

# ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL AND OLEORESIN OF BLACK PEPPER FROM SUPERCRITICAL CARBON DIOXIDE AND CONVENTIONAL EXTRACTIONS IN GROUND PORK

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## INTRODUCTION

The utilization of isolated essential oils (EOs) and oleoresins (ORs) as an alternative to crude spices has increased in the meat industry. The extracts have several advantages over dried spice materials: microbial safety, better stability during storage, high concentration of flavor components, ease of handling, reduced storage space, no seasonal variation, and standardization. The process for obtaining EO results in the elimination of highly volatile compounds through evaporation and the loss of water-soluble compounds. Likewise, solvent in the production of ORs leave undesirable residues and piperine can be degraded during extraction and subsequent removal of solvent. Supercritical fluid carbon dioxide (SF-CO<sub>2</sub>) is being used for the extraction of various botanicals to obtain aroma concentrates and EOs. The process offers the advantages of low-temperature processing, recovery of solvent-free extract, rapid extraction resulting from high mass transfer due to higher diffusivity, and lower viscosity in comparison to organic solvents.

## OBJECTIVES

The objective of this study were to evaluate the conditions of SF-CO<sub>2</sub> to selectively obtain EO and OR from black pepper, with a goal of complete recovery of volatile oil compounds and piperine, to compare the quantitation of piperine between spectrophotometric method and HPLC technique, and to determine the antioxidant activity of EO and OR of black pepper from SF-CO<sub>2</sub> extraction compared to conventional extracts and ground black pepper when added to ground pork.

## EXPERIMENTAL

### Preparation of Materials

Ground black pepper (*Piper nigrum* L.) was obtained from MNE's Herbs & Spices mesh No. 40 and stored at 4 °C. EO was then removed by using a Clevenger distillation apparatus for 6 h as described in AOAC (1995) method 962.17. Pepper (20 g) was also extracted by refluxing for 4 h with dichloromethane (98% v/v) to obtain OR, with exclusion of light as far as possible. After cooling, the extract was evaporated with a rotor evaporator. Both EO and OR were collected in amber-colored bottles and stored at 4 °C. The separation techniques were duplicated with three replications. Commercial extracts of EOs and ORs were obtained from commercial sources (St. Louis Flavors, The Lebermuth Co., Inc. and CHR Hansen Spice Extracts) and stored at 4 °C.

The SF-CO<sub>2</sub> extractions were carried out on 1.1 (±0.1) g black pepper powder in an ISCO (model SFX 2-10) extractor and 260 D syringe pump. The EO extraction was carried out at 8 MPa or 10 MPa and at 40 °C or 60 °C with 40 ml of CO<sub>2</sub> used. The OR extraction conditions were 20-32 MPa and 45-65 °C with 60 ml of CO<sub>2</sub> used. The length and diameter of restrictors used were 20 cm and 50 cm or 100 mm for EO and OR respectively. Both extracts were collected in tubes placed in amber-colored bottles and stored at 4 °C. Each SF-CO<sub>2</sub> extraction parameter was duplicated three times for quantitation and repeated until at least 2.0 ml EO and 6.0 g OR were collected. Each SF-CO<sub>2</sub> extract was combined prior to quantification and application.

Ground pork (600 g) was mixed with 1.6% salt and ground black pepper or three different EOs and ORs (commercial, lab and SF-CO<sub>2</sub>) at the levels of 0.4%, 0.0043% and 0.0216%, respectively. The samples were cooked to 65 °C and stored at 4 °C for up to 8 days. The control treatment was ground pork. Each treatment was divided into 2 groups of 50 g and 1 group of 150 g for the analysis of volatiles and TBA value, and sensory evaluation, respectively.

### Analysis

The EO fractions were injected (2 µl) and analyzed by gas chromatography in a Perkin-Elmer model 8500 gas chromatograph (FID detector) on a capillary column (Supelcowax 10), 60 m x 0.25 mm i.d. with helium as the carrier gas. The gas chromatography was programmed at 50 °C (0.00 min. isothermally) to 250 °C at 4 °C/min. with isothermal period of 10.00 min. at 250 °C. The gas chromatography conditions were: injector 250 °C; detector 265 °C; carrier gas flow rate 2ml/min.; split ratio 1:50; and column inlet pressure 30.00 Psi. The data were analyzed using the built-in processor of the gas chromatograph. Quantitation of volatiles was completed in duplication and performed using relative peak areas compared to peak areas of the internal standard (n-nonanol). The peaks were identified using retention time and the knowledge of previous mass spectral data of black pepper volatiles.

Determination of piperine fractions was carried out by the spectrophotometric method as described in AOAC (1995) Method 987.07 and HPLC method. Each fraction was determined in duplicate for three times in both methods. In HPLC technique, each OR fraction was first softened by gentle warming, homogenized by stirring and accurately weighed (±0.0001g) as a 1.0000 g well-mixed sample into 100 ml volumetric flask. Methanol (HPLC grade) was added to volume and the mixture was well shaken until dissolved. A 10 ml well-dissolved solution was pipetted into 100 ml volumetric flask and diluted to volume. A 20 ml 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 micron, and the length 15 cm with the use of guard column. The mobile phase was methanol and water at a ratio of 60:40 with flow rate of 1.5 ml/min. Identification of piperine was obtained by comparison to the retention time of standard and by enrichment fractions with an authentic piperine at the wavelength of 343 nm.

After storage for 0, 2, 4, 6 or 8 days, 5 g of each treatment was weighed into a 500 ml round bottom flask. Distilled deionized water (50 ml) and 10 ml of internal standard (2-methyl-4-octanone in pentane) were added to the flask. The flask was immediately fitted to the purge and trap apparatus containing a glass tube filled with a solid adsorbant (Tenax TA, 60/80 mesh, Alltech Associates, Inc., Deerfield, IL). The flask was heated using a sand bath at 100-200 °C and refluxed for 30 min. while the contents were stirred using a magnetic stirrer 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 with two replications.

The volatiles were thermally desorbed onto a gas chromatographic column using thermal desorption unit model 890 (Dynotherm Analytical Instruments, Inc., Kelton, PA). The desorption temperature, transfer line temperature and the valve temperature were 230, 200 and 180 °C respectively. Desorption time was 5 min.

The volatiles were analyzed by gas chromatography in a Perkin Elmer model 8500 gas chromatograph (Perkin Elmer Corporation, 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 0.5 mm. Helium carrier gas flow rate was 3 ml/min with split ratio of 1:7.6. Column oven temperature program of 35 °C hold for 5 min, 8 °C/min to 220 °C and 2.5 °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 peaks were identified using retention time and the knowledge of previous mass spectral data of meat flavor volatiles.

The TBA test was carried out in duplicate with two replications as per Tarladgis et al. (1960).

Five g of each treatment was evaluated for warmed-over flavor (WOF) intensity by six trained panelists after storage of 0, 2, 4, 6 or 8 days. The terms used for off-flavors were stale, cardboard, painty and fishy. The intensities were ranked on the scale of 0-10 (least acceptable to most acceptable).

## RESULTS AND DISCUSSION

### Recovery of Volatile Compounds and Piperine

SF-CO<sub>2</sub> extraction has been found to result in superior EO compared to conventional extraction, regardless of the lower oil yield. In addition to lower classification index (the ratio of sesquiterpene to monoterpene hydrocarbons), the superiority of EO obtained from SF-CO<sub>2</sub> was also due to the higher recovery of the oxygenated hydrocarbons as they provide the main constituent of aroma of pepper oil. The EO obtained at 10 MPa and 60 °C, with the density of CO<sub>2</sub> at 0.294 g/ml, was considered superior to all extracts, as per classification index and the recovery of oxygenated hydrocarbons (Table 1). The highest recovery of volatile oil compounds was found at 10 MPa and 40 °C, with the density of CO<sub>2</sub> at 0.60 g/ml. The SF-CO<sub>2</sub> EO extracted at 10 MPa was found superior in quality than 8 MPa due to the lower classification index value. Consequently, the higher recovery of high boiling fractions from SF-CO<sub>2</sub> extraction was related to increasing of solubility of dense CO<sub>2</sub>.

Table 1. Recovery of pepper volatile oil obtained by steam distillation and SF-CO<sub>2</sub>

	Steam distillation	SF-CO <sub>2</sub>			
		8MPa	8MPa	10MPa	10MPa
		40 °C	60 °C	40 °C	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
Oxygenated hydrocarbons(%)	3.05	5.72	6.18	6.47	7.63
Monoterpenes(%)	71.95	57.53	63.55	59.64	48.71
Sesquiterpenes(%)	23.85	40.82	34.75	40.38	51.19

The increases of OR yields were less consistent with increasing of solubility of dense CO<sub>2</sub>. However, the increases of solubility tended to allow higher OR yield with complete recovery of piperine when compared to solvent extraction. Pepper OR extracted with SF-CO<sub>2</sub> is as feasible as solvent-extracted OR recovery (6.1%) and was not different from OR fractions obtained at pressures of 28 and 32 MPa and at temperatures of 55 and 60 °C (6.13, 6.55, 6.05 and 6.33% respectively). SF-CO<sub>2</sub> ORs obtained at 28 MPa (55 and 60 °C) and at 32 MPa (60 °C) were found comparable to conventional extracts, using yield and recovery of piperine (HPLC method) as criteria. The HPLC conditions used provided a means for determining piperine in OR extracts with a good degree of specificity and with fair precision compared to the AOAC official method.

### Antioxidant Activity

The key volatiles detected by GC were pentanal, hexanal, heptanal, benzaldehyde, octanal, 2,3-octanedione and 5 hydroxy methyl furfural. Hexanal was found predominant among these aldehydes and giving most consistent results for interpretation of antioxidant properties among treatments. In comparison to conventionally prepared oleoresins, cooked ground pork mixed with SF-CO<sub>2</sub> OR showed lower increases in hexanal levels during storage for up to 8 days (Fig. 1). A similar result was also reported in TBA values. SF-CO<sub>2</sub> EO was less consistent in reducing aldehydes after 2 days compared to conventional EO samples, possibly due to lesser monoterpene hydrocarbons. Nonetheless, the increases in TBA value during storage for up to 8 days were less in SF-CO<sub>2</sub> EO compared to conventional extracts. The smallest increase in aldehyde level and TBA value after storage for 4 days was found in the ground black pepper sample. As reported by Farrel (1990), spice extracts seem to possess less stability in providing flavor, color and heat intensity than ground spices. Ground black pepper, SF-CO<sub>2</sub> OR and EO were indicated most acceptable in retarding meat flavor deterioration as a result of low WOF intensity. This was consistent with the lower aldehyde levels and TBA values.

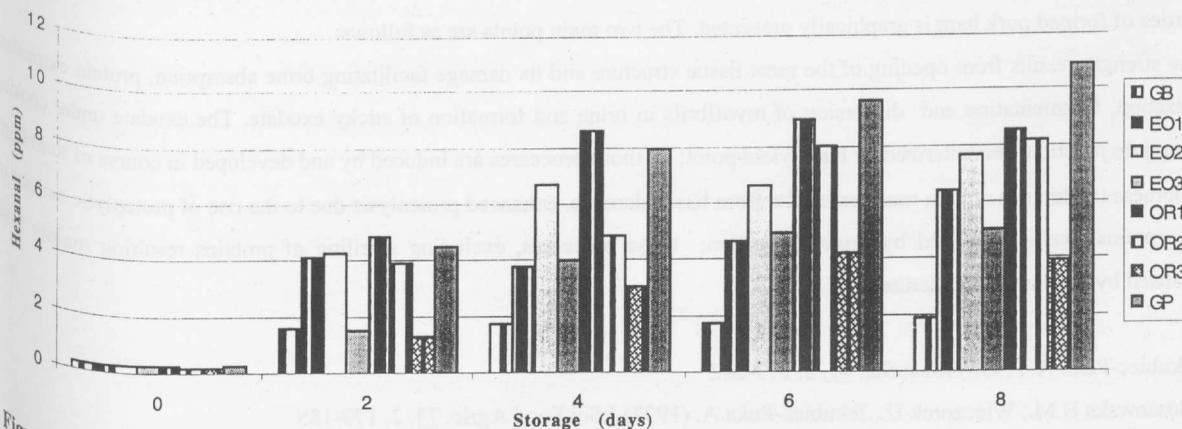


Figure 1. Changes in hexanal levels of cooked ground pork with black pepper oils and oleoresins from SF-CO<sub>2</sub> and conventional extractions during storage at 4 °C for 8 days.

Noted: GB, EO1, EO2, EO3, OR1, OR2, OR3 and GP represent ground black pepper, laboratory essential oil, commercial essential oil, SF-CO<sub>2</sub> essential oil, laboratory oleoresin, commercial oleoresin, SF-CO<sub>2</sub> oleoresin and control respectively.

### CONCLUSIONS

In practice, EO and OR from SF-CO<sub>2</sub> extraction may be suitable for seasoning meat and other products. SF-CO<sub>2</sub> extracts have been found superior in reducing lipid oxidation compared to conventional prepared or extracts. Additionally, antioxidant action of SF-CO<sub>2</sub> EO and OR was found comparable to ground black pepper for up to 2 days. Both SF-CO<sub>2</sub> EO and conventionally prepared EO, nonetheless, were less effective as antioxidants than ground black pepper and both SF-CO<sub>2</sub> OR and conventionally prepared OR.