CAROTENE CONTENTS OF PROCESSED MEATS BLENDED WITH PALM FATS

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ABSTRACT

The contents of carotene, a pre-cursor of vitamin A of processed meats (beef burgers and chicken frankfurters) blended with palm fats was studied. Alpha-carotene and β-carotene decreased significantly (P<0.05) by 62-66% and 72-80% respectively in beef burgers where the fats were substituted with red palm fat (RPF35) and a mixture of red palm fat and palm fat (FB) when the meats were stored at -18°C for 6 month after cooking. Alpha-carotene lost by 53% and 33% while beta-carotene by 67% and 47%, respectively in raw beef burger where the fats were replaced with RPF35 and FB After storage for 6 months (-18°C). Alphacarotene concentrations lost by 66% and 62%, respectively in beef burger substituted with RPF35 and FB during storage for 6 months at -18°C after cooking. Alpha-carotene in retorted chicken frankfurter (RC) substituted with RPF48 only lost 25% while oven-cooked chicken frankfurter (OC) containing RPF48 lost 61%. Beta-carotene was degraded faster compared to α -carotene in RC. OC and cooked beef burger indicating that the beef burger was more stable after cooking and storage. Even though OC, RC frankfurters and cooked beef burgers substituted with red palm fat showed the highest percent loss in β -carotene concentrations after storage, the value retained was still the highest (23.0, 42.0 and 23.8 μg/g, respectively). In summary, the effect of processing, cooking, frozen storage and the type of fats used could influence the stability of alpha- and beta-carotenes and their content in meat products.

Key words: carotene, oven cooked, retort, Vitamin A, chicken frankfurter, beef burger, red palm fat, palm fats

INTRODUCTION

Animal fats are added to meat products reasons of cost-saving, texture and flavour. Animal fats and skin are useful raw materials but they are also high in saturated fats, cholesterol and harmful microorganisms. As more evidence concerning the benefits and risks associated with dietary nutrients are emerging in both the scientific field and the mass media, today's consumers are more informed on the link between health and diet. Levels of saturated fat and cholesterol have been a major problem, resulting in meat products becoming the subject of scrutiny by nutritional, medical, and consumer groups.

Crude and red palm oil contain between 500 and 700 ppm of carotenoids (Ooi *et al.*, 1996). The major components are α -carotene (35-37%) and β -carotene (47-56%) (Ooi *et al.*, 1996). These carotenoids have pro-vitamin A activity. Carotenoids are often thermally degraded and removed during the deodorization stage of the refining process. In crude palm oil, these carotenoids appear to offer some protection against oxidation by themselves being oxidized first prior to the oxidative attack on the triglycerides (Choo *et al.*, 1993).

The use of functional palm fats, which are cholesterol free and naturally containing carotenoids, tocopherol and tocotrienols, may generate safer, nutritious and better quality of processed meat products to the market. Researchers believe that palm fats make sausages and other meat products better and healthful (Babji *et al.*, 2001; Wan Sulaiman *et al.*, 2001). Alina *et al.* (2000) and Tan *et al.* (2001) suggested the potential of palm oil products, especially palm olein as fat sources in the production of comminuted meat products. Despite these benefits, they still contain a high concentration of saturated fats.

Carotenoids have been cited as responsible for the reduction of the risk of developing degenerative diseases such as cancer, cardiovascular disease and macular degeneration (Marcela *et al.*, 2004). Carotenoids have also been shown in a number of studies to be able to act as a radical scavenging antioxidant. It has been suggested that β-carotene scavenges peroxyl radicals by forming an adduct between β-carotene and the peroxyl radical, yielding a resonance-stabilized carotenoid radical (Burton and Ingold, 1984). The carotenoid pigments can be decolorized by bleaching alone or with high temperature treatment (110-149°F). Conventional deodorization is done at 182-218°C (Onyewu *et al.*, 1986). The carotenoid also can be degraded at deodorization temperatures of 260°C and 219°C.

Carotenoids are like other antioxidants, and are degraded by radicals when functioning as antioxidants and the presence of other antioxidants is thus important for the preservation of colour (Mortensen and Skibsted, 2000). Study of oxidation in chicken liver showed that α -tocopherol decreased the formation of hemichrome, an oxidized form of hemoglobin and myoglobin and a marker of early oxidation events, initiated by ferrous ion, whereas β -carotene showed a slight antioxidative effect at some ferrous ion concentrations and was a prooxidant at other concentrations (Mortensen and Skibsted, 2000). Beta-carotene was found not to decrease the formation of hemichrome and thiobarbituric reactive substances (TBARS) initiated by ferrous sulfate in chicken liver slices (Mortensen and Skibsted, 2000).

Carotenoids of processed/cooked foods have greater bioavailability than those of raw commodities (Rock *et al.*, 1998; Stahl and Sies, 1996). Onyewu *et al.*, (1986) reported that frying of fats and oils could also lead to considerable losses of β-carotene, other carotenoids and vitamin A (Onyewu *et al.*, 1986). The losses of carotenoid could be due to the heat during cooking with disintegrate tissue if coupled with exposure to oxygen,

light and acid, can result in the destruction of the provitamin A carotenoid (Gayathri et al., 2004).

In recent years, there are few studies reported on the effects of cooking/processing on carotenoid composition in vegetables (Gayathri *et al.*, 2004; Marcela *et al.*, 2004 and Padmavathi *et al.*, 1992) but lacking in processed meat products. Tee and Lim (1992) only reported vitamin A contents in chicken burger and chicken frankfurter formulated with animal fats which accounted for 16.1 and 11.7 μg/100 g respectively.

This research focused on the carotene contents of processed meats blended with palm fats in raw and cooked beef burgers and chicken frankfurters substituted with palm fat and red palm fat (RPF35 and RPF48) during cooking and storage. The concentration and reduction of α - and β -carotenes during cooking and storage were also monitored.

MATERIALS AND METHODS

The beef batter formulations are shown in **Table 1**. The fat source was varied. The fats (15% of the formulation) consisted of Beef Fat (control), Palm Fat (PF with Slip melting point (SMP) 41-44°C, Iodin value (IV) 45-

50), Red Palm Fat (RPF35 with SMP 33-37°C, IV 48-53) or a blend of PF with RPF35. The chicken frankfurter formulations shown in Table 2, also contained 15% fats which were varied between treatments and consisted of Chicken fat (control), PF, Red Palm fat (RPF48 with SMP 46-50°C, and IV 42-46) or a blend of PF with RPF48 at a fixed level of fat (15%). Palm Fat (white in colour) was supplied by Carqill Fats & Oils Specialty Company while Red Palm Fat (yellow in colour) by Carotino Company. The different between RPF35 and RPF48 is their degree of saturation. lodin value (IV) is a measure of the total number of unsaturated double bonds present in an oil or fat. The higher the IV value, the lower the degree of saturation. The slip melting point (SMP) of a fat is defined as the temperature at which a column of fat in an open capillary tube moves up the tube when it is subjected to controlled heating in a waterbath. The fat with higher in SMP (temperature range) is physically more solid than the fat with lower in SMP. RPF48 and RPF35 wasn't used for both beef burger and chicken frankfurter studies because these fats were not stable in the finished products. Beef, chicken breast, chicken trimming and other dry materials were purchased from local suppliers. The finished meat batters were then weighed into 70g portions, then manually stamped to produce an uniform beef burger. Beef burgers were cooked for 7 min (internal temperature, 74 ± 1°C). Meanwhile, the finished chicken meat batters

were manually stuffed into 26mm Viscofan Cellulose casings using a stuffer (FDIC, Germany). The cooking schedule was 55°C for 20 min, 65°C for another 20 min, 75 min for 20 min and 80°C for 15 min. After cooking, the frankfurters were cooled, weighed, peeled, and stored in freezer at –18°C. Another half of stuffed batters were manually placed into a 17 x 13 cm retort pouches and kept in chiller at 2-5°C until ready for sterilized. chicken frankfurters were then sterilized/retorted (Clutch Retort, Model H60) at 121°C until F_o reached 3.2. After retorting, the frankfurters were cooled, stored in room temperature.

Fat extraction

Fat was extracted using a method based on Kinsella *et al.*, (1977). The lipid extract was stored at -18°C for further analysis for carotene components.

Carotene analyses

Carotenes were determined using HPLC developed by Hart and Scott (1995) with some modifications. Before extraction of carotenes, the lipid extracts were saponified for 16 hrs prior to extraction (Hart and Scott, 1995).

Saponification and Extraction

Duplicated 5 g of lipid extracts were placed in 500 ml saponification flasks (covered with aluminium foil) together with 70 ml 1% (w/v) ethanolic pyrogallol and 20 ml 50% (w/v) KOH. The flask was then purged with nitrogen gas for 30 min prior to agitated/shaked for 16 hours at 20°C. All procedures were conducted in the dark. The carotenes were extracted from the KOH/ethanolic phase by careful shaking with 120 ml diethyl ether and 100 ml 10% NaCl solution in a separating funnel. The lower phase was removed to another separating funnel and was extracted one more time with 120 ml diethyl ether. The diethyl ether phases were combined in a separating funnel and washed with water until washings were neutral to around 7. The organic phase was transferred to a 250 ml evaporating flask and evaporated by using rotary evaporator at 40°C just to dryness. The residue was redissolved by agitation in 20 ml acetonitrile for HPLC analysis and filtered through a 0.45 µm syringe filter (Hamilton 705 (Reno, Nevada USA). All procedures were carried in the dark room. All glasswares were also covered with aluminium foil.

Preparation of standard α - and β -carotene

Alpha-carotene, β-carotene were dissolved in HPLC grade hexane and made to volume with hexane to give a final solvent ratio of 1:9 v/v. All

solvents contained 0.1% butylated hydroxytoluene (BHT). Individual working solutions of around 0.5-1.0 ug/ml were prepared from stock solutions by evaporating an aliquot under nitrogen and making to volume with mobile phase and their purity assessed by HPLC analysis. A mixed working standard solution was prepared, in mobile phase, from individual stock solutions (Hart and Scott, 1995). A calibration graph was prepared from the HPLC standard concentrations versus peak areas.

Chromatography

Carotene content was analysed using HPLC developed by Hart and Scott (1995). The HPLC system was a isochratic solvent delivery pump (Waters model 1515) coupled with UV detector (Waters model 2487). The column system consisted of 250 mm x 4.6 mm, 5 μm μBondapak octadecylsilane ODS (C₁₈) analytical column (SGE) modified by the placement of metal frits. The mobile solvent system consisted of acetonitrile, methanol and dichlorometane (75:20:5 v/v/v) containing 0.1% BHT. The prepared mobile phase was filtered through a 0.45 μm Whatman membrane filter and degassed using ultrasonic agitation. The flow rate was 2.5 ml/min. Samples were injected via a micrometer syringe (model 705 Hamilton) loading injector fitted with a 20 μL loop loop. Peak responses were

measured at 450 nm using a variable wavelength UV/Vis with an output to a chromatographic data handling system (Breeze system).

Statistical analyses

Data obtained were tested for significance using ANOVA and Duncan Multiple Range Test with SAS version 6.12 (SAS, 1989). Significance was established at $P \le 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Carotene content in Raw Beef Burgers

Carotene concentrations in both palm fat (PF) and chicken fat accounted less than $1\mu g/g$, respectively (**Table 3**). After processing, the carotene was completely depleted in both raw beef burgers formulated with PF and beef fat. Alpha-, beta- and total carotene concentration in raw beef burger substituted with red palm fat (RPF35) and fat blend (FB) were decreased in line with time of storage. Alpha-carotene in raw beef burger substituted with RPF35 decreased from 135.5 to 64.0 $\mu g/g$ (53%) while raw beef burger substituted with FB only decreased by 33% (from 50.3 to 33.6 $\mu g/g$) after storage for 6 months at -18° C. After storage for 6 months (-18°C), both raw beef burgers formulated with RPF35 and fat blend were

decreased significantly (P<0.05) from 239 to 79.7 μ g/g (67% loss) and from 86.0 to 45.5 μ g/g (47%) in β -carotene concentrations. Even though raw beef burgers substituted with RPF35 showed the highest percent loss in β -carotene concentrations, the value retained was still high compared to other treatments. Total carotene decreased from 374.3 to 143.7 μ g/g (62%) and from 136.3 to 79.1 μ g/g (42%), respectively for both raw beef burger substituted with RPF35 and fat blend after storage for 6 months at -18° C.

Carotene content in Cooked Beef Burgers

Alpha-carotene concentrations significantly decreased (P<0.05) from 135.5 to 45.7 μ g/g (66%) and from 50.3 to 19.1 μ g/g (62%), respectively in beef burger substituted with RPF35 and FB during storage for 6 months (-18°C) after cooking (Table 4). Beta-carotene concentration in cooked beef burgers containing RPF48 and FB also decreased with storage time. Even though cooked beef burgers substituted with RPF35 showed the highest percent loss in β -carotene concentrations, the content retained was still high compared to other treatments. This treatment decreased significantly (P<0.05) from 239 to 48.5 μ g/g or 80% reduction followed by cooked beef burger formulated with FB which decreased from 86.0 to 23.8

 μ g/g (72%) after 6 months of storage in β-carotene concentrations. The rate loss of β-carotene in cooked beef burger was higher than α -carotene.

Total carotene in cooked beef burger recorded higher percent loss in the range from 69 to 75% or reduction from 374.3 to 94.2 μ g/g and from 136.3 to 42.9 μ g/g. Even though cooked beef burgers substituted with RPF35 recorded the highest percent loss (75%) in total carotene concentrations, the value retained was still high (94.2 μ g/g) compared to other treatments after 6 months of storage.

Carotene content in chicken frankfurter

Alpha-carotene content in red palm fat (RPF48) and raw fat blend (FB) before adding into frankfurter formulation accounted for 31.1 and 13.2 μ g/g, respectively **(Table 5)**. Meanwhile, palm fat (PF) and chicken fat accounted less than 1 μ g/g, respectively. The percent loss of α -carotene in both RPF48 and FB retorted chicken frankfurters were lower than in both oven cooked chicken frankfurter substituted with RPF48 and FB after cooking (0 month). Alpha-carotene in retorted chicken frankfurter substituted with RPF48 only lost 25% (from 31.1 to 23.4 μ g/g) while oven cooked chicken frankfurter containing RPF48 lost 61% (from 31.1 to 12.2

 μ g/g). A similar trend of α-carotene reduction was also detected in FB retorted chicken frankfurters which lost 21.2% or from 13.2 to 10.4 μ g/g lower than in oven cooked frankfurters, which lost 41% (13.2 to 7.8 μ g/g).

Beta-carotene was present in the highest amount in both raw RPF48 and raw fat blend. They accounted for 41.3 and 19.8 μ g/g, respectively. The rate loss in β -carotene concentrations was higher than α -carotene. Beta-carotene decreased significantly (P<0.05) to 27.5 and 11.5 μ g/g (33.4 and 42.0%), respectively in retorted chicken frankfurters containing RPF48 and fat blend after cooking (0 month). They decreased further to 23.8 and 9.7 μ g/g (42 and 51% reduction) after 6 months of storage. However the percent loss of β -carotene in retorted chicken frankfurter was lower than oven cooked chicken frankfurter substituted with palm based fat. Cooking in the oven destroyed 62% and 54% or retained 15.6 and 9.1 μ g/g of β -carotene in chicken frankfurters containing RPF48 and FB at 0 month. They significantly decreased (P<0.05) to 10.8 and 4.9 μ g/g (74% and 75%), respectively after 6 months of storage.

Total carotene concentrations in retorted chicken frankfurter decreased in the range of 18.0-51.1 μ g/g (33-42% loss). However total carotene

concentrations in oven cooked chicken frankfurters substituted with RPF48 and FB were significantly reduced (P<0.05) by 62 and 54% or dropped to 27.8 and 16.9 μ g/g after 0 month storage, and by 69 and 64% or dropped to 22.7 and 12.0 μ g/g, respectively after 6 months of storage. Total carotene concentrations in retorted chicken frankfurters formulated with RPF48 and FB decreased only from 72.4 to 42.3 μ g/g (42% loss) and from 33.0 to 18.0 μ g/g (45% loss), respectively after 6 month of storage. These results indicate that the rate loss of total carotene concentrations in retorted chicken frankfurter substituted with RPF48 and FB was lower than in oven cooked chicken frankfurters.

Alpha-, beta- and total carotene degraded faster in cooked beef burger than in raw beef burger substituted with palm based fat. The result also shows that β -carotene degraded faster compared to α -carotene in beef burger and chicken frankfurter indicated that the latter was more stable after processing and cooking. Anguelova and Warthesen (2000) also reported that β -carotene degraded with a lower rate than lycopene but faster than α -carotene. The faster degradation of β -carotene compared to α -carotene in this study could be due to their chemical structure which differ from each other in the number of conjugated double bonds (Stahl and Sies, 1996). Carotenoids act as antioxidants against lipid

peroxidation by quenching singlet oxygen and trapping free peroxyl radicals (Palozza and Krinsky, 1992). Lycopene, α -carotene and β -carotene have all 11 double bonds that in the straight molecule of lycopene fully overlap. However, α -carotene has 9 fully overlapping double bonds plus one β -ring conjugated double bond as the double bond of the ϵ -ring but is not part of the conjugated double bond system (Anguelova and Warthesen, 2000). As a result, the order for their free radical scavenging abilities was: lycopene > β -carotene > α -carotene (Anguelova and Warthesen, 2000).

CONCLUSION

Alpha-carotene, β -carotene and total carotene degraded faster in oven cooked chicken frankfurter than in retorted chicken frankfurter substituted with palm based fat, suggesting that carotene was more stable with retorting/sterilizing compared to oven cooking. Beta-carotene degraded faster compared to α -carotene in retorted and oven cooked chicken frankfurter and cooked beef burger indicating that the latter was more stable after cooking and storage. Even though cooked beef burgers, retorted and oven cooked chicken frankfurters substituted with red palm fat (RPF35 and RPF48) showed the highest percent loss in β -carotene

concentrations after storage for 6 months but the content retained was still high. In summary, the effect of cooking, frozen storage and the type of fats used could influence carotenes stability and content in meat products. This study showed the potential of utilizing red palm fats as animal fat analogues in improving the nutritional quality (carotenes) of processed meat.

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Table 1: Beef burger formulations

Ingredient	Percent
Beef	49.0
Fat (beef fat, palm fat, red palm fat or fat blend)	15.0
Water	22.5
Textured vegetable protein	5.0
Potato starch	3.0
Isolated soy protein	3.0
Salt	1.1
Sodium tripolyphosphate	0.3
Spices and seasoning	1.1
Total	100

Table 2: Chicken frankfurter formulations

Ingredient	Percent
Chicken meat (breast)	10.00
Chicken trimming	42.00
Fat (chicken fat, palm fat, red palm fat or fat blend)	15.00
Water	24.91
Potato starch	2.50
Isolated soy protein	3.00
Salt	1.10
Sodium tripolyphosphate	0.30
Spices and seasoning	1.14
Natrium erithrobate	0.03
Nitrate	0.02
Total	100.00

Table 3. Carotene content of raw beef burgers containing palm fat and red palm fat during storage for 6 months (-18°C)

		FATS				
Carotene (μg/g)	Storage time (month)	Beef fat (control)	Palm fat (PF)	Red palm fat (RPF35)	Palm fat + red palm fat (FB)	
	Raw fat	< 1.0 ^c	< 1.0 ^c	^p 135.3 ^a	^p 50.3 ^b	
	0	0.0 ^c	0.0 ^c	^q 80.8 ^a	^q 36.9 ^b	
α-	2	0.0^{c}	0.0^{c}	^r 70.5 ^a	^{qr} 36.2 ^b	
Carotene	4	0.0^{c}	0.0^{c}	^s 66.2 ^a	^{qr} 34.0 ^b	
	6	0.0^{c}	0.0^{c}	^s 64.0 ^a	^r 33.6 ^b	
	Raw fat	< 1.0 ^c	< 1.0 ^c	^p 239.0 ^a	^p 86.0 ^b	
	0	0.0 ^c	0.0 ^c	^q 109.4 ^a	^q 50.0 ^b	
β-	2	0.0^{c}	0.0^{c}	^r 94.0 ^a	^q 49.2 ^b	
Carotene	4	0.0^{c}	0.0^{c}	^s 86.0 ^a	^q 46.1 ^b	
	6	0.0^{c}	0.0 ^c	^t 79.7 ^a	^q 45.5 ^b	
	Raw fat	< 1.0°	< 1.0 ^c	^p 374.3 ^a	^p 136.3 ^b	
	0	0.0 ^c	0.0 ^c	^q 190.2 ^a	^q 86.9 ^b	
Total	2	0.0^{c}	0.0^{c}	^r 164.5 ^a	^q 85.4 ^b	
carotene	4	0.0^{c}	0.0^{c}	s152.2a	^q 80.1 ^b	
	6	0.0 ^c	0.0°	^t 143.7 ^a	^q 79.1 ^b	

^{a-c} Mean values within the same row bearing different superscripts differ significantly (P<0.05) ^{p-t} Mean values within the same column bearing different superscripts differ significantly (P<0.05)

Table 4. Carotene content of beef burgers containing palm fat and red palm fat during storage for 6 months (-18°C) after cooking

		FATS				
Carotene (μg/g)	Storage time (month)	Beef fat (control)	Palm fat (PF)	Red palm fat (RPF35)	Palm fat + red palm fat (FB)	
	Raw fat	< 1.0 ^c	< 1.0 ^c	^p 135.3 ^a	^p 50.3 ^b	
	0	0.0 ^c	0.0 ^c	^q 71.7 ^a	^q 32.0 ^b	
α-	2	0.0^{c}	0.0^{c}	^r 59.1 ^a	^r 26.0 ^b	
Carotene	4	0.0^{c}	0.0^{c}	^r 54.7 ^a	^s 20.0 ^a	
	6	0.0^{c}	0.0^{c}	^t 45.7 ^a	^s 19.1 ^b	
	Raw fat	< 1.0 ^c	< 1.0 ^c	^p 239 ^a	^p 86.0 ^b	
β-	0	0.0°	0.0 ^c	^q 90.5 ^a	^q 44.6 ^b	
Carotene	2	0.0^{c}	0.0^{c}	^r 74.6 ^a	^r 35.2 ^b	
	4	0.0^{c}	0.0^{c}	^r 70.3 ^a	^s 25.1 ^b	
	6	0.0 ^c	0.0 ^c	^s 48.5 ^a	^s 23.8 ^b	
	Raw fat	< 1.0 ^c	< 1.0 ^c	^p 374.3 ^a	^p 136.3 ^b	
	0	0.0 ^c	0.0^{c}	^q 162.2 ^a	^q 76.6 ^b	
Total Carotene	2	0.0^{c}	0.0^{c}	^r 133.7 ^a	^r 61.2 ^b	
341313110	4	0.0^{c}	0.0^{c}	^s 125.0 ^a	^s 45.1 ^b	
	6	0.0 ^c	0.0 ^c	^t 94.2 ^a	^s 42.9 ^b	

^{a-c} Mean values within the same row bearing different superscripts differ significantly (P<0.05) ^{p-t} Mean values within the same column bearing different superscripts differ significantly (P<0.05)

Table 5. Carotene content of retorted and oven cooked chicken frankfurters containing palm fat and red palm fat during storage for 6 months (-18°C)

			FATS				
Carotene (μg/g)	Cooking method	Storage time (month)	Chicken fat	Palm fat (PF)	Red palm fat (RPF48)	Palm fat + red palm fat (FB)	
	Raw fat		< 1.0 ^c	< 1.0 ^c	^p 31.1 ^a	^p 13.2 ^b	
α-	Retort	0	0.0 ^c	0.0 ^c	^q 23.4 ^a	^q 10.4 ^b	
Carotene		6	0.0^{c}	0.0^{c}	^r 18.5 ^a	^r 8.3 ^b	
	Oven cooked	0	0.0^{c}	0.0^{c}	s12.2a	^r 7.8 ^b	
		6	0.0°	0.0 ^c	^s 11.9 ^a	^r 7.1 ^b	
β- Carotene	Raw fat		< 1.0 ^c	< 1.0 ^c	^p 41.3 ^a	^p 19.8 ^b	
	Retort	0	0.0 ^c	0.0 ^c	^q 27.5 ^a	^q 11.5 ^b	
		6	0.0^{c}	0.0^{c}	^r 23.8 ^a	^r 9.7 ^b	
	Oven cooked	0	0.0^{c}	0.0^{c}	^s 15.6 ^a	^s 9.1 ^b	
		6	0.0 ^c	0.0 ^c	^t 10.8 ^a	^t 4.9 ^b	
Total carotene	Raw fat		< 1.0 ^c	< 1.0 ^c	^p 72.4 ^a	^p 33.0 ^b	
	Retort	0	0.0 ^c	0.0 ^c	^q 51.1 ^a	^q 21.9 ^b	
		6	0.0^{c}	0.0 ^c	r42.3 ^a	^r 18.0 ^b	
	Oven cooked	0	0.0^{c}	0.0 ^c	^s 27.8 ^a	^s 16.9 ^b	
		6	0.0 ^c	0.0^{c}	^t 22.7 ^a	^t 12.0 ^b	

^{a-c} Mean values within the same row bearing different superscripts differ significantly (P<0.05) ^{p-t} Mean values within the same column bearing different superscripts differ significantly (P<0.05)