Optimisation of extraction protocol for *Carica papaya* L. to obtain phenolic rich phyto-extract with prospective application in chevon emulsion system

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Abstract The present study optimized the extraction protocol conditions to obtain the antioxidant-rich bioactive extracts from *Carica papaya* L. (Papaya) leaves. A Box and Behnken Design (BBD) consisting of three different extracting variables viz. extracting temperature (60–70 °C), time (10–20 min) and solvent concentration (55–65%) was used. Antioxidant efficacy was recorded by evaluating four responses viz. ABTS, DPPH, SASA and total phenolic contents. The optimized model predicted, solvent concentration of 60% with extraction time of 15 min and extracting temperature of 65 °C with bioactive-rich antioxidants having highest total phenolic activity. The efficacy of obtained bioactive-rich papaya leaves extracts (PLE) were subjected for in-vivo evaluation in chevon emulsion added with the level of T-1 (0.10%); T-2 (0.25%); T-3 (0.50%) and control (without extract) stored under refrigeration (4 ± 1 °C) for 9 days by evaluating various physicochemical, microbiological, sensory quality characteristics. The pH was significantly higher for control than all treatments and water activity (a_w) showed decreasing trend throughout storage period. Oxidation efficiency values showed an increasing drift during storage period, irrespective of added level of PLE, showing lowest oxidation in samples treated with 0.5% PLE. Sensory panellists awarded comparatively higher scores to all PLE treatments than control. Microbiological quality of emulsion incorporated with different levels of papaya leaves

extract successfully improved and was lower in 0.5% PLE treated samples. It was concluded that extraction of bioactive antioxidants from *Carica papaya* L. leaves improved by optimising extraction parameters using RSM. *Carica papaya* L. leaves extracts have proven prospects as natural anti-oxidants in chevon emulsion as a meat emulsion system.

Keywords *Carica papaya* L. · Papaya leaves extracts · Lipid oxidation · Chevon emulsion

Introduction

Chevon also termed, as goat meat, which is widely accepted by the consumers throughout the World. According to 19th Livestock Census (GOI 2012) population of goat in India is 135.2 million with 5.05 MT chevon production (FAO 2016). Chevon is considered as lean meat distinguished with desirable nutritional qualities having a good source of vitamins and minerals and showing an ideal choice for the health-conscious customers (Haenlein 2004). Chevon has been characterised with lower fat and cholesterol, but also lower in saturated fats than other meat of food animals. Besides, having higher levels of iron, magnesium and potassium contents with lower sodium levels. As far as essential amino acid concern, chevon closely resembles that of lamb and beef. There are various popular chevon products such as chevon nuggets, kababs, kofta etc. however, these chevon products have limited storage life due to the rapid formation of metmyoglobin, lipid peroxidation etc. (Noor et al. 2018).

Lipid oxidation, important lab think era of meat industry, which is leading deteriorative causes for quality deterioration. Lipid oxidation not only imparts adverse effects

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on sensory attributes like colour, texture, odour, and flavour but also adversely affecting the nutritional quality of product (Wagh et al. 2015). Predisposing factors for lipid oxidation are when polyunsaturated fatty acids counter with available O₂ molecules via free radical chain mechanism which results in peroxide formation (Kagan 1988) initiating deteriorative changes in colour as well as production of toxic compounds affecting nutritive value and ultimately the health of the consumers. Hence, it is hour need to establish processing technologies for the development of healthier chevon meat and meat products so-called functional meat foods (Beriaín et al. 2018).

Functional foods are foods with known nutritional benefits explore to prevent and cure certain diseases and disorders (Jimenez-Colmenero 2007). The incorporation of antioxidant-rich bioactive phyto-ingredients/phyto-extract can be an effective approach for development of functional meat foods which also improves the nutritive value and combat the quality issues related to storage and oxidation. The common natural antioxidants used in meat products are extracts of sea buckthorn seeds, grape seeds, green tea and *Acacia catechu* (Wagh et al. 2015), tomato puree, tomato pulp, pink guava pulp (Joseph et al. 2014), curry and mint leaf extracts (Biswas et al. 2012).

Carica papaya L. commonly known as “Papaya”, family *Caricaceae* found in tropical regions like South Africa, Australia, Sri Lanka and India (Sorwar et al. 2016). Papaya leaves, pulp and seeds also contain a variety of bioactive phyto-chemicals, including polyphenols. In tropical parts of the World, papaya leaves are made into beverages like tea especially for as a curative agent for malaria and fever of unknown origin (Asase et al. 2010). Young leaves of papaya are potent source flavonoid like *myricetin*, phenolic compounds viz., *caffeic*, *chlorogenic* and *ferulic acid*, alkaloids viz. *carpaine*, *pseudocarpaine*, cynogenetic compounds viz. *benzylglucosinolate* (Miean and Mohamed 2001; Sorwar et al. 2016; Yogiraj et al. 2014) and shown medicinal possessions like anti-fertility/inflammatory, hypoglycaemic and chiefly as potent antioxidant etc. However, the scientific evidences are almost silent for the utilization of papaya leaves extract as a natural bioactive antioxidant in a meat model system.

In the light of above discussion, studies were planned with the optimization of extraction protocols to obtain bioactive-rich extracts from *Carica papaya* L. using BBD. The candidature as a novel antioxidant of the obtained PLE was further assessed using in-vivo meat model system as chevon emulsion by evaluating various physicochemical, oxidative efficacy and instrumental colour traits, microbiological and sensory attributes at refrigeration storage (4 ± 1 °C) for 9 days.

Materials and methods

Source of plant material

Papaya leaves were directly purchased from local market of Ludhiana, India, which were subjected to drying (Industrial Cabinet dryer, MAC, New Delhi). Powdered (size of 60–80 mesh) in Willey Grinder, Mumbai, India and stored in black coloured (to avoid sunlight) plastic bags at room temperature.

Extraction protocols

Papaya leaves were sequentially extracted according to the optimized process protocols defined by RSM employing Box and Behnken design (BBD). Variables used in present experiments were extracting solvent concentration; process extracting time and temperature which were predetermined using Design Expert software, USA in view of experimental design. Ten grams of papaya leaves powder was weighed and mixed in 100 mL of designed extracting solvent concentration in a glass flask, which was then shaken (200 rpm) employing a shaker (Narang Pvt. Ltd., India) for overnight. Obtained solution then filtered properly to get extracts without any powder particle. Vacuum evaporation was done using a Rota-evaporator, (Yamato Sci. Pvt. Ltd, USA) at designed extracting temperature (Table 1) and speed (50–80 rpm). Finally, extracted solution were collected in an amber coloured glass bottle and stored at -20 °C till further analysis.

Preparation of chevon emulsion

Castrated Beetal buck of age 12–15 months, weighing 25–30 kg were bought from the University Goat Farm, Guru Angad Dev Veterinary and Animal Sciences University, Punjab, Ludhiana, India. The animals were humane slaughtered as per standard procedure in the experimental abattoir of department of Livestock Products Technology, GADVASU University, Ludhiana, India with due consideration of the animal welfare and ethical aspects. The carcasses then hot deboned manually and all connective tissues viz. fat, fascia etc. were separated and boneless tissues were collected and stored in polyethylene packages and stored at -18 ± 1 °C till further use. The thawed (4 ± 1 °C) deboned chopped and minced by using 6 mm grinder plate in a meat mincer (Meat grinder, ESKIMO, MADDO GmbH, Germany) to obtain chevon emulsion added with 2% water dissolved salt. The obtained chevon emulsion was divided into four different batches as control (without PLE) while other three treatments containing 0.1% PLE (T-1), 0.25% PLE (T-2) and 0.5% PLE (T-3).

Table 1 ANOVA and regression coefficients of the second-order polynomial model for the response variables of papaya leaves extracts

Source	Df	ABTS (%)			DPPH (%)			SASA (%)			TP (mg GAE/100 g)		
		Coefficient	Sum of squares	p value	Coefficient	Sum of squares	p value	Coefficient	Sum of squares	p value	Coefficient	Sum of squares	p value
Model	9	68.13	2219.62	< 0.0001	55.81	2389.71	< 0.0001	61.18	1981.34	< 0.0001	534.39	1.602E+05	< 0.0001
Linear													
A1	1	6.77	366.82	< 0.0001	7.15	408.92	< 0.0001	5.94	282.31	< 0.0001	53.51	22,909.36	0.0002
B2	1	− 1.48	17.54	0.0974	1.70	23.06	0.0739	− 0.8913	6.36	0.1361	17.65	2490.97	0.0522
C3	1	− 4.79	183.68	0.0004	− 5.04	202.87	0.0004	− 4.63	171.32	< 0.0001	− 36.37	10,583.73	0.0019
Quadratic													
A1 B2	1	− 0.20	0.1615	0.8596	− 0.1452	0.0843	0.9025	− 1.39	7.76	0.1052	33.07	4275.82	0.0174
A1 C3	1	− 6.82	185.88	0.0004	− 7.16	205.31	0.0004	− 6.58	173.37	< 0.0001	− 62.18	15,467.32	0.0007
B2 C3	1	− 1.12	5.03	0.3400	− 1.18	5.55	0.3371	− 1.08	4.69	0.1914	4.45	79.12	0.6897
Interaction													
A2	1	− 12.60	688.22	< 0.0001	− 9.99	419.86	< 0.0001	− 11.63	569.29	< 0.0001	− 92.58	36,090.45	< 0.0001
B2	1	− 4.63	90.20	0.0034	− 1.61	10.92	0.1917	− 3.93	65.08	0.0010	− 5.21	114.44	0.6321
C2	1	− 11.66	572.33	< 0.0001	− 11.81	1012.4	< 0.0001	− 11.80	586.09	< 0.0001	− 91.49	61,686.30	< 0.0001
Residual	7		33.56			36.60			15.69			3197.81	
Lack of fit	3		25.67	0.0953		27.88	0.0976		8.33	0.3413		261.64	0.9445
Pure error	4		7.90			8.72			7.37			2936.17	
Total	16		2253.1			2426.3			1997.03			1.634E+05	
Adj. R ²		0.9660			0.9655			0.9820			0.9553		
Pred. R ²		0.8123			0.8105			0.9275			0.9463		
C.V. %		4.02			5.31			3.10			4.95		

ABTS (%): 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical activity; **DPPH (%)**: 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity; **SASA (%)**: superoxide anion scavenging activity; **TP**: Total phenolic content (mgGAE/100 g)

Experimental design

A Box and Behnken design (BBD) subjected for designing the experiments comprising three variables with three levels each viz. ethanolic solvent conc. of 55, 60 and 65%; Extraction time 10, 15 and 20 min and extraction temperature of 60, 65 and 70 °C. Total 17 experiments were conducted and design was employed for systematic investigation of how different extracting time, temperature and solvent concentration of ethanol influence antioxidants assays (Total phenolic, ABTS, DPPH and SASA activity) parameters.

Physico-chemical and oxidative parameters

The pH estimation was done using digital pH meter (Lab-IAI Pvt Ltd, New Delhi, India). Water activity, which indicates microbial stability, determined using Digital a_w meter (Hygro/palm, Rotronic, Switzerland). Protein oxidation and lipid oxidation was measured by Koniecko (1979) and Witte et al. (1970) respectively with slight modifications. The instrumental colour profile Instrumental colour profile was measured using Konica CR-400 Chroma meter (Konica Milota, Japan) and 'L*', a*, and b* value was noted. 'L*' value lightness, a* represents redness and b* for yellowness.

Antioxidant efficacy

The antioxidant efficacy in term of 1,1diphenyl-2picrylhydrazyl (DPPH) radical testing in samples was estimated by adopting the method of Kato et al. (1988). The 2,2-azino-bis radical scavenging activity (ABTS) reducing efficacy examined by methodology implemented by Shirwaikar et al. (2006). The Total phenolic content (TP) was estimated using Folin–Ciocalteu's reagent by adopting methodology by (Yuan et al. 2005). Super-oxide anionic scavenging ability i.e. SASA estimated by method given by Wagh and Chatli 2017.

Sensory evaluation

The sensory evaluation was performed by 7–12 food scientist along with trained members including postgraduate students of department. The test samples were offered to the panellists by conveying appropriate codes and evaluated on 1, 3, 5, 7 and 9 days of storage for colour, odour and overall acceptability using Five-point Hedonic scale having 1 as extremely undesirable to 5 as extremely desirable (Keeton 1983). Sensory evaluation conducted in three successive sitting and 21 observations were recorded.

Microbiological qualities

Microbiological quality was evaluated using standard/total plate counts (SPC), psychrophilic count, coliforms, yeast and mould Counts using methods formulated by American Public Health Association (APHA 2001) and data were expressed as $\log^{10}$ colony forming units/numbers (CFU) per gram sample.

Statistical analysis

Design Expert 10.2 (Stat-Ease Pvt. Inc., USA) programme was used for response surface plotting keeping level of 95.0%. Data obtained during chevon emulsion storage analysis was analysed using IBM-SPSS version 24, USA software package and differences among mean values were obtained by Duncan's test. The complete set of the experiment was triplicated and each parameter was noted in duplicate. Obtained results were indicted as mean $\pm$ standard error ($n = 6$) and the significance was defined at a level of $p < 0.05$.

Results and discussions

Extraction protocols

The extraction processing parameters viz. solvent concentration, temperature and time for papaya leaves were optimized implementing RSM. A Box–Behnken methodology was used in order to develop the finest extraction conditions to obtain phenolic-rich antioxidants from papaya leaves extracts (PLE), demonstrated by TP, ABTS, SASA and DPPH. The results are presented in the text with the support of statistically analysed Table 1 and Fig. 1.

Fitting the models

Table 1 and Fig. 1 depicts a linear, quadratic and interaction relation function of independent variables viz. ABTS inhibition, DPPH scavenging ability, SASA activity and TP in papaya leave extracts obtained from experiments. The polynomial equations (second-order) represent fitted experimental data and models coefficients were determined by analysis variance. The quality of the generated model was evaluated by R^2 and lack of fit.

In addition, high R^2 values and non-significant 'Lack of Fit' ($p > 0.05$) were observed in Table 1, with highly ($p > 0.05$) significant quadratic model and capable of describing the relationship between extraction conditions and responses. It showed that the models (polynomial with second-order) were satisfactorily justified by the respective experimental data. The coefficients i.e. (R^2) of 0.9960,

(a) Response Surface plots for ABTS (%)

(b) Response Surface plots for DPPH (%)

(c) Response Surface plots for SASA (%)

(d) Response Surface plots for TP (mgGAE/100gDW)

Fig. 1 Response surface plots for **a** ABTS (%), **b** DPPH (%), **c** SASA (%) and **d** TP (mgGAE/100 gDW) of Papaya leaves extract (PLE) in function of solvent concentration (%), extraction temperature (C) and extraction time (min)

0.9655, 0.9820 and 0.9553 were obtained for design responses of ABTS, DPPH, SASA and TP activity, respectively.

The coefficients i.e. R^2 of 0.9960, 0.9655, 0.9820 and 0.9553 were obtained for the response of ABTS, DPPH, SASA and TP activity, respectively. It indicated that polynomial models (second-order) were satisfactorily denoted by the corresponding resultant data.

ABTS inhibition ability

The optimization study using response surface as revealed in Table 1 validated the relationship between antioxidant efficiency measured by ABTS scavenging activity and in relation to processing parameters of extraction having quadratic interaction with a good regression coefficient ($R^2 = 0.9960$), and studied quadratic model exhibited the relationship as per equation as given below;

$$\begin{aligned} \text{ABTS} = & 68.12 + 6.77A - 1.48B - 4.79C - 0.20AB \\ & - 6.81AC - 1.12BC - 12.59A^2 - 4.62B^2 \\ & - 11.62C^2 \end{aligned}$$

The ANOVA of the regression models i.e. quadratic had a significant effect ($p < 0.05$) in relation to p values of < 0.0001 . The statistical analyses revealed that extraction processing in particular ethanol concentration had both linear as well as interaction effects ($p < 0.0001$) on the ABTS inhibition activity of PLE extracts followed by the linear term effects of process temperature during extraction. Thus, predicated model results suggested that a higher solvent concentration significantly increased the extraction of phenolic compounds, which can effectively scavenge free radicals. Furthermore, non-significant ($p > 0.05$) quadratic term effect between ethanol concentration, time and temperature were detected (Table 1).

The 3D graphs (Fig. 1a) showed the effect of extraction processing responses i.e. solvent concentration and temperature on the content of ABTS activity. ABTS activity showed an increasing trend with the increase of ethanol conc. at a fixed temperature and almost reached a peak at highest solvent concentration verified. It was also concluded that, increasing temperature of vacuum evaporator at a static solvent concentration results in a gradual increase in the ABTS of the resultant extracts. This may be justified due to less thermo-labile compounds in present extracts, which resist the degradation of active compounds (Wagh and Chatli 2017). The effect of extracting solvent conc. and time (Fig. 1a) confirmed that ABTS activity increased with successive increase in ethanolic solvent concentration at a static extracting time, while an increase in time i.e. extracting at a fixed ethanol concentration also led to a marked increase in ABTS activity, these findings

are in agreement with research finding of Wagh and Chatli 2017.

The maximum response for ABTS inhibition proven by response surface analysis was 68.12% of papaya leaves extracts under processing condition; keeping ethanol solvent concentration of 60%, temperature of 65 °C and time of 15 min.

DPPH scavenging ability

DPPH ability influenced by independent variables was reported by coefficient of the polynomial regression equation (second-order) as follows, which shows that model was significant ($p < 0.05$) with p values of < 0.0001 .

$$\begin{aligned} \text{DPPH} = & 41.77 + 5.42A + 1.29B - 3.82C - 5.43AB \\ & - 1.5AC - 10.54A^2 - 4.25B^2 - 1.62C^2 \end{aligned}$$

In terms of antioxidant capacity of PLE, linear as well as interaction terms of ethanol solvent concentration had positive ($p < 0.05$) influence on DPPH. However, quadratic term of processing temperature and ethanolic solvent concentration had ($p < 0.05$) effects at the $p < 0.0001$ level. The R square was 0.9655 for DPPH scavenging ability, and measure for lack of fit was ($p > 0.05$) non-significant, confirming a good suitability of model.

The RSM plots (Fig. 1b) postulated the pooled effect of ethanol as a solvent concentration, temperature and time on the DPPH, and found that at a lower concentration of ethanolic solvent concentration as well as temperature and time, the DPPH ability was minimal, which was subsequently increased with increasing independent variables. As a rule, higher DPPH of the extracts can be obtained with continuous increasing extracting temperature (Wagh and Chatli 2017). Silva et al. (2007) reported, increasing processing temperature could result in increasing solubility of resultant solute, which ultimately responsible to increase in antioxidant efficacy.

The response surface analyses indicated that maximum DPPH activity could be predicated at defined extraction time of 15 min, extraction temperature of 65 °C and ethanol conc. of 60%, leading to 55.81% DPPH activity.

SASA scavenging ability

Table 1 shows that a high regression value ($R^2 = 0.9820$) for the scavenging ability in terms of SASA in papaya leaves extracts and polynomial equation (second-order) explained the relationship for the same. The analysis of quadratic models was significant ($p < 0.05$) with p values of < 0.0001 .

$$\begin{aligned} \text{SASA} = & 61.18 + 5.94A - 0.89B - 4.62C - 1.39AB \\ & - 6.58AC - 1.08BC - 11.62A^2 - 3.93B^2 \\ & - 11.79C^2 \end{aligned}$$

Process extracting ethanol conc. had significant ($p < 0.0001$) linear as well as interaction term dependency on studied scavenging activity, whereas quadratic term effect of solvent conc. and temperature ($p < 0.05$) followed less effect on the same (Table 1). Equation term (linear) for processing temperature also found significantly ($p < 0.005$) affecting overall SASA activity (Fig. 1c).

F-value of 98.19 suggests predicted model is highly ($p < 0.05$) significant, which indicates only 0.01% chances that an F-value is larger than predicated one. p values in the obtained equation was found < 0.05 indicating model values are highly ($p < 0.005$) significant. In present study, A, C, AC, A^2 , B^2 and C^2 are significant ($p < 0.005$) model terms (Table 1).

The optimal conditions determined by RSA were ethanol concentration of 60%, extraction temperature of 65 °C and time of 15 min exhibiting a maximum SASA scavenging ability of 61.18%.

Total phenolics content (TP)

The polynomial equation (second-order) explained the envisaging the effect of extracting variables on TP is as follows:

$$\begin{aligned} \text{TP} = & 534.38 + 53.51A + 17.64B - 36.37C + 33.07AB \\ & - 62.18AC + 4.44BC - 92.58A^2 - 5.21B^2 \\ & - 121.03C^2 \end{aligned}$$

The studied model found highly significant ($p < 0.005$) having only 0.01% possibility that this large F-value might occur owing to model noise. In the present study, p values indicated model are significant ($p < 0.005$) for evaluation. The variable i.e. A, C, AB, AC, A^2 and C^2 are significant model values (Table 1).

The 3D RSM plots indicated interacts of solvent i.e. ethanol concentration, processing temperature as well as time, and it revealed that at low levels of the ethanol concentration, temperature, TP was minimal (Fig. 1d). This was subsequently increased with increasing the independent variables. These results are similar to studied DPPH and SASA scavenging activity with relation to processing variables.

Processing solvent concentration showed both linear and interaction model term effect ($p < 0.01$); whereas processing time didn't have any significant effect ($p > 0.05$) on total phenolics content of obtained extracts. Processing temperature along with interaction model term of solvent conc. i.e. ethanol had most significant effects on TP as

shown in Table 1, followed quadratic model term effect with relation to ethanolic solvent conc. and temperature (Fig. 1d).

The maximal TP predicted by response surface analysis was 534.38% with selected optimum ethanolic conc. of 60%, extraction time of 15 min and extraction temperature of 65 °C.

Optimization of extraction processing conditions

RSM optimization was demonstrated using Design-Expert 11 software to get optimum level of independent attribute/variables for papaya leaves extracts having highest ABTS, DPPH, SASA scavenging ability and TP. The optimized protocols using the model was as follows: solvent (ethanol) concentration, 60%; extraction temperature, 65 °C; and extraction time, 15 min. Keeping these processing conditions, the model predicted a supreme responses. The experimental values of 534.38 mg GAE/gm for TP, 68.12% for ABTS, 55.81 for DPPH%, and 61.18% for SASA. By employing, the optimized extraction protocol condition papaya leaves with maximum efficiency as an antioxidant can be obtained, which was further used in the study to evaluate its efficacy in meat model system.

Optimization of the level of incorporation of papaya leaves extract into chevon emulsion as a meat model system

Four different groups of chevon emulsion were prepared with incorporation of papaya leaves extract (PLE) obtained from optimization experiment done using RSM viz. control (without PLE), T-1 = 0.1%; T-2 = 0.25% and T-3 = 0.5% and stored under aerobic refrigeration temperature for 9 days and their physico-chemical, instrumental colour profile, oxidative stability parameters and sensory parameters were evaluated and with results (Tables 2, 3).

Physico-chemical quality parameters

The critical analyses of Table 2 showed that pH of chevon emulsion varied significantly ($p < 0.05$) amongst treatments and storage (days). The pH of treated chevon emulsion showed cumulative trend-set during storage days, irrespective of the type of phyto-extract added. The pH was comparable in all the treatments including control on 1st and 3rd day of storage. The pH was significantly ($p < 0.05$) lesser for T-3 as well as in T-2 than T-1 on last day of storage.

Control showed a higher ($p < 0.05$) pH values than all the treatments throughout the storage period. Demeyer et al. (1986) reported this influence due to the less carbohydrate content in the muscle, ultimately formation of

Table 2 Effect of different levels of papaya leaves extract (PLE) on the physico-chemical qualities of chevon emulsion during refrigerated aerobic storage (Mean $\pm$ S.E.)

Treatments/days	Day 1	Day 3	Day 5	Day 7	Day 9
<i>pH</i>					
C	5.74 $\pm$ 0.04 ^{Aa}	5.75 $\pm$ 0.01 ^{Ab}	5.77 $\pm$ 0.01 ^{Ac}	5.81 $\pm$ 0.08 ^{Ad}	5.84 $\pm$ 0.04 ^{Ae}
T1	5.75 $\pm$ 0.04 ^{Aa}	5.76 $\pm$ 0.08 ^{Ab}	5.78 $\pm$ 0.01 ^{ABc}	5.82 $\pm$ 0.07 ^{Ad}	5.85 $\pm$ 0.02 ^{ABe}
T2	5.76 $\pm$ 0.05 ^{Aa}	5.78 $\pm$ 0.06 ^{ABb}	5.79 $\pm$ 0.03 ^{Bbc}	5.84 $\pm$ 0.01 ^{Bd}	5.86 $\pm$ 0.01 ^{Bc}
T3	5.76 $\pm$ 0.02 ^{Aa}	5.79 $\pm$ 0.08 ^{Bb}	5.82 $\pm$ 0.09 ^{Cc}	5.85 $\pm$ 0.01 ^{Bd}	5.88 $\pm$ 0.07 ^{Cc}
<i>Water activity (a_w)</i>					
C	0.920 $\pm$ 0.003 ^{Ac}	0.907 $\pm$ 0.002 ^{Ad}	0.808 $\pm$ 0.006 ^{Ac}	0.707 $\pm$ 0.002 ^{Ab}	0.701 $\pm$ 0.004 ^{Aa}
T1	0.922 $\pm$ 0.002 ^{Ad}	0.909 $\pm$ 0.002 ^{Ad}	0.832 $\pm$ 0.007 ^{Bc}	0.792 $\pm$ 0.002 ^{Ab}	0.734 $\pm$ 0.001 ^{Ba}
T2	0.925 $\pm$ 0.003 ^{Ad}	0.933 $\pm$ 0.002 ^{Bd}	0.853 $\pm$ 0.004 ^{BCc}	0.810 $\pm$ 0.001 ^{Bb}	0.782 $\pm$ 0.007 ^{Ca}
T3	0.934 $\pm$ 0.002 ^{Be}	0.946 $\pm$ 0.002 ^{Bd}	0.890 $\pm$ 0.004 ^{Cc}	0.814 $\pm$ 0.003 ^{Bb}	0.789 $\pm$ 0.002 ^{Ca}
<i>SPC (log₁₀ cfu/g)</i>					
C	5.81 $\pm$ 0.04 ^{Bc}	6.30 $\pm$ 0.74 ^{Cd}	6.79 $\pm$ 0.54 ^{Cc}	7.13 $\pm$ 0.16 ^{Cd}	7.91 $\pm$ 0.51 ^{Cc}
T1	4.15 $\pm$ 0.07 ^{Aa}	4.85 $\pm$ 0.14 ^{Bb}	5.76 $\pm$ 0.18 ^{Bc}	6.19 $\pm$ 0.42 ^{Bd}	6.85 $\pm$ 0.34 ^{Bc}
T2	4.18 $\pm$ 0.15 ^{Aa}	4.35 $\pm$ 0.01 ^{Ab}	5.05 $\pm$ 0.17 ^{Ac}	5.65 $\pm$ 0.11 ^{Ac}	6.15 $\pm$ 0.16 ^{Ad}
T3	4.19 $\pm$ 0.26 ^{Aa}	4.38 $\pm$ 0.02 ^{Ab}	5.02 $\pm$ 0.03 ^{Ac}	5.59 $\pm$ 0.02 ^{Ac}	6.09 $\pm$ 0.19 ^{Ad}
<i>Coliforms count (log₁₀ cfu/g)</i>					
C	1.15 $\pm$ 0.01 ^{Ac}	1.34 $\pm$ 0.01 ^{Cd}	1.95 $\pm$ 0.02 ^{Cc}	2.11 $\pm$ 0.03 ^{Cd}	2.42 $\pm$ 0.01 ^{Cc}
T1	1.09 $\pm$ 0.02 ^{Aa}	1.19 $\pm$ 0.03 ^{Bb}	1.42 $\pm$ 0.01 ^{Bc}	1.62 $\pm$ 0.01 ^{Bd}	1.96 $\pm$ 0.02 ^{Bc}
T2	1.05 $\pm$ 0.05 ^{Aa}	1.16 $\pm$ 0.04 ^{Ab}	1.23 $\pm$ 0.05 ^{Ac}	1.57 $\pm$ 0.01 ^{Ac}	1.90 $\pm$ 0.03 ^{Ad}
T3	1.04 $\pm$ 0.03 ^{Aa}	1.15 $\pm$ 0.02 ^{Ab}	1.24 $\pm$ 0.02 ^{Ac}	1.56 $\pm$ 0.02 ^{Ac}	1.89 $\pm$ 0.02 ^{Ad}
<i>Psychrophilic count (log₁₀ cfu/g)</i>					
C	4.32 $\pm$ 0.08 ^{Ba}	4.85 $\pm$ 0.07 ^{Cb}	5.47 $\pm$ 0.01 ^{Cc}	5.64 $\pm$ 0.18 ^{Bd}	5.82 $\pm$ 0.05 ^{Be}
T1	4.09 $\pm$ 0.04 ^{Ba}	4.61 $\pm$ 0.09 ^{Bb}	5.01 $\pm$ 0.02 ^{Bc}	5.37 $\pm$ 0.08 ^{Bcd}	5.49 $\pm$ 0.17 ^{Bd}
T2	2.46 $\pm$ 0.01 ^{Aa}	3.82 $\pm$ 0.04 ^{Ab}	4.06 $\pm$ 0.07 ^{Ab}	5.21 $\pm$ 0.04 ^{Ac}	5.59 $\pm$ 0.16 ^{Ac}
T3	2.27 $\pm$ 0.02 ^{Aa}	3.01 $\pm$ 0.02 ^{Aab}	3.18 $\pm$ 0.05 ^{Ab}	4.15 $\pm$ 0.24 ^{Ac}	4.34 $\pm$ 0.01 ^{Ac}
<i>Yeast and molds count (log₁₀ cfu/g)</i>					
C	ND	ND	1.03 $\pm$ 0.02 ^a	1.16 $\pm$ 0.02 ^a	2.96 $\pm$ 0.01 ^{Bb}
T1	ND	ND	ND	ND	1.06 $\pm$ 0.12 ^A
T2	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND

Mean $\pm$ S.E. with different superscripts column wise (small alphabets) and row wise (capital alphabets) differ significantly ($p < 0.05$)

n = 6; C = control (without extracts); T-1 = 0.1% PLE, T-2 = 0.25% PLE; T-3 = 0.5% PLE

nitrogenous non-protein compounds and along with basic ammonium ions infused with actions of protein. This could be attributed to the accretion of basic compounds like ammonia, resulting from microbial action. These findings were justified by Kumar et al. (2015) in pork emulsion added with different phyto-extracts like sea buckthorn seed extracts, grape seed extracts etc.

Water activity (a_w) varied significantly ($p < 0.05$) with the progression of days during storage (Table 2). The a_w was comparable in all treatments on day one, found in consonance with Kumar et al. 2015, during storage of pork nuggets treated with different synergies of phyto-extracts. The rate of diminution in water activity during storage was lower in chevon emulsion samples treated followed by T-2 and T-1. Water activity in samples treated with highest

level of incorporation i.e. T-3 was maximum and found least in control at last day of storage. However, the water activity was significantly ($p < 0.05$) higher than control in all treated products throughout storage period. Microbial inhibition qualities of the incorporated papaya leave extracts into the chevon emulsion could be responsible. Antimicrobial properties of papaya leaves have already been documented by various scientists (Sorwar et al. 2016; Mian and Mohamed 2001; Yogiraj et al. 2014).

Microbiological quality

Microbiological qualities in terms of SPC of the chevon emulsion incorporated with different levels of papaya leaves extract (Table 2) varied significantly ($p < 0.05$)

Table 3 Effect of different levels of papaya leaves extract (PLE) on the sensory and instrumental colour profile of chevon emulsion during refrigerated aerobic storage (Mean $\pm$ S.E.)

Treatments/days	Day 1	Day 3	Day 5	Day 7	Day 9
<i>L*</i>					
C	52.15 $\pm$ 0.30 ^{Da}	51.01 $\pm$ 0.18 ^{CDa}	50.53 $\pm$ 0.15 ^{BCa}	50.03 $\pm$ 0.17 ^{Ba}	48.02 $\pm$ 0.35 ^{Aa}
T1	52.07 $\pm$ 0.15 ^{Da}	51.87 $\pm$ 0.26 ^{Ca}	51.87 $\pm$ 0.26 ^{Bb}	50.85 $\pm$ 0.30 ^{ABa}	50.45 $\pm$ 0.33 ^{Aab}
T2	52.80 $\pm$ 0.14 ^{Ca}	51.58 $\pm$ 0.29 ^{BCa}	51.53 $\pm$ 0.14 ^{BCb}	51.13 $\pm$ 0.16 ^{ABb}	50.95 $\pm$ 0.24 ^{Aab}
T3	52.53 $\pm$ 0.23 ^{Ba}	51.43 $\pm$ 0.15 ^{Aa}	51.33 $\pm$ 0.18 ^{Ab}	51.27 $\pm$ 0.25 ^{Ab}	51.20 $\pm$ 0.14 ^{Ab}
<i>a*</i>					
C	14.00 $\pm$ 0.20 ^{Da}	13.98 $\pm$ 0.27 ^{CDa}	12.87 $\pm$ 0.13 ^{Ca}	11.77 $\pm$ 0.15 ^{Ba}	10.40 $\pm$ 0.32 ^{Aa}
T1	14.10 $\pm$ 0.17 ^{Da}	13.81 $\pm$ 0.20 ^{Ca}	12.66 $\pm$ 0.28 ^{BCa}	12.87 $\pm$ 0.30 ^{Bab}	12.35 $\pm$ 0.20 ^{Ab}
T2	14.13 $\pm$ 0.19 ^{Ca}	13.76 $\pm$ 0.31 ^{Ba}	13.21 $\pm$ 0.29 ^{ABb}	13.20 $\pm$ 0.17 ^{ABb}	12.40 $\pm$ 0.16 ^{Ab}
T3	14.25 $\pm$ 0.32 ^{Ca}	13.75 $\pm$ 0.16 ^{BCa}	13.65 $\pm$ 0.16 ^{ABCb}	13.50 $\pm$ 0.23 ^{Ab}	13.48 $\pm$ 0.22 ^{Ac}
<i>b*</i>					
C	15.17 $\pm$ 0.44 ^{Ca}	15.01 $\pm$ 0.09 ^{BCa}	14.84 $\pm$ 0.01 ^{Ba}	14.09 $\pm$ 0.64 ^{ABa}	13.86 $\pm$ 0.46 ^{Aa}
T1	15.59 $\pm$ 0.51 ^{Ca}	15.23 $\pm$ 0.10 ^{BCa}	15.06 $\pm$ 0.10 ^{Bb}	14.58 $\pm$ 0.46 ^{ABa}	14.19 $\pm$ 0.16 ^{Aab}
T2	15.54 $\pm$ 0.57 ^{Ba}	15.46 $\pm$ 0.40 ^{Ba}	15.11 $\pm$ 0.51 ^{ABb}	14.63 $\pm$ 0.54 ^{Aa}	14.38 $\pm$ 0.96 ^{Aab}
T3	15.85 $\pm$ 0.16 ^{Ca}	15.44 $\pm$ 0.55 ^{BCa}	15.26 $\pm$ 0.85 ^{Bb}	15.04 $\pm$ 0.55 ^{ABb}	14.85 $\pm$ 0.12 ^{Ab}
<i>Colour</i>					
C	4.34 $\pm$ 0.03 ^{Ac}	3.99 $\pm$ 0.03 ^{Ad}	3.60 $\pm$ 0.03 ^{Ac}	3.03 $\pm$ 0.03 ^{Ab}	2.26 $\pm$ 0.02 ^{Aa}
T1	4.37 $\pm$ 0.02 ^{ABe}	4.21 $\pm$ 0.02 ^{Bd}	4.09 $\pm$ 0.03 ^{Bc}	3.82 $\pm$ 0.02 ^{Bb}	2.93 $\pm$ 0.02 ^{Ba}
T2	4.41 $\pm$ 0.02 ^{Be}	4.23 $\pm$ 0.03 ^{Bd}	4.02 $\pm$ 0.03 ^{Bc}	3.80 $\pm$ 0.03 ^{Bb}	3.62 $\pm$ 0.02 ^{Ca}
T3	4.49 $\pm$ 0.02 ^{Cc}	4.40 $\pm$ 0.02 ^{Cd}	4.29 $\pm$ 0.02 ^{Cc}	4.16 $\pm$ 0.01 ^{Cb}	3.64 $\pm$ 0.03 ^{Ca}
<i>Odour</i>					
C	4.40 $\pm$ 0.01 ^{Ac}	4.16 $\pm$ 0.03 ^{Ad}	3.73 $\pm$ 0.01 ^{Ac}	3.28 $\pm$ 0.01 ^{Ab}	2.85 $\pm$ 0.03 ^{Aa}
T1	4.59 $\pm$ 0.02 ^{Be}	4.43 $\pm$ 0.01 ^{Bd}	4.20 $\pm$ 0.01 ^{Bc}	3.96 $\pm$ 0.02 ^{Bb}	3.68 $\pm$ 0.02 ^{Ba}
T2	4.64 $\pm$ 0.02 ^{Be}	4.45 $\pm$ 0.02 ^{Bd}	4.23 $\pm$ 0.02 ^{Bc}	3.99 $\pm$ 0.03 ^{Bb}	3.76 $\pm$ 0.02 ^{BCa}
T3	4.78 $\pm$ 0.02 ^{Cc}	4.63 $\pm$ 0.02 ^{Cd}	4.41 $\pm$ 0.03 ^{Cc}	4.19 $\pm$ 0.02 ^{Cb}	3.95 $\pm$ 0.01 ^{Ca}
<i>Overall acceptability</i>					
C	4.44 $\pm$ 0.02 ^{Ac}	4.12 $\pm$ 0.02 ^{Ad}	3.63 $\pm$ 0.02 ^{Ac}	3.17 $\pm$ 0.02 ^{Ab}	2.72 $\pm$ 0.01 ^{Aa}
T1	4.56 $\pm$ 0.02 ^{Ac}	4.42 $\pm$ 0.01 ^{Bd}	4.15 $\pm$ 0.02 ^{Bc}	3.86 $\pm$ 0.01 ^{Cb}	3.13 $\pm$ 0.02 ^{Aa}
T2	4.72 $\pm$ 0.02 ^{Ac}	4.72 $\pm$ 0.02 ^{Cd}	4.54 $\pm$ 0.02 ^{Cc}	4.39 $\pm$ 0.02 ^{Db}	3.23 $\pm$ 0.01 ^{BCa}
T3	4.81 $\pm$ 0.02 ^{Be}	4.43 $\pm$ 0.03 ^{Bd}	4.12 $\pm$ 0.02 ^{Bc}	3.79 $\pm$ 0.02 ^{Bb}	3.66 $\pm$ 0.03 ^{Ca}

Mean $\pm$ S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($p < 0.05$). (5-point Hedonic descriptive scale; 1 as extremely undesirable to 5 as extremely desirable)

n = 6; C = control (without phyto-extract); T-1 = 0.1% PLE, T-2 = 0.25% PLE; T-3 = 0.5% PLE

amongst the treatments. Table 2 showed significantly ($p < 0.05$) lower values in T-3 than all the other treatments, which might be due to strong anti-microbial effect exerted by a higher level of PLE into chevon emulsion compared to other treatments. SPC was higher ($p < 0.05$) in untreated samples as compared to treatments during all storage days.

There was regular increase in SPC of all the products throughout storage. However, it was lower in treated products than control throughout storage period.

Psychrophilic count (PC) followed a similar trend to that of SPC in chevon emulsion during storage days. Psychrophilic count was found comparable in T-2 along with T-3 on 3rd day and onward throughout storage period of 9 days. Coliforms count followed increasing drift with storage progression and were ($p < 0.05$) higher in untreated samples. T-3 showed significantly ($p < 0.05$) lower coliforms count throughout storage period.

Yeast/mold count found at 5th day in control, T-1 at last day, while not detected even last day of storage, in reaming

treatments. In control samples, it increased significantly ($p < 0.05$) on further storage. Various workers, Wagh et al. (2015), Wagh et al. (2014) found regular increase in standard plate count and psychrotrophic count during refrigerated storage in chevon patties.

These results can be justified as leaves of papaya are potent source flavonoid like *myricetin*, phenolic compounds viz., *caffeic*, *chlorogenic* and *ferulic acid*, alkaloids viz. *carpaine*, *pseudocarpaine* (Miean and Mohamed 2001; Yogiraj et al. 2014) which are proven antimicrobial agent, responsible for anti-oxidant as well as bacteriostatic in studied chevon emulsion.

Colour parameters

Instrumental colour profile (Table 3) of chevon emulsion with the incorporation of PLE varied significantly ($p < 0.05$) within treatments as well as with storage days. L^* values were comparable between treatments and control on day 1st and 3rd and 5th day of storage. L^* value followed a declining trend with progress of storage periods, however, the decrease was not statistically significant ($p < 0.05$) among treatments. The rate of reduction in treatments were lower than control, which might be due to the capability of the PLE to preserve the colour of the product by delaying the oxidation reaction as T-3 contains higher amount of PLE.

All the treatments, as well as control, showed declining trend during days of storage, which accredited to the evidence that salt greatly accelerated the process of discolouration of meat due to the oxidative activity particularly pro-oxidative, due to release iron from heme-ion pigments and other hem-binding molecules (Wagh et al. 2015).

The a^* value is an indicator of redness of the meat, which is characteristic of raw meat and criteria for quality evaluation by the consumers. Redness attribute showed pattern as $T-3 > T-2 > T-1$ among treatments and continued a declining drift in treatments until the storage days continue. This is attributed to myoglobin oxidation and accumulation of Met-myoglobin with storage time (Pietrasik et al. 2006; Mancini and Hunt 2005). However, the a^* value was higher in treated samples than control during the storage and rate of decrease in a^* value was significantly ($p < 0.05$) lower in treated products than control.

The b^* values also found in declining trend for all the treated samples as well as control during storage regardless of the level of integration of PLE (Table 3). The b^* values were ($p < 0.05$) higher for T-3 than control and T-1 on seventh day of storage. Similarly, Realini et al. (2015) also reported a decrease in b^* values of beef patties comprising *Acerola* fruit extract, which is proving the presence of the metmyoglobin brown pigment. Reducing enzymes are

necessary to transform metmyoglobin back to oxymyoglobin, which is well known to contribute to the fresh meat colour (Pietrasik et al. 2006).

Sensory quality parameters

Data pertaining to various sensory attributes of chevon emulsion combined with different PLE levels are presented at Table 3. Highest colour, odour and overall acceptability scores were reported to treatments than control from first day, which was maintained till end of storage.

However, rate of decline of sensory score was ($p < 0.05$) higher in control than treated products during storage. T-3 showed highest colour score during entire storage, followed by T-1 and T-2. Meat discoloration particularly in control samples indicated, oxidative stability of control was poor than PLE treatments. Greene and Cumuze (1982) documented that the colour discolouration resulted from metmyoglobin which act as an indicator for oxidation of meat and meat products. Odour score followed similar trend as that of the colour score during storage and followed a trend as $T-3 > T-2 > T-1$ amongst the treatments (Table 3). In the earlier studies, research scientists (Kumar et al. 2015; Wagh and Chatli 2017) reported the significant improvement in sensory scores with the incorporation of natural antioxidants (Aloe vera and apple peel extract) into meat emulsion. From above discussion, it was concluded that 0.5% incorporation level of PLE was best to maintain sensory qualities of chevon emulsion.

Oxidative efficiency parameters

Results of oxidative stability attributes are presented in Figs. 2, 3. The quality changes produced from the oxidation of lipids were responsible for deterioration of colour, flavour and aroma as well as development of toxic complexes like aldehyde, ketones etc. During initial phase of lipid oxidation, peroxides and hydroperoxides are the predominant reactions and so the peroxide formation occurs in the initial step of lipid oxidation. It is used as a sign of the extent of oxidative rancidity.

It was observed (Fig. 2) that TBARS values were increased ($p < 0.05$) during storage period regardless of added PLE levels in studied emulsion. However, it was least in T-3 and reached a maximum in T-1 and similar trend continued during storage. TBARS was ($p < 0.05$) higher in control than treatments on day one and as long as storage period of 9 days. Witte et al. 1970 postulated that TBARS value should be in range of permissible level i.e. below 1.0 mgMDA/Kg, which is regarded as acceptability value for minced meat. Such a lower values were attributed to oxidative capabilities delivered by phyto-extracts. TBARS values perceived in the current study are related to

Fig. 2 Effect of different levels of papaya leaves extract (PLE) on the TBARS (mg malonaldehyde/kg) of chevon emulsion during refrigerated (4 ± 1 °C) aerobic storage (Mean $\pm$ S.E.)

findings described by Devatkal et al. (2014) for raw chicken meat incorporated with sapodilla peel during refrigerated storage.

Peroxide value (Fig. 3) of control sample remained significantly higher during studied days as compared to treatments. T-3 showed lowermost peroxide content which was then shown by T-2 > T-1. This can be correlated to oxidative stability provided by added PLE. Also due to, phenolic groups present in PLE delivers a hydrogen atom (labile) for abstraction by radicals developed in particular free radicals viz. peroxy or alkoxyl radicals which then get converted into phenoxyl radical (Miean and Mohamed 2001; Yogiraj et al. 2014; Wagh et al. 2015). PV followed an increasing trend in both treated as well as control samples during storage. Botsoglou et al. (2014) who evaluated the efficacy of olive leaf extracts on oxidative changes of chevon patties observed similar escalation in

Fig. 3 Effect of different levels of papaya leaves extract (PLE) on the Peroxide value (meq/Kg) of chevon emulsion during refrigerated (4 ± 1 °C) aerobic storage (Mean $\pm$ S.E.)

peroxide value during an aerobic storage period of 15 days at 4 ± 1 °C. Our results are in justification with an earlier study done by Wagh and Chatli 2017; Kumar et al. 2015.

FFAs are the products of enzymatic or microbial lipolysis of fat determined by titration of FFAs present in meat. There was non-significant ($p < 0.05$) effect on FFA content between the treatments on 1st day, however (Data not shown), it tracked an increasing trend during storage in both the control as well as PLE treated chevon emulsion. These results clearly indicate that 0.5% PLE can act as an antioxidant in meat system by controlling the oxidative deterioration changes.

Hence, it was concluded from above results and discussions that the incorporation of 0.5% level of PLE extract in the chevon emulsion led to the improvement in the various qualities like physico-chemical, antioxidant potential, microbiological and sensory quality during 9 days of refrigerated storage (4 ± 1 °C).

Conclusion

The processing conditions of ethanol concentration, 60%; extraction temperature, 65 °C; and extraction time, 15 min of Response Surface Methodology (RSM) optimization design we can get phenolic rich bioactive extracts from papaya leaves (*Carica papaya* L.) with highest antioxidants potency. It was concluded that in-vivo meat model system i.e. chevon emulsion can be successfully implemented to test the efficacy of papaya leaves (*Carica papaya* L.) extracts as novel natural antioxidants, which can be further used for the development of functional meat products from goat meat. As compared to added levels i.e. 0.10%, 0.25% and 0.50%, incorporation at the level of 0.5% level of papaya leaves extract in the chevon emulsion lead to the improvement in the various physico-chemical qualities, antioxidant potential, microbiological and sensory quality during 9 days refrigerated storage. We stand first in reporting evidence to utilize papaya leaves (*Carica papaya* L.) extracts as a natural bioactive phyto-extracts, can be further used for the development of functional meat products.

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