

EFFECT OF COMBINATION OF DIFFERENT HUMECTANTS AND ANTIOXIDANTS ON THE SHELF LIFE OF PORK NUGGETS

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ABSTRACT

Increasing the shelf life of meat and meat products is considered to be an important aspect of achieving success in the meat industry. This could be achieved by adopting several preservation techniques including the use of different additives. Five different formulations were prepared using combinations of humectants and antioxidants along with a control group. Nuggets were packaged in aerobic packaging and stored at refrigerated temperature (4±1 °C). The parameters were studied on the day 1 and subsequently on the 7th, 15th and 30th day. A significant increase in pH and Thiobarbituric Acid Reactive substance found between the control and the treated groups. The moisture percentage was significantly lower (P<0.01) in the natural humectants and antioxidants added group. The Total Viable Plate Count revealed a significant increase (P<0.01) in bacterial load from the 1st to 30th day. Yeast & mold, Coliform, and *Staphylococcus aureus* were not detected during the entire storage period. Colour profile studies revealed a significant increase (P<0.01) in L* and b* values with the progression of storage. Natural antioxidants added nuggets recorded high ratings for different sensory parameters. In contrast, the control products were only acceptable up to the 15th day. Therefore, from the present study it can be concluded that pork nuggets incorporated with natural humectants and natural antioxidants is considered to be the best formulation in improving its shelf life compared to other formulations.

Keywords: Humectants, Antioxidants, Pork, Nuggets, Aerobic packaging

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Shelf stability of fresh/processed meat and meat products are considered to be an important aspect of achieving success and growth in meat industry. This could be achieved by adopting use of a number of existing preservation techniques and other processing aids to prevent the microbial spoilage of meat and meat products. The primary source of contamination in any kind of meat or food product is the free water which can be made unavailable by use of humectants thus stabilizing the moisture content and reducing the water activity (aw) of the product. Considering the adverse effects of chemical additives on human health and sensory qualities, the present trends in consumer acceptability indicate a preference for natural products, which are safer and healthier than synthetic ones (Rajalakshmi and Narasimhan, 1996). A number of synthetic humectants are now available in market but their incorporation is not preferred in meat and meat products due to their residual effect and reduced consumer preference.

Minced meats undergo oxidative changes and develop rancidity more quickly than intact muscle as grinding exposes more muscle surface to air and allows microbial contamination readily (Mitsumoto *et al.*, 2005). The use of natural antioxidants are more supported than the synthetic ones primarily because of the presence of

phenolic compounds, a potent factor in preventing lipid oxidation, besides they don't require any safety tests before their incorporation into food stuff.

Considering the above facts, the present work was undertaken to study the physico-chemical, microbiological and shelf-life of pork nuggets prepared by incorporating humectants and antioxidants and then packaged with aerobic packaging and stored under refrigeration (4±1 °C) temperature.

MATERIALS AND METHODS

The pork nuggets incorporated with humectants and antioxidants were prepared according to the basic recipe (Table 1). The pork utilised in the present study was obtained from the Pork Processing Plant, College of Veterinary Science, Khanapara, of 7-8 months old Hampshire local cross bred of 70-85 kg live weight. The present research work was approved by the Institutional Ethics Committee of the Assam Agricultural University vide approval number: 770/ac/CPCSEA/FVSc/AAU/IAEC/16-17/416 dated 30.07.2016. The lean pork and fat were cut into small cubes of 2-3 cm, packaged in food-grade polyethylene bags, and kept at a temperature of -20 °C until further use. The pork lean was minced twice in a mechanical meat mincer by passing through a 6 mm pore size plate and the fat cubes were melted aseptically in a pan

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to obtain the lard. The lean, lard, non-meat ingredients, spices and condiments, and ice flakes were then bowl chopped at low speed until a uniform emulsion was formed.

Addition of humectants and antioxidants

The emulsion was divided into five parts- Control (without humectants and antioxidants), T₁ (chemical humectants and chemical antioxidants), T₂ (chemical humectants and natural antioxidants), T₃ (natural humectants and chemical antioxidants) and T₄ (natural humectants and natural antioxidants).

The humectants and antioxidants used in the study were:

Chemical humectants: Glycerol @ 5% and Sorbitol @ 1%

Chemical antioxidant: BHA @ 0.02%

Natural humectants: Soya protein @ 3%, Skim Milk powder @ 1%, Skim milk powder & Honey @ 1% each

Natural antioxidants: Pomegranate rind powder extract @ 5%, Green Tea Extract @ 0.2% and Ghost chilly in paste form (5g per kg)

The concentration of different natural humectants and antioxidants used in the present study was determined based on preliminary studies conducted in the laboratory. All the emulsions were then bowl chopped separately for 15-30 seconds for proper mixing.

Preparation of pork nuggets

The meat emulsion of the control and all the treated groups were stuffed into suitable moulds and cooked to an internal temperature of 72 °C for 45 minutes, followed by rapid cooling in chilled water to about 4 °C (Seo *et al.*, 2019). The cooked meat loaves were then sliced and then given the shape of nuggets, after which they were dipped for a few seconds in 1% warm potassium sorbate solution and then dried in an oven at around 60 °C for a few seconds.

Packaging and storage of the pork nuggets

The prepared nuggets were finally aerobic packaged (AP) in food-grade High-Density Polyethylene (HDPE) packages and then each set of control and treated products were stored at refrigeration temperature (4±1 °C). The samples were assessed on the day of preparation, the 7th day and fortnightly to determine their shelf life.

The pH (Pippen *et al.*, 1965), Thiobarbituric Acid Reactive Substance (TBARS) (Witte *et al.*, 1970), Moisture (AOAC, 1970), Total viable plate count (TVPC), Yeast & Mold count, Coliform count and Staphylococcal count (Harrigan and McCance, 1976), Colour by CIE Lmambm system in Cary 100 UV Visible Spectrophotometer were determined. Sensory evaluation was carried out by semi-trained panellists consisting of 7 members using the 7-point Hedonic scale (Ingham *et al.*, 2005).

STATISTICAL ANALYSIS

Five batches of the products were prepared for the

Table 1. Basic recipe of pork nuggets

S.No.	Name of ingredients	Percentage
1.	Pork lean	70
2.	Lard	10
3.	Corn flour	5
4.	Salt	1.7
5.	Dry spice mix	2.5
6.	Condiments paste	4.0
7.	Sodium Tripolyphosphate (STPP)	0.30
8.	Ice cubes	6.3
9.	Sodium Nitrite	150 ppm
10.	Monosodium Glutamate (MSG)	50 ppm
TOTAL		100

ppm: parts per million

present study. The data generated in the present study were analysed by using the Statistical Package for Social Sciences (SPSS, version 20.0 Chicago, USA) by two-way ANOVA and a comparison of means was tested using Duncans multiple range test (Duncan, 1995). The effects were considered to be significant at P<0.01.

RESULTS AND DISCUSSION

Physico-chemical characteristics

In the present study (Table 2), the pH values showed a uniform, increasing trend (P<0.01) in control and treated products for up to 30 days. Significant differences (P<0.01) could be observed between the control and the treated groups on the 1st, 7th, 15th and 30th day of storage. Such increase in the pH values might be due to the accumulation of various metabolites by bacterial action on the carbohydrate contents in meat, besides proteolysis of meat protein resulting in the formation of ammonia and consequent rise in pH values. The highest pH values were recorded in the control products. However, lower values were recorded in the treated products with the lowest pH value in the T4 group during the entire storage period. The pH lowering might be attributed to the phenolic and bioactive compounds of the natural antioxidants and the acidic pH of extracts added as natural antioxidants. The findings of the present study corroborated well with the reports of Jauhar *et al.* (2021) who showed that the addition of pomegranate peel extract and green tea leaves extract significantly reduced (P<0.05) the pH in chilled chicken meat.

The TBARS of control and treated pork nuggets gradually increased along with the storage days. However, these values were well below the permissible limit of 1 mg malonaldehyde per kilogram for all the products prepared with the addition of antioxidants. The lower values (p<0.05) were registered in natural antioxidant added pork

Table 2. Physico-chemical properties of pork nuggets incorporated with humectants and antioxidants (Mean±SE)

Parameters	Treatment	Storage days			
		1	7	15	30
pH	Control	^A 6.12±0.01	^B 6.26 ^d ±0.02	^C 6.43 ^f ±0.02	^D 6.67 ^d ±0.02
	T ₁	^A 6.13±0.02	^B 6.23 ^d ±0.02	^B 6.24 ^d ±0.02	^C 6.44 ^c ±0.04
	T ₂	^A 6.09±0.05	^A 6.14 ^{abc} ±0.01	^B 6.19 ^{cd} ±0.01	^C 6.39 ^{bc} ±0.01
	T ₃	^A 6.11±0.01	^{AB} 6.17 ^{bc} ±0.04	^B 6.22 ^{cd} ±0.02	^C 6.42 ^c ±0.02
	T ₄	^A 6.09±0.09	^A 6.10 ^{ab} ±0.02	^B 6.19 ^{cd} ±0.02	^C 6.31 ^{ab} ±0.04
TBARS (mg malonaldehyde/kg)	Control	^A 0.10 ^b ±0.01	^B 0.43 ^b ±0.03	^C 1.00 ^e ±0.05	^D 1.36 ^f ±0.06
	T ₁	^A 0.07 ^a ±0.00	^B 0.14 ^a ±0.02	^C 0.53 ^c ±0.02	^D 0.88 ^{cd} ±0.03
	T ₂	^A 0.06 ^a ±0.00	^A 0.11 ^a ±0.01	^B 0.49 ^{abc} ±0.03	^C 0.85 ^{bcd} ±0.02
	T ₃	^A 0.08 ^{ab} ±0.01	^B 0.13 ^a ±0.01	^C 0.51 ^{bc} ±0.02	^D 0.92 ^d ±0.02
	T ₄	^A 0.06 ^a ±0.01	^B 0.12 ^a ±0.02	^C 0.47 ^{abc} ±0.02	^D 0.75 ^{ab} ±0.03

N=5; Means with different superscript within column (abc) and subscript within row (ABC) differ significantly (P<0.01)

Table 3. Moisture percentage of pork nuggets incorporated with humectants and antioxidants (Mean±SE)

Parameters	Treatment	Storage days			
		1	7	15	30
pH	Control	64.02 ^c ±0.41	65.00 ^c ±0.13	65.28 ^{fg} ±0.24	65.73 ^{cd} ±0.32
	T ₁	^A 62.83 ^{bc} ±0.53	^B 63.28 ^{bcd} ±0.32	^B 64.43 ^{ef} ±0.36	^C 65.34 ^{bcd} ±0.56
	T ₂	^A 62.60 ^{bc} ±0.38	^A 62.89 ^{bc} ±0.58	^B 64.16 ^{dc} ±0.29	^B 64.75 ^{abc} ±0.33
	T ₃	^A 61.43 ^b ±0.34	^B 62.94 ^{bc} ±0.43	^C 63.27 ^{cd} ±0.32	^C 63.92 ^a ±0.55
	T ₄	^A 58.87 ^a ±0.30	^B 60.52 ^a ±0.37	^B 60.87 ^a ±0.34	^C 63.85 ^a ±0.06

N=5; Means with different superscript within column (abc) and subscript within row (ABC) differ significantly (P<0.01)

Table 4. Microbiological qualities of pork nuggets incorporated with humectants and antioxidants (Mean±SE)

Parameters	Treatment	Storage days			
		1	7	15	30
TOTAL PLATE COUNT (log cfu per gram)	Control	2.64 ^b ±0.02	4.83 ^f ±0.03	5.31 ^f ±0.02	NP
	T ₁	^A 2.49 ^a ±0.03	^B 3.75 ^d ±0.04	^B 3.83 ^d ±0.04	^C 4.39 ^e ±0.08
	T ₂	^A 2.52 ^a ±0.03	^B 3.54 ^c ±0.05	^B 3.63 ^c ±0.05	^B 3.69 ^c ±0.05
	T ₃	^A 2.52 ^a ±0.03	^B 3.66 ^d ±0.03	^B 3.91 ^d ±0.03	^C 4.15 ^d ±0.04
	T ₄	^A 2.48 ^a ±0.03	^B 3.34 ^b ±0.05	^B 3.48 ^b ±0.04	^B 3.50 ^b ±0.04
Yeast and Mold Count Coliform Count <i>Staphylococcus aureus</i>	Not detected in any treatment group in entire storage period at refrigerated temperature				

N=5; NP: Not Performed

Means with different superscript within column (abc) and subscript within row (ABC) differ significantly (P<0.01)

nuggets which might be due to inhibition of lipid peroxidation and redox properties attributed to polyphenolic constituents present in pomegranate rind extract (PRE) and GTE which play an important role in absorbing and neutralising free radicals, quenching singlet oxygen or decomposing peroxides (Cao *et al.*, 1997). The higher TBARS value in the control group is due to auto-oxidation of lipids during storage. Similar findings were

also reported by Jamwal *et al.* (2015) in chicken patties incorporated with GTE, fig and red pepper and found that the GTE incorporated patties had the lowest TBARS values. Reddy *et al.* (2017a) reported that the TBA values of chicken sausages with GTE at 0.2% were significantly (P<0.01) lower than the control and RE and BHT incorporated sausages during refrigeration storage.

The moisture percentage (Table 3) was significantly

Table 5. Colour profile of pork nuggets incorporated with humectants and antioxidants (Mean±SE)

Colour profile	Treatment	Storage days				
		1	7	15	30	
L*	Control	^A 64.68 ^d ±0.33	^A 64.88 ^c ±0.33	^B 66.32 ^h ±0.40	^B 66.45 ^f ±0.27	
	T ₁	^A 60.01 ^c ±0.14	^A 60.45 ^b ±0.11	^B 62.04 ^f ±0.24	^B 62.22 ^c ±0.23	
	T ₂	^A 57.42 ^a ±0.34	^A 58.16 ^a ±0.34	^{BC} 9.40 ^{bcd} ±0.41	^C 59.74 ^{abc} ±0.59	
	T ₃	59.03 ^b ±0.42	60.38 ^b ±0.38	60.44 ^{de} ±0.59	60.59 ^{cd} ±0.38	
	T ₄	56.97 ^a ±0.15	57.88 ^a ±0.19	58.97 ^{bc} ±1.12	59.14 ^{ab} ±0.39	
	a*	Control	^C 8.30 ^b ±0.15	^C 7.76 ^a ±0.18	^B 6.57 ^a ±0.13	^A 5.73 ^a ±0.27
	T ₁	^B 7.78 ^{ab} ±0.26	^B 7.48 ^a ±0.17	^B 7.33 ^{bc} ±0.21	^A 6.63 ^b ±0.10	
	T ₂	7.36 ^a ±0.13	7.13 ^a ±0.24	7.10 ^{abc} ±0.17	6.79 ^b ±0.06	
	T ₃	7.52 ^a ±0.31	7.27 ^a ±0.34	7.07 ^{abc} ±0.17	6.56 ^b ±0.17	
	T ₄	7.23 ^a ±0.25	7.09 ^a ±0.23	7.03 ^{abc} ±0.24	6.87 ^b ±0.09	
	b*	Control	16.68 ^c ±0.15	16.87 ^c ±0.15	17.13 ^c ±0.10	17.35 ^d ±0.50
	T ₁	^A 15.31 ^b ±0.30	^A 15.78 ^d ±0.35	^{BC} 16.31 ^{cd} ±0.19	^C 16.79 ^{cd} ±0.12	
	T ₂	^A 13.85 ^a ±0.16	^A 14.69 ^{ab} ±0.33	^{BC} 14.18 ^a ±0.21	^C 14.96 ^a ±0.11	
	T ₃	^A 14.91 ^b ±0.24	^A 15.29 ^{bcd} ±0.26	^A 15.59 ^b ±0.24	^B 16.54 ^{bc} ±0.18	
	T ₄	^A 13.79 ^a ±0.29	^B 14.92 ^{abc} ±0.24	^B 14.61 ^a ±0.29	^B 14.89 ^a ±0.22	

N=5; Means with different superscript within column (abc) and subscript within row (ABC) differ significantly (P<0.01)

Table 6. Sensory properties of pork nuggets incorporated with humectants and antioxidants (Mean±SE)

Sensory profile	Treatment	Storage days			
		1	7	15	30
Appearance	Control	^D 6.91 ^b ±0.06	^C 6.24 ^a ±0.05	^B 5.42 ^a ±0.18	^A 4.78 ^a ±0.09
	T ₁	^D 6.62 ^{ab} ±0.07	^C 6.44 ^a ±0.07	^B 6.06 ^c ±0.12	^A 5.72 ^b ±0.04
	T ₂	^C 6.35 ^a ±0.09	^C 6.28 ^a ±0.03	^B 5.94 ^{bc} ±0.10	^A 5.55 ^b ±0.12
	T ₃	^C 6.88 ^b ±0.05	^B 6.39 ^a ±0.08	^B 6.16 ^c ±0.21	^A 5.65 ^b ±0.08
	T ₄	^C 6.44 ^a ±0.15	^B 6.28 ^a ±0.02	^{AB} 5.88 ^{ab} ±0.17	^A 5.52 ^b ±0.06
Flavour	Control	6.76±0.17	5.70 ^a ±0.25	5.25 ^{ab} ±0.22	NP
	T ₁	^C 6.72±0.10	^C 6.04 ^a ±0.19	^B 5.62 ^{abc} ±0.26	^A 4.43±0.12
	T ₂	^C 6.32±0.12	^B 5.98 ^a ±0.19	^B 5.82 ^{bc} ±0.25	^A 4.52±0.17
	T ₃	^C 6.67±0.16	^B 5.89 ^a ±0.25	^B 5.67 ^{abc} ±0.10	^A 4.30±0.11
	T ₄	^C 6.41±0.18	^C 6.12 ^a ±0.10	^B 5.91 ^c ±0.04	^A 4.50±0.13
Texture	Control	6.41 ^{ab} ±0.12	5.83 ^a ±0.15	4.62 ^a ±0.08	NP
	T ₁	^C 6.52 ^b ±0.09	^C 6.31 ^{bcd} ±0.06	^B 5.53 ^b ±0.10	^A 4.23 ^a ±0.04
	T ₂	^C 6.13 ^a ±0.11	^B 5.91 ^{ab} ±0.22	^B 5.39 ^b ±0.10	^A 4.21 ^a ±0.04
	T ₃	^C 6.74 ^b ±0.15	^C 6.51 ^{cd} ±0.05	^B 5.62 ^b ±0.04	^A 4.20 ^a ±0.03
	T ₄	^C 6.45 ^{ab} ±0.11	^C 6.22 ^{abcd} ±0.04	^B 5.45 ^b ±0.14	^A 4.53 ^{bc} ±0.14
Juiciness	Control	6.62±0.14	5.55 ^a ±0.15	4.48 ^a ±0.08	NP
	T ₁	^C 6.59±0.08	^C 6.31 ^b ±0.06	^B 5.47 ^b ±0.10	^A 4.40±0.26
	T ₂	^C 6.60±0.15	^C 6.17 ^b ±0.23	^B 5.33 ^b ±0.14	^A 4.40±0.18
	T ₃	^C 6.51±0.17	^C 6.42 ^b ±0.19	^B 5.16 ^b ±0.17	^A 4.31±0.10
	T ₄	^C 6.47±0.11	^C 6.25 ^b ±0.04	^B 5.37 ^b ±0.22	^A 4.52±0.17
Overall Acceptability	Control	6.69 ^b ±0.07	5.76 ^a ±0.12	4.87 ^a ±0.09	NP
	T ₁	^D 6.61 ^{ab} ±0.06	^C 6.29 ^c ±0.04	^B 5.62 ^b ±0.06	^A 4.59±0.05
	T ₂	^D 6.37 ^a ±0.09	^C 6.10 ^{bc} ±0.17	^B 5.55 ^b ±0.11	^A 4.64±0.04
	T ₃	^D 6.69 ^b ±0.11	^C 6.33 ^c ±0.10	^B 5.57 ^b ±0.07	^A 4.56±0.06
	T ₄	^C 6.41 ^{ab} ±0.08	^C 6.24 ^c ±0.03	^B 5.56 ^b ±0.09	^A 4.70±0.04

N=5; Means with different superscript within column (abc) and subscript within row (ABC) differ significantly (P<0.01)

($P < 0.01$) lower in T_4 group followed by T_3 , T_2 , T_1 and the highest percentages in the control group during the entire storage period up to 30 days. The lower moisture content values in treated pork nuggets might be attributed to the humectant's binding property of free water. In the present study, the moisture content of T_3 and T_4 groups containing natural humectants *viz.* soya protein, honey and skim milk powder were lower than T_2 and T_1 groups prepared by adding chemical humectants. This again might be because the natural humectants are more efficient in binding free water than the chemical ones. A significant increase ($P < 0.05$) in moisture percentages was recorded as the storage days progressed in control and treated products, which might be due to the slow moisture permeability of the packaging material. Decrease in moisture content was also recorded by Sharma and Yadav (2020) in chicken meat patties incorporated with pomegranate peel extract.

The microbiological qualities of the pork nuggets prepared with different humectants and antioxidants are presented in Table 4. The TVPC showed an increasing trend ($P < 0.01$) both in control as well as in the treated pork nuggets with the progression of storage. It was also observed that the T_4 and T_2 products recorded the lowest TVPC than those of T_1 and T_3 products. This might be attributed to the antimicrobial effect of phytoextracts used in nugget making (Ahn *et al.*, 2004 and Michel *et al.*, 2012). A similar increasing trend in total plate count during the storage periods was recorded by Reddy *et al.* (2017b), Nashi *et al.* (2015), Nath *et al.* (2016), Jamwal *et al.* (2015) and Sharma and Yadav (2020) in chicken meat sausages, beef sausages, chevon meat patties, chicken meat sausages and chicken meat patties in their respective studies. Yeast and molds were not detected in any of the product samples included in the present study. This might be due to the adoption of hygienic processing practices besides the potassium sorbate (antifungal agent) treatment of the products before packaging. The results agreed with the reports of Reddy (2017), who carried out their studies in chicken meat patties. Coliforms and Staphylococci counts were not detected in all the product samples throughout the study period. It reflects the strict hygienic conditions followed during the preparation of pork nuggets and the high heat treatment employed during the cooking process (Kumar and Sharma, 2004).

During storage, the L^* values of pork nuggets increased gradually (Table 5), indicating oxidation of myoglobin and accumulation of metmyoglobin and which might be attributed to the gradual oxidation the storage days (Sarkar *et al.*, 2021). Presence of pigments in natural phytoextracts might have increased the L^* values. A significantly higher ($P < 0.01$) L^* values were recorded in

the control group, followed by T_1 , T_3 , T_2 products and the least values in the T_4 products. The a^* values of pork nuggets decreased significantly with storage except for the T_1 and control group indicating a change in colour from red to brown due to metmyoglobin formation. Qin *et al.* (2013) reported that redness (a^* value) was significantly ($p < 0.05$) higher in pomegranate rind powder (PRP), pomegranate juice (PJ) and pomegranate seed powder (PSP) patties as compared to the control and BHT groups raw ground pork.

The mean b_m values increased with storage and were significantly higher ($P < 0.01$) in the control products followed by T_1 , T_3 , T_2 and lastly T_4 ones and maintained the trend throughout the storage period. The nuggets incorporated with natural antioxidants (i.e., T_2 and T_4 groups) exhibited lower b_m values, which might be due to the presence of phenolic compounds with antioxidant properties. The results obtained were in agreement with the reports of Gramatina *et al.* (2014), who also reported an increase in the yellowness during storage of venison jerky.

SENSORY CHARACTERISTICS

All the sensory quality attributes *viz.* appearance, flavour, texture, juiciness and overall acceptability of pork nuggets recorded higher scores on day 1, which gradually declined as the storage period progressed (Table 6). The lowest scores were recorded on the 30th day of storage. On storage, the scores for control products were acceptable up to the 15th day at refrigeration temperature. No significant difference could be seen in overall acceptability of treated pork nuggets at the end of storage period i.e. on the 30th day. On the whole, pork nuggets prepared with natural antioxidants (T_2 and T_4) recorded a higher rating than the T_1 and T_3 products for different sensory parameters at 30th day of storage. Similar findings were also recorded by Devatkal *et al.* (2010) who reported that panelists did not find any significant difference for flavour and overall acceptability of chicken patties prepared with the addition of extracts of KRP, PRP and PSP.

CONCLUSION

Based on the results obtained in the present study, it may be concluded that incorporating natural humectants and antioxidants during preparation of pork nuggets is relatively more advantageous than using their synthetic counterparts. Microbial loads can be reduced in pork nuggets with the addition of both natural and chemical antioxidants. Overall, the study concludes that the T_4 products, i.e., pork nuggets added with natural humectants and natural antioxidants, are considered to be the best formulation, followed by T_2 , T_3 and T_1 , respectively.

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