IECN 2024 Conference

The 4th International Electronic Conference on Nutrients

16–18 October 2024 | Online

MDPI

Assessment of Superoxide Dismutase Enzyme Activity in Various Plant Residues

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INTRODUCTION & AIM

- Superoxide dismutase (SOD EC 1.15.1.1) is a metalloprotein catalysing the dismutation of the superoxide free radical ($O2^-$) to molecular oxygen and hydrogen peroxide[1].
- Superoxide dismutase is considered one of the important enzymatic antioxidants present in plant and animal cells to protect the cellular components against the deleterious effects of free radicals.
- Fruits are good sources of antioxidant compounds, such as phenolic, vitamins, carotenoids, and minerals[2].
- Large amounts of these fruits are consumed worldwide, from which huge quantities of their waste such as the peels result.
- Plant peels are organic waste that accumulates in large quantities and is a great environmental threat.

- **Aims**

1. Screening for SOD activities on five plants waste (plant peel) including local Iraqi watermelon, bitter orange, melon, banana, and potato.
2. Looking out the possible variations in this enzyme forms, among the different peel of plants.
3. Identifying types of extracted SOD isoenzymes.

METHOD

- Extraction buffer: Potassium phosphate buffer [50 mM KH_2PO_4, pH 7.8, 0.1 mM EDTA, and 1% Triton X-100].
- Total protein concentration of all crude extract samples was determined according to the modified biuret method[3].

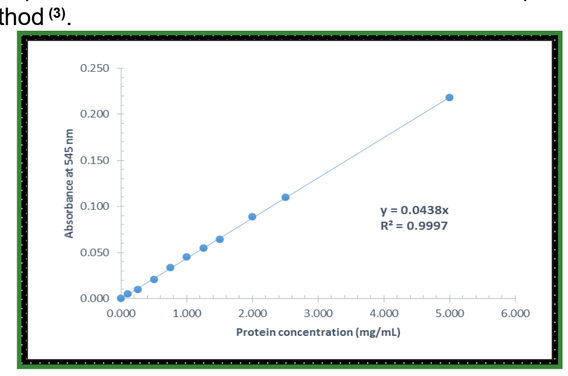
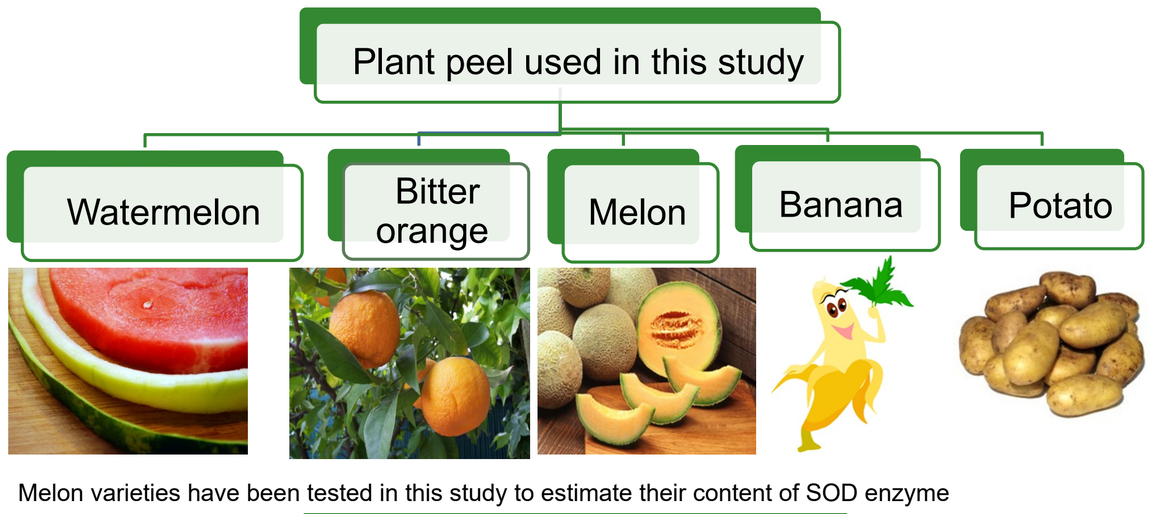


Figure 1: Standard curve of protein determination

Figure 2; Biuret solution

- The activity of SOD has been determined in solution using an indirect method; riboflavin/NBT method[4].
- Electrophoresis: conventional polyacrylamide gel electrophoresis (PAGE) was carried out to look out the variations in the different SOD isoenzymes[4].
- Plant material:



- Melon varieties have been tested in this study to estimate their content of SOD enzyme

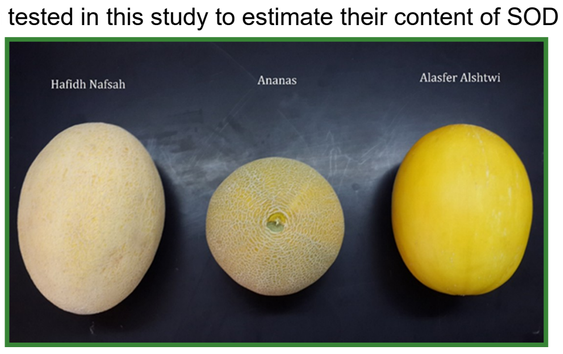


Figure 3: Different varieties of melon that tested for SOD activity [5]

RESULTS & DISCUSSION

- Table (1) Screening plant's waste for SOD activity.

:Peel extract of	SOD Activity (U/mL)	Specific activity (U/mg)
Watermelon	12.006	6.860
Melon	15.881	7.936
Banana	11.041	5.220
Bitter orange	18.570	5.042
Potato	13.622	6.807

- Table 2: Comparison of SOD activity & specific activity among peels of different melon varieties as the source of the enzyme using two different buffers for SOD extraction

Melon varieties	Extraction buffers	SOD activity (U/ml)	Specific activity (U/mg)
Ananas	Buffer A	17.89	6.50
	Buffer B	16.84	8.17
Hafidh Nafsah	Buffer A	9.04	4.39
	Buffer B	10.32	4.26
Alasfer Alshtwi	Buffer A	16.23	7.77
	Buffer B	11.64	4.64

Where; Buffer (A) [50 mM KH_2PO_4, pH 7.8 and 0.1 mM EDTA].
Buffer (B) [50 mM KH_2PO_4, pH 7.8, 0.1 mM EDTA, and 1% Triton X-100].

- Eight SOD active bands were obtained from a mixture of the extracts of the three plant peels in a single electrophoretic run. These bands are numbered in order of increasing relative mobility.
- In bitter orange peel, six bands were observed, while in melon, five bands and in watermelon rind, four bands that exhibited SOD activity were observed.

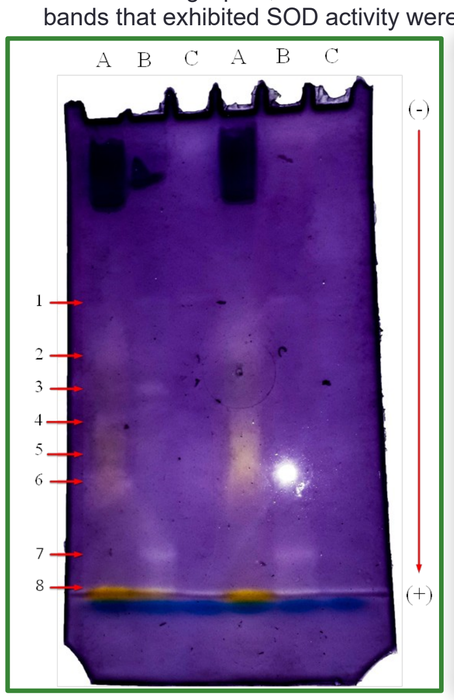


Figure 4: Conventional polyacrylamide gel electrophoresis, the gel was stained for SOD activity and the samples used were: (A) Bitter orange peel, (B) melon peel, (C) Watermelon peel

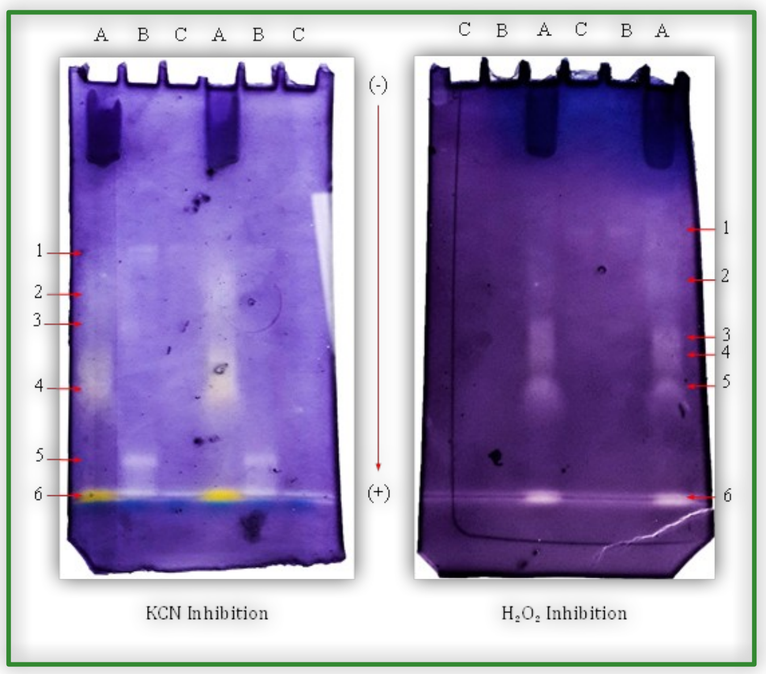


Figure 5: Conventional polyacrylamide gel electrophoresis, two gels were incubated for 20 min in KCN and H_2O_2 inhibitors, then stained for SOD activity and the samples used were: (A) Bitter orange peel, (B) melon peel, (C) Watermelon peel.

- Bitter orange peel has five isoenzymes of MnSOD and one isoenzyme of CuZnSOD. While melon peel has two isoenzymes of both MnSOD and FeSOD and one isoenzyme of CuZnSOD. Watermelon has two isoenzymes of CuZnSOD, and one isoenzyme of both FeSOD and MnSOD.

CONCLUSION

- The peels of watermelon, melon, and bitter orange which are considered as environmental pollutents, can be considered as a good source of SOD
- The Ananas melon variety demonstrated the highest SOD activity and specific activity among various local melon varieties. This makes its peels an exceptionally efficient source for extracting an enzyme with numerous pharmaceutical and cosmeceutical applications.

FUTURE WORK / REFERENCES

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