EFFECTS OF COOKING ON CAROTENE AND FREE FATTY ACID CONTENTS OF CHICKEN BURGER INCORPORATED WITH PALM FATS

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Background

Oxidative rancidity is one of the limiting feature of prolonged storage of fresh meats at frozen temperatures as lipid peroxidation contributed towards the nutrititional deterioration of such products. Processing techniques prior to freezing burgers involved comminuting the meat, disrupting the tissue membranes and dislodgement of iron from heme compounds, thus exposing the fats to oxygen and prooxidants such as sodium chloride and free iron. Heating/cooking release iron from heme compounds and disrupt the cellular integrity of muscle food, accelerating the process of oxidation. As chicken fat is highly unsaturated, studies of chicken fat being replaced with palm fat (PF) were done (Babji et al 2000, Babji et al 2001) to delay oxidation. Palm fats are a natural source and provider of vitamins A and E. However, the retention of carotene after cooking in processed meats with PF substitution had not been studied.

Objectives

The purpose of this study is to evaluate the changes in peroxide value (PV), free fatty acid (FA), thiobarbituric acid (TBA) and carotene content of raw and cooked chicken burgers incorporated with red palm fat, palm fat and control.

Materials and methods

Palm stearin (PS) and red palm stearin (RPS) burger were compared with control chicken fat burgers (C) at a fixed level of fats (15%). The burgers at 70 g each were cooked on an open flat pan by flipping every 3 minutes until the final internal temperature of 70° C was reached. The duration of heat treatments were three and ten minutes. The fats from the samples were extracted at the 1, 2 and 3 months of storage at -18° C. Chloroform/methanol lipid extraction of the samples was done, based on the method of Folch et al., (1957). Test done were on the free fatty acid content (PORIM 1995) and thiobarbituric acid values (TBA) as described by Tarladgis et al (1960). Carotene content analysis was carried out using the method of Hart and Scott (1995). Data were subjected to analysis of variance (ANOVA), using the SAS software package (SAS 1985).

Results and discussion

Free fatty acid (FA) was the highest in burgers with chicken fat (C), followed by palm stearin (PS) and red palm stearin (RPS) treated chicken burgers (Table 1). Prolonged heating resulted in increasing values of FA, peroxide (PV) and thiobarbituric acid (TBA) (Table 2). On the first month of storage (-18^oC), carotene content of RPS burgers were at 298 ppm (raw) and 86 ppm (10 min of cooking). After the third month of storage, the carotene content were significantly reduced to 180 and 38 ppm (raw and 10 min of cooking) respectively. The Red Palm Stearin (RPS) treatment had the lowest FA, PV and TBA values throughout the 3 months of shelf life study.



Free Fatty Acid (%)			Caro	tene content	(ppm)		
Treatment		Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
	C	0.33 a	0.14 c	0.33 a	0 b	0 b	0 b
Raw	PS	0.29 b	0.43 a	0.29 b	0 b	0 b	0 b
	RPS	0.15 c	0.29 b	0.15 c	298 a	194 a	180 a
Heat	C	0.44 a	0.24 c	0.44 a	0 b	0 b	0 b
Treated	PS	0.31 b	0.59 a	0.31 b	0 b	0 b	0 b
(3mins)	RPS	0.27 c	0.44 b	0.27 c	96 a	86 a	87 a
Heat	C	0.60 a	0.80 a	0.60 a	0 b	0 b	0 b
Treated	PS	0.24 b	0.60 b	0.24 b	0 b	0 b	0 b
(10 mins)	RPS	0.25 b	0.42 b	0.25 b	86 a	67 a	38 a

Table 1. Free fatty acid and carotene content of chicken burger at 1, 2 and 3 months of storage at -18^{0} C.

Means within the same column (a-c) with different small letters are significantly different (P<0.05)

Table 2. Peroxide value and thiobarbituric acid value of chicken burger at 1, 2 and 3 months of storage at -18° C.

Peroxide value			TBA value				
Treatment		Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
	С	13.28 a	24.35 b	26.77 a	0.68 a	0.69 a	0.77 a
Raw	PS	10.05 b	25.50 a	19.20 c	0.59 b	0.60 b	0.64 b
	RPS	9.30 c	22.35 c	21.44 b	0.32 c	0.37 c	0.40 c
Heat	C	14.98 a	28.63 a	34.54 a	1.08 a	2.00 a	1.29 a
Treated	PS	9.31 c	23.43 b	25.37 b	0.96 b	0.96 b	1.24 b
(3mins)	RPS	10.28 b	15.77 c	24.88 c	0.41 c	0.42 c	0.47 c
Heat	C	15.06 a	47.43 a	46.13 a	1.79 a	1.85 a	1.88 a
Treated	PS	13.19 b	33.78 b	29.80 b	0.98 b	1.30 b	1.71 b
(10 mins)	RPS	11.53 c	25.73 с	26.56 c	0.48 c	0.52 c	0.60 c

Means within the same column (a-c) with different small letters are significantly different (P<0.05)

Conclusions

Substitution of 15% chicken fat with palm stearin and red palm stearin improved the products' shelf life quality with decreased values of free fatty acids, peroxide and thiobarbituric acid values. The presence of carotene in RPS treatments further enhanced the oxidative stability of the products.

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THE EFFECT OF SODIUM CHLORIDE, SOYBEAN PROTEIN AND STARCH ON GEL PROPERTIES AND COLOR OF BEEF SURIMI

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Background

The quality of meat surimi products is determined by the functional properties of muscle protein, which are water-holding capacity, gel and emulsification properties. These functional properties are based on the solubility of protein and can also determine the binding properties and physical stability of meat surimi. Myofibril protein, which is composed of myosin and actin, is not only the major protein in muscle, but also the main component of extractable protein. Myosin is insoluble at normal ion strength (0.15 to 0.20M) in

the main component of extractable protein. Myosin is insoluble at normal ion strength (0.15 to 0.20M) in muscle of live animals, while it can dissolve at higher ion strength and proper pH. Adding sodium chloride and phosphate can increase the ion strength range from 0.3 to 0.6M. As a result, myosin dissolved can be extracted effectively. Furthermore, the increased pH is also helpful for myosin extraction. Stanley (1994) pointed out that increasing ion strength to a specific extent would improve gel properties and water-holding capacity of beef significantly. Siegal (1979) reported that phosphate and sodium chloride could improve cohesiveness of myosin thus, improve gel properties. Liu et al. (1996) studied the influence of ion strength on heat gel properties of myofibril protein of chicken myosin. The result indicated that the gel formed by protein solution with low ion strength (0.2M KCl, pH6.0) is very soft, while hardness of gel increased gradually along with the increasing ion strength. LesioÂw et al(2001) reported mechanism of rheological changes in poultry myobrillar proteins during gelation.

Non-meat proteins have been widely used in surimi-based meat products to improve gelation strength. The most commonly used were beef plasma protein (BPP), soybean protein, egg white protein, and whey protein concentration (Porter, 1993; Park, 1994). Soybean protein plays an important role in increasing fat usage factor and modifying the texture and emulsification properties of meat products. Soybean protein concentration or soybean protein isolate are usually added to meat surimi in the form of powder or emulsion. These merits make it widely used in meat industry in these days. Although the emulsification properties of soybean protein have been well documented, there is little study on its influence on beef gel properties. Starch worked as a thickening ingredient in meat production. It is widely used in both traditional Chinese meat products and Western meat products. Natural starch contains two kinds of starch, namely straight-chain starch and branch-chain starch. Generally speaking, the more branch-chain starch added to the product, the better the thickening function is, the greater the gel strength is, and vice versa. Zhou (2003) compared the properties of sausages with added potato starch, cassava starch, wax cornstarch, and cornstarch. It suggested that the palatability, color, springiness and texture of sausages with added potato starch were the best. Adding starch in the production of meat surimi can improve water-holding capacity and modify texture of final products. This is mainly because starch can adsorb water, swell and form gel in the process of heating. According to earlier study, the gelation temperature of starch particles was higher than the denaturation temperature of meat proteins. As a result, meat proteins had been denatured and formed matrix when starch particles began to gelatinize. Starch particles captured weaker-binding water and immobilized it to improve the strength of matrix thus, enhanced the water-holding capacity. At the same time, starch particles swelled, moistened as it adsorbed water. Therefore, starch can improve elasticity and texture of final products by binding minced meat and stuffing the holes between it. Furthermore, adding starch particles can reduce fat loss and increase yield of final product by binding melted lipid in the process of heating as well (Kong, 1996).

Objectives

The objective of this research was to determine the effect of varying concentrations of the additives, such as sodium chloride, soybean protein and four starches (cornstarch, mung bean starch, potato starch and modified starch) on the quality of a beef based meat surimi product. Gel properties and color were measured as indicators of quality.



Materials and methods

The basic formula of the beef sausage was as follows, 1kg of loin from 3 years old local Mongolian cattle, 30g sodium chloride, 100g potato starch, 50g soybean protein, 10g carrageenan, 4g monosodium glutamate, 10g sugar, 3g pepper, 20g garlic, 10g ginger powder.

The sodium chloride, soybean protein and starch content of the product were modified by the following changes to the basic formula. The product was prepared with 1%, 2%, 4% and 5% (w/w) sodium chloride per kg of raw beef in stead of the basic formula content of 3%. The product was prepared with 1%, 2%, 3% and 4% (w/w) soybean protein per kg of raw beef in stead of the basic formula content of 5%. The product was prepared with 5%, 15% and 20% (w/w) of starch per kg of raw beef in stead of the basic formula content of 10%. Additionally, product was prepared with mung bean, modified starch and cornstarch replacing potato starch at all four concentrations. Sausages produced following the basic formula but without corresponding materials in the specific experiment, such as sodium chloride, soybean protein and starch, were taken as controls.

Raw beef was cut into pieces and minced after removing connective tissue. Then the minced meat was cured with sodium chloride at 4 for 24h. The cured meat was chopped and blended with additives and filled into nature casing. Then the sausages was cooled to 4 after cooking at 90 for 30min. Sausages produced by this procedure were taken as samples in this research.

Samples were prepared for texture analysis by removing the product casing and into cylinders of 3cm length. Texture analysis was performed using a TA.XT2i/25 texture analyser, supplied by Stable Micro System Company, England. The sample was penetrated twice with a 50 mm diameter probe and double apex profiles were generated automatically for each sample. Distance, time and force measured by the texture analyser were used to calculate the parameters of hardness, springiness and cohesiveness of the meat surimi products.

The color of samples was measured using a WSC-S color difference instrument, supplied by Shanghai Physical Optical Instrument Factoryi, China. Samples were prepared for color measurements by removing the casing and cutting to form a 1cm long cylinder. The sample was then placed into the sample case and the brightness (L^*) and redness (a^*) values determined.

Data were analyzed by one way analysis of variance. When significant differences among treatments were detected, treatment means were compared using the least significant difference method. The comparison error was 0.05.

Results and discussion

The effect of sodium chloride on gel properties of beef surimi was shown in table 1. The hardness, springiness and cohesiveness increased from 1890.06, 0.649, 0.359 to 2937.10, 0.722 and 0.471 respectively along with the amount of sodium chloride increasing. It had been well documented that the solubility of myofibril protein, cohesiveness and amount of actomyosin, which was formed by the connection of myosin and actin, would increase with the amount of sodium chloride increasing from 1.7 percent to 11.2 percent. As a result, gel strength increased as well. Although in theory, the more sodium chloride added, the more salt-soluble protein extracted, it was unacceptable for consumer to add so much sodium chloride in meat product. So the amount of sodium chloride in meat products is usually no more than 3.5 percent of meat weight, or the products will be too salty for consumption.

The effect of sodium chloride on the color of beef surimi was presented in table 2. Brightness of beef surimi decreased significantly (from 46.72 to 42.70) with the amount of sodium chloride increase, while the effect of sodium chloride on redness was not significant. So increasing the amount of sodium chloride added to beef surimi would decrease brightness of final products.

The stability of gel is mainly determined by the amount of functional proteins. So it is necessary to add some functional proteins for the purpose of strengthening the gel in meat surimi products. As the optimum pH for the formation of soybean protein gel is in the range of that of meat products, it is a suitable functional protein additive in the production of meat surimi. Soybean protein can form gel before myofibril shrinkage in the



cooking process of sausage and other meat surimi products. It can also form a compact film surrounding muscle tissue. As a result, there is less water as well as water-soluble vitamin and minerals loss during cooking process. The effect of soybean protein on beef gel properties was shown in table 3. Hardness of gel increased from 1955.02 to 2890.66, while springiness reduced from 0.829 to 0.779 with the amount of soybean protein isolate increasing. There was no significant change in cohesiveness (from 0.513 to 0.467). Soybean protein was able to disperse fat, form matrix, concrete and gelate while heating, thus increasing gel strength of product after cooking. But the more soybean protein added, the more solute in final products. High content of soybean protein may compete limited water with meat proteins and thus reduce springiness of the final products.

The effect of soybean protein on the color of beef surimi products was shown in table 4. Brightness of beef sausages decreased significantly(from 40.91 to 38.72) with increasing amount of soybean protein(P < 0.05). But the influence of soybean protein on redness was not significant.

The effect of different kinds of starch on gel strength of beef surimi is shown in table 5. The gel strength of beef surimi made with various kinds of starch increased compared with that of control. The more starch added, the greater the gel strength is. The main reason is that starch particles absorbed water and swelled, thereby increased water-holding capacity and enhanced the strength of matrix during heating. Starch can bind proteins, fat and water together and make beef surimi more viscous. The capacity of starch increasing gel strength varies from kinds to kinds (Ma L. et al., 1996). The capacity of mung bean starch and modified starch is the largest because mung bean starch has a higher content of branch-chain starch and higher water binding capacity. So it could swell to a relatively large volume (Wu et al., 1987); the swelled starch particles would bring about greater stress on beef protein. The most obvious characteristic of modified starch is that hydroxymethyl group and hydroxyethyl group endow modified starch with excellent hydrophilic and lipophilic properties, thus improve the quality of surimi-based meat products. As the branched chain starch content of cornstarch was relatively lower than others, the gel formed by it was relatively weaker (Okada, 1985).

The effect of different starches on gel springiness of beef surimi was shown in table 6. The springiness decreased gradually compared with that of control when the amount of starch added to beef surimi increased gradually. The extent to which the springiness of beef surimi decreased varies from kinds to kinds as well. The springiness of beef surimi made with mung bean starch was the best, while that of beef surimi with cornstarch was the worst.

The effect of different starches on gel cohesiveness of beef surimi is shown in table 7. Adding starch decreased the gel cohesiveness of beef surimi in comparison with that of control. The gel cohesiveness decreased with gradually increasing amount of starch added to beef surimi. The effect of different starches on cohesiveness varied from kinds to kinds. Cohesiveness of beef surimi made with modified starch is the best, while that of beef surimi with cornstarch and potato starch was relatively worse.

The effect of different starches on the brightness (L^* value) and redness (a^* value) of beef surimi products was shown in table 8 and 9.Adding starch decreased the L^* value and a^* value of beef surimi in comparison with that of control. With the gradually increasing amount of starch added to beef surimi, the L^* value and a^* value decreased gradually. The effect of different starches on L^* value made some differences, among them, the lightness of beef surimi made with mung bean starch and potato starch was better, and that of with cornstarch and modified starch was lower. On the other hand, the effect of different starches on the a^* value made some differences, but the difference was insignificant (P > 0.05).

Conclusions

Adding sodium chloride and soybean protein had a great influence on the gel properties and color of beef surimi products. The hardness, springiness, cohesiveness of beef surimi gel increased, while brightness decreased with the amount of sodium chloride increasing. But there is no significant influence on redness (P > 0.05). The gel hardness increased gradually with increasing amount of soybean protein added to beef surimi, but cohesiveness and a* value changed little, and *L** value decreased significantly(P<0.05). Adding various starches increased gel strength of beef surimi products; on the other hand, gel springiness and



cohesiveness decreased. The effect of different kinds of starch on gel properties of final products varied greatly. The mung bean starch was the best as meat additive, while cornstarch is the worst for that use. Adding starch decreased the L^* value of beef surimi products, and the more starch added to beef surimi, the more L^* value decreased. The effect of starch on L^* value varied from kinds to kinds. The effect of beef surimi made with mung bean starch and potato starch was better, while that of cornstarch and modified starch was worse. No matter what kind of starch added, a* value of beef surimi products decreased.

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Table 1. Effect of solitain emotion of get properties of beer						
sodium chloride	Hardness	Springiness	Cohesiveness			
Control (no sodium chloride)	1890.06±113.38 ^d	0.649±0.033 ^c	$0.359 {\pm} 0.036^{b}$			
1%	2175.92±126.69 ^c	$0.651 \pm 0.046^{\circ}$	0.362 ± 0.031^{b}			
2%	2342.12±82.59 ^c	0.653 ± 0.032 °	$0.354{\pm}0.024^{b}$			
3%	2558.16±84.51 ^b	0.703 ± 0.032^{bc}	$0.428{\pm}0.024^{a}$			
4%	2760.84±123.27 ^a	$0.769{\pm}0.069^{ab}$	0.474 ± 0.054^{a}			
5%	2937.10±89.94 ^a	0.722 ± 0.032^{a}	0.471 ± 0.028^{a}			

Table 1. Effect of sodium chloride on gel properties of beef

a, b, c Means in the same row with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).

Table 2. Effect of sodium chloride on colour properties of beef

Salt	Brightness	Redness
Control (no sodium chloride)	46.72±0.91 ^a	$11.74{\pm}0.28^{a}$
1%	44.29±0.73 ^b	11.79±0.41 ^a
2%	43.42 ± 0.54^{bc}	11.89±0.31 ^a
3%	$42.93 \pm 0.74^{\circ}$	11.52 ± 0.40^{a}
4%	41.77±1.37 ^c	11.36 ± 0.37^{a}
5%	42.70±0.37 ^c	11.39±0.25 ^a

a, b, c Means in the same row with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).

Soybean protein	Hardness	Springiness	Cohesiveness
Control(no sodium chloride)	1955.02±102.71°	0.829 ± 0.028^{a}	0.513±0.051
1%	2153.94±218.12 ^c	0.847 ± 0.054^{a}	0.467 ± 0.043
2%	2480.18±112.16 ^b	0.823 ± 0.055^{a}	0.479 ± 0.034
3%	2776.60±164.32 ^a	$0.860{\pm}0.037^{a}$	0.513±0.036
4%	2722.20±252.92 ^a	$0.784{\pm}0.062^{b}$	0.474 ± 0.046
5%	2890.66±188.83 ^a	0.779 ± 0.082	0.467 ± 0.074

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Table 3. Effect of so	ybean protein on	gel characteristics of beef	

a, b, c Means in the same line with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).

Table 4. Effect of soybean protein on colour characteristics of beef

Soybean protein	Brightness (L^*)	Redness (a*)
Control (no sodium chloride)	40.91 ± 0.55^{a}	11.73±0.58
1%	$40.70{\pm}0.59^{a}$	12.03±0.17
2%	$40.28{\pm}0.49^{ m ab}$	11.19±0.47
3%	39.48 ± 0.53^{b}	11.65±0.49
4%	39.61 ± 0.48^{b}	11.49±0.51
5%	38.72 ± 0.51^{b}	11.39±0.35

a, b, c Means in the same line with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).

Table 5. Effect of different starches on hardness of beef

	mung bean	Potato	Modified	Corn
Control(no starch) 5% 10% 15% 20%	1055.00±127.75 ^d 1196.80±113.45 ^{d xy} 1655.48±70.94 ^{cx} 2151.82±111.59 ^{b x} 2725.56±148.06 ^{a x}	1055.00±127.75 ^d 1276.70±109.58 ^{c x} 1558.52±97.92 ^{b xy} 2109.82±63.21 ^{a x} 2173.90±53.78 ^{a z}		$\begin{array}{c} 1055.00{\pm}127.75^{d} \\ 1027.6{\pm}54.48^{dz} \\ 1450.92{\pm}140.65^{cy} \\ 1843.36{\pm}141.57^{by} \\ 2067.74{\pm}156.38^{az} \end{array}$

a, b, c Means in the same line with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05), x, y, zMeans in the same row with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05).

Table 6. Effect of starch on springiness of beef

	θ					
	mung bean	Potato	Modified	Corn		
Control(no starch) 5% 10%	0.865±0.015 ^a 0.778±0.042 ^{b x} 0.705±0.037 ^c 0.687±0.058 ^{c xy}	$\begin{array}{c} 0.865{\pm}0.015^{a} \\ 0.745{\pm}0.042^{b\;xy} \\ 0.703{\pm}0.082^{b} \\ 0.708{\pm}0.035^{b\;x} \end{array}$	$\begin{array}{c} 0.865{\pm}0.015^{a}\\ 0.821{\pm}0.036^{ax}\\ 0.712{\pm}0.105^{b}\\ 0.642{\pm}0.053^{cy} \end{array}$	$\begin{array}{c} 0.865 {\pm} 0.015^{a} \\ 0.731 {\pm} 0.047^{b \ y} \\ 0.672 {\pm} 0.061^{bc} \\ 0.623 {\pm} 0.056^{cd \ y} \end{array}$		
15% 20%	$0.639 \pm 0.050^{c x}$	0.631±0.088 ^{c x}	0.623±0.012 ^{c xy}	$0.605 \pm 0.086^{d y}$		

a, b, c Means in the same line with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05), x, y, zMeans in the same row with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).

Table 7. Effect of starch on cohesiveness of beef

	mung bean	Potato	Modified	Corn
Control 5% 10% 15%	$\begin{array}{c} 0.372{\pm}0.044^{a}\\ 0.353{\pm}0.014^{a}{}^{xy}\\ 0.367{\pm}0.027^{a}{}^{x}\\ 0.305{\pm}0.026^{b}{}^{xy}\\ 0.288{\pm}0.040^{b}{}^{x} \end{array}$	$\begin{array}{c} 0.372{\pm}0.044^{a}\\ 0.333{\pm}0.052^{axy}\\ 0.323{\pm}0.015^{aby}\\ 0.281{\pm}0.022^{by}\\ 0.231{\pm}0.036^{cy} \end{array}$	$\begin{array}{c} 0.372{\pm}0.044^{a} \\ 0.396{\pm}0.079^{ax} \\ 0.326{\pm}0.035^{bx} \\ 0.320{\pm}0.024^{bx} \\ 0.312{\pm}0.009^{bx} \end{array}$	0.372±0.044 ^a 0.327±0.022 ^{ab y} 0.319±0.033 ^{b y} 0.287±0.009 ^{b y} 0.232±0.049 ^{c y}
20%				

a, b, c Means in the same line with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05), x, y, z Means in the same row with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05).



Table 6. Effect of staten on originaless value of beer						
	mung bean	Potato	Modified	Corn		
Control	40.16 ± 1.02^{a}	40.16±1.02 ^a	40.16±1.02 ^a	40.16±1.02 ^a		
5%	40.47 ± 0.74^{a}	39.60±0.71 ^{ab}	38.16±0.54 ^b	40.31±0.68 ^a		
10%	40.05±0.61 ^{a x}	39.30±0.49 ^{ab x}	37.46±0.51 ^{b y}	38.24 ± 1.16^{by}		
	39.15±0.66 ^{ab x}	38.66±0.81 ^{bc x}	35.67±1.25 ^{c y}	36.81±0.75 ^{b y}		
15%	$38.00\pm0.72^{b x}$	$37.80 \pm 0.62^{c x}$	33.68±0.58 ^{d y}	34.95±1.22 ^{c y}		
20%						

Table 8. Effect of starch on brightness value of beef

a, b, c Means in the same line with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05), x, y, zMeans in the same row with same superscripts letter do not differ(P > 0.05), with different superscripts letter differ (P < 0.05).

Table 9. Affect of starch on red value of beef

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	mung bean	Potato	Modified	Corn		
Control	13.63±1.06 ^a	13.63±1.06 ^a	13.63±1.06 ^a	13.63±1.06 ^a		
5%	12.62 ± 0.49^{ab}	13.28±0.57 ^{ab}	13.49±0.20 ^a	13.69±0.27 ^a		
	12.53±1.20 ^{ab}	12.33 ± 0.78^{b}	13.12±0.39 ^{ab}	13.68±0.55 ^a		
10%	12.12±0.83 ^b	12.29±0.95 ^b	12.72±1.28 ^b	13.11±0.54 ^{ab}		
15%	11.57±0.65 ^b	11.93 ± 1.12^{b}	12.11 ± 1.07^{b}	12.87±0.71 ^b		
20%						

a, b, c Means in the same line with same superscripts letter do not differ (P > 0.05), with different superscripts letter differ (P < 0.05).