Antioxidant effect of seasonings derived from wine pomace in refrigerated and frozen beef patties

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ABSTRACT

The food industry is constantly looking for natural solutions to retard lipid oxidation and to extend the shelf-life of foodstuffs. Beef patties made with three natural seasonings derived from red wine pomace showed higher stability against lipid oxidation than control patties stored under refrigeration conditions in a high-oxygen atmosphere. The seasoning produced from seedless wine pomace presented the highest activity in comparison with the other seasonings. It showed higher efficacy than sulfites in delaying the formation of thiobarbituric acid reactive substances (TBARS) and other volatile organic compounds related to lipid oxidation. The seasoning also retarded the development of lipid oxidation in cooked and raw beef patties stored under freezing conditions. In the frozen samples, sulfites presented a contradictory effect, since it inhibited hexanal formation, but promoted the formation of TBARS. The results of the present study reveal that the seasoning is able to limit the development of lipid oxidation, extending the shelf-life of meat products without using synthetic antioxidants.

KEYWORDS: vegetal seasoning, lipid oxidation, wine pomace, beef patties, sulfites

1. INTRODUCTION

Lipid oxidation is one of the major factors that limits the shelf-life of meat products. It involves the formation of hydroperoxides that are easily broken down to form low molecular weight compounds (Kanner, 1994; Zhao, Wells, & McMillin, 1994). Different volatile organic compounds (VOCs) such as alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids, are extensively formed, causing rancid and unpleasant flavors and reducing the sensorial quality of meat products (Frankel, 1983; Kanner, 1994). Furthermore, lipid oxidation decreases the nutritional value of foodstuffs due to the loss of essential compounds such as polyunsaturated fatty acids (Kanner, 1994). Meat products are affected by lipid oxidation, as they usually contain high levels of fat and pro-oxidants such as salt. Furthermore, processes such as grinding or cooking also decrease the stability against lipid oxidation, due to structural degradation and the release of pro-oxidants (Alfawaz, Smith, & Jeon, 1994; Kanner, 1994).

Nowadays, meat products for storage under refrigerated conditions are usually packaged under high-oxygen atmospheres (70-80% O_2, 20-30% CO_2). This type of atmosphere maintains the attractive bright red color associated with fresh meat and present antimicrobial effect. However, storage with high levels of oxygen also accelerates the process of lipid oxidation and the appearance of off-flavors (Zhao et al., 1994).

The development of a global meat market and the increase of distance between producers and consumers have increased the use of freezing as a preservation technique (Leygonie, Britz, & Hoffman, 2012). Low temperatures and low levels of available water drastically reduce microbial and chemical reactions, thereby extending the shelf-life of these products. However, freezing also modifies the homeostasis of the meat

system, due to the cryoconcentration of solutes in the unfrozen phase. These changes may damage cell membrane leading to leakage of intracellular pro-oxidants such as lipases and metals, affecting the chemical stability of the product (Leygonie et al., 2012). Moreover, the catering and retail sector is increasingly interested in ready-to-eat foods that can be stored over long periods. Some consumers also prefer cooked products due to their convenience and their shorter preparation times. However, storage of cooked products is usually linked to extensive lipid oxidation and the development of the unpleasant "warmed-over flavor" (Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007; Igene, Pearson, Merkel, & Coleman, 1979).

Several chemical additives can be used to inhibit lipid oxidation and consequently extend the shelf-life of meat products (Cornelius & Lilian, 2005). For instance, sulfites are mainly applied as antimicrobial agents but also exert an important function as antioxidant. However, their consumption has been linked to allergic reactions and safety concerns over their long-term consumption have yet to be clarified (Cornelius & Lilian, 2005). Moreover, consumer rejection of chemical additives is growing and the food industry is constantly looking for natural additives. Natural products, such as spices and plant extracts, have been proposed as natural antioxidants to replace the use of chemical additives (Brewer, 2011; Lindberg Madsen & Bertelsen, 1995; Yanishlieva, Marinova, & Pokorný, 2006). Due to the complex reactions involved in lipid oxidation of foodstuffs, the efficacy of natural antioxidants may be affected under different storage conditions (Lindberg Madsen & Bertelsen, 1995). For instance, different herbs and spices such as curry and cinnamon showed high antioxidant protection in refrigerated storage of minced chicken meat, but not at the frozen storage (El-Alim, Lugasi, Hóvári, & Dworschák, 1999). Then, if their full potential is to be evaluated, natural antioxidants should be tested under different conditions.

Different seasonings recently developed from wine pomace presented excellent properties to be used as food ingredients. The seasonings are rich in fiber, minerals, especially potassium, and in phenolic compounds, including extractable and non-extractable fractions such as catechins, anthocyanins and proanthocyanidins, among other phenols (García-Lomillo, González-SanJosé, Del Pino-García, Rivero-Pérez, & Muñiz-Rodríguez, 2014). They have successfully been applied in different food matrices and have shown a preservative effect in low salt patties with high levels of consumer acceptance (González-SanJosé et al., 2014). Furthermore, the seasonings were found to successfully inhibit lipid oxidation under accelerated oxidative conditions in the Rancimat test, which points out to their potential use as natural antioxidants in the food industry (García-Lomillo et al., 2014).

The main aim of this work is to study the potential of red wine pomace seasonings (RWPSs) to retard or inhibit lipid oxidation in beef patties under different storage conditions (raw refrigerated under high-oxygen atmosphere as well as raw and cooked frozen vacuum-packaged patties), comparing the antioxidant effect with the antioxidant capacity of sulfites, a common additive in meat patties.

2. MATERIALS AND METHODS

2.1.Material

Beef meat was obtained from a local supplier (Gros Mercat, Burgos, Spain). Common salt, food grade starch and a commercially available mixture of phosphates provided by Doscadesa (Murcia, Spain) and sodium metabisulfite ($Na_2S_2O_5$) (Panreac, Barcelona, Spain) were used in the formulation of beef patties. The seasonings under study were prepared at the pilot plant of the Food Technology Department of the University of Burgos (Spain), following the process described by González San José, García Lomillo, Del Pino García, Dolores Rivero, & Muñiz Rodríguez (2015). The

seasoning obtained directly from whole red wine pomace was labeled as WRWPS (whole red wine pomace seasoning). A second seasoning was obtained from seedless red wine pomace and was labeled as SkRWPS (skin red wine pomace seasoning). The third seasoning, obtained from the seeds that had been separated was labeled SdRWPS (seed red wine pomace seasoning). The chemical and phenolic composition as well as their antimicrobial capacity of the three seasonings were previously described (García-Lomillo et al., 2014).

For lipid oxidation assessment, perchloric acid was purchased from VWR International (Barcelona, Spain), and 2-thiobarbituric acid, cyclopentanone, dichloromethane, hexanal, 1,1,3,3-tetraethoxypropane were purchased from Sigma (St. Louis, USA).

2.2.Patty preparation

Control beef patties were prepared by mixing 920 g of previously chopped and minced meat, 12 g of potato starch and 50 mL of water in which 3 g of a commercially available mix of food grade phosphates had previously been dissolved, and the corresponding quantity of salt to obtain a final concentration of 1.5 g of salt per kg of patty. Beef patties with seasonings were formulated in the same way, but suspending the corresponding amount of seasoning in water to obtain a final concentration of 2 % (w/w). Sulfite samples were similarly prepared obtaining a final concentration of 300 mg of SO_2 per kg of patty (300 ppm). Ingredients were mixed in a food processor for 5 minutes and patties of 100 grams were manually formed and packaged or cooked and then packaged. Cooked samples were processed according to the procedure described by Carpenter, et al. (2007) in a fan-assisted oven (Berto´s, Padova, Italy) previously heated at 180ºC, until an internal temperature of 72ºC was reached (8 minutes) and subsequently maintained for 8 minutes.

126 Raw patties were stored under two different conditions, high-oxygen atmosphere
127 (70% O_2/30% CO_2) and refrigeration, and vacuum packing and freezing. In the high-
128 oxygen atmosphere, samples were packaged in trays of polyethylene/ethylene vinyl
129 alcohol/polystyrene (Sanviplast, Barcelona, Spain) with permeability to oxygen of 0.99
130 $cm^3/(m^2$ day atm). They were then sealed using a polyethylene terephthalate
131 polyvinylidene chloride/polyethylene film with an oxygen permeability of 7 $cm^3/(m^2$
132 day atm) (Amcor, Burgos, Spain). The vacuum-packaged samples (raw and cooked),
133 were sealed in polyamide/polyethylene bags (20/70 μm) (Vacioplast, Salamanca, Spain)
134 with an oxygen permeability lower than 40 $cm^3/(m^2$ day atm). The samples were frozen
135 using a conventional freezer working at -30 ºC stored over 6 months at -18ºC.

136 Sampling of the refrigerated patties (conserved at 4ºC) was conducted at days 0,
137 4, 8, 12 and 15 days of storage, and at months 0, 2, 4 and 6 of the frozen samples.
138 Frozen samples were thawed at 4ºC for 12 hours before analysis. This study was
139 performed in duplicate and, in each experiment, three independent replicates (trays or
140 bags) were considered on each sampling date.

141 The chemical composition of the beef patties was analyzed using a FoodScan™
142 near-infrared spectrophotometer (Foss Electric A/S, Hillerød, Denmark) and data
143 processing done with ISIscan™ Software.

144 **2.3.Thiobarbituric acid reactive substances (TBARS) analysis**

145 TBARS method was conducted according to the method proposed by Tarladgis,
146 Watts, Younathan, & Dugan (1960) with minor modifications. Briefly, samples were
147 homogenized in 3.86% perchloric acid, filtered and distilled. Three mL of the resulting
148 distillate were mixed with 1 mL of 0.04 M of 2-thiobarbituric acid in perchloric acid
149 (10%) and incubated at 100ºC for 45 minutes. After incubation, samples were cooled

and their absorbance was measured at 532 nm using a U-2000 Hitachi spectrophotometer (Tokyo, Japan) against a blank where sample was replaced with 3 mL of perchloric acid. Quantification was conducted by preparing a standard curve with tetraethoxypropane and the results were expressed in ppm of malondialdehyde (MDA). The quantification limit (0.08 ppm of MDA) of the method was calculated using the DETARCHI software (Sarabia & Ortiz, 1994).

2.4.Analysis of volatile organic compounds (VOCs)

Volatile organic compounds were evaluated by a headspace solid-phase dynamic extraction (HS-SPDE) coupled with gas chromatography–mass spectrometry (GC-MS) (Agilent Technologies 6890N) fully controlled by a CTC-CombiPAL autosampler (Bender and Hobein, Zurich, Switzerland), following the method optimized by Corcuera-Tecedor (2013) Samples were introduced in a glass vial with 10 mL of headspace (Chromacol Ltd. Herts, United Kingdom), mixed with 5 µl of a ciclopentanone:dichloromethane (1:9) solution and sealed using a metallic cap with a chlorobutyl/polytetrafluoroethylene seal (Chromacol Ltd. Herts). Extraction was conducted in an incubation station at 55ºC, using a previously conditioned SPDE-syringe with a non-polar PDMS/AC (90% polydimethylsiloxane and 10% activated carbon) needle (60 extraction strokes). After extraction, VOCs were then desorbed from the fiber in the injection port at 250ºC, using helium as the carrier agent. Separation was conducted in 007-WAX capillary column (Quadrex Corporation, New Haven, USA) (60 m length, 0.32 mm inside diameter and 1µm film thickness). Compounds were identified by comparing their mass spectra with those found in the Wiley 7th and NIST 98 libraries and confirmed with their retention times. The retention index was calculated in relation to a series of standard alkanes (C6-C18) that were used for calculating Kovats indexes. The results were expressed as relative areas of each chromatographic

peak (compounds) in relation to the peak area of the internal standard (arbitrary units, AU) that was used.

2.5.Statistical analysis

Statistical analysis was performed using StatGraphics ® Centurion XVI. Fisher´s least significant difference test was performed in order to identify significant differences between different formulations and at different days of storage (p-value < 0.05).

3. RESULTS AND DISCUSSION

3.1.Chemical analysis of beef patties

The main compositional parameters of the raw and the cooked samples were measured and significant differences were not observed between the different batches. Raw samples averaged 63.0 ± 1.3% of moisture, 16.2 ± 0.3% of total protein and 13.4 ± 0.9% of total lipid. Cooked samples presented values of 64.3 ± 1.3% moisture, 23.8 ± 1.2% of protein and 9.9 ± 0.9% of total lipid content.

3.2.Lipid oxidation under high-oxygen atmosphere and refrigeration conditions

TBARS analysis is a widely used indicator to evaluate secondary lipid oxidation during the storage of meat products. It is usually considered a measure of the MDA content, although other aldehydes may also participate in the reaction (Kanner, 1994).

Previous studies reported that SkRWPS showed a higher antioxidant effect in the Rancimat test in comparison to SdRWPS and WRWPS (García-Lomillo et al., 2014). This study also showed different inhibition capacities for each seasoning (Figure 1).

TBARS values of the control patties increased during refrigerated storage, due to the lipid instability of meat products stored under a high-oxygen atmosphere

(Frankel, 1983). Patties are minced meat products with high fat and relatively high salt contents, factors that enhance lipid oxidation (Alfawaz et al., 1994; Kanner, 1994). These facts could explain the extensive formation of TBARS not only during storage but also during patty preparation. The three seasonings under study were able to prevent lipid oxidation during patty processing and subsequent storage, but with different levels of efficacy. SkRWPS was the most effective inhibiting lipid oxidation completely until the end of storage (two weeks). WRWPS and SdRWPS were also able to delay the onset of lipid oxidation for around one week and to reduce the oxidation rate significantly. Then, patties made with these seasonings showed TBARS values that were 55% lower than the control patties, and no significant differences were observed between them.

In view of the high effectiveness of SkRWPS against lipid oxidation of patties, further experiments were conducted to compare the effect of this seasoning with sulfites (Figures 2a and 2b). The results obtained in the two assays showed that sulfite was less efficient than SkRWPS, which totally inhibited TBARS formation. Sulfites were not able to retard the onset of the oxidation, but they were able to reduce the intensity of lipid oxidation, and lower TBARS values than in control samples were obtained on all sampling dates. At the end of storage, formulation with sulfites presented 55% lower TBARS values than the control samples.

Besides the information provided by TBARS value, which quantifies those aldehydes (mainly MDA) that react to thiobarbituric acid, other low molecular weight compounds, formed by the breakdown of the fatty acids, are also considered good indices of this process. Among these Volatile Organic Compounds (VOCs), saturated aldehydes and especially hexanal are extensively recognized as indicators of the lipid oxidation. They play a major role in the development of rancid odors, due to their

224 extensive formation and their relatively low threshold (Calkins & Hodgen, 2007). Other
225 VOCs of interest are alcohols, 2,3-octanedione and 2-pentylfuran which also contribute
226 to the development of rancid off-odors (Calkins & Hodgen, 2007; Gravador et al.,
227 2015).

228 Different VOCs were detected in all the patties, although they were mainly
229 present in control patties, and, there was a general increase throughout the storage
230 period (Figures 3 and 4). Hexanal content of control samples increased notably
231 throughout the storage and similar results were obtained in the two assays carried out
232 (Figure 3A and 3B). This result indicated an advanced stage of lipid oxidation, in
233 agreement with trends of TBARS values for these samples. These results coincide with
234 previous studies reporting high correlations between TBARS values and hexanal
235 formation (Frankel, 1983; Kanner, 1994). In agreement with hexanal levels, significant
236 increases of levels of 1-pentanol, 1-hexanol, 1-octen-3-ol, 2,3-octanedione and 2-
237 pentylfuran were also detected in control samples (Figure 4), corroborating the
238 development of secondary lipid oxidation.

239 Patties made with sulfites showed lower levels of hexanal and the other analyzed
240 VOCs in comparison to control patties (Figures 3 and 4), which is in agreement with the
241 antioxidant activity of this additive (Günther, König, Habicher, & Schwetlick, 1997).
242 The antioxidant activity of sulfites is usually linked to their capacity to reduce lipid
243 hydroperoxides without producing radicals (Günther et al., 1997). Moreover, reactive
244 oxygen species (ROS) can also be scavenged by sulfites leading to radical termination
245 processes (Kaneda, Takashio, Osawa, Kawakishi, & Tamaki, 1996). It might also
246 involve the reduction of other pro-oxidants such as H_2O_2 and the ability of sulfites to
247 avoid the accumulation of protein radicals that could act as lipid pro-oxidants (Garcia-
248 Lomillo, González-SanJosé, Skibsted, & Jongberg, 2016).

According to TBARS results, SkRWPS patties showed no hexanal except for very low levels at the end of storage. The seasoning was also able to inhibit the formation of 1-octen-3ol, and to reduce the formation of the other VOCs (Figure 4). These results showed higher effectiveness of SkRWPS than sulfites, pointing out the high potential of the new seasoning as an antioxidant in beef patties stored under a high-oxygen atmosphere.

The results obtained agree with those reported by other authors. Sáyago-Ayerdi, Brenes, & Goñi, (2009) reported that grape antioxidant dietary fiber was capable of inhibiting lipid oxidation in chicken hamburger and Garrido, Auqui, Martí, & Linares (2011) observed the protective effect of different grape pomace extracts in pork burgers stored under aerobic conditions. Herbs and spices have also reported antioxidant activity in refrigerated meat products (Lindberg Madsen & Bertelsen, 1995; Yanishlieva et al., 2006). El-Alim, et al.(1999) tested different spices observing that the highest inhibition was produced by clove, nutmeg, curry and cinnamon. However, the inhibition was lower than the protection reported for SkRWPS. The protection exerted by SkRWPS was also higher than the antioxidant activity of other spices such rosemary, sage or ground mustard (Lindberg Madsen & Bertelsen, 1995).

The strong inhibition produced by the seasonings can be ascribed to their high content of phenolic compounds (García-Lomillo et al., 2014). According to Pazos et al. (2005) grape polyphenols present an optimal degree of polymerization and galloylation to delay lipid oxidation in muscle systems. Some authors pointed out the importance of simple extractable compounds such as flavanol monomers, phenolic acids or anthocyanins (Yu & Ahmedna, 2013). In contrast, Ursini et al. (2001) reported the relevance of polymerized compounds in comparison to their correspondent monomers. Most probably, synergistic reactions between the high content in extractable

polyphenols and polymerized proanthocyanidins of the seasoning could explain the observed antioxidant activity (García-Lomillo et al., 2014). The antioxidant mechanisms of polyphenols are usually ascribed to their capacity to donate electrons to free radicals formed during lipid oxidation and to their capacity to stabilize their structure by the resonance delocalization of an electron within their aromatic ring (Brewer, 2011). Moreover, grape phenolics are able to reduce highly-oxidizing ferryl species (Kanner, Frankel, Granit, German, & Kinsella, 1994) as well as to scavenge superoxide anions (Chen, Zheng, Jia, & Ju, 1990) and hydroxyl radicals (Rafat Husain, Cillard, & Cillard, 1987). Other proposed mechanisms include the chelation of metal initiators and the inhibition of the enzymatic activity of lipoxygenase (Duque, Pinto, & Macias, 2011).

3.3.Lipid oxidation under frozen storage of raw and cooked patties

Meat products stored under different conditions are affected by different oxidative mechanisms and natural antioxidants may show different efficacy in products stored under different storage conditions (Lindberg Madsen & Bertelsen, 1995). Then, the potential efficacy of the SkRWPS against lipid oxidation was also tested during frozen storage of raw and cooked patties.

TBARS content increased during storage in both the cooked and the raw control samples (Figure 5). At the end of storage, TBARS levels were 0.18 ppm in the raw and 0.08 ppm in the cooked control patties. The observed values were relatively low (ten times lower than in patties stored under refrigeration) indicating only slight lipid oxidation. This fact agrees with the stability of beef, in comparison to other meats, during frozen storage (Igene et al., 1979). The slight formation of TBARS correlated well with the low levels of hexanal that were detected (Figure 6).

The cooked patties showed higher stability against lipid oxidation than the control patties (Figures 5 and 6). Although cooking usually produces pro-oxidant effects due to the disruption of cellular organization and the release of iron with catalyst activity (Kanner, 1994). Utrera, Morcuende, Ganhão, & Estévez (2015) also showed lower oxidative stages in cooked samples, ascribing this fact to potential reactions between formed aldehydes and meat proteins. Other authors also hypothesized that Maillard reaction products formed during cooking may have antioxidant effect (Alfawaz et al., 1994). Moreover, the inactivation of pro-oxidant enzymes such as lipases could also contribute to the higher stability of cooked rather than raw samples (Kanner, 1994).

SkRWPS was able to reduce lipid oxidation in both the raw and the cooked frozen patties significantly, which showed lower TBARS and hexanal values than the control samples through the storage (Figures 5 and 6). No TBARS were formed in the cooked patties and the raw patties showed a 60% reduction in comparison to the control patties. The seasoning also inhibited hexanal formation by around 80% in both types of patties. The formation of other VOCs such as alcohols and ketones was also inhibited by the seasoning (data not shown) in a similar way to hexanal.

Similar products derived from wine pomace or herbs and spices also showed capacity to inhibit lipid oxidation during frozen storage. For instance, Sánchez-Alonso et al. (2006) found that red grape antioxidant dietary fibre was able to minimize the development of primary and secondary oxidation in minced fish muscle stored at -20º C. These authors highlighted the importance of the so-called "non-extractable polyphenols" in the observed antioxidant capacity. Cagdas & Kumcuoglu (2015) also found that grape seed powder added into the batter inhibited oxidation in precooked chicken nuggets. Wine pomace extracts lowered TBARS values in vacuum packaged chicken (Selani et al., 2011). El-Alim et al. (1999) tested different spices at 1% and

found that the most effective spices were marjoram, caraway and rosemary oleoresin preventing TBARS formation in frozen meat products (Lindberg Madsen & Bertelsen, 1995), but the inhibition was lower than the protection exerted by SkRWPS.

The results observed in patties with sulfites were controversial. Sulfites promoted the formation of TBARS in both cooked and raw hamburgers (Figure 5), which suggests a pro-oxidant activity. However, sulfites successfully delayed the formation of other VOCs derived from lipid oxidation and assessed by GC-MS including hexanal (Figure 6). In this case, their efficacy was even higher than in samples with SkRWPS. The discrepancy between TBARS and VOCs analysis as well as the different behavior of sulfite samples stored under different conditions may be ascribed to diverse factors. In frozen samples, the amount of water decreases in the liquid phase and the solutes (as sulfites) are cryoconcentrated in the liquid phase of water, and may reach pro-oxidant levels (Leygonie et al., 2012). In this regard, sulfites may induce direct intramolecular cleavage of polyunsaturated fatty acids and induce the formation of low molecular weight compounds (Southerland, Akogyeram, Toghrol, Sloan, & Scherrer, 1982). From among the predominant polyunsaturated fatty acids in beef muscle, sulfites are more likely to attack linolenic acid, (that is MDA precursor) than linoleic acid (whose degradation forms hexanal) (Daley, Abbott, Doyle, Nader, & Larson, 2010; Southerland et al., 1982). Then, cryoconcentrated sulfites in frozen samples may directly attack double bonds from linolenic leading to MDA formation. The chemistry of sulfites in food is rather complex and depends on several factors such as concentration, temperature, matrix effect, etc. (Günther et al., 1997; Kaneda et al., 1996). Further experiments should therefore be conducted for a complete evaluation of the effect of sulfites on lipid oxidation in frozen samples.

4. CONCLUSIONS

Taken together, the results of the present study suggest that, from among the three seasonings, the seasoning derived from seedless red wine pomace presented the highest activity. It inhibited the formation of compounds derived from lipid oxidation in patties under different storage conditions. This seasoning also showed higher efficacy than sulfites that showed contradictory results in frozen samples, suggesting a possible pro-oxidant activity.

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FIGURE CAPTIONS

Figure 1. Formation of TBARS in beef patties without antioxidants (Control), with the addition red wine pomace seasonings (Seed RWPS, Whole RWPS or Skin RWPS at 2% w/w) stored under high-oxygen atmosphere (70% O_2/30% CO_2) for 15 days at 4 °C. Points show mean values of three replicates, and bars indicate standard deviations at each sampling point.

Figure 2. Formation of TBARS in beef patties without addition of any antioxidant (Control), with addition red skin wine pomace seasonings (Skin RWPS at 2% w/w) or sulfites (300 ppm) stored under high-oxygen atmosphere (70% O_2/30% CO_2) for 15 days at 4 °C in two independent experiments (A and B). Points show mean values of three replicates, and bars indicated standard deviation at each sampling point.

Figure 3. Formation of hexanal in beef patties without addition of antioxidants (Control), with the addition of skin red wine pomace seasoning (SkRWPS at 2% w/w) or with addition of sulfites (300 ppm) stored under high-oxygen atmosphere (70% O_2/30% CO_2) for 15 days at 4 °C in two independent experiments (A and B). Results are expressed in Arbitrary Units (AU). Points show mean values of three replicates, and bars indicated standard deviations at each sampling point.

Figure 4. Formation of five volatile organic compounds: 1-pentanol, 1-hexanol, 1-octen-3-ol, 2,3-octanedione and 2-pentylfuran in beef patties without addition of antioxidants (Control), with the addition of skin red wine pomace seasoning (SkRWPS at 2% w/w) or with the addition of sulfites (300 ppm) stored under high-oxygen atmosphere (70% O_2/30% CO_2) for 15 days at 4 °C. Results are expressed in Arbitrary Units (AU). Points show mean values of three replicates, and bars indicate standard deviations at each sampling point.

Figure 5. Formation of TBARS in raw and cooked beef patties without addition of antioxidants (Control), with the addition of sulfites (300 ppm SO_2) or skin red wine pomace seasoning (SkRWPS, 2% w/w) stored in vacuum packaging for 6 months at -18 °C. Points show mean values of three replicates, and bars indicate standard deviations at each sampling point.

Figure 6. Formation of hexanal in raw and cooked beef patties without addition of antioxidants (Control), with the addition of sulfites (300 ppm SO_2) or skin red wine pomace seasoning (SkRWPS, 2% w/w) stored in vacuum packaging for 6 months at -18 °C. Results are expressed in Arbitrary Units (AU). Points show mean values of three replicates, and bars indicate standard deviations at each sampling point.

Figure 1

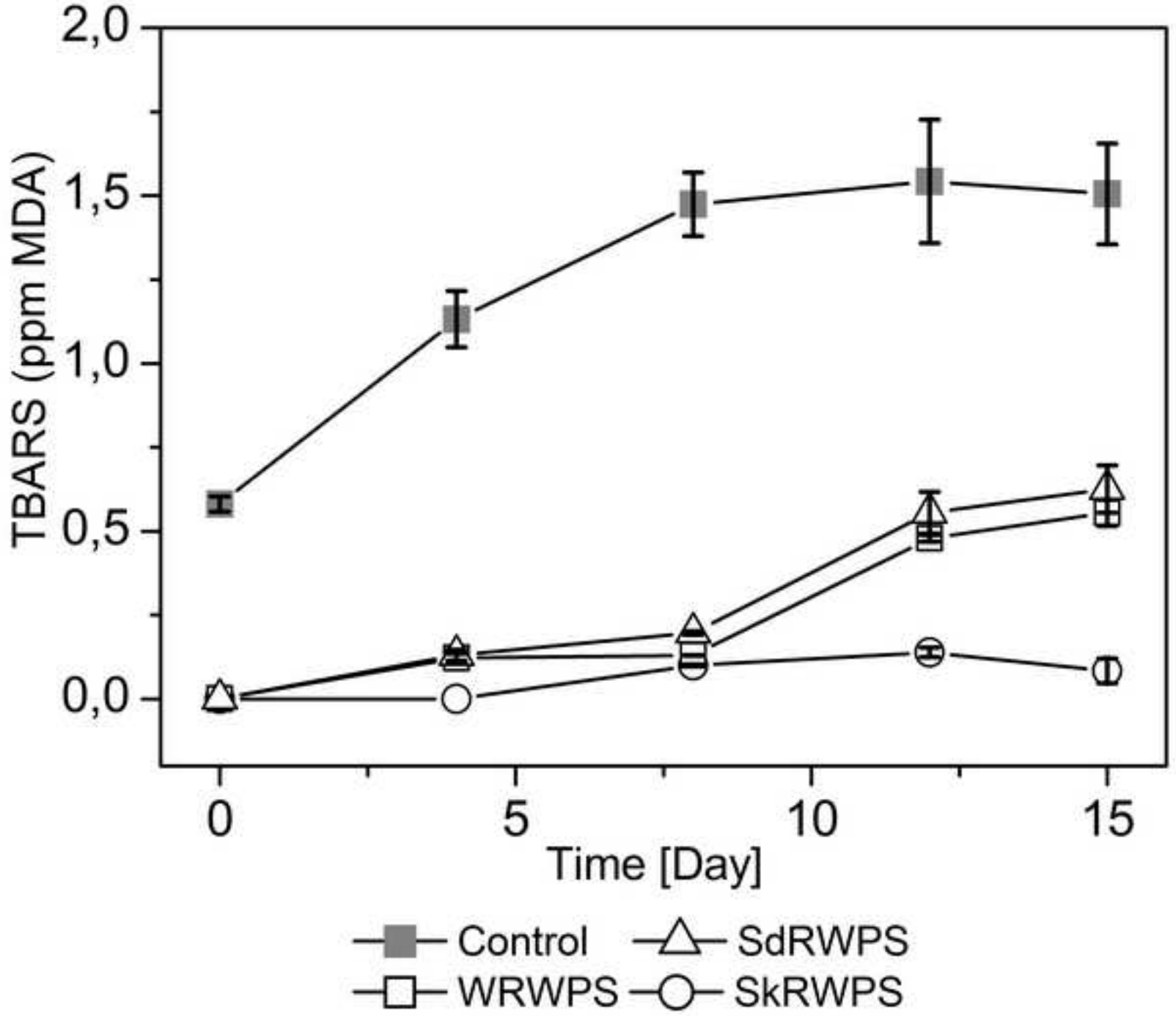

2,0
1,5
1,0
0,5
0,0
TBARS (ppm MDA)
0
5
10
15
Time [Day]
Control
SdRWPS
WRWPS
SkRWPS

Figure 2

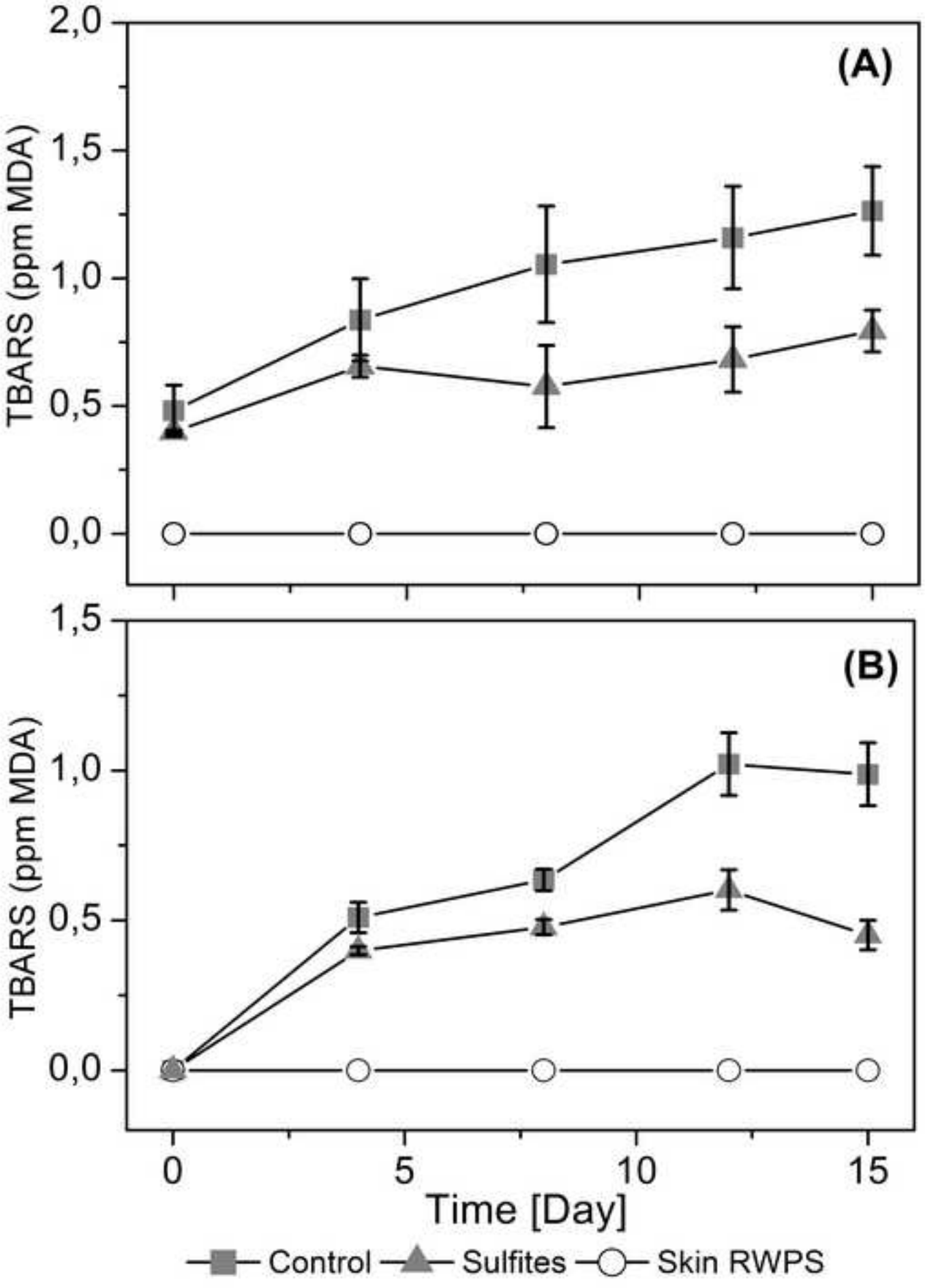

(A)
TBARS (ppm MDA)
2,0
1,5
1,0
0,5
0,0
(B)
TBARS (ppm MDA)
1,5
1,0
0,5
0,0
0
5
10
15
Time [Day]
Control
Sulfites
Skin RWPS

Figure 3

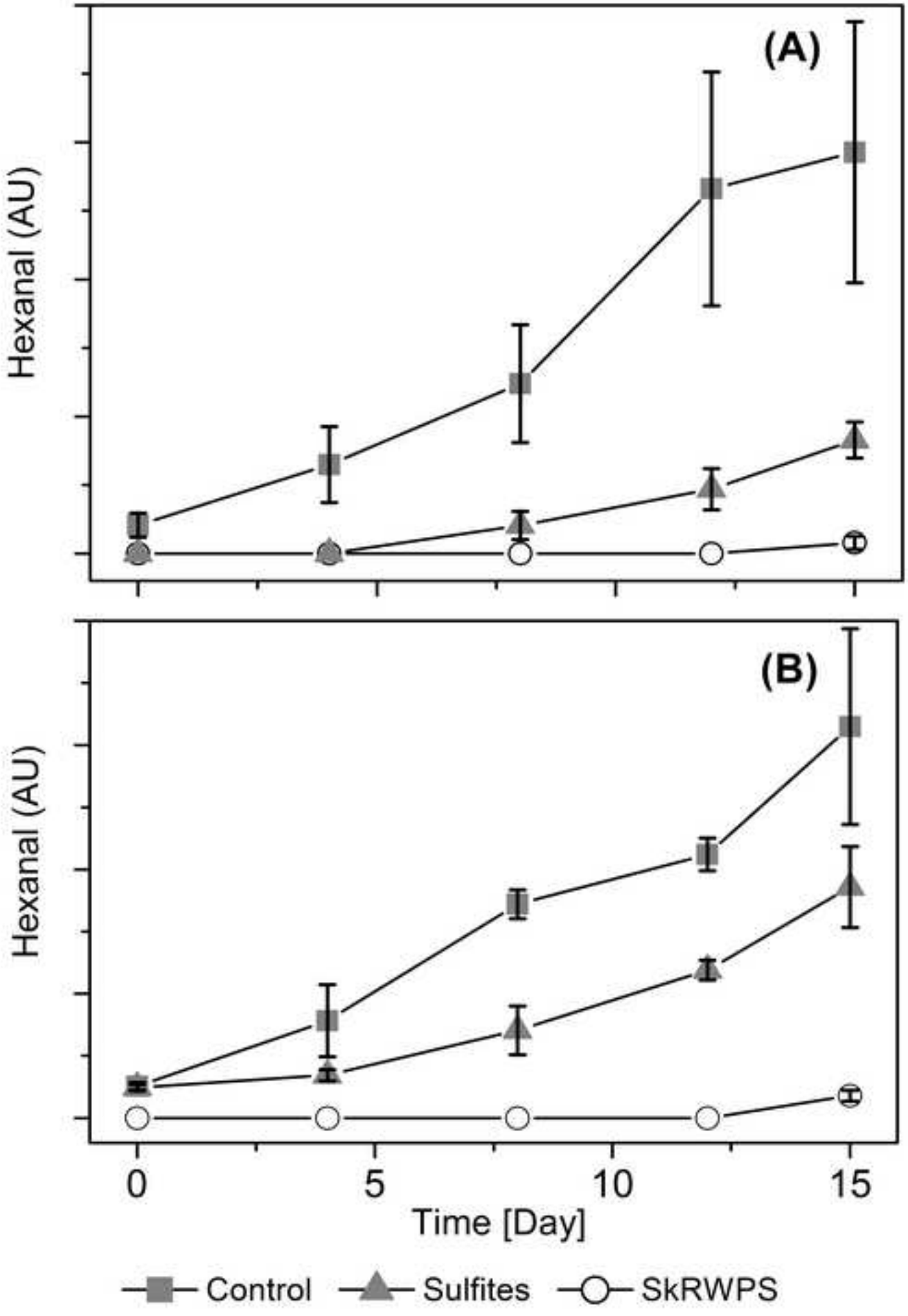

(A)
Hexanal (AU)
(B)
Hexanal (AU)
0
5
10
15
Time [Day]
Control
Sulfites
SkRWPS

Figure 4

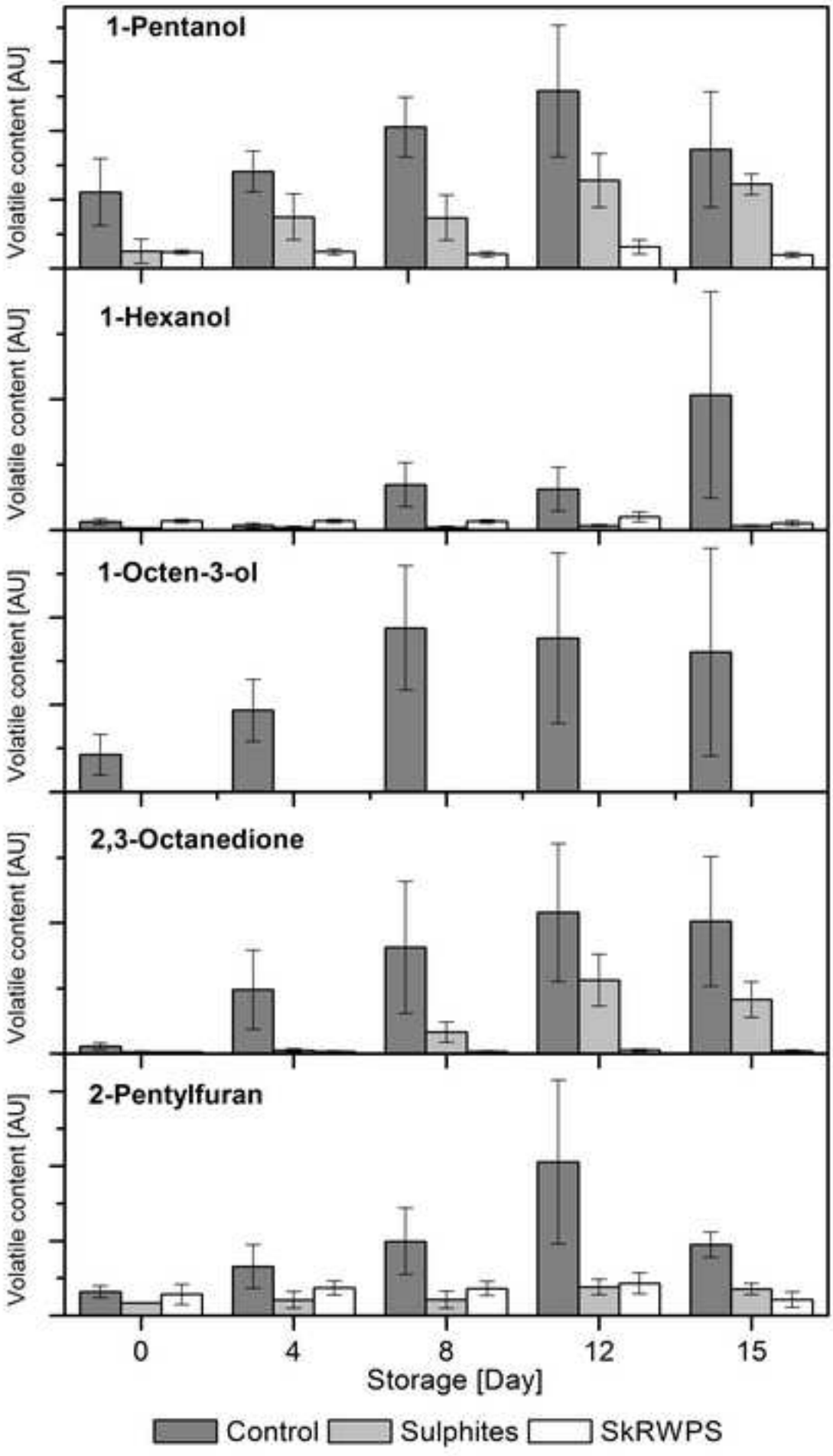

1-Pentanol
1-Hexanol
1-Octen-3-ol
2,3-Octanedione
2-Pentylfuran
Volatile content [AU]
0
4
8
12
15
Storage [Day]
Control
Sulphites
SkRWPS

Figure 5

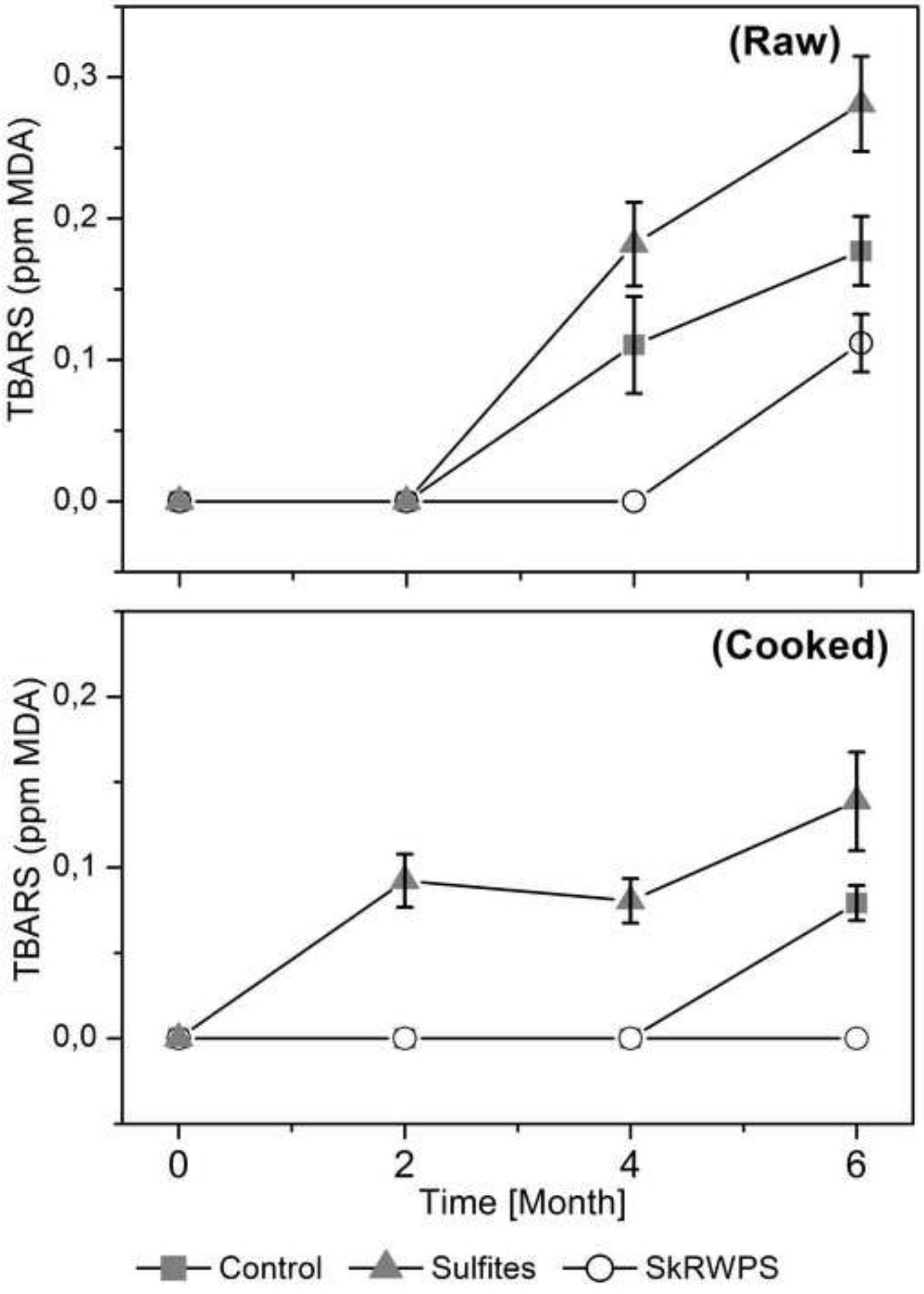
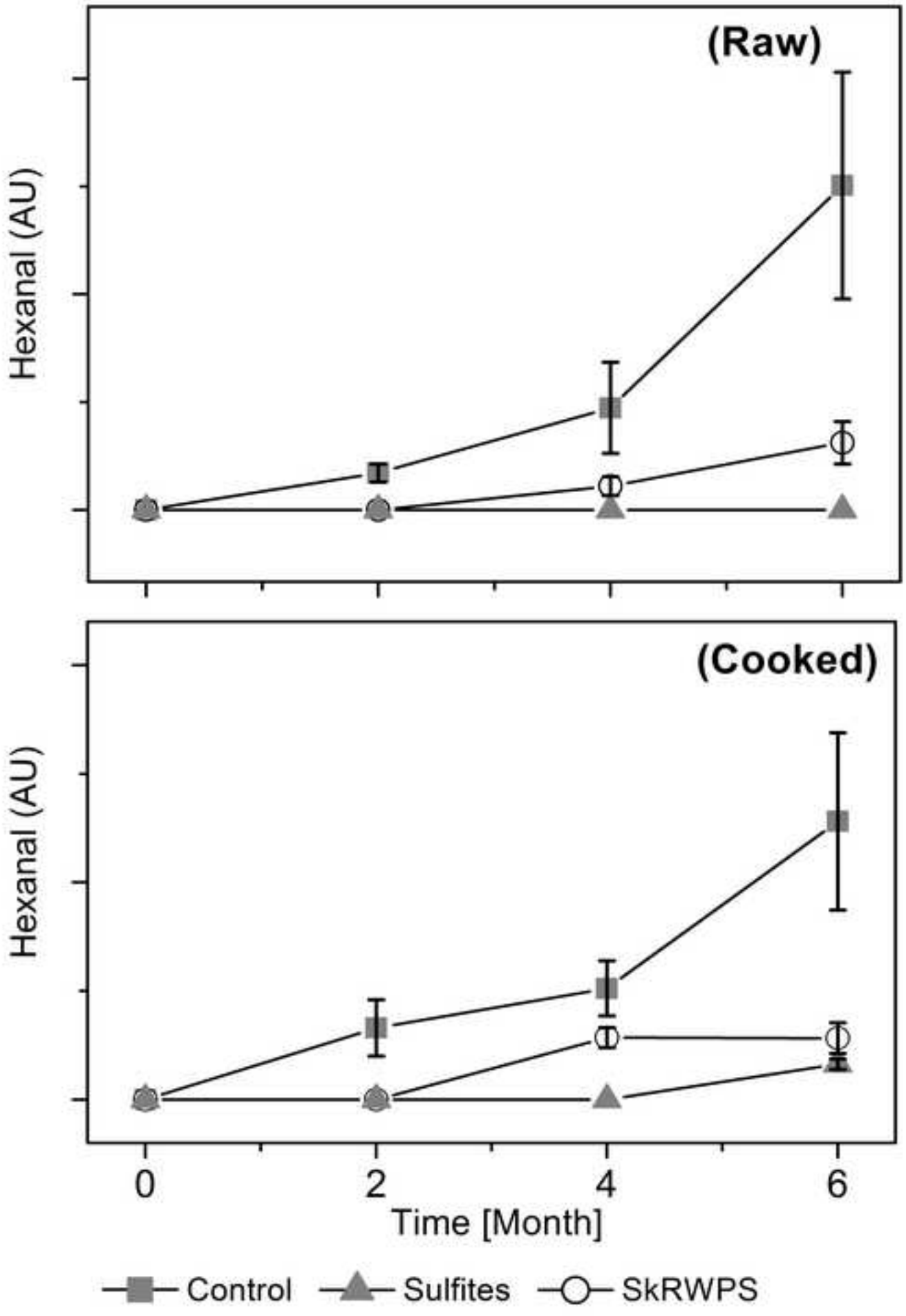

(Raw)
TBARS (ppm MDA)
0,3
0,2
0,1
0,0
(Cooked)
TBARS (ppm MDA)
0,2
0,1
0,0
0
2
4
6
Time [Month]
Control
Sulfites
SkRWPS

Figure 6


(Raw)
Hexanal (AU)
(Cooked)
Hexanal (AU)
0
2
4
6
Time [Month]
Control
Sulfites
SkRWPS