



# Antimicrobial and Antioxidant Potential of Papain Liver Hydrolysate in Meat Emulsion Model at Chilling Storage Under Aerobic Packaging Condition

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## Abstract

**Purpose** Meat byproduct is rich source protein for hydrolysis. Pork liver is low value byproduct obtained from meat industry. Therefore, utilization of low value slaughterhouse byproduct enhances the income of meat industry, reduces the cost of disposal and environmental pollution.

**Methods** Physico-chemicals, colour profiles, lipid oxidation, the antioxidant and antimicrobial potential of liver hydrolysate (at three levels T1 = 0.03, T2 = 0.06 and T3 = 0.09% w/w meat emulsion) were comparatively investigated with control (without hydrolysate C = 0) and positive control (PC (BHT = 0.02% w/w meat emulsion)) in meat emulsion. Samples were stored at  $4 \pm 1$  °C under aerobic packaging condition and were drawn at 2 days intervals for analysis.

**Results** Results revealed that pH values increased significantly ( $P < 0.05$ ) however, water activity, extract release volume and emulsion stability decreased significantly through storage. The sample having a higher concentration of hydrolysate significantly ( $P < 0.05$ ) showed higher antioxidant activity except BHT treated sample. Comparatively lower lipid oxidation and colour profile deterioration were recorded in PC and T3 than other groups. Meat emulsion prepared with the addition of 0.09% liver hydrolysate exhibited significantly ( $P < 0.05$ ) higher antimicrobial activities (SPC, Coliforms and, Yeast and mould) than another sample during storage. Meat emulsion treated with hydrolysate also examined for the microbial challenge test for *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli*, and *Bacillus cereus* microbes showed comparatively lower microbial proliferation than control during refrigerated storage.

**Conclusion** Therefore, liver protein hydrolysate maybe used as natural preservatives having improved antioxidant and antimicrobial activities for the shelf-life enhancement of meat products.

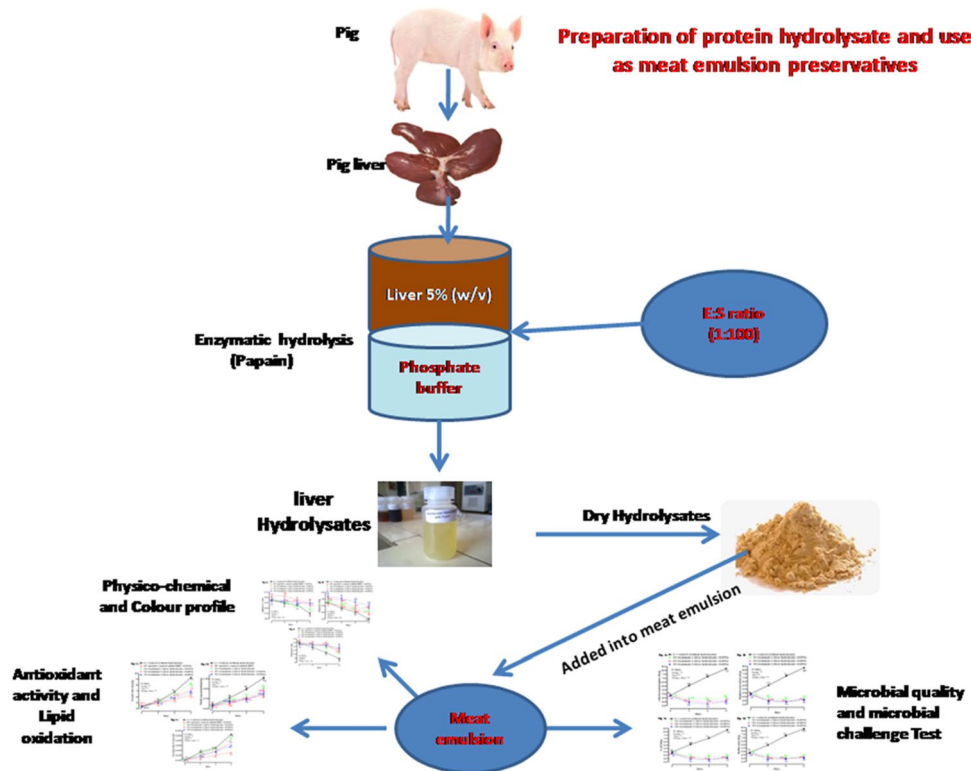
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## Graphic Abstract



**Keywords** Liver · Papain · Hydrolysate · Lipid oxidation · Microbial quality

## Statement of Novelty

Slaughterhouse, low-value, by-product hydrolysate was used as natural preservative in meat model system. Limited studies on byproduct hydrolysates in meat model system and its effect on quality attributes encouraged us to envisage physico-chemical properties, antioxidant activity, lipid oxidation, colour profiles, microbial quality and microbial challenge test of porcine liver hydrolysate in meat emulsion. Outcomes of the study revealed that hydrolysate obtained from by-products of meat industry may become natural source of food preservative and its efficient utilization may also enhance economic returns and reduce the cost of waste disposal.

## Introduction

Most common cause of meat and meat products deterioration is the microbial and lipid oxidation. Mincing of meat leads to the disintegration of the cellular membrane of muscle and facilitates the interaction of pro-oxidant as well as

oxygen molecule with fatty acids. Food spoilage microbes and food-borne pathogens that are survived to refrigerated temperature may also equally responsible for quality deterioration of meat and meat products. Oxidation of lipid and protein molecules is continues process with a cascade of radical reactions that entail with initiation, propagation, and termination process with concurrent generation of free radicals. Oxidative and microbial deterioration leads to the formation of the large quantity of smaller molecular compounds and some oxidative compounds are often volatile in nature. These oxidative products are finally accountable for the development of a rancid flavour and decreased the acceptability of the products. Extensive oxidation of the meat and meat products may produce very harmful products that may have mutagenic and carcinogenic potential [1] which is a serious health concern.

Quality deterioration of the meat and meat products may be retarded or prevented by inclusion of antimicrobial and antioxidants compounds in optimum concentration that prevent the growth of microbes as well stabilize/or slow down formation of free radicals/propagation of oxidative reactions. The free radicals are highly unstable compounds and react with antioxidants frequently possessing an aromatic

ring configuration in the way sequestering them and making free radicals unavailable for series of reaction with the unsaturated fatty acids. The series of free radical reaction terminated by the inclusion of potential anti-oxidative compounds in meat and meat products.

Alongside conventional preservation approach such as chilling, freezing, salting, drying, synthetic/natural preservative also increased their shelf life. Studies on the exploit of natural preservatives for meat products have been carry out and efforts are persistently being made to find out potential natural preservatives. The preservation of highly perishable meat products by natural preservatives is an imperative concern in terms of protecting consumer health and reducing losses. Today's consumers are health conscious and demand nutritious, synthetic additive-free, ready-to-cook, and longer keeping quality of foods. These demands of customer create interest on researchers to explore alternative preservative substances which is safe and healthy for consumer. Protein hydrolysate in general have gained much interest as preservative substance for food due to its functional activity, potential health benefits and prolonging shelf life of food. Recently few protein hydrolysates from meat and marine byproducts have been studied as antioxidant and antimicrobial agents in in-vitro condition such as eel By-Products [2], tuna liver [3] and liver hydrolysates [4]. Therefore, in this study we want to explore liver protein hydrolysate as natural preservative in meat emulsion at refrigeration temperature.

## Material and Methods

### Chemicals and Enzyme

Reagents used in this study like 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were procured from Sigma–Aldrich. 2, 4, 6-tripyridyl-s-triazine (TPTZ) and enzyme papain were procured from MP Biomedical, phenozoniummetho sulphate (PMS), NADH, and Nitro blue tetrazolium (NBT) were purchased from S. D. Fine Chemicals. Microbial culture used for the microbial challenge test viz. *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 2991), *Bacillus cereus* (MTCC 6728), and *Listeria monocytogenes* (MTCC 657) were procured from the Institute of Microbial Technology, Chandigarh, India. Bacteriological media and other reagents (analytical grade) used for exploration in this study were procured from standard firms.

### Liver Hydrolysate of Liver and Preparation of Meat Emulsion

Clean porcine liver minced and takes 5 g in 100 mL of phosphate buffer (pH 6.5) followed by homogenization for proper

mixing [5]. The homogenized porcine liver was heated in a water bath at  $100 \pm 2$  °C for 5 min destroyed the activity of native enzymes present in the liver. Cooled the sample up to °C followed by addition of the enzyme (substrate: enzyme ratio = 100:1) and the sample was kept at 50 °C in the water bath for hydrolysis of native protein of liver up to 6 h. After the completion of hydrolysis, process samples were heated at 85 °C for 15 min to inactivation of residual enzyme in the solution followed by cooling the sample. The liver hydrolysate was centrifuged at  $4 \pm 1$  °C at  $11,200 \times g$  rpm for 25 min for separation. Supernatant was collect and dried in a vacuum oven at 600 mm Hg vacuum at  $50 \pm 2$  °C overnight. Recovered dried liver hydrolysate was milled to make fine powder kept in vials at  $-20$  °C for further use.

Prior to the mincing of meat (pork) connective tissue and fascia was removed from meat and thawed overnight at  $4 \pm 1$  °C. Meat was cut into the smaller cubes for mincing and minced twice through 6 mm plate. A separate batch of meat emulsion was prepared with meat (83.5%), salt (1.5%), water (5%) and refined oil (10%). Three levels of liver hydrolysate at 0.03% (T1), 0.06% (T2), and 0.09% (T3) on w/w meat emulsion basis were analyzed in comparison with control (no liver hydrolysate = C), 0.02% w/w butylhydroxyl toluene of meat emulsion (PC = positive control). The emulsion was prepared as per the method described by [6]. Each group of emulsion were packed in aerobic packaging condition in LDPE (low-density polyethylene, 200 gauge) and stored at  $4 \pm 1$  °C for further analysis up to 6 days and analysed for physico-chemicals, colour profiles, anti-oxidant activity, lipid oxidation and microbial quality viz. MCT for (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes*) and aerobic plate count (APC), E. coli count, and yeast and moulds count.

### Technique used for Determination of Physico-Chemical Qualities

Firstly 10 g meat emulsion was properly homogenized in 100 mL distilled water and pH values were measured with a digital pH meter. Water activity of meat emulsion was measured at 25 °C with Rotronix digital portable water activity meter. Emulsion stability (ES) analysis was estimated with the method described by [7]. Extract release volume (ERV) of meat emulsion was determined as per the method described by [8].

### Antioxidant Attributes

#### DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of meat emulsion was assayed following the method of [9]. Take 1 mL (freshly prepared) of DPPH (100 µM) in eppendorf tubes and blend

with 0.25 mL of Tris–HCl (0.1 M) followed by the addition of 25  $\mu$ L sample solution. The absorbance of the sample was recorded at (time  $t=0$  min ( $t_0$ )) at 517 nm with a multimode reader. Samples were incubated for 20 min ( $t_{20}$ ) in a dark place followed by absorbance was recorded at the same wavelength and ethanol was taken as blank.

$$\text{DPPH Scavenging activity (\% inhibition)} = 100 - \left[ \frac{A_{t_{20}}}{A_{t_0}} \times 100 \right]$$

### ABTS<sup>+</sup> Radical Scavenging Activity

This assay was determined by spectrophotometric method as per procedure given by [10]. The ABTS<sup>+</sup> stock solution (fresh) was prepared in distilled water to a 7 mM concentration prior to estimation scavenging activity of ABTS<sup>+</sup> solution was mixed with equivalent quantity of potassium persulphate (2.45 mM) solution and incubated in dark place for 16 h. Before use stock solution ABTS<sup>+</sup> was diluted with distilled water and absorbance was adjusted at 0.70 at 734 nm. For the determination of radical scavenging activity take 1 mL of ABTS<sup>+</sup> working solution followed by mixed with 10  $\mu$ L of taster solution and kept in dark place for 20 min and reading was measured at 734 nm with multimode reader.

$$\text{ABTS}^+\text{activity (\% inhibition)} = \frac{0.7 - A_{t_{20}}}{0.7} \times 100$$

### FRAP and SASA Assay

The FRAP assay was determined according to the procedure described by [11]. Freshly prepared 900  $\mu$ L of FRAP solution was mixed with 300 mM acetate buffer, pH 3.6: 20 mM ferric chloride: 10 mM TPTZ in 40 mM hydrochloric acid: 10:1:1. Followed by the addition of 100  $\mu$ L of taster solution and was kept under the dark condition at 37 °C for 40 min. Absorbance was measured at 593 nm. Method described with [12] was followed for the estimation of SASA.

### Lipid Oxidation Indices

Lipid oxidative parameter of meat emulsion i.e. Peroxide value and free fatty acid values were estimated as per procedure illustrated by [13] with little changes. Whereas, for the determination of TBARS value in meat emulsion was followed the method illustrated by [14].

### Meat Emulsion Colour Profiles

Instrumental colour profiles of the meat emulsion was measured with the Lovibond Tintometer (Model: RT-300) at 2° of cool white light ( $D_{65}$ ). Prior to the measure of colour

profiles (lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ )) the equipment was calibrated with black and white standard.

### Microbiological Quality of Meat Emulsion

Microbial quality of meat emulsion stored at refrigerated temperature was determined for the Total Plate Counts, Coliforms count, *Bacillus cereus*, *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and Yeasts and Mold counts) as per modus operendi described by [15]. Total microbial counts in each group was calculated with counted colonies multiply with the inverse of dilution factor and presented as log cfu/g. Microbial challenge test for *Bacillus cereus*, *E. coli*, *Staphylococcus aureus* and *Listeria monocytogenes* was determined as per methodology previously illustrated by [16]. Microbial concentration for the inoculum dose ( $10^4$ – $10^5$  cfu/g) was optimized as procedure described by [6]. For the study of MCT against these microbes separate batch of meat emulsion was formulated and packed in aerobic packaging and stored at  $4 \pm 1$  °C for analysis.

### Analysis of Data

Three trails (3) were conducted and reading was recorded twice (2) for each attributes ( $n=(3 \times 2=6)$ ). Analysis of data was carried out by using Two-way analysis of variance as groups and storage time as a fixed factors with general linear model of SPSS (Statistical Package for the Social Sciences) package (SPSS 17.0 for Windows, IBM SPSS Inc, Chicago, Ill, USA) and consequences of the analysis have been illustrated in the tabular and graphical forms. Means of attributes were also analyzed using Duncan's multiple range tests, at  $P < 0.05$ .

## Results and Discussion

### Change in Physico-Chemical Quality of Meat Emulsion Prepared with Liver Protein Hydrolysates

The different physico-chemical parameters of pork emulsion incorporated with protein hydrolysate as well as control and positive control have been arranged in tabular form as Table 1.

The pH of pork emulsion did not differ significantly ( $P > 0.05$ ) among the groups on 0 and 2nd day of storage. However, pH values varied significantly ( $P < 0.05$ ) for treatments than control on 6th day of storage. The difference in pH values might be due to the inclusion of liver protein hydrolysate. The pH value of meat emulsion increased significantly ( $P < 0.05$ ) among all groups except T3 with storage. Results evinced that the pH remained more stable in T2 and T3 than control, PC and

**Table 1** Effect of incorporation of different levels of porcine liver protein hydrolysates (Papain) on physico-chemical quality parameters of pork emulsion during refrigerated aerobic storage (Mean  $\pm$  S.E.)

Groups	0 day	2 day	4 day	6 day	P-values		
					Group (G)	Day (D)	G $\times$ D
<b>pH</b>							
C	5.95 $\pm$ 0.03 <sup>Aa</sup>	6.02 $\pm$ 0.03 <sup>Ab</sup>	6.18 $\pm$ 0.02 <sup>Cc</sup>	6.24 $\pm$ 0.01 <sup>Cd</sup>			
PC	5.92 $\pm$ 0.03 <sup>Aa</sup>	5.98 $\pm$ 0.02 <sup>Ab</sup>	6.15 $\pm$ 0.03 <sup>Cc</sup>	6.22 $\pm$ 0.03 <sup>Cd</sup>			
T1	5.91 $\pm$ 0.02 <sup>Aa</sup>	5.96 $\pm$ 0.03 <sup>Ab</sup>	6.07 $\pm$ 0.03 <sup>Bc</sup>	6.15 $\pm$ 0.02 <sup>Bd</sup>	**	**	*
T2	5.92 $\pm$ 0.04 <sup>Aa</sup>	5.95 $\pm$ 0.02 <sup>Aa</sup>	6.01 $\pm$ 0.02 <sup>Ab</sup>	6.11 $\pm$ 0.01 <sup>Bc</sup>			
T3	5.98 $\pm$ 0.02 <sup>Aa</sup>	5.96 $\pm$ 0.03 <sup>Aa</sup>	6.02 $\pm$ 0.02 <sup>Aa</sup>	6.04 $\pm$ 0.03 <sup>Aa</sup>			
<b>Water activity (<math>a_w</math>)</b>							
C	0.942 $\pm$ 0.002 <sup>Ad</sup>	0.933 $\pm$ 0.002 <sup>Ac</sup>	0.918 $\pm$ 0.002 <sup>Ab</sup>	0.909 $\pm$ 0.002 <sup>Aa</sup>			
PC	0.939 $\pm$ 0.001 <sup>Ad</sup>	0.935 $\pm$ 0.002 <sup>ABc</sup>	0.922 $\pm$ 0.003 <sup>Ab</sup>	0.913 $\pm$ 0.001 <sup>Aa</sup>			
T1	0.942 $\pm$ 0.004 <sup>Ab</sup>	0.935 $\pm$ 0.001 <sup>ABb</sup>	0.918 $\pm$ 0.002 <sup>Aa</sup>	0.911 $\pm$ 0.002 <sup>Aa</sup>	**	**	*
T2	0.943 $\pm$ 0.003 <sup>Ac</sup>	0.938 $\pm$ 0.001 <sup>Bc</sup>	0.932 $\pm$ 0.001 <sup>Bb</sup>	0.924 $\pm$ 0.002 <sup>Ba</sup>			
T3	0.945 $\pm$ 0.001 <sup>Ad</sup>	0.940 $\pm$ 0.002 <sup>Bc</sup>	0.933 $\pm$ 0.001 <sup>Bb</sup>	0.925 $\pm$ 0.001 <sup>Ba</sup>			
<b>Extract release volume (mL)</b>							
C	44.83 $\pm$ 1.08 <sup>Ad</sup>	41.83 $\pm$ 1.01 <sup>Ac</sup>	38.66 $\pm$ 1.09 <sup>Ab</sup>	35.75 $\pm$ 0.63 <sup>Aa</sup>			
PC	45.16 $\pm$ 0.83 <sup>Ac</sup>	43.08 $\pm$ 1.00 <sup>Abc</sup>	41.58 $\pm$ 0.69 <sup>Aab</sup>	40.50 $\pm$ 0.76 <sup>Ca</sup>			
T1	45.58 $\pm$ 1.08 <sup>Ac</sup>	42.33 $\pm$ 0.70 <sup>Ab</sup>	39.58 $\pm$ 1.37 <sup>Aab</sup>	37.58 $\pm$ 0.82 <sup>ABa</sup>	**	**	NS
T2	46.50 $\pm$ 0.99 <sup>Ac</sup>	42.83 $\pm$ 0.98 <sup>Ab</sup>	40.16 $\pm$ 0.98 <sup>Aab</sup>	38.83 $\pm$ 1.19 <sup>BCa</sup>			
T3	45.66 $\pm$ 0.71 <sup>Ac</sup>	43.66 $\pm$ 1.80 <sup>Abc</sup>	41.16 $\pm$ 1.80 <sup>Aab</sup>	39.58 $\pm$ 0.78 <sup>Ca</sup>			
<b>Emulsion stability (%)</b>							
C	73.43 $\pm$ 0.87 <sup>Ac</sup>	71.74 $\pm$ 0.75 <sup>Ac</sup>	68.89 $\pm$ 0.49 <sup>Ab</sup>	66.47 $\pm$ 0.73 <sup>Aa</sup>			
PC	73.17 $\pm$ 0.67 <sup>Ab</sup>	71.65 $\pm$ 0.83 <sup>Ab</sup>	70.53 $\pm$ 0.87 <sup>ABb</sup>	67.72 $\pm$ 0.90 <sup>ABa</sup>			
T1	74.62 $\pm$ 0.99 <sup>Ab</sup>	73.26 $\pm$ 1.38 <sup>ABb</sup>	72.26 $\pm$ 1.14 <sup>Bab</sup>	69.65 $\pm$ 0.55 <sup>BCa</sup>	**	**	NS
T2	75.30 $\pm$ 0.56 <sup>Ac</sup>	74.69 $\pm$ 0.55 <sup>Bc</sup>	72.45 $\pm$ 0.68 <sup>Bb</sup>	70.04 $\pm$ 0.67 <sup>Ca</sup>			
T3	75.47 $\pm$ 0.55 <sup>Ac</sup>	74.01 $\pm$ 0.71 <sup>ABbc</sup>	72.59 $\pm$ 0.76 <sup>Bab</sup>	71.45 $\pm$ 0.53 <sup>Ca</sup>			

Means with different superscripts (small letters in the same row and capital letters in the same column) indicate significant difference ( $P < 0.05$ ),  $n = 6$ ; P-value is the observed significance level of the F-test for Group, Day and  $G \times D$ , \*P-Value:  $< 0.05$ , \*\*P-Value:  $< 0.001$  and NS non significant. C control (without hydrolysate), PC positive control (added BHT = 0.02%), T1 treatment-1 (liver hydrolysate = 0.03%), T2 treatment-2 (liver hydrolysate = 0.06%), T3 treatment-3 (liver hydrolysate = 0.09%)

T1 throughout the storage. The increase in pH with storage might be due to the growth of aerobic micro-organism in meat emulsion, presence of bacterial metabolites and deamination of meat proteins [17]. Aksu and Kaya [18] also reported that ammoniacal compounds are formed during proteolysis process which might contribute to rise in pH during storage. Similar trends for the pH values also observed by [19] during the refrigerated storage of Pork Patties.

Water activity of pork emulsion remained comparable on day of processing however, it varied significantly ( $P < 0.05$ ) across all groups on 2nd, 4th and 6th day of storage. The difference in  $a_w$  values among groups might be due to the addition of various level of liver protein hydrolysate and variation in microbial load during storage. Water activity value decreased with the progression of storage as shown in (Table 1). However, water activity was higher in treatment group attributed to antimicrobial action of liver protein hydrolysate and retention of higher moisture due to

interaction between protein hydrolysate and water molecule in treated group.

The ERV values differ significantly ( $P < 0.05$ ) among groups on 6th day of storage. Results revealed that ERV value followed a decreasing trend among groups; however it was better maintained in treatments than control. This might be due to lower protein break down and lipid oxidation molecules in treatment groups than control. Jay et al. [20] also reported decrease in ERV with the increase in spoilage of meat. Similar results were also noticed by [21] for pork stored at refrigeration. Ingram and Dainty [22] observed that hydration and increase in pH during storage, as well as generation of amino sugar by spoilage microbes in food [21] is responsible for decrease in ERV. The ERV values also decreased in this study throughout the storage period, although the values of ERV remained within the recommended limit of 17 ml [23]. Anandh [24] has also postulated that the increase in microbial growth has negative correlation with ERV.

Emulsion stability of liver protein hydrolysate incorporated samples was higher than control. It decreased significantly ( $P < 0.05$ ) among all groups during storage. However, ES was better maintained in treatment group than control. Protein hydrolysate might have formed protective film around lipid molecule in treated samples. Presence of hydrophilic and hydrophobic peptides in hydrolysate may have also interacted with water and fat molecule in meat matrix system, which prevented leaching of lipid and water molecule during cooking. The decrease in emulsion stability with storage might be due to microbial breakdown of protein, enzymatic and non-enzymatic oxidation of lipid molecules. Verma et al. [25] also reported that addition of meat hydrolysates in meat batter decreased the surface tension and acts as emulsifier therefore, it reduced the formation of fat pockets decreased the loss of water and fat during cooking as well as increase the quality of products.

## Antioxidant Parameters of Meat Emulsion Prepared with Liver Protein Hydrolysates

Pork emulsion was used as medium for *in-vivo* investigation of antioxidant activity of porcine liver protein hydrolysate. The antioxidant parameters used in this study were ABTS, DPPH, FRAP and SASA and the data obtained were statistically analysed and are presented in Table 2.

ABTS<sup>+</sup>% inhibition was significantly higher ( $P < 0.05$ ) in PC followed by T3 > T2 > T1 > control and similar pattern was observed among groups throughout storage. Results as expressed in (Table 2) revealed that ABTS<sup>+</sup>% inhibition significantly ( $P < 0.05$ ) decreased throughout storage across all samples. In our study the ABTS<sup>+</sup>% inhibition increased in concentration dependent manner among hydrolysate incorporated samples, similar to results reported previously by [26]. Davies [27] reported that antioxidative peptides are donates electron and terminate the cascade of formation of free radicals. The antioxidant activity of the peptides varied with types and hydrophobicity nature of the amino acid,

**Table 2** Effect of incorporation of different levels of porcine liver protein hydrolysates (Papain) on antioxidant parameters of pork emulsion during refrigerated aerobic storage (Mean  $\pm$  S.E.)

Groups	0 day	2 day	4 day	6 day	P-values		
					Group (G)	Day (D)	G $\times$ D
ABTS (% inhibition)							
C	55.05 $\pm$ 0.24 <sup>Ad</sup>	45.37 $\pm$ 0.23 <sup>Ac</sup>	40.11 $\pm$ 0.17 <sup>Ab</sup>	31.24 $\pm$ 0.34 <sup>Aa</sup>			
PC	87.21 $\pm$ 0.23 <sup>Ed</sup>	85.30 $\pm$ 0.36 <sup>Ec</sup>	81.33 $\pm$ 0.36 <sup>Db</sup>	78.76 $\pm$ 0.37 <sup>Da</sup>			
T1	77.97 $\pm$ 0.19 <sup>Bc</sup>	76.73 $\pm$ 0.21 <sup>Bb</sup>	76.04 $\pm$ 0.21 <sup>Ba</sup>	75.57 $\pm$ 0.16 <sup>Ba</sup>	**	**	**
T2	81.47 $\pm$ 0.13 <sup>Cd</sup>	79.61 $\pm$ 0.16 <sup>Cc</sup>	78.47 $\pm$ 0.15 <sup>Cb</sup>	76.47 $\pm$ 0.15 <sup>Ca</sup>			
T3	84.95 $\pm$ 0.09 <sup>Dd</sup>	80.57 $\pm$ 0.10 <sup>Dc</sup>	78.59 $\pm$ 0.23 <sup>Cb</sup>	76.23 $\pm$ 0.17 <sup>Ca</sup>			
DPPH (% inhibition)							
C	15.25 $\pm$ 0.14 <sup>Ad</sup>	12.04 $\pm$ 0.12 <sup>Ac</sup>	9.43 $\pm$ 0.32 <sup>Ab</sup>	6.41 $\pm$ 0.12 <sup>Aa</sup>			
PC	42.42 $\pm$ 0.31 <sup>Dc</sup>	41.69 $\pm$ 0.42 <sup>Ec</sup>	37.82 $\pm$ 0.32 <sup>Eb</sup>	34.17 $\pm$ 0.36 <sup>Ea</sup>			
T1	30.46 $\pm$ 2.59 <sup>Bc</sup>	27.30 $\pm$ 0.48 <sup>Bbc</sup>	25.31 $\pm$ 0.28 <sup>Bab</sup>	22.52 $\pm$ 0.62 <sup>Ba</sup>	**	**	NS
T2	33.84 $\pm$ 0.58 <sup>BCd</sup>	30.49 $\pm$ 0.41 <sup>Cc</sup>	29.28 $\pm$ 0.26 <sup>Cb</sup>	25.43 $\pm$ 0.23 <sup>Ca</sup>			
T3	36.16 $\pm$ 0.15 <sup>Cd</sup>	33.28 $\pm$ 0.36 <sup>Dc</sup>	31.05 $\pm$ 0.40 <sup>Db</sup>	28.59 $\pm$ 0.56 <sup>Da</sup>			
SASA (% inhibition)							
C	22.52 $\pm$ 0.24 <sup>Ad</sup>	18.04 $\pm$ 0.48 <sup>Ac</sup>	13.89 $\pm$ 0.26 <sup>Ab</sup>	10.77 $\pm$ 0.43 <sup>Aa</sup>			
PC	29.21 $\pm$ 0.57 <sup>Bc</sup>	27.79 $\pm$ 0.56 <sup>Bbc</sup>	26.19 $\pm$ 0.53 <sup>Bb</sup>	24.36 $\pm$ 0.54 <sup>Ba</sup>			
T1	57.86 $\pm$ 0.29 <sup>Cd</sup>	45.02 $\pm$ 0.22 <sup>Cc</sup>	41.44 $\pm$ 0.36 <sup>Cb</sup>	39.14 $\pm$ 0.52 <sup>Ca</sup>	**	**	**
T2	65.50 $\pm$ 0.19 <sup>Dd</sup>	63.54 $\pm$ 0.34 <sup>Dc</sup>	60.04 $\pm$ 0.71 <sup>Db</sup>	54.31 $\pm$ 0.29 <sup>Da</sup>			
T3	66.81 $\pm$ 0.34 <sup>Ed</sup>	64.82 $\pm$ 0.18 <sup>Ec</sup>	61.68 $\pm$ 0.16 <sup>Eb</sup>	58.02 $\pm$ 0.51 <sup>Ea</sup>			
FRAP (mM equivalent to FeSO <sub>4</sub> ·7H <sub>2</sub> O)							
C	10.41 $\pm$ 0.25 <sup>Ad</sup>	8.28 $\pm$ 0.18 <sup>Ac</sup>	7.32 $\pm$ 0.16 <sup>Ab</sup>	6.38 $\pm$ 0.14 <sup>Aa</sup>			
PC	18.99 $\pm$ 0.24 <sup>Bd</sup>	17.86 $\pm$ 0.18 <sup>Bc</sup>	17.22 $\pm$ 0.05 <sup>Bb</sup>	15.65 $\pm$ 0.12 <sup>Ba</sup>			
T1	19.52 $\pm$ 0.11 <sup>Cd</sup>	18.21 $\pm$ 0.08 <sup>Cc</sup>	17.29 $\pm$ 0.07 <sup>Bb</sup>	15.75 $\pm$ 0.23 <sup>Ba</sup>	**	**	**
T2	19.98 $\pm$ 0.06 <sup>Dd</sup>	18.80 $\pm$ 0.05 <sup>Dc</sup>	17.98 $\pm$ 0.08 <sup>Cb</sup>	16.84 $\pm$ 0.20 <sup>Ca</sup>			
T3	21.57 $\pm$ 0.18 <sup>Ed</sup>	19.24 $\pm$ 0.06 <sup>Ec</sup>	18.72 $\pm$ 0.06 <sup>Db</sup>	17.30 $\pm$ 0.18 <sup>Ca</sup>			

Means with different superscripts (small letters in the same row and capital letters in the same column) indicate significant difference ( $P < 0.05$ ),  $n = 6$ ; P-value is the observed significance level of the F-test for Group, Day and G  $\times$  D, \*\*P-Value:  $< 0.001$  and NS non significant. C control (without hydrolysate), PC positive control (added BHT = 0.02%), T1 treatment-1 (liver hydrolysate = 0.03%), T2 treatment-2 (liver hydrolysate = 0.06%), T3 treatment-3 (liver hydrolysate = 0.09%)

their location in sequence of peptide plays major roles in efficacy of the antioxidant activity of peptides. The ABTS activity of protein hydrolysate might be due to effective proton or electron donors and /or metal sequestering activity that could react with unstable free radicals and changed into a stable form as well as terminate further radical formation.

As shown in Table 2, the DPPH scavenging activities among groups varied significantly ( $P < 0.05$ ). Positive control and T3 showed the highest DPPH activities by scavenging 42.42% and 36.16% DPPH in meat emulsion on 0 day of storage; whereas control had the lowest activity quenching only 15.25% of the radicals. However, DPPH radical scavenging activity decreased significantly ( $P < 0.05$ ) among all groups with storage as expected. The hydrolysate concentration has been shown to have a dose-dependent effect on the DPPH radical scavenging activities in meat model system. Similarly, Je et al. [3] also reported that DPPH values increased with increase in the concentration of hydrolysate for the tuna liver hydrolysate. Results were in accordance with the findings of [28] for tannery fleshings hydrolysate. The DPPH activities of hydrolysate incorporated samples were higher than control, which might be due to the presence of antioxidant peptides/free amino acid residues, which have ability to react with oxidants. Je et al. [3] reported that tuna liver hydrolysate showed 90% DPPH scavenging activities at 5 mg/ml.

The SASA (%) activity was significantly higher ( $P < 0.05$ ) in T3 and followed a decreasing pattern for others  $T2 > T1 > PC > C$  and similar trend was evident throughout entire storage. Among all groups, T3 retained significantly ( $P < 0.05$ ) highest SASA (%) inhibition and control the lowest on 6th day of storage. In general across all groups SASA% inhibition significantly ( $P < 0.05$ ) decreased during storage. Similarly, Sakanaka and Tachibana [29] estimated SASA of egg-yolk protein hydrolysates and postulated their activities as dose-dependent. The present result implies that liver protein hydrolysate has strong superoxide scavenging activity and may contribute to its antioxidant property. These results were in harmony with findings of [30] for blood hydrolysate and [31] for chicken skin hydrolysate showed antioxidant activity of hydrolysate significant increase with increased the levels of protein hydrolysate.

Antioxidant activity, based on FRAP assay, was observed in porcine liver protein hydrolysate included pork emulsion as shown in (Table 2). The FRAP activity varied significantly ( $P < 0.05$ ) among groups during storage. However, highest FRAP assay was observed for T3 and lowest for control throughout storage. FRAP values decreased significantly ( $P < 0.05$ ) among all groups with storage. Hydrolysate incorporated samples had higher FRAP activity that might be due to presence of more peptides/free amino acids, so it have more reducing power for converting ferric to ferrous ion. Young et al. [32] also reported that antioxidant activity

of protein hydrolysate was primarily attributed to the ability of chelating ion such as cobalt, copper and zinc.

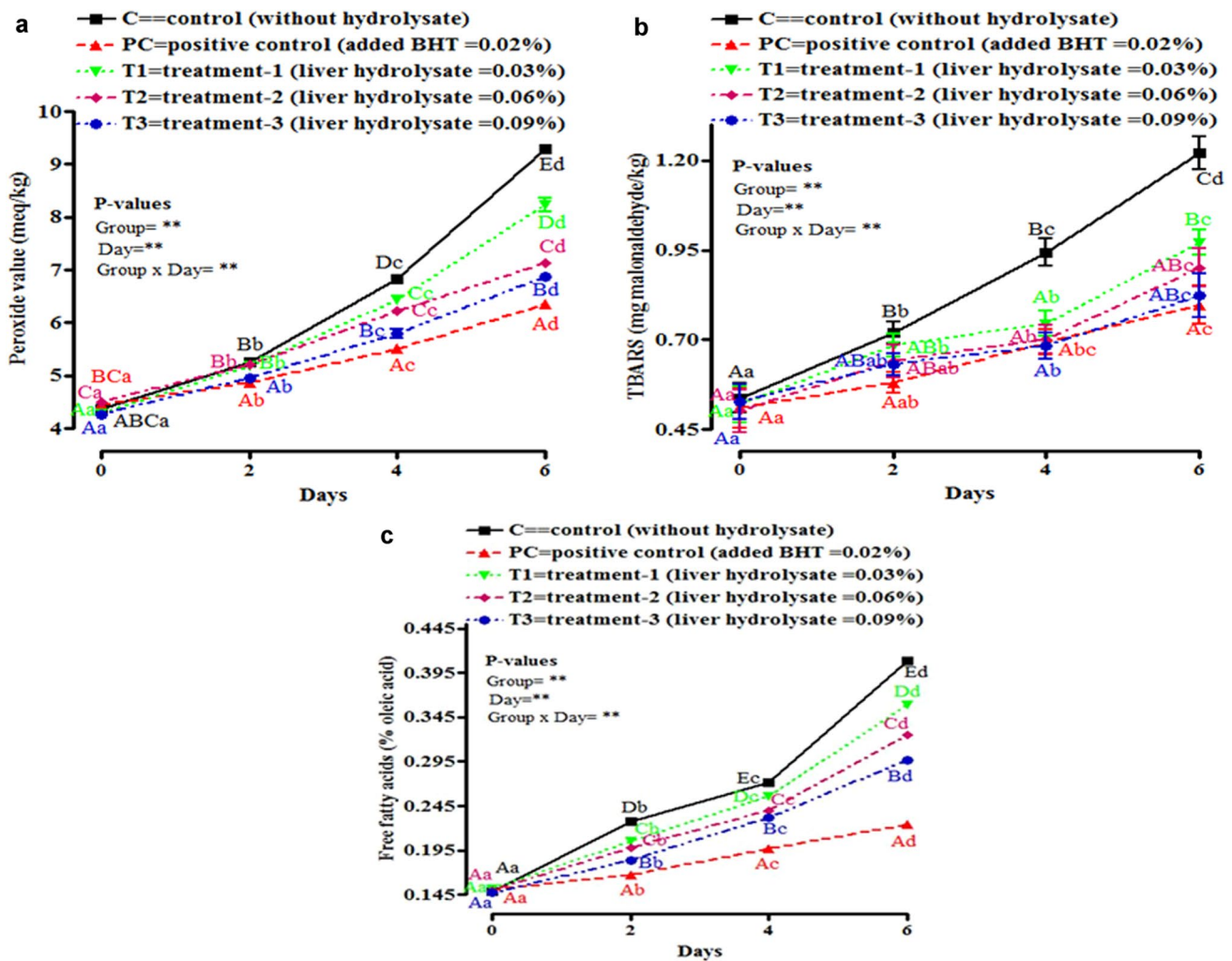
### Lipid Peroxidation Parameters of Meat Emulsion Prepared with Liver Protein Hydrolysates

The parameters such as PV (meq/kg sample), TBARS (mg malonaldehyde/kg) and FFA (% oleic acid) were assessed and statistically analyzed data are presented in (Fig. 1a–c).

Peroxide values varied significantly ( $P < 0.05$ ) among groups with storage. Peroxide values increased from  $4.36 \pm 0.06$  to  $9.30 \pm 0.07$  meq/kg in case of control and  $4.27 \pm 0.06$  to  $6.88 \pm 0.06$  meq/kg in case of T3 during storage (Fig. 1a). The result evinced that antioxidant efficacy of the liver protein hydrolysate have positive correlation with the level of incorporation of hydrolysate in pork emulsion. The antioxidant activity of the protein hydrolysate can be attributed to the presence of radical scavenging peptides and metal chelating properties of peptides/free amino acids. Kawashima et al. [33] noted that some di- and tri-peptides containing aromatic amino acid residues, as well as peptides containing Tyr, Pro and His, showed strong antioxidant activity. Various anti-oxidative peptides have also been identified from variety of food proteins, such as royal jelly protein [1] and egg yolk [34].

Perusal of TBARS results, it is evident that there was significant ( $P < 0.05$ ) increase in lipid oxidation in pork emulsion, which progressively increased with storage period (Fig. 1b). Among groups PC and T3 showed significantly ( $P < 0.05$ ) lower TBARS than other groups during storage. These observations indicated that PC and T3 had (34.80% and 32.60%) lower TBARS values on last day of storage than control. However, levels and compositions of free amino acids and peptides were reported to determine the antioxidant activities of protein hydrolysates [35]. Bougatef et al. [2] stated that the incorporation of protein hydrolysate at different concentration in to the minced meat decreased the formation of MDA and therefore it may be concluded that protein hydrolysate act as antioxidative substance and decreased the rate of lipid oxidation. Liu et al. [36] also reported 25–40% lower TBARS values for porcine plasma hydrolysate incorporated in liposome model system. Similarly Farvin et al. [37] documented crude cod hydrolysate inhibited 20% TBARS formation.

FFA values varied significantly ( $P < 0.05$ ) among groups from 2nd day of storage, which were lower in treatments and PC than control (Fig. 1c). In general, FFA value increased significantly ( $P < 0.05$ ) among all products with an increase in storage time. The significant ( $P < 0.05$ ) variation in FFA contents amongst all groups might be due to level of oxidation and decomposition of fat by bacterial multiplication as well as enzymatic oxidation. Enzymatic digestion of the native protein with proteolytic enzymes resulted in



**Fig. 1** a–c Effect of incorporation of different levels of porcine liver protein hydrolysates (Papain) on the lipid oxidation parameters of pork emulsion during storage (Mean  $\pm$  S.E.). Means with different superscripts (small letters in the same row and capital letters in the same column) indicate significant difference ( $P < 0.05$ ),  $n = 6$ ;

$P$ -value is the observed significance level of the  $F$ -test for Group, Day and  $G \times D$ , \*\* $P$ -Value:  $< 0.001$ . C control (without hydrolysate), PC positive control (added BHT = 0.02%), T1 treatment-1 (liver hydrolysate = 0.03%), T2 treatment-2 (liver hydrolysate = 0.06%), T3 treatment-3 (liver hydrolysate = 0.09%)

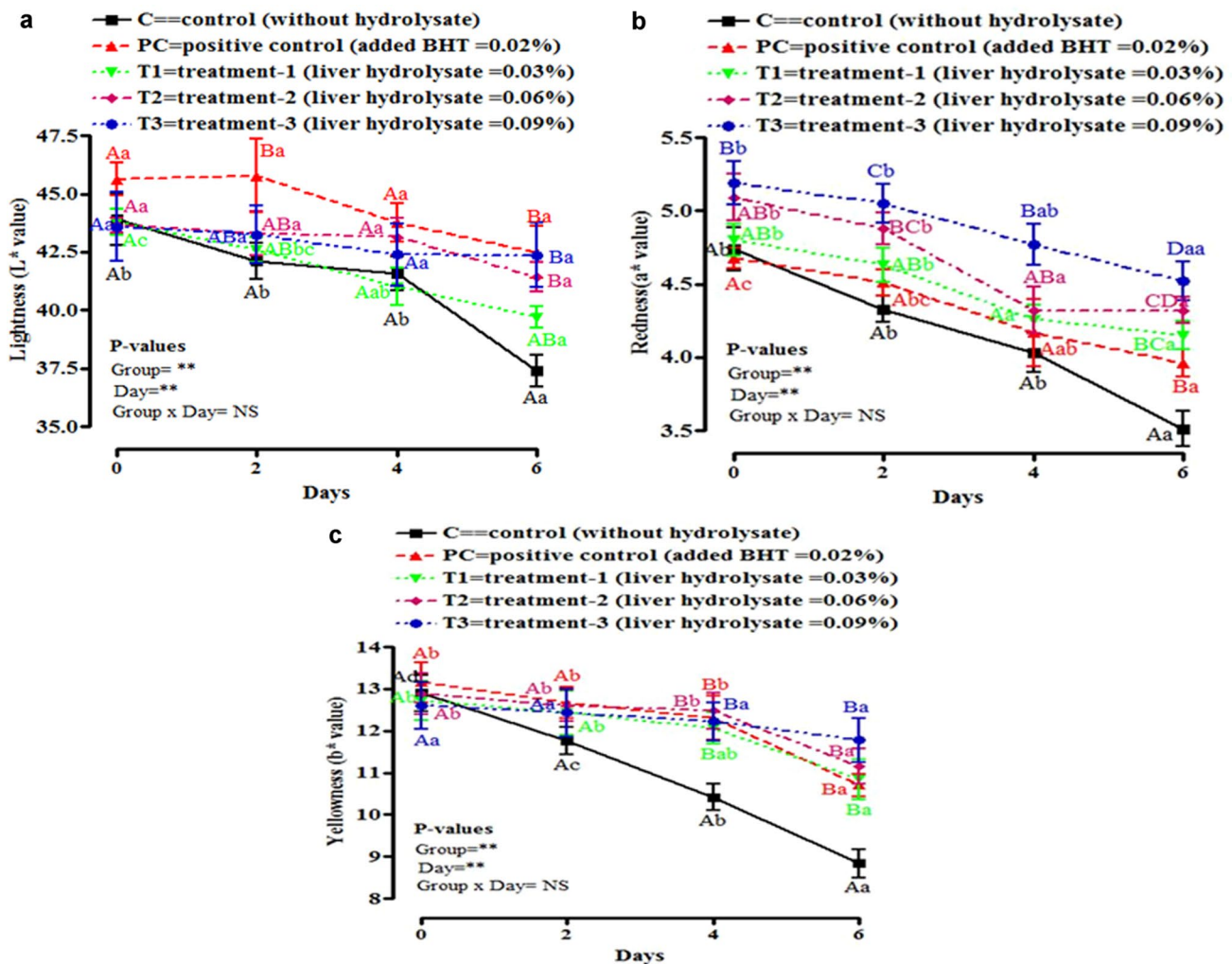
production of the bioactive peptides/free amino acids residues capable of reacting with oxidants. Hirose and Miyashita [38] suggested that besides acting as a free radical scavenger, protein hydrolysate also work as protective membrane around lipid molecule against oxidants. Probably, amphoteric and structurally flexible smaller peptides, hydrolysate fragments could readily diffuse to the water–oil interface, where they would adsorb or loosely bind to the phospholipids membrane in the liposome, site of oxidation.

### Colour Profile of Meat Emulsion Prepared with Liver Protein Hydrolysates

The statistically analyzed data recorded for instrumental colour profile of pork emulsion are presented in Fig. 2a–c.

Instrumental colour profile viz.  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*_{ab}$  and  $H^0_{ab}$  were significantly ( $P < 0.05$ ) affected with the addition of porcine liver protein hydrolysate and their interaction with the meat matrix system. The lightness values ( $L^*$ ) decreased significantly ( $P < 0.05$ ) in control by the end of the storage; however it remained comparable in PC, T2 and T3 during entire storage (Fig. 2a). The  $L^*$  values decreased with slower rate in case of treatments and PC than control, this might be due to the ability of the porcine liver hydrolysate and positive control to maintain the colour of pork emulsion by retarding the oxidation reaction. The decrease in  $L^*$  values also might be due to the innate reddish-brown colour of liver hydrolysate and lipid hydroperoxides interaction with other macromolecules in pork emulsion causing discoloration. Redness values slightly increased in treated





**Fig. 2 a–c** Effect of incorporation of different levels of porcine liver protein hydrolysates (Papain) on the instrumental colour quality parameters of pork emulsion during refrigerated aerobic storage (Mean  $\pm$  S.E.). Means with different superscripts (small letters in the same row and capital letters in the same column) indicate significant difference ( $P < 0.05$ ),  $n = 6$ , P-value is the observed significance level

groups than control and PC (Fig. 2b). Amongst treatments, redness values varied significantly ( $P < 0.05$ ) from control and PC during storage. Higher redness values in hydrolysate incorporated groups might be due to the inherent reddish-brown colour of the liver protein hydrolysate. The increase in redness values in treated group evinced positive correlation with concentration of protein hydrolysate added in pork emulsion. Redness ( $a^*$ ) value decreased significantly ( $P < 0.05$ ) among groups throughout storage. The decrease in redness values might be due to microbial, enzymatic and non-enzymatic lipid and myoglobin oxidation during storage. Similar results were also reported by [39] for mahi-mahi red muscle. However,  $a^*$  value was comparatively higher in treated samples than control throughout storage and the rate

of the F-test for Group, Day and  $G \times D$ , \*\*P-Value:  $< 0.001$  and NS non significant. C control (without hydrolysate), PC positive control (added BHT=0.02%), T1 treatment-1 (liver hydrolysate=0.03%), T2 treatment-2 (liver hydrolysate=0.06%), T3 treatment-3 (liver hydrolysate=0.09%)

of decrease in  $a^*$  value was significantly ( $P < 0.05$ ) lower in treated products than control. Results were in line with the finding of [40] for meat sausages formulated with incorporation of mechanical deboned chicken meat hydrolysates. Yellowness values ( $b^*$ ) were lower in the samples incorporated with liver protein hydrolysate than control and PC (Fig. 2c). Similar results were also reported by [41] in poultry meat batters incorporated with dairy protein hydrolysate. However, throughout storage yellowness values decreased significantly ( $P < 0.05$ ) among all groups except T3. At the end of storage, the  $b^*$  value of the control was significantly ( $P < 0.05$ ) lower as compared to treatments and PC, however  $b^*$  values were better maintained in T3 than control throughout study.

### Changes in Microbiological Quality of Pork Emulsion Treated with Liver Protein Hydrolysates

Standard plate count was lower in treated samples than control and PC (Table 3). SPC values varied significantly ( $P < 0.05$ ) among all samples throughout storage, however in liver protein hydrolysate added samples it decreased upto 2nd day of storage. It reached 6.41 log cfu/g in control and 6.34 log cfu/g in PC pork emulsion on 6th day, while it was only about 4.78 log cfu/g in T1, 4.54 log cfu/g in T2 and 4.42 in T3, respectively. Antimicrobial activity of peptides varied with dose, configuration of peptides and their size as well as molecular weight. Antimicrobial activity of protein hydrolysate might be due to the metal chelation and interaction of peptides with bacterial cell wall/membrane, which led to increase in permeability of bacterial membrane. Similar results were also reported by [42]. They postulated that antimicrobial peptides can directly contact and disrupt the bacterial membrane by permeating lipid bilayers, and ultimately leads to cell death. Lei et al. [43] reported that cationic peptides persuade antimicrobial action via the interruption of membrane integrity and succeeding interface with vital intracellular elements. Coliforms counts remained comparable among groups on day of preparation of meat emulsion. However, coliform counts were lower in treated group than control and PC. Coliforms count decreased in treated

groups, whereas it increased in case of C and PC throughout storage. Antimicrobial activity of protein hydrolysate increased with the level of incorporation of hydrolysate in meat model system. Some antimicrobial peptides were found to interfere with the metabolic processes of microbes. Daoud et al. [44] have also been identified antimicrobial peptide from bovine haemoglobin against various microorganisms. Yeast and mould counts were not detected on 0 day in T2 and upto 2nd days of storage in T3. Yeast and mould counts increased significantly ( $P < 0.05$ ) with storage between control and PC, and were comparable among treatments. This might be due to the antifungal activity of the liver protein hydrolysate. Similarly, Liu et al. [45] also reported anti-fungal activity of peptides, CgPep33, obtained from by enzymatic hydrolysis of oyster (*Crassos treagigas*). Protein hydrolysate obtained from various sources, such as eel byproducts waste [2], meat byproducts [25] and egg yolks [29] are used as antimicrobial substance for the preservation of meat and meat products.

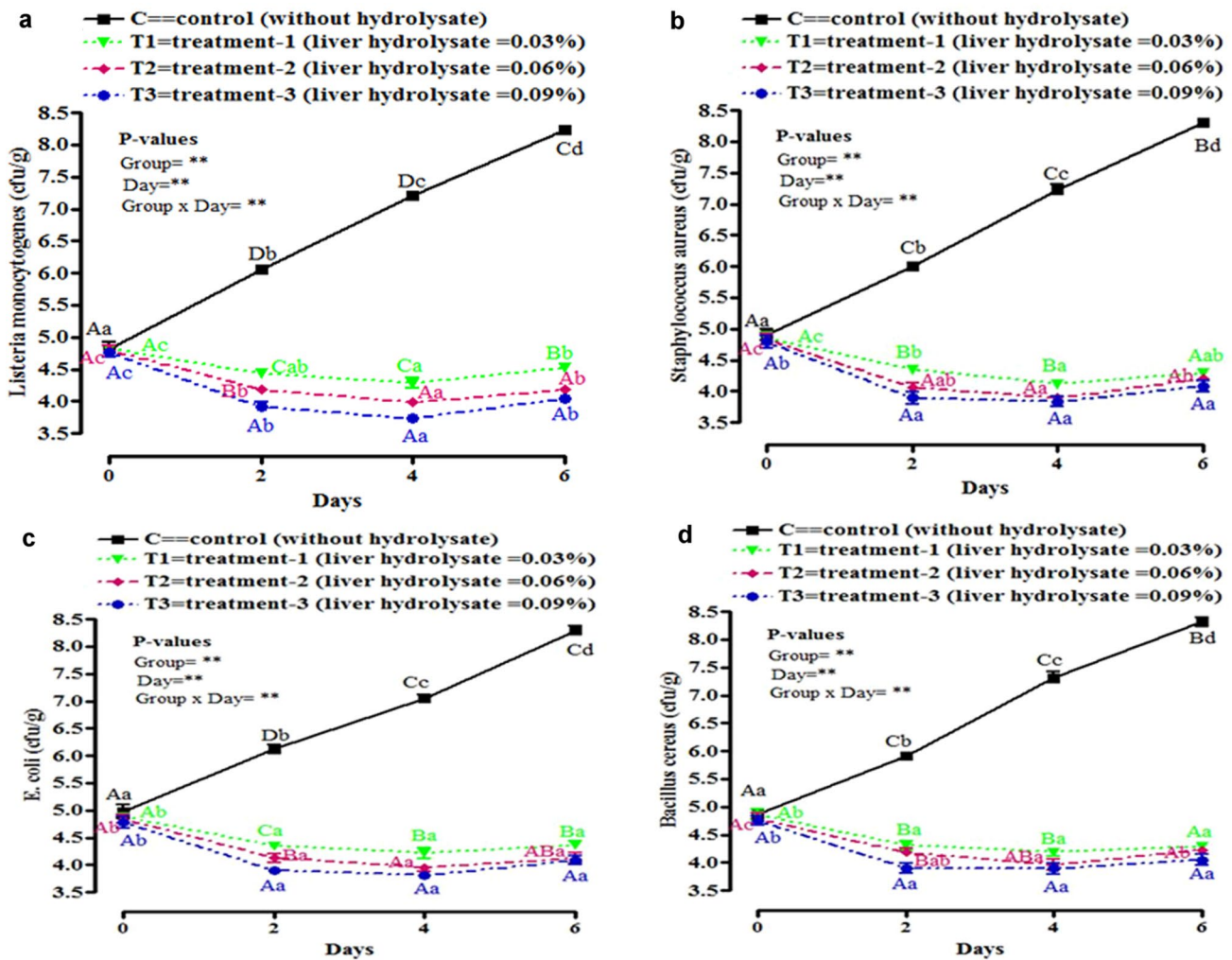
### Microbial Challenge Test for Papain Liver Protein Hydrolysate

The MCT was performed for various food spoilage organisms against liver protein hydrolysate at different concentration under *in-vivo* condition (Fig. 3a–d). Across all groups

**Table 3** Effect of different levels of porcine liver hydrolysate protein (Papain) on the SPC, coliforms and Yeast and Moulds of pork emulsion during refrigerated aerobic storage (Mean  $\pm$  S.E.)

Groups	0 day	2 day	4 day	6 day	p-values		
					Group (G)	Day (D)	G $\times$ D
<b>SPC cfu/g</b>							
C	4.31 $\pm$ 0.24 <sup>Aa</sup>	4.81 $\pm$ 0.17 <sup>Cb</sup>	5.25 $\pm$ 0.26 <sup>Cc</sup>	6.41 $\pm$ 0.03 <sup>Cd</sup>			
PC	4.28 $\pm$ 0.23 <sup>Aa</sup>	4.73 $\pm$ 0.18 <sup>Cb</sup>	5.18 $\pm$ 0.19 <sup>Cc</sup>	6.34 $\pm$ 0.05 <sup>Cc</sup>			
T1	4.24 $\pm$ 0.19 <sup>Ab</sup>	3.98 $\pm$ 0.12 <sup>Ba</sup>	4.27 $\pm$ 0.11 <sup>Bb</sup>	4.78 $\pm$ 0.15 <sup>Bc</sup>	**	**	NS
T2	4.21 $\pm$ 0.20 <sup>Ab</sup>	3.83 $\pm$ 0.15 <sup>Ba</sup>	4.15 $\pm$ 0.13 <sup>Bb</sup>	4.54 $\pm$ 0.13 <sup>Ac</sup>			
T3	4.15 $\pm$ 0.18 <sup>Ac</sup>	3.41 $\pm$ 0.17 <sup>Aa</sup>	3.84 $\pm$ 0.15 <sup>Ab</sup>	4.42 $\pm$ 0.11 <sup>Ad</sup>			
<b>Coliforms cfu/g</b>							
C	1.98 $\pm$ 0.07 <sup>Aa</sup>	2.25 $\pm$ 0.04 <sup>Cb</sup>	2.36 $\pm$ 0.02 <sup>Dbc</sup>	2.41 $\pm$ 0.04 <sup>Cc</sup>			
PC	1.99 $\pm$ 0.06 <sup>Aa</sup>	2.23 $\pm$ 0.03 <sup>Cb</sup>	2.38 $\pm$ 0.04 <sup>Dc</sup>	2.44 $\pm$ 0.02 <sup>Cc</sup>			
T1	1.96 $\pm$ 0.07 <sup>Ab</sup>	1.65 $\pm$ 0.04 <sup>Ba</sup>	1.94 $\pm$ 0.03 <sup>Cb</sup>	2.17 $\pm$ 0.04 <sup>Bc</sup>	**	**	NS
T2	1.98 $\pm$ 0.04 <sup>Ac</sup>	1.49 $\pm$ 0.05 <sup>Aa</sup>	1.78 $\pm$ 0.03 <sup>Bb</sup>	1.96 $\pm$ 0.03 <sup>Ac</sup>			
T3	1.92 $\pm$ 0.05 <sup>Ab</sup>	1.45 $\pm$ 0.06 <sup>Aa</sup>	1.52 $\pm$ 0.08 <sup>Aa</sup>	1.88 $\pm$ 0.08 <sup>Ab</sup>			
<b>Yeast and Mould cfu/g</b>							
C	1.25 $\pm$ 0.08 <sup>Aa</sup>	1.33 $\pm$ 0.08 <sup>Ba</sup>	1.46 $\pm$ 0.05 <sup>Ba</sup>	1.76 $\pm$ 0.04 <sup>Cb</sup>			
PC	1.23 $\pm$ 0.07 <sup>Aa</sup>	1.30 $\pm$ 0.06 <sup>Bab</sup>	1.43 $\pm$ 0.04 <sup>Bb</sup>	1.69 $\pm$ 0.03 <sup>Cc</sup>			
T1	1.20 $\pm$ 0.06 <sup>Aa</sup>	1.23 $\pm$ 0.07 <sup>Ba</sup>	1.35 $\pm$ 0.03 <sup>Bab</sup>	1.46 $\pm$ 0.05 <sup>Bb</sup>	**	**	**
T2	ND	0.71 $\pm$ 0.02 <sup>Aa</sup>	1.17 $\pm$ 0.08 <sup>ABb</sup>	1.38 $\pm$ 0.08 <sup>Bb</sup>			
T3	ND	ND	0.93 $\pm$ 0.06 <sup>Aa</sup>	1.23 $\pm$ 0.07 <sup>Aa</sup>			

Means with different superscripts (small letters in the same row and capital letters in the same column) indicate significant difference ( $P < 0.05$ ),  $n = 6$ ; P-value is the observed significance level of the F-test for Group, Day and  $G \times D$ , \*\*P-Value:  $< 0.001$  and NS non significant. C control (without hydrolysate), PC positive control (added BHT=0.02%), T1 treatment-1 (liver hydrolysate=0.03%), T2 treatment-2 (liver hydrolysate=0.06%), T3 treatment-3 (liver hydrolysate=0.09%)



**Fig. 3 a–d** Microbial challenge test against meat spoilage micro-organism with Papain liver protein hydrolysate in meat emulsion. Means values bearing different superscripts in row- and column-wise differ significantly ( $P < 0.05$ )  $n = 6$ ; P-value is the observed significance level of the F-test for Group, Day and  $G \times D$ ,

\*\*P-Value:  $< 0.001$ . **C** control emulsion without hydrolysate, **PC** emulsion containing 0.02% BHT, **T1** emulsion containing 0.03% hydrolysate, **T2** emulsion containing 0.06% hydrolysate, **T3** emulsion containing 0.09% hydrolysate

during storage the microbial counts (cfu/g) varied significantly ( $P < 0.05$ ) for all test microorganisms.

Microbiological count (cfu/g) showed significant ( $P < 0.05$ ) increase in control group with storage however, it decreased significantly ( $P < 0.05$ ) among treatment groups up to 4th day than day of processing and there after increased by last day of storage. Results evinced that microbiological counts decreased with the level of incorporation of protein hydrolysate in meat emulsion. It was observed that highest reduction for all test microbes was in emulsion containing 900  $\mu\text{g/g}$  (T3) protein hydrolysate on 4th day than control. This might be due antimicrobial properties of the protein hydrolysate and its activity increased with concentration of hydrolysate.

Various researchers have also reported reduction of various microbes tested against peptides/hydrolysate such as [46] for *S. aureus* and *E. coli* in comminuted meats, [47] against *L. monocytogenes* in cheddar cheese by an anionic peptides-enriched extract from whey proteins and [48] against *E. coli* and *L. monocytogenes* in lamb meat with protective culture. Jin et al. [49] also reported that blood hydrolysate also showed antimicrobial efficacy against the *B. cereus*. The variation in the antimicrobial activity of peptides under *in-vitro* conditions and *in-vivo* (meat model system) conditions attributed to the interaction of highly charged peptides present in hydrolysates to different food components, extrinsic factors during storage, intrinsic effects contributed by meat model system (pH,  $a_w$  etc.).

## Conclusion

The utilization of meat byproducts is a main challenge to the meat industry therefore; the generation of the protein hydrolysate from the low value meat byproducts is a sustainable solution. In this work, three different levels of protein hydrolysate recovered from hydrolysis of porcine liver with (papain enzyme) were assessed and compared. The results exhibited significantly better maintained physico-chemical attributes in treated groups during storage. Antioxidant capacity (DPPH, ABTS, SASA and FRAP), lipid oxidation (PV, TBARS and FFA) and colour profiles ( $L^*$ ,  $a^*$  and  $b^*$ ) of the meat emulsion declined with lower rate in sample containing 0.09% hydrolysate and positive control than other groups. Microbial quality (SPC, Coliforms and Yeast and moulds) alongside with microbial challenge test for microbes (*L. monocytogenes*, *S. aureus*, *E. coli*, and *B. cereus*) showed comparatively lower microbial growth in treated groups. The outcomes of the study suggest that protein hydrolysate can decrease the lipid oxidation, showed antioxidant activity, maintained colour of meat emulsion, also exerted antimicrobial efficacy therefore; hydrolysate can find a useful application in the meat industry. These results further suggest that protein hydrolysate could be transformed into value-added substances that would enhance the meat byproducts market in the food, pharmaceutical as well as in cosmetic products.

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**Author Contributions** AKV and MKC have contributed equally in designing the research. AKV carried out the experiments, recorded data and prepared the manuscript. PK and NM helped in interpretation and analysis of the data.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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