Egyptian Journal of Food Science
http://ejfs.journals.ekb.eg/

The Use of Cauliflower Waste Extracts As a Natural Antioxidant to Improve Quality of Beef Sausage

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CAULIFLOWER waste is an excellent source of health-promoting phytochemicals such as antioxidants as phenols, flavonoids, vitamins and minerals, which are generally added to avoid or stop the process of oxidation in processed foods. This work was done to encourage the use of cauliflower waste extracts as a natural antioxidant. Cauliflower stalks waste powder was high value of total phenolic compounds (16.12 mg gallic acid equivalents/100g) and total flavonoid content (2.13 mg catechin equivalent /100g), respectively. The extracts of cauliflower waste were prepared by aqueous, ethanol absolute, and water:ethanol (1:1). The high scavenging activity was observed at a mixture of water and ethanol (1:1) extract. Registered the high potential antibacterial activity against Gram-negative bacteria at concentrated 0.5 % of water:ethanol (1:1) extract.Total volatile basic nitrogen (TVBN), thiobarbituric acid (TBA), water holding capacity (WHC), pH, cooking loss and microbiological tests of sausage samplesprepared with water and ethanol extract (1:1) of cauliflower waste at various ratios during frozen storage at -18°C for 90 days were evaluated. TVBN, TBA values, pH, water holding capacity (WHC), cooking loss, and microbiological analysis were reduced. Furthermore, as storage time increased, the TVBN, TBA, and pH values of sausage samples increased. While microbial loadings of selected samples tested were decreased with rising addition rate of water:ethanol (1:1) extract (0.5 %) during the storage period -18°C for 90 days.

Keywords : Cauliflower Waste, Antioxidant, Antimicrobial, Sausage.

Introduction

Waste from the fruit and vegetable processing industry is the second largest environmental waste generator. Different fruits and vegetables contain between 25% and 30% of non-edible items with different amounts of waste (Ajila et al., 2010). During harvesting, the waste material can be in the form of leaf and straw. Meanwhile, the peels, seeds and stones produced by the processing of fruit and vegetables (Joshi & Devraj, 2008), which can be used as source of phytochemicals, and antioxidants (Ayala-Zavala et al., 2010). Food losses and waste can occur in the food supply chain, starting from harvest up to retail and consumption levels. Food wastes can also be categorised as to unprocessed and processed onesAccording to FAO (2019). On a global scale, an average of 30–40% of annual food production is wasted; Huang et al., 2019).

Cauliflower (*Brassica oleracea var. Botrytis*) is one of the main family of Brassicaceae, with the highest waste index, which amounts to around 45-60 % of the vegetable's total weight, remains a key issue that produces a foul odour of decomposition (Oberoi et al., 2007; Cabello-Hurtado et al., 2012).

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Received: 15/9/2020; accepted: 27/5/2021
DOI: 10.21608/EJFS.2021.43075.1079

Fresh sausages are the world's most popular processed meat products and have been a staple in many places for a long time (Sharma et al., 2017). Fresh sausages are composed of meat and amount of fats, which are minced and mixed with a variety of non-meat ingredients such as salt, spices, herbs, coloring agents and preservatives depending on preparations (Salinas et al., 2014). As they are made from fresh minced meat, these items are extremely perishable and favourable for the microbial growth of pathogenic agents and spoilage. In addition, one of the huge problems during the processing and storage of fresh sausages is decoloration and lipid oxidation, which leads to a decrease in food quality, adversely affecting the product's colour, taste and texture, causing a limited shelf life (Baldin et al., 2016).

Synthetic or natural antioxidant and antimicrobial agentscan reduce lipid oxidation and microbial growth by applying it to the meat product processing for increasing the product quality, shelf-life, and safety (Kim et al., 2013). However, the negative consequence of using synthetic antioxidants has emphasized the value of natural antioxidants. Therefore, search on naturally occurring antioxidant and antimicrobial compounds have increased dramatically (Lorenzo et al., 2013). Lipid oxidation depending on the product type and added amountof natural antioxidants, must be taking into concern that natural antioxidants could also have a negative effect on the color and sensory characteristics of the meat products (Tomović et al., 2017).

The goal of this study was to produce antioxidants and antimicrobials extracted from the powder of cauliflower waste and thus to shed light on the effect of integrating extraction on the characteristics of chemical, physicochemical consistency, microbiological and sensory assessment of beef sausage.

Materials and Methods

All chemicals

All standards, chemicals and solvents were purchased from Sigma-Aldrich, U.S.A.

Microbiological strains

All strains were obtained from MERCN of the Faculty of Agriculture, Ain Shams University. *Escherichia coli* (RCMB 010052) ATCC 25955, *Salmonella typhimurium* (RCMB 006) ATCC 14028, and *Pseudomonas aeruginosa* (NCIM 5029), *Staphylococcus aureus* (ATCC 13565), and *Bacillus cerues* (ATCC 11778) *Aspergillus flavus and Candida albicans RCMB 005003 (ATCC 10231)*

Sample collection and preparation

Cauliflower waste (stalks) was brought from Almasria Pickles, Abu Zaabal industrial zone, Kalubia governorate, Egypt.

Dehydration of stalks and preparation of powder

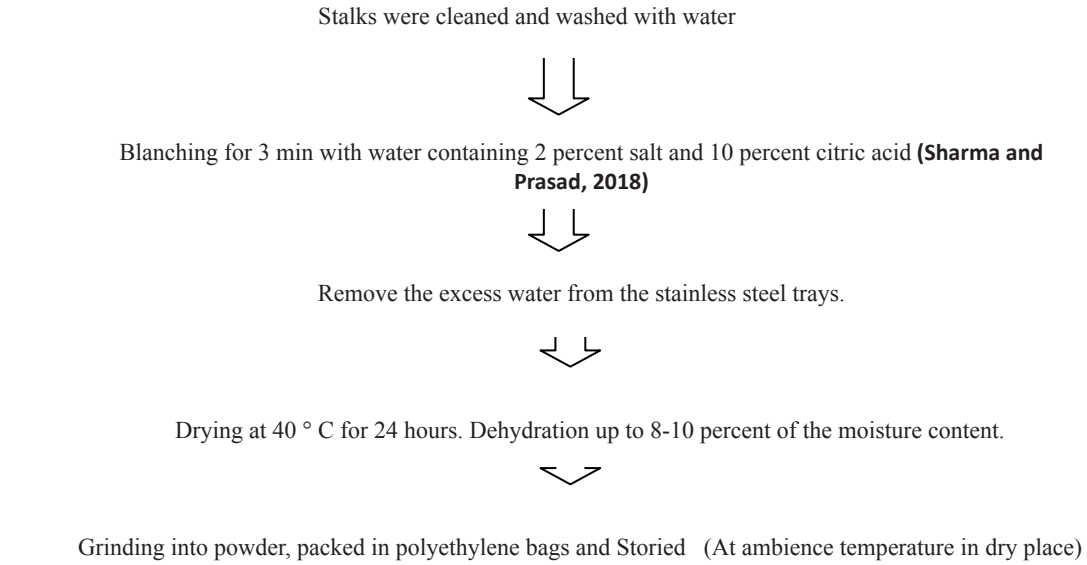

Fig. 1. Preparation of cauliflower stalks powder.

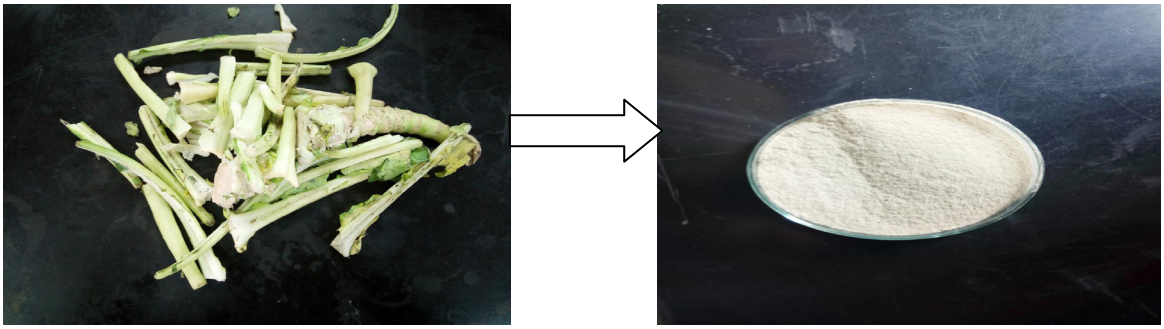

Fig. 2. Stalks of cauliflower **Cauliflower wastes powder**

Preparing extracts

The three extracts were prepared by soaking of dried cauliflower powder (50.0 g) (in triplicate) using 100% water, 100% ethanol and water:ethanol (1:1) (500 mL for each) separately. The extractions were stirred 3 times / day at room temperature and then the extracts were filtered through filter paper (Whatman No. 1). The residue was re-extracted with two additional fresh solvent aliquots using the same technique. The combined extracts were evaporated to dryness using a rotary evaporator. The crude concentrated extract was stored at 4°C until use (Anwar et al., 2013).

Preparation of beef sausage

Frozen beef, animal fat and natural sheep intestinal casings were purchased from the local market in Giza, Egypt, and used for the preparation of beef sausage samples. Visible fat, tendons and other connective tissues were removed from the meat and placed in the refrigerator at 4±1 ° C overnight. The casing was soaked in 5 percent lactic acid solution for 20 min before filling as described by Gök et al. (2011). All spices mixtures were purchased from a local retail spice market in Giza, Egypt.

Sausage samples were prepared by mixing minced meat with the other ingredients specified in Table 1 according to the requirements of the preparation (Azizkhani & Tooryan 2014). Four batches of sausage were prepared using three percentage levels of water: ethanol (1:1) extract (0.1, 0.3, and 0.5% dissolved in Tween 80), as a substitution of ice water and control (with no addition). Formulated sausage mixtures were filled into sheep casing using filler (Moulinex, France). Sausage samples of each treatment (triplicates) were stored in the freezer at -18°C further use and analysis. A part of every sample was separated for the analysis at zero time, and a part of each batch was cooked for sensory evaluation.

TABLE 1. **Basal beef sausages formula (100g).**

Ingredients	%
Minced frozen beef meat	60.00
Animal fat	15.20
Iced water	10.00
Rusk	1.10
Wet soy (1 soy: 2 water)	7.00
Whey protein powder	1.50
Minced garlic	1.00
Spices**	1.90
Corn starch	0.80
Salt	1.50

**Spices mixture%: Fennel (60.00), coriander (27.00), Chinese Kobeba (3.00), Black pepper (3.00), Clove (3.00), Laurie paper (2.00), Cardamom (2.00)

Proximate and mineral analysis

Moisture, ash, protein ($N \times 6.25$), fat, and crude fibers contents were tested according to the methods described by the A.O.A.C. (2012). Total carbohydrates content were calculated by differences.

Minerals (Iron, Calcium, Potassium, Sodium, Zinc, and Sulfur) were measured in a dilute solution of the ashed sample using Perkin Elmer atomic absorption spectrometer (Model 2380) as according to the A.O.A.C. (2012). The results were presented as dry weight (DW) basis.

Determination of total phenolic compounds

The total phenolic compounds of cauliflower waste extracts were calorimetrically calculated according to the method defined by the Folin-Ciocalteu reagent (Singleton et al., 1999). The absorbance of the mixture was measured at 725 nm against blank using a spectrophotometer (Jenway 6705 UV /vis). Gallic acid was used as a standard.

Determination of total flavonoids

Total flavonoids of the extracts of cauliflower waste were calculated in accordance with the methods of Zhishen et al. (1999). The absorbance of the mixture was measured at 510 nm against blank using a spectrophotometer (Jenway 6705 UV /vis). Quercetin was used as a standard.

Determination of antioxidant activity

The antioxidant activity of cauliflower waste extracts was determined based on the radical scavenging ability in reacting with a stable DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) free radical as described by Brand-Williams et al. (1995). DPPH (2.4 mg) in 100 mL methanol was prepared and 3.9 ml of this solution was added to 0.1 mL of sample extract. The mixture was shaken well and left at room temperature for 30 min in the dark. Then the absorbance was measured at 515 nm using a spectrophotometer (Jenway 6705 UV/vis). The radical scavenging percentage was calculated by the following equation:

$$\text{Inhibition \%} = (\text{Ac}(0) - \text{AA}(t)) / \text{Ac}(0) \times 100$$

Where:

Ac (0) is the absorbance of the control at time = 0 min.

AA (t) is the absorbance of the antioxidant at time =30 min.

Identification of phenolic acids and flavonoids compounds by HPLC

Using HPLC, phenolic and flavonoid compounds were fractionated according to the technique of Goupy et al. (1999) and Mattila et al. (2000). The different treatments were injected into Agilent HPLC (Series 1200) 5HC-C18, 250 x 4.6 mm, and ultraviolet detector which adjusted at 280 nm for phenolic acids and 330 nm for flavonoids compounds. Gradient separation was done with methanol and acetonitrile as a mobile phase at a flow rate of 1 mL/min. The column temperature was set at 35°C. The fractionated phenolic and flavonoids compounds were identified comparison of its retention time compared with those of automatic areas.

Antimicrobial activity of plant extracts

Dimethyl sulfoxide (DMSO) (3% w/v) was used to prepare the stock of water, absolute ethanol, and 50 % ethanol extract solutions to evaluate their activities against the standard pathogenic bacteria and fungal that used in this study. 0.2, 0.3, and 0.5mg/mL concentration of three extracts were used according to the method of Fadeyi et al. (2015).

The antibacterial activity of prepared extracts was determined using agar well diffusion method against *E. coli, S. typhimurium, P. aeruginosa, S. aureus,* and *B. cerues* (Daoud et al., 2019). Nutrient agar plate was prepared and 100 µL of overnight grown culture was spread over the media, wells were bored and sealed with soft agar. 0.2, 0.3 and 0.5 mg/mL of prepared extracts were added to respective wells. The antibacterial activity present in the extracts were let to diffuse out into the medium and interact in a plate freshly seeded with the test organisms by incubating the plates overnight at 37°C. The resulting diameter of the inhibition zone was measured in millimeters using zone measuring scale. DMSO at a concentration of 10% was employed as a negative control.

The antifungal activity of prepared extracts was tested using agar well diffusion method against *A. flavus* and *C. albicans* (Nejad et al., 2010). SDA media was prepared, autoclaved for 15 minutes at 121°C, cooled, and poured in laminar flow cabinet in Petri plates. After allowing the media to solidify at room temperature, one hundred microliters of inoculums (106 FU/mL; 0.5 McFarland) of every tested fungus evenly spread onto separate agar plates using a sterile glass spreader. The agar plates were dried and punch wells were shaped by sterile borer of 6 mm in diameter. The wells were filled with 100 µL of 0.1-0.3-0.5 % of prepared extracts. The extract was allowed to diffuse into agar media for 2 h and incubated at 27°C for 72 hours. Dimethyl sulfoxide (DMSO) was used as a negative control.

Chemical quality attributes

Total volatile nitrogen (T.V.N.) determination was achieved according to the method published by Winton & Winton (1958). According to the procedure performed by Kirk & Sawyer (1991), the Thiobarbituric acid value (TBA) was calculated spectrophotometrically. The values for TBA were expressed as malonaldehyde/ kg mg.

Physical analysis

The water holding capacity (WHC) of beef sausage samples was done by Soloviev's (1966) filter paper press process. A 10% dispersion of the sausage sample in distilled water using a Jenway Automated pH metre (Model 3510) was used to measure the pH value. Cooking loss, as defined by Alesson-Carbonell et al., (2005), was determined as follows

$$\textbf{\% cooking loss} = \frac{\text{uncooked sausage weight} - \text{cooked sausage weight}}{\text{uncooked sausage weight}} \times 100$$

Bacteriological methods

Ten gram of a representative sausage sample was mixed with 90 ml of sterile buffered 0.1 % peptone water in a sterile blender, under sterile conditions, Serial dilutions were planned to be used for counting total bacteria count, Coliform bacteria, *psychrophilic bacteria*, *Staphylococcus aureus,* and yeast & mold counts.

All samples were analyzed for aerobic plate count, *E. coli*, *Salmonella*, *S. aureus*. *Psychrophilic* bacteria, yeast and mold. All analyses were tested by using the standard procedures outlined in the American Public Health Association (A.P.H.A., 1992)

Water Activity (^{a}w)

Water activity (^{a}w) was measured at 25°C using Decagon Aqualab Meter Series 3TE (Pullman, WA, USA). Three replicate readings (three different samples) were taken for ^{a}w measurements of sausage.

Sensory evaluation

Sausage samples were boiled for 15 min and then fried at 150 ° C for 5 min in deep sunflower oil. According to Mansour & Khalil (1999), ten professional panelists from the staff of the meat and fish research technology department carried out the sensory properties of sausage samples. For each trait, five sensory attributes (taste, odor, color, texture and overall acceptability) were assessed using a nine-point hedonic scale where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.

Statistical analysis

All data from the current study is expressed as mean values ±SD The Statistical Analysis System (SAS) software program (SAS Institute, 2004) was carried out by (ANOVA) followed by a statistically significant difference using tests (LSD) at $P \leq 0.05$.

Results and Discussion

Chemical composition of powdered cauliflower waste

The chemical composition of cauliflower waste powder was shown in Table (2), whereas the total protein, fiber and carbohydrate content of cauliflower waste powder was relatively high (18.90, 17.23 and 46.84% respectively) but contained a moderate moisture content (9.67 %).The total fat and ash content, meanwhile, reported the lowest value. These results were in good agreement with Baloch et al. (2015), who recorded that the gross chemical composition of cauliflower waste powder were 19.09 % protein, 18.56 % fiber, 42.44 % total carbohydrates, and 9.99 % moisture.

The mineral composition of cauliflower waste powder was presented at the same table. For the potassium, sodium, calcium and sulphur content of cauliflower waste powder, the highest values (3120.7, 1055.3, 956.2, and 647.1 mg/100 g) were, respectively. In cauliflower waste powder, the lowest value of iron and zinc was contained.

TABLE 2. Chemical composition and mineral content of cauliflower waste powder.

Parameters %	Cauliflower waste powder
Moisture	9.67±0.13
Protein	18.90±0.29
Fat	2.23±0.07
Ash	5.23±0.21
Fiber	17.23±0.35
Carbohydrate	46.84±0.28
Minerals (mg/ 100g)	
Potassium	3120.7
Sodium	1055.3
Calcium	956.2
Sulfur	647.1
Iron	0.596
Zinc	0.49

*Means ± SE: Means of triplicate determinations for the chemical component being tested ± standard error
** Total carbohydrates: calculated by differences

Fractionation of phenolic and flavonoids Compounds of cauliflower waste powder

HPLC isolated and classified the phenolic and flavonoid compounds of cauliflower waste powder and presented the findings in the table (3). Seventeen phenolic compounds have been identified and Pyrogallol, followed by benzoic acid, Catechein (11.29, 9.48, and 9.62 ppm, respectively), and Ellagic acid, was the major phenolic compound contained in cauliflower waste powder (31.12 ppm). Cauliflower waste powder, meanwhile, had a trace amount of Cinnamic acids and gallic acids (0.70 and 0.52 ppm, respectively). The results are consistent with those reported by Podsedk et al. (2006).

The range of flavonoids that occur in plant materials is generally large; the flavonoid fraction components come from different aglycone, mono, and polyglycoside or acylated compound groups. The HPLC was isolated and characterized by six flavonoid compounds as shown in Table 3.

Quercetrin was the prevalent flavonoid compound present in cauliflower waste powder (37, 97 ppm). Whereas the second flavonoid compounds found in a high amount of Rutin and Rhamentin were (28.13 and 28.03 ppm). The lowest values of flavonoid compounds were found in Quercetin and Kampeferol.

These results are close to Ahmed &Ali (2013) who stated that the predominant phenolics of raw cauliflower were protocatechuic acid (192.45 mg/100g), quercetin (202.4 mg/100g), pyrogallol (18.9 mg/100g), vanillic acid (11.90 mg /100g), coumaric acid (6.94 mg /100g), and kaempferol (25.91 mg/100g), respectively on dry weight.

Total phenols, flavonoids content, and antioxidant activity content of cauliflower waste extracts

Three types of extraction procedures (aqueous, absolute ethanol, and water:ethanol (1:1) v/v) were used to extract the biologically active compounds from cauliflower waste powderand the data illustrated in Table 4.

Water:ethanol (1:1) extract of cauliflower waste powder reported the highest values of total phenolic and flavonoid content, with an increase in concentrations (0.1 to 0.5 percent) from 21.08 to 73.67 mg/100g and 0.026 to 0.064 mg/100g, respectively, followed by absolute ethanol at the same concentrations, varying from 19.21 to 67.96 mg/100g and 0.021to 0.058 mg /100g, respectively

Meanwhile, the aqueous extract had the lowest values of total phenolic and flavonoid contents. These results were generally in agreement with Shehata et al. (2014) who noticed that the total phenolic and flavonoid content in cauliflower cold-water extract were 61.75 and 299.0 mg/ 100g, respectively on fresh weight.

Subsequently, their capacity to inhibit the development of free radicals was reflected in the antioxidant activity of cauliflower waste extracts (aqueous and absolute ethanol and water:ethanol (1:1) v/v). The most used method for screening the antioxidant potential of plant extract is DPPH scavenging operation. The highest antioxidant quality, after statistically comparing the results of all assays, the highest content of the antioxidant activity observed at sample extracted with water:ethanol (1:1) which increased with an increase in concentration (0.1 to 0.5 %) from 63.16 to 72.29 %, respectively compared to absolute and aqueous extracts at the same concentration.

The probability of using 0.5% extracts as a good source of antioxidants was supported by these findings. These findings were not in line with those of Shehata et al. (2014), who found that the water extract of cauliflower leaves had the greatest potential to neutralize DPPH radicals (31.5 %).

TABLE 3. **Fractionation of phenolic and flavonoids Compounds of cauliflower waste powder (dry matter).**

Phenolic compounds	Concentrations Ppm	Flavonoids compounds	Concentrations ppm
Gallic acid	0.52	Naringin	18.56
Pyrogallol	31.12	Rutin	28.13
Protocatchuic	3.27	Quercetrin	37.97
Catechein	9.48	Quercetin	8.035
Chlorogenic acid	4.08	Rhamentin	28.03
Catechol	2.78	Kampeferol	6.011
Caffeine	2.11		
P- OH- benzoic	6.86		
Caffeic acid	0.87		
Vanillic acid	2.35		
P- Coumaric	1.55		
Ferulic acid	1.23		
Ellagic acid	9.62		
Benzoic acid	11.29		
Coumarin	1.25		
Salycilic acid	3.44		
Cinnamic acid	0.70		

TABLE 4. Total phenols, flavonoids content and antioxidant activity of cauliflower waste extracts.

Treatments		Total phenols mg gallic acid equivalents/100g	Total Flavonoids mg catechin equivalent/100g	DPPH %
Aqueous	**0.1%**	$12.75^{g} \pm 0.90$	$0.013^{d} \pm 0.002$	$39.98^{e} \pm 1.14$
	0.3%	$30.33^{e} \pm 1.44$	$0.016^{cd} \pm 0.001$	$55.90^{d} \pm 2.90$
	0.5%	$63.75^{c} \pm 0.54$	$0.024^{c} \pm 0.003$	$66.95^{b} \pm 1.15$
Absolute ethanol	**0.1%**	$19.21^{f} \pm 0.90$	$0.021^{cd} \pm 0.002$	$54.35^{d} \pm 1.38$
	0.3%	$43.88^{d} \pm 2.83$	$0.038^{b} \pm 0.002$	$61.42^{c} \pm 1.62$
	0.5%	$67.96^{b} \pm 2.06$	$0.058^{a} \pm 0.004$	$71.63^{a} \pm 1.15$
Water: Ethanol (1:1)	**0.1%**	$21.08^{f} \pm 2.02$	$0.026^{c} \pm 0.011$	$63.16^{c} \pm 0.93$
	0.3%	$45.21^{d} \pm 0.89$	$0.044^{b} \pm 0.004$	$67.65^{b} \pm 4.00$
	0.5%	$73.67^{a} \pm 0.85$	$0.064^{a} \pm 0.003$	$72.29^{a} \pm 0.54$
L.S.D.		**2.6555**	**0.0083**	**3.3014**

Antibacterial and antifungal activity of cauliflower waste powderaqueous, absolute and 50 % ethanol extracts

Antibacterial activity of aqueous, absolute, and water:ethanol (1:1) extracts of cauliflower waste powder was measured against six selected-foodborne pathogenic bacteria, *E. coli*, *Salmonella Sp*., and *p. aeruginosa* as Gram-negative as well as, *S. aureus*, *B. cereus*, and *L. monocytogen* as Gram-positive bacteria as well as, antifungal activity of the same extracts against *A. flavus* and *C. albicans* and the results are showed in Table (5).

The data showed that water:ethanol (1:1) extract from cauliflower waste powder showed more potent antibacterial agents against Gram-negative bacteria among the three different extracts, which reported the highest inhibition zones against *P aeruginosa*, *E. coli*, and *S.typhimurium* at 0.5% percent (76, 66.6, and 55.1 mm, respectively), followed at the same concentration by absolute ethanol extract against absolute ethanol extract at the same concentration against *P aeruginosa*, *Salmonella*sp, and*E. coli*, and Compared to Gram-positive bacteria, coli (55.5, 51, and 49.5 mm, respectively) recorded 72.6, 68 and 33 mm, respectively, against *L. monocytogen*, *S. aureus and B.cereus* for water at 0.5%: ethanol (1:1) followed by absolute ethanol extract (49, 28 and 23 mm, respectively) against *S. aureus*, *L. monocytogen,* and *B. cereus*, respectively. Meanwhile, the aqueous extract had the lowest antimicrobial activity, especially against Gram-positive bacteria, especially at 0.1mg/mL. Khadiga (2016) found that agro-industrial wastes showed antimicrobial activity against some strains of pathogenic microorganisms.

On the other side, water:ethanol (1:1)extract of cauliflower waste powder showed the greatest antifungal activity 23, 31, and 40 mm, respectively at 0.1, 0.3, and 0.5% against *A. flavus*and 30, 49 and 60, respectively against *C. albicans*. as well as, absolute ethanol extract exhibited moderate activity at 0.1%, 0.3%, and 0.5 % against *A. flavus* (3, 9, and 12mm respectively) and (7, 19, and 25mm, respectively) against *C. albicans*. Meanwhile, the aqueous extract exhibited the lowest activity against all fungal strain at 0.5%.

Sensory properties of beef sausage prepared by aqueous, absolute, and water:ethanol(1:1) extracts of cauliflower waste at different ratios

Aqueous, absolute, and water:ethanol (1:1) extracts of cauliflower waste with different ratios of 1000, 3000, and 5000 ppm were applied to the beef sausage samples (0.1, 0.3 and 0.5 percent). Based on its sensory properties, all samples were tested and all samples showed no major variations between all parameters (odor, taste, color, texture, and overall acceptability).

According to the data of the antioxidant activity and the antimicrobial effect of aqueous, absolute ethanol, and water:ethanol (1:1) extractsof cauliflower waste at different ratios, The best acceptable results were for the beef sausage prepared by water:ethanol (1:1) extract of all concentrations (0.1, 0.3 and 0.5 %).

TABLE 5. **Antibacterial and antifungal activity of cauliflower aqueous, absolute ethanol and Water:Ethanol (1:1) extracts using agar well diffusion method.**

	*inhibition zone*of the cauliflower extract diameter (mm)								
Concentration / Pathogenic	Aqueous			Absolute Ethanol			Water:Ethanol (1:1)		
	0.1%	0.3%	0.5%	0.1%	0.3%	0.5%	0.1%	0.3%	0.5%
Gram-negative									
Escherichia coli	-	3.2	9.8	18	36	49.5	9	12.5	66.6
Salmonella typhimurium	-	5	7	-	30	51	-	30	55.1
Pseudomonas aeruginosa	1.5	8	12	25	45	55.5	-	40	76
Gram-positive									
Bacillus cerues	-	-	-	-	10.5	23	7	21	33
Staphylococcus aureus	-	6	9.2		38	49	-	40	68
Listeria monocytogen	-	5.8	9	-	12	28	20	47	72.6
Fungi									
Aspergillus flavus	-	-	4	3	9	12	23	31	40
Candida albicans	-	-	2.5	7	19	25	30	49	60

Changes in chemical quality and physical properties

It could be observed from Table (6) that the total volatile basic nitrogen (TVBN) of samples of all-beef sausage decreased significantly (P ≤ 0.05) due to increased extract ratios. With a significant difference (P ≤ 0.05) during frozen storage, TVBN values increased gradually by increasing the storage time. Increased TVBN values may be due to bacterial degradation during meat storage (Osheba & Abd Elbar, 2007). In all treatments, the TVBN values were within the acceptable level range reported by Egyptian Standards Specification (2005) which indicated the content of the TVBN content should not exceed 20 mg/100 g.

Thiobarbituric acid (TBA) test is an index for the measurement of oxidative rancidity (formation of malonaldehyde) in meat. Furthermore, the TBA-test is a sensitive test for highly unsaturated fatty acid decomposition products that do not appear in peroxide determination (Melton, 1983). From the results represented in the same Table 6, it could be observed that the lowest thiobarbituric acid values (TBA) values with significant difference (P ≤ 0.05) recorded for beef sausage prepared by adding 0.5% of extract during storage period this could be due to the antioxidant effect of phenol compounds in the extract as mentioned above. Meanwhile, the highest increase in TBA values observed for control sample with significant difference (P ≤ 0.05) recorded for control samples reached 0.708 mg malondhyde/kg (after 90 days) but still within the range which is 0.9 mg malonaldehyd/kg according to Egyptian Standards Specification (2005). During frozen storage, TBA values were increased gradually for all-beef sausage samples with significant difference (P ≤ 0.05) were progressively increased during frozen storage period.

To maintain color, holding water, and improving tenderness, the pH of meat is important (Oshibanjo, 2010). The results of the same Table 6 showed that there was a small variation between samples in pH values with no significant difference (P >0.05) between samples except at the end of storage time. Meanwhile, the pH values increased linearly with significant difference (P ≤ 0.05) during storage time in all different sausages sampled. The rise of pH valuesmay be attributed to the production of volatile basic components such as ammonia and total volatile nitrogen by meat spoilage bacteria (Osheba, 2013). In addition, nearly all meat microbiota bacteria catabolize lactic acid and some amino acids accompanied by nucleotides, urea, and water-soluble proteins, resulting in the development of alkaline radicals (ammonia and amines), leading to a rise in pH values (Nychas et al., 2008). In addition, a higher pH may have a beneficial effect on the color of meat and the ability to retain water (Jee et al., 1999). Therefore, the ideal pH for meat is ranged between 5.8 and 6.3 (Pearson & Gillette, 1996). Also, (Kaba, 2006) announced that a positive relationship between the values of TVBN and TBA and the storage duration was found.

TABLE 6. Changes in chemical quality attributes, physiochemical and physical properties of beef sausage prepared by water:ethanol (1:1) extract of cauliflower waste at different ratios during frozen storage.

Parameters	Storage time "days"	Control	0.1%	0.3%	0.5%	L.S.D
TVBN mg N /100g	0	11.67^{Ca}±0.404	10.50^{Cb}±0.000	9.57^{Cc}±0.404	7.00^{Cd}±0.000	**0.5381**
	45	14.00^{Ba}±0.000	12.37^{Bb}±0.404	10.50^{Bc}±0.000	9.80^{Bd}±0.000	**0.3805**
	90	17.73^{Aa}±0.404	14.93^{Ab}±0.808	12.20^{Ac}±0.361	11.43^{Ac}±0.404	**0.9918**
L.S.D		**0.6593**	**1.0424**	**0.6247**	**0.4662**	
TBA mg malondhyde/ kg	0	0.421^{Ca}±0.019	0.333^{Cb}±0.030	0.271^{Cc}±0.275	0.189^{Cd}±0.001	**0.0358**
	45	0.616^{Ba}±0.047	0.416^{Bb}±0.020	0.338^{bC}±0.025	0.250^{Bd}±0.018	**0.0563**
	90	0.708^{Aa}±0.016	0.594^{Ab}±0.012	0.410^{Ac}±0.017	0.348^{Ad}±0.020	**0.0309**
L.S.D		**0.0613**	**0.0441**	**0.0379**	**0.0309**	
pH	0	5.63^{Ca}±0.075	5.62^{Ca}±0.021	5.61^{Ca}±0.021	5.61^{Ca}±0.006	**0.0765**
	45	6.14^{Ba}±0.006	6.09^{Bb}±0.02	6.07^{Bb}±0.026	6.06^{Bb}±0.006	**0.0322**
	90	6.34^{Aa}±0.01	6.27^{Ab}±0.006	6.20^{Ac}±0.023	6.16^{Ad}±0.015	**0.0261**
L.S.D		**0.0881**	**0.0339**	**0.0452**	**0.0199**	
WHC Cm2/0.3g	0	2.70^{Ca}±0.173	2.33^{Cb}±0.058	2.10^{Cbc}±0.173	2.03^{Cc}±0.058	**0.2431**
	45	3.47^{Ba}±0.058	3.33^{Bab}±0.115	3.20^{Bb}±0.173	2.93^{Bc}±0.115	**0.2306**
	90	4.63^{Aa}±0.115	4.17^{Ab}±0.058	3.97^{Ac}±0.153	3.73^{Ad}±0.058	**0.1960**
L.S.D		**0.2492**	**0.1631**	**0.3330**	**0.1631**	
Cooking loss %	0	18.94^{Ca}±0.030	18.94^{Ca}±0.017	18.95^{Ca}±0.006	19.93^{Ca}±0.050	**0.0578**
	45	21.93^{Ba}±0.03	21.92^{Ba}±0.025	21.95^{Ba}±0.044	21.95^{Ba}±0.023	**0.0593**
	90	24.52^{Aa}±0.055	24.54^{Aa}±0.070	24.52^{Aa}±0.040	24.52^{Aa}±0.029	**0.0554**
L.S.D		**0.0802**	**0.0873**	**0.0686**	**0.0770**	

TVBN = Total volatile basic nitrogen,TBA = Thiobarbituric acid ,WHC = water holding capacity
Each value in the table is the mean of three replicates ± standard deviation.
Small letter= Means in the same column with different letters are significantly different at p ≤ 0.05 (comparison between control and other samples).
Capital letter= Means in the same column with different letters are significantly different at p ≤ 0.05 (comparison between

WHC (water holding capacity) is characterized as the ability to bind water to meat and meat products, (Pearce et al., 2011). The variation of water holding capacity were shown in Table (6) and concluded that water holding capacity values sausage samples were increased (p ≤ 0.05) during frozen storage period. The lowest water holding capacity (highest value, 2.70 cm2/0.3g) was recorded for control sample and the highest water holding capacity (lowest value, 2.03 cm2/0.3g) was recorded for sausage sample which treated by 0.5% of water:ethanol (1:1) extract of cauliflower waste at the begging of storage. During storage, the rise in WHC values of frozen sausage samples may be due to protein denaturation and protein solubility loss (El-Kordy, 2006).

Data in Table 6 showed that cooking loss value of sausage samples did not significantly (P >0.05) change between treatments until the endof storage time. Moreover, cooking loss values of sausage samples were increased (p ≤ 0.05) during storage period. This increase was due to a decrease in water binding capacity.

Microbiology of sausage samples at -18°C from zero time to three months (at fresh basis)

Sausage is exposed to high temperatures during the heating process, but this temperature is notenough to inactivate all of the microorganisms presents.Therefore, the sausages with variable concentrations of cauliflower water:ethanol (1:1) extracts were evaluated microbiologically at -18±2 °C for zero, 45, and 90 days and the results are represented in Table 7.

In the present study, the number of total counts increased gradually during the storage period from 9×10^2 to 40×10^8cfu after 90 days. In addition, the total bacterial count increased from 7×10^1 to 15×10^6 cfu at 0.1 and from 5×10^1 0.1 to 0.3 cfu at 0.5. As well as, the yeasts and molds of

beef sausage samples which contained different concentrations of cauliflower water:ethanol (1:1) extracts as well as control has no growth at zero time and during the storage period (45 and 90 days). On the other hand, no viable cells from *psychrophilic, salmonella*, *Staph*, *E. coli*, and coliform could be detected at zero time and during the storage period (45 and 90 days).

Sensory properties of beef sausage prepared by water:ethanol(1;1)extract of cauliflower waste at different ratios during frozen storage

Sensory qualities of any product affect the acceptability of sausages and the customer's choice. Sensory properties of water-prepared beef sausage: ethanol (1:1) extract of cauliflower waste in various proportions during frozen storage are shown in Table 8.

TABLE 7. Microbiology analysis of sausage samples at -18°C from zero time to three months (at fresh basis).

Sausage Samples	Total count	Yeast& mold	Psychrophilic	Salmonella	Staph	E.coli	coliform	Water activity
Zero time								
0.0%	9×10^2	ND	ND	ND	ND	ND	ND	0.894
0.1%	7×10^1	ND	ND	ND	ND	ND	ND	0.860
0.3%	5×10^1	ND	ND	ND	ND	ND	ND	0.835
0.5%	ND	ND	ND	ND	ND	ND	ND	0.820
45 Day								
0.0%	20×10^4	ND	ND	ND	ND	ND	ND	0.934
0.1%	8×10^3	ND	ND	ND	ND	ND	ND	0.880
0.3%	4×10^1	ND	ND	ND	ND	ND	ND	0.889
0.5%	ND	ND	ND	ND	ND	ND	ND	0.896
90 Day								
0.0%	40×10^8	ND	4x10	ND	ND	ND	ND	0.981
0.1%	15×10^6	ND	2x10	ND	ND	ND	ND	0.869
0.3%	8×10^2	ND	ND	ND	ND	ND	ND	0.883
0.5%	ND	ND	ND	ND	ND	ND	ND	0.820

ND: not detect

TABLE 8. Changes in sensory properties of beef sausage prepared by water:ethanol (1:1) extract of cauliflower waste at different ratios during frozen storage.

Parameters	Storage time "days"	Control	0.1%	0.3%	0.5%	L.S.D
Odor	**0**	$8.45^{Aa}\pm0.85$	$8.40^{Aa}\pm0.92$	$8.50^{Aa}\pm1.11$	$8.60^{Aa}\pm1.02$	0.9383
	45	$7.90^{ABa}\pm0.57$	$7.80^{ABa}\pm0.63$	$7.70^{ABa}\pm0.48$	$7.70^{Ba}\pm0.95$	0.6177
	90	$7.50^{Ba}\pm0.53$	$7.50^{Ba}\pm0.53$	$7.50^{Ba}\pm0.71$	$7.50^{Ba}\pm0.53$	0.5237
L.S.D		0.6275	0.6724	0.7281	0.8093	
Taste	**0**	$8.25^{Aa}\pm1.27$	$8.30^{Aa}\pm0.68$	$8.40^{Aa}\pm1.07$	$9.05^{Aa}\pm1.07$	0.9483
	45	$7.50^{ABa}\pm0.97$	$7.50^{Ba}\pm0.53$	$7.50^{Ba}\pm0.53$	$8.00^{Ba}\pm0.67$	0.6306
	90	$7.20^{Ba}\pm0.42$	$7.30^{Ba}\pm.0.48$	$7.40^{Ba}\pm0.52$	$7.40^{Ba}\pm0.70$	0.4898
L.S.D		0.8781	0.5209	0.6907	0.7623	
Color	**0**	$8.40^{Aa}\pm0.84$	$8.75^{Aa}\pm1.18$	$8.80^{Aa}\pm0.92$	$9.15^{Aa}\pm0.88$	0.8558
	45	$7.50^{Ba}\pm0.85$	$7.75^{Ba}\pm1.18$	$8.10^{Ba}\pm0.57$	$8.20^{Ba}\pm0.63$	0.7652
	90	$6.70^{Cb}\pm0.48$	$7.30^{Ca}\pm0.82$	$7.80^{Ca}\pm0.42$	$7.90^{Ca}\pm0.42$	0.5796
L.S.D		0.6839	0.9888	0.6143	0.6958	
Texture	**0**	$8.45^{Aa}\pm1.01$	$8.40^{Aa}\pm1.07$	$8.50^{Aa}\pm0.85$	$8.60^{Aa}\pm1.07$	0.8882
	45	$7.80^{Ba}\pm0.42$	$7.70^{Ba}\pm0.48$	$7.70^{Ba}\pm0.48$	$7.80^{Ba}\pm0.63$	0.4635
	90	$7.00^{Ca}\pm0.47$	$7.00^{Ca}\pm0.47$	$7.00^{Ca}\pm0.00$	$7.10^{Ca}\pm0.32$	0.3346
L.S.D		0.6324	0.6724	0.5179	0.6817	
Overall acceptability	**0**	$8.50^{Aa}\pm0.88$	$8.50^{Aa}\pm0.78$	$8.60^{Aa}\pm1.02$	$8.60^{Aa}\pm0.84$	0.8042
	45	$7.90^{Ba}\pm0.57$	$7.90^{Ba}\pm0.32$	$7.90^{Ba}\pm0.57$	$7.90^{ABa}\pm0.57$	0.4684
	90	$7.10^{Ca}\pm0.32$	$7.10^{Ca}\pm0.32$	$7.10^{Ca}\pm0.32$	$7.20^{Ca}\pm0.91$	0.4851
L.S.D		0.5803	0.4771	0.6416	0.7260	

Each value in the table is the mean of three replicates ± standard deviation
Small letter= Means in the same column with different letters are significantly different at $p \leq 0.05$ (comparison between control and other samples). Capital letter= Means in the same column with different letters are significantly different at $p \leq 0.05$ (comparison between samples and during storage period).

It should be noted that there was not a substantial difference with no significant difference ($P > 0.05$) for all the properties (odor, taste, color, texture and overall acceptability) between samples by various ratios with which this result disagreed (Tomović et al., 2017), noting that different natural antioxidants could also have a negative effect on the color and sensory properties of the meat products. The results of all sensory parameters decreased with a low significant difference ($P \leq 0.05$) by increasing the storage period.

Conclusion

We concluded that cauliflower stalks waste powder was represent a good source of natural antioxidants and antimicrobial they could be considered as useful sources of bioactive compounds, which can be added to some food products to prolong their preservation period instead of using syntheticantioxidants that have harmful effects. The extraction ofvaluable bioactive compounds present in vegetable by-products should be recovered.

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