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Decision on submission to Food Hydrocolloids - [EMID:43c8330c3d5b090a]

Carmen Petkowicz <em@editorialmanager.com> Sun, Jan 23, 2022 at 9:22 PM
Reply-To: Carmen Petkowicz <clop@ufpr.br>
To: Novita Ika Putri <novita.ika.putri@gmail.com>

Manuscript Number: FOODHYD-D-21-03459
Functionalization of pectin-depleted residue from different citrus by-products by high pressure homogenization

Dear Ms Putri,

Thank you for submitting your manuscript to Food Hydrocolloids.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by 02/24/2022.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/foodhyd/, and navigate to the "Submissions Needing Revision" folder.

Food Hydrocolloids values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Carmen Petkowicz
Editor
Food Hydrocolloids

Editor and Reviewer comments:

Reviewer 1: The manuscript entitled "Functionalization of pectin-depleted residue from different citrus by-products by high pressure homogenization" is well structured and nicely written. The authors reported on functionalization of side streams from citrus fruit processing, which is an area of interest for industry and food scientists. However, before it can be accepted for publication there are some issues that need to be addressed. A main drawback of the paper is that the authors claim in the text in several places, including highlights, that the removal of pectin is key for the functionalization of these materials however, there are no control samples with pectin. Therefore, such claim is not funded on the results obtained in this study.

-Abstract: It is not clear what the authors mean by 'The microstructural characteristics, specifically particle morphology and size, did not directly correlate with the storage modulus (G') of the suspensions' and the 'polymer content and structural characteristics were correlated to the G' of the suspensions before HPH. However, after HPH, fragmentation and subsequently aggregation of the particles were observed'. It is well known that for these materials the particle morphology and size are not enough to explain their rheological properties. On the other hand, polymer content is related to G', or do they refer to specific polymers and molecular structures? If so, which ones?

-Line 65: The authors wrote "The CWM can be developed into a natural ingredient that can be used in food production". These ingredients are already commercially available and used by industry in food products. The sentence should be rewritten and references to recent research on citrus fibres as food ingredients included here. Some examples: J Food Sci Technol. 2016 Dec; 53(12): 4197-4204.; Int J Mol Sci. 2011; 12(4): 2174-2186.; Front. Nutr., 16 April 2020 | https://doi.org/10.3389/fnut.2020.00046; Appl. Sci. 2020, 10(19), 6633; https://doi.org/10.3390/app10196633, Journal of Food Engineering Volume 125, March 2014, Pages 97-104 among others.

-The authors should clarify in the abstract, results and discussion that the samples are pre- homogenised in an ultraturrax. Did the authors measure samples after ultraturrax and before HPH? This should be included to understand

the effect of HPH, how significant is HPH compared to ultraturrax? This is of relevance as HPH it is a costly technology, perhaps this data could be added as supplementary information.

-The rheology results are convincing regarding the effect of HPH (20 MPa), since the authors included a non-HPH as control. However, the discussion and conclusions regarding pectin are misleading as there are no controls with pectin. All samples are pectin-depleted and only minor (not significant) are observed in pectic compounds. The authors referred to a paper by Su et al., 2019, and concluded that pectin extraction is key to valorised these materials, however Su et al did not use the same raw materials and methods. If conclusions are to be drawn regarding the role of pectin, then the samples with pectin (prior to pectin extraction) should be used as control. Otherwise, is highly speculative as during pectin extraction not only pectin changes occur. Therefore, the authors should include rheological, chemical, and microstructural data on samples prior to pectin removal. Alternatively they should refrain/soften the conclusions regarding pectin, which implies rewriting the discussion.

-The microstructural discussion needs to be rewritten to help the reader. Whilst clear differences are observed after HPH, the differences between peel and pulp are not clearly observed in the LM images, rod like particles are present in all samples and the difference between peel and pulp are not obvious to this reviewer. Could the authors perform image analysis to quantify rod like particles in the samples? The authors talked about 'rough edges', this reviewer fails to understand what do the authors mean, these features should be indicated in the micrographs. LM is probably not the best technique to determine particle aggregation of suspensions after HPH, samples were diluted and mixed for LM, this could influence microstructure. This should be addressed in the discussion.

-Are there any references for the standardized tap water containing 0.2% NaCl and 0.015% CaCl2.H2O in ultrapure water? Why not use deionized water? Was this done to have constant ionic strength? Salts can have an effect on the interactions and rheological properties, this should be addressed in the text.

-The authors include a discussion about LAOS and referred to Hyun 2002. Although interesting it should be highlighted that in these complex systems more than one type of interactions are taking place. Here the study from on Shear Elastic Deformation and Particle Packing in Plant Cell Dispersions (2011) Food Biophysics 7(1):1-14 DOI:10.1007/s11483-011-9237-9, could be of interest for the authors to explain the rheological behaviour of these systems.

-The journal has the option to include supplementary information therefore the frequency sweeps (which are key to understand the rheological behaviour of these systems) as well as particle size measurements (to understand particle size distributions) should be included as supplementary figures. These results will help the readers.

-Regarding the chemical composition, the results on monosaccharide analysis, cellulose, and hemicellulose and pectin should be compared to available literature data for these citrus fruits.

-The pectic related molecules composition (GalU, Gal, Rham) showed no correlation with rheology. This should be clearly stated in the highlights and abstract.

-The authors stated in line 449-450 ´´... solubilize the pectin and eventually improved the G`` _ Do the authors mean that the solubilized pectin in the liquid phase will contribute to increase the viscosity? What is the estimated concentration of pectin in the liquid phase? Or do they mean that the solubilized pectin will lead to changes in the particles themselves , and those changes will increase G' ? This should be clarified.

-The authors found a weak correlation with pectin DM, but they conclude that 'This supported the hypothesis that the structural characteristics of the pectin may determine the rheological properties before HPH'. If all differences between materials are taken into account, and considering that the pectin content is very low in these materials from the beginning, the conclusion is not supported and should be reconsidered.

-The discussion about cellulose/xyloglucan is confusing. The authors seemed to indicate that the xyloglucan interactions with cellulose might occur as result of structural changes during HPH. It is more likely that strong interactions between cellulose and xyloglucan are broken as result of the high shear/pressure. Are there any supporting references for the propose creation of new XG/cellulose interactions ?

-Although interesting to measure protein content, the correlation with rheological properties seems highly speculative. What is the relative protein content compare to polysaccharides? The authors said: "It can be hypothesized that the existence of protein, especially the structural protein in the CWM, can inhibit the functionalization of the HPH. In this study, the peel ARs, which have significantly lower protein content, have higher G' compared to the pulp ARs. Orange ARs also have significantly higher protein content than the other citrus species and consequently have a lower G'. A previous study supported the hypothesis, showing that both heated pumpkin pomace (therefore denaturation of protein occurred) and deproteinate " However, when heating pomace more than just protein denaturation occurs and therefore protein effects cannot be isolated from the rest of factors. Could the authors elaborate on how they envisage a mechanism in which proteins inhibit the functionalisation of these materials?

-The conclusion " Pectin extraction from the CWM prior to the functionalization is essential to the improvement of the

rheological properties since the removal of pectin leads to a more open structure which can encourage the fragmentation and aggregation / network formation of the particles." As mentioned above, this is not based on the results shown by the authors as they did not include samples prior to pectin depletion.

Reviewer 2: The article evaluates the effect of high pressure homogenization on the rheological properties and particle size and aggregation of acid treated citrus pulp and peel (orange, lemon and grapefruit) waste streams. Differences in rheology are explained based on the chemical and compositional characterization. The subject is novel and worthy of investigation for future valorisation of citrus residues into texturizing materials. There are some questions and small mistakes, however, which need to be revised prior to publication.

Overall, it is clear that high pressure homogenization improves the texturizing potential of the acid treated residues. This is a strength.

However, the discussion on why rheological properties differ from one sample to another is only based on composition (amount of protein, homogalacturonan, RG-I, methylation degree), these showing only minor differences among samples (table 1, statistical analysis missing here) and median particle size. Crucial factors affecting rheology, such as molecular weight or the presence of salts are not analysed or taken into account. In fact, the authors perform dialysis of the samples (3.5kDa cut-off) before compositional characterization, but not before rheological analysis. Dialysis might not only remove salts and minerals incorporated in the process, but also low molecular weight compounds, such as oligosaccharides or simple sugars, peptides, small apolar compounds, etc, either present in the samples or released during the acid treatment, all of which can affect rheology.

Minor comments

Valorisation of waste streams depends a lot on cost-efficiency. Please add a justification for the pre-treatment washing step with ethanol and acetone (AIR).

In FTIR, the presence of protein can cause amide I bands to overlap with carboxylate bands, leading to errors in the determination of the degree of esterification. Was this taken into account?

It seems size distribution has been calculated (title and discussion in section 3.2.2). Why not show these size distribution data but only median size instead?

Line 422 for this conclusion to be made, the initial amount of pectin in the samples has to be determined. Where are these data?
Line 52 in the interest
Line 68 "could" or reference needed
Line 256 three times
Line 425-426 there is pectin in the ARs and the presence of pectin probably has an influence. I would rephrase to: "In this study, the small differences in the amount of pectin, as indicated…, among the different samples… "
Line 488-490 do the authors have an explanation for this negative correlation between xyloglucan content and G'?
Line 499 polysaccharide
Line 510 have also shown
Although methylation prevails in pectin compared to other esterification types, esterification is more accurate

Reviewer 3: This is an interesting piece of work in an area of relevance. The paper belongs to a scope of "Food Hydrocolloids"." This research paper focus on the functionalization of cell wall materials (CWM) from different citrus by-products into texturizing agents using high pressure homogenization. In the paper, the authors discussed two issues : (1) the difference in the CWM characteristics obtained from different citrus fruit and from different parts of the fruit, and (2) the rheological properties of the CWM suspensions and their correlation to the CWM characteristics in order to give insight into the functionalization potential of the by-products as a texturizing ingredient. Overall, the manuscript is clearly presented and written. However, some parts of the manuscript need to be modified and/or clarified. Additionally, some aspects of the discussion are not currently convincing and need to be addressed. I recommend therefore a minor revision of the article,
considering the following remarks and/or questions.
1- In the part where the authors are listed (see lines 4-7), the letter "a" associated to the name of each author should be removed as the authors are all affiliated to the same laboratory.
2- In section 2 (see pages 4 -11), the authors should specify:
* how does the residue after the final filtration collected as AIR (see lines 124-125) was dried? Even though it is mentioned that the residue was dried overnight at 40 °C, the drying mode/technology need to be disclosed.
* why adjusting the pH of the suspension at 4.5 prior HPH treatment? (see lines 143-145) Is there any reason behind the choice of this pH?
* the pre-shear magnitude (or the level of shear rate) and time (or duration) applied to pre-shear the sample loaded in the rheological measuring cell (or the cup) to avoid loading history (see lines 156-157).

3- It would have been interesting to:
* Evaluate the impact of HPH by varying the applied pressure magnitude (e.g., 20, 30, 40 and 50 MPa) instead of considering only one level of pressure (at 20 MPa) or vary the number of passes of HPH at fixed level of pressure.
* Complete the light microscopy observations with high-resolution microscopy (such TEM or FEG-SEM) in order to clearly observe what really happens at meso-scale after applying the HPH treatment at 20 MPa.
Could you please explain why this approach was not carried out, and discuss in depth how might some aspects of this could affect the discussion made on the effects of HPH treatment at 20 MPa?
4- The authors argued that the change in the microstructure observed after homogenizing the suspension at 20 MPa could also result from cellulose particle fragmentation or breaking down? (see pages 12-14). Is the word "fragmentation" related to the well-known "cellulosic fibers defibrillation" while applying HPH treatment and/or a breaking down of the cellulosic fiber in their lateral direction? If the authors assume that the cellulosic fiber breaking-down takes place in their lateral direction, then they should discuss if the level of the mechanical energy generated by applying a pressure of 20 MPa is sufficient enough to break down the glucosidic bonds of cellulosic fibres?
All this needs to be explained/clarify and discussed further in the revised manuscript.

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Data in Brief (optional):

We invite you to convert your supplementary data (or a part of it) into an additional journal publication in Data in Brief, a multi-disciplinary open access journal. Data in Brief articles are a fantastic way to describe supplementary data and associated metadata, or full raw datasets deposited in an external

repository, which are otherwise unnoticed. A Data in Brief article (which will be reviewed, formatted, indexed, and given a DOI) will make your data easier to find, reproduce, and cite.

You can submit to Data in Brief when you upload your revised manuscript. To do so, complete the template and follow the co-submission instructions found here: www.elsevier.com/dib-template. If your manuscript is accepted, your Data in Brief submission will automatically be transferred to Data in Brief for editorial review and publication.

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MethodsX (optional)

We invite you to submit a method article alongside your research article. This is an opportunity to get full credit for the time and money spent on developing research methods, and to increase the visibility and impact of your work. If your research article is accepted, we will contact you with instructions on the submission process for your method article to MethodsX. On receipt at MethodsX it will be editorially reviewed and, upon acceptance, published as a separate method article. Your articles will be linked on ScienceDirect. Please prepare your paper using the MethodsX Guide for Authors: https://www.elsevier.com/journals/methodsx/2215-0161/guide-for-authors (and template available here: https://www.elsevier.com/MethodsX-template) Open access fees apply.

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Functionalization of pectin-depleted residue from different citrus by-products by high pressure homogenization

Manuscript number : FOODHYD-D-21-03459

Dear Editor and Reviewers,
We would like to thank you for the comments and inputs on our manuscript. We highly appreciate the remarks and suggestions which we believe will improve the quality of this manuscript.

Each comment has been carefully considered and the manuscript has been revised accordingly. Please find below our reply for the comments from the reviewers. *The reviewer comments are in italic* and the authors response can be found under every comment written in normal style. Changes made in the paper will be indicated in red. The line numbers of these modifications are indicated in this rebuttal letter in between brackets and in *red*.

Thank you for reconsidering our manuscript and we are looking forward to your response.

Yours sincerely,
On behalf of all authors
Novita Ika Putri

Reviewer 1 :

The manuscript entitled ''Functionalization of pectin-depleted residue from different citrus by-products by high pressure homogenization" is well structured and nicely written. The authors reported on functionalization of side streams from citrus fruit processing, which is an area of interest for industry and food scientists. However, before it can be accepted for publication there are some issues that need to be addressed. A main drawback of the paper is that the authors claim in the text in several places, including highlights, that the removal of pectin is key for the functionalization of these materials however, there are no control samples with pectin. Therefore, such claim is not funded on the results obtained in this study.

1. *Abstract: It is not clear what the authors mean by 'The microstructural characteristics, specifically particle morphology and size, did not directly correlate with the storage modulus (G') of the suspensions' and the 'polymer content and structural characteristics were*

correlated to the G' of the suspensions before HPH. However, after HPH, fragmentation and subsequently aggregation of the particles were observed'.
It is well known that for these materials the particle morphology and size are not enough to explain their rheological properties. On the other hand, polymer content is related to G', or do they refer to specific polymers and molecular structures? If so, which ones?

Authors response : The abstract has been revised. The polymer content and structural characteristic that are corelated to the G' have been detailed *(Line 33-35).*

2. *Line 65: The authors wrote "The CWM can be developed into a natural ingredient that can be used in food production". These ingredients are already commercially available and used by industry in food products. The sentence should be rewritten and references to recent research on citrus fibres as food ingredients included here. Some examples: J Food Sci Technol. 2016 Dec; 53(12): 4197-4204.; Int J Mol Sci. 2011; 12(4): 2174-2186.; Front. Nutr., 16 April 2020 | https://doi.org/10.3389/fnut.2020.00046; Appl. Sci. 2020, 10(19), 6633; https://doi.org/10.3390/app10196633, Journal of Food Engineering Volume 125, March 2014, Pages 97-104 among others.*

   Authors response : Thank you for suggesting recent research in the application of citrus fibre. Indeed, citrus fibre is commercially available and has been applied in food productions. Some research on the application of citrus fibre has been added in the introduction section as background information *(Line 58-64)*. However, this paper intends to show the potential of different materials with different functionality than the citrus fibre as such.

   This study focuses on the CWM **left-over after pectin extraction** to show that the residue can still be valorised and be used in the context of food production. To add clarity to the focus of this study, a paragraph in the introduction section has been revised *(Line 66-73)*.

3. *The authors should clarify in the abstract, results and discussion that the samples are pre-homogenised in an ultraturrax. Did the authors measure samples after ultraturrax and before HPH? This should be included to understand the effect of HPH, how significant is HPH compared to ultraturrax? This is of relevance as HPH it is a costly technology, perhaps this data could be added as supplementary information.*

   Authors response : The effect of pre-homogenization has been studied and published in another paper (Willemsen et al., 2018). The paper showed the optimum process of

functionalization, which is the pH adjustment, followed by ultraturrax mixing and then HPH. Thus, this paper followed the same condition.

The authors took samples of the suspensions after ultraturrax mixing before HPH as the non-homogenized samples. This is done to show the effect of only HPH on the system. Therefore, throughout the paper, when the author discussed the effects of HPH, they are solely changes that were brought about by HPH, not by UT. The sampling process of the non-homogenized sample (that happened after UT missing) has been clearly stated in the method section *(Line 157-158)*.

4. *The rheology results are convincing regarding the effect of HPH (20 MPa), since the authors included a non-HPH as control. However, the discussion and conclusions regarding pectin are misleading as there are no controls with pectin. All samples are pectin-depleted and only minor (not significant) are observed in pectic compounds. The authors referred to a paper by Su et al., 2019, and concluded that pectin extraction is key to valorise these materials, however Su et al did not use the same raw materials and methods. If conclusions are to be drawn regarding the role of pectin, then the samples with pectin (prior to pectin extraction) should be used as control. Otherwise, is highly speculative as during pectin extraction not only pectin changes occur. Therefore, the authors should include rheological, chemical, and microstructural data on samples prior to pectin removal. Alternatively, they should refrain/soften the conclusions regarding pectin, which implies rewriting the discussion.*

   Authors response : In this study, the authors performed an experiment where the rheological properties of the cell wall suspensions from AIR (before acid pectin extraction) and AR (after pectin extraction) of lemon peel and pulp were compared. However, the lemon peel and pulp used were from different type of samples (from industrial origin); thus, previously it was decided not to include the data in this manuscript. Furthermore, as the AIR suspensions showed a very poor rheological properties, it was decided not to perform HPH and analysis on them on the subsequent samples.

   However, as the reviewer pointed out, the conclusion about the importance of pectin extraction became weak. Therefore, the authors revised the manuscript adding the data that show the difference in the rheological properties of the functionalized residues without pectin extraction (AIR samples) and with pectin extraction (AR samples). This data is added as *Figure 1* in the manuscript.

A specific discussion section has been added to the manuscript to address this result and discuss the possible role of pectin extraction in the functionalization of citrus residue *(Section 3.1. The role of pectin extraction and HPH on the functionalization of lemon CWM; Line 282)*. The discussion has also been strengthened with findings from a previous study (Willemsen et al., 2017), that studied the effect of different levels of pectin extraction on the functionalization of lemon peel residue in order to support the conclusion.

5. *The microstructural discussion needs to be rewritten to help the reader. Whilst clear differences are observed after HPH, the differences between peel and pulp are not clearly observed in the LM images, rod like particles are present in all samples and the difference between peel and pulp are not obvious to this reviewer. Could the authors perform image analysis to quantify rod like particles in the samples? The authors talked about 'rough edges', this reviewer fails to understand what do the authors mean, these features should be indicated in the micrographs.*

   Authors response : When referring to the rod-like particles, the authors meant the fibrous particles and when referring to the rough edges, we meant the irregularly shaped particles. The authors changed the term rough-edges into irregularly shaped particles to make it clearer. We also added different arrows on the microscopy visualization to indicate the fibrous (rod-like) shaped particles and the irregularly shaped particles (Figure 4). We hope that it became clear that the rod-like / fibrous particles are present more prominently in the pulp samples compared to the peel. The microstructural properties discussion has also been revised to become clearer *(Line 408, 418-424)*.

6. *LM is probably not the best technique to determine particle aggregation of suspensions after HPH, samples were diluted and mixed for LM, this could influence microstructure. This should be addressed in the discussion.*

   Authors response : The limitation of LM to show aggregation has been addressed in the discussion section *(Line 428-433)*

7. *Are there any references for the standardized tap water containing 0.2% NaCl and 0.015% CaCl2.H2O in ultrapure water? Why not use deionized water? Was this done to have constant ionic strength? Salts can have an effect on the interactions and rheological properties, this should be addressed in the text.*

Authors response : The standardized tap water was used in the previous study (Willemsen et al., 2017) that was referred to in the method section. The standardized tap water was used to simulate or resemble the conditions commonly practiced where tap water is used in the production of food.

8. *The authors include a discussion about LAOS and referred to Hyun 2002. Although interesting it should be highlighted that in these complex systems more than one type of interactions are taking place. Here the study from on Shear Elastic Deformation and Particle Packing in Plant Cell Dispersions (2011) Food Biophysics 7(1):1-14 DOI:10.1007/s11483-011-9237-9, could be of interest for the authors to explain the rheological behaviour of these systems.*

   Authors response : The authors thank the reviewer for the additional insight on the interactions of particle in plant dispersions with different microstructure. Indeed, as the article suggests, there are various interactions that may be presents between particles which exhibit different rheological behaviour at different concentration and this interaction can be affected by the microstructure of the particles as well. This information has been added to the discussion section of the manuscript *(Line 373-379).*

9. *The journal has the option to include supplementary information therefore the frequency sweeps (which are key to understand the rheological behaviour of these systems) as well as particle size measurements (to understand particle size distributions) should be included as supplementary figures. These results will help the readers.*

   Authors response : Supplementary file is added which contains the frequency sweep results and particle size distributions.

10. *Regarding the chemical composition, the results on monosaccharide analysis, cellulose, and hemicellulose and pectin should be compared to available literature data for these citrus fruits.*

    Authors response : There is limited literature that studied the same type of materials as in the present study (various citrus residue after pectin extraction), thus a direct comparison is difficult. However, the authors have tried to add a relevant comparison with the results of previous studies that used citrus fibre (without pectin depletion) in the discussion section *(Line 491-493 ; 516-519 ; 537-538).*

11. *The pectic related molecules composition (GalU, Gal, Rham) showed no correlation with rheology. This should be clearly stated in the highlights and abstract.*

Authors response : This observation has been added to Highlights *(point 3)* and Abstract *(Line 28-30).*

12. *The authors stated in line 449-450 ´´... solubilize the pectin and eventually improved the G`` _ Do the authors mean that the solubilized pectin in the liquid phase will contribute to increase the viscosity? What is the estimated concentration of pectin in the liquid phase? Or do they mean that the solubilized pectin will lead to changes in the particles themselves , and those changes will increase G' ? This should be clarified.*

    Authors response : In this part the authors would like to suggest that HPH may alter the interaction between cellulose and the strongly-bound residual pectin which may cause changes on the characteristics of the particle, allowing them to have a more open structure and thus improving the G' of the suspension after HPH. The discussion section in the manuscript has been revised in order to clarify this argument *(Line 504-510)*.

13. *The authors found a weak correlation with pectin DM, but they conclude that 'This supported the hypothesis that the structural characteristics of the pectin may determine the rheological properties before HPH'. If all differences between materials are taken into account, and considering that the pectin content is very low in these materials from the beginning, the conclusion is not supported and should be reconsidered.*

    Authors response : There are approximately 16% of pectin left in the acid residue (AR), as indicated by the GalA content, and the authors think that the pectin contribution on the characteristics of AR is not insignificant. Thus, the structural properties of the pectin are still relevant to be discussed in the context of its correlation with rheological properties.

    Furthermore, in the discussion regarding the DM of the pectin and its correlation to the rheological properties, the authors wish to emphasize the fact that the correlation between the DM and the G' of the suspensions before HPH is much stronger compared to the correlation between the DM and the G' after HPH. This was also observed on other properties of the AR. Therefore, we would like to conclude that the structural properties of the CWM in the ARs may affect the G' before HPH, however HPH changed their structure causing the correlation to disappear.

14. *The discussion about cellulose/xyloglucan is confusing. The authors seemed to indicate that the xyloglucan interactions with cellulose might occur as result of structural changes during HPH. It is more likely that strong interactions between cellulose and xyloglucan are broken as*

result of the high shear/pressure. Are there any supporting references for the propose creation of new XG/cellulose interactions ?

Authors response : The authors agree that the cellulose-xyloglucan interactions cannot be formed due to HPH. The confusing statement has been deleted from the discussion section of the manuscript *(Line 566-571).*

15. *Although interesting to measure protein content, the correlation with rheological properties seems highly speculative. What is the relative protein content compare to polysaccharides? The authors said: ''It can be hypothesized that the existence of protein, especially the structural protein in the CWM, can inhibit the functionalization of the HPH. In this study, the peel ARs, which have significantly lower protein content, have higher G' compared to the pulp ARs. Orange ARs also have significantly higher protein content than the other citrus species and consequently have a lower G'. A previous study supported the hypothesis, showing that both heated pumpkin pomace (therefore denaturation of protein occurred) and deproteinate '' However, when heating pomace more than just protein denaturation occurs and therefore protein effects cannot be isolated from the rest of factors. Could the authors elaborate on how they envisage a mechanism in which proteins inhibit the functionalisation of these materials?*

Authors response : The protein content ranged from 3-8% from the total dry matter and the rest is expected to be polysaccharides.

The authors agree that the heating of pumpkin pomace in that specific study may cause other changes in the system. However, deproteinated samples (that were treated with proteinase, without heating) also showed an improvement of storage modulus albeit not statistically significant.

The hypothesized effect of the protein to possibly inhibit the functionalisation has been written in the discussion section, where the authors stated that : "Structural proteins in the cell wall provide binding sites with other polysaccharides. For example, extensin, a structural protein found in cell wall, has been found to interact with acidic pectin, resulting in protein-polysaccharide crosslinks (Showalter, 1993; Sommer-Knudsen et al., 1998). These crosslinks may act as barrier in the unfolding and breaking down of the particles, a mechanism proposed for the improvement of the rheological properties after HPH of CWM suspensions discussed above."

16. *The conclusion " Pectin extraction from the CWM prior to the functionalization is essential to the improvement of the rheological properties since the removal of pectin leads to a more open structure which can encourage the fragmentation and aggregation / network formation of the particles." As mentioned above, this is not based on the results shown by the authors as they did not include samples prior to pectin depletion.*

    Authors response : To address this issue, we have included a comparison between AIR (without pectin extraction) and AR (after pectin extraction). The new added data should support this conclusion.

Reviewer 2 :

The article evaluates the effect of high pressure homogenization on the rheological properties and particle size and aggregation of acid treated citrus pulp and peel (orange, lemon and grapefruit) waste streams. Differences in rheology are explained based on the chemical and compositional characterization. The subject is novel and worthy of investigation for future valorisation of citrus residues into texturizing materials. There are some questions and small mistakes, however, which need to be revised prior to publication.

Overall, it is clear that high pressure homogenization improves the texturizing potential of the acid treated residues. This is a strength.

1. *However, the discussion on why rheological properties differ from one sample to another is only based on composition (amount of protein, homogalacturonan, RG-I, methylation degree), these showing only minor differences among samples (table 1, statistical analysis missing here) and median particle size.*

   Authors response : The statistical analysis (Two-way ANOVA) results for all the data are presented in Table 3. In the discussion section, we always consider the statistically significant difference and the correlation coefficient in order to give a sound argument. Indeed, there are some properties that are similar between the different residues, for example the GalA content, however we always stated that they are not different and do not draw any conclusion on why rheological properties differ from one sample to another based on this insignificant difference.

2. *Crucial factors affecting rheology, such as molecular weight or the presence of salts are not analysed or taken into account. In fact, the authors perform dialysis of the samples (3.5kDa cut-off) before compositional characterization, but not before rheological analysis. Dialysis might not only remove salts and minerals incorporated in the process, but also low molecular weight compounds, such as oligosaccharides or simple sugars, peptides, small apolar compounds, etc, either present in the samples or released during the acid treatment, all of which can affect rheology.*

   Authors response : The small molecules that are present in the by-products are expected to be removed during the AIR separation. The acidic treatment (pH 1.6) of the extraction

also ensured that the pectin was not charged and did not bind any salts or minerals. Small molecules released during the acid treatment are expected to be present in the extractable fraction (filtrate) of the samples during the centrifugation after the extraction process. The filtrates were discarded and the residues were further washed with water and filtered before use. Therefore, it is safe to assume that the acid residue (AR) did not contain relevant amounts if small molecules and the dialysis process are not expected to affect the monosaccharide content, protein and DM analyses.

3. *Valorisation of waste streams depends a lot on cost-efficiency. Please add a justification for the pre-treatment washing step with ethanol and acetone (AIR).*

   Authors response : The purpose of the pre-treatment has been added to the method section *(Line 127-128)*. It is needed to obtain a more refined cell wall materials from the residue. This step can be removed in the practical application in the industry, but in the experimental setup it is important to have samples with only cell wall materials in order to investigate the effect of the treatment.

4. *In FTIR, the presence of protein can cause amide I bands to overlap with carboxylate bands, leading to errors in the determination of the degree of esterification. Was this taken into account?*

   Authors response : Yes, the authors were aware of the interference of protein on the FT-IR testing and we deconvoluted the spectra prior to analysis. This has been mentioned in the methodology section, however, to make this clearer, the methodology section has been revised *(Line 228-234)*.

5. *It seems size distribution has been calculated (title and discussion in section 3.2.2). Why not show these size distribution data but only median size instead?*

   Authors response : Only the median particle size was presented in the main body of the paper because the same conclusion can be reached by comparing the median and by comparing the whole particle size distribution. Graphs with full particle size distribution of the samples were added to the supplementary file.

6. *Line 422 for this conclusion to be made, the initial amount of pectin in the samples has to be determined. Where are these data?*

   Authors response : The data related to the calculation of the %GalA extracted were added as Table 1 in the manuscript

7. *Line 52 in the interest*

   Authors response : revised *(Line 51)*

8. *Line 68 "could" or reference needed*

   Authors response : revised by adding a reference *(Line 71-74)*

9. *Line 256 three times*

   Authors response : revised *(Line 270)*

10. *Line 425-426 there is pectin in the ARs and the presence of pectin probably has an influence. I would rephrase to: "In this study, the small differences in the amount of pectin, as indicated…, among the different samples… "*

    Authors response : revised *(Line 481-482)*

11. *Line 488-490 do the authors have an explanation for this negative correlation between xyloglucan content and G'?*

    Authors response : A postulated explanation of the negative correlation have been added to the discussion section *(Line 552-555).* Xyloglucan may act as a link between the cellulose microfibril. Prior to HPH, the bounds still have a strong influence on the matrix and they prevent the cell wall particles to open up.

12. *Line 499 polysaccharide*

    Authors response : revised *(Line 564)*

13. *Line 510 have also shown*

    Authors response : revised *(Line 569)*

14. *Although methylation prevails in pectin compared to other esterification types, esterification is more accurate*

    Authors response : The term “degree of methylation” in the manuscript has been revised to “degree of methyl-esterification” (DM) in order to be more accurate and clear.

Reviewer 3 :

This is an interesting piece of work in an area of relevance. The paper belongs to a scope of "Food Hydrocolloids"." This research paper focus on the functionalization of cell wall materials (CWM) from different citrus by-products into texturizing agents using high pressure homogenization. In the paper, the authors discussed two issues : (1) the difference in the CWM characteristics obtained from different citrus fruit and from different parts of the fruit, and (2) the rheological properties of the CWM suspensions and their correlation to the CWM characteristics in order to give insight into the functionalization potential of the by-products as a texturizing ingredient. Overall, the manuscript is clearly presented and written. However, some parts of the manuscript need to be modified and/or clarified. Additionally, some aspects of the discussion are not currently convincing and need to be addressed. I recommend therefore a minor revision of the article, considering the following remarks and/or questions.

1. *In the part where the authors are listed (see lines 4-7), the letter "a" associated to the name of each author should be removed as the authors are all affiliated to the same laboratory.*

   Authors response : the letters are removed *(Line 4-5)*

2. *In section 2 (see pages 4 -11), the authors should specify:*

- *how does the residue after the final filtration collected as AIR (see lines 124-125) was dried? Even though it is mentioned that the residue was dried overnight at 40 °C, the drying mode/technology need to be disclosed.*

  Authors response : The AIR was dried with an oven. This information has been added to the method section *(Line 134).*

- *why adjusting the pH of the suspension at 4.5 prior HPH treatment? (see lines 143-145) Is there any reason behind the choice of this pH?*

  Authors response : The decision to adjust the pH to 4.5 was based on previous study (Willemsen et al., 2018). In this paper, it was shown that compared to other pH level, an optimum storage modulus can be reached at pH 4.5. The justification has been written in the method section of the paper *(Line 154-155).*

- *the pre-shear magnitude (or the level of shear rate) and time (or duration) applied to pre-shear the sample loaded in the rheological measuring cell (or the cup) to avoid loading history (see lines 156-157).*

  Authors response : The pre-shear was carried out at 10 s^{-1} for 30 s and followed by 30 s rest. This information has been added to the method section of the paper *(Line 169).*

3. *It would have been interesting to:*

- *Evaluate the impact of HPH by varying the applied pressure magnitude (e.g., 20, 30, 40 and 50 MPa) instead of considering only one level of pressure (at 20 MPa) or vary the number of passes of HPH at fixed level of pressure.*

  Authors response : It is indeed interesting to study the suggested parameter by the reviewer. However, the impact of different pressure level had been studied in other paper (Willemsen et al., 2018). The varying number of passes of HPH will be considered for the next experiments.

- *Complete the light microscopy observations with high-resolution microscopy (such TEM or FEG-SEM) in order to clearly observe what really happens at meso-scale after applying the HPH treatment at 20 MPa. Could you please explain why this approach was not carried out, and discuss in depth how might some aspects of this could affect the discussion made on the effects of HPH treatment at 20 MPa?*

  Authors response : The authors have attempted SEM analysis on the non-homogenized and homogenized suspension samples. However, the SEM visualisations were difficult to interpret and did not result in additional information on the mechanism of the rheological properties improvement after HPH.

4. *The authors argued that the change in the microstructure observed after homogenizing the suspension at 20 MPa could also result from cellulose particle fragmentation or breaking down? (see pages 12-14). Is the word "fragmentation" related to the well-known "cellulosic fibres defibrillation" while applying HPH treatment and/or a breaking down of the cellulosic fibre in their lateral direction? If the authors assume that the cellulosic fibre breaking-down takes place in their lateral direction, then they should discuss if the level of the mechanical energy generated by applying a pressure of 20 MPa is sufficient enough to break down the glucosidic bonds of cellulosic fibres?*

Authors response : Fragmentation of the particles does not means cellulosic defibrillation. The authors also believe that the HPH at 20 MPa is not sufficient to break down the cellulose microfibril. The fragmentation of the particles in this manuscript meant the breakdown of the cell wall materials into clusters of smaller cell wall fragment. The fragmentations could happen due to weakened interactions between cell wall constituents, i.e. cellulose, hemicellulose and pectin.

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Decision on submission to Food Hydrocolloids - [EMID:055bdc3b6fc4de8c]

Carmen Petkowicz <em@editorialmanager.com> Wed, Mar 2, 2022 at 7:53 PM
Reply-To: Carmen Petkowicz <clop@ufpr.br>
To: Novita Ika Putri <novita.ika.putri@gmail.com>

Manuscript Number: FOODHYD-D-21-03459R1
Functionalization of pectin-depleted residue from different citrus by-products by high pressure homogenization

Dear Ms Putri,

Thank you for submitting your manuscript to Food Hydrocolloids.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.
Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Food Hydrocolloids and hope you will consider us again for future submissions.

Kind regards,

Carmen Petkowicz
Editor
Food Hydrocolloids

Editor and Reviewer comments:

Reviewer 1: The authors have adequately addressed the comments and significantly improved the presentation of results and conclusions drawn from their study.The recommendation is Accept for publication.

Reviewer 3: The manuscript number FOODHYD-D-21-03459R1 has been correctly revised following all comments and suggestions. The quality of the new version of this research paper is significantly improved and therefore acceptable for publication in Food Hydrocolloids. I therefore recommend its publication in this journal.

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