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Graduation Research Project

Comparison of Enzymatic-Assisted, Heat-Assisted, and Ultrasound-Assisted Extraction of the Polysaccharides from Okra (*Abelmoschus esculentus*) and their Physicochemical and Functional Properties.

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Resumen

La okra se exporta fresca, por lo que debe cumplir normas de calidad que generan residuos y uso limitado. El fruto que no cumple estos parámetros aún contiene nutrientes y componentes funcionales que pueden ser revalorizados. El objetivo del estudio fue evaluar tres métodos de extracción de goma de okra, los cuales fueron asistidos por medios enzimáticos, calor y ultrasonidos. El polvo obtenido se caracterizó fisicoquímicamente (color, análisis proximal, aw y pH) y funcionalmente (solubilidad, propiedades emulsionantes, retención de agua y menor concentración de gelificación). Las propiedades funcionales se utilizaron para elegir un método de extracción eficaz. El análisis proximal mostró que las gomas extraídas estaban compuestas principalmente por carbohidratos, 65-75%, un contenido proteico de 6-11%, y aw 0.35 -0.48. Las gomas extraídas con enzimas presentaron un EAI elevado (7.11 m^2/g) y capacidad de retención de agua (8.61g H_2O/g) con una concentración mínima de gelificación del 0.5% (p/v). Las gomas extraídas por calor presentaron el mayor ESI (16.48 minutos) con una concentración mínima de gelificación del 3.0% (p/v). Por último, las gomas obtenidas por ultrasonidos presentaron la mayor solubilidad (27.71%) con una concentración de gelificación del 1.0% (p/v). El método enzimático presentó propiedades funcionales superiores para su uso como goma alimentaria. Los subproductos obtenidos pueden ser revalorizados como ingredientes potencial en la industria alimenticia, ya que presentaron contenidos de carbohidratos de 55 a 78%, con un contenido de proteínas de 13 a 19%, dependiendo del método de extracción. Se recomienda extraer las gomas utilizando una combinación de los métodos evaluados.

Palabras clave: gelificación, gomas alimentarias, revalorización, solubilidad.

Abstract

Okra is exported fresh, so it must meet quality standards, generating waste and limited use. The fruit that does not meet these parameters still contains nutrients and functional components that can be revalued. The objective of the study was to evaluate three methods to extract okra gums, which were enzymatic-, heat- and ultrasound-assisted. The powder obtained was characterized physicochemically (color, proximal analysis, a_w and pH) and functionally (solubility, emulsifying properties, water retention and lower gelation concentration). Functional properties were used as criteria to choose an efficient extraction method. The proximal analysis showed that the extracted gums were composed mainly of carbohydrates in a range between 65 to 75%, a protein content around 6-11%, and a_w 0.35 -0.48. The gums extracted with enzymes an elevated EAI (7.11 m^2/g) and water holding capacity (8.61g H_2O/g) with a minimum gelation concentration of 0.5% (w/v). The heat-extracted gums had the highest ESI (16.48 minutes) with the least gelation concentration of 3.0% (w/v). Finally, the gums obtained by ultrasound had the highest solubility (27.71%) with a least gelation concentration of 1.0% (w/v). The enzymatic method presented superior functional properties for use as a food gum. The by-products obtained can be used for revalorization as potential ingredients in food industry, as they presented carbohydrates content of 55 to 78%, with a protein content of 13-19%, depending on the extraction method. It is recommended to extract the gums using a combination of the evaluated methods.

Keywords: food gums, gelation, revalorization, solubility.

Introduction

Okra (*Abelmoschus esculentus*) belongs to the Malvaceae family, and it is cultivated worldwide (Dantas et al., 2021). It is frequently consumed as a cooked vegetable or as add-on. According to the Food and Agriculture Organization (2016a), okra production is estimated to be approximately seven million tons, with India being the world leader producing 70% of the total, followed by Mexico, the United States, and countries in Central America (Honduras, Nicaragua, Guatemala). Okra is commercialized by local traders and co-operative institutions and its peak production occurs between April and June. As far as availability of okra, it accounts for 60% of the export of fresh vegetables (Varmudy, 2011). Premium quality okra are the ones that have green color, tenderness, 4–5 ridged and about 6–8 cm in length (Singh y Pandley, 1993). However, when these quality standards are not met, rejection and waste of okra vegetables are expected and it has been reported that okra waste accounts for 40-50% of the 48.4% total food waste globally, a significant volume across its processing value chain (FAO, 2016b). Food losses and waste imply the quantity or quality of food along the food supply chain (FAO, 2019) and recapturing it as a resource to the creation of new materials, ingredients, and new products embraces a sustainable model which protects the environment, improves the economy, and elevates social justice. As a result, an increasing interest in research of total utilization and upcycling of commodities has been developing.

This crop has many benefits for the food industry. Okra is a powerhouse of valuable nutrients (Adetuyi y Osagie, 2011) and antioxidant content such as vitamin C, polyphenols, polysaccharides, and mineral elements (Yuan et al., 2018). The composition of okra pods per 100 g edible portion is: protein 2.10 g, fiber 1.70 g, Ca 84.00 mg, P 90.00 mg, Fe 1.20 mg, 60 μg of folate (Habtamu Fekadu Gemede et al., 2014). Okra is a natural source of gums such as a polysaccharide-rich hydrocolloid which contain acidic polysaccharides comprising glucose, galactose, rhamnose, galacturonic acid and glucuronic acid (Aziz et al., 2018). Indeed, gums are popular ingredients in the food industry with multiple applications such as thickeners, stabilizers, and as suspending agents in many foods such as batter and bakery

products, dairy products, salad dressings, sauces, soups, among others (Habibi y Khosravi-Darani, 2017). However, by-products are produced as a result of okra processing to obtain gum products, which can contain protein, fiber, and others, but they have not been sufficiently explored as food ingredients. These can potentially be used as functional food additives or aids to improve the nutritional value of foodstuff as dietary fiber ingredients, flavoring agents, and others (Karam et al., 2016).

To extract okra gum and its by-products it is required to use the right method (including the correct equipment and materials). In general, vacuum drying is a method with high drying rate that requires low drying temperatures which may help to maintain the qualities such as shape, color, aroma, flavor, and nutritive values of the dried product (Artnaseaw et al., 2010). For this project, vacuum dying was considered as the best option due to its attractive characteristics including scalability. On the other hand, enzyme-assisted extraction of natural ingredients from plants has been widely investigated in terms of the advantages that it offers over conventional procedures, such as operational simplicity, high efficiency, and environmental friendliness (Chen et al., 2018). Alternatively, hot water-assisted extraction (HWE) is the main method for polysaccharides extraction because it has the advantages of convenient extraction, no special equipment is required and it considered a low-cost method (Xue et al., 2022). Also, the ultrasonic-assisted extraction method has been of interest because of its low energy consumption, short time and high efficiency (Wang et al., 2023). However, there is much interest in finding a cost effective and efficient method to extract the gums and solids from okra. Consequently, the combination of both techniques (drying and extraction) is attractive as a novel method for the extraction of gum and by-products of okra.

Therefore, the objective of the research is to develop a scalable protocol to process okra wastes in a rational, sustainable way, to obtain value-added ingredients that can be used in the food industry. This protocol allowed to utilize the whole okra pods by extracting its emulsifying and gelling agents (gum) and by-products (fiber, seeds, among others) as powders with three different methods.

Next, the powders obtained were characterized: physicochemically (proximate analysis, moisture content, and water activity); and functionally (solubility in water, emulsifying properties, water holding capacity and least gelation concentration). Finding a scalable protocol to process okra will allow farmers to produce okra all year long and process value-added food ingredients that will give importance to the production chain.

Based on the above, the following specific objectives were established:

Determine the physicochemical and functional characteristics of the gum obtained from the three extraction methods.

Determine the most efficient extraction method according to the results of the characterizations.

Analyze the physicochemical and functional properties of the by-products of the process for further use in food industry.

Materials and Methods

Study Location

The research was carried out at North Carolina State University Research Campus at the Food Science, Bioprocessing and Nutrition Laboratory, in the city of Kannapolis, North Carolina.

Materials

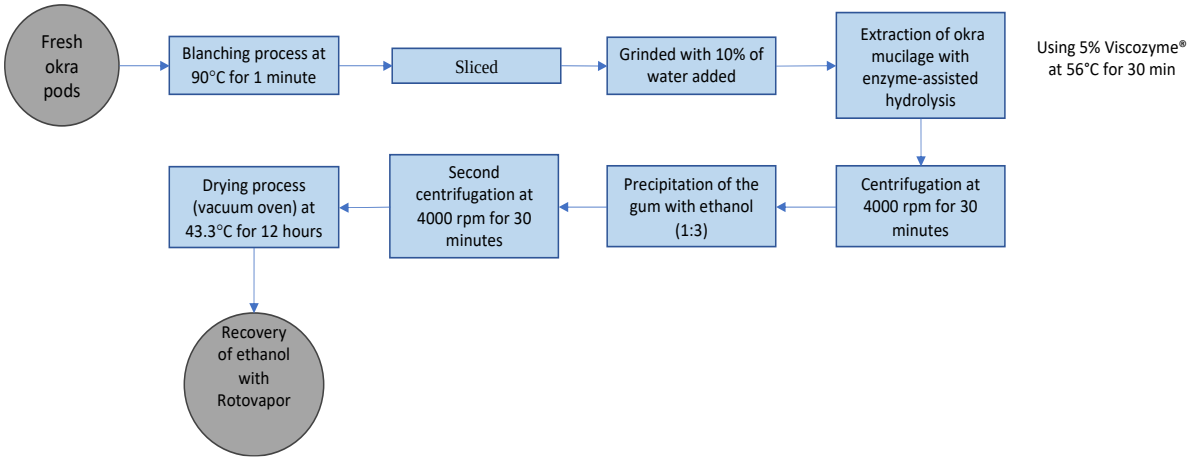
Fresh okra pods used in this study were purchased from local grocery stores in Kannapolis, North Carolina, USA. The enzyme (Viscozyme®) was gently donated by Novozymes®, Franklinton, NC, USA.

Enzyme-Assisted Extraction

The enzyme-assisted extraction (ENZ) was based in the combination of Zaharuddin et al. (2014) and Ogaji y Hoag (2014) methods, with some modifications. As shown in Figure 1, one tray of fresh okra pods went through the blanching process for 1 minute at 90°C and then sliced using a knife. Then, the sliced pods were ground with 10% of water added (w/v). After mixing it, the puree was hydrolyzed with 5% of Viscozyme® (w/v) for 30 minutes using a water bath set to 56°C, which is the enzyme's optimal operating temperature range. Then, the mixture was boiled to inactivate the enzyme used. Subsequently, the mixture was centrifuged at 4000 rpm for 15 minutes. Meanwhile, the precipitate (okra by-products) was kept to be dried after. Ethanol was added into the supernatant solution (ethanol to supernatant ratio, 3:1) to help precipitate the gum. Continuous stirring with a magnetic stir bar was required. Next, the mixture was centrifuged at 4000 rpm for 30 minutes to completely precipitate the gum. The precipitate obtained was dried at 43.3 °C for 12 hours in the vacuum oven and stored for further analyzes. The by-products were also dried at the same conditions. Ethanol in the supernatant was recovered with Rotovapor® R-300, Büchi, Labortechinick AG, Meiseggstrasse, to be reused.

Figure 1

Flowchart of enzyme-assisted extraction method of okra gum

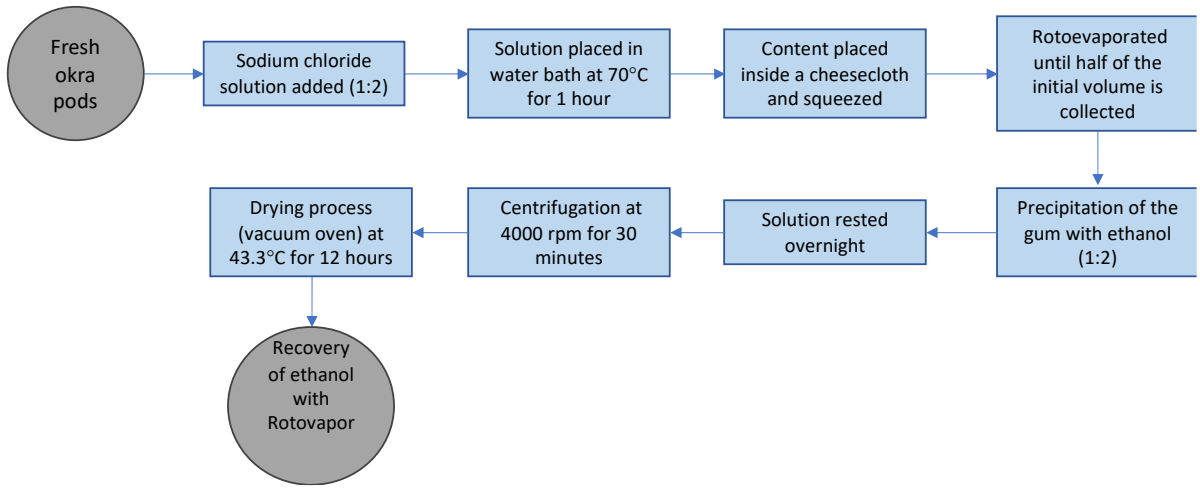


Heat-Assisted Extraction/ Hot Water Extraction (based on 500 g)

The heat-assisted extraction (HEAT) was based in Samavati (2013) method with modifications. As shown in Figure 2, 500 g of fresh okra were weighted, and a solution of sodium chloride (2.92 g in 1,000 mL) was added in a 1:2 ratio (okra: solution). The mixture was placed in a water bath at 70°C for 1 hour. Then, the content was placed inside a cheesecloth and squeezed until no liquid came out. The extraction was repeated with the same ratio of okra and solution. After, the extracted solution was rotoevaporated until half of the initial volume was collected. Ethanol was added (ethanol to solution ratio, 2:1) into the solution to help precipitate the gum. Continuous stirring with a magnetic stir bar was required. The solution needed to undergo an overnight resting period to be centrifuged the next day at 4000 rpm for 30 minutes. The precipitate (okra gum) was collected and after that, it was dried at 43.3°C for 12 hours in the vacuum oven. The by-products were also dried at the same conditions. Finally, ethanol was recovered with Rotovapor® R-300, Büchi, Labortechinick AG, Meiseggstrasse, to be reused.

Figure 2

Flowchart of heat-assisted extraction method of okra gum

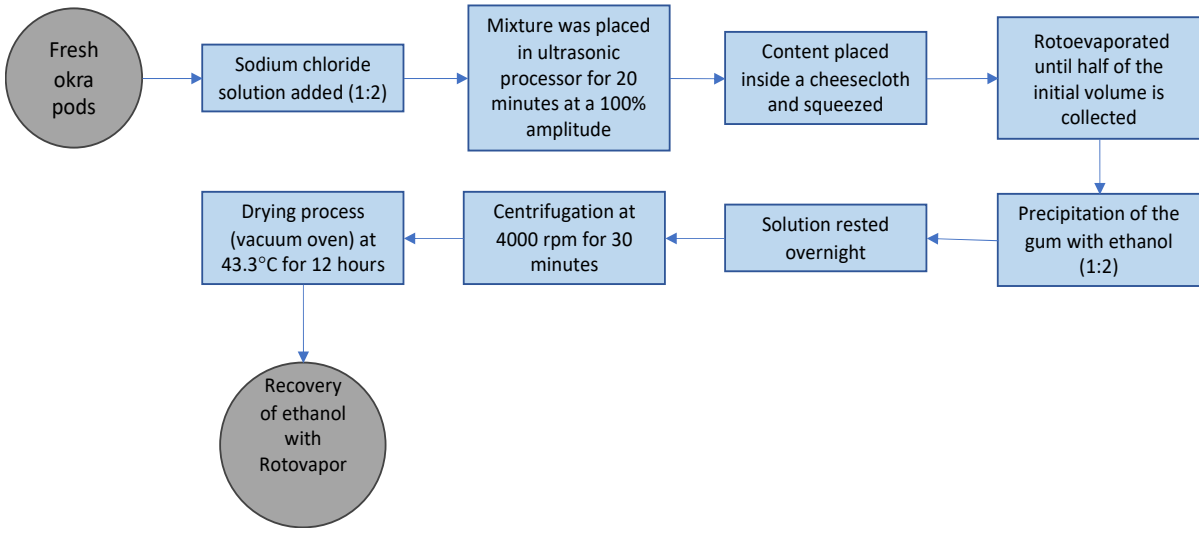


Ultrasound-Assisted Extraction (based on 500 g)

The ultrasound-assisted extraction (U-SOUND) was based in Ormanli et al. (2018) method with modifications. As shown in Figure 3, 500 g of fresh okra were weighted, and a solution of sodium chloride (2.92 g in 1,000 mL) was added in a 1:2 ratio (okra: solution). The mixture was placed in the ultrasonicator and the treatment was carried in pulses (5 seconds ON, 5 seconds OFF) for 20 minutes with an amplitude of 100%. Then, the content was placed inside a cheesecloth and squeezed until no liquid came out. The extraction was repeated with the same ratio of okra and solution. After, the extracted solution was rotoevaporated until half of the initial volume was collected. Ethanol was added (ethanol to solution ratio, 2:1) into the solution to precipitate the gum. Continuous stirring with a magnetic stir bar was required. The solution needed to undergo an overnight resting period to be centrifuged the next day at 4000 rpm for 30 minutes. The precipitate (okra gum) was collected and after that, it was dried at 43.3 °C for 12 hours in the vacuum oven. The by-products were also dried at the same conditions. Finally, ethanol was recovered with Rotovapor® R-300, Büchi, Labortechinick AG, Meiseggstrasse, to be reused.

Figure 3

Flowchart of ultrasonic-assisted extraction method of okra gum



Characterization of Okra Gum and By-products

Physicochemical analysis

Color.

The color of each sample was analyzed using a colorimeter (Chroma Meter CR-5, Konica Minolta, USA) which estimates lightness (L*), where 100 is white and 0 is black, a* (greenness to redness), where the highest value (+60) represents red and the lowest value (-60) is green, and b* (blueness to yellowness) where the highest value (+60) represents yellow and the lowest value (-60) is blue.

Proximate analysis

Proximate composition of both powders was performed using the AOAC official methods (Association of Official Agricultural Chemists [AOAC], 2006) to determine the moisture, fat, protein, and ash content. Carbohydrates were calculated considering the sum of the chemical components previously mentioned and subtracting them from the total weight of the sample (Code of Federal Regulations (CFR), 2023). The moisture content (MC) was determined by a Microwave Infrared

Moisture and Solids Analyzer, CEM, USA. Fat content was determined by Oracle™ a Rapid NMR Fat Analyser, CEM, USA. Ash content was determined with a Hi-Temp Oven DR200 at 550 °C. For the protein content, the samples were sent to the Medallion Labs in Minneapolis (MN, USA) to be quantified.

Water Activity (a_w).

The water activity (a_w) of each sample was analyzed using a water activity meter (AQUALAB® 4te, METER Group Inc., WA, USA). This method measures the surface temperature of samples by infrared and the dew point on a cooled mirror (Voelker et al., 2020). The sample was placed in the plastic cup and waited until the equipment indicated that the reading was finished.

pH.

The pH was measured using a calibrated pH meter (Thermo Scientific Orion Star A211, Thermo Fisher Scientific, USA) at 25°C (room temperature).

Functional analysis

Solubility in Water.

Samples (0.5 g) were mixed with 50 mL of distilled water, vortexed at 4000 rpm for 1 minute, and centrifuged at 4000 rpm for 15 minutes. An aliquot (25 mL) of the supernatant was transferred to aluminum trays and dried in vacuum oven at 43.3 °C overnight. The solubility was calculated gravimetrically as in Equation 1.

$$solubility\ (\%) = \frac{(m\ after\ (pan{+}soluble\ solids)){-}m\ pan}{25\ mL} \times \frac{50\ mL}{weight\ sample} \times 100 \quad [1]$$

Where:

m after = weight of aluminum tray with soluble solids after drying in vacuum oven, g .

m pan = weight of empty aluminum tray, g.

mL = milliliters

Emulsifying Properties

The emulsifying properties were determined according to Hoskin et al. (2022) with some modifications. First, dispersions of 1% (w/v) of the okra gum and by-products were prepared in water. Then, an emulsion was prepared by mixing 15 mL of the 1% (w/v) dispersion and 5 mL of sunflower oil using a high shear homogenizer (HQ-2509, Mxbaoheng, USA) at 1600 rpm for 2 min. After this, 50 μl of the emulsion was collected from the bottom of the tube and transferred to another tube containing 4.9 mL 0.1% sodium dodecyl sulfate (SDS) solution. Aliquots were collected immediately after the homogenization (T_0) and after 10 min (T_{10}). The absorbance of both aliquots was measured at 500 nm using a spectrophotometer (Epoch 2, BioTek, VT, USA) and a 96-well microplate. The emulsifying activity index (EAI) and emulsion stability index (ESI) were calculated as in Equations 2 and 3, respectively:

$$EAI = \frac{2 \times 2.303 \times A_0}{0.25} \quad [2]$$

$$ESI = \frac{A_0 \times \Delta T}{\Delta A} \quad [3]$$

Where A_0 is the absorbance at 0 minutes, A_{10}, absorbance at 10 minutes, ΔT=10 minutes, $\Delta A = A_0 - A_{10}$.

Water-Holding Capacity (WHC).

The water-holding capacity was determined according to Aziz et al. (2018) with some modifications. For this, 0.5 g samples were vortexed at 4000 rpm for 1 minute and allowed to stand for 1 hour at room temperature, followed by centrifugation and 4000 rpm for 15 minutes. Samples were submitted to decantation and the water-holding capacity was determined by difference with the Equation 4, respectively:

$$WHC = \frac{(m\ after\ (tube + solids\ with\ water) - m\ tube - m\ sample)}{m\ sample} \quad [4]$$

Where:

m after = mass of tubes + solids with water

m tube = mass of empty tube

m sample = mass of the sample

Least Gelation Concentration (LGC).

The least gelation concentration was determined based on Hoskin et al. (2022) with some modifications. Dispersions of the samples were prepared in 5 mL of distilled water at different concentrations using 15-mL falcon tubes. For protein-based aggregates, the following concentrations are recommended: 1, 5, 10, 15, 20, 25, 30% (w/v). From preliminary tests, it was observed that the concentration of 1% of polysaccharides formed a gel, which is why the concentrations were decreased to: 0.5, 1.0 and 3% (w/v). Then, the dispersions were vortexed for 5 minutes and placed in a water bath at 60°C for 1 hour. After, the tubes were cooled down on ice and stored at 4°C overnight. The lowest concentration (%) at which the sample did not slip when the tube was inverted was denoted as LGC.

Selection Criteria

In order to determine the most efficient extraction method a selection criterion was stablished. It was based on the functional analysis which are considered to be the most important properties of food gums.

Experimental Design and Statistical Analysis

The results of the research were analyzed statistically using a Completely Randomized Design (CRD). The data obtained was analyzed by the statistical program SAS® (Statistical Analysis System), version 9.4, through an analysis of variance (ANDEVA) and a DUNCAN separation of means in order to identify the most efficient treatment for extraction of okra gums.

Results and Discussion

Proximate Analysis

Proximate composition is important in determining the quality of raw materials and often to establish its nutritional value (Kavitha y Parimalavalli, 2014). Results presented in Table 1 and Table 2 show the nutrient composition of powdered okra gum and its by-products. The research revealed that the dominant component of okra gum is carbohydrates with a range of 65-75% for all treatments. Also, the minor component of okra gum is fat with a range of 0.005-0.065% for all treatments. Moreover, there were not significant differences between the fat components ($P \geq 0.05$). In ash, moisture and carbohydrate contents, there was a significant difference between the enzymatic treatment (ENZ), the heat and ultrasound treatment (HEAT and U-SOUND, respectively) since the probability was ($P \leq 0.05$). In addition, protein content was statistically different for all treatments ($P \leq 0.05$). In a study by Aziz et al. (2018), it was stated that the proximate composition on extracted okra gum in an aqueous form, is 66.15% moisture, 31.49% carbohydrate, 2.13% protein, 0.12% crude fat and 0.11% ash. Since the form of the gum was different in this study (powder), the proximate composition differs from the one found in this project. This explains why the moisture content decreased and the carbohydrate content increased.

On the other hand, the research also revealed that the dominant component from the okra by-products was also carbohydrates with a range of 55-78% for all treatments. Additionally, the minor component of okra by-products was also fat with a range 0.5-0.7% for all treatments. Furthermore, there were not significant differences between the fat components ($P \geq 0.05$). Ash, moisture, and carbohydrate showed a significant difference between the first treatment (ENZ), the second and third treatment (HEAT and U-SOUND, respectively) since the probability was ($P \leq 0.05$). In this case, protein content was also statistically different for all treatments ($P \leq 0.05$). As reported by Habtamu Fekadu Gemede et al. (2014), the proximate composition of 100 g of okra pods is 9.69-13.33% moisture, crude protein 10.25-26.16%, crude fat 0.56-2.49%, crude fiber 11.97-29.93%, crude ash 5.37-11.30% and

carbohydrates 36.66-50.97%. These results are similar with the ones obtained in this study, with a slight variation in the carbohydrate and moisture content, where the moisture content decreased for the heat-assisted method and the ultrasound-assisted method, respectively. Additionally, the carbohydrate content increased for all treatments. This could be due to the fact that in this research the analyzed product was powder instead of the okra pods which affects its features.

Table 1

Proximate composition of okra gum powder

Treatments	Fat (%) ± S.D.	Ash (%) ± S.D.	Moisture content (%) ± S.D.	Protein (%) ± S.D.	Carbohydrate (%) ± S.D.
ENZ	0.005 ± 0.007^{a}	12.54 ± 0.28^{b}	10.61 ± 2.24^{a}	11.6 ± 0.28^{a}	65.25 ± 2.81^{b}
HEAT	0.035 ± 0.02^{a}	14.88 ± 0.40^{a}	3.61 ± 0.08^{b}	8.51 ± 0.13^{b}	72.98 ± 0.47^{a}
U-SOUND	0.065 ± 0.02^{a}	14.68 ± 0.14 ^{a}	3.17 ± 0.16^{b}	6.71 ± 0.38^{c}	75.38 ± 0.66^{a}
P	0.17	0.05	0.05	0.009	0.07
%CV	46.66	2.58	21.11	3.06	2.35

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Fat, Ash, Moisture content, Protein and Carbohydrate represents the mean of two repetitions. $^{a-c}$: Different letters in each column indicate significant difference (P <0.05) between treatments.

Table 2

Proximate composition of okra by-products

Treatments	Fat (%) ± S.D.	Ash (%) ± S.D.	Moisture content (%) ± S.D.	Protein (%) ± S.D.	Carbohydrate (%) ± S.D.
ENZ	0.69 ± 0.12^{a}	10.47 ± 0.32^{a}	11.62 ± 0.88^{a}	18.95 ± 0.07^{a}	55.42 ± 4.43^{b}
HEAT	0.54 ± 0.03^{a}	5.43 ± 0.06^{c}	3.49 ± 0.098^{b}	12.96 ± 0.13^{c}	77.59 ± 0.12^{a}
U-SOUND	0.71 ± 0.007^{a}	8.9 ± 0.21^{b}	3.19 ± 0.16^{b}	15.60 ± 0.29^{b}	71.61 ± 0.08^{a}
P	0.38	0.008	0.008	0.004	0.03
%CV	12.48	3.31	8.36	1.32	3.67

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Fat, Ash, Moisture content, Protein and Carbohydrate represents the mean of two repetitions. $^{a-c}$: Different letters in each column indicate significant difference (P <0.05) between treatments.

Functional Analysis

In food industry, a functional analysis of the gums is important, since it provides an overview of key mechanical and rheological properties of food products to obtain information about the quality and the parameters that are critical for guaranteeing the product consistency.

Emulsifying Properties

In general, the emulsifying property is measured by the Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI). EAI is used to determine the amount of oil that can be emulsified per unit of protein or polysaccharides, and the ESI measures the resistance of the emulsion to aggregate as it maintains its homogeneous texture during specific storage time and process (Ai, 2023; Aryee et al., 2018). Table 3 shows the EAI and ESI for okra gum and its by-products. For EAI of gum, there were significant differences between the HEAT treatment and the other two (ENZ, U-SOUND respectively) since the probability is ($P \leq 0.05$). The EAI value for the enzyme-assisted method obtained an elevated value with 7.11 m^2/g. In the study conducted by Randall et al. (1988), they evaluated the emulsifying properties of arabic gum in which they found that it had an EAI of 0.004 m^2/g. This value was lower compared to the ones obtained in the present study, showing that okra gums have potential to be used as an emulsifier in food industry. On the other hand, Olawuyi et al. (2022) studied the emulsifying properties of okra pectin after an enzymatic treatment with glycoside hydrolases and reported emulsion activity indexes in the range of 68.45 – 333.65 m^2/g; these values are higher than the ones reported in the present study. For the ESI of okra gum there were significant difference between HEAT treatment and the other ones since the probability is ($P \leq 0.05$). This treatment was also the one that obtained the highest value, showing that it can hold the emulsion for 16.48 minutes. According to Oh y Kim (2022), the mucilage of *Corchorus olitorus L*. had an ESI of 8 hours when the concentration was 8 mg/mL. This result differs from the one in this project even though both plants belong to the Malvaceae family. Also, the concentration used was not the same as the study, in this project we used

10 mg/mL which could explain the lower values obtained for the ESI parameter for okra gum powder and the influence that the extraction method can have in the ESI parameter.

Table 3

Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) for okra gum and solids powder

	Gum		By-products	
Treatments	EAI (m^2/g) ± S.D.	ESI (min) ± S.D.	EAI (m^2/g) ± S.D.	ESI (min) ± S.D.
ENZ	7.11 ± 0.14^a	13.77 ± 0.05^b	1.57 ± 0.22^a	5.77 ± 0.14^b
HEAT	4.63 ± 0.02^b	16.48 ± 0.62^a	1.71 ± 0.035^a	46.08 ± 1.31^a
U-SOUND	6.91 ± 0.05^a	13.59 ± 0.007^b	1.85 ± 0.042^a	45.75 ± 1.97^a
P	0.008	0.04	0.24	0.004
%CV	2.33	2.55	6.10	5.11

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of EAI (m^2/g) and ESI (min) represents the mean of two repetitions. $^{a-c}$: Different letters in each column indicate significant difference ($P < 0.05$) between treatments.

Water Holding Capacity (WHC)

Water Holding Capacity (WHC) is the ability of food to hold its own or added water during the application of force, pressure, centrifugation, or heating (Gyawali y Ibrahim, 2016). Table 4 displays the results for WHC parameter for okra gum and its by-products. There were not significant differences between the ENZ and the HEAT treatments ($P \geq 0.05$). Additionally, there were not significant differences between the HEAT and the U-SOUND treatments ($P \geq 0.05$). The highest value was obtained by the ENZ treatment, which can hold up to 8.61 g H_2O/g sample. In general, most of the plant polysaccharides gums are water-soluble polymers. They can absorb water and swell up to form a gel or provide highly viscous solutions when added to water (Amid et al., 2013). In a study conducted by Dogan et al. (2011), it was found that the WHC for Guar Gum was 28.06 g H_2O/g gum. These latter values were higher compared to the ones found in the present study. This could be since both gums have different origins.

For the okra by-products, there were not significant differences between all treatments since the probability was ($P \geq 0.05$). All the values obtained were low, in the range of 1.5-2 g H_2O/g sample;

this could be since the by-product does not have emulsifying effects, and they cannot hold a good amount of water.

Table 4

Water Holding Capacity of okra gum and by-products

Treatments	Gum	By-products
	g H_2O/g sample ± S.D.	
ENZ	8.61 ± 1.42[a]	1.56 ± 0.64[a]
HEAT	6.68 ± 0.09[ab]	1.81 ± 0.04[a]
U-SOUND	5.01 ± 0.16[b]	1.97 ± 0.05[a]
P	0.11	0.54
%CV	11.06	19.17

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Water Holding Capacity (WHC) represents the mean of two repetitions. [a-c]: Different letters in each column indicate significant difference (P <0.05) between treatments.

Solubility

Solubility is defined as the maximum quantity of a substance that can be completely dissolved in a given amount of solvent (Gong et al., 2007). Table 5 displays the results for solubility for okra gum and its by-products. There were significant differences between all treatments for okra gum ($P \leq 0.05$). The highest value was obtained by the U-SOUND treatment with 27.71%. According to Xu (2017), arabic gum showed an excellent water solubility, up to 30% at room temperature. The higher value obtained was similar to the one found in this study, which reinforces the fact that okra gum has excellent water solubility.

For okra by-products, there were significant differences between the HEAT treatment and the other two treatments ($P \leq 0.05$). The values obtained were high due to the fact that the components of okra by-products are soluble in water such as the fiber according to Gemede et al. (2015).

Table 5

Solubility (%) of okra gum and by-products

Treatments	Gum	By-products
	Percentage ± S.D.	
ENZ	19.36 ± 2.31[c]	27.01 ± 0.7[a]
HEAT	23.19 ± 0.95[b]	19.62 ± 1.5[b]
U-SOUND	27.71 ± 0.69[a]	27.83 ± 1.6[a]
P	0.03	0.009
%CV	3.72	2.01

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Solubility (%) represents the mean of two repetitions. [a-c]: Different letters in each column indicate significant difference (P <0.05) between treatments.

Least Gelation Concentration (LGC)

The LGC was performed for all treatments of okra gum in different concentrations: 0.5 % (w/v), 1.0 % (w/v) and 3.0 % (w/v). Figure 4, 5 and 6 show the LGC for okra gum extracted with the different methods. The LGC for okra gum extracted using the enzyme-assisted method was 0.5 % (w/v). For okra gum extracted using the heat-assisted method, the LGC was 3.0 % (w/v). Finally, for okra gum extracted using the ultrasound-assisted method, the LGC was 1.0 % (w/v). Okra gum obtained by the enzyme-assisted method is the one that required lower concentration to form gel.

According to Alam et al. (2021), the LGC for xanthan and guar gum is 6 and 8 %, respectively. The values obtained in this study were lower, which means that okra gum can form gel at a lower concentration than xanthan and guar gum. Gelation is an important functional property that is directly related to the texture and viscoelasticity of food components (Awuchi Chinaza Godswill et al., 2019). It usually helps to produce a variety of foods with distinct textures such as: sauces, dairy products, and many others. Since the values obtained are lower it means that the ability of gelating is better. This is beneficial for the food industry since it can provide an economical benefit since less okra gum is needed for it to form gelation which represents savings in the process because less amount will be used.

Figure 4

Least Gelation Concentration of okra gum extracted using enzyme-assisted method.

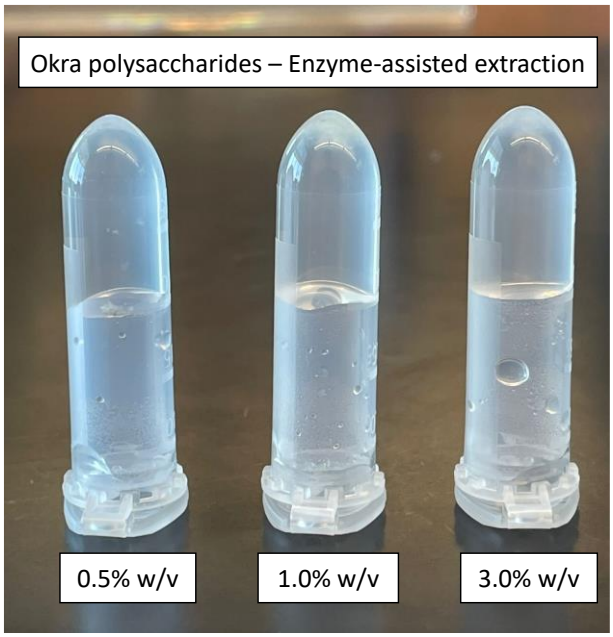


Figure 5

Least Gelation Concentration of okra gum extracted using heat-assisted method

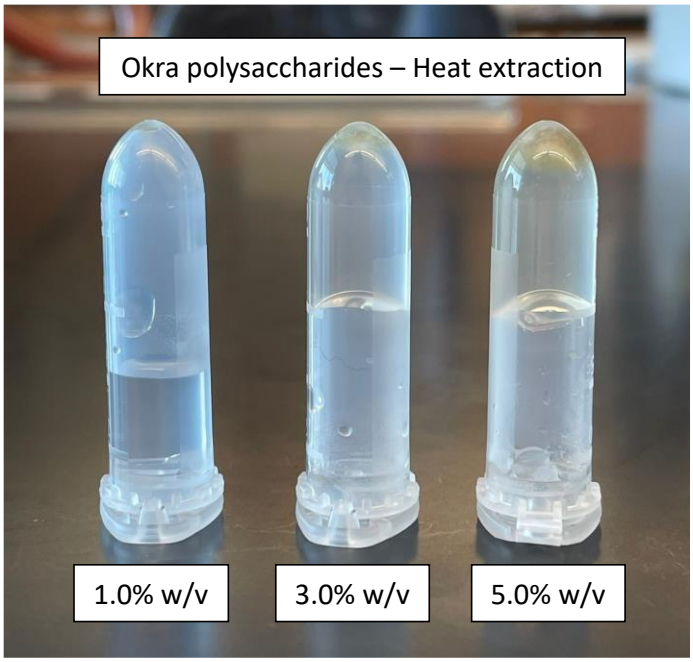
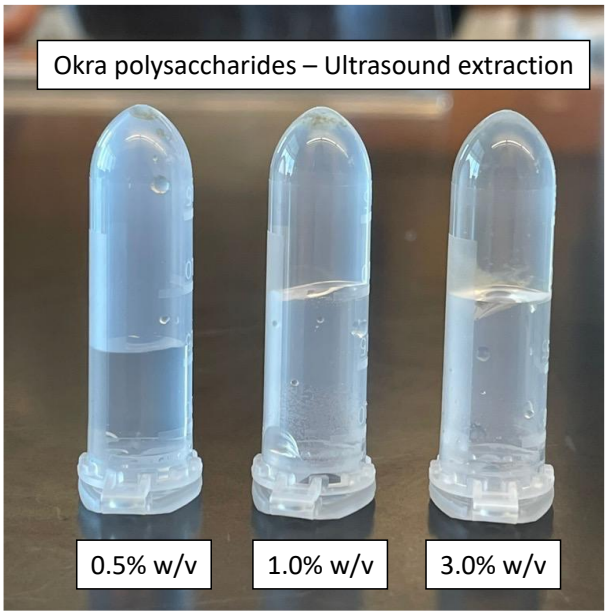


Figure 6

Least Gelation Concentration of okra gum extracted using ultrasound-assisted method.



Physicochemical analysis

Color

The human eye can detect visible light, which is composed of various wavelengths that are perceived as colors. The color humans perceive is a result of those wavelengths that are reflected to the eyes, they usually go from 400 nm to 700 nm (Grzybowski y Kupidura-Majewski, 2019).

For the color analysis, the values L, a and b were obtained (Table 6). Where, a higher value in "L" indicated that there is a high luminosity. A higher value of "a" is closer to red tones and a lower value is closer to green tones. On the other hand, a higher value of "b" indicates that the product is closer to yellow shades and a lower value to blue shades.

For the treatments evaluated for okra by-products powder there were no significant differences since the probability is ($P \geq 0.05$). The value of "a" was the same for all treatments. As a result, an opaque greenish color was obtained, which was similar for all treatments. In general, fresh okra samples contain values of L, a and b of 42.69, -3.6 and 8.24, respectively. Also, depending on the

drying method utilized, the color parameters will be affected. When using vacuum oven as a drying method, the values of L a and b obtained were 44.71, 6.80 and 9.39 in that order (Ismail et al., 2019). According to the values previously mentioned, for all treatments the luminosity increased significantly, the “a” values were closer to the greenish tones and decreased compared to the one dried with the vacuum oven. Finally, the “b” values also increased significantly compared to the ones obtained from the fresh okra.

Table 6 also shows that for the treatments evaluated for okra gum there was no significant difference for the “a” value ($P \geq 0.05$). For the “L” and “b” parameters there were significant differences between the ENZ treatment, the HEAT and U-SOUND treatments. According to Sidkey, M et al. (2020), xanthan gum is a white to cream color powder. The okra gum powder obtained has in general high values of luminosity, low values of “a” and a high value of “b”, which results in a cream color powder except for the HEAT and U-SOUND treatments that have more greenish tones.

Table 6.

Color measurements on the L, a and b scale for okra gum and solids powders.

Treatments	Gum			By-products		
	L ± S.D.	a ± S.D.	b ± S.D.	L ± S.D.	a ± S.D.	b ± S.D.
ENZ	72.23 ± 2.31[a]	3.19 ± 1.48[a]	16.52 ± 0.50[b]	57.08 ± 3.82[a]	1.81 ± 0.47[a]	19.72 ± 0.66[a]
HEAT	67.08 ± 3.82[b]	1.81 ± 0.47[a]	19.72 ± 0.66[a]	57.08 ± 3.82[a]	1.81 ± 0.47[a]	19.72 ± 0.66[a]
U-SOUND	68.75 ± 1.73[b]	1.81 ± 0.47[a]	20.92 ± 0.007[a]	58.75 ± 1.73[a]	1.81 ± 0.74[a]	20.92 ± 0.007[a]
P	0.11	0.63	0.04	0.11	0.69	0.15
%CV	5.38	1.86	0.67	2.09	0.84	1.91

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of L, a and b represents the mean of two repetitions. [a-b]: Different letters in each column indicate significant difference (P <0.05) between the L, a and b scale.

pH

Stability and food safety depends on the pH in the food environment (Sandulachi, E, 2012). In Table 7, the results for the pH of okra gum and its by-products are shown. For the okra gum there were significant differences between the ENZ treatments and the other two treatments evaluated. The higher value of 7.4 was obtained by the ENZ treatment. In general, the results of pH obtained for gums were neutral (~ 7). In a study conducted by Matyjaszewski y Möller (2012), it was found that the pH of Xanthan Gum is between 6.5 and 7.5. The values found in this research were between the preceding range.

For okra by-products, there were no significant differences between the pH values ($P \geq 0.05$). The higher value of 5.82 was obtained by the U-SOUND treatment. In general, the results of pH obtained for the solids were slightly acid. In general, the okra has a pH of 6 (Kontogiorgos et al., 2012). The values obtained were close to the pH obtained in this study.

Table 7

pH values for okra gum and by-products.

Treatments	Gum	By-products
	pH ± S.D.	
ENZ	7.4 ± 0.25[a]	5.24 ± 0.71[a]
HEAT	6.72 ± 0.02[b]	5.72 ± 0.12[a]
U-SOUND	6.29 ± 0.03[b]	5.82 ± 0.13[a]
P	0.04	0.5
%CV	1.95	7.65

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of pH represents the mean of two repetitions. [a-b]: Different letters in each column indicate significant difference (P <0.05) between treatments.

Water Activity (a_w)

Stability and food safety also de depend on water activity in the food environment (Sandulachi, E, 2012). The range for water activity for okra gums powder presented in Table 8, goes from 0.35-0.48. There were significant differences between the U-SOUND treatment and the HEAT

treatment (P ≤ 0.05). On the other hand, the range of water activity for okra by-products was between 0.36-0.41. They did not present a significant difference since the probability was (P ≥ 0.05).

Both powders enter in the category of low-water activity foods. According to Lian et al. (2015), low-water activity foods are those with a_w levels lower than 0.85. They are usually believed to have the advantages of controlling the growth of pathogenic and spoilage microorganisms since the a_w is low.

Table 8

Water Activity (a_w) values for okra gum and solids powder.

Treatments	Gum	By-products
	a_w ± S.D.	
ENZ	0.35 ± 0.05[b]	0.37 ± 0.02[a]
HEAT	0.47 ± 0.004[ab]	0.41 ± 0.01[a]
U-SOUND	0.48 ± 0.001[a]	0.36 ± 0.003[a]
P	0.1	0.23
%CV	6.46	3.82

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Water Activity (a_w) represents the mean of two repetitions. [a-b]: Different letters in each column indicate significant difference (P <0.05) between treatments.

Yield

Yield was determined for both powders. Product recovery was determined to know how much okra by-products and gum powder was recovered in comparison to the quantity of okra used at the beginning. Table 9 shows the yield values obtained for okra gum and its by-products.

For okra gum powder, there was a significant difference obtained (P ≤ 0.05). The highest yield was obtained by the enzyme-assisted extraction with 5.77% of gum recovered. Furthermore, for the okra by-products powder there was no significant difference since the probability was (P ≥ 0.05). The highest yield was also obtained by the enzyme-assisted method with 42.21%. In a study conducted by Nagpal et al. (2018), it was found that the combination of ultrasound and microwave-assisted technology led to an improvement in the yield extraction of gums of about 31.52 ± 0.22%. This differs

to the results obtained in this study because there were no combinations of techniques used which can also explain the low values obtained for the gum extraction yield.

Table 9

Yield values for okra gum recovery and by-products recovery.

Treatments	Gum	By-products
	Recovery of products (%) ± S.D.	
ENZ	5.77 ± 0.14[a]	42.21 ± 4.35[a]
HEAT	1.36 ± 0.23[b]	34.32 ± 1.60[a]
U-SOUND	0.88 ± 0.07[b]	42.06 ± 3.02[a]
P	0.004	0.35
%CV	7.04	9.42

Note. S.D.= Standard Deviation. %CV = Coefficient of variation. ENZ= enzyme-assisted method. HEAT= heat-assisted method. U-SOUND = ultrasound-assisted method. Each measurement of Yield represents the mean of two replicates. [a-b]: Different letters in each column indicate significant difference (P <0.05) between treatments.

Conclusions

Okra gum obtained through an enzyme-assisted extraction method exhibited high protein content and water holding capacity (WHC), as well as superior LGC. Additionally, okra gum extracted using a heat-assisted method displayed the highest Emulsifying Stability Index (ESI) and the ultrasound-assisted extraction method delivered the most water-soluble okra gum.

The powder that best fits these criteria is the one obtained by the enzyme-assisted method which was selected because of its LGC outcomes, highest water holding capacity (WHC) and Emulsifying Activity Index (EAI).

The by-product derived from the enzyme-assisted exhibits favorable attributes, such as a high amount of protein which makes it a viable candidate for revalorization as a potential ingredient in food industry for protein bars or animal feed.

Recommendations

Given that the ultrasound-assisted method gave noteworthy results, such as in solubility, the highest among the selected criteria. It can be considered for subsequent investigations to merge its attributes with the enzyme-assisted method to explore potential enhancements.

Evaluate if the level of maturity of okra affects the amount of gum extracted.

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Appendices

Appendix A

Colors obtained of okra gum and by-products powders for all treatments.