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RETORT PROCESSED, INDIAN TRADITIONAL TYPE CHETTINAD CHICKEN PRODUCT

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Abstract : Chettinad chicken was prepared using boneless meat derived from spent chicken and broiler breeder packed in retort pouches (250g), and processed in retort at the product temperature of 121.1ºC and the corresponding F_0 value of 5.2. The product was stored at ambient temperature (35 ± 2°C) up to 180 days. The sensory scores for texture of the Chettinad chicken prepared from spent chicken and broiler breeder meat decreased significantly however the scores were rated very acceptable even on 180th day. The thiobarbituric acid (TBA), tyrosine values and acid value increased gradually during storage but E. *coli, Salmonella spp, Clostridium spp, Staphylococci spp, Streptococci spp,* yeast and mould could not be detected during the entire storage period. The cost of production of Chettinad chicken (250 g) prepared from spent chicken meat and broiler breeder meat was Rs.37 and Rs.50, respectively. It was concluded that the retort processed Chettinad chicken prepared from spent chicken and broiler breeder meat can be safely stored up to 180 days at ambient temperature.

Keywords: Broiler breeder meat, Spent chicken meat, Chettinad chicken, Retort, Storage stability.

INTRODUCTION

Spent chickens either layer or broiler breeder are the by-products of layer / broiler poultry industry. Spent chicken meat is tough, dry and sinewy and chickence the demand for marketing of spent chickens for meat is limited. With proper processing or value addition the tough meat can be converted in to acceptable meat product and thereby can increase the profit margin of the poultry farmers. Chettinad chicken is a famous traditional meat product of Tamil Nadu originated from Karaikudi region. High perishability of meat and meat products is a serious problem in developing countries including India due to climatic condition and limited refrigeration facilities (Bachhil, 1982). It is necessary to develop shelf stable product to attract consumers. A ready-to-serve consumer product of good shelf-life will definitely have good market. This situation naturally demands appropriate technologies for its profitable and proper utilization (Bindu *et al*., 2004). Retort processing enables to providing ready to eat

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processed food products which are stable at ambient temperature. Present study was planned to assess the storage stability of retort processed Chettinad chicken at ambient temperature up to 180 days.

MATERIALS AND METHODS

Preparation of Chettinad chicken

Layer spent chicken birds and broiler breeder birds weighing about 1.5 kg and 4 kg, respectively were purchased from Private poultry farms and birds were slaughtered by Halal method. Deboned meat from breast and thigh region was packed in low density polyethylene (LDPE, 250 gauge) bags and kept under frozen storage (-20^{o}C). The recipe for Chettinad chicken was standardized (Table 1). Dry spices, onion, garlic and ginger were fried in refined oil and mixed with the precooked meat. The whole mix was fried for about 10 min and cooled.

Retort processing

Retort processing was done at Varsha Fresh Meat Products Limited, processing plant near by Govindapuram in Kerala. Chettinad chicken (250 g) was carefully filled in four laminated retort pouch (M/s. Pradeep Laminators Pvt. Ltd., Pune). The hermetically sealed pouches on the trays were loaded in the retort machine (M/s.Lakshmi Engineering Works, Cchickennai). For heat penetration studies, a sample pouch was fixed with thermo couple glands through which thermocouple was inserted into meat pieces for recording the core temperature during heat processing. The filled and sealed pouches (along with sample pouch) were subjected to thermal processing at 121.1ºC and 5.2. F_0 value for 36 min. Pressure was maintained at 20 psi throughout the process. Rapid cooling was done to 55^{0} C. The processed retort pouches were stored in cool dry place for further storage studies at ambient temperature (35 ± 2 ^{o} C).

The samples were analyzed for moisture, crude protein, ether extract and total ash as per the standard procedures (AOAC, 1995).

pH of Chettinad chicken was determined as per AOAC (1995). Thiobarbituric acid (TBA) value was estimated by extraction method described by Witte *et al.* (1970) and was expressed as mg malonaldehyde (MA) per kg of pet food. The procedure of Strange *et al.* (1977) was followed for the tyrosine value. Tyrosine value was calculated and expressed as mg of tyrosine per 100 g of sample. Estimation for the free fatty acid content, acid value and peroxide value was done as per AOAC (1995). The microbiological quality of pet foods was assessed in respect of total plate count (TVC), *E. coli, Staphylococci spp, Clostridium spp, Salmonella spp, yeast and mould* count as per the method prescribed by Quinn *et al*. (1994)

and expressed as the log cfu /g of sample. Sensory quality of Chettinad chicken during storage was assessed through an 8-point hedonic scale ranging from 8 (extremely acceptable) to 1 (extremely unacceptable) by the in house semi trained taste panelists. The cost of production of the Chettinad chicken prepared from spent chicken meat and broiler breeder meat was calculated based on the market price of the live birds and ingredients. The data (3 replicates) were subjected to analysis of variance (ANOVA) as per Snedecor and Cochran (1989) using Statistical Analytical System (SPSS, 1999) version 10.0 for Windows. Significant differences ($p<0.05$) were tested by Duncan's multiple range test.

Results and discussion

The average live weight of spent chicken and broiler breeder birds was 1.12 kg and 3.89 kg and the average boneless meat yield per bird was 0.24 kg and 1.24 kg per bird, respectively. The yield of boneless meat from thigh and breast region of spent chicken and broiler breeder was 21.06 and 31.82 per cent live weight, respectively.

Retort processing

The thermal processing conditions adopted in the present study were in agreement with Frott and Lewis (1994), who reported that recommended F_0 value for retort processed fish products ranges from 5-20. Gopal *et al*. (2001) maintained the retort temperature at 121.1°C and the traditional Kerala style fish curry, filled in pouches were heat processed to F_0 values of 6.56 and 8.43. Devadason (2000) also studied thermal processing of shelf stable buffalo meat blocks in retort pouches and the corresponding F_0 value determined was 6.52 (5D of *Clostridium sporogenes* PA 6379). While Rajkumar (2008) reported that retort processed Chettinad goat meat product with F_0 value of 12.10 was rated best by the taste panelists on sensory evaluation. The cooking yield of Chettinad chicken prepared from spent chicken and broiler breeder meat was 56.07 ± 0.03 and 55.64 ± 0.03, respectively.

Proximate composition

There was no significant difference in the proximate composition of Chettinad chicken prepared from spent chicken and broiler breeder meat (Table 2). The proximate composition of the retort processed products were determined by Thankamma *et al*. (1998) in fish paste, Devadason (2000) in buffalo meat block, Bindu *et al.* (2007) in ready to eat black calm product and Rajkumar (2008) in Chettinad goat meat. Abdullah (2007) reported that in luncheon meat the chemical composition (moisture, protein and fat) did not influence the overall acceptability of the different formulation after retort processing. Variation in the proximate principles might be due to type of meat and product.

Storage stability of Chettinad chicken

The moisture content decreased significantly (P<0.01) in the Cheittinad chicken prepared from spent chicken meat and from broiler meat, respectively during the storage period (Table 3). This is in conformity with report of Rajkumar (2008), who stored retort processed chevon up to 10 months. The release of moisture in the gravy during heat denaturation of proteins might have contributed for sampling error during storage studies. Leander *et al*., (1980) also observed that when chicken meat is cooked, water soluble proteins and fats are expelled from the tissue. This was again supported by Martens *et al.*, (1982).

The pH also decreased significantly (P<0.01) in the Cheittinad chicken prepared from spent chicken meat and from broiler meat, respectively during storage period (Table 3). Similar trend was also observed during storage by Devadason (2000) in retort processed buffalo meat block and Rajkumar (2008) in retort processed Chettinad goat meat .This might be due to degradation of proteins and liberation of free amino acids.

As expected tyrosine values increased significantly (P<0.01) during the storage (Table 3). The increase was in agreement with the observations of Devadasan (2000) in retort processed buffalo meat block and Rajkumar (2008) in retort processed Chettinad goat meat. Tyrosine value increases during storage time until deamination of amino acid limits the formation of free amino acids (Pearson, 1968). Similarly an increase of tyrosine value during storage was also reported in buffalo meat patties (Kulkarni *et al.,*1993).

The TBA values increased significantly (P<0.01) during the storage (Table 3). A significant and linear increase of TBRS (ThioBarbituric acid Reacting Substances) during storage was reported by Devadason (2000) in retort processed buffalo meat block and Rajkumar (2008), and in retort processed Chettinad goat meat, respectively. The increase in TBA value might be due to residual oxygen remaining in the pouch as the pouches were not vacuum sealed. The development of slow oxidative rancidity was also reported by Chia *et al.,* (1983) in retort pouch fish products. However Zipser and Watts (1961) stated that the production of antioxidants in meat itself at high temperature was responsible for stability against oxidative rancidity.

The free fatty acid decreased significantly (P<0.01) during storage in the Cheittinad chicken (Table 3). Free fatty acids are the products of enzymatic or microbial lipolysis of lipids. Increase in FFA in retort processed black clam meat was also observed by Bindu *et al*., (2007) during a storage period up to 12 months.

The acid values increased significantly (P<0.01) in the Cheittinad chicken prepared during the storage period (Table 3). In the present study acid value increased with the decrease in the pH during the storage, this might be due to degradation of proteins and liberation of free amino acids (Rajkumar 2008). Increase in the free amino acids content was reported by Devadason (2000) in retort processed buffalo meat block during room temperature storage of 135 days. Peroxide value was nil during the entire storage period.

Microbial quality

Total bacterial counts including *E. coli, Salmonella* spp*., Clostridium* spp*., Staphylococci* spp*,* yeast and mould could not be detected in the Chettinad chicken samples during the entire storage. The results were in agreement with Terajima and Nonaka (1996) who reported that commercial heat sterilization of foods in retort pouches reduced undesirable microorganism. Thankamma *et al.,* (1998) reported that E*. coil, Staphylococcus. V. cholera* and *Salmonella* were absent in retort processed and stored fish paste. Absence of anaerobe colonies in the thermal processing of shelf stable buffalo meat blocks in retort pouches during storage period was reported by Devadason (2000). Manju *et al*., (2004) also observed that seer fish curry packed in retort pouch remained sterile throughout storage period of 19 months. Rajkumar (2008) reported that *coliforms, clostridium botulinum, staphylococcus*, yeast and mould were absent in retort processed Chettinad goat meat during storage up to 10 months.

Sensory Evaluation

The sensory scores of the Chettinad chicken prepared from both broiler spent chicken and broiler breeder meat revealed that the texture scores (only in spent chicken meat) and overall acceptability scores decreased significantly (P<0.01), however the values for all the other sensory parameters of Chettinad chicken did not change significantly during the entire storage period. (Table 4). The significant decrease in the texture scores from very desirable (7.6) to moderately desirable (6.2) was also reported by Devadason (2000) in retort processed buffalo meat block during room temperature storage of 135 days. This might be also due to slight degradation of proteins and oxidative changes in the product. Rajkumar (2008) observed that the texture scores of the Chettinad goat meat processed in retort pouch decreased from 8.20 to 7.07 during storage up to 10 months, but the scores were above the acceptable limits. The decrease in the texture scores as observed in the present study might be a reason for decrease in the overall acceptability of the Chettinad chicken, however the scores ranged from 7.77 to 7.33 (very acceptable) during the entire storage period and no toughness was detected during sensory evaluation.

Cost of production

The cost of production of Chettinad chicken (250 g pouch) prepared from spent chicken meat and broiler breeder meat was Rs.37 and Rs. 50, respectively. This was lower than the cost of the commercially available retort processed meat products.

Conclusion

The tough meat from spent chicken and broiler breeder bird can be used in making value added meat product Chettinad chicken and retort processing can be effectively used for tenderizing tough meat of spent chicken and broiler breeder while retaining the sensory quality during the entire storage period of 180 days. This could help in popularizing traditional styled products like Chettinad chicken in different places of the country.

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Table 1. Chettinad chicken recipe

Ingredients	%
Chicken meat (boneless)	100
Chilly power	7.5
Coriander powder	5.0
Turmeric powder	0.3
Big onion	50
Tomato	50
Curry leaves	0.3
Coriander leaves	0.4
Garlic	5.0
Ginger	2.5
Anise seed	2.0
Salt	2.5
Refined oil	50
Spice mix (cinnamon, cloves, cardamom powders mixed in equal parts)	0.6

Table 2. Proximate composition of Chettinad chicken

Moisture (%)		Crude protein (%)		Ether extract (%)		Total ash (%)	
Spent chicken	Broiler breeder	Spent chicken	Broiler breeder	Spent chicken	Broiler breeder	Spent chicken	Broiler breeder
51.21 ± 0.02	53.83 ± 0.04	19.87 ± 0.08	25.51 ± 0.17	36.63 ± 0.25	33.12 ± 0.32	3.42 ± 0.09	3.45 ± 0.11

NS-Not significant, n= 6.

Table 3. Physicochemical composition of Chettinad chicken during storage at ambient temperature

Storage (days)	Moisture (%)	pH	Tyrosine (mg/100g)	TBA (mg MA/kg)	FFA (%)	Acid value (ml/g)
Spent chicken meat						
0	$52.80^{k} \pm 0.10$	$6.26^{a} \pm 0.01$	$82.96^{a} \pm 11.49$	$4.05^{a} \pm 0.72$	$0.08^{e} \pm 0.00$	$0.63^{a} \pm 0.05$
15	$52.48^{j} \pm 0.11$	$6.24^{eg} \pm 0.00$	$89.42^{b} \pm 1.45$	$4.19^{b} \pm 0.22$	$0.08^{e} \pm 0.00$	$0.65^{b} \pm 0.05$
30	$51.88^{i} \pm 0.09$	$6.22^{e} \pm 0.02$	$90.48^{bc} \pm 0.89$	$4.43^{bc} \pm 0.10$	$0.08^{e} \pm 0.01$	$0.65^{b} \pm 0.05$
45	$51.64^{h} \pm 0.05$	$6.21^{cde} \pm 0.01$	$91.13^{bc} \pm 1.07$	$4.60^{cd} \pm 0.15$	$0.07^{e} \pm 0.01$	$0.75^{bc} \pm 0.05$
60	$51.38^{g} \pm 0.10$	$6.21^{g} \pm 0.01$	$94.02^{bcd} \pm 0.73$	$4.79^{de} \pm 0.05$	$0.07^{d} \pm 0.00$	$0.77^{bc} \pm 0.05$
75	$51.21^{f} \pm 0.05$	$6.20^{cd} \pm 0.01$	$95.32^{cd} \pm 0.45$	$4.94^{def} \pm 0.09$	$0.07^{d} \pm 0.00$	$0.82^{bcd} \pm 0.04$
90	$50.88^{e} \pm 0.06$	$6.16^{bcd} \pm 0.08$	$96.70^{de} \pm 0.25$	$5.03^{ef} \pm 0.04$	$0.07^{d} \pm 0.00$	$0.87^{bcde} \pm 0.05$
105	$50.85^{de} \pm 0.09$	$6.18^{abc} \pm 0.00$	$97.39^{de} \pm 0.33$	$5.04^{ef} \pm 0.01$	$0.06^{cd} \pm 0.00$	$0.88^{bcde} \pm 0.04$
120	$50.55^{d} \pm 0.07$	$6.18^{abc} \pm 0.01$	$97.57^{de} \pm 0.23$	$5.11^{ef} \pm 0.01$	$0.06^{bc} \pm 0.00$	$0.97^{bcde} \pm 0.05$
135	$50.16^{c} \pm 0.07$	$6.17^{ab} \pm 0.00$	$98.20^{de} \pm 0.08$	$5.15^{gf} \pm 0.02$	$0.06^{a} \pm 0.00$	$0.98^{bcde} \pm 0.04$
150	$49.81^{b} \pm 0.14$	$6.16^{ab} \pm 0.01$	$98.39^{de} \pm 0.26$	$5.24^{gf} \pm 0.02$	$0.06^{a} \pm 0.00$	$1.02^{cde} \pm 0.08$
165	$49.76^{b} \pm 0.09$	$6.15^{a} \pm 0.00$	$99.18^{de} \pm 0.06$	$5.21^{g} \pm 0.10$	$0.06^{a} \pm 0.00$	$1.13^{de} \pm 0.05$
180	$49.60^{a} \pm 0.11$	$6.15^{a} \pm 0.00$	$99.33^{ade} \pm 0.10$	$5.36^{g} \pm 0.01$	$0.05^{a} \pm 0.00$	$1.20^{e} \pm 0.00$
Broiler breeder meat						
0	$53.83^{m} \pm 0.09$	$6.28^{h} \pm 0.01$	$79.41^{a} \pm 8.4$	$4.03^{a} \pm 0.56$	$0.11^{i} \pm 0.01$	$0.67^{a} \pm 0.05$
15	$53.30^{l} \pm 0.13$	$6.25^{h} \pm 0.01$	$85.58^{a} \pm 2.48$	$4.17^{abc} \pm 0.24$	$0.11^{ghi} \pm 0.00$	$0.65^{a} \pm 0.05$
30	$52.84^{k} \pm 0.09$	$6.25^{h} \pm 0.01$	$88.61^{a} \pm 0.24$	$4.14^{ab} \pm 0.39$	$0.11^{hi} \pm 0.00$	$0.67^{a} \pm 0.05$
45	52.28j ± 0.08	$6.24^{g} \pm 0.01$	$89.93^{b} \pm 0.67$	$4.37^{bc} \pm 0.13$	$0.11^{i} \pm 0.01$	$0.68^{ab} \pm 0.08$
60	52.10i ± 0.04	$6.24^{g} \pm 0.01$	$92.42^{bcd} \pm 0.71$	$4.57^{cd} \pm 0.06$	$0.11^{fgh} \pm 0.00$	$0.73^{c} \pm 0.05$

75	$51.88^{h} \pm 0.10$	$6.21^{f} \pm 0.01$	$95.44^{cdef} \pm 0.47$	$4.72^{de} \pm 0.02$	$0.10^{efg} \pm 0.00$	$0.70^{b} \pm 0.00$
90	$51.79^{g} \pm 0.11$	$6.20^{ef} \pm 0.00$	$98.21^{defgh} \pm 0.15$	$4.88^{ef} \pm 0.05$	$0.10^{efg} \pm 0.00$	$0.77^{d} \pm 0.05$
105	$51.45^{f} \pm 0.02$	$6.20^{de} \pm 0.00$	$98.45^{efgh} \pm 0.37$	$5.01^{ef} \pm 0.03$	$0.10^{def} \pm 0.00$	$0.83^{de} \pm 0.05$
120	$50.91^{e} \pm 0.07$	$6.19^{cde} \pm 0.01$	$99.07^{fgh \pm} 0.27$	$5.19^{fg} \pm 0.05$	$0.10^{cde} \pm 0.00$	$0.88^{ef} \pm 0.04$
135	$50.78^{d} \pm 0.11$	$6.19^{bcd} \pm 0.00$	$99.81^{ghi} \pm 0.49$	$5.38^{gh} \pm 0.03$	$0.10^{cd} \pm 0.00$	$0.92^{fg} \pm 0.04$
150	$50.48^{c} \pm 0.08$	$6.19^{abc} \pm 0.00$	$101.83^{hij} \pm 1.62$	$5.39^{gh} \pm 0.15$	$0.09^{bc} \pm 0.00$	$0.98^{gh} \pm 0.04$
165	$50.16^{b} \pm 0.10$	$6.18^{a} \pm 0.01$	$103.23^{ij} \pm 0.11$	$5.62^{hi} \pm 0.01$	$0.09^{ab} \pm 0.00$	$1.02^{h} \pm 0.04$
180	$49.83^{a} \pm 0.07$	$6.18^{a} \pm 0.00$	$103.76^{j} \pm 0.08$	$5.82^{i} \pm 0.01$	$0.09^{a} \pm 0.00$	$1.1^{i} \pm 0.00$

Mean with different superscript in a row differ significantly (*P< 0.01 ;** p<0-05), n=6

Table 4. Sensory scores of Chettinad chicken during storage at ambient temperature

Spent chicken meat

Storage days	Appearance	Flavour	Juiciness	Texture	Saltiness	Mouth coating	Overall acceptability
0	7.71 ± 0.14	7.83 ± 0.08	7.58 ± 0.15	7.67^{b} ± 0.1	7.79 ± 0.08	7.50 ± 0.15	7.77^{ab} ± 0.53
15	7.92 ± 0.06	7.96 ± 0.04	7.88 ± 0.07	7.92^{b} ± 0.06	7.63 ± 0.10	7.63 ± 0.10	7.92^{ab}± 0.28
30	7.96 ± 0.04	7.96 ± 0.04	7.92 ± 0.06	7.96^{b} ± 0.04	7.63 ± 0.10	7.54 ± 0.10	7.96^{ab}± 0.2
45	7.96 ± 0.04	7.92 ± 0.06	7.88 ± 0.09	7.92^{b} ± 0.06	7.63 ± 0.10	7.50± 0.10	7.92^{ab} ± 0.28
60	7.92 ± 0.06	7.83 ± 0.10	7.75 ± 0.12	7.79^{ab} ± 0.12	7.63 ± 0.12	7.58 ± 0.12	7.83^{ab}± 0.48
75	7.92 ± 0.06	7.83 ± 0.10	7.79 ± 0.12	7.71^{ab} ± 0.11	7.67 ± 0.12	7.58 ± 0.15	7.79^{ab}± 0.59
90	7.88 ± 0.07	7.79 ± 0.10	7.79 ± 0.10	7.75^{ab} ± 0.11	7.75 ± 0.11	7.46 ± 0.15	7.75^{ab} ± 0.61
105	7.83 ± 0.08	7.79 ± 0.12	7.75 ± 0.12	7.71^{ab} ± 0.14	7.88 ± 0.07	7.54 ± 0.12	7.79^{ab}± 0.59
120	7.83 ± 0.08	7.79 ± 0.12	7.63 ± 0.15	7.67^{ab} ± 0.14	7.71 ± 0.11	7.50 ± 0.15	7.71^{ab} ± 0.69

135	7.92 ± 0.06	7.83 ± 0.10	7.75 ± 0.11	7.79^{ab} ± 0.1	7.71 ± 0.13	7.50 ± 0.13	7.79^{ab} ± 0.51
150	7.92 ± 0.06	7.75 ± 0.09	7.75 ± 0.09	7.63^{ab} ± 0.12	7.67 ± 0.12	7.50 ± 0.12	7.71^{ab}± 0.46
165	7.83 ± 0.10	7.58 ± 0.13	7.58 ± 0.13	$7.5O^{ab}$ ± 0.15	7.63 ± 0.12	7.50 ± 0.12	7.58^{ab}± 0.58
180	8.00± 0.00	7.54 ± 0.10	7.75 ± 0.09	7.13^{a} ± 0.14	7.83 ± 0.08	7.83 ± 0.08	7.33^{a} ± 0.48
Broiler breeder meat							
0	7.75 ± 0.14	7.88 ± 0.07	7.75 ± 0.09	7.73 ± 0.10	7.82 ± 0.08	7.73 ± 0.10	7.95^{b} ± 0.05
15	7.83 ± 0.08	8.00 ± 0.00	7.92 ± 0.06	7.75 ± 0.09	7.67 ± 0.10	7.67 ± 0.10	7.83^{b} ± 0.08
30	7.96 ± 0.04	7.88 ± 0.07	7.67 ± 0.10	7.79 ± 0.08	7.67 ± 0.10	7.67 ± 0.10	7.85^{b} ± 0.06
45	7.88 ± 0.07	7.88 ± 0.07	7.88 ± 0.07	7.79 ± 0.08	7.63 ± 0.10	7.46 ± 0.13	7.88^{b} ± 0.07
60	7.88 ± 0.07	7.88 ± 0.07	7.83 ± 0.10	7.79 ± 0.10	7.67 ± 0.10	7.54 ± 0.13	7.88^{b} ± 0.07
75	7.92 ± 0.06	7.92 ± 0.06	7.88 ± 0.07	7.71 ± 0.09	7.71 ± 0.09	7.54 ± 0.12	7.92^{b} ± 0.06
90	7.92 ± 0.06	7.88 ± 0.07	7.83 ± 0.10	7.75 ± 0.09	7.71 ± 0.11	7.54 ± 0.12	7.88^{b} ± 0.07
105	7.75 ± 0.09	7.88 ± 0.07	7.88 ± 0.07	7.88 ± 0.07	7.88 ± 0.07	7.58 ± 0.12	7.88^{b} ± 0.07
120	7.79 ± 0.08	7.88 ± 0.07	7.79 ± 0.10	7.79 ± 0.10	7.75 ± 0.11	7.54 ± 0.13	7.79^{b} ± 0.10
135	7.88 ± 0.07	7.83 ± 0.10	7.83 ± 0.10	7.88 ± 0.09	7.75 ± 0.12	7.58 ± 0.12	7.92^{b} ± 0.06
150	7.92 ± 0.06	7.79 ± 0.08	7.75 ± 0.09	7.67 ± 0.12	7.67 ± 0.12	7.54 ± 0.12	7.75^{b} ± 0.09
165	7.83 ± 0.10	7.67 ± 0.13	7.67 ± 0.13	7.58 ± 0.15	7.71 ± 0.11	7.54 ± 0.12	7.67^{ab} ± 0.12
180	7.71 ± 0.09	7.67 ± 0.10	7.92 ± 0.06	7.71 ± 0.13	7.88 ± 0.07	7.46 ± 0.10	7.42^{a} ± 0.10

Mean with different superscript in a row differ significantly (*P< 0.01 ;** p<0-05), n=18