TECNOCIENCIA CHIHUAHUA
Revista de ciencia y tecnología

ISSN-e: 2683-3360

UACH
UNIVERSIDAD AUTÓNOMA DE CHIHUAHUA

Research Article

Effect of a black garlic extract combined with ascorbic acid on the colour and lipid oxidative stability of patties

Efecto de un extracto de ajo negro combinado con ácido ascórbico sobre el color y la oxidación lipídica de hamburguesas

Nuria Fernández Fernández-Valladares [1], Irma Caro[2], Javier Mateo[1] y Mauricio Fernando Mariño-Almache[1*]

[1] Departamento de Higiene y Tecnología de los Alimentos, Universidad de León. Campus Vegazana s/n, 24007 León, Spain

[2] Facultad de Medicina, Universidad de Valladolid. Avenida Ramón y Cajal 7, 47005 Valladolid, Spain

*Correspondencia: mmaria00@estudiantes.unileon.es (Mauricio Fernando Mariño-Almache)
DOI: https://doi.org/10.54167/tch.v18i4.1683
Recibido: 30 de septiembre de 2024; **Aceptado**: 16 de diciembre de 2024
Publicado por la Universidad Autónoma de Chihuahua, a través de la Dirección de Investigación y Posgrado.
Editor de Sección: Dr. Diego Eloy Carballo-Carballo

Abstract
Oxidation of meat products limits their quality and shelf life and is prevented by antioxidant additives. Natural ingredients rich in antioxidants are proposed as an alternative to these additives. In this study, the antioxidant effect of an aqueous extract of black garlic, as a natural ingredient rich in polyphenols, was evaluated in pork patties. 20 ml of extract, obtained from a homogenate prepared with one-part garlic and three parts water, was used per kg of patty in combination with three levels of ascorbic acid (from 0.125 to 0.5 g/kg). The effect of different combinations to prevent discolouration in raw patties and lipid oxidation stability in cooked patties during aerobic refrigerated storage was tested. The use of black garlic extract darkened the patties and increased the red and yellow indices. Ascorbic acid potentiated the antioxidant effect of black garlic in cooked patties and vice versa. The black garlic extract combined with ascorbic acid, even in an amount four times lower than that commonly used in the meat industry, was the treatment with the highest antioxidant effect.

***Keywords*: natural antioxidant, patty, meat quality, black garlic, shelf life**

Resumen
La oxidación de los productos cárnicos limita su calidad y vida útil y se evita mediante aditivos antioxidantes. Como alternativa a estos aditivos, se propone el uso de ingredientes naturales ricos en antioxidantes. En este estudio, se evaluó el efecto antioxidante de un extracto acuoso de ajo negro, como ingrediente natural rico en polifenoles, en hamburguesas de cerdo. Se utilizaron 20 ml de extracto, obtenido de un homogeneizado preparado con una parte de ajo y tres de agua, por kg de hamburguesa en combinación con tres diferentes niveles de ácido ascórbico (entre 0,125 a 0,5 g/kg). Se comprobó el efecto de las distintas combinaciones para prevenir la decoloración en las hamburguesas crudas y la estabilidad de la oxidación lipídica en las hamburguesas cocinadas durante el almacenamiento aeróbico refrigerado. El uso de extracto de ajo negro oscureció las hamburguesas y aumentó los índices de rojo y amarillo. El ácido ascórbico potenció el efecto antioxidante del ajo negro en las hamburguesas cocidas y viceversa. El extracto de ajo negro combinado con ácido ascórbico, incluso en una cantidad cuatro veces inferior a la utilizada habitualmente en la industria cárnica, fue el tratamiento con mayor efecto antioxidante.

Palabras clave: **antioxidante natural, hamburguesa, calidad de carne, ajo negro, vida útil.**

1. Introduction

One of the limiting factors in the quality of meat and meat products is lipid auto-oxidation (Morrissey *et al.,* 1998). This oxidation is an irreversible process that occurs by a chain reaction mechanism mediated by the formation of free radicals. The initiation of this reaction and its extent over a given time depends on a balance between pro-oxidant and antioxidant factors in the product and the environment (Choe and Min, 2006). In the autoxidation process, peroxides and secondary oxidation compounds of low molecular weight are generated, and chemical changes occur in proteins such as cross-linking or oxidation of metals such as iron bound to myoglobin. As a result, the sensory attributes of the meat are altered, and potentially toxic compounds such as free radicals or cholesterol oxides are formed (Morrissey *et al.,* 1998; Sampels, 2013). In response to this alteration process, meat processing can resort to the use of antioxidant additives, which are substances that, at low concentrations, prevent or avoid oxidative damage to specific molecules by scavenging reactive oxygen species (ROS), enhancing biological antioxidant defences or inhibiting ROS production (Khlebnikov *et al.,* 2007).

Research and the food industry are actively working on incorporating antioxidant-rich natural ingredients derived from condiments, spices, fruits, vegetables or seeds containing polyphenols or other bioactive compounds (Karre *et al.,* 2013; Falowo *et al.,* 2014; Shahidi and Ambigaipalan, 2015). Antioxidant compounds encompass a wide range of sources, including conventional and non-conventional edible materials and by-products such as fruit peels, seeds, and medicinal herbs. The most frequent bioactive compounds with antioxidant power present in plants are phenolic compounds, although there are also other natural antioxidant compounds such as non-phenolic terpenoids, vitamins, metal-chelating ions, proteins and peptides (Falowo *et al.,* 2014). In addition to their antioxidant properties, antioxidant-rich ingredients could contribute to the nutritional improvement of foods, including meat products, by providing fibre, vitamins or minerals (Falowo *et al.,* 2014), as well as antimicrobial compounds (Hygreeva *et al.,* 2014). However, on the other hand, dietary phenolic compounds can exert a negative impact on the digestion processes such as enzyme inhibition (Cirkovic Velickovic and Stanic-Vucinic, 2018).

Black garlic is the result of the transformation or blackening of common garlic through the Maillard reaction. Its use as a food ingredient or for medical purposes dates to ancient times in regions of the Asian continent. Kimura *et al.* (2017) reported that the consumption of black garlic as a condiment in culinary preparations or for direct consumption is spreading and that it is associated with functional antioxidant, anti-inflammatory, anti-cancer or anti-diabetic properties, indicating its beneficial health properties.

To obtain the black garlic, fresh garlic is kept at a constant temperature of 45-70 °C with a relative humidity of 70-80 % for a variable period of time, usually between 15-40 days, after which it is cooled and dried for approximately one week (Dewi and Mustika, 2018). The process results in a product with 40-50 % moisture content, a mild aroma, and a tender and creamy texture. It has been determined that the optimal ageing period for garlic to maximise its antioxidant capacity is 21 days (Choi *et al.,* 2014). Black garlic is a stable product at room temperature, with a shelf life of about 18 months, provided the storage conditions are appropriate (dry, cool and away from light).

The use of black garlic as an antioxidant ingredient in meat derivatives has scarcely been studied. In recent studies (Lee *et al.,* 2019; Barido *et al*., 2022; Santos *et al.,* 2024), it has been found that the use of black garlic in meat patties revealed a significant lipid antioxidant effect. The antioxidant effect has been attributed to active compounds such as reducing sugars resulting from enzymatic hydrolysis flavonoids, polyphenols, alkaloids, S-allyl-cysteine, and some of the Maillard reaction intermediates, i.e. 5-hydroxymethylfurfural (Choi *et al.,* 2014; Yuan *et al*., 2016; Kimura *et al.,* 2017). This effect could be comparable or synergistic with that of ascorbic acid, an antioxidant additive commonly used in the meat industry.

This study aimed to assess the impact of combining black garlic with ascorbic acid, a commonly used antioxidant additive in the meat industry, on lipid oxidation and colour stability in pork patties stored under aerobic conditions.

2. Materials and methods

2.1 Experimental design

The study comprised two main phases: an *in vitro* analysis and an *in-situ* application. In the in vitro part, the antioxidant capacity of a black garlic aqueous extract was evaluated by determining the total polyphenol content and performing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. The antioxidant capacity of the garlic extracts obtained by the DPPH test was compared with that of two antioxidant additives used in the food industry: ascorbic acid and butylated hydroxytoluene (BHT). In the *in-situ* phase, the focus was on assessing the colour stability of raw pork patties and the oxidative stability of lipids in cooked pork patties containing different levels of the black garlic extract combined with different amounts of ascorbic acid, during a six-day period of aerobic refrigerated storage. The colour stability was determined by measuring the change in instrumental colour, and the oxidative stability of lipids was determined by changes in the concentration of thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation.

2.2 Preparation of the black garlic extract and patties

Two samples of black garlic were purchased at the local market. The garlic cloves were minced with a domestic food processor, and 25 g of minced garlic was mixed with 75 g of distilled water with an Ultraturrax T18 IKA Werke (Staufen, Germany) for 2 min at 13,500 revolutions per minute. The pH was then adjusted with 0.1 M NaOH to 7.0 and centrifuged for 25 min at 12,000 rpm with a Beckman J2-21 centrifuge (Palo Alto, U.S.A). The supernatant was first filtered through a Whatman No. 1 filter (Darmstadt, Germany) and then through a 0.45 mm pore size polytetrafluoroethylene filter (Membrane Solutions, LLC, Auburn, WA, USA).

Frozen pork loin meat purchased from a local market produced the patties. A 2 kg meat piece was thawed for 48 h at 4 °C, minced in a Mainca mincer (Mainca PM-12; Granollers, Spain) with a 6 mm plate, salt was added at a rate of 15 g/kg and mixed by hand for 6 min. The different patties were prepared in duplicate (two batches, each prepared on different days). Two sets of patties were prepared inside each replication, one with each sample of black garlic purchased. Five treatments (formulations) were prepared for each set (Table 1): i) a control sample without added antioxidants; ii) a formulation containing 2 % (v/w) black garlic extract, attempting to use a high quantity without compromising sensory characteristics (Augustynska-Prejsnar *et al.* 2024); iii) patties with 2 % (v/w) of an ascorbic acid solution (25 mg/mL), a level commonly used in the food industry (0.5 mg ascorbic acid/kg patty); and iv-vi) formulations combining 2 % (v/w) black garlic extract with 2 % (v/w) ascorbic acid with low (6.25 mg/mL), medium (12.5 mg/mL), and high (25 mg/mL) concentrations, respectively.

Tabla 1. Formulaciones de hamburguesas y cantidades de antioxidantes para cada tratamiento experimental.
Table 1. Patty formulations and amounts of antioxidants for each experimental treatment

	Amounts used (g or ml/100 g of patty mix)				Concentration (g/kg of patty mix)	
	Meat+salt	**Water**	**Ascorbic acid solution**	**Black garlic extract**	**Black garlic (g)***	**Ascorbic acid**
CON	90	10	0	0	0	0
Asc$_h$	90	8	2	0	0	0.5
BG	90	8	0	2	5	0
BG+Asc$_l$	90	6	2	2	5	0.12
BG+Asc$_m$	90	6	2	2	5	0.25
BG+Asc$_h$	90	6	2	2	5	0.5

CON: control treatment; Asc$_h$: patty to which a 25 mg/mL ascorbic acid solution was added; BG: patty containing black garlic; BG+Asc$_l$: patty containing black garlic and a 6.25 mg/mL ascorbic acid solution: BG+Asc$_m$: patty containing black garlic and a 12.5 mg/mL ascorbic acid solution; BG+Asc$_h$: patty containing black garlic and a 25 mg/mL ascorbic acid solution.
* g equivalents, i.e. grams of black garlic to prepare the amount of garlic extract needed to produce 1 kg of patty mixes.

Two patties, 80 g, 5-6 cm diameter and 1.3-1.6 cm thick patties covered with cellophane plastic were prepared for each treatment and set. One of the patties was used for colour determination, which was determined on the day of the patty forming (day 1) and after 6 days of refrigerated storage (day 7). The other patty was vacuum packaged, cooked for 40 min at 70 °C, removed from the packaging and used for TBARS determination just after cooking (day 1) and after six days of storage (day 7).

Raw and cooked samples were stored in plastic trays covered with polyethylene cling film at 4 °C under darkness.

2.3 Polyphenol content and antioxidant activity of black garlic extract

The total polyphenol content of the garlic extracts was performed, in duplicate, by the Folin-Ciocalteu test following the indications described by Musci and Yao (2017). 0.25 mL of a 1/50 (v/v) aqueous dilution of garlic extract was added to 1 mL of 0.2 M Folin-Ciocalteu reagent, the mixture was shaken and kept for 5 min at room temperature (20 °C) in the dark. Then 1 mL of a 10 % (w/v) Na_2CO_3 solution was added, stirred and kept in the dark for 60 min. Finally, the absorbance of the sample was determined at 765 nm in a Miltonroy spectrophotometer (Spectronic 40, Ivyland, USA). Quantification was performed using gallic acid standard solutions. The results were expressed as mg gallic acid equivalents/L of black garlic extract.

The DPPH test was carried out in duplicate, according to Habinshuti *et al.* (2019). A 1/800 dilution of black garlic extracts (v/v) was prepared, and 1 mL of each dilution was mixed with 2.0 mL of a solution of DPPH (0.1 M) in ethanol. The mixture was vortexed and kept in the dark for 20 min at room temperature (22 °C). The absorbance was read at 517 nm using a Miltonroy spectrophotometer (Spectronic 40, Ivyland, USA). The absorbance of control with 1 mL of distilled water and 2 mL of DPPH solution was also determined. The colour reduction in percentage was calculated as a measure of reducing activity (%) = [blank A517 - sample A517) / blank A517]×100. Sample A517 represents the absorbance of the sample solution and blank A517 represents the absorbance of 1.0 mL of water mixed with the DPPH solution. Furthermore, the antiradical capacity of ascorbic acid and BHT was determined from 5 and 10 mg/L ethanol dilutions, respectively. One mL of each solution was mixed with 2 mL of the DPPH solution, and the colour reduction was compared against the blank. Finally, the antioxidant capacity of the extracts was compared with that of the ascorbic acid and BHT solutions, obtaining the equivalent reducing capacity of the garlic extract (1 mL of extract) to 1 mg of ascorbic acid or 1 mg of BHT.

2.4 Patty quality measurements

Colour determination was carried out in duplicate with a CM-700d colorimeter (Konica-Minolta, Oxaca, Japan) in specular component included (SCI) mode, illuminant D65, measuring aperture 11 mm and observer angle 10°. Colour was expressed in the coordinates of lightness, L*; red index, a*; and yellow index, b* (AMSA, 2012).

The TBARS test was carried out in duplicate following Nam and Ahn (2003). Half of each cooked patty was minced with a domestic mincer, 2 g were taken, to which 20 mL of water was added, homogenized for 1 min at 9,500 rpm with an IKA Ultra-turrax (Staufen, Germany) and filtered with a strainer into a beaker. Then 1 mL of homogenate was placed into a tube, 50 μL of a solution of BHT in 7.2 % (w/v) ethanol was added, and the mixture was shaken, and 2 mL of 20 mM thiobarbituric acid in a 15 % (w/v) trichloroacetic acid solution was added and shaken again. The tubes were then placed in a water bath for 20 minutes at 80 °C. After this time, the tubes were cooled in a water bath and then centrifuged for 20 minutes at 3000 rpm and 5 °C using a Beckman centrifuge model J2-21 (Palo Alto, U.S.A). Finally, the absorbance of the supernatant was measured at 531 nm in a spectrophotometer. In addition, a series of standards were prepared to make a calibration curve from a stock solution of tetraethoxypropane (TEP) 3 x 10^{-5} M, which were analyzed following the steps described above, omitting filtration and centrifugation.

2.5 Statistical analysis

The mean values obtained from the analytical replicates of patty characteristics (colour and TBARS) were analyzed by univariate analysis of variance (general linear model; software SPSS v.26; IBM, Somers, NY, USA). Treatment and time were used as fixed factors, and garlic type as a random factor. When the analysis was significant ($P<0.05$), a pairwise analysis was carried out with the posthoc Tukey test to assess differences between treatments within the same day or days within a treatment ($P<0.05$).

3. Results and discussion

3.1 Polyphenol content and antioxidant activity of black garlic extract

The antioxidant potential of natural antioxidants depends on the polyphenol content. However, total polyphenol content has limitations in predicting antioxidant capacity, as the antioxidant activity of polyphenolic compounds will depend on their chemical structure (Sang *et al.,* 2002). Table 2 shows the polyphenol content of the black garlic used in the experiment. In addition, several studies have shown how the ageing process to which fresh garlic is subjected to obtain black garlic has an impact on the polyphenol content, leading to an increase in polyphenols (Kimura *et al.,* 2017, Choi *et al.,* 2014). The amounts obtained in this study are lower than those of Toledano-Medina *et al.* (2016), who found 2-4 times higher amounts. The variability between studies may be due not only to the variety or maturity of garlic (Škrovánková *et al.,* 2018), but also to the extraction method.

Tabla 2. Contenido en polifenoles solubles y capacidad antioxidante de un extracto acuoso de ajo negro (BG) comparado con el del ácido ascórbico (Asc) y el hidroxitolueno butilado (BHT).
Table 2. Soluble polyphenol content and antioxidant capacity of a black garlic aqueous extract (BG) compared to that of ascorbic acid (Asc) and butylated hydroxytoluene (BHT)

	Mean ± standard deviation (n = 2)
Polyphenol content[$]	408 ± 116
Antioxidant capacity of BG extract compared to Asc[&]	0.480 ± 0.100
Antioxidant capacity of BG extract compared to BHT[&]	0.169 ± 0.036

[$] expressed as mg gallic acid equivalents/L black garlic extract (prepared from 25 g of garlic and 75 ml of water)
[&] expressed as mL of garlic extract with an antioxidant capacity equivalent to 1 mg of ascorbic acid as determined with the 2,2-diphenyl-1-picrylhydrazyl method.

Table 2 also shows the antioxidant (antiradical) capacity of garlic extracts relative to the antioxidant capacity shown by ascorbic acid and BHT, two antioxidants used in the food industry. Overall, the results show that 0.48 and 0.17 mL of garlic extract showed an anti-radical effect equivalent to 1 mg of ascorbic acid and BHT, respectively. The antioxidant effect of black garlic has been mainly attributed to polyphenols and the compound S-allyl-cysteine, derived from alliin (Toledano-Medina *et al.*, 2016). These authors compared the antiradical activity of fresh and black garlic using the DPPH method and found that the activity of black garlic was about 10 times higher than that of fresh garlic.

3.2 Patty quality measurements (colour and lipid oxidation)

The CIELAB colour parameters of the patties with different formulations are shown in Table 3. Treatment significantly affected the brightness of the patties, but time showed no significant effect. Considering the mean L* values of both days, the use of black garlic decreased the brightness compared to the control patties, with significant differences being observed when the addition of garlic was accompanied by medium or high values of ascorbic acid. This tendency can be attributed to the black hue of black garlic.

The redness index (a*) of the patties was significantly affected by treatment, storage and the interaction between the above factors was also significant. On day 1 of storage, the addition of garlic together with medium and high amounts of ascorbic generated a more intense red colour than the control patties. It appears that black garlic has some synergy with ascorbic acid in the reddening of the patties, which could be considered advantageous from a colour acceptance point of view. In potential agreement, in a previous study, Augustynska-Prejsnar *et al.* (2024) found that the addition of black garlic in amounts of 2 % in meat products made with minced meat (higher than the amount used in this study) improved their sensory acceptability. However, sensory acceptability in terms of colour, flavour and overall acceptability dropped with amounts higher than 4 %.

Overall, with storage time, the value of a* in the patties tended to increase. This increase might be attributed to water increased oxymyoglobin concentration due to water evaporation from the patty surface during storage or increased oxygen penetration (Callejas-Cárdenas *et al*., 2014). This increase in a* indicates that the eventual oxidation of the meat pigment due to oxidation reactions leading to discolouration was more than compensated by water evaporation or oxygen diffusion. However, the reddening was not as marked in either patty, being more marked and significant in the CON patties, those to which black garlic was added and those to which black garlic with high ascorbic acid was added. After seven days of storage, however, no significant differences were observed between the control and any of the other treatments, but it was observed that the addition of ascorbic acid to the patties with black garlic made the patties redder than the patty with black garlic and no ascorbic acid.

Concerning the yellowness index (b*), the addition of black garlic produced an increase compared to the CON patty and with ascorbic acid, which was significant when the addition of black garlic was accompanied by ascorbic acid and more evident after 7 days of refrigerated storage. The storage time had no influence on this colour coordinate.

Tabla 3. Efecto del tratamiento y del tiempo de almacenamiento refrigerado en el color de las hamburguesas crudas (medias y desviaciones estándar).
Table 3. Effect of treatment and refrigerated storage time on the colour of raw patties (means and standard deviations)

	Storage			**P-level**		
	Day 1 (n = 4)	**Day 7 (n = 4)**	**Mean value**	**Treatment**	**Day**	**Treatment x day**
L*						
CON	45.2±0.6	45.4±0.1	45.28±2.50^{a}			
Asc_h	45.6±0.9	45.7±0.4	45.68±2.44^{a}			
BG	41.7±3.5	43.6±5.4	42.65±2.28^{ab}	0.022	0.246	0.230
BG+Asc_l	41.6±2.0	42.8±4.3	42.21±3.30^{ab}			
BG+Asc_m	40.8±1.0	41.4±2.0	41.14±2.28^{b}			
BG+Asc_h	40.9±0.4	41.2±0.2	41.04±2.21^{b}			
a*						
CON	5.86±0.03^{b,y}	9.20±0.06^{ab,x}	7.71±1.57			
Asc_h	6.43±1.01^{ab,y}	9.00±0.65^{ab,x}	7.53±1.83			
BG	7.10±0.81^{ab}	7.32±1.70^{b}	7.21±1.09	<0.001	0.046	<0.001
BG+Asc_l	7.28±0.13^{ab}	9.69±1.40^{a}	8.27±1.32			
BG+Asc_m	7.62±1.19^{a}	8.92±1.33^{ab}	8. 49±1.53			
BG+Asc_h	7.46±0.54^{a,y}	10.18±0.40^{a,x}	8.82±1.57			
b*						
CON	14.97±0.42	15.38±0.70^{b}	15.17±0.95^{b}			
Asc_h	15.28±2.07	15.64±0.19^{b}	15.43±1.43^{b}			
BG	16.88±0.94	16.40±0.78^{ab}	16.64±1.13^{ab}	<0.001	0.166	0.0.30
BG+Asc_l	17.80±2.51	18.22±0.37^{a}	18.02±1.60^{a}			
BG+Asc_m	17.57±0.58	18.40±0.35^{a}	17.98±1.00^{a}			
BG+Asc_h	17.12±1.06	18.46±1.11^{a}	17.79±1.44^{a}			

CON: control treatament; Asc_h: patty to which a 25 mg/mL ascorbic acid solution was added; BG: patty containing black garlic; BG+Asc_l: patty containing black garlic and a 6.25 mg/mL ascorbic acid solution: BG+Asc_m: patty containing black garlic and a 12.5 mg/mL ascorbic acid solution; BG+Asc_h: patty containing black garlic and a 25 mg/mL ascorbic acid solution.
^{ab} Mean values followed by different superscript letters in the same columns are statistically significant ($P < 0.05$). ^{xy} Mean values followed by different superscript numbers in the same row are statistically significant ($P \leq 0.05$).

The values of TBARS, an indicator of lipid oxidation, in the patties cooked at the beginning and end of aerobic refrigerated storage, are shown in Table 4. On day 1 of storage, TBARS values were generally lower than 0.5 mg of malondialdehyde/kg of patty in all treatments and significant differences were observed. The patty with ascorbic acid showed the lowest oxidation rate, with significant differences compared to the patty with black garlic and high levels of ascorbic acid. Ascorbic acid, as a reducing agent, can act as an electron donor in free radical-mediated oxidative processes by stabilizing, to some extent, the lipid oxidation of meat (Min *et al.*, 2008) during the patty-making and cooking processes. On the other hand, black garlic may contain coloured substances such as Maillard compounds or phenolics, which could interfere in TBARS analysis, increasing the absorbance and, thus, the concentration (Pérez and Estévez, 2020). Maybe that is why the average TBARS values of black garlic samples on day 1 of storage are, on average, somewhat higher than patties without black garlic.

Refrigerated aerobic storage of the cooked patties clearly caused lipid oxidation in all treatments, a characteristic phenomenon of cooked meat during aerobic refrigerated storage (Min *et al.*, 2008). However, results showed that the intensity of oxidation depended on the treatment. The prevention of oxidation by ascorbic acid and black garlic was also observed after seven days of storage. The control patties were the most oxidized; in the second position, the patties with ascorbic acid or black garlic as the only antioxidants and in the third position were the patties with both antioxidants, black garlic and ascorbic acid.

The antioxidant activity of ascorbic acid in the patties after storage is attributable to electron donation or free radical inhibition (Min *et al.*, 2008). This activity may be limited because it decreases over time due to ascorbic acid degradation or exposure to adverse environmental conditions such as light and oxygen (Bellucci *et al.*, 2022). The antioxidant effect observed in the patties with black garlic could be exerted by a range of antioxidant substances, including polyphenols or Maillard reaction products, which may act by anti-radical mechanism and by mechanisms other than hydrogen donation (Choi *et al*., 2014; Kimura *et al.*, 2017). Interestingly, the results show a clear synergistic effect of ascorbic acid and black garlic independent of the amount of ascorbic acid used. The patties with black garlic and ascorbic acid, unlike the rest and regardless of the level of ascorbic acid, showed TBARS values below 2 mg/kg on day 7, which, according to Domínguez *et al.* (2017) can be considered a TBARS threshold indicative of rancid flavour in meat.

Tabla 4. Efecto del tratamiento y del tiempo de almacenamiento refrigerado sobre la estabilidad oxidativa lipídica de las hamburguesas cocidas determinada por las sustancias reactivas del ácido tiobarbitúrico expresadas en mg de malondialdehído/kg de hamburguesa (medias y desviaciones estándar).
Table 4. Effect of treatment and refrigerated storage time on the lipid oxidative stability of cooked patties determined by the thiobarbituric acid reactive substances expressed as mg of malondialdehyde/kg patty (means and standard deviations)

	Storage			**P-level**		
	Day 1 (n = 4)	**Day 7 (n = 4)**	**Mean value**	**Treatment**	**Day**	**Treatment x day**
CON	$0.30±0.07^{ab,x}$	$3.97±0.69^{c,x}$	2.13±1.95	0.002	0.023	0.005
Asc_h	$0.23±0.07^{b,x}$	$2.45±0.20^{b,x}$	1.34±1.16			
BG	$0.42±0.07^{a,x}$	$2.78±0.97^{b,x}$	1.60±1.42			
$BG+Asc_l$	$0.39±0.04^{ab,x}$	$1.72±0.38^{a,x}$	1.06±1.42			
$BG+Asc_m$	$0.38±0.10^{ab,x}$	$1.77±0.50^{a,x}$	1.07±0.81			
$BG+Asc_h$	$0.43±0.17^{a,x}$	$1.66±0.50^{a,x}$	1.04±0.74			

CON: control treatment; Asc_h: patty to which a 25 mg/mL ascorbic acid solution was added; BG: patty containing black garlic; $BG+Asc_l$: patty containing black garlic and a 6.25 mg/mL ascorbic acid solution: $BG+Asc_m$: patty containing black garlic and a 12.5 mg/mL ascorbic acid solution; $BG+Asc_h$: patty containing black garlic and a 25 mg/mL ascorbic acid solution.

^{abc} Values followed by different superscripts in the same columns are statistically significant (P < 0.05).

^{xy} Values followed by different superscripts in the same row are statistically significant (P ≤ 0.05).

The synergy of ascorbic acid with other antioxidants, such as tocopherols, in the prevention of oxidation of minced meat has been previously demonstrated (Bruun-Jensen *et al.,* 1996), but no previous studies on the synergistic effect of black garlic and ascorbic acid in meat products have been found. According to Day *et al*. (2008), the ascorbic acid could have increased the stability of black garlic polyphenols by reducing the phenoxyl radicals formed during oxidation, i.e. regenerating polyphenols.

4. Conclusions

The addition of the aqueous black garlic extracts in the conditions used in this experiment influences the colour of the patties, decreasing their lightness and increasing the red and yellow indices. The combination of ascorbic acid with black garlic extract increases the redness and yellowness after seven days of storage. The effect of black garlic extract on colour acceptability could be positive, although sensory analysis is needed to confirm the latter. Adding aqueous black garlic extract shows synergy with ascorbic acid in preventing lipid oxidation in cooked patties. The combination of 0.5 % black garlic extract and ascorbic acid amounts of 0.12 g/kg in the patty formulation showed optimal antioxidant activity in this study. Further research could test whether this antioxidant activity is maintained by decreasing the amount of black garlic and the effect of black garlic on the patty sensory properties.

Conflict of interest

There is no conflict of interest for the publication of these research results.

5. References

Augustyńska-Prejsnar, A., Kačániová, M., Ormian, M., Topczewska, J., Sokołowicz, Z., & Hanus, P. (2024). Quality Assessment of Minced Poultry Products Including Black Fermented Garlic. *Foods*, 13(1), 70. https://doi.org/10.3390/foods13010070

AMSA. (2012). http://www.meatscience.org

Barido, F. H., Jang, A., Pak, J. I., Kim, Y. J., & Sung, S. K. (2022). Combined effects of processing method and black garlic extract on quality characteristics, antioxidative, and fatty acid profile of chicken breast. *Poultry Science*, 101(4): 101723. https://doi.org/10.1016/j.psj.2022.101723.

Bellucci, E. R. B., Bis-Souza, C. V., Domínguez, R., Bermúdez, R., & Barretto, A. C. d. S. (2022). Addition of Natural Extracts with Antioxidant Function to Preserve the Quality of Meat Products. *Biomolecules*, 12(10): 1506. https://doi.org/10.3390/biom12101506

Bruun-Jensen, L., Skovgaard, Ib M., Madsen, E. A., Skibsted, L. H., & Bertelsen, G. (1996). The combined effect of tocopherols, L-ascorbyl palmitate and L-ascorbic acid on the development of warmed-over flavour in cooked, minced turkey. *Food Chemistry*, 55(1): 41-47. https://doi.org/10.1016/0308-8146(95)00070-4

Callejas-Cárdenas, A. R., Caro, I., Blanco, C., Villalobos-Delgado, L. H., Prieto, N., Bodas, R., Giráldez, F. J., & Mateo, J. (2014). Effect of vacuum ageing on quality changes of lamb steaks from early fattening lambs during aerobic display. *Meat Science*, 98(4): 646-651. https://doi.org/10.1016/j.meatsci.2014.06.036

Cirkovic Velickovic, T. D., & Stanic-Vucinic, D. J. (2018). The Role of Dietary Phenolic Compounds in Protein Digestion and Processing Technologies to Improve Their Antinutritive Properties. *Comprehensive Reviews in Food Science and Food Safety*, 17(1): 82-103. https://doi.org/10.1111/1541-4337.12320

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, 5(4): 169-186. https://doi.org/10.1111/j.1541-4337.2006.00009.x

Choi, I. S., Cha, H. S., & Lee, Y. S. (2014). Physicochemical and Antioxidant Properties of Black Garlic. *Molecules*, 19(10): 16811-16823. https://doi.org/10.3390/molecules191016811

Dai, F., Chen, W. F., & Zhou, B. (2008). Antioxidant synergism of green tea polyphenols with α-tocopherol and l-ascorbic acid in SDS micelles. *Biochimie*, 90(10): 1499-1505. https://doi.org/10.1016/j.biochi.2008.05.007

Dewi, N. N. A., & Mustika, I. W. (2018). Nutrition content and antioxidant activity of black garlic. *International Journal of Health Sciences*, 2(1): 11-20. http://dx.doi.org/10.29332/ijhs.v2n1.86

Domínguez, R., Pateiro, M., Agregán, R., & Lorenzo, J. M. (2017). Effect of the partial replacement of pork backfat by microencapsulated fish oil or mixed fish and olive oil on the quality of frankfurter type sausage. *Journal of Food Science and Technology*, 54: 26-37. https://doi.org/10.1007/s13197-016-2405-7

Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International*, 64: 171-181. https://doi.org/10.1016/j.foodres.2014.06.022

Habinshuti, I., Chen, X., Yu, J., Mukeshimana, O. Duhoranimana, E., Karangwa, E., Muhoza, B., Zhang, M., Xia, S., & Zhang, X. (2019). Antimicrobial, antioxidant and sensory properties of Maillard reaction products (MRPs) derived from sunflower, soybean and corn meal hydrolysates. *LWT*, 101: 694-702. https://doi.org/10.1016/j.lwt.2018.11.083

Hygreeva, D., Pandey, M. C., & Radhakrishna, K. (2014). Potential applications of plant-based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Science*, 98(1): 47-57. https://doi.org/10.1016/j.meatsci.2014.04.006

Karre, L., Lopez, K., & Getty, K. J. (2013). Natural antioxidants in meat and poultry products. *Meat Science*, 94(2) : 220-227. https://doi.org/10.1016/j.meatsci.2013.01.007

Kimura, S., Tung, Y. C., Pan, M. H., Su, N. W., Lai, Y. J., & Cheng, K. C. (2017). Black garlic: A critical review of its production, bioactivity, and application. *Journal of Food and Drug Analysis*, 25(1): 62-70. https://doi.org/10.1016/j.jfda.2016.11.003

Khlebnikov, A. I., Schepetkin, I. A., Domina, N. G., Kirpotina, L. N., & Quinn, M. T. (2007). Improved quantitative structure-activity relationship models to predict antioxidant activity of flavonoids in chemical, enzymatic, and cellular systems. *Bioorganic & Medicinal Chemistry*, 15(4): 1749-1770. https://doi.org/10.1016/j.bmc.2006.11.037

Lee, H. J., Yoon, D. K., Lee, N. Y., & Lee, C. H. (2019). Effect of aged and fermented garlic extracts as natural antioxidants on lipid oxidation in pork patties. *Food Science of Animal Resources*, 39(4): 610-622. https://pmc.ncbi.nlm.nih.gov/articles/PMC6728816/

Min, B. R., Nam, K. C., Cordray, J. C., & Anh, D. U. (2008). Factors affecting oxidative stability of pork, beef, and chicken meat. *Iowa State University Animal Industry Report*, 5(1). https://doi.org/10.31274/ans_air-180814-1046

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49(1): S73-S86. https://doi.org/10.1016/S0309-1740(98)90039-0

Musci, M., & Yao, S. (2017). Optimization and validation of Folin–Ciocalteu method for the determination of total polyphenol content of Pu-erh tea. *International Journal of Food Science and Nutrition*, 68(8): 913–918. https://doi.org/10.1080/09637486.2017.1311844

Nam, K. C., & Ahn, D. U. (2003). Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. *Journal of Food Science*, 68(5): 1686-1690. https://doi.org/10.1111/j.1365-2621.2003.tb12314.x

Pérez-Palacios, T., & Estévez, M. (2020). Analysis of lipids and lipid oxidation products. In Biswas, A. K., & Mandal, P. K. (Eds.) Meat Quality Analysis: Advanced Evaluation Methods, Techniques, and Technologies. (pp. 217-239). Academic Press. https://doi.org/10.1016/B978-0-12-819233-7.00013-6

Santos, L. C. R., Santos, E. N. F., Oliveira, C. C., Nascentes, G. A. N., Saldaña, E., Bastos, L. M., Martins, M. M., Campagnol, P.C.B., Cunha, L. C. S., & Jardim, F. B. B. (2024). Black garlic extract: Phytochemical characterisation and application as natural antioxidant in burgers. *International Food Research Journal*, 31(3). https://doi.org/10.47836/ifrj.31.3.14

Sampels, S. (2013). Oxidation and antioxidants in fish and meat from farm to fork. In Muzzalupo, I (Ed). Food Industry. Intech Open. https://dx.doi.org/10.5772/53169

Sang, S., Cheng, X., Stark, R. E., Rosen, R. T., Yang, C. S., & Ho, C. T. (2002). Chemical studies on antioxidant mechanism of tea catechins: analysis of radical reaction products of catechin and epicatechin with 2,2-Diphenyl-1-picrylhydrazyl. *Bioorganic & Medicinal Chemistry*, 10(7): 2233-2237. https://doi.org/10.1016/S0968-0896(02)00089-5

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, 18(Part B): 820-897. https://doi.org/10.1016/j.jff.2015.06.018

Škrovánková, S., Mlček, J., Snopek, L., & Planetová, T. (2018). Polyphenols and antioxidant capacity in different types of garlic. *Potravinarstvo Slovak Journal of Food Sciences*, 12(1): 267–272. https://doi.org/10.5219/895

Toledano-Medina, M. A., Pérez-Aparicio, J., Moreno-Rojas, R., & Merinas-Amo, T. (2016). Evolution of some physicochemical and antioxidant properties of black garlic whole bulbs and peeled cloves. *Food Chemistry*, 199: 135-139. https://doi.org/10.1016/j.foodchem.2015.11.128

Yuan, H., Sun, L., Chen, M., & Wang, J. (2016). The comparison of the contents of sugar, amadori, and heyns compounds in fresh and black garlic. *Journal of Food Science*, 81(7): C1662-C1668. https://doi.org/10.1111/1750-3841.13365