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Tasty laboratory for cellular respiration: A preliminary integration of molecular gastronomy into general biology education

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Abstract

Dietary carbohydrates are broken down in cells to release energy through glycolysis, the Krebs cycle, and oxidative phosphorylation. The Krebs cycle, essential for energy production, is often difficult for students to understand due to its molecular complexity. Although numerous pedagogical strategies have been employed to improve learning outcomes, this study introduced a novel hands-on experiment that combines tasting activities with biology education. Students sampled organic acids central to the Krebs cycle, identifying distinct taste profiles beyond simple sourness. The sensory activity enhanced engagement and understanding, with participants describing it as both educational and intriguing. Post-activity feedback indicated improved conceptual retention by linking biochemical processes to sensory experience. This approach demonstrates that incorporating sensory perception activities inspired by molecular and physical gastronomy into laboratory exercises can make complex metabolic pathways more accessible and memorable, offering an innovative and experiential strategy for enhancing biology education.

Keywords

cellular respiration, hands-on experiment, Krebs cycle, molecular physical gastronomy, organic acid, sensory perception, sourness

Introduction

A significant proportion of the organic acids consumed through foodstuffs play crucial roles in various biological processes (Sun *et al.*, 2020). Organic acids are integral to energy metabolism, serving as substrates in biochemical pathways that produce energy. They also contribute to the regulation of pH levels and participate in

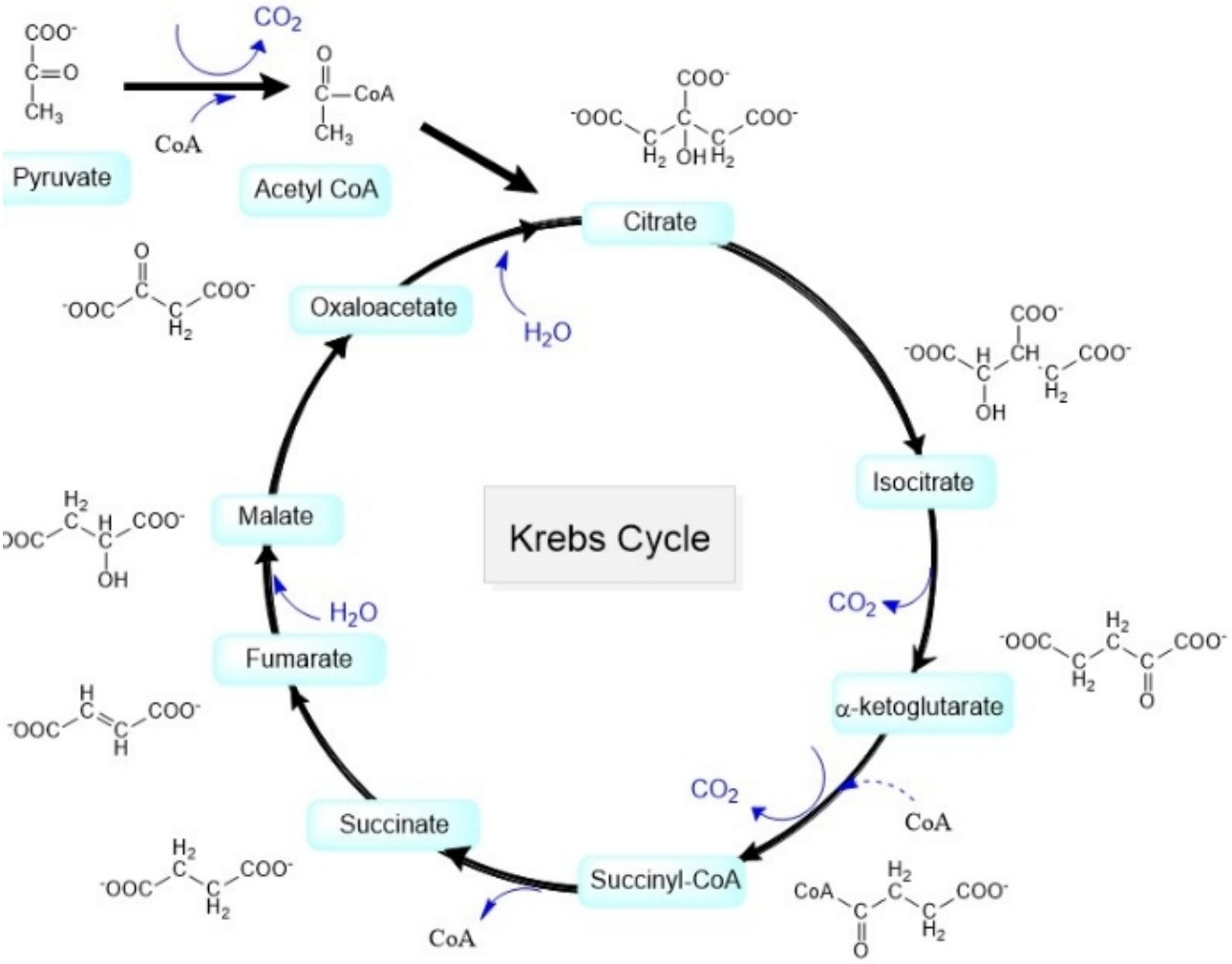

Figure 1. Pyruvate oxidation and the Krebs cycle. First, pyruvate is oxidized to acetate. Then, acetate is activated by coenzyme A, yielding acetyl-CoA. Acetyl-CoA then enters the Krebs cycle. The Krebs cycle, also known as the citric acid cycle, is a closed loop in which the final product reformats the initial molecules into citric acid.

essential metabolic processes necessary for sustaining life.

In biology education, cellular respiration is a central topic for studying energy generation, oxidation–reduction reactions, electron transport systems, and metabolic biochemical pathways (Akeroyd, 1983; Chris, 1985). Cellular respiration for energy production from D-glucose proceeds through three major stages (Cain *et al.*, 2017). The first stage, glycolysis, involves the breakdown of one glucose molecule into two pyruvate molecules. The second stage, also referred to as the Krebs cycle or citric acid cycle, completes the breakdown of pyruvate. The third stage, oxidative phosphorylation, generates the majority of adenosine triphosphate (ATP), the principal energy currency of living organisms.

The Krebs cycle enables cells to produce ATP through a series of oxidation-reduction and decarboxylation reactions. It is also known as

the tricarboxylic acid (TCA) cycle or citric acid cycle, as it begins with the formation of citric acid, which is subsequently oxidised to carbon dioxide (CO_2) (Akeroyd, 1983; Chris, 1985; Cain *et al.*, 2017). The cycle comprises multiple organic acid intermediates, including citrate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate (Figure 1).

Several of these compounds are familiar as organic acids encountered in everyday foods (Berlitz *et al.*, 2009; Coultrate, 2016). For instance, citric acid and malic acid are major contributors to the sourness of most fruits and fruit juices, and blackberries (*Rubus fructicosus*) are particularly rich in isocitric acid (Coultrate, 2016). Citric acid and acetic acid are among the most widely used organic acids in the food industry (Criminna *et al.*, 2017). Moreover, acetic acid, in the form of acetyl-CoA, also enters the Krebs cycle and contributes to ATP production.

Molecular and physical gastronomy has emerged as a field dedicated to the study of the chemical and physical transformations of molecules during cooking (This, 2002). It employs experimental and theoretical approaches to elucidate the mechanisms underlying culinary processes (This, 2009). According to This, molecular and physical gastronomy is proposed as a convergent scientific discipline for understanding culinary phenomena, analogous to how molecular biology provides a comprehensive framework for understanding biological processes (This, 2009; 2013). Molecular and physical gastronomy has also been successfully integrated into educational practices across various disciplines (Burke *et al.*, 2016; Burke and Danaher, 2018; Serna-Gallén *et al.*, 2022).

Biology is particularly well-suited to integration with molecular and physical gastronomy, not only because biological processes such as fermentation are closely linked to cooking, but also because flavour is fundamentally a biological process. Furthermore, cooking is a fundamental biological characteristic of humans (Wrangham and Conklin-Brittain, 2003). Consequently, a range of biological topics, including the Krebs cycle—the focus of this study—can be effectively explored through sensory-based approach grounded in molecular gastronomy principles. This approach provides opportunities to reflect on the origins of culinary ingredients, the sequential nature of biochemical reactions, and the broader significance of cycles in biological systems.

This study aims to develop a hands-on experiment for use in general biology classes to enhance students' understanding of the Krebs cycle and to increase their familiarity with its constituent molecules through a tasting activity involving the relevant compounds.

Materials and Methods

All materials used in this tasting experiment were of certified food-grade quality. D-Glucose and D-fructose were purchased from ES Ingredients (Korea), and the organic acid molecules involved in the Krebs cycle were obtained from Sigma-Aldrich (USA), including pyruvic acid (W297003), citric acid (W230618), succinic acid (W471920), malic acid (W265501), fumaric acid (W248800), and acetic acid (W200603). However, food-grade α-ketoglutaric acid and oxaloacetic acid were not available from this supplier and were therefore sourced from iHerb, Korea.

All ingredient materials required for the tasting test experiment were prepared as 2 % (w/v) solutions using bottled water (Nongshim Co., Korea), pH 6.9. Prior to the experiment, students drew the molecular structures on a worksheet (see Appendix) and familiarised themselves with the molecular configurations. The tasting was conducted following a modified protocol for the sensory evaluation of foods (Weaver and Daniel, 2003).

Before each tasting, students rinsed their mouths with bottled water, sampled one drop of the solution using either a cooking dropper or a cotton swab, rinsed again with drinking water, and then proceeded to the next solution. Students were instructed to compare and describe the perceived sourness of each solution on the worksheet.

Procedure

The objective of the experiment was to explore the range of tastes—particularly sourness—that can be elicited by various organic acid molecules, rather than to quantitatively determine the acidity of the substances tested. The tasting sequence followed the metabolic progression of cellular respiration, encompassing glycolysis and the Krebs cycle, and was conducted in the following order:

1. Taste one drop each of 2 % glucose, 2 % fructose, and 2 % pyruvic acid solutions; describe the sweetness and sourness.
2. Taste one drop each of 2 % acetic acid, 2 % pyruvic acid, 2 % succinic acid, 2 % α-ketoglutaric acid, and 2 % citric acid solutions; describe the perceived sourness.
3. Taste one drop each of 2 % acetic acid, 2 % α-ketoglutaric acid, and 2 % succinic acid solutions; describe the perceived sourness.
4. Taste one drop each of 2 % fumaric acid, 2 % malic acid, and 2 % oxaloacetic acid solutions; describe the perceived sourness.

Certain solutions, such as pyruvic acid and oxaloacetic acid, may cause notable irritation. Therefore, students were advised to have drinking water available for rinsing their mouths immediately after each tasting and prior to proceeding to the next sample. If a student experienced an adverse or overly sensitive reaction, they were encouraged to omit tasting that particular solution.

Results and Discussion

Cellular respiration constitutes a fundamental component of biology education, as it elucidates how cells generate essential energy from the materials ingested and digested by organisms. Using Campbell's Biology as the primary textbook (Cain *et al.*, 2017), the course emphasises theoretical understanding, supported by structured worksheets designed to promote autonomous student engagement. This study aimed to motivate students to connect sensory perception grounded in molecular gastronomy principles to their understanding of cellular respiration by adapting the worksheet to incorporate the tasting of molecular compounds.

The results were based on a case study analysis of 21 students enrolled in a general biology course during the first semester of the 2021 academic year at the Korea Science Academy of KAIST. Prior to the study, a pilot experiment was conducted with 10 students during the second semester of 2020. The pilot study served to identify procedural issues and was not included in the final analysis.

Before tasting the molecules involved in the Krebs cycle, students began with glucose, the most important sugar in the human diet. Glycolysis, the first stage of cellular respiration, involves the conversion of glucose to fructose and the subsequent breakdown into two pyruvate ions. Accordingly, students sampled 2 % glucose, 2 % fructose, and 2 % pyruvic acid solutions — the key molecular products of glycolysis. Tasting these sweet compounds also served to ease students' initial apprehension about sampling pure chemical substances. Most students found fructose to be sweeter than glucose, although two students reported no discernible difference. All students unanimously agreed on the pronounced sourness of pyruvic acid.

The Krebs cycle proceeds with the transfer of an acetyl group from pyruvate to oxaloacetate, mediated by acetyl CoA, resulting in the formation of citrate within the mitochondria (Cain *et al.*, 2017). The sequence of intermediates includes citrate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. To explore the relationship between the structure of Krebs cycle intermediates and their taste profiles, students sampled five selected molecules categorised according to their number of carbon atoms and carboxyl groups: a two-carbon molecule with one carboxyl group (C2, acetic acid); a three-carbon molecule with one carboxyl group (C3, pyruvate); a four-carbon molecule with two carboxyl groups (C4, succinic acid); a five-carbon molecule with two carboxyl groups

(C5, α-ketoglutaric acid); and a six-carbon molecule with three carboxyl groups (C6, citric acid) (see Table 1). The ranking results for perceived sourness varied considerably among students (*n* = 21). For example, 4 students ranked the acids in the following order of increasing sourness: C6 < C3 < C5 < C4 < C2. Meanwhile, 3 students reported the order as C2 < C6 < C4 < C5 < C3. All the other students answered in a different order. When identifying the most sour molecule, student responses were distributed as follows: C2 (*n* = 9), C3 (*n* = 5), C5 (*n* = 3), C6 (*n* = 2), and C4 (*n* = 1). For the least sour molecule, the responses were: C2 (*n* = 5), C6 (*n* = 3), C5 (*n* = 5), C3 (*n* = 4), and C4 (*n* = 4).

Table 1. The example of organic acids with different carbon numbers in this study.

Number of carbon atoms	**Example of organic acids**	**Formula**
Carbon two (C2)	Acetic acid	CH_3-COOH
Carbon three (C3)	Pyruvic acid	CH_3-CO-COOH
Carbon four (C4)	Succinic acid	HOOC-CH_2-CH_2-COOH
Carbon five (C5)	α-Ketoglutaric acid	HOOC-CH_2-CH_2-CO-COOH
Carbon six (C6)	Citric acid	HOOC-CH_2-C(OH)(COOH)-CH_2-COOH

Students' responses varied considerably and did not show a clear correlation with the number of carbon atoms or carboxyl groups present in the molecules. Consequently, it was difficult to establish a definitive ranking of sourness.
In the subsequent experiment, the tasting sessions were organised into two groups of organic acids based on their sequential appearance in the Krebs cycle. The first group included three organic acids: acetic acid (A), α-ketoglutaric acid (K), and succinic acid (S). The second group comprised fumaric acid (F), malic aid (M), and oxaloacetic acid (O). As shown in Table 2, the rankings of sourness from least to most sour for the first group yielded the following student responses: S < K < A (*n* = 5), K < S < A (*n* = 6), A < S < K (*n* = 4), S < A < K (*n* = 2), A < K < S (*n* = 2), and no response (NR) (*n* = 2).
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Table 2. Comparison of students' responses to sourness after tasting the three solutions S stands for succinic acid, K for α-ketoglutaric acid, A for acetic acid.

Rank of sourness	**Number of students (*n* = 21)**
S < K < A	5
K < S < A	6
A < S < K	4
S < A < K	2
A < K < S	2
No response (NR)	2

The most acidic compound was reported with the following frequencies: acetic acid (*n* = 11), α-

ketoglutaric acid (*n* = 6), succinic acid (*n* = 2), and no response (NR) (*n* = 2). The least acidic compound was reported as follows: succinic acid (*n* = 7), acetic acid (*n* = 6), α-ketoglutaric acid (*n* = 6), and NR (*n* = 2). These results may be attributed to the familiarity of the acetic acd flavor, whereas α-ketoglutaric and succinic acids represent unfamiliar flavors that are more difficult to describe. In particular, succinic acid exhibits a salty and bitter taste, which may have complicated students' evaluations (Da Conceicao Neta *et al.*, 2007). As shown in Table 3, the ranking of sourness from most to least sour for the second group of organic acids produced the following results: F < O < M (*n* = 5), F < M < O (*n* = 6), O < M < F (*n* = 4), M < F < O (*n* = 2), and NR (*n* = 4).

Table 3. Comparison of students' responses to sourness after tasting the three solutions. F stands for fumaric acid, M for malic acid, O for oxaloacetic acid.

Rank of sourness	**Number of students (*n* = 21)**
F < O < M	5
F < M < O 6	6
O < M < F 4	4
M < F < O 2	2
No response (NR)	4

Students reported that distinguishing between different sour flavours and articulating their taste experiences was challenging, with four students providing no response (*n* = 4). These students expressed considerable difficulty in describing the perceived flavours. As a result, the varied responses observed in the tasting experiments reflected differing sequences of perceived sourness, thereby preventing the identification of a definitive pattern.

Following the tasting experiment, students participated in a structured survey designed to evaluate learning outcomes and levels of engagement. A summary of selected student reactions is presented in Table 4. Although students' rankings did not correspond precisely with the actual sequence of the Krebs cycle, they reported that the tasting activity was both engaging and helpful in facilitating their understanding of the cycle.

Table 4. Student's responses to each question of the questionnaire

Survey question	**Examples of students' answers**
1. Did this experiment help you to remember the intermediates of the Krebs cycle?	1. It helped me memorize the order of the Krebs cycle intermediates. 2. The taste itself did not help, but it helped the memory by attracting interest. 3. Taste was very sour or weird but it helps to remember.
2. Did this experiment increase your interest in the Krebs cycle?	1. It was interesting to make the connection between taste and the Krebs cycle. 2. 2) It was interesting to know that such small changes to the structure makes these huge differences in taste and its role. 3. It intrigued me a lot about making cycle. I would like to make the artificial cycle with chemical compound if possible.
3. Was there any fear associated with tasting the chemicals used in this experiment? If so, please describe it.	1. I didn't have any fear about chemicals since it is not that different with what we eat every day but still some of them tasted so bad.

	2. Yes, we don't know how exactly, and in what condition the purification process the chemicals took, so despite being food level, we fear tasting them. 3. Yes, it is. Because the taste is too sour or weird.
4. Do you prefer the citric acid found in lemons or food-grade citric acid? If you have a preference, please explain why.	1. I would personally prefer the natural products. I prefer natural feeling but I can't exactly explain this 2. Since it has the same chemical formula, the substance itself wouldn't have any difference. 3. Chemical citric acid may be better since there's less chance of contamination.
5. Would you like to participate in other experiments involving the tasting of molecules? If so, which subject would you like to explore?	1. Yes, may be molecules in photosynthesis. 2. Yes, I'm interested in protein and amino acids 3. Yes, I want to try tasting the different carbohydrates (mono, di, oligo, poly-saccharides)

The first survey question asked whether the experiment assisted in memorising the intermediates of the cycle. The majority of students responded positively (n = 20), while one student indicated that the experiment did not aid their memory at all (n = 1). Regarding the second question, all respondents stated that the experiment was engaging, particularly due to its association with taste. One student provided a notably creative and insightful comment, suggesting the possibility of artificially constructing a similar cycle.

The third question addressed whether students experienced fear related to tasting chemical solutions. Most students reported no significant fear (n = 17), although four students expressed reluctance to taste chemical substances (n = 4). The fourth question explored students' preferences between citric acid derived from lemons and food-grade citric acid. Eight students reported no preference, acknowledging that both are chemically identical. However, 13 students preferred citric acid from lemons, perceiving it as more natural and healthier. This preference highlights a cultural tendency in Korea to regard whole foods as healthier than chemically derived products. A well-known example of this perception is the belief that monosodium glutamate (MSG) is harmful, despite longstanding scientific evidence affirming its safety (FDA, 2012). Although glutamate in natural foods and MSG are chemically identical, public scepticism persists (Niaz *et al.*, 2018).

In response to the final questionnaire item, students expressed interest in conducting future experiments on a range of topics, including molecules involved in photosynthesis, sweetness perception, various spicy compounds, biological polymers discussed in textbooks, amino acids and proteins, and the diverse flavours associated with fermentation.

Conclusion and Perspectives

The Krebs cycle - also known as the citric acid cycle or the tricarboxylic acid (TCA) cycle - features citric acid, a six-carbon compound, as a key intermediate. It represents the cyclical transformation of glucose, another six-carbon molecule, into various intermediates before regenerating into oxaloacetate (Cain *et al.*, 2017). This elegant biological process contrasts markedly with the direct and irreversible breakdown typical of chemical combustion. Its complexity posed a significant challenge to early researchers and ultimately led to its naming after Hans Krebs, who elucidated its mechanisms (Hagen, 1996). Consequently, understanding the numerical changes in carbon atoms is crucial for comprehending the cycle. The organic acids that

constitute the cycle vary in the number of carbon atoms and functional groups, and this experiment was designed to help students grasp these differences through taste.

However, in all of the taste experiments with three or five organic acids conducted in this study, the results failed to show any consistent relationship between students' perceived order of sourness and the actual sequence of intermediates in the cycle (Table 1, 2 and 3) This outcome likely stems from the inherent difficulty in evaluating sourness, which involves complex sensory processing and is challenging for untrained individuals (Da Conceicao Neta *et al.*, 2007). As such, students struggled to arrange the acids in order of perceived sourness. Nevertheless, despite the inability to correlate taste perception with molecular sequence, student feedback was overwhelmingly positive: 20 out of 21 participants reported that the tasting experience enhanced their understanding of the molecules they had previously studied in theory (Table 4). Future studies involving a larger number of participants across multiple classes may yield more statistically significant and meaningful results. Additionally, conducting taste tests after providing accurate information on the sensory characteristics of sweetness and sourness, along with the theoretical and measured pH values of the organic acid solutions, would enhance the experiment's educational value.

This study demonstrated the pedagogical value of integrating sensory perception and experiential tasting activities into biology education to support students' understanding of the Krebs cycle. By incorporating a structured taste experiment into a standard worksheet, the activity encouraged students to engage with the material both intellectually and experientially. Furthermore, the findings suggest that this form of experiential learning could be extended to other metabolic pathways, such as fatty acid synthesis and amino acid synthesis. For example, α-ketoglutarate is converted into glutamate via transamination, and glutamate is associated with the umami taste. This highlights the connection between metabolic intermediates and sensory–nutritional experiences, thereby broadening students' appreciation of biochemical processes.

This novel educational intervention bridged theoretical biochemical content with direct sensory perception, transforming the abstract concept of metabolic intermediates into tangible and memorable learning experiences.

Pedagogical Implications

Cellular respiration is a cornerstone of biology education, yet it is often taught through abstract theoretical instruction and the memorisation of structural formulas. A major limitation of this approach is that students frequently learn molecular sequences for the sole purpose of passing examinations, without developing a deep understanding of their biological relevance. By introducing a sensory dimension - specifically the tasting of organic acids - this study offered an experiential alternative.

Nevertheless, accurately distinguishing sourness proved to be challenging for the students because the perception of sour taste is not solely determined by the presence of hydrogen ions (H^+) but rather involves complex physiological mechanisms and multidimensional sensory input (Da Conceicao Neta et al., 2007). Furthermore, since the students had no prior experience with sensory testing, they struggled to express their impressions of the flavors. Training students to develop familiarity with these molecular flavours may, over time, enhance their memory retention and learning efficiency regarding the metabolic intermediates involved in biological pathways.

The transition from sweetness to sourness, experienced during the tasting experiments, exemplifies a fascinating molecular transformation that aligns with the principles of molecular gastronomy (This, 2009, 2013). It provides a novel perspective for interpreting intracellular processes such as glucose metabolism and parallels the transformative chemical reactions observed in culinary practices like fermentation. These reflections also prompt broader biochemical and evolutionary questions: Why does this cycle

exist? How is it so efficiently structured for energy production in living organisms?
In this context, molecular gastronomy, as an inspired interdisciplinary framework offers valuable opportunities for enriching biology education. It promotes innovative, multisensory teaching strategies that enhance student engagement, deepen conceptual understanding, and foster critical thinking across both classroom and laboratory settings.

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Appendix

Molecular Gastronomic Biology Worksheet: Glycolysis and Krebs Cycle

ID and Name:
(Please fill in.)

Objective
To understand the processes of cellular respiration and to experience the molecular components of glycolysis and the Krebs cycle through a tasting activity.

Procedure
Students will explore the molecular structures of intermediates in glycolysis and the Krebs cycle through tasting tests. Following the tasting session, students will review and reinforce their understanding of these metabolic processes by recalling the flavors associated with each molecule.

Notice: All tasting materials are prepared using certified food-grade molecules. If you have any dietary or other restrictions, that might preclude your participation, please inform your instructor prior to the activity.

Materials for Tasting Test
All solutions were prepared at a concentration of 2 % (w/v) using bottled water:
- Glycolysis intermediates: D- glucose, D-fructose (ES Ingredients, Korea), and pyruvic acid (Sigma-Aldrich, USA)
- Krebs cycle intermediates: α-ketoglutaric acid, oxaloacetic acid (iHerb, Korea), acetic acid, citric acid, succinic acid, fumaric acid, malic acid, and (Sigma-Aldrich, USA)

Grouping for Tasting Tests:
- Group 1: Glucose, fructose, pyruvic acid
- Group 2: Acetic acid, pyruvic acid, succinic acid, α- ketoglutaric acid, citric acid
- Group 3: Acetic acid, α- ketoglutaric acid, succinic acid
- Group 4: Fumaric acid, malic acid, oxaloacetic acid

Procedure:
Place one drop of each solution onto the provided small spoon, or wet a cotton swab and taste. Be sure to rinse between samples. If you feel any discomfort, rinse your mouth thoroughly with water.

Tasty Lab Activities

1. Molecular Structures and Sourness Prediction
Draw the chemical structures of the following molecules:
Glucose, fructose, pyruvate, acetate (substitute for acetyl-CoA), citrate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, oxaloacetate.
Predict the degree of sourness for each molecule.

2. Carboxylic Acids and Krebs Cycle Intermediates
Draw the chemical structures of the following carboxylic acids: Oxalic acid, maleic acid, malic acid, succinic acid, tartaric acid.

Identify which of these molecules serve as intermediates in the Krebs cycle.
3. Taste Testing Group 1: Glycolysis Molecules
Taste glucose, fructose, and pyruvic acid. Rinse between samples.
Rank them in ascending order according to:
Sourness:
Sweetness:

4. Taste Testing: Intermediates by Carbon Number
Taste the following intermediates in order:
Acetic acid (C2), pyruvic acid (C3), succinic acid

(C4), α-ketoglutaric acid (C5), citric acid (C6).

4.1 Sourness Rating:
Rate each sample using the following descriptive terms: none / slight / moderate / strong / extreme.

4.2 Sourness Comparison:
- Which sample is the most sour?
- Which sample is the least sour?

4.3 Krebs Cycle Sequencing:
List the molecules in the correct order of the Krebs cycle from acetate.

5. Taste Testing Group 3: Acetic acid, α-Ketoglutaric acid and Succinic acid
Rank the three samples in ascending order of sourness.
Rate each sample using the following descriptive terms: none / slight / moderate / strong / extreme.

After tasting, review and reinforce the Krebs cycle by associating the flavors with each intermediate.

6. Taste Testing Group 4: Fumaric acid, Malic acid and Oxaloacetic acid
Focus on sourness.
Rank the three samples in ascending order of sourness.
Rate each sample using the following descriptive terms: none / slight / moderate / strong / extreme.

After tasting, review and reinforce the Krebs cycle by associating the flavors with each intermediate.

7**. Comparative Analysis**
Compare your tasting results to the order and characteristics of intermediates as presented in the textbook.

8. Reference textbook
Cain ML, Wasserman SA, Minorsky PV, Reece JB. 2017. *Campbell Biology (*11th ed), Pearson Education, 236–258.