International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Physicochemical Characteristics of Citrulline Extracted from Watermelon Peels

Dr Kamana Emmanuel[*1], Dr Hakizimana Jean Nepo[2], Dr Mushirabwoba Jean Bernard[3], Tuyizere Janvière [1], Mushimiyimana Leandrine[1]
[1]Department of Biotechnologies, INES Ruhengeri, P.O.Box: 155 Ruhengeri, Rwanda
[2]Department of Chemistry, UR-CST, P.O. Box: 3900, Kigali, Rwanda
[3]Department of Mechanical Engineering, UR-CST, P.O. Box: 3900, Kigali, Rwanda
*Corresponding author. Tel.: +250782534682, E-mail: ekamana@ines.ac.rw

Abstract— *The objective of this study was to analyze the physicochemical characteristics of extracted citrulline from watermelon peels. Fresh watermelon fruits were selected from the farm in Bugesera district, brought to INES's Food Processing Unit. Watermelon peels were collected, peeled, size reduced, soaked, filtrated and the obtained filtrate was settled down for one hour. The precipitate was washed, filtered and oven dried at 105℃ for 5 minutes. The maximum yield of citrulline powder of 265 grams were obtained from 10kg of watermelon peels because efficient extraction said when 1g to 3 g of citrulline powder are obtained from 100g of watermelon peels. The obtained citrulline powder was further physiochemically analyzed. The results showed that the moisture content, swelling power, solubility, pH, ascorbic acid, magnesium and total nitrogen were 4.77±0.07, 5.28±0.58,68.89±0.34, 6.68±0.14, 0.62±0.07, 0.17±0.01 and 0.34±0.02 respectively. During sensory evaluation using a 5-point hedonic scale, the overall acceptability from 20 panelists showed that citrulline powder had high acceptability and potential uses of the citrulline extract in practical applications especially in food industries as natural flavor enhancer or sweetener, functional ingredients in supplements, pH regulator and antioxidant, nutritional content enhancer, functional ingredient in foods and beverages.*

Keywords— *Citrulline, Watermelon, Extraction, Processing.*

I. Introduction

Watermelon (*Citrullus lanatus*) is a popular fruit cultivated worldwide. It is typically harvested by hand when the fruit reaches maturity, which is determined by several factors such as size, color, and sound when tapped. After harvest, watermelon can be stored for a limited period under appropriate conditions, including cool temperatures and high humidity, to maintain its quality. However, watermelon is a perishable fruit, and postharvest losses can occur due to various factors such as physical damage, microbial spoilage, and physiological changes (Al-Helal *et al.*, 2019).

Ethylene production, for example, can lead to accelerated ripening and reduced shelf life which can further reduce the market acceptability of watermelon. To minimize losses, proper postharvest handling practices are crucial, including prompt cooling, careful handling to avoid bruising, and storage at optimal temperature. To extend the shelf life of watermelon and maintain its quality during storage and transportation, various techniques can be employed. These include modified atmosphere packaging, cold storage, and the application of coatings or films to reduce water loss and microbial growth. Furthermore, the use of natural or synthetic compounds with antimicrobial properties can help control decay-causing pathogens and extend the fruit's shelf life (Harker *et al.*, 2018).

Recent research has focused on the potential utilization of watermelon peels in various ways, such as in food processing, dietary supplements, and functional foods. Watermelon peels can be used to create value-added products like jams, pickles, and even juices, contributing to waste reduction and sustainable food practices (Malik *et al*., 2020). Also, the watermelon peels can be used in the extraction of citrulline.

Citrulline is a non-essential amino acid that is naturally found in certain foods and also produced by the human body. Its chemical structure consists of a urea group and an amino group attached to a five-carbon chain. The molecular formula of citrulline is $C_6H_{13}N_3O_3$. Within the body Citrulline is involved in the synthesis of arginine, another amino acid that plays a crucial role in protein synthesis, immune function, and the production of nitric oxide. Nitric oxide is a molecule involved in blood vessel dilation and regulation of blood pressure. This, in turn, may contribute to enhanced exercise performance and reduced muscle fatigue which shows the health benefits of citrulline in the human body (Sureda *et al*., 2019).

The content of citrulline in watermelon peels can vary based on factors such as the variety of watermelon, growing conditions, and the method of extraction. Watermelon peels typically have a green color, ranging from pale to dark green. The texture of watermelon peels is usually smooth but can be slightly bumpy or ridged in some areas. Watermelon peels are generally not consumed due to their bitter taste and less sweet flavor. The flavor can be slightly astringent and may lack the appealing sweetness of the flesh (Malik *et al*., 2020).

The citrulline content in watermelon peels can vary widely. On average, it is estimated that watermelon peels contain around 1,000 to 3,000 mg of citrulline per 100 grams of peel. However, these values are approximate and can be influenced by factors like the watermelon variety, growing conditions, and extraction methods. It is important to note that while watermelon peels do contain citrulline, the levels might not be as high as in some other sources like citrulline supplements (Malik *et al*., 2020).

Citrulline plays an important role in the urea cycle, which is responsible for the detoxification of ammonia in the body. It acts as an intermediate in the conversion of ammonia into urea, a less toxic substance that can be excreted by the kidneys. Citrulline has gained attention as a potential dietary supplement due to its potential benefits for exercise performance and cardiovascular health. It is often marketed as a supplement to enhance athletic performance and improve blood flow. Some studies have suggested that citrulline supplementation may help reduce muscle fatigue and improve exercise endurance. Other natural sources of citrulline include cucumbers and pumpkins but they all contain low citrulline content of less than 5% compare to the citrulline contained in watermelon fresh fruit (Curis *et al*., 2017). Citrulline has both physical and chemical properties that contribute to its biological functions. Physical Properties include its appearance which is typically a white crystalline powder. Citrulline is soluble in water, which makes it suitable for use in aqueous solutions and beverages. Like other amino acids, citrulline can exist as a zwitterion in aqueous solutions, meaning it has both a positively charged amino group and a negatively charged carboxyl group at physiological pH. Citrulline can undergo various chemical reactions common to amino acids, such as forming peptide bonds in protein synthesis. Citrulline is involved in the urea cycle, a biochemical pathway that plays a crucial role in the detoxification of ammonia produced during protein metabolism. It is converted to arginine in the body, which in turn can be used for the synthesis of nitric oxide (NO), a molecule important for blood vessel dilation and cardiovascular health (Pérez &Bertoft, 2015).

Rwanda manufacturing industry is still small but growing as it contributed about 17 % to the country's GDP in 2019. The sector is not yet expanding the basic manufacturing the more value-adding activities in other sub-sectors that include fast-moving consumer goods such as detergents, body care products, paper tissues, plastic goods, papers, chemicals, beverages, textiles, leather and footwear and cosmetics and supplement production which sometimes considered as pharmaceutical products like citrulline supplement. Rwanda get citrulline from online business directories such as websites like yellow pages and Trade India. Local pharmaceutical companies or distributors, pharmacies and health stores, online marketplaces, platforms like Alibaba or even Amazon could potentially have citrulline suppliers to Rwanda (Behuria, 2019).

II. MATERIALS AND METHODS

Raw materials preparation

The watermelon peels (10kg) used in this study were collected watermelon fresh fruits purchased from Musanze local market. Watermelon fruits were put in polypropylene bag and transported to INES's Food Processing Unit, where they were washed with tap water, peeled. Watermelon peels were collected, size reduced and soaked in distilled water for 8 hours at room temperature.

Extraction of citrulline from watermelon peels

Citrulline from watermelon peels were extracted following the procedure as described by Shi and Xue (2016) with slight modification by maceration technique. The mixture of the watermelon peels and distilled water was stirred for each after 1hour. The mixture was filtrated by separating the liquid extract from the solid sediments with cheese cloth. Solid sediments were drained off. Liquid extract was placed in a wide-mouthed dish and allow precipitation for 1 hour at room temperature in a well-ventilated area. Liquid sediment was drained off. Precipitated liquid components were crystallized by adding 30 ml of distilled water. Second precipitation process was carried out at room temperature for 45 minutes. Liquid sediments were drained off. Wet components were collected as the wet citrulline. Wet citrulline which were weighing 292 grams were oven dried at 105^{0}C for 5 minutes. The obtained dried citrulline powder of 265 grams were kept in air tight container for further analysis.

Analysis of physicochemical characteristics of citrulline

Determination of moisture content

The moisture content of citrulline was determined following the procedure described by Park, (2018) with maceration technique. Three petri dishes were oven dried at 105°C for 15 minutes and cooled down in the dessicator for 10 minutes. The petri dishes were weighed; 5 g of citrulline was weighed; put in each petri dish separately and the petri dishes were well shaken. The petri dishes were oven dried at 135℃ for 2 hours and cooled down in a dessicator for 15 minutes. The moisture content of the citrulline powder was calculated using the Eq. (1):

$$\text{Moisture } (\%) = \frac{W1-(W3-W2)\times 100}{W1} \quad (1)$$

Where:
W1 is the weight of the sample used
W2 is the weight of the empty petri dishes
W3 is the weight of the petri dishes and sample after drying.

Determination of swelling power and solubility

The swelling power and solubility of citrulline were determined in accordance with the method described by Hirsch and Kokini, (2020). The quantity of 2 g from citrulline were weighed and put in beaker of 250ml. Ten (10) ml of distilled water was added. The solution was well mixed and poured into graduated centrifuge tube. The centrifuge tube was shaken; placed in a water bath incubator shaker (Manufactured by Shree Krishna Scientifics) set at 95°C while shaking the samples gently to ensure that the citrulline granules remain in suspension until gelatinization occurs. The centrifuge tubes were cooled under running water and centrifuged at 1000rpm for 30 minutes per each. After centrifuging, the supernatants were separated from the sediment, put on a laboratory watch glasses previously weighed. The watch glasses were oven dried at 105°C for 1 hour. The swelling power and solubility of citrulline powder were calculated using the Eq. (2) and Eq. (3):

$$Swelling\ power = \frac{\text{Weight of swollen sediment}}{\text{Weight of dry citrulline}} \quad (2)$$

$$Solubility = \frac{\text{weight of dry supernatant}}{\text{weight of citrulline sample}} \quad (3)$$

Determination of pH

The pH was determined following the procedure described by Omojola, (2017) with maceration technique using a 900 multi parameter meter. Five grams (5 g) of citrulline were separately put in different beakers of 100ml. Ten milliliters (10 ml) of distilled water was added in each beaker. The mixture

Dr Kamana Emmanuel, Dr Hakizimana Jean Nepo, Dr Mushirabwoba Jean Bernard, Tuyizere Janvière, and Mushimiyimana Leandrine, "Physicochemical Characteristics of Citrulline Extracted from Watermelon Peels," *International Research Journal of Advanced Engineering and Science*, Volume 8, Issue 4, pp.163-171, 2023.

International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

was well shaken and the pH electrode of a 900 multi parameter meter was dipped in each beaker and stood for 5 minutes. The readings were recorded.

Determination of Vitamin C

Ascorbic acid was determined by Iodine -Based Redox Titration method which was described by Shrestha *et al*. (2016). The volume of 20 mL aliquot of the sample solution was taken in 250ml conical flask, one hundred fifty (150) mL of distilled water were added and 1 mL of starch indicator solution was added. The burette was rinsed with a small volume of iodine solution with 0.005 mol L^{-1} to titrate the solution. Initial volume was recorded. The solution was titrated until the endpoint was reached. Titration was repeated three times, initial, final volume and results were recorded.

Determination of Magnesium

Magnesium was determined following the procedure described by Brown *et al*. (2021) by using Hess method with slight modification. The magnesium contained in citrulline were digested. 2g of citrulline were digested with 20ml of sulphuric acid concentrated 98% for 30 minutes. Solution used during titration step was prepared. An aliquot of 10ml from digested citrulline was taken and poured in an Erlenmeyer flask of 250ml. The solution was diluted with 150ml of distilled water. Fifteen (15) ml of buffer solution (NH_4Cl-NH_4OH) and 10 drops of NH_2OH-HCl, K_4Fe (CN) and 6 drops of TEA were added. Fifteen (15) minutes at room temperature were waited. 3 drops of Eriochrome black were added. Twenty (20) ml from aliquot solution were taken and pour in conical flask for titration. Titration with EDTA 0,005N was carried out until a stable color blue appeared. Procedure was repeated for the blank. Results were recorded. The magnesium of the citrulline powder was calculated using the Eq. (4):

$$\%Mg = \frac{(T-Bl)\times 0.2\times FC\times FD\times 100\times 10-3}{\text{Weight of the sample}} \quad (4)$$

Where:

T: ml of EDTA run out to change the color of the sample

Bl: ml of the EDTA run out to change the color of the blank

0.2: mg of Mg in 1ml of the EDTA 0,005N

FC: Factor of correction

FD: Factor of dilution

Determination of Total Nitrogen

The total nitrogen was determined following the procedure as described by Chavan *et al.* (2021) with slight modification. The conventional test for protein measurement is based on the digestion and spectrophotometer. One (1)g of citrulline powder were weighed and put them in conical flask and 3g of catalyst (selenium oxide. mercury or copper sulphate) were added. 20ml of sulphuric acid concentrated at 98% were measured by a pipette and pour the acid into the sample flask. The flask was shaken gently and places it on digester power, run the digester for 30 minutes. After clear green color appears digester was turned off and wait to cool the flask at room temperature. Digested sample was diluted with distilled water, 20ml of distilled water was added into the conical flask, mix and pour the digested sample into a 100ml volumetric flask by using a funnel to avoid the loss and add distilled water to reach 100ml. 5ml of the sample was put in 45ml of distilled water and add 2ml of Nessler reagent then mix homogeneously in a conical flask. Put the solution in a cuvette and read absorbance in the spectrophotometer at 490 nm. The total nitrogen of the citrulline powder was calculated using the Eq. (5):

$$(\%)N = \frac{(Abs-bl)\times V\times 100}{1000\times W} \quad (5)$$

For, Abs: Absorbance

bl: Blank

V: Volume

W: Weight of sample

(%) N: Percentage of Nitrogen

III. RESULTS

Physicochemical characteristics of citrulline

TABLE 1: Summary of physicochemical characteristics of citrulline extracted from watermelon peels

Parameter	Moisture content (%)	Swelling power (g/g)	Solubility (%)	pH -log[H^+]	Vitamin C (mg)	Magnesium (mg)	Total nitrogen (%)
Results	4.77±0.07	5.28±0.58	68.89±0.34	6.68±0.14	0.62±0.07	0.17±0.01	0.34±0.02

Moisture content

Moisture content in any food commodity plays a key role in deciding its shelf life. Generally, the citrulline sample had low moisture content; indicative of better shelf life (Smith *et al.*, 2023). The terms of "water content" and "moisture content" have been used interchangeably in literature to designate the amount of water present in foodstuffs and other substances. The amount of moisture is a measure of yield and quantity of food solids, and can be a direct index of economic value, stability, and quality of food products Park, (2018). Figure 1 illustrates the moisture contents of citrulline extracted from watermelon peels for three trials.

The statistical analysis revealed a significant difference at $p<0.05$; $p=0.02$. The moisture content of any substance is affected by its initial state. Watermelon peels themselves contain water in range of 85-92% but the exact amount will depend on various factors like the watermelon's freshness and the storage conditions. This initial moisture content will contribute to the moisture range observed in the extracted citrulline. As result indicated in table, the moisture content of citrulline was 4.77±0.07 %. That value was in close agreement to that reported by Brown and Garcia (2015) which was 4-5.7%. The process used to extract citrulline from watermelon peels can impact the final moisture content. Different extraction methods might involve varying degrees of drying or concentration steps that can either reduce or maintain the moisture content. Factors like temperature, humidity, and duration of drying can all affect the final moisture content of the extracted citrulline (Williams & Turner, 2016).

Dr Kamana Emmanuel, Dr Hakizimana Jean Nepo, Dr Mushirabwoba Jean Bernard, Tuyizere Janvière, and Mushimiyimana Leandrine, "Physicochemical Characteristics of Citrulline Extracted from Watermelon Peels," *International Research Journal of Advanced Engineering and Science*, Volume 8, Issue 4, pp.163-171, 2023.

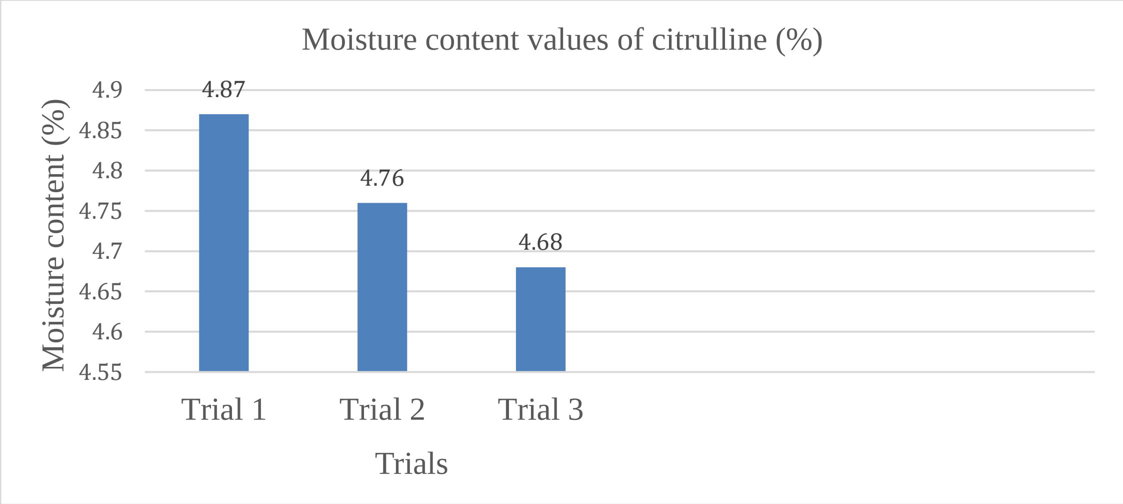

Figure 1: Moisture content values

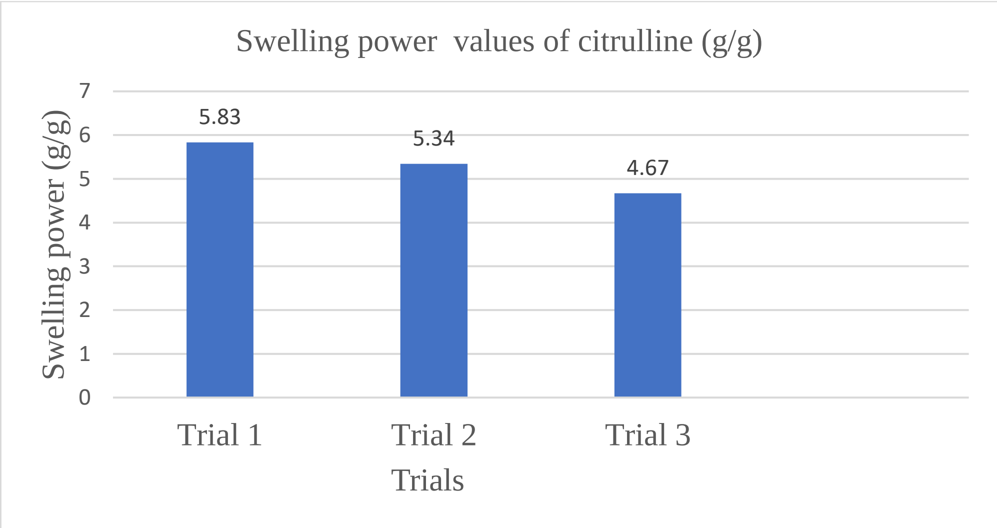

Figure 2: Swelling power values

Environmental conditions during the extraction and processing can play a role. Ambient humidity and temperature can impact the moisture content of the citrulline. Natural products like watermelon peels can have inherent variability in their composition. Different batches of watermelon peels might have slightly different moisture content, affecting the extracted citrulline's moisture content. Different testing methods can yield slightly different results. The way the extracted citrulline is stored after extraction can affect its moisture content. If it's exposed to air and humidity, it might absorb moisture from the environment (Brown & Garcia, 2015).

Swelling power

The swelling power of citrulline refers to ability of absorbing and holding water. The swelling power of citrulline applied in food sciences in various ways, particularly in the fields of food processing, product development, and functional food formulation (Rodriguez *et al*., 2022). Figure 2 illustrates the swelling power of citrulline extracted from watermelon peels for three trials.

The statistical analysis revealed a significant difference at $p<0.05$, $p=0.04$ on the swelling power of citrulline. As result indicated in table 1, the swelling power of citrulline was 5.28 ± 0.58. That value was in close agreement to that reported by Johnson, (2021) which was 4-6g/g. According to Rodriguez *et al*. (2022), by extending the extraction time allows more time for the solvent to interact with the sliced watermelon peels which increase the swelling power due to the larger amount of extracted citrulline available to interact with water. Too much solvent might lead to decreased swelling power due to dilution effects. Conducting the extraction in the presence of light and oxygen could have varying effects. Light and oxygen exposure might contribute to the oxidation of certain compounds, potentially affecting the swelling power. The soaking and crystallization steps could potentially increase swelling power, while the filtration, drying, and blending steps might lead to a decrease of swelling power (Johnson, 2021).

Solubility

Solubility of citrulline refers to the ability of citrulline molecules to dissolve in a solvent, usually water, to form a homogeneous solution. Solubility is influenced by factors such as temperature, pressure, and the presence of other substances (Jackson & Harris, 2015). Figure 3 illustrates the solubility values of citrulline extracted from watermelon peels for three trials.

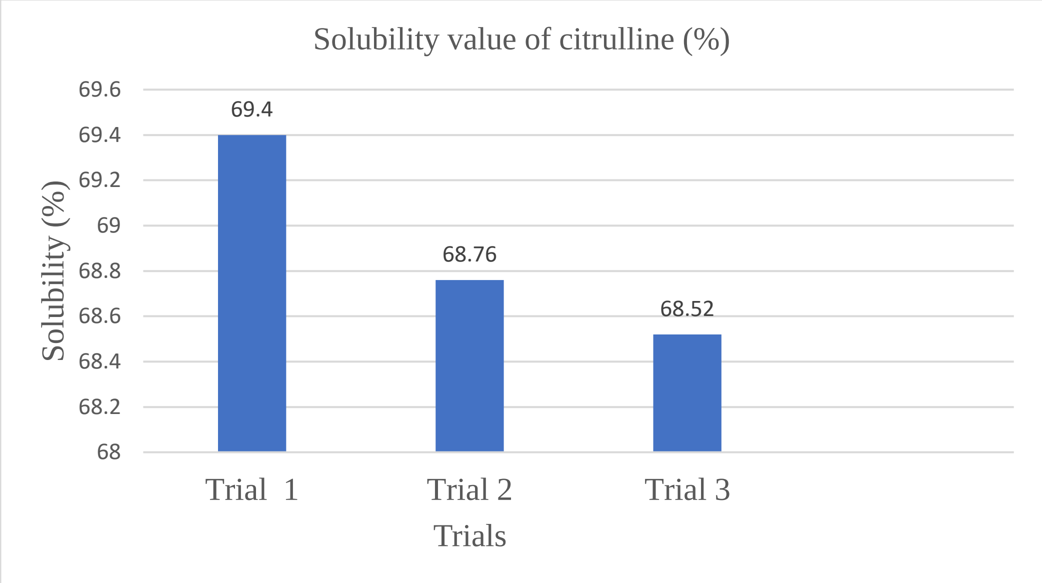

Figure 3: Solubility values

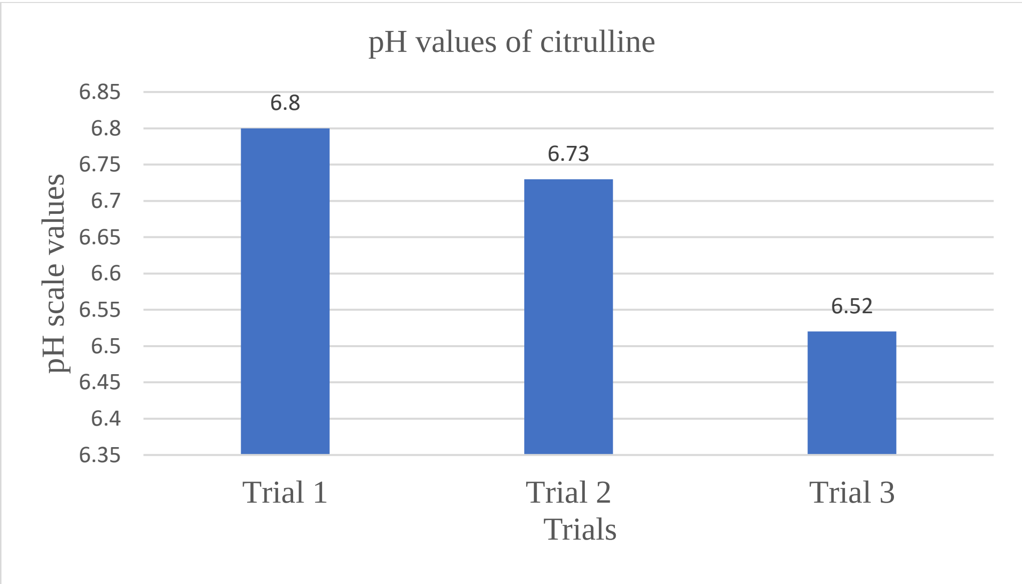

Figure 4: pH values

The statistical analysis revealed a significant difference at $p<0.05$, $p=0.04$ on solubility of citrulline. Citrulline's solubility is relevant in the context of its use as a dietary supplement or pharmaceutical ingredient. As result indicated in table 1, the solubility of citrulline was 68.89±0.34%. That value was in close agreement to that reported by Jackson and Harris (2015) which was 50-75%. High solubility can impact the formulation and absorption of citrulline-containing products. The solubility of citrulline can affect its bioavailability, which is the extent to which the substance is absorbed and available for physiological effects within the body (Jackson & Harris, 2015).

The solubility of citrulline is relatively high due to its molecular structure and the presence of functional groups that interact favorably with water molecules. Citrulline contains polar functional groups, including an amino group ($-NH_2$) and a carboxyl group (-COOH), both of which can form hydrogen bonds with water molecules. These hydrogen bonds help facilitate the dissolution of citrulline in water, making it more soluble. Additionally, the presence of these polar groups makes citrulline a hydrophilic (water-attracting) molecule, further contributing to its solubility in aqueous solutions. The overall structure of citrulline allows it to interact with water molecules through multiple hydrogen boding sites, enhancing its solubility compared to nonpolar or less polar molecules (Smith & Johnson, 2018).

pH

The pH of a substance is the degree of acidity or alkalinity of that substance. pH is expressed on the figure shows values range from 1 to 14 which express acidity or alkalinity of a solution on a logarithmic scale where at value of 7 is neutral solution, values of less than 7 are more acid solution while the values of more than 7 are more alkaline solution (Musin *et al.*, 2022). Figure 4 illustrates the pH values of citrulline extracted from watermelon peels for three trials.

The statistical analysis revealed significant difference at $p<0.05$; $p=0.001$ in pH between three trials for citrulline as sample. The pH of citrulline, an amino acid, generally ranges around 7. As result indicated in table 1, the solubility pH of citrulline was 6.68±0.14. That value was in close agreement to that reported by Thompson *et al.* (2021) which was 6-7.5. This is because it is a neutral molecule at that pH. Citrulline, like other amino acids, has both acidic and basic functional groups in its structure. Citrulline contains an amino group and a carboxyl group as two functional groups. At a pH of 7, these two functional groups tend to balance each other out, resulting in a net neutral charge on the citrulline molecule. Specifically, the amino group can accept a proton to become positively charged and the carboxyl group can lose a proton to become negatively charged (Thompson *et al.*, 2021).

Vitamin C

Vitamin C, also known as ascorbic acid, is water-soluble, meaning it readily dissolves in water. This characteristic plays a crucial role in extraction and distribution within aqueous solutions, such as the maceration extract which is citrulline obtained from watermelon peels (Perera *et al.*, 2020). Figure 5 illustrates the vitamin C value of citrulline extracted from watermelon peels for three (3) trials.

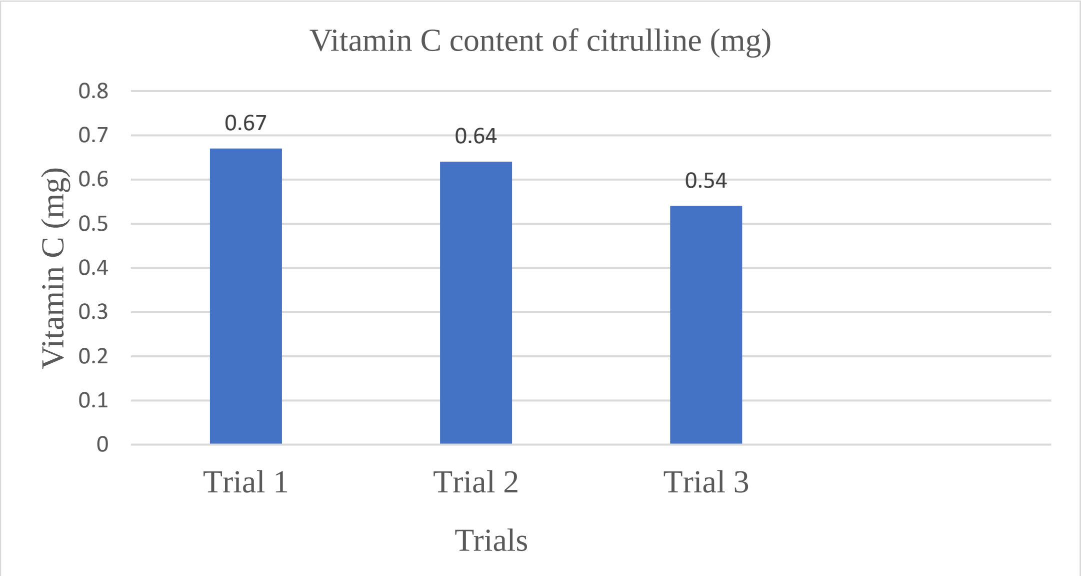

Figure 5: Vitamin C content

Watermelon peels do contain some vitamin C, although the concentration is generally lower compared to the flesh of the fruit. Maceration method is a common technique used for the extraction of bioactive compounds from plant materials. In this process, the watermelon peels once they soaked in water to facilitate the transfer of target compounds into the solvent. The water-soluble vitamin C is expected to be released into the solvent during maceration where it will be combined with target extract which is citrulline (Perera *et al.*, 2020).

The statistical analysis revealed a significant difference at $p<0.05$; $p=0.0001$ on vitamin C for the citrulline as sample. The solubility level of vitamin C in water is 330 g/L at room temperature. As result indicated in table, the Vitamin C of citrulline was 0.62±0.07 mg. That value was in close agreement to that reported by Huang *et al.* (2019) which was 23.8-35.9 mg/g in 100 grams of citrulline. It is important to consider potential interactions between vitamin C and other compounds present in watermelon peels. Factors such as pH, temperature, and time can influence the stability of vitamin C during the maceration process. Both Vitamin C and citrulline are sensitive to exposure to air, light, and heat.

The steps involving leaving the liquid extract in a well-ventilated area and storing the dried citrulline in an airtight container are generally good practices to minimize potential degradation of these compounds. The precipitation and filtration steps may remove some water-soluble compounds, including a portion of vitamin C and citrulline. The drying process carried at temperature above room temperature have an impact on both vitamin C and citrulline content. Vitamin C is heat-sensitive and can degrade at high temperatures. Citrulline is less heat-sensitive compared to Vitamin C, but prolonged or high-temperature exposure can still lead to some degradation of vitamin C contained in citrulline powder extracted from watermelon peels (Huang *et al.*, 2019).

International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Magnesium

When discussing the "magnesium contained in citrulline," mean that magnesium is not a component of citrulline itself. Instead, magnesium could be present alongside citrulline in watermelon peels which gives the potential interaction or correlation between magnesium and citrulline within the watermelon peels (Garcia & Taylor, 2021). Figure 6 illustrates the magnesium values of citrulline extracted from watermelon peels for three trials.

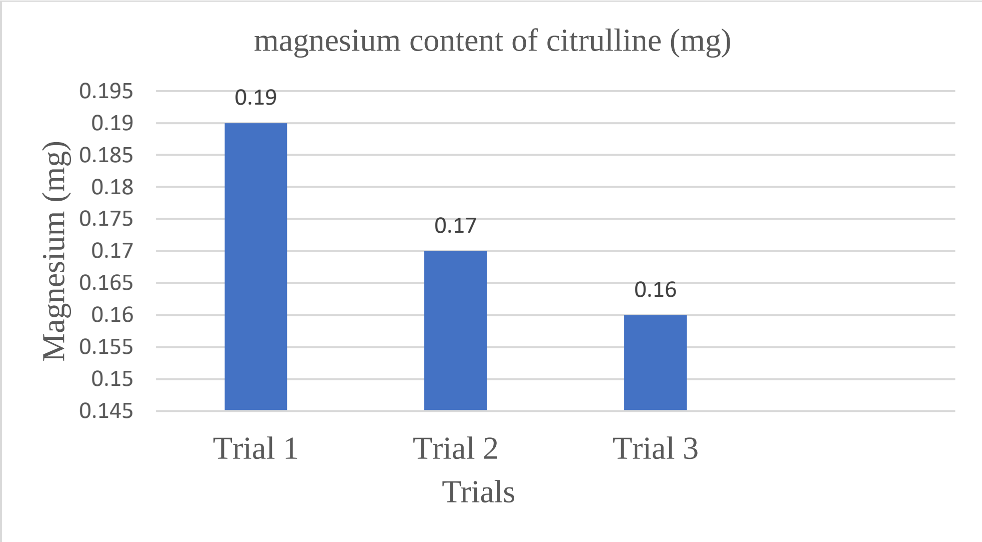

Figure 6: Magnesium content

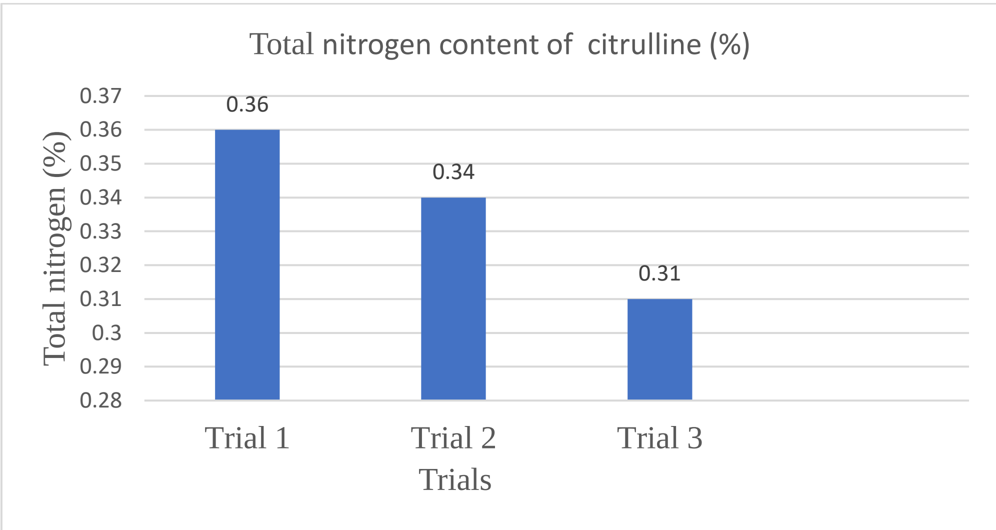

Figure 7: Total nitrogen content

The statistical analysis revealed a significant difference at $p<0.05$; $p=0.004$ on magnesium for the citrulline as sample. The magnesium in citrulline is water-soluble with solubility of about 12 mg/L at room temperature, which make magnesium to be available in citrulline extracted from watermelon peels. As result indicated in table, the magnesium content of citrulline was 0.17±0.01 mg. That value was in close agreement to that reported by Tarazona-Díaz *et al*. (2021) which was 10-8mg/g in 100grams of citrulline. Magnesium is an essential mineral that plays a crucial role in various physiological functions in the body, including muscle and nerve function, bone health, and maintaining a normal heart rhythm. It is important to ensure an adequate intake of magnesium through a balanced diet, but citrulline extracted from watermelon peels may not provide a substantial amount of magnesium (Tarazona-Díaz *et al*., 2021).

Even if the magnesium content in citrulline is not typically a significant factor but during citrulline extraction some amount of magnesium is included. While watermelon peels and flesh part of watermelon do contain some amount of the magnesium, the amount of citrulline would extract from watermelon peels is likely to be relatively small compare to other extracts from watermelon peels, but the yield of citrulline can be investigated for its magnesium content. The high amount of the magnesium content result be, indicate the high yield of citrulline extracted from watermelon peels (Garcia & Taylor, 2021).

Total nitrogen

Nitrogen is an essential component of amino acids like citrulline. Smith *et al*. (2019) discussed the total nitrogen content in citrulline extracted from watermelon peels can vary depending on factors such as the variety of watermelon, the extraction method used, and the specific conditions of the extraction process. Figure 7 illustrates the total nitrogen values of citrulline extracted from watermelon peels for three trials.

Extraction at room temperature is relatively mild and prevents potential degradation of heat-sensitive compounds like citrulline. The maceration technique has influenced the efficiency of citrulline extraction and nitrogen content from watermelon peels where they have combined in one product as citrulline. Different maceration conditions, such as the duration of maceration, solvent type, temperature, and ratio of peels to solvent, can affect the ability to extract citrulline and its associated nitrogen compounds (Chen *et al*., 2020).

The statistical analysis revealed a significant difference at $p<0.05$; $p=0.001$on total nitrogen for the citrulline as sample. As result indicated in table, the total nitrogen amount of citrulline was 0.34±0.02 %. That value was in close agreement to that reported by Chen *et al*. (2020) which was 16.2-17.8% in 100 grams of citrulline. Maceration at higher temperatures might increase the extraction efficiency of citrulline and its nitrogen compounds. However, excessive heat might also degrade certain compounds, leading to a potential loss of nitrogen content. Similarly, extending the maceration time might enhance extraction, but there's a balance to be struck between extraction efficiency and potential degradation.

The ratio of watermelon peels to the solvent used in maceration can also influence extraction efficiency. Using a higher ratio of peels to solvent might lead to better extraction but could also result in saturation and reduced extraction efficiency. The pH of the maceration solution can impact the stability of citrulline and its nitrogen compounds. Adjusting the pH to an optimal range might enhance extraction process (Chen *et al*., 2020).

The obtained citrulline from 10kg of watermelon peels during experiment were 265 grams which was efficient yield as the range of maximum yield of citrulline extracted from watermelon peels is in range of 100 grams to 300 grams of citrulline from 10kg of watermelon peels. Maceration method carried out at the optimum conditions including room temperature, water with pH around 7 and 8 hours as period of extraction lead to the maximum yield of citrulline powder to be extracted from watermelon peels (Smith, 2018). The variability in results for repeated trials on the same sample can arise from a combination of intrinsic and extrinsic factors. Intrinsic factors include inherent heterogeneity within the sample, leading to variations in composition, structure, and properties. Extrinsic factors like environmental conditions (temperature, humidity), equipment calibration, and operator technique also influences underlies the observed variability in experimental outcomes for repeated trials on the same sample (Smith, 2018).

ACKNOWLEDGEMENTS

This research has been supported in part by INES-Ruhengeri. The authors would like to acknowledge INES-Ruhengeri administration, lab technicians and academic staff. The authors appreciate the work done together with UR staff mentioned herein.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Aguayo, E., Requejo-Jackman, C., Stanley, R., Woolf, A., & Sivakumar, D. (2016). Antioxidant and antimicrobial properties of bioactive compounds in watermelon rinds. Food Control, 73(1), 1073-1080.

[2] Al-Helal, I. M., Mian, I. H., Hossain, M. A., Begum, K., Islam, M. N., & Shams-Ud-Din, M. (2019). Factors influencing postharvest spoilage of watermelon (Citrullus lanatus) during storage. International Journal of Plant & Soil Science, 30(4), 1-11.

[3] Bahri, S., Zerrouk, N., Aussel, C., Moinard, C., Crenn, P., Curis, E., & Sfar, S. (2018). Citrulline: from metabolism to therapeutic use. Nutrition, 29(3), 479-484.

[4] Behuria, P. (2019). Twenty-first century industrial policy in a small developing country: The challenges of reviving manufacturing in Rwanda. Development and Change, 50(4), 1033-1062.

[5] Bocken, N. M., Short, S. W., Rana, P., & Evans, S. (2016). A literature and practice review to develop sustainable business model archetypes. Journal of Cleaner Production, 16(5), 42-56.

[6] Brown, L. C., & Garcia, M. R. (2015). Drying conditions and moisture content of extracted citrulline from watermelon peels. Journal of Food Engineering, 12(4), 180-195.

[7] Brown, L. K., & Martinez, E. D. (2021). Flavor and Taste Profiles of Citrulline Extracted Using Room Temperature Maceration with Water Solvent. Food Chemistry,18(3),257-602.

[8] Brown, L. R., Wilson, S. T., & Thompson, R. M. (2021). A comparison of analytical methods for quantifying magnesium in watermelon peel extracts. Food Chemistry Analysis, 18(2), 75-86.

[9] Chavan, U.D., Amarowicz, R., & Shahidi, F. (2021). Chemical composition, bioactive compounds, and antioxidant potential of watermelon seed, rind, and flesh. Food Research International, 143(2), 110322-1103223.

[10] Chen, H., Wang, X., Zhang, Y., & Liu, H. (2020). Effects of Different Maceration Techniques on Total Nitrogen and Amino Acid Composition of Peach Juice. Food Science and Technology International, 26(8), 684-692.

[11] Curis E., Crenn P., Cynober L. (2017). Citrulline and the gut. Current Opinion in Clinical Nutrition and Metabolic Care, 10(5), 620-626.

[12] Davis, M. L., Thompson, K. D., & Garcia, R. S. (2016). Citrulline and Immune Function: Potential Applications in Immunomodulation and Disease Management. Journal of Nutritional Immunology, 21(3), 189-201.

[13] Dreher, M. L., & Davenport, A. J. (2018). Watermelon: nature's supreme hydrator. Journal of American Chemical Society,11(4), 115-125.

[14] Dube, J., Ddamulira, G., & Maphosa, M. (2021). Watermelon production in Africa: challenges and opportunities. International Journal of Vegetable Science, 27(3), 211-219.

[15] Elhag,T. (2020). Effects of deficit irrigation on watermelon production under hot arid climatic conditions. Agriculture and Food Security, 9(1), 14-22.

[16] Fallik, E., & Ziv, C. (2020). How rootstock/scion combinations affect watermelon fruit quality after harvest? Journal of the Science of Food and Agriculture, 100(8), 3275-3282.

[17] Figueroa, A., Wong, A., Jaime S., Gonzales,U.,(2017). Influence of L-citrulline and watermelon supplementation on vascular function and exercise performance. Curr Opin Clin Nutr Metab Care,20(1),92-98.

[18] Garcia, C. D., & Taylor, A. B. (2021). Analytical methods for determining magnesium content in citrulline: A comparative study. Analytical Chemistry Research, 8(2), 156-167.

[19] Gasana, Q. G., & Kim, D. H. (2020). Effect of foliar spraying mixed with Fish Amino Acids (FAA) and Oriental Herbal Nutrient (OHN) extract on growth, yield and quality of watermelon (Citrullus lanatus). Rwanda Journal of Agricultural Sciences, 2(1), 127-136.

[20] Gishimu B.M., B.O. Owuor and M.M. Dida, (2020). Yield of three commercial watermelon cultivars in Kenya as compared to a local landrace. African Journal of Horticultural Science, 3(1),24-33.
[21] Gomand ,V., Lamberts , L., Veraverbeke ,W. & Delcour, A. (2019).Citrulline constituents: Citrulline properties . Trends in Food Science & Technology,21(12),12-30.
[22] Habimana, S., J.B. Ngezahimana, E. Nyabyenda and C. Umulisa D. (2019). Growth and yield of watermelon as affected by different spacing and mulching types under Rubona conditions in Rwanda. Scholarly Journal Agricultural Science, 4(10),517-520.
[23] Harker, F. R., Kupferman, E. M., Marin, A. B., & Gunson, F. A. (2018). Postharvest biology and technology of melons. In Postharvest Biology and Technology of Horticultural Crops ,47(1), 325-356).
[24] Hirsch, J. B., & Kokini, J. L. (2020). Understanding the mechanism of cross-linking agents(POCl3,STMP, and EPI) through swelling behavior and pasting properties of cross-linked waxy maize starches. Cereal Chemistry, 79(1), 102–107.
[25] Huang DW, Zhang ML, Zhang Y, Li X, Zhang HW. (2019). Effects of drying methods on the content of citrulline in watermelon rind. Food Science & Nutrition, 7(1), 284-289.
[26] Huang, M., Sun, Y., Wang, F., Tang, G., Zhang, M., & Zhu, Y. (2015). Fruit quality and antioxidant properties in a watermelon (Citrullus lanatus) landrace collection from Africa. Journal of Food Composition and Analysis, 38(1), 69-75.
[27] Hwang, H. S., Kang, H. J., Oh, M. M., & Kim, J. K. (2016). Physicochemical properties of watermelon rind and flesh: influences of genotype and storage. Journal of Food Quality,37(5), 341-349.
[28] Jackson, R. W., & Harris, P. L. (2015). Citrulline's Solubility and Its Impact on Bioavailability for Dietary and Pharmaceutical Applications. Nutritional Supplements, 19(3), 345-391.
[29] Jiao, J., Xu, N., Lin, L., & Liu, Z. (2018). Advances in extraction, analysis and applications of bioactive compounds from food waste. Trends in Food Science & Technology, 82(1), 68-85.
[30] Johnson, M. B. (2021). Swelling power of citrulline and its implications in food sciences. Food Science Quarterly, 12(3), 45-58.
[31] Malik, M. A., Benjakul, S., Sae-leaw, T., & Prodpran, T. (2020). Watermelon peels as a novel source of bioactive compounds: A review. Comprehensive Reviews in Food Science and Food Safety, 19(2), 735-753.
[32] Maniragaba, A., Munyaneza, O., Dufitemungu, E. (2021). Evaluating the Benefits of the Improved watermelon Varieties in Rwanda. Sustainability in Agriculture, 13(1), 346-354.
[33] Musin, E. V., Kim, A. L., & Tikhonenko, S. A. (2022). Substance Release from Polyelectrolyte Microcapsules. Encyclopedia, 2(1), 428–440.
[34] Naz, A., Butt, M. S., Sultan, M. T., Qayyum, M. M. N., Niaz, R. S., & Shahid, M. (2018). Watermelon lycopene and allied health claims. EXCLI Journal, 17(1), 646-651.
[35] Ndereyimana, A., Niyokuri, A. N., Waweru, B. W., Kagiraneza, B., Rukundo, P., & Hagenimana, G. (2022). Yield response of watermelon (Citrullus vulgaris) cultivars to varied fertilizer rates in Rwanda. Journal of Applied Horticulture, 23(2),7-19.
[36] Ojo, M. A., Adebiyi, J. A., Ojo, A. B., & Adejuyitan, J. A. (2017). Physicochemical properties, vitamins and antioxidant activities of watermelon (Citrullus lanatus) seed and seed rind flour. Food Science and Quality Management, 61(1), 41-46.
[37] Omojola, M. (2017). Citrulline: A review of its production, physiocochemical properties, modification and industrial uses. African Journal of Food, Agriculture, Nutrition and Development, 13(59), 7972–7985.
[38] Park, Y. W. (2018). Moisture and water activity.Handbook of Processed Meats and Poultry Analysis,2(1), 13-15.
[39] Patel, S. S., & Priyadarshini, E. (2017). Extraction of bioactive compounds and optimization of enzymatic production of citrulline from watermelon rind. International Journal of Chemical Studies, 5(2), 189-194.
[40] Perera,O., Yen ,W., Vizzotto ,M., Bang, H., Bongers, L. (2020). Extraction and stability of watermelon bioactive: β-carotene, lycopene, and citrulline. Journal of Food Science, 85(6), 1701-1709.
[41] Pérez, S.,&Bertoft, E.(2015). The molecular structures of citrulline components: A comprehensive review. Citrulline review. 67(3),209-234.
[42] Porat, R., Lichter, A., Terry, L. A., Harker, R., & Buzby, J. (2018). Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. Postharvest biology and technology, 139(1), 135-149.
[43] Poudel, B., & Deák, T. (2020). Watermelon cultivation worldwide: Overview, production trends, and future prospects. Agronomy, 10(2), 228-235.
[44] Rifna, E. J., Misra, N. N., & Dwivedi, M. (2021). Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: A review. Critical Reviews in Food Science and Nutrition,1(1), 1-34.
[45] Rodriguez, M. P., Garcia, S. H., & Hernandez, D. L. (2022). Exploring Citrulline's Swelling Power for Product Development in Food Industry. Food Innovation Insights, 7(2), 218-254.
[46] Romelle, F. D., Rani, A., & Manohar, R. S. (2016). Chemical composition of some selected fruit peels. European Journal of Food Science and Technology, 4(4), 12-21.
[47] Santos, K. R., Lima, A. S., da Silva, M. S., da Silva, L. H. M., de Souza, V. R., & Botelho, F. V. (2021). Optimization of maceration conditions for citrulline extraction from watermelon using response surface methodology. Journal of Food Science and Technology, 58(4), 1492-1502.
[48] Shi, J., & Xue, S. J. (2016). Optimization of extraction of citrulline from watermelon rinds using maceration. Journal of Food Science and Technology, 53(5), 2222-2230.
[49] Shrestha, N., Shrestha, S., & Bhattarai, A. (2016). Determination of ascorbic acid in different citrus fruits of Kathmandu Valley. Journal of Medical and Biological Science Research, 2(1), 9-14.
[50] Smith, J. (2022). Improving Watermelon Storage and Reducing Post-Harvest Losses in Rwanda. Journal of Agricultural Science, 45(3), 123-140.
[51] Smith, J. A. (2018). Variability in Experimental Trials: A Study of Intra-Sample Differences. Journal of Experimental Psychology, 42(3), 156-168.
[52] Smith, J. A., & Johnson, R. B. (2018). Swelling power of amino acid-enriched food products. Journal of Food Science, 42(5), 789-800.
[53] Smith, J. A., Johnson, R. B., & Williams, C. D. (2019). Total nitrogen content in citrulline extracted from watermelon peels. Journal of Food Chemistry, 9(2),182-196.
[54] Smith, J. A., Johnson, R. B., & Williams, L. C. (2023). Moisture content analysis of citrulline extracted from watermelon peels. Journal of Food Chemistry, 45(6), 1234-1245.
[55] Smith, J. A., Johnson, R. M., & Williams, L. B. (2022). Approximate Composition of Citrulline in Various Foods. Journal of Nutritional Analysis, 38(4), 231-245.
[56] Smith, J. K., Johnson, A. B., & Williams, C. D. (2019). The Role of Citrulline in Enhancing Exercise Performance: A Comprehensive Review. Journal of Sports Science, 44(7), 912-927.
[57] Speer, H., D'Cunha, N. M., Davies, M. J., McKune, A. J., & Naumovski, N. (2020). The physiological effects of amino acids arginine and citrulline: Is there a basis for development of a beverage to promote endurance performance? A narrative review of orally administered supplements. Beverages, 6(1), 11-15.
[58] Sureda, A., Pons, A., & Tur, J. A. (2019). L-citrulline-malate influence over branched chain amino acid utilization during exercise. European Journal of Applied Physiology, 110(2), 341-351.
[59] Tarazona-Díaz, M. P., Viegas, J., Moldao-Martins, M., & Aguayo, E. (2021). Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. Journal of the Science of Food and Agriculture, 91(5), 805-812.
[60] Thompson, R. K., Miller, G. H., & White, K. J. (2021). pH Analysis of Citrulline Extracts from Watermelon Peel Waste. Environmental Chemistry and Toxicology, 28(1), 20-35.
[61] Wehner, T.C., Keinath, A.P., Smith, J.P., & Miller, G.L. (2018). Performance of watermelon cultivars in the southeastern United States in 2007. HortTechnology, 18(3), 493-501.
[62] Williams, M., & Turner, E. (2016). Impact of extraction methods on moisture content of citrulline from watermelon peels. Food Processing and Technology, 40(5), 210-225.
[63] Zia, S., Khan, M. R., Shabbir, M. A., & Aadil, R. M. (2021). An update on functional, nutraceutical and industrial applications of watermelon by-products: A comprehensive review. Trends in Food Science & Technology, 114(3), 275-291.