The topic of the paper is of great interest, as demonstrated by the literature in this field.

My main concern is the analytical method used by the authors to “analyze through an agnostic untargeted metabolomic approach a wide range of coffee brews” that allowed to consider a reduced panel coffee metabolites, excluding some compounds with significant impact on human health, as trigonelline and caffeine. To obtain an exhaustive work, these compounds should be evaluated, setting the appropriate experimental conditions to reach this goal. Moreover, the adverse effects of some important coffee metabolites (caffeine) should be considered in this work.

Reply: Similarly to reviewer #2, the reviewer stresses inherent limitations of untargeted metabolomic analyses. With untargeted metabolomics, the number of features detected can be high, but a single analytical approach (reversed-phase column, positive ionization) cannot measure all chemicals in a particular biological sample. These limitations have been underlined in the conclusions of the manuscript (last paragraph of the discussion). Some important coffee compounds such as trigonelline and caffeine are not covered in the present work. To include all such coffee compounds, a targeted analytical approach would have been needed as now mentioned in this last paragraph. However, this was not necessary to demonstrate that we could differentiate different types of coffee brews with untargeted metabolomics and identify compounds characteristic of the main types, which was the main objective of this work.

The aim of the paper is clearly stated, but it is not clear if it was fulfilled or not. The authors should explain more extensively the implications of their results, especially concerning the influence of the brewing method on the chemical composition of coffee, with particular focus on the bioactive metabolites. In this perspective, conclusions paragraph could be more incisive.

Reply: The impact of coffee brew methods on the content of coffee bioactive compounds was already mentioned in the discussion (‘These coffee compounds showing wide variations in their concentrations are not only abundant in coffee but have also biological properties which contributes to explain health effects of coffee’; 5th paragraph of the discussion section). This was detailed in particular for two major compounds in coffee (chlorogenic acid and caffeine. A sentence has been added as suggested at the end of the discussion: ‘In particular it will be of particular importance in future epidemiological studies on health effects of coffee, to develop targeted methods of analysis to specifically measure in human biospecimens, coffee compounds specific for different types of coffee brews as described here, but also all major bioactive compounds known in coffee that may explain the effects of coffee intake on health’.

Minor comments:

Reply: References have been added.

2. Line 67. Title of paragraph 2.2 could be more informative.

Reply: The title has been modified.

3. Line 81. Please clarify the meaning of “Association of coffee compound profiles with different types of coffee brews.”.

Reply: This was a title for a subsection. It has been corrected.

4. Line 100. “Caffeinated and decaffeinated coffee brews could be differentiated along PC3 (accounting for 7.7% of the variability), a result consistent with high loadings of paraxanthine and theobromine on this PC (Supplementary figure S3).” Please move this sentence to main text and explain why in fig S3 A the PC2 and PC3 score plots of coffee brews are reported: do they contain additional information respect to PC1 and PC2?

Reply: The sentence was in the figure caption by mistake. It has been moved to the main text. PC3 was not reported in the core of the manuscript. Therefore it corresponds to novel information. It is shown together with PC2 (which also appears in Figure 1A and 1B) for ease of visualization.

5. Figures 2, 3, 4 and 5 can be rationalised and merged. The quality of the pictures should be improved.

Reply: Merging the four figures into one figure would be justified if the same compounds would appear in the different figures. However they all differ in the four figures, and for this reason, we think that it is more appropriate to show these graphs in four different figures, each corresponding to one separate character of coffee brews. All graphs were plotted again to harmonize formats and high resolution files are now provided.


Reply: Literature on coffee and health has accumulated quickly over the last few years. We have added in particular the following reference (ref 11 cited in 1st paragraph of the introduction): Poole, R.; Kennedy, O. J.; Roderick, P.; Fallowfield, J. A.; Hayes, P. C.; Parkes, J., Coffee

We preferred to cite in the introduction (1st paragraph) diseases where there is strongest evidence for an association of coffee intake with disease risk. For this reason, we left aside the following references showing either the lack of evidence with some disease outcomes [1], or focused on preclinical work [2]. The two references on biological properties of chlorogenic acids [3,4] are now cited in the discussion (now references 31 and 32).

7. Line 215. “The triplicate preparations were combined to make a single pooled sample for each coffee type.” The authors should explain the reason of pooling the triplicate samples and not analysing them in three different experiments.

*Reply:* The reason was to limit the total number of analyses and corresponding costs. This is now mentioned in the experimental section (section 4.2, 4th paragraph)

8. Line 220. “Coffee selection and preparation are described in further detail elsewhere (Loftfield et al, in preparation).” Please provide the details in this paper, not in a manuscript in preparation.

Regarding the coffee selection, an explanation of the rationale behind the selection of coffee samples should be added. Could the authors also clarify the reason of applying a different set of brewing methods for the different brands? Consequently, different numbers of samples are produced for each type of coffee brew: could this have any impact on the results?

*Reply:* Details on the selection and preparation of coffee samples have been added in the experimental section (Section 4.2; three new paragraphs).

9. Line 224. “Samples were centrifuged at 120g.” Please correct 120g.

*Reply:* 120g has been corrected into 120 x g. It is otherwise correct.

10. Line 238 - 239. Please correct 2 uL and 45oC

*Reply:* This has been corrected.

**References**

