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Effect of Application of Natural Polyphenol Containing Polymer Coatings on Quality of Pork Balls

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ABSTRACT

An attempt was made to evaluate the quality of pork balls coated with starch (T1), starch incorporated with 1% Tannic acid (T2), and starch incorporated with 1% Quercetin (T3) and were analyzed at regular intervals of 5 days and 15 days under refrigeration (4 ± 1^{O}C) and frozen storage temperatures (-18 ± 1^{O}C) respectively. The values of pH, TBA, PV, TPC and per cent water loss of the coated pork balls increased significantly (P<0.05) during the storage period for all the treatment groups, but the rate of increase was at a lower level in natural polyphenols incorporated formulations when compared to control and starch coated ones. They were well acceptable up to 15 days under refrigerated storage and up to 75 days under frozen temperature. The polyphenol enriched starch coated products showed significantly (P<0.05) lower total plate counts when compared to non coated control irrespective of type of polyphenols incorporated. The coatings were tested for their antimicrobial ability against various antibiotics by disc diffusion method. The zone of inhibition was significantly higher for 1% Tannic acid 1% quercetin incorporated starch coatings when compared to the control. The present study demonstrated that tannic acid and quercetin can be successfully incorporated into biodegradable starch coatings and films and can exhibit excellent antioxidant and antimicrobial activities as a part of active packaging, to extend the shelf life of the product.

Keywords: *Pork balls, Polymer coatings, Quercetin, Starch, Tannic acid*

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INTRODUCTION

Fresh meat and meat products are the major protein source and are perishable. Maintenance of quality and delivery of safe food to consumers is a major challenge today. Edible films and coatings prepared from polysaccharides, proteins and lipid materials besides serving as gas and moisture barriers can also act as carriers for functional food additives such as antioxidants and antimicrobials (Cuppett, 1994). Starch is one of the most important polysaccharide that is used to develop biodegradable films because of its capability to form a continuous matrix and is renewable with abundant resource (Talja et al., 2007). Various natural polyphenols could be incorporated as food additives into edible films to broaden their antimicrobial properties. Natural polyphenols are complex mixtures of numerous compounds from various parts of the plants. Tannic acid has antibacterial, antifungal, insecticidal, anti oxidant activities. These natural polyphenols upon direct addition into the food may alter the flavour of the product, because of their strong odour. Among the antioxidants, Polyphenols are considered to be major group because of their excellent free radical scavenging activity and protection against oxidation of transition metals and lipid peroxidation (Zhou & Elias, 2013). Among Polyphenols, tannins received most attention due to their wide spectrum and higher antimicrobial activity against bacteria like Staphylococcus aureus, Escherichia coli in comparison with other Polyphenols (Rodriguez et al., 2010). Quercetin has a variety of actions besides antioxidant activity, highlighted by antimutagenic (Gupta et al., 2010) and antitumorigenic (Wang *et al.,* 2012) properties.

With this background, the present work has been designed to develop and evaluate starch based polymer coatings and films incorporated with natural polyphenols i.e. tannic acid and quercetin on the quality of pork balls with objectives of studying the apparent characteristics of these coating and films on pork balls and to study the physicochemical and microbiological characteristics of the pork balls coated with above polymers at refrigerated (4 ± 1oC) and frozen (-18 ± 1oC) storage temperature at regular intervals.

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MATERIALS AND METHODS

Chicken meat: Giriraja spent hens of about 60 weeks of age were procured from the Instructional Livestock Farm Complex, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER) and birds were slaughtered following standard hygienic method in the Department of Livestock Products Technology (LPT), RIVER, Puducherry. All the carcasses were deboned manually. Deboned meat was packed in LDPE bags and stored in freezer (Blue star) at -18±10C to prepare patties.

Preparation of spent hen meat patties using food processor

***Standardization of running time for emulsion formation using food processor*:** A total of one kg batter with 70% meat was prepared using food processor. First proteins were extracted by running the machine after adding curing ingredients and chilled water. Then oil was added and the machine was run at different running time (2, 3 and 4 minutes) to know about the best running time for emulsion formation using food processor. The suitable running time was selected based on emulsion stability.

Standardization of meat level for emulsion formation using food processor: Three combinations of emulsions were prepared, with 65, 70 and 75% meat by keeping all other ingredients same. The best meat level was selected based on the physico-chemical characteristics like cooking yield of patties, pH of emulsion, pH of patties and sensory characteristics.

Standardization of added oil level for emulsion formation using food processor: Three combinations of emulsions were prepared by using 7, 10 and 13% oil in the recipe and keeping all other ingredients same. The best oil level was selected based on the physico-chemical characteristics like cooking yield of patties, pH of emulsion, pH of patties and sensory characteristics.

Physico-chemical analyses: The cooking yield (%) of patties was calculated by adopting the procedure laid down by Murphy et al. (1975). The pH of emulsion and patties was determined by following AOAC (1995) using combined glass electrode of the pH meter. Emulsion stability was determined following the method described by Townsend et al(1975).

Sensory Evaluation: A 10 member semi-trained panel recorded their preference on 8 point hedonic scale (8=extremely desirable, 1=extremely undesirable) (Keeton, 1983) for the attributes viz. appearance, flavor, texture, juiciness and overall palatability. Plain water was provided to each panelist to rinse the mouth in between the samples.

Statistical Analysis: Each experiment was replicated thrice and each parameter was analyzed in duplicate. The data were analyzed using SPSS version 16.0 (SPSS, Chicago, U.S.A). One way analysis of variance (ANOVA) was used to analyze the effect of different levels of oil, meat on patties for physico-chemical quality and sensory attributes. The level of significance was tested using the least significant difference (LSD) test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSIONS

Effect of running time of the food processor on the quality of emulsion: The emulsion stability with 2, 3 and 4 minutes running time for emulsion formation in food processor were 87.19 ± 0.04, 89.06±0.07 and 86.26±0.07%, respectively. This revealed that there was a significant increase (P<0.05) in emulsion stability from 2 minutes to 3 minutes running time due to increase in fat emulsification, however, further increase of time (4 minutes) resulted in significant decrease (P<0.05) in emulsion stability. Mandal et al (1996) prepared spent hen meat balls by mixing minced meat and fat for two minutes in a Hobart mixer with desirable quality. Since the emulsion stability was significantly (P<0.05) higher for 3 minutes running time compared to 2 and 4 minutes time, it was selected for emulsion formation using food processor.

Effect of different levels of meat on physico-chemical quality of patties: The cooking yield, emulsion stability (Table-1) of spent hen meat patties prepared with different levelsof meat ranged between 83.71 to 85.91% and 93.78 to 95.22%, respectively. Patties prepared with 70 and 75% meat levels had significantly (P<0.05) higher cooking yield and emulsion stability than 65% meat in the formulation and there was no significant difference between the patties containing 70 and 75% meat. This might be due to higher protein content in 70 and 75% meat to form stable emulsion. The pH of emulsion and patties prepared with different levels meat ranged between6.10 to 6.11 and 6.23 to 6.24, respectively (table-2). There was no significant (P>0.05) difference in pH of emulsion and patties prepared with 70 and 75% meat. Emulsion and patties prepared with 65% meat had significantly (P<0.05) higher pH than products containing 70 and 75% meat. Mandal et al. (1996) prepared spent hen meat balls by mixing minced meat (71%) and fat in a Hobart mixer with pH of 6.0 - 6.14 and cooking yield of 83.0 - 88.0%. All most similar results on cooking yield, pH and emulsion stability of chicken meat patties with different extenders prepared by hand mixing has been reported by Gawdaman et al. (2009).

Table 1: Proximate composition (Mean ± SE) values of pork balls as influenced by starch coatings with different polyphenols

	Proximate Composition		
Parameters	**Moisture**	**Protein**	**Fat**
C	68.54±0.91a	21.42±0.34a	4.08±0.21a
T 1(S)	69.09±1.22b	21.48±0.55a	4.03±0.30a
T 2(S+T)	69.75±1.67c	21.51±0.74a	4.06±0.20a
T 3(S+Q)	69.90±2.69c	21.46±0.42a	4.06±0.16a
Overall mean	69.32 ±1.62	21.47±0.51	4.06±0.22

Means bearing at least one common superscript in the same column do not differ significantly (P<0.05). S: Corn T: Tannic Acid, Q: Quercetin

Table 2: Physiochemical and microbial count (Mean ± SE) values of pork balls as influenced by different coatings at refrigeration storage (4±1^{0}C)

		Level of Giriraja spent hen meat			
Parameters	**Coating treatments**	**Day 0**	**Day 5**	**Day 10**	**Day 15**
pH	C	6.25±0.01aA	6.38±0.02bA	*6.42±0.01cA	*6.47±0.02dA
	T 1	5.96±0.01aB	6.24±0.01bB	6.31±0.03cB	*6.41±0.03dB
	T2	5.84±0.04aC	6.17±0.05bC	6.29±0.02cB	6.37±0.08dC
	T 3	5.93±0.01aB	6.11±0.01bD	6.23±0.01cC	6.34±0.02dC
TBARS	C	1.18±0.09aA	1.34±0.09bA	*1.98±0.12cA	*2.12±0.03dA
	T 1	1.12±0.05aB	1.29±0.04bB	1.59±0.08cB	*1.95±0.02dB
	T2	1.08±0.06aC	1.20±0.09bC	1.52±0.15cC	1.74±0.03dC
	T 3	1.06±0.06aC	1.21±0.06bC	1.48±0.03cD	1.76±0.06dC
Peroxide value	C	0.84±0.03aA	1.57±0.01bA	*2.96±0.08cA	*3.21±0.07dA
	T 1	0.77±0.02aB	1.41±0.02bB	2.17±0.09cB	*2.89±0.11dB
	T2	0.72±0.01aC	1.35±0.02bC	2.09±0.02cC	2.61±0.04dC
	T 3	0.71±0.02aC	1.32±0.06bC	2.05±0.04cC	2.62±0.03dC
TPC	C	4.92±0.02aA	5.31±0.06bA	*5.88±0.06cA	*6.32±0.05dA
	T 1	4.85±0.16aB	5.24±0.03bB	5.75±0.03cB	*6.05±0.10dB
	T2	4.70±0.03aC	5.17±0.15bC	5.53±0.01cC	5.67±0.02dC
	T 3	4.60±0.01aD	5.13±0.01bC	5.50±0.02cC	5.71±0.02^{dC}

(P<0.05); Means bearing at least one common superscript (small letter) in the same row do not differ significantly. * spoiled

Means bearing at least one common superscript (capital letters) in the same column (treatment wise) do not differ significantly

Physico-chemical quality: pH, TBARS, peroxide value and percent water loss of coated and uncoated pork balls differed significantly P<0.05).The coated balls showed lower values when compared to uncoated controls. Among the coated formulations the values were lower for natural polyphenols incorporated counter parts. During storage, irrespective of formulations pH, percent water loss increased both at refrigerated and frozen storage temperatures. pH values and percent water loss were lower in T2 and T3 when compared with C and T1. This might be due the effect of phenolic compounds such as flavonoids and phenolic acids present which exhibit a wide range of antimicrobial effect and the hydrogen and covalent interactions between the starch network and phenolic compounds (Ghasemlou et al., 2013) limiting the availability of hydrogen groups might be responsible for lower water losses. (Shen et al., 2010) However the rate of increase in pH and water loss was lower for natural polyphenols incorporated coating solution containing pork balls. The development of desiccated surface layer over coating in cold storage might produced a further resistance to mass transfer thus bringing about a slow increase in water loss. The results were in accordance with the result of Yongling et al. (2011). Lowered cooking loss and thawing loss might be associated with lower evaporation rate as well as respiration rate due to wrapping in films. (Naga mallika et al. 2018). Decrease in peroxide values was likely due to presence of phenolic compounds i.e. Tannic acid and Quercetin could retard the initiation and propagation of lipid oxidation reactions by scavenging lipid radicals (Maqsood & Benjakul, 2010a) and forming low-energy antioxidant radicals that do not readily promote oxidation of unsaturated fatty acids. The TBARS values of T2 and T3 were significantly (P<0.05) lower when compared to T1 and C which might be due to the polyphenols acting as free radical scavengers (Perumulla and Hettiarachchy 2011). The starch polymer based coatings on the surface of the product might have resisted oxygen diffusion and retarded lipid oxidation. These results were in accordance with Chidanandaiah et al. (2009).The values of all treatments along with controls increased continuously during storage period irrespective of temperature of storage but the rate of increase was lower for T2 and T3 when compared to T1 and C. This observation was similar to the results of Yongling et al. (2011), (Pratyusha et al., 2016). These results were in agreement with those of Rostami et al. (2010).

Table 3: Physiochemical and microbial (Mean ± SE) values of pork balls as influenced by different coatings at frozen storage (-18 ± 1°C)

Para meters	Coating treatments	Day 0	Day 15	Day 30	Storage period Day 45	Day 60	Day 75	Day 90
pH	C	6.25±0.01^{aA}	6.42±0.01^{bA}	6.49±0.02^{cA}	6.54±0.01^{dA}	*6.74±0.01^{eA}	*6.79±0.01^{fA}	*6.84±0.02^{gA}
	T1	5.96±0.01^{aB}	6.49±0.01^{bB}	6.47±0.02^{cA}	6.53±0.02^{dA}	6.57±0.01^{eB}	*6.73±0.01^{fB}	*6.76±0.04^{gB}
	T2	5.84±0.04^{aC}	6.46±0.01^{bB}	6.48±0.01^{cA}	6.51±0.02^{dA}	6.56±0.03^{eB}	6.60±0.01^{fC}	*6.74±0.04^{gB}
	T3	5.93±0.01^{aB}	6.39±0.01^{bC}	6.46±0.01^{cA}	6.52±0.01^{dA}	6.55±0.02^{eB}	6.64±0.01^{fC}	*6.75±0.03^{gB}
TBARS	C	1.18±0.09^{aA}	1.21±0.01^{bA}	1.44±0.01^{cA}	1.53±0.11^{dA}	*1.84±0.11^{eA}	*1.92±0.17^{fA}	*2.07±0.21^{gA}
	T1	1.12±0.05^{aB}	1.14±0.12^{bB}	1.38±0.13^{cB}	1.48±0.07^{dB}	1.66±0.07^{eB}	*1.78±0.19^{fB}	*1.90±0.11^{gB}
	T2	1.08±0.06^{aC}	1.09±0.21^{bB}	1.33±0.11^{cB}	1.43±0.09^{dC}	1.61±0.04^{eC}	1.76±0.30^{fB}	*1.83±0.13^{gC}
	T3	1.06±0.06^{aC}	1.08±0.12^{bB}	1.33±0.11^{cB}	1.42±0.07^{dC}	1.62±0.08^{eC}	1.76±0.09^{fB}	*1.82±0.11^{gC}
PV	C	0.84±0.03^{aA}	1.22±0.30^{bA}	1.42±0.11^{cA}	2.32±0.07^{dA}	*2.77±0.19^{eA}	*2.97±0.11^{fA}	*3.20±0.09^{gA}
	T1	0.77±0.02^{aB}	1.18±0.15^{bB}	1.39±0.13^{cB}	2.27±0.08^{dB}	2.68±0.15^{eB}	*2.92±0.22^{fB}	*2.93±0.07^{gA}
	T2	0.72±0.01^{aC}	1.15±0.12^{bB}	1.38±0.09^{cB}	2.24±0.12^{dB}	2.58±0.21^{eC}	2.67±0.07^{fC}	*2.81±0.21^{gB}
	T3	0.71±0.02^{aC}	1.16±0.17^{bB}	1.37±0.09^{cB}	2.25±0.11^{dB}	2.57±0.22^{eC}	2.66±0.06^{fC}	*2.79±0.21^{gB}
TPC	C	4.92±0.02^{aA}	5.20±0.21^{bA}	5.57±0.31^{cA}	5.82±0.21^{dA}	*5.93±0.09^{eA}	*6.14±0.19^{fA}	*6.32±0.13^{gA}
	T1	4.85±0.16^{aB}	5.10±0.22^{bB}	5.33±0.22^{cB}	5.58±0.17^{dB}	5.82±0.11^{eB}	*5.93±0.21^{fA}	*6.05±0.18^{gA}
	T2	4.70±0.03^{aC}	5.11±0.21^{bB}	5.27±0.29^{cC}	5.49±0.13^{dC}	5.54±0.13^{eC}	5.57±0.22^{fB}	*5.81±0.11^{gA}
	T3	4.60±0.01^{aD}	5.11±0.33^{bB}	5.28±0.21^{cC}	5.49±0.11^{dC}	5.59±0.11^{eC}	5.63±0.18^{fC}	*5.89±0.21^{gB}

(P<0.05); Means bearing at least one common superscript (small letter) in the same row do not differ significantly. * spoiled
Means bearing at least one common superscript (capital letters) in the same column (treatment wise) do not differ significantly.

Microbial quality: Total plate counts (TPC) were significantly (P<0.05) lower in coated samples when compared to the control samples. This might be due to the antimicrobial effect of polyphenols in synergy with the ability of starch to produce strong gels resulting in formation of semi permeable layer reducing microorganism infiltration into the coated samples accompanied by lowering water activity. During storage the total plate counts increased with increasing storage period. However lower counts were noticed in treatment samples in comparison with controls. Further TPC of T2 and T3 were significantly (P<0.05) lower than the control and T1.

The marginal improvement in microbial quality of the product might be due to the effect of phenolic compounds that are present in natural poly phenols which were proved to possess certain antimicrobial activity in vitro. These results were in agreement with the reports of Chidanandaiah et al. (2009) and

Yongling et al. (2011).The pork balls coated with starch based coating incorporated with polyphenols recorded significantly ($P<0.05$) higher sensory scores than the control pork balls which were coated with only starch based coatings. This could be due to the reduced loss of volatile compounds in these products while cooking. These sensory results were in accordance with Chidanandaiah et al. (2009), Yongling et al. (2011).

Sensory evaluation: It is obvious that the sensory scores decreased significantly (P<0.0.5) in both treatment and control groups with increasing storage time. This might be due to microbial effect, oxidation of lipid and degradation of protein in the pork balls. The results were well in correlation with those of Moawad et al. (2017) who worked on the effect of combination of Olive oil extract and Tannic acid on quality of refrigerated beef patties, Rosireddy *et al.* (2015) in mutton balls applied with polymer coatings enriched with natural spice oils and Pratyusha et al. (2014) in chicken nuggets coated with natural antioxidants enriched edible polymer coatings.

Table 4: Sensory (Mean ± SE) values of pork balls as influenced by different coatings at refrigeration storage (4 ± 1^0C)

		Level of Giriraja spent hen meat			
Parameters	Coating treatments	Day 0	Day 5	Day 10	Day 15
Colour	C	8.07 ± 0.31^{aA}	6.50 ± 0.43^{bA}	$*4.00\pm0.26^{cA}$	$*3.50\pm0.22^{dA}$
	T 1	7.50 ± 0.22^{aB}	7.18 ± 0.17^{bB}	5.87 ± 0.21^{cB}	$*5.07\pm0.33^{dB}$
	T2	7.57 ± 0.21^{aB}	7.22 ± 0.13^{bB}	5.50 ± 0.31^{cB}	5.37 ± 0.43^{dB}
	T 3	7.60 ± 0.21^{aB}	7.24 ± 0.20^{bB}	5.48 ± 0.33^{cB}	5.43 ± 0.31^{dB}
Flavour	C	8.17 ± 0.3^{aA}	6.16 ± 0.30^{bA}	$*3.66\pm0.21^{cA}$	$*3.16\pm0.16^{dA}$
	T 1	7.83 ± 0.17^{aB}	6.83 ± 0.16^{bB}	6.16 ± 0.30^{cB}	$*4.16\pm0.30^{dB}$
	T2	7.50 ± 0.22^{aB}	6.83 ± 0.16^{bB}	5.66 ± 0.21^{cC}	5.16 ± 0.21^{dC}
	T 3	7.66 ± 0.21^{B}	6.93 ± 0.21^{bB}	5.50 ± 0.22^{cC}	5.06 ± 0.21^{dC}
Tenderness	C	8.66 ± 0.21^{aA}	6.83 ± 0.40^{bA}	$*3.50\pm0.22^{cA}$	$*3.17\pm0.17^{dA}$
	T 1	8.36 ± 0.21^{aB}	7.03 ± 0.17^{bA}	5.66 ± 0.21^{cB}	$*4.83\pm0.31^{dB}$
	T2	8.00 ± 0.17^{aB}	7.76 ± 0.17^{bB}	5.83 ± 0.40^{cB}	5.07 ± 0.21^{dC}
	T 3	7.97 ± 0.22^{aB}	7.66 ± 0.21^{bB}	5.86 ± 0.33^{cB}	5.33 ± 0.42^{dC}
Juiciness	C	8.50 ± 0.22^{aA}	6.63 ± 0.17^{bA}	$*4.17\pm0.05^{cA}$	$*3.17\pm0.17^{dA}$
	T 1	8.53 ± 0.22^{aA}	6.70 ± 0.22^{bA}	5.33 ± 0.33^{cB}	$*4.17\pm0.17^{dB}$
	T2	8.60 ± 0.21^{aB}	6.83 ± 0.21^{bA}	5.67 ± 0.21^{cB}	5.33 ± 0.21^{dC}
	T 3	8.67 ± 0.21^{aB}	6.87 ± 0.17^{bA}	5.50 ± 0.22^{cB}	5.20 ± 0.22^{dC}
Overall acceptability	C	8.35 ± 0.31^{aA}	6.53 ± 0.22^{bA}	$*3.83\pm0.22^{cA}$	$*3.25\pm0.17^{dA}$
	T 1	8.05 ± 0.17^{aB}	6.93 ± 0.22^{bB}	5.75 ± 0.21^{cB}	$*4.42\pm0.17^{dB}$
	T2	7.91 ± 0.22^{aB}	7.16 ± 0.31^{bB}	5.66 ± 0.37^{cB}	5.23 ± 0.22^{dC}
	T 3	7.97 ± 0.33^{aB}	7.17 ± 0.21^{bB}	5.58 ± 0.26^{cB}	5.25 ± 0.21^{dC}

($P<0.05$); Means bearing at least one common superscript (small letter) in the same row do not differ significantly. * spoiled

Means bearing at least one common superscript (capital letters) in the same column (treatment wise) do not differ significantly

Anti-microbial activity: The results of modified disc diffusion test revealed that starch coatings containing tannic acid and quercetin can effectively inhibit the growth of two tested bacteria with varying magnitude. Both gram positive and gram negative bacteria were sensitive to the potent natural polyphenols. Tannic acid exhibited better effect when compared to the moderate effect seen with quercetin at 1% level. The zone of inhibition of tannic acid and quercetin incorporated films ranged from 9.75 to 9.45 mm in diameter

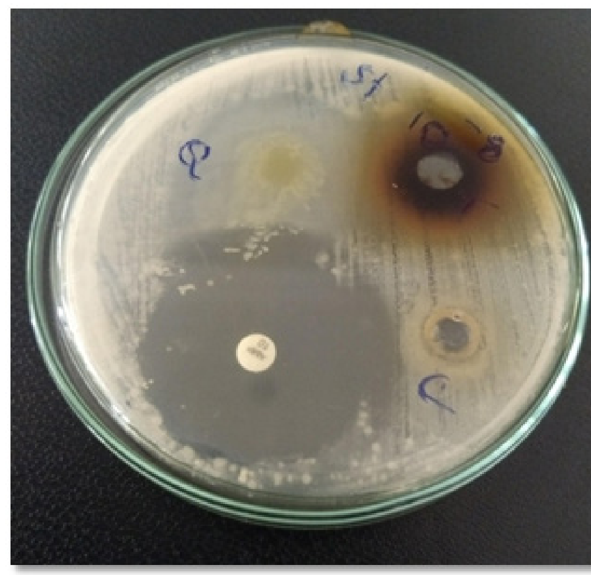

Fig.1: Showing the inhibitory effect of Tannic acid and Quercetin containing starch coatings in comparison with the standard antibiotic

CONCLUSION

The salient features of the study revealed that application of starch coatings with incorporation of tannic acid and quercetin had significant effect in reducing the TBARS values and total plate counts of pork balls and can improve the quality and shelf-life of the product without compromising the sensory quality.

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COMPETING INTERESTS

No

ETHICS STATEMENT

Not Applicable

Table 5: Sensory (Mean ± SE) values of pork balls as influenced by different coatings at frozen storage (-18 ± 1^{o}C)

Para meters	Coating treatments	Storage period Day 0	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
Colour	C	8.07±0.31^{aA}	7.17±0.30^{bA}	6.67±0.21^{cA}	5.17±0.30^{dA}	*4.83±0.30^{eA}	*3.67±0.21f^{A}	*3.12±0.11^{gA}
	T1	7.50±0.22^{aB}	7.33±0.42^{bA}	6.77±0.21^{cA}	5.67±0.33^{dB}	5.33±0.21^{eB}	*5.04±0.16f^{B}	*5.01±0.30^{gB}
	T2	7.57±0.21^{aB}	7.33±0.42^{bA}	6.83±0.16^{cA}	6.17±0.30^{dC}	5.50±0.22^{eB}	5.30±0.22f^{B}	*5.09±0.03^{gB}
	T3	7.60±0.21^{aB}	7.33±0.42^{bA}	6.83±0.16^{cA}	6.00±0.36^{dC}	5.50±0.22^{eB}	5.32±0.22f^{B}	*5.05±0.03^{gB}
Flavour	C	8.17±0.3^{aA}	7.03±0.33^{bA}	6.50±0.22^{cA}	5.33±0.33^{dA}	*4.66±0.21^{eA}	*3.50±0.22f^{A}	*3.05±0.03^{gA}
	T1	7.83±0.17^{aB}	7.17±0.40^{bA}	6.67±0.21^{cA}	5.66±0.21^{dA}	5.16±0.16^{eB}	*4.33±0.42f^{B}	*3.65±0.24^{gB}
	T2	7.50±0.22^{aB}	7.23±0.33^{bA}	7.00±0.25^{cB}	6.33±0.33^{dB}	5.66±0.21^{eC}	5.33±0.21f^{C}	*4.20±0.14^{gC}
	T3	7.66±0.21^{B}	7.33±0.42^{bA}	6.93±0.16^{cB}	6.20±0.36^{dB}	5.58±0.22^{eC}	5.40±0.22f^{C}	*4.33±0.18^{gC}
Tenderness	C	8.66±0.21^{aA}	6.83±0.33^{bA}	6.50±0.22^{cA}	5.33±0.33^{dA}	*4.66±0.21^{eA}	*3.50±0.22f^{A}	*3.16±0.17^{gA}
	T1	8.36±0.21^{aB}	6.96±0.40^{bA}	6.66±0.21^{cA}	5.66±0.21^{dA}	5.16±0.17^{eB}	*5.03±0.42f^{B}	*3.83±0.31^{gB}
	T2	8.00±0.17^{aB}	7.03±0.33^{bA}	7.00±0.26^{cB}	6.33±0.33^{dB}	5.76±0.22^{eC}	5.50±0.22f^{C}	*4.16±0.40^{gB}
	T3	7.97±0.22^{aB}	7.13±0.42^{bA}	6.93±0.17^{cB}	6.20±0.37^{dB}	5.72±0.21^{eC}	5.56±0.21f^{C}	*4.20±0.43^{gB}
Juiciness	C	8.50±0.22^{aA}	7.16±0.31^{bA}	6.66±0.21^{cA}	5.16±0.31^{dA}	*4.83±0.31^{eA}	*3.66±0.21f^{A}	*3.16±0.17^{gA}
	T1	8.53±0.22^{aA}	7.33±0.42^{bA}	6.76±0.21^{cA}	5.66±0.33^{dB}	5.33±0.21^{eB}	*5.06±0.17f^{B}	*4.16±0.17^{gB}
	T2	8.60±0.21^{aB}	7.38±0.42^{bA}	6.83±0.17^{cA}	6.16±0.31^{dC}	5.52±0.22^{eB}	5.30±0.22f^{B}	*4.33±0.21^{gB}
	T3	8.67±0.21^{aB}	7.39±0.42^{bA}	6.80±0.17^{cA}	6.00±0.37^{dC}	5.50±0.22^{eB}	5.32±0.22f^{B}	*4.50±0.22^{gB}
Overall acceptability	C	8.35±0.31^{aA}	7.04±0.31^{bA}	6.58±0.21^{cA}	5.24±0.30^{dA}	*4.74±0.30^{eA}	*3.58±0.21f^{A}	*3.12±0.16^{gA}
	T1	8.05±0.17^{aB}	7.20±0.42^{bA}	6.71±0.21^{cA}	5.66±0.33^{dB}	5.24±0.21^{eB}	*4.86±0.25f^{B}	*4.16±0.42^{gB}
	T2	7.91±0.22^{aB}	7.24±0.42^{bA}	6.91±0.16^{cA}	6.16±0.30^{dC}	5.61±0.22^{eC}	5.35±0.30f^{C}	*4.44±0.22^{gB}
	T3	7.97±0.33^{aB}	7.29±0.42^{bA}	6.87±0.16^{cA}	6.10±0.37^{dC}	5.57±0.22^{eC}	5.40±0.22f^{C}	*4.52±0.21^{gB}

($P<0.05$); Means bearing at least one common superscript (small letter) in the same row do not differ significantly. * spoiled
Means bearing at least one common superscript (capital letters) in the same column (treatment wise) do not differ significantly.

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