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Quality Evaluation of Chicken Sausages treated with Betle and Spinach Leaves Extract under Aerobic Packaging Condition Stored at 4±1°C

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ABSTRACT

The goal of this study was to explore antioxidant and antimicrobial effectiveness of ultrasound assist extractedbetel (*Piper betle spp.*) and spinach leaves (*Spinacia oleracea spp.*) extract. Spent hen chicken sausages (SHCS) were prepared as Control (T-1), SHCS with 100 ppm BHT (T-2); positive control, SHCS with 1.5% ultrasonic assisted ethanolic betelleaves extract (T-3) and SHCS supplemented with 2% ultrasonic assisted ethanolic spinach leaves extract (T-4). For 25 days under refrigeration (4±1°C), SHCS were aerobically packed and scientifically tested various oxidative andmicrobiological stability characteristics. Thiobarbituric acid reactive substances (T.B.A.R.S), Free Fatty Acids (F.F.A) and Peroxide Value (P.V) are all indications of lipid peroxidation. The pH and water activityof both control and treated SHCS products increased significantly; however, treated products (extracts) had a substantially lower value ($P<0.05$) than control. The microbiological and sensory properties of the extracts incorporated treatments were better preserved throughout the storage period. Because of its rich antioxidant and antimicrobial properties, ultra-sonic extracted betel and spinach leaves have the potential to improve the storage sustainability of aerobically packaged SHCS stored at refrigerator temperature (4±1°C). It was revealed that stored meat products, such as chicken sausages, may be effectively preserved without any substantial microbial/sensory quality degradation.

HIGHLIGHTS

- Ultrasonicassisted betel (*Piper betle spp.*) and spinach leaves (*Spinaciaoleracea spp.*) extract having rich bioactive components, antioxidants propertiesand effective improving functionality of the meat products.
- Functional meat or processed food industries can assuredly use betel leaves (*Piper betle spp.*) as natural antioxidants for the development of functional meat products, instead of synthetic antioxidant due to their toxic and hazardous effects.

Keywords: Storage, Chicken sausages, Aerobic packaging, Betel leaves, Spinach leaves

The rapid industrialization and urbanization resulted in rapid increase in nuclear families and more working women, thus having less time to prepare food at home. It is a driven factor for drastic inclination towards convenient and fast functional meat products (Floros *et al*., 2010). Oxidation of meat lipids and protein is considered as very important factor in spoilage of these products. Synthetic antioxidants such as BHT etc. are mostly used in the meat industry to inhibit both, the process of lipid oxidation and microbial growth, but in recent trend is to decrease their use because of the growing concern and hazardous effects on consumer's health due to chemical additives (Wagh *et al*., 2015). Hence, alternate search for natural additives, especially of plant origin, has notably increased. Scientific Epidemiological studies have also allocated that frequently utilization of natural antioxidants is affiliated with a lower risk of cardiovascular diseases and cancers. Various phyto-extracts could be a potential alternative to these synthetic

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antioxidants due to their strong antioxidants potential. The use of extract of various plant components is preferred over raw/powder form due to getting desired effect at very low concentration as well as not modifying organoleptic properties of food products (Jagtap*et al*., 2019).

The antioxidant efficacy of plant extracts depends of processing parameters such as extraction media, method of extraction, time, temperature etc, by increasing the amount/ concentration of ingredients in active stage in the extract (Wagh *et al*., 2015). The ultrasound assisted extraction is a novel method for extraction of various bioactive compounds from plant biomass. Under this method, the biomass is exposed to high intensity ultrasonic waves creating tiny cavitations/bubbles around the cells. The sudden collapse of these bubbles release shockwaves that disrupt the cell wall releasing intracellular contents. This technology is one of the most simple, inexpensive extraction systems and can be operated rapidly in a broad range of solvents for large-scale preparations suited for industrial purposes with suitable modifications (Ali *et al*., 2018).

Piper betle commonly known as Pan, belong to Piperaceae, is widely cultivated in India, Indonesia, Phillipine and East Africa (Guzman*et al*., 2021). Its leaves are rich source of large number of bioactive molecules like lutein, zeaxanthin, steroids, saponins and tanninsthan other parts of the plant(Salehi*et al*., 2019). *Spinaciaoleracea* Linn (Family- Chenopodiaceae) commonly known as "Spinach", is a native to South-West Asia and cultivated through the world as vegetable. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic effects like laxative, diuretic, carminative and cooling. It is a rich source of Vitamins A, C, E, K, B6, B12 and minerals like magnesium, manganese, folate, betaine etc. (Wagh*et al*., 2015). *Spinaciaoleracea*(Spinach) is also well known to be packed with number of anti-oxidants like polyphenols, flavonoids and carotenoids, which have shown to possess anti-inflammatory and anti-neoplastic effect (Ali *et al*., 2018). The published study is lacking on utilization of betel and spinach leaves extract in meat products as antioxidant.

Thus, present research study was undertaken to assess betel and spinach leaves extracts gained by ultrasound assisted extraction method, as natural antioxidant in spent hen chicken sausages stored at refrigeration temperature under aerobic packaging condition.

MATERIALS AND METHODS

Ultrasound-assisted extraction of *Piper betle* and *Spinacia oleracea* leaves

Fresh *Piper betle*and *Spinaciaoleracea*leaves were purchased from a local market (Ludhiana, India). These leaves were properly cleaned to remove impurities, dirt, then dried overnight at 40°C in an industrial dryer (MAC industrial drying oven, New Delhi, India), before being finely pulverised in a mechanical blender. The homogenized fine powder was kept at room temperature in a sterile, sealed amber-colored container.

In 50 mL glass bottles, one gram of powdery leaves was combined with 30 mL of 90% ethanol solution. To observe the influence of extraction temperature, the mixtures were heated to 50°C, 60°C and 70°C for each run in an ultrasonic bath system (Nayar Scientific, Ultra-52, New Delhi, India). Extraction was place at a frequency of 37-50 kHz and a constant power of 400 W. To keep the ultrasonic bath system at a constant temperature of 2°C, recycled distilled water was introduced at regular intervals.After that, the samples were put through a vacuum rotary evaporator with condition of 100 RPM for 20 min (Yamata Rotary evaporator, RE-300, Tokyo, Japan). All of the preceding extractions were done in triplicate, and the crude extracts were kept separately at 4°C till it's needed.

SOURCE OF MATERIALS

Spent hen chicken (Birds) was procured from College Poultry Farm, Department of (LPM) Livestock Production Management, GADVASU, Ludhiana. The animals were slaughtered according to standard protocol at the Depart. of (LPT) Livestock Product Technology, (COVS) College of Veterinary Science, (GADVASU) Guru Angad Dev and Veterinary Animal Sciences University, Ludhiana, Punjab, with animal welfare and ethical considerations taken into mind. The dressed Flesh (meat) was takenfrom the laboratory and deboned it manually by skilled persons. After the skin, exterior fascia, fat and all separable connective tissues were removed, boneless meat was recovered. The boneless meat and fat were separately packed in 1 kg unit packs in low density polyethylene (LDPE) bags and stored at -18±1ºC until needed. The required amount of frozen spent hen chicken meat packs were taken out of the freezer and thawed overnight in refrigeration (4±2ºC) before being used within three months.

Preparation of spent hen chicken sausages

In a meat mincer, partially thawed spent hen chicken was chopped into small cubes and double crushed it by using a 4 mm plate (Mado Eskimo Mew-714, Mado, Germany). The meat emulsion was made with a bowl chopper (Seydelmann K20, Ras, Germany). After that adding a pre-weighed amount of minced chicken, salt, sodium tripolyphosphate and sodium nitrite. The mixture was chopped for 2-3 minutes. It was chopped for another 2 minutes after adding the ice flakes. The refined vegetable oil was gradually incorporated into the batter while cutting until it was evenly distributed. Condiment paste, dry spice mix, refined wheat flour and other substances were also used in meat product formulations. Chopping was continued until all of the ingredients were uniformly distributed and the emulsion attained the desired consistency. Around 2 kg of spent hen chicken meat emulsion was put into stainless-steel sausage filler. These were baked for 30 minutes at 115°C in a hot air oven. A sharp knife was used to cut uniform flesh slices for the spent hen chicken sausages. These were wrapped in plastic and stored in the refrigerator.

pH and Water activity (a_w)

The pH of spent hens chickensausages (n=6) was resolutewith a alphanumeric pH metre (SAB,INDIA) with a combination glass rod (Trout *et al*., 1992). In homogenizer, ten grammes of sample were homogenised for one minute with 50 ml distilled water (T-25D S22 digital ultra-TURRAX Germany). A combination glass electrode was dipped into the suspension to record the pH.

A hand-held portable digital water activity metre (RotronicHygro Palm A_w1 Set/40) was used to evaluate the water activity of the emulsion as well as the product. Flesh (Spent henchicken sausages) was loaded to the brim (80%) in a moisture-free sample cup, and water activity was measured according to the manufacturer's instructions. Duplicate readings were taken.

Determination of antioxidant parameters

Lipid oxidation indices such as TBARS (Folch *et al*., 1957), FFA and Peroxide Value (Koniecko *et al*., 1979) of chicken sausages were utilised to study the antioxidant activity of watermelon rind extract. Using the method of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, the ability of added antioxidants to scavenge 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radicals in samples was assessed (Kato *et al*., 1998).

Microbiological analysis or evaluation for storage products

The samples were counted by using the methods recommended by the American Public Health Association (APHA, 1984) for Standard Plate Count, Yeast and Mould Count, and Coliforms Count.

Sensory evaluation

A panel of 14 experienced members, comprising of scientists and postdoctoral scholars from the department, evaluated Spent hen chicken sausages multiple sensory aspects including appearance and colour, texture, flavour, juiciness, and overall acceptability by using a 8-point descriptive scale (Keeton, 1983). Samples were warmed (40-45°C) in a microwave oven (LG Electronics India Pvt.Ltd., Mumbai) for 1-2 minutes before being served to panellists in the departmental sensory laboratory. A control sample was used twice in the evaluations, randomised among the other samples, to test the reliability.

Statistical analysis

The IBM Corporation's SPSS-20.0 software packages were used to analyse the data acquired throughout the investigations. Duplicate samples were drawn for each parameter and the complete experiment was repeated thrice (n=6). To compare mean values between storage periods, a two-way analysis of variance was utilised (ANOVA). The statistical significance at the 5% level ($P<0.05$) was determined using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

pH

As astoring period extended, the pH value in all samples significantly declined until a 25th days of storage (Table 1). This possibly will be owing to the productfermentable

Table 1: Effects of betel (1.5%) and spinach (2%) extracts, as well as BHT (100 ppm), on the physicochemical and oxidative properties of chicken meat sausages during refrigerated storage (4±1°C) in aerobic packing

Treat/Days	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25
			pH			
T-1	5.65±0.16^{Fa}	5.50±0.16^{Ea}	5.21±0.10^{Da}	5.17±0.09^{Ca}	5.09±0.08^{Ba}	5.01±0.04^{Aa}
T-2	5.74±0.02^{Fc}	5.68±0.09^{Ec}	5.59±0.09^{Dc}	5.48±0.08^{Cb}	5.27±0.02^{Bb}	5.12±0.02^{Ab}
T-3	5.66±0.04^{Fb}	5.61±0.09^{Eb}	5.58±0.09^{Db}	5.53±0.08^{Cc}	5.33±0.02^{Bc}	5.17±0.04^{Ac}
T-4	5.88±0.07^{Fd}	5.75±0.09^{Ed}	5.68±0.08^{Dd}	5.55±0.08^{Cd}	5.29±0.02^{Bd}	5.20±0.07^{Ad}
			Water Activity (A_w)			
T-1	0.973±0.012^{Ba}	0.964±0.011^{Ba}	0.852±0.005^{Aa}	0.895±0.007^{ABa}	0.843±0.001^{Aa}	0.811±0.009^{Aab}
T-2	0.988±0.003^{Fc}	0.976±0.006^{Eb}	0.969±0.006^{Da}	0.921±0.003^{Cc}	0.881±0.009^{Bc}	0.872±0.006^{Ab}
T-3	0.991±0.027^{Fd}	0.982±0.001^{Ec}	0.973±0.001^{Da}	0.937±0.007^{Cd}	0.909±0.002^{Bd}	0.893±0.001^{Ab}
T-4	0.982±0.006^{Cb}	0.976±0.006^{Cb}	0.961±0.006^{BCa}	0.914±0.003^{BCb}	0.867±0.003^{Bb}	0.718±0.003^{Aa}

n=6**, T-1**: Without extracts, **T-2:** BHT (100 ppm), **T-3**: betel leave extract (1.5%), **T-4**: spinach leave extract (2%)*Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly (*p*<0.05).

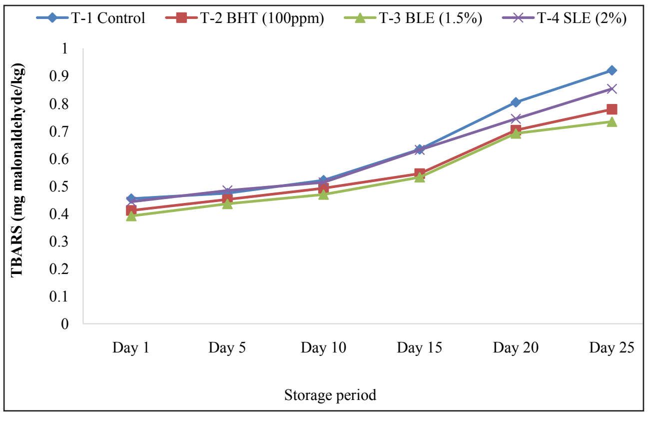
carbohydrates, which trigger the production of lactic acid. After incorporating papaya leavesextracts into chevon nuggets (Jagtap *et al*., 2019) discovered a comparable reduction in pH, whereas (Wagh *et al*., 2015) discovered a similar decrease trendin pH in pork frankfurters after incorporating different phyto-extracts. The drop in pH could be attributable to increase Psychrophilic and lactobacillus counts during storage, which produce lactic acid through carbohydrate breakdown, according to (Nisar *et al*., 2019). Compounds such as vitexin 4′-O-galactoside and isovitexin were detected as important components in betel leaves ethanolic extractdechlorophyllized utilising the sedimentation procedure, according to (Tagrida and Benjakul, 2020). Elevation of pH is also caused by these chemical substances.

Water activity (A_w)

Water activity reduced significantly (P<0.05) as the storage period proceeded due to the adding *Piper betle* (Betel) leaves and *Spinacia oleracia* (Spinach) leaves. Water activity of spent hen chickensausages diminished dramatically as storage time increased (P<0.05). Until, tenth day of chilled storage, the water activity of sausages did not differ substantially between treatments, after which it diminished significantly (P<0.05). T-3 and T-2 had higher water activity at the end of the storage period than T-1 and T-4, which might be due to added phyto-extracts and synthetic antioxidant, BHT, providing oxidative stability (Carvalho *et al*., 2019). Scientist (Gupta and BD Sharma, 2016) observed, the considerable increase in the psychrophilic counts throughout consecutive storage periods could be attributed to the favourable physical environment for feeding or consumption for development & growth of bacteria.

TBARS value

From day 1 to day 25th, TBARS measurements (values) were increased considerably (P>0.05) for all products (Graph 1).

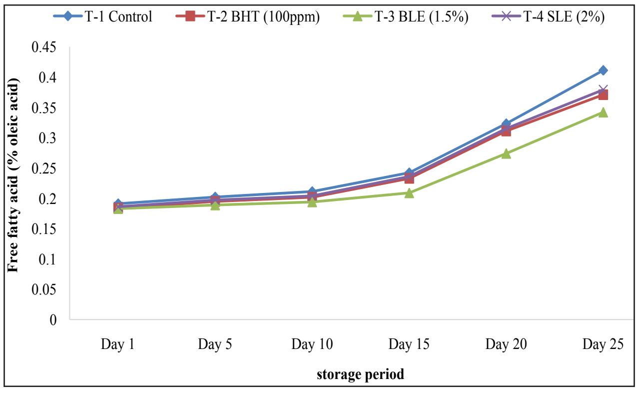


Graph 1: Effect of betel (1.5%) and Spinach (2%) extract and BHT (100 ppm) on TBARS (mg malonaldehyde/Kg) of Spent hen chicken sausagesduring refrigerated storage (4±1°C) under aerobic packagingconditions

This might be due to increased fat oxidation and a production of volatile metabolites in the presence of oxygen. Goat meat nuggetsduring storage studies (Jagtap *et al*., 2019) reported an increase in TBARS value. Researcher (Sharma *et al*., 2017) was observed similar findings in fresh chicken sausages. The mean TBARS values were below the threshold value of 1-2 mg malonaldehyde/kg meat throughout the storing period (Folch *et al*., 1957). The antioxidant effects of the combined phyto-extract ofbeteland spinach leaves may have contributed to the decreased in TBARS value of treated products related to both control and aerobic packaging group of chicken meat sausages. T-1 samples without antioxidants had the highest TBARS values throughout the storage period (Graph 1). All overin storage period, T-3 samples (betel leaves extract) showed the lowest TBARS value. It's possible that this was due to the extract's higher antioxidant potency and present active ingredients like Hydroxychavicol which defend & decline value of malonaldehydein meat (Kavitha *et al*., 2019).

Free fatty acids (FFA)

Free fatty acid levels followed a similar pattern as TBARS readings, with a positive correlation with fat oxidation (Graph 2). Throughout the storage period in aerobic packing, FFA values in T-1 were considerably higher (P>0.05) than in all other treatments (Graph 2).

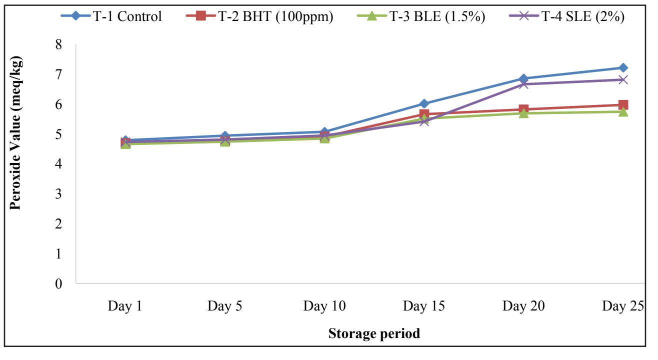


Graph 2: Effect of betel (1.5%) and Spinach (2%) extract and BHT (100 ppm) on Free fatty acid of Spent hen chicken sausagesduring refrigerated storage (4±1°C) under aerobic packagingconditions

This could be because these spent hen chicken sausages lack plant extracts (UAE betel leaves and spinach leaves), as well as synthetic antioxidants, resulting in a lower antioxidant potential (BHT). The T-3 exhibited a significantly lower (P<0.05) FFA value than the other treatments throughout the storing period, which might be owing to the strong antioxidant activity of betel leaves extract due to the presence of phenolic metabolites. This could be attributed to the extract's strong antioxidant efficacy and an active ingredient like Hydroxychavicol, which prevent and directly affects on fat oxidation and FFA values meat product (Kavitha *et al*., 2019).

Peroxide Value

The peroxide value of both the controls and treatment products improved in a similar way to the TBARS and FFA value, however it was considerably lower (P<0.05) in T-2, T-3, and T-4 samples through the storage period, but of T-2 were exception (Graph 3).



Graph 3: Effect of betel (1.5%) and Spinach (2%) extract and BHT (100 ppm) on Peroxide Value *(mEq/Kg)* of Spent hen chicken sausagesduring refrigerated storage (4±1°C) under aerobic packagingconditions

It possibly because polyphenolic contents of UAE phyto-extract *Piper betle* and *Spinacia oleracia* and their potent Safrol, Hydroxychavicol compounds terminating free radical chain-type processes, reduce lipid peroxidation. Despite the fact that the peroxide value (PV) increased during storage, it was lowest in T-3 on the 25th day. The synthesis of hydro peroxides during storage, rather than their breakdown into secondary oxidation products, could explain the increase in peroxide value with time (Graph 3). Researcher (Jagtap *et al*., 2019) investigated the influence of papayaleaves extract on protein and fat oxidation in goat meat nugget and found similar results. Meat scientist (Wagh and Chatli, 2017), noticed a comparable rise in PV during aerobic storage when researching sea buckthorn seed extract as an antioxidant in meat foodstuffs.

Microbiological quality

The critical observation of Table 2 showed that the microbiological quality of chicken sausages containing *Piper betle* leaves extract (PBL), *Spinacia oleracea* leaves extract (SOL), and BHT (100ppm) differed significantly ($p<0.05$) across the treatments. That was substantially ($p<0.05$) lower in T-3 than in T-1 and T-4, which could be attributed to the PBL extracts' stronger antioxidant and antibacterial efficacy. Polyphenols are organic chemicals derived from the *Piper betle* plant that have a variety of chemical configurations. *Piper betle* contains a number of sub-compounds, such as flavonoids (Safrol and hydroxychevicol), that have biological properties such as antioxidant and antibacterial activity (Salehi *et al*., 2019).

At every subsequent storage interval, the mean values of the standard plate count (SPC) for T-1 and treatment products grew significantly ($P<0.05$). T-1 and T-4 had significantly ($p<0.05$) increased value of standard Plate Counts. In the current investigations, the reduced microbial count in phyto-extract treated chicken sausages compared to control could be attributed to the products being cooked using dry heat (internal core temperature of products throughout cooking 72±1ºC), which killed significant microflora (Kumar *et al*., 2015).

Absence of hazard causing Coliforms in the products can be attributed due to hygienic processing conditions, cooking time and temperature combinations used antibacterial and antifungal activity of various phyto-extracts used, and packaging material and methods used. This could be owing to the presence of Kaempferol derivatives, hydrocinnamic acid, citric acid, and epigallocatechin within *Piper betle*. These chemical substances have a wide range of bioactivities, including antimicrobial, antifungal activity (Babaei *et al*., 2020). Those data are compatible with (Wagh and Chatli, 2015), respectively. T-2, T-3, and T-4 treated samples showed no yeast or mould counts until the 15th day of storage, but T-1 revealed yeast and mould counts from the 15th day of storage onwards. T-3 chicken sausages had the lowest counts at the end of storage, followed by T-2, T-4, and T-1 chicken sausages.

Table 2: Effect of Piper betle (1.5%) and *Spinacia oleracea* (2.0%) extract on microbiological characteristics of chicken meat sausages during refrigerated storage (4±1°C) under aerobic packaging conditions

Treat/ Days	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25
Standard Plate count (log_{10}cfu/g)						
T-1	1.05 ± 0.07^{Aa}	1.52 ± 0.08^{Bb}	2.71 ± 0.09^{Cc}	3.97 ± 0.11^{Dd}	6.63 ± 0.19^{Ed}	6.98 ± 0.19^{Fd}
T-2	1.01 ± 0.03^{Aa}	1.23 ± 0.08^{ABa}	2.16 ± 0.12^{Ba}	3.42 ± 0.15^{Cb}	4.61 ± 0.11^{Db}	4.82 ± 0.19^{Db}
T-3	1.04 ± 0.05^{Aa}	1.15 ± 0.05^{ABa}	2.24 ± 0.12^{Ba}	3.17 ± 0.15^{Ca}	4.28 ± 0.16^{Da}	4.31 ± 0.19^{Da}
T-4	1.03 ± 0.07^{Aa}	1.34 ± 0.08^{ABb}	2.65 ± 0.09^{Bb}	3.87 ± 0.11^{Cc}	5.39 ± 0.19^{Dc}	6.08 ± 0.19^{Ec}
Coliforms count (log_{10}cfu/g)						
T-1	ND	ND	ND	ND	ND	ND
T-2	ND	ND	ND	ND	ND	ND
T-3	ND	ND	ND	ND	ND	ND
T-4	ND	ND	ND	ND	ND	ND
Yeast and Mould Count (log_{10}cfu/g)						
T-1	ND	ND	ND	1.01 ± 0.02^{A}	1.84 ± 0.02^{A}	2.47 ± 0.02^{B}
T-2	ND	ND	ND	ND	1.05 ± 0.04^{A}	2.01 ± 0.04^{B}
T-3	ND	ND	ND	ND	ND	1.72 ± 0.05^{A}
T-4	ND	ND	ND	ND	1.42 ± 0.05^{A}	2.21 ± 0.02^{B}

n=6, T-1: Control samples without extracts, T-2: BHT (100 ppm), T-3: *Piper betle* leave extract (1.5%) and T-4:*Spinacia oleracea*leave extract (2%) *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly ($p<0.05$). ND: Not detected.

Sensory quality

Overall scores for all SHCSproducts specimenevaluated revealed a gradual significant (P< 0.05) loss as storage time extend, but the rate of decline in T-1 and T-2 score were lower (Table 3). Pigments and fat oxidation may be to blame for the drop in appearance scores. On the 25th day of storage, the treatment product (T-3) had the best appearance score. In terms of appearance, the flavour scores followed the same diminishing trend as the control and treatment product scores. A rise in TBARS values and FFA in meat food products might be connected to a progressive drop in flavour ratings under aerobic settings. In 25th days of storage, T-3 had the highest flavour scores, followed by T-4, which had a higher value than T-1. This could be due to product lower amounts of TBARS, FFA and Peroxide Value (Kumar *et al*., 2015).

Through-outstoring period, juiciness and texture scores declined regardless of treatment; however, the rate of reduction in T-1 product was higher (P>0.05) than the other antioxidant-infused foodstuffs. The progressive decline juiciness and texture scoring could be linked to a loss of surface moisture and subsequent pH change, which causes protein denaturation and disintegration of muscle fibre protein as a result of bacterial assault, resulting in poorer water retention. T-2, T-3 and T-4 had considerably higher overall acceptance values than control (P>0.05). As the storage time extended, it did, follow a diminishing pattern in both groups. As on 25th days of storage, T-3 score was significantly increase (P>0.05) than T-4. The overall acceptability of the control and all treatment commodities had decrease considerably by the 25th day of storage (P<0.05). Other sensory factors, such as appearance,

Table 3: Effect of betel (1.5%) and spinach (2%) extract and BHT (100 ppm) on sensory quality attributes of Spent hen chicken sausages during refrigerated storage (4±1°C) under aerobic packaging conditions

Treat/Days	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25
			Colour and Appearance			
T-1	$6.80±0.16^{Fa}$	$6.50±0.16^{Ea}$	$6.11±0.10^{Da}$	$5.67±0.09^{Ca}$	$5.40±0.08^{Ba}$	$4.80±0.04^{Aa}$
T-2	$7.30±0.05^{Fc}$	$7.14±0.06^{Ec}$	$6.66±0.16^{Dc}$	$6.09±0.06^{Cc}$	$5.74±0.16^{Bc}$	$5.35±0.05^{Ab}$
T-3	$7.51±0.18^{Fd}$	$7.31±0.16^{Ed}$	$6.80±0.06^{Dd}$	$6.50±0.06^{Cd}$	$5.91±0.09^{Bd}$	$5.50±0.09^{Ac}$
T-4	$7.22±0.09^{Fb}$	$7.03±0.09^{Eb}$	$6.43±0.06^{Db}$	$5.71±0.06^{Cb}$	$5.50±0.06^{Bb}$	$4.80±0.16^{Aa}$
			Flavour			
T-1	$6.88±0.05^{Fc}$	$6.71±0.06^{Ec}$	$6.53±0.06^{Dc}$	$5.88±0.06^{Cb}$	$5.51±0.07^{Bc}$	$4.28±0.09^{Aa}$
T-2	$6.81±0.04^{Fb}$	$6.58±0.18^{Eb}$	$6.24±0.06^{Db}$	$6.01±0.09^{Cc}$	$5.13±0.12^{Bb}$	$5.01±0.10^{Ac}$
T-3	$7.11±0.09^{Fd}$	$7.02±0.06^{Ed}$	$6.90±0.06^{Dd}$	$6.44±0.06^{Cd}$	$6.25±0.08^{Bd}$	$5.84±0.08^{Ad}$
T-4	$6.66±0.18^{Fa}$	$6.44±0.16^{Ea}$	$6.14±0.08^{Da}$	$5.67±0.05^{Ca}$	$5.08±0.06^{Ba}$	$4.88±0.05^{Ab}$
			Texture			
T-1	$6.50±0.06^{Fa}$	$6.34±0.05^{Ea}$	$6.10±0.05^{Da}$	$5.80±0.06^{Ca}$	$5.51±0.06^{Ba}$	$4.92±0.09^{Ac}$
T-2	$7.29±0.05^{Fc}$	$7.07±0.06^{Ec}$	$6.85±0.06^{Dc}$	$6.35±0.11^{Cc}$	$5.90±0.08^{Bb}$	$4.81±0.16^{Ab}$
T-3	$7.38±0.11^{Fd}$	$7.13±0.04^{Ed}$	$6.91±0.09^{Dd}$	$6.67±0.08^{Cd}$	$6.45±0.06^{Bc}$	$5.44±0.08^{Ad}$
T-4	$7.07±0.16^{Fb}$	$7.00±0.05^{Eb}$	$6.45±0.06^{Cb}$	$6.13±0.06^{Bb}$	$6.88±0.08^{Dd}$	$4.12±0.06^{Aa}$
			Juiciness			
T-1	$6.77±0.07^{Fa}$	$6.71±0.06^{Eb}$	$6.31±0.15^{Db}$	$5.89±0.05^{Cb}$	$5.25±0.05^{Bb}$	$4.39±0.06^{Aa}$
T-2	$7.11±0.11^{Fc}$	$6.77±0.09^{Eb}$	$6.51±0.09^{Dc}$	$6.22±0.01^{Cc}$	$5.78±0.05^{Cc}$	$4.93±011^{Ac}$
T-3	$7.23±0.06^{Fd}$	$7.12±0.05^{Ec}$	$6.84±0.09^{Dd}$	$6.56±0.11^{Cd}$	$6.11±0.06^{Bd}$	$5.85±0.18^{Ad}$
T-4	$6.95±0.05^{Fb}$	$6.28±0.09^{Ea}$	$5.85±0.05^{Da}$	$5.32±0.05^{Ca}$	$4.80±0.06^{Ba}$	$4.55±0.09^{Ab}$
			Overall Acceptability			
T-1	$6.73±0.05^{Fa}$	$6.51±0.05^{Ea}$	$6.19±097^{Da}$	$5.81±0.06^{Ca}$	$5.52±0.81^{Ba}$	$4.40±0.66^{Aa}$
T-2	$7.33±0.15^{Fc}$	$7.14±0.07^{Ec}$	$6.93±0.09^{Dc}$	$6.71±0.08^{Cc}$	$6.13±0.05^{Bc}$	$5.81±0.13^{Ac}$
T-3	$7.55±0.09^{Fd}$	$7.39±0.05^{Ed}$	$7.05±0.06^{Dd}$	$6.69±0.05^{Cc}$	$6.28±0.05^{Bd}$	$5.92±0.09^{Ad}$
T-4	$7.22±0.11^{Fb}$	$7.01±0.06^{Eb}$	$6.77±0.06^{Db}$	$6.49±0.07^{Cb}$	$5.88±0.07^{Bb}$	$4.79±0.05^{Ab}$

n=6**, T-1**: Without extracts, **T-2: BHT** (100 ppm), **T-3**: betle leave extract (1.5%), **T-4:** spinach leaves extract (2%) *8-point descriptive scale, whereas 8 = extremely desirable and 1 = extremely undesirable *Mean ± SE. with different superscripts row wise (Capital alphabets) and column wise (small alphabets) differ significantly (*p*<0.05).

colour, flavour, juiciness and texture, could be contributory to the overall fall in acceptability. Furthermore, a steady increase in TBARS, FFA and Peroxide levels explains the deteriorating trend in the meat product's sensory quality evaluationthroughout storage. According meat study scientists, natural antioxidants (Aloe vera and apple peel extract) enriched in minced emulsified meat dramatically increased sensory ratings (Wagh and Chatli, 2017). The sensory qualities of meat dishes are improved by high-temperature cooking, which includes a particular flavour, golden brown colour, appearance and crispy texture (Nayak *et al*., 2016).

CONCLUSION

The physicochemical and sensory properties of an aerobically packaged Ultrasonic assist extractedbetel, spinach leaves extract and BHT supplementedspent hen chickensausage were found to be stable for 25 days in refrigerated storage excluding control samples. Spent henchicken sausages infused with 1.5 percent betel leaves extracts & BHT treated samples showed best results in terms of physicochemical, microbiological and sensory qualities. The effect of phyto-extracts in aerobic packed successfully sustained the color, odor and overall appropriateness of spent hen chickensausages during storage findings, so the research studiesare strongly recommended that functional meat or processed food industriesassuredly use potent betel leaves (*Piper betle spp.*) as natural antioxidantsfor the development of functional meat products.

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