HIGH SCHOOL EDITION
Journal of Student Research

Volume 12 Issue 4 (2023)

A Thorough Dissection on Lactic Acid Enantiomers: The Nutritional, Environmental, and Medical Aspect

Virginia Ding

Shanghai American School Puxi, China

ABSTRACT

The fermentation process is a wonder of nature. It has been applied by our ancestors for transforming food, such as converting milk to yogurt and cheese, grapes to wine, and wheat to beer. In this study, three brands of store-bought yogurt, Ahmoulé, Junlebao, and Classy Kiss, will be analyzed to quantitatively determine the total lactic acid (LA) concentration in yogurt, as well as the ratio of L to D lactic acid enantiomers using liquid and gas chromatography. The novelty of this paper lies in the versatility of lactic acid and its enantiomers. Understanding lactic acid also stems out into the environmental and medical fields. In the environmental aspect, the polymerized form of lactic acid, polylactic acid, is proficient for producing biodegradable plastics, alleviating urgent issues like plastic waste. In the medical aspect, lactic acid enantiomers can be studied to improve medicine and medical polylactic acid (PLA) can be used in various ways, such as drug-controlled release materials [1,2]. The conclusions drawn from the sample preparation of the total lactic acid concentrations supported previously published results [3]. However, there was a shocking discovery in the data regarding the L to D enantiomer ratio in commercially available yogurts that differed from past studies, showing significant progress that manufacturers have made in the nutritional value of yogurt. These novel findings and routes of thinking about how to fully utilize lactic acid are beneficial advancements for the scientific field.

Introduction

In the case of making yogurt, certain types of bacteria convert various forms of sugar, a type of hydrocarbon, into lactic acid. Lactic acid is the key ingredient to support the thickness, flavor, shelf life of yogurt, and more importantly, it has health benefits to humans. Fermented milk is coagulated and thickened by lactic acid due to the decrease in pH. Past studies show that there are always two different lactic acids in yogurt: L and D [3]. The L type and D type lactic acids are enantiomers due to a carbon atom within lactic acid that is connected to four groups, giving the chirality trait to the molecule [4]. Each enantiomer has a different impact on our bodies' health once consumed. The L enantiomer has a multitude of health benefits. For example, antibacterial properties and better metabolism comparative to the D enantiomer. On the other hand, D lactic acid is a toxic metabolite, which can cause issues for our health. Surprisingly, past studies on the enantiomer ratio of pointed out that yogurt had a high concentration, approximately 40%, of the D enantiomer. Such a high concentration of the “toxic” enantiomer is an alarming fact, bringing importance to this investigation. However, the study is relatively old, therefore shedding light on the current nutritional value of yogurt is important for consumer comfortability and clarity in the food industry.

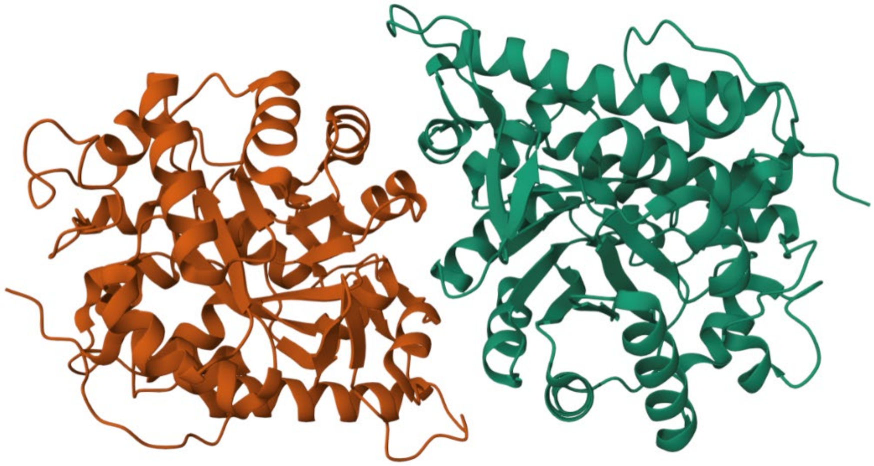

Figure 1

This figure is L –lactase oxidase with D-lactate in Aerococcus viridans, a member of the bacterial genus Aerococcus (Figure 1). As can be observed, the structure of the L and D lactase enantiomers are notably similar and are non-superimposable mirror images.

- **Method:** X-RAY DIFFRACTION
- **Resolution:** 1.38 Å
- **R-Value Free:** 0.224
- **R-Value Work:** 0.196
- **R-Value Observed:** 0.197

Methods

Liquid and Gas Chromatography

Gas chromatography and liquid chromatography are instruments for quantitative measurement of components in a mixture. The use of liquid chromatography is to separate and identify compounds in a sample. In this investigation, it is used to quantify the concentration of lactic acid in a sample of yogurt. The components move through the column at different rates, allowing them to be separated and identified. In the case of utilizing gas chromatography, the apparatus is efficient in recognizing the difference in the enantiomers' thermodynamic properties. This causes a difference in retention times, the length of time it takes for the sample to reach the end of the liquid chromatography column, between the two enantiomers. Important advantages of the separation of enantiomers by gas chromatography are high efficiency, sensitivity, and rapid separation [5,6].

HIGH SCHOOL EDITION
Journal of Student Research

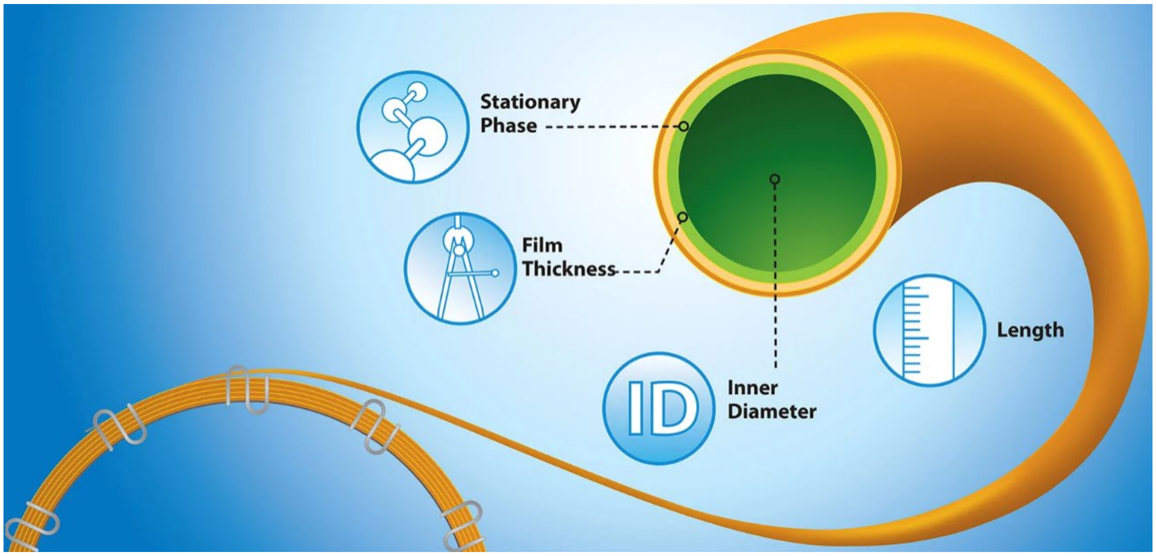

Figure 2

Sections of a LC/GC apparatus (Figure 2)

1. Injection port: This section of the liquid/gas chromatography pushes a sample through the chiral column.
2. Chiral column: A long tube that separates chiral molecules like lactic acid, consisting of two enantiomers. The chiral column achieves the separation through the stationary phase.
3. Stationary phrase: A very thin chemical coating on the inside of the tube that separates enantiomers by interacting with molecules differently, causing them to have different retention timers.
4. Detector: This last section is responsible for displaying the quantitative data of the LC/GC process.

Sample preparation (determining the total LA concentration)

Lactic acid is only a minor component in yogurt [3], so to determine the LA concentration with no interference of all other components (like proteins), a sample preparation method must be developed. The process is to extract LA by water and acetonitrile, causing precipitation of organic matters such as proteins to be removed by filtration. 2mL of purified water and 0.1 mL of concentrated HCl are added into a 0.5g sample of yogurt, which is then put into a sonic bath for 5 minutes. Then, 2mL of acetonitrile is added to the resulting mixture, achieving phase separation. Finally, filtration is performed to result in a clear sample for lactic acid analysis. For sample preparation, the liquid chromatography process was used, since the sample is yogurt.

HIGH SCHOOL EDITION
Journal of Student Research Volume 12 Issue 4 (2023)

Results

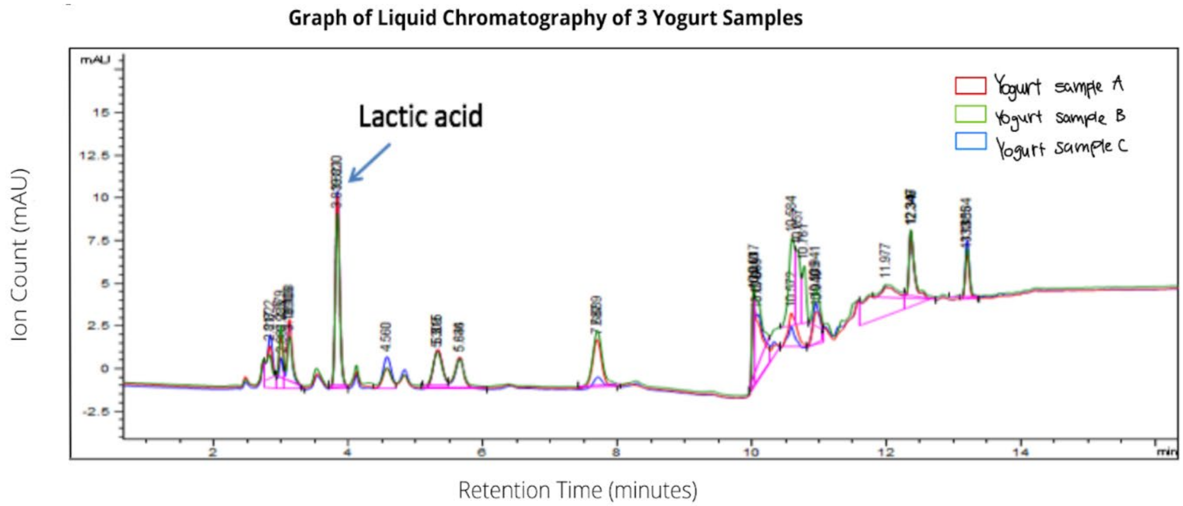

Figure 3

Annotations for Figure 3:

- Retention time of lactic acid is approximately 4 minutes (as seen with the peak in the graph).
- The other smaller peaks are the other minor components in the yogurt samples.
- The yogurt brands, Ahmoulé, Junlebao, and Classy Kiss, are Yogurt sample A, B, and C, respectively.

Liquid Chromatography Results (3 Yogurt Samples and 3 Calibrations with Standard Lactic Acid)

	Sample Name	Actual Added Value (%)	Theoretical Value (%)	Recovery Rate (%)	Sample Amount (g)	Peak Area	Concentration of Lactic Acid (%)
Sample (2μL)	Yogurt A: 2mL Water, 0.1mL HCl, 3mL Acetonitrile	0	0	0	0.5267	51.4	0.6147
	Yogurt B: 2mL Water, 0.1mL HCl, 3mL Acetonitrile	0	0	0	0.4790	45.9	0.6036
	Yogurt C: 2mL Water, 0.1mL HCl, 3mL Acetonitrile	0	0	0	0.4826	53.0	0.6917
	Yogurt A: 2mL Water, 0.1mL HCl, 3mL Acetonitrile + Standard	0.4181	0.4285	97.5649	0.4812	78.9	1.0327
	Yogurt B: 2mL Water, 0.1mL HCl, 3mL Acetonitrile + Standard	0.4212	0.4285	98.2887	0.4782	77.8	1.0247
	Yogurt C: 2mL Water, 0.1mL HCl, 3mL Acetonitrile + Standard	0.4111	0.4285	95.9468	0.4883	85.5	1.1029

Figure 4

Annotations for Figure 4:

- The peak area is linear to the amount of lactic acid.
- The concentrations of lactic acid in all three yogurts are remarkably similar (around 0.6-0.7%), which were the concentrations described in past studies [3].

With the quantitative data for the total lactic acid concentration in the three yogurt brands, the experiment can proceed onto the experimental procedure using gas chromatography to determine the L vs. D lactic acid enantiomer concentration in each yogurt sample. Besides gas chromatography recognizing the difference in the enantiomers' thermodynamic properties, causing a difference in retention times, gas chromatography produces exceptionally clear data peaks.

Experimental procedure for Gas Chromatography (L vs. D lactic acid concentration)

Through gas chromatography, the L and D lactic acid can be separated and measured with a chiral column. The column was 200 degrees Celsius, 30 meters long, a diameter of 0.25mm, with a stationary phase of 0.25 micrometer thickness. The chiral column was first doped with pure L lactic acid, and then pure D lactic acid to see what their retention times are. Then, the three yogurt samples are put into the column to see if they have a retention time at only pure L lactic acid, only pure D lactic acid, or both L and D. Those three possible scenarios will lead to different conclusions about the lactic acid enantiomer ratio thus the nutritional value in those commercially available yogurts. 3 trials were conducted, and results were consistent with the representative graph below (Figure 5).

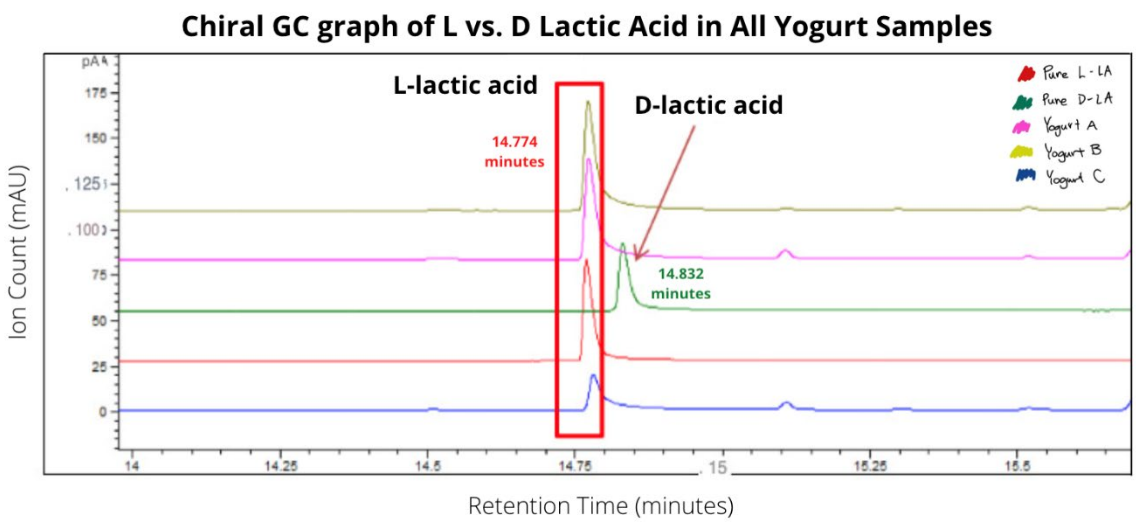

Figure 5

Discussion

Nutritional Aspect

With liquid chromatography, the concentration of lactic acid in the yogurt samples was quantified. The values were all remarkably similar to past studies, verifying the data [3]. This shows that liquid chromatography is a precise and reliable instrument for quantitative analysis. However, the novel finding is the ratio of the two enantiomers rather than the overall concentration. As seen in the graph, the retention time of pure L lactic acid is 14.77 minutes. Similarly, the 3 yogurt samples all have a retention time of 14.77 minutes as well. Therefore, it can be concluded that yogurts' lactic acid concentration is purely dominated by the L enantiomer. To further support this conclusion, the line graphs of the yogurt samples did not peak when pure D lactic acid peaked (14.83 retention time), showing that there is no D lactic acid in any of the yogurts. This also demonstrates that the GC method with the chiral column is reliable and can achieve complete separation of two lactic acid enantiomers. Finding that the three yogurts all have only L lactic acid is a fascinating discovery as it suggests that since the last studies on lactic acid in commercially available yogurts, yogurt companies have been very selective and careful with the yeasts they choose to make their yogurts. Essentially, yogurt manufactures were able to eliminate the D enantiomer from commercially available yogurt. The graph completely refutes the background information and the first part of my hypothesis, where it was discussed how all yogurts tested produced lactic acid with both enantiomers. Even so, Alm stated that "In yogurt, about 400 mg/100 g, or about 40%, of the total lactic acid was D (−) isomer" [3].

This data indicates that the yogurt samples are all equally nutritious in terms of lactic acid, as it contains only L, the enantiomer with many health benefits. This discovery calls for further investigation, where the next possible step could be to find the best yeast with not just the healthiest lactic acid enantiomer composition but also the right bacteria, as the variety of bacteria in yeasts have different effects on nutritional value. Lactic acid is also applicable to give benefits to humans and animals when creating medicine and biodegradable plastics with polylactic acid.

Environmental Aspect

Plastic waste is a prevalent issue, and its effect can be huge. Not only is it bringing damage to bodies of water and the creatures living there, sunlight and heat causes the plastic to release powerful greenhouse gases, thus worsening global warming as time goes on. Yet, "the fossil fuel industry plans to increase plastic production by 40 percent over the next decade. These oil giants are rapidly building petrochemical plants across the United States to turn fracked gas into plastic. This means more toxic air pollution and plastic in our oceans" [7]. This notorious material is used so often, and we rely on it so much; but since it is lightweight, plastic floats from landfill into drains and bodies of water, like our oceans. Biodiversity, specifically habitat and species diversity are damaged by plastic in the ocean, and animals like sea turtles choke on it or get tangled in plastic waste. The consumed plastic blocks the intestinal route of sea animals, disallowing them to feed, and can lead to fatality; but even when they do not die, the blockages will take a toll on their growth and reproduction rates.

A realistic solution that has already been implemented, utilizing lactic acid, is Polylactic Acid biodegradable plastic (Figure 6). Lactic acid can be made into PLA which can then be transformed to biodegradable plastic. Polylactic acid is initially made from plants that are fermented into plant starch. The plants can range from corn to maize to sugarcane, etc. The sugar components in these plants are turned into lactic acid, which is then made into polylactic acid through a process called polymerization. This process is when a large quantity of monomer molecules reacts together and chemically combines to become a chainlike molecule, which is also known as a polymer.

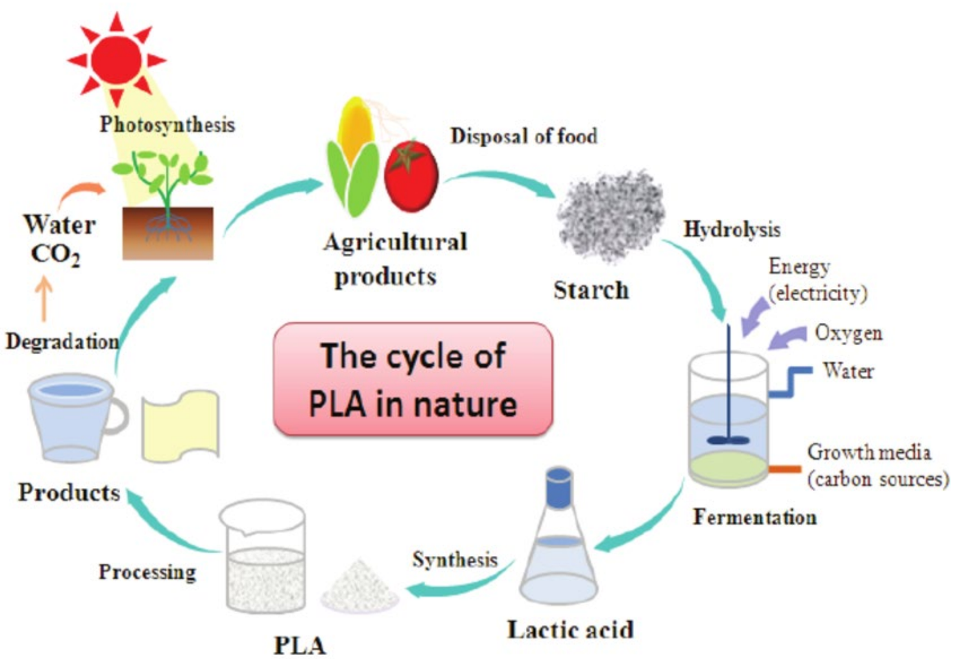

Figure 6

A major win of PLA is that it naturally degrades when it becomes exposed to our environment, so it naturally lessens the severity of the issue regarding climate change. A benefit that PLA serves to ocean animals is that it does not create intestinal blockages if consumed. A "PLA bottle left in the ocean would typically degrade in six to 24 months", while typical plastic will take up to thousands of years [2]. In addition, PLA is cost efficient and PLA production uses 65% less energy than it would for regular plastics and generates 68% fewer greenhouse gases.

Yet, PLA also has its downsides. PLA has low heat resistance and lower strength than conventional plastic. However, a recent study published by Purac Bioplastics stated that through stereochemistry, the resulting PLA homopolymers from L and D lactide (the monomers from PLA, from lactic acid that comes in two enantiomers/chiral molecules) were much more heat resistant and impact resistant [8]. Another drawback of PLA is its higher permeability compared to other plastics. This characteristic of PLA indicates that moisture and oxygen can pass through PLA more easily. Therefore, food spoilage can occur faster. So, PLA is not recommended for long-term food storage and related applications. However, researchers are exploring ways to approach this drawback to reduce the permeability of PLA and thus improve its applications.

Despite the many benefits PLA can provide to our world, conventional plastic is popular for a reason: it is arguably the most accessible, cheap, handy, useful material. From grocery bags to water bottles to candy wrappers, conventional plastic is visible everywhere. It is impossible to take over the existing plastic industry, because it has undeniably made all our lives so much easier, but that does not mean we cannot build and grow a new one for the better of our community.

Medical Aspect

The fact that D lactic acid is difficult to digest and can be poisonous to humans [9] seems like a negative fact on surface, but there is a clever use for this trait. Since D lactic acid is not easily metabolized in the body, it can serve as an extremely effective carrier for medicine. The antibody drug conjugate (ADC) made from D lactic acid can last long, and in other words, is very stable. This means that the effect of the drug may last longer and is able to enter bad cells to kill them without harming other cells [1]. This value of D lactic acid can potentially help increase the productivity of cancer medicine such as ADC. Medical polylactic acid can also be used as a drug-controlled release material, which has special application value in the upgrading and development of modern drugs and dosage forms, such as drug molecular encapsulation and sustained/controlled release preparations, vaccine encapsulation, and sustained/controlled release preparations for positioning implanted drug. In addition, bio-grade polylactic acid directly synthesized by enzymatic method or whole-cell catalysis has certain prospects in the manufacture of medical polylactic acid materials in the future [10].

Further understanding of the L to D enantiomer ratio and positions in the polylactic acid chain can potentially help to design better PLA-related medical devices or disposable materials. It is also widely used in the direction of medical materials, such as absorbable screws and absorbable surgical threads made of polylactic acid, which can be used as fiber braids or membrane materials for human tissue repair, internal fixation materials for fractures, ophthalmic implant materials, tissue engineering scaffold materials, and more. Stitches, for example, or fixtures for bone fractures with PLA can be absorbed into our body tissue without secondary surgery. For such medical purposes, PLA must have the right properties, such as toughness, flexibility, and decomposition time [12, 13, 14]. Therefore, the study of these enantiomers for the optimal composition ratio is of immense value.

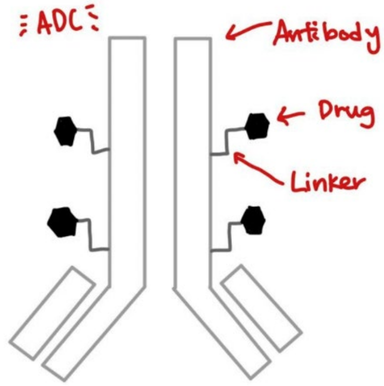

Figure 7

Antibody Drug Conjugate (Figure 7) Key Factors [11]:
- High potency
- Low immunogenicity
- Low cytotoxicity to off-target cells
- High cancer cell specificity
- Long circulating life (D lactic acid is valuable for this because of its stability)

Conclusion

While the data for overall lactic acid concentration verified past studies, the novel discovery that the yogurts contained only the L-type enantiomer was surprising. In the span of a few years, food industries were able to eliminate the presence of the toxic metabolite, the D-type enantiomer, from yogurt. This information brings clarity and sheds light on such a staple food in many households all around the world. Yet, the investigation on lactic acid and its enantiomers does not end here. As discussed in the environmental and medical section, scientists can apply the study of lactic acid to continuously improve its ability to serve as an environmental-friendly alternative to plastic in its polymerized form. Additionally, scientists can take further steps to improve the medical applications of lactic acid, especially testing to find ideal ratios of the two enantiomers for various purposes, whether it is for medicine or suturing. From yogurt to environmental protection to medicine, there is no doubt that lactic acid is a versatile component in the scientific field.

Acknowledgements

I would like to thank Dr. Chongyin Zhang, Director of Central Research and Development at WANHUA Chemicals for assisting the data collection from liquid and gas chromatography. I would also like to express gratitude to Erik Luijten, Professor and Associate Dean at Northwestern University, for exposing me to computational analysis, allowing me to model the L and D lactase in the Introduction section. Lastly, I want to thank Dr. Tao Yu, Global Technology Director at WANHUA Chemicals North America for his advice and enthusiasm for this paper.

References

[1] McCollum, D. (2017, October 16). Sutro Biopharma. Sutro Biopharma Inc. Retrieved April 10, 2022, from https://www.sutrobio.com/technology/xpress-cf/

[2] Rogers, T. (2015, October 7). Everything you need to know about polylactic acid (PLA). Everything You Need To Know About Polylactic Acid (PLA). Retrieved April 10, 2022, from https://www.creativemechanisms.com/blog/learn-about-polylactic-acid-pla-prototypes

[3] Alm, L. (2010, April 21). Effect of fermentation on L(+) and D(−) lactic acid in milk. Journal of Dairy Science. Retrieved April 10, 2022, from https://www.sciencedirect.com/science/article/pii/S0022030282822285

[4] Inaki, Mikiko et al. "Cell chirality: its origin and roles in left-right asymmetric development." *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* vol. 371,1710 (2016): 20150403. doi:10.1098/rstb.2015.0403

[5] Pitt, James J. "Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry." *The Clinical biochemist. Reviews* vol. 30,1 (2009): 19-34.

[6] Maier, N M et al. "Separation of enantiomers: needs, challenges, perspectives." *Journal of chromatography. A* vol. 906,1-2 (2001): 3-33. doi:10.1016/s0021-9673(00)00532-x

[7] CENTER for BIOLOGICAL DIVERSITY. (n.d.). Ocean plastics pollution. Ocean Plastics Pollution. Retrieved April 14, 2022, from https://www.biologicaldiversity.org/campaigns/ocean_plastics/

[8] Bie, F. de. (1961, January 1). Heat resistant PLA for Coffee Cups. Plastics. Retrieved April 14, 2022, from https://www.plastics.gl/packaging/hot-stuff/

[9] Pohanka, M. (2020, June 18). D-lactic acid as a metabolite: Toxicology, diagnosis, and detection. BioMed Research International. Retrieved April 10, 2022, from https://www.hindawi.com/journals/bmri/2020/3419034/

[10] Nanaki, Stavroula et al. "Risperidone Controlled Release Microspheres Based on Poly(Lactic Acid)-Poly(Propylene Adipate) Novel Polymer Blends Appropriate for Long Acting Injectable Formulations." *Pharmaceutics* vol. 10,3 130. 13 Aug. 2018, doi:10.3390/pharmaceutics10030130

[11] Tsuchikama, K., & An, Z. (2016, October). Antibody-drug conjugates: Recent advances in conjugation ... Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Retrieved April 10, 2022, from https://www.researchgate.net/publication/309141197_Antibody-drug_conjugates_recent_advances_in_conjugation_and_linker_chemistries

[12] Balla, Evangelia, et al. "Poly(Lactic Acid): A Versatile Biobased Polymer for the Future with Multifunctional Properties—From Monomer Synthesis, Polymerization Techniques and Molecular Weight Increase to PLA Applications." *Polymers*, vol. 13, no. 11, May 2021, p. 1822. *Crossref*, https://doi.org/10.3390/polym13111822.

[13] Vouyiouka, S. (2017, October 6). A review on enzymatic polymerization to produce polycondensation polymers: The case of aliphatic polyesters, polyamides and polyesteramides. Progress in Polymer Science. https://www.sciencedirect.com/science/article/abs/pii/S0079670016300855

[14] Albertsson, Ann-Christine, and Rajiv K Srivastava. "Recent developments in enzyme-catalyzed ring-opening polymerization." *Advanced drug delivery reviews* vol. 60,9 (2008): 1077-93. doi:10.1016/j.addr.2008.02.007