EFFECT OF STORAGE CONDITIONS ON MEAT SEASONING PHENOLIC PROFILE AND ANTIOXIDANT

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Abstract

In recent years, consumers interest for food with great nutritional value and potential health effects, known as functional food, has grown. The food industry has adapted to these demands increasing the nutritional value of its products and replacing artificial additives for natural extracts. This natural additives, like white pomaces, have a high polyphenol content and antioxidant activity which, aside from increasing food shelf life, are bioaccesible and have healthy properties. In previous studies, was observed that white wine pomace used as chicken breast seasoning during different storage conditions increased shelf life of meat. The aim of this study was to evaluate if the white-wine pomace used as meat seasoning and stored under different conditions maintains healthy properties. The chicken breast meat was seasoned with 3% of white-wine pomace and 2% of salt. The influence of different conditions of storage, vacuum and modified atmosphere during 7 and 15 days, on seasoning health properties were studied. Phenolic profile and antioxidant capacity of seasoning was evaluated by HPLC-MS and ABTS respectively. Cytotoxicity and effect on biomarkers of lipid and protein oxidation (MDA and carbonyl group) of the bioaccesible fraction of seasonings were evaluate on Caco-2 cells. The storage conditions changed the stored seasoning phenolic profiles, resulting in a decrease in the number of flavonoids compounds compared with control sample. Stored seasonings significantly increased their antioxidant capacity with respect to control. No cytotoxicity of the bioaccesible fractions at different concentrations obtained by gastrointestinal digestion of the seasonings were observed. The MDA and carbonyl groups values of Caco-2 cells treated with bioaccesibles fractions of the stored samples of seasoning did not change with respect to control. These results indicate that the stored condition applied to seasoning did not showed toxicity and did not increased the cell damage evaluated as lipid and protein oxidation.

Key words: White grape pomace, seasoning, modified atmosphere, vacuum, antioxidant activity, phenolic profile

1. INTRODUCTION

The relationship between dietary habits and health problems is a growing concern between consumers, who are demanding food with a high nutritional value and potential health effects. To adapt to this demands for functional foods the industry is changing to add nutritional value, fortify dietary fiber content, replace artificial additives… Artificial additives main role is to stop lipid and protein oxidation during storage, but have been reported to show potential damaging side effects. For this reason, the industry is replacing them with natural additives [1], [2].

Natural additives are plant extracts with a high content on antioxidants and dietary fiber that have a technological function, increasing the shelf time of food, and potential health effects due to their composition. Several studies have tested their efficacy and compared them to artificial additives with positive results, and some of them are already in the market [3]–[5].

Wine pomace is a residue from the wine industry, up to 25% of the grape crushed mass used, that historically has been used for distillation, composting, gasification and combustion [6]. Grape pomace is rich in polyphenols and dietary fibers, which increases its nutritional value. Because of their composition, wine pomace presents antioxidant and anti-inflammatory effects, antimicrobial activities and modulates various signal pathways, endothelial dysfunction… [7]–[11].

Furthermore, the grape pomace is being studied to use as seasoning to improve microbial stability, product quality and maintain sensory properties to replace artificial additives [12]–[14]. This is an economically affordable and environmentally friendly potential new use for this natural product and a way to incorporate this potential functional food in the diet.

The method of storage can modify the additive potential of the pomace. Time, temperature and storage atmosphere are able to modify the antioxidant properties of the seasoning and the properties of the food it is stored with [15]–[17]. Studies have shown changes caused by vacuum packaging (VP) and modified atmosphere packaging (MAP) in the polyphenol content of food stored with plant extracts used as natural additives [14], [18], [19]. Other studies have shown the effects of storage methods in the seasoning when it is stored alone [20]. However, we have not found studies that determine the effect of packaging methods on the pomace while it is being used as a seasoning. This determination is important because the pomace is not only promising as seasoning to maintain food quality during storage, but also as functional food, therefore, its composition and antioxidant activity after storage are important. In view of the above, the aim of this study was to determine the phenolic profile and antioxidant activity of white wine pomace used as seasoning submitted to different storage methods, vacuum and modified atmosphere packaging, to study its potential effects as a functional ingredient. Additionally, based on previous studies in which changes in structure and activity of polyphenols were observed after digestion of WPP [21], an in vitro gastrointestinal digestion of the pomace was performed.

2. MATERIALS AND METHODS

2.1. White wine pomace products

The white wine seasoning used in this studied was obtained at the University of Burgos from seedless white wine pomace products (wWPP) following a previously described method [22].

Chicken breasts were seasoned with 2g of seasoning and 3g of salt by 100g of meat. The seasoned meat was stored at 4ºC for 7 or 15 days under two methods, modified atmosphere-packaging method, with 70% N_2 and 30% CO_2 atmosphere, or vacuum-packaging method. After storage, the wWPP (pomace stored 7 days in MAP (7M), 15 days in MAP (15M), 7 days in VP (7V) and 15 days in WP (15V)) were obtained scrapping the seasoning of the fillets surface. For the control wWPP the seasoning was scrapped after 30 minutes of storage with both these methods to replicate water conditions of the pomace obtained after 7 and 15 days.

2.2. *In vitro* gastrointestinal digestion

In vitro digestion of the pomaces obtained (control, 7V, 15V, 7M and 17M) were performed in order to mimic the physiological processes following a previously described method with some modifications [23]. The digestion, with an oral, a gastric and an intestinal phase, was performed in 300 mg of the pomace. After the *in vitro* digestion, the gastrointestinal fractions were obtained (WPGI) which are the fractions available for absorption at the small intestine.

2.3. *In vitro* analysis of the phenolic profile and antioxidant capacity of the pomaces and digested fractions

2.3.1. Assessment of the phenolic profile by high-performance liquid chromatography (HPLC)

The wWPP and wWPGI were analyzed following a method previously described [24]. Stilbenes, flavan-3-ols and flavonols were identified and quantified employing calibration curves of each corresponding polyphenols standards.

The phenolic profile assessment was carried out using analytical reversed-phase HPLC with an Agilent 1100 series HPLC system (Agilent Technologies Inc., Palo Alto, CA, USA) coupled to a diode array detector and a Spherisorb3® ODS2 reversed phase C18 column (250 mm × 4.6 mm, 3 µm particle size; Waters Cromatografia S.A., Barcelona, Spain). The results were expressed in µg of biochemical component/g of pomace

2.3.2. Assessment of Total Antioxidant Capacity (TAC)

The TAC was determined by and ABTS assay previously described [25]. This assay measures the ability of the antioxidants present on the sample to neutralize the radical of the ABTS·+ cation. This reaction produces a color change detected measuring the absorbance at 734 nm. The results were expressed in mmol TE (Trolox equivalent)/ 100g WPP using a Trolox calibration curve.

2.4. Cell culture and treatment

Human colon adenocarcinoma cell line Caco-2 (ATCC® HTB37™) was used as a model of the epithelial intestinal barrier. The cells were cultured in a monolayer using Minimum Essential Medium (MEM) supplemented with 20% (v/v) heat inactivated Fetal Bovine Serum, 1% non-essential amino acids, 100 u/ml Penicillin, 100 mg/ml Streptomycin and 0,5 µg/ml Amphotericin B. They were incubated at 37ºC and 90% humidity in a 5% CO2 atmosphere. For experiments, cells were exposed for 24h to bioaccesible fractions (0,07 µg GAE/ml).

2.4.1. Cell viability assessment

The effects of the pomaces and their fractions in the viability of the Caco-2 cells were determined using an MTT assay previously described [26]. The cells were incubated with the digested fractions (0,07 µg GAE/ml) for 24 hours, then they were incubated with MTT solution, the supernatant was removed and replaced with 100 µl of DMSO and the cell viability was assessed measuring absorbance at 570 nm. Results were expressed in percentage of cell viability of the treated cells compared with non-treated control cells.

2.4.2. Assessment of oxidative stress cell biomarkers

The formation of carbonyl groups (CGs) is the main biomarker for protein oxidation due to their stability [27]. A previously described colorimetric method was used for measuring at 373 nm the absorbance of the hydrazone chromophore generated by the reaction of the protein carbonyls and 2,4-dinitrophenylhydrazine [28]. The levels were normalized with the protein content of the sonicated homogenate and the results were expressed in µmol CGs/ mg of protein. Protein content was determined using the Bradford method [29].

The malondialdehyde (MDA) is a marker of lipid peroxidation generated by the peroxidation of polyunsaturated fatty acids, and it is potentially carcinogenic and mutagenic. MDA levels were determined by HPLC-DAD following a previously described method. The aldehyde MDA reacts with the thiobarbituric acid (TBA) forming a Schiff base adduct, the MDA-TBA_2 complex, and then it is separated from the other compounds that may also react with the TBA using reverse phase HPLC [30]. The flow rate was 1 ml/ min and the absorbance was measured at 532 nm. To determine the concentration we used a calibration curve of standard 1,1,3,3-tetramethoxypropane (TMP) and the results were expressed in µM of MDA equivalents [31].

3. RESULTS AND DISCUSION

White wine pomace is an abundant natural by-product of the wine industry that has been highlighted as a seasoning and as a functional ingredient due to its antioxidants properties associated a high concentration in polyphenols. We studied the effects of two different packaging methods, MAP and VP, on the pomace composition and antioxidant activity after 7 and 15 days

as chicken breast seasoning. The WPGI cytotoxicity in Caco-2 cells and their effects on lipid and protein biomarkers were studied.

3.1. Phenolic profile and antioxidant capacity of bioaccesible fractions of chicken breast seasoning stored with modified atmosphere and vacuum

Phenolic compounds (phenolic acids, flavan-3-ols and flavonols) of the wWPP stored 7 or 15 days in a modified atmosphere or vacuum packaging, the control pomace and their bioaccesible fractions are presented in Table 1.

Table 1. Phenolic profiles and total antioxidant capacity (TAC) of the control wWPP, seasoning wWPP and their bioaccesible gastrointestinal fraction (WPGI).

		Phenolic profiles (µg/ g muestra)						TAC (mmol TE/ 100g wWWP)	
		Non-Flavonoids		Flavonoids					
		Phenolic acids		Flavan-3-ols		Flavonols			
Control pomace									
	wWPP	467,79 ± 4,0	bε	97,21 ± 0,205	bβ	86,05 ± 0,3	δ	0,4576 ± 0,067	aα
	WPGI	444,63 ± 9,1	a	78,63 ± 1,3	a	ND		14,587 ± 0,901	b
Modified atmosphere									
7M									
	wWPP	102,80 ± 0,1	aα	24,39 ± 0,4	aα	4,70 ± 0,0	γ	1,69134 ± 0,135	aγδ
	WPGI	203,38 ± 1,9	c	ND		ND		15,1173 ± 0,516	b
15M									
	wWPP	279,76 ± 4,5	dβ	23,77 ± 0,1	aα	3,89 ± 0,0	β	1,21592 ± 0,205	aβ
	WPGI	167,09 ± 2,9	b	69,95 ± 1,2	b	ND		15,0765 ± 0,238	b
Vacuum									
7V									
	wWPP	424,58 ± 0,6	dδ	23,66 ± 0,3	aα	4,58 ± 0,0	γ	1,5296 ± 0,102	aγ
	WPGI	220,59 ± 1,1	b	ND		ND		15,023 ± 0,399	b
15V									
	wWPP	316,18 ± 1,7	cγ	153,27 ± 2,8	cγ	3,44 ± 0,0	α	1,79427 ± 0,059	aδ
	WPGI	197,50 ± 1,4	a	78,91 ± 0,3	b	ND		14,7255 ± 0,114	b

Values represents mean ($n \geq 3$) ± SD. Significant difference between the fractions used for different storage methods are indicated with Greek letter and the one between the different fractions with the same treatment with a Latin letter (ANOVA Variance test, $p<0.05$). wWPP: white wine pomace products, WPGI: wine pomace gastrointestinal fraction, 7M: 7 days as chicken seasoning in a modified atmosphere; 15M: 15 days as chicken seasoning in a modified atmosphere; 7V: 7 days as chicken seasoning in a vacuum atmosphere; 15V: 15 days as chicken seasoning in a vacuum atmosphere.

We tested for non-flavonoid and flavonoid polyphenols. In the first group we tested for phenolic acids, and in the second group we tested for flavonols and flavan-3-ols. Our pomace had a higher number of non-flavonoid polyphenols than flavonoids.

Different studies had characterized red and white grape polyphenols by HPLC [31]–[33] and how conditions and storage methods affect the total polyphenol content, anthocyanin content and antioxidant activity [19], [20], [31], [34], [35].

The phenolic acids were the most abundant compounds detected in the wWPP and wWPGI. They were significantly higher in the wWPP control than the wWPPs after 7 and 15 days as chicken breast seasoning. The concentration of phenolic acids in the wWPP was higher than in the WPGI in all samples analyzed except in the 7M, where the concentration of phenolic acids was doubled in the WPGI.

Regarding flavonoids, the flavan-3-ols significantly decreased in the wWPPs, after 7 and 15 days as chicken breast seasoning compared to the control pomace except in the 15V seasoning where they significantly increased. The flavan-3-ols content was similar for 7M, 15M and 7V (24.39 μg/g, 23.77 μg/g and 23.66 μg/g, respectively). In digested fractions the flavan-3-ols only were detected in both fractions after 15 days stored. Previous reports have shown that flavonoid content changes with storage time and that it is capable of increasing after an initial decrease [36]. The total content in flavonols also significantly decreased in every seasoning compared with the control and were not detect in any WPGI. The changes in the composition of the wWPP and the WPGI of the different pomaces are due to the processes of the digestion that change the phenolic profile [37].

Studies that analyzed the effect of storage methods on polyphenols in food showed the reduction of catechin an epicatechin, two flavan-3-ols, in strawberry purées with MAP conditions [38]. However, in another study with strawberry spreads they found that catechin did not significantly change ant that vanillic acid, a characterized grape phenolic acid, significantly decreased [39]. This results indicate that the phenolic profile is dependent of the food matrix, storage conditions and its bioaccessibility.

The total antioxidant capacity was assessed by ABTS method. Undigested pomace products (wWPP) and their digested fractions (WPGI) present in vitro total antioxidant capacity (Table 1). The stored seasoning had a significantly higher TAC than the control whereas in the digested fractions there were not any significant changes. In all the storage conditions and the control, the antioxidant capacity of digested fractions (WPGI) was significantly higher than in the wWPP. Previous studies have reported a significant increase of the total antioxidant capacity of the bioaccesible digested fractions compared to their pomaces [21].

S.Wang et al. (2017), using meat seasoned with red wine pomace, stored at 4ºC during 3 months in a normal atmosphere did not show any significant changes in the antioxidant capacity determined with the ABTS method [40], whereas. When stored in modified atmosphere conditions, meat seasoned with red pomace increased its ability to neutralize ABTS·+ [14] and it slightly decreased at room temperature in normal atmosphere conditions [41]. the results show that the antioxidant capacity might be altered by the storage conditions. Our studies show an increased the antioxidant capacity of wWPP after storage in MAP and VP , which might indicate that it maintains its potential as a functional ingredient when used as seasoning in meat.

Summarizing, the storage of grape pomace as seasoning with meat increased or did not affect the antioxidant capacity of the pomace and its fractions. Both MAP and VP reduce the phenolic profile compared to the control pomace, but in both cases after 7 and 15 days the seasoning still presented quantifiable concentrations of several polyphenol groups reported to have healthy effects [42], [43].

3.2. Effect of bioaccesible pomace products on the cell viability and biomarkers of oxidative stress in Caco-2 cells

3.2.1. Effect on the cell viability of the WPGI

After exposure of Caco-2 cells to each digested fractions (control pomace WPGI, 7M WPGI, 15M WPGI, 7V WPGI and 15V WPGI) (0,07 µg GAE/ml), the cell viability was measured by MTT assay in a 24-h study (Figure 1). Not viability changes were observed between the control, non-treated cells, and the cells treated with the WPGI under normoglycemic conditions which means they could be safe to use as a functional ingredient.

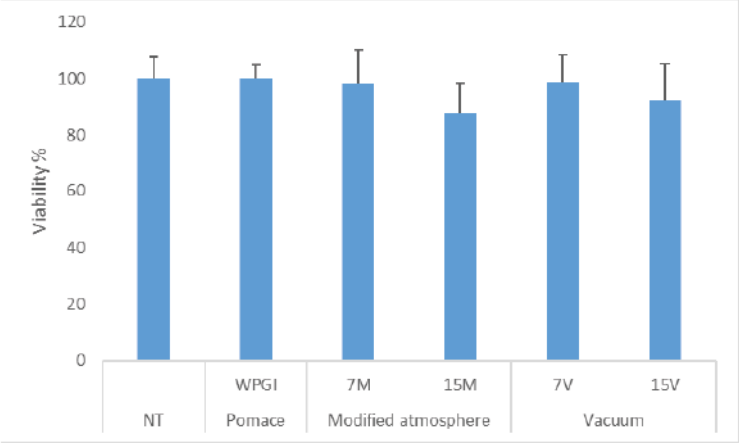

Figure 1. Viability of Caco-2 cells treated with the digested soluble fraction of the control pomace and the ones used as seasoning in vacuum and modified atmosphere stored chicken. Values represents mean (n ≥3) ± SD. Values represents mean (n ≥3) ± SD. ANOVA Variance test, $p<0.05$. NT: non-treated cells; WPGI: wine pomace digested fraction; 7M: 7 days as chicken seasoning in a modified atmosphere; 15M: 15 days as chicken seasoning in a modified atmosphere; 7V: 7 days as chicken seasoning in a vacuum atmosphere; 15V: 15 days as chicken seasoning in a vacuum atmosphere.

3.2.2. Effect on oxidative stress biomarkers of WPGI

To determine the potential healthy effect of the seasoning when used as a functional ingredient, two cell biomarkers for oxidative stress were analyzed: malondialdehyde (MDA) levels as indicator of lipid peroxidation and carbonyl groups (GC) as a biomarker of protein damage.

We tested for MDA to determine lipid peroxidation generated by the peroxidation of polyunsaturated fatty acids and we did not appreciate any significant changes between the cells incubated with a WPGI and non-treated cells (NT). We did not detect any flavonols in the WPGI and any flavan-3-ols in the 7 days' fractions but all of them had a high concentration of phenolic acids. Some phenolic acids like the vanillic acid have been reported to reduce lipid peroxidation markers in diabetic hypertensive rats [42].

We also tested for a biomarker of protein oxidation, carbonyl groups. The bioaccesible fractions of the stored wWPP significantly reduced protein oxidation between a 17 and 31%.

The reduction on carbonyl groups could be due to the changes in the phenolic profile after the contact with the meat for 7 or 15 days and fiber-bound polyphenols that we cannot detect with the HPLC and have been proven to scavenge carbonyl radicals [44],

In summary, we demonstrated that the packaging methods and the time of storage do not change the protective effects of WPGI in epithelial cells, and these positive outcomes revealed that the wWPP might have an antioxidant effect when ingested as meat seasoning.

4. CONCLUSIONS

The outcomes of this study show that modified atmosphere packaging or vacuum packaging change the antioxidant capacity of the grape pomace and its WPGI when used as meat seasoning for 7 or 15 days. The pomace and digested fractions phenolic profiles are modified by storage conditions but high concentrations of diverse phenolic acids are still maintained after storage.

The biological relevance of our data is that a WPP seasoning was tested after *in vitro* gastrointestinal digestion to approach the real physiological conditions. The digested fractions decreased the formation of protein carbonyl groups. Therefore, the data suggested that packaging methods maintain health effects of WPP as seasoning during storage, that it might have advantage in foods

5. ACKNOWLEDGMENTS

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