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**YIELD AND QUALITY OF ONION FLAVOR OIL OBTAINED BY
SUPERCRITICAL FLUID EXTRACTION AND OTHER METHODS**

By

Aditi Dron

A THESIS

**Submitted to
Michigan State University
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1994

ABSTRACT

YIELD AND QUALITY OF ONION FLAVOR OIL OBTAINED BY SUPERCRITICAL FLUID EXTRACTION AND OTHER METHODS

By

Aditi Dron

Methods of extraction of onion flavor oil were studied including supercritical fluid extraction using carbon dioxide (CO₂), liquid CO₂ extraction and steam distillation-solvent extraction. The effect of using entrainers with supercritical fluid extraction was also studied. The yield and the quality of onion extracts obtained from the different methods were compared. The maximum yield of 0.0324% was obtained by supercritical CO₂ extraction at 3600 psi (24.5 MPa), 37°C at a CO₂ flow rate of 0.5 l/min. Ethyl alcohol used as entrainer enhanced the yield of onion oil over that obtained by supercritical CO₂ experiment without entrainer at the CO₂ flow rate of 1.0 l/min. Gas chromatography and combined gas chromatography-mass spectrometry of the extracts indicated that the flavor profiles were different for extracts obtained by different methods. Supercritical and liquid CO₂ extracts had fresh onion-like flavor as opposed to a cooked flavor of the extract obtained by steam distillation-solvent extraction.

DEDICATED TO MY PARENTS

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1. INTRODUCTION

1.1 OVERVIEW

Onions (*Allium cepa* L.) and other members of the genus *Allium* are among the oldest of the cultivated plants. Their origins, most likely in Central Asia, predate written history (Block, 1985). They possess strong, characteristic aromas and flavors which have made them important ingredients of food. A remarkable property of this genus is that most members have no odor unless the plant tissue is cut or otherwise damaged. Stoll and Seebeck (1951) found that these characteristic volatiles are absent from intact tissues and that the volatiles are enzymatically produced when injury occurs. Apart from their use in food, certain extracts of onions and garlic have been found to be antibacterial, antifungal and antithrombotic (Block, 1985).

Onion and onion flavors (onion oil) are important seasonings widely used in food processing. Currently the majority of onion oils used in U.S. food production are imported. These products, which are usually steam distilled, lack the fresh onion flavor. Their quality varies depending on the origin of production and is sometimes undesirable or inconsistent. Thus, for production of onion flavor oil on a commercial scale, a detailed study is required into the various possible methods for extraction and their comparative evaluation for yield and product quality.

Extraction using carbon dioxide (CO₂) as solvent is gaining attention because it is nontoxic, easily separated from the extract, non-flammable, inexpensive and available in high purity. CO₂ in liquid state has been used for extraction of many natural

products (Schultz and Randall, 1970). In addition, CO₂ in supercritical state is also being used for extraction of natural products. The critical temperature and pressure of CO₂ are 31°C and 1070.7 psi (7.4 MPa). Thus, supercritical CO₂ extractions can be carried out under relatively moderate conditions with minimal degradation of thermally labile flavor components (Rizvi et al., 1986). This technique has the ability to change and "fine tune" its solubilizing power by controlling pressure and temperature. Supercritical CO₂ is generally a more powerful solvent than liquid CO₂. However, liquid CO₂ tends to be more selective (Grimmett, 1981).

While supercritical CO₂ has many desirable properties, its polarizability is very low. Therefore, small amounts of co-solvents, which are referred to as modifiers or entrainers, may be added to modify the polarity and solvent strength of supercritical CO₂ to increase the solute solubility (and/or selectivity). The entrainers used are commonly polar or non-polar organic compounds which are miscible with supercritical CO₂.

This study was undertaken to research various methods for extraction of onion flavor oil and to compare the yield and quality of the product obtained by each method with the goal of evaluating SFE-CO₂ (supercritical fluid extraction using CO₂) feasibility in extracting a unique fresh onion flavor.

1.2 OBJECTIVES

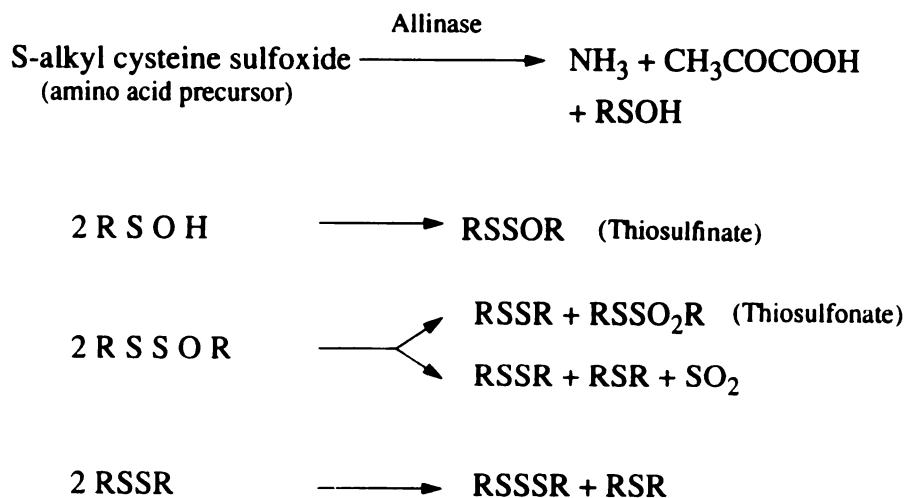
1. Compare the yields of onion flavor oil obtained by Supercritical CO₂ Extraction, Liquid CO₂ Extraction, and Steam Distillation-Solvent Extraction under the conditions studied.
2. Investigate the effect of using entrainers with Supercritical Fluid Extraction on yield of onion flavor oil.
3. Compare the quality of extracts obtained by the various methods.

2. LITERATURE REVIEW

2.1 ONION CHEMISTRY

2.1.1 Development of Flavor in Onion

The characteristic flavor of onions comes primarily from volatile organic sulfur compounds released enzymatically by the action of allinase (alliin alkyl-sulfenatase; EC 4.4.1.4) on several nonvolatile, odorless amino acid precursors, namely (+)-S-methyl-, (+)-S-propyl-, and trans-(+)-S-1-propenyl-L-cysteine sulfoxides, when the onion bulbs are chopped or crushed (Whitaker, 1976). The primary reaction products of these amino acids are thiosulfinates, which dissociate to produce various sulfides containing methyl, propyl, and propenyl groups, thiophene derivatives, and other sulfur-containing heterocycles (Carson, 1987). The formation of various sulfur compounds can be summarized as follows:



The alkyl thiosulfonates (methyl methane-, propyl methane-, and propyl propanethiosulfonates) have been associated with fresh onion-like flavors, while propyl- and propenyl- containing di- and trisulfides have been associated with cooked onions or steam distilled onion oils (Boelens et al., 1971).

Previous studies (Brodnitz et al., 1969; Boelens et al., 1971; Mazza et al., 1980; Kallio and Salorinne, 1990; Kuo et al., 1990) have reported flavor components of head space, solvent extracts, and distilled oils. Block (1985) reviewed the chemistry of garlic and onion. Block et al. (1986) reported synthesis of antithrombotic organosulfur compounds from garlic.

2.1.2 Properties of Onion Oil

Fenaroli (1971) described the onion oil as a yellowish liquid with a characteristic onion odor whose main constituents are di-n-propyl and methyl n-propyl disulfide with a specific gravity of 1.047-1.098 and solubility of 1:10 (in 90% ethyl alcohol).

Fenwick and Hanley (1985) described onion oil as a brown-amber liquid obtained in 0.002 to 0.03% yield by the distillation of minced onions which had been allowed to stand for some hours prior to distillation. It was reported that the oil comprises a complex mixture of (mainly) sulfur containing volatiles. The product possesses (on a weight basis) 800 to 1000 times the strength of odor of fresh onions, but its commercial value may be many thousand times that of the onion. The product is used for its solubility, lack of color, and strong aroma.

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Sinha et al. (1992) reported extraction of onion oil using supercritical carbon dioxide extraction. They described the oil as having the characteristic fresh onion-like flavor. Several new components were reported, including diallyl thiosulfinate and propyl methanethiosulfonate.

Onion oil has been granted the status "GRAS" (generally recognized as safe) by the Food and Drug Administration (Fenaroli, 1971). Onion oil forms part of the "White list" of the FDA which means that in order for a product to be safe, the conditions of intended use must be known and reasonable assurance given that the actual use conforms to the intended conditions.

Besides being commonly used as a food ingredient, onion oil shows other potential uses. Wit et al. (1979) found that onion oil (or garlic oil), when used in the proportion of 1.5 mg/g in meat slurry, inhibited toxin production by *Clostridium botulinum* type A (strain 73A). However, the inhibition of toxin production was not complete since the oil solution did not inhibit toxin production by *Clostridium botulinum* type B (strain R1V1) and type E (strain R1V2). Some studies were conducted on the inhibition of aflatoxin-producing fungi by onion extracts by Sharma et al. (1979), who reported the effect of an onion extract (2% v/v), onion oil solution (10% v/v), lachrymatory factor solution (1% v/v), and dipropyl disulfide on the growth rate of cultures of *Aspergillus flavus* and *Aspergillus parasiticus*. They found that the onion extract, the onion oil solution, and the lachrymatory factor solutions possess similar fungi-growth inhibitory properties and that the dipropyl disulfide solution hardly

inhibited the culture growth. Apparently the lachrymatory factor was the main component responsible for the inhibition of the aflatoxin-producing fungi growth.

2.2 SUPERCRITICAL FLUID EXTRACTION

2.2.1 Properties of Supercritical Fluids

Supercritical fluid extraction makes use of a supercritical fluid (SCF) as a solvent. A SCF is at a temperature above its critical temperature and at a pressure above its critical pressure. The supercritical region for a pure component is shown in Figure 1. This compressed gas has characteristics of both gases and liquids. It has the density of a liquid and functions like a liquid solvent, but it diffuses easily like a gas. A SCF has viscosity and diffusivity lying between that of a gas and a liquid. It is particularly attractive as an extracting agent because the solvent power can be manipulated by small changes in temperature and/or pressure.

Another important feature of extracting aroma concentrates with supercritical gases is that both the enhancement of vapor pressure and phase separation play a role in the process. In other words, two unit operations are carried out simultaneously, namely, distillation and solid-liquid or liquid-liquid extraction. Additionally, the zero surface tension of SCFs allows facile penetration into microporous materials. A reduction in solvent density with changes in temperature and/or pressure allows the recovery of solute and solvent. The result is a highly efficient extracting solvent.

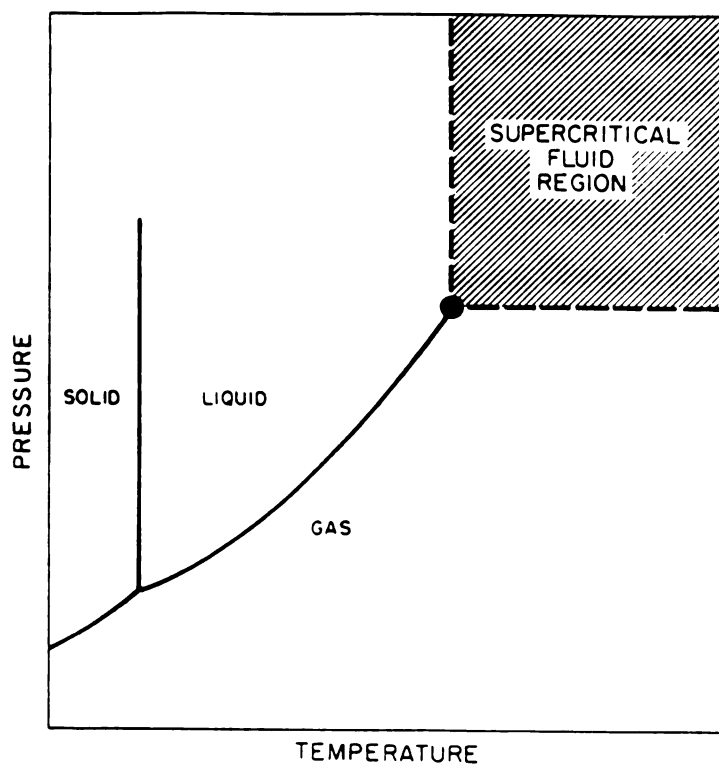


Figure 1. Pressure-Temperature Diagram of a Pure Component. From McHugh and Krukonis (1994).

2.2.2 Supercritical Fluid Extraction Process

2.2.2.1 How the Process Works

Supercritical extraction is a unique process that uses the special properties of fluids above their critical temperatures and pressures. In short, the solvent fluid is pressurized and heated to its supercritical state. Then it is introduced into the extraction vessel at the selected extractor operating conditions. The material in the extractor can be in either solid or liquid phase. In the extractor, the supercritical solvent extracts one or more components from the source material. The solute-rich supercritical fluid exits the extractor and undergoes a temperature and/or pressure change. This change decreases the solubility of the solute in the solvent fluid and, due to the change in solubility, a solute/solvent separation takes place in the separator vessel. Solute is removed and solute-lean fluid is pressurized and recycled in a continuous flow (Cohen, 1984).

2.2.2.2 System Components

- A supercritical fluid supply system
- An extraction vessel
- A pressure/ temperature control system
- A separation system
- A recycling system

Figure 2 shows a flow chart of a supercritical fluid extraction system.

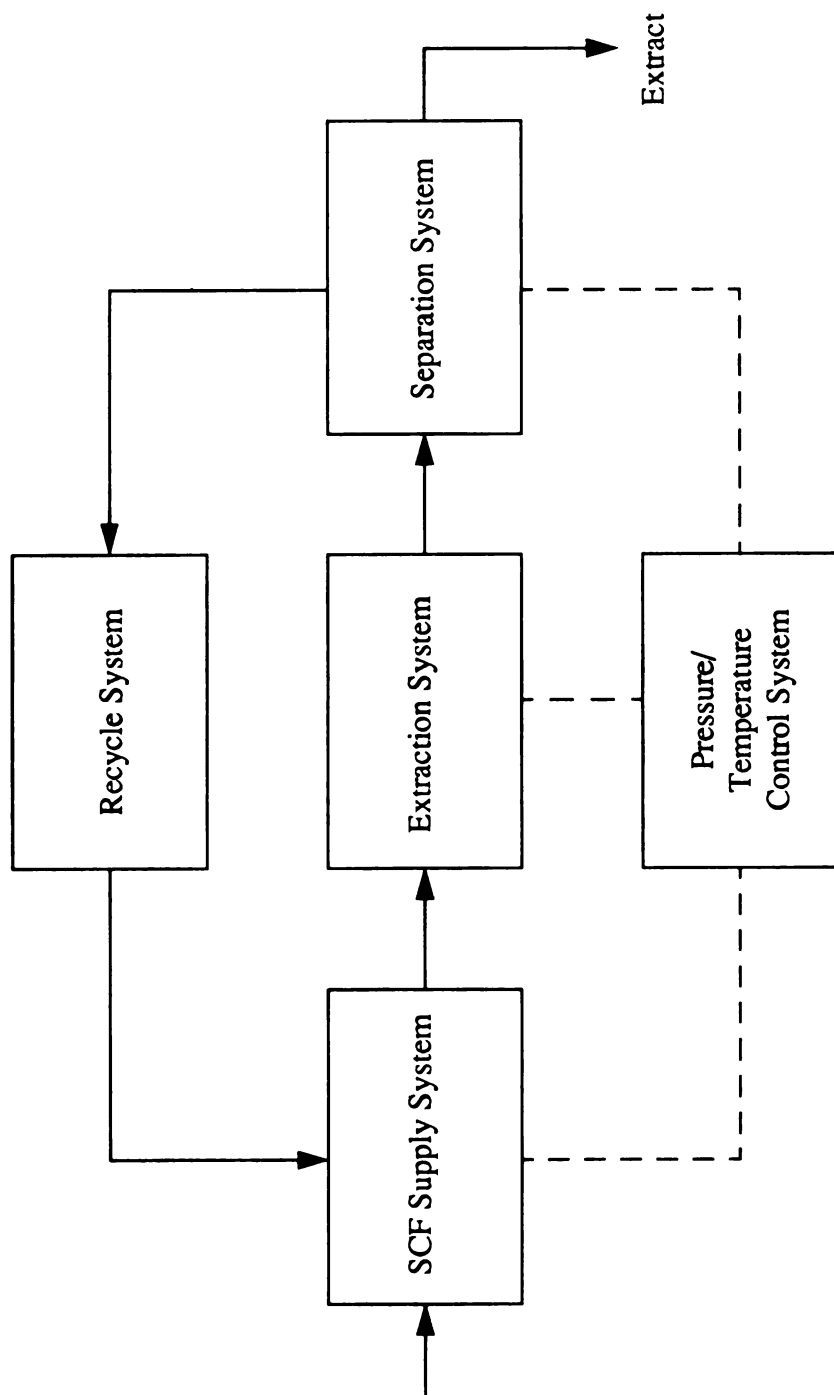


Figure 2. Supercritical Fluid (SCF) Extraction System.

2.2.2.3 Advantages of Supercritical Fluid Extraction

The advantages of supercritical fluid extraction are (Cohen, 1984):

1. Heat-sensitive compounds with low vapor pressure may be more successfully separated without damage or contamination.
2. The extracted material may be separated from the supercritical fluid by changing the temperature or pressure, an energy efficient process.
3. High selectivity can be achieved in the removal of specific components of multicomponent mixtures.
4. By selecting the proper extractor and multiseparator operating conditions, a fractionation of multicomponent solutes can be made.
5. Many supercritical fluids are more environmentally acceptable than organic solvents, thereby reducing workplace and environmental hazards.
6. Many supercritical fluids do not leave solvent residue. This is an important consideration in food applications.
7. Supercritical extraction processes often work where other separation techniques fail.

However, this method of extraction presents two basic drawbacks: the physicochemical principles involved in the theory of this process are highly sophisticated and the technology to develop the high pressure system necessary to carry out the extraction operation is expensive.

2.2.2.4 Historical Developments and Applications

The first observations that supercritical fluids dissolve unexpectedly large quantities of relatively non-volatile materials were reported in the literature about 100 years ago (Hannay and Hogarth, 1879; Andrews, 1887) when it was noted that metal halides became soluble in supercritical tetrachloromethane and ethanol. Buchner (1906) subsequently reported that the solubilities of low volatility organic materials in CO₂, under supercritical conditions, were orders of magnitude higher than would be expected from vapor pressure considerations alone.

Beginning in the 1930s, the effort to improve petroleum refining technology led to acquisition of vapor-liquid equilibrium data on hydrocarbon mixtures at high pressures (Sage et al., 1936; Kay, 1938). Messmore (1947) obtained a patent for deasphalting of oils using SFE. In the 1950s the Residuum Oil Supercritical Extraction process was developed for the removal of lighter products from the residue of commercial distillation of crude oil (Basta, 1984).

In studies on solubilities, Francis (1954, 1955) established the technical feasibility of using liquid CO₂ just below the critical point as a solvent for organic materials. Elgin and Weinstock (1959) presented a method for separating a number of mixtures into water-rich and organic-rich fractions.

Intensive study of supercritical fluids for extraction of food components began in the early 1970s. Many patents resulted from these first studies covering the SFE of hops, coffee, tea, tobacco, and spices (Roselius et al., 1972a, b; Vitzthum and Hubert,

1976, 1979; Zosel, 1972), among others. Built by HAG A.G. (Germany) and currently operated by General Foods Corp., the first large-scale production plant using SFE for food systems was designed to remove caffeine from green coffee beans. The plant, which uses supercritical CO₂, has been operating since 1979 (Rizvi et al., 1986).

Supercritical fluid extraction has been applied to a wide variety of foods. Research and applications include: decaffeination of coffee and tea (Vitzthum and Hubert, 1979; Zosel, 1982), fractionation of fish oils, hops extraction, oleoresin and essential oil extraction from spices and herbs (Hubert and Vitzthum, 1978), deodorization and hydrogenation of fats and oils, flavor extraction (Nguyen et al., 1991; Sinha et al., 1992), food coloring extraction from plant material (Degnan et al., 1991), citrus oil extraction from peels (Calame and Steiner, 1982; Copella and Barton, 1987), oil extraction from snacks, oilseed extraction (Stahl et al., 1980), and cholesterol removal from eggs (Rossi et al., 1989).

Other applications include:

- Separation of organic-water solutions (Paulitis et al., 1981; Moses et al., 1982; Kuk and Montagna, 1983).
- Polymers and monomers processing (Krase, 1945; Cottle, 1966; Wild et al., 1982; DeSimone et al., 1990; Guckes et al., 1990).
- Regeneration of activated carbon (Modell et al., 1978).

The design of commercial supercritical fluid extraction plants has been discussed by several authors (Eggers and Tschiersch, 1978; Schneider et al., 1980; Stahl et al., 1988; Novak and Robey, 1989). According to Novak and Robey, important design

considerations are: raw material preparation, extraction conditions, separation conditions, and supercritical solvent recycle and treatment.

2.3 USE OF ENTRAINER WITH SUPERCRITICAL FLUID EXTRACTION

Small amounts of co-solvents, which are referred to as modifiers or entrainers may be added to modify the polarity and solvent strength of the primary supercritical fluid to increase the solute solubility (and/or selectivity) and to minimize operating costs. The entrainers are commonly polar and non-polar organic compounds which are miscible with the supercritical fluid solvent (Dobbs et al., 1986; Dobbs et al., 1987).

The increase in solvent power when a co-solvent is added has been noted by several authors: Brunner and Peter (1982); Kurnik and Reid (1982); Ely and Baker (1983); Paulaitis et al. (1983); Dandge et al. (1985); Gopal et al. (1985); and Kim et al. (1985). For many systems the increase in solvent power is due to an increase in the density of the solvent mixture, and does not lead to improved selectivity. VanAlsten et al. (1984) and Schmitt and Reid (1986) measured the solubility of a single solid compound in a pure supercritical fluid and in a mixture of the supercritical fluid with a small amount of co-solvent. The study by VanAlsten et al. focused on the functionality of the solute while the study by Schmitt and Reid focused on the functionality of the co-solvent.

The addition of a small amount of entrainer into a primary supercritical fluid tends to increase both the critical temperature and pressure of the resulting solvent

mixture. To ensure operation in the critical region of a binary solvent mixture of defined composition, knowledge of the gas-liquid critical point for the mixture is essential (Gurdial et al., 1993). These authors have presented data for binary mixtures containing CO₂ as primary supercritical fluid and many different entrainers.

The majority of the information in the literature dealing with entrainers focuses on the increased solubility of solids in supercritical fluids containing small amounts of co-solvents. There are few examples of liquid-fluid equilibrium data for liquid-entrainer-supercritical fluid systems. Most deal with the separation of two organic compounds with similar boiling points that are difficult to separate by conventional distillation. Peter and Brunner (1978) increased the concentration of glycerides in propane by adding small amounts of acetone. They also found that the distribution coefficient of palm oil in CO₂ doubled with the addition of ethanol (Brunner and Peter, 1982). Brunner (1983) studied the effect of entrainers on the separation factor of hexadecanol, octadecane, and salicylic acid phenyl ester in several supercritical fluids including CO₂. He found that, depending on the temperature and pressure, the solubility as a function of entrainer concentration may decrease, increase, or run through a maximum. Roop and Akgerman (1989) proposed a method of predicting the effect of adding a small amount of an entrainer to a supercritical fluid for the extraction of organic compounds from aqueous systems.

2.4 EXTRACTION WITH LIQUEFIED GASES

If the pressure is raised sufficiently, many substances which are gaseous at ambient pressure either liquefy or begin to behave like liquids in that they exert appreciable solvent power, even for solutes of low volatility. For example, at temperatures up to 31°C, the critical temperature, CO₂ can be liquefied by raising the pressure and this liquid can be used to dissolve natural oils and quite a wide range of non-polar or slightly polar materials (King and Bott, 1993).

Liquid carbon dioxide has been found to be a very selective solvent for the extraction of flavor compounds such as terpenes, aldehydes and ketones, while other components of foods such as sugars, fruit acids, salts, amino acids, fats and water are practically insoluble (Schultz and Randall, 1970). Early research on liquid CO₂ was carried out by Francis (1955), who devised a process using liquid carbon dioxide to increase the dissolving power of conventional solvents and also conducted an extensive study on the mutual solubilities of liquid carbon dioxide with 261 different substances (Francis, 1954). One of the first uses of liquid carbon dioxide was on the extraction of coffee aroma (Sivetz, 1963). Schultz and Randall (1970) carried out a liquid CO₂ extraction of aroma components from apple, pear and orange juices, orange pieces and roasted ground coffee. They also listed data about the distribution of alcohol and esters between liquid carbon dioxide and water. The authors claimed that they obtained highly concentrated essence extracts, i.e., up to 100,000 fold in the case of apple juice.

The greatest use of the liquid CO₂ extraction method is probably in the preparation of hop extracts for the brewing industry. Currently hop extracts are prepared mainly by direct solvent extraction. The solvents are generally methylene chloride, trichloro-ethylene, hexane or methanol. Laws et al. (1977) carried out an extensive study on the extraction of a solvent-free isomerized concentrate from hops, and claimed a recovery of extractables of up to 90%.

In general, the aromas and flavors of extracts obtained by liquid CO₂ extraction bear a closer resemblance to the original material than those obtained by organic solvent extraction. This is ascribed to the very mild conditions of the process and to the lack of oxygen in the extraction system (Grimmett, 1981).

2.5 TRADITIONAL METHODS OF EXTRACTION OF FLAVOR CONCENTRATES

Teranishi et al. (1971) provided a comprehensive review of the methods for the isolation and concentration of volatile food constituents. Distillation is by far the most widely used method. The authors described two most utilized variations of this method: flash distillation and high vacuum (steam) distillation. These methods of extraction of flavor components are most commonly used commercially. Flash distillation is used mainly in the recovery of essences in the fruit industry (Milleville and Eskew, 1946) while steam distillation is widely used in the extraction of concentrates and essential oils of seasoning and aromatic herbs (Heath, 1973). Actually, in commercial applications more than one method of flavor extraction is practiced in a process. For instance, in steam distillation the distillate is subjected to a liquid-liquid

extraction in order to separate the flavor components from the aqueous solution or emulsion.

In the food industry, solvent extraction is an important method for isolation of soluble spice essences and other oleoresins. It has to be followed by distillation to remove the solvent. Spiro and Kandiah (1989) described the kinetics of extraction of ginger oleoresin by solvent extraction.

2.5.1 Steam Distillation

Steam distillation is the oldest and still the most important method for obtaining essential oils, i.e., the characteristic smelling volatile oils contained in plant material. These relatively volatile oils are separated from other material with the help of the carrier, steam. Live steam is injected into the liquid or solid mixture which contains the flavor volatiles. When the steam separates from the mixture, it carries the flavor volatiles with it. The steam is subsequently condensed and subjected to a liquid-liquid extraction with a solvent or is further concentrated (Teranishi et al., 1971).

In steam distillation, the starting material is subjected to a temperature of 100°C. This can lead to artifacts of the flavor oil components which are often thermolabile. In addition, water can exert a hydrolytic influence, bringing about chemical changes in the oils (Stahl et al., 1988).

Steam distillation can also be conducted under reduced pressure (vacuum distillation). It has the advantage of a lower temperature, and thus yields a higher oil quality. Distillation at a pressure above atmospheric, using superheated steam, is

sometimes applied to plant material containing oils which are difficult to distill. The higher temperature leads to large amounts of decomposition products (Stahl et al., 1988).

2.5.2 Classical Solvent Extraction

Extraction with classical organic solvents is an important procedure for obtaining lipophilic plant components. The selection of a suitable solvent is a critical decision. Each technical step in the extraction, separation and recovery of the solvent from the solution of the extract, and from the extraction residue, has to be considered.

The extractions need to be followed by an energy-expensive solvent-stripping stage. The last traces of solvent are difficult to remove completely, and even small amounts of organic solvents in foods are now coming under critical scrutiny for health reasons.

2.6 ANALYTICAL METHODS USED FOR ASSESSMENT OF QUALITY OF EXTRACTS

2.6.1 Gas Chromatography and Combined Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) is the most widely used analytical method in flavor research because of its ability to separate compounds. It has been a favorite analytical method of researchers working on flavor components of onion oil either alone or in combination with mass spectrometry (Brodnitz et al., 1969; Brodnitz and Pollock, 1970; Boelens et al., 1971; Block et al., 1992; Kuo and Ho, 1992).

Mass spectrometry (MS) has been considered an important method of compound identification. Since GC is not a direct identification technique and only provides component separation, researchers directly link the GC to MS. This is successful because of the compatibility of sample size requirements of both methods (Teranishi et al., 1971).

The method is imperfect in that it measures secondary compounds of the enzymatic action, and the relative contribution of these compounds to overall flavor and aroma is not known. It could give a distorted picture of the actual sequence of events, since the compounds found are those that are thermostable, thus surviving the separation process, and those that arise as a result of the heat (Whitaker, 1976).

2.6.2 Headspace Volatiles Analysis

Headspace analysis has been the tool of several researchers (Saghir et al., 1964; Bernhard, 1968; Boelens et al., 1971; Bandyopadhyay et al., 1970; Freeman and Mossadeghi, 1970; Freeman and Whenham, 1974; Tewari and Bandyopadhyay, 1977; Mazza and LeMaguer, 1979; Mazza et al., 1980; Yagami et al., 1980) who worked with *Alliums*. Mazza et al. (1980) assessed several procedures suitable for qualitative and quantitative determination of the volatiles of fresh and dehydrated onions. They emphasized the fact that for obtaining detectable amounts of minor constituents important to the aroma profile, concentration of the headspace vapor is required by means of external cold traps or adsorbents. They found that adsorbents are suitable for this purpose in the case of onions.

2.6.3 Other Methods

Appraisal of flavor or pungency of *Alliums*, such as onion and garlic, can be based on either subjective sensory analysis or detection of compounds generated by cysteine sulfoxide lyase (C-S lyase; EC 4.4.1.4) activity after tissue disruption. The typical flavor of *Allium* species is due to the conversion of endogenous alk(en)yl-L-cysteine sulfoxide flavor precursors to pyruvate, ammonia, and thiosulfinates by C-S lyase (Nock and Mazelis, 1987). The determination of pyruvate as an indicator of pungency is perhaps the most established method of appraisal although it is cumbersome and time consuming.

An alternative method for the evaluation of pungency in *Allium* species involved the determination of the thiosulfinates (Carson and Wong, 1959; Nakata et al., 1970). The procedure involved derivatizing the thiosulfinates with N-ethylmaleimide and measuring the absorbance of the conjugate at 515 nm. This procedure was quite specific for thiosulfinates. The method has been further modified by Thomas et al. (1992).

3. MATERIALS AND METHODS

3.1 ONIONS AND JUICE PREPARATION

Onions (*Allium cepa* L.) MSU experimental variety 3506 grown at the Michigan State University (Muck Farm) were obtained in November 1993. They were stored at 2.8°C.

The pH of the onion juice was 5.45. It was measured using a Corning pH meter (Model: 610A). The soluble solids content, measured using a hand held refractometer with automatic temperature compensation (Kernco Instruments Co., Inc.), was 7.7 °Brix.

The onion bulbs were peeled, cut and immediately processed with an Acme Juicerator (Model: 11JE21) which uses filter paper to separate the pulp from the juice. The juice was stored in a covered container and kept at ambient temperature (26 - 29°C) for one hour to facilitate enzymatic action for flavor development.

3.2 EXTRACTIONS

3.2.1 Supercritical CO₂ Extraction

Supercritical CO₂ extractions were conducted at 3600 psi (24.5 MPa) and 37°C. The density of CO₂ under these conditions is 0.89 g/cm³ (Angus et al., 1976).

Industrial grade CO₂ (AGA gas, 99.5% purity) from a gas cylinder was compressed with a gas booster (Haskel, Inc.) and stored in a 2.0 L reservoir. A pressure regulator positioned between the reservoir and the extraction vessel controlled the extraction pressure. A two-liter stirred autoclave (Model: Magnedrive II bolted closure autoclave, Autoclave Engineers) was used as the extraction vessel. The vessel was filled with 800 g onion juice prior to pressurization. Onion oil was collected in a 25 ml test tube. CO₂ was monitored with a flow meter and a dry test meter. The extraction was commenced by slowly raising the pressure in the extraction vessel while the system outlet was closed. After reaching the extraction pressure, the heated micrometering outlet valve was opened to commence flow. Figure 3 shows the supercritical CO₂ extraction system. The collection trap was weighed after 1100 liter CO₂ (STP) had passed through the system. The experiments described in the next paragraph had shown that passing larger volumes of CO₂ do not increase the yield appreciably beyond 1100 liter. Hence, this amount was chosen for the experiments. For this study, CO₂ was vented and not recycled.

To study the effect of the CO₂ flow rate on the extraction process, similar experiments were conducted in triplicate at two different CO₂ flow rates, namely 1.0 l/min and 0.5 l/min. The collection trap was weighed periodically during the extractions.

3.2.2 Supercritical CO₂ Extraction with Entrainer

The following additions were made to the supercritical CO₂ extraction system:

- A high pressure metering pump (Model: A-30-S, Eldex Laboratories Inc.) was

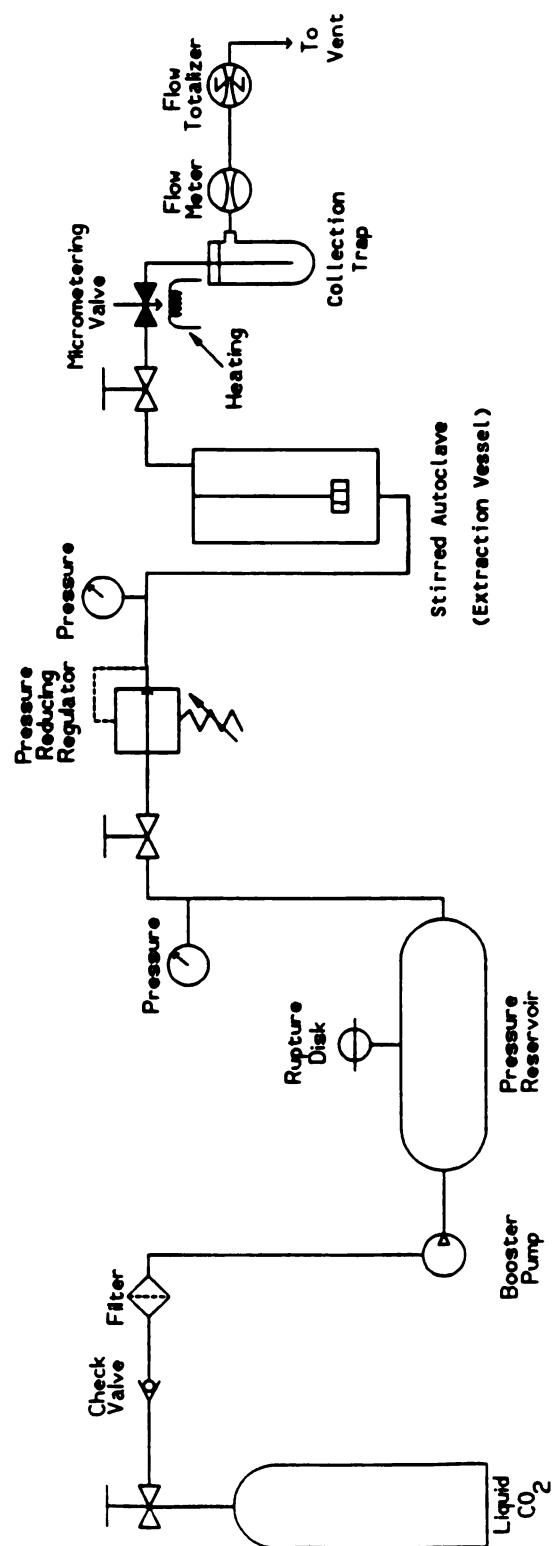


Figure 3. Supercritical CO₂ Extraction System.

attached using a tee fitting in the line between the pressure regulator and the CO₂ inlet of the extraction vessel.

- A static mixer, consisting of a steel tubing filled lightly with glass beads restricted to remain inside the tubing by closing both ends with steel filings, was installed between the extraction vessel and the high pressure metering pump. Its purpose was to ensure that CO₂ and entrainer entering the extraction vessel were mixed properly.

Figure 4 shows the supercritical CO₂ extraction system with modifications mentioned above for adding entrainer. Ethanol (200 proof dehydrated alcohol, U.S.P. punctilious, obtained from Quantum Chemical Corporation, USI Division) was used to evaluate the effect of a polar entrainer on the extraction of onion oil. Octane (obtained from J.T. Baker, Inc.) was used to evaluate the effect of a non-polar entrainer.

Extractions were conducted in triplicate at 3600 psi (24.5 MPa) and 37°C. Two different amounts of each were used, namely 50 ml & 75 ml, to evaluate the effect of different concentrations of entrainer. These amounts were chosen on the basis of preliminary experiments. In each case, 10 ml entrainer was added initially to the juice before extraction. The remaining was added continuously during the time when the system was at the desired extraction pressure. Information regarding critical conditions of supercritical CO₂-entrainer mixtures at various concentrations of entrainer was obtained from Gurdial et al. (1993). It was used to ensure that the extractions were conducted within the supercritical region of the binary mixtures of CO₂ and entrainer.

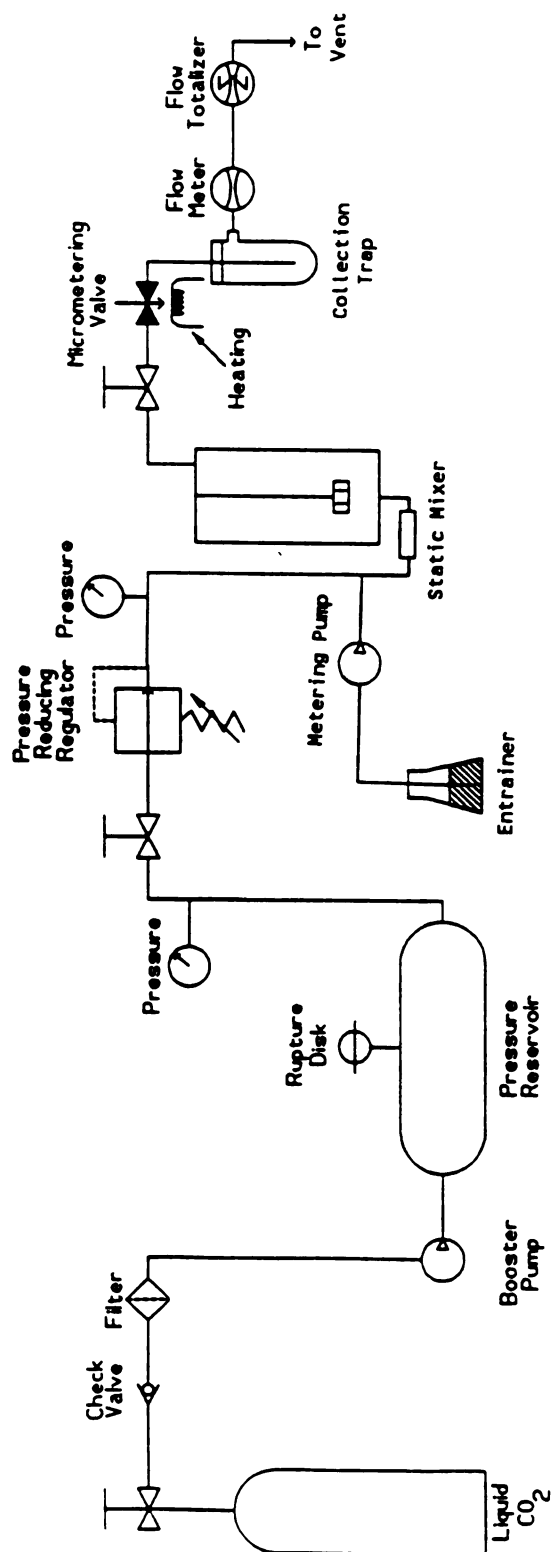


Figure 4. Supercritical CO₂ Extraction System with Modifications for Adding Entrainer.

In addition, an experiment was conducted at a lower pressure, i.e. 2600 psi (17.7 MPa), with 75 ml ethyl alcohol as entrainer to evaluate the effect of entrainer at a lower pressure (though still in the supercritical region). The temperature was kept the same at 37°C.

3.2.3 Liquid CO₂ Extraction

The same equipment as for supercritical CO₂ extraction was used. Extraction was conducted at 2900 psi (19.7 MPa) and 27°C. The density of CO₂ is 0.90 g/cm³ under these conditions. The above conditions were chosen such that the density of CO₂ for the supercritical CO₂ and liquid CO₂ experiments was nearly equal so that a meaningful comparison of the two methods could be made.

3.2.4 Steam Distillation-Solvent Extraction

200 ml onion juice was mixed with 100 ml distilled water. The volatile components were extracted by 20 ml dichloromethane for one hour in a modified Likens-Nickerson apparatus (Likens and Nickerson, 1964, Schultz et al., 1977). The apparatus is shown in Figure 5. Trace water in the extracted volatile solution was removed by anhydrous sodium sulfate and excess solvent was removed by nitrogen purging.

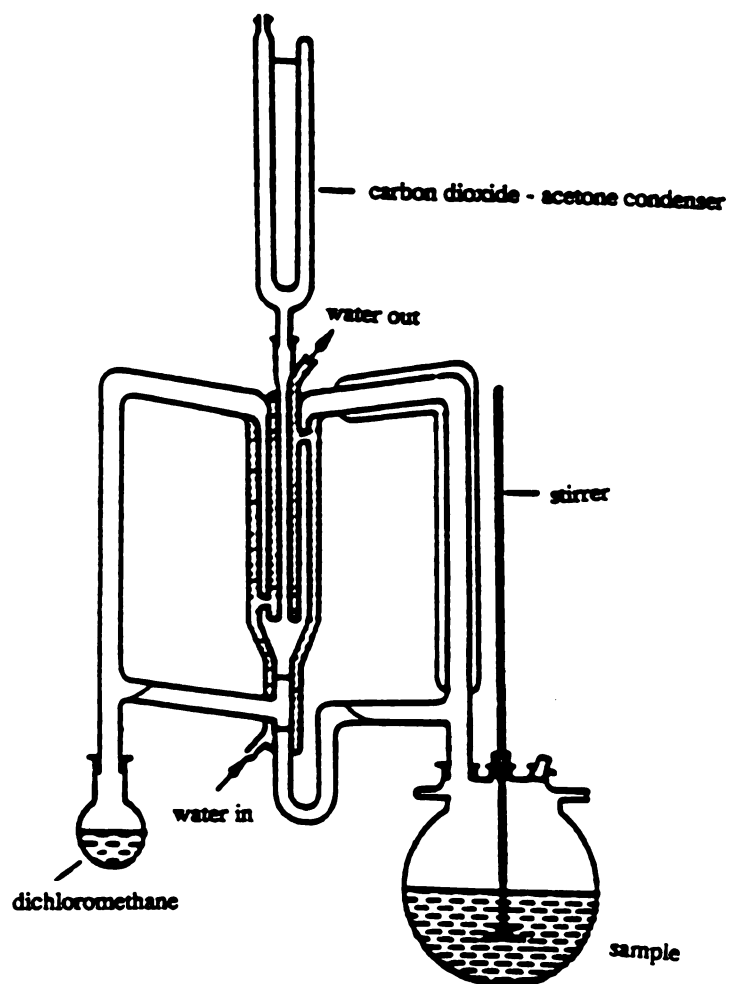


Figure 5. A Modified Likens-Nickerson Apparatus for Distillation-Solvent Extraction.

3.3 ANALYSIS

3.3.1 Estimation of Onion Oil Yield

3.3.1.1 Gravimetric Method

Onion oil yield was estimated gravimetrically in the case of supercritical CO₂ extraction and liquid CO₂ extraction. The collection trap was weighed after passing 1100 liter of CO₂ under desired conditions. However, in the case of (supercritical CO₂ + entrainer) and steam distillation-solvent extraction this was not possible because the extract contained entrainer and moisture (in some cases). The entrainer present in the extracts could have been removed by nitrogen purging, and then the gravimetric yield determined. However, nitrogen purging could result in a loss of some volatile flavor components. Hence, an alternative method, quantitative GC analysis, was used for estimating the yield of extracts containing entrainer.

3.3.1.2 Quantitative Gas Chromatographic Analysis

Gas chromatography was done in duplicate on the samples of extracts using GC (Model: Hewlett-Packard 5890 Series II). A 30 m HP-1 methyl silicone column of 0.53 mm inner diameter was used to separate the flavor components. A 1 µl dichloromethane dilution (containing 0.0045 g extract/ml dichloromethane) of each extract was injected for analysis. The operating conditions were as follows: injector temperature, 220°C; helium carrier gas flow rate, 6 ml/min; oven temperature, 35 to 200°C at a linear rate of 5°C/min and 15 min holding time at 200°C. A flame ionization

detector at temperature 240°C was used. HP 3365 Series II Chemstation Version A.03.21 by Hewlett Packard was used to control the GC and to record and integrate the data.

Sample preparation for GC analysis involved dilution of the extract with methylene chloride. In addition, the samples from (supercritical CO₂ + ethanol) experiments had to be dried using anhydrous sodium sulfate. Abraham et al. (1976) and Sinha et al. (1992) reported that the characteristic flavor of onions is due to the volatile oil, which consists chiefly of sulfur compounds. Sulfur-compound peaks were identified using gas chromatography-mass spectrometry (GC-MS). Summation of these peak areas was done for each sample.

A known amount (0.005 g) of an internal standard, benzyl disulfide, was added to the juice before extraction. The peak area of the internal standard, the amount of internal standard added and the sum of peak areas of sulfur peaks were used to calculate the amount of sulfur compounds present in each sample using the following equation:

$$\text{Total Wt. of S Compounds} = \frac{\langle \sum \text{Peak Area of S Compounds} \rangle \times \text{Wt. of IS}}{\text{Peak Area of IS}} .$$

The following simplifying assumptions were made:

1. The internal standard and the onion flavor compounds have similar extraction properties and GC and MS responses.

2. The total weight of sulfur compounds present in the extracts was considered as an indicator of the yield of onion oil.

The quantitative GC analysis will give information regarding the yield obtained by different extraction methods on a relative basis and not the absolute yield of onion oil.

3.3.2 Estimation of Quality of Extracts

3.3.2.1 Headspace Volatiles Analysis

30 ml onion juice was purged with purified nitrogen gas at a flow rate of 75 ml/min for 2.5 hours. The headspace volatiles were adsorbed onto an activated coconut charcoal trap. For gas chromatography analysis, the onion headspace volatiles were eluted from the trap using 1 ml carbon disulfide. This analysis was done to evaluate the efficiency of the various extraction methods studied with regard to the quality of extract obtained. Headspace samples contain most of the volatile compounds responsible for fresh onion flavor. By comparing the profiles of extracts obtained from the various methods with the headspace volatiles profile, it can be determined if the specified method is capable of extracting the components found in the headspace of fresh onion juice.

3.3.2.2 Thiosulfinate Analysis

Thiosulfinate analysis was done by the method described by Thomas et al. (1992). The analysis was performed on the following samples: (1). Onion oil obtained

from SFE-CO₂ with ethyl alcohol as entrainer, (2). Onion oil obtained from SFE-CO₂ with octane as entrainer, and (3). Commercial onion oil obtained by steam distillation.

The samples were purged with nitrogen to remove any traces of solvent/entrainer present. 5 ml isopropyl alcohol was added to equal amounts of the samples. 1 ml of 0.05 M N-ethylmaleimide in isopropyl alcohol, 1 ml of 0.25 M KOH in isopropyl alcohol, and 1.5 ml of 10 g liter⁻¹ ascorbic acid in distilled water were added. After vortexing the solution for approximately 10 seconds, the absorbance at 515 nm was recorded using a LKB Biochrom spectrophotometer (Model: Ultrospec II). The purpose of this analysis was to get information about the quality of the extract obtained from supercritical CO₂ extraction and compare it with commercial onion oil obtained from distillation. In addition, it is also a way of evaluating whether the extracts possess a fresh-like or cooked flavor. The samples containing high level of thiosulfinates tend to have a fresh-like flavor.

3.3.2.3 Gas Chromatography

Extracts obtained from the extraction methods were analyzed in duplicate by GC. The chromatograms were compared.

3.3.2.4 Gas Chromatography-Mass Spectrometry

Extracts from different methods were analyzed by GC-MS to identify the sulfur compounds present. Analysis by GC-MS was carried out on a JEOL AX-505H double-focusing mass spectrometer coupled to a Hewlett-Packard 5890J gas

chromatograph via a heated interface. GC separation employed a SPB-1 fused silica capillary column (30m length, 0.25mm i.d. with a 0.25 μ m film coating) supplied by Supelco, Inc. Direct (splitless) injection was used. 3 μ l methylene chloride solutions of different extracts were injected for analysis. Helium gas flow was approximately 1 ml/min. The GC temperature program was initiated at 35°C, held at this temperature for 5 min then heated at 5°C/min to 200°C and held for 15 min at this temperature. MS conditions were as follows: interface temperature 280°C, ion source temperature ca. 220°C, and the scan rate of the mass spectrometer was 1 s/scan over the m/z range 35/500. The mass spectra were obtained by electron ionization at 70 eV.

3.3.2.5 Experiments on Post-Extraction Residue and Fresh Juice

Dichloromethane extracts of onion juice that had undergone supercritical extraction were analyzed using GC to establish how much of the flavoring material remained in the post-extraction residue. GC analysis of dichloromethane extract of fresh onion juice was also done. This provides information regarding the amount of flavoring material that was present in the juice before extraction. Comparison of these two profiles would provide information regarding the efficiency of extraction of flavor compounds using supercritical CO₂. 800 ml fresh onion juice was kept in a covered beaker for one hour before doing solvent extraction with dichloromethane. The extract was concentrated using nitrogen purging and analyzed by GC. The dichloromethane extract of post-extraction residue was also concentrated.

4. RESULTS AND DISCUSSION

4.1 QUANTITATIVE ANALYSIS

4.1.1 Gravimetric Yield of Onion Oil by Different Methods

Gravimetric yield of onion oil from SFE-CO₂ with a CO₂ flow rate 1.0 l/min, SFE-CO₂ with a CO₂ flow rate 0.5 l/min and liquid CO₂ extraction was obtained by weighing the extract collected in the collection trap after passing 1100 liter CO₂ through the extraction system. Table 1 shows the gravimetric yield for these three cases. SFE-CO₂ extraction with the CO₂ flow rate of 0.5 l/min gave the maximum onion oil yield.

Table 1: Gravimetric yield of onion oil obtained by different methods.

Method	Oil Yield ^a (g)	% Oil Yield ^b (wt. basis)
SFE-CO ₂ , CO ₂ flow: 1.0 l/min	0.2287 ^c	0.0286
SFE-CO ₂ , CO ₂ flow: 0.5 l/min	0.2592 ^d	0.0324
Liquid CO ₂ extraction	0.1719 ^e	0.0215

a. Mean of triplicate samples

b. On basis of weight of onion juice used

c, d, e. Indicate that the yields are significantly different at the 1% level.

Gravimetric yield data were subjected to analysis of variance and Fisher's protected least significant difference analysis. It was found that the yields for all three methods (Table 1) were significantly different at the 1% level. The ANOVA table and least significant difference calculations are presented in Appendix C. Gravimetric yield

could not be evaluated for the other methods due to the presence of small amounts of entrainer/solvent in the extracts.

Fenwick and Hanley (1985) reported 0.002 to 0.03% yield of onion oil by distillation. In this study a maximum of 0.0324% yield of onion oil was obtained.

4.1.2 Quantitative GC Analysis for Comparison of Onion Oil Yield from All Methods

The total weight of sulfur compounds contained in a sample was calculated as follows:

$$\text{Total Wt. of S Compounds} = \frac{(\sum \text{Peak Area of S Compounds}) \times \text{Wt. of IS}}{\text{Peak Area of IS}}$$

Where 'IS' is the abbreviation for the internal standard and 'S' for sulfur. The total weight of sulfur compounds present in a sample was assumed to be representative of the yield of onion oil. Abraham et al. (1976) and Sinha et al. (1992) reported that the characteristic flavor of onions is due to the volatile oil, which consists chiefly of sulfur compounds. Figure 6 shows the total weight of sulfur compounds present in the extracts from all the methods studied. The standard deviation of yield obtained from triplicate experiments is shown on top of each bar in Figure 6. The relative proportions of yield obtained by SFE-CO₂ at 0.5 l/min and by SFE-CO₂ at 1.0 l/min were similar for both the gravimetric and the quantitative GC methods for estimation of yield. However, in the case of Liquid CO₂ Extraction, quantitative GC method indicated less yield than the gravimetric method. The difference between the gravimetric and the quantitative GC methods for estimation of yield could be due to the presence of small amounts of moisture in the extract, which may affect the gravimetric yield data but have no effect on

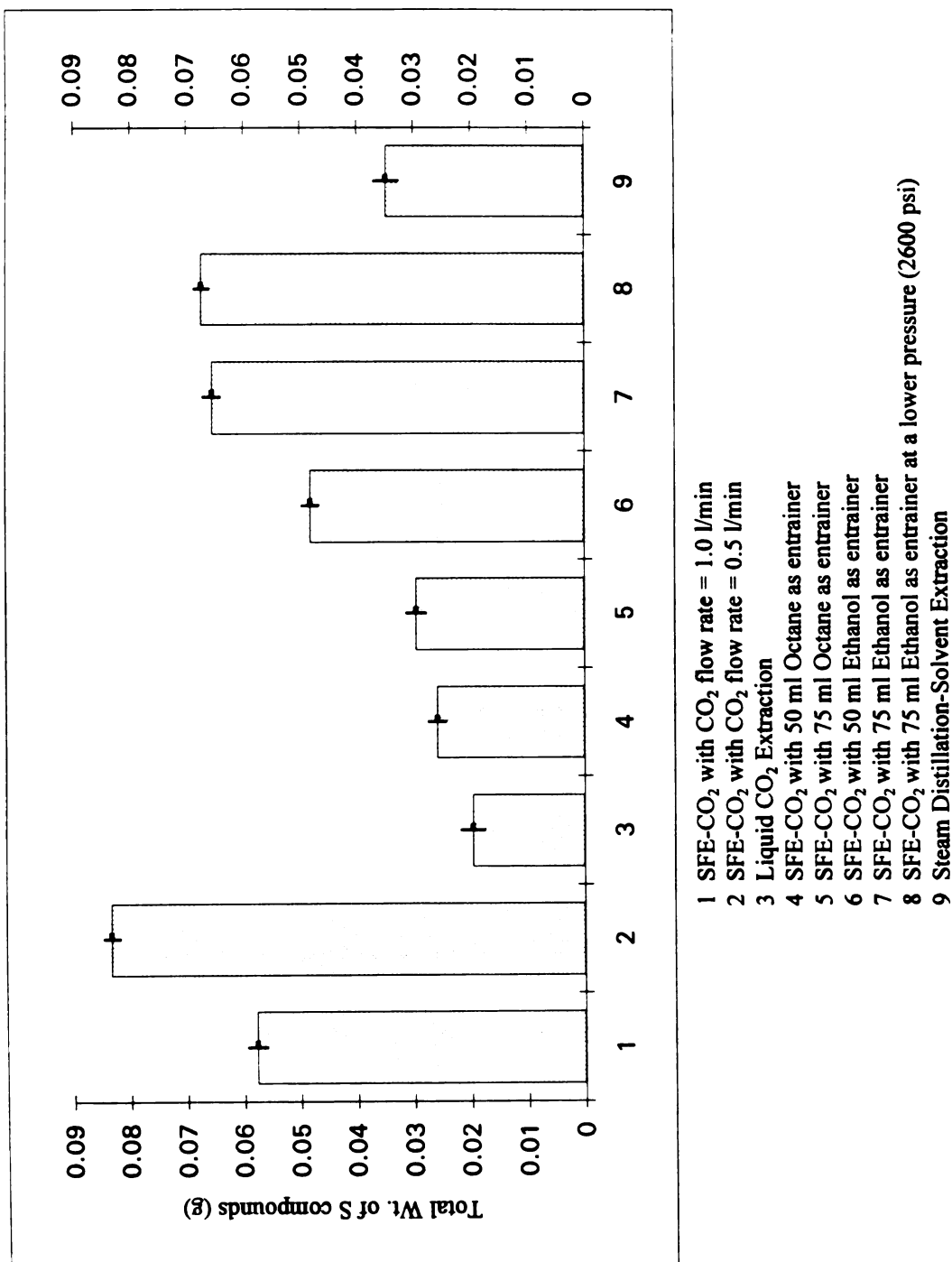


Figure 6. Total Amount of Sulfur Compounds in the Extracts obtained by Quantitative GC Analysis.

quantitative GC data since water is not detected by a flame ionization detector.

The extract obtained by SFE-CO₂ with a CO₂ flow rate of 0.5 l/min contained the maximum quantity of sulfur compounds thus indicating that the onion oil yield would be maximum in this case. Liquid CO₂ extract was found to contain the minimum quantity of sulfur compounds. Statistical analysis showed that the onion oil yield obtained by the different methods was significantly different at the 5% level except in the case of the following pairs: SFE-CO₂ with 50 ml octane and SFE-CO₂ with 75 ml octane; SFE-CO₂ with 75 ml octane and steam distillation-solvent extraction; and SFE-CO₂ with 75 ml ethyl alcohol at 3600 psi and at 2600 psi (Appendix C). The steps followed in the calculation of the total weight of sulfur compounds in the different extracts are explained in Appendix A.

4.1.3 Effect of Volume of CO₂ Passed Through the Extraction System on the Yield of Onion Oil

Figure 7 shows the effect of volume of CO₂ passed through the extraction system on the yield of onion oil under the conditions of SFE-CO₂ at 3600 psi (24.5 MPa) and 37°C with CO₂ flow rate 0.5 l/min. The yield increases at a higher rate during the initial part of the extraction as indicated by the initial steepness of the curve. This is followed by a region of lower extraction rate as indicated by the flattening of the curve during the latter part of the extraction. The lower extraction rate during the latter part of the extraction may be explained in terms of depletion of the solute (onion flavor oil) in the substrate (onion juice).

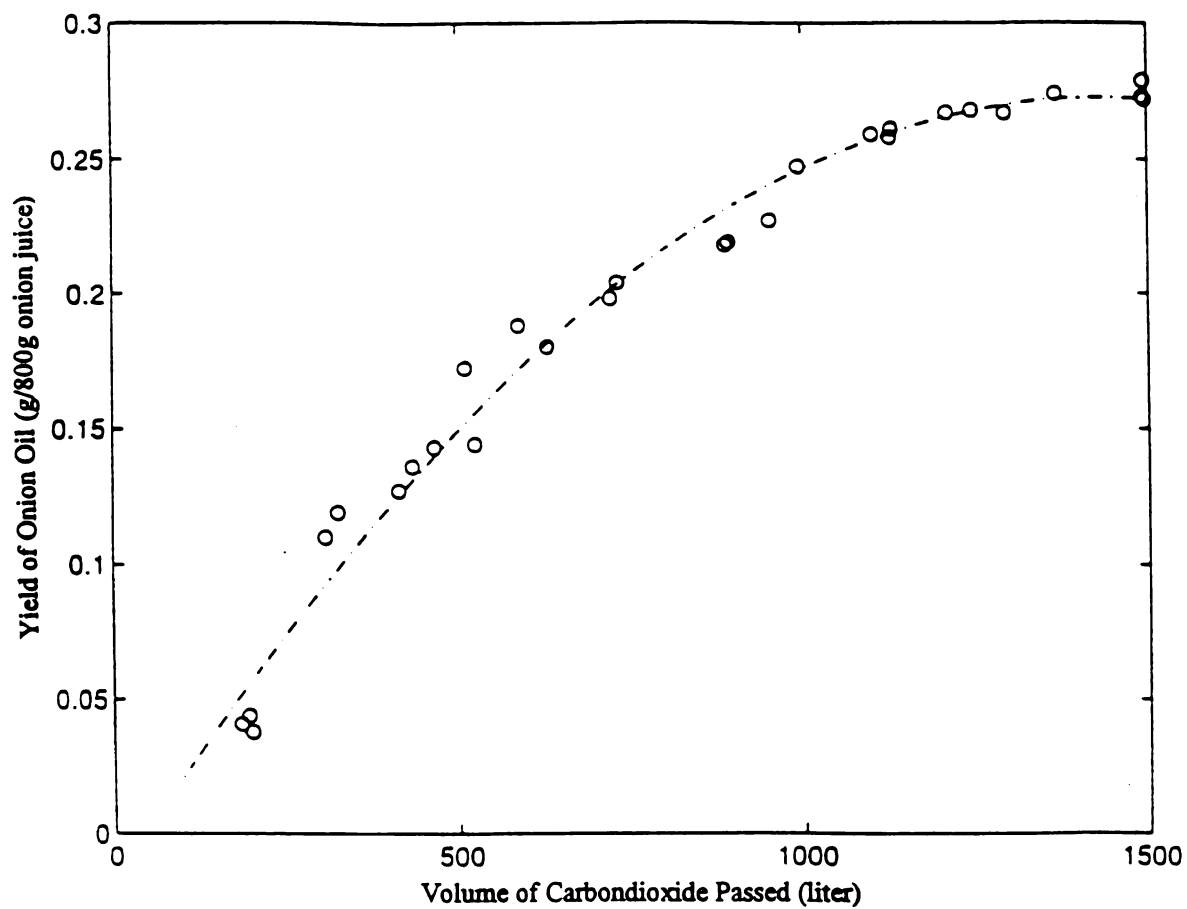


Figure 7. Yield of Onion Oil versus Volume of CO₂ passed at 3600 psi, 37°C.

Figure 8 shows the yield of onion oil obtained at different volumes of CO₂ passed through the system at two different CO₂ flow rates, namely, 0.5 l/min and 1.0 l/min. The data points in the initial part of the curves indicate similar extraction rates. A possible reason for this could be the presence of headspace (more than 1 liter) in the extraction vessel, which was the same during both the experiments. In the later part of extraction, 0.5 l/min CO₂ experiment resulted in a higher yield than 1.0 l/min experiment. The higher yield in the case of the experiment with 0.5 l/min flow rate could be due to greater residence time of the solvent (CO₂) in the extraction system. The two curves also indicate that the process does not come to an equilibrium state under the conditions tested. In Figures 7 and 8, polynomials of second degree were used to fit the experimental data.

4.1.4 Effect of Entrainer

The effect of entrainer was studied under the following subheadings:

- Effect of polar entrainer
- Effect of non-polar entrainer
- Effect of entrainer at different pressures

4.1.4.1 Effect of Polar Entrainer

The polar entrainer, ethyl alcohol, was added to the SFE-CO₂ system in two different amounts, i.e. 50 ml and 75 ml. As indicated in Figure 6, 26.4% greater yield was obtained in the case of 75 ml ethyl alcohol as compared with 50 ml ethyl alcohol.

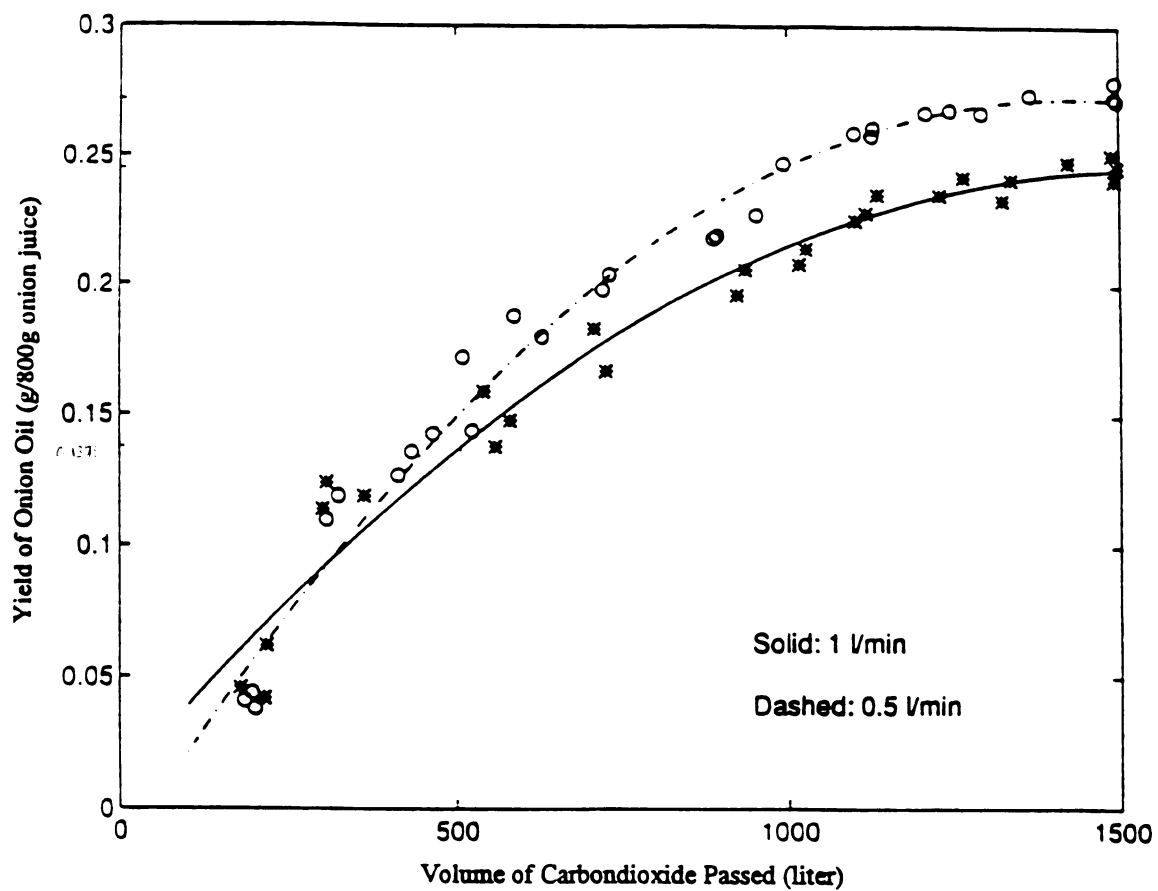


Figure 8. Yield of Onion Oil at Different Volumes of CO₂ Passed at Different Flow Rates of CO₂.

The 75 ml ethyl alcohol experiment enhanced the yield of onion oil over the experiment without entrainer (at same CO₂ flow rate, i.e. 1.0 l/min) by 16.6%. In the case of the 50 ml ethyl alcohol experiment, the yield was lower than SFE-CO₂ without entrainer (Figure 6).

4.1.4.2 Effect of Non-polar Entrainer

The non-polar entrainer, octane, was added to the SFE-CO₂ system in two different amounts, i.e. 50 ml and 75 ml. The yield of onion oil obtained from experiments with 75 ml octane as entrainer with SFE-CO₂ extraction was higher than that obtained from 50 ml octane experiments. However they were both less than the yield of SFE-CO₂ experiments without any entrainer. Thus, under the conditions studied, octane failed to enhance the yield of onion oil from SFE-CO₂.

4.1.4.3 Effect of Entrainer at Different Pressures

The SFE-CO₂ system was found to behave almost identically in the case of 75 ml ethyl alcohol used as entrainer at 3600 psi (24.5 MPa) and at 2600 psi (17.7 MPa) in terms of the onion oil yield (Figure 6). Although the solvent power of supercritical CO₂ is known to be higher at higher pressures, in these experiments the yield of onion oil was 2.7% higher at 2600 psi than that at 3600 psi. However, statistical analysis showed that the yields at 2600 psi and at 3600 psi were not significantly different at the 5% level (Appendix C).

This is a very interesting result because it indicates the possibility of lowering the extraction pressure from 3600 psi to 2600 psi with no reduction in the yield of onion oil by using ethyl alcohol as entrainer in the above mentioned quantity. This could have positive effects on the economics of this process. This result supports the findings of Brunner and Peter (1982) that supercritical fluid extraction can be conducted at a lower pressure in the presence of an entrainer. This happens because the solubility of the solute in the SCF under the same temperature and pressure conditions is greatly enhanced in presence of an entrainer. Lower operating pressures are also desirable for safety reasons.

Experiments were not done at 2600 psi without entrainer. Thus, it cannot be proved that the yield enhancement in the case of 2600 psi experiment over the 3600 psi experiment was solely due to the presence of entrainer. The enhancement of yield could also be due to the solvent characteristics of supercritical CO₂ at 2600 psi and 37°C.

4.2 QUALITATIVE ANALYSIS

4.2.1 Quality of Extracts Obtained by Various Methods

The onion extracts produced through SFE-CO₂ (with or without entrainer) and liquid CO₂ extraction had characteristic fresh onion-like smell in contrast to the rather unpleasing cooked onion-like smell of steam distilled extracts. Fresh onion-like aroma of supercritical CO₂ extracts can be confirmed by the report of a trained panel organoleptic evaluation of similar extracts (Appendix D). Gas chromatography-mass spectrometry was used to identify the chemical components of the various extracts and

headspace volatiles of onion juice. The emphasis was on sulfur compounds which are the main flavor components of onions (Abraham et al., 1976, Sinha et al., 1992). These results are presented in Table 2. The identification of the flavor compounds was based on the comparison of mass spectral data with published works. In one case tentative identification was made by direct interpretation of mass spectral data. Where definitive characterization could not be made, the mass number is indicated.

Considering the CO₂ extraction methods studied (SFE-CO₂ without entrainer, SFE-CO₂ + Ethanol, SFE-CO₂ + Octane and Liquid CO₂ extraction), the maximum number of flavor compounds were identified in SFE-CO₂ + Ethanol extracts. SFE-CO₂ + Ethanol and Liquid CO₂ extracts contained all the compounds identified in the headspace of onion juice except molecular sulfur (31), which is most likely an artifact resulting from the processing or GC-MS analysis, and dipropyl trisulfide (24). SFE-CO₂ (without entrainer) extracts contained all the compounds identified in the headspace of onion juice except molecular sulfur (31), dipropyl trisulfide (24) and 1-propenyl propyl trisulfide (25). SFE-CO₂ + Octane did not contain other compounds that were identified in headspace, like methyl trans-propenyl disulfide (9) and dimethyl trisulfide (10). This indicates that SFE-CO₂ + Ethanol, SFE-CO₂ without entrainer, and Liquid CO₂ Extraction produce more fresh onion-like flavor than other methods studied as indicated by the presence of the headspace flavor compounds.

The SFE-CO₂ without entrainer, SFE-CO₂ + Ethanol and SFE-CO₂ + Octane extracts contained diallyl thiosulfinate (22) or its isomer, di-1-propenyl

Table 2: Onion Flavor Compounds Identified by GC-MS in Supercritical CO₂ Extracts and Headspace Samples

no.	Compound	Formula, Mass	SFE-CO ₂	SFE-CO ₂ + Ethanol	SFE-CO ₂ + Octane	Liq. CO ₂	Headspace
1	dimethyl disulfide	C ₂ H ₆ S ₂ , 94	✓	✓			
2	methyl ethyl disulfide	C ₃ H ₈ S ₂ , 108	✓	✓		✓	
3	2,5-dimethyl thiophene	C ₆ H ₈ S, 112	✓	✓		✓	
4	2,4-dimethyl thiophene	C ₆ H ₈ S, 112	✓	✓		✓	
5	3,4-dimethyl thiophene	C ₆ H ₈ S, 112	✓	✓	✓	✓	✓
6	diallyl sulfide	C ₆ H ₁₀ S, 114	✓	✓		✓	
7	methyl propyl disulfide	C ₄ H ₁₀ S ₂ , 122		✓	✓		
8	methyl cis-propenyl disulfide	C ₄ H ₈ S ₂ , 120	✓	✓	✓	✓	✓
9	methyl trans-propenyl disulfide	C ₄ H ₈ S ₂ , 120	✓	✓		✓	✓
10	dimethyl trisulfide	C ₂ H ₆ S ₃ , 126	✓	✓		✓	✓
11	unknown compound	unknown, 130	✓	✓	✓		
12	dipropyl disulfide	C ₆ H ₁₄ S ₂ , 150	✓	✓			
13	1-propenyl propyl disulfide	C ₆ H ₁₂ S ₂ , 148	✓	✓	✓	✓	✓
14	propyl methane thiosulfonate	C ₄ H ₁₀ S ₂ O ₂ , 154		✓			
15	3-ethenyl-1,2-dithi-5-ene	C ₆ H ₁₀ S ₂ , 146	✓	✓	✓	✓	✓
16	methyl propyl trisulfide	C ₄ H ₁₀ S ₃ , 154	✓	✓	✓	✓	✓

Table 2 (Continued)

no.	Compound	Formula, Mass	SFE-CO ₂	SFE-CO ₂ + Ethanol	SFE-CO ₂ + Octane	Liq. CO ₂	Headspace
17	methyl 1-propenyl trisulfide (<i>E/Z</i>)	C ₄ H ₈ S ₃ , 152	✓	✓	✓	✓	✓
18	methyl 1-propenyl trisulfide (<i>E/Z</i>)	C ₄ H ₈ S ₃ , 152	✓	✓	✓	✓	✓
19	3-ethenyl-1,2-dithi-4-ene	C ₆ H ₁₀ S ₂ , 146	✓	✓	✓	✓	✓
20	3,4-dihydro-3-vinyl-1,2-dithiin	C ₆ H ₈ S ₂ , 144	✓	✓	✓	✓	✓
21	methyl 5-methylfuryl sulfide	C ₆ H ₈ OS, 128		✓			
22	diallyl thiosulfinate	C ₆ H ₁₀ OS ₂ , 162	✓	✓	✓		
23	1-propenyl propyl trisulfide (<i>E/Z</i>)	C ₆ H ₁₂ S ₃ , 180	✓	✓	✓	✓	✓
24	dipropyl trisulfide	C ₆ H ₁₄ S ₃ , 182			✓		✓
25	1-propenyl propyl trisulfide (<i>E/Z</i>)	C ₆ H ₁₂ S ₃ , 180		✓	✓	✓	✓
26	diallyl trisulfide	C ₆ H ₁₀ S ₃ , 178		✓	✓		
27	dibenzothiophene	C ₁₂ H ₈ S, 184	✓	✓		✓	
28	methyl 3,4-dimethyl-2-thienyl disulfide	C ₇ H ₁₀ S ₃ , 190	✓	✓	✓	✓	
29	4,6-diethyl-1,2,3,5-tetrathiane	212		✓			
30	2,4-dimethyl-5,6-dithia-2,7-nonadienal 5-oxide	218		✓			
31	molecular sulfur	S ₈ , 256					✓
32	5,7-diethyl-1,2,3,4,6-pentathiepane	264	✓	✓	✓		

thiosulfinate. Sinha et al. (1992) reported the presence of diallyl thiosulfinate or its isomer, di-1-propenyl thiosulfinate, in supercritical CO₂ onion extracts for the first time. However, they mentioned that the identification of diallyl thiosulfinate was tentative due to the absence of a synthesized reference compound or supporting NMR or IR spectra. Hence, the identification of diallyl thiosulfinate (22) is tentative.

Brodnitz et al. (1971), using IR and NMR analysis, reported diallyl thiosulfinate to be a major constituent of fresh garlic extracts. The authors indicated that during gas chromatography diallyl thiosulfinate undergoes dehydration, forming two isomeric disulfides: 3-vinyl-1,2-dithi-5-ene and 3-vinyl-1,2-dithi-4-ene. Nishimura et al. (1988) reported 3,4-dihydro-3-vinyl-1,2-dithiin in *Allium victorialis* L. The authors indicated that the decomposition of diallyl thiosulfinate in methanol at room temperature for 7 days produced 3,4-dihydro-3-vinyl-1,2-dithiin, 2-vinyl-4*H*-1,3-dithiin, diallyl sulfide and diallyl trisulfide. In the headspace of crushed onions, Kallio and Salorinne (1990) reported the tentative presence of 3-ethenyl-1,2-dithi-4-ene and 3-ethenyl-1,2-dithi-5-ene.

In the present study, diallyl thiosulfinate (or its isomer) (22), 3-ethenyl-1,2-dithi-4-ene (19), 3-ethenyl-1,2-dithi-5-ene (15), 3,4-dihydro-3-vinyl-1,2-dithiin (20) and diallyl trisulfide (26) have been found in each of the supercritical extracts (SFE-CO₂ without entrainer, SFE-CO₂ + Ethanol, SFE-CO₂ + Octane). The presence of the above mentioned compounds in the extracts supports the identification of diallyl thiosulfinate since these compounds have been reported to be produced by decomposition of diallyl thiosulfinate.

Thiosulfonates are secondary products arising from thiosulfinates (Abraham et al., 1976). The presence of methyl methanethiosulfonate, propyl methanethiosulfonate, and propyl propanethiosulfonate in dichloromethane extracts of freshly chopped onions has been reported by Boelens et al. (1971). These authors suggest that the absence of these compounds in steam-distilled onion oil is a result of their being soluble in water; thus, they are present in the water layer and do not get transferred to the organic phase during the steam distillation process. In this study, only propyl methanethiosulfonate (14) was identified and only in SFE-CO₂ + Ethanol extracts (Table 2).

Molecular sulfur (31) is being reported in headspace of onion juice for the first time. The identification is based on spectral comparisons with data from Heller and Milne (1978). It is expected to be an artifact resulting from rearrangement of sulfur atoms during processing or GC-MS analysis due to heat.

The identification of 4,6-diethyl-1,2,3,5-tertathiane (29), 2,4-dimethyl-5,6-dithia-2,7-nonadienal 5-oxide (30), and 5,7-diethyl-1,2,3,4,6-pentathiepane (32) was based on mass spectral data from Kuo and Ho (1992).

An unknown compound of molecular weight 130 was detected in SFE-CO₂, SFE-CO₂ + Ethanol and SFE-CO₂ + Octane extracts. The MS data is provided in Appendix B.

The SFE-CO₂ + Ethanol sample contained the maximum amount of thiosulfinates as indicated by the maximum absorbance recorded in this case (Table 3).

Its value was almost five times that for commercial onion oil. The absorbance for SFE-CO₂ + Octane was also many times higher than that for commercial onion oil. This justifies the fresh-like flavor of supercritical CO₂ extracts of onion (with the use of entrainer) as opposed to cooked flavor in case of commercial oils (which are steam distilled).

Table 3: Results of Thiosulfinate Analysis.

Sample	Absorbance ^a
SFE-CO ₂ + Ethanol	1.673 ^b
SFE-CO ₂ + Octane	1.573 ^b
Steam distilled commercial oil	0.308 ^c

a. Measured at 515 nm in triplicate

b, c Numbers followed by different letters are significantly different at the 1% level.

By smelling the extracts it was found that SFE-CO₂ extracts (with and without entrainer) had a similar, fresh-onion like, smell. On the other hand, steam distilled extracts had the unpleasant smell of cooked onions. Hence, on the basis of this paragraph and the previous one, we can say that SFE-CO₂ extracts (with or without entrainer) have the unique and pleasant smell of fresh onions.

Figures 9 and 10 show typical gas chromatograms of SFE-CO₂ extract of onion and of steam distilled commercial onion oil respectively. The two flavor profiles

are very different. There are more peaks in the chromatogram of SFE-CO₂ extract than in the chromatogram of steam distilled extract. This is an evidence of the presence of extra compounds in SFE-CO₂ extracts which are either absent or present in very small amounts in the steam distilled extracts.

Figures 11 and 12 show gas chromatograms of the dichloromethane extract of fresh onion juice and the dichloromethane extract of onion juice after it has undergone supercritical fluid extraction. The sample that has undergone SFE-CO₂ has very small peaks indicating very small amounts of the compounds present. This indicates that supercritical fluid extraction using CO₂ is an efficient method of isolating onion flavor oil.

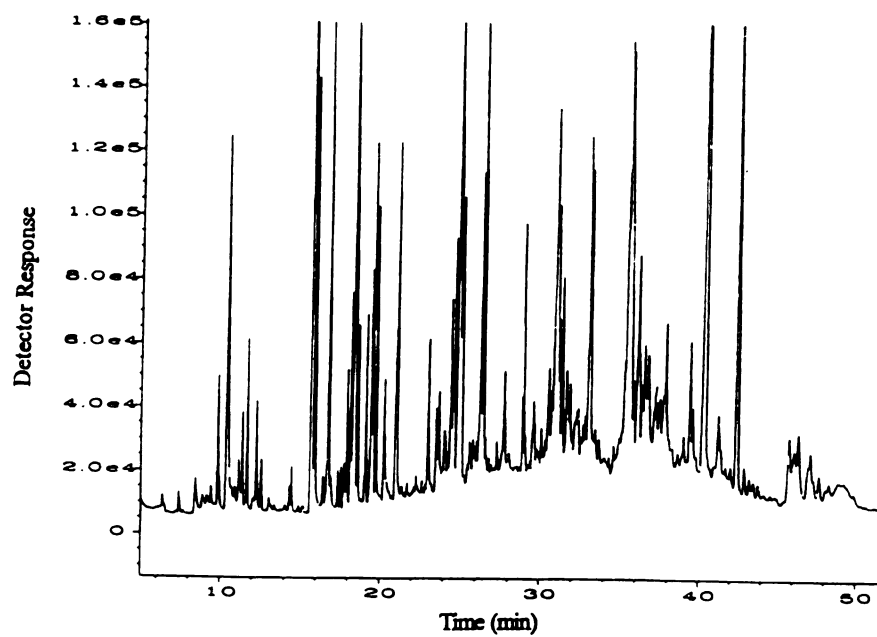


Figure 9. Gas Chromatogram of Onion Oil Obtained by Supercritical CO₂ Extraction.

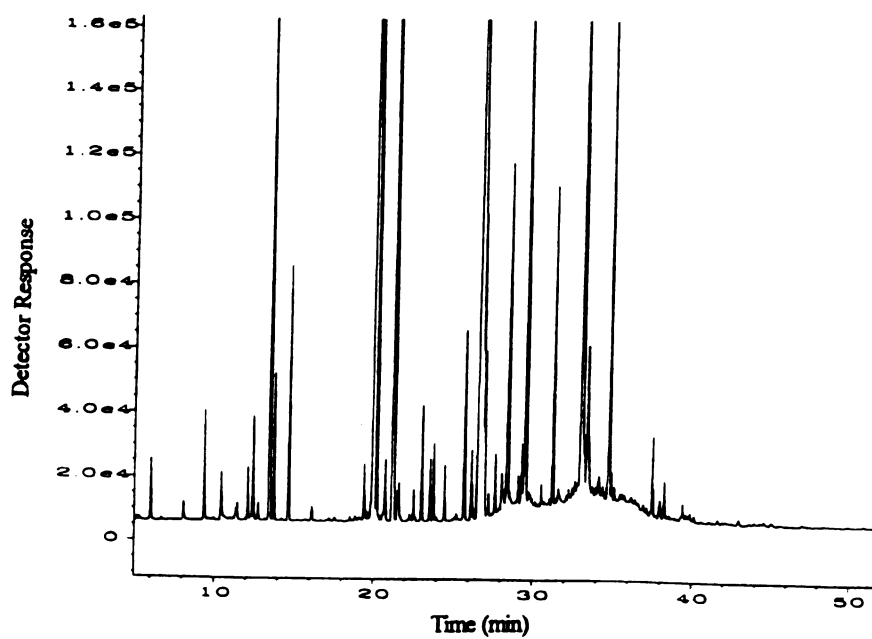


Figure 10. Gas Chromatogram of Commercial Onion Oil Obtained by Steam Distillation.

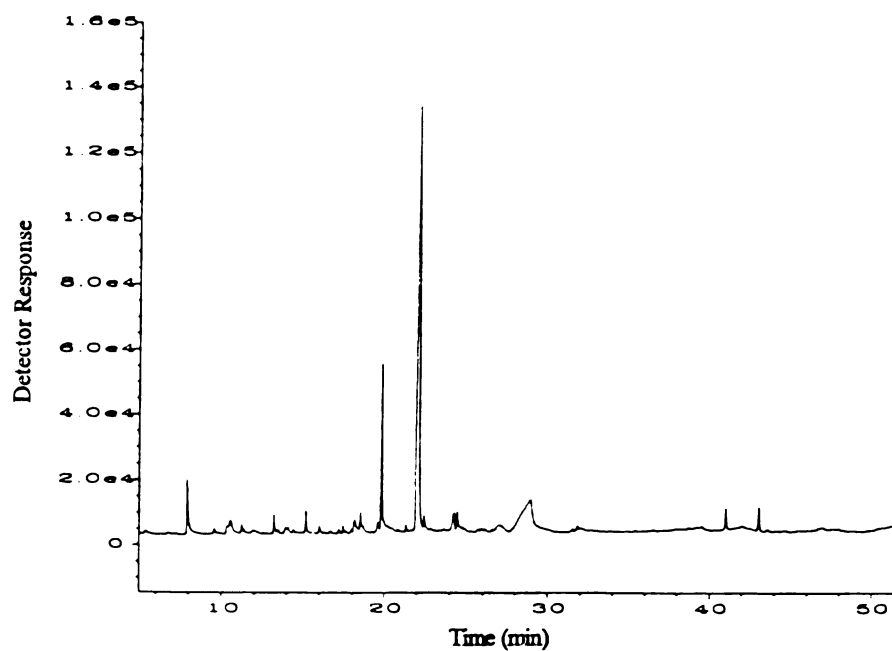


Figure 11. Gas Chromatogram of a Dichloromethane Extract of Fresh Onion Juice.

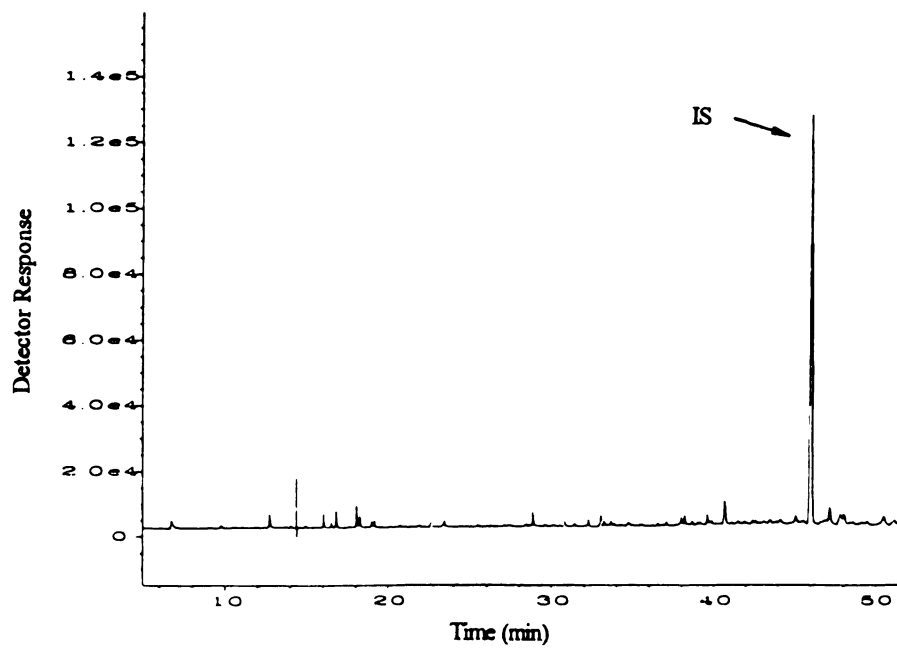


Figure 12. Gas Chromatogram of a Dichloromethane Extract of Onion Juice after SFE-CO₂.

5. SUMMARY & CONCLUSIONS

A comparison between various methods of extracting onion flavor oil has been made with respect to the yield and the quality of the extracts. Supercritical fluid extraction with CO₂ resulted in higher yield and better quality extracts than steam distillation-solvent extraction (the latter is the most widely used method for commercial production of onion flavor oil). The use of polar entrainer, ethyl alcohol with SFE-CO₂ was found to enhance the yield of SFE-CO₂. Ethyl alcohol is considered a food-grade solvent. Thus, onion flavor oil produced by SFE-CO₂ with ethyl alcohol as entrainer will result in a high yielding, safe (free from organic solvent residues) and unique fresh onion-like flavor. The commercial potential of the unique fresh onion-like flavor extracted by supercritical CO₂ extraction can be supported by the results of a trained panel organoleptic evaluation of similar extracts (Appendix D).

1. Supercritical fluid extraction, using carbon dioxide as solvent, is a promising alternative to steam distillation-solvent extraction which is the most widely used method for extraction of onion flavor oil. It is possible to produce high yielding and better quality extracts using supercritical fluid extraction.
2. Lower flow rate of carbon dioxide in supercritical fluid extraction of onion oil from onion juice, under the conditions studied, resulted in a higher yield of onion oil.
3. The use of 75 ml ethyl alcohol as entrainer in supercritical CO₂ extraction enhanced the yield of onion oil obtained from the extraction without entrainer

under similar conditions (3600 psi (24.5 MPa), 37°C, 1.0 l/min CO₂ flow rate).

4. The yield of onion oil was greater at 2600 psi (17.7 MPa) than at 3600 psi (24.5 MPa) in experiments with 75 ml ethyl alcohol as entrainer. This indicates that the supercritical extractions can be conducted at lower pressures.
5. Supercritical and liquid CO₂ extracts had fresh onion like flavor as opposed to cooked flavor obtained by steam distillation-solvent extraction.

6. SUGGESTIONS FOR FURTHER RESEARCH

This study has indicated that supercritical fluid extraction using carbon dioxide as solvent is a promising alternative to the traditional methods used for extraction of onion flavor oil. There is a need for a detailed study to optimize the solvent power of CO₂ with respect to extraction of onion oil in which experiments are conducted at various temperatures and pressures.

Use of ethyl alcohol and octane as entrainers in the SFE-CO₂ process have been studied in this work. Information on the use of other solvents as entrainers could help understand the effect of entrainers on the process more clearly. In addition, the possibility of obtaining same or higher yields at lower pressures in the presence of an entrainer needs further study.

GC and GC-MS techniques may preferably be replaced by high performance liquid chromatography (HPLC), cryogenic GC, SCF chromatography or other analytical techniques which employ lower temperatures. This is desirable because some onion flavor components are thermolabile and there is a possibility of their degradation or rearrangement under high temperatures which are usually employed in GC injectors and detectors (Block et al., 1992; Block, 1993).

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APPENDICES

APPENDIX A

Quantitative GC Analysis to Estimate Onion Oil Yield

Addition of an appropriate internal standard in the beginning of the analysis is an important step in quantitative GC analysis. 0.005 g of benzyl disulfide (internal standard) was added to onion juice before extraction in each experiment. The following simplifying assumptions were made:

1. The internal standard and the onion flavor compounds have similar extraction properties and GC and MS responses.
2. The total weight of sulfur compounds present in the extracts was considered as an indicator of the yield of onion oil.

GC-MS was used to identify the sulfur compound peaks. This was done by matching corresponding peaks on the total ion chromatogram (TIC) obtained from GC-MS and the chromatogram obtained from GC. Figure 13 shows the TIC for SFE-CO₂ + Ethanol, 3600 psi, 37°C, extract. Figure 14 shows the GC chromatogram of the same extract. Twenty large sulfur peaks were chosen for the analysis. One of these was the internal standard peak. These twenty peaks were identified on GC chromatograms of all extracts and their peak areas were obtained from the Area Percent Reports. The Area Percent Report for SFE-CO₂ + Ethanol at 3600 psi (24.5 MPa), 37°C is shown in Table 4. Each extract was analyzed in triplicate. Table 5 shows the peak areas of the twenty selected peaks for experiment SFE-CO₂ + Ethanol at 3600 psi (24.5 MPa), 37°C.

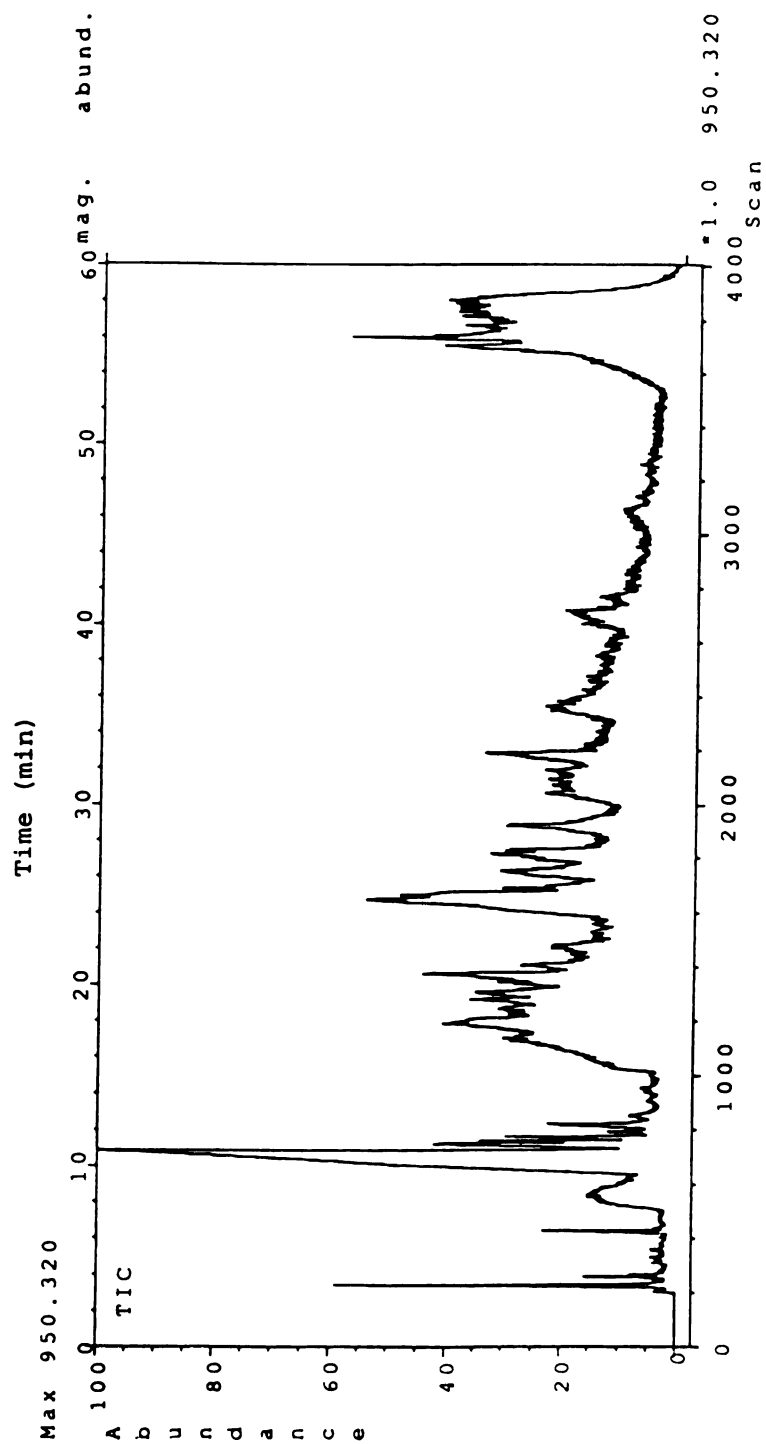


Figure 13. Total Ion Chromatogram of SFE-CO₂ + Ethanol (75 ml, 3600 psi, 37°C) Extract.

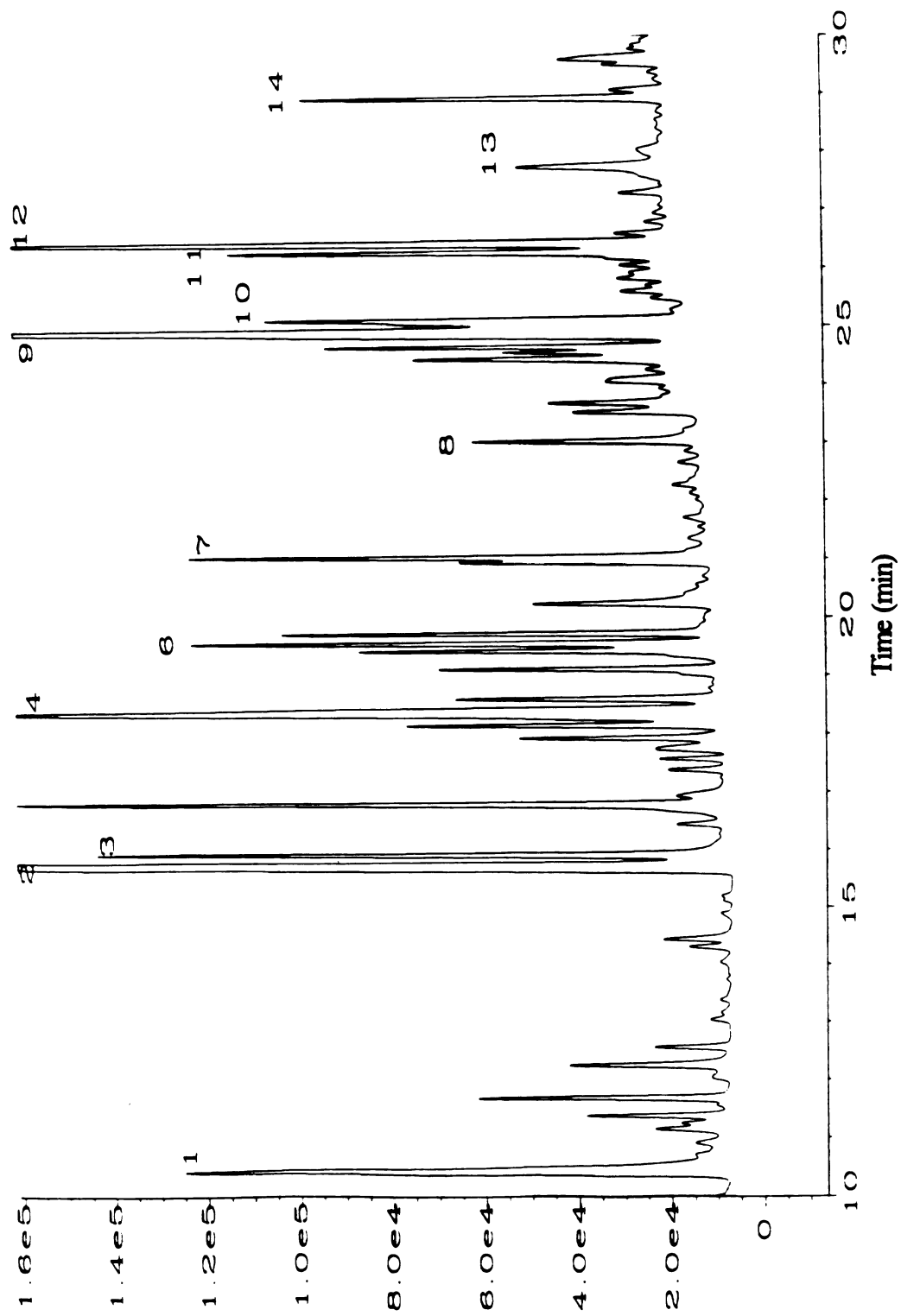


Figure 14. Gas Chromatogram of SFE-CO₂ + Ethanol (75 ml, 3600 psi, 37°C) Extract Showing the Selected Peaks

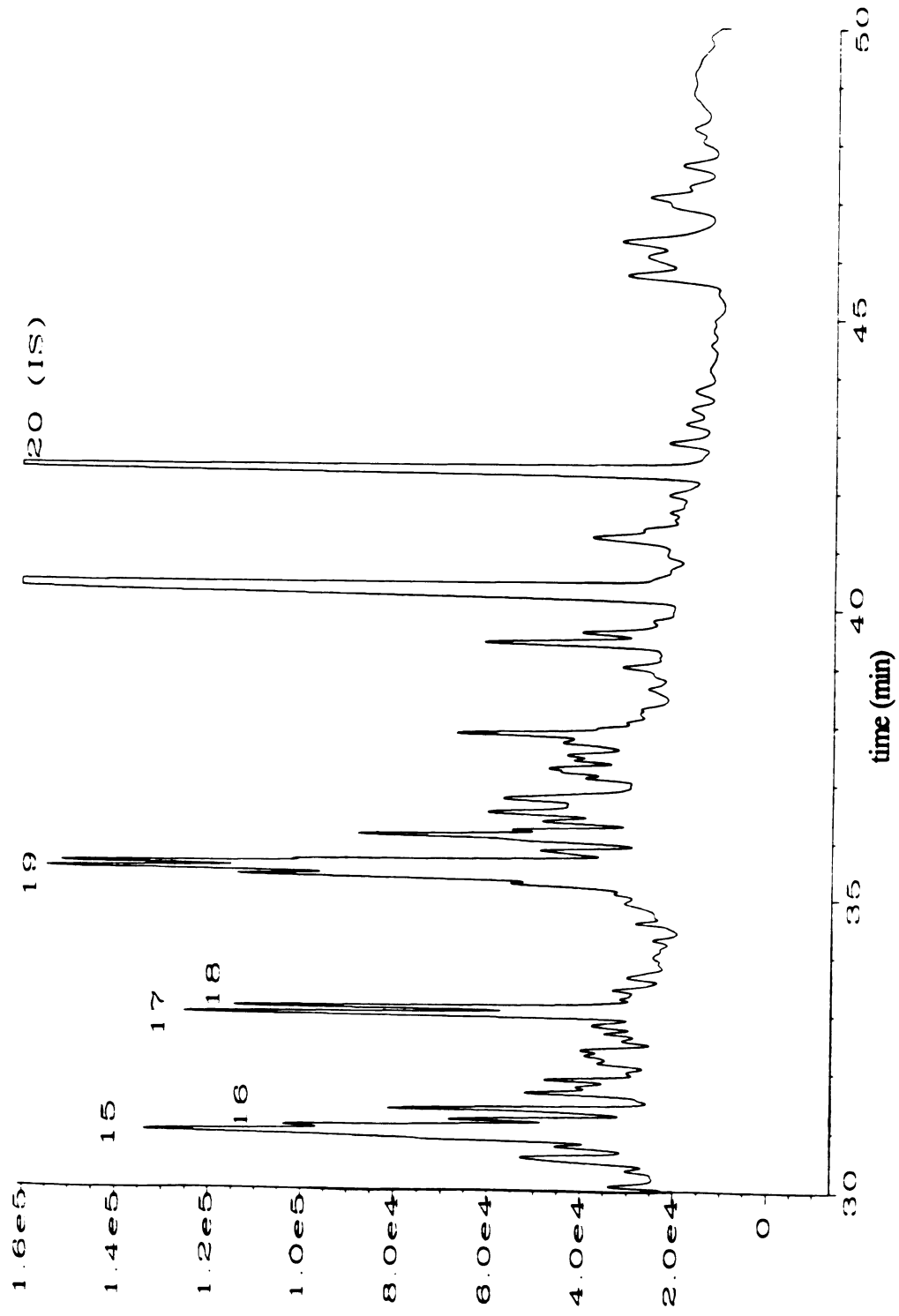


Figure 14 (Continued)

Table 4: Area Percent Report of SFE-CO₂ + Ethanol (75 ml, 3600 psi, 37°C) Extract
obtained by GC

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	10.421	934847	117442	BV	0.122	2.4638
2	11.381	151642	31051	VV	0.074	0.3996
3	11.690	239716	54178	VV	0.069	0.6318
4	12.257	205729	34694	VV	0.089	0.5422
5	15.749	2336415	388523	VV	0.086	6.1575
6	15.917	666972	136856	VV	0.073	1.7578
7	16.795	811847	155937	VV	0.076	2.1396
8	17.929	216169	44874	VV	0.072	0.5697
9	18.146	308840	69181	VV	0.069	0.8139
10	18.348	1401112	158269	VV	0.128	3.6926
11	18.599	290850	58570	VV	0.075	0.7665
12	19.111	306386	61727	VV	0.074	0.8075
13	19.427	407259	78805	VV	0.076	1.0733
14	19.557	534907	115321	VV	0.071	1.4097
15	19.727	433759	95480	VV	0.068	1.1432
16	20.241	281759	41145	VV	0.099	0.7426
17	20.954	240565	56839	VV	0.063	0.6340
18	21.047	577784	115266	VV	0.074	1.5227
19	23.034	326196	53113	VV	0.089	0.8597
20	23.536	211044	31335	VV	0.096	0.5562
21	23.693	237371	36526	VV	0.093	0.6256
22	24.063	233582	23990	VV	0.129	0.6156
23	24.449	408606	65377	VV	0.089	1.0769
24	24.563	196847	46187	VV	0.063	0.5188
25	24.663	386788	84492	VV	0.069	1.0194
26	24.943	1906242	282319	VV	0.096	5.0238
27	25.130	703588	97232	VV	0.099	1.8543
28	26.292	651115	104729	VV	0.089	1.7160
29	26.459	1016782	182740	VV	0.088	2.6797
30	26.609	167926	21229	VV	0.109	0.4426
31	27.298	167346	20049	VV	0.114	0.4410
32	27.752	537771	41925	VV	0.177	1.4173
33	28.050	231060	15960	VV	0.192	0.6090
34	28.946	477919	88107	VV	0.080	1.2595
35	29.607	268521	32241	VV	0.112	0.7077
36	30.095	195117	23491	VV	0.115	0.5142
37	30.562	455163	42452	VV	0.147	1.1996
38	30.750	216656	35105	VV	0.087	0.5710
39	30.980	1219514	123281	VV	0.137	3.2140
40	31.083	464663	93400	VV	0.074	1.2246
41	31.196	299098	57770	VV	0.077	0.7883
42	31.382	477812	70685	VV	0.095	1.2593
43	31.653	354552	41365	VV	0.118	0.9344
44	31.756	169203	30331	VV	0.078	0.4459
45	31.877	229706	37073	VV	0.087	0.6054
46	32.168	182923	25515	VV	0.103	0.4821
47	32.297	196808	28330	VV	0.095	0.5187
48	32.386	193982	29209	VV	0.095	0.5112
49	32.816	230845	26717	VV	0.125	0.6084
50	33.003	627609	114185	VV	0.083	1.6540

Table 4 (Continued)

Pk#	Ret Time	Area	Height	Type	Width	Area %
51	33.116	560045	103693	VV	0.079	1.4760
52	33.274	154937	20447	VV	0.101	0.4083
53	33.431	199617	22085	VV	0.123	0.5261
54	33.649	216390	18938	VV	0.160	0.5703
55	34.593	168730	16923	VV	0.134	0.4447
56	34.938	272775	19221	VV	0.185	0.7189
57	35.264	301184	43609	VV	0.097	0.7938
58	35.387	602018	102595	VV	0.085	1.5866
59	35.492	812547	143575	VV	0.083	2.1414
60	35.589	1061836	140390	VV	0.102	2.7984
61	35.846	301263	37324	VV	0.110	0.7940
62	36.106	668814	76495	VV	0.118	1.7626
63	36.201	203183	43249	VV	0.069	0.5355
64	36.355	268015	36765	VV	0.107	0.7063
65	36.506	432865	48381	VV	0.129	1.1408
66	36.741	525072	45016	VV	0.159	1.3838
67	37.099	175498	27333	VV	0.090	0.4625
68	37.264	364710	35193	VV	0.139	0.9612
69	37.401	158133	29680	VV	0.078	0.4168
70	37.489	232993	31219	VV	0.104	0.6140
71	37.701	286684	32065	VV	0.122	0.7555
72	37.854	514187	54921	VV	0.128	1.3551
73	38.642	210090	13658	VV	0.199	0.5537
74	39.013	287220	19045	VV	0.198	0.7570
75	39.416	433173	48733	VV	0.129	1.1416
76	39.590	256805	27611	VV	0.129	0.6768
77	40.396	2481299	223121	VV	0.147	6.5394
78	41.227	313073	25111	VV	0.170	0.8251
79	42.403	1491929	199127	VV	0.118	3.9319

area = 3.7944E+007

Table 5: Peak Areas of Sulfur Compounds and Internal Standard for SFE-CO₂ + Ethanol
(75 ml, 3600 psi, 37°C).

Peak No.	Peak Area 1	Peak Area 2	Peak Area 3
1	934847	3620787	821680
2	2336415	2726697	2250953
3	666972	580099	712804
4	1401112	1421560	1369923
5	-	816678	1028352
6	534907	866853	672291
7	577784	1199303	781626
8	326196	1008938	442970
9	1906242	3447582	2206183
10	703588	1112474	811043
11	651115	840105	832081
12	1016782	1287419	1206924
13	537771	797538	538039
14	477919	719733	476052
15	1219514	2128427	140915
16	464663	749909	639012
17	627609	1378408	585982
18	560045	1087334	816499
19	1061836	3110072	1373058
20 (IS)	1271368	2103342	1364128
Σ Peak Area (1-19)	16005317	28899916	17706387

The following equation was used to evaluate the total weight of sulfur compounds present in the extracts and thus provide an estimate of the onion oil

$$\text{Total Wt. of S Compounds} = \frac{(\sum \text{Peak Area of S Compounds}) \times \text{Wt. of IS}}{\text{Peak Area of IS}}$$

For Peak Area 1:

$$\Sigma \text{ Peak Area of S Compounds} = 16005317$$

$$\text{Peak Area of IS} = 1271368$$

$$\text{Weight of IS} = 0.005 \text{ g}$$

$$\begin{aligned} \text{Thus, Total Wt. of S Compounds} &= 16005317 * 0.005/1271368 \\ &= 0.0629 \text{ g} \end{aligned}$$

Similarly,

For Peak Area 2:

$$\Sigma \text{ Peak Area of S Compounds} = 28899916$$

$$\text{Peak Area of IS} = 2103342$$

$$\text{Weight of IS} = 0.005 \text{ g}$$

$$\begin{aligned} \text{Thus, Total Wt. of S Compounds} &= 28899916 * 0.005/2103342 \\ &= 0.0687 \text{ g} \end{aligned}$$

Similarly,

For Peak Area 3:

$$\Sigma \text{ Peak Area of S Compounds} = 17706387$$

$$\text{Peak Area of IS} = 1364128$$

$$\text{Weight of IS} = 0.005 \text{ g}$$

$$\begin{aligned} \text{Thus, Total Wt. of S Compounds} &= 17706387 * 0.005/1364128 \\ &= 0.0649 \text{ g} \end{aligned}$$

$$\text{Total Wt. of S Compounds (Average)} = 0.0655 \text{ g}$$

Similarly, the total weight of sulfur compounds (average of triplicate experiments) were calculated for the other extracts and these data were presented in the form of a bar chart in Figure 6.

APPENDIX B

Table 6: Mass Spectral and Retention Data for Onion Flavor Compounds Detected in Various Extracts Studied.

No.	Compound, Mass	I _k ^a	MS Data ^b
1	dimethyl disulfide, 94	706	96(13.1), 95(5.7), 94(100) , 79(48.0), 64(3.0), 61(9.2), 48(20.4), 46(41.6), 45(62.3)
2	methyl ethyl disulfide, 108	831	110(8.0), 109(4.2), 108(30.0), 79(13.8), 66(15.0), 64(3.2), 47(15.8), 45(11.1), 43(100)
3	2,5-dimethylthiophene, 112	862	114(7.5), 113(12.3), 112(74.6), 111(100) , 97(45.0), 79(5.4), 77(12.6), 69(10.2), 67(10.9), 51(3.8), 45(27.7)
4	2,4-dimethylthiophene, 112	863	114(3.1), 113(12.3), 112(100) , 111(91.5), 97(68.2), 79(8.0), 77(15.0), 69(10.2), 67(10.0), 51(6.3), 45(29.0)
5	3,4-dimethylthiophene, 112	866	114(4.8), 113(12.9), 112(100) , 111(97.2), 97(100), 79(9.4), 77(15.0), 69(48.2), 67(9.1), 51(21.0), 45(84.7)
6	diallyl sulfide, 114	870	116(4.8), 115(7.0), 114(33.2), 113(18.4), 101(8.6), 99(100) , 84(4.9), 73(9.5), 72(13.0), 71(11.9), 65(15.3), 59(13.8), 55(15.1), 45(35.4), 41(24.7)
7	methyl propyl disulfide, 122	910	124(15.3), 123(5.0), 122(100) , 80(88.4), 64(13.1), 60(6.2), 47(12.2), 46(12.2) , 45(44.0), 43(36.8), 41(29.4)
8	methyl cis-propenyl disulfide, 120	933	122(4.2), 121(6.1), 120(76.8), 105(3.9), 80(26.1), 75(32.2), 72(26.5), 61(16.6), 47(17.5), 46(11.0), 45(100) , 41(29.4)
9	methyl trans-propenyl disulfide, 120	942	122(15.0), 121(12.6), 120(100) , 105(9.7), 80(20.0), 75(44.8), 72(34.1), 61(5.1), 47(14.8), 46(24.7), 45(90.2), 41(14.2)
10	dimethyl trisulfide, 126	953	128(9.8), 127(4.7), 126(38.3), 111(9.9), 80(7.0), 79(42.5), 64(30.0), 61(5.1), 47(32.1), 46(29.8), 45(100)

Table 6: Mass Spectral and Retention Data for Onion Flavor Compounds Detected in Various Extracts Studied.

No.	Compound, Mass	I _k ^a	MS Data ^b
11	unknown, 130	1017	132(4.8), 131(10.3), 130(100) , 115(23.0), 114(2.3), 113(12.2), 101(83.1), 85(14.9), 69(26.8), 68(14.0), 67(39.6), 59(23.0), 53(13.9), 45(20.0), 41(31.1), 39(18.2)
12	dipropyl disulfide, 150	1057	152(3.2), 151(4.0), 150(28.3), 108(17.9), 75(10.6), 73(7.8), 66(6.4), 61(8.9), 47(27.3), 45(68.5), 43(75.0), 41(100)
13	1-propenyl propyl disulfide, 148	1062	150(11.4), 149(8.8), 148(100) , 106(30.1), 73(40.1), 72(42.2), 71(11.0), 61(22.5), 47(22.4), 45(26.3), 43(35.0), 41(67.2)
14	3-ethenyl 1,2-dithi-5-ene, 146	1068	147(4.6), 146(37.5), 113(100) , 112(8.0), 111(11.8), 97(16.2), 85(7.9), 79(19.6), 77(7.2), 59(5.7), 45(8.4), 41(6.0)
15	methyl propyl trisulfide, 154	1072	156(12.0), 155(38.3), 154(100) , 114(49.0), 113(23.8), 112(32.8), 79(15.6), 64(16.1), 47(69.0), 46(38.5), 45(13.0), 43(70.3), 41(71.2)
16	propyl methane thiosulfonate, 154	1075	156(13.8), 155(5.7), 154(98.0), 139(6.5), 138(3.4), 112(33.1), 97(7.0), 79(53.8), 64(43.2), 47(90.0), 45(80.5), 43(100) , 41(99)
17	methyl 1-propenyl trisulfide (E/Z), 152	1079	153(2.0), 152(100) , 88(69.8), 73(40.0), 64(5.6), 47(11.3), 46(14.5), 45(68.2), 41(7.8), 40(9.1), 39(20.0)
18	methyl 1-propenyl trisulfide (E/Z), 152	1081	154(2.0), 153(5.6), 152(100) , 111(5.1), 88(67.2), 73(45.0), 64(6.3), 47(7.1), 46(9.4), 45(66.8), 41(11.0), 39(15.8)
19	3-ethenyl 1,2-dithi-4-ene, 146	1084	148(2.8), 147(4.0), 146(35.0), 113(100) , 112(8.1), 111(14.4), 98(16.1), 97(16.1), 85(6.9), 79(18.3), 77(8.2), 59(5.1), 45(14.0), 41(6.3), 39(7.5)
20	3,4 dihydro-3-vinyl-1,2-dithiin, 144	1091	146(8.5), 145(23.8), 144(100) , 129(20.0), 111(70.4), 99(12.8), 85(6.7), 77(19.2), 70(11.5), 69(18.0), 68(26.3), 67(10.0), 59(8.3), 41(19.1)
21	methyl 5-methylfuryl sulfide, 128	1220	130(0.8), 129(1.1), 128(100) , 113(10.0), 100(28.3), 99(36.4), 85(98.0), 67(97.0), 66(25.0), 65(24.7), 59(38.1), 55(8.8), 51(48.2), 45(67.8), 41(37.0)

Table 6: Mass Spectral and Retention Data for Onion Flavor Compounds Detected in Various Extracts Studied.

No.	Compound, Mass	I_k^a	MS Data ^b
22	diallyl thiosulfinate, 162	1225	164(7.6), 162(14.6), 129(70.0), 99(7.1), 87(4.2), 86(4.6), 85(11.0), 69(100) , 59(25.4), 55(12.1), 45(18.7), 41(24.3)
23	1-propenyl propyl trisulfide (E/Z), 180	1249	182(18.5), 181(12.0), 180(100) , 164(11.8), 151(5.1), 116(32.2), 115(40.0), 106(41.0), 101(6.3), 8(9.1), 83(34.2), 75(7.0), 74(47.3), 73(15.7), 64(14.6) , 59(13.8), 47(7.4), 45(23.0), 41(52.5)
24	dipropyl trisulfide, 182	1254	184(15.2), 183(20.3), 182(100) , 140(8.2), 117(5.0), 98(6.2), 89(3.3), 75(76.0), 47(9.1), 45(10.8), 43(51.8), 41(31.7)
25	1-propenyl propyl trisulfide (E/Z), 180	1261	182(12.6), 181(8.1), 180(100) , 138(4.3), 116(47.2), 105(25.0), 75(8.8), 74(49.6), 73(74.7), 61(17.5), 59(15.2), 47(9.8), 45(57.0), 43(32.2), 41(48.4), 39(34.3)
26	diallyl trisulfide, 178	1263	182(13.0), 181(8.2), 180(65.1), 179(8.5), 178(100) , 147(18.0), 131(2.0), 114(84.8), 113(8.3), 99(61.6), 79(2.2), 73(58.3), 61(27.7), 47(15.8), 45(81.0), 43(42.5), 42(10.0)
27	dibenzothiophene, 184	1265	185(22.8), 184(100) , 169(35.0), 155(15.9), 151(35.0), 140(50.8), 139(47.6), 125(39.7), 112(70.4), 91(53.2), 85(13.3), 79(22.4), 69(12.0), 68(12.7), 64(17.5), 59(51.4), 54(11.0), 45(80.4), 41(49.1)
28	methyl 3,4-dimethyl-2-thienyl disulfide, 190	1429	192(14.3), 191(4.8), 190(69.0), 143(100) , 11(9.8), 97(5.5), 85(5.7), 69(5.4), 67(10.1), 65(5.8), 59(5.8), 55(3.6), 45(10.0), 41(3.4), 39(10.8)
29	4,6-diethyl-1,2,3,5-tetrathiane, 212	1484	213(4.2), 212(27.3), 192(12.0), 146(22.8), 139(5.0), 138(8.1), 115(10.7), 99(25.0), 74(100) , 73(62.6), 64(45.8), 59(7.4), 55(32.2), 46(37.0), 45(35.4), 43(7.7), 42(12.1), 41(84.0)
30	2,4-dimethyl-5,6-dithia-2,7-nonadienal-5-oxide, 218	1688	218(2.5), 129(20.6), 73(22.5), 71(9.6), 70(6.0), 69(100) , 59(17.5), 47(2.3), 45(44.5), 41(59.2)

Table 6: Mass Spectral and Retention Data for Onion Flavor Compounds Detected in Various Extracts Studied.

No.	Compound, Mass	I_k^a	MS Data ^b
31	5,7-diethyl 1,2,3,4,6 pentathiepane, 264	1817	180(25.8), 170(21.9), 139(22.0), 106(63.2), 99(54.0), 74(70.2), 73(27.7), 64(84.6), 59(18.1), 45(67.0), 41(100)
32	molecular sulfur, 256	1878	257(41.2), 256(100) , 224(3.0), 194(7.4), 193(1.8), 192(31.3), 162(13.2), 161(3.8), 160(52.2), 130(11.0), 129(0.8), 128(56.4), 98(3.7), 97(0.7), 96(19.5), 66(8.2), 65(1.8), 64(64.2)

a. Kovats retention indices

b. m/z (intensity)

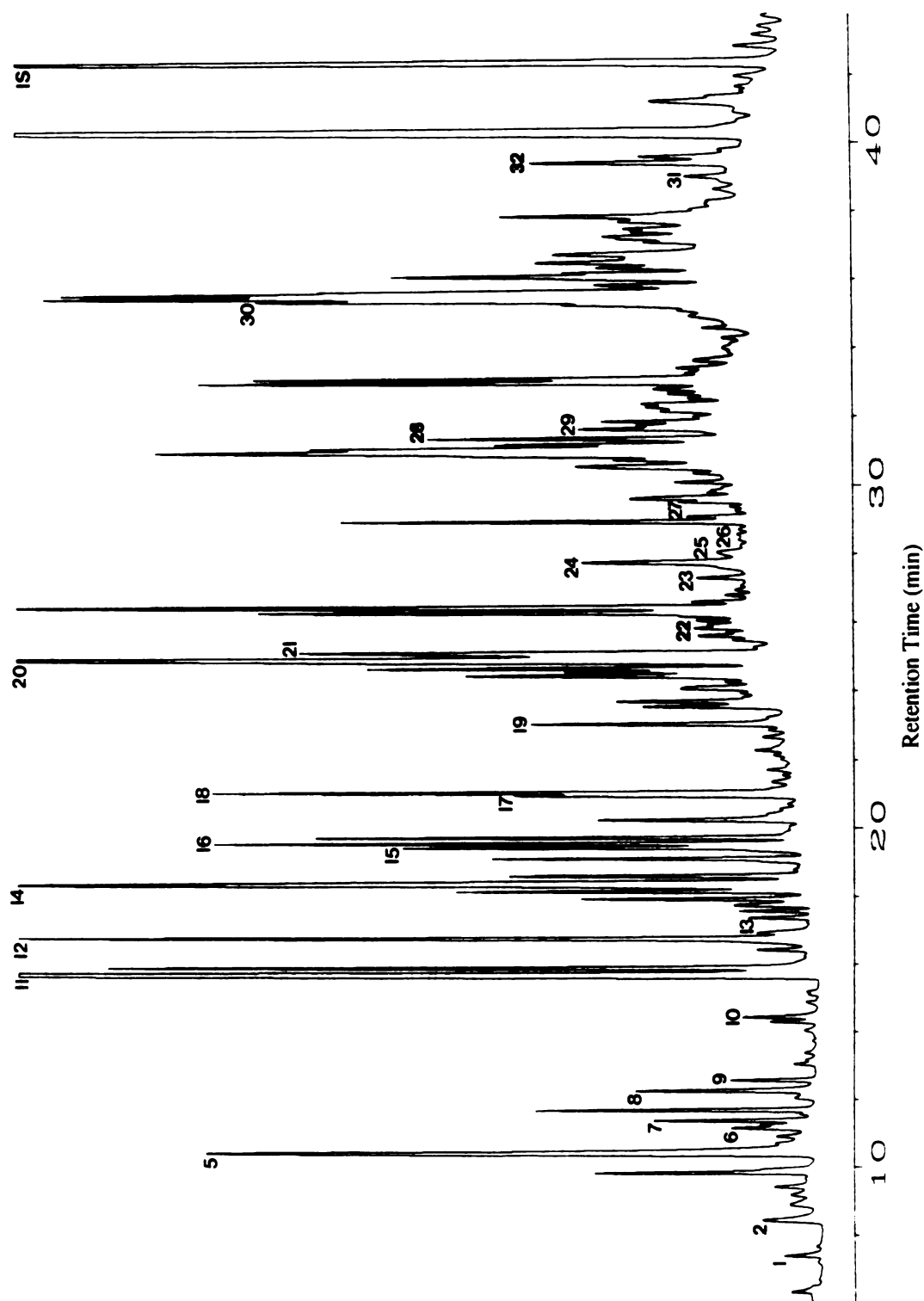


Figure 15. Gas Chromatogram of SFE-CO₂ + Ethanol (75 ml, 3600 psi, 37°C) Extract. Peak Numbers correspond with Table 6.

APPENDIX C

Statistical Analysis of Gravimetric Yield Data

Gravimetric yield data:

SFE (1)	SFE (0.5)	Liq CO2
0.225	0.2607	0.1843
0.2341	0.2579	0.164
0.227	0.259	0.1675

ANOVA Table:

Anova: Single-Factor

Summary (alpha = 0.01)

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
SFE (1)	3	0.6861	0.2287	2.29E-05
SFE (0.5)	3	0.7776	0.2592	1.99E-06
Liq CO2	3	0.5158	0.171933	0.000118

ANOVA

Source of Variation

	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Gr	0.011768	2	0.005884	123.7684	1.33E-05	10.92485
Within Gro	0.000285	6	4.75E-05			
Total	0.012053	8				

Fisher's Protected Least Significant Difference (LSD) Analysis:

LSD= 0.0209

Since all the pairwise differences of means were found to be greater than the LSD, hence, the population means for the three methods are different at the 1% level.

Statistical Analysis of Quantitative GC Data

Yield Data from Quantitative GC Analysis:

	1	2	3	Avg	StDev
1	0.0525	0.0613	0.0592	0.0577	0.0046
2	0.0854	0.0813	0.0796	0.0833	0.003
3	0.0228	0.0148	0.0214	0.0197	0.0043
4	0.0231	0.0254	0.0292	0.0259	0.0031
5	0.0262	0.0329	0.0297	0.0296	0.0034
6	0.0516	0.0476	0.0454	0.0482	0.0031
7	0.0629	0.0687	0.0649	0.0655	0.003
8	0.0645	0.0677	0.0698	0.0673	0.0027
9	0.0396	0.0334	0.0314	0.0348	0.0043

Analysis of Variance: Single-Factor ($\alpha = 0.05$)

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
1	3	0.173	0.057667	2.11E-05
2	3	0.2463	0.0821	8.89E-06
3	3	0.059	0.019667	1.83E-05
4	3	0.0777	0.0259	9.49E-06
5	3	0.0888	0.0296	1.12E-05
6	3	0.1446	0.0482	9.88E-06
7	3	0.1965	0.0655	8.68E-06
8	3	0.202	0.067333	7.12E-06
9	3	0.1044	0.0348	1.83E-05

ANOVA

Source of Variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Gr	0.01122	8	0.001403	111.7572	9.47E-14	2.510156
Within Gro	0.000226	18	1.25E-05			
Total	0.011446	26				

Fisher's Least Significant Difference Analysis:

$$\text{LSD} = 2.101$$

All the pairwise differences were found to be significantly different at the 5% level except the following pairs: 4-5, 5-9, and 7-8.

Statistical Analysis of Thiosulfinate Analysis Data

Thiosulfinate analysis data:

SC-EtOH	SC-Oct	Stm Dst.
1.784	1.556	0.322
1.524	1.447	0.286
1.711	1.717	0.315

ANOVA Table:

Anova: Single-Factor

Summary (alpha = 0.01)

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
SC-EtOH	3	5.019	1.673	0.017983
SC-Oct	3	4.72	1.573333	0.01845
Stm Dst.	3	0.923	0.307667	0.000364

ANOVA

Source of Variation

	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Gr	3.475981	2	1.73799	141.693	8.91E-06	10.92485
Within Gro	0.073595	6	0.012266			
Total	3.549576	8				

Fisher's Protected Least Significant Difference (LSD) Analysis:

LSD= 0.3352

The first two means were not significantly different from each other but they both were found to be significantly different from the third mean at 1% level.

APPENDIX D

Organoleptic Evaluation of Supercritical CO₂ Onion Extracts



KALSEC[®], Inc.

P.O. Box 511, KALAMAZOO, MICHIGAN 49005-0511 • USA PHONE: (616) 349-9711 • TELEX: 295181 • FAX (616) 382-3060 • SPICEX

TO: Dr. Daniel Guyer
Department of Agricultural Engineering, MSU

FROM: James A. Guzinski
Kalsec, INC.

DATE: February 26, 1983

FAX #: 1-517-353-8982

RE: Onion flavor extract

Dear Dr. Guyer:

Thank you for sending the sample of onion oil you obtained through carbon dioxide extraction. I submitted the material to our sensory division for comparison to a typical, commercially available oil manufactured by steam distillation of onions. The sample was diluted by 1 part in 10,000 and rated on our standard flavor attributes.

The results of the organoleptic evaluation show that the sample, which was described as having 2.5% onion oil, was weaker than a commercial onion oil tasted at the same level. However, the flavor profile was distinctly different. It was described as having less of the burnt and metallic flavor notes of typical onion oil. As one of the panelists, I thought it was a very good flavor. I am going to resubmit the sample for testing at 50% higher concentration. I think there would be a market for a better, fresher onion flavor. If possible, I would like a larger sample to test in some flavor applications, possibly in an uncooked application such as a salad dressing.

I encourage you to continue in this line of research. We are definitely interested in a fresh onion flavor and are willing to evaluate any samples you can provide.

Regards,

A handwritten signature in cursive script, appearing to read "J. Guzinski".

Dr. James A. Guzinski
Senior Chemist

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