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Effect of Added Grape Seed and Skin on Chicken Thigh Patties during Chilled Storage

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Abstract

The effect of 2% grape seed and 2% grape skin powder added to chicken thigh patties stored at 4°C was assessed by measuring lipid oxidation, total phenolic content, pH, color changes and sensory attributes. The addition of these grape by-products to the patties lowered pH values and significantly reduced in lightness, redness and yellowness compared with the control. However, the addition of grape seed and skin significantly improved the oxidative stability of raw chicken patties due to higher total phenolic content, grape seed being more effective than skin in this regard. The phenolic content of these samples remained stable even after cooking. The acceptability of the chicken meat in general was not affected by the addition of grape by-products. These results show that grape seed and grape skin could potentially be used as natural antioxidants in raw chicken patties and would be accepted by consumers.

Keywords: Grape seed; Grape skin; Total extractable polyphenol; Chicken patties; Lipid oxidation

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Introduction

By-products from the winemaking industry are widely available in Mediterranean countries. In many cases, these products cause an important environmental problem in terms of storage, processing or disposal (Brenes *et al*., 2016). However, grape pomace (seeds, skin, and stems) contains a wide range of polyphenols and is one of the major sources of phenolic compounds (Yildrim *et al*., 2005; Brenes *et al*., 2016). Many positive effects on human health have been described for these compounds including anti-inflammatory, anti-carcinogenic, cardio protective and vasodilatory properties (Georgiev *et al*., 2014). A positive effect on animal health has also been described by Brenes *et al*. (2016). The antioxidant and antimicrobial properties of grape polyphenols have been widely reported (Etxeberria *et al*., 2013; Georgiev *et al*., 2014). Polyphenols often act as free radical scavengers and stop radical chain reactions that occur during the oxidation of triglycerides. Today the meat industry is interested in replacing traditionally used synthetic antioxidants (BHT, BHA, TBHQ, etc.) which are potentially toxic and harmful to health with natural products or ingredients of plant origin containing bioactive compounds with potential health benefits (Ayo *et al*., 2007; Rojas & Brewer, 2008). This is particularly important for the meat industry which is anxious to improve its image which has been tarnished over the last decade (Jiménez-Colmenero *et al*., 2010).

Lipid oxidation and color are important factors for consumer acceptance of meat and meat products. Several studies have found a reduction in oxidation in different meats (pork, beef, lamb, chicken and goat) after the addition of different natural compounds such as those found in oregano, sage, rosemary, thyme, marjoram, caraway, basil, ginger, kinbow, pomegranate, cereal, walnut and seaweed (Botsoglou *et al*., 2003; Naveena *et al*., 2008; Ayo *et al*., 2007; López-López *et al*., 2009;Devatkal *et*

al., 2010). Grape by-products, mainly grape pomace, has proven effective in delaying lipid oxidation by reducing the accumulation of primary (e.g. lipid hydroperoxide) and secondary products of lipid oxidation [e.g., Thiobarbituric Acid Reactive Substances (TBARS)] in turkey, chicken, beef, pork, liver pate and dry cured sausage (Lau & King, 2003; Rojas & Brewer, 2008; Brannan, 2009; Lorenzo *et al*., 2014; Pateiro *et al*., 2014). Sáyago-Ayerdi *et al*. (2009) also demonstrated that the addition of grape antioxidant dietary fiber significantly improved the oxidative stability and radical scavenging activity in raw and cooked chicken burgers. Skin and seed are a rich source of flavonoids including monomeric phenolic compounds such as (+)- catechins, (-)- epicatechin, and (-)- epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins. Grape seed has exhibited higher antioxidant capacity compared with grape skin, mostly due to higher levels of oligomeric and polymeric procyanidins (Shahidi & Wanasundara, 1992). However, the effect of a certain potential antioxidants could vary considerably depending on a complex interaction between various factors involving the type and concentration of active compound(s) and the nature of the food system (Schwarz *et al*., 2001). There is little information on the individual effect of the principal components of grape pomace (skin and seed) on meat products. Hence, the aim of this paper is to research the effect of grape skin and grape seed on the physico-chemical and sensorial properties of chicken thigh patties.

Materials and Methods

Grape Seed (GS) and Grape Skin (SS) (Vitis vinifera variety: Cencibel, La Mancha, Spain) were provided from Explotaciones Hermanos Delgado winery (Socuéllamos, Ciudad Real, Spain). GS and SS were collected from a tank during the vinification process after 15 days of alcoholic fermentation, dried (indirect air with Tª less than 80ºC), mechanically separated and grounded at 0.5 mm. Seeds were subjected to cool press oil extraction process before grounding. The other ingredients (eggs, breadcrumbs and salt) were obtained from a local market in Madrid (Spain). Fresh chicken thighs (± 4 kg) were likewise obtained from a local market in Madrid (Spain) and stored in the refrigerator for approximately 12h. The proximate composition of the chicken thighs was: moisture 73.95%, protein 18.43%, fat 6.63% and ash 0.97%). Thigh meat was chosen because it is darker than breast meat and to compensate for the change in color that was expected from the addition of these by-products. The boneless chicken thighs were cut and then ground (4 mm plate) using a grinder (Mainca, Granollers, Spain). The ground meat was then used to prepare the patties.

Preparation of patties

Three formulations were used to prepare patties consisting of 85.4% meat, 6.8% whole egg, 1% salt and 6.8% breadcrumbs in the control sample (PC), 4.8% breadcrumbs and 2% grape seed in the PGS sample and 4.8% breadcrumbs and 2% grape skin in the PSS sample following the formulation of Sáyago-Ayerdi *et al*. (2009). The ground meat was blended in a bowl mixer (Hobart, Model N50, USA) for 60 s and then the salt was added and mixed a further 30 s. The eggs and breadcrumbs were then put into the mixer and mixed for 60s. A total of 25 patties were made from the mixture (~50g per patty) for every treatment using a conventional burger maker (Mini steak burger maker, O.L. Smith Co. Ltd., Italy). The patties were placed in high oxygen barrier vacuum bags (nylon/polyethylene, 9.3 ml $O_2/m^2/24$ h at 0ºC, Koch Kansas City, MO). Each bag contained 2 patties which were refrigerated at 4º C. The patties were analyzed on day 0 and after 3, 6 and 9 days of storage.

Proximate analysis

Moisture and ash were analyzed in meat and patties in quadruplicate according to AOAC methods (2005). Fat content was evaluated in triplicate according to the Bligh and Dyer (1959) method. Protein content was measured in quadruplicate using a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI, USA). Protein content was analyzed in GS and SS. The crude fiber of these ingredients was also analyzed according to AOAC methods (2005).

pH

pH was determined by means ofa pH meter (827 pH Lab Methrom, Herisau, Switzerland) on 10 g of sample homogeneously mixed with 100 ml of distilled water. pH was measured for each sample.

Color measurement

Color [CIE-LAB tristimulus values, lightness (L*), redness (a*) and yellowness (b*)] was measured on the surface of raw patties using a CR-400 Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan). Ten measurements were taken per sample.

Determination of total extractable polyphenols compounds

Total Extractable Polyphenols (TEP) was extracted from GS and SS, raw and cooked patties (prepared in an electric pan 1.5 min per side at 210 ± 4ºC) by means of double aqueous-organic extraction according to the method of Sáyago-Ayerdi *et al*. (2009). Extractable polyphenols were determined by the Folin–Ciocalteu procedure using gallic acid (Sigma-Aldrich, St. Louis, MO) as standard. Results were expressed as gallic acid equivalents (mg GAE/kg of sample).

Lipid oxidation evaluation

Lipid oxidation was determined by measuring the Thiobarbituric Acid Reactive Substances (TBARS) described by López-López *et al*. (2009). A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to Measure Malonaldehyde (MDA). TBARS determinations were performed in triplicate. Values were expressed as mg of malondi aldehyde/kg of sample.

Sensory evaluation

Patties were assessed by a 15-member panel of individuals who regularly consume these types of products. The panel was selected after preliminary training (two sessions) on the products and terminology. Samples were cooked in an electric pan 1.5 min per side at 210 ± 4ºC. Patties were cut into pieces of uniform size (2 x 2 cm) and were then immediately presented to the judges who were instructed to evaluate color, flavor, hardness, juiciness and general acceptability. All parameters were measured on unstructured scales (0 = dislike very much, 10 = like very much) except general acceptability which was

measured on a hedonic scale rating test. Sensory analysis was performed 2 days after preparation of the patties.

Statistical Analysis

Data was analyzed using SPSS V.13.0 software (SPSS Institute Inc., Cary, NC). The experimental design was done in duplicate. Differences between the two replicates were not significant ($P < 0.05$). Results were expressed by mean values and standard error of the mean of three or more separate determinations. One-way and two-way Variance Analysis (ANOVA) was used to determine the effect of formulation and storage time on the physico-chemical and sensorial properties of chicken thigh patties. Comparison of means was performed using the Tukey HSD test and differences were declared at $P < 0.05$.

Results and Discussion

Proximate analysis

The proximate composition of SS and GS is shown in Table 1. Protein content was similar for both ingredients. The main difference was observed in the polyphenolic content. Grape seed showed higher TEP and crude fiber content (approximately 4 and 2 times respectively) than grape skin. Similar results were reported by Ky *et al*. (2014).

Table 1: Proximate composition (%) and total extractable polyphenols expressed as (g of Gallic Acid Equivalents (GAE)/100 g of Grape Seed (GS) and Grape Skin (SS).

Parameters	GS	SS
Protein	19.64 ± 0.41	16.27 ± 0.01
Fiber	25.28 ± 1.87	14.40 ± 1.33
Total extractable polyphenols	8.23 ± 0.16	2.35 ± 0.14

Note: Means ± SD. GS: grape seed and SS: grape skin.

Table 2 shows the proximate composition of the chicken patties. No significant differences were found in moisture, fat or protein. The addition of grape seed and skin resulted in higher ($P < 0.05$) ash content compared to the control sample due to the presence of grape by-product powder in the treatments. Similar results were found in ground chicken thigh meat treated with grape seed extract (Brannan, 2008).

Table 2: Proximate analysis (%) of different chicken thigh patties.

Parameters	PC	PGS	PSS
Moisture	68.80 ± 0.09^a	69.99 ± 0.27^a	69.08 ± 0.02^a
Protein	18.30 ± 0.33^a	18.72 ± 0.06^a	18.44 ± 0.02^a
Fat	6.45 ± 0.45^a	6.16 ± 0.34^a	6.25 ± 0.18^a
Ash	1.90 ± 0.04^b	2.04 ± 0.01^a	2.03 ± 0.08^a

Note: Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape Skin)
Means ± SD. Different letters in the same row (a, b) indicate significant differences ($P < 0.05$).

pH

Table 3 shows the pH of the different chicken thigh patties during chilled storage. Initial pH levels were equal to or higher than 6.20 in all samples, the lowest levels being observed in the PSS. These results are similar to those obtained for chicken patties by other authors (Calliari *et al*., 2015) and in cooked chicken patties elaborated with extract of kin now rind, pomegranate rind and pomegranate seed powder (Devatkal *et al*., 2010).

During storage time, a significant decrease in the pH was observed, with lower levels in samples containing GS (PGS) and SS (PSS). pH decreased significantly in all samples during storage time. The lowest pH levels were detected in PGS and PSS samples. Other authors (Lorenzo *et al*., 2014) also found that the addition of grape seed extract leads to lower pH values in pork patties. This lower pH is associated with microbial growth, mainly lactic acid bacteria (Triki *et al*., 2013), and the polyphenol content of these grape by-products.

Total extractable polyphenolic compounds

Initial levels of total extractable polyphenolic compounds were highest ($P < 0.05$) in the Patties with Grape Seed (PGS) and lowest in the control sample (Table 3). These results coincide with the polyphenol content of GS and SS, which was highest in the GS (Table 2). Similar results were obtained by other authors (Thorn gate& Singleton, 1994; Zhao *et al*., 1999; Yilmaz *et al*., 2015). Thorngate & Singleton (1994) estimated approximately 60 – 70% of grape polyphenols in grape seeds and about 5% in the skin. However, the phenolic composition of grapes and different grape parts depends on multiple factors, including climate, degree of ripeness, grape size, grapevine variety and viticulture practices (Rodríguez-Montealegre *et al*., 2006).

Polyphenol content observed in the control patties and in those formulated with grape by-products was similar to that found by other authors in patties enriched with fruit extracts (Devatkal *et al*., 2010) and in the thigh meat of broilers fed wild grapes (Yong *et al*., 2013). However, polyphenol content in the patties of this study was lower than that observed by other authors in low-salt meat pork emulsion containing seaweed (López-López *et al*., 2009) and walnut (Ayo *et al*., 2007).

An increase in the levels of polyphenolic compounds in all the samples was observed during storage, the highest levels found in PGS samples followed by PSS in relation with initial levels. A similar pattern of total phenolic content increase during chilled storage was observed in chicken patties elaborated with extract of kin now rind, pomegranate rind and pomegranate seed powder and in cooked chicken patties treated with pomegranate juice and rind extract (Naveena *et al*., 2008; Devatkal *et al*., 2010).

In order to evaluate the effect of heat treatment on polyphenol levels, only an initial study was conducted on the second day of patty storage to simulate consumer behavior. Polyphenol levels in the cooked patties were higher ($P < 0.05$) in the sample with grape seed PGS (739.1 mg GAE/kg) compared with PSS (527.9 mg GAE/kg) and the control (435.9 mg GAE/kg) samples. This was in relation with the polyphenolic content of raw patties (Table 3). As expected, the polyphenol levels were higher in cooked patties compared to the raw ones due to decreased moisture after cooking. The fact that patties maintain their polyphenol content after cooking suggests that this product could have health benefits for consumers.

Table 3: pH, Total Extractable Polyphenols (TEP) and Thiobarbituric Acid-Reactive Substances (TBARS) values of different chicken thigh patties during chilled storage (4ºC).

Parameters	Sample	Storage (days) at 4 °C			
		0	3	6	9
	PC	6.64 ± 0.01^{a1}	6.39 ± 0.06^{a2}	5.30 ± 0.05^{a3}	4.99 ± 0.03^{a4}
pH	PGS	6.52 ± 0.01^{b1}	6.26 ± 0.01^{a2}	5.12 ± 0.01^{b3}	4.78 ± 0.05^{b4}
	PSS	6.20 ± 0.01^{c1}	6.05 ± 0.01^{b2}	5.14 ± 0.01^{b3}	4.83 ± 0.02^{c4}
	PC	387.9 ± 0.6^{c3}	397.8 ± 0.6^{b3}	424.6 ± 0.5^{c2}	642.6 ± 1.4^{c1}
TEP (mg gallic acid equivalents/kg)	PGS	575.5 ± 0.6^{a3}	597.1 ± 1.3^{a3}	955.5 ± 0.6^{a2}	1148.1 ± 1.0^{a1}
	PSS	400.1 ± 1.0^{c3}	411.2 ± 0.4^{b3}	577.0 ± 1.3^{b2}	841.8 ± 0.2^{b1}
	PC	0.96 ± 0.12^{a1}	0.39 ± 0.03^{a3}	0.67 ± 0.01^{a2}	0.70 ± 0.01^{a2}
TBARS (mg malondialdehyde/kg)	PGS	0.57 ± 0.06^{b1}	0.21 ± 0.04^{b3}	0.39 ± 0.01^{c2}	0.46 ± 0.01^{c2}
	PSS	0.64 ± 0.04^{b1}	0.40 ± 0.03^{a3}	0.49 ± 0.01^{b2}	0.57 ± 0.01^{b2}

Note: Sample denomination: PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape Skin)
Means ± SD. Different letters in the same column (a, b, c) and numbers in the same row (1, 2, 3) indicate significant differences ($P < 0.05$).

Lipid oxidation

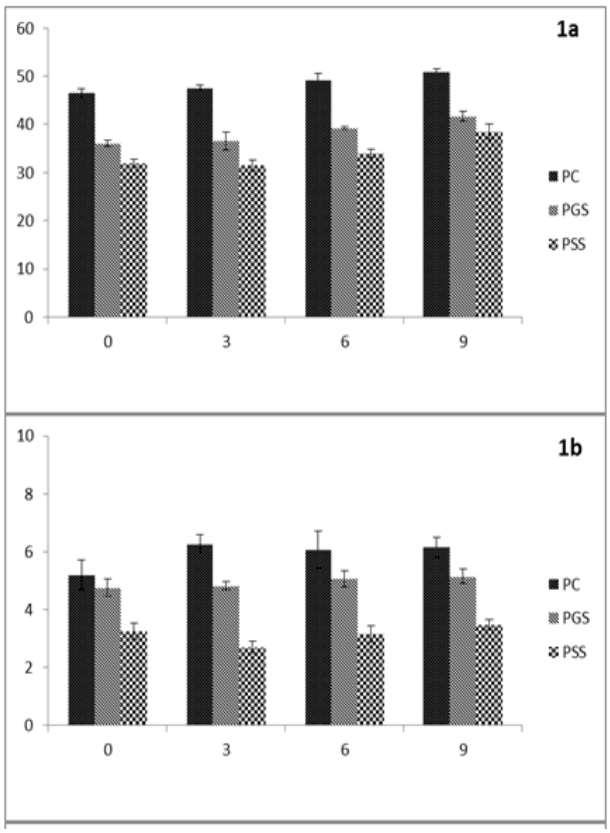
Initial patty TBARS values were very low (0.57-0.96 mg MDA/kg sample) with significantly lower TBARS levels in the patties formulated with GS and SS (Table 3). These low TBARS were similar to the ones found by other authors (Sáyago-Ayerdi *et al*., 2009). TBARS generally decreased during storage in all samples. Decreases in TBARS in meat have been described at different stages of storage (Brewer & Wu, 1993; Bhattacharya *et al*., 1998; Delgado-Pando *et al*., 2011; Salcedo-Sandoval *et al*., 2015), presumably due to intermolecular reactions in the malonaldehyde formed (polymerization) and reactions with other constituents, especially amino acids/proteins (Jamora & Rhee, 2002). This phenomenon is easier to observe due to low oxidation levels in the conservation conditions used (high oxygen barrier vacuum bags). However, in general, the most significant fact of this study is that while low levels of this parameter were found in all samples, the initial differences between the samples with regard to TBARS remained constant during chilled storage with the lowest ($P < 0.05$) values found in the patties treated with the grape by-products, mainly in the PGS followed by PSS. This effect correlates to the polyphenol content of grape seed and grape skin, i.e. lower TBARS values corresponded to the higher polyphenol content of the reformulated patties (Table 3). Yilmaz *et al*. (2015) have also described higher antioxidant activity in seed than in skin. The antioxidant effect of grape polyphenols in ground dark turkey meat, beef, pork, fish and chicken has also been described by many authors (Lau & King, 2003; Brannan, 2008; Brenes *et al*. 2010; Lorenzo *et al*. 2014). Flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and to stop oxidative reactions (Shahidi & Wanasundara, 1992). Grape skin and seed are a rich source of flavonoids including phenolic acids and mono meric compounds such as (+)- catechins, (-)- epicatechin, and (-)- epicatechin-3-O-gallate and oligomeric and polymeric procyanidins. Therefore, the increased effectiveness of GS versus SS in the reduction of lipid oxidation could be due not only to the different concentrations of phenolic compounds present in these by-products, but also to their different phenolic profile. Yilmaz & Toledo (2004) found lower concentrations of gallic acid, monomeric catechin and epicatechin in winery by-product grape skin than in seed due to the possible release of polyphenols contain edin grape skin into the wine during the winemaking process.

In general, chicken thighs are more susceptible to oxidation than breast meat due to the high fatty acid content in the thigh and also the presence of pro-oxidative agents such as Fe-hemo in chicken parts where blood flow is greater. This makes it all the more interesting to include grape by-products in meat products elaborated with these cuts of meat.

Color

Color parameter results (L*, a* and b*) are shown in Figure 1 (a, b and c). The significant changes observed in the color parameters were attributed mainly to the grape by-products added to the patties. The addition of GS and SS in chicken meat caused a significant reduction in L*, and b*, the lowest values found in patties formulated with SS. In this latter case a significant reduction of a* was likewise found but the reduction was not significant between control and PGS patties. A general increase in all the color parameters except b* was observed during the storage period with lower color values for the samples treated with grape by-products compared to control patties (Figure 1). The darkening (mainly a significant reduction in L* and b*) of meat products following the addition of grape extract was also reported in ground chicken thigh and breast meat treated with grape seed extract (Brannan, 2009) and chicken burgers prepared with grape pomace dietary fiber,Sáyago-Ayerdi *et al*. (2009). This was also the case of cooked chicken meat treated with grape seed and peel extracts (Selani *et al*., 2011) and cooked pork sausages formulated with grape pomace powder (Ryu *et al*., 2014). This color reduction in PGS and PSS samples could be due to the dark color of both grape seed and skin. This dark color could be due to polyphenol, mainly anthocyanins, which are responsible for many of the fruit and floral colors, observed in nature.These anthocyanins are water-soluble scarlet, magenta, purple, blue and red pigments. Grapes are among the fruits with the highest levels of phenolic substances with different concentrations of total anthocyanins depending on the variety and the part of the grape (skin, pomace, etc.). In this regard, seeds contain higher levels of polyphenol than skin (Table 1) but have lower concentrations of anthocyanins and phenolic acids than skin as reported by Pinelo *et al*. (2006). However, despite the vividred color of grape skin and seed used in this study, a reduction of a* was observed in the patties containing these

grape by-products (Figure 1b). In consistent results have been reported regarding the behavior of this parameter. An increase in the value of a* has been observed in chicken burgers prepared with grape pomace (Sáyago-Ayerdi *et al*., 2009). However, other authors have observed a significant decrease in CIE a* values in cooked products such as pork sausages reformulated with grape pomace powder (Ryu *et al*., 2014). This reduction was explained by the loss of anthocyanin present in grape skin when the grape juice was removed (Rababah *et al*., 2008). Also, Jin *et al*., (2013) observed a reduction of a* in cooked sausage after adding schisandrachinensis powder which is a rich source of polyphenolic compounds, including anthocyans and flavonols. These authors noted that a reduction in a* could be due to the unstable nature of anthocyanin. On the other hand, other authors (Selani *et al*., 2011) did not observe significant changes in L*, a* or b* color parameters when grape seed and peel extracts were added to chicken or pork meat. These contrasting results could be due to the higher concentration of grape by-products (2%) added in this study compared to other experiments (Rojas & Brewer, 2008) where lower amounts of grape seed extract were added (0.01% - 0.02%). We would further note that the wine making process also affects levels of anthocyanins as does drying, increasing color intensity and the concentration of these phenolic compounds due to the effect of dehydration of grape by-products and diffusion of the colored compounds from the skin to the pulp due to structural alterations of the skin (Mazza, 1995). In our experiment, the reduction of a* could be attributed to different factors such as: the limited presence of these substance in animal tissue, their instability in such a medium or possible interferences of theinteraction components of GS and SS with the other components in the meat products (proteins, myoglobin, water or fat) influencing the color of meat products (Pietrasik & Janz, 2009).



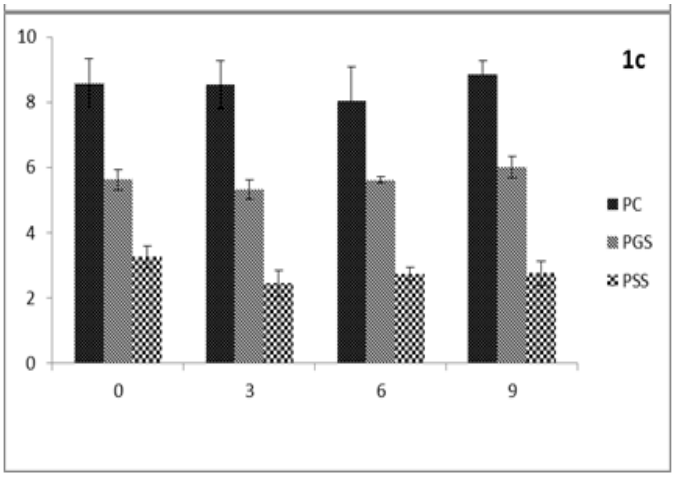


Figure 1: Lightness (L*) (1a), Redness (a*) (1b), Yellowness (b*) (1c) of different chicken patties [PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape Skin)] during refrigerated storage at 4 ºC.

Sensory evaluation

Sensory attributes of different chicken patties are shown in Figure 2. The addition of grape by-products significantly affected the sensorial color and overall acceptability parameters of the patties. No significant differences among treatments were found for other parameters. The color score could be explained by the direct addition of these grape by-products at a concentration of 2% and because of the dark color of these ingredients as already mentioned. In general, the low score ($P < 0.05$) was observed for the Patties Containing Grape Skin (PSS), improbably because of the darker color of these samples. A very similar observed for the control sample and in Patties with Grape Seed (PGS). All the samples received an acceptable by the judges. In other words, the panel did not negatively evaluate the addition of grape seed powder into the patties. These results coincide with those reported by Sáyago-Ayerdi *et al*. (2009) and Brannan (2009).

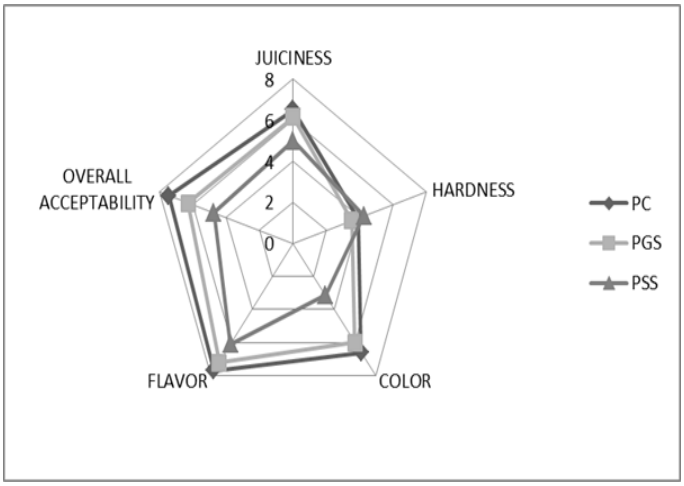


Figure 2: Sensory evaluation of different cooked chicken patties[PC (patties Control); PGS (patties Grape Seed); PSS (patties Grape Skin)].

Conclusion

The use of grape by-products such as skin and seed in the formulation of patties limited the lipid oxidation of these products. Grape seed was considered more effective in retarding

lipid oxidation than grape skin. In general, the patties elaborated with grape seed were evaluated as being more similar to the control sample than those elaborated with grape skin. The marketing of these patties could also have a positive effect on human health due to their polyphenolic content. Therefore, these results are relevant for the meat and the agri-food industries. Wine processing by-products were traditionally considered as waste material. However, this environmental impact can be minimized thanks to this new use as natural antioxidants in meat products. Moreover, the use of these grape by-products in the development of meat products could also have positive effects for consumers thanks to their polyphenol content thus opening a new avenue in the development of healthier foods.

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