

Antioxidant Effects of Essential Oil and Oleoresin of Black Pepper from Supercritical Carbon Dioxide Extractions in Ground Pork[†]

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Supercritical CO₂ (SF-CO₂) extracts of ground black pepper have been found superior in reducing lipid oxidation of cooked ground pork compared to conventional extracts as measured by TBARS and hexanal concentrations. Oleoresin extracted by SF-CO₂ at 28 MPa (60 °C) was most effective in reducing hexanal concentration for up to 2 days. Essential oil extracted by SF-CO₂ at 10 MPa (60 °C) and essential oil prepared by a conventional method were less effective as antioxidants than ground black pepper, oleoresin extracted by SF-CO₂, and oleoresin prepared by a conventional method. TBARS and hexanal analyses were considered reliable indicators of meat flavor deterioration.

Keywords: *Black pepper; essential oil; oleoresin; supercritical CO₂ extraction; antioxidant*

INTRODUCTION

The utilization of isolated essential oils (EO) and oleoresins (OR) as an alternative to crude spices has increased in the meat industry. The extracts have several advantages over dried spice materials, including microbial safety, better stability during storage, high concentration of flavor components, ease of handling, reduced storage space, no seasonal variation, and standardization (Govindarajan, 1977; Farrel, 1990). Supercritical fluid carbon dioxide (SF-CO₂) is being used for the extraction of various botanicals to obtain aroma concentrates and EO (Vidal and Richard, 1987; Rizvi et al., 1986b; Sankar, 1994; Kerrola, 1995). Vidal and Richard (1987) reported that the EO fraction of black pepper was best extracted at 8 MPa and 30 °C and that the extraction level of piperine increased with the CO₂ density. Volatile oil obtained at 10 MPa and 60 °C was considered superior to the steam-distilled oil, using aroma, taste, and the ratios of sesquiterpene to monoterpene hydrocarbons as the criteria (Sankar, 1994). The amount of volatile oil recoverable from black pepper increased with increasing density of CO₂.

Lipid oxidation is one of the major causes of quality deterioration in frozen meat products. Oxidative deterioration of meat products can directly affect many characteristics such as color, flavor, texture, nutritive value, and safety. Spices have received particular attention as sources of antioxidants. The antioxidative action of black pepper, for instance, has been attributed to its piperine and piperine isomers, such as chavicine, isopiperine, and isochavicine, and monoterpene content (Chipault et al., 1956; Milbourne, 1987). However, antioxidative action of ground black pepper compared to its conventional and SF-CO₂ extracts has yet to be investigated.

The objectives of this study were to determine optimal conditions of the SF-CO₂ to selectively obtain EO and OR from black pepper and to determine the antioxidant activity of pepper extracts obtained by SF-CO₂ and conventional methods in ground pork.

MATERIALS AND METHODS

Conventional and Commercial Extracts. Ground black pepper (*Piper nigrum* L.) was obtained from MNE's Herbs & Spices (Columbia, MO) through 40 mesh screen and stored at 4 °C. EO was then removed by a multiple step process as described in AOAC (1995) method 962.17. Pepper (20 g) was also extracted by Soxhlet extraction for 4 h with dichloromethane (98% v/v) to obtain OR. After cooling, the solvent was evaporated using a rotor evaporator. Both EO and OR were stored at 4 °C in amber-colored bottles. The extractions were carried out in duplicate. These laboratory extracts were to be compared with commercial extracts. Commercial extracts of EOs and ORs were obtained from commercial sources (St. Louis Flavors, St. Louis, MO; The Lebermuth Co., Inc., South Bend, IN; and CHR Hansen Spice Extracts, Milwaukee, WI) and stored at 4 °C.

SF-CO₂ Extracts. The SF-CO₂ extractions were carried out on 1.1 (±0.1) g of black pepper powder using an ISCO (model SFX 2-10) extractor equipped with a 260 D syringe pump. The EO extraction was carried out at 8 or 10 MPa and at 40 or 60 °C using 40 mL of CO₂ (SFE/SFC grade CO₂; Mathenson Gas Product, Inc., Columbia, MO). The OR extraction conditions were 20–32 MPa and 45–65 °C with 60 mL of CO₂ used. The length and diameter of restrictors used were 20 cm and 50 or 100 μm for EO and OR, respectively. Both extracts were collected in tubes placed in amber-colored bottles and stored at 4 °C. Each SF-CO₂ extraction treatment, i.e., extraction of EO and extraction of oleoresin, was carried out in duplicate, and both experiments were repeated three times (*n* = 3).

Cooked Ground Pork Mixed with Ground Pepper and Various Pepper Extracts. Ground pork (2 kg) obtained from a local supermarket was mixed with 1.6% salt and divided into eight groups. Group 1 (GB) was mixed with ground black pepper at the level of 0.4% (w/w). Groups 2 (EO1), 3 (EO2), and 4 (EO3) were mixed with commercial, laboratory, and SF-CO₂ (10 MPa and 60 °C) EOs at the level of 0.0043% (w/w). A level of 0.4% added ground black pepper is typical for the

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ground pork sausage industry. The levels of extracts used were equivalent to the level of ground black pepper used to provide aroma characteristics (Pearson and Gillett, 1996). Groups 5 (OR1), 6 (OR2), and 7 (OR3) were mixed with commercial, laboratory, and SF-CO₂ (28 MPa and 60 °C) ORs at the levels of 0.0216% (w/w). The control treatment was ground pork without extract addition (group 8). The samples were then cooked in a preheated (180 °C) forced air convection oven (Wimco model 27-2DOX; Keating of Chicago Inc., Chicago, IL) to an internal temperature of 65 °C. The temperature was monitored with a model T102-25B-02 temperature probe (MetriCor Inc., Woodinville, WA). Each treatment was divided into 2 groups of 50 g for the analysis of volatiles and 2-thiobarbituric acid-reactive substances (TBARS), respectively. Each sample was then transferred into a 10-in. × 12-in. vacuum pouch (KOCH Supplies Inc., Kansas City, MO), sealed (Multivac model AG800; West Germany), and stored at 4 °C for up to 8 days. The experiment was repeated twice ($n = 2$).

Analyses of Volatile Oil. After each EO fraction from duplicates and replications was mixed, 2 μ L of the EO fractions was injected and analyzed in duplicate by gas chromatography (Perkin-Elmer model 8500 gas chromatograph equipped with a FID detector). A fused silica capillary column (Supelcowax 10), 60-m × 0.25-mm i.d., was used with helium as the carrier gas. The gas chromatographic conditions were column temperature program at 50–250 °C at 4 °C/min with a hold time of 10.00 min at 250 °C; injector 250 °C; detector 265 °C; carrier gas flow rate 2 mL/min; split ratio 1:50; and column inlet pressure 207 kPa. The data were analyzed using the built-in processor of the gas chromatograph. The relative abundance of each peak was expressed as a percent of the total peak area after duplicate injections. The isolated EO volatiles were tentatively identified using retention time as compared to previous literature values (Govindarajan, 1977).

Piperine Determination. Piperine content of each fraction was determined in duplicate for every replication. Each OR fraction was first softened by warming in a 40 °C water bath and then mixed well by stirring. An accurately weighed (1 ± 0.001 g) and well-mixed sample was transferred to a 100-mL volumetric flask. Methanol (HPLC grade) was added to volume, and the mixture was well-shaken until dissolved. Next, 10 mL of well-dissolved solution was pipetted into a 100-mL volumetric flask and diluted to volume. A 20- μ L solution was then analyzed for piperine recovery on a Perkin-Elmer series 410 LC pump and Perkin-Elmer LC 90 UV detector on a column of Supelco packing (LC-18, i.d. 4.6 mm, particle size 5.0 μ m, and length 15 cm) with the use of guard column. The mobile phase was methanol and water in the ratio of 60:40 with a flow rate of 1.5 mL/min. Identification of piperine was obtained by comparison to the retention time of standard piperine (Aldrich Co., Milwaukee, WI) at a wavelength of 343 nm.

Trapping of Cooked Ground Pork Volatiles. Trapping of cooked ground pork volatiles was performed using a modified procedure of Dupuy et al. (1987). After storage of sample for 0, 2, 4, 6, or 8 days, 5 g from each treatment was weighed into a 500-mL round-bottom flask. Distilled deionized water (50 mL) and 10 μ L of internal standard (1.56 μ g of 2-methyl-4-octanone in pentane) were added to the flask. Individual flasks were immediately fitted to the purge and trap apparatus (Clarke and Fernando, 1995) containing a glass tube filled with a solid adsorbent (Tenax TA, 60/80 mesh; Alltech Associates, Inc., Deerfield, IL). The flask was heated using a sand bath at 100–120 °C and refluxed for 30 min while the contents were stirred using a magnetic stir bar. The volatiles were swept onto the Tenax trap using prepurified nitrogen gas at a flow rate of 50 mL/min. Trapping of volatiles was determined in duplicate.

Desorption of Cooked Ground Pork Volatiles. The volatiles were thermally desorbed onto the gas chromatographic column using thermal desorption unit model 890 (Dynerm Analytical Instruments, Inc., Kelton, PA). The desorption temperature, transfer line temperature, and valve

Table 1. Characteristics of Pepper Volatile Oil Obtained by Steam Distillation and SF-CO₂^a

	steam distillation	SF-CO ₂			
		8 MPa 40 °C	8 MPa 60 °C	10 MPa 40 °C	10 MPa 60 °C
yield of oil	1.05	0.43	0.52	0.71	0.65
total terpene hydrocarbons	82.19	74.86	71.33	79.54	75.85
monoterpenes	71.95	57.53	63.55	59.64	48.71
sesquiterpenes	23.85	40.82	34.75	40.38	51.19
oxygenated hydrocarbons	3.05	5.72	6.18	6.47	7.63

^a LSD = 0.0123, $\alpha = 0.01$ for yield of oil (%). Total terpene hydrocarbons, monoterpenes, sesquiterpenes, and oxygenated hydrocarbons are reported as relative abundance (%) from duplicates.

temperature were 230, 200, and 180 °C, respectively. Desorption time was 5 min.

Analysis of Cooked Ground Pork Volatiles. The volatiles were analyzed by gas chromatography using a Perkin-Elmer model 8500 gas chromatograph (Perkin-Elmer Corp., Norwalk, CT) equipped with a flame ionization detector. The chromatographic column used was a 50M SE-54 fused silica capillary (Hewlett-Packard, Palo Alto, CA) with internal diameter 0.32 mm and film thickness of 0.5 μ m. Helium carrier gas flow rate was 3 mL/min with split ratio of 1:7.6. Column oven temperature program of 35 °C held for 5 min and then 8 °C/min to 220 °C and 2.0 °C/min to 250 °C was used. The data were analyzed using the built-in processor of the gas chromatograph. Quantitation of volatiles was performed using relative peak areas compared to peak areas of the internal standard. The isolated cooked ground pork volatiles were tentatively identified using retention time as compared to previous literature values (Bailey et al., 1987).

Determination of 2-Thiobarbituric Acid-Reactive Substances (TBARS). The TBARS test was carried out in duplicate as per Tarladgis et al. (1960) and as modified by Bailey et al. (1987). The TBARS values were expressed in mg of malonaldehyde/kg of meat.

Statistical Analyses of Data. All statistical analyses of the data were performed using the general linear model procedure of Statistical Analysis System (SAS, 1989). All data were analyzed in a completely randomized design using analysis of variance for each experiment. The means comparison in each experiment was performed using least significant difference (LSD) at $\alpha = 0.01$. The correlation analysis and linear regression between density and essential oil yield and recovery of piperine were also performed at $\alpha = 0.01$ or 0.05, as well as between the content of hexanal and TBARS values. The extractions and piperine determination experiment were conducted three times. The analyses of volatiles and TBARS experiments were conducted twice. All results, then, were pooled for statistical analysis. The analyses of EO volatiles were conducted in duplicate after each extract was mixed; therefore, the statistical analyses were not completed.

RESULTS AND DISCUSSION

Relative Extraction Rate of Volatile Compounds and Piperine. SF-CO₂ extracted oils were found to have greater concentrations of sesquiterpene and oxygenated hydrocarbons than EO obtained by conventional method (Table 1). These hydrocarbons have been reported to be important to the aroma characteristics of pepper oil (Govindarajan, 1977; Salzer, 1977). The greatest relative extraction rate (%) of black pepper volatiles ($P < 0.01$) was found in the case of steam distillation (Table 1). This was inconsistent with the result found by Sankar (1994) and Vidal and Richard (1987). The lower oil yield from SF-CO₂ in the present experiment may be due to the fact that the extraction was not fully completed. The classification index is

Table 2. Relative Abundance (%) of Certain Important Constituents of Essential Oil from Black Pepper^a

process code	α -pinene	β -pinene + sabinene, X	δ -3-carene + phellandrene + limonene, Y	caryophyllene, Z	classification index (X + Y)/Z
A	9.85	20.33	35.47	17.70	3.15
B	5.52	13.33	36.64	18.22	2.74
C	2.85	9.73	31.43	20.13	2.04
D	4.67	13.64	32.74	16.03	2.89
E	3.68	12.58	32.50	16.81	2.68
F	3.29	11.35	31.01	26.01	1.63

^a A and B represent conventional extracts (commercial and lab samples, respectively), whereas C, D, E, and F represent SF-CO₂ extracts obtained at 8 MPa (40 °C), 8 MPa (60 °C), 10 MPa (40 °C), and 10 MPa (60 °C), respectively. Percent relative abundance is reported from duplicates.

Table 3. Relative Extraction Rate (%) of Black Pepper Oleoresin and Piperine^a by SF-CO₂ Extractions

pressure (MPa)		temperature (°C)				
		45	50	55	60	65
20	oleoresin	4.60	4.74	4.84	5.21	5.07
	piperine	35.7	35.0	35.3	35.9	33.2
24	oleoresin	4.93	5.14	5.24	6.06	5.97
	piperine	36.7	36.5	36.6	37.4	35.7
28	oleoresin	5.44	6.02	6.13	6.47	6.10
	piperine	37.6	37.1	37.1	39.1	34.6
32	oleoresin	5.43	5.82	6.05	6.33	6.03
	piperine	39.4	38.0	35.9	38.4	32.8

^a Relative extraction rate of piperine obtained from oleoresin. N = 3, LSD = 0.0574 and 0.2761 for oleoresin and piperine, respectively, α = 0.01.

reported in Table 2 as described by Salzer (1977). The major constituents of the terpene fraction are α - and β -pinene, sabinene, δ -3-carene, and limonene, whereas β -caryophyllene is the major sesquiterpene (Govindarajan, 1977). A high total monoterpenes content results in a strong "peppery" top-note, and a predominantly pinene content of the terpene fraction gives a turpentine-like note, while a high caryophyllene content results in a sweet, flowery note, which is more desirable in the flavor industry (Govindarajan, 1977; Salzer, 1977). In essence, the classification index (Salzer, 1977) distinguishes the quality of the volatile oil reflecting from the ratio of monoterpenes (i.e., β -pinene, sabinene, δ -3-carene, phellandrene, and limonene) to sesquiterpenes (i.e., caryophyllene). The lower the classification index value, the better the quality of EO (Salzer, 1977). In addition to the ratio of monoterpenes to sesquiterpenes, the oxygenated compounds are supposed to provide the heart of the aroma of oil of pepper (Govindarajan, 1977; Salzer, 1977). However, only the ratio of monoterpenes to sesquiterpenes represents the classification index of the black pepper oil and indicates the aroma value (Salzer, 1977). The lowest classification index was found in oil extracted by SF-CO₂ at 10 MPa and 60 °C (Table 2). In addition to lower classification index, the superiority of EO obtained from SF-CO₂ was also due to the greater recovery of the oxygenated hydrocarbons (Table 1). At each pressure (8 and 10 MPa), the increase in temperature (40–60 °C) increases the extraction of oxygenated hydrocarbons.

Table 3 showed that pepper OR extracted with SF-CO₂ is as feasible as solvent-extracted OR recovery (6.1%) and was not different ($P > 0.01$) from OR fractions obtained at pressures of 28 MPa and 55 °C (6.13%). SF-CO₂ ORs obtained at 28 and 32 MPa (60 °C) were significantly greater than the conventional extract ($P < 0.01$). Sankar (1994) and Vidal and

Richard (1987) also reported that greater relative extraction rates of OR were observed with SF-CO₂ extraction at selected conditions compared to solvent extraction. The percent relative extraction rate of piperine from SF-CO₂ OR at 28 MPa and 60 °C was not significantly different from conventional oleoresin ($P > 0.01$) (Table 3). The SF-CO₂ OR at 32 MPa and 45 °C resulted in the greatest percent relative extraction rate of piperine when compared to other SF-CO₂ extraction conditions ($P < 0.01$).

Relationship between CO₂ Density and Relative Extraction Rate of EO Volatiles and Piperine. The correlation was found between EO yield and recovery of piperine and CO₂ density ($r = 0.64$ and 0.78 , at $\alpha = 0.05$ and 0.01 for EO yield and relative extraction rate of piperine, respectively). The predicted equations for EO yield and relative extraction rate of piperine from obtained oleoresin were then calculated using linear regression and shown as follows. The predicted equation for EO yield can possibly provide an early start for those who may look for the extraction condition even though the equation was developed from few densities.

$$\text{essential oil yield (\%)} = 0.4724 + 0.3004(\text{density})$$

$$\text{piperine relative extraction rate (\%)} = 24.3024 + 14.8621(\text{density})$$

It was found that the amount of extracted EO increased with the CO₂ density. In the present experiment, the greatest relative extraction rate (%) of volatile oil compounds by extraction with SF-CO₂ was found at 10 MPa and 40 °C ($P < 0.01$). This condition provided the highest experimental density of CO₂ at 0.6 g/mL. On the other hand, a less relative extraction rate of total terpene hydrocarbons was found at 8 MPa (40 and 60 °C) or 10 MPa and 60 °C than at 10 MPa and 40 °C, with the lower density of CO₂ at 0.300, 0.194, or 0.294 g/mL. As reported by Rizvi et al. (1986a,b) and Taylor (1996), the solvent power of dense CO₂ increases with increasing CO₂ density, resulting in high relative extraction rate of the compounds being extracted. In contrast, the low density of CO₂, which is dependent upon temperature, pressure, and vapor pressure, leads to high selectivity of the compounds being extracted. As a consequence, the solubility of caryophyllene tended to decrease with an increase in CO₂ density, resulting in a less relative extraction rate of this compound at 10 MPa and 40 °C than at 10 MPa and 60 °C or 8 MPa and 40 °C (Table 2).

It was found that the reduction in piperine relative extraction rate was likely due to a decrease in CO₂ density. This was consistent with the results observed by Vidal and Richard (1987). Vidal and Richard (1987) reported that in the supercritical region, as the density of CO₂ is reduced, the relative extraction rate of piperine decreases, and with increasing temperature it decreases still further. In the present experiment, at 20 MPa, the piperine relative extraction rate (experimental values) was 35.7, 35.0, 35.3, 35.9, and 33.2% at 45, 50, 55, 60, and 65 °C, respectively. Their corresponding densities were 0.81, 0.79, 0.77, 0.73, and 0.60 g/mL, respectively.

Antioxidant Activity. The EO extracted by SF-CO₂ at 10 MPa and 60 °C was used because it resulted in the highest quality of EO when classification index and oxygenated compounds were used as the criteria. SF-CO₂ OR obtained at 28 MPa and 60 °C was also chosen due to the high recovery of yield and piperine content.

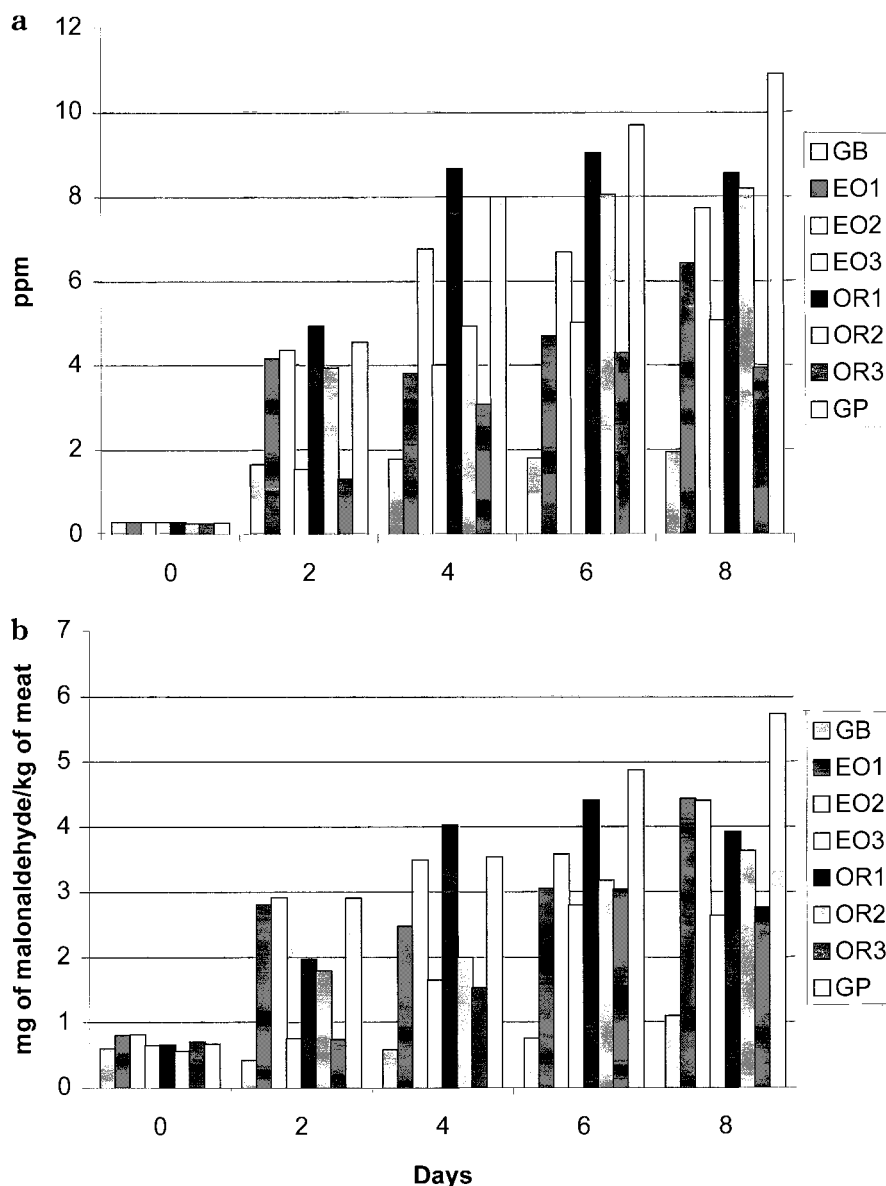


Figure 1. Changes in hexanal levels (a) and TBARS value (b) of cooked ground pork with black pepper oils and oleoresins from SF-CO₂ and conventional extractions during storage at 4 °C for 8 days. Note: LSD = 0.0663, 0.2447, 0.234, 0.4373, and 0.6067 for 0, 2, 4, 6, and 8 days, respectively, $\alpha = 0.01$ (a); LSD = 0.05, 0.0112, 0.0095, 0.0141, and 0.0223 for 0, 2, 4, 6, and 8 days, respectively, $\alpha = 0.01$ (b). Note: GB, EO1, EO2, EO3, OR1, OR2, OR3, and GP represent ground pork mixed with ground black pepper, ground pork mixed with commercial essential oil, ground pork mixed with laboratory essential oil, ground pork mixed with essential oil obtained from supercritical carbon dioxide extraction, ground pork mixed with commercial oleoresin, ground pork mixed with laboratory oleoresin, and ground pork mixed with oleoresin obtained from supercritical carbon dioxide extraction, and ground pork without extract or ground spice added, respectively.

In the present experiment, 4 hydrocarbons, 14 carbonyls, and 5 alcohols from all isolated volatiles were the compounds that were reported to be related to lipid oxidation products found in cooked pork (Ho et al., 1994). The key volatiles detected by GC were pentanal, hexanal, heptanal, octanal, and 2,3-octanedione. Hexanal was predominant among the aldehydes and gave the most consistent results for interpretation of antioxidant properties among treatments. Hexanal is the only aldehyde that arises from both the 9- and 13-hydroperoxides of linoleate, which is the unsaturated fatty acid found in the greatest concentration in pork (Shahidi and Pegg, 1994). In addition, hexanal has been reported to be one of the most potent flavor volatiles derived from linoleic acid and has been related to warmed-over flavor (Handley et al., 1997; Shahidi and Pegg, 1994; Shahidi, 1994). Despite its potential further degradation into

hexanoic acid, the content of hexanal has been reported to be a good indicator for determination of flavor deterioration in meat due to autoxidation after storage for up to 10 days (Shahidi et al., 1987).

In comparison to conventionally prepared oleoresins, cooked ground pork mixed with SF-CO₂ OR showed lower increases in hexanal levels during storage for up to 8 days (Figure 1a). A similar result was also reported in TBARS (Figure 1b). These may be because of the higher piperine content. As postulated by Chipault et al. (1956), Govindarajan (1977), and Milbourne (1987), the antioxidative action of black pepper has been attributed to its piperine, piperine isomers, and monoterpene contents. However, the most potent compound exhibiting an antioxidant property was reported to be the piperine. SF-CO₂ EO was less consistent in reducing aldehydes after 2 days compared to conventional EO

samples, possibly due to lesser monoterpene hydrocarbons (Figure 1a). Nonetheless, the increases in TBARS during storage for up to 8 days were less after addition of SF-CO₂ EO as compared to conventional extracts (Figure 1b). The large increase in hexanal concentration and TBARS of cooked ground pork mixed with conventional EO extracts may be due to high thermal oxidation products contained in extracts during production. Govindarajan (1977) reported that the ratio of unsaturated fatty acids to saturated fatty acids in black pepper was approximately 65:35%, and oleic and linoleic acids were the most predominant. Therefore, the elevated temperature during steam distillation in the production of conventional black pepper EO can contribute to thermal oxidation of those unsaturated fatty acids. The smallest increase in aldehyde level and TBARS value after storage for 4 days was found in the ground black pepper sample. These results agree with those of Farrel (1990) and Govindarajan (1977) who reported that spice extracts seem to possess less stability in providing flavor, color, and heat intensity than ground spices. In addition, it may be because the spice extracts used in the present experiment were not encapsulated, resulting in less stability of the antioxidant compounds. A correlation between the content of hexanal and TBARS value has been found ($r = 0.64$, $\alpha = 0.05$). Shahidi et al. (1987) has also found that a linear relationship existed between the TBA numbers and hexanal levels of cooked ground pork.

CONCLUSIONS

In practice, EO and OR from SF-CO₂ extraction (at 10 MPa and 60 °C and at 28 MPa and 60 °C for EO and OR, respectively) may be suitable for seasoning meat and other products. These SF-CO₂ extracts have been found superior in reducing the content of hexanal and TBARS value compared to conventional extracts. The hexanal content of ground pork mixed with SF-CO₂ OR was significantly lower than that from other treatments for up to 2 days. Additionally, antioxidant action of SF-CO₂ EO was found comparable to ground black pepper for up to 2 days. Both SF-CO₂ EO and conventionally prepared EO, nonetheless, were less effective as antioxidants than ground black pepper and both SF-CO₂ OR and conventionally prepared OR.

ABBREVIATIONS USED

SF-CO₂, supercritical carbon dioxide; CO₂, carbon dioxide; OR, oleoresin; EO, essential oil; GB, ground pork mixed with ground black pepper; EO1, ground pork mixed with commercial essential oil; EO2, ground pork mixed with laboratory essential oil; EO3, ground pork mixed with essential oil obtained from supercritical carbon dioxide extraction; OR1, ground pork mixed with commercial oleoresin; OR2, ground pork mixed with laboratory oleoresin; OR3, ground pork mixed with oleoresin obtained from supercritical carbon dioxide extraction; GP, ground pork without extract or ground spice added; LSD, least significant difference.

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