

# Supercritical Fluid Application Notes

## SFE543: Enzyme-Assisted Supercritical Carbon Dioxide Extraction of Piperine in Black Pepper Oleoresin

Black pepper (*Piper nigrum* L.) is known as the King of Spices and is the most popular spice internationally. The active ingredient of black pepper is piperine, a potent therapeutic compound.

Piperine is typically extracted by methods that may promote thermal degradation, hydrolysis of useful pharmaceutical compounds, and traces of solvents in the extracts. Supercritical CO<sub>2</sub> extraction offers an alternative, green extraction technique suited for solvent free extracts of spices for therapeutic applications.

In this work, enzyme-assisted supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of black pepper oleoresin was investigated using  $\alpha$ -amylase (from *Bacillus licheniformis*) for enhanced yield of piperine-rich extract possessing a good combination of phytochemical properties.

Black pepper contains approximately 58% carbohydrate and starch (30.4 %) is the predominant carbohydrate in the sample. Since starch may inhibit the extraction of piperine, the use of starch degrading enzymes, such as  $\alpha$ -amylase for pre-treatment of the pepper matrix prior to extraction renders extraction easier and improves the piperine yield of the extracts.

### Materials

(Please refer to references for detailed procedures)

Malabar Garbled black pepper

Standard piperine (97% pure)

$\alpha$ -amylase from *B. licheniformis*  
(lyophilized powder)

### Sample Preparation

Black pepper berries were ground using an electric mixer to dp of 0.42

For enzyme-assisted extraction of black pepper oleoresin, the powdered pepper sample was mixed with the lyophilized enzyme in optimized ratio (enzyme: black pepper powder 1: 5000) and subjected to continuous modes of extraction.

### Equipment

Applied Separations Supercritical Extraction  
Equipment: *Spe-ed* SFE 2



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## Extraction Conditions

Extraction vessel: 50 mL  
Sample: 20 g  
Pressure: 300 Bar  
Temperature: 60 °C  
CO<sub>2</sub> Flow Rate: 2 L/min (gas)  
Static time: 30 minutes  
Dynamic time: 15 minutes  
Collection: 60 mL pre-weighed vial

## Analysis:

Densitometric analyses of black pepper extracts were conducted to estimate the total piperine content using a Camag TLC. The plates were scanned with Camag TLC Scanner 3 at 337 nm using a Deuterium lamp at a scanning speed of 20 mm/s. Amount of piperine present in the extracts was determined from the standard curve prepared using pure piperine.

## Results

Enzyme-assisted SC-CO<sub>2</sub> vs SC-CO<sub>2</sub> extraction of black pepper at 60C and 300 bar.

Method	Yield piperine (g extract/100 g dry black pepper)
Enzyme Assisted SC-CO <sub>2</sub>	5.3 (SD .2 )
SC-CO <sub>2</sub>	4.6 (SD .2)

## Conclusion

Alpha-amylase assisted SC-CO<sub>2</sub> extraction of black pepper oleoresin enhanced the yield of piperine-rich extract with a good mix of phytochemical properties (antioxidant activity, total phenolic content, reducing power, and anti-inflammatory activity).

## References

Dutta S, Bhattacharjee P. Enzyme-assisted supercritical carbon dioxide extraction of black pepper oleoresin for enhanced yield of piperine-rich extract. Journal of bioscience and bioengineering. 2015 Jul 1;120(1):17-23.

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