

Response to Reviewer 2

The work described in this paper is of interest to the readers of this journal. Nonetheless, there are several issues to be addressed. The manuscript is not acceptable for publication in its present form.

Thank you for the feedback. The manuscript has been extensively edited and enriched to improve its scientific quality and readability.

- The whole manuscript: *Lactobacillus lactis* or *Lactococcus lactis*?

***Lactobacillus Lactococcus*. Corrections were made.**

- Abstract: The text does not contain any information about the use of the species *Lactobacillus lactis*, while the species *Lactococcus lactis subsp cremoris*, *lactis*, *lactis biovar diacetylactis* are mentioned. I propose to include in the summary those microorganisms that actually participated in experiments and also present important test results. The summary should not contain any abbreviations.

The correct species were listed in the abstract: *Propionibacterium freudenreichii subsp. globosum*, *Lactococcus lactis subsp. cremoris*, *lactis*, *lactis biovar diacetylactis*, and *Leuconostoc*. Page 1, Lines 14-16.

- Keywords: They are not consistent with the manuscript title, abstract, and analytical scope of the research presented. I suggest you choose more appropriate keywords.

Keywords were edited: protein digestibility; peanuts; legumes; probiotics; fermented food; *Lactococcus*; *Propionibacterium*; cheese; amino acids; texture. Page 1, Lines 23-24.

- Why wasn't *Propionibacterium* mentioned in the title and abstract?

***Propionibacterium freudenreichii globosum* was added to the title (Page 1, Lines 2-4) and the abstract (Page 1, Line 14).**

- Introduction: In my opinion, the introduction should be expanded to include the importance of LAB and *Propionibacterium* for the digestibility and bioavailability of legume proteins. There are numerous studies in this area. In addition, it would be valuable to demonstrate the digestibility and bioavailability of peanuts and legumes unfermented. The purpose of the research should be well presented. The introduction and overall manuscript would benefit from using more recent references for general topics.

Information on unfermented and fermented legume protein was added. Pages 1-2, Lines 38-54.

- 2.2 pH: I propose to detail the preparation of the fermented peanut sample for this analysis.

Details added: Page 2, Lines 79-80.

- 2.3 Microbial Enumeration: Lactobacillus or Lactococcus? Why was MRS used for analysis? What was the pH of this medium? I propose to detail the preparation of the fermented peanut sample for this analysis. Was the final result expressed in 1mL sample or 1g sample? Why could the *Propionibacterium* number not be determined?

***Lactococcus*. MRS was chosen as selective growth media for lactic acid microorganisms. The pH of the medium was 5.7. Results were expressed as CFU/g. Page 2, Lines 82-88.**

· 2.4.1 Preparation of Sample Solutions: For what purpose was distilled water (of pH 7.0) added to the solution adjusted to pH 2.0? What was the final pH of the sample?

The solution used to adjust the pH was 1 mol/L HCl, for a final pH value of 2.0.

· 2.5 Free Amino Acids: I propose to describe in more detail the preparation of samples for analysis. Merely citing references does not represent the methodology well.

Methodology was described in detail for both total and free amino acids. Pages 3,4: Lines 132-150.

· 2.6 Texture Profile Analysis: How does this relate to the digestibility of peanuts?

Digestibility of protein is also affected by their texture and structure. Therefore, Texture Profile Analysis was performed on peanut slurry. Section 2.7: Page 4, Lines 152-156.

· Lines 157-159: The results of the presented microorganism population do not look good. What about the population of *Propionibacterium*? Why were the cultures performed with such a low sample dilution? The population at 10^3 is insufficient for fermentation samples. Normally, the microorganism population in fermentation reaches 10^6 . There are references to this. I propose to discuss this critically in the manuscript.

The dose of *Propionibacterium* was chosen based on manufacturers' recommendation. The amount chosen was intentionally low to avoid inhibition of lactic acid bacteria. In addition, the goal of this study was to modify protein, with minimal affect on sensory, thus a low doses were chosen.

· 3.2. Protein Digestibility and 3.3. Free Amino Acid Profile: In my opinion, the results should be correlated with the *Lactobacillus* or *Lactococcus* and *Propionibacterium* population. Also, the pH change should correlate in these results.

Sections 3.2, 3.3 and 3.4 were edited, implementing microorganisms in the presentation of protein digestibility and amino acids.

· 3.4. Texture: What can you explain the SD values in Table 2? I suggest removing "1 TMC = Too Many to Count" because it does not apply in this table. In my opinion, the results should be correlated with the *Lactobacillus* or *Lactococcus* and *Propionibacterium* population. Also, the pH change should correlate in these results.

Unfermented slurries were rich in both oil and water soluble nutrients, thus resulting in inhomogeneous structure. Page 11, Lines 390-394. TMC was removed. Discussion was implemented with consideration of metabolites released by fermentation. Page 11, Lines 393-403.

· Lines 219-227: Does LAB only produce lactic acid? I propose to discuss the composition of the starter microorganisms used, including the homo/hetero fermentation of this LAB. What about *Propionibacterium*? What sugars are in peanuts and are used by LAB and *Propionibacterium* for fermentation?

Postbiotics (metabolites synthesised by lactic bacteria) were included in the discussion.

· Lines 229-237: The discussion of the results should be implemented by deepening the findings that may be an advancement of knowledge. There are aspects that are brought as findings of the study that are actually long-established evidence. On what basis do the authors conclude that microbial growth was exponential, since the results received are "Too Many to

Count"? Lactobacillus and Propionibacterium species are not probiotic bacteria. How important was the Propionibacterium starter for the samples? What benefits did it bring? I propose to discuss the use of Propionibacterium to ferment plant-based products by other authors. There are references for this. What does the fragment mean: „This is due to peanuts nutritional value, delivering as much as 24 g of protein per 100 g sample, as well as 4.9 g of sugar [16]. In addition, peanuts contain nutritionally relevant levels of micronutrients such as vitamins B1, B2, B3, B6, B9 and choline [16] and minerals calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, manganese, and selenium [17].”?

Discussion as implemented. *Lactococcus lactis* produces lactic acid, 2,3-butanediol (a plasticizer) and ornithine (an umami-tasting amino acid). Page 5, Lines 255-262.

***Propionibacterium f.* metabolises lactic acid into propionic acid, pyruvic acid, carbon dioxide and vitamin B12. Page 12, Lines 394-397.**

· Lines 253-288: Mesophilic lactic acid bacteria enzymes work very slowly and are only important for the digestibility of proteins in cheese production during the cheese maturing phase. There are references for this. Proteolytic enzymes from lactic acid bacteria are normally intracellular and are only released after cell death. These enzymes need water, or do you know how high the water content was in the samples? Their importance for protein digestibility is only important with the high lactic acid bacteria population. I propose to discuss it closely using literature data. Likewise, there is much evidence of the use of Propionibacterium for the digestibility of proteins in cheese and plant-based analogues.

Discussion on water dosage and metabolic pathways was implemented.

The moisture content of the slurry was 70% for all samples treated, providing sufficient moisture for the proteolysis to take place. Page 10, Lines 316-318.

Discussion on metabolic pathway was enriched. Pages 10-11, Lines 319-331.

- Line 261: *Leuconostoc* in italics.

Corrected. Thanks.

- Line 305: *P. globosum* in italics. Ensure that all names of microorganisms appear in italics throughout the document.

Corrected. Thanks.

- Lines 315-324: Free amino acids are also a matter of taste. Does peanut protein digestibility matter for the flavor profile of peanuts? What about bitter peptides? They are an important issue in the production of rennet cheese for aging. I suggest deleting this paragraph or expanding it to include a reliable discussion of all consequences of peanut protein digestibility.

Taste of peanut protein, peptides and amino acids was implemented. Fermentation of peanut protein typically resulted in sweet and umami taste. Page 11, Lines 367-380.

- Lines 328-337: The texture of peanuts is not only due to the conversion of proteins, but also of fat. I suggest discussing it. How does starter microorganism selection affect the texture of peanuts? There is ample evidence of the use of lactic acid bacteria and *Propionibacterium* in cheese and vegetable analogues. The authors should focus on interpreting the results in the context of lactic acid bacteria and *Propionibacterium* data. What about pH changes?

The role of fat and the emulsifying ability of fermented peanut protein was discussed. Page 11, Lines 384-398.

· 5. Conclusions: I propose to expand this part of the manuscript, taking into account the purpose of the research and the title of the manuscript. What are the potential benefits of the results obtained? What are the limitations of these experiments and results? Both the summary and the conclusions should be supplemented with the specific results of the analyses.

Conclusions section was edited introducing benefits (higher digestibility and consumer acceptance, potential synthesis of vitamin B12) and limitations (fermentation time and cost). Page 12, Lines 413-425.