

QUALITATIVE IMPROVEMENT OF RABBIT BURGERS USING *ZINGIBER OFFICINALE* ROSCOE POWDER

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Abstract: The object of this study was to evaluate the effect of *Zingiber officinale* powder on physical-chemical traits, microbiological growth and sensory properties of rabbit burger. Raw burgers (only meat and meat added with 1 and 2% w/w ginger powder) were stored at 4°C for 1, 4 and 7 d and then cooked. Ginger modified the colour of both raw and cooked burgers, leading to more yellow hue and reducing lightness. Aspect of burgers were affected by ginger powder addition, leading to a noticeable difference between the samples. During storage time, the highest modifications were recorded for control samples, followed by burgers with added ginger. Sensory evaluation highlighted that ginger enhanced the juiciness of the burgers; moreover, burgers with ginger powder presented a significant delay in microbial growth. Ginger powder might be considered as a potential ingredient in rabbit meat products to increase their quality and extend their shelf-life.

Key Words: burger, rabbit meat, ginger, meat quality, colour, rabbits.

INTRODUCTION

Nowadays ready-to-cook and ready-to-eat products represent important food production items in terms of market share. Ready-to-cook meat products can meet the demand of the consumers for a nutrient food (rich in proteins, essential amino acids, minerals, vitamins and poor in fats) that does not need time-consuming preparation. Rabbit meat could be a suitable matrix from which to obtain meat products with functional value, due to its high content of protein and amino acids, with an important biological value and for its low content of fat and high percentage of unsaturated fatty acids (Dalle Zotte 2002; Dalle Zotte and Szendrő, 2011).

World annual production of rabbit meat for the year 2014 was estimated at 1.6 million tonnes, with China the main producer (763 thousand tonnes), followed by Italy and the Democratic People's Republic of Korea (269 and 152 thousand tonnes, respectively) (FAO, 2017). In the market, rabbit meat is currently sold as whole carcass or cut-up parts, while the sale of processed products is low (Cavani *et al.*, 2009; Petracci and Cavani, 2013). However, in recent years, to promote the sale of rabbit meat and make more attractive products for consumers, the interest from industry in ready-to-cook and ready-to-meal rabbit products has increased.

Typically, processed meat products could be subject to a rapid deterioration of quality due to enzymatic and microbial degradation (Davies *et al.*, 1998; Hui *et al.*, 2001). Improvements in packaging and in the use of natural additives have been thoroughly studied in order to enhance shelf-life (Falowo *et al.*, 2014; Gómez-Estaca *et al.*, 2014; Shah *et al.*, 2014; Overholt *et al.*, 2016). Plant products, such as essential oils and spices, could enhance shelf-life and add antioxidant properties to meat products. Moreover, they are usually well accepted by consumers, both for their good taste and their natural origin. Several studies have documented the positive effects of a few plant belonging

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to the Zingiberaceae family, such as *Curcuma longa* L. (turmeric) and *Alpinia galanga* (galangal), added both to diet and in products, on antioxidative properties and meat quality (Cheah and Abu Hasim, 2000; Juntachote *et al.*, 2007; Mancini *et al.*, 2016; Mancini *et al.*, 2017a).

Like turmeric and galangal, *Zingiber officinale* Roscoe (ginger) is widely used as a spice and as a medicament in traditional Chinese herbal medicine (Tapsell *et al.*, 2006). Ginger has a pleasant aroma and a pungent taste and its main compounds have shown various physiological effects (Ali *et al.*, 2008). Ginger root, powder and extracts have been studied for their antioxidant and antimicrobial properties both in diet supplementation (Zomrawi *et al.*, 2012; Herawati and Marjuki, 2011; Zhao *et al.*, 2011) and food preservation (Abdel-Naeem and Mohamed, 2016; Cao *et al.*, 2013; Naveena and Mendiratta, 2004). Information on the effect of adding ginger to meat products is limited (Cao *et al.*, 2013; Mi *et al.*, 2016; Mancini *et al.*, 2017b), particularly in rabbit meat (Mancini *et al.*, 2017c). The aim of this study was to assess the effect of 2 different concentrations of ginger powder on the physical characteristics, sensory properties and microbial growth of raw and cooked rabbit burgers.

MATERIAL AND METHODS

Animals

Meat was obtained from 9 hybrid rabbits reared under intensive conditions and fed commercial pelleted feed. Rabbits (2.5±0.10 kg) were slaughtered in a farm abattoir by electrical stunning followed by cutting of the carotid arteries and jugular veins. After chilling for 24 h at 4±0.5°C, the carcasses were transported to the laboratory (Department of Veterinary Science, Pisa) and meat was obtained by careful dissection and deboned following standard procedures (Blasco and Ouhayoun, 1996).

Experiment design and preparation of burgers

Meat obtained from each carcass was ground and proximate analyses were conducted; the ground meat from each rabbit was considered as an experimental unit (9 experimental units). Three meat ground batches were assigned to each formulation (F), and 3 formulations were prepared: meat with no additives (control, C), meat with ginger powder at concentrations of 1% (10 g ginger kg⁻¹ of meat, Z1) and 2% (20 g ginger kg⁻¹ of meat, Z2).

Ten burgers of 50 grams per batch were formed in Petri dishes (85 mm diameter) for a total of 90 burgers, 30 per F. Burgers were packaged in single Styrofoam trays, overwrapped with polyethylene film and stored at 4±0.5°C for up to 7 d.

The pH, colour, drip loss, cooking loss and microbial growth of raw burgers were determined at day 1, 4 and 7 (T1, T4 and T7). Furthermore, pH, colour and sensory evaluation were also determined in cooked samples.

Physical-chemical analysis

Moisture and crude fat (ether extract) in ground meat were determined according method AOAC (1995).

Drip loss was calculated as proposed by Lundström and Malmfors (1985) within the F between T1-T4 and T1-T7. Cooking loss was calculated as percentage of the decrease of weight before and after cooking in a preheated oven at 163°C to an internal temperature of 71°C and were turned every 4 min to prevent excess surface crust formation (AMSA 1995).

Raw and cooked samples were used for the determination of pH and colour indexes.

The pH was measured using a pH meter (Hanna pH 211, Hanna Instruments, Padova, Italy) equipped with a glass electrode (Hanna FC 200B, suitable for meat penetration) and an automatic temperature compensator.

Colour was measured using a Minolta CR300 chroma meter (Minolta, Osaka, Japan) and was expressed as L* (lightness), a* (redness), and b* (yellowness) according to the CIElab system (CIE 1976). Hue (H*) and chroma (C*) were calculated as function of a* and b*. Moreover, in order to evaluate the perceptible differences in colour, the numerical total colour difference (ΔE) was calculated as proposed by Sharma (2002) as $\Delta E_{1-2} = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{0.5}$,

where L*, a* and b* referred to 2 different formulations at the same storage time or 2 different storage times for the same formulation. ΔE differences were calculated for both raw and cooked samples. Moreover, changes in colour before and after cooking were determined for each F at each T. The threshold of a human noticeable difference was fixed at 2.3 points, as proposed by Sharma (2002).

Microbial growth

Microbial growth was assessed in 10 g of samples. Total aerobic count, β -glucuronidase-positive *Escherichia coli*, Enterobacteriaceae, coagulase positive and negative staphylococci, *Pseudomonas* spp. and *Enterococcus* spp. were enumerated as reported by Mancini *et al.* (2017a) and microbial counts were expressed as log colony forming units (CFU) g⁻¹.

Sensory evaluation

For the sensory analysis, rabbit burgers were cooked in an electrical clamshell grill covered with aluminium foil until they reached an internal temperature of 71°C, measured by a portable thermocouple thermometer (HI 92704C, Hanna Instruments, Padova, Italy). Immediately after cooking, each burger was cut into 8 wedges that were individually wrapped in aluminium foil and kept at 60°C until serving. In each session, samples were presented following a balanced design (Macfie *et al.*, 1989) to a panel formed by 6 trained assessors chosen among the staff of the Department of Veterinary Science of Pisa University. Six sensory properties were assessed using a 10 cm long unstructured scale. The 6 parameters were: appearance (typical appearance of rabbit burgers), aroma intensity (defined as the intensity of the characteristic aroma of rabbit burgers), off-odours (uncharacteristic or undesirable odours, usually associated with transformation or degradation of the sample), flavour intensity (defined as the intensity of the characteristic flavour of rabbit burgers), off-flavours (uncharacteristic or undesirable flavours, usually associated with transformation or degradation of the sample) and juiciness (amount of juice released in the initial stages of mastication). Moreover, the panellist was asked to give a global evaluation to the samples using a 9-point structured scale (1, extremely negative; 5, neither negative nor positive; 9 extremely positive).

Statistical analysis

Data on drip loss, cooking loss, pH, colour, microbial growth and sensory were analysed with the following linear model: $Y_{ijz} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijz}$, where Y_{ijz} is the dependent variable of the z^{th} observation; μ is the overall mean; α_i is the effect of the formulation, F (i=C, Z1, Z2); β_j is the effect of the storage time, T (j=1, 4, 7 d); $\alpha\beta_{ij}$ is the effect of the interaction between F and T, and e_{ijz} is the random error.

A two-way ANOVA was conducted and when the interaction F×T was not significant the results were reported as the mean of the fixed effects F and T; 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 ($P < 0.05$).

The analyses were performed with the free statistical software R (R Core Team, 2015).

RESULTS AND DISCUSSION

No significant differences were observed in proximate analyses on the meat batches between the experimental units (moisture: 72.67±0.77%; ether extract: 2.75±0.67%; data not shown).

Physical-chemical characteristics

Table 1 presents the results for physical characteristics of the raw and cooked burgers. No statistical significances were revealed for the interaction F×T. Considering the main effects, the formulation affected the colour parameters of both raw and cooked burgers, while the storage time affected the lightness and the redness index of raw burgers, the water holding capacity (drip loss and cooking loss) and the pH of both raw and cooked samples ($P < 0.05$ for L* and a* of raw burgers; $P < 0.01$ for cooking loss; $P < 0.001$ for drip loss and pH of both raw and cooked).

Table 1: Physical characteristics of raw and cooked burgers.

	Formulation (F)			Storage time (T, d)			RMSE	P-value	
	C	Z1	Z2	T1	T4	T7		F	T
Raw burgers (n)	30	30	30	30	30	30			
Drip loss (%)	3.78	3.78	2.67	2.00 ^x	2.67 ^x	5.56 ^y	1.333	0.154	<0.001
Cooking loss (%)	24.67	23.67	23.22	25.22 ^y	23.56 ^x	22.78 ^x	1.305	0.082	0.003
L*	58.59 ^b	56.52 ^{ab}	55.53 ^a	58.05 ^y	57.08 ^{xy}	55.50 ^x	1.884	0.009	0.032
a*	9.17 ^b	8.85 ^{ab}	7.76 ^a	9.14 ^y	8.76 ^{xy}	7.88 ^x	1.012	0.022	0.045
b*	9.21 ^a	13.48 ^b	16.20 ^c	13.2	12.98	12.71	1.339	<0.001	0.744
C*	13.01 ^a	16.13 ^b	18.00 ^c	16.18	15.8	15.15	1.548	<0.001	0.382
H*	45.27 ^a	56.69 ^b	64.40 ^c	54.53	55.00	56.83	2.547	<0.001	0.156
pH	5.58	5.58	5.59	5.53 ^x	5.46 ^x	5.76 ^y	0.095	0.970	<0.001
Cooked burgers (n)	9	9	9	9	9	9			
L*	70.74 ^c	67.11 ^b	63.17 ^a	68.08	66.77	66.16	2.352	<0.001	0.237
a*	5.59 ^a	6.78 ^{ab}	7.85 ^b	6.42	6.99	6.81	1.049	<0.001	0.508
b*	12.93 ^a	15.81 ^b	19.93 ^c	16.15	15.86	16.67	1.569	<0.001	0.547
C*	14.10 ^a	17.22 ^b	21.47 ^c	17.4	17.33	18.03	1.720	<0.001	0.643
H*	66.63	66.94	68.55	68.05	66.08	67.99	2.314	0.197	0.151
pH	5.76	5.70	5.78	5.67 ^x	5.66 ^x	5.91 ^y	0.094	0.197	<0.001

C: only meat; Z1: meat+1% ginger powder; Z2: meat+2% ginger powder; RMSE: root mean square error.

^{a, b, c} in the same row indicate significant differences for F; ^{x, y} in the same row indicate significant differences for T.

The raw C burgers showed higher values of L* and a* than raw Z2 burgers and the lowest values of b*, C* and H*. Modifications were also shown after cooking regarding L*, b* and C*; moreover, the redness index of cooked burgers increased in relation to the amount of ginger. This differential effect of ginger, in redness index of raw and cooked samples, could be ascribable to a dilution effect of the myoglobin colour in raw burgers, or to a protection effect against myoglobin oxidation and discoloration in cooked burgers.

Lightness and yellowness indexes, as well as chroma and hue, of both raw and cooked samples could be related to the presence of natural pigment in the spice, which might modify the meat colour. Meat colour changes were also reported by Mansour and Khalil (2000), who studied the effect of extracts of plant materials (potato peel, fenugreek seeds and ginger rhizomes) on meat quality of beef patties, and by Mi *et al.* (2016) who evaluated the effect of 6-gingerol on red drum fillets during storage time.

As regards the T, at T7 the raw burgers showed lower values of L* and a*. The differences in L* may be explained by the increase in pH, as pH and lightness are linked by a negative correlation. Similar results were also reported by Mancini *et al.* (2015) and Dal Bosco *et al.* (2014); these authors, who studied the effect of natural antioxidants and storage time on meat quality, observed a decrease in L* and an increase in pH during short storage times.

The significant decrease in a* value at T7 may be related to oxidation of the raw burgers. Several studies found reductions in the redness of ground meat during storage time and ascribed these effects to the formation of metmyoglobin produced by myoglobin oxidation (Choe *et al.*, 2011).

The storage time also affected drip loss; the burgers showed the capacity to hold the water naturally present in the meat for 4 d, and after this period this capacity decreased, as shown by the drip loss value (T1=T4<T7, P<0.001).

The T also affected the cooking losses, which were higher at T1 than T4 and T7, with an overall range of variability from 20% to 27% (P<0.01); at T1, the highest value of cooking loss may be ascribed to the amount of moisture present in the sample.

These results are in agreement with those of previous research carried out on the addition of antioxidant ingredients in meat products, where the storage time was found to increase exudate losses and reduced the cooking losses, mainly in burgers with natural powder added (Mancini *et al.*, 2015; Moroney *et al.*, 2013). No significant modification in cooking loss was caused by addition of the ginger powder, and increasing the ginger content tends to reduce

the cooking loss anyway. Soltanizadeh and Ghiasi-Esfahani (2015) and Reihani *et al.* (2014) showed a decrease in cooking loss in beef burgers respectively containing *Aloe vera* or *Cosmos caudatus* extract.

At T7, both raw and cooked burgers showed higher pH values than at T1 and T4 ($P<0.001$). The increase of the pH in raw burgers may be attributable to hydrolysis of the proteins by the metabolisms of bacteria and the consequent increase in the level of ammoniacal nitrogen, amines and other basic compounds, as reported by Karabagias *et al.* (2011) and Rodríguez-Calleja *et al.* (2005) during the storage of meat products refrigerated at 4°C. As a consequence of the increased level of alkalisation of raw burgers, the cooked ones were found to be affected with the same trend.

In Table 2, the total colour differences (ΔE) are reported. As rabbit meat is very light in colour, with a natural pale pink hue, the addition to the burgers of an ingredient with a strong characteristic colour, such as ginger powder, could lead to a discernible modification of the products. Indeed, considering the colour differences (ΔE) between the F at the same T of analysis, only the combination Z1-Z2 at T1 between raw burgers reported ΔE value below the threshold (value above 2.3 points), with no noticeable difference. As expected, the greatest differences were recorded between C and Z2 both for raw and cooked samples.

The ΔE between different times for the same F showed that all the raw samples, as well as cooked Z1 and Z2 burgers, reported a noticeable change of colour in the total time of the trial (T1-T7). Z1 raw and cooked burgers were shown to modify their colour between the T4-T7 period, whereas cooked Z2 burgers changed colour in the T1-T4 period. As mentioned previously, the inclusion of a spice with an intrinsic colour may lead to a noticeable variation of the aspect of the product, and this modification could be used for recognisability of the product. However, no general deduction could be formulated as different ingredients may cause different colour modifications.

The overall evaluation of colour modification as difference in raw-cooked samples with both F and T fixed showed that all the samples displayed a large modification in colour; interestingly, the highest modifications were recorded for C samples, followed by Z1 and Z2.

Microbiological evaluation

Microbiological growth in burgers is reported in Table 3. The complete absence of *Enterococcus* spp. and *Escherichia coli* was shown in all the samples at all the tested times. The total aerobic count and evaluation of the *Enterobacteriaceae*, *Pseudomonas* spp. and staphylococci were affected by the interactive effects of F×T ($P<0.001$).

Table 2: Numerical total colour difference (ΔE) calculated between formulations at the same storage time and between storage times for the same formulation and between raw and cooked samples.

	ΔE Formulation					
	Raw burgers			Cooked burgers		
Storage time (T, d)	C-Z1	C-Z2	Z1-Z2	C-Z1	C-Z2	Z1-Z2
T1	5.45*	7.10*	1.77	3.36*	6.87*	3.60*
T4	3.57*	7.70*	4.19*	4.64*	12.89*	8.28*
T7	5.41*	8.54*	3.78*	6.69*	12.33*	5.77*
ΔE Storage time (T, d)						
Formulation	T1-T4	T4-T7	T1-T7	T1-T4	T4-T7	T1-T7
C	1.15	2.19	2.87*	1.73	1.09	1.72
Z1	1.62	2.48*	2.56*	1.40	2.43*	2.49*
Z2	1.96	1.97	3.60*	5.28*	0.34	5.15*
Raw-Cooked burgers storage time (T, d)						
Formulation	T1	T4	T7			
C	11.30*	13.43*	14.98*			
Z1	11.20*	10.58*	11.44*			
Z2	10.09*	7.38*	8.49*			

C: only meat; Z1: meat+1% ginger powder; Z2: meat+2% ginger powder.

*value over the threshold (2.3 points) with a noticeable difference in colour between the samples.

Table 3: Microbial analysis of raw burgers (log colony forming units g⁻¹).

Formulation (F)	C			Z1			Z2			RMSE	P-value		
	T1	T4	T7	T1	T4	T7	T1	T4	T7		F	T	F×T
Total aerobic count	5.54 ^a	8.35 ^c	8.73 ^e	5.97 ^b	8.46 ^d	8.70 ^e	5.62 ^a	8.43 ^{cd}	8.71 ^e	0.030	<0.001	<0.001	<0.001
Enterobacteriaceae	0 ^a	0 ^a	3.22 ^d	0 ^a	0 ^a	2.60 ^c	0 ^c	0 ^a	1.29 ^b	0.014	<0.001	<0.001	<0.001
<i>Pseudomonas</i> spp.	3.24 ^a	4.94 ^c	5.48 ^e	3.59 ^a	4.86 ^c	5.09 ^d	3.30 ^a	4.25 ^b	5.39 ^d	0.030	<0.001	<0.001	<0.001
Staphylococci	0 ^a	2.31 ^c	3.18 ^e	0 ^a	2.01 ^b	3.28 ^e	0 ^a	2.16 ^b	2.94 ^d	0.024	<0.001	<0.001	<0.001
<i>Enterococcus</i> spp.	0	0	0	0	0	0	0	0	0	-	-	-	-
<i>E. coli</i>	0	0	0	0	0	0	0	0	0	-	-	-	-

C: meat only; Z1: meat+1% ginger powder; Z2: meat+2% ginger powder. RMSE: root mean square error.

^{a, b, c, d, e} in the same row indicate significant differences for F×T.

The total aerobic count showed that for the first 4 d of storage time the C burgers maintained a log CFU g⁻¹ comparable to or lower than Z1 and Z2. At T7, all the F reported the highest values for quantification of the total aerobic bacteria. *Enterobacteriaceae* bacteria were undetectable until T7, and the F reported higher values of log CFU g⁻¹ inversely proportional to the percentage of ginger added (C>Z1>Z2). As for *Enterobacteriaceae*, the coagulase positive and negative staphylococci were likewise undetectable at T1; at T4 all the F showed the presence of these bacteria, with higher values for C than for Z1 and Z2; at T7, no difference was highlighted between C and Z1, with greater values than Z2. As common surface contaminant bacteria, *Pseudomonas* spp. were detected at T1 with equal log CFU g⁻¹ between the F. As function of the interaction F x T, the values of bacteria increased during T with the major quantity for C and Z1 at T4, and for C at T7. The effectiveness of an ingredient in meat products to inhibit or delay bacterial growth is a key factor in enhancing shelf-life. Ginger powder seems to delay the growth of all the tested bacteria. Other research into natural ingredient addition showed that plant extracts, powders or essential oils were highly effective against the microbial growth in meat products. Pork burgers/patties supplemented with different plant products, such as passion fruit co-products and tea or grape extracts, showed a lower bacterial growth than the respective control treatment (López-Vargas *et al.*, 2014; Lorenzo *et al.*, 2014).

Chicken meat with added spice extracts (*Syzygium aromaticum*, *Cinnamomum cassia* and *Origanum vulgare*) was shown to delay the growth of the total viable count of Enterobacteriaceae and *Pseudomonas* spp. (Radha Krishnan *et al.*, 2014).

Sensory

The sensory analysis results are reported in Figure 1. The main factor F affected the juiciness and the global score of the burgers (both $P<0.05$); C burgers had lower juiciness than in Z1 and Z2 burgers, and had the lowest global score. Z1 burgers had a lower global score than Z2 burgers (global score Z1<Z2, $P<0.05$). Storage time (T) influenced the appearance, aroma intensity and flavour intensity of rabbit burgers and the global score ($P<0.01$, $P<0.05$, $P<0.01$ and $P<0.05$, respectively). The highest global score was recorded at T1, with a subsequent decrease at T4 and then at T7. No differences were shown by either F or T for the presence of off-odours and off-flavours ($P>0.05$), with the lowest scores trending to 0. Ginger, as a spice, seems to be well accepted and to enhance the sensory features of meat products. Naveena *et al.* (2004) reported that buffalo meat treated with ginger received better scores for appearance, flavour, tenderness and overall acceptability and Mansour and Khalil (2000) reported that beef patties with ginger extract after 12 d at 5°C had the lowest value of rancid odour.

Other spices such as rosemary and oregano have been reported to extend shelf-life of beef patties (Sánchez-Escalante *et al.*, 2003) without changing the typical odour and flavour of the patties. Karabagias *et al.* (2011) reported how thyme and oregano essential oils enhanced the shelf-life of lamb meat, although a higher concentration of 0.3% gave a very strong odour/taste to the product, making it unacceptable.

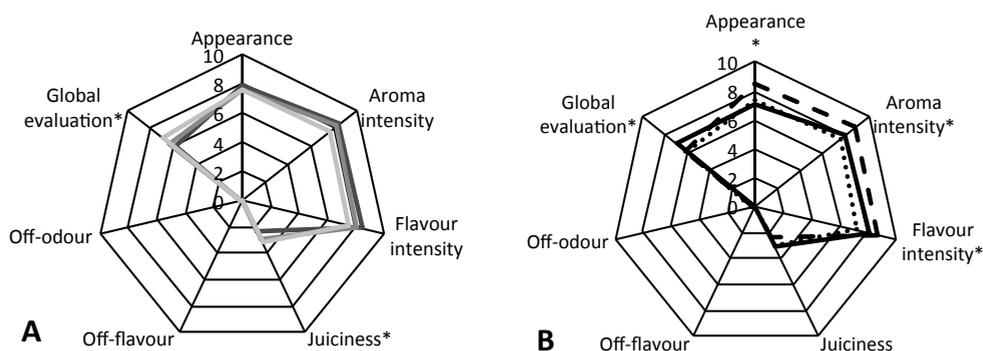


Figure 1: Sensory evaluations. A. Sensory evaluations as function of the formulation (F): C, only meat (dark grey); Z1, meat+1% ginger powder (grey); Z2, meat+2% ginger powder (light grey). B. Sensory evaluations as function of the storage time (T, days): T1, solid line; T4, dashed line; T7, dotted line. *significant differences between the F or the T ($P < 0.05$).

CONCLUSIONS

Ginger powder as a food additive in rabbit burgers proved to have significant effects on burger colour and microbial growth. Moreover, ginger powder partially modified the sensory appraisal of burgers, leading to higher global evaluation and juiciness than meat-only samples. In ready-to-eat products, the addition of ginger to meat affects the colour, leading to a richer hue. Further studies might be interesting to test the effect of ginger powder on fatty acids oxidation and antioxidant capacity.

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