Peer Review Report

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Message from the Peer Reviewer

Thank you for choosing Enago to assist you in peer reviewing. I have carefully reviewed your manuscript and have performed a comprehensive evaluation of your manuscript. Based on this evaluation, I have prepared this report that gives you my assessment of your paper, along with a list of problem areas and suggested revisions organized in an order of priority, to minimize chances of journal rejection.

Delivery Checklist

Items	Status
Peer Review Report	Delivered

Manuscript Details

Assignment Code	ABCDE-1	Number of Figures	3
Total Word Count	4,206	Target Journal	Archives of Oral Biology

Manuscript Title	----------
Journal URL	http://www.journals.elsevier.com/archives-of-oral-biology/

Detailed Review of Each Section

- **Title Page**

  Title:

  You also need to emphasize novelty, and the use for food industry. We suggest:

  "----------".

- **Abstract**

  Abstract: The abstract is not structured, but you still have to present these 4 sections:

  1) Background:

  After the text:

  Add the goal of the paper:

  "The most efficient method to produce L-cysteine is whole-cell assays using Pseudomonas strains, which is problematic given the infectious nature of these bacteria. ----------"

  2) Methods.

You forgot to include the Method section:

"The metabolites of γ-EC were identified by HPLC analysis following thermal treatment of purified compounds and yeast extract."

3) Results.

To indicate where the Results section begins, write:

"Results show that purified γ-EC was almost completed converted into L-cysteine in 2h by thermal treatment under acidic conditions (90°C; pH 5.0). Thermal treatment of yeast extract also supported the liberation of L-cysteine from γ-EC. Investigation of the mechanisms supporting this conversion supports primarily a two-step process..."

Do not describe all possible pathways. This is an Abstract. Stick to the major findings.

4) Conclusion

"This study suggests that the thermal treatment of a yeast strain overproducing γ-EC may constitute a new mechanism to generate L-cystenine for the food industry.

- **Introduction**

General statement:

We understand the comments of the reviewers very well. Most of the text is a list of foodstuff applications for cysteine, while you devote little time to explain the rationale and problematic to justify your study. Given the critical importance of this section, we devoted considerable time to show you how to improve the text in terms of focus and impact.

Suggestions:

Here is the general structure of an Introduction:

1) First Paragraph: Introduce the importance of cysteine in the food industry. Reduce this paragraph by ½. Remember, this is not a review of literature.

As a general guideline, here is our suggestion:

"L-Cysteine, one of the 20 natural amino acids, plays important roles in foodstuffs, with respect to food texture, color and flavor. For example, L-cysteine improves the rheological properties of bread, crackers, and cookies (Narpinder and others 2002; Bollain and Collar 2004) by reducing disulfide bonds in the dough, which relaxes gluten interactions (Bloksma and others 1990). In fruit juices, L-cysteine prevents browning of the product (Skalski and Sistrunk 1974; Montogmery 1983) and preserves flavor during during storage (Naim and others 1993a, 1993b). As new applications are continuously emerging for L-cysteine in the food industry (Starkenmann et al. 2008), the development of efficient production methods is becoming a priority."

2) Second Paragraph: Explains the importance of your study by explaining the current problem with L-cysteine production for food products. Consult the Introduction of this article as a source:

Huang Y. Et al. 2011. Optimization of enzyme-producing conditions of micrococcus sp. S-11 for Lcysteing

production. African J. Biotechnol. 10(4): 615-623

(http://www.academicjournals.org/AJB/PDF/pdf2011/24Jan/Huang%20et%20al.pdf)

We suggest a content along this line:

"Currently, four methods are being used to produce L-cysteine: hair hydrolysis (Hee et al., 1997), microbe fermentation, chemical synthesis (Maier and Winterhalter, 2001) and bioconversion of 2-amino-2-thiazoline-4-carboxylic acid by whole-cell catalysis (Sano and Mrrsugi, 1978). While the latter technique is favored for its high yield and low energy requirements, most bacterial strains belong to the highly infectious Pseudomonas family

(Hiroshi et al., 2002). For this reason, considerable effort is currently deployed to identify other high-yield sources of L-cysteine. "

3) Third paragraph: Explains the rationale of your study, which is essentially why the approach that you use to solve the problem is justified and plausible.

"We recently isolated a strain of yeast (Saccharomyces cerevisiae haploid strain Nα3) that accumulates γ-glutamylcysteine (γ-EC) (Nishiuchi et al., 2010), which is a precursor of Lcysteine (Ristoff and Larrson, 2007). ----------

In the present study, we tested the hypothesis that γ-EC is the metabolic source of L-cysteine in yeast extract, and that a strain which accumulates this precursor could represent a safe and high yield alternative to Pseudomonas-derived sources of the amino acid."

Ristoff E. & Larrson A. 2007. Inborn errors in the metabolism of glutathione. Orphanet J Rare Dis. 2: 16. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1852094/pdf/1750-1172-2-16.pdf

- **Methods**

General Guideline:

Start each paragraph by explaining why you are doing this.

Also, the sections are not in a comprehensive order. We suggest the following:

A - Chemicals

B - Thermal degradation of each compound

1) Start the text with a statement to explain why you did this.

2) Justify the list of metabolites. Why did you decide to detect these molecules? Are they abundant proteins in yeast? Your HPLC profiles do not show most of them.

3) Justify why you used a wide range of pH (3.0 to 7.0). Is it to represent the various pH conditions that would be encountered in yeast and food?

4) Justify 90ºC and pH 5.0. Are these the conditions used in the study "Munch et al. 1997"?

If this is the case, mention it here and add the reference. This will reassure the reviewers that you chose valid conditions for your study.

C - Measurements of cysteine, α-EC, γ-EC, and PyrCys contents

1) This first sentence would clarify your intentions:

"The concentrations of L-cysteine, α-EC, β-EC, and PyrCys derived from ABD-F were determined by derivatization of the samples, followed by HPLC analysis as we previously reported (Nishiuchi and others 2011a, 2011b)."

2) Since you already published the HPLC method, do not give details. Only mention what is not in your previous papers. You need to remove the rest of the text, so that the above sentence is all that you have in this section.

D - Measurements of glutamic acid, Leu, Phe, and Gly content

Explain why you measured these compounds in one sentence. Give a reference for this method. Do you mean "γ -GluLeu, γ -GluGlu, γ -GluPhe, and γ -GluGly"?

E - Measurements of of pyroglutamic acid content

Explain why you measured these compounds in one sentence, then give a reference for this

method. Do you mean "glutamic acid"?

F - Dynamics of γ-EC in yeast extract

1) Again, start by reminding us why you do this.

"Experiments were conducted to test whether heat degrades γ-EC into L-cysteine in the yeast strain we identified as a rich source of this precursor (Nishiuchi et al. 2010). "

2) Question: In your description of the culture protocol for yeast, what was the pH of the culture medium? Was it different than 5.0? "

3) Clarify your protocol because you use 2 different incubation temperatures. Does this

text make sense?

"The cell suspension was heated at 70°C for 10 min to cause lysis, and the yeast extract was collected by centrifugation. The supernatant was adjusted to pH 5.0 with 1N HCl, and heated at 90°C for up to 4h (not 1h) to determine the impact of heating on γ-EC metabolism".

3) Question: Justify 90ºC and pH 5.0. Are these the conditions used in the study “Munch et al., 1997”? If this is the case, mention it here and add the reference. This will reassure the reviewers that you chose valid conditions for your study.

4) Finish the last sentence by specifying the method of analysis:

“...cooled, the samples were analyzed by HPLC.”

Conclusion: By now, you should understand that your use of a range of pH (3.0-7.0) to analyze the metabolites is confusing during the entire paper. Justify!!

G - Statistical Analysis

Add that the degradation efficiency of α-EC, γ-EC, glutamic acid, PyrCys, γ -GluLeu, γ - GluGlu, γ -GluPhe, and γ -GluGly into L-cysteine will be compared by One-way ANOVA, followed by T-tests.

Discussion

A - General Guideline:

To know how to organize this section, remember your hypothesis:

“Heat denaturation converts yeast γ-EC into L-cysteine”.

Then, go through your data in a logical order to verify this hypothesis.

Here are the new sections we propose for the “Results and Discussion”:

1) Standardization of Metabolite Identification by HPLC Demontrate that you can identify by HPLC the major metabolites of γ-EC using the ABD-F derived preparations. Replace Figure 2 by HPLC profiles showing single peaks for all the molecules that are potential sources of L-cysteine in yeast.

2) Production of L-cysteine by Thermal Treatment

These are the heat degradation that you conducted for each purified cysteine-rich molecule proven to be abundant in your yeast strain:

Question 1: Why did you test 3 different pH conditions?

“Figure 1” shows degradation profiles at pH 3, 5 and 7, which is irrelevant because you only conduct the yeast extract experiments at pH 5.0.

Question 2: Why did you conduct time-courses for all molecules, but not pyroglutamic?

Suggestion 1: Prepare a new Figure 1 comparing the heat degration profiles of the sources of L-cysteine in yeast (α-EC, γ-EC, glutamic acid, PyrCys, γ -GluLeu, γ -GluGlu, γ -GluPhe, and γ -GluGly) at a concentration of 1 mM and pH 5.0.

Suggestion 2: Prepare a new Table comparing the concentration of L-cysteine generated from each substrate (α-EC, γ-EC, glutamic acid, PyrCys, γ -GluLeu, γ -GluGlu, γ -GluPhe, and γ -GluGly) at a

concentration of 100 mM and pH 5.0 after 4h. Since you conducted these experiments at 3 times, conduct statistical analysis to determine which product generates the most L-cysteine (one-way ANOVA, followed by T-tests).

3) Thermal Treatment of Yeast Extract Converts γ-EC into L-Cysteine.

Figure 3 only monitors γ-EC and L-cysteine concentrations in yeast extract during 4h (90ºF; pH 5.0) because you identified γ-EC as the predominant source of the amino acid in Figure 1 and the new Table 1. This is confirmed by the fact that the total concentration of the two molecules decreases by < 10%, and the loss in γ-EC corresponds to the gain in L-cysteine.

The text is in your original section named "Expanding the degradation for Foodstuff".

4) Mechanism of γ-EC Degradation into L-cysteine

Paragraph 1: Here is how we would arrange it to clarify the text for the reviewers:

"The current literature supports the following mechanism for the thermal degradation of γ-EC into Lcysteine in yeast extract: pyroglutamylation of the N-terminal glutamic acid resulting in the formation of PyrCys, followed by hydrolysis of the peptide bond in PyrCys to form pyroglutamic acid and L-cysteine. First, studies showed that pyroglutamic acid N-terminal groups form spontaneously or by enzymatic catalysis, mostly from N-terminal glutaminyl residue and N-terminal glutamyl residue

(REF). ---------- Therefore, we hypothesized that the acidic conditions (pH 5.0) of the thermal treatment used in

the present study would support this two-step process in the production of L-cysteine from of γ-EC. "

This order allows the reader to follow you through the literature.

Under this first paragraph, show the 2 equations that support the two-step process for Lcysteine

production to justify the different moieties shown in Table 1, 2 and 3. Without these equations, it is impossible to follow the text in pages 10-12:

- Investigation on the mechanism of cysteine liberation from γ-EC (2): the relation with pyroglutamylation of N-terminal glutamic acid

- Investigation on the mechanism of cysteine liberation from γ-EC (3): comparison

with other g-glutamyl-di-peptides

Remove these titles and combine the texts in this new section we named: "----------

" (see above).

Paragraph 2: Focus on testing your hypothesis.

- In a new Table, place the γ-EC data from Table 1 and the data of PyrCys from Table 2.

Together, they show that the thermal degradation of γ-EC results in equivalent accumulation of L-cysteine and pyroglutamic acid, which acccounts for ≥ 95% of the substrate after 4 h.

The fact that the thermal treatment also supports the degradation of PyrCys into L-cysteine is consistent with the proposed two-step mechanism for the accumulation of L-cysteine in yeast extract, and that γ-EC is the primary source of the amino acid for this strain of yeast.

Question: Why didn't you measure PyrCys accumulation in the γ-EC assay?

B - Sections to Remove from the Original "Results and Discussion"

Based on the above guidelines, certain sections must be removed or merged with others.

1) Cysteine liberation from γ-EC in buffer solution (page 8)

This entire paragraph is irrelevant. All you did is confirm the study of Binkley et al. 1950 showing that thermal treatment under acidic condition supports the conversion of γ-EC into L-cysteine. Remove this entire paragraph and add this reference (Binkley et al. 1950) to the Methods ("Thermal degradation of each compound") to justify the experimental conditions.

2) Investigation on the mechanism of cysteine liberation from γ-EC (1): comparison with the structural isomer α-EC

The justification that you provide for conducting this experiment is not that α-EC may be an endogenous source of L-cysteine, but that you were curious about the structure requirement for the conversion. This experiment does not bring any information to test the hypothesis we present in the new "Introduction". While I understand that this project is part of your degree, not all your data can fit in this paper without losing focus on the goal and hypothesis. This was a major argument by the reviewers who rejected your paper. So you need to remove the entire section, and modify Figure 1-2 and Table as recommended in "General Guidelines".

3) Investigation on the mechanism of cysteine liberation from γ-EC (2): the relation with pyroglutamylation of N-terminal glutamic acid

- The small accumulation of glutamic acid is not enough to warrant your attention. Remove the entier paragraph starting with: "To support this hypothesis, firstly we need to evaluate a negligible amount of glutamic acid...".
- Remove the entire following paragraph starting with: "It was demonstrated cysteine liberation will generate pyroglutamic acid as mentioned above. Therefore,..." The data on Table 2 regarding PyrCys was incorporated in the new Table 1 and discussed above. As for the data on α-EC, we already argued that this is not a major source of L-cysteine in yeast, unless you have a reference for that. Remember, this is not a thesis. Focus on the goal of this
paper: "To test whether γ-EC is a major source of L-cysteine for the food industry."
- The entire idea of another pathway may be interesting for a thesis, but it seems to account for a minor fraction of the overall production of L-cysteine, which accumulates at 95% of the rate of γ-EC degradation. In table 3, do you mean that there may be a common first step and 2 second steps in your 2-step process? Be clear on that. Also, is that second mechanism is important? If it is less than 10%, remove Table 3 and all the associated text.

- **Conclusion**

  Condense the text presented in pages 13 and 14:
  "In this study, we have demonstrated that cysteine was efficiently liberated....Takagi, 2006."
  Include the remark:
  - Thermal treatment of Saccharomyces cerevisiae haploid strain Nα3 yeast extract liberates L-cysteine from γ-EC, which may constitutes a significant source of the amino acid for the food industry.
  Then finish by changing the text:
  "Our potential method...under progress."
  by a text more focused on a safer mechanism of L-cysteine production than the current Pseudomonas-based high-yield bioconversion method. This statement shows that you addressed the problem raised in the Introduction (chapter 2).

- **References**

  The reference list is complete. However, the format of the references is not correct. For example, the in-text citations should be superscript Arab numerals instead of Roman numerals.

- **Figures**

  The figures lack legends. Please provide them is a separate file.

- **Tables**

  No tables are present in this manuscript.

- **Format**

  Papers should be as concise as possible and, in view of the international character of the journal, English usages that may present difficulties to readers whose first language is not English should be avoided. The spellings used can be British or American, but must be consistent within the manuscript. Authors should express their own findings in the past tense and use the present tense where reference is made to existing knowledge, or where the author is stating what is known or concluded. Original papers should follow the pattern of: Introduction, Materials and Methods, Results or Findings, Discussion. Follow this order when typing manuscripts: Title, Authors, Affiliations, Abstract, Keywords, Main text (Introduction, Materials & Methods, Results, Discussion for an original paper), Acknowledgments, Appendix, References, Figure Captions and then Tables.

Quality of Research

- **Originality of research** [Rating: Good]

  The subject matter is intriguing, and the examples and supporting evidence are illustrative and engaging. Another observation is that the references seem "thin," and the paper could be strengthened in this

regard, too—more sources and more current sources (some of the journals listed in the References don't seem to exist any longer, as I could not find any websites for them online; other sources seem outdated). Also, it's not entirely clear what some of these sources actually are. All of this is to say, the level of scholarship could be raised/improved.

- **Significance to field** [Rating: Excellent]

The manuscript sheds significant light to the existing research. However, we strongly suggest you incorporate the proposed modifications.

- **Soundness of study design** [Rating: Fair]

The Methods section is missing. Also the Discussions section is not sufficiently detailed. We suggest you add the following to this section to make it stronger:
"Results show that purified γ-EC was almost completed converted into L-cysteine in 2h by thermal treatment under acidic conditions (90°C; pH 5.0). Thermal treatment of yeast extract also supported the liberation of L-cysteine from γ-EC. Investigation of the mechanisms supporting this conversion supports primarily a two-step process..."
"L-Cysteine, one of the 20 natural amino acids, plays important roles in foodstuffs, with respect to food texture, color and flavor. For example, L-cysteine improves the rheological properties of bread, crackers, and cookies (Narpinder and others 2002; Bollain and Collar 2004) by reducing disulfide bonds in the dough, which relaxes gluten interactions (Bloksma and others 1990). In fruit juices, L-cysteine prevents browning of the product (Skalski and Sistrunk 1974; Montogmery 1983) and preserves flavor during during storage (Naim and others 1993a, 1993b). As new applications are continuously emerging for L-cysteine in the food industry (Starkenmann et al. 2008), the development of efficient production methods is becoming a priority."

- **Overall Rating** [Rating: Fair]

The paper requires Advance Editing to remedy the many sentence-level errors as well as the lack of cohesiveness. I think there is much that is good about the paper in terms of the author's ideas and the supporting details that are provided.

Manuscript Quality

- **Clarity of presentation [Rating: Poor]**

The expression of ideas is very weak. This aspect of the paper requires a lot of attention. It is difficult to follow the author's line of thought because of the lack of clarity at the sentence level: missing words, misuse/lack of articles, punctuation errors, issues with verb tense, and grammatical and mechanical

errors. The formatting also could use some work, as it also hinders understanding. Some of the lists should possibly be bulleted. The sections and paragraphs could be made smoother, too, with better transitions and more explanations that tie together the author's thoughts. The writing is choppy, in other words, and this also contributes to the difficulty in understanding the paper.

- **Organization and Structure [Rating: Poor]**

  The Methods section is missing. Also the Discussions section is not sufficiently detailed. Also a detailed cover page needs to be prepared which should include the paper title, author names, author affiliations, running title, no. of words and figures/tables, address for correspondence and financial disclosures, if any.

- **Adequacy of literature review [Rating: Excellent]**

  The references present are sufficient.

- **Overall Rating [Rating: Fair]**

  The paper requires Advance Editing to remedy the many sentence-level errors as well as the lack of cohesiveness. I think there is much that is good about the paper in terms of the author's ideas and the supporting details that are provided.

Suitability to Journal

- **Journal Scope**

  This journal publishes high-quality scientific papers on food science and technology. The article will be suitable with the new abstract and introduction.

- **Journal Coverage**

  The journal is indexed in the following databases:

  Citebase

  Embase

  Index Copernicus

  MEDLINE

  Medscape

  OAIster PubMed

  PubMed Central

  Science Citation Index Expanded

  SCImago

  Scirus

- **Journal Quality**

  The impact factor of the journal is 3.97.
  It is international and interdisciplinary; it is a source used in other articles that are similar to this one.

- **Manuscript Compatibility**

  With your given requirement for fast publication, this is best suited with regard to acceptance, journal quality, and publication frequency. However, the current paper in its state needs to be modified for journal compatibility. For example, the paper should be double spaced. However, the current one is single spaced. Also the references need to be limited to 20.

Next Steps

The following are the three most important improvements that the author needs to make.

- Rewrite the Abstract and Introduction, as suggested above, to describe the importance of the study and clearly state the goal and hypothesis.
- Reorganize the Results and Discussion section as suggested to transform this “Thesis design” into a Scientific Paper.
- Emphasize in the conclusion the usefulness for the Food industry in terms of identifying a safer process to generate L-cysteine to improve food texture, color and flavor.

The following are the three most important strengths of this paper which the author should not lose in the process of revision.

- Recent identification of a new strain of yeast accumulating the precursor of L-cysteine.
- That yeast extract is not toxic or infectious like Pseudomonas.
- That L-cysteine production from yeast is a simple and efficient mechanism.

Current Manuscript Status and Recommendation

- Although your manuscript is well written, it needs to be formatted according to the journal guidelines before submission.
- Several grammatical errors were found that should be corrected before publication.
- Overall flow of the manuscript and writing style should be corrected before publication.

Recommendation: Use Advance Editing to resolve the above issues.

Our Commitment

Enago recognizes the need to deliver high-quality services and to guarantee the security of your information at all times. This belief is reflected in our commitment to establishing and maintaining systems processes that are compliant with the ISO quality and IT security management standards.

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