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OPEN PEER REVIEW

Comparison of Portable and Benchtop Near-Infrared Spectrometers for the Detection of Citric Acid-adulterated Lime Juice: A Chemometrics Approach

Reza Jahani, Arash Mahboubi, Saskia van Ruth, Yannick Weesepoel, Martin Alewijn, Farzad Kobarfard, Mehrdad Faizi, Mohammad Hossain Shojaee AliAbadi, Azadeh Nasiri, Hassan Yazdanpanah

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1 Original submission

1 comment · 19 Jul 2022

Zahra Tamiji Reviewer

MINOR REVISION 19 Jul 2022

15-July-2022

Dear Dr. Samira Eslamizad
I am writing to you regarding the manuscript entitled " Comparison of portable and benchtop near-infrared spectrometers for the detection of citric acid-adulterated lime juice: A chemometrics approach".
The paper reports the application of NIRS to detect citric acid-adulterated lime juice. I have some suggestions for the authors to enhance the paper, as it can provide a useful reference for future work but needs some further details and information.

Methods:
How many samples were in the training and test sets? In the results, 60%-40% is mentioned, but please add this information to this section and mention the number of samples.
Is no preparation at all needed to analyze the samples? Is no filtration needed to remove the remaining pulp?
Can it be expected that the lime juice samples in this work represent the natural variability of limes? Why were the samples not collected from different locations? Analyzing them individually would help to get an idea about the natural variability. The process of collecting 16 samples is ambiguous and needs further explanation. Please explain the 16 samples of lime juice in more detail.
Results:
The percentage of correct classification (%CC) is the criterion used to compare classification results obtained with chemometric methods. I suggest that the authors also calculate correct classification and include it in the discussion. If the authors feel comfortable, I suggest a reference in which the authors use this parameter to discuss correct classification, but the authors may feel free to search elsewhere:
Ciosek, P., et al. "Direct and two-stage data analysis procedures based on PCA, PLS-DA and ANN for ISE-based electronic tongue—Effect of supervised feature extraction." Talanta 67.3 (2005): 590-596.

The use of variable selection methods leads to improved results for quantitative and qualitative analysis. The variable selection could be done on the basis of the study of spectral variance, which may directly influence classification. Spectral variance in one group and spectral variance for all data are important. Similarly, I suggested that the authors expand this discussion a little with more information about the main wavelengths and their link to organic bonds, elaborating on how they could influence the spectra and differentiate the samples.
In Table 2, the results of MSC and SNV preprocessing are completely equal. Why is SNV preferred?
Considering that the LC-MS/MS method is included in this article, it would be interesting to see its results and compare them with those of the proposed method as well.
In this paper, the proposed method has not been compared with other methods and other articles in terms of figures of merit.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

We sincerely thank the reviewers for their constructive criticisms and valuable comments, which were of great help in revising the manuscript. Accordingly, the revised manuscript has been systematically improved with new information and additional interpretations. We hope our revision has improved the paper to a level of their satisfaction. Number-wise answers to their specific comments/suggestions/queries are as follows. Necessary to note that the edited/modified items are highlighted in the text.

Response to Reviewer #1 Comments

Comment 1: *This manuscript presented the capability evaluation of a benchtop Fourier-Transformation-NIRS apparatus (FT-NIRS, 1000–2500 nm) and a portable short wave NIRS device (SW-NIRS, 740–1070 nm) for the discrimination of genuine and citric acid-adulterated lime juice samples. The paper is correctly written and presented. The results are quite interesting and the experiments were carried out pretty systematically. An interesting contribution was done by the authors and there is a good balance between the results and the rest of the work.*

Response: We are grateful to the reviewer for his positive and encouraging comment.

Comment 2: *Why the authors have not used unknown market samples to test and verify the developed methods in comparison with the LC-MS analysis?*

Response: We appreciate the reviewer's comment on this point. In this preliminary study, we aimed to compare the performance between the benchtop FT-NIRS and portable SW-NIRS approaches in combination with chemometrics techniques for the authenticity assessment of lime juice. In this regard, the results obtained from internal validation and external validation were compared. Samples were divided into the training set and test set and the models were constructed using only the training set. Since the test set samples are not used in the model construction, we believe that the test set could be almost representative for the unknown market samples.

Comment 3: *Figure 1. A. Correct the axes labels.*

Response: We are sorry for this. In the revised version, it was corrected.

Comment 3: *Several typos and grammatical errors have been marked in the manuscript that can be considered.*

Response: As per the reviewer's comment, it was corrected in the text.

Response to Reviewer #2 Comments

The paper reports the application of NIRS to detect of citric acid-adulterated lime juice. I have some suggestions for the authors to enhance the paper, as it can provide a useful reference for future works, but needs some further details, information.

Comment 1: *How many samples were in the training and test sets? In the result it is mentioned 60%-40%, but please add to this section, and mention the number of samples.*

Response: We are sorry for this missed information. In the revised version, it was included.

Comment 2: *Is no preparation at all needed to analyze the samples? No filtration to remove the remaining pulp*

Response: Juices were prepared using a cold press juicer machine and then were carefully homogenized to prepare a uniform blend of juice. Since the lime juice was completely homogenized and uniform, there was no need for any filtration step.

Comment 3: *Can it be expected that the lime juice samples in this work is representing the natural variability of limes? Why were the samples not collected from different locations? Analyzing them individually would help to get an idea about the natural variability. The process of collecting 16 samples is ambiguous and needs further explanation. Please explain the 16 samples of limes juice in more detail.*

Response: We appreciate the reviewer's view on this point. Since the lime fruits originated from Jahrom (Fars Province, Iran) are the most commonly used limes in the lime juice factory, all samples used in this study were originated from Jahrom. Samples were then authenticated by a botanist at the herbarium department of Shahid Beheshti University of Medical Sciences (Tehran, I.R. Iran). However, this is a preliminary study and further experiments are required with considerably more samples to confirm the real potential of this technology and to develop more robust prediction models.

Comment 4: *The percentage of correct classification (%CC) is the criterion used to compare classification results obtained with chemometric methods. I suggest the authors to also calculate correct classification, and include in the discussion. If the authors feel comfortable, I suggest some reference where the authors use these parameter to discuss the correct classification, but the authors may feel free to search somewhere else:*

Ciosek, P., et al. "Direct and two-stage data analysis procedures based on PCA, PLS-DA and ANN for ISE-based electronic tongue—Effect of supervised feature extraction." Talanta 67.3 (2005): 590-596.

Response: It is a good suggestion but one point to consider about the percentage of correct classification (%CC) and accuracy (%) is that normally both parameters are calculated based on the same equation. The correct classifications rate is obtained as follows: CC = Nc/(Nc + Nnc) where Nc is the number of correct classifications and Nnc is the number of incorrect classifications. Similarly, accuracy is obtained as follows: Accuracy = (TP + TN) / (TP + TN + FP + FN) where (TP+TN) is the number of correct classifications and (TP + TN + FP + FN) is the sum of correct classifications and incorrect classifications. Please kindly take a look at the following table where the obtained results for the percentage of correct classification and accuracy are exactly equal. However, in the current study, we decided to use accuracy to maintain uniformity when comparing our results to that of other studies.

		Benchtop FT-NIRS		Portable SW-NIRS	
Preprocessing (Number of LVs, Explained variance)		**Internal validation (Training set)**	**External validation (Test set)**	**Internal validation (Training set)**	**External validation (Test set)**
Raw data	Sensitivity (%)	53	70	41	64
FT-NIR (3, 100%)	Specificity (%)	56	83	67	57
SW-NIR (2, 100%)	Accuracy (%)	54	75	50	61
	Correct classifications (%)	54	75	50	61
	AUROC	0.58		0.49	
Smooth	Sensitivity (%)	80	80	41	64
FT-NIR (4, 100%)	Specificity (%)	67	86	67	57
SW-NIR (2, 100%)	Accuracy (%)	75	82	50	61
	Correct classifications (%)	75	82	50	61
	AUROC	0.71		0.42	
1st derivative	Sensitivity (%)	73	60	94	100
FT-NIR (3, 99.40%)	Specificity (%)	67	100	67	100
SW-NIR (4, 99.99%)	Accuracy (%)	71	76	85	100
	Correct classifications (%)	71	76	85	100
	AUROC	0.73		0.80	
2nd derivative	Sensitivity (%)	67	80	81	100
FT-NIR (3, 99.40%)	Specificity (%)	44	57	56	86

SW-NIR (2, 99.89%)	Accuracy (%)	58	71	72	94
	Correct classifications (%)	58	71	72	94
	AUROC	0.59		0.82	
MSC	Sensitivity (%)	80	90	94	73
FT-NIR (3, 100%)	Specificity (%)	100	100	67	86
SW-NIR (3, 100%)	Accuracy (%)	88	94	85	78
	Correct classifications (%)	88	94	85	78
	AUROC	0.87		0.84	
SNV	Sensitivity (%)	**80**	**90**	94	73
FT-NIR (3, 99.96%)	Specificity (%)	**100**	**100**	67	86
SW-NIR (3, 99.99%)	Accuracy (%)	**88**	**94**	85	78
	Correct classifications (%)	**88**	**94**	85	78
	AUROC	**0.87**		0.84	
SNV + 2nd derivative	Sensitivity (%)	67	70	**88**	**91**
FT-NIR (3, 95.26%)	Specificity (%)	56	57	**100**	**100**
SW-NIR (3, 99.97%)	Accuracy (%)	63	65	**92**	**94**
	Correct classifications (%)	63	65	**92**	**94**
	AUROC	0.66		**0.95**	

Comment 5: *The use of variable selection methods leads to improved results for quantitative and qualitative analysis. The variable selection could be done on the basis of the study of spectral variance, which may directly influence classification. Spectral variance in one group and spectral variance for all data are important. Similarly, I suggested the authors to enhance a little this discussion with more information about the main wavelengths and their link to organic bonds, elaborating on how they could influence the spectra and differentiate the samples.*

Response: We agree with the reviewer's comment on this point. However, following variable selection, we could not find any improvement in the obtained results. Discussion about the main wavelengths and their link to organic bonds are highlighted in the manuscript.

Comment 6: *In Table 2, the results of MSC and SNV preprocessing are completely equal. Why is SNV preferred?*

Response: Thanks for pointing this out. It was mentioned and discussed in the text that MSC and SNV preprocessing techniques deliver the same accuracy since both are scatter correction techniques and are applied to get rid of all effects that are unrelated to the chemical nature of the sample such as multiplicative effects.

Comment 7: *Considering that the LC-MS/MS method is included in this article, it would be interesting to see result of this and compare it with the proposed method as well.*

Response: Following LC-MS/MS measurement of citric acid and iso-citric acid as a gold standard method in the authenticity assessment of lime juices, samples were divided into two groups of genuine and adulterated based on the citric acid to iso-citric acid ratio. According to the Association of the Industry of Juices and Nectars (AIJN) reference guideline for lime juice, samples with citric acid to iso-citric acid ratio over 300 are considered non-authentic (adulterated) samples. This ratio for each sample is presented in Table 1.

Please kindly find more about the nature of samples in the article published by our team entitled "Detection of lime juice adulteration by simultaneous determination of main organic acids using liquid chromatography-tandem mass spectrometry".

Comment 8: *In this paper, the proposed method has not been compared with other methods and other articles in terms of figures of merit.*

Response: We agree with the reviewer's comment on this point. Accordingly, it was included in the result and discussion section.

2 Revision 1

2 comments · 6 Sep 2022 – 7 Sep 2022

Zahra Tamiji Reviewer

ACCEPT 6 Sep 2022

Dear Dr. Samira Eslamizad
The authors have satisfactorily responded to all my questions and made the necessary changes to the manuscript. This revision has significantly improved the manuscript. I would recommend the manuscript for publication.

Associate Editor

ACCEPT 7 Sep 2022

Dear Editor,
The comments were addressed, and the requested changes were made correctly. Thus, I agree to publish this paper.
Best regards,