









distribution, and activity of polyphenol oxidase enzyme as well as phenolics accumulation in plants.<sup>[6]</sup> Moreover, it has been shown that a group of phenolics, flavonoids, play an important role in development of plants due to regulation of auxin transport.<sup>[53]</sup>

## CONCLUSION

The objective of this study was to evaluate the correlation between polyphenol oxidase activity and phenolics content in different physiological stages of saffron corm. Despite the importance of polyphenol oxidase enzyme in plants, there have been limited studies on saffron corm. As described above, polyphenol oxidase has been purified using DEAE-Sephadex A<sub>25</sub> column and kinetic characteristics of enzyme were determined. Also, two distinct isoforms of enzyme have been identified in both dormant and waking corms with molecular masses of 70 and 54 kDa. The findings presented here show an inverse correlation between polyphenol oxidase enzyme activity and phenolics content in dormancy and waking stages of *C. sativus* corms. The higher amount of phenols in waking stage suggests the importance of phenolics in germination. In addition, the lower content of phenolics in dormancy can be due to oxidation by polyphenol oxidase enzyme, which showed higher activity and catalytic efficiency during dormancy.

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## Conflicts of interest

There are no conflicts of interest

## REFERENCES

- Esmaeili N, Ebrahimzadeh H, Abdi K, Safarian S. Determination of some phenolic compounds in *Crocus sativus* L. corms and its antioxidant activities study. *Pharmacogn Mag* 2011;7:74-80.
- Pitsikakos N, Boultsadakis A, Georgiadou G, Tarantilis P, Sakellariadis N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of anxiety. *Phytomedicine* 2008;15:1135-9.
- Vatankhah E, Niknam V, Ebrahimzadeh H. Activity of antioxidant enzyme during *in vitro* organogenesis in *Crocus sativus*. *Biol Plant* 2010;54:509-14.
- Amin B, Hosseinzadeh H. Evaluation of aqueous and ethanolic extracts of saffron, *Crocus sativus* L., and its constituents, safranal and crocin in allodynia and hyperalgesia induced by chronic constriction injury model of neuropathic pain in rats. *Fitoterapia* 2012;83:888-95.
- Saiedian S, Keyhani E, Keyhani J. Polyphenol oxidase activity in dormant saffron (*Crocus sativus* L.) corm. *Acta Physiol Plant* 2007;29:463-71.
- Ayaz F, Demir O, Torun H, Kolcuoglu Y, Colak A. Characterization of polyphenoloxidase (PPO) and total phenolic contents in medlar (*Mespilus germanica* L.) fruit during ripening and over ripening. *Food Chem* 2008;106:291-8.
- Dicko M, Gruppen H, Traore AS, Voragen AGJ, van Berkel WJH. Phenolic compounds and related enzymes as determinants of sorghum for food use. *Biotechnol Mol Biol Rev* 2006;1:21-38.
- Sanchez-Ferrer A, Rodriguez-Lopez JN, Garcia-Canovas F, Garcia-Carmona F. Tyrosinase: a comprehensive review of its mechanism. *Biochim Biophys Acta* 1995;1247:1-11.
- van Gelder CW, Flurkey WH, Wichers HJ. Sequence and structural features of plant and fungal tyrosinases. *Photochemistry* 1997;45:1309-23.
- Dicko MH, Hilhorst R, Gruppen H, Traore AS, Laane C, van Berkel WJH, *et al.* Comparison of content in phenolic compounds, polyphenol oxidase, and peroxidase in grains of fifty sorghum varieties from Burkina Faso. *J Agric Food Chem* 2002;50:3780-8.
- Rivero RM, Ruiz JM, Garcia PC, Lopez-Lefebvre LR, Sanchez E, Romero L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci* 2001;160:315-21.
- Garcia-Rodriguez R, Romero-Segura C, Sanz C, Sanchez-Ortiz A, Perez AG. Role of polyphenol oxidase and peroxidase in shaping the phenolic profile of virgin olive oil. *Food Res Int* 2011;44:629-35.
- Yoruk R, Marshall MR. Physicochemical properties and function of plant polyphenol oxidase: a review. *J Food Biochem* 2003;27:361-422.
- Sherman TD, Vaughn KC, Duke SO. A limited survey of the phylogenetic distribution of polyphenol oxidase. *Phytochemistry* 1991;30:2499-506.
- Murata M, Kurokami C, Homma S. Purification and some properties of chlorogenic acid oxidase from apple (*Malus pumila*). *Biosci Biotechnol Biochem* 1992;56:1705-10.
- Vaughn KC, Duke SO. Function of polyphenol oxidase in higher plants. *Physiol Plant* 1984;60:106-12.
- Mayer AM. Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry* 2006;67:2318-31.
- Trebst A, Depka B. Polyphenol oxidase and photosynthesis research. *Photosynth Res* 1995;46:41-4.
- Constabel CP, Yip L, Patton JJ, Christopher ME. Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiol* 2000;124:285-96.
- Gawlik-Dziki U, Zlotek U, Swieca M. Characterization of polyphenol oxidase from butter lettuce (*Lactuca sativa* var capitata L.). *Food Chem* 2008;107:129-35.
- Altunkaya A, Gokmen V. Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chem* 2008;107:1173-9.
- Lee CY, Kagan V, Jaworski AW, Brown SK. Enzymatic browning in relation to phenolic compounds and polyphenol oxidase activity among various peach cultivars. *J Agric Food Chem* 1990;38:99-101.
- Li L, Steffens J. Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* 2002;215:239-47.
- Carbonaro M, Mattered M. Polyphenol oxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv Williams). *Food Chem* 2001;72:419-24.
- Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot M, Aubert SY. Enzymatic browning reactions in apple and apple products. *Crit Rev Food Sci Nutr* 1994;34:109-57.
- Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem* 2006;99:191-203.
- Tomas-Barberan FA, Espin JC. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J Sci Food Agric* 2001;81:853-76.
- Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. *Molecules* 2013;18:2328-75.
- Wesche-Ebeling P, Montgomery MW. Strawberry polyphenol oxidase: extraction and partial characterization. *J Food Sci* 1990;55:1320-4.
- Arslan O, Erzenin M, Sinan S, Ozensoy O. Purification of mulberry (*Morus alba* L.) polyphenol oxidase by affinity chromatography and investigation of its kinetic and electrophoretic properties. *Food Chem* 2004;88:479-84.
- Aydemir T. Partial purification and characterization of polyphenol oxidase from artichoke (*Cynara scolymus* L.) heads. *Food Chem* 2004;87:59-67.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Ho KK. Characterization of polyphenol oxidase from aerial roots of an orchid, *Aranda 'Christine 130'*. *Plant Physiol Biochem* 1999;37:841-8.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Lineweaver H, Burk D. The determination of enzyme dissociation constants. *JACS* 1934;56:658-66.
- Folin O, Ciocalteu V. On tyrosine and tryptophane determinations in proteins. *J Biol Chem* 1927;73:627-50.
- Marri C, Frazzoli A, Hochkoeppler A, Poggi V. Purification of a polyphenol oxidase isoform from potato (*Solanum tuberosum*) tubers. *Phytochemistry* 2003;63:745-52.
- Nagai T, Suzuki N. Partial purification of polyphenol oxidase from Chinese cabbage *Brassica rapa* L. *J Agric Food Chem* 2001;49:3922-6.
- Halder J, Tamuli P, Bhaduri A. Isolation and characterization of polyphenol oxidase from Indian tea leaf (*Camellia sinensis*). *JNB* 1998;9:75-80.
- Paul B, Gowda LR. Purification and characterization of a polyphenol oxidase from the seeds of field bean (*Dolichos lablab*). *J Agric Food Chem* 2000;48:3839-46.
- Ortega-Garcia F, Blanco S, Peinado MA, Peragon J. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. Picual trees during fruit ripening. *Tree Physiol* 2008;28:45-54.

42. Dincer B, Colak A, Aydin N, Kadioglu A, Güner S. Characterization of polyphenoloxidase from medlar fruits (*Mespilus germanica* L., Rosaceae). *Food Chem* 2002;77:1-7.
43. Flurkey WH. Polypeptide composition and amino-terminal sequence of broad bean polyphenoloxidase. *Plant Physiol* 1989;91:481-3.
44. Colak A, Ozen A, Dincer B, Guner S, Ayaz FA. Diphenolases from two cultivars of cherry laurel (*Laurocerasus officinalis* Roem.) fruits at an early stage of maturation. *Food Chem* 2005;90:801-7.
45. Mishra BB, Gautam S, Sharma A. Purification and characterization of polyphenol oxidase (PPO) from eggplant (*Solanum melongena*). *Food Chem* 2012;134:1855-61.
46. De Jesus Rivas N, Whitaker JR. Purification and some properties of two polyphenol oxidases from Bartlett pears. *Plant Physiol* 1973;52:501-7.
47. Mazzafera P, Robinson SP. Characterization of polyphenol oxidase in coffee. *Phytochemistry* 2000;55:285-96.
48. Cevallos-Casals BA, Cisneros-Zevallos L. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chem* 2010;119:1485-90.
49. Ding CK, Chachin K, Ueda Y, Imahori Y. Purification and properties of polyphenol oxidase from loquat fruit. *J Agric Food Chem* 1998;46:4144-9.
50. Orak HH. Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. *Sci Horticult* 2007;111:235-41.
51. Burda S, Oleszek W, Lee CY. Phenolic compounds and their changes in apples during maturation and cold storage. *J Agric Food Chem* 1990;38:945-8.
52. Lima VL, Melo EA, Maciel MIS, Prazeres FG, Musser RS, Lima DE. Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. *Food Chem* 2005;90:565-8.
53. Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, *et al.* Flavonoids act as negative regulators of auxin transport *in vivo* in Arabidopsis. *Plant Physiol* 2001;126:524-35.