Review Article **Open-Access**

AFBC
Agriculture and Food Bioactive Compounds

An overview of the extraction methods of anthocyanins from local African roselle and their use as potential functional foods and food additives colorants

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Submission Date: December 23rd, 2024; **Acceptance Date:** January 30th, 2025; **Publication date:** January 31st, 2025

Please cite this article as: Please cite this article as: Ndoye B., Mbow B., Faye P. G., Kane C., Cissé M., Ayessou N. An overview of the extraction methods of anthocyanins from local African roselle and their use as potential functional foods and food additives colorants. *Agriculture and Food Bioactive Compounds* 2025; 2(1): 15-31.
DOI: https://doi.org/10.31989/afbc.v2i1.1525

ABSTRACT

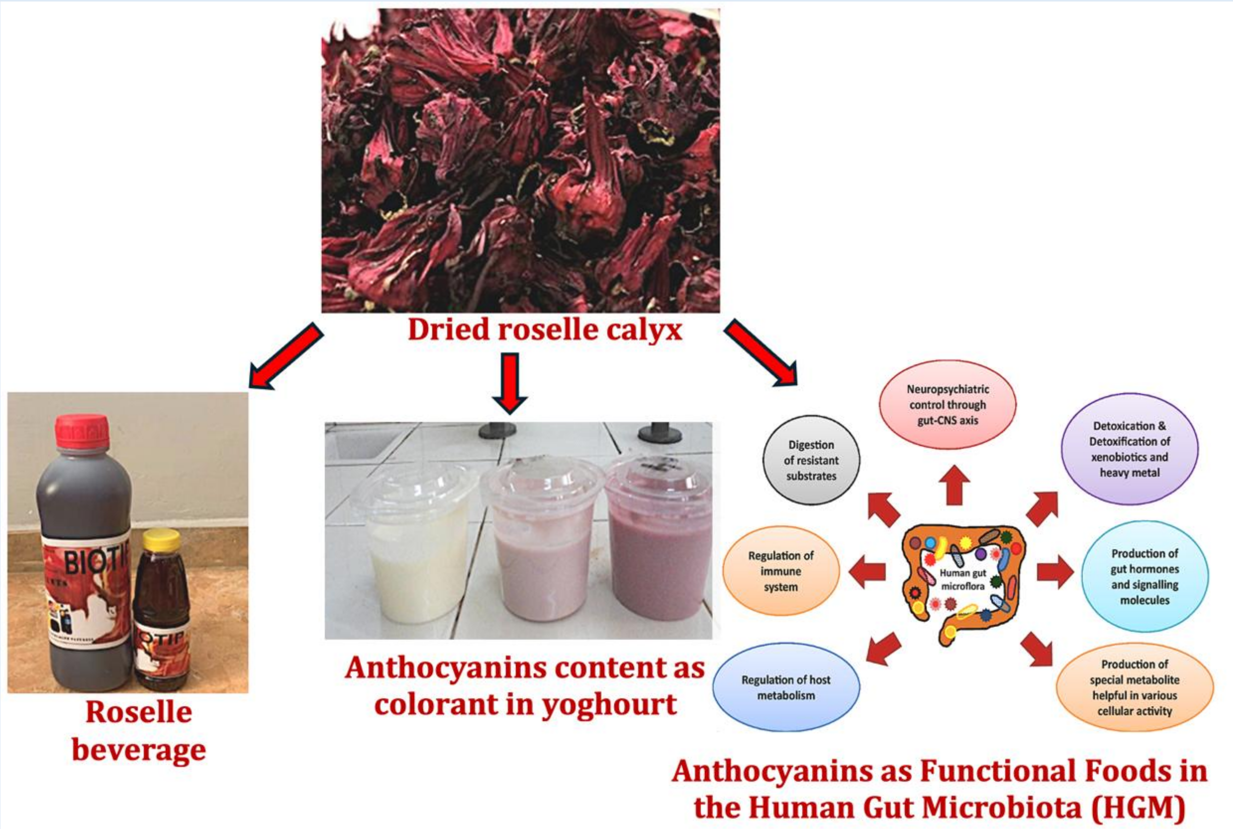
This review describes the summary of studies related to anthocyanins extracted from a Senegalese *Hibiscus sabdariffa* plant. In sub-Saharan Africa, dried *H. sabdariffa* is often used to prepare a beverage juice that provides diverse health benefits. Many studies have already shown its advantages as a prebiotic compound that could be added or mixed with other food ingredients. It is sold worldwide in the form of either freeze-dried and spray-dried powder or raw-dried roselle leaf. Anthocyanins of *H. sabdariffa* are placed in the internal part of the roselle calyx and, therefore, need efficient extraction tools based on temperature, pressure, and flow rate to recover the highest possible concentration.

Scientific paper searches were conducted using a combination of the following keywords: “roselle”, “*Hibiscus sabdariffa*”, “anthocyanins”, “extraction methods”, “food additives”, and “stabilization”, by order of preference in Web of Science, Scopus, Scimago, PubMed, Science direct, Functional Food Science Publisher (https://www.ffhdj.com), research4life, Google Scholar and Google. Inclusion criteria were generally studies written either in English or French.

In this regard, we develop here an overview of studies related to the extraction methods and chemical characterization of anthocyanins to optimize their concentration and stabilization from the roselle. Their potential use as functional foods and food additive colorants will be discussed. Particular attention will be given to their possible role as functional foods in human gut microbiota as highlighted by recent studies for other anthocyanins extracted from cranberries, for instance. Anthocyanins contained in roselle calyx can be better used as both bioactive compounds and functional foods that are healthy ingredients. However, further research is needed to better optimize their extraction and provide scientific and clinical evidence of their role in human gut microbiota.

Novelty: This review summarizes the current most used methods of extraction, concentration, purification and stabilization of anthocyanins from African roselle (*Hibiscus sabdariffa*). However, most of them are employed at a laboratory scale in Africa with low productivity yield. In addition, most of the roselle calices benefits are focused on beverage and coloring additives. This paper brings a new orientation on recovering the highest possible concentration of roselle anthocyanins and their health benefits on human gut microbiota, which are assumed to be functional foods and/or bioactive compounds.

Keywords: Anthocyanins, Roselle, *Hibiscus sabdariffa* L., extraction, stabilization, functional foods, food additives, bioactive compounds.



Graphical Abstract: main applications of dried roselle calyces as beverages, food additives colorants and functional foods in Human Gut Microbiota

INTRODUCTION

Anthocyanins are natural pigments present in different deep colors (red, purple, blue, etc.) in plants [1]. They are part of a category of plant-based chemicals named flavonoids and were proven to have exceptional human health benefits and be against a few diseases that are classed as public health risks, such as diabetes, hypertension, cholesterol, leukemia, coronary heart, cancer, etc. [2-4]. Roselle, from its scientific name *Hibiscus sabdariffa,* belongs to the family Malvaceae and is grown in tropical regions of Africa, Asia, and America. In Senegal (West Africa), it is called "Bissap", and the crop is available all over the year. Calyces are traditionally used to produce beverages or mixed in food [5-6] as active pharmaceutical ingredients (ApI) and cosmetical products [7-8].

Anthocyanins present in *Hibiscus* are flavonoid molecules with antioxidative properties that counteract cancer, inflammation, and degenerative diseases and regulate gut microbiota [9-13]. Anthocyanins are carrying a charge on the oxygen of the heterocycle [3]. A "flavone" ring characterizes the basic structure of anthocyanins, generally glycosylated at the Carbone "3" position [3, 7]. Anthocyanins act mainly as primary antioxidants stabilizing radical peroxides, but they can also deactivate reactive oxygen species (superoxide ion, OH radical, singlet oxygen), inhibit enzymes such as lipoxygenase or even chelate metals [3, 7-8].

Therefore, to enhance the antioxidant activity of the roselle, it would need to come up with optimal methods of recovery that include extraction, filtration, stabilization, pasteurization, and purification, as well as scaling up from laboratory level to industrial scale processes.

In the literature, many peer-reviewed reports on roselle *H. sabdariffa* were mainly focused on the extraction step due to the localization of the anthocyanins in the internal part of the roselle calyx [14]. The widest method of extraction used is the polar solvent extraction, which involves current fluid such as water, methanol, ethanol, etc. [15]. Aqueous or solvent extraction with high temperature as the conventional technique has been conducted by Cisse et al. [1] to recover the highest concentration of anthocyanins. However, the heat in aqueous extraction discolorizes the redness of the extract, and hence its quality, innocuity (antimicrobials) and acceptability (consumers). Subsequent complex methods to extract a higher concentration of phenolic compounds from roselle were investigated. One method, such as subcritical fluid, has received particular attention because it has demonstrated better advantages, such as efficient extraction and separation phases, less energy, higher selectivity and purity, free solvent, etc., than solvent extraction [16]. In this system, pressure and temperature are critical parameters that may affect the solvent selectivity and effectiveness. In subcritical fluid, acetone is widely used as a co-solvent instead. More recently, a combination of two methods of extraction was applied to extract the highest concentration of anthocyanins in roselle. The first step uses carbon dioxide to break down the outer part of the calyx and recover the anthocyanins/antioxidants from the roselle, and the last method involves applying water extraction to elute the extract of anthocyanin products [15].

Another challenge to face after extracting the anthocyanins extract from the roselle is both its filtration and stabilization of the red color. A few small pieces of debris may occur in the extracted fluids due to the breakdown of the outer layer of the roselle during extraction, but they are generally removed with centrifugation. The high temperature used in the aqueous extraction may also decrease the red color and hence downgrade the final quality of the extracts. Therefore, research on filtration and stabilization of the roselle extracts was strongly needed and reported [1]. The calyces of the roselle contain organic compounds (e.g., antioxidants, E300-399) such as alkaloids, phenolics, fatty acids, flavonoids, etc., and are used to produce a variety of foods and beverages [17]. Therefore, they are considered to belong to important classes of food additives, and a strong filtration process is needed to preserve their texture and taste. A crossflow microfiltration (CFM) process was performed specifically to outfit the impacts of the high temperatures that were major concerns in the extraction process of the roselle calices [1].

In the CFM process, membrane technologies are used as an alternative to replace high-temperature treatments. The resulting products are cold pasteurized, and their storage stability evaluated at 4 and 20 °C is for 90 days. This study is interesting in providing pasteurized roselle extracts meeting the microbiological safety requirement with an acceptable level of nutritional and sensory qualities. Food additives are essential chemical compounds used in the formulation of ultra-processed foods. They may be involved to enhance the appearance, taste, and texture of these foods as well as to increase their shelf life [18]. However, a few of them, emulsifiers, for example, are a high risk of cardiovascular disease [19].

This review highlights the novel findings in developing methods of optimization and characterization of anthocyanins from roselle (*Hibiscus sabdarifa* L.) extract. It will further describe the possible industrial and health applications of the stabilized and purified product in the development of *the Hibiscus value chain, such as food additives, colorants,* and functional foods owing to provide successful industrial scale.

Methodology: Scientific paper searches were conducted using a combination of the following keywords: "roselle", "Hibiscus sabdariffa", "anthocyanins", "extraction methods", "food additives", and "stabilization", by order of preference in Web of Science, Scopus, Scimago, PubMed, Science Direct, Functional Food Science Publisher (https://www.ffhdj.com), research4life, Google Scholar and Google. Inclusion criteria were generally studies written either in English or French. This review, covering the period from 2002 to 2024, allowed us to refer to 71 traceable and thrustable sources. Relevant articles on roselle anthocyanin extraction methods, as well as those related to their uses in animals randomized clinical trials to determine their effective impact on gut microbiota, are considered for the references of choice. Otherwise, anthocyanins from other food sources are also considered for reference.

Optimization and characterization of anthocyanins: Extracting anthocyanins from roselle for a sustainable ecological transition with less energy is more than a challenge for researchers and industrial scientists to promote their efficient industrial application. Different methods are being used to optimize the extraction, concentration, purification, stabilization, and pasteurization of anthocyanins from roselle calyces, as well as to recover the highest possible concentration. [**20**] (Table 1).

Extraction methods of anthocyanins

Conventional or Solvent Extraction: Conventional extraction or solvent extraction method was applied to calices from roselle to make beverages (Table 1) [6, 9].

These authors considered the simplicity of the process that delivers the freshness of the juice extract as usually performed by African households. In this conventional technique, water is used as a solvent, and there is no need to use temperature as a critical parameter. From there, Ndong et al. [16] applied further methods for subsequent optimization processes. For instance, these authors used the pasteurization method with potassium sorbate (E202) at 70 °C for 30 min to stabilize the roselle extracts and follow their shelf life when stored at 4, 30, and 45°C for 3 months.

Anthocyanin's extraction seems to depend on several parameters, such as the size of the calyces, the temperature of the water, and the unit operations (infusion, maceration, decoction). For instance, solvent extraction of roselle calyces could be influenced by the particle size, temperature, and extraction time [21]. These latter are the operating parameters that must be optimized to increase the extraction yield. For instance, the variations in the temperature and the extraction time are closely related, as well as the evolution of the anthocyanin concentration and the color of the extract [22]. The extraction procedure at a given temperature during storage clearly influences the extract's stability. On the maceration process at 60°C, color modifications were studied during storage over a period of 6 months [22]. The consequence is the changes in the color of the extract according to the pasteurization temperature. Therefore, solvent extraction is limited by the frequent instability of the roselle extract due to temperature sensitivity and, hence, a low extraction yield.

A subsequent technique is the use of microwave-assisted extraction (MAE) to disrupt the cell membranes and reach the content of the roselle calyx by quick temperature and pressure increase via a microwave process (Table 1). MAE was successfully used to extract different bioactive compounds from plant materials intended for the food industry [23]. An optimized method consisted of combining MAE with an enzymatic compound such as cellulase in enzyme-microwave assisted extraction (EMAE) to enhance the anthocyanin recovery efficiency compared to microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and enzyme-assisted conventional extraction (EAE), respectively [23- 26].

Subcritical fluids extraction: We have highlighted above the main concern of the solvent extraction process for roselle antioxidants: high temperatures and pressure increase and, to some extent, the presence of intrinsic enzymes [22]. While solvent extraction uses water as a solvent, the extraction of antioxidants using subcritical fluid is processed with acetone as a solvent to generate less energy, higher selectivity and purity, extraction time increase, etc. While subcritical fluid extraction is scarce in roselle, Lukmanto et al. [2] have developed a powerful extraction tool with acetone and CO2 as co-solvents to extract antioxidants from dried roselle calyces. However, slight variations in the operating parameters, such as temperature and pressure, decreased the extraction yield and the effectiveness of the process. The corresponding equipment developed by Lakmanto et al. [2] is described in a semi-pilot scale process extractor (figure 1). With this described equipment and optimized method, the increase of temperature and pressure increased in the same order the amount of the antioxidants and the extraction yield, which is better than the subcritical water extraction used by Rizkiyah et al. [24]. In this process, even at a very high temperature of 95°C, anthocyanins would be cleaved, and the cleavage reaction would be able to recover a high number of phenolic compounds.

Combination of supercritical carbon dioxide extraction and subcritical water extraction: A combination of the previous methods was applied to optimize the maximum recovery of anthocyanins from roselle [5]. This is a robust strategy to extract the anthocyanins from the entire plant of the roselle and recover the highest concentration without altering the quality of the extract. Both stages have already shown their effectiveness in solvent extraction, but their challenges were leveraged when combined. Supercritical carbon dioxide (ScCO2) was chosen as the first stage to destroy the root parts and stems of the entire Hibiscus plant [2, 27]. Then, large debris, impurities, and cellular extracts were removed with nanofiltration and ultrafiltration membranes in the second stage with subcritical water extraction (SWE) [5, 28]. This combined procedure is unique to promote a concentrated, stabilized and pasteurized roselle extract without altering the color of the finished product [27, 28].

The first stage technology (ScCO2) was described in the previous sub-chapter. The SWE equipment constituted of a semi-pilot laboratory scale is shown in Figure 1.

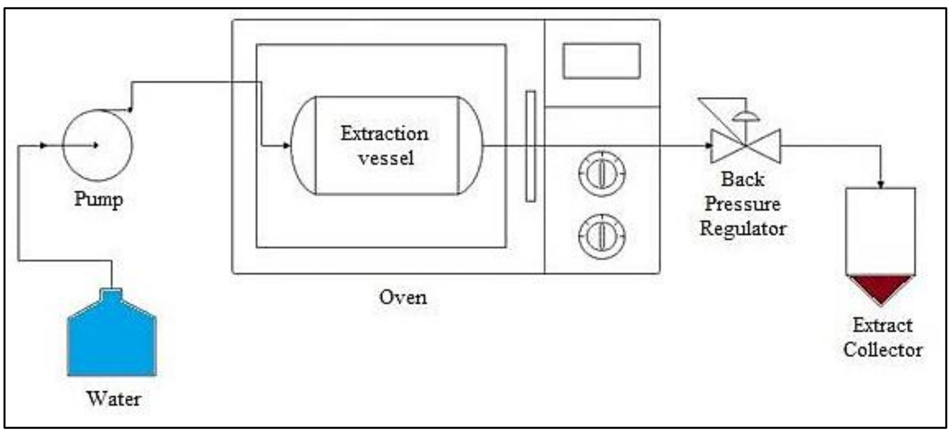


Figure 1. Semi-pilot laboratory scale scheme of subcritical water extraction (SWE). Adapted from Rizkiyah et al. [13].

In this system, three operating parameters drive the anthocyanin extraction: the water flow controlled by a pump, the temperature monitored by an oven that comprises the extraction chamber, and a pressure pump that releases the roselle extract. These three parameters work together to optimize the extraction yield with an appreciable concentration.

Ultra-High Pressure Assisted Extraction: Ultra-high pressure assisted extraction (UHPAE) is a membrane purification process that is being used in the extraction phase to improve the filtration and stabilization of the extract [19]. The need to use UHPAE on anthocyanin extraction is demonstrated by studying the influence of pressure, time, temperature, and other parameters [20, 28, 30-32]. Then, due to the multiple parameters involved, a suitable design of experiment (DOE), such as RSM (response surface method) or ODE (orthogonal design of experiment), is associated with UHPAE to select the best extraction operating parameters. This described system has been successfully used in different outsourced pigments such as purple corn seeds or sliced strawberries [28, 30, 32]. Unfortunately, UHPAE remains an expensive equipment that limits its application in developing countries for pigment extraction [20].

Filtration, stabilization, and pasteurization: The following challenging step after the extraction of the concentrated anthocyanins is undoubtedly the filtration of the remaining debris and the stabilization of the red pigment of anthocyanins from roselle. This step is crucial in maintaining free bacterial contamination of the finished product as well as in promoting its microbial safety (innocuity). Therefore, research has been focused on the following optimization techniques:

Crossflow Microfiltration (CMF): A cheaper and more ecological alternative to UHPE is Crossflow Microfiltration (CMF). This is a membrane filtration process that is used to enable the filtration and stabilization of fruit juices as well as a cold concentration of liquid extract at low temperatures. Often used to filtrate cloudy and pulpy juices as well as to perform cold sterilization or pasteurization, crossflow microfiltration also allows the concentration of dry materials such as anthocyanins from *Hibiscus sabdariffa* [1]. Therefore, the operating conditions were optimized at the laboratory scale for a simple model at a volumetric reduction rate and a relative pressure exerted on the membrane cells. Crossflow microfiltration allowed a good shelf-life of the permeate. Experiments showed that results obtained are further optimized if preliminary ultrafiltration, nanofiltration [26, 33, 34], as well as osmotic concentration phases, are applied to aqueous extracts of *H. sabdariffa* anthocyanins [35]. From these results, membrane technologies have become a new priority for processed dietary products, improving knowledge of green processes to produce fruit concentrates from fresh foods.

Chemical stabilization by tannins: Anthocyanin stability tests were performed at 4, 20, and 37 °C with an aqueous extract containing tannins extracted from *Acacia nilotica* and *Casuarina equisetifolia* over a period of two months: a control batch and a batch containing standard gallic tannin [34]. The results of these batch experiments showed that storing the beverage batches at 20 °C and 37 °C with the addition of gallic tannin from *Acacia nilotica* delayed the degradation of anthocyanins more than in the control batch while the tannins from *Casuarina equisetifolia* had no significant effect on the stabilization of anthocyanins [35]. Prolonged stability results were recorded with *Moringa oleifera* tannins and gum Arabic.

Electrochemical stabilization of anthocyanins: Numerous stabilization techniques of anthocyanins use thermal methods, while the latter is thermolabile [35-36]. Platinum electrode oxygen reduction is a new thermal technique using a two-compartment electrolysis cell separated by a cationic membrane [36]. It has been applied for the stabilization of roselle juice by electro-reduction [36]. The latter consists of an electrochemical sweep of the roselle juice in a determined time. The roselle extract, thus electro-reduced, was then stored for one month at room temperature to monitor the effective stabilization of the roselle juice. The evaluation of the anthocyanin concentration of the electro-reduced roselle juice showed the very important role of dissolved oxygen as an electro-active element of the extract.

Table 1. Extraction methods of anthocyanins from different material sources.

Extraction method	Conditions		Sources	Classes of anthocyanins identified or quantified	Additives	References
	Range of operations	Yield				
Aqueous two-phase extraction (ATPE)	40% ethanol and 0.24 g/mL (NH4)2SO4	4.12 mg/g	Roselle (*Hibiscus sabdariffa*)	Cyanidin Delphinidin Pelargonidin Malvidin	Antioxidants (E300-399); Food colorings (E100-199)	[9, 17, 19, 45]
Organic solvent	Extraction temperature 48 ◦C, extraction time 4 min, static extraction pressure 8 MPa, cycle two times, maceration	19.89 mg/g	*Lycium ruthenicum* Murr Grape seeds	Cyanidin Peonidin	Antioxidants (E300-399); Potassium sorbate (E202); Food colorings (E100-199)	[9, 17, 19, 45]
Subcritical liquid Extraction / Supercritical Carbone Dioxide)	Extraction temperature 40 ºC, pressure 20 MPa, flow rate 10 mL/min	19.60 mg/g	Blueberry (*Vaccinium corymbosum* L.)	Cyanidin Delphinidin Pelargonidin Peonidin	Antioxidants (E300-399); Food colorings (E100-199); potassium sorbate (E202)	[9, 13, 17, 19, 45]
Ultra-High Pressure Assisted Extraction (UHPAE)	time 25 min, solid-to-liquid ratio 1:58 g/mL	200.13 mg/100 g	Purple potato (*Ipomoea batatas* L.)	Delphinidin Cyanidin Peonidin	Food colorings (E100-199); Antioxidants (E300-399)	[45, 57, 67, 69]
Microwave-assisted Extraction (MAE)	Microwave power 469 W, liquid-to-solid ratio 25:1 g/mL, microwave time 4 min	2.18 ± 0.06 mg/g	Blackberry (*Rubus fruticosus*)	Peonidin Cyanidin Malvidin	Food colorings (E100-199); Antioxidants (E300-399)	[45, 57, 67, 70]
Enzyme-Microwave Assisted Extraction (EMAE)	Cellulase addition 2.8%, liquid-to-solid ratio 21:1 mL/g, microwave power 494 W, microwave time 6.5 min	56.98 mg/100 g	*Rhodomyrtus Tomentosa*	Cyanidin	Food colorings (E100-199);	[45, 57, 67]
Ultrasound-Microwave Assisted Extraction (UMAE)	Ultrasonication time 24 min, liquid-to-solid ratio 21:1 mL/g, ultrasound power 210 W, microwave time 1 min, microwave power 500 W	1.11 mg/g	Purple potato (*Ipomea batatas* L.)	Delphinidin Cyanidin Peonidin	Food colorings (E100-199);	[45, 57, 67, 70]

Scaling up from laboratory to industrial scales: Roselle production and innovation of the value chain in developing countries such as Africa requires a mandatory scale-up process from laboratory to industry [37-38]. One of the most valuable benefits is its potential as a food additive colorant due to the presence of compound dye in the calyces [39-40]. A successful downstream process of the roselle extract includes an optimal pasteurized concentrate and a subsequent drying process by freeze-drying or spray-drying to create a powdered or encapsulated finished product [41]. The industrial scale of the roselle value chain is an opportunity to increase householders' revenues and reduce poverty alleviation, especially in Africa. Today, roselle's anthocyanins are exploited at the academic R&D level on a laboratory scale to yield a very low productivity. Activities in the laboratory include separation and concentration of the roselle extract. One of the main concentration techniques used in the laboratory scale is rotary evaporation to discard aqueous solution from extracts using weak temperature and pressure [36, 42]. Due to its thermosensitivity characteristics, roselle extract has undergone a spray-drying process to obtain a marketable finished product [39, 43- 48]. In the spray-drying process, maltodextrin (10%, most of the time) is used as an encapsulating agent that preserves the core material's physicochemical properties against heating or other parameters in which the temperature is a critical factor. A high inlet temperature (>150 °C) may affect the shelf-life and decrease the activity of the anthocyanin of dried roselle powder. In addition to temperature, other process parameters need to be optimized to obtain stable powders with better acceptability [49]. Unfortunately, the high cost of the equipment represents a pitfall to its development for the industry in some countries [49]. A less expensive and laboratory-scale technique is the use of the drying oven. The latter is effective in evaporating liquid from samples of fruits, vegetables, and some cereal grains [49-50]. The main parameter to consider in a drying oven is the temperature variation. Drying ovens are used across different industries, including pharmaceuticals, food processing, agriculture, textiles, and research laboratories [51]. They come in different types and sizes: convection drying ovens, conveyor drying furnaces, flotation and roll support web dryers, rotary drying ovens, vacuum drying ovens, and vertical tower dryers [50- 52]. Except for oven and convective drying ovens, they are ideal for preserving thermosensitive materials and dehydrating foods to increase their shelf life [53-56]. Oven and convective drying, however, depend on temperature, exposition to high temperature and oxygen that induce oxidation of anthocyanin.

In terms of industrial applications of roselle extracts such as food colorant dye, formulation tests for stirred yogurt were carried out with the incorporation of *H. sabdariffa*'s anthocyanins as a colorant [55]. Physicochemical analyses and sensory tests, as well as sensory characteristics and acceptability, were carried out and evaluated to designate the most characteristic attributes [57]. As a result of this work, a classification of yogurts into five classes is obtained. Nevertheless, the sensory profile showed that colored yogurts at a rate of 0.3% and 1% (ml/100ml) of anthocyanin concentrate (figure 2) gave more satisfaction to consumers according to the assigned characteristics of H. sabdariffa such as taste, color, and acidity. These results confirmed previous work that demonstrated that the acceptability of derivative products from *Hibiscus* was positively correlated to the taste of *Hibiscus* drink and anthocyanin content [55].

Figure 2. Anthocyanins content as a colorant in yogurt [55].

As potential colorants for common foods such as bread, pigmented wheat of durum and soft wheat are used in bread formulations. Anthocyanin levels consistently increased in bread and induced significant reductions in the estimated glycemic index of these breads in the ranges of 46% and 30%. Finally, purple durum wheat-enriched bread had a pleasant aroma and good taste [57- 59].

Anthocyanins as Functional Foods in the Human Gut Microbiota: Human gut microbiota (HGM) is populated by a considerable number (billions or trillions) of microorganisms through the human gastrointestinal tract (GIT) [20]. It has been demonstrated in randomized clinical trials (RCT) that specific ingredients named prebiotics and bacterial supplements called probiotics can help modulate the gut microbiota [60-63]. Various studies indicate that the billions of microorganisms lining the human intestine provide good health to the host by promoting proper regulation of the immune system. [60, 61].

Diet and the intestinal microbial ecosystem are interdependent. Prebiotic and probiotic substances have demonstrated their role in maintaining intestinal balance [62]. A few randomized clinical trial studies in functional food research with anthocyanins extracted from fruits were noticed in humans, fish, and poultry [63-66]. In the case of roselle (*H. sabdariffa*), a limited number of reviews have been conducted regarding its functionality with the human gut microbiota. The research results have led to the creation of SMEs or spin-offs around the world and have begun to potentially exploit bacterial mechanisms in health. Today these companies have largely contributed to the health of HGM, notably in disease surveillance (figure 3).

So far, few studies have been developed to embrace anthocyanin's potential use as a functional food or bioactive compound [67-70]. A recent review study in Malaysia highlighted its potential in industrial applications as functional compounds [10]. In Africa, roselle is a huge source of vitamin C, antioxidants, amino acids, and minerals as a beverage juice. In Africa, roselle seeds are often discarded as waste during processing despite their considerable amounts of protein, oil, carbohydrates, and dietary fiber. It would be a huge benefit for Africa to industrially exploit the roselle seeds to produce value-added bioactive compounds based on a sustainable circular economy concept.

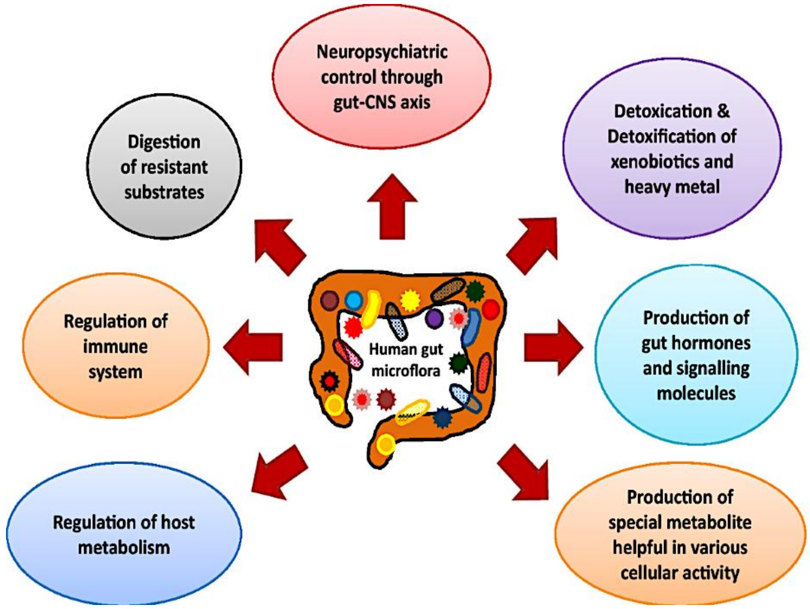


Figure 3. Anthocyanins as functional food ingredients in the human gut microbiota: Therapeutic applications

Hibiscus sabdariffa L., so-called "red bissap" in Senegal, has an ancient tradition as a juicy and laxative beverage in West Africa. People use *Hibiscus sabdariffa* to regulate high blood pressure, high cholesterol, and many other conditions, but there is not much scientific evidence to support most of these uses [70]. Its availability all over the year, as well as its richness in phenolic compounds, antioxidants, and anti-inflammatory properties beneficial to rural populations' health, proved to make Hibiscus the beverage of choice in West African countries. Cissé et al. [1] have developed studies in primary extraction, filtration, and stabilization methods to optimize the concentration of the soluble fractions [1, 4, 16, 20, 21]. Further, Cissé et al. [27] have also proven evidence of the presence of phenolic compounds such as anthocyanins. Their rapid absorption in the mouse gastrointestinal tract in a clinical trial showed its bioavailability in the body [71]. However, the main challenge of the use of roselle extracts in Africa remains the anthocyanin fractions instability, which discards the red color in high storage temperatures [22, 27, 35-37, 42]. Therefore, Faye et al. [55] led studies in the stabilization of the red color and hence, drove the use of anthocyanins as substantial colorants in the local food industries.

Our present and future research directions in roselle anthocyanins shall focus on studying how they are beneficial to human health, mainly the gut microbiota. This latter is the microbial ecosystem that naturally colonizes the human and/or animal intestine and may provide protection against infections in the immune system. It has been proven and widely demonstrated its optimal activities and health benefits when dietary food is supplemented with probiotics, which are microorganisms such as Lactobacillus, Bifidobacteria, etc., that enhance gut microbiota metabolism and hence, the immune system, when consumed in sufficient quantity [62]. By contrast to emulsifiers present in ultra-processed foods, roselle anthocyanins may enhance antioxidant activity, metabolic functions in chickens 'gut [61], and antimicrobial activity [60, 68, 69, 70, 71]. For these purposes, simple techniques, or culture-dependent such as ELISA, were recently used to identify the microorganisms colonizing the gut microbiota in chickens after a diet with anthocyanins [68]. A recent study on cranberries rich in polyphenols and oligosaccharides has newly been published in Nature publishing to showcase the impacts of these antioxidants in human gut microbiota [63]. By using specific omics techniques such as transcriptomics and metabolomics to analyze the presence of microorganisms, polyphenols content, oligosaccharides composition, and short-chain fatty acids profiles of the cranberries extract and determine their bifidogenic effect [63]. This kind of study would be needed on roselles anthocyanins with the use of high-throughput Next Generation Sequencing (NGS) technology and high-resolution mass spectrometry to clinically determine the presence of probiotics and their effects on human gut microbiota. These future studies would demonstrate the effective role of dietary roselle anthocyanins in gut microbiota and hence drive them to a lucrative animal feed agribusiness. Further, the use of artificial intelligence (AI) through machine learning would be of great importance for the food industry in West Africa. By leveraging AI, the functional food industry can meet consumer demands with safer, more nutritious, and personalized products while optimizing efficiency and reducing waste.

Concluding remarks Roselle calyces are a reservoir of phenolic compounds, mainly anthocyanin, which can be used in food industries and pharmaceuticals as a natural antioxidant and functional food for the benefit of human health. However, its localization in the inner part of the roselle made it extremely challenging to perform the highest level of extraction yield. Therefore, a few extraction techniques were optimized from conventional or aqueous extraction, which is a traditional approach usually performed by householders, to stirring-assisted extraction, subcritical fluids extraction, and supercritical CO2 extraction techniques used alone or combined, were processed at a laboratory level to improve the extraction yield. Subsequent chemical processes were performed to sustain the stabilization of the red color of the anthocyanin and its microbiological safety by pasteurization. A technique such as crossflow microfiltration (CMF) has proven successful in stabilizing the red color and preserving the extracts' microbial safety by bypassing the roselle anthocyanins' heat sensitivity. For their best industrial applications, spray drying techniques might be available at a smaller scale for the benefit of small and medium-sized enterprises (SMEs) in developing countries. Therefore, dried anthocyanins production at an industrial scale exhibits great futures in the agribusiness over the *Hibiscus sabdariffa* value chain development in developing countries.

The novelty of this review: This paper highlighted the health benefits of roselle anthocyanins as potential functional foods and bioactive compounds. When properly extracted from the calyces with high quality, innocuity, and acceptability, their mixture with other foods in humans may enhance the gut microbiota functionality by generating probiotics and producing special metabolites (postbiotics) to regulate the immune system and boost various cellular activities.

Abbreviations: AI, Artificial Intelligence, API, active pharmaceutical ingredients; CFM, crossflow microfiltration**;** ECS, Electrochemical stabilization; EMAE, enzyme-microwave assisted extraction; HGM, Human Gut Microbiota; GIT, Gastro Intestinal Tract; MAE, microwave-assisted extraction; NGS, Next generation sequencing; qPCR, quantitative polymerase chain reaction; Ruforum, Regional universities forum for capacity building in agriculture in Africa; SCFA, short-chain fatty acid; SME, small and medium-sized enterprises; SWE, subcritical water extraction; SCO_2, Supercritical Carbone Dioxide; TPC, Total phenolic compounds; RCT, randomized clinical trials; R&D, research and development; UHPAE, ultra-high pressure assisted extraction

Acknowledgments: The authors pay great thanks to Pr. Mama Sakho and Pr. Codou Mar Diop for their huge contribution to the field of *Hibiscus sabdariffa*.

Conflict of interest: The authors declare no conflict of interest.

Author contribution: BN conceived the structure of the manuscript and coordinated the writing and reviewing. BM completed the whole revisions and proofreading, and PGF, CK, and MC contributed to the writing of the subsections. NA supervised and approved the whole work.

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