Peer Review File

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Reviewer A

Comment 1: They have to explain that crocin is not a single substance that is responsible for the color of saffron, but rather that it is a set of carotenoids from the same family, these are crocetin glycosidic esters of which up to 15 of them have been identified, this group is called "crocins" in the scientific literature. To explain this, the authors should better study the article numbered (45) by Carmona M, Zalacain A, Sánchez AM, et al. ("Crocetin esters, picrocrocin and its related compounds present in Crocus sativus stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS". J Agric Food Chem 2006;54:973-9) where they are detected 5 new crocins.

Reply 1: Thank you for your kind suggestion. As the reviewer commented, we added the following sentence;

"Crocins, crocetin ester derivatives, are found in saffron and in the fruit of Gardenia jasminoides. These compounds are the main coloring and bioactive constituents of saffron. Five types of crocin derivatives are known as crocin-1, crocin-2, crocin-3, crocin-4, and crocin-5 that are distinguished by the number and position of the sugar moieties bound to the crocetin. Crocin-1, the main component, comprises two gentiobiose groups bound to crocetin. Crocins are also distinguished into two stereoisomers, where all double bonds in the crocetin chain are trans and the other is formed with one cis double bond on C13 of the chain. Carmona et al. have identified 15 crocins in saffron" (page 10, lines 167-175). Furthermore, figure 2 was recreated.

Comment 2: They must critically review the standardization and when they cite the ISO3632 standard, comment that the determination of the parameters A1%1cm 330nm and A1%1cm 257nm are not related to the concentration with the concentration of safranal and picrocrocin, respectively, in this section they must cite the conclusions of the study by García-Rodriguez et al. "Comparative evaluation of an ISO3632 method and an HPLC-DAD method for safranal quantity determination in saffron". Food Chemistry 221 (2017) 838–843.

Reply 2: As the reviewer suggested, we added the following sentence;

"However, these analytical methods have several limitations for the determination of saffron quality. The determination of safranal, the substance responsible for the aroma of saffron, is essential for its quality control because safranal is specific to saffron. Crocins are also present in gardenia fruit. Safranal content has been determined using UV-vis spectrophotometry at a wavelength of 330 nm with a water-soluble extract as the analyte. In the method employed in ISO 3632, some crocetin esters (mainly *cis*-isomers) are reported to interfere with the analysis of safranal, because they have an

absorption wavelength of near 330 nm. However, the degree of interference is not clear. In addition, while crocins and picrocrocin are graded according to their content, the maximum and minimum amount of safranal is the same for all grade categories. Therefore, safranal, which importantly determines the aroma of saffron, is not reflected with high precision in the saffron grade classification, and the ISO 3632 standard cannot be said to accurately reflect the quality of saffron. In fact, a quantitative analysis method for safranal contents using a HPLC coupled diode array detector (HPLC-DAD) has been reported for the quality control of saffron, but a comparison of its result with those of the quantitative analysis method used in ISO 3632 reveals that there is no correlation. They concluded that the HPLC-DAD method is suitable for the quality assessment of saffron, but there is a limitation: safranal is easily decomposed and the purity of the safranal reagent is low or unclear. In the method using an absolute calibration curve, the reliability of the calibration curve is poor due to the degradation of safranal. Although an analytical method has also been developed for the determination of these quality control markers using LC-MS/MS, this method has not been widely tested, and thus, no effective method has been established." (pages 15-16, lines 265-288).

Comment 3: Page 5 line 78 Change "six pale purple petals" by "six pale purple tepals". When in a plant the petals and the sepals are confused, they are called tepals.
Reply 3: As the reviewer commented, we revised it to the tepals in our manuscript.

Comment 4: Page 6 line 106 Change "crocin" by "crocins", since it is not a single compound, rather it is a group of compounds from the same chemical family. This change must be made throughout the manuscript each time crocin is cited.
Reply 4: As the reviewer commented, we changed all "crocin" by "crocins" in this article.

Comment 5: Page 7 line 114 Change "three stigmas" by "a stigma formed by three filaments".
Reply 4: As the reviewer commented, we changed "three stigmas" by "a stigma formed by three filaments" in our manuscript (page 7, line 113).

Comment 6: Page 9 line 162 Change "aqueous" by "hydrophilic"
Reply 6: As the reviewer commented, we rewrote "aqueous" by "hydrophilic" in the text (page 10, line 176).

Comment 7: Page 14 line 239 The statement "..., is of the best quality" This statement should be deleted because it is not objective, the authors do not provide scientific data for it.
Reply 7: As the reviewer suggested, we deleted this sentence and rewritten it as follows;
"In particular, the Iranian (Persian) saffron, which accounts for the majority of the global supply, is categorized in order of quality as follows; Super Negin, Sargol, Negin, and Poshal (page 14, lines 250-252)."

Comment 8: Page 14 lines 240-241 The statement “The categories are evaluated by color for the balance of red and yellow.” It should be removed as it is not correct.
Reply 8: As the reviewer commented, this sentence was deleted.

Comment 9: Page 14 Line 249 The sentence "Therefore, in 2010–2011, the standardization system for saffron was clarified by ISO 3632" is not correct and the authors must correct it, the first edition of the ISO3632 standard was made in 1993, since then this standard has been used in commercial saffron transactions.
Reply 9: As the reviewer commented, the author revised this sentence as follows;
“Therefore, in 1993, the first edition of the standardization system for saffron was established by the ISO. ISO3632 was applied to the international saffron market as a universal standard. The standard was further tightened between 2010-2011” (page 15, lines 259-262).

Comment 10: Page 15 line 267 Change “petal” by “tepal”
Reply 10: As the reviewer commented, we changed “petal” by “tepal” in this article (page 17, line 296).

Comment 11: line 393 Change “Jose Bagur M” by “Bagur MJ”
Reply 11: As the reviewer commented, we revised it to “Bagur MJ” (page 24, line 420).

Reviewer B
Comment 1 and 8: “Manuscript needs some editing for scientific English (comment 1)” and “Manuscript can be improved by care in editing (comment 8).”
Reply 1 and 8: As the reviewer suggested, our manuscript was revised and proofread by a native check. In addition, the authors edited carefully this manuscript overall.

Comment 2: Line 86 .. what are scales on a plant?
Reply 2: Scale on plant indicates the bud scale and scale leaf. However, this expression was rewritten to avoid ambiguity as follows; Several cases of poisoning and death due to accidental ingestion of parts of this plant have been reported, because its bulbs and leaves resemble those of onions and garlic plants (page 5, lines 85-87).

Comment 3: Lines 113-114. The calculation seems reversed ... 70,000 flowers or 210,000 stigmas.
Reply 3: Thank you for your kind point out about our mistake. Thus we revised this sentence as follows;
“Approximately 70,000–200,000 flower stigmas are required to produce 1 kg of saffron; one stigma formed by three filaments is obtained from each flower” (page 7, lines 112-113).

Comment 4: Lines 166-167. Rewrite
Reply 4: As the reviewer commented, we rewrote this sentence as follows;
“Although picrocrocin, which is reported to contain up to approximately 26% in dry saffron” (page 10, lines 179-180).

Comment 5: Line 226 strenuous is not the right word here
Reply 5: As the reviewer commented, we rewrote as fllows;
“The saffron harvest process is tedious as it is done manually, given that the crocins and picrocrocin content reache a maximum during the full bloom term” (page 14, lines 239-240).

Comment 6: Line 230 use fraud not fraudulent damage
Reply 6: As the reviewer commented, this word was revised to “fraud” (page 14, line 242).

Comment 7: Line 231 for this write Genus don't just use C.
Reply 7: As the reviewer commented, this word rewrote to be “*Crocus sativus*” (page 14, line 243).