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Use of *Hibiscus sabdariffa* Calyxes in Meat Products

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In the search for new ingredients that counteract some of the problems associated with the consumption of meat and meat products like high contents of saturated fat, salt, cholesterol, the absence of dietary fiber, and the presence of synthetic additives, *Hibiscus sabdariffa* calyxes have shown good colorant, antimicrobial, and antioxidant properties. This research paper studies the use of *H. sabdariffa* roselle calyxes directly or by means of extracts in meat and meat products. Although its application is incipient, the results are promising. The vibrant red color of the calyxes makes calyxes suitable for their use in meat products even though the concentration must be optimized since the acid taste can detract from the overall acceptance. The antimicrobial properties contribute to safer meat products, and antioxidant effects, helping to extend the shelf life of meat products and reducing oxidative processes. Nonetheless, achieving the desired effects is still challenging since several factors can affect these functional properties.

Keywords: *Hibiscus sabdariffa*, antimicrobial properties, antioxidant properties, healthy meat products, natural colorants

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INTRODUCTION

In the last decades, consumer demand has experienced a transition toward healthier nutrition, which helps in the prevention or treatment of illnesses provoked by unhealthy lifestyles. In this sense, there is a growing trend to search for natural or processed foods which meet nutritional demands with a positive impact on health. Although meat was historically considered a good source of protein and its inclusion in diet was not only desirable but also for economic or social status, this perception has been threatened in recent times (González et al., 2020; Pintado and Delgado-Pando, 2020). Besides the claims about sustainability, especially in the production of beef, the high contents of saturated fat, salt, cholesterol, the absence of dietary fiber, and the presence of synthetic additives (Bronzato and Durante, 2017) have driven the food industry to look for alternatives to decrease the negative impact of meat and meat products while preserving the high-quality meat protein. One of the most adopted strategies has been the inclusion of ingredients coming from plants or vegetable origin in meat products since these materials are a source of functional compounds in addition to being considered healthier (Viuda-Martos et al., 2010; Pateiro et al., 2018; Fernández-López et al., 2019).

Hibiscus sabdariffa is one of these newfangled ingredients. This plant has an uncertain origin (India, tropical Africa, or Saudi Arabia) but is widely cultivated in tropical and subtropical climates of Asia, Africa, and Central and South America at present

(Guardiola and Mach, 2014; Riaz and Chopra, 2018). It is known as roselle in English-speaking countries, Bissap in Senegal, Jamaica in Mexico and Spain, and Congo in France to mention some (Riaz and Chopra, 2018). The brilliant red calyx is the more used part of the plant (Romano Mendonça et al., 2021), and it has been exploited in traditional gastronomy, foods, medicines, and even in animal feeding or in cosmetics (Da-Costa-Rocha et al., 2014; Riaz and Chopra, 2018). Taking advantage of the ancestral functional properties attributed to this plant, several studies have been published lately about the introduction of these calyxes or their extracts in foods including meat products (Jung and Joo, 2013; Paim et al., 2017; Márquez-Rodríguez et al., 2020). In this study, some of these potential uses of *H. sabdariffa* calyxes in meat products, such as its antimicrobial, antioxidant, and colorant properties, have been explored.

FIGURE 1 | Dried *H. sabdariffa* calyxes.

GENERAL CHARACTERISTICS AND NUTRITIONAL COMPOSITION

Hibiscus sabdariffa belongs to the *Malvaceae* family, and it is an annual, bushy plant with a height of up to 2–2.5 m that is characterized by smooth, cylindrical red stems, reddish veins, and long green leaves (Mohamed et al., 2007; Sindi et al., 2014). The flowers are borne singly, are different shades of yellow or buff with a rose or brown eye, and turn pink during maturation. The calyx is brilliant red, consisting of five valves, each containing between three and four light-brown, kidney-shaped seeds (Sindi et al., 2014).

Generally, roselle calyxes have a sour flavor and after desiccation (**Figure 1**) are commercially exploited in the production of drinks (juices or teas), jams, jellies, sauces, and wines (Jabeur et al., 2017). Even though the calyxes are the most used part of the plant, the tender leaves and stems can be consumed in salad as vegetables or used for making chutney as a side dish (Singh et al., 2006; Ismail et al., 2008; Riaz and Chopra, 2018). Also, seeds can be used after oil extraction in the elaboration of soups or even as a coffee substitute or meat condiment after fermentation (Yagoub and Mohammed, 2008; Riaz and Chopra, 2018).

In addition to its culinary use, *H. sabdariffa* has been widely used in folk medicine to alleviate and treat some illnesses like colds, toothaches, urinary tract infections, and kidney and urinary bladder stones (Riaz and Chopra, 2018). Other uses have been antihypertensive, to relieve indigestion (Da-Costa-Rocha et al., 2014), and reduce flatulence in cattle; additionally, it can be mixed with salt to help in the treatment of diarrhea and dysentery in humans and animals, and it can even cure waist pain and some gynecological disorders (Singh et al., 2006). These therapeutic effects have encouraged research into the bioactive molecules that possess antioxidant, anti-inflammatory, antiobesity, antihyperlipidemic, antihypertensive, diuretic, antiurolithiatic, antimicrobial, anticancer, hepatoprotective, renoprotective, antitumor, and immunomodulatory properties and the ability to inhibit blood platelets aggregation (Fernández-Arroyo et al., 2011; Yin et al., 2011; Da-Costa-Rocha et al., 2014; Guardiola and Mach, 2014; Cid-Ortega and Guerrero-Beltrán, 2015). The review of Riaz and Chopra (2018) offers a good revision of the studies focusing on the use of extracts of roselle against hypertension, hyperlipidemia, inflammation, liver disorders, diabetes, renal diseases, and cancer.

Nutritionally, the calyxes are rich in carbohydrates, dietary fiber, proteins, vitamins, minerals, and bioactive compounds (Cid-Ortega and Guerrero-Beltrán, 2015; Jabeur et al., 2017). Despite the large number of compounds reported in *H. sabdariffa* calyxes, the composition of ground calyxes may vary depending on the variety, cultivar geographical area, climate, harvest conditions, soil nutrients, season as well as the drying, milling, and sieved processes of the raw material (Da-Costa-Rocha et al., 2014; Ifie et al., 2018). The preparation of the extracts is also another source of metabolome profile variation (Rasheed et al., 2018). Despite its intrinsic variation, the main component in dry matter is the carbohydrate content, which ranges from 65 to 70% according to Riaz and Chopra (2018), where the content of dietary fiber is remarkable (33.9–41.37%). In general, insoluble fiber content is twice as least the soluble fiber (Sáyago-Ayerdi et al., 2014; Amaya-Cruz et al., 2018). The protein content is 5.5% (Jabeur et al., 2017), while the presence of fat is a minority (0.4%).

Also, the presence of some organic acids such as oxalic, malic, shikimic, and fumaric has been reported, where malic acid is the most abundant (9.10 g/100 g) according to the reported by Jabeur et al. (2017). Da-Costa-Rocha et al. (2014) and Ifie et al. (2018) have also reported the presence of other acids like citric, hydroxycitric, tartaric, and ascorbic acid. The presence of phenolic acids like caffeic, protocatechuic, chlorogenic, or hibiscus acids has been reported in some works (Fernández-Arroyo et al., 2011; Kuo et al., 2012; Rasheed et al., 2018; Izquierdo-Vega et al., 2020). The elevated presence of acids gives the calyxes a very low pH (2.10 $\pm$ 0.02) that together with a low moisture content of the dried calyxes (around 10 %) contributes to its preservation and stability during storage (Salazar-González et al., 2012). In addition to chlorogenic acid and protocatechuic acid, other flavonoids have been reported to be present such as pelargonic acid, hibisicitrin, sabdaritrin, gossypetin, gossitryn, eugenol, quercetin, luteolin, and the sterols β-sitosterol and ergosterol (Da-Costa-Rocha et al., 2014). Apart from some flavonoids, the most interesting compounds from *Hibiscus* are anthocyanins. Anthocyanins are aqueous soluble pigments, responsible for intense colorations ranging from pink, magenta, red to violet, and blue. The presence of anthocyanins in *Hibiscus* calyx gives a brilliant red characteristic color which can be exploited by the food industry as an alternative natural food dye substituting synthetic colorants in foods (Cid-Ortega and Guerrero-Beltrán, 2015). The anthocyanins content is around 1.5 g per 100 g of dry matter of calyxes and gives a color intensity similar to synthetic red No. 2, also known as amaranthus red, because of the presence of delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside; these are are the major anthocyanins present in hibiscus, while delphinidin 3-glucoside and cyanidin 3-glucoside are regarded as minor pigments (Du and Francis, 1973; Sindi et al., 2014; Borrás-Linares et al., 2015; Ifie et al., 2018; Pragalyaashree et al., 2018; Rasheed et al., 2018). The polyphenols content contributes to the antioxidant properties reported in several studies (Sindi et al., 2014; Borrás-Linares et al., 2015).

HIBISCUS SABDARIFFA CALYXES AS NATURAL COLORANT ADDITIVE

Color is responsible for 62–90% of the consumer's assessment of food and is one of the most important attributes in the acceptance of foods (Teixeira et al., 2022). Tartrazine, sunset yellow, and ponceau 4R are synthetic colorants extensively applied in foodstuff. However, the current trend of replacing synthetic dyes with natural ones has led to the use of flavonoid derivatives (anthocyanins), isoprenoid derivatives (carotenoids), and nitrogen-heterocyclic derivatives (betalains) (Viera et al., 2019). In the case of *H. sabdariffa* calyxes, the anthocyanin pigments are used as natural colorants although they are likely to be unstable during their storage and use. First, the extraction conditions should be directed to obtain the highest recovery of pigment. For Pragalyaashree et al. (2018), extraction with warm temperatures up to 60°C can improve the recovery of pigments. However, temperatures over 60°C can favor the degradation of the pigments converting anthocyanins into a colorless chalcone form. Sipahli et al. (2017) suggested HCl acidified ethanol as the recommended solvent for anthocyanin extraction to have a safe additive and stable when it is subjected to pH, heat, and light. Pragalyaashree et al. (2018) confirmed that 1.5 N HCl acidified ethanol was the best solvent to release more pigment. Also, pigments from *H. sabdariffa* are more stable at low pHs, so a pH between 1 and 6 is recommended by Sipahli et al. (2017) and Pragalyaashree et al. (2018) for extraction and application. Also, the application of emerging technologies like ultrasound (Pinela et al., 2019) can improve extraction yields. Pimentel-Moral et al. (2018, 2019, 2020) have studied the use of microwaves (Pimentel-Moral et al., 2018), supercritical CO_2 (Pimentel-Moral et al., 2019), natural deep eutectic solvents (Alañón et al., 2020), and pressurized GRAS solvents (Pimentel-Moral et al., 2020) in the extraction of phenolic compounds, analyzing the impact of several variables involved to maximize the anthocyanin extraction.

When the pigments are added to food matrices, the stability of these natural compounds will depend on several factors like anthocyanin structure, pH, temperature, oxygen concentration, light, and water activity (Sipahli et al., 2017). Besides, they are susceptible to enzymatic degradation and interactions with other food components like ascorbic acid, some sugars, metallic ions, and other pigments altering their stability. According to Sipahli et al. (2017), heat treatments (50°C and 80°C for 6 h) provoked a gradual decrease in intensity measured as pigment retention suggesting that increasing the anthocyanin concentration, removing oxygen, and inactivating enzyme stability could be improved. Pragalyaashree et al. (2018) reported high percentage retention of anthocyanins (over 90%) with thermal treatments of 60°C for 75 min. However, the percentages dramatically decreased to 69 and 49% when temperatures of 80°C and 100°C were applied, respectively. In general, the storage of pigments in amber containers or in the absence of light under refrigeration should be recommended to have longer half-life periods (Pragalyaashree et al., 2018; Paraíso et al., 2020).

Bozkurt and Belibagli (2009) used 1 g of dried ground roselle calyxes per 1 kg of beef meat in the elaboration of a Turkish cooked meat product known as kavurma (**Table 1**). Despite differences between control samples and added samples were observed in Hunter color parameters after the cooking process, the color of *Hibiscus* added samples was not significantly sensorially different ($p > 0.05$) from control samples. When the extracts were added as antimicrobials in meat products, a color modification was also reported in some studies. Higginbotham et al. (2014) reported an increase in red color in hot dogs while Márquez-Rodríguez et al. (2020) indicated there was a diminution of color parameters in beef meat slices sprayed with 250–1,250 mg/L gallic acid equivalents of roselle extracts. When 2% of ground roselle calyxes were added to beef patties by Villasante et al. (2020), a lower percentage of metmyoglobin was reported to stabilize the metmyoglobin formation although modifications of color were observed in the chroma and Hue angle. On the other hand, Jung and Joo (2013) observed that the addition of 0.1−1.3% of aqueous roselle extract in pork patties provoked an increase in redness values while lightness and yellowness decreased. But the cooked patties with roselle extract showed a higher preference in quality attributes, including color, flavor, juiciness, tenderness, and overall quality than the control group. The encapsulation of a lyophilized water/ethanol/acetic acid roselle extract with maltodextrin gave the characteristic pink color to cooked ham, similar to the use of E-120 colorant when it was added to the brine in a concentration of 3.6 g/kg of ham (Dias et al., 2020). These results suggest that the addition of roselle calyxes to improve the meat product's color will be effective as long as the concentration and encapsulation procedure are both optimized.

ANTIMICROBIAL ACTIVITY OF *HIBISCUS* CALYXES IN MEAT PRODUCTS

The antimicrobial activity of aqueous extracts from *H. sabdariffa* against several pathogens has been reported by several authors in different foods. Gutiérrez-Alcántara et al.

TABLE 1 | Application of *Hibiscus sabdariffa* calyxes in meat, meat products and diets for animal feeding.

Application form	Concentration	Meat product/animal	Studied effect	Results	References
Aqueous extracts	0, 2, 4, 6, and 8%	Lean pork frankfurter-type sausages	Colorant and Antioxidant	Samples with 4% extract scored in color similar to control samples Lipid oxidation increased as the roselle extract concentration increases (0.23 and 0.32 mg MDA/kg sample, for 0 and 8%, respectively), also affecting negatively physicochemical and sensory properties.	Pérez-Báez et al., 2020a; Perez-Baez et al., 2021
Ethanolic extract	2.5 mg/mL	Tilapia fish surimi	Antimicrobial and colorant	Microbial reduction (*Salmonella typhimurium, S. aureus, E. coli* and *P. aeruginosa*) in surimi surface was higher (4-5 Log CFU/g) than in the inner (2-4 Log CFU/g). *Hibiscus* extract increased the sensorial characteristics (appearance, odor and color) of surimi, compared to the control, after cold storage for 7 d.	Tayel et al., 2021
Ethanolic extract obtained by ultrasound method	CMC +1,000 ppm of free extract CMC +1,000 ppm nanoencapsulated extract	Chicken nuggets	Antioxidant	Addition of 1,000 ppm of nanoencapsulated extract showed the lowest peroxide and TBA values during 9 d of storage, being similar to the effect of TBHQ and in some cases even more effective.	Bahrami Feridoni and Khademi Shurmasti, 2020
Lyophilized water/ethanol/acetic extract, free and encapsulated in maltodextrin	Free, 3.6 g/kg of ham Encapsulated, 6.93 g/kg of ham	Cooked ham	Colorant	Encapsulated hibiscus extract, showed best coloring properties, similar to those obtained in samples with E120 dye, used as control sample.	Dias et al., 2020
Ethanolic extract	50, 500, 750, 1,000, and 1,250 mg/L of gallic acid equivalents of the hibiscus extract.	Beef steak	Antimicrobial and colorant	Meat with 500 mg/L of gallic acid equivalents showed lower counts of mesophiles (10^6 CFU/g) and psychrophiles (10^5 CFU/g) compared to the control during 9 d (10^9 and 10^{10} CFU/g, respectively).	Márquez-Rodríguez et al., 2020
Flower powder	Roselle, 2% (w/w) Roselle, 2% (w/w/) + pecan shell, 2 % (w/w) + red pepper, 0.35 %, (w/w) Roselle, 2% (w/w/) + pecan shell, 4 % (w/w) + red pepper, 0.35 %, (w/w)	Beef patties	Antimicrobial and antioxidant	All treatments after 6 d, showed lower bacterial counts (4-5 log CFU/g meat), than those observed in control sample (6–7 CFU/g meat). On the last day, TBARS values on all treatments, were significantly lower (0.351, 0.473 and 0.333 mg MDA/kg meat) than in the control group (1.31 mg MDA/kg meat).	Villasante et al., 2020
Roselle flower powder	Roselle, 5% (w/w) Roselle, 5% (w/w) + pecan nut, 5% (w/w)	Sardine (*Sardina pilchardus*) patties	Antimicrobial and antioxidant	Roselle powder alone, reduced microbial growth and combined with pecan nut maintain mesophilic bacteria under 10^2 UFC/g After 66 h of storage (4°C), both treatments were effective in relation to the control, showing TBARS values of 2.33, 2.18, and 4.29 mg MDA/kg, respectively.	Villasante et al., 2019
Methanolic or acetonic extract		Pork sausages	Antimicrobial	Decrease of up to 3 log CFU of inoculated *L. monocytogenes* after 7 days of incubation	Cruz-Gálvez et al., 2018

(Continued)

TABLE 1 | Continued

Application form	Concentration	Meat product/animal	Studied effect	Results	References
Aqueous extract	1, 2, and 3%	Se'i Rotenesse (smoked beef meat)	Antimicrobial and Antioxidant	Total plate count decreased 1 log CFU/g, for 2 and 3% treatments. TBARs values reduced from 0.77 in control to 0.57 mg MDA/kg in 2 and 3% roselle extract marinate.	Malelak et al., 2017
Hydroethanolic extract	5, 10, 15, or 20% for samples inoculated with *E. coli* 20 or 30% for mesophilic counts	Ground beef	Antimicrobial	Efficacy against *E. coli* was observed in all the treatments, reducing the microbiological limit to 10^4 CFU/g. Extract at 30% had a significant antibacterial effect against mesophilic count, remaining stable during 9 d of evaluation.	Paim et al., 2017
Sterilized aqueous extract	120 y 240 mg/mL in rinse solution	Beef hot dogs	Antimicrobial and colorant	Extracts at 240 mg/mL were more effective inhibiting *L. monocytogenes* and *S. aureus* reducing of up to 4 Log CFU at 60 min of rinsing and 24 h of storage under refrigeration.	Higginbotham et al., 2014
Aqueous extracts	0.1–1.3 %	Pork patties	Colorant	Decrease in L and b color parameters, a increase 0.7% of Hibiscus combined 12.5 % of soybean oil gave the maximum overall quality score.	Jung and Joo, 2013
Commercial water-soluble extracts (Plant extrakt, Germany)	0.2, 0.4, 0.6, 0.8 g/100 g of oil-based marinades	Fried beef patties	Antioxidant	Concentration of an aromatic amine was reduced by about 50% and 40%, in marinated meat at the highest amount of extract, compared to sunflower oil and control marinade, respectively.	Gibis and Weiss, 2010
Ethanolic extracts	0.05, 0.1, 0.2, and 0.3%	Chinese-style pork sausages	Antioxidant	Extracts tended to exhibit higher peroxide values and TBARS during storage compared with control without extract, suggesting that in some situations, antioxidants can also act as pro-oxidants.	Parinyapatthanaboot and Pinsirodom, 2010
Ground dried flowers	0.1%	Kavurma (cooked beef meat)	Antioxidant and colorant	*H. sabdariffa* reduced lipid oxidation (TBARS) during kavurma cooking process (from 0.60 to 0.47 mg MDA/kg). Sensorially, color was not affected although differences were observed in Hunter color parameters	Bozkurt and Belibagli, 2009
Aqueous extract	300–600 ppm	Sucuk (Turkish dry-fermented sausage)	Antioxidant	Extracts were ineffective in TBARS reduction maybe due to its low phenolic content (69.04 mg gallic acid equivalent/L of extract), even at high concentration, compared to the effect showed by BHT.	Karabacak and Bozkurt, 2008
Seeds	7.5, 15.0, and 22.5% in diet	Broiler chickens, during 6 weeks	Animal feeding	A negative response of dietary feeding graded levels of rosella seed was observed, decreasing feed intake, weight gain and feed conversion with the increase of roselle seed in diets.	Ahmed Mukhtar, 2007
Ground dried calyxes	0, 1.5, 3.0, 4.5, and 6.0% in diet	Broiler chickens, 4 weeks	Animal feeding	Oxidation of meat decreased with increasing levels of dietary *H. sabdariffa* calyces. Bacteria load of meat decreased according levels of dietary *H. sabdariffa* calyces and length of refrigeration increase.	Onibi and Osho, 2007

(2016a,b) demonstrated that roselle extracts exhibited a great reduction in concentration of multidrug-resistant *Salmonella* strains inoculated in carrots (Gutiérrez-Alcántara et al., 2016a) and tomatoes (Gutiérrez-Alcántara et al., 2016a) over conventional antimicrobial like sodium hypochlorite, acetic acid, and colloidal silver. The same research group has evidenced the antimicrobial activity against other pathogens (*Listeria monocytogenes, Shigella flexneri, Staphylococcus aureus, E. coli, and Vibrio cholerae*) in several vegetables and fruits as avocado (Gómez-Aldapa et al., 2017), strawberries (Gómez-Aldapa et al., 2018a), lettuce, spinach, coriander (Gómez-Aldapa et al., 2018b), and peppers (Rangel-Vargas et al., 2017).

In meat and meat products, less information is available, but that which is available shows remarkable achievements. Several works have used ethanolic, acetonic, or aqueous extracts to inhibit the growth of pathogen or spoilage microorganisms in meat products. Higginbotham et al. (2014) used two sterilized roselle aqueous extract concentrations (120 and 240 mg/mL) to rinse (5, 15, 30, and 60 min) beef hot dogs to prevent the development of *L. monocytogenes* and *S. aureus* inoculated over 5 log CFU/g. Concentrated extracts were more effective in the control of both microorganisms, showing reductions of up to 4 log CFU after 60 min of rinsing and 24 h of storage time. However, *S. aureus* was more sensitive than *L. monocytogenes*, possibly due to the ability of *Listeria* to grow at refrigeration temperatures, in contrast with *S. aureus*, which can survive at low temperatures, but typically does not grow. Malelak et al. (2017) observed a decrease of 1 log CFU/g in total plate count number when 2 and 3% of a *Hibiscus* aqueous extract was added to Se'i Rotenesse smoked beef meat, going from 1.62 CFU/g in the control samples to 0.65 and 0.56 log CFU/g in the treated samples, respectively.

Hydroethanolic extracts obtained from roselle were tested to control the *E. coli* population and to evaluate the mesophilic stability in ground beef under cooling storage by Paim et al. (2017). In the case of *E. coli*, 10 ml of the 5, 10, 15, or 20% extracts applied in a range of 10^4 to 10^8 CFU/g inoculated samples (200 g) reached two logarithmic reduction levels independently of the initial infecting doses, although the 20% extract presented the greatest reduction. Regarding mesophilic counts, a reduction to acceptable levels (around 10^6 CFU/g) compared to the control was observed, remaining stable during the 9 evaluation days of storage. Márquez-Rodríguez et al. (2020) achieved lower psychrophiles and mesophiles counts in beef meat stored for 10 days at 4°C by spraying concentrations of 500 mg/L (gallic acid equivalents) roselle ethanolic extract compared to control samples. However, sensorially, the color of fresh meat was affected by lower L, a, and b color parameters, though this showed more stability depending on the storage time, and the taste became slightly acidic, though it was still qualified as being pleasant by panelists. The immersion of tilapia fish surimi in an aqueous solution of 2.5 mg/mL of a previously dried ethanolic roselle extract was very effective to reduce the counts of inoculated samples with *Salmonella* Typhimurium, *Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa* (Tayel et al., 2021). Samples treated with *Hibiscus* solution presented a reduction of around 3–4 log CFU/g, reaching a reduction of 5 log on the surface of *E. coli* inoculated samples.

The antimicrobial effect of ethanolic and acetonic *H. sabdariffa* extracts has been tested also against *L. monocytogenes* by incorporating the extracts into a film using pork sausages as a food model Cruz-Gálvez et al. (2018). According to the authors, samples covered with the films containing the extracts exhibited an antimicrobial activity from time zero, and a decrease of up to 3 log CFU of *L. monocytogenes* after 7 days of incubation. Lower reductions against this pathogen were reported by Ravishankar et al. (2012) through the application of an edible film using pectin-based roselle film containing carvacrol (2.22 log with 3% of carvacrol) and cinnamaldehyde (<0.5 log). The addition of roselle extracts in meat products can be considered as an additional barrier in a hurdle preservation procedure to assure meat product safety and contribute to a longer shelf-life.

ANTIOXIDANT PROPERTIES OF *H. SABDARIFFA* IN MEAT PRODUCTS

Antioxidant molecules help to neutralize the free radicals, responsible for oxidative stress and are related to some sufferings such as cancer, cardiovascular disease, cognitive impairment, immune system dysfunction, and muscle deterioration to mention a few. In the food industry, the addition of antioxidants reduces the chemical spoilage by rancidity, especially in foods with high-fat contents or rich in polyunsaturated fats, since these compounds are quite sensitive to autooxidation phenomena in the presence of oxygen. The oxidation processes lead to nutritional losses and the development of rancid odor and flavor, negatively affecting the quality and shelf-life of the foods. The usual strategy of adding synthetic additives to lessen the oxidation reactions including propyl gallate, BHA, or BHT is being replaced by the use of natural antioxidants since synthetic additives have been questioned about their toxicity and the risk to consumer health (Shahidi and Zhong, 2010). The antioxidant activity of the *H. sabdariffa* calyxes has been attributed to phenolic compounds, being the flavonoids able to reduce the free radicals and modulate oxidation reactions.

The inclusion of ground roselle calyxes (1 g/kg meat) in the kavurma elaborated by Bozkurt and Belibagli (2009) resulted also in lower TBARS, demonstrating the antioxidant effect during the cooking process compared to the use of butylated hydroxytoluene (BHT, 3,300 mg/kg of meat) and the absence of antioxidants (control samples). According to the authors, the natural antioxidants from *H. sabdariffa* have a potential to reduce lipid oxidation like synthetic antioxidants (BHT), even at a lower concentration. On the other hand, when Karabacak and Bozkurt (2008) tested the antioxidant effect of a *H. sabdariffa* aqueous extract (300–600 ppm) along with other plant extracts, during the ripening of sucuk, a Turkish dry-fermented sausage, roselle extracts were ineffective in the reduction of TBARS compared to other antioxidants. Authors attributed the poor performance of *Roselle extract* to its low phenolic content (69.04 mg gallic acid equivalent/L of extract).

Gibis and Weiss (2010) reported a preventive effect in the inhibition of heterocyclic aromatic amines (HAA) formation in fried beef patties by using oil-based marinades added with different concentrations of a roselle extract (0.2, 0.4, 0.6, 0.8 g/100 g of marinade). After frying, patties that had been marinated with the highest amount of extract presented a reduction between 50 and 40% of MelQx HAA. Also antioxidant capacity and total phenolic compounds were the highest, without sensory significant differences ($p > 0.05$) with control samples. Good reduction TBARS values from 0.77 to 0.57 mg malonaldehyde/kg sample were reported also by Malelak et al. (2017) when a *Hibiscus* aqueous extract at 1, 2, and 3% was added to Se'i Rotenesse smoked beef meat, even though lipid oxidation values found were lower than the malonaldehyde limit for acceptability (2 mg malonaldehyde/kg sample).

The combination of shredded roselle calyxes (5% w/w) with pecan nuts (5 and 10% w/w) was evaluated by Villasante et al. (2019) in sardine patties. While the incorporation of roselle was very effective as an antimicrobial, inhibiting development of mesophilic bacteria, the antioxidant effect was potentiated by the combination with pecan nuts, showing lower TBARS values even than the product added with BHA. In a later work, these authors (Villasante et al., 2020) tried the combination of roselle calyxes with pecan shell and red pepper in beef patties. Again, the antimicrobial effect of roselle was demonstrated in samples containing 2% of the calyxes, which presented counts between 4 and 5 log CFU/g while samples without *Hibiscus* presented counts over 6 log CFU/g after 6 days of cold storage. The presence of roselle (alone or combined) contributed also to diminishing TBARS values compared to control samples and lower hexanal formation, a by-product generated mainly by the oxidation of polyunsaturated fatty acids. Pérez-Báez et al. (2020b) recently tested the inclusion of roselle aqueous extracts (0–1%), potato peel flour (0–2%), and beef fat (0–15%) on physicochemical properties, antioxidant capacity, and total phenols in beef patties. The addition of high roselle extract concentrations increased the antioxidant capacity and total phenols in formulations, but decreased pH, instrumental color, cooking yield, moisture retention, springiness, and cohesiveness. Using surface response methodology, they concluded that optimal beef patties formulation was reached with 0.61% of roselle extract, 1.27% of potato peel flour, and 3.04% of beef fat without a loss of physicochemical quality. The research group Perez-Baez et al. (2021) also evaluated the effect of roselle aqueous extracts (which showed a high content of anthocyanins) addition on the lipid oxidation of lean pork frankfurter-type sausages. Although, sensorially, 4% roselle extract resulted in adequate sensory properties, the addition of roselle extract slightly increased the TBARS content from 0.23 mg malonaldehyde/kg sample in control samples to 0.32 mg malonaldehyde/kg sample for 8% roselle extract samples. These results were unexpected since the addition of a roselle aqueous extract had the purpose to stabilize the product and protect it from lipid oxidation. The increase in peroxide values and TBARS because of the addition of 0.05–0.3% of roselle anthocyanins ethanolic extracts in Chinese-style pork sausages were also reported by Parinyapatthanaboot and Pinsirodom (2010), who attributed this phenomenon to extracts can act as pro-oxidants in some situations. It seems that antioxidant properties in meat products are not only highly related to phenolic compound content but also to the extract concentration added. These factors should be considered to succeed in the prevention of rancidity.

The strategy proposed by Bahrami Feridoni and Khademi Shurmasti (2020) to assure the antioxidant effect of the extracts was to nanoencapsulate the aqueous extracts (with a phenolic compound's concentration of 626.57 mg/g gallic acid and anthocyanin concentrations of 379.11 μg/mL) and include these in a carboxy-methylcellulose (CMC) biofilm. According to the authors, chicken nuggets treated with 1,000 ppm of nanoencapsulated *Hibiscus* tea extract with CMC showed the lowest peroxide and TBA values during the 9 days of cold storage, being similar to the effect of TBHQ or even more effective in some cases. The nanocapsules delayed oxidative spoilage and extended the sensory properties during storage, presenting better color, odor, and overall acceptance scores than the control samples.

SENSORIAL IMPLICATIONS OF ROSELLE ADDITION

The incorporation of roselle extracts into meat and meat products provokes sensory changes that can improve or negatively affect the consumer's acceptance. As shown previously, one of the most visible effects of roselle application is the impact on color. Anthocyanins from roselle can improve the color of meat products such as beef patties (Gibis and Weiss, 2010), fish surimi (Tayel et al., 2021), sucuk (Karabacak and Bozkurt, 2008), or hot dog sausages (Higginbotham et al., 2014), and color remained stable even in storage conditions (Bahrami Feridoni and Khademi Shurmasti, 2020). However, other works have reported unfavorable effects from the addition of roselle in beef slices (Márquez-Rodríguez et al., 2020) and cooking pork patties (Jung and Joo, 2013). Regarding lightness, while an increase of this parameter is expected when a low concentration of roselle extract is added (Bozkurt and Belibagli, 2009; Malelak et al., 2017), high extract concentrations tend to provoke the opposite, especially when the product is subjected to thermal treatment (Jung and Joo, 2013; Malelak et al., 2017; Perez-Baez et al., 2021). It seems that during the cooking process the presence of denatured-globin hemochromes, colored Maillard products, and protein and other meat compounds modifications could negatively change the color (Jung and Joo, 2013). During the storage, the antimicrobial and antioxidant properties of roselle extracts usually contribute to reducing spoilage and give better color scores compared to control samples (Bahrami Feridoni and Khademi Shurmasti, 2020).

Textural modifications measured through different attributes (resistance to cut, granularity, juiciness, firmness, and hardness) have been studied in some works. Bahrami Feridoni and Khademi Shurmasti (2020) reported a decrease in firmness because of the addition of roselle extract combined with carboxymethylcellulose. When several concentrations of aqueous extract (2–8%) were added to frankfurters, hardness, springiness, and chewiness tended to decrease as the concentration increased (Perez-Baez et al., 2021). The same effect was observed by

Jung and Joo (2013) in pork patties. According to Iqbal et al. (2016), the acids as those present in roselle extracts could affect the meat structure improving proteolysis by breaking off peptide bonds of amino acids resulting in increased tenderness. Although this effect may be beneficial in fresh meat, could be undesirable in emulsified meat products like frankfurters.

Finally, roselle extracts are associated with an acidic taste due to the natural phenolic acids of Roselle extracts and it could negatively affect the sensorial parameters giving acidity notes to the products, resulting in unacceptable for consumers. For example, in the work of Pérez-Báez et al. (2020a), frankfurter-type sausages added with 8% of an aqueous Roselle extract scored very low sensorially compared to control samples or samples with 4% of the extract, which were not significantly different ($P > 0.05$). In most cases, when these extracts were incorporated into meat products at low concentrations, the sour flavor was hardly detectable (Karabacak and Bozkurt, 2008; Gibis and Weiss, 2010; Perez-Baez et al., 2021). Even though the acid was detected, it helped to enhance taste and aroma and can be found pleasant by consumers (Jung and Joo, 2013; Malelak et al., 2017; Márquez-Rodríguez et al., 2020; Villasante et al., 2020).

ANIMAL FEEDING USES

Apart from the nutraceutical and functional functions of *H. sabdariffa* in humans, there are few works focused on the use of calyxes and by-products in animal feeding looking for potential effects on meat quality. Onibi and Osho (2007) analyzed the effect of five animal diets containing different levels (0, 1.5, 3.0, 4.5, and 6.0%) of dried *H. sabdariffa* calyces, in broiler chickens for 4 weeks. According to their results, the oxidation of refrigerated meat decreased ($P < 0.01$) with increasing levels of dietary *H. sabdariffa* calyxes and meat presented a lower bacteria load confirming the antimicrobial effect of this plant. However, according to Ahmed Mukhtar (2007), the supplementation of broiler chicken diet with higher concentrations of *H. sabdariffa* seeds (7.5–22.5 %) decreased the feed intake, weight gain, and feed conversion with the increase of roselle seed in the diets although no antioxidant effect was tested in the meat.

CONCLUSIONS

There is no doubt about the interesting properties of *H. sabdariffa* calyxes when used directly or as an extract to improve the safety and shelf-life of meat and meat products. Despite the brilliant red color of these calyxes making them especially suitable in meat products, also increasing their acceptability, the acid taste associated with these extracts could be a handicap in their incorporation in meat products. The antimicrobial properties, especially against pathogens, as well as the antioxidant properties, are remarkable, although differences can be expected according to several factors such as growing conditions, storage, or incorporation process. In this sense, the evaluation of the additive performance becomes essential, not only in the meat product characteristics but also in the potential effect on the consumer's health should be primordial. The application of *H. sabdariffa* calyxes, dried or extracts, is incipient, but the promising published results open a new opportunity to test not only the whole calyx but also the extraction of specific biocompounds or the use of byproducts to improve the functional properties of meat products.

AUTHOR CONTRIBUTIONS

ER-V gave the idea for the article's topic and established the general outline of it. ES reviewed the structure of the article and reinforced some of the topics. IS-O made the article's table, checked the bibliography, and made a general revision. JL and RD reviewed the topic's relevance and contributed to the article's general revision. PM contributed to the review and relevance of the article's topics. RF-C contributed to the literature review and general review of the article. II proofread the article and made the general editing. All authors contributed to the article and approved the submitted version.

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