vilia paramita



@gmail.com>

Linnea Xie @mdpi.com>	Tue. Mar 24. 2020 at 3:12 PM
To: vilia paramita < ''' @gmail.com>	
Cc: ijms@mdpi.com, Naksit Panyoyai <pre>cmci:_nan@cmru.ac.th></pre>	, Stefan Kasapis <@gmail.com>
Dear Dr. paramita,	
Thank you for your kind reply. I have received the revised man We will help you upload the revised revision to our system and to process it. If there is a follow-up message, we can communi-	uscript. continue cate in time.
Kind regards,	
Ms. Linnea Xie	
Assistant Editor	
SAVE THE DATE: 4th International Symposium on Frontiers in Science — Biochemistry, Molecular Biology and Druggability of (ISFMS 2020) will take place in Florence, Italy from 8-11, Septe	Molecular Proteins ember 2020.
Information and registration: https://sciforum.net/conference/ISFMS2020 *Abstract Submission Deadline*: 15 April 2020 *Early Bird Registration Deadline*: 30 June 2020	
On 2020/3/24 14:40, vilia paramita wrote: Dear Linnea,	
Ms. Ref. No.: ijms-727663 - Title: Molecular functionality of proteins from low- to high-solid systems with ligand and co-s	olant solute
We would like to submit our revised manuscript with the resp	onse-to-reviewer comments attached to this email.
Hope to hear a piece of good news from you soon.	
Regards	
Vilia	
On Thu, Mar 19, 2020 at 2:38 PM vilia paramita	n>> wrote:
Dear Linnea,	
Thank you for your reminder. We will send you the revision a possible.	s soon as
Regards	
Vilia	
On Wed, Mar 18, 2020 at 6:01 PM Linnea Xie @r	ndpi.com <mailto @mdpi.com="">> wrote:</mailto>
Dear Dr. Paramita,	

Gmail - [IJMS] Manuscript ID: ijms-727663 - Revision Reminder

We sent a revision request for the following manuscript on 28 February 2020.

Manuscript ID: ijms-727663 Type of manuscript: Review Title: Molecular functionality of plant proteins from low- to high-solid systems with ligand and co-solute Authors: Vilia Darma Paramita. Naksit Panyoyai, Stefan Kasapis * Received: 7 February 2020 E-mails: .@gmail.com <mailto @gmail.com>, @cmru.ac.th <mailto @cmru.ac.th> @gmail.com <mailto: _____@gmail.co m> Submitted to section: Molecular Plant Sciences, https://www.mdpi.com/journal/ijms/sections/molecular plant sciences Structural/Functional https://www.mdpi.com/journal/ijms/sections/molecular_plant_sciencesStructural/Functional Characterization of Plant Proteins https://www.mdpi.com/journal/ijms/special issues/plant proteins May we kindly ask you to update us on the progress of your revisions? If you have finished your revisions, please upload the revised version together with your responses to the reviewers as soon as possible. You can find your manuscript and review reports at this link: https://susy.mdpi.com/user/man uscripts/resubmit/8f882b07a6c130e706e70930ea01a653 Thank you in advance for your kind cooperation and we look forward to hearing from you soon. Kind regards, Ms. Linnea.Xie Assistant Editor MDPI Branch Office, Wuhan E-Mail. @mdpi.com <mailte " @mdpi.com>

[Quoted text hidden]





Response to Reviewers Ms. Ref. No.: ijms-727663 - Title: Molecular functionality of plant proteins from low- to highsolid systems with ligand and co-solute

Reviewer #1

Comment:

Ref [1] underlines that "potential health concerns do exist from a diet of protein consumed from primarily animal sources" but does not state that animal derived proteins are bad. Response

On page 1, line 30, it is stated: "Increasingly, however, animal-based proteins are seen with a growing concern due to potential meat-transmitted disease [1], meat-intolerance leading, for example, to abdominal pain or cramping [2], and dairy allergies [3]." Thus, we only suggested that increasing consumption of animal based protein has become "a growing concern" among the populace. This is now reflected in cited literature that discusses the above concerns:

- Biswas, A.K.& Mandal, P.K. Meat-borne Pathogens and Use of Natural Antimicrobials for Food Safety. In Foodborne Pathogens and Antibiotic Resistance, O.V. Singh (Ed.). doi:<u>10.1002/9781119139188.ch10</u> (2017).
- Nicoletti, C. F., de Oliveira, B. A. P., Barbin, R., Marchini, J. S., Junior, W. S., & Nonino, C. B. Red meat intolerance in patients submitted to gastric bypass: a 4-year follow-up study. *Surg Obes Relat Dis.* 11(4), 842-846 (2015).
- De Lima Binsfeld, B., Pastorino, A. C., Castro, A. P. B., Yonamine, G. H., Gushken, A. K. F., & Jacob, C. M. A. Knowledge of industrialized dairy products labels by parents of patients allergic to cow's milk. *Rev Paul Pediatr.* 27(3), 296-302 (2009).

Comment:

Pag1 Line 34 "are SOMETIMES preferred"

Response:

Page 1, line 34, the rewritten sentence is "Although, sometimes perceived as lacking optimal textural properties, plant proteins are sometimes preferred over the animal-based proteins due to safety or health considerations, biocompatibility and sustainability of production [4]."

Comment:

Page1 Line 39 please add a reference for crosslinking of biological tissues using genipin <u>Response:</u>

The reference (ref 6) below is added on page 1, line 40 of the revised manuscript.

6. Johnston, S. P., Nickerson, M. T., & Low, N. H. The physicochemical properties of legume protein isolates and their ability to stabilize oil-in-water emulsions with and without genipin. *J Food Sci Tech.* **52**, 4135-4145 (2015).

Comment:

Pag1 Lines 42-43 It does not look like that the references 4-6 speak about a "favourable comparisons with dairy proteins and BSA"

Response:

We have added ref 10 on the comparison between soy glycine with dairy protein and BSA. 10. Savadkoohi, S. & Kasapis, S. High pressure effects on the structural functionality of condensed globular-protein matrices. *Int. J. Biol. Macromol.* **88**, 433–442 (2016).

Comment:

Page1 Line 43 References 4-6 refers to BSA and milk protein not to vegetable proteins. Response:

These references (previously ref 4-6) have been changed to ref 7-9 to reflect the structural properties of plant protein due to high pressure, ultrahigh-temperature and ultrasound treatments as follows:

- Condés, M. C., Speroni, F., Mauri, A. & Añón, M. C. Physicochemical and structural properties of amaranth protein isolates treated with high pressure. *Innov. Food Sci. Emerg. Technol.* 14, 11–17 (2012).
- Jian, H. et al. Gelation enhancement of soy protein isolate by sequential low- and ultrahigh-temperature two-stage preheating treatments. Int. J. Food Sci. Technol. 49, 2529–2537 (2014).
- Xiong, T. *et al.* Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. *Food* Res. Int. 109, 260–267 (2018).

Comment:

Page 2 Lines 9-10 This sentence misses something. Maybe you meant "In the presence of plasticizers the water binding capacity is altered and the overall mechanical properties of edible biofilms are improved [9,10]". Moreover, are you sure it is an "edible biofilms" they are writing about? To me, it seems more a protein-based plastic they are talking about.

Response:

Page 2 line10, the text has been changed to "Addition of plasticizers altered the water binding capacity and improved the overall mechanical properties of pea protein films".

Comment:

Page2 line29 Since it is a review you should write" to explore the actual knowledge concerning the relationship among"

Response:

Page2 lines 29-31, the sentence has been rewritten as "we shall attempt to explore the actual knowledge concerning the relationship among gel formation, phase separation and vitrification of plant protein matrices and controlled release of bioactive ingredients in added value food systems."

Comment:

Page 2 line31"will be complemented"

Response:

Page 2 line31, the word "complimented" has been replaced with "complemented" in the sentence.

Comment:

Page 2 line38, since ref 25 is a book can you please provide the chapter in which the sentence in lines 36-38 is reported?

Response:

Ref 29 (previously ref 25) has been replaced with:

 Savadkoohi, S., Bannikova, A. & Kasapis, S. Glass Transition of Globular Proteins from Thermal and High Pressure Perspectives. In *Glass Transition and Phase Transitions in Food and Biological Materials*, Jasim Ahmed, Ed, Wiley, New York, USA, 49-117 (2017).

Comment:

Page3 line 28, please replace "signified" with "indicated as/called"

Response:

Page 3 line 28 (now line 34), the word "signified" is replaced with "indicated" in the sentence.

Comment:

Page 4 lines 9-10 Please add a reference.

Response:

Page 4 lines 9-10 (now line 13), ref [49] is added.

49. Manassero, C. A., Vaudagna, S. R., Añón, M. C. & Speroni, F. High hydrostatic pressure improves protein solubility and dispersion stability of mineral-added soybean protein isolate. *Food Hydrocoll.* **43**, 629–635 (2015).

Comment:

Page 4 lines 24-25 I do not understand the sentence "that parallels the behaviour of the atmospheric (non thermally treated) counterparts"

Response:

Page 4 lines 31-32 (previously lines 25-28), the sentence means that either pressurised or non thermally treated samples largely maintained their native state with only 20% denaturation.

Comment:

Page 4 line 28 From Figure 1 it does not look like that there is a "preservation of native conformation, soy glycinin at 80% (w/w) solids"

Response:

From Figure 1, it is clear that the percentage of denaturation is about 20% at 80% (w/w) solids, which is well below the percentage of denaturation of samples with < 70% (w/w) solids, being almost 100%.

Comment:

Page 5 line 30 since it is the first time you use this acronym in the text you should explain it : Ultra-high-pressure homogenization (UHPH)

Response:

Page 5 lines 21-23, text explaining the Ultra-high-pressure homogenization (UHPH) is added: "Plant proteins from cereals, legumes and nuts have been subjected to high pressure homogenization prior to UHT treatment, in an effort to reduce their particle size, known as ultra high pressure homogenization (UHPH) at 300 MPa [57–59]."

<u>Comment:</u> Page 6 line 12 "oxidation." <u>Response:</u> Page 6 line 24 (previously line 12), is now "oxidation." - thank you.

<u>Comment:</u> Page 6 Line 19" 30 °C" <u>Response:</u> Page6 line 31(previously line 19), is now "30 °C" – thank you.

Comment:

Page 8 line 17 reference [81] is about sodium caseinate, does it somewhere state that plant-sourced proteins have quality at parity to that of animal sources"?

Response:

Page 9 lines 5 (previously page 8 line 17), this reference has been removed. The comparison between the functional properties of plant and animal proteins is discussed in ref 79 (previously ref 80).

<u>Comment:</u> Page8 line17 "focuses on" <u>Response:</u> Page 9 line 5 (previously page 8 line 17), is now "focuses on"; thank you.

<u>Comment:</u> Page 8 Line18 "C. album" <u>Response:</u> Page 9 Line 7 (previously page 8 line 18), the text has been extended to "... protein isolate from <u>Chenopodium album seed...</u>"

Comment:

Page 8 Line 21 Ref [79] is about "black bean protein isolates" not C. album.

Response:

References for the black bean and *Chenopodium album* protein isolate have been arranged accordingly, as reflected in page 9 lines 7-9 (previously page 8 line 21).

Comment:

Page 8 Line 44 The dissociation constant for equation (1) is $K_d=[PL]/([P][L])$, this equilibrium constant is related to the forward and backward rate constants, k_{on} and k_{off} of the reactions involved in reaching equilibrium: $K_d=k_{on}/k_{off}$. Thus k_{on} and k_{off} are not K_b and K_d . Unless those under quoted "b" and "d" means something different to you.

Response:

 K_b and K_d have been removed from page 9 line 34 (previously page 8 line 44) to avoid symbol confusion.

<u>Comment:</u> Page 9 line 9 check the symbols. <u>Response:</u> Page 9 line 44 (previously line 9), the symbols have been corrected.

<u>Comment:</u> Page 9 lines 14-15 remove the colon "Gibbs free energy which," <u>Response:</u> Page 10 lines 22 (previously page 9 lines 14-15), done, as requested.

<u>Comment:</u> Page 9 lines 23 why "argues"? <u>Response:</u> The word "argues" (now in page11 line 25) was replaced with <mark>"suggests"</mark>

<u>Comment:</u> Pag10 line 8 "bonding" <u>Response:</u> The text " ... the negative value of the Gibbs free energy of binding" (previously on page 10 line 7, now in page 10 line 22) is correct. Comment:

Page 18 line 11 Please check the capital letters for the authors' name Response:

Previously ref 18 (now ref 22), the capital letters for the authors' name are correct.

Comment:

Line 35 ref [39] is a chapter $(n^{\circ}3)$ of a book, please modify the reference.

Response:

The modified reference format is given below (now ref 29):

29. Savadkoohi, S., Bannikova, A. & Kasapis, S. Glass Transition of Globular Proteins from Thermal and High Pressure Perspectives. In *Glass Transition and Phase Transitions in Food and Biological Materials*, Jasim Ahmed, Ed, Wiley, New York, USA, 49-117 (2017).

Reviewer #2

Comment:

The authors did a good review. Generally, it is interesting and well written. However, the following major points should be fixed.

Response:

We thank the Reviewer for the positive opening remarks and have addressed the remainder of his comments as follows:

Comment:

1.Figure 1 is very confusing. The authors should explicitly introduce the meanings of the curves. <u>Response:</u>

The explanation of the figure has been given on page 4, lines 30-39 (previously lines 22-32): "As shown in Figure 1, this source of plant protein remained largely in the native state (about 20% denaturation) in condensed preparations of 70-80% (w/w) solids that parallels the behaviour of the atmospheric (nonthermally treated) counterparts. This behavior is intermediate of whey proteins undergoing almost complete denaturation up to 70% (w/w) solids and BSA that retains native conformation due to the presence of seventeen disulphide bridges in the molecule [10]. Despite the preservation of native conformation, soy glycinin at 80% (w/w) solids is able to form coherent networks exhibiting comparable mechanical strength and glass transition temperature to that of the thermally denatured counterparts, arguing that pressurisation provides both structural functionality and bioactivity in formulation engineering [12]." The figure clearly makes the comparison of the % denaturation of soy glycinin to whey protein (shown as the \blacktriangle symbol), ovalbumin (shown as the \blacklozenge symbol).

Comment:

2.Protein-ligand interactions play an important role. However, there are some computational systems can analyze the proteins sequences, such as BioSeq-Analysis, BioSeq-Analysis2.0, DeepSVM-fold, etc. These related tools should be introduced and discussed.

Response:

Computational systems such as BioSeq-Analysis, BioSeq-Analysis2.0, DeepSVM-fold are useful tools to automatically generate various predictions for amino acid sequence analysis at both residue level and sequence level for the primary structure of the protein. As for the protein-ligand interactions, changes occur at the secondary and tertiary structures of the protein. These protein sequencing

softwares are also complex and require a paper-length discussion on their own. In this part, we focus on molecular docking in relation to selected software utilised in literature.

Page 11, lines 34-41, we added text and references as follows: "In this model, the protein tends to form a flexible structure and the interacting ligand will induce conformational changes on the protein structure towards a state of thermodynamic equilibrium to accommodate the ligand [97]. Currently, there are over 75 types of software available to simulate molecular docking of protein. Some of them namely AutoDock, DOCK, GOLD, ICM, Glide, Surflex, Affinity, LigandFit, Discovery Studio and many others are well reviewed in literatures [102, 104, 105]. The most recent docking programs in the last 5 years are CABS-dock, FlexAID, GalaxyPepDock, LightDock and MOLS 2.0 [106-110]. Some popular molecular docking simulations use the CDOCKER protocol that identifies a number of possible binding configurations, known as poses."

104. Glaab, E. Building a virtual ligand screening pipeline using free software: a survey. *Brief. Bioinform.* 17, 352-366 (2016).

- 105. Jamkhande, P. G., Ghante, M. H., & Ajgunde, B. R. Software based approaches for drug designing and development: A systematic review on commonly used software and its applications. *Bull. Fac. Pharm. Cairo Univ.* 5, 203-210 (2017).
- 106. Blaszczyk, M., Ciemny, M. P., Kolinski, A., Kurcinski, M., & Kmiecik, S. Protein-peptide docking using CABSdock and contact information. *Brief. Bioinform.* 20, 2299-2305(2019).
- 107. Morency, L. P., Gaudreault, F., & Najmanovich, R. Applications of the NRGsuite and the Molecular Docking Software FlexAID in Computational Drug Discovery and Design. In *Computational Drug Discovery and Design*. Humana Press, New York, NY. 367-388 (2018).
- 108. Lee, H., & Seok, C. Template-based prediction of protein-peptide interactions by using GalaxyPepDock. In *Modeling Peptide-Protein Interactions*. Humana Press, New York, NY, 37-47 (2017).
- 109. Jiménez-García, B., Roel-Touris, J., Romero-Durana, M., Vidal, M., Jiménez-González, D., & Fernández-Recio, J. LightDock: a new multi-scale approach to protein–protein docking. *Bioinformatics* **34**, 49-55. (2018).
- 110. Paul, D. S., & Gautham, N. MOLS 2.0: software package for peptide modeling and protein-ligand docking. J Mol. Model. 22, 239 (2016).

Comment:

3. The format of the paper should be improved, such as on page 8, line 43.

Response:

The format of equation (1) has been improved in the revised manuscript, as follows:

$$P + L = \frac{k_{on}}{\overline{k_{off}}} P L$$

Comment:

4. The software tools used for drawing the figure 5 should be given.

Response:

Information on the software tool is given now in the legend of Figure 5 "3D docking model of rice glutelin-EGCG interaction simulated by Discovery Studio 3.0 (Accelrys Inc., USA)".

Comment:

5. In the conclusion section, I suggest the authors to give more a deeper discussion on the impact of this review paper on the related field.

Response:

We strongly feel that Conclusions concisely describe the impact of the review by emphasizing the importance of thermal and non-thermal processing of plant based materials in changing their structural characteristics leading to improvements in functional properties for novel product development. We further point to the various classical and modelling approaches that predict the affinities of plant protein molecules to ligands leading to model systems for further exploitation in various food and pharma applications.