**Response to reviewers**

**Manuscript ID Number**: 9217-52888-2-RV

**Manuscript Title**: “The use of mucilage extracted from *Opuntia ficus indica* as microencapsulating shell”

**Article Type**: Research Article

**Dear Prof. Olgica Nedić**

**Editor in chief of Journal of the Serbian Chemical Society**

We would like to thank you for the time you have spent to handle our paper and the reviewer for their constructive comments and suggestions. Please find below, the replies to the reviewers (point to point). We have incorporated all the comments and recommendations into the newly submitted version, all changes are written in red.

**Reviewer #A**

The manuscript 9217-52888-2-RV entitled “The use of mucilage extracted from *Opuntia ficus indica* as microencapsulating shell” deals with extraction and potential application of heteropolysaccharide from Opuntia ficus indica. Extracts were characterized in terms of chemical composition, morphology and the ability to act as encapsulating material for sunflower oil encapsulation. However, there are several inconsistencies throughout the manuscript, and the general comment is that the manuscript is not easy to read. English has to be improved too.

Thank you for taking the time to review our paper and for your valuable comments and suggestions, they were very insightful. The whole manuscript has been through roughly proofread by a native English speaker with respect to the scientific meaning of the text.

1. INTRODUCTION: The introduction is too general, especially the first paragraph (lines34-42), and should be completely revised.

**Reply**:

Thank you. All the part of introduction was revised carefully.

1. Please avoid and do not repeat the general statements like these given in Lines 49-50, 55-57, 60-65, 76-77.

**Reply**:

Thank you for this suggestion. These parts were excluded and new paragraphs were added.

1. Furthermore, please be more specific regarding the microencapsulation of edible oils as the focus of your study. Please state the pros and cons of mucilage from OFI as encapsulating material.

**Reply**:

Thank you for this suggestion. This was corrected.

1. Please explain why chitosan and carboxymethyl cellulose are chosen as co-encapsulating wall materials?

**Reply**:

Thank you. Due to the growing demand of biobased materials for future sustainability, chitosan and carboxymethyl cellulose based material and their applications will bring good future perspective in fundamental and applied aspects of new product formulations. Both chitosan and carboxymethyl cellulose present several properties which are affected by the change of pH, such as the swelling behavior, which allows increasing the matrix volume of polyelectrolyte and polyelectrolyte complexe upon changing the pH from acidic to basic conditions. This concept is applied in during this study. These details will be added in the revised text.

1. M&M Abbreviations used should be uniform throughout the manuscript, so please introduce the abbreviation in the first place of appearance. For instance, In the Introduction section mucilage from Opuntia was abbreviated as OFI, while in the Materials and Method section the abbreviation CM (cactus mucilage) was used!

**Reply**:

Thank you. This was corrected. CM was used for the abbreviation of cactus mucilage, and *OFI* for *Opuntia ficus indica*.

1. I assumed that CMC stands for critical micellar concentration of TTAB in Line 94, but in Lines 123-124 the authors said:“Complex coacervation was basically the same as simple coacervation except that the CM and CMC solutions were added to the pre-emulsion vessel.” CMC in this sentence is referred to carboxymethyl cellulose.

**Reply**:

Thank you. Very sorry for these mistakes. This was corrected.

1. Lines 138-139, please add the name and the producer of the used particle size analyzer and describe more in detail the protocol of this analysis.

**Reply**:

Thank you. The particle size distribution of the powdered CM, the emulsions, and the suspensions of microcapsules was investigated by laser diffraction using a Mastersizer X Malvern (laser Cilas1190, France). The sample was dispersed in aqueous medium under mechanical stirring for 2 min. The technique is based on measurement of the intensity of light scattered as a [laser beam](https://www.sciencedirect.com/topics/physics-and-astronomy/laser-beams) passes through a dispersed particulate sample. The test was repeated at least in triplicate and the difference between the various values obtained was within an experimental error of 5%. These details were added in the revised text.

1. Lines 155-157, please provide more details on calculation of encapsulation efficiency. How did you determine the amount of surface oil?

**Reply**:

Thank you. Following your request, more details were added in the revised text.

1. Description of scanning electron microscopy used in this study in M&M section is missing?

**Reply**:

Thank you. You are right. This was added.

1. There is no scientific work without statistics. Please describe the statistical analysis used in this manuscript.

**Reply**:

Thank you. This was corrected.

1. RESULTS: The particle size analyzer provides the data on particle size distribution, not their morphology. In line with that, please revise the sentence in Line 197 “The average particle size, as determined by laser diffraction analysis, indicated that the particles were fine and smooth in appearance”, since the SEM analysis revealed the opposite.

**Reply**:

Thank you. You are right. We totally agree that the particle size analyser provides only the size distribution and not their description morphology. This was correct in the revised manuscript.

1. Is there a statistically significant difference between the OFI powders' size distribution?

**Reply**:

Thank you. The test of particle size of *OFI* was repeated at least in triplicate and the difference between the various values obtained was within an experimental error of 5%.

1. Line 221, please provide the experimental data (as supplementary file) which confirms your statement on the stability of the primary emulsion. Which test did you use to investigate the stability during six months of storage?

**Reply**:

Thank you. Excellent remark. We have only some photos taking at different months and we observed that no sedimentation was taking place and we have usually kept a good emulsion. In all case, these sentences were removed in order to remove any ambiguities.

1. There is a typo in Figure 5, x-axis should be labeled as Particle size.

**Reply**:

Thank you. This was corrected.

1. Many statements in the Result and Discussion section are given without verification and are confusing. In Line 285 the authors said ‘’ The encapsulation efficiencies of the microcapsules obtained by complex coacervation and crosslinked with Chi and different CM ranged from 87% to 95%.’’ The authors did not provide the results of EE when only CM or CM with CMC was used but stated in the CONCLUSION that “The microcapsules crosslinked with Chi and different cactus extracts had sufficient stabilities needed to maintain their structures, with good entrapment efficiency (87 to 95%) compared to those crosslinked with CMC or only with CM compared to those crosslinked with CMC or only with CM.

**Reply**:

Thank you. You are right the part reporting the obtained encapsulation efficiencies was missed. Consequently, a new figure of encapsulation efficiencies was added and more details were introduced in the revised text.

1. CONCLUSIONS: Please revise the conclusion in accordance with the obtained results. For instance, in Line 292, the authors said: “It can be highlighted from the results that CM is a promising agent for microencapsulation and protection of bioactive molecules”. If the encapsulation is achieved it doesn’t mean direct protection, and the authors did not investigate the stability of encapsulated vs non-encapsulated oil to prove the statement.

**Reply**:

Thank you. This was corrected.

1. In my opinion, this manuscript should: be published after major revision and additional review

**Reply**:

Thank you. We sincerely thank you for your constructive comments. We hope that this revised version is satisfactory and appropriate to be published in the “**Journal of the Serbian Chemical Society**” journal.

------------------------------------------------------

**Reviewer #F**

On my opinion, the topic of this work could be interesting to the readers of Journal of the Serbian Chemical Society but the paper needs a complete revision, taking into account comments and suggestions.

We would like to thank you for the interest that you have shown for this paper and for your useful comments. Thank you.

1. In the introduction: It is not clear the composition of sunflower oil

**Reply**:

Thank you. Sunflower oil is composed by: Palmitic acid: 5-7.6%; Stearic acid: 2.7 – 6.5%; Oleic acid: 14-39.4%; Linoleic acid 48.3 – 74%. These details were added in the revised text.

1. It is not clear the composition of carboxymethyl cellulose and chitosan.

**Reply**:

Thank you. The introduction part was revised as well as all whole manuscript was revised and others schema were added.

1. Some insights about the components of cactus mucilage are needed in order to confirm the reaction with carboxymethyl cellulose and chitosan.

**Reply**:

Thank you. Following your request this point was taking in the revised part.

1. In the experimental: In this manuscript, in the introduction, the authors described the preparation of the coacervates studying the effect of pH and the ratio between wall materials, however, it not show in the methodology, which will make readers confusing. The methodology in general not is clear.

**Reply**:

Thank you. All the text was revised and we hope now that the revised manuscript meets your suggestion.

1. In the results and discussion: Why is SEM “fine and smooth” meaning there is “presence of carboxyl groups in the pH different.”? SEM is only presented to show the surface morphology rather than mucilage structure.

**Reply**:

Thank you. Absolutely you are right. This part was revised.

1. There is no thermal stability result for the used microcapsules. DSC and TGA would be needed.

**Reply**:

Thank you.Due to the COVID-19 issue, all the Universities in Tunisia are closed, thus we did not get any opportunity to do the suggested experiments.

We would like to submit our edited revised manuscript. We have taken into account all the necessary time for the correction of the article by all the authors. We have taken into account the reviewers’ suggestions and we thank them, because we believe that the quality of our paper was improved. We hope that now our revised paper can meet the required standard of publication in “**Journal of the Serbian Chemical Society**”.

We thank you in advance and we look forward to hearing from you.

Thank you again for your cooperation.

Best regards,

Prof. Younes Moussaoui