Original Research

Storage Stability of Pork Emulsion Incorporated with Arjuna *(Terminalia arjuna)* Tree Bark Extract

Ritika Birla, Om Prakash Malav*, Rajesh V. Wagh, Nitin Mehta, Manish Kumar Chatli and Pavan Kumar

Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, INDIA

***Corresponding author:** drommalav@gmail.com

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Abstract

Arjuna tree bark extract was incorporated at three different levels separately in pork emulsion after replacing the lean meat in the pre-standardized formulation. The control and treatment pork emulsion was packaged in LDPE pouches and stored aerobically under refrigeration (4±1°C) for 9 days and analyzed at regular interval of 1, 3, 5, 7 and 9 days of storage. Water activity decreased significantly (P<0.05) with the advancement of storage period. Redness (a) value, lightness (L* value) and yellowness (b* values) of control and treatment emulsion, followed a declining trend with the increase in storage time. Oxidative stability parameters followed an increasing (P<0.05) trend throughout the storage period. Microbiological count followed an increasing trend in control and treatment emulsion. Sensory scores showed declining trend with increase in storage time.*

Key words: Antioxidant, Colour Profile, Extract, Storage, Sensory

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Introduction

The pig population of India is 10.29 million as the latest livestock census (19th Livestock Census, 2012) it constitutes about 1% of world pig population. In the year 2015-16, pork production was 0.38 million tonnes which constitutes around 6% of total meat produced in India (BAHS, 2016). Pig meat (pork) is a very tasty and popular meat worldwide. But the pork has high fat content with higher proportion of unsaturated fatty acids, which oxidizes more rapidly than chevon and mutton. Therefore pork is more susceptible to lipid oxidation leading to development of warmed-over flavour (WOF) and deteriorative changes in quality thus leading to decreased quality of meat. Lipid peroxidation, the oxidative deterioration of the polyunsaturated lipids of meat and meat products, leads through formation of hydroperoxides to short-chain aldehydes, ketones and other oxidized compounds, which are responsible for causing flavour, texture, colour and nutritional deterioration of meat and meat products. Several adverse health effects, due to the presence of free reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical, peroxyl radical, during lipid oxidation, have been reported (Nissen *et al.*, 2004). Therefore, to avoid or delay the lipid oxidation processes in meat, meat product manufacturers are using several synthetic food additives in the past few decades such as nitrites, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) etc.

Terminalia arjuna, commonly known as Arjuna, (Family: Combretaceae), contains useful phytoingredients like triterpenoids, β-sitosterol, flavonoids, glycosides etc. Triterpenoids and flavonoids are responsible for its beneficial antioxidant and cardioprotective effect (Dwivedi and Chopra, 2014). Bark of Arjuna has been widely used in Indian system of medicine for a variety of purposes and the bark is particularly effective useful in cardiovascular therapy. Phytochemical screening showed higher concentration, such as phytosterol, lactones, flavonoids, phenolic compounds, tannins and glycosides. The antimicrobial activity of extract showed that greater inhibition zone against gram negative bacteria than gram positive bacteria and the extract showed a promising antioxidant activity (Mandal *et al.*, 2013). Arjuna tree bark contains a very high level of flavonoids compared to other commonly used plant item. Flavonoids detected from its bark are namely arjunolone, flavones, bicalein, quercetin, kempferol and pelorgonidin. The aqueous extract of Arjuna contains 70% polyphenols having a molecular weight greater than 3.5 kDa. Flavonoids are very important because of its antimutagenic and antibacterial property. It is also act as strong anti- proliferative and antioxidant agents due to presence of free radical scavenging action of various phenolic contents (Gupta *et al.*, 2018).

The antioxidant and antimicrobial efficacy of developed Arjuna tree bark extract (ATBE) was valuated in model meat system (*In vivo*) using pork emulsion in relation to physico-chemical qualities, oxidative stability and instrumental colour attributes microbiological and sensory characteristics during refrigeration (4 ±1C) under aerobic packaging conditions.

Materials and Methods

Preparation of Arjuna Tree Bark Extract

Arjuna tree bark was dried and powdered, crushed in mixer and passed through stainless steel sieve. It was powdered into a particle size of 60-80 mesh and successively extracts were prepared, Arjuna tree bark powder was added to a glass thimble, which was placed inside a glass Sohxlet Appratus. This was attached to a condenser and a pre-weighed 500 ml round-bottomed flask (quick-fit, UK) containing 200 ml solvent (distilled water or ethanol) and a few anti-bumping granules. The apparatus was placed on electro mantle, according to Response Surface Methodology (RSM) taken three variants (time, temperature and solvent concentration) were taken. The solvents were distilled off in a rotary vacuum evaporator (RE-300 Yamato

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Rotary Evaporator, Japan) at different time-temp combination on 210 rpm, leaving the extract in the flask. Re-weighing the flask allowed determination of the weight of the extract. This process was carried out using aqueous and ethanol solvents. The extracts were filtered through Whatman's filter paper No. 1 after doing all procedure the collected liquid extract was poured in glass petri plates and kept in incubator for 36-48 hrs for drying. Dry extract from glass petri plates was collected and stored in amber coloured bottles at 4±1°C till further use.

Analytical Methods

pH and Water Activity (a_w)

The pH of emulsion was determined as per the method described by Trout *et al.* (1992) with digital pH meter (FE-20-1-KIT, Mettler-Toledo India Pvt. Ltd., Mumbai) equipped with a combined glass electrode. Ten gram of sample was homogenized with 50 ml of distilled water for 1 min using pestle and mortar. The electrode was dipped into the suspension and the pH value of the sample was recorded. Water activity was determined by using hand held portable digital water activity meter (ROTRONIC HygroPalm AW1, Bassersdorf, Switzerland). Samples were filled up to 80% in moisture free sample cup and water activity was recorded as per specifications.

Peroxide Value (PV) and Free Fatty Acid (FFA)

The peroxide value and free fatty acid were measured as per procedure described by Koniecko (1979).

Thiobarbituric Acid Reactive Substances (TBARS)

The extraction method described by Witte *et al.* (1970) was used with suitable modifications for the determination of TBARS values of cooked pork nuggets. 10g of sample was triturated with pestle and mortar with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) prepared in 2 M orthophosphoric acid solution for 2 min. The content was then transferred quantitatively to a beaker by rinsing with 25 ml of cold distilled water, well mixed and filtered through ashless filter paper (Whatman filter paper No. 1). Then, 3 ml of TCA extract (filtrate) was mixed with equal amount of 2-thiobarbituric acid (TBA) reagent (0.005 M) in test tubes and placed in dark cabinet for 16 hrs. A blank sample was prepared by mixing 3ml of 10% TCA and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 to 533 nm using UV-VIS spectrophotometer (SL-159 Elico India Ltd., Mumbai). TBA value was calculated as mg malonaldehyde per Kg of sample by multiplying O.D. value with K factor of 5.2.

Instrumental Colour Profile

Colour profile was measured using CR-400 Konica Chroma meter (Konica Minolta, Japan) set at 2° of cool white light (d_{65}) and known as '*L*', *a*, and *b* values. '*L*' value denotes (brightness 100) or lightness (0), *a* (+

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redness/- greenness), *b* (+ yellowness/-blueness) values were recorded on/in a hundreds of pork nugget kept in a plate. The instrument was calibrated using light trap (black hole) and white tile provided with the instrument. Then the above colour parameters were selected. The instrument was directly put on the surface of pork nugget at three different points. Mean and standard error for each parameter were calculated. Delta e (total colour difference) can be calculated by using following formula-

$$\Delta e = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

Microbiological Counts

Standard plate counts (SPC), Psychrophilic count, Yeast and Mould counts and Coliforms count in the samples were enumerated following the methods as described by American Public Health Association (APHA 2001) and data were expressed as log10 colony forming units (CFU) per gram sample.

Sensory Evaluation

A seven member experienced panel of judges consisting of teachers and postgraduate students of department of LPT, of College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University evaluated the samples for the sensory attributes viz colour and appearance, flavour, juiciness, texture and overall acceptability using 5-point descriptive scale (Keeton, 1983), where 5=excellent and 1=extremely poor. The test samples were presented to the panelists after assigning the suitable codes. The samples were warmed in a microwave oven for 20 sec before serving to the sensory panelists. The water was served for rinsing the mouth between the samples.

Statistical Analysis

Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran, 1994). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have total number of observations. Sensory evaluation was performed by a panel of seven member judges, Total observations of all parameters were six (n=6) except sensory parameters where n=21. The average values were reported along with standard error. The statistical significance was estimated at 5% level ($P<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

Results and Discussion

Three different levels of selected phyto-extracts of Arjuna *(Terminalia arjuna)* tree bark (ATB) viz: (T-1=0.2%, T-2=0.5%, T-3=1.0%) was incorporated separately in pork emulsion after replacing the lean meat in the pre-standardized formulation. The control and treatment pork emulsion was packaged in LDPE

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pouches and stored aerobically under refrigeration (4±1°C) for 9 days and analysed at regular interval of 1, 3, 5, 7 and 9 days of storage.

The results for physico-chemical properties i.e. (emulsion pH, water activity (a_w), instrumental colour profile, oxidative parameters {(Thiobarbituric acid reactive substances (TBARS)}, free fatty acid contents (FFA) and peroxide value), microbiological quality (SPC, coliforms, psychrophilic, yeast and molds count) and sensory evaluation (5-point descriptive scale), (APPEARANCE, odour and overall acceptability) of pork emulsion incorporated with the three different levels of i.e. 0.20%, 0.50% and 1.0% ethanolic Arjuna tree bark extract (ATBE) are presented in Tables 1 to 5. The control and treatment emulsion was stored aerobically under refrigeration (4±1°C) for 9 days and analyzed at regular interval of 1, 3, 5, 7 and 9 days of storage.

Physico-chemical Properties

The pH of pork emulsion varied significantly (P<0.05) amongst treatments and days of storage with the incorporation of 0.20, 0.50 and 1.0 % Arjuna (*Terminalia arjuna*) tree bark extract (ATBE). In the present study the pH values were comparable in all treated samples on 1st day of storage, thereafter followed an increasing trend during storage, irrespective of the added amount of phyto-extract. The pH was significantly (P<0.05) lower for T-3 and T-2 than T-1 on the (9th) day of storage and significantly (P<0.05) higher in control than all the treatments throughout the storage period. The increase in the pH during storage might be due to the low carbohydrate content, formation of N non-protein compounds and basic ammonium ions coupled with buffering action of protein (Rathour *et al.*, 2017 and Kumar *et al.*, 2015). Similarly, Villalobos-Delgado *et al.*, (2017) reported that an increase in pH is associated with the accumulation of ammonia caused by the degradation of proteins and amino acids. It has been widely recognized that the concept of water activity (a_w) is of great importance in meat preservation, because measured values generally correlated well with the potential for growth and metabolic activity of microorganisms (Chirife and Buera, 1995).

Water activity decreased significantly (P<0.05) with the advancement of storage period (Table 1). The variation in a_W of treatment pork emulsion, might be due to the addition of 0.20, 0.50 and 1.0 % ATBE and microbial growth during storage. Similar findings were reported by Verma *et al.* (2018) in pork emulsion incorporated with blood hydrolysate. The a_W was highest in T-2 and lowest in control on last (9th) day of storage. The values in the present study were in conformity with the earlier findings on ground raw pork by Wagh and Chatli (2017).

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Table 1: Effect of different levels of Arjuna tree bark extract (ATBE) on the physico-chemical parameters of pork emulsion during refrigerated (4±1°C) aerobic storage (Mean±S.E.)*

Treatments/Days	Refrigerated Storage period (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
pH					
C (Control)	5.77±0.06^{A}	5.78±0.06^{AB}	5.85±0.01^{AB}	5.90±0.05^{B}	5.92±0.05^{Aa}
T1 (0.20%)	5.80±0.05^{A}	5.83±0.02^{A}	5.87±0.09^{A}	5.93±0.03^{AB}	6.06±0.06^{Bb}
T2 (0.50%)	5.78±0.05^{A}	5.81±0.06^{A}	5.84±0.06^{A}	5.89±0.04^{AB}	6.02±0.01^{Bb}
T3 (1.0%)	5.78±0.045^{A}	5.81±0.06^{A}	5.84±0.06^{A}	5.89±0.04^{AB}	6.02±0.01^{Bb}
Water Activity (a_W)					
C (Control)	0.92±0.006^{C}	0.90±0.005^{B}	0.89±0.005^{ABa}	0.88±0.007^{Aa}	0.87±0.006^{Aa}
T1 (0.20%)	0.93±0.006^{B}	0.92±0.010^{AB}	0.91±0.006^{Ab}	0.90±0.005^{Ab}	0.90±0.006^{Ab}
T2 (0.50%)	0.93±0.007^{A}	0.92±0.006^{A}	0.92±0.005^{Ab}	0.91±0.005^{Ab}	0.92±0.006^{Ab}
T3 (1.0%)	0.93±0.005^{B}	0.91±0.011^{A}	0.90±0.005^{Aab}	0.90±0.006^{Ab}	0.91±0.006^{Ab}

*n=6; C= Control (without phyto-extract); T-1= 0.2% Arjuna tree bark extract, T-2= 0.50% Arjuna tree bark extract; T-3= 1.0 % Arjuna tree bark extract. *Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).*

Instrumental Colour Profile

The results of instrumental colour profile of pork emulsion incorporated with the three different levels of i.e. 0.20%, 0.50% and 1.0% ethanolic Arjuna tree bark extract (ATBE) are presented in Table 2.

Table 2: Effect of different levels of Arjuna tree bark extract (ATBE) on the instrumental colour profile of pork emulsion during refrigerated (4±1°C) aerobic storage (Mean±S.E.)*

Treatments/ Days	Refrigerated Storage Period (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
L* (Lightness)					
C (Control)	55.76±0.64^{B}	54.16±0.58^{AB}	54.08±0.53^{AB}	53.89±0.58^{AB}	52.78±0.64^{A}
T1 (0.20%)	54.81±0.60^{A}	54.59±0.52^{A}	54.15±0.60^{A}	54.10±0.35^{B}	53.03±0.24^{A}
T2 (0.50%)	54.38±0.18^{B}	54.14±0.19^{AB}	53.99±0.25^{AB}	53.59±0.33^{A}	53.74±0.30^{AB}
T3 (1.0%)	54.55±0.41^{AB}	54.76±0.25^{B}	54.69 ± 0.59^{B}	53.73±0.38^{AB}	53.35±0.32^{A}
a* (Redness)					
C (Control)	12.83±0.56^{C}	12.19±0.50^{C}	11.93±0.53^{BC}	10.34±0.23^{AB}	9.38±0.21^{Aa}
T1 (0.20%)	12.11±0.56^{A}	12.03±0.50^{A}	12.06±0.53^{A}	11.79±0.23^{A}	11.48±0.21^{Ab}
T2 (0.50%)	11.89±0.61^{A}	11.85±0.56^{A}	11.67±0.59^{A}	10.88±0.61^{A}	10.91±0.53^{Ab}
T3 (1.0%)	11.94±0.63^{A}	11.78±0.55^{A}	11.66±0.66^{A}	10.98±0.33^{A}	10.80±0.63^{Ab}
b* (Yellowness)					
C (Control)	15.83±0.59^{C}	15.19±0.53^{C}	14.93±0.56^{BC}	13.34±0.26^{AB}	12.39±0.24^{Aa}
T1 (0.20%)	15.11±0.59^{A}	15.03±0.53^{A}	15.06±0.58^{A}	14.79±0.26^{A}	14.48±0.24^{Ab}
T2 (0.50%)	14.89±0.64^{A}	14.85±0.59^{A}	14.67±0.62^{A}	13.88±0.64^{A}	13.91±0.56^{Ab}
T3 (1.0%)	14.94±0.60^{A}	14.78±0.58^{A}	14.66±0.69^{A}	13.98±0.35^{A}	13.80±0.66^{Ab}

*n=6; C= Control (without phyto-extract); T-1= 0.2% Arjuna tree bark extract, T-2= 0.50% Arjuna tree bark extract; T-3= 1.0% Arjuna tree bark extract. *Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).*

Lightness (*L** value) of control and treatment emulsion, followed a declining trend with the increase in storage time however colour scores decreased significantly (P<0.05) for treatments on the 9th day of the

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storage which is attributed to the fact that salt greatly enhanced the process of meat discoloration due to the pro-oxidative activity as well as its ability to release iron from heme pigments (Devatkal *et al.*, 2014). The rate of decrease in treatments was lower than control, which might be due to the ability of the ATBE to maintain the colour of the product by retarding the oxidation reaction (Kumar *et al.*, 2015). Calvo *et al.* (2008) also reported a decrease in *L** value in tomato peel incorporated beef and beef products. Similar observations were recorded in raw beef hamburgers incorporated with dried tomato peel (Garcia *et al.*, 2009), in pork patties incorporated with tomato powder (Kim *et al.*, 2009), in beef patties containing commercial lycopene preparation (Escalante *et al.*, 2003) and Rathour *et al.* (2017) in chevon rolls incorporated with aloe vera and cinnamon bark extracts stored under aerobic packaging at 4 ± 1ºC.

Redness (*a** value) is an indicator of freshness of the meat and criteria for quality evaluation by the consumers. Redness (*a**) value showed the pattern T-3>T-2>T-1 amongst treatments and followed a decreasing trend in all the treatments throughout the storage. This might be due to gradual oxidation of myoglobin and accumulation of metmyoglobin with storage time (Mancini and Hunt, 2005). However, *a** value was higher in ATBE treated samples than control throughout the storage period and rate of decrease in *a** value was significantly (P<0.05) lower in treated products than control which might be due to antioxidant properties of ABTE. Several authors have studied the colour of meat and meat products and reported that meat oxidation causes a decrease in *a** value (Lavieri and Williams, 2014; Kumar *et al.*, 2015).

Yellowness (*b** values) also followed a decreasing trend for all the treated emulsion as well as control throughout the storage period irrespective of the level of incorporation of ATBE. A decrease in *b** values for meat is an indicative of the development or presence of the metmyoglobin brown pigment (Lin *et al.*, 2015). Realini *et al.* (2015) also reported decrease in *b** values of beef patties containing Acerola fruit extract. Selgas *et al.* (2009) reported same trend for *a** and *b** values in hamburgers containing dried tomato powder. Similar observations were recorded in oxidative stability of pork emulsion containing tomato products and pink guava pulp during refrigerated (4±1ºC) aerobic storage by Joseph *et al.* (2014).

Oxidative Quality Parameters

The oxidative stability of pork emulsion during storage was evaluated on the basis of TBARS, peroxide value and free fatty acid content (Table 3). The deteriorative changes produced from the oxidation of lipids are responsible for colour deterioration, rancid flavour and aroma as well as formation of toxic compounds. TBARS values followed an increasing (P<0.05) trend throughout the storage period irrespective of added level of ATBE in the pork emulsion (Fig. 1). TBARS value was measured lowest in T-3 and highest in T-1 among treatments and similar trend continued throughout the storage. However, TBARS was significantly (P<0.05) higher in control than ATBE treated products throughout the storage period. TBARS values were well below the permissible level of 1.0, indicator of acceptability of meat products (Witte *et al.*, 1970).

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Table 3: Effect of different levels of Arjuna tree bark extract (ATBE) on the oxidative parameters of pork emulsion during refrigerated (4±1°C) aerobic storage (Mean±S.E.)*

Treatments/ Days	Refrigerated Storage Period (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
TBARS Value (mg malonaldehyde/Kg)					
C (Control)	$0.38±0.009^{Ab}$	$0.56±0.005^{Bd}$	$0.85±0.013^{Cc}$	$1.24±0.052^{Dc}$	$1.75±0.013^{Eb}$
T1 (0.20%)	$0.38±0.007^{Ab}$	$0.48±0.007^{Bc}$	$0.65±0.008^{Cb}$	$0.79±0.007^{Db}$	$0.87±0.013^{Ea}$
T2 (0.50%)	$0.35±0.011^{Aa}$	$0.44±0.004^{Bb}$	$0.63±0.006^{Cb}$	$0.73±0.004^{Db}$	$0.86±0.003^{Ea}$
T3 (1.0%)	$0.33±0.006^{Aa}$	$0.41±0.010^{Ba}$	$0.52±0.008^{Ca}$	$0.65±0.010^{Da}$	$0.79±0.017^{Ea}$
Free Fatty Acid (%)					
C (Control)	$0.19±0.008^{Ac}$	$0.29±0.016^{Bc}$	$0.342±0.008^{Cd}$	$0.46±0.004^{Dd}$	$0.48±0.008^{Dc}$
T1 (0.20%)	$0.17±0.003^{Ab}$	$0.23±0.005^{Bb}$	$0.267±0.003^{Cc}$	$0.29±0.004^{Dc}$	$0.30±0.008^{Db}$
T2 (0.50%)	$0.11±0.004^{Aa}$	$0.14±0.003^{Ba}$	$0.189±0.003^{Cb}$	$0.24±0.011^{Db}$	$0.29±0.006^{Eb}$
T3 (1.0%)	$0.10±0.004^{Aa}$	$0.14±0.004^{Ba}$	$0.174±0.003^{Ca}$	$0.19±0.007^{Da}$	$0.21±0.002^{Ea}$
Peroxide Value (meq/Kg)					
C (Control)	$5.93±0.15^{Ab}$	$6.24±0.13^{Bc}$	$6.757±0.059^{Cc}$	$7.59±0.04^{Dd}$	$7.96±0.09^{Ed}$
T1 (0.20%)	$5.49±0.09^{Aa}$	$5.99±0.14^{Bc}$	$6.45±0.065^{Cb}$	$6.88±0.09^{Dc}$	$7.09±0.07^{Dc}$
T2 (0.50%)	$5.29±0.03^{Aa}$	$5.67±0.06^{Bb}$	$6.25±0.067^{Cb}$	$6.64±0.04^{Db}$	$6.73±0.02^{Db}$
T3 (1.0%)	$5.22±0.16^{Aa}$	$5.32±0.03^{Aa}$	$5.45±0.079^{Aa}$	$5.73±0.07^{Ba}$	$5.81±0.04^{Ba}$

*n=6; C= Control (without phyto-extract); T-1= 0.2% Arjuna tree bark extract, T-2= 0.50% Arjuna tree bark extract; T-3= 1.0% Arjuna tree bark extract. *Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).*

The lower TBARS value in treated products can be attributed to oxidative stability provided by ATBE which contain high levels of antioxidant components, such as polyphenol, flavonoid, tannins. The results of the present study were in consonance with the findings reported by Biswas *et al.* (2012) and Verma *et al.* (2018) who incorporated curry (*Murraya koenigii L.*) and mint (*Mentha spicata*) leaf extracts and porcine blood protein hydrolysate in pork emulsion stored under aerobic packaging condition at 4±1ºC respectively.

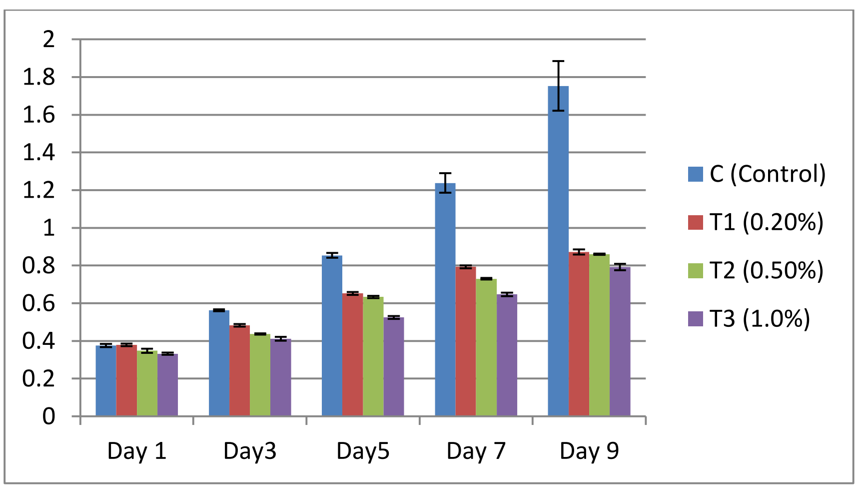

Fig. 1: Effect of different levels of ATBE on TBARS values of pork emulsion during refrigerated storage. (Control: Without any phytoextracts; T-1: 0.2% ATBE; T-2: 0.5% ATBE; T-3: 1.0% ATBE. Bar represents the standard error (n = 6))

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Peroxide value of pork emulsion followed an increasing trend in control and treatments (Fig. 2). The Peroxide value (PV) was significantly (P<0.05) higher in control than all the other treatments throughout storage period of 9 days. It might be due to the formation of hydroperoxides during storage than their degradation into secondary oxidation products and antioxidant properties of added amount of phytoextracts. T-3 measured lowest PV followed by T-2 and T-1. The lower peroxide value in treatment emulsion is an indication of antioxidant potential of ATBE, attributed to the phenolic groups i.e. polyphenols (mainly flavonoids) which provides a labile hydrogen atom by free radicals like alkoxyl or peroxyl radicals and get transformed into phenoxyl radical. Similar observations were recorded in pork patties incorporated with sea buckthorn extract (Wagh *et al.*, 2017) and in pork emulsion containing tomato products and pink guava pulp (Joseph *et al.*, 2014) stored under aerobic packaging condition at 4±1ºC respectively.

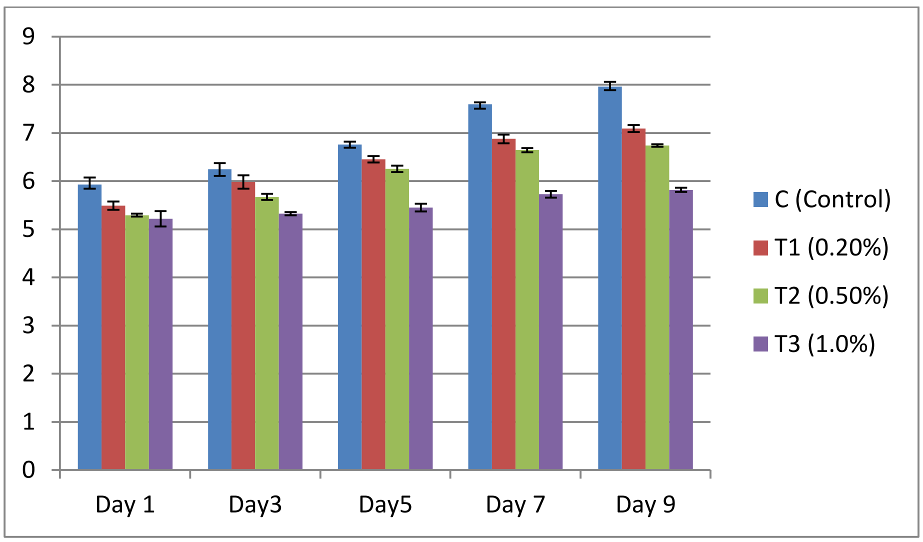

Fig. 2: Effect of different levels of ATBE on peroxide value of pork emulsion during refrigerated storage. (Control: Without any phytoextracts; T-1: 0.2% ATBE; T-2: 0.5% ATBE; T-3: 1.0% ATBE. Bar represents the standard error (n = 6))

Microbiological Quality

The results of microbiological quality of pork emulsion incorporated with the three different levels of i.e. 0.20%, 0.50% and 1.0% ethanolic Arjuna tree bark extract (ATBE) are presented in Table 4. The standard plate count (SPC) of pork emulsion followed an increasing trend in control and treatment emulsion. The standard plate count (SPC) was significantly (P<0.05) higher in control than all the treatments throughout storage period of 9 days. It might be due to the antimicrobial properties of phytoextract incorporated in the treatment emulsion. ATBE contains phenolic compounds, flavonoids, tannins, steroids, alkaloids, and triterpenoids which exhibited a strong antimicrobial effect on fresh pork (Zhang *et al.*, 2009). Similarly, Reddy *et al.* (2017) reported that rosemary and green tea extract in chicken meat sausages and Wagh and

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Chatli (2017). Sea buckthorn extract in raw ground pork, were found to be significantly (P<0.05) effective in decreasing the standard plate count in treatment products.

Table 4: Effect of different levels of Arjuna tree bark extract (ATBE) on the microbiological parameters of pork emulsion during refrigerated (4±1°C) aerobic storage (Mean±S.E.)*

Treatments/ Days	Refrigerated Storage Period (Days)				
	Day 1	Day 3	Day 5	Day 7	Day 9
Standard Plate Count (log_{10} cfu/g)					
C (Control)	$2.56±0.06^{Ad}$	$4.42±0.06^{Bc}$	$5.83±0.05^{Cc}$	$5.20±0.05^{Dc}$	$6.88±0.13^{Ec}$
T1 (0.20%)	$2.04±0.07^{Ac}$	$3.46±0.07^{Bb}$	$4.19±0.07^{Cb}$	$5.55±0.05^{Db}$	$5.80±0.06^{Eb}$
T2 (0.50%)	$1.35±0.04^{Aa}$	$3.51±0.06^{Ba}$	$3.92±0.05^{Ca}$	$4.41±0.07^{Da}$	$4.74±0.06^{Ea}$
T3 (1.0%)	$1.68±0.05^{Ab}$	$3.56±0.06^{Ba}$	$3.93±0.04^{Ca}$	$4.54±0.06^{Da}$	$4.72±0.05^{Ea}$
Coliforms Count (log_{10} cfu/g)					
C (Control)	$1.99±0.009^{Ab}$	$2.05±0.01^{Ac}$	$2.20±0.02^{Bb}$	$2.26±0.03^{Bb}$	$3.02±0.05^{Cc}$
T1 (0.20%)	$1.90±0.02^{Ab}$	$1.98±0.03^{Bb}$	$2.05±0.02^{Ca}$	$2.00±0.02^{BCa}$	$2.33±0.02^{Db}$
T2 (0.50%)	$1.80±0.06^{Aa}$	$1.88±0.03^{ABa}$	$2.00±0.02^{BCa}$	$2.05±0.03^{Ca}$	$2.14±0.07^{Ca}$
T3 (1.0%)	$1.89±0.02^{Ab}$	$1.96±0.01^{ABb}$	$2.04±0.03^{BCa}$	$2.08±0.03^{Ca}$	$2.29±0.02^{Db}$
Psychrophilic Count (log_{10} cfu/g)					
C (Control)	$2.44±0.03^{Ad}$	$2.90±0.02^{Bd}$	$3.24±0.02^{Cd}$	$4.20±0.02^{Dd}$	$4.66±0.10^{Ec}$
T1 (0.20%)	$2.11±0.01^{Ac}$	$2.84±0.03^{Bc}$	$2.93±0.02^{Cc}$	$3.28±0.02^{Dc}$	$3.75±0.02^{Eb}$
T2 (0.50%)	$1.15±0.02^{Aa}$	$1.91±0.03^{Ba}$	$2.29±0.03^{Ca}$	$3.14±0.04^{Da}$	$3.19±0.03^{Ea}$
T3 (1.0%)	$1.31±0.03^{Ab}$	$2.05±0.07^{Bb}$	$2.88±0.04^{Cb}$	$3.25±0.02^{Db}$	$3.33±0.02^{Ea}$
Yeast and Molds Count (log_{10} cfu/g)					
C (Control)	ND	ND	$1.36±0.06^{Bb}$	$2.76±0.05^{Cb}$	$2.93±0.05^{Dc}$
T1 (0.20%)	ND	ND	ND	ND	$1.05±0.06^{Bb}$
T2 (0.50%)	ND	ND	ND	ND	ND
T3 (1.0%)	ND	ND	ND	ND	ND

*n=6; C= Control (without phyto-extract); T-1= 0.2% Arjuna tree bark extract T-2= 0.50% Arjuna tree bark extract; T-3= 1.0% Arjuna tree bark extract. *Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).*

Psychrophilic count (PC) followed similar increasing trend as that of SPC in pork emulsion throughout the storage period. Psychrophilic count was found comparable in T-2 and T-3 on 3rd day of storage and onward throughout storage period of 9 days. These findings are in consonance with Dhanze *et al.* (2013) who found that there was a considerable reduction in the numbers of pathogens on raw chicken meat incorporated with sea buck thorn leave extract during refrigeration storage of 7 days. Coliforms count followed an increasing trend with the progress of storage period. Coliform count were significantly (P<0.05) higher in control than treatment emulsion which might be due to the antimicrobial properties of ATBE. T-2 showed significantly lower (P<0.05) coliforms count throughout storage period of 9 days.

Yeast and mold count were detected on 5th day of storage in control and 9th day in T-1 whereas, in all other treatments it was not detected even on 9th day of storage. Similarly, Verma *et al.* (2018) reported the slower growth rate of yeast and mould counts in pork emulsion incorporated with porcine blood hydrolysate than control which might be due to the anti-fungal properties of the protein hydrolysate.

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Sensory Quality Parameters

The results of sensory quality parameters of pork emulsion incorporated with the three different levels of of *i.e.* 0.20%, 0.50% and 1.0% ethanolic Arjuna tree bark extract (ATBE) are presented in Table 5 and Fig. 3.

Table 5: Effect of different levels of Arjuna tree bark extract (ATBE) on the sensory parameters of pork emulsion during refrigerated aerobic storage (Mean±S.E.)

Treatments/Days	Refrigerated Storage Period (Days)				
	Day 1	**Day 3**	**Day 5**	**Day 7**	**Day 9**
Appearance					
C (Control)	$4.25{\pm}0.06^{C}$	$4.01{\pm}0.05^{BCa}$	$3.74{\pm}0.08^{Ba}$	$3.03{\pm}0.04^{Aa}$	$2.97{\pm}0.07^{Aa}$
T1 (0.20%)	$4.28{\pm}0.11^{B}$	$4.25{\pm}0.06^{Bb}$	$4.19{\pm}0.04^{Bb}$	$3.12{\pm}0.04^{Aa}$	$3.08{\pm}0.07^{Aa}$
T2 (0.50%)	$4.29{\pm}0.06^{C}$	$4.22{\pm}0.05^{Cb}$	$4.18{\pm}0.04^{BCb}$	$4.02{\pm}0.06^{ABb}$	$3.86{\pm}0.06^{Ab}$
T3 (1.0%)	$4.36{\pm}0.06^{C}$	$4.29{\pm}0.05^{Cb}$	$4.25{\pm}0.04^{BCb}$	$4.09{\pm}0.06^{ABb}$	$3.93{\pm}0.056^{Ab}$
Odour					
C (Control)	$4.05{\pm}0.02^{Da}$	$3.81{\pm}0.05^{Ca}$	$3.75{\pm}0.02^{Ca}$	$2.47{\pm}0.03^{Ba}$	$2.06{\pm}0.03^{Aa}$
T1 (0.20%)	$4.11{\pm}0.07^{Da}$	$4.07{\pm}0.04^{Db}$	$3.84{\pm}0.04^{Ca}$	$3.25{\pm}0.05^{Bb}$	$2.98{\pm}0.05^{Ab}$
T2 (0.50%)	$4.73{\pm}0.01^{Cb}$	$4.68{\pm}0.02^{Cc}$	$4.59{\pm}0.02^{Cb}$	$4.21{\pm}0.05^{Bc}$	$3.91{\pm}0.03^{Ac}$
T3 (1.0%)	$4.80{\pm}0.01^{Cb}$	$4.75{\pm}0.02^{Cc}$	$4.66{\pm}0.02^{Cb}$	$4.28{\pm}0.05^{Bc}$	$3.98{\pm}0.03^{Ac}$
Overall Acceptability					
C (Control)	$4.16{\pm}0.05^{Ca}$	$4.05{\pm}\ 0.05^{Ca}$	$3.75{\pm}0.06^{Ba}$	$3.07{\pm}0.05^{Aa}$	$2.95{\pm}0.05^{Aa}$
T1 (0.20%)	$4.235{\pm}0.06^{Ba}$	$4.16{\pm}\ 0.05^{Bab}$	$4.00{\pm}0.09^{Bb}$	$3.46{\pm}0.04^{Ab}$	$3.33{\pm}0.10^{Ab}$
T2 (0.50%)	$4.44{\pm}0.06^{Bb}$	$4.33{\pm}\ 0.06^{Bbc}$	$4.27{\pm}0.08^{Bc}$	$3.98{\pm}0.05^{Ac}$	$3.87{\pm}0.05^{Ac}$
T3 (1.0%)	$4.51{\pm}0.06^{Bb}$	$4.40{\pm}\ 0.06^{Bc}$	$4.34{\pm}0.08^{Bc}$	$4.05{\pm}0.05^{Ac}$	$3.94{\pm}0.05^{Ac}$

*n=21; C= Control (without phyto-extract); T-1= 0.2% Arjuna tree bark extract, T-2= 0.50% Arjuna tree bark extract; T-3= 1.0% Arjuna tree bark extract. *Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).*

Sensory scores for the entire parameters showed declining trend with increase in storage time. The rate of decrease of sensory score was significantly (P<0.05) higher in control than treatment emulsion during storage. T-3 showed highest sensory scores for appearance than other treatments and control emulsion during the storage period. The slower rate of decrease in appearance might be due to the antioxidant properties of incorporated phyto-extract, these results are also supported with the findings of instrumental colour profile. Similar findings were reported by Pogorzelsk *et al.* (2018) during refrigerated storage of raw ground pork meat incorporated with *Haematococcus pluvialis* extract rich in astaxanthin. Joseph *et al.* (2014) also reported that the colour scores decreased (P<0.05) throughout the storage period in control and treatment pork emulsion incorporated with tomato products and guava pulp.

On day one of storage study sensory scores of treatment emulsions were higher than control emulsion. Similarly, Selgas *et al.* (2009) reported increase in visual colour scores with the incorporation of lycopene in raw hamburgers. Colour and appearance are among the most important sensorial properties by which consumers judge the quality of meat and influence them when they decide to purchase (Villalobos-Delgado

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et al., 2017). Odour score followed similar decreasing trend as that of the appearance score during storage. The results revealed significantly higher (P<0.05) odour score of T-3 followed by T-2 and T-1 pork emulsion throughout the storage period. Joseph *et al.* (2014) also reported that odour scores decreased significantly (P<0.05) throughout storage period. Villalobos-Delgado *et al.* (2017) reported that odour was most affected during the storage period, probably because of lipid oxidation and ammonia production from protein breakdown.

The score of overall-acceptability of pork emulsion incorporated with 0.20, 0.50 and 1.0 % ATBE (Fig. 3) was very important for the selection of level of incorporation of phytoextract in the present study. The critical analysis of sensory quality attributes revealed that the sensory panelists awarded highest overall acceptability scores to T-3 that was significantly (P<0.05) higher than T-2 and T-1 and graded it as best among all the treatments and control emulsion. Liang *et al.* (2018) reported that the score of overall-acceptability of dried minced pork slices with mulberry polyphenols was higher than control. The values in the present study were in conformity with the earlier findings on ground raw pork by Wagh and Chatli (2017).

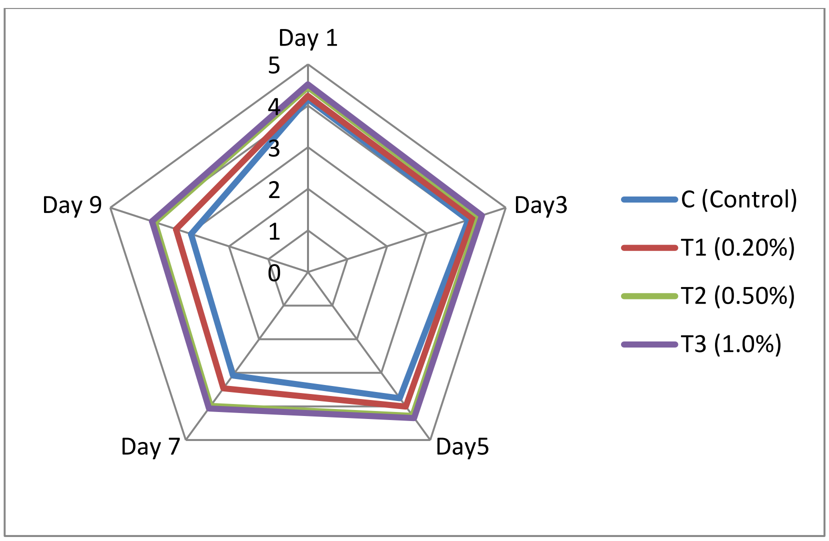

Fig. 3: Effect of different levels of ATBE on over acceptability of pork emulsion during refrigerated storage. (Control: Without any phytoextracts; T-1: 0.2% ATBE; T-2: 0.5%ATBE; T-3: 1.0% ATBE. Bar represents the standard error (n = 21)).

Conclusion

Hence, it was concluded from above results and discussions that the incorporation of 1.0 % level of Arjuna tree bark extract (T-3) in the pork emulsion lead to the improvement in the various physico-chemical qualities, antioxidant potential, microbiological and sensory quality during 9 days refrigerated storage. Development of shelf-stable pork nuggets incorporated with Arjuna tree bark extract at 1.0 % level might

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be novel approach to provide a product which will provide all the appropriate nutrients in a convenient form to the consumers.

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