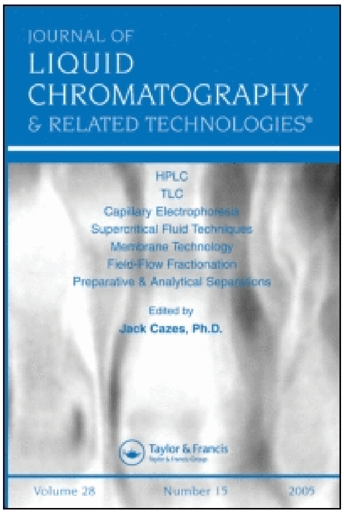

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COMPARISON OF ACETALS AS IN SITU MODIFIERS FOR THE SUPERCRITICAL FLUID EXTRACTION OF β-CAROTENE FROM PAPRIKA WITH CARBON DIOXIDE

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COMPARISON OF ACETALS AS IN SITU MODIFIERS FOR THE SUPERCRITICAL FLUID EXTRACTION OF β-CAROTENE FROM PAPRIKA WITH CARBON DIOXIDE

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ABSTRACT

This study investigated various acetals for the purpose of improving the supercritical fluid extraction (SFE) using carbon dioxide of β-carotene from an aqueous matrix. Results showed that the presence of water decreases the extraction efficiency of β-carotene from paprika by a factor of 2. Upon the addition of 2,2-dimethoxypropane (DMP), the extraction efficiency of β-carotene from wet paprika was increased by a factor of about 2.5 and from dry paprika a factor of 1.5. Diethoxypropane (DEP) and triethylorthoformate (TEOF) proved to be less effective for improving the extraction of β-carotene from paprika. Although the addition of an acid catalyst enhances the reaction between DMP or TEOF with water under SFE conditions, an actual decrease in the extraction of carotene from paprika was found. It is possible, even without an acid catalyst, that DMP can remove a little water, improving extraction efficiency. However, its main impact on improving SFE seems to be as a modifier.

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INTRODUCTION

Supercritical fluid extraction (SFE) has three main advantages over conventional liquid extraction. Because fluids used for SFE are usually nontoxic, waste removal and environmental safety concerns hardly ever come into question. SFE is performed in considerably less time (10-60 min) due to enhanced mass transfer characteristics of supercritical fluids. Finally SFE yields samples that are not extensively diluted but instead ready for analysis.

Studies have shown that the presence of water in biological samples can interfere with the effectiveness of supercritical fluid extraction with $CO_{2.}$[1] Some analytes (particularly organic amines) can have more of an affinity for the aqueous sample matrix than the supercritical CO_2 which is only moderately polar.[2] The presence of water can also alter the sample matrix in such a way as to hinder diffusion of the supercrititcal fluid through it. In an effort to counteract the problem of water in samples, some researchers have dried samples by dehydration in an oven at 50 - 100 ^{o}C.[1] Samples containing volatile or unstable components cannot be dehydrated. Freeze drying has also been used to counteract water in samples.[3] However, freeze drying is time consuming and requires additional equipment. Adsorbents such as diatomaceous earth[1,4] and sodium sulfate[5] have also been used to alleviate water problems. The problem with the use of adsorbents is that the undesirable adsorption of the analyte to the sample matrix can occur. Since adsorbents are powder-like and are mixed with the sample, it is impossible to recover the sample for further analysis.

Acetals such as 2,2-dimethoxypropane (DMP) and diethoxypropane (DEP) can react with water to form acetone and an alcohol (Reactions 1 and 2).

$$(CH_3)_2C(OCH_3)_2 + H_2O \rightarrow 2CH_3OH + (CH_3)C{=}O(CH_3) \qquad (1)$$

$$(CH_3)_2C(OCH_2CH_3)_2 + H_2O \rightarrow 2CH_3CH_2OH + (CH_3)C{=}O(CH_3) \qquad (2)$$

Triethylorthoformate (TEOF) can give a more complete reaction with water than DMP forming methanol and ethyl formate (Reaction 3).

$$HC(OCH_2CH_3)_3 + H_2O \rightarrow 2CH_3CH_2OH + HC{=}O(OCH_2CH_3) \qquad (3)$$

Analytically DMP has been used to remove water prior to FT-IR detection[6] and to remove water from normal phase HPLC mobile phases.[7] To the best of our knowledge, DEP has not been investigated previously for the analytical removal of water. The determination of water in solvents using TEOF by GC has been reported.[8,9]

β-Carotene is a precursor to Vitamin A which falls into the class of nutrients called antioxidants. Antioxidants are believed to counteract the effects of oxidative processes in the body which can cause chronic diseases such as cancer.[10,11] Fruits and vegetables that are orange or red in color most commonly have a high concentration of β-carotene. Although other isomers are present, β-carotene represents the dominant form. β-Carotene has been extracted from many fruits and vegetables using SFE with CO_2.[3,4,12] All of the procedures described use some type of drying method such as the addition of adsorbents or freeze drying to minimize the effects of water that may be present in the sample. Two of the procedures described use ethanol as a modifier during extraction.[3,4] Recoveries of β-carotene were 15-25 % higher using SFE than traditional liquid solvent extraction.

In this study we have chosen to use paprika as a model SFE system for extracting β-carotene from foods that may contain moisture. Paprika serves as an excellent model system because of its dry and powdery texture, not unlike most fruits and vegetables after homogenization prior to extraction. The relatively dry nature of paprika also proves to be an advantage because it is possible to control the water content of the sample more easily. A comparison of the acetals DMP, TEOF, and DEP each as an additive for the in situ removal of water during SFE of paprika is the primary purpose of this study.

EXPERIMENTAL

Materials

The three acetals DMP, DEP, TEOF, as well as ethyl formate were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Spectranalyzed grade toluene, ethanol, methanol, and acetone, as well as glacial acetic acid (17.4 M) were supplied by Fisher Scientific (Fair Lawn, NJ). Industrial grade CO_2 gas cylinders with dip tubes were purchased from Air Products and Chemicals, Inc. (Allentown, PA) and Doansco, Inc. (Hamilton, OH). A SFE/SFC grade CO_2 gas cylinder with a dip tube was purchased from AGA Specialty Gas (Maumee, OH). McCormick® brand paprika was purchased from a local grocery store. Trans β-carotene was purchased from Sigma Chemical Co. (St. Louis, MO).

Instrumentation

A Hewlett Packard Model 7680A supercritical fluid extractor (Avondale, PA) equipped with a stainless steel bead trap was used for all extractions. The

Table 1

Summary of SFE Extraction Conditions

Fluid Delivery
Density: 0.93 g/mL
Pressure: 338 bar
Flowrate: 1.5 mL/min

Extraction Chamber
Temperature: 40°C
Equilibration time: 10.0 min
Thimble size: 7.0 mL
Thimble volumes swept: 2.1

Analyte Trap
Nozzle temperature: 45°C; Trap temperature: 20ºC
Trap packing: stainless steel beads
Void volume compensation: 1.0 mL
Fraction output: two 1.7 mL volumes of toluene at 2.0 mL/min
with trap at 40°C

absorbance spectra of the carotenes in the SFE extracts were measured using a Hewlett Packard Model 8452A photodiode array spectrophotometer. All absorbance measurements were taken at 464 nm. All gas chromatography was performed using a Perkin Elmer 8500 gas chromatograph (Norwalk, CT) with flame ionization detection and equipped with a J&W (Folsom, CA) 30 m x 0.32 mm I.D. DB5 (1.0 µm) fused silica capillary column. The GC injector and detector temperatures were 250^{o}C.

SFE Procedure

Paprika samples, approximately 26 mg, were weighed on filter paper. Dry paprika samples were used as is. Wet paprika samples were prepared by spiking dry samples with 100 µL of H_2O. The paprika samples and any reagents used were placed into the thimble which contained a glass wool or Kimwipe plug. DMP, TEOF, or DEP was added to the samples using an airtight syringe. Paprika samples, in the presence of various volumes of DMP, TEOF, or DEP, were subjected to SFE with CO_2. SFE conditions were as shown in Table 1. In some studies we chose to use glacial acetic acid as a catalyst; methanesulfonic acid proved to be corrosive particularly to the thimble caps. The extracts were washed from the stainless steel bead trap using toluene. One milliliter of the extract from the first toluene wash was diluted to 10 mL and the absorbance spectrum of the carotenes (assumed principally to be β-carotene) was measured. A previous HPLC study of paprika pigments has indicated the yellow color is due principally to β-carotene and to a lesser extent capsanthin.[13] The first derivative spectra of a β-carotene standard and an extract of paprika were found to be fairly similar over the visible wavelength range. A 24 mg/L solution of trans β-carotene was used for reference purposes. This solution was stored in the dark in the refrigerator and prepared fresh daily.

GC Procedure

The reaction of DMP or TEOF under SFE conditions was performed using stoichiometric equivalents of DMP or TEOF with or without 100 μL of water and with or without an acetic acid catalyst. Injections of 1 μL each of the undiluted SFE extract from the first toluene wash were made into the gas chromatograph. Temperature programs were designed to permit the resolution of low boiling compounds first before stripping the higher boiling toluene solvent off the column. The temperature program for the DMP reaction extract was: 50°C held for 0.7 min, ramped at 30°C/min until 75°C which was held for 0.9 min, and then ramped at 30°C/min to 225°C. The temperature program for the TEOF reaction extract was: 33°C held for 0.5 min, ramped at 30°C/min until 80°C which was held for 2.0 min, and then ramped at 30°C/min to 220°C. For peak identification and development of the optimum GC conditions, reaction mixtures of 1 mL of each reagent and/or the reaction products were diluted to 10 mL and injected into the gas chromatograph.

RESULTS AND DISCUSSION

A comparison of the extraction of dry and wet paprika (26 mg) by sonication in 10 mL of toluene for 30 min was made. The absorbance data at 464 nm showed the recovery of β-carotene was about 15% less from wet paprika as compared to dry paprika. It was noted that this presence of 100 μL of water which dampened the paprika sample completely caused the particulate sample to clump up when toluene was added. It is likely the available surface area of the wet paprika sample in contact with toluene during the sonication is lessened causing the reduced recovery of β-carotene. It seems reasonable that a similar process could be occurring during the SFE of wet paprika.

The optimum pressure at which β-carotene was extracted from dry paprika was determined by monitoring the absorbance at 464 nm of the β-carotene extract as shown in Figure 1. Extraction efficiency was dramatically reduced at pressures less than 250 bar. It is well known that increasing the pressure

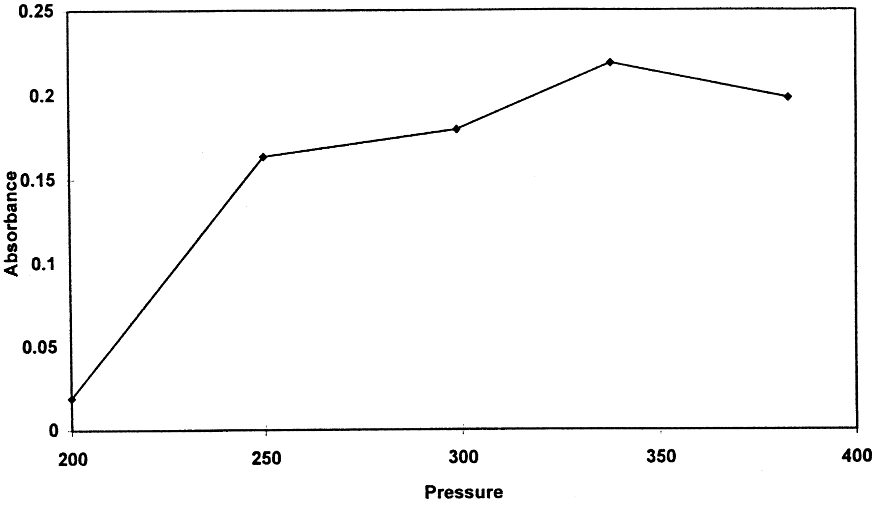

Figure 1. Absorbance of β-carotene extracted by SFE from dry paprika vs. SFE pressure.

Table 2

Summary of Absorbance Data for Different Paprika SFE Conditions

Extraction Condition	**Absorbance at 464 nm**	
	Dry Paprika	**Wet Paprika**
No modifier	0.198 ± 0.022[a]	0.103 ± 0.014[b]
DMP (500 μL)	0.300 ± 0.003[a]	0.245 ± 0.001[a]
DMP (500 μL) + acetic acid (20%)	ND	0.111
Methanol (467 μL)	0.140	ND
Acetone (420 μL)	0.127	ND

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[a] Average Deviation (n = 2).
[b] Standard Deviation (n = 4).
ND: Not determined.

increases the density and supercritical extraction efficiency of the chosen fluid.[14] The trap temperature during the fraction output step was varied from 5 to 50°C in increments of 10°C. Temperatures below 35°C decreased slightly the extraction efficiency of β-carotene. In all other studies, temperature and

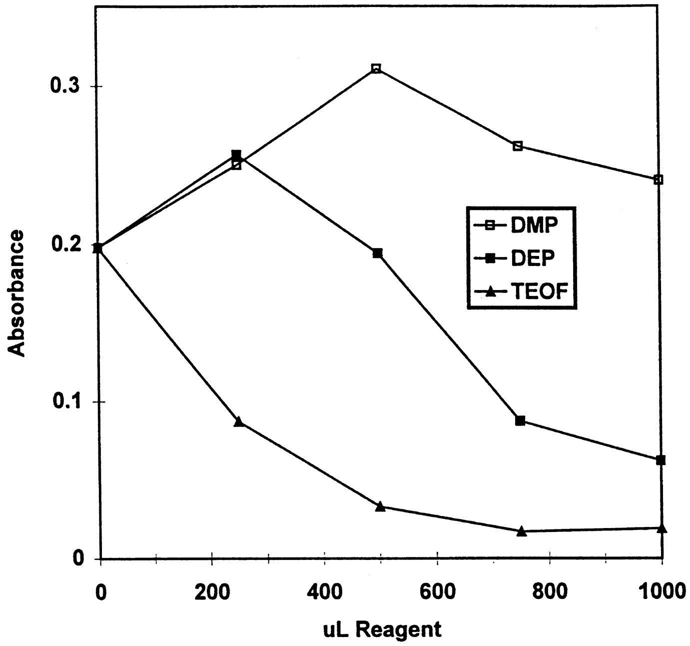

Figure 2. Absorbance of β-carotene extracted by SFE from dry paprika vs. μL of DMP, DEP, or TEOF.

pressure were chosen to be 40°C and 338 bar, respectively. These optimum SFE conditions were the same as those used by Marsili and Callahan.[4] Table 2 summarizes absorbance data for β-carotene extracted from paprika under SFE conditions. First the absorbance spectra of SFE extracts originating from dry and wet paprika were compared. On average, the absorbance at 464 nm of β-carotene extracted from dry paprika is 0.198 while that from wet paprika is 0.103. In general, this presence of water caused the extraction of β-carotene to be approximately half that of β-carotene from dry paprika. The difference in extraction efficiency as measured by carotene absorbance when DMP is added without an acid catalyst to dry and wet paprika samples was determined.

Upon the addition of 500 μL of DMP, the extraction efficiency of dry paprika was increased by a factor of nearly 1.5 which indicates that DMP can act as a favorable modifier for the extraction of β-carotene. The addition of 500 μL of DMP (near stoichiometric to the 100 μL of water) to a wet paprika sample increased the extraction efficiency by a factor of about 2.5 when compared to wet paprika with no reagents. The extraction efficiency of β-carotene from wet

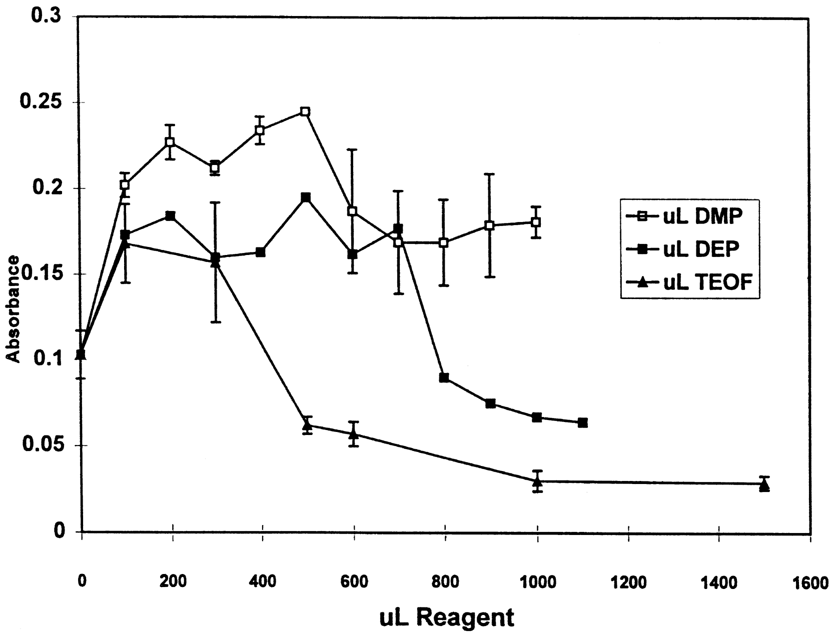

Figure 3. Absorbance of β-carotene extracted by SFE from wet paprika vs. μL of DMP, DEP, and TEOF.

paprika in the presence of DMP was also greater that that of β-carotene extracted from dry paprika in the presence of no reagents. The effect of acetals on the extraction of β-carotene from dry and wet paprika was studied over a volume range from about 0 to 1000 or 1500 μL.

Figure 2 shows the effect of the addition of various volumes of DMP, DEP, and TEOF on the extraction efficiency of β-carotene from dry paprika. Extraction efficiency of dry paprika is improved initially as DMP or DEP is added before a decrease is seen. The optimum DMP and DEP volumes are close to 500 μL and 250 μL, respectively. The presence of TEOF consistently causes a decrease of β-carotene recovery to near zero at 800 μL. An explanation for why this TEOF profile is significantly different from those of DMP or DEP is not obvious.

Figure 3 shows the effect of the addition of various volumes of DMP, DEP, and TEOF on the extraction efficiency of β-carotene from wet paprika without an acid catalyst. DMP enhances the extraction of β-carotene from wet paprika as well with a profile similar to that for dry paprika. The profiles for

TEOF and DEP in Figures 2 and 3 appear significantly different. All three reagents appear to enhance the extraction up to a volume of 300 μL. While DMP and DEP enhance the extraction of β-carotene from dry paprika to 500 μL, TEOF generally causes the extraction efficiency of β-carotene to be decreased. The enhanced recovery of β-carotene is consistently greater using DMP than DEP. The extractability of β-carotene from paprika is always improved by the presence of DMP up to 1000 μL. Above 700 μL, the presence of DEP causes the extraction of β-carotene to drop below that for no DEP added. The results of reproducibility studies, when various volumes of DMP or TEOF are added to wet paprika, are summarized below. For 0-1000 μL of DMP the average or standard deviations were in the range of 0.001-0.036; the RSD was in the range of 0.4-21.2% with an average of 9.3%. Reproducibility was best (1-5%) in the 100 – 500 μL DMP range. For 0-1500 μL of TEOF standard deviations were in the range 0.004-0.035; the RSD was in the range of 13.7-22.3% with an average of 15.0%.

Gas chromatography effectively separated the acetone and alcohol products due to the catalyzed and uncatalyzed reactions of DMP or TEOF and water under SFE conditions from the starting material and the toluene wash solvent. Figure 4 shows a comparison of the acetone peak areas plotted against DMP volume for the catalyzed and uncatalyzed DMP-water reaction under SFE conditions. The peak area of the acetone produced by the acid catalyzed reaction is remarkably greater, by a factor of 15 on average, than that of the same reaction performed without an acid catalyst for all concentrations shown. However, even without an acetic acid catalyst, DMP particularly when in a stoichiometric excess can react with the water to form some acetone. Similar results were shown by the TEOF with water reaction when run under SFE conditions. A comparison of the ethyl formate peak area versus volume of TEOF for catalyzed and uncatalyzed reactions of TEOF with water was made. The peak area of ethyl formate produced by the acid catalyzed reaction was much greater, by a factor of 35 on average, than that of the of the ethyl formate produced by the uncatalyzed reaction at 300-1500 μL of TEOF.

The previous acid catalyzed reactions discussed were performed with 100% of the stoichiometric equivalent of the acetic acid catalyst. The optimum acetic acid concentration required for both the DMP or TEOF water reaction and the stability of β-carotene was of interest. The catalytic effect of acetic acid was equivalent to that of 100% when as low as 20% of the stoichiometric equivalent of acetic acid is added to the DMP- or the TEOF-water reaction under SFE conditions. Under ambient conditions, the effect of various acetic acid concentrations corresponding to 0, 10, 20, and 100% of the stoichiometric equivalent on the absorbance profile for a β-carotene standard was determined.

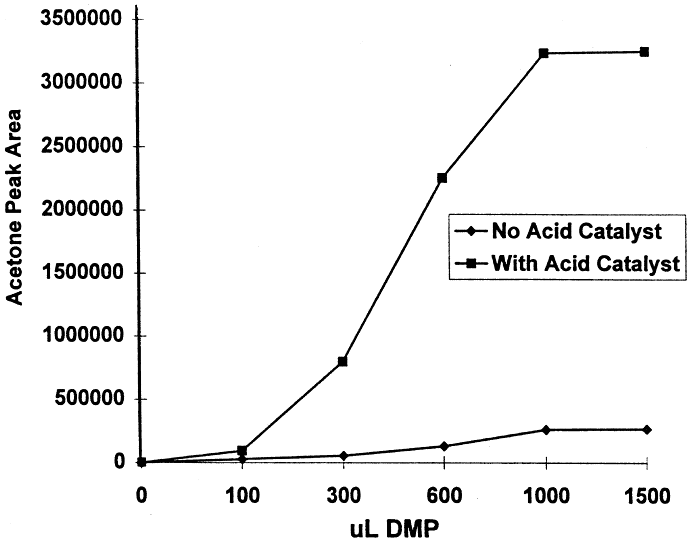

Figure 4. Comparison using acetone peak area of acid catalyzed and uncatalyzed DMP (0 -1500 μL) - H_2O (100 μL) reactions under SFE conditions. Acetic acid was at the 100 % stoichiometric equivalent level.

At low acid concentrations the absorbance spectra and the first derivative spectra of β-carotene remained unchanged. However, at 100% of the stoichiometric equivalent of acetic acid both the absorbance spectrum and the first derivative spectrum of β-carotene were changed. Since β-carotene is known to be unstable in the presence of acids,[15] it is reasonable to expect these spectral differences. Therefore a 20% acetic acid stoichiometric equivalent was considered optimum.

SFE results with the acid catalyzed reactions of DMP or TEOF with wet paprika are described below. The absorbance of β-carotene extracted in the presence DMP, water, and 20% of the acetic acid catalyst was a factor of 2 lower than when β-carotene is extracted in the presence of DMP and water with no acid catalyst (Table 2). The first derivative spectrum of β-carotene extracted in the presence of acetic acid, DMP, and water was slightly changed as compared to that in the absence of acetic acid. It is possible that β-carotene may be unstable even in the presence of low concentrations of acetic acid during SFE. When β-carotene is extracted in the presence of TEOF, water, and 20% of the stoichiometric equivalent of acetic acid, the absorbance was 0.039 at 464 nm which was slightly improved over that with no acetic acid but still low. One reason the extraction of β-carotene from wet paprika in the presence of an acetal and acetic acid is not improved or reduced may be because of the methanol or acetone formed. Both these reagents decreased the recovery of β-carotene from paprika (Table 2) as compared to that with no modifier. Even though water may be removed, the acetic acid remaining and the organic product formed may still hinder the extraction ability of CO_2. Since neither of the reaction products enhances the extraction efficiency of β-carotene from paprika, it is likely that DMP is acting more as a modifier during the extraction.

CONCLUSION

In this study we have demonstrated that water does, in fact, reduce the extraction efficiency of β-carotene from paprika by a factor of approximately 2. DMP is successful in minimizing the effects of moisture in paprika samples during supercritical fluid extraction of β-carotene with CO_2 by increasing extraction efficiency by a factor of about 2.5. DMP also proved to act as a favorable modifier during the extraction of β-carotene from dry paprika samples by increasing extraction efficiency by a factor of roughly 1.5 similar to that found for sonication of paprika in toluene. Upon the addition of methanol or acetone to dry paprika, extraction efficiency was reduced which further suggests the use of DMP as a modifier. Although the addition of an acid catalyst enhanced the reaction between DMP and water, no advantage in extractability was found. Perhaps even without an acid catalyst, DMP removed a little water improving SFE efficiency. However, its main impact on improving SFE efficiency is as a modifier. In the future, it is hoped that DMP could be proven as an effective modifier for other SFE applications.

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