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Pre-submission Review Report

Message from the Peer Reviewer

Thank you for choosing Enago to assist you in peer reviewing. We have carefully reviewed your manuscript and have performed a comprehensive evaluation of your manuscript. Based on this evaluation, we have prepared this report that gives you an 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.

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Manuscript Details

Assignment Code	ABCDE-1	Total Word Count	4206
Manuscript Title	----------		
Journal URL	http://www.journals.elsevier.com/archives-of-oral-biology/		

Detailed Review of Each Section

- **Title Page**

  The title is concise and accurately conveys the principle content of the document. No contact details or affiliations are provided. Please make sure that a contact email address is added to the title page along with the affiliation of the author.

- **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-cysteine 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

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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 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 L-cysteine production. African J. Biotechnol. 10(4): 615-623
(http://www.academicjournals.org/AJB/PDF/pdf2011/24Jan/Huang%20et%20al.pdf)
We suggest 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 L-cysteine (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.
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.

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2) 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?
3) Justify 90ºC and pH 5.0. Are these the conditions used in the study "Munch et al. 1997"?
4) 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 - Dynamics of γ-EC in yeast extract

1) Again, start by reminding us why you do this.
2) "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). "

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 makes 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".

4) Question: Justify 90°C 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.
5) 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.

- **Results and Discussion**

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 demonstrates 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.

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Question 2: Why did you conduct time-courses for all molecules, but not pyroglutamic?
Suggestion 1: Prepare a new Figure 1 comparing the heat degradation 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) 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 L-cysteine 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 L-cysteine
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.
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.

- **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**

We have checked the journal guidelines in terms of the references and made some adjustments (see separate file of references for this article). One of the references (reference no. 17) seems to be missing in the text. I have placed additional comments in this regard in the document that lists the references. Please check and cite it in appropriate location in the text.

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- **Figures**

The figures lack legends. Please provide them is a separate file. A single figure is cited in the text and the figure is placed at respective location in the text. However, figures should be prepared in .pdf format and uploaded during the submission process.

- **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.

- **Style**

The document is adequately written. I have made a number of changes and suggestions in the document that will improve the general level of language. As mentioned above, the document is written in a clear and concise manner.

- **Authorship**

Please ensure that the authorship meets the criteria as described here http://wustl.edu/policies/authorship.html)

Quality of Research

- **Originality of research** [Rating: Excellent, Good, Fair, or Poor]

Fair. 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, Good, Fair, or Poor]

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

- **Soundness of study design** [Rating: Excellent, Good, Fair, or Poor]

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

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(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 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."

- **Ethical soundness** [Rating: Excellent, Good, Fair, or Poor]

This section is not applicable for this research.

- **Sufficiency of data analysis** [Rating: Excellent, Good, Fair, or Poor]

Good. There is sufficient data presented in the results and discussed afterwards. However, substantive editing is needed to enhance clarity.

- **Overall Rating** [Rating: Excellent, Good, Fair, or Poor]

Good. Overall, the research quality is good. The study presents an interesting objective and is designed to address and meet the aims of the research project.

Manuscript Quality

- **Clarity of presentation** [Rating: Excellent, Good, Fair, or Poor]

Poor. The expression of ideas is very weak. This aspect of the paper requires a lot of attention. While there are grammatical issues such as misspelled words, the clarity of the text is fair. English editing is suggested to make the text more readable and ensure it is clear what is the main idea and overall impact of the work.

- **Organization and Structure** [Rating: Excellent, Good, Fair, or Poor]

Good. The manuscript has all the key sections but would benefit from English editing to enhance flow and readability. Please check with the journal guidelines in case the total no. of words and figures/tables is to be provided in the title page.

- **Evidence supports** [Rating: Excellent, Good, Fair, or Poor]

Good. The author(s) sufficiently explains and interweaves contemporary and previous studies in the discussion. Ensure it is clear via submitting this text for English editing.

- **Adequacy of literature review** [Rating: Excellent, Good, Fair, or Poor]

Good. It seems the author has done a good job in referencing previous works and discussing previous studies in light of the currently presented research. Ensure all referencing requirements are met before final submission to whichever chosen journal.

- **Overall Rating** [Rating: Excellent, Good, Fair, or Poor]

Good. The manuscript's quality is good. Ensure the manuscript is submitted for English editing to address clarity issues stemming from grammatical errors.

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Suitability to Journal

- **Journal Scope and manuscript compatibility**

  This journal publishes high-quality scientific papers on food science and technology. The article will be suitable with the new abstract and introduction. Overall, the text is compatible with the journal's scope. With substantive English editing and ensuring all journal requirements are met, the text has a nice chance of being accepted.

- **Journal Coverage and manuscript compatibility**

  The journal is indexed in the following databases:
  Abstracts in Anthropology, Abstracts on Hygiene and Communicable Diseases, Aquatic Sciences and Fisheries Abstracts, BIOBASE, BIOSIS, Elsevier BIOBASE, Cancerlit, Current Contents/SciSearch Database, Current Contents/Science Citation Index, MEDLINE, Index Veterinarius, Science Citation Index, Biological Abstracts, CABI Information, Gale Database of Publications & Broadcast Media, Scopus, Global Health, ISI Science Citation Index, BIOSIS Toxicology, CSA Life Sciences Abstracts

- **Journal Quality and manuscript compatibility**

  The journal has an impact factor of 1.748 and takes 19.6 weeks from submission to first decision. It is an international and interdisciplinary journal. 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

- Overall flow of the manuscript, grammatical errors, and writing style should be corrected before publication.
  Recommendation: See Advance Editing

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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 and processes that are compliant with the ISO quality and IT security management standards.

Our relationship with you does not end here. This is just the beginning!

Enago is committed to help you get your research published and we will ensure that your future needs are met. For any assistance, simply mail us at publish@enago.com. Your dedicated assignment manager will be happy to help you with all your needs and queries.

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