Dear Editor,

Please see attached the new version of our manuscript “Natural Red Pigment Production by Monascus purpureus in Submerged Fermentation Systems Using a Food Industry Waste: Brewer's Spent Grain”, with corrections based on the reviewer’s minor revision comments. A detailed response to each comment is presented below.

Authors believe that corrections have improved the quality of the manuscript and we hope this new version is suitable for publication in Foods Journal.

Sincerely yours,

Yekta Goksungur, on behalf of all authors
Response to reviewers' comments:

**Reviewer #1:**

"Line 51-56: It's good to characterize Monascus purpureus!"

The authors agree with the reviewer and appreciate the suggestion. Some characteristics of *Monascus purpureus* were added to the manuscript according to reviewer comments (Lines 38-47):

"Monascus is a xerophilic fungus which grows in a wide variety of natural substrates including rice and other cereals (Babitha et al 2006). The genus Monascus is divided into three species: purpureus, ruber and pilosus which are mainly isolated from oriental foods. The most important characteristic of this genus is their ability to synthesize pigments from polyketide chromophores and β-keto acids by esterification. The pigments produced by Monascus purpureus are classified into at least six types of pigments based on color; (1) red pigment (rubropunctamin, C21H26NO4, and monascurubramin, C23H27NO4); (2) orange pigment (rubropunctatin, C21H22O5 and monascorubrin, C23H26O5) and (3) yellow pigment (monascin, C21H26O5 and ankaflavin, C23H30O5). The structure of pigments produced by Monascus species depends on factors such as the type of substrate and nitrogen source, pH, temperature and agitation (Dufosse, 2005, Haque et al., 2016, Kim et al., 2002)."

"Line 222-224: A very interesting observation, I think it needs a better discussion."

The authors agree with the reviewer and following discussion was added to the manuscript (Line 231):

"This showed that *Monascus purpureus* did not synthesize cellulase to utilize the cellulose moiety of BSG. The rigid lignocellulosic structure of BSG also prevented the utilization of cellulose and therefore pigment production by *Monascus purpureus*."

"Line 233-235: I understand, therefore, that the best growing medium is a liquid substrate."

The reviewer is totally right since good fermentation characteristics were observed with dilute acid hydrolyzed medium, hence the best fermentation medium is a liquid substrate (Line 242-244)

"Line 251-255: Pretty confusing text regarding the speed of shaking."

Lines 251-255 describes the effect of shaking speed of 250-400 rpm on pigment production. It was stated in the manuscript that at low shaking speed (250 rpm), large pellets were formed leading to mass transfer problems. High shaking speeds (400 rpm) caused high shear stress on the Monascus mycelium leading to low pigment production values. Hence, the highest pigment production was observed at moderate shaking speed of 350 rpm.
“Line 260-267: It is clear from the text that, in general, the method is quite unpredictable regarding the efficiency of pigment production.”

The effect of medium volume on pigment production was given in Lines 277-284:

25, 50 and 75 ml of fermentation media were used and the highest pigment production was observed with 50 ml of fermentation medium. Insufficient mixing was observed when pigment production was done in 25 ml of fermentation medium and this resulted in low pigment production. Vortex formation leading to poor oxygen transfer in 75 ml of fermentation medium was also observed which resulted in low pigment production values.

“Line 286-302: The text needs to be clarified, now it is quite unclear.”

The authors agree with the reviewer. The expression was corrected as suggested by the reviewer. (Line 305-314):

“The biomass and pigment concentration of Monascus mycelia is affected by the initial inoculum concentration. To observe the effect of inoculum concentration on pigment production, 50 ml of BSG fermentation medium was inoculated with 1, 2, 3 and 4 % (v/v) spore suspension solution, corresponding to $0.5 \times 10^6$, $1.0 \times 10^6$, $1.5 \times 10^6$ and $2.0 \times 10^6$ spores/50 ml of fermentation medium. The highest pigment production (22.25 UA500) was observed in the pretreated BSG medium inoculated with 2% (v/v) spore suspension. The pigments produced in the fermentation media inoculated with 1, 3 and 4% spore suspensions were 15.87, 18.15 and 11.36 UA500, respectively (Figure 3F). Our results showed that low inoculum ratio reduced the amount of biomass leading to lower concentration of pigment. However, high inoculum ratio yielded high biomass concentration that resulted in rapid consumption of nutrients in the fermentation medium required for pigment synthesis.”

“Line 362-365: These differences, however, are very large.”

The reviewer stated an important point that the difference in pigment production data in the literature were large. But the values given in the manuscript are obtained from the literature survey, from the published articles and the probable reasons of this variation were given in the manuscript as:(Line 381-384):

“The variation in the literature data for Monascus pigment production may be related to several factors such as the strain of microorganism, the type of substrate and nitrogen source, the fermentation system, the method of pigment estimation, conditions used during fermentation.”

“Line 381-384: Probably? Certainly, these are the key issues.”

The authors agree with the reviewer. The word “probably” was removed from the manuscript according to reviewer comments (Line 400-402).

“This was due to the deamination of amino acids present in the medium by M. purpureus and the production of ammonia, which increased the pH of the fermentation broth.”