

Antioxidant Effect of Litchi (*Litchi chinensis* Sonn.) Seed Extract on Raw Ground Chicken Meat Stored at 4 ± 1 °C

K. Yogesh*, Tanbir Ahmad, D.N. Yadav and R.K. Vishwakarma

Central Institute of Post Harvest Engineering and Technology, Ludhiana, Punjab, India, 141 004

Abstract: In this study, total phenolics (TP), trolox equivalent antioxidant capacity (TEAC), reducing power (RP) and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (SA) were estimated in litchi (*Litchi chinensis* Sonn.) seed extracts (LSE). The antioxidant potential of the LSE in raw chicken ground meat (GM) during refrigerated (4 ± 1 °C) storage was also evaluated. In the LSE, TP and TEAC were 7.30 ± 0.06 mg TAE/gdw and 42.26 ± 0.28 mg TE/gdw respectively. The LSE also showed remarkable SA ($56.07 \pm 0.51\%$) and RP. Addition of LSE to GM significantly altered the cooking losses, water holding capacity and pH of GM. During storage (4 °C), the thiobarbituric acid reactive substances (TBARS) values were observed significantly ($P < 0.01$) more (1.32 ± 0.03 mg malonaldehyde/kg) in the control than the treated samples; and the antioxidant efficacy was more in vacuum packaged than aerobically packaged GM samples. Thus, LSE prevents lipid oxidation in meat, and could be used in place of synthetic antioxidants, which have been proved for their negative health implications.

Keywords: Antioxidant activity, DPPH, Litchi chinensis seeds, Meat, Reducing power, TBARS.

INTRODUCTION

Meat and meat products are high-fat food items, which contain high level of mono and polyunsaturated fatty acids (MUFA/PUFA). These are more vulnerable for the oxidative attack during various processing and storage conditions [1]. Oxidative deterioration of the meat products is the major cause for quality deterioration, and ultimate rejection of final products by consumers. Antioxidants are added to prevent this oxidative damage; and in the past, several synthetic antioxidants (BHA/BHT) were used to enhance the oxidative stability of meat and meat products. However, the negative health consequences of synthetic antioxidants have been proven; thus, the meat industry now chooses natural antioxidants for various products to delay oxidative degradation of lipids, maintain quality and nutritional value of meat and meat products [2-5].

In general, fruits, vegetables, plant by-products, spices and herbs are known to contain a wide variety of phytochemicals, such as polyphenols, carotenoids and vitamin C which possess antioxidant activity. Moreover, these sources have been evaluated in different meat products to improve oxidative stability during various processing and storage conditions [6-8].

However, there is no published literatures concerning use of litchi fruit seed, as a source of natural antioxidants, to reduce oxidative damage in the meat and meat products. Therefore, in view of the presence of high content of phenolics and flavonoids in the litchi seeds, which may contribute to antioxidant activity; the

objectives of this study were: (i) to estimate total phenolics (TP), trolox equivalent antioxidant capacity (TEAC), reducing power (RP) and 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging activity (SA), and (ii) to determine the effect of litchi seed powder extract on physicochemical, color and oxidative properties of chicken ground meat during eight days (d) refrigerated storage period.

MATERIALS & METHODS

Materials

Litchi and chicken breast meat (6 wks of age) were obtained from local market. All reagents and solvents were purchased from Merck, Sigma and SD Fine chem Ltd. unless otherwise mentioned. All chemicals used in the experiments were of analytical grade.

Preparation of Litchi Seed Extract

The litchi seeds were dried in hot air oven at 55 °C for 48 h, powder of dried seeds was made in heavy duty kitchen grinder, and sieved through 0.6 mm mesh size sieve. About 10 g of litchi seed powder was mixed with 200 ml boiled sterilized distilled water and left for 2 h with frequent stirring. This was centrifuged at 5000 RPM for 10 min; the supernatant called as litchi seed extract (LSE) was collected in another sterile tube, and stored at 4 °C till further experimentation. This LSE was prepared in triplicate with three measurements at each time.

Treatment of Chicken Meat with LSE

Chicken breast meat was minced through mincer (5 mm plate). One kg minced meat was grinded with 20 g

*Address correspondence to this author at the Central Institute of Post Harvest Engineering and Technology, Ludhiana, Punjab, India, 141 004; Tel: +91-0161-2313125; E-mail: ysomvanshi@gmail.com

NaCl, 50 ml LSE (4 °C) and 50 ml vegetable oil (soybean oil) for 5 min in a kitchen grinder, this ground meat (GM) was filled in low density polyethylene (LDPE) and stored at 4 ± 1 °C for further studies; whereas, in control samples 50 ml LSE was replaced with 50 ml distilled water, keeping other contents same as that of treated samples. Three groups were made as GM + LSE aerobically packaged (AP) at 4 ± 1 °C; GM + LSE vacuum packaged (VP) at 4 °C; and control with no added LSE at 4 ± 1 °C aerobically packaged.

Total Phenolic

Total phenolic (TP) in the extract was determined by the Folin Ciocalteus (FC) assay as reported by Escarpa & Gonzalez [9], with slight modifications. Suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water, followed by the addition of 0.25 ml FC (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance was recorded (UV-1800 PharmaSpec, SHIMADZU, Japan) after 40 min at 725 nm. The results were expressed as milligrams of (tannic acid equivalent) TAE per gram dry weight (gdw) of powder.

Trolox Equivalent Antioxidant Capacity (TEAC)

This method is based on the ability of antioxidant molecules to quench 2,2'-azino-bis(3)-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of trolox, a water-soluble vitamin E analog. The addition of antioxidants to the pre-formed radical cation reduces it to ABTS radical resulting in decolorization. A stable stock solution of ABTS was prepared by the reaction of a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration), and allowing the mixture to stand in the dark at room temperature for 16 h before use [10]. The ABTS working solution was obtained by the dilution of the stock solution in ethanol to an absorbance of 0.70 ± 0.02 AU at 734 nm. Two ml diluted ABTS solution was added to the appropriately diluted LSE. The contents were mixed well and absorbance was read after 2 min. The percent inhibition was calculated against standard trolox standard curve (prepared using 0 – 2.5 mM trolox) and the results were expressed as mg trolox equivalent (TE)/gdw.

DPPH Radical Scavenging Activity

The method of Singh, Murthy and Jayaprakasha [11], with slight modifications, was employed to assess

the ability of LSE to scavenge 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radicals. The 400 µl LSE was diluted with 1600 µl, 0.1 M Tris-HCl buffer (pH 7.4) and this was mixed with 2 ml of DPPH (500 µM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min. The absorbance was measured at 517 nm, and the scavenging activity (SA) was calculated [1].

Reducing Power

The reducing power was quantified by the method described by Jayaprakasha, Singh and Sakariah [12]. The LSE (2.5 ml) was mixed with 2.5 ml phosphate buffer (200 µM, pH 6.6) and incubated with 2.5 ml potassium ferricyanide (1% w/v) at 50 °C for 20 min. At the end of incubation, 2.5 ml of 10% trichloroacetic acid solution was added and centrifuged at 9700 g for 10 min. The supernatant was mixed with 5 ml distilled water and 1 ml ferric chloride (0.1% w/v) solution. The absorbance was measured at 700 nm. Increase in the absorbance of the reaction indicated more reducing power of the sample.

Cooking Loss, WHC and pH of Ground Meat

For cooking loss determination 20 g sample was sealed in a plastic bag and cooked in a water bath at 100 °C for 20 min. Each piece was cooled, removed from the bag and then weighed. The weights of samples were recorded before and after cooking, and the cooking loss was expressed in percentage.

Water holding capacity (WHC) was determined according to Wardlaw, Maccaskill and Acton [13]. Ground meat (20 g) was placed in a centrifuge tube containing 30 ml of NaCl (0.6 M) and was stirred with glass rod for 1 min. The tube was kept at 4 °C for 15 min, stirred again and centrifuged (3000 g) for 25 min at 4 °C. The supernatant was measured and WHC was expressed in percentage.

The pH was determined by blending 5 g sample in 50 ml of double distilled water. The mixture was filtered, and the pH of the filtrate was measured using a digital pH meter (Eutech instruments cyberscan pH tutor, India).

Instrumental Color

The measurement of CIE color values was conducted on the surface of the samples from day zero to day 8, with a miniscan XE plus (Hunter Associated Labs, Inc, Reston, VA, USA) that had been calibrated

against black and white reference tiles ($X=78.6$, $Y=83.4$, $Z=89.0$). An average value from four random locations from duplicate samples was taken.

Thiobarbituric Acid Reactive Substances (TBARS)

The thiobarbituric acid reactive substances (TBARS) were determined from day zero to day 8, by extraction method described by Witte, Krauze and Bailey [14], with slight modifications. Four g sample was homogenized with 20% trichloroacetic acid solution (20 ml) and the slurry was centrifuge at 3000 g (MP 400R Eltek Ltd, India) for 10 min. Two ml of supernatant was mixed with equal volume of freshly prepared 0.1% thiobarbituric acid in a glass test tubes and heated in water bath 100 °C for 30 min, followed by cooling under tap water. The absorbance of the mixture was measured at 532 nm using UV-VIS spectrophotometer (UV-1800 PharmaSpec, SHIMADZU, Japan). The TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg of meat.

Statistical Analysis

All measurements were done in triplicate and obtained results were subjected to analysis of variance (ANOVA). Duncan's multiple range procedure was applied to determine the significant differences among treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Total Phenolic, Trolox Equivalent Antioxidant Capacity (TEAC), DPPH Radical Scavenging Activity and Reducing Power of LSE

Total phenolic in LSE were 7.30 ± 0.06 mg TAE/gdw; the trolox equivalent antioxidant capacity (TEAC) activity was 42.26 ± 0.28 mg TE/g; the DPPH radical SA of LSE was $56.07 \pm 0.51\%$ and the reducing power of extract was 3.27 ± 0.02 for LSE. The high phenolic content in natural antioxidants is responsible for the scavenging of free radicals and chelating of metal ions (prooxidants) [15]. The reduction of the free radicals is an important factor, because free radicals are mainly responsible for initiation as well as propagation reaction during oxidation of lipid and their fatty acids. The more TEAC was also attributed to higher phenolic content in the LSE samples. It is important here to discuss the nature of this phenolic compound, as only aqueous extraction was carried out during preparation of LSE samples; thus, these phenolics are more water soluble (hydrophilic) in

nature. Some of the water-soluble phenolic compounds such as, anthocyanins and proanthocyanidins present abundantly in litchi seed may contributed to the total phenolic content and TEAC of the LSE samples.

Free radical scavenging ability by hydrogen donation is a known mechanism for oxidation inhibition. The data obtained in this study revealed that the LSE samples were free radical scavengers, which reacted with DPPH radical by their electron-donating ability. In a previous study Prasad, Yang and Zhao [16] reported SA of 48.9% and 42% for litchi seed, and BHT, respectively at 100 µg/ml extract concentration. Similarly, higher SA (91.3%) has been reported for anthocyanins present in litchi pericarp at 50 µg/ml [17]. Moreover, higher reducing activity at lower concentration indicates potential antioxidant application at commercial level; and some previous studies have also reported that the higher reducing power was associated with more antioxidant activity [18, 19].

Cooking Loss, WHC and pH of Ground Meat

The Cooking losses, WHC and pH of ground meat are shown in Figure 1. pH, WHC and Cooking losses were differed significantly ($P < 0.05$) between treated and control group. pH was significantly lower in LSE treated ground meat which may also contribute toward more WHC of the final product. WHC of treated ground meat was 19.52 ± 0.32 which was significantly better than control group, likewise cooking losses were higher in control group, which might be due to some emulsifying capacity of LSE.

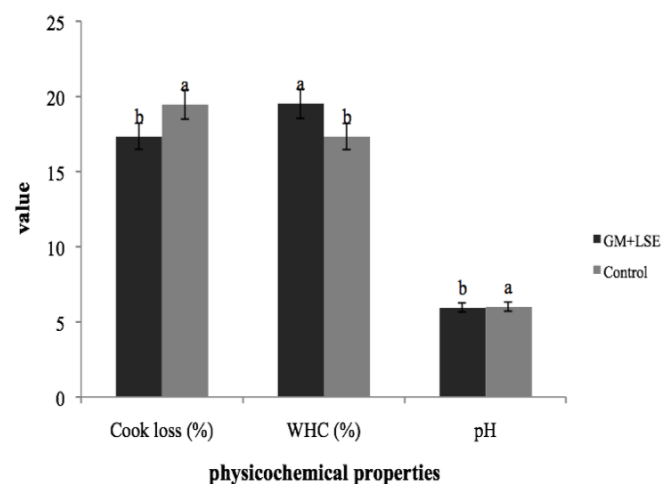


Figure 1: Effect of LSE treatment on Cooking loss, WHC, and pH of raw chicken ground meat.

Mean \pm standard deviation (triplicate); different letters on the top of columns indicate significant difference at $P < 0.05$; GM-ground meat; LSE-litchi seed extract.

Instrumental Color

The lightness (L^*) values were reduced (Table 1) gradually from d 0 to d 8 in the LSE treated samples. Higher reduction was observed in vacuum packaged (VP) than aerobically packaged (AP) samples; while, in the control, L^* values were more stable. Thus, LSE treatment was resulted in darkness of the meat samples and the darkness was increased as a function of storage time. The redness (a^*) was increased gradually during storage and differed significantly ($P < 0.01$) among different samples. The yellowness (b^*) was increased during first two days of storage in all the samples, however after second day, b^* values were decreased in all the samples. The packaging condition (VP or AP) has no impact on b^* values of LSE treated samples. However, as compared to the control b^* values were observed more in LSE treated samples, at any point during refrigerated storage period.

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS value correlates with oxidative stability of meat products and determines the extent of lipid oxidation. TBARS value increases with storage period, as the inappropriate storage conditions, action of light and presence of oxygen accelerate the oxidation reactions. The trends in the TBARS values among different samples are shown in Figure 2. The initial concentration of TBARS in the control as well as in all the treated meat samples were between 0.48 ± 0.01 and 0.51 ± 0.01 mg malonaldehyde/kg and not significantly different. However, during storage, the LSE treated samples showed significant ($P < 0.01$) less

TBARS as compared to the control samples. The values were also differed significantly between VP and AP samples. In the AP samples, the TBARS were higher than the VP samples during the whole storage period. Thus, LSE treatment as well as vacuum packaging resulted in more oxidative stability of refrigerated ground meat.

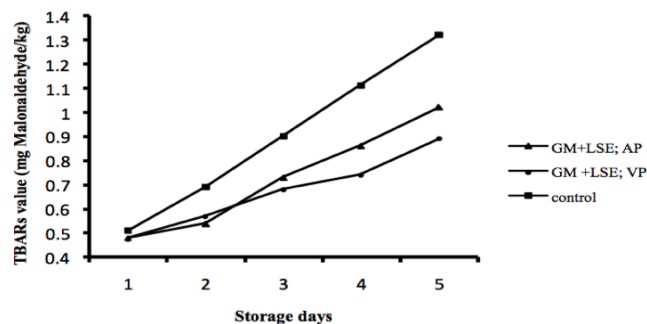


Figure 2: Effect of LSE treatment on TBARS (mg Malonaldehyde/kg) values of raw chicken ground meat (triplicate).

GM-ground meat; LSE-litchi seed extract; AP-aerobically packaged and VP-vacuum packaged.

It has been already discussed in previous sections that LSE contains more phenolic compounds which was also demonstrated by Shan *et al.* [20] and Shan *et al.* [21]. These phenolic compounds might be involved in the inhibition of lipid oxidation, by inhibiting free radical formation, by preventing propagation of free radical chain reactions through the chelation of transition metal ions, particularly those of iron and copper [22]. Similarly, Lee and Ahn [23] found that phenolic compounds such as gallate and sesamol were

Table 1: Effect of LSE Treatment on Color L^* , a^* and b^* Values of Raw Chicken Ground Meat Stored at 4 ± 1 °C

Group		d 0	d 2	d 4	d 6	d 8
L^*	GM+LSE; AP	57.58±1.76	54.99±1.48	45.20±1.78 ^b	45.32±31.80 ^b	43.3±31.80 ^b
	GM+LSE; VP	54.08±1.36	54.49±1.79	43.97±0.98 ^b	43.53±1.20 ^c	42.53±1.20 ^c
	Control	56.32±1.63	56.17±1.98	49.09±1.82 ^a	49.01±1.61 ^a	49.01±1.61 ^a
Treatment effect		NS	NS	**	**	**
a^*	GM+LSE; AP	8.12±0.40 ^a	10.61±0.55	13.12±0.58 ^a	14.47±0.84 ^a	15.34±0.81 ^a
	GM+LSE; VP	7.79±0.41 ^a	10.58±0.69	13.86±1.11 ^a	14.47±0.42 ^a	15.23±0.62 ^a
	Control	6.31±0.69 ^b	10.62±0.93	11.48±2.44 ^b	12.56±0.24 ^b	10.56±0.24 ^b
Treatment effect		**	NS	**	NS	**
b^*	GM+LSE; AP	16.93±1.13 ^a	22.36±0.73 ^a	20.36±0.52 ^a	19.86±0.53 ^a	17.57±0.24 ^a
	GM+LSE; VP	16.55±0.98 ^a	22.40±0.42 ^a	20.05±0.70 ^a	19.56±0.46 ^a	17.41±0.41 ^a
	Control	15.07±1.86 ^b	21.17±1.21 ^b	18.60±0.55 ^b	18.75±0.32 ^b	16.32±0.16 ^b
Treatment effect		*	**	**	*	*

All values are expressed as Mean \pm standard deviation of eight determinations (triplicate). GM- ground meat; LSE-litchi seed extract; AP-aerobically packaged; VP-vacuum packaged. Values bearing different superscript in a column differ significantly; ** $P < 0.01$; * $P < 0.05$.

effective antioxidants in reducing TBARS in turkey breast during 5 days storage period. Chen *et al.* [24] reported that phenolic compounds such as quercetin were also effective in preventing lipid oxidation in both raw and cooked turkey during 7 days of storage period.

CONCLUSION

This study revealed that litchi seed extract contains high amount of total phenolics, which contribute positively to the antioxidative potential. The addition of LSE in the ground meat was resulted in the lower TBARS values; hence, indicated more oxidative stability of LSE treated meat samples. The LSE treatment was also resulted in improved WHC which was positively correlated with cooking losses of ground meat. The LSE treatment was also associated with improved color values (L^* , a^* , b^*) of ground meat. Thus, LSE prevents lipid oxidation in meat, and could be used in place of synthetic antioxidants, which have been proved for their negative health implications.

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