College of Science, University of Babylon, Iraq) following peripheral venous puncture. Heparinized test tubes were used to prevent coagulation and whole blood samples were centrifuged at $400 \times g$ for 10 min. Subsequently, plasma and buffy coat were discarded after centrifugation and 500- μl aliquots of the resulting RBCs were washed three times in 5-ml aliquots of 0.9% NaCl solution and were centrifuged at $400 \times g$ for 10 min after each wash. Two-ml aliquots of ice cold double distilled water were then transferred into test tubes containing 500 μl of erythrocyte mixtures (five-fold dilution), and were vortexed for 5 s and incubated for 15 min at 4°C in the dark. Finally, the resulting 2.5 ml of five-fold, re-suspended stock hemolysates in phosphate buffer solution (0.05 M) were diluted to a dilution factor of 500. The resulting hemolysate solutions were used as a source of catalase activity.

Tissue preparations

Four-week-old broiler chickens were purchased from a local market at Hilla city, Iraq. Male albino mice and rats were purchased from the

Table 1
Procedure for assessments of catalase activity.

Reagents	Test	Standard	Blank
Sample	1000 μl	–	–
Distilled water	–	1000 μl	3000 μl
Hydrogen peroxide	2000 μl	2000 μl	–
After mixing, test tubes were incubated for 2 min at 37°C and the following reagent was added thereafter:			
Ammonium metavanadate reagent	2000 μl	2000 μl	2000 μl
After mixing, test tubes were incubated for 10 min at 25°C and absorbance was recorded at 452 nm against a reagent blank.			

central animal house at the College of Science, University of Babylon, Iraq. Immediately before measurements of tissue catalase activities, rats were sacrificed and kidney and liver tissues were surgically excised. Livers were immediately washed in 0.9% (w/v) NaCl solution to remove contaminating blood and were then homogenized using a glass homogenizer and ice cold 1.15% (w/v) potassium chloride solution. Homogenate solutions were then filtered and diluted (at a ratio of 1:500) with 0.05 M phosphate buffer for analyses of catalase activity, which were performed immediately.

Procedure

Enzyme activity procedure was elucidated in (Table 1). The rate constant (k) of the first-order reaction equation for catalase activity was calculated using the following formula:

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * \log \frac{S^0}{S} \quad (2)$$

where t is time, S^0 is the absorbance of the standard solution, and S is the absorbance of the sample.

Results and discussion

In the present method, the ammonium metavanadate/sulfuric acid reagent acted as a catalase reaction stopper by denaturing the protein. Subsequently, H_2O_2 molecules reacted with ammonium metavanadate to form the peroxovanadium complex (equation (1)) and wavelength scans from 200 to 700 nm revealed absorption maxima at 452 nm (Fig. 1A). Catalase activity is directly proportional to the rate of H_2O_2 catalysis, and decreases in absorbance of the reduced vanadium complex were proportional to catalase activity (Fig. 1B). Catalase standard solution was prepared by dissolving 20 mg of catalase enzyme powder (HiMedia; Product code: TC037, New Delhi, India) in 100 ml of 50-mM phosphate buffer solution (pH 7.0). Catalase was diluted to 8 U mL^{-1} and final catalase activity was adjusted using the dichromate method as described by Sinha [20] and modified by Hadwan [26].

The present observations of the colored peroxovanadium complex showed that it has high stability at room temperature. In agreement, Nogueira et al. [21], reported no significant changes in the absorbance of peroxovanadium at 450 nm for 180 h at 25°C .

Probable interferences of chemicals that could affect catalase activity measurements were assessed using the methods described by Hadwan and Abid [24]. Briefly, 9-ml solutions of various chemicals (Table 2) in 50 mM phosphate buffer (pH 7.4) were mixed with 1 ml catalase solutions of known activity (5 U/mL). Subsequently, catalase activity was determined using the dichromate method described by Sinha [20] and modified by Hadwan [25] and deviations from the final activity of 0.5 U mL^{-1} were recorded (Table 2).

Catalase activities in homogenized diluted red blood cell solutions were assessed using the present novel method and were compared with those determined using the dichromate method described by Sinha [20] and modified by Hadwan [25]. Matching samples and buffers were used

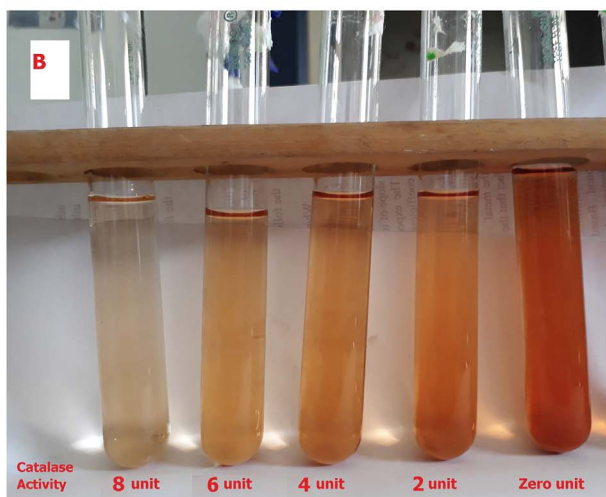
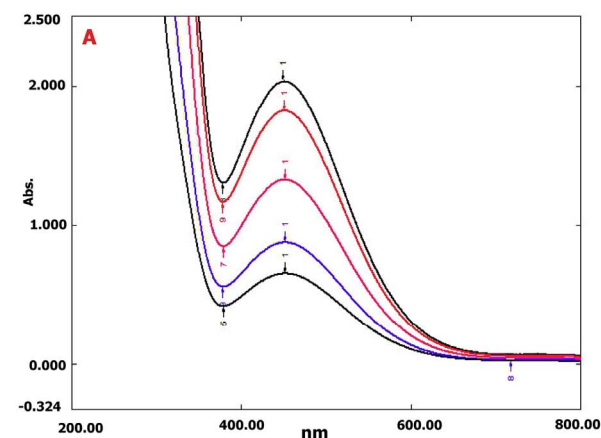


Fig. 1. (A). Absorption spectra of the colorimetric assay product; (B) absorbance decreases are proportional to increases in catalase activity.

Table 2

Effects of various chemicals on assessments of the catalase activity using the present novel assay.

Substance	Concentration of substance	Added catalase U mL ⁻¹	Found catalase U mL ⁻¹	Relative error (%)
Glucose	0.35 mg mL ⁻¹	0.5	0.503	-0.68
Ascorbic acid	20.0 μM	0.5	0.484	3.03
Albumin	0.5 mg mL ⁻¹	0.5	0.513	-2.649
Histidine	50 μM	0.5	0.493	1.36
Lysine	50 μM	0.5	0.509	-1.834
Arginine	50 μM	0.5	0.51	-2.04
Uric Acid	50 μM	0.5	0.484	3.03
Heparin	78.4 USP/10 mL	0.5	0.513	-2.649
EDTA	20.0 μM	0.5	0.512	-2.5

Table 3

Precision of the peroxovanadium method.

	No.	Mean (± SD): U mL ⁻¹	CV %
Within-run	20	2.26 ± 0.058	2.56%
Between-run	20	2.22 ± 0.091	4.09%

in both assays and the peroxovanadium complex assay showed good precision (as shown in Table 3). Moreover, data from peroxovanadium assays were strongly correlated with those from the dichromate assays (Table 4).

Table 4

Statistical comparisons of the dichromate method and the present peroxovanadium method for determining catalase activity (U mL⁻¹).

No. of Measurements	20
Mean of the present method	2.755
Mean of dichromate Method	2.762
Mean of both methods	2.758
Regression coefficient B	0.992
Regression coefficient A	0.008
Correlation coefficient	0.993

The accuracy of the present peroxovanadium method was assessed by determining recovery of added catalase enzyme activity (HiMedia; Product code: TC037, New Delhi, India). In these experiments, catalase was prepared in 0.05 mM phosphate buffer solution (pH 7) and was standardized using the dichromate method. Recovery of catalase activity was greater than 95% when the enzyme was added at 1–8 U mL⁻¹ and decreased to 87.33% in the presence of 10 U mL⁻¹ of enzyme (Table 5).

The conversion of H₂O₂ to H₂O and oxygen in the presence of catalase was monitored by determining the absorbance of the red–orange peroxovanadate end product over time (Fig. 2). The optimal time point for measurements was 120 s after addition of ammonium metavanadate, and absorbance plateaued thereafter, reflecting optimal catalase activity.

Catalase activity is inversely proportional to the absorbance of the formed vanadium complex. (Fig. 3) shows the regression straight line that obtained by plotting the absorbance reading of the formed vanadium complex at 452 nm against enzyme's substrate concentrations.

The sensitivity of the peroxovanadate method was assessed in the presence of varying dilutions of red blood cell homogenates by comparing measured and expected catalase activities (Fig. 4). The expected activities were determined using the peroxovanadate method; while, the found activities were adjusted using the dichromate method. Measured catalase activities in the presence of red blood cell homogenates were linearly correlated ($r = 0.9986$) with expected activities and the best fit linear curve passed through the origin.

Catalase activities of liver tissue homogenates from broiler chickens, male albino mice, and male albino rats were determined using the peroxovanadate method. Liver homogenates had expectedly high catalase activities (Fig. 5). In agreement, liver catalase activities were previously used to evaluate oxidative stress in broilers chickens [26–28]. Moreover, Ajuwon et al., 2010 used catalase enzyme assessments of liver broiler chickens to characterize the protective roles of ascorbic acid against copper-induced oxidative injury [27]. It is widely accepted that oxidative stress damages cells, tissues, and biomolecules, and the resulting reductions in antioxidant system efficiency, immunity, and growth rates of broiler chickens have been shown previously [28]. In addition, because catalase activity is considered a proxy for liver function, multiple previous studies show catalase activities in livers from albino mice and rats [29–31]. The present comparisons of peroxovanadate and dichromate methods (Fig. 5) with tissue homogenates showed compatibility of the methods.

To further assess potential applications of the present peroxovanadate method, we performed catalase assays with lysates of bacterial laboratory strains *Escherichia coli* and *Staphylococcus aureus*. These experiments showed similar catalase activities to those reported by Iwase et al. [32], who determined catalase activities in these bacterial strains using Triton X-100 to trap oxygen by-products in bubbles. In their study, oxygen generation was determined according to foam heights, which were calibrated using standard curves of bacterial catalase activity. In agreement with their results, our experiments (Fig. 6) demonstrate that *Staphylococcus aureus* has higher catalase activity than *Escherichia coli*.

The present peroxovanadate method has many advantages over

Table 5
Percentage recovery of catalase activity after addition to reaction solutions.

Catalase enzyme contents	Catalase enzyme activity added U mL ⁻¹	Catalase enzyme calculated activity U mL ⁻¹	Catalase enzyme observed activity ^a U mL ⁻¹	Recovery %
Enzymatic sample	–	–	5	–
Enzymatic sample + catalase enzyme added	1	6	5.87	97.84%
Enzymatic sample + catalase enzyme added	3	8	7.82	97.75%
Enzymatic sample + catalase enzyme added	5	10	9.77	97.7%
Enzymatic sample + catalase enzyme added	7	12	11.9	99.17%
Enzymatic sample + catalase enzyme added	8	13	12.71	97.77%
Enzymatic sample + catalase enzyme added	9	14	13.1	93.57%
Enzymatic sample + catalase enzyme added	10	15	13.1	87.33%

^a Mean of triplicate determinations.

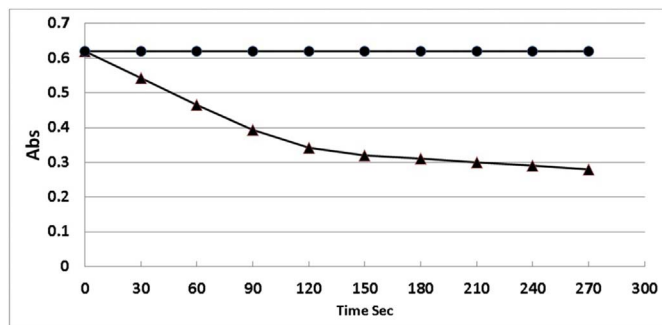


Fig. 2. Absorbance of the peroxovanadate complex over time in the presence of H₂O₂ alone (●) and H₂O₂ with 0.86-U mL⁻¹ catalase (▲).

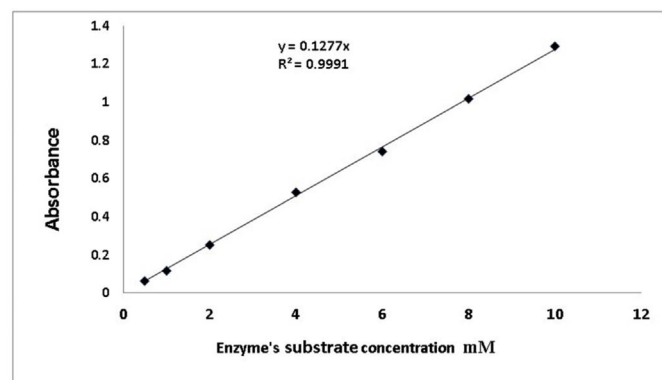


Fig. 3. The regression straight line that obtained by plotting the absorbance reading of the formed vanadium complex at 452 nm against a range of enzyme's substrate concentrations.

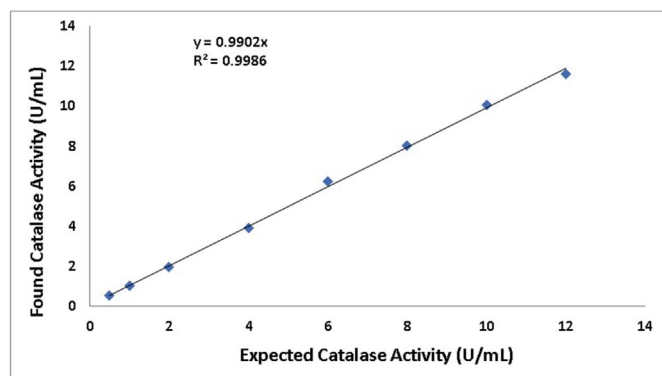


Fig. 4. Catalase activities of red blood cell homogenates were measured using the peroxovanadate method and were graphed against expected activities over a range of dilutions.

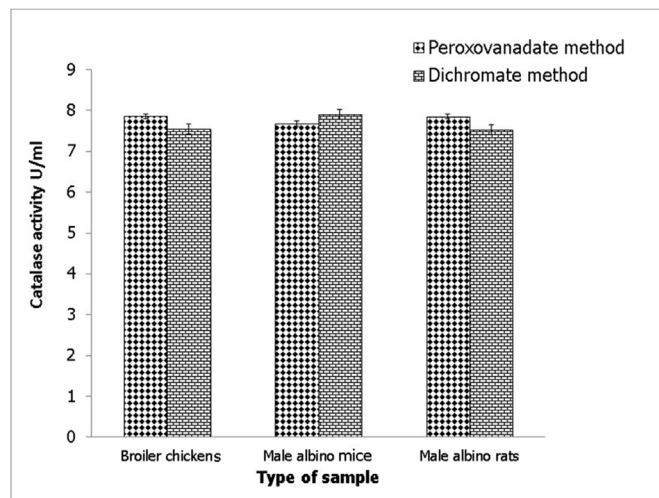


Fig. 5. Catalase activities of diluted tissue homogenates (1:500) were assessed using the peroxovanadate method and were compared with those determined using the dichromate method.

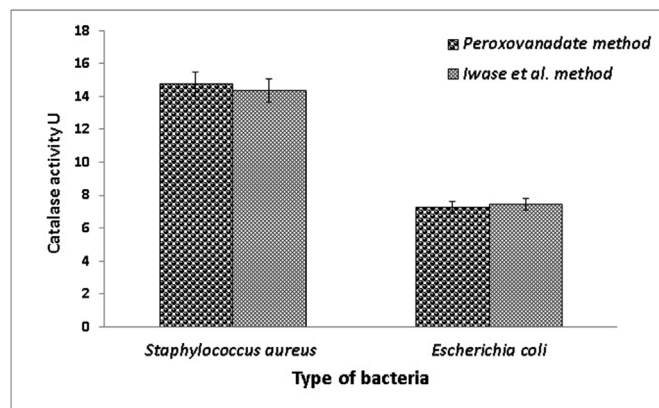


Fig. 6. Catalase activities of *Escherichia coli* and *Staphylococcus aureus* homogenates were measured using the peroxovanadate method and were compared with those determined using the dichromate methods described by Iwase et al.

previous methods for determining catalase activities in bacterial extracts. Among these, the time required for analyses is significantly decreased compared with that required for previous methods, allowing rapid analyses of large numbers of samples. In addition, compared with many previously developed methods, the present peroxovanadate method is inexpensive, does not require cumbersome procedures, and could be made available as assay kits [32].

In conclusion, we validated a simple method for assessments of catalase activity that show high precision and accuracy in the presence of high concentrations of several types of biomolecules, and at low

H₂O₂ concentrations. These data indicate that ammonium metavanadate in sulfuric acid is a sensitive reagent for measurements of hydrogen peroxide concentrations, and allows assessments of catalase activity at low substrate concentrations.

Competing financial interests

The author declares no competing financial interests.

Acknowledgements

We thank our colleagues in University of Babylon/College of Science for their technical supporting, comments and help regarding our study, especially Dr. Hussein O.M. Al-Dahmashi, Dr. Noor S. K. Al-Khafaji and Dr. Ala Tariq.

References

- [1] I. Ceballos-Picot, A. Nicole, M. Clement, J.M. Bourre, P.M. Sinet, Agerelated changes in antioxidant enzymes and lipid peroxidation in brains of control and transgenic mice overexpressing copper-zinc superoxide dismutase, *Mutat. Res.* 275 (3–6) (1992 Sep) 281–293.
- [2] A.D. Bolzan, M.S. Biachi, N.O. Bianchi, Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking, *Clin. Biochem.* 30 (1997) 449–454.
- [3] P.C. Loewen, J. Switala, B.L. Triggs-Raine, Catalases HPI and HPII in *Escherichia coli* are induced independently, *Arch. Biochem. Biophys.* 243 (1985) 144–149.
- [4] W.A. Day Jr., J.L. Sajecki, T.M. Pitts, L.A. Joens, Role of catalase in *Campylobacter jejuni* intracellular survival, *Infect. Immun.* 68 (2000) 6337–6345.
- [5] A.C. Chance, Maehly, Assay of catalases and peroxidases, *Methods Enzym.* 2 (1955) 764–775.
- [6] A.L. Deisseroth, Dounce, catalase: physical and chemical properties, mechanism of catalysis, and physiological role, *Physiol. Rev.* 50 (1970) 319–375.
- [7] L.M. Magalhaes, M.A. Segundo, S. Reis, J.L.F.C. Lima, Methodological aspects about in vitro evaluation of antioxidant properties, *Anal. Chim. Acta* 613 (2008) 1–19.
- [8] S. Mueller, H.-D. Riedel, W. Stremmel, Determination of catalase activity at physiological hydrogen peroxide concentrations, *Anal. Biochem.* 245 (1997) 55–60.
- [9] H. Aebi, Catalase in vitro, *Methods Enzym.* 105 (1984) 121–126.
- [10] A.J. Siqueira, J.O. Remião, A.M. Azevedo, C.R. Azambuja, A gasometric method to determine erythrocyte catalase activity, *Braz J. Med. Biol. Res.* 32 (9) (1999) 1089–1094.
- [11] R.G. Kroll, E.R. Frears, A. Bayliss, An oxygen electrode based assay of Catalase activity as a rapid method for estimating the bacterial contents of food, *J. Appl. Bacteriol.* 66 (1989) 209–217.
- [12] R.B. Setlow, The inactivation of catalase by deuterons and heat, *Archives Biochem. Biophys.* 34 (2) (1951 Dec 1) 396–408.
- [13] M. Richardson, L.F. Huddleson, R. Bethea, Study of catalase in erythrocytes and bacteria. I. Procedure for the determination of the catalase activity of erythrocytes, *Archives Biochem. Biophys.* 42 (1953) 114–123.
- [14] J. Maral, K. Puget, A.M. Michelson, Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals, *Biochem. Biophys. Res. Commun.* 77 (4) (1977 Aug 22) 1525–1535.
- [15] S. Mueller, H.D. Riedel, W. Stremmel, Determination of catalase activity at physiological hydrogen peroxide concentrations, *Anal. Biochem.* 245 (1) (1997 Feb 1) 55–60.
- [16] A. Rigo, G. Rotilio, Simultaneous determination of superoxide dismutase and catalase in biological materials by polarography, *Anal. Biochem.* 81 (1) (1977 Jul 1) 157–166.
- [17] A. Shivakumar, P. Nagaraja, N.A. Chamaraja, H. Krishna, K. Avinash, Determination of catalase activity using chromogenic probe involving Iso-Nicotinic acid Hydrazide and Pyrocatechol, *J. Biotechnol.* 155 (4) (2011 Oct) 406–411.
- [18] R.M. El Nashar, Flow injection catalase activity measurement based on gold nanoparticles/carbon nanotubes modified glassy carbon electrode, *Talanta* 96 (2012 Jul) 161–167.
- [19] L.A. Goth, Simple method for determination of serum catalase activity and revision of reference range, *Clin. Chim. Acta* 196 (2–3) (1991 Feb) 143–151.
- [20] A.K. Sinha, Colorimetric assay of catalase, *Analytical Biochemistry* 47 (2) (1972 Jun) 389–394.
- [21] R. Nogueira, M. Oliveira, W. Paterlini, Simple and fast spectrophotometric determination of H₂O₂ in photo-fenton reactions using metavanadate, *Talanta* 66 (2005) 86–91.
- [22] M.S. Abdul Galil, M.S. Yogendra Kumar, M.A. Sathish, G. Nagendrappa, Simple spectrophotometric method for the determination of sulfur dioxide by its decolorizing effect on the peroxovanadate complex, *J. Anal. Chem.* 63 (3) (2008 Mar 1) 239–243.
- [23] E. Sandel, Colorimetric Determinations of Trace Metals, third ed., Interscience Publishers, New York, NY, 1959, p. 929.
- [24] M.H. Hadwan, H.N. Abed, Data supporting the spectrophotometric method for the estimation of catalase activity, *Data brief* 6 (2016 Mar 31) 194–199.
- [25] M.H. Hadwan, New method for assessment of serum catalase activity, *Indian J. Sci. Technol.* 9 (4) (2016 Jan 12).
- [26] L.S. Reddy, A. Thangavel, V. Leela, K.V. Raju, Antioxidant enzyme status in broilers: role of dietary supplementation of Tulasi (*Ocimum sanctum*) and selenium, *Tamilnadu J. Veterinary Animal Sci.* 5 (6) (2009) 251–256.
- [27] O.R. Ajuwon, O.M. Idowu, Vitamin C attenuates copper-induced oxidative damage in broiler chickens, *Afr. J. Biotechnol.* 9 (44) (2010) 7525–7530.
- [28] S.R. Keshavamurthy, S. Kumar, C.B. Manohar, K.C. Sharadamma, Effect of antioxidant formulation supplementation through water on antioxidant status of broiler chicken, *Stress* 5 (2013) 6.
- [29] R. Polavarapu, D.R. Spitz, J.E. Sim, M.H. Follansbee, L.W. Oberley, A. Rahemtulla, A.A. Nanji, Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil, *Hepatology* 27 (5) (1998 May 1) 1317–1323.
- [30] S. Szymonik-Lesiuk, G. Czechowska, M. Stryjecka-Zimmer, M. Słomka, A. Mądro, K. Celiński, M. Wielosz, Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication, *J. Hepato-Biliary-Pancreatic Sci.* 10 (4) (2003 Aug 1) 309–315.
- [31] E.N. da Silva Pereira, R.R. Silveira, E.E. Flores, K.L. Rodrigues, I.P. Ramos, L.J. Da Silva, M.P. Machado, R.A. Miranda, C.C. Pazos-Moura, C.F. Gonçalves-de-Albuquerque, H.C. de Castro Faria-Neto, Hepatic microvascular dysfunction and increased advanced glycation end products are components of non-alcoholic fatty liver disease, *PLoS One* 12 (6) (2017 Jun 19) e0179654.
- [32] T. Iwase, A. Tajima, S. Sugimoto, K.I. Okuda, I. Hironaka, Y. Kamata, K. Takada, Y. Mizunoe, A simple assay for measuring catalase activity: a visual approach, *Sci. Rep.* 3 (2013 Oct 30) 3081.