Enhanced L-citrulline in parboiled paddy rice with watermelon (Citrullus lanatus) juice for preventing Sarcopenia: A preliminary study

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Aging related muscle loss and sarcopenia are public health problems. The objective of this study was to investigate the potential of rice parboiled with watermelon juice as a source of L-citrulline and as a means of slowing or preventing sarcopenia. In the western and industrialized countries, preventive and curative treatments for sarcopenia include physical exercise, pharmacological approach and dietary supplementation. Currently, L-citrulline supplementation plays a central role against decreasing muscle mass and may contribute in sarcopenia protection in the elderly. However, in developing countries, preventive and curative approaches are constrained by poverty and poor access to pharmaceutical supplements. Paddy rice was soaked in watermelon juice at temperatures of 80°C and steamed for 18 h. Rice was then hulled, dried and used to determine L-citrulline and L-arginine contents by HPLC. L-citrulline content increased to 46.6 mg/100 g (on dry basis) and L-arginine content increased to 17.7 mg/100 g (on dry basis) when rice was soaked with watermelon juice. This study shows that parboiled rice with watermelon juice could be a source of L-citrulline and L-arginine, as a means of slowing or preventing sarcopenia for elderly people where access to medical care and pharmaceutical supplements is poor.

Key words: Parboiled rice, watermelon, L-citrulline, sarcopenia, L-arginine.

INTRODUCTION

Sarcopenia is a pathology characterized by a decrease in muscle mass, strength and function that imparts frailty to the elderly. This condition is generally caused by a low protein intake or protein metabolism alteration and reduced physical activity (Cruz-Jentoft et al., 2010; Rolland et al., 2009). Sarcopenia is thought to be a complex multifactorial
The process facilitated by a combination of factors including the adoption of a more sedentary life style and a less than optimal diet (Boirie, 2009; Paddon-Jones et al., 2008). Various studies estimate that 25% of people over 70 years old and 40% of those over 80 years would be sarcopenic. Preventive or curative measures must be maintained during the whole life of the affected person. As such, sarcopenia and weight loss are a major public health challenge around the world (Renoud et al., 2014).

In western and industrialized countries, physical exercise, hormones and drugs therapy approaches have been proposed as mechanisms for treatment of sarcopenia (Iolascon et al., 2014; Beas-Jiménez et al., 2011; Yarasheski et al., 1999). Currently, no pharmacological protocols have been consensually validated in the prevention of sarcopenia and no pharmaceutical treatment is available (Kishida et al., 2015; Beas-Jiménez et al., 2011). Although, physical activity has well documented benefits in many older populations, the ability to exercise can become compromised by physical disability or disease (Paddon-Jones et al., 2008). It has been shown that an adequate protein intake is necessary for the elderly to benefit from resistance exercise, because protein consumption is the major nutritional determinant for protein synthesis activation due to increased blood amounts of amino acids (hyperamino-acidemia) derived from the proteinic digestion of foods (Beas-Jiménez et al., 2011). Treatment of sarcopenia with anabolic hormones has many side effects (Sakuma and Yamaguchi, 2013). The success of postprandial aminoacidemia depends on the ability of the intestine and the liver to retain amino acids from protein digestion. While some amino acids are retained by the splanchnic tissue, splanchnic sequestration is accentuated with age and reduces the availability of amino acids for muscle biosynthesis (Jahan-Mihan et al., 2011; Fouillet et al., 2003).

Promoting muscle anabolism with a protein rich food has several advantages over supplementation. Many plant and animal based protein containing foods are readily accessible and relatively inexpensive, whereas supplements such as essential amino acids frequently are not (Paddon-Jones et al., 2008). One alternative to slow or prevent muscle protein catabolism is the therapeutic use of L-citrulline. Unlike other amino acids, L-citrulline possesses a highly specific metabolism that bypasses splanchnic (internal organ) extraction. Because L-citrulline is not used by the intestine or taken up by the liver, it is made available throughout the body rapidly after ingestion and thus may act directly (Bahri et al., 2013). L-citrulline is a non protein amino acid that is produced predominantly in the intestines (Betue et al., 2013) and metabolized to L-arginine in the vascular endothelium, renal and other cells. L-arginine is a semi-essential amino acid and participates as an intermediary compound in the urea cycle and is a precursor for endogenous synthesis of nitric oxide due to the activity of nitric oxide synthase, which releases L-citrulline as a byproduct (Wu et al., 2009). L-citrulline increases the blood concentration of L-arginine more effectively than oral L-arginine, as L-citrulline undergoes neither intestinal nor hepatic metabolism, is not a substrate for arginase, and does not induce the expression or activity of the enzyme (Orozco-Gutiérrez et al., 2010). Using L-citrulline to combat sarcopenia has been assessed in clinical trials with promising results (Bahri et al., 2013; Moinard and Cynober, 2007) and plays a key role in the immune system (Yu et al., 1995).

Several pharmacokinetic studies have confirmed that L-citrulline is efficiently absorbed when administered orally (Bahri et al., 2013). Oral L-citrulline supplements, readily available in industrialized countries, could be used to deliver L-arginine to the systemic circulation or as a protein anabolic agent in specific clinical situations for sarcopenia (Bahri et al., 2013). Although L-citrulline represents an interesting nutritional strategy, delivery in the African sub-Saharan countries faces constraints of poverty, poor access to pharmaceutical supplements and lack of adequate medical care.

Watermelon (Citrullus lanatus) is a natural and rich source of the non-essential amino acid L-citrulline and is present in watermelon flesh at concentrations ranging from 0.7 to 3.6 g/kg (Collins et al., 2007). L-citrulline has a high bioavailability, with 80% of the ingested amount quickly absorbed in the blood (Mandel et al., 2005).

Watermelon is a common crop grown in West Africa, yet fruit consumption is relatively low because it is not uniformly available and accessible at any time (Layade and Adeoye, 2014). Rice is a staple food for millions of people, including those in Senegal, and there is ongoing research to increase both yield and protein content (Manful, 2010). Parboiling paddy rice is a process developed to improve rice quality by giving higher milling yields and higher nutritional value (Manful et al., 2009; Bleoussi et al., 2009; Sareepuang et al., 2008; Derycke, 2007). It consists of soaking in water, steaming and drying of the paddy rice (Dutta and Mahanta, 2014; Parnsakhorn et al., 2008). Parboiled rice is more nutritious as compared to raw brown rice because the proteins and vitamins are released at the center of the grain after parboiling (Derycke, 2007). Rice parboiled with watermelon offers a means to incorporate L-citrulline and L-arginine into diets as a source of protein to avoid muscle mass wasting.

This study was done to investigate if parboiled rice with watermelon juice could be a source of L-citrulline.

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MATERIALS AND METHODS

Plant material

Red-fleshed watermelons were grown from seed in greenhouses and seedlings transplanted one week after germination to the Senegalese Agricultural Research Institute plots in Dakar (Senegal). At maturity, watermelons were harvested and stored in a cold place (at 15°C) for three days. Each watermelon was gently and completely hollowed and all components of the fruit (flesh, peels, juice, and seeds) except rinds were collected. Content of each watermelon was labeled and placed in a polyethylene bag, samples were sealed and packed in another black plastic bag and frozen. After removing from storage, samples were brought to room temperature by holding for one day. Thawed samples were pureed until completely changed into juice.

An irrigated paddy rice ‘Sahel 108’ newly harvested from the Senegal River Valley was used in this study. Paddy rice harvested too late has a great number of cracked grains and a high percentage of broken rice in the milled product (Bhattacharya, 1969). Paddy rice was first cleaned by winnowing and then washed with water in order to remove impurities (stones, sand and other foreign bodies) and immature seeds. Washing of paddy rice was performed in a basin containing two paddy volumes for three volumes of water. Immature grains that floated to the surface and heavy stones that fell to the bottom by densimetric separation were removed using a sieve. Paddy rice was then drained for a few minutes with a stainless steel drainer. Washed paddy rice was spread on cotton gauze fabric laid on trays as a solar dryer and exposed to the sun until the moisture content was below 13%. After sun drying, rice was kept in bags to prevent contamination.

Treatments

Treatments were done as outlined in Figure 1. The parboiling process described by Houssou and Amonsou (2005) was followed in our study as the most appropriate to ensure a better quality of parboiled rice. The same steps and procedure described for conventional parboiling paddy rice were followed except the soaking water which was replaced by watermelon juice. The study design consisted of a time/temperature combination where rice samples were parboiled to a temperature of 80°C then allowed to soak in the water or watermelon juice up to 18 h.

Samples of 3 kg (compared to the average amount of a hulled rice family’s daily intake) of paddy rice were soaked (using a butane gas heater) in a pot without a lid and containing water or watermelon juice slightly exceeding the level of rice and was stirred occasionally. Temperature was monitored using a thermometer and once the desired temperature was reached, the operation was stopped, and the pot was removed from the stove. The paddy rice was kept in the covered pot and placed at room temperature (average 27°C) for 18 h, the soaking time programmed to allow cooling overnight and migration of some nutrients inside seeds. Preheating and soaking operations were performed in early afternoon in order to obtain soaking time indicated and start steaming operation, solar drying in the next morning. After 18 h, paddy rice was removed from soaking water and rinsed with fresh clean water and then drained.

Steaming paddy rice

Steaming was done using a perforated steamer and a pot full of water. Paddy rice was poured into the steamer, covered with a transparent fabric and the whole was placed on the pot. The charged device was warmed up to the appearance of steam followed by the bursting of some paddy grains, for about 25 to 30 min.

Sun drying of parboiled paddy rice

Parboiled paddy rice was spread again on trays to solar dry (28°C) for two hours, then dried in the shade in a ventilated storage (25°C). This system reduced moisture content of paddy rice below 12% for its conservation and shelling. The steamed paddy rice was then dried at room temperature (25°C). Samples were packed and stored (in a ventilated storage at 25°C).

Protocol for analysis of L-citrulline and L-arginine using HPLC

All rice samples were dehulled. About 5 g of paddy rice per
treatment were gently peeled by hand to ensure the integrity of the grain, and ground using a coffee mill. About 0.1 g of the rice powder was vortexed for 1 min with 1.2 ml 0.03 M H₂PO₄ in microfuge tubes, sonicated for 30 min and left at room temperature for 10 min. Samples were centrifuged at 14000 rpm (5417 R, Eppendorf, USA) at 4°C for 20 min. Supernatants were saved and pellet re-extracted with 1.2 ml of 0.03 M H₂PO₄ as above. Combined supernatants were placed in 5 ml tubes, mixed well and 1 ml of the supernatant was filtered into HPLC vials with a nylon syringe filter (17 mm, 0.2 µm, F2513-2, Thermo Scientific). Vial headspace was washed with N₂, sealed, and stored at -80°C until run on HPLC.

L-citrulline and L-arginine contents were quantified following the methods of Jayaprakasha et al. (2011). The analyses were made using a HPLC (System-Elite LaChrom, Hitachi, Japan) equipped with a DAD detector set at 195 nm, and autosampler. Sample injections of 10 µl were done on a Gemini 3u C18, 110 A, 250 X 4.6 mm column (Phenomenex, CA, USA) with Security Guard Cartridges (C18 4 x 2.0, Phenomenex) at an oven temperature of 25°C. The mobile phase was 0.015 M H₂PO₄ with a flow rate of 0.5 ml/min. L-citrulline and L-arginine contents were calculated using standard curves developed with L-citrulline and L-arginine standards (Sigma, USA).

Data analysis

All the measurements were replicated three times and the data are presented as mean ± SD.

RESULTS AND DISCUSSION

L-citrulline and L-arginine contents were increased (21.5 and 10.2 mg/100g dry weight, respectively) in parboiled rice with watermelon juice samples (WmPR) when compared with non-parboiled and traditional parboiled rice (WaPR) (Table 1). The unparboiled rice contained trace amounts of free L-citrulline (0.45 mg/100 g dw) and free L-arginine (3.27 mg/100 g dw). In comparison, rice parboiled with water contained even less of these amino acids (0.10 and 2.50 mg/100 g dw, L-citrulline and L-arginine, respectively). These results suggest that rice parboiled with watermelon juice could be a good food vehicle for L-citrulline intake supplementation. Several studies have found that L-citrulline administration using watermelon puree or juice was associated with increased plasma concentrations of L-citrulline, and metabolically related amino acids such as L-arginine (Moinard et al., 2008; Schwedhelm et al., 2008; Mandel et al.; 2005; Collins et al. 2007).

To our knowledge, this is the first study that helps increase the presence of L-citrulline and L-arginine in rice using parboiling technique with watermelon juice. Tarazona-Diaz et al. (2013) reported that L-citrulline is an excellent candidate to reduce muscle soreness; their study investigated the potential of watermelon juice as a functional drink for athletes. In the same way, consumption of WmPR could bring likely advantages as L-citrulline food vehicle. Rice parboiled with watermelon juice has high levels of L-citrulline and L-arginine that may help alleviate the effects of sarcopenia in the elderly. This assertion requires a further study to determine bioavailability of L-citrulline and frequency of consumption of WmPR which produces beneficial effects.

Parboiling rice with watermelon juice process also may be an application to minimize post harvest losses in the African sub-Saharan countries.

Fresh watermelon contains around 4 mg/g of L-citrulline, while the WmPR contained 21 mg/100g dw. Optimizing parboiling parameters should be achieved to establish best performance, and trialing yellow or orange flesh types, which may have more L-citrulline than red-fleshed watermelon (Rimando et al., 2005) should be done.

Dietary requirements for protein and amino acids are characteristics of an individual (WHO/FAO/UNU, 2002). Multiple posologies exist about L-citrulline supplements and some of them are dosed at 250 mg. The present results show that rice parboiled with watermelon juice could contain around 25 mg/100, hence consumption of 350 g WmPR could provide 87.5 mg of L-citrulline and 3 times a daily intake of 350 g WmPR should exceed supplement dose. L-arginine supplementation is also widespread in industrialized countries as compared to Sub-Saharan countries. L-arginine is extensively metabolized by the liver that calls in question of the efficacy of L-arginine supplementation. However, L-citrulline is not captured by the liver and passes freely to the kidneys where it is metabolized into L-arginine (Wijnands et al., 2015). Our method is therefore a good strategy to generate L-arginine and improve the nutritional status of the elderly who do not have access to basic health services.

In this study, results showed that WmPR samples contain more L-arginine than unparboiled or samples parboiled with water (Figures 1 and 2). Furthermore, it has been demonstrated that plasma concentration of L-arginine and L-citrulline are low during illness in children and normalize again after recovery. Plasma L-arginine and L-citrulline are strongly related to the severity of inflammation indicated by plasma CRP concentration (Van Waardenburg et al., 2007). Also, it is necessary to increase consumption level of these amino acids in illness situations. Hence we recommend consumption of rice parboiled with watermelon juice that could be used for undernourished children.

Access to sufficient food with adequate quality to maintain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L-Arginine</th>
<th>L-Citrulline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unparboiled</td>
<td>3.27±1.45</td>
<td>0.45±0.31</td>
</tr>
<tr>
<td>Parboiled with water</td>
<td>2.50±0.20</td>
<td>0.10±0.28</td>
</tr>
<tr>
<td>Parboiled with watermelon</td>
<td>10.51±1.06</td>
<td>21.19±3.20</td>
</tr>
</tbody>
</table>

Data represent averages of three independent repeats ± standard deviation.

Table 1. Free amino acid content of L-arginine and L-citrulline (mg/100 g dw) in rice after treatments.
normal body composition and function throughout the life-cycle is fundamental to maintaining health. A source of protein is an essential element of a healthy diet (WHO/FAO/UNU, 2002). In consideration of this guidance from WHO and FAO, parboiled rice with watermelon juice may be useful in daily dietary protein intake, managing and preventing specific diseases in addition to its likely positive effects on mechanisms that are involved in the decrease of the muscle mass in humans. According to WHO and FAO, adequate amounts of amino acids of a suitable pattern must be provided in the diet, either in a preformed state, or as appropriate precursors that can be used to generate a suitable mix of amino acids following endogenous transformations, in order to match the demand for protein synthesis and other metabolic pathways.

Parboiling rice with watermelon juice could be considered as a type of food fortification strategy. Fortification is defined as the practice of deliberately increasing the content of an essential micronutrient in a food so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health (Allen et al., 2006). Our new technical approach of parboiling using watermelon juice brings nutrients, improves the protein nutritional quality of rice and then could be a food fortification strategy against malnutrition, weight loss and sarcopenia considered as major public health challenge around the world.

However, future research should be focused on the bioavailability of L-citrulline in rice parboiled with watermelon juice due to the fact that protein utilization is generally discussed in terms of digestibility and biological value (WHO/FAO/UNU, 2002).

**Conclusion**

Watermelon is a natural source of L-citrulline that bypasses splanchnic sequestration and has a direct effect on muscle protein synthesis, thereby enabling the increase of muscle mass. This work presents the first use of watermelon juice in rice parboiling process. Results suggest that rice parboiled with watermelon juice could be a good food vehicle for L-citrulline and L-arginine. In consequence, an adequate consumption of this food may increase plasma L-citrulline, and a means of slowing or preventing sarcopenia for elderly people in a context of poor access to pharmaceutical supplements.

**Conflict of interests**

The author(s) did not declare any conflict of interest.

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