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ARTICLE

Biological Activities and Food Applications of *Citrulline* from Watermelon (*Citrullus lanatus*).

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ABSTRACT

Citrulline is considered an amino acid not essential for the human body. However, recently, the interest on this compound has increased, because of the different performance benefits that it could offer to athletes, who consume it as a dietary supplement. Among these benefits are: increase of energy synthesis, muscle development, increasing blood flow, delaying fatigue and preventing catabolism. Citrulline is synthesized and sold as citrulline malate, however, it is found naturally and abundantly in fruits from the Cucurbitaceae family, and in greater proportion in the watermelon *(Citrullus lanatus)* shell. On the other hand, in the last decade, demand for consumption of functional foods has increased, thereby, the food industry has developed new foods with high nutritional value, which provide health benefits, such as: snacks, energy-protein bars, yogurt, cereals, gums, truffles, etc. The objectives of this document are to describe the health benefits of consumption of natural citrulline and its application for development of functional foods. Among the scientific advances to be highlighted are: citrulline is reported to be of great ergogenic help, which also provides benefits for diseases treatment. Supplements with citrulline significantly increase health benefits, among the effects reported are: decrease of insulin resistance, body weight, arterial stiffness, blood pressure, muscle mass, vasodilation, blood flow, and elimination of excess of ammonia. In conclusion, the food industry is constantly at the forefront so, by developing a functional food from a natural source (watermelon), not only will it be taking advantage of its nutrients from the watermelon shells, but it will also be minimizing the environmental problems by using an agro-industrial waste.

KEYWORDS

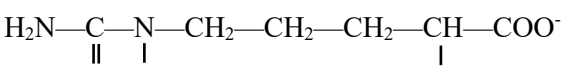
Citrullus lanatus, functional food, food supplement, health benefits, snacks.

1 Introduction

Fruit consumption provides nutrients such as: carbohydrates, protein, fiber, vitamins, and minerals [1]. However, during its consumption, some plant structures such as shells and seeds are discarded, which may contain bioactive compounds [2]. In most cases, fruit shells are considered an agro-industrial waste, and are thrown into the environment, although, they are sometimes used as livestock feed [1]. However, fruit shell extracts contain phytochemicals that can be used to treat or prevent diseases [2]. These phytochemicals can also be incorporated into foods in order to confer them added-value. Currently, an important market has developed for this type of foods, which are marketed as: juices, breads, candies, yogurts, custards, truffles, etc.

Citrulline (Table 1) is a compound present in fruit pulp and shells from the Cucurbitaceae family, mainly in watermelon (*Citrullus lanatus*). This compound may have different benefits to human health when it is incorporated to foods. This molecule has not received much importance, and it was classified only as a non-essential amino acid for the human body, where its importance lay only on being an intermediary in the urea cycle [3], but as knowledge about this molecule accumulated, it was found to have other metabolic contributions, which are beneficial to human health. Recently, it has been associated with antioxidant and anti-cancer effects [4]. While in plants, citrulline is involved in nutrient transport, signaling, and the interaction process with pathogens and environmental stress. In human beings, this compound promises to be a good ergogenic aid, improving physical conditions, sports performance, delaying fatigue, and improving synthesis of nitric oxide (NO), which help to eliminating toxic compounds, and decreasing oxidative damage [5;6]. However, it is necessary to determine the maximum amount of citrulline that can be added to each food to avoid an excess in l-arginine production, since excess l-arginine consumption has been associated with gastrointestinal side effects, such as nausea and diarrhea, because of rapid increase in NO production in the gastrointestinal tract [7]. The objectives of this document are to discuss the health benefits of citrulline and the possibilities of its application on development of functional foods.

Table 1. Physical characteristics and biological description of L-Citrulline US National Library of Medicine [8].

Features	
1.1 Chemical formula	$C_6H_{13}N_3O_3$
1.2 Molar mass	175.19 /mol
1.3 Chemical Structure	

O H $^{+}NH_3$

2. Biological description

2.1 Imagen SVG	H—Cit—OH
2.2 IUPAC	DL-Citrulline

1. Name and Identification

3.1 Name IUPAC	2-amino-5-(carbamoylamino)pentanoic acid.

1.1 Citrulline synthesis

L-Citrulline was isolated about 100 years ago from *Citrullus lanatus*, where its name came from. It has also been identified in other species from the Cucurbitaceae family [9], including melon, cucumbers, and pumpkin [10]. This amino acid can be synthesized by two pathways, from the amino acids L-glutamine (Figure 1) and L-arginine. The pathway from L-glutamine is:

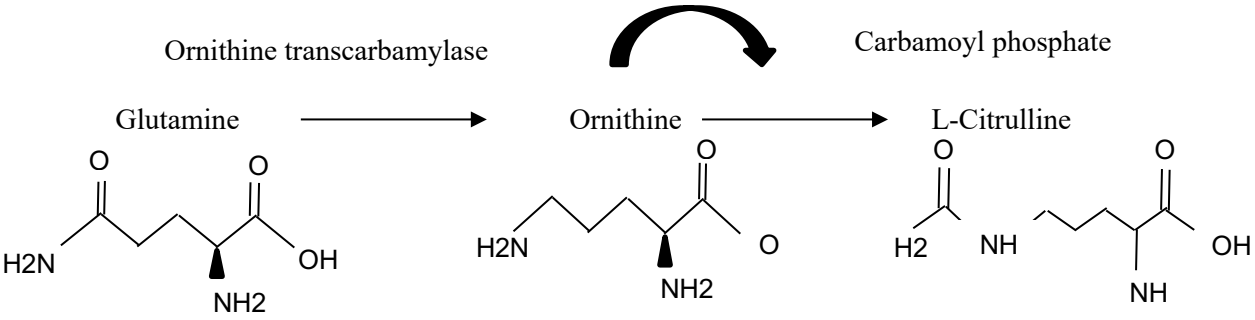


Figure 1. Citrulline synthesis in mitochondria during the urea cycle.

The second route of citrulline synthesis is carried out by using L-arginine [12], as a precursor, which is catalyzed by nitric oxide synthase and co-producer of nitric oxide (NO) synthesis [3; 10]:

L-arginine + 3 NADPH + H^+ + 4 O2 → 2 *L-citrulline* + 2 NO + 4 H2O + 3 *$NADP^+$*

It is not yet known at the cellular level which is the best nitric oxide precursor, arginine or citrulline [3], but, the best alternative could be citrulline, as it is more efficient for increasing the availability of arginine, obtaining a higher yield [11]. However, the human body is capable of synthesizing citrulline from other amino acids (from glutamine, glutamate, and proline catabolism in the small intestine), making direct consumption unnecessary [12].

Endogenous NO synthesis occurs through the L-arginine-NO pathway, whereby L-arginine is converted to NO and L-citrulline by nitric oxide synthase (NOS) enzymes [13].

1.2 Metabolic pathway of citrulline in the human body

Citrulline is important during the urea cycle, because it converts ammonia into urea, a process that takes place in the liver. Therefore, people with problems by excreting ammonia into the blood have received citrulline to assist them, in this elimination [10]. In addition, combination of L-citrulline with malate (amino acid) can act as a mediator in the tricarboxylic acid cycle, thus increasing the rate of adenosine triphosphate (ATP) production cost of muscle contractile force [14], improving mitochondrial efficiency, and calcium handling [13]. However, excessive amounts of citrulline may cause the known disorder called citrullination, a chemical process that occurs when there are high amounts of citrulline, and where a process of post-translation hydrolysis of arginine residues occurs to create citrulline and ammonia residues, with catalyzation by peptidylarginine deiminases (PAD), causing extensive protein modification, which could trigger a cascade of diseases such as multiple sclerosis, autoimmune disorders, and even cancer [10]. Being citrullination a promising therapeutic use for autoimmune, neurodegenerative diseases and certain cancers [15].

1.3 Nitric oxide (NO) and citrulline ratio

Citrulline increases the availability of arginine, and consequently increases the synthesis of nitric oxide (NO) which is a gas synthesized from L-arginine and oxygen, by the endothelial nitric oxide synthetase (eNOS) enzyme. Nitrite (NO_2-) and nitrate (NO_3-) anions are NO metabolites, which are substitutivity measured to know endothelial NO production [16]. This gas is secreted by the endothelium and activates the soluble guanylate cyclase (SGC), increases production of cyclic guanosine monophosphate (cGMP), and promotes vasodilation of blood vessels [17]. This process aids blood circulation and effectively improves mitochondria function [18], especially during physical exercise [14].

NO regulates mitochondrial biogenesis, with increased regulation of the receptor the activation of N-Methyl-D-Aspartate (NMDA) receptors through the excitatory action of glutamate, which induces Ca2+ entry into the cell. In conjunction with calmodulin, the enzyme nitric oxide synthase (NOS) is activated, and in the presence of other cofactors such as oxygen, NADPH, FAD, FMN and biopterin, the substrate L-Arginine is converted to NO and L-Citrulline by the peroxisome proliferator-γ co-activator-1α (PGC-1α), which acts to regulate mitochondrial function. NO has a series of physiological functions among which the following stand out: increase the effectiveness of muscle contraction, control of oxygen consumption, relax of capillaries, and increase of exercise tolerance (therefore, it is an intercellular messenger, and forms reactive nitrogen species). In addition, this gas has certain benefits of dietary nitrite (NO_3-) supplements, and their beneficial effects on sports performance. In contrast, other authors report no improvement in

endurance performance, following NO3- supplementation in highly trained athletes [18]. Nitric oxide deficiency has been implicated in several health problems, including mitochondrial disease, and this deficiency can be better remedied, with citrulline supplementation than with arginine [10].

1.4 Disease prevention and treatment

Citrulline has been used as therapy for mitochondrial diseases, and it is important to note that this compound is well tolerated, unlike arginine supplementation [10], despite the fact that citrulline is a precursor of L-arginine (itself a precursor of nitric oxide) [14;17]. In addition, effectiveness and benefits of L-citrulline administration have been proven as a therapeutic use for multiple diseases, however, its major application has been in athletes to increase their improvement during continuous physical activity. These diseases are triggered by overweight and obesity, therefore, in order to reduce weight, several hypocaloric diets have been proposed, which reduce weight in fat mass, preserving lean mass, as the restitution of muscle mass is very complicated. Leucine and citrulline are two amino acids that help preserve muscle function during restrictive diets.

Recently, interest on non-essential plant-based amino acids (NPAA) has intensified, as they have been associated with health benefits, including antioxidant, cardioprotective and anti-inflammatory activities [17] (Table 2), and their incorporation into dietary supplements, and pharmacology has been investigated [4]. In addition, citrulline has capacity to increase availability of arginine in the plasma, and later the synthesis of nitric oxide, performing in the process of vasodilation, blood flow, respiration of the mitochondria and oxidation of glucose [10], it has been suggested to be involved in physiological processes, where nitrogen transport occurs [4]. Citrulline is recognized as an amino acid with great contributions to cardio-metabolic health. Although, research continues to confirm the potential of this substance, not only as ergogenic on vascular function and physical performance, but also in the treatment of various diseases.

Table 2. Function and effect of citrulline by system.

Systems	Function	Effect	Reference
Metabolism	Increased glucose results in muscle vasodilation.	Decreases insulin resistance.	[27;28]
	Cellular activity increases		
	Lipolysis is increased.	Energy synthesis is increased.	
		Low body weight.	

Musculoskeletal	Activates muscle synthesis.	Muscle development.	[5;7]
	Muscle blood flow increases significantly.	It anticipates catabolism.	
Immunological	It reduces the number of white blood cells circulating.	The white blood cells look midway, in the face of endothelial damage.	[31]
Cardiovascular	Synthesis of NO	Vasodilation.	[7; 16; 23; 25]
	Arginine Synthesis	It lowers arterial stiffness.	
		It lowers blood pressure.	
		Prevents myocardial damage and endothelial dysfunction.	
		Improve blood flow to the male member (helping to develop erections).	
Liver	In the metabolism of urea.	In the blood, it removes ammonia excess.	[29]

1.5 Citrulline and central nervous system

The brain is made up of, among other things, astrocytes, which are cells responsible for detoxification of ammonia, by the amidation of glutamate to form glutamine. Presence of citrulline in the central nervous system (CNS) has been reported for more than 50 years, and it was thought that origin of citrulline was only hepatic, however, it was discovered over the years that this amino acid can be synthesized at the brain level. In the central nervous system (CNS), specifically, within the astrocytes, there is a key mechanism for regulation of excitatory neurotransmission in a normal way. This is evidence that citrulline is present in the central nervous system (CNS). In addition, presence of citrulline has been reported in neurons, microglia and oligodendrology, and it is suggested that this amino acid is involved in the physiology, and pathophysiology of the CNS [19].

1.6 Citrulline and cardiovascular disease

Cardiovascular diseases are result of heart and blood vessels disorders, due to: tobacco use, lack of physical exercise and poor diet [20]. Currently, this type of disease is increasing at an alarming rate. According to the global forecast for 2030, it is estimated that 23.6 million people will die from these diseases [21]. The first risk factor for cardiovascular disease is hypertension, which can trigger myocardial infarction, vascular accidents and even death. For treatment of hypertension, use of L-citrulline alone or in combination with L-arginine has been suggested for increase of plasma nitric oxide. However, in other qualitative and quantitative studies where L-citrulline is supplemented, overall, no significant effect on aortic systolic and aortic diastolic blood pressure was found. In order to promote the effectiveness of citrulline and obtain its benefits, physical activity is important, and further research and clinical trials are needed to confirm these effects [21]. For proper endothelial health, proper NO production is needed. When this production decreases, low density lipoprotein (LDL) oxidation increases and plaques form in the arteries, which trigger the development of cardiovascular disease [16].

On the other hand, it has been observed that citrulline, added to nutritional supplements, can help the blood flow in the male reproductive system, benefiting the impulse of erections [22;23]. The control of erectile dysfunction and subjective improvement in sexual relations, are some of the benefits that have been observed when administering this amino acid, proposing itself as a more natural alternative for those who suffer from mild or moderate dysfunction [24;25].

1.7 Citrulline and Diabetes Mellitus

Diabetes is a disease that occurs when pancreas is not producing enough insulin, or when the body cannot effectively use the insulin that it is producing. There are 3 types of diabetes: type 1, type 2, and gestational diabetes, where the main problem is hyperglycaemia (increased blood glucose), which occurs when the disease is not controlled [26]. This metabolic disease is one of the leading causes of death in most countries. In 2015, the International Diabetes Federation mentioned that 8.8% of adults between 20 and 79 years of age suffered from this disease, and they state that by 2040 these data will increase to 10.4%, which is alarming, since diabetes brings with it both micro and macro vascular conditions, when patients are in chronic hyperglycemia [27]. Hyperglycemia produces some compounds that cause inflammation, chronic stimulating cytokines such as TNF-α are secreted, and over expressed in fatty and muscular tissue, giving a resistance to insulin. It has been mentioned that decrease of NO, affects both directly and indirectly the metabolism of skeletal muscle, and insulin resistance will develop. Some reports mention that citrulline can significantly decrease the cytokines responsible for inflammation, and also increase anti-inflammatory cytokines [28], this being the main reason why citrulline supplementation is believed to bring benefits in

diabetes [17], by increasing insulin signaling, thus improving oxidative stress, glucose tolerance, and lipid metabolism to prevent mitochondrial deterioration [17;28].

A meta-analysis on diabetes mentions that citrulline and watermelon extract supplementation have an antioxidant and anti-inflammatory effect. In some analyzed articles, where different metabolic variables related to diabetes were evaluated, it was found that supplementation offered various benefits in patients with diabetes, including: reduction of inflammation, improvement of glycemic status with hypolipidemic, and antioxidant effect [17]. On the other hand, diabetes has been associated with the risk of suffering cardiovascular diseases (CVD), such as hypercholesterolemia, hypertension and even activating inflammation, and it has been mentioned that with very low levels of citrulline, production of NO by the endothelium will decrease in patients with diabetes mellitus (apparently physically healthy) [16]. However, more studies are needed to establish the role of citrulline in patients with diabetes, as well as to determine optimal doses of citrulline for this condition.

1.8 Citrulline and liver diseases

The disease known as non-alcoholic fatty liver (NAFLD), is characterized by an accumulation of 5-10% of lipids in the liver tissue, without chronic alcohol consumption, viral infection, or other specific liver disease conditions. It has been reported that, 20% of patients with NASH, may have progressive liver fibrosis, leading to liver cirrhosis and hepatocellular carcinoma [28]. This disease is associated with obesity, insulin resistance, hypertension, dyslipidemias, and metabolic syndrome NASH which begins with steatosis, where a liver lesion is observed, and a series of inflammatory reactions and lobular bulging begins, which, if not treated in a timely manner, can lead to cirrhosis (irreversible disease) [29].

The rate of inflammation, liver elasticity, and changes in an ecogenesis are some of the effects sought to be tested, using pancreatic enzymes in patients with NASH disease. Thus, in one study, 2 g/day were administered to newly diagnosed patients with NASH, without any previous treatment, significant changes in activity (TNF-α) (a marker of chronic inflammation) were observed. In addition to a relevant decrease in hepatic steatosis. However, more evidence is lacking to determine the maximum doses of the amino acid, which can be supplemented to patients [28].

1.9 Citrulline and exercise performance

Recently, people's physical activity has increased, as it has been shown that regular exercise contributes to maintaining good physical health, and preventing disease [20]. In addition, the sports culture aims to develop athletes with greater mental and physical endurance, which is why the demand for ergogenic supplements has increased. Secondly, these supplements are intended to improve emotional stress [7].

Development of nutritional supplements, that help to improve individuals' exercise performance, delay fatigue, or enable early recovery, must be within the legal framework [5]. Since during physical exercise, there are hormones that accelerate fatigue such as cortisol, lactate, ammonia, etc., supplements are intended to reduce the levels of these compounds (e.g. lactate) [7]. Lactate is an indicator of physical exhaustion, since during exercise it increases the amounts of hydrogen ions, this due to the accumulation of lactic acid. Citrulline activates muscle fatigue, by decreasing ammonia, promoting NO production, and increasing blood oxygenation [30]. It has also been reported that L-citrulline from watermelon is necessary to suppress activation of phosphofructokinase (PFK), and the enzyme of the glycolytic system, inhibiting the re-synthesis of adenosine triphosphate (ATP) [7]. Citrulline supplementation increases concentration of l-arginine more than when pure l-arginine is administered. However, excess of l-arginine generates a rapid production of nitric oxide (NO), which can cause gastrointestinal discomfort such as nausea and diarrhea [31].

Branched-chain amino acids (BCAAs) have been reported to help relieve post-training fatigue [32], and this is another application of citrulline in sports practice, as it decreases muscle pain [10]. Arginine and NO levels increase thanks to citrulline, therefore, more blood flows through the muscle, which increases the transfer of oxygen and nutrients, providing more muscle energy, and ammonia is eliminated from the body [5]. In addition, glucose uptake, contractility, blood flow and muscle repair are increased, thus benefiting sports activity [14]. On the other hand, it has been reported that women may have a greater response to citrulline malate (CM) supplementation, because women produce more estrogen, which regulates NO production [14].

Citrulline malate (CM) is the combination of citrulline with malic acid (malate), this mixture is part of certain chemical reactions useful for energy (ATP) production [33], therefore, it helps those people who in addition to improving their muscle composition, seek to replenish strength (more energy) in their day to day activity [34]. CM supplementation has been proposed to improve sports performance, since it reduces muscle fatigue, CM being an intermediate in the tricarboxylic acid (TCA) cycle, and when consumed, it improves muscle energy production and therefore physical performance. The administration of 6 g during 15 days increases up to 20% recovery of creatine phosphate post training, controlling 40% of the sensation of fatigue or muscular pain, and increasing 34% production of adenosine triphosphate (ATP) [5].

In watermelon pulp juice, there are bioactive metabolites in addition to citrulline, which have a synergistic interaction with other amino acids, carotenoids, micronutrients and antioxidant enzymes, and can be used during production of ATP (energy), which can be used during exercise, and after training can speed up the recovery process. This increase production of NO, which in turn increases blood flow, that causes vasodilation and increased blood perfusion, thus, eliminating more toxic metabolites, such as lactate and

ammonia [7]. Oral consumption of citrulline and arginine mixture, has been shown to increase the concentration of arginine in plasma more effectively, and efficiently than if either of these compounds were supplied separately. Administering citrulline and arginine mixture (1:1) at a dose of 2.4 g/day for one-week, improved perception of "legs muscle pain" and "ease of pedaling" during an ergometry test [18].

1.10 Sources of citrulline

Citrulline is a free amino acid found in plants of the Cucurbitaceae family, among which watermelon stands out [17; 35], but, it has also been identified, although in smaller quantities, in pumpkin, melon, cucumber, and other agricultural commodities such as: almond, cocoa (dark chocolate), chickpea, garlic, onion, nuts, peanuts, mushrooms, in aquaculture products such as salmon and even in certain fermented foods and beverages, such as: soybean, wine [30], meat, fish and vegetables [35].

1.11 Citrulline supplements

Citrulline is considered as a drug-nutrient for the human organism, since it may be able to transform itself into other compounds [35]. In Table 3, different supplements available in the market are shown.

Table 3. Different citrulline products

Product name	Attributes	Price, company name (city, state and country where it is located)
Amazing Formulas L-Citrulina 750 mg 90 tablets	-Excellent cardiovascular and circulatory support. - Helps reduce fatigue and promotes endurance. - Promotes sexual health. - Increases physical endurance.	$1,139.00 MXN Amazing nutrition Jersy City, Nueva York, U.S.A.
Nutricost L-Citrulline 600 mg, 195 scoops.	-Increases physical endurance.	US $27.99 Simaro Nutricost, Vineyard, Utah. U.S.A.
L- Citulline 120 mg Tablets	-Support the extra force. -Aids in the function of the immune system. -Supports protein metabolism. -GMP certified, vegan/vegetarian.	US $18.35 Now sports U.S.A.

The recommended doses for each objective have not been established, yet. However, studies are continuing. According to previously applied clinical trials, tentative amounts of citrulline ranging from 1 to 10 grams per day have been proposed [35]. The Mexican Supplements Guide proposed recommended doses for certain objectives, for cardiovascular health 6 to 10 g/day, sexual health 1.5 g/day, for increasing muscle mass, and for sports exercise performance 6 to 10 g/day.

Information about the time of day when most absorption of the citrulline supplement is performed, is still lacking. For athletes, it is recommended that the intake is made 1 or 2 hours before physical exercise, and if the dose exceeds 1-1.5 grams, should be divided, until to complete the recommended daily grams.

Supplements can be taken with food, since food will not affect citrulline absorption. In some supplements, the effects can be seen within a month or more of its consumption, however, in the case of citrulline, its effects have been reported since the first week of supplementation [33]. In addition, as with any dietary supplement, it is suggested that the product should be stored properly, tightly closed, in a cool place away from strong odors and sunlight. Its effectiveness may be preserved for up to two years after the date of manufacture [33].

1.12 Watermelon

Watermelon *[Citrullus lanatus* Thunb. Matsum and Nakai*]* is the fruit with the highest amount of citrulline and is among the most important vegetable crops worldwide, its annual production reaching approximately 118 million tons. This fruit is consumed for its great taste and nutritional content. It is primarily constituted of sugar, fiber, vitamin C, and minerals such as calcium, magnesium, potassium, sodium, zinc, etc. [36], lycopene and amino acids (citrulline and arginine) [37;38]. It is estimated that watermelon contains 0.7 and 3.6 mg/g fresh weight [13] arginine and citrulline, respectively, and 40% more lycopene than raw tomato [17], other watermelon compounds are lutein and sucrose. Watermelon fruit presents a great diversity in terms of shape, size, color, texture, flavor, and nutritional composition [37]. However, its cropping can be affected by many environmental conditions such as temperature, heavy metals, soil salinity, drought, and pests or soil-borne pathogens [39], coupled with the fact that, it is composed of 95% water, which accelerates its deterioration [37;38]. In addition, at least 18 polyphenolic compounds have been reported in the watermelon's shell [34]. These compounds have anti-mutagenic properties that are directly related to cancer [40], or they promote cardiovascular health, during treatment of diabetes, etc., [41].

Watermelon contains large amounts of L-citrulline [31]. Although it has been reported that in watermelon, L-citrulline is found in seeds, leaves, xylem and phloem sap, fruit pulp and shell, although, most of this amino acid is observed in the shell on a dry weight basis [30]. Several authors mentioned that amount of

citrulline in watermelon, is increased progressively as the fruit approaches its maturity, and then, decrease after ripening, and this does not depend on the used watermelon cultivar [30], while the red fleshed watermelon has 16 mg / 100 g. It has also been reported that citrulline levels increase in the plant under abiotic stress conditions [4]. Watermelon chemicals have been reported to improve exercise performance when are orally supplied [7].

1.12 Food applications

Watermelon citrulline has been reported as an ergogenic compound [31], since it helps to improve performance during exercise, and it is in greater amount in watermelon shell. At industrial level, citrulline is produced using microbial strains. However, this amino acid is produced naturally and in large quantities in watermelon. From one ton of watermelon pulp and rind, 19 kg of citrulline was extracted, which makes this process economically viable [30]. In addition, watermelon rind is characterized by its high dietary fiber content, a compound used for treatment and prevention of chronic diseases [42] and bioactive compounds, that is why, it has been proposed for incorporation in products mainly of bakery, as substitute of flours [41], including in these foods also pectins [43]. In addition, flour from watermelon shell has a low energy value, and could help to reduce fatigue and tiredness after physical effort, and improve the cardiovascular system [41].

It has been reported that watermelon shell contains large amounts of total phenolic compounds, which are powerful antioxidants. This is why its incorporation into foods could be a very promising application, generating foods rich in natural dietary fiber, with better final product, in terms of its organoleptic characteristics (color, flavor, texture, aroma), with higher nutritional quality and low energy inputs [44]. In some countries, the lack of organization in the dates of sowing watermelon, lead to harvest concentration in a short time of the year, which causes that much fruit is not harvested because it is not affordable, and watermelon is lost [45], also in most cases, only fruit pulp is utilized, and shell or bark is only used in some cases as livestock feed and mostly only thrown into the environment as waste, causing serious ecological problems of pests, and pollution, therefore use of watermelon shells during food development is a great alternative.

There are companies that aim to develop new foods which include improvements in its nutritional quality. According to this, in most cases, plant extracts are incorporated into foods, since these compounds have powerful antioxidant activity [46] and anti-cancer [47] effects, so their incorporation provides added value with health benefits. However, the recommended doses of its bioactive compounds in food to provide the desired functionality are still unknown. Therefore, it is important to establish the appropriate food

formulations to meet the desired functionality, which leads to the design of foods with different proportions of their active ingredients.

1.13 Healthy eating

Consumers' food choices have changed over the years. Nowadays, the choice of their food is based on the dietary preferences of each individual, as they look for low-calorie foods which may provide an extra health benefit, so the food industry has created a trend towards healthy food consumption [48].

The Codex Alimentarius defines a food as a substance prepared for human consumption from the time it is manufactured, prepared or treated on the food and mentions that a healthy diet protects against malnutrition, and a range of chronic and degenerative diseases [49]. Over the years, life style has accelerated and habits have been modified, including food, increasing consumption of industrialized products, high-calorie fast food, but in addition, without a great nutritional contribution, because its composition includes saturated fats, free sugars, sodium, etc. [49]. The consumption of this type of food is greater in some regions where "snack" is consumed, since it is a food for daily consumption due to its practicality, and can be consumed at any time of the day [50]. In addition, if vegetables and fruits are included daily, the risk of chronic diseases will be reduced [42].

The food industry has developed functional foods, which are classified as substances that have a basic nutritional value, but also provide an extra health benefit. The European Food Safety Authority requires that if any food is declared as functional, it must have an accepted scientific basis and justification. Therefore, evaluation of a food for the identification of the bioactive compound is important. However, many foods claimed to be functional do not have studies that validate their functionality and safety. In addition, there is no institution that regulates and determines the appropriate dosage, efficacy, and bioavailability of the active ingredient of each functional food, deliberately allowing the sale of foods with these characteristics. These foods are known as dietary supplements, and excessive consumption of some of them is associated with hepatocellular lesions and high levels of transaminases, about certain untested products [51].

1.15 Incorporation of citrulline during development of functional foods and supplements

The selection of foods by consumers has changed over the years, they generally choose them based on their dietary preferences, number of calories, and benefits that they provide for health. Therefore, the food industry maintains a trend in the development of healthy foods [48]. Accordingly, citrulline isolated from agro-industrial residues of plants has the potential to formulate functional foods or food supplements.

Consequently, countries producing *C. lanatus* such as Mexico could take advantage of plant material for its preparation, since *C. lanatus* is a promising specie because it is the most studied to recover this precious amino acid.

The citrulline content that developed foods or supplements should contain is still unknown. For that reason, food formulations must be established to fulfill the desired function. Therefore, it is proposed to prepare the product with the functional doses of synthetic citrulline established in previous studies. Since according to clinical trials to evaluate the biological activities of citrulline, the amino acid has a dose-effect of 1 to 10 grams per day [35] In Table 4 is showed the recommended daily doses of citrulline to improve some health conditions.

Table 4. Recommended daily doses to improve some health conditions.

Property	**Doses g / day**	**Reference**
Cardiovascular health	6 to 10	[18;36;52].
Improve erectile function Sexual health	1.5	[2;23;53].
Increase muscle mass and improve athletic performance	1-8	[4;22;23;27;28;38].
Hypoglycemic treatment in Diabetes mellitus II	1	[16;54;55].

Athletes should ingest citrulline in divided doses 1 to 2 hours before physical exercise to complete daily requirements. In addition, the effects of citrulline are manifested from the first week of consumption [33] Consumption of the amino acid is considered safe but should not be consumed for more than six months without medical supervision [56].

Information on the incorporation of citrulline in food is scarce. Although, some researchers propose its incorporation as a substitute for flour in bakery products [41], while others incorporate *C. lanatus* husk flour. Since it has a low energy value, and due to its citrulline content, it can reduce fatigue after physical effort and improve the cardiovascular system [41]. Alternatively, development of food supplements should include storage tests that have conditions such as a cool and dry place free of unpleasant odors and sunlight to keep it in excellent condition [33]. Besides, it is necessary to evaluate its effectiveness for two years after the date of manufacture.

According to the Codex Alimentarius, a healthy diet protects from both, malnutrition and a variety of chronic and degenerative diseases WHO [49]. Therefore, development of a "Snack" containing citrulline from *C. lanatus* could be promising, given that this type of food has a high daily consumption due to its practicality [50]. Since, intake in the daily diet of plant derivatives reduces risk of chronic diseases [51;45]. As well, the European Food Safety authority declares it a functional food, the product developed with a scientific basis. As a result, evaluating food composition, its biological activities, and the reduction of the risk to a disease is important. However, in Mexico, many food products affirm their functionality without having studies validate their function and safety. Consequently, their study represents an area of opportunity to investigate since currently, no institution regulates the adequate dose, efficacy, and bioavailability of the active ingredient of each functional food.

2. Conclusion

Citrulline is an amino acid, to which they have been attributed different health benefits and has been proposed to treat diseases such as diabetes mellitus, liver disease, heart disease, CNS and even cancer. Among the positive effects of the administration of this compound are: decreases insulin resistance, body weight, arterial rigidity, blood pressure, thus increasing energy synthesis, muscle mass, vasodilation, blood flow, and eliminating ammonia excess, besides being known as a natural Viagra. Currently, citrulline is added to dietary supplements, and most of the one used for this purpose is synthesized chemically or from microbiological origin. Citrulline is used as a sport ergogenic, because of its benefits in sports performance. However, watermelon is rich in citrulline, which offers the advantage that, it is of natural origin and could be used for development of functional foods, which is undoubtedly an excellent proposal, since it would be taking full advantage of natural resources that were considered agro-industrial waste (shells), and minimizing environmental pollution.

Conflict of interest

The authors declare that they have no conflict of interest.

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Referencias

1. Bhol, S., Lanka, D., Bosco, S.J.D. (2016). Quality characteristics and antioxidant properties of breads incorporated with pomegranate whole fruit bagasse. *Journal Food Sci Technol, 53,* 1717-1721.
2. Nagulb, D. M., Tantawy, A. A. (2019). Anticancer effect of some fruits peels aqueous extracts. *Oriental Pharmacy and Experimental Medicine, 19*, 415-420.
3. Ahmadi, H., Babalar, M., Sarcheshmeh, M. A. A., Morshedloo, M. R., Shokrpour, M. (2020). Effects of exogenous application of citrulline on prolonged water stress damages in hyssop *(Hyssopus officinalis L.):* Antioxidant activity, biochemical indices, and essential oils profile. *Food Chemistry.* 127433.
4. Joshi, V., Joshi, M., Silwal, D., Noonan, K., Rodriguez, S., et al. (2019). Systematized biosynthesis and catabolism regulate citrulline accumulation in watermelon. *Phytochemistry, 162,* 129–140.
5. Kiyici, F., Eroğlu, H., Kishali, N. F., & Burmaoglu, G. (2017). The Effect of Citrulline/Malate on Blood Lactate Levels in Intensive Exercise. *Biochemical Genetics, 55(5-6),* 387–394.
6. Valenzuela, Pedro L.; Morales, Javier S.; Emanuele, Enzo; Pareja-Galeano, Helios; Lucia, Alejandro (2019). *Supplements with purported effects on muscle mass and strength. European Journal of Nutrition.*
7. Ridwan, R., Abdul Razak, H. R., Adenan, M. I., & Md Saad, W. M. (2019). Supplementation of 100% flesh watermelon [Citrullus lanatus (Thunb.) matsum. And nakai] juice improves swimming performance in rats. *Preventive Nutrition and Food Science, 24(1),* 41-48.
8. Saulskaya, N. B., Fofonova, N. V., Sudorgina, P. V., & Komarova, A. S. (2011). Danger-associated sound signals activate the nitrergic system of the medial part of the nucleus accumbens. *Neuroscience and Behavioral Physiology, 41(5), 452–458.*
9. Joshi, V., & Fernie, A. R., (2017). Citrulline metabolism in plants. *Amino Acids, 49*(9), 1543–1559.
10. Caruso, A., Rossi, M., Gahn, C., & Caruso, F. (2017). A structural and computational study of citrulline in biochemical reactions. *Structural Chemistry, 28(5),* 1581–1589.
11. Umang Agarwal, Inka C Didelija, Yang Yuan, Xiaoying Wang, Juan C Marini. (2017). Supplemental citrulline is more efficient than arginine in increasing systemic arginine availability in mice, *The Journal of Nutrition*, 147(4):596–602, https://doi.org/10.3945/jn.116.240382
12. McNeal, C. J., Meininger, C. J., Reddy, D., Wilborn, C.D., & Wu, G. (2016). Safety and Effectiveness of Arginine in Adults. The *Journal of Nutrition, 146(12),* 2587S-2593S.
13. Gonzalez, Adam M.; Trexler, Eric T. (2020). *Effects of Citrulline Supplementation on Exercise Performance in Humans. Journal of Strength and Conditioning Research, 34(5), 1480–1495.* doi:10.1519/JSC.0000000000003426
14. Glenn, J. M., Gray, M., Wethington, L. N., Stone, M. S., Stewart, R. W., et al. (2017). Acute citrulline malate supplementation improves upper- and lower-body submaximal weightlifting exercise performance in resistance-trained females. *European Journal of Nutrition, 56(2),* 775–784.

15. Mondal, S., & Thompson, P. R. (2021). *Chemical biology of protein citrullination by the protein A arginine deiminases. Current Opinion in Chemical Biology, 63,* 19–27.

16. Hess, S., Baker, G., Gyenes, G., Tsuyuki, R., Newman, S., et al. (2017). Decreased serum L-arginine and L-citrulline levels in major depression*. Psychopharmacology, 234(21),* 3241–3247.

17. Azizi, S., Mahdavi, R., Vaghef-Mehrabany, E., Maleki, V., Karamzad, N., et al. (2019). Potential roles of Citrulline and watermelon extract on metabolic and inflammatory variables in diabetes mellitus, current evidence, and future directions: A systematic review. *Clinical and Experimental Pharmacology and Physiology.*
18. Suzuki, I., Sakuraba, K., Horiike, T., Kishi, T., Yabe, J., et al. (2019). A combination of oral l-citrulline and l-arginine improved 10-min full-power cycling test performance in male collegiate soccer players: a randomized crossover trial. *European Journal of Applied Physiology, 119(5),* 1075–1084.
19. Pérez-Neri, I., Diéguez-Campa, C. E. (2018). Relevancia de la citrulina para el sistema nervioso central. *Archivos de Neurociencia, 23(1)*, 50-54.
20. World Health Organization. (2020a). Cardiovascular diseases. https://www.who.int/cardiovascular_diseases/es/ Accessed 25 March 2020.
21. Mirenayat, M. S., Moradi, S., Mohammadi, H., Rouhani, M. H. (2018). Effect of L-Citrulline Supplementation on Blood Pressure*:* A Systematic Review and Meta-Analysis of Clinical Trials. *Current Hypertension Reports, 20(11).*
22. Davies, K. P. (2015). Development and therapeutic applications of nitric oxide-releasing materials to treat erectile dysfunction. *Future Science OA, 1(1).*
23. Hotta, Y., Shiota, A., Kataoka, T., Motonari, M., Maeda, Y., et al. (2013). Orall L-citrulline supplementation improves erectile function and penile structure in castrated rats. *International Journal of Urology, 21(6)*, 608–612.
24. Miyake, M., Kirisako, T., Kokubo, T., Miura, Y., Morishita, K., et al. (2014). Randomised controlled trial of the effects of L-ornithine on stress markers and sleep quality in healthy workers. *Nutrition Journal, 13(1),* 1-8.
25. Morita, M., Hayashi, T., Ochiai, M., Maeda, M., Yamaguchi, T., et al. (2014). Oral supplementation with a combination of L-citrulline and L-arginine rapidly increases plasma L-arginine concentration and enhances NO bioavailability. *Biochemical and Biophysical Research Communications, 454(1),* 53–57.
26. World Health Organization. (2020b). Global Strategy on Diet, Physical Activity and Health. https://www.who.int/dietphysicalactivity/pa/es/ Accessed 26 March 2020.
27. Ogurtsova, K, da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., et al. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice, 128,* 40-50.
28. Darabi, Z., Darand, M., Yari, Z., Hedayati, M., Faghihi, A., et al. (2019). Inflammatory markers response to citrulline supplementation in patients with non-alcoholic fatty liver disease: A randomized, double blind, placebo-controlled, clinical trial. *BMC Research Notes, 12(1),* 89.
29. Briseño-Bass, P., Chávez-Pérez, R., López-Zendejas, M. (2018). Prevalence and relation of hepatic steatosis with lipid and hepatic profile in check-up patients. *Journal of Gastroenterology of Mexico. 84:* 290-295.
30. Joshi, V., & Fernie, A. R., (2017). Citrulline metabolism in plants. *Amino Acids, 49*(9), 1543–1559.
31. Kuropteva, Z. V., Baider, L. M., Nagler, L. G., Bogatyrenko, T. N., & Belaiac, O. L. (2019). l-Arginine and nitric oxide synthesis in the cells with inducible NO synthase. *Russian Chemical Bulletin*, *68(1),* 174-180.

32. Chen, I-Fan., Wu, Huey-June., Chen, C. Y., Chou, K. M., & Chang, C. K. (2016). Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated matches in taekwondo athletes: A randomized controlled trial. *Journal of the International Society of Sports Nutrition, 13*(1), 28.
33. Guía de suplementos. (2020). Citrulina: ¿Cuál es el mejor suplemento del 2020?. Última actualización 21/02/2020. https://www.guiadesuplementos.mx/citrulina/ Accessed 06 April 2020.
34. Gonzalez, A. M., Spitz, R. W., Ghigiarelli, J. J., Sell, K. M., & Mangine, G. T. (2017). Acute effect of citrulline malate supplementation on upper-body resistance exercise performance in recreationally resistance-trained men. *Journal of Strength and Conditioning Research, 1.*
35. Agarwal, U, Didelija, I. C., Yuan, Y., Wang, X., & Marini, J. C. (2017). Supplemental Citrulline is More Efficient Than Arginine in Increasing Systemic Arginine Availability in Mice. *The Journal of Nutrition, 147(4),* 596–602.
36. Romdhane M., Haddar A., Ghazala I., Ben Jeddou K., Boisset H. C., et al. (2017). Optimization of polysaccharides extraction from watermelon rinds: Structure, functional and biological activities. *Food Chemistry, 216*, 355-364.
37. Grumet, R., Katzir, N., & Garcia-Mas, Jorgi. (2017). Genetics and genomics of cucurbitaceae, plant genetics and genomics: crops and models. *Springer International Publishing*, *20*, 199.
38. Bellary, A. N., Indiramma, A. R., Prakash, M., Baskaran, R., & Rastogi, N. K. (2016). Anthocyanin infused watermelon rind and its stability during storage. *Innovative Food Science & Emerging Technologies, 33*, 554–562.
39. Garcia-Lozano, M., Dutta, S. K., Natarajan, P., Tomason, Y. R., Lopez, C., et al (2019). Transcriptome changes in reciprocal grafts involving watermelon and bottle gourd reveal molecular mechanisms involved in increase of the fruit size, rind toughness and soluble solids. *Plant Molecular Biology, 102(1-2),* 213-223.
40. Zamora-Ros, R., Cayssials, V. , Jenab M., Rothwell, J. A., Fedirko, V., Aleksandrova, K., et al. (2018). Dietary intake of total polyphenol and polyphenol classes and the risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *European Journal of Epidemiology, 33(11),* 1063-1075.
41. Durán, R., Villegas, M. E., & Nieves, I. (2017). Caracterización y extracción de citrulina de la corteza de la sandía (Citrullus lanatus “thunb”) consumida en Valledupar. *Temas Agrarios, 22(1),* 60-67.
42. Naguib, D. M., Tantawy, A. A. (2019). Anticancer effect of some fruits peels aqueous extracts. *Oriental Pharmacy and Experimental Medicine, 19*, 415-420.
43. Petkowicz, C., Vriesmann L., Williams P. (2017). Pectins from food waste: Extraction, Characterization and proprieties of watermelon rind pectin. *Food Hydrocolloids.* 65:57-67
44. Naknaen, P., Itthisoponkul, T., Sondee, A., Angsombat, N. (2016). Utilization of watermelon rind waste as a potential source of dietary fiber to improve health promoting properties and reduce glycemic index for cookie making. *Food Science and Biotechnology, 25(2),* 415–424.
45. *Gobierno de México* . (10 de Julio de 2017). Recuperado el 2019 de Octubre de 12, de https://www.gob.mx/senasica/galerias/produccion-de-sandia-en-mexico.
46. Bassani, B., Rossi, T., De Stefano, D., Pizzichini, D., Corradino, P., et al. (2016). Potential chemopreventive activities of a polyphenol rich purified extract from olive mill wastewater on colon cancer cells. *Journal of Functional Foods, 27*, 236-248.

47. Gomez-Cadena, A., Martinez-Usatorre, A., Urueña, C., Prieto, K., Barreto, A., Romero, P., Fiorentino, S. (2015). Immune system activation through immunogenic cell death and tumor recruitment of dendritic cells is required for anti-tumor activity of a plant derived polyphenol rich fraction. *Journal for ImmunoTherapy of Cancer*. 3(Suppl 2): 301-301. DOI: 10.1186/2051-1426-3-S2-P301.
48. Altamimi, J. Z. (2019). Awareness of the Consumption of Dietary Supplements among Students in a University in Saudi Arabia. *Journal of Nutrition and Metabolism, 2019*(10).
49. World Health Organization. (2018). Healthy eating https://www.who.int/es/news-room/fact-sheets/detail/healthy-diet/ Accessed 12 October 2019.
50. Chacón Orduz, G., Muñoz Rincón, A., & Quiñónez Mosquera, G. A. (2017). Description of the healthy snack market in Villavicencio, Meta. *Libre Empresa*, *14*(2), 33-45.
51. Ettel, M., Gonzalez, G.A., Gera, S., Eze, O., Sigal, S., Park, J.S., Xu, R. (2017) Frequency and pathological characteristics of drug-induced liver injury in a tertiary medical center. *Hum Pathol*.68:92-98.
52. Bouillanne, O., Melchior, J.-C., Faure, C., Paul, M., Canouï-Poitrine, et al. (2018). Impact of 3-week citrulline supplementation on postprandial protein metabolism in malnourished older patients: The Ciproage randomized controlled trial. *Elsevier Ltd and European Society for Clinical Nutrition and Metabolism,* 1-11.
53. Chen, I-Fan., Wu, Huey-June., Chen, C. Y., Chou, K. M., & Chang, C. K. (2016). Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated matches in taekwondo athletes: A randomized controlled trial. *Journal of the International Society of Sports Nutrition, 13*(1), 28.
54. Gonzalez Martinez, D. W. (2017). *Obtención de fitoquímicos mediante metolodogía emergentes: microondas y ultrasonido de corteza de melón (Cucumis melo L.), corteza de sandia (Citrullus lanatus) y orujo de uva (Vitis vinídera)* Universidad Autónoma de Coahuila. Saltillo, Coahuila. Tesis de Licenciatura 111p.
55. Figueroa, A., Wong, A., Jaime, S. J., & Gonzales, J. U. (2017). Influence of L-citrulline and watermelon supplementation on vascular function and exercise performance. *Current Opinion in Clinical Nutrition and Metabolic Care, 20(1),* 92–98.
56. Mohan, S., Wu, C. C., Shin, S., & Fung, H. L. (2012). Continuous exposure to L-arginine induces oxidative stress and physiological tolerance in cultured human endothelial cells. *Amino Acids, 43*(3), 1179–1188.