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EFFECT OF TURMERIC POWDER (*CURCUMA LONGA* L.) AND ASCORBIC ACID ON ANTIOXIDANT CAPACITY AND OXIDATIVE STATUS IN RABBIT BURGERS AFTER COOKING

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Abstract: The aim of this study was to evaluate the effects of turmeric powder and ascorbic acid on lipid oxidation and antioxidant capacity in cooked rabbit burgers. The burgers were derived from 3 different formulations (C, control, with no additives; Tu with 3.5% of turmeric powder and AA with 0.1% of ascorbic acid) and were stored at 4°C for 0 and 7 d and cooked. The lipid oxidation (thiobarbituric acid reactive substances [TBARS]) and antioxidant capacity (2,2-azinobis-[3 ethylbenzothiazoline-6-sulfonic acid] {ABTS}, 1,1-diphenyl-2-pircydrazyl [DPPH] and ferric reducing ability [FRAP]) were evaluated. A significant interaction between storage time and formulation ($P<0.001$) was observed for DPPH, FRAP and TBARS in cooked burgers. At day 0 and day 7, the DPPH value was higher in Tu and AA compared to C burgers. At day 0, C showed a lower level of FRAP than the Tu and AA burgers. At day 7, the FRAP values tended to decrease but remained significantly higher in Tu and AA compared to C burgers. Lipid oxidation at day 0 in Tu and AA showed lower TBARS values compared to C burgers. The addition of 3.5% turmeric powder in rabbit burgers exerts an antioxidant effect during storage and it seems more effective in controlling lipid oxidation than ascorbic acid after cooking.

Key Words: rabbit, meat quality, antioxidant, turmeric, ascorbic acid, cooked burger.

INTRODUCTION

Modern trends in the food market have introduced and increased ready-to-cook products that are easy to prepare and consume. In recent years, several studies have been carried out on antioxidant additives to increase the shelf-life of raw and cooked meat products (Sánchez-Escalante *et al.,* 2001; Rojas and Brewer, 2007; Moroney *et al.,* 2012). Natural antioxidant additives could serve as an alternative to synthetic antioxidants (which are sometimes associated with toxic effects) due to their great acceptance by the consumers and for their demonstrated efficiency (Zhang *et al.,* 2010; Velasco and Williams, 2011; Shah *et al.,* 2014). Among natural antioxidants, *Curcuma longa* rhizome powder, called turmeric, is a spice used in food preparations for its flavour, colour and antioxidant properties due to the presence of curcumin. Curcumin is a curcuminoid and one of the major components of turmeric. It has a primary antioxidant effect against oxygen free radicals as it has the ability to break the oxidant chain reaction by its conjugated structure (Naganuma *et al.,* 2006, Puangsombat *et al.* 2011; Sharma *et al.,* 2012).

Several studies have already been conducted to analyse the shelf-life of raw or cooked meat products derived from animals fed diets supplemented with natural antioxidants (Botsoglou *et al.,* 2003; Sáyago-Ayerdi *et al.,* 2009; Yan *et al.,* 2011; Cardinali *et al.,* 2012; Dal Bosco *et al.,* 2014) or from minced meat supplemented with natural antioxidants (Haak *et al.,* 2009; López-López *et al.,* 2009).

Moreover, studies have reported the effects of natural antioxidants in meat products and are focused on the shelf-life of raw and cooked burgers, in particular burgers cooked at day 0 and after refrigerated storage (Carpenter *et al.,* 2007; Ganhão *et al.,* 2010; Cheng *et al.,* 2013; Kılıç *et al.,* 2014).

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In the market, burgers are generally sold raw, and consumers buy and cook them after different storage periods, during which various changes of meat quality might occur. In particular, rabbit meat is easily subjective to lipid oxidation, being characterised by a high polyunsaturated fatty acids content (PUFAs). For this reason, tends to produce an off-flavour (Dalle Zotte, 2002; Petracci and Cavani, 2013).

Due to the fact that there is no information available on the antioxidant capacity and lipid oxidation in rabbit burgers cooked after being stored raw for different periods, the aim of the present study was to evaluate the effects of turmeric powder and ascorbic acid on lipid oxidation and the antioxidant capacity in cooked rabbit burgers refrigerated for different storage periods as raw burgers.

MATERIALS AND METHODS

Burger manufacture

A detailed description of the manufacture of raw rabbit burgers is provided by Mancini *et al.* (2015). For the experiment, 6 batches (B) of meat produced from the hind legs of 36 hybrid rabbits were used (6 rabbits for batch); rabbits were reared under intensive conditions, and fed a commercial pelleted feed. From each batch 3 burger formulations (F) were produced: a Control (C) formulation was made only with meat, while the other 2 different meat formulations were produced after the addition of 3.5% turmeric powder (Tu) or 0.1% ascorbic acid (AA). The quantities of turmeric powder and ascorbic acid were chosen to make them comparable for antioxidant capacity; preliminary evaluations were performed with 2,2-azinobis-(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) reducing activity, 1,1-diphenyl-2-pircydrazyl (DPPH) radical scavenging activity, and ferric reducing ability (FRAP) methods on the 2 additives (data not shown). After manufacturing, the burgers were packaged in Styrofoam trays and wrapped with polyethylene film. Raw burgers were stored at 4±1°C for 0 and 7 d (storage time, ST). At day 0 and 7, 6 burgers per F (three burgers from each batch, one per F) were cooked in a preheated oven at 163°C to an internal temperature of 71°C; every 4 min burgers were turned to prevent excess surface crust formation.

Antioxidant capacity (ABTS, DPPH and FRAP) and lipid oxidation (thiobarbituric acid reactive substances [TBARS]) of cooked burgers were assessed after the samples reached room temperature.

Antioxidant capacity (ABTS, DPPH and FRAP) and lipid oxidation (TBARS)

Antioxidant capacity was assessed using ethanol extracted samples following the methods of Re *et al.* (1999) for ABTS reduction activity, Jung *et al.* (2010) for DPPH radical scavenging activity and Descalzo *et al.* (2007) for FRAP reduction ability with modification as reported in Mancini *et al.* (2015).

TBARS were evaluated using a spectrophotometer at 532 nm (V-530 Jasco International, Milan, Italy). TBARS value were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle) with a calibration curve with TEP (1,1,3,3-tetraethoxypropane) as reported by Dal Bosco *et al.* (2009).

Statistical analysis

Data were analysed with the following linear model: $Yijkz = \mu + \alpha i + \beta j + \gamma k + \alpha\beta ij + eijkz$, where Yijkz is the dependent variable of the z^{th} observation; μ is the overall mean; αi is the effect of the F (i =C, Tu, AA); βj is the effect of the ST (j=0, 7 d); γk is the effect of the block B (k=1, 2, 3, 4, 5, 6); αβij is the effect of the interaction between ST and F, and eijkz is the random error. No block B significant difference was found and B was removed from the final model.

Two-ways ANOVA was conducted, and the data are reported as the mean of the fixed effects F and ST; the variability was expressed as root mean square error (RMSE). The significance level was set at 5% (statistically significant for $P<0.05$) and if statistical significance was found, the differences were assessed using Tukey's test ($P<0.05$).

Probabilities and correlation coefficients between TBARS on cooked samples and the variables TBARS and antioxidant capacity of the raw samples were calculated. The normality of residuals was tested with Shapiro-Wilk test. R free statistical software was used (R Core Team 2013).

RESULTS AND DISCUSSION

The effects of F, ST and of the interaction of ST by F on antioxidant capacity (ABTS, DPPH and FRAP) and lipid oxidation (TBARS) are presented in Table 1 and Figure 1.

Regarding the main effects, the F and the ST significantly affected all parameters (Table 1). The formulations indicated that Tu had higher values of ABTS than AA burgers and AA had higher values than C burgers; the storage time showed that at day 7 ABTS level was lower than those observed at day 0.

A significant ($P<0.001$) ST×F interaction was observed for DPPH, FRAP and TBARS (Figure 1). At day 0 and day 7, the DPPH values were higher in Tu and AA burgers than those in C burgers. The ST×F interaction indicated that at day 0, the meat derived from C formulation produced burgers had a lower level of FRAP than meat derived from Tu and AA cooked burgers. At day 7, FRAP values tended to decrease but always remained significantly higher in Tu and AA burgers than in C burgers. The higher FRAP value observed in AA burgers at day 0 might be explained by the high sensibility of this analytic method for the antioxidant properties of ascorbic acid (Benzie and Strain, 1996).

The interaction revealed differences attributable to the formulations in TBARS; at day 0, the cooked burgers derived from the formulations supplemented with antioxidants showed lower values of TBARS than the burgers derived from the control formulation, while at day 7 the TBARS of cooked meat derived from Tu and AA formulations increased and AA samples showed a content similar to that observed in C burgers.

The reduction in lipid oxidation was mainly evident in cooked burgers supplemented with antioxidants at day 0, whereas after 7 d, the lipid oxidation values increased and only cooked burgers supplemented with turmeric powder showed lower values.

These results are in agreement with those observed in a previous study (Mancini *et al.,* 2015), in which low lipid oxidation values were observed in Tu and AA raw samples at day 0, but during storage time the lipid oxidation increased in all formulations. The higher level of TBARS after 7 d of storage in AA cooked samples might be attributed to the partial reduction of antioxidant activity observed in raw samples. These findings suggested that the antioxidant capacity was evident even after cooking and over time in Tu and AA burgers.

Few studies have reported antioxidant capacity on meat products after cooking. The results revealed that the burgers derived from formulations supplemented with antioxidants had higher antioxidant capacity and lower lipid oxidation values than those with no addition. An effective reduction of lipid oxidation values was also observed by Mitsumoto *et al.* (2005) and Tang *et al.* (2001) in cooked beef and chicken patties supplemented with natural antioxidants and ascorbic acid, respectively. A lack of significance for ABTS values was found in pork meat supplemented with seaweeds and heated in a water-bath (López-López *et al.,* 2009). The addition of natural antioxidants to goat, pork

Table 1: Effect of storage time (ST), formulation (F) and the ST×F interaction on lipid oxidation values (thiobarbituric acid reactive substances [TBARS]) and antioxidant capacity values (2,2-azinobis-[3 ethylbenzothiazoline-6-sulfonic acid] {ABTS}, 1,1-diphenyl-2-pircydrazyl [DPPH] and ferric reducing ability [FRAP]) of cooked burgers.

	Formulation			Storage time		*P*-value			
	C	Tu	AA	day 0	day 7	F	ST	ST×F	RMSE
Burgers n	6	6	6	6	6				
ABTS	1.86^{c}	3.80^{a}	2.95^{b}	3.06^{x}	2.69^{y}	***	**	ns	0.348
DPPH	0.30^{b}	0.94^{a}	0.93^{a}	0.68^{y}	0.76^{x}	***	***	***	0.054
FRAP	0.31^{c}	2.51^{b}	3.41^{a}	2.40^{x}	1.75^{y}	***	***	***	0.291
TBARS	0.30^{a}	0.13^{c}	0.18^{b}	0.15^{y}	0.25^{x}	***	***	***	0.035

ABTS and DPPH in mmol of Trolox equivalents per kilogram of fresh meat; FRAP in mmol of Fe^{II} equivalents per kilogram of fresh meat; TBARS expressed in mg of MDA per kilogram of fresh meat; C: control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder; ns: not significant; RMSE: root mean square error.

$^{a, b, c}$ different letters in the same row indicate significant differences for F at $P<0.05$; $^{x, y}$ different letters in the same row indicate significant differences for ST at $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

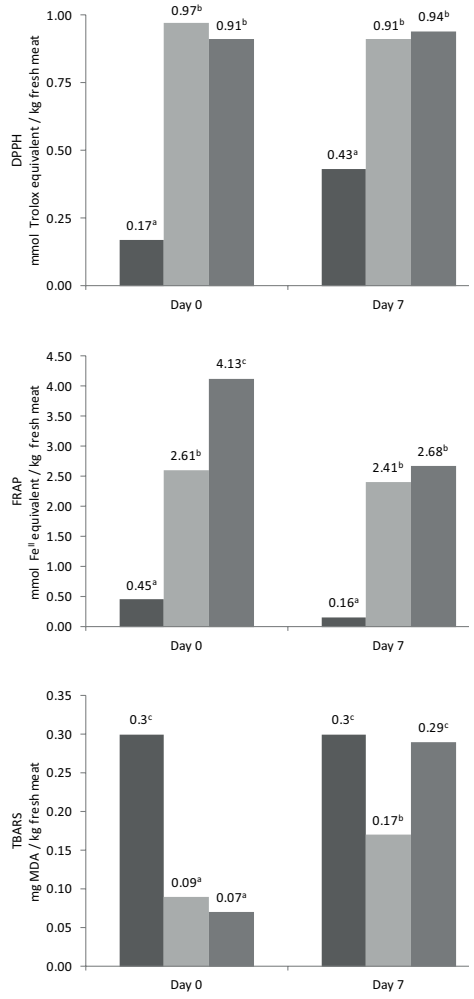

Figure 1: Interaction effect ST×F (storage time×formulation) on 1,1-diphenyl-2-pircydrazyl (DPPH), ferric reducing ability (FRAP) and Thiobarbituric acid reactive substances (TBARS).
■: C; ■: Tu; ■: AA; [a, b, c] Bars with different letter are significantly different at $P<0.001$.

and beef meat products before cooking resulted in higher DPPH values after cooking than the controls (Fasseas *et al.,* 2008; Banerjee *et al.,* 2012; Sánchez-Muniz *et al.,* 2012). A similar trend was reported for FRAP values in goat (Banerjee *et al.,* 2012) and pork (López-López *et al.,* 2009) meat products supplemented with broccoli powder and seaweed, respectively. Natural antioxidant additives such as green tea leaf extract (Jo *et al.,* 2003) or lotus and barley leaf powder (Choe *et al.,* 2011) were shown to reduce lipid oxidation in cooked meat products during storage.

To better understand the relationship between lipid oxidation values and antioxidant capacity, the TBARS of cooked burgers were related to the TBARS and antioxidant capacities of raw burgers and the antioxidant capacities of cooked burgers for the 3 formulations and the storage time (Table 2). The data showed a strong correlation between some variables and the oxidative status of cooked burgers as indicated by the r values that were often close to 1.

Regarding the formulation, a positive and significant correlation was observed between the TBARS values of cooked burgers and the TBARS of raw burgers supplemented with antioxidants ($P<0.001$ for both Tu and AA).

Considering the antioxidant capacities, the DPPH, FRAP values of Tu raw burgers presented a negative and significant correlation with the TBARS of cooked burgers ($P<0.001$ and $P<0.05$, respectively), while the AA raw burgers showed a negative and significant correlation between ABTS, DPPH, FRAP and TBARS of cooked meat. In the AA cooked burgers we also found a negative and significant correlation between the FRAP and TBARS of cooked burgers ($P<0.001$).

At day 0, ABTS, DPPH, and FRAP of raw and cooked burgers showed negative and highly significant ($P<0.001$) correlations with the TBARS of cooked samples. At day 7, all of the parameters were negatively correlated with the TBARS values of cooked samples, but only the ABTS, DPPH, and FRAP of raw burgers and the ABTS of cooked burgers showed a significant correlation ($P<0.001$, $P<0.05$, $P<0.01$ and $P<0.05$, respectively). It is difficult to compare our results with existing literature, as there are no studies available on pairwise comparisons between TBARS of cooked samples and antioxidant capacity of raw and cooked samples. However, a great number of articles reported a remarkable trend between low TBARS values of cooked samples and the presence of a natural antioxidant. This trend was reported in different meat products derived from pork meat (Rey *et al.,* 2005; Salminen *et al.,* 2006; Bastida *et al.,* 2009), poultry and beef (Nam *et al.,* 2004; Mitsumoto *et al.,* 2005; Naveena *et al.,* 2013). Similar results were also observed in raw samples derived after natural antioxidant dietary supplementation (Botsoglou *et al.,* 2002; Goliomytis *et al.,* 2014; Nkukwana *et al.,* 2014).

Table 2: Pairwise comparison correlation coefficients and the significance between the thiobarbituric acid reactive substances (TBARS) values of cooked burgers and TBARS values of raw burgers and antioxidant capacity values (2,2-azinobis-[3 ethylbenzothiazoline-6-sulfonic acid] {ABTS}, 1,1-diphenyl-2-pircydrazyl [DPPH] and ferric reducing ability [FRAP]) of raw and cooked burgers.

		TBARS cooked burgers				
		Formulation			Storage time	
Burgers	Parameter	C	Tu	AA	day 0	day 7
Raw	TBARS	–0.3441	0.8881***	0.8843***	–0.0622	–0.3710
Raw	ABTS	–0.0370	0.3441	–0.7342**	–0.8908***	–0.7869***
Raw	DPPH	–0.4850	–0.8275***	–0.7864**	–0.9770***	–0.5471*
Raw	FRAP	0.1690	–0.6585*	–0.9649***	–0.9613***	–0.6717**
Cooked	ABTS	–0.3221	0.1972	–0.3766	–0.7311***	–0.5841*
Cooked	DPPH	–0.1145	0.0991	0.3226	–0.9632***	–0.3320
Cooked	FRAP	–0.0987	–0.3054	–0.8237***	–0.9087***	–0.3459

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

Considering both formulations supplemented with antioxidant powders and the storage time, the results indicated that as the antioxidant capacities decreased the lipid oxidation of cooked burgers increased, mainly when the correlations are considered in raw burgers.

CONCLUSIONS

In conclusion, the results confirm that the addition of 3.5% turmeric powder to rabbit burgers exerts an antioxidant effect during storage time and seems more effective in controlling lipid oxidation than ascorbic acid after cooking. The findings indicated a strong relation between the oxidative status of cooked burgers and the antioxidant capacity (of raw and cooked samples). These results indicate that turmeric powder might be profitably used in the market for the preparation of burgers. This finding is important, mainly for rabbit meat, which is rich in polyunsaturated fatty acids.

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