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Research Article

OPTIMIZATION OF ETHANOL-ASSISTED EXTRACTION OF KIWI PEEL AND ANTIOXIDANT ACTIVITY IN CHICKEN EMULSION

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ABSTRACT

The present study optimized the extraction protocol conditions to obtain the antioxidant-rich bioactive extracts from Kiwi fruit peel and *in-vivo* studies to test its antioxidant efficacies in chicken meat emulsion. The optimized model predicted that ethanol concentration of 60 % with extraction time of 15 min and extracting temperature of 65 °C yielded extracts with highest total phenolic, DPPH and ABTS activity. The obtained kiwi peel extracts (KPE) was then added at the level of T-1 (1.5%); T-2 (2.0%); T-3 (2.5%) and control (without extract) in spent hen chicken emulsion and stored under refrigeration (4±1°C) for 9 days. Storage study revealed that pH showed increasing trends in all treatments throughout storage period. TBARS, FFA and PV values showed an increasing drift during storage period, irrespective of added level of KPE, showing lowest oxidation in samples treated with best sensory qualities in 2.5% KPE treated samples. The study concluded that 2.5 % KPE extract could be successfully employed for extending shelf life of meat model systems with improved sensory and physicochemical properties.

Keywords: Chicken emulsion, Kiwi fruit, Kiwi fruit peel extracts, Lipid oxidation

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Poultry development in India has taken a quantum jump since last few decades, with annual growth rate of 11.44 percent in broiler production (Ponnampalam *et al.*, 2017). In India, poultry meat is widely consumed due to scads of health benefits and being relatively cheaper compared to red meats. Spent hen meat protein is a complete protein rich in essential amino acids, a good source of B-complex vitamins besides micro minerals like Zn, Fe, and Se. Products of animal origin are mostly prone to lipid oxidation and warmed-over flavour production due to presence of unsaturated nature of fat. Development of meat products involves the use of synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) etc., to retard lipid oxidation. However, the consumer has concerns about the safety of synthetic antioxidants, which had led to the utilization of natural antioxidants (Wagh *et al.*, 2015).

In this context, plant derivatives and their by-products which are rich sources of antioxidant and nutraceutical substances were used in comminuted meat products to enhance quality and shelf life. The incorporation of antioxidant rich bioactive phyto-extract can be an effective approach for development of functional meat foods (Jagtap *et al.*, 2020). Kiwi fruit (*Actinidia deliciosa*) also known as the Chinese gooseberry, is an edible berry widely grown in India. On an average of a fresh weight basis, kiwi fruit contains about 1-2 times more vitamin C than orange, 10 times as much as a banana or up to 15 times as much as an apple (Ferguson and Ferguson, 2002). The utilization of kiwi by-products, viz., kiwi peel extract and kiwi pomace extract left after fruit processing as a source of functional compounds and their application in meat science is a promising field in coming era.

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In the light of above discussion, present study was planned to optimize the extraction protocol conditions to obtain the antioxidant-rich bioactive extracts from Kiwi fruit peel and *in-vivo* studies to test its antioxidant efficacies in chicken meat emulsion at refrigerated storage condition.

MATERIALS AND METHODS

Source of meat and kiwi fruit peel : Spent hen chicken meat were procured from the University Poultry Farm, Department of Livestock Production Management, GADVASU, Ludhiana. Kiwi fruits were procured from local market, Ludhiana and were washed, cleaned and peeled off. The obtained peels were subjected to drying at Industrial dryer at temperature of 55 °C for the period of 10 hrs. The dried peel was powdered into a particle size of 60-80 mesh using grinder and stored in low density polyethylene (LDPE) bags.

Extraction protocols : Kiwi peel was sequentially extracted according to the optimized process protocols defined by Response Surface Methdology (RSM) employing Box and Behnken design (BBD). Variables used were extracting ethanol concentration; extracting time and temperature of vacuum evaporator, which were predetermined using Design Expert software, USA in view of experimental design. Ten grams of kiwi peel 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) 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 and speed (50-80 rpm). Finally, extracted solution were collected in an amber coloured glass bottle and stored at -20 ºC till further analysis

Preparation of spent hen chicken emulsion : The thawed (at 4±1°C) deboned chicken meat was minced by using 6 mm grinder plate in a meat mincer (Meat grinder, ESKIMO, MADO GmbH, Germany) to obtain spent hen chicken emulsion added with 2% water dissolved salt. The obtained chicken emulsion was divided into four different batches as control (without KPE) while other three treatments containing 1.5% KPE (T-1), 2.0% KPE (T-2) and 2.5% KPE (T-3).

Physico-chemical and oxidative parameters : The pH estimation was done using digital pH meter (Lab IAI Pvt Ltd, New Delhi, India). The instrumental colour profile was measured using Konica CR-400 Chroma meter (Konica Milota, Japan) and 'L*' (lightness), a*(redness) and b*(yellowness) values were noted.

Antioxidant and oxidative efficacy : The antioxidant efficacy in term of 1, 1-diphenyl-2 picrylhydrazyl (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) and reducing efficacy was examined by methodology implemented by Shirwaikar *et al.* (2006). The Total phenolic content (TP) was estimated using Folin–Ciocalteau's reagent by adopting methodology of Yuan *et al.* (2005). The method described by Witte *et al*. (1970) was used for the determination of Thiobarbituric acid reactive substances (TBARS). Peroxide value (PV) and free fatty acid (FFA) were measured as per the procedure described by Koniecko (1979). The sensory evaluation was performed by 12 food scientists including postgraduate students of department for colour, odour and overall acceptability using Five-point Hedonic scale having 1 as extremely undesirable to 5 as extremely desirable (Keeton, 1983).

Statistical analysis : Design Expert 12.0 (Stat-Ease Pvt. Inc., USA) programme was used for response surface plotting keeping level of 95.0%. Data obtained during spent hen chicken emulsion storage analysis were analysed using IBM-SPSS version 24, USA software package. Obtained results were indicted as mean ± standard error (n=6) and the significance was defined at a level of $p>0.05$.

RESULTS AND DISCUSSION

Extraction protocols

The extraction processing parameters, viz., ethanol concentration, temperature and time of vacuum evaporator for kiwi peel was optimized implementing RSM to obtain phenolic-rich antioxidants from kiwi peel extracts (KPE), demonstrated by TP, ABTS and DPPH.

Fitting the models

ABTS inhibition ability : Results validated the relationship between ABTS inhibition and extraction parameter as quadratic with a good regression coefficient. Predicated model resulted that a higher solvent concentration significantly increased the extraction of phenolic compounds, which can effectively scavenge free radicals. The statistical analyses revealed that extracting ethanol had both significant linear and interaction effects ($p < 0.0001$) on the ABTS inhibition activity of kiwi peel extracts. ABTS increased gradually with the increase of ethanol conc. at a fixed extraction temperature, and nearly reached a peak at the highest ethanol concentration tested (Fig. 1). Similarly, the increase in extraction temperature at a fixed ethanol concentration led to a gradual increase in the ABTS activity. The maximum ABTS inhibition predicted by response surface methodology was 32.66% of kiwi peel extracts under the extraction condition; the ethanol concentration of 60%, extraction temperature of 65 °C and extraction time of 15 min.

DPPH scavenging ability : The linear and interaction terms of ethanol concentration had significant effect ($p<0.05$) on DPPH scavenging ability. The R square was 0.9655 for DPPH scavenging ability and the lack of fit was highly non-significant, verifying a good fitness of the model. It was found that at low levels of the ethanol concentration, extracting temperature and extracting time, the DPPH scavenging ability was minimal, which was subsequently increased with increasing the independent variables (Fig. 2). Wagh *et al.* (2015) documented that increasing the extraction temperature lead to increase the solubility of solute, which ultimately lead to increase in antioxidant efficacy, leading to yield 43.53% DPPH activity.

Total phenolic content (TP) : Solvent concentration i.e. ethanol had both significant linear and interaction effects ($p<0.01$); whereas extraction time didn't have any significant effect ($p>0.05$) on total phenolic contents. Extraction temperature as well as the interaction effect of ethanol conc. had the most significant effects on TP, which increased linearly with the increase in extraction temperature from 60 to 70 °C. Likewise, it was found that

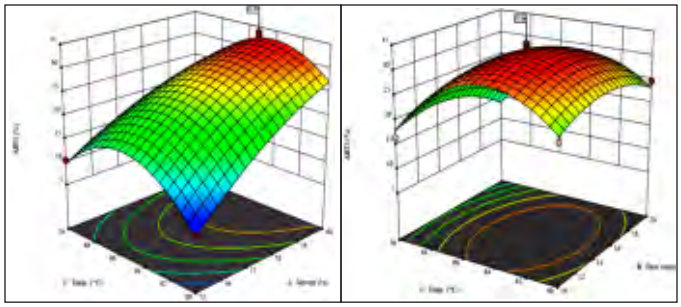

Fig. 1. Response Surface plots for ABTS (%) of kiwi peel extract in function of solvent concentration (%), extraction temperature (°C) and extraction time (min)

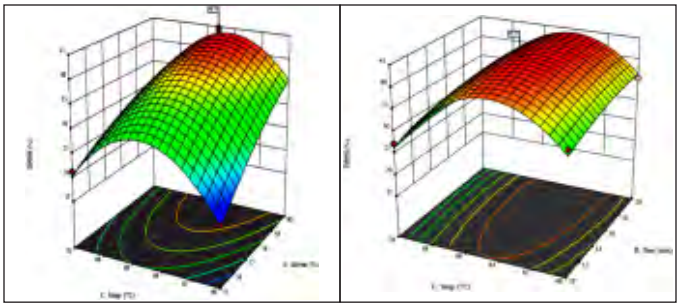

Fig. 2. Response Surface plots for DPPH (%) of kiwi peel extract in function of solvent concentration (%), extraction temperature (°C) and extraction time (min)

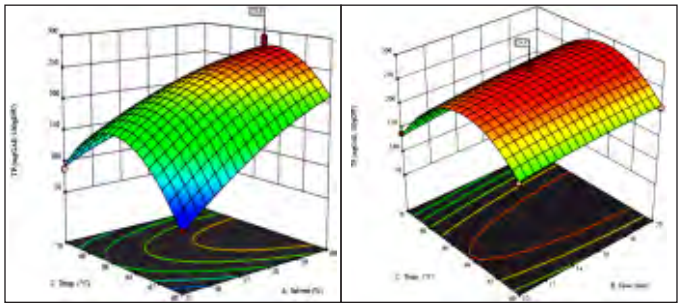

Fig. 3. Response Surface plots for TP (mgGAE/100gDW) of kiwi peel extract in function of solvent concentration (%), extraction temperature (°C) and extraction time (min)

increasing ethanolic conc. from 55 to 65% promoted linear increase in TP level (Fig. 3). The optimized model showed highest TP of 274.67 (mg GAE/100gDW) with optimum ethanolic conc. of 60%, extraction time of 15 min. and extraction temperature of 65°C.

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

Physico-chemical quality parameters : The pH of chicken emulsion incorporated with i.e. 1.5%, 2.0% and

Table 1

Effect of different levels of kiwi peel extract (KPE) on the physio-chemical and oxidation parameters of chicken 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	5.78±0.05^{A}	5.77±0.06^{AB}	5.86±0.01^{AB}	5.91±0.05^{B}	5.92±0.05^{Aa}
T-1	5.81±0.05^{A}	5.83±0.02^{A}	5.87±0.09^{A}	5.93±0.03^{AB}	6.06±0.06^{Bb}
T-2	5.79±0.05^{A}	5.81±0.06^{A}	5.84±0.06^{A}	5.89±0.04^{AB}	6.02±0.01^{Bb}
T-3	5.87±0.04^{A}	5.81±0.06^{A}	5.84±0.06^{A}	5.89±0.04^{AB}	6.02±0.01^{Bb}
			Thiobarbituric acid reactive substances (mg malonaldehyde/Kg)		
C	0.39±0.09^{Ab}	0.55±0.04^{Bd}	0.86±0.03^{Cc}	1.25±0.05^{Dc}	1.76±0.04^{Eb}
T-1	0.39±0.04^{Ab}	0.47±0.07^{Bc}	0.66±0.08^{Cb}	0.79±0.07^{Db}	0.88±0.06^{Ea}
T-2	0.36±0.01^{Aa}	0.43±0.08^{Bb}	0.64±0.06^{Cb}	0.74±0.04^{Db}	0.87±0.09^{Ea}
T-3	0.34±0.04^{Aa}	0.40±0.06^{Ba}	0.53±0.08^{Ca}	0.66±0.06^{Da}	0.80±0.07^{Ea}
			Free fatty acid (%)		
C	0.19±0.008^{Ac}	0.29±0.016^{Bc}	0.34±0.008^{Cd}	0.46±0.004^{Dd}	0.48±0.008^{Dc}
T-1	0.17±0.003^{Ab}	0.23±0.005^{Bb}	0.27±0.003^{Cc}	0.29±0.004^{Dc}	0.30±0.008^{Db}
T-2	0.11±0.004^{Aa}	0.14±0.003^{Ba}	0.19±0.003^{Cb}	0.24±0.011^{Db}	0.29±0.006^{Eb}
T-3	0.10±0.004^{Aa}	0.14±0.004^{Ba}	0.17±0.003^{Ca}	0.19±0.007^{Da}	0.21±0.002^{Ea}
			Peroxide value (meq/Kg)		
C	5.93±0.15^{Ab}	6.24±0.13^{Bc}	6.76±0.06^{Cc}	7.59±0.04^{Dd}	7.96±0.09^{Ed}
T-1	5.49±0.09^{Aa}	5.99±0.14^{Bc}	6.45±0.06^{Cb}	6.88±0.09^{Dc}	7.09±0.07^{Dc}
T-2	5.29±0.03^{Aa}	5.67±0.06^{Bb}	6.25±0.07^{Cb}	6.64±0.04^{Db}	6.73±0.02^{Db}
T-3	5.22±0.16^{Aa}	5.32±0.03^{Aa}	5.45±0.08^{Aa}	5.73±0.07^{Ba}	5.81±0.04^{Ba}

n=6; C= Control (without phyto-extract); T-1= 1.5% kiwi peel extract, T-2= 2.0% kiwi peel extract; T-3= 2.5 % kiwi peel extract.
*Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly (P<0.05).

2.5% kiwi peel (KPE) varied throughout the storage period (Table 1). Through out the storage period, there was an increase in pH values of all treatments, which could be attributed to the formation of N non-protein compounds and basic ammonium ions coupled with buffering action of protein (Kumar *et al.*, 2015). Similar findings were reported by Wagh *et al.* (2014) in raw ground pork added with *Matteuccia struthiopteris* extracts during refrigerated storage.

Instrumental colour profile : The results of instrumental colour profile of chicken emulsion incorporated with kiwi peel extract (KPE) are presented in Table 2. Redness (a*) value showed significantly ($p<0.05$) declining trend for control and treatment emulsions during the storage which might be due to gradual oxidation of myoglobin and accumulation of metmyoglobin with storage time (Mancini and Hunt 2005). a* value was higher in KPE treated samples than control on the 9th day of storage (Table 2). Similar results were reported by Jagtap *et al.* (2020) on quality indices of chevon meat infused with *Origanum vulgare* leaf extracts. Assimilation of KPE with the level of 1.5, 2.0% and 2.5% affected the yellowness (b* values) and the rate of decrease was significantly ($P<0.05$) lower

Table 2

Effect of different levels of kiwi peel extract (KPE) on the Sensory parameters of chicken 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	4.27±0.09^{C}	4.03±0.07^{BCa}	3.76±0.08^{Ba}	3.05±0.04^{Aa}	2.99±0.09^{Aa}
T-1	4.30±0.15^{B}	4.27±0.06^{Bb}	4.21±0.04^{Bb}	3.14±0.04^{Aa}	3.10±0.07^{Aa}
T-2	4.31±0.09^{C}	4.24±0.05^{Cb}	4.21±0.04^{BCb}	4.04±0.06^{ABb}	3.88±0.06^{Ab}
T-3	4.38±0.04^{C}	4.31±0.05^{Cb}	4.27±0.04^{BCb}	4.11±0.06^{ABb}	3.95±0.06^{Ab}
			Odour		
C	4.07±0.02^{Da}	3.83±0.05^{Ca}	3.75±0.02^{Ca}	2.47±0.03^{Ba}	2.06±0.03^{Aa}
T-1	4.13±0.07^{Da}	4.09±0.04^{Db}	3.84±0.04^{Ca}	3.25±0.05^{Bb}	2.98±0.05^{Ab}
T-2	4.75±0.01^{Cb}	4.70±0.02^{Cc}	4.59±0.02^{Cb}	4.21±0.05^{Bc}	3.91±0.03^{Ac}
T-3	4.82±0.01^{Cb}	4.75±0.02^{Cc}	4.66±0.02^{Cb}	4.28±0.05^{Bc}	3.98±0.03^{Ac}
			Overall Acceptability		
C	4.18±0.05^{Ca}	4.05± 0.05^{Ca}	3.75±0.06^{Ba}	3.07±0.05^{Aa}	2.95±0.05^{Aa}
T-1	4.25±0.06^{Ba}	4.16± 0.05^{Bab}	4.00±0.09^{Bb}	3.46±0.04^{Ab}	3.33±0.10^{Ab}
T-2	4.46±0.06^{Bb}	4.33± 0.06^{Bbc}	4.27±0.08^{Bc}	3.98±0.05^{Ac}	3.87±0.05^{Ac}
T-3	4.51±0.06^{Bb}	4.40± 0.06^{Bc}	4.34±0.08^{Bc}	4.05±0.05^{Ac}	3.94±0.05^{Ac}
			L* (Lightness)		
C	55.66±0.64^{B}	54.16±0.58^{AB}	54.08±0.53^{AB}	53.89±0.58^{AB}	51.78±0.30^{A}
T-1	54.71±0.60^{A}	54.59±0.52^{A}	54.15±0.60^{A}	54.10±0.35^{B}	52.03±0.24^{A}
T-2	54.48±0.18^{B}	54.14±0.19^{AB}	53.99±0.25^{AB}	53.59±0.33^{A}	52.74±0.28^{AB}
T-3	54.65±0.41^{AB}	54.76±0.25^{B}	54.69± 0.59^{B}	53.73±0.38^{AB}	52.35±0.87^{A}
			a* (Redness)		
C	13.73±0.76^{C}	12.19±0.40^{C}	11.93±0.53^{BC}	10.34±0.23^{AB}	9.38±0.21^{Aa}
T-1	13.21±0.26^{A}	12.03±0.60^{A}	12.06±0.53^{A}	11.79±0.23^{A}	11.48±0.21^{Ab}
T-2	12.99±0.21^{A}	11.85±0.84^{A}	11.67±0.59^{A}	10.88±0.61^{A}	10.91±0.53^{Ab}
T-3	12.84±0.43^{A}	11.78±0.14^{A}	11.66±0.66^{A}	10.98±0.33^{A}	10.80±0.63^{Ab}
			b* (Yellowness)		
C	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}
T-1	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}
T-2	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}
T-3	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= 1.5% kiwi peel extract, T-2= 2.0% kiwi peel extract; T-3= 2.5 % kiwi peel extract.
*Mean±S.E. with different superscripts row wise (capital alphabets) and column wise (small alphabets) differ significantly ($P<0.05$).

for all the treated samples throughout the storage period. This could be attributed to the fact of the development of the metmyoglobin brown pigment (Lin *et al.*, 2015).

Oxidative quality parameters : TBARS values followed a significant ($P<0.05$) increasing trend throughout from Day 1 to 9 for all the treatments as well as control chicken emulsion (Table 1). TBARS value was observed lowest in T-3 and highest in T-1 among treatments on 5th day of storage and later followed a similar trend throughout the storage. An increasing trend in FFA values was observed in both control and treatment emulsions during storage of 9 days but increase in value of FFA in all treatments is comparatively lower ($P<0.05$) than control throughout the storage period which might be due to strong antioxidant activity of KPE extract due to the presence of polyphenols. Among the treatments, T-3 showed the lowest FFA value. Peroxide value (PV) of chicken emulsion followed the similar significantly increasing trend as that of TBARS values and FFA values for control and treatments, but the value was significantly ($P<0.05$) higher in control than all the other treatments throughout storage period of 9 days (Table 1). The higher oxidative stability of treatments added with KPE as compered to control samples attributed to natural antioxidant efficacies of kiwi peel extracts.

CONCLUSIONS

It was concluded that as incorporation at the level of 2.5% level of kiwi peel extract in the spent hen chicken emulsion lead to the improvement in the various physico-chemical qualities, antioxidant potential and sensory quality during 9 days refrigerated storage.

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