A Potential Liquid Supplement to Promote Cardiovascular Health

A Project Paper

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Biological & Environmental Engineering

by

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May 2021

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ABSTRACT

L-citrulline is an amino acid which has been investigated for having potential human health benefits. It is of particular interest for its impacts on smooth muscle, skeletal muscle, and the cardiovascular systems. This project is the production of a liquid supplement that incorporates a L-citrulline formula in a way that will be suitable for consumers. Trial formulations were performed in food labs at Cornell University. The purpose was to make a fluid that has permissible hedonic properties and is well suited to abide by FDA regulation of dietary supplements. Hundreds of combinations were tried and tested for pH, temperature manipulation, and stability. The resulting product is one that mimics the flavor profile of lemonade, possesses the bioavailability of amino acids found in watermelon, and L-citrulline levels that are enhanced in comparison to anything found in nature.

BIOGRAPHICAL SKETCH

Joe Hashmall attended Cornell University as an undergraduate and studied applied economics and management, cellular and molecular biology, and environmental, energy, and resource economics. He began his graduate studies at Cornell University in January 2020, just before the global onset of the COVID-19 pandemic. He plans to dedicate his professional career to using business to bring scientific innovations into the lives of people whom can benefit from them.

Dedicated to Mom, Dad, Pat, and Maggie

ACKNOWLEDGMENTS

Janet Anderson

Minglin Ma

Olga Padilla-Zakour

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

FL-CP Functional L-citrulline Powder

Description: This powder consists of FDA-approved ingredients and aims to increase the bioavailability of L-citrulline in the gastrointestinal tract. The proportion of L-citrulline, malic acid, and other components is not disclosed in this paper on account of Joe Hashmall filing for legal protection of the ratio.

PREFACE

This project combines biology, food science, engineering, law, and economics, and will require information and expertise from other fields for it to progress in the future. The global onset of the COVID-19 pandemic impacted us all in big ways. In my case, my ability to participate in in-person lab work throughout my three semesters of graduate studies in biological and environmental engineering was limited. I learned a lot from literature and from talking with Professor Minglin Ma about the lab projects in preparation for when I could start contributing in-person to his breakthrough research initiatives. When it became apparent at the start of 2021 that I would not be able to perform lab work during my final semester due to the pandemic, I knew that I needed to adapt quickly to make the most of my remaining time. I had made good use of my time outside of class during quarantine by working independently on a project that I started several years ago. I knew it would be borderline impossible to switch projects with less than three months left at Cornell, but I was able to do it because of the amazing professionals here. Thanks to the innovative, self-starter mindset of the graduate school and the biological and environmental engineering department, I was not only permitted but encouraged to transition my efforts toward creating a liquid supplement that I believe will someday improve the lives people with hypertension, help to lower healthcare costs, and become a sought-after product market.

I wanted to work on this project not only to take a step toward fighting hypertension and its associated diseases, but also to demonstrate how much I have benefited from the integrative education Cornell has helped me pursue. I came here as an undergraduate interested in business and biology; I wanted to learn the life sciences so that I could help researchers bring their innovations out of the lab and into the lives of those whom can benefit using business. My experience in this program has reinforced why I came to Cornell to begin with. I could work

across departments, meld the expertise of diverse faculty, and take an interdisciplinary approach to building a solution that may help people around the world.

Introduction:

American Hypertension:

Hypertension (high blood pressure) is defined by having a systolic blood pressure of 130 mm Hg and above *or* by having a diastolic blood pressure of 80mm Hg or above [9]. It has become the most prevalent disease among American adults. An estimated 45% of American adults either have hypertension or are actively taking medication to prevent hypertension [4]. Every year, over 450,000 deaths in the United States have hypertension as the primary cause or as a contributing factor. Several factors play into the rising rates of hypertension in the U.S. including diet, exercise, and genetic risk. Financially, hypertension costs the United States $131 billion annually. The costs primarily stem from medications, health care services, and foregone productivity from disease and premature death [9]. The factors that play into the production of this supplement are biological, cultural, social, and financial.

L-citrulline Significance:

L-citrulline is an amino acid that is investigated widely because of the potential benefits it holds for human cardiovascular systems. Cardiovascular health is of the utmost importance every demographic across the world, but this work focuses on two broad categories of particular interest: pre-hypertensive individuals and athletes in the United States. L-citrulline is found naturally in several fruits and is sold widely as a supplement in the United States and abroad.

Physiologically, L-citrulline is promising amino acid in the fight against hypertension because of its ability to increase nitric oxide (NO) levels in the bloodstream. When L-citrulline is consumed, it is processed in both the liver and the kidneys as a part of the urea cycle. The citrulline is converted to arginosuccinate which is converted to arginine in both organs [6]. Although the arginine in the liver does not progress directly onto other processes, the

arginine that is produced from citrulline in the kidneys is released into the blood and drives key downstream cascades. In the blood, the arginine is taken up by the endothelial cells lining the blood vessels. The endothelial cells use nitric oxide synthase (NOS) to convert the arginine to into NO (MPDI). The gaseous NO is powerful once released from the endothelial cells into the smooth muscle that lines arteries and blood vessels because it spurs the relaxation of smooth muscle which leads to vasodilation (widening of the blood vessels) [1, 2, 5]. Molecularly, NO triggers smooth muscle relaxation by activating guanylate cyclase which converts GTP to cyclic guanosine monophosphate (cGMP) and this cGMP drives muscular relaxation as a second messenger [1,2]. The resulting vasodilation can help reduce blood pressure because with wider channels for the same amount of blood to flow through, less stress is put on the heart to pump it and the cardiovascular system at-large benefits.

Current solutions:

This work aims to successfully integrate the functionalized L-citrulline powder into a liquid supplement that can be further explored as a mechanism to preemptively protect against hypertension in pre-hypertensive individuals and people who are at-risk of developing hypertension but who are not yet hypertensive. In order to understand the work discussed in this paper, it is essential to understand the current choices that Americans at-risk of developing hypertension have today. The Center for Disease Control (CDC) has several recommendations for the prevention of hypertension, they include diet choices, weight management, physical activity, not smoking, proper sleep, and limiting alcohol intake.

The Dietary Approach to Stop Hypertension (DASH) is the CDC's official outline for how to slow and prevent the progression of hypertension [9]. This plan is outlined on the CDC website as follows (excerpt):

- Eating vegetables, fruits, and whole grains
- Including fat-free or low-fat dairy products, fish, poultry, beans, nuts, and vegetable oils
- Limiting foods that are high in saturated fat, such as fatty meats, full-fat dairy products, and tropical oils such as coconut, palm kernel, and palm oils
- Limiting sugar-sweetened beverages and sweets

The plan touts no requirement for special foods and "instead provides daily and weekly nutritional goals" (CDC) and key focus on constructing a diet low in saturated fats, rich in potassium, calcium, magnesium, fiber, and protein, and lower in sodium [9].

Next, the CDC provides guidance for maintaining healthy weights. There are myriad links between obesity and hypertension and current research investigates the many dimensions to their relationship [4]. Similar lifestyle habits drive the development of each and research has provided evidence that the two also have causative impacts on one-another [9]. Weight maintenance is often viewed as a conglomerate of the other guidelines because CDC recommendations for achieving a healthy weight run parallel to those for hypertension prevention. The physical activity guidance for hypertension suggests adults "get at least 2 hours and 30 minutes of moderate-intensity exercise" a week [9]. Despite this, only 53.3% of American adults reach this [9]. Similarly, the CDC recommends not smoking, yet 15.1% of American adults were smoking tobacco in 2015 [10]. The CDC recommends men consume no more than two alcoholic drinks per day and women consume no more than one per day, yet in 2019 25.8% of adults reported having engaged in drinking alcohol in quantities that are classified as binge drinking in the past month [11]. These examples are included to convey the extent to which Americans continue to engage in activities that have been highlighted as factors that could contribute to them developing hypertension. These statistics exist on a foundation of much more intricate issues involving socioeconomic structures and corporate strategies that make it more difficult for many demographics to live in accordance to the CDC guidelines, but this does not

change the reality of the epidemiology of hypertension. Outside of lifestyle choices, pharmaceuticals are widely used in the United States to manage hypertension once it has progressed in patients. In lieu of lifestyle changes and as an alternative to more expensive pharmaceuticals prescribed after the onset of the condition, a convenient way to promote cardiovascular health before developing hypertension could be impactful.

Currently, L-citrulline supplements exist in various forms, but they are largely just pure L-citrulline or L-citrulline combined with malic acid and they do not offer bioavailability levels that support their effectiveness [5]. The alternative method for consuming as much of the amino acid as a supplement contains is to eat approximately 4.5kg of watermelon. America stands to benefit from a supplement that has the potential to provide prehypertensive individuals with a convenient and cost-effective way to promote their cardiovascular health. The intuition behind this work is to strive to provide such a mechanism in the form of a liquid supplement that is accessible and affordable to all Americans and eventually the broader global community to diminish the diverse tolls the condition has on society. Like many supplements, the health benefits of the FL-CP are not yet satisfactorily substantiated or noted by the FDA, but with further research, this may become the case. This would allow the liquid supplement to be moved out of the supplement arena, and into the consumer health product arena.

Formulation:

Development Process:

The process for formulation testing consisted of approximately 216 iterations with varying ingredients, proportions of ingredients, and serving size over the course of two and half months. The ratio of the core ingredients which drive the functional properties of the drink were not altered in the iterations throughout this work, only the inclusion and proportion of auxiliary ingredients were altered. The components that were tested are found in Appendix 1.

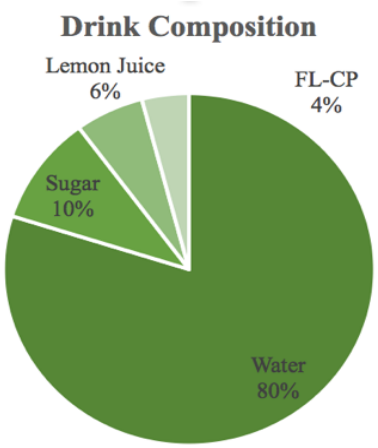
Included in this paper is an overview of the process of formulation as well as a granular breakdown of the shifts and transitions involved in the evolution of the beverage.

The pH of this beverage was at the center of the process from the beginning. For reference, the average pH of lemon juices that were explored as ingredients was 2.62; the functional L-citrulline powder (FL-CP) has a pH below 2.5 when dissolved in a 1:1 ratio with water. The FL-CP pH was treated as an unchangeable factor because this is deemed necessary for the optimization of the functional properties. This pH remained problematic throughout the process because it is unacceptable for a product due to its intolerable taste, dangerous impact on dental health, and ability to cause digestive discomfort. The other side of piece of significance regarding the pH is its role in shelf stability and heat processing. A key feature and limiting factor to the production of a product containing FL-CP is evidence that high-temperature heat-treatment alters the functional properties of L-citrulline [6]. Heat-treatment is widely used for pasteurization, sensory quality adjustment, and ingredient isolation [8]. In the case of the formulation of this beverage, it is surmised that the heat used to kill microbes and achieve shelf stability inadvertently alters the chemical composition of the drink by binding proteins with sugars or by changing the configuration of the L-citrulline itself; irrespective of the mechanism for heat-driven change, the result is a decrease in the bioavailability of the functional components as was seen in Aguayo, et al 2013. The 3.05 pH of FL-cP therefore imposes not solely negative consequences, because of the support it provides in the prevention of microbes compromising product. This aids in the upholding the bioavailability of the L-citrulline in the drink. For the most recent formulation of the drink (pH 3.05), a safe processing temperature was determined to be achieved by pre-bottled heating of the liquid to 82°C for three minutes followed by rapid in-bottle cooling. Despite heat-treatment being mild, it has still been shown to decrease

bioavailability by approximately 35% around this level of heat exposure (Tarazona-diaz2). This is far from optimal and will be an area for improvement going forward but it is acceptable given the high gross weight of functional ingredients included to begin with.

Current Ingredient Formula:

It was determined prior to this work that a beverage containing FL-cP would have a low-volume serving size. This was maintained for two reasons: the taste was so poignant that only a small serving would be tolerated by consumers; and the ingredients are effective at low enough volumes to have their full impact in small amounts relative to typical beverage size. Following myriad candidate ingredients and ratios, in order to attenuate the unpalatable taste that comes with the low FL-cP pH, it was decided that the beverage formulation would assume a flavor profile that closely mimics that of fresh lemonade. The reasoning behind this is three-fold and factors in food science, market viability, and consumer health. First, and perhaps most obvious, the familiar sourness that is expected from lemonade conceals the sour bite of the low pH FL-cP. Second, the lemonade flavor is an extraordinary base-profile that can be easily altered by additional flavors to bolster the beverage with a discrete taste for consumers to associate with the functional properties of the supplement. Third, it allows for seamless product flavor expansions in the future.



Ingredient	Weight (mg)
Water	799
Sugar	100
Lemon Juice	60
FL-CP	41

Figure 1: While composed primarily water and adequately sweetened, the beverage still maintains an acidic profile (attributed to 6% lemon juice and 4% FL-cP). Percentage breakdown seen above is by weight.

Developing a flavor that is more novel and less like a typical lemonade may prove to be a key factor for market success in the future. The goal is to make use of the propensity people have for processing olfactory information and associating it with the subsequent effects the substance they consume has. No supplemental or consumer beverage has been brought to market possessing the ability to support cardiovascular health by dilating blood vessels. But it is important to note that developing the first product to do this does not ensure success; the process of developing a distinct flavor that consumers can easily associate with the health benefits of the supplement will help fend-off competition in the future. Developing such a flavor is an area of continued work in pursuit of bringing the drink to market.

Production & Results:

The production of the most recent candidate formulas was performed in a test kitchen at Cornell University. The variations being evaluated were orange juice with no added sugar and four variations using lemon juice with varying sugar content. Orange juice was not chosen because it did not mask the acidity of the FL-cP and it had drastic flavor change following the heat treatment necessary for bottling.

Each ingredient – lemon juice, sugar, room temperature tap water, and pre-homogenized functional powder was weighed and combined into a glass container. The preparation was for 1-liter of the beverage at a time. The powders were stirred until completely dissolved in the liquid. The liquid was heated in the facility's microwave for 40 seconds, and then repeats of 10 seconds until a temperature of 82°C was obtained for two minutes. The liquid was then poured into a 1-liter plastic bottle and sealed. The bottle was then submerged in ice

water to cool. Due to the accelerated timeframe of this study, getting the regulatory approval necessary for *in vivo* or *in vitro* testing of the resulting L-citrulline bioavailability was not realistic and it was not performed. There is prior research supporting the decline, but not complete depletion of L-citrulline bioavailability following heat-treatment and this is assumed to hold in this processing as well [7]. The 1-liter bottles of liquid supplement that were produced each contained what will be approximately five 200mL serving sizes.

Liquid supplement metrics:

The supplement has a degree Brix (°Bx) of 10. This indicates that there are 10 grams of sucrose for every 100 grams of aqueous solution. The use of added sugar in this supplement is notable because consumer beverages are formulated to have hedonic properties, liquid supplements such as this are not. In many earlier prototype formulations, the supplement had degrees Brix in the range of 0-2. The increase to higher degrees up to 10°Bx occurred in order to conceal the harsh acidity and get the supplement to a point where consumers are less likely to avoid it due to taste.

Dental Risks:

Currently, there is no way to produce the supplement without high acidity while maintaining its functional properties. This is unfortunate not only because of factors of taste, but also because of the negative impacts liquids of such low acidity can have on human teeth. Leading sports drinks, sodas, and energy drinks which have comparable pH values to that of the liquid supplement in this work have been shown to drive erosion and enamel lesions [3]. In this study, Ehlen et al. showed that Gatorade®, Red Bull®, and Coke® (pH values of 2.84, 2.76, and 2.65, respectively) caused lesions in enamel and roots surfaces while teeth were suspended in them *in vitro* [3]. This will remain a problem for the liquid supplement but the limited serving size relative to the aforementioned beverages will help to reduce the contact time with teeth.

Future Work:

Regulatory Dynamics:

The relationship between supplements, consumer products, and pharmaceuticals is complex – as is how the FDA regulates each. The regulation is continuously evolving and improving and properly navigating the regulatory environment will be key to bringing this supplement to market. It is important to note again that distinctions exist clearly between a supplement and a pharmaceutical product and most obviously supplements are not permitted to prevent, treat, or relieve symptoms of diseases [13]. Additionally, while evaluating whether a liquid product ought to be classified as a liquid supplement versus a consumer beverage, the FDA considers numerous factors. These factors include product marketing and labeling, the product name, the packaging size and intended perception, packaging and serving size, recommendations and directions for use, and other more nuanced ingredient features [13]. Positioning this liquid supplement securely within the appropriate supplement parameters is a priority moving forward to ensure a safe entrance into the market.

Bottling:

Small bottles will be chosen: with serving size between 200-300mL. This is important for how the FDA will view this as a liquid supplement. When discerning whether a product ought to be classified as a supplement as opposed to a consumer product, the FDA accounts for many factors – including the packaging and serving sizes. Further liquid supplement producers, like food and beverage producers, have immense environmental responsibility. More work will be done to understand how to help consumers while concurrently doing as little harm to the environment as possible. Regarding the bottling method and the shelf stability of the supplement, current heat treatment methods are not optimal for FL-CP bioavailability. New

methods of protecting against microbes such as ultraviolet and electromagnetic pulse treatment will be explored to assess their potential to better maintain the molecular integrity of the formula.

Ancillary Supplements:

The FL-cP in this liquid supplement has the potential to have other beneficial health impacts at higher dosages. This will be investigated as an extension of this supplement. Potential benefits via other mechanisms should be considered and pursued upon further research.

Flavoring:

As previously mentioned, to enhance the market viability of this supplement, it will be important to finalize a flavor profile that is distinguishable from typical drink and supplement flavors. With a composition that currently mimics that of most lemon-based drinks, a more unique combination of flavors should be implemented.

Non-B2C Approaches:

An exciting prospect exists with this supplement. More work will be done toward substantiating health impacts related to hypertension to the FDA in order to achieve the use of health claims. In the future, this presents a unique opportunity to account for multiple stakeholders in the American healthcare system. It could be in the interest of insurers to subsidize the cost of a subscription to this supplement in order to systematically improve the blood pressure of customers to lower their broader cost structures. This also has the potential to accelerate adoption.

APPENDIX

I.)

Candidate Juices
Lemon Juice
Orange Juice
Mango Juice
Cherry Juice
Blueberry Juice
Watermelon Juice
Watermelon Rind Juice
Peach Nectar
Apple Juice
Grape Juice
Cranberry Juice
Lime Juice
Grapefruit Juice

Candidate Flavorings
Mint Extract
Jasmine Extract
Basil
Honey
Ginger
Turmeric

Candidate Additives
Sugar
Maltodextrin
Stevia extract
Honey
Simple Syrup

II.)

Figure 2: Trial formula with a low pH

III.)

Figure 3: Most recent formulation post-bottling

IV.)

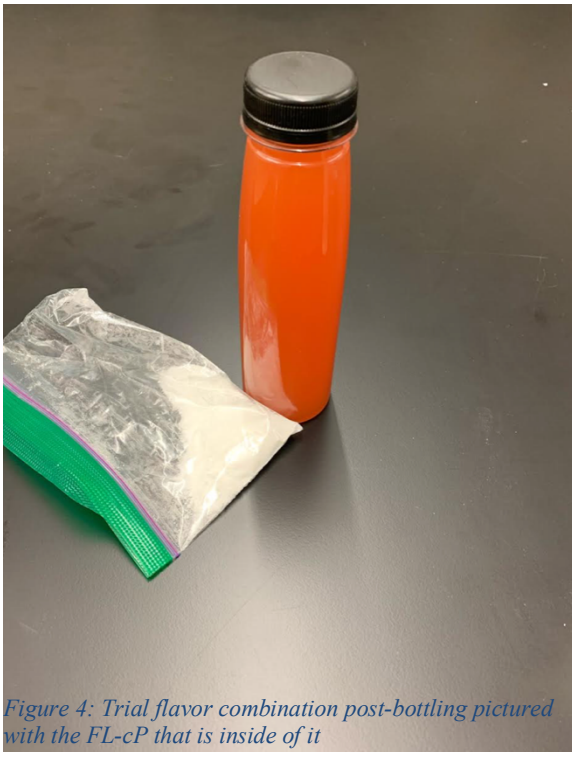


Figure 4: Trial flavor combination post-bottling pictured with the FL-cP that is inside of it

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