This is to certify that the

thesis entitled

"Supercritical Fluid Extraction of
Quercetin from Onion Skins"

presented by

Karina Gorostiaga Martino

has been accepted towards fulfillment
of the requirements for

M.S. degree in Biosystems Engineering

Daniel Guyer
Major professor

Date Aug 21, 2002

SUPERCRITICAL FLUID EXTRACTION OF QUERCETIN FROM ONION SKINS

By

Karina Gorostiaga Martino

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER IN SCIENCE

Department of Biosystems Engineering

2002

ABSTRACT

SUPERCRITICAL FLUID EXTRACTION OF QUERCETIN FROM ONION SKINS

By

Karina Gorostiaga Martino

Supercritical fluid extraction with ethanol-modified carbon dioxide of quercetin from onion skins (red and yellow varieties) was studied. Static and dynamic modes were investigated as extraction methods, with the static mode yielding the highest amount of quercetin recovered. The total amount of 0.024 g of quercetin per kg of onion skin was obtained for the red variety, and 0.020 g per kg for the yellow variety, at 5700 psi, 40°C, an average of 7.6% (molar concentration) of ethanol, and in an extraction period of 2.5 h.

The extraction of pure quercetin from spiked diatomaceous earth was also investigated, extraction conditions were the same as for the onion skins. A maximum of 0.036 g of quercetin per kg of matrix was extracted.

The modifier was found to have an important influence on quercetin recovery. The greater the amount of ethanol collected in the trap, the greater the amount of quercetin recovered. This trend was found for both varieties and for the pure quercetin.

Identification and quantification analysis were conducted in a High Performance Liquid Chromatography system.

ACKNOWLEDGMENTS

I would like to give my special thanks to the Department of Food Science and Human Nutrition for letting me use its facilities. I am specially appreciative to Dr. Maurice Bennink for his guidance and generosity in letting me access to his laboratory equipment, and to Dr. Kirk Dolan and Dr. Mark Uebersax for also permitting me to use their laboratory equipment.

I would also like to thank to the Department of Civil and Environmental Engineering, especially to Dr. Tomas Voice, Dr. Paul Loconto and Mr. Joseph Nguyen for their valuable contribution to my work, with their guidance and letting me use their laboratory equipment, at the beginning of this project.

I would like to give my appreciation and respect to my committee members, Dr. Carl Lira and Dr. Kirk Dolan for their technical support, and Dr. Daniel Guyer for giving me the opportunity to pursue my degree, for all his support and guidance and his strong confidence in my work.

Finally, thanks to all my friends from Agricultural Engineering and Food Science departments for making the last two years very enjoyable and productive. A very special thanks to my lovely husband and family for their unending support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

1.1. Flavonoids

Flavonoids are plant polyphenols found frequently in fruits, vegetables, and grains. Divided into several subclasses, they include the anthocyanidins, pigments chiefly responsible for the red and blue colors in fruits, fruit juices, wines, and flowers; the catechins, concentrated in tea leaves; the flavonones and flavanone glycosides, found in citrus and honey; and the flavones, flavonols and flavonol glycosides, found in tea leaves, fruits, vegetables, and honey. Flavonoids are known for their hydrogen-donating antioxidant activity as well as their ability to complex divalent transition metal cations. Moreover, they promote human health. These compounds are active against allergies, inflammation, viruses, hypertension, arthritis, and are reported to prevent mutations, carcinogens, cancer, and AIDS (Merken et al., 2000). The supercritical fluid extraction of quercetin (one member of the flavonol group) from onion skins, was the main focus of this study.

Flavonoids, derived biosynthetically from phenylalanine, are pigments found widespread in plants. Three moles of malonyl-coenzyme A (CoA) from glucose metabolism condense to form ring A, a reaction catalyzed by chalcone synthetase (Figure 1). Rings B and C are also derived from glucose metabolism, but via the shikimate pathway in which phenylalanine is converted to cinnamic acid and then to coumaric acid. Coumaric acid CoA and three malonyl CoAs then condense in a single enzymatic step to form naringenin chalcone. The C-ring closes and becomes hydrated to form 3-hydroxiflavonoids (e.g. catechins), 3,4-diol flavonoids (e.g. quercetin), and procyanidins.

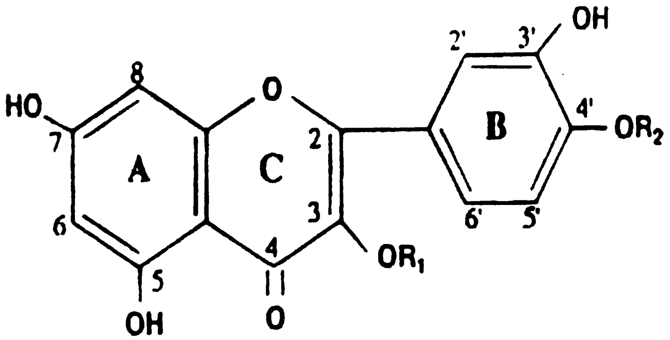

Figure 1. Flavonoids structure. Quercetin (free aglycone): $R_1$and R_2 = Hydrogen

Flavones and flavonols are usually found in plants as *O*-glycosides. The flavonols have a hydroxyl group at C_3, where the flavones have a hydrogen atom. Glycosides of the flavonol quercetin predominate in vegetables, whereas glycosides of the flavonol kaempferol and of the flavones apigenin and luteolin also exist.

The formation of flavone and flavonol glycosides depends normally on the action of light, so that in general the highest concentrations of these compounds are nearly always found in the free-standing leaves. Onions are the only exception (Herrmann, 1976). Flavones and flavonols do not contribute markedly to the coloration of the plant except where they occur in very high concentration, as in the skins of onions, or when they are complex with metals (Herrmann, 1976).

1.2. Quercetin

Hayashi (2000) has demonstrated that tumor growth in mice is significantly inhibited using a combination of quercetin and chalcone (open chain flavonoid). Furthermore, Caltagirone (2000) reported that quercetin and apigenin inhibit the growth, the invasiveness, and metastatic potential of melanoma; therefore, flavonols may constitute a valuable tool in combination therapy for metastatic melanoma. Finally, Xing et al. (2001) stated that quercetin has the potential to become a chemopreventive and/or chemotherapeutic agent for prostate cancer.

Unlike other vegetables and in contrast to white-skinned varieties, onions with pigmented skins have exceptionally high flavonol content. Quercetin appears mainly in the free form (3, 3', 4', 5, 7-Pentahydroxyflavone), i.e. as the aglycone, 67-86% of total quercetin (aglycone and glycosides), and to a smaller extent as spiraeoside (quercetin-4'-glycoside). The epidermis of onion scales contains quercetin glycosides exclusively, mainly as spiraeoside, which is formed first, the diglycosides are formed later and increase continuously during storage. The flavonol concentration decreases from the outer to the inner scales, with higher levels in the outer epidermis (Herrmann, 1976).

Bilyk et al. (1984), found that the skin of the Yellow Globe hybrid variety has the highest free quercetin (aglycone) content in the dry skin (53%, w/w, of the total quercetin). Other varieties studied were Sweet Spanish Utah, Early Yellow Globe, Yellow Globe Hybrid, Sweet Spanish Hybrid, Red Hamburger, Walla Walla and Evergreen Long White Bunching, the range of free quercetin content in these varieties was 23 to 51% (w/w, of the total quercetin).

In 1997, Price et al. investigated different varieties, such as Red Baron, Rijnsburger, Rose and Albion. They found that Rijnsburger variety had the highest amount of free quercetin (0.039 g per kg fresh weight).

Extraction of flavonols from onion tissue, in the above noted studies, was accomplished by solvent extraction with methanol. Unfortunately at industrial level, problems arise with this technique when removing organic solvent residues from the final product, disposing of waste methanol, and exposing personnel to the extracting solvent. Therefore, there is an important need for rapid and clean methods for the extraction and determination of this highly valuable natural product, quercetin. Supercritical fluid extraction provides several advantages over traditional liquid-solvent-based extraction methods including improved selectivity, expeditiousness, automation and environmental safety. The avoidance of organic solvents residues is a major goal in the isolation of natural products which may be commercialized as food additives (Tena et al., 1998).

The present study investigated the hypothesis that quercetin could be extracted from a natural matrix (onion skins), using odorless, tasteless, inert and nontoxic supercritical carbon dioxide.

In order to test this hypothesis, the following objectives were proposed.

1. To develop a technique for the extraction of quercetin.
2. To identify quercetin and quantify the amount extracted, from red and yellow onion varieties.
3. To compare the extraction of quercetin in the presence of other similar compounds from the natural matrix (onions), with the extraction of the pure quercetin from the spiked inert matrix (diatomaceous earth).

2. Literature Review

2.1. Theories of Supercritical Fluid (SF)

SFs utilize the ability of certain chemicals to become excellent solvents for certain solutes under an appropriate combination of temperature and pressure. There are several advantages of these fluids compared to liquid organic solvents. They have a higher diffusion coefficient and a lower viscosity. The absence of surface tension allows for rapid penetration into the pores of heterogeneous matrices helping to enhance extraction efficiencies. Depending on the conditions of temperature and pressure, the solubility of the various components in the SF can be varied. Therefore, selectivity during extraction may be manipulated. Finally, SFs do not leave a chemical residue (Rozzi et al., 2002).

2.1.1. Carbon dioxide as a supercritical solvent

In her latest work, Mukhopadhyay (2000) reviewed relevant characteristics and properties of supercritical carbon dioxide. For example, when a gas is compressed to a sufficiently high pressure, it becomes liquid. A gas can also be heated beyond a specific temperature, at which point no amount of compression will cause it to become a liquid. This temperature is called the critical temperature and the corresponding vapor pressure is called the critical pressure. These values of temperature and pressure define a critical point, which is unique to a given gas or liquid. Figure 2 shows a phase diagram that describes the state of gas or liquid, called a SF, when both the temperature and pressure exceed the critical point (CP) values.

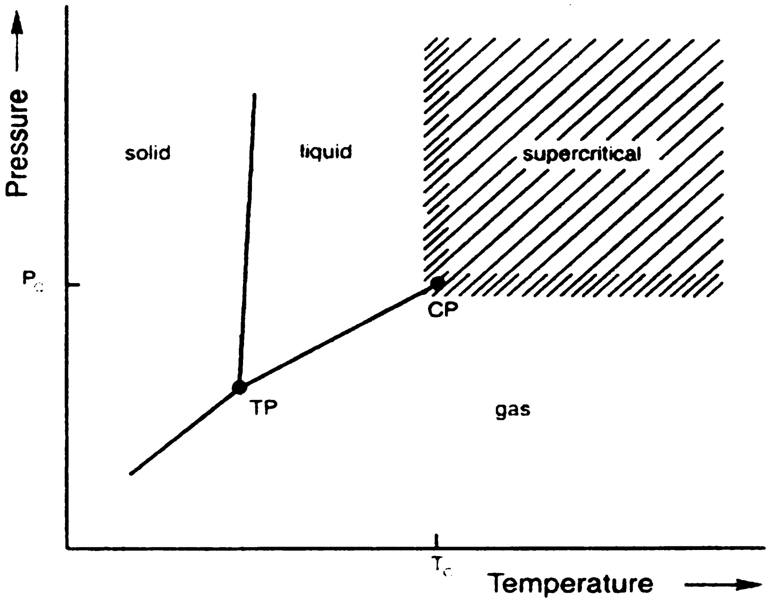

Figure 2. Phase diagram of a pure component. Mukhopadhyay (2000).

The SF assumes many of the properties of both gas and liquid. In the supercritical region (Figure 2), with only small changes in temperature and pressure, maximum solvent capacity and the widest range of variations in solvent properties can be achieved (Mukhopadhyay, 2000).

Carbon dioxide is one of the most desirable SF solvents for extraction of natural products for use in foods and medicines. It is inert, inexpensive, easily available, odorless, tasteless, environment-friendly, and GRAS (generally regarded as safe). Furthermore, no additional "green house effect" results from using carbon dioxide as the supercritical fluid solvent since it is already present in the environment. It is obtained as a by-product from fermentation processes or fertilizer manufacture; therefore, its use as an extractant does not cause any further increase in the amount of carbon dioxide present in the earth's atmosphere (Mukhopadhyay, 2000).

2.1.2. Supercritical fluid extraction (SFE) system

The four primary steps involved in SFE are compression, extraction, expansion, and separation; the pieces of equipment commonly utilized are a compressor, a high-pressure extractor, a pressure reduction valve and a low pressure separator (Figure 3).

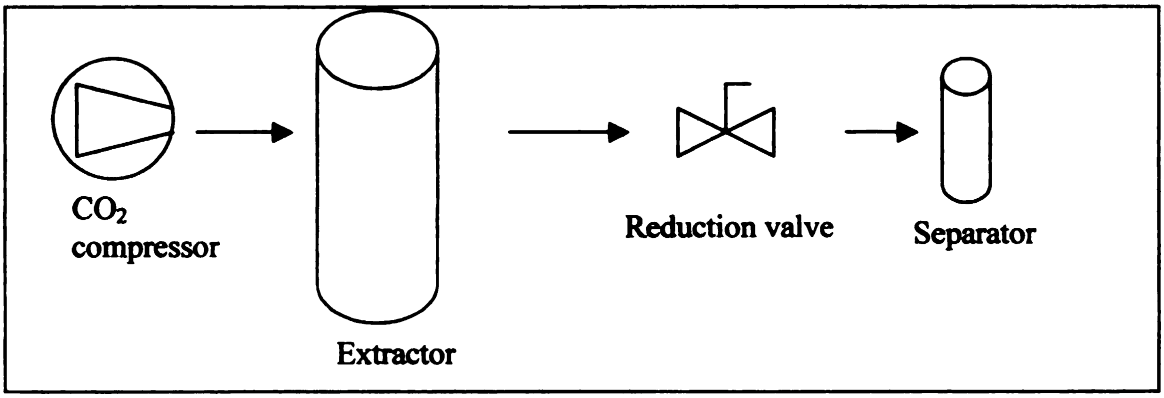

Figure 3. SFE steps

2.1.3. Extraction modes

SFE can be performed in a static, dynamic or coupled static/dynamic mode. When a fixed amount of SF interacts with the analyte and the matrix, a static extraction takes place. The extraction vessel containing the matrix is pressurized with the chosen SF at a certain temperature. The SF remains in the vessel, without flow, until the extraction is completed. No SF enters or leaves the vessel during the extraction. The high diffusivity of the fluid causes it to permeate the matrix and remove some portion or all of the analyte. Typically, a static mode is used when modifiers and derivatizing reagents are added (Taylor, 1995).

If fresh SF is continuously passed over or through the sample matrix, a dynamic extraction takes place (Taylor, 1995). This mode permits the continuous flow of SF until the extraction is completed and uses as much SF as is needed for that period of time.

The coupled mode is especially useful when the analyte must diffuse to the matrix surface to be extracted (Taylor, 1995). In this mode, there is a combination of no flow and continuous flow through the vessel for a fixed period of time. For example, dynamic/static mode, means first there is a period of time when the SF passes through the vessel continuously. Then, the inlet and outlet valves of the vessel are closed and the SF remains in the vessel without flow for a fixed time. Extraction can also occur in static/dynamic mode, in which the steps are reversed.

2.1.4. Modifier introduction

An entrainer (or modifier) is a cosolvent, such as ethanol, water or various gases, which can be used in the food industry (Palmer, 1995). The entrainer increases the polarity and solvent strength of the SF while it retains the sensitivity of solubility with respect to pressure and temperature. Furthermore, a cosolvent can improve the selectivity of separation by preferentially interacting with one or more components and facilitating selective fractional separation. For example, for systems consisting of polar solutes, solubility is significantly enhanced with polar cosolvents due to dipole-dipole interactions and hydrogen bonding (Mukhoapadhyay, 2000).

2.2. Related research

There has been no previous research related to the extraction of quercetin from a natural matrix with modified SF carbon dioxide.

However, Tena et al. (1998) studied the SF extraction of spiked diatomaceous earth (inert matrix) with phenolics including gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, p-coumaric acid, myricetin, t-resveratrol, quercetin and salicylic acid. Pure carbon dioxide and methanol carbon dioxide was used. Low-molecular-weight phenolics containing 2-3 polar groups such as t-resveratrol, p-coumaric acid and salicylic acid were recovered with pure carbon dioxide (mean recovery ≥ 95%). However, the inclusion of methanol was necessary to recover more polar compounds such as gallic acid, caffeic acid, catechins and quercetin; the recovery rates ranged between 30% and 70%. Myricetin was not recovered.

Palma et al. (1999), studied the extractability of eight polyphenolic compounds, found naturally in grapes, from spiked sand samples. Such compounds included benzoic acids, cinnamic acids, benzoic aldehydes, catechin and resveratrol. Gallic acid was the most polar and the least polar was resveratrol. They used high density and high percentage of modifier, due to the high polarity of phenols. The average recovery of the eight phenols was 88%. After modified conditions of extraction, such as modifier content and restrictor/trap temperature, recoveries for all eight compounds ranged from 92% to 100%.

Resveratrol was successfully extracted from grape skin using supercritical fluid, and coupled static (30 seconds)/dynamic (15 min) mode. The operating conditions were

150 bars, 40°C and 7.5% ethanol. They recovered almost 100% in 15 min (Pascual-Marti et al., 2001).

Grape glycosides were recovered from spiked earth and real grapes (100% in both cases) using methanol-modified supercritical carbon dioxide and coupled static/dynamic mode as an extraction method. Extraction conditions were: gas density, 0.95 g/ml; temperature, 40°C; supercritical fluid flow, 1.5 ml/min; static time, 15 min; dynamic time, 20 min; restrictor temperature, 50°C; liquid trap temperature, 30°C; liquid trap solvent, water; liquid trap volume, 8 ml (Palma et al., 2000).

The last two papers mentioned above studied the extraction of compounds similar to quercetin from natural matrices. All of them used modified-carbon dioxide as a supercritical fluid and tried static/dynamic mode as an extraction method. For the present study, similar extraction conditions were tested.

Bilyk et al. (1984) investigated the presence of quercetin and kaempferol of eight onion varieties (Table 1). They separated the dry skins (portion A), outer rings (portion B) and inner rings (portion C) and extracted, with methanol, the flavonol glycosides. Flavonols were detected and quantified by thin-layer chromatography, high-performance liquid chromatography, and spectrophotometric analyses. They concluded that the skin of all onion varieties contained quercetin in both the aglycone and the glycosides form. Some specific varieties also contained small amounts of kaempferol.

Table 1. Quercetin content (g/kg of onion portion, fresh weight)

		Quercetin	
Variety	*Portion* [a]	*free*	*Total* [b]
Carmer hybrid	A	7.73 (23%)	34.15 ± 0.460
	B		0.062 ± 0.006
	C		0.027 ± 0.001
Sweet spanish Utah	A	3.87 (24%)	16.53 ± 0.410
	B		0.295 ± 0.005
	C		0.002 ± 0.001
Early yellow globe	A	6.18 (39%)	16.06 ± 0.040
	B		0.053 ± 0
	C		ND [c]
Yellow Globe hybrid	A	7.54 (53%)	14.16 ± 0.380
	B		0.055 ± 0
	C		0.010 ± 0
Sweet spanish hybrid	A	4.73 (50%)	9.51 ± 0.090
	B		0.113 ± 0.012
	C		0.032 ± 0.001
Red hamburger	A	2.61 (40%)	6.6 ± 0.180
	B		ND
	C		ND
Walla walla	A	2.69 (51%)	5.30 ± 0.090
	B		0.082 ± 0.008
	C		0.001 ± 0
Evergreen long white bunching	leaves	0.06 (5%)	1.14 ± 0.007
	bulbs	ND	ND

[a] A = dry skin; B = outer rings (scales 1-3); C = inner rings (remaining rings)
[b] Results are given as mean ± standard deviation for triplicate determinations.
[c] ND = not detectable.
Adapted from Bylik et al., 1984.

In a different study, extraction of flavonols from four onion varieties was investigated (Price et al., 1997. Table 2). The major flavonoids of mature onion bulbs were confirmed to be the quercetin diglycosides and quercetin monoglycoside, using methanol extraction and a combination of chromatographic comparisons, mass spectrometry and nuclear magnetic resonance spectroscopy.

Table 2. Flavonol level and composition

Variety	*Content (mg/kg fresh weight)* [a]				*% total flavonols*
	Qdg	*Qmg*	*Q*	*Total*	
Red baron	1375	394	9	1778	88.30
Rijnsburger	1117	360	39	1516	93.00
Rose	1052	302	15	1369	94.20
Albion	50	36	3	89	61.00

[a] Abbreviations: Qdg, quercetin 3,4'-O-diglycoside; Qmg, quercetin 4'-O-monoglycoside; Q, quercetin. Price et al., 1997.

2.2.1. Analytical Method

High performance liquid chromatography (HPLC) is used as an analytical method for quantification of flavonoids, including quercetin. Columns used in the system are almost exclusively reversed-phase (RP), ranging from 100 to 300 mm in length, 4.6 mm internal diameter, and packed with C_{18} column material. Mobile phase systems are usually binary, with an aqueous acidified polar solvent (solvent A) such as aqueous acetic acid, perchloric acid, phosphoric acid, or formic acid, and a less polar organic solvent (solvent B) such as methanol or acetonitrile. Flow rates range between 1.0 and 1.5 mL/min. Thermostatically controlled columns are normally kept at ambient or slightly above ambient temperatures. Injection generally ranges from 1 to 100 μL. Phenols absorb in the ultraviolet (UV) region; flavones, flavonols and flavonol glycosides are usually detected at wavelengths 270, 280, 350, 365, and 370 nm (Merken et al., 2000).

2.2.2. Modeling the supercritical fluids

There are many methods that describe the solubility of substances in SFs. Two of them are the solubility parameter and process modeling. The first one describes the solubility of the solute under laboratory conditions and the second method is to model the process itself with experiments (Rozzi et al., 2002). In the present study the combination of the theory (solubility parameter) and the practice was attempted. The extraction conditions were determined with the solubility parameter, and then these extraction conditions were adapted to the equipment limitation and operation.

3. Materials and Methods

The study was divided in two parts. Preliminary experiments explored methods for extraction and analysis of quercetin, and familiarization with the supercritical fluid (SF) and high performance liquid chromatography (HPLC) system. The preliminary results lead to the second study phase, where primary experiments and analytical procedures were focused and replicated.

3.1. Reagents and samples

Industrial grade carbon dioxide (CO_2, BOC Gases, Murray Hill, N.J.) was used as a SF. Yellow (Spartan Banner 80 and Sweet Sandwich) and red (Mars) onion varieties were obtained from the Muck Farm (Michigan State University, East Lansing, MI). Methanol was purchased from Honeywell International Inc. Burdick and Jackson (Muskegon, MI). Ethyl alcohol 190 Proof was purchased from Pharmco (Brookfield, CT). Phosphoric acid 86.3% was obtained from J. T. Baker (Phillisburg, NJ). Quercetin standard and diatomaceous earth were obtained from Sigma (St. Louis, MO).

3.2. SF extraction equipment

The SF equipment is shown in Figure 4.

Extractions were conducted in a 500 ml (10) stainless steel high-pressure vessel (Autoclave Engineers, Inc., Erie, PA). The vessel was wrapped with heating tape (120 volts) and insulated with foil bubble wrap to maintain constant temperature. The temperature was monitored and controlled with a thermocouple and temperature controller (Omega Engineering, Inc., Stamford, CT).

Carbon dioxide from a gas cylinder (1) was compressed by an air-driven booster compressor (3, Haskel, Inc, Burbank, CA) and stored in a 2 L reservoir (4). The gas cylinder had a CO_2 regulator (2) attached to it. Pressure gauges (5, 8) were used to monitor the pressure in the reservoir and extraction vessel, respectively. A forward pressure regulator (7, Tescom Corporation, Elk River, MN), positioned between the reservoir and the extraction vessel, controlled the extraction pressure. The flow of gas was monitored using a dry test meter (14, American Dry Test Meter Model DTM-200A-3, American Meter Co., Philadelphia, PA). Two shutoff valves (6, 11, Autoclave Engineers, Inc., Erie, PA) were used to control the CO_2 stream in the system. For the preliminary experiments, a piston pump (9, Model 305, Gilson, Middletown, WI) was used to inject the modifier in the CO_2 feed line, and later, to recirculate the extract to that same line (Figure 5). The micrometering valve (12, Autoclave Engineers, Inc., Erie, PA) was opened, the pressure was reduced to atmospheric, the extract was then separated from the gas and collected in a glass tube trap (13). The trap consisted of a glass vial (3.7 ml) inserted in a glass tube, surrounded by dry ice. Cartridge heaters embedded in steel saddle blocks were mounted to the micrometering valve and set at a temperature just high enough to keep the valve from freezing due to the instantaneous expansion of CO_2.

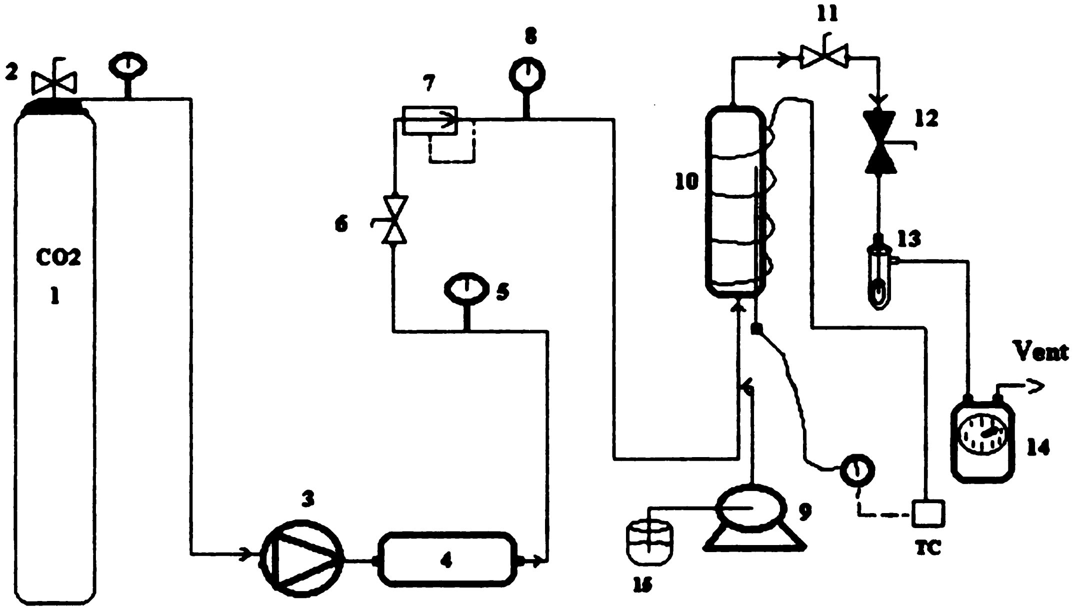

Figure 4. SF equipment. Carbon dioxide cylinder, 1. Carbon dioxide regulator, 2. Booster compressor, 3. Compressed gas reservoir, 4. Pressure gauges, 5, 8. Shut-off valves, 6, 11. Forward pressure regulator, 7. Piston pump, 9. Vessel, 10. Micrometering valve, 12. Trap, 13. Gas meter, 14. Entrainer reservoir, 15. TC: temperature control

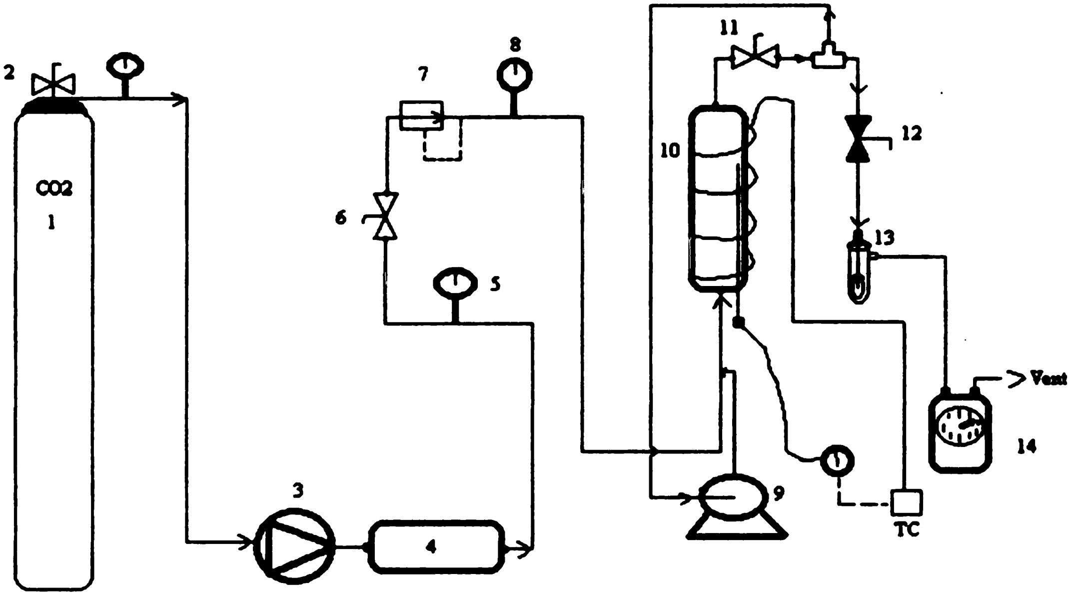

Figure 5. SF equipment with recirculation. Carbon dioxide cylinder, 1. Carbon dioxide regulator, 2. Booster compressor, 3. Compressed gas reservoir, 4. Pressure gauges, 5, 8. Shut-off valves, 6, 11. Forward pressure regulator, 7. Piston pump, 9. Vessel, 10. Micrometering valve, 12. Trap, 13. Gas meter, 14. TC: temperature control

3.3. Liquid chromatographic systems

Preliminary analysis of the extracts was conducted using an HPLC system consisting of Turbochrom chromatography software (version 4.1), and a Perkin Elmer binary LC pump (Model 250, Norwalk, CT) connected to a Rheodyne (Cotati, CA) manual injector (5 µL injection loop). The chromatographic column used for this analysis was a BetaBasic C_{18}, 250 x 4.6 mm, 5 µm particle size, and a 150 Å pore size (Keystone Scientific, Inc., Bellefonte, PA). A guard column (packed with C_{18}, 1 cm x 4 mm, 5 µm particle size) was connected in front of the column. Compounds were detected and identified using a Perkin Elmer Diode Array detector (Model 235 C).

During initial development of SF extraction and analysis methods, problems were encountered with the above HPLC system. Therefore, a different HPLC system was implemented at the time the primary experiments began. The system consisted of PEAKSIMPLE for WINDOWS '95 software. A Dionex gradient pump/QUAT (Model AGP-1, Cotati, CA) was connected to a Rheodyne manual injector (10 µL injection loop). The same column and guard column as in the preliminary analysis were used. An LC spectrophotometer was used as a detector (Waters, Lambda-Max, Model 481, Milford, MA).

3.4. Sample storage

In order to preserve the raw material during the total research period, onions were stored at -20°C. Extracts were stored at 6°C.

3.5. Supercritical fluid extraction

3.5.1. Sample preparation

3.5.1.a. Natural sample: the onion's outer dry skin layers were manually separated from the bulb and diced (broken) into small flakes approximately 0.5 x 0.5 in.

3.5.1.b. Spiked model: quercetin (≈0.1 g) was dissolved in 20 ml of absolute methanol. This solution was spiked into 50 g of diatomaceous earth. The spiked solid was oven-dried at 50°C for 2 h and then stored at room temperature for at least 12 h to evaporate methanol. The ratio of quercetin loaded per weight of earth was chosen based on the minimum ratio found in the methanol extraction of quercetin from the onion skins. The minimum ratio, assumed to be the worst-case scenario (smallest amount of quercetin in natural matrix), was used to investigate if small amounts of quercetin can be extracted in higher amounts from the inert than from the natural matrix with similar quercetin concentration.

3.5.2. Experimental Procedure

3.5.2.a. Preliminary experiments: static and dynamic extraction modes were conducted separately. Figure 6 shows the schema of this study phase.

Three different *static mode* experiments were performed. A range of 9 to 12 g of diced onion skins was used. The entrainer concentration was approximately 3% (molar concentration). Both entrainer and onion skins were added directly to the vessel before it was sealed. System pressure was set to 7700 psi, and the heating tape was set to 40°C. To start up the extraction, the vessel was slowly filled (to avoid onion skins plugging the vessel's outlet and to prevent freezing the CO_2 feed line) with CO_2. The system reached the desired temperature and pressure in 25 to 30 min. Once the system reached the desired conditions, to collect the extract, the micrometering valve was opened, and the desired amount of CO_2 (Table A1) passed the dry test meter, as fast as possible in order to maintain the static mode method. The micrometering valve was then closed, and the vial was changed for a new one. The extraction collection plus waiting period between collections lasted 10 – 20 min, in order to collect higher volumes of extract for sample analysis. Next, the micrometering valve was opened again, and the next extract was collected as described above. The extraction period lasted 2.5 h. The extract consisted of ethanol and polyphenols, including quercetin.

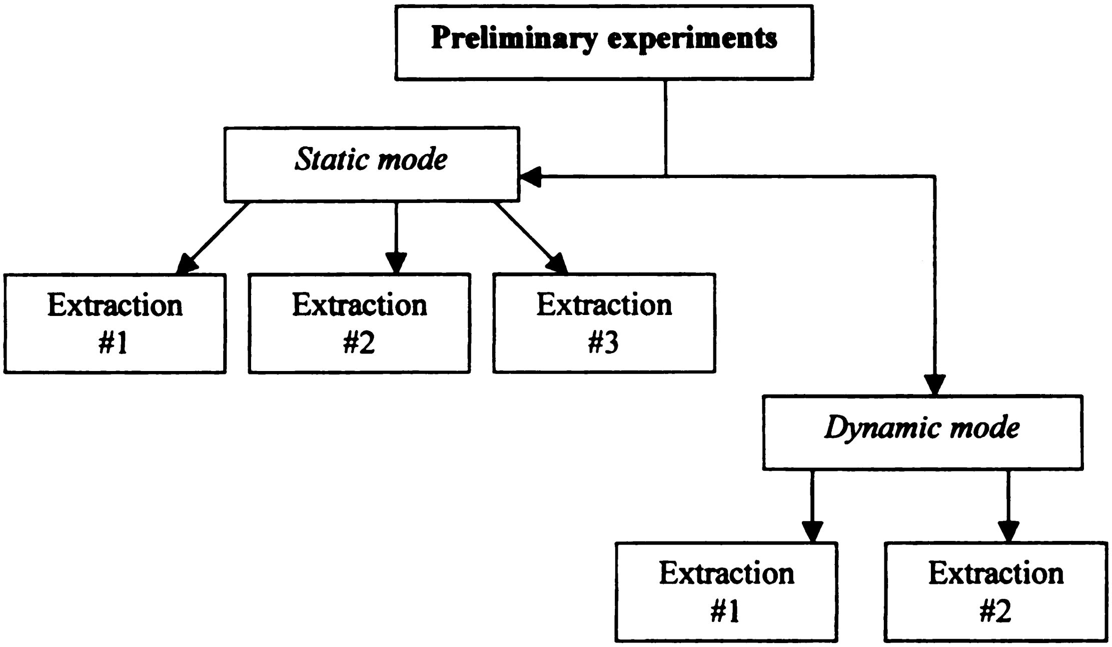

Figure 6. Preliminary experiments schema

Based on early extractions (before preliminary experiments) conducted with the same SF equipment, the amount of ethanol added was first estimated, and then confirmed (once the actual volume of CO_2 in the system was determined), with the following equation:

$$\% \, EtOH = \frac{EtOH \ moles}{CO_2 \ moles + EtOH \ moles} * 100 \qquad \text{Eqn.1}$$

Where:

$$EtOH \ moles = \frac{\rho \left(C_2H_5OH\right) * ml \left(C_2H_5OH\right)}{Molecular \ Weight \left(C_2H_5OH\right)} \qquad \text{Eqn.1a}$$

$$CO_2 \ moles = \frac{L \left(CO_2\right)}{Molar \ Volume} \qquad \text{Eqn.1b}$$

$\rho \left(C_2H_5OH\right) = 0.8 \ g / ml$

$Molecular \ Weight \left(C_2H_5OH\right) = 46 \ g / mole$

$Molar \ Volume \left(CO_2\right) = 24.34 \quad L / mole$ (at 298 K and 1 atm)

EtOH = ethanol

ml (C_2H_5OH) = ml of ethanol used during the extraction period

L (CO_2) = liters of carbon dioxide used to fill the system.

Two different *dynamic mode* extractions were conducted. The amount of diced onion skins on average was 11 g. Part of the total amount (2.0 to 6.6 %, molar concentration, based on Equation 1) of ethanol was added to the vessel, the rest of it was pumped to the CO_2 feed line with a piston pump during the extraction period (flow rate range: 0.1 to 0.5 ml/min). The vessel pressure and temperature were set to 7700 psi and 40°C, respectively. Once extraction conditions were reached, the micrometering valve was opened, the CO_2 flow started, and the piston pump started to pump the modifier. To collect the extract, CO_2 passed through the trap for 10 – 20 min (Table A2), then the micrometering valve was closed, and the vial was changed for a new one. The micrometering valve was immediately opened again for the next collection. Extraction period was about 3 h total.

3.5.2.b. Primary experiments: static mode experiments were conducted for the yellow and red onion varieties. The schema can be seen in figure 7.

The vessel pressure and temperature were set to 5700 psi (maximum pressure allowed by the piston pump to recirculate) and 40°C, respectively. The 500 ml vessel was wrapped with the heating tape and heated 24 h before the extraction, in order to have uniform vessel temperature by the time of extraction. Diced onion skins and ethanol (% calculated based on Equation 1) were added to the vessel before it was sealed. The piston pump was set to 5 ml/min for recirculation. Once the desired conditions were reached, the micrometering valve was opened, 15 L of CO_2 passed through the trap (as fast as possible to maintain the static mode), then the micrometering valve was closed, and the vial was changed for a new one. This procedure and the waiting period between collections lasted 10 – 18 min. Then, the next sample collection started once again with the opening of the micrometering valve (Table A4 and A5). After the collection, the extract volume was measured with a 5 or 1 ml pipette.

3.5.2.c. Spiked procedure: the same conditions and procedure as the static mode described above were used (Table A6). The filling procedure of the vessel was different. First, a piece of glass wool was introduced, followed by a filter paper, then, ethanol was added (which filtered through the paper). Finally, a mixture of the spiked earth and glass wool was introduced. The filter paper and glass wool acted as a support for the spiked earth, it also prevented the direct contact of the earth with ethanol. A piece of glass wool was then added again to the top of the vessel before sealing.

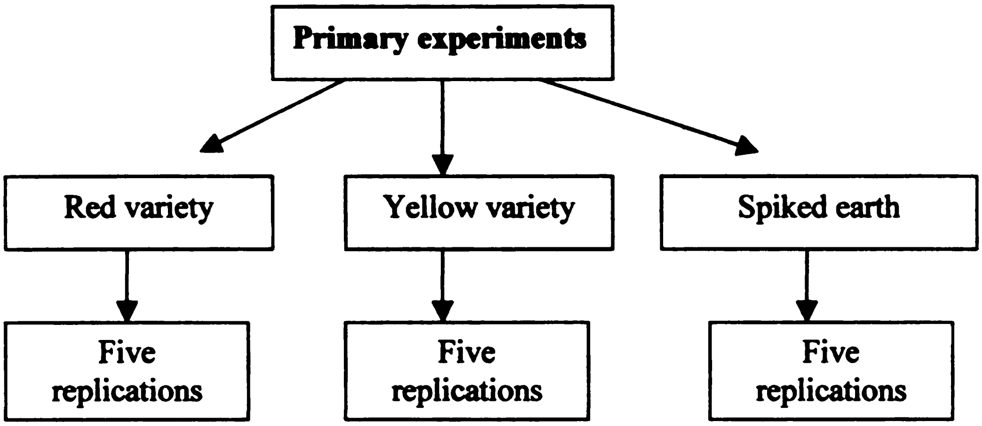

Figure 7. Primary experiments schema

3.6. Sample analysis

Extracts collected during SF extraction were injected into an offline HPLC system, without any further preparation. Analysis of the extracts from the preliminary and primary experiments was conducted as follows. Mobile phase consisted of a mixture of 40% phosphoric acid (0.5%, v/v, water solution) and 60% absolute methanol (v/v). Flow rate was maintained constant at one ml/min, and the detector was set at a wavelength of 280 nm. Period range of analysis was 10 to 15 min for each sample. Calibration curves were developed prior to analysis with quercetin standards each time that a new mobile phase was prepared. Five standards of known concentration were prepared and injected into the HPLC system. Retention time and area of the peak were recorded. Simple linear regression was applied to obtain the calibration curves (Figure A1.Table A3).

Before extracts were injected into the HPLC, standards were run at least twice in order to confirm retention time and area under the curve for quercetin. Then, samples were run once (since the volume of the extracts were not enough to run several times).

Mass spectrometry analysis was conducted at the Department of Biochemistry, Mass Spectrometry facility (Michigan State University) to identify and confirm the presence of quercetin in the extract. Matrix Assisted Laser Desorption Ionization (MALDI) Time of flight Mass Spectrometry was the method used.

3.7. Solid-liquid extraction

Along with each SF extraction, a methanol extraction was conducted. From the skins prepared for the SF extraction, a portion of it was separated and steeped in absolute methanol (Table 3), 1:40 (w/v) at room temperature, for 24 hours (Bilyk et al., 1984). Then, methanol was separated from the skins by filtering under reduced pressure (aspirator) through filter paper, grade 601, size 7 cm (Ahlstrom, Mt. Holly Springs, PA).

Methanol extracts were analyzed with the same method and HPLC system as the SF extracts.

Table 3. Methanol extraction data

Extraction #	Onion skins weight (g)	Methanol added (ml)	Extraction #	Onion skins weight (g)	Methanol added (ml)
Preliminary experiments					
Static Mode			Dynamic Mode		
1	1.103	44	1	0.464	19
2	0.475	19	2	0.502	20
3	0.247	10			
Primary experiments					
Red variety			Yellow variety		
1	0.429	17	1	0.284	11
2	0.442	18	2	0.483	19
3	0.371	15	3	0.465	19
4	0.794	32	4	0.238	10
5	0.825	33	5	0.221	8

4. Results and discussion

4.1. Optimal extraction conditions

Extraction conditions were chosen based on the solubility parameter and equipment limitations. In theory, maximum solubility should be attained when the solubility parameter of the extracting fluid (CO_2) is equivalent to that of the solute, in this case quercetin (King, 1989). The solubility parameter of CO_2 can be estimated based on the Giddings equation (Giddings et al., 1968),

$$\delta = 1.25 P_c^{1/2} (\rho_r / 2.66) \qquad \text{Eqn. 2}$$

Where: δ = solubility parameter, P_c = critical pressure, and ρ_r = reduced density.

From this equation, to maximize the solubility parameter, maximum reduced density should be attained. Based on Giddings' reduced states curve (Figure B1), for a given reduced pressure, lower reduced temperature gives higher reduced density. Therefore, during extraction, the goal was to maintain higher pressure at lower temperature. The effect was made to maximize the solubility parameter of CO_2, since quercetin is a very polar compound (five hydroxyl groups), and therefore has a high solubility parameter value. In theory, the solubility parameter is the quantitative meaning of polarity (Schoenmakers et al., 1982).

Exact numbers could not be calculated since other variables could also affect both quercetin and CO_2 solubility parameters, such as the presence of other similar compounds in the onion matrix, in which case, extraction must be optimized for groups of compounds rather than a single target analyte (Hawthorne, 1990). Additionally, Giddings relationship is useful when target analytes represent a large percentage of the bulk sample (Hawthorne, 1990), whereas quercetin is found in minor concentrations in onion skins.

The solubility parameter of CO_2 is assumed to be affected also by the solubility parameter of the entrainer, as is reported with liquid mixtures (Karger et al., 1973).

However, this theory was still used in this project as an acceptable estimation of optimal operation conditions, based on the results of caffeine extraction predictions done by King et al. (1990).

4.2. Preliminary experiments

4.2.1. Static mode

The total ratio of quercetin extracted per liter of CO_2 (extraction ratio) was found to be in the range of 0.19 to 0.57 (Table 4). Experimental data collected during each extraction can be found in Table A1. Variation in this ratio is assumed to be the result of non-uniform distribution of solute concentration through the vessel, and it might be possible that gas channeling inside the vessel had been formed. The collected data (Table A1) and the results (Table 4) suggest that there is a relationship between volume of CO_2 passed through the trap, and total quercetin extracted. At the highest volume of CO_2 passed, the highest amount of quercetin was extracted. However, this proportional relationship is not found between extraction duration and total quercetin extracted.

An example of the chromatograms and the UV absorbance chart that confirmed the presence of quercetin can be seen in Figures B2 and B3.

Table 4. Results from static mode

Extraction #	Extraction duration (min.)	Conc.[a] (μg/μl)	Total quercetin (μg)	Extraction ratio (μg of quercetin/L of CO_2) [b]	g of quercetin/kg of onion skins	Total CO_2 (L) [c]
1	132	0.017	71.57	0.57	0.0062	129
2	136	0.004	11.45	0.19	0.0010	60
3	192	0.012	49.16	0.41	0.0054	121

[a, b] Values shown are average in time

[a] Concentration of quercetin per ethanol volume collected in the trap

[c] Carbon dioxide passed through trap during extract collection

4.2.2. Dynamic mode

Table 5 shows the results from the dynamic mode. The experimental data collected during each extraction can be seen in Table A2. It can be observed from the results that at highest volume of CO_2 passed through the trap, the highest total quercetin is extracted. However, total quercetin appears to be independent from extraction ratio and extraction duration.

Table 5. Results from dynamic mode

Extraction #	Extraction duration (min.)	Conc.[a] (μg/μl)	Flow rate (L/min) [b]	Total quercetin (μg)	Extraction ratio (μg of quercetin/L of CO_2) [c]	g of quercetin/kg of onion skins	Total CO_2 (L) [d]
1	174	0.005	1.11	9.9	0.0481	9.80E-04	207
2	169	0.005	2.03	12.81	0.0365	1.03E-03	403

[a, b, c] Values shown are average in time

[a] Concentration of quercetin per ethanol volume collected in the trap

[d] Carbon dioxide passed through trap during extract collection

An example chromatogram can be seen in Figure B4.

4.2.3. Comparison between modes

Static and dynamic modes were conducted separately in order to compare their performance (total quercetin extracted). As seen in Table 6 the static mode had a higher total quercetin and extraction ratio than the dynamic mode. This result can be due to higher contact time between the solute and the dense gas in the static mode. Continuous flow during dynamic mode may not provide sufficient time for interaction between the solute and the gas.

Table 6. Comparison between modes

Mode	**Extraction #**	**Conc. [a] (µg/µl)**	**Total quercetin (µg)**	**Extraction ratio (µg of quercetin/L of CO_2) [b]**
Static	1	0.017	71.57	0.569
	2	0.004	11.45	0.187
	3	0.012	49.16	0.408
Dynamic	1	0.005	9.90	0.048
	2	0.005	12.81	0.037

[a] Concentration of quercetin per ethanol volume collected in the trap

[a, b] Average values in time

Based on these preliminary results, static mode was the extraction method chosen to compare the SF extraction of quercetin between yellow and red onion varieties. Moreover, to improve reproducibility and to achieve uniform distribution of solute through the vessel, a recirculation pump was added to the SF equipment.

4.3. Static Mode

4.3.1. Red variety

In five replicate extractions (Table 7) on average, 287.86 μg of quercetin could be extracted (in 150 min), with a ratio of 1.91 μg of quercetin per liter of CO_2; this generates a total amount of quercetin of 0.024 g per kg of onion skins. Even though there is a small variation in the extraction duration (143 to 157 min), weight of onion skins (10.50 to 13.90 g), and percentage of ethanol (6.80 to 7.78%), the amount of quercetin extracted had no relationship with any of these factors. Specific details of each extraction can be seen in Table A4.

Table 7. Results for red variety

Extraction #, (date)	**Extraction duration (min)**	**Conc. (μg/μl) [a]**	**Total quercetin (μg)**	**Extraction ratio (μg of quercetin/L of CO_2) [b]**	**g of quercetin/kg of onion skins**	**Onion skins weight (g)**	**% Ethanol (molar conc.)**
1, (11/08/01)	145	0.037	313.22	2.09	0.027	11.80	7.09
2, (11/12/01)	143	0.037	322.64	2.09	0.027	13.90	7.78
3, (11/26/01)	152	0.035	290.91	1.94	0.023	12.80	6.80
4, (01/22/02)	152	0.029	286.57	1.91	0.024	10.50	7.78
5, (01/25/02)	157	0.029	225.98	1.51	0.019	12.00	7.78
	150	***0.034***	***287.86***	***1.91***	***0.024***	***12.20***	***7.45***

[a] Concentration of quercetin per ethanol volume collected in the trap.
[a, b] Values shown are average in time

An example of the calibration curve for analysis is shown in Figure A1 (Table A3). An example of the chromatograms from HPLC analysis and results from the Mass Spectrometry analysis are shown in Figures B5, B6, and B7.

Since the results suggest no effect of time, onion weight and ethanol concentration (within the range that these parameters varied) on total quercetin extracted, the influence of ethanol volume collected in the trap was investigated. In order to evaluate any significant correlation between the quercetin extracted and the ethanol volume collected in the trap, a statistical analysis was performed. To conduct this analysis, the average of the five replications for each of the 10 vials collected, was calculated (Appendix C, analysis C1). The regression analysis showed that, at 95% confidence level, a significant linear relationship exists between these two variables (R^2=0.82, $p< 0.001$). This result suggests that there is a limiting factor in the collection of the solute of interest, with less ethanol volume less quercetin is collected per vial (Figure 8).

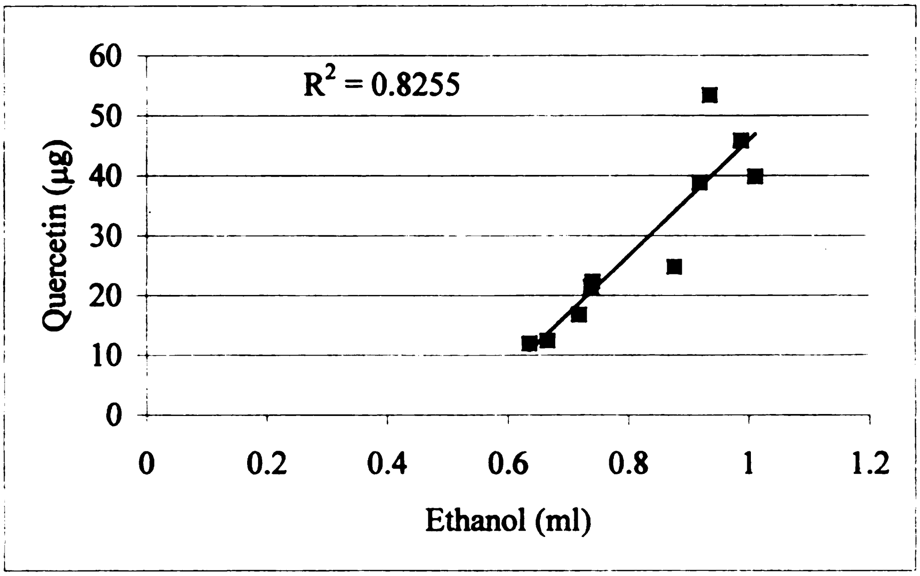

Figure 8. Correlation between quercetin extracted and ethanol volume per vial

Furthermore, regression analysis of ethanol volume (average of five replications for each of the ten vials) versus time, and extraction ratio (average of five replications for each of the ten vials) versus time were done in order to find any possible trend (Appendix C, analyses C2 and C3). Both analyses suggest the same trend, as time increases, volume of ethanol and extraction ratio decrease. At 95% confidence level, a significant linear relationship was found for both regressions (Figures 9 and 10), at p=0.001 for ethanol versus time, and p< 0.001 for extraction ratio versus time.

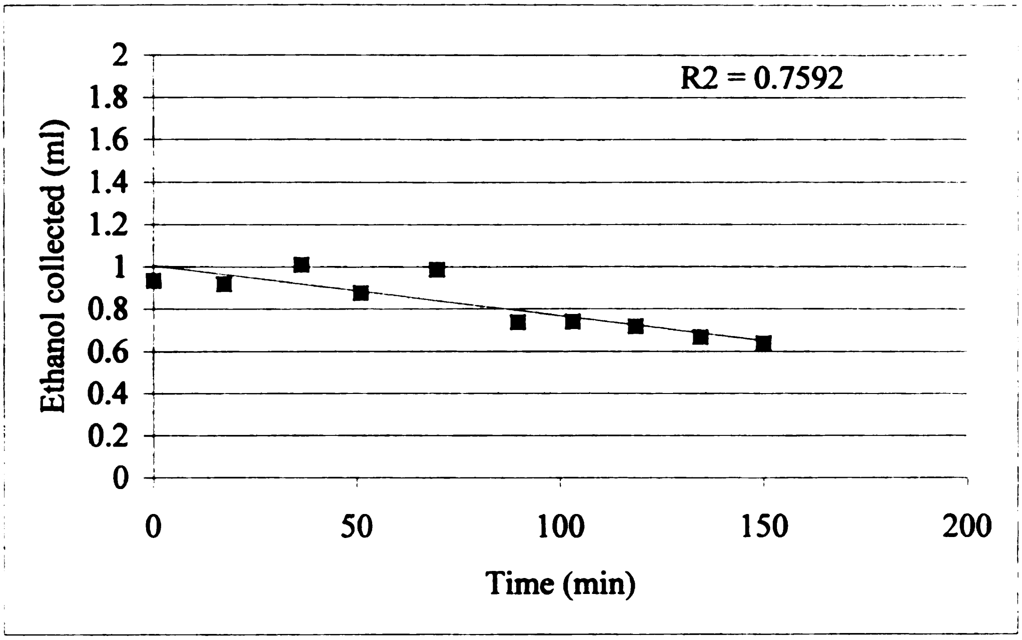

Figure 9. Correlation between ethanol volume per vial and time

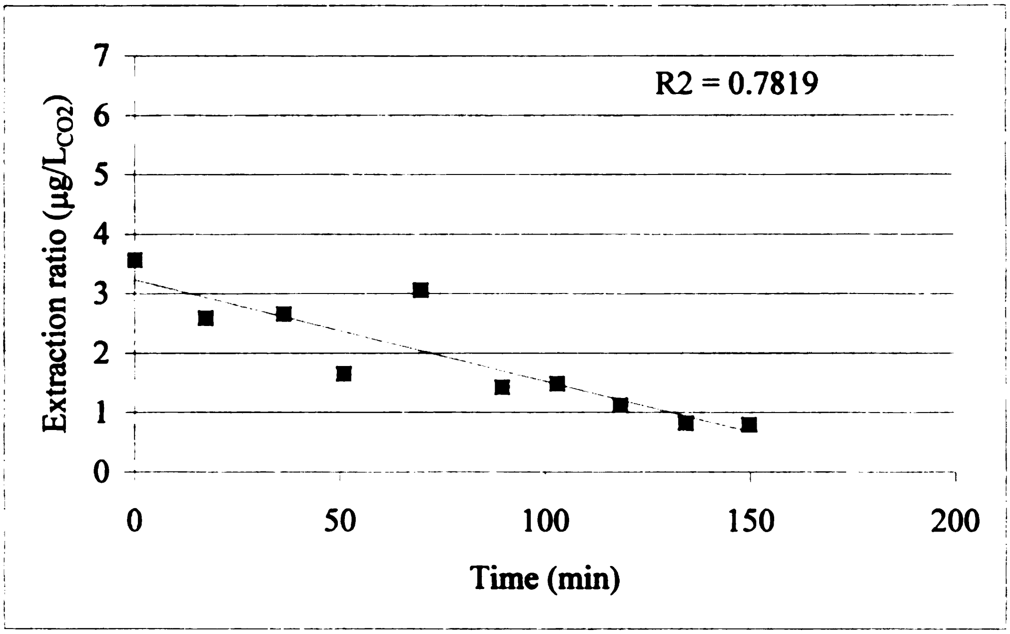

Figure 10. Correlation between extraction ratio per vial and time

From these results, the influence that the entrainer volume collected in the trap has on the amount of quercetin extracted can be observed. The reasons for the trends shown in Figures 8, 9, and 10, could be the following. First, that ethanol was saturated with quercetin therefore, no more quercetin could be trapped in the collection trap (direct relationship shown in Figure 8); second, not all the ethanol that was coming out with the gas stream was trapped (trend shown in Figure 9); third, within the vessel, ethanol has better affinity with the onion skins than with the gas stream, therefore, after certain time no more ethanol was pulled out of the vessel (Figure 9); and finally, quercetin has better affinity with ethanol than with CO_2, so the dense gas cannot compete with the entrainer and quercetin stays where ethanol stays (Figure 10).

The above reasoning also suggests that saturation of the CO_2 with quercetin could not be reached or detected during the extraction, since constant concentration of quercetin (μg/L of CO_2) in time was not achieved. Other similar compounds, such as resveratrol, gallic acid, catechins, p-coumarin, grape glycosides, etc., reached saturation fast (15 min), as it is reported by Palma et al. (2000), Pascual-Marti et al. (2001), and Tena et al. (1998). These studies were conducted in a much smaller vessel (≈7 ml) than the present study (500 ml). This fact is assumed to be one of the causes of a better understanding of the early stages of the extraction process, since they had a better control, for example, of the filling time (seconds), pressure and temperature, less void volume, less potential for channeling formation inside the vessel.

There are also other factors that may influence the results, for instance, the presence of other similar compounds in the onion skins, such as kaempferol and myricetin. These compounds may be competing with quercetin for a place in the gas stream and ethanol. On the other hand, it might be possible, that forces in the onion skin , due to bonds with these similar compounds, are stronger than the solvent power of CO_2, so extraction of quercetin becomes difficult.

The results of this study show that it is possible to overcome the difficulties mentioned above, and the experimental limitations (vessel volume, trap collection, ethanol introduction, etc.), since the extraction of quercetin from the natural matrix was successfully accomplished.

4.3.2. Yellow variety

In five different extractions (Table 8) on average, a total of 173.87 μg of quercetin could be extracted, with an extraction ratio of 1.142 μg of quercetin per liter of CO_2 (in 156 min); this generates 0.02 g of quercetin per kg of onion peels. Again, as in the red variety case, small variations in extraction durations (151 to 164 min), weight of onion skins (8.10 to 8.60 g) and ethanol concentration (7.69 to 8.17%) have no relationship with the extraction ratio of solute per gas (within the range that these parameters varied). Experimental data collected of each extraction can be found in Table A5.

Table 8. Results for yellow variety

Extraction #, (date)	**Extraction duration (min.)**	**Conc. [a] (μg/μl)**	**Total quercetin (μg)**	**Extraction ratio (μg of quercetin/L of CO_2) [b]**	**g of quercetin/kg of onion skins**	**Onion skins weight (g)**	**% Ethanol (molar conc.)**
1, (02/07/02)	156	0.015	192.523	1.268	0.022	8.6	7.69
2, (02/13/02)	151	0.016	201.448	1.324	0.025	8.1	7.69
3, (02/20/02)	159	0.020	225.076	1.461	0.023	9.6	8.17
4, (02/28/02)	164	0.020	149.193	0.989	0.018	5.8	7.69
5, (03/05/02)	150	0.014	101.098	0.668	0.012	8.2	7.69
	156	***0.017***	***173.868***	***1.142***	***0.020***	***8.06***	***7.79***

[a] Concentration of quercetin per ethanol volume collected in the trap

[a, b] Values shown are average on time

An example of the chromatograms can be found in Figure B8.

Again, the relationship between ethanol collected and quercetin extracted per vial was tested (regression can be seen in Figure 11). At 95% confidence level, a significant linear relationship was found, with a $p< 0.001$ and $r^2=0.877$ (Appendix C, analysis C4).

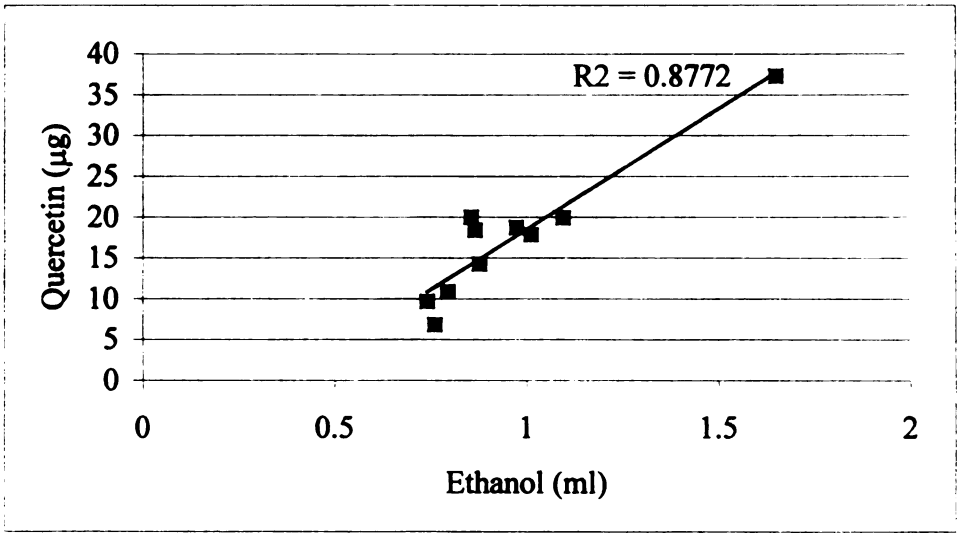

Figure 11. Correlation between quercetin extracted and ethanol volume collected per vial

Figures 12 and 13 show the same trend as in the red variety, as time increases, ethanol volume collected and the extraction ratio per vial decrease. The same assumptions as those for the red variety were made in this case. Statistical analysis (Appendix C, analyses C5 and C6) showed a significant linear relationship at 95% confidence level, with p=0.004 for ethanol versus time, and p=0.001 for extraction ratio versus time.

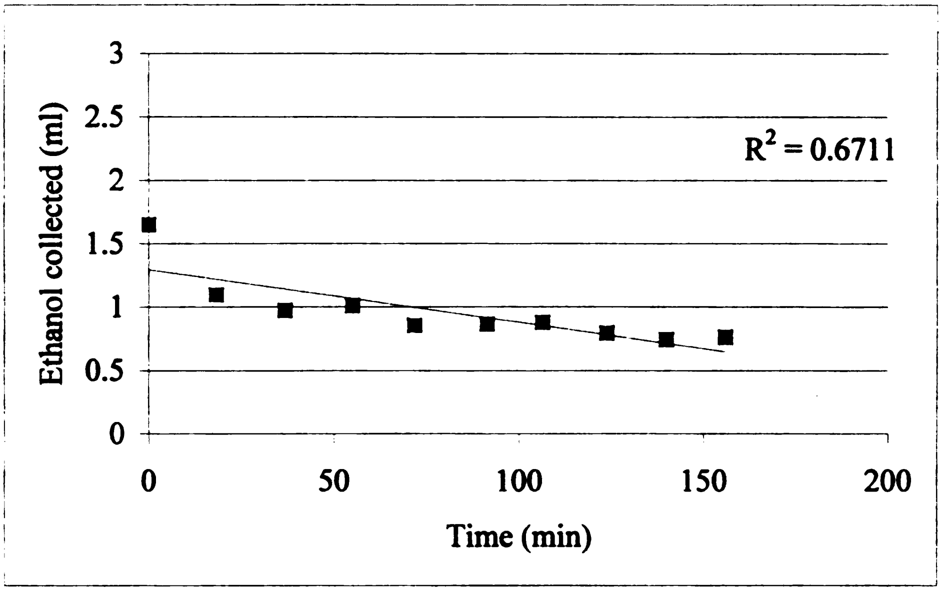

Figure 12. Correlation between ethanol volume collected per vial and time

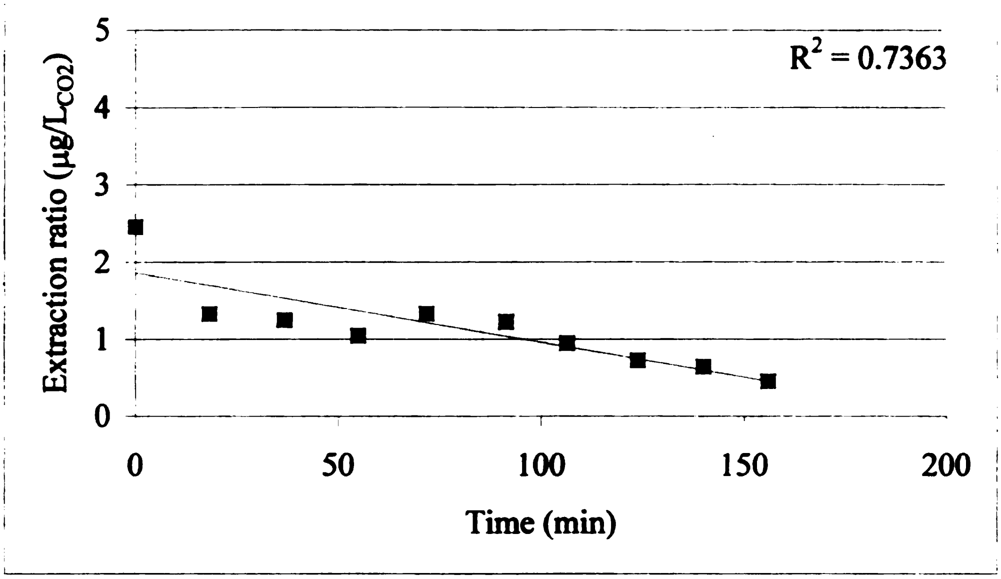

Figure 13. Correlation between extraction ratio per vial and time

4.3.3. Comparison between varieties

A statistical analysis was performed (Appendix C, analysis C7) in order to test any significant difference between g of quercetin extracted per kg of onion skins from each variety. At 95% confidence level there is no significant difference (p=0.182) between them, with less standard deviation (0.003) for the red than the yellow variety (0.005). Results from the yellow and red onion varieties extractions (average of five replications) are shown in Table 9.

Table 9. Results for the red and yellow varieties

Extraction duration (min)	g of quercetin/kg of onion skins
Red	
145	0.027
143	0.027
152	0.023
152	0.024
157	0.019
Yellow	
156	0.022
151	0.025
159	0.023
164	0.018
150	0.012

4.3.4. Comparison of the preliminary and primary experiments conducted with yellow variety

Statistical analysis was conducted to the values shown in Table 10 (Appendix C, analyses C8, C9 and C10). It was found that at 95% confidence level the total quercetin extracted ($p=0.007$), g of quercetin per kg of onion skins ($p=0.003$), and ethanol volume collected per vial ($p=0.004$) had a significant difference between the preliminary and the primary experiments conducted. This means that primary experiments have significantly higher values than preliminary ones. However, the extraction ratio did not show a significant difference between the preliminary and primary experiments ($p=0.01$, appendix C, analysis C11). From these results it can be observed that, although the extraction ratio did not present a statistical difference, the ethanol collected in the primary experiments was higher, which gave higher amount of quercetin extracted and g of quercetin per kg of onion skins. These results also suggest that the recirculation pump and dry ice added to the collection trap may have improved the amount of quercetin extracted.

Table 10. Results from preliminary and primary experiments for yellow variety

	Extraction #	**Extraction duration (min.)**	**Conc. [a] (μg/μl)**	**Ethanol volume collected in the trap (ml)**	**Total quercetin (μg)**	**Extraction ratio (μg of quercetin/L of CO_2) [b]**	**g of quercetin/kg of onion skins**
Preliminary	1	132	0.017	0.468	71.57	0.57	0.006
	2	136	0.004	0.234	11.45	0.19	0.001
	3	192	0.012	0.206	49.16	0.41	0.005
Primary	1	156	0.015	1.107	192.52	1.27	0.022
	2	151	0.016	1.180	201.45	1.32	0.025
	3	159	0.020	1.090	225.08	1.46	0.023
	4	164	0.020	0.733	149.19	0.99	0.018
	5	150	0.014	0.706	101.10	0.67	0.012

[a] Concentration of quercetin per volume of ethanol collected in the trap
[a, b] Values shown are average in time

4.3.5. Comparison of SF with solid-liquid extraction

Since SF and methanol extractions were not performed in the same time basis (3 and 24 h, respectively), it is not possible to directly compare the amount of quercetin extracted in each extraction. However, it was found (Appendix C, analyses C12 and C13) that standard deviation is higher for methanol extraction (3.034 g of quercetin per kg of onion skin, red variety; 3.214 g of quercetin per kg of onion skin, yellow variety) than for

SF extraction (0.003 g of quercetin per kg of onion skin, red variety; 0.005 g of quercetin per kg of onion skin, yellow variety). Results from both extractions can be seen in Table 11.

Table 11. SF and methanol extractions results

Variety	**Extraction #**	**SF (g of quercetin/kg of onion skins)**	**Methanol (g of quercetin/kg of onion skins**
Red	1	0.027	10.14
	2	0.027	15.90
	3	0.023	8.73
	4	0.024	10.82
	5	0.019	8.33
Average =		***0.024***	***10.78***
Yellow	1	0.022	3.80
	2	0.025	10.41
	3	0.023	3.07
	4	0.018	3.48
	5	0.012	7.62
Average =		***0.020***	***5.68***

4.4. Static mode for pure quercetin

Five replications were done using the same extraction conditions and procedure (Table A6) as in the previous red and yellow variety cases. A maximum amount of 1800 μg was extracted from the spiked inert matrix with a maximum extraction ratio of 12 μg of quercetin per liter of CO_2 (Table 12).

Table 12. Results from the pure quercetin extraction

Extraction #, (date)	**Conc. [a] (μg/μL)**	**Total quercetin (μg)**	**Extraction ratio (μg of quercetin/L of CO_2) [b]**
1, (03/26/02)	0.015	107.79	0.70
2, (04/01/02)	0.104	650.43	4.34
3, (04/03/02)	0.040	247.06	1.65
4, (04/10/02)	0.158	1832.50	12.02
5, (04/17/02)	0.087	706.02	4.69

[a, b] Values shown are average in time

[a] Concentration of quercetin per volume of ethanol collected in the trap

Even though the results were not as reproducible as with the natural onion matrix, similar trends were found. After a regression analysis (Appendix C, analysis C14) was applied to quercetin extracted versus ethanol volume collected per vial (average values of five replications for each vial), at 95% confidence level, a significant linear relationship was found ($p < 0.001$, Figure 14).

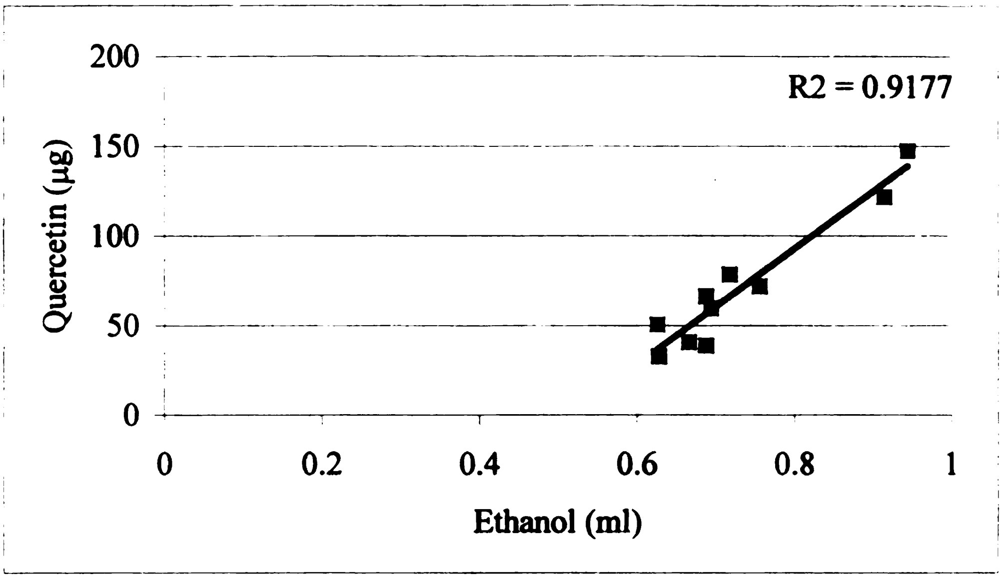

Figure 14. Correlation between quercetin extracted and ethanol volume collected per vial

A decreasing trend was also found between ethanol collected versus time and extraction ratio versus time (Appendix C, analyses C15 and C16). However, they did not have a significant linear relationship as they had with the natural matrix. These results suggest that the collection trap was not efficient enough to collect all the ethanol and solute that were coming in the gas stream. No ethanol-matrix and/or solute-matrix interaction is supposed to occur, since the matrix is a spiked earth.

Extraction ratios were also compared (Appendix C, analysis C17). Extraction ratios from the yellow variety presented less standard deviation (0.314 µg/L) than the pure quercetin (4.444 µg/L). The difference between the first three extraction ratios from the pure quercetin was assumed to be due to varying degrees of channeling formation inside the vessel. To avoid the potential of this channeling glass wool was mixed with the earth for the last two extractions. Extraction ratios values can be seen in Table 13.

Table 13. Extraction ratios for yellow variety and pure quercetin

Extraction #	**Extraction ratio (μg of quercetin/L of CO_2)** [a]
	Pure quercetin
1	0.7
2	4.34
3	1.65
4	12.02
5	4.69
	Yellow variety
1	1.27
2	1.32
3	1.46
4	0.99
5	0.67

[a] Average values in time

Analysis of variance was performed (Appendix C, analysis C18) to the values presented in Table 14. The amount of quercetin extracted per kg of onion skins showed less standard deviation (0.005 g of quercetin per kg of onion skin) than the amount of quercetin extracted per kg of earth (0.013 g of quercetin per kg of earth). However, the values shown (g of quercetin extracted/kg of matrix) in Table 14 did not show significant difference at 95% confidence level ($p=0.375$). These results suggested that there is no effect due to matrix, quercetin collected is the same for both, natural and inert matrices (for this study and under the conditions that it was conducted).

An example of the chromatograms for pure quercetin can be seen in Figure B9.

Table 14. Comparison of the results from the yellow variety and pure quercetin

Extraction #	g of quercetin extracted/kg of matrix	Quercetin present before extraction (g of quercetin/kg of matrix)
	Onion skins	
1	0.022	3.80 [a]
2	0.025	10.41
3	0.023	3.07
4	0.018	3.48
5	0.012	8.13
	Earth	
1	0.002	2.50
2	0.013	2.78
3	0.005	2.56
4	0.036	2.42
5	0.014	2.47

[a] Based on methanol extraction

5. Conclusions

This study confirms the potential of supercritical fluid extraction as an alternative method to extract quercetin from a natural matrix, in this case, onion skins. Essential data on the extraction process have been provided for the first time. After comparing the amount of quercetin that can be extracted with dynamic (11 µg total, average of two replications) and static (44 µg total, average of three replications) modes, the static mode was found to be the best method.

There is no statistical difference between the amount of quercetin recovered from the red (0.024 ± 0.003 g of quercetin/kg of onion skins, average of five replications) and yellow (0.020 ± 0.005 g of quercetin/kg of onion skins, average of five replications) onion varieties. The overall amount and reproducibility (g of quercetin per kg of onion skins), for both varieties, has been improved by adding a recirculation system to the SF equipment.

The modifier has an important influence on the amount of quercetin extracted. At the highest ethanol volume collected per extract, the highest amount of quercetin was extracted. This trend is found in both varieties and the pure solute.

Even though the extraction ratio (µg of quercetin/L of CO_2) from the natural matrix has less standard deviation (1.142 ± 0.314 µg/L) than the extraction ratio from the spiked inert matrix (4.680 ± 4.444 µg/L), higher values were obtained from the spiked inert matrix (as much as 8.23 times higher, comparison between maximum values, 12.02 µg/L on earth and 1.46 µg/L on onion skins).

When the amount of quercetin extracted per kg of onion skins is compared to the amount extracted per kg of earth, there is no statistical difference between them, meaning that there is no effect due to matrix (under the extraction conditions of this study).

6. Recommendations

6.1. Experimental improvements

It might be helpful for future experiments:

- Freeze-dry the onion skins before the extraction to ensure no water is present in the sample.
- The current method of recirculation can be improved. For this study the flow rate of this recirculation was not known exactly. An online flow meter would be helpful to ensure what portion of the gas stream and solute are been recirculated.
- The current collection trap could be improved in order to ensure the total collection of ethanol and solute. A solid trap is a possibility, such as C_{18} column. A liquid trap can be also implemented such as specific volume of ethanol or water. Furthermore, a combination of both solid and liquid might also improved the collection system.

6.2. Future work

It would be beneficial to further investigate:

- If storage period and temperature had any influence on quercetin content or extraction.
- The saturation point (when concentration of quercetin is constant in time) of the pure quercetin, in order to estimate the equilibrium constant, and develop a mass transfer model.
- Application of the above model to the natural matrix.

- How to extract the maximum ethanol volume and quercetin from the vessel if they tend to interact with the onion skins.
- Identify and quantify the other similar compounds that are extracted along with quercetin, in order to find any possible pattern that they are following.
- Once the other compounds are identified, how to improve selectivity of SFE by further separation of these compounds (by different traps).

APPENDICES

APPENDIX A. Experimental Data

Preliminary results

Table A1. Experimental data collected during static mode

Extraction #	Time (min.)	Vial #	Volume per extract (ml)	Conc.[a] (μg/μl)	Total quercetin (μg)	Volume CO_2 (L)	Extraction ratio (μg of quercetin/L of CO_2)	g of quercetin/kg of onion skins
1	5	1	0.62	0.015	9.36	11.91	0.79	8.10E-04
	20	2	0.48	0.014	6.86	9.57	0.72	5.90E-04
	35	3	0.37	0.012	4.44	10.24	0.43	3.80E-04
	53	4	0.46	0.022	10.26	25.27	0.41	8.80E-04
	70	5	0.27	0.016	4.29	10.60	0.41	3.70E-04
	85	6	0.44	0.012	5.41	15.54	0.35	4.70E-04
	100	7	0.53	0.013	7.10	15.63	0.45	6.10E-04
	117	8	0.54	0.021	11.34	15.32	0.74	9.80E-04
	132	9	0.50	0.025	12.50	15.08	0.83	1.08E-03
Total/Average			**4.21**	**0.017**	**71.57**	**129.16**	**0.57**	**6.17E-03**
2	3	1	0.32	0.004	1.18	6.00	0.20	1.00E-04
	20	2	0.18	0.000	0.00	4.00	0.00	0.00E+00
	35	3	0.28	0.001	0.39	4.00	0.10	3.40E-05
	55	4	0.21	0.000	0.00	5.00	0.00	0.00E+00
	69	5	0.29	0.003	0.84	6.00	0.14	7.20E-05
	81	6	0.26	0.005	1.25	5.00	0.25	1.10E-04
	93	7	0.20	0.005	1.02	5.00	0.20	8.70E-05
	102	8	0.26	0.006	1.48	5.00	0.30	1.30E-04
	111	9	0.20	0.006	1.28	5.00	0.26	1.10E-04
	119	10	0.20	0.006	1.18	5.00	0.24	1.00E-04
	128	11	0.21	0.010	2.08	5.00	0.42	1.80E-04
	136	12	0.20	0.004	0.74	5.00	0.15	6.30E-05
Total/Average			**2.81**	**0.004**	**11.45**	**60.00**	**0.19**	**9.80E-04**

[a] Concentration of quercetin per ethanol volume per extract

Table A1. Continuation

3	3	1	0.20	0.000	0.00	6	0.00	0.00E+00
	12	2	0.23	0.012	2.69	6	0.45	3.00E-04
	23	3	0.22	0.013	2.79	6	0.47	3.10E-04
	32	4	0.21	0.012	2.46	5	0.49	2.70E-04
	41	5	0.21	0.014	2.92	6	0.49	3.20E-04
	52	6	0.27	0.014	3.78	7	0.54	4.20E-04
	62	7	0.23	0.015	3.36	6	0.56	3.70E-04
	71	8	0.22	0.014	3.15	6	0.52	3.50E-04
	81	9	0.22	0.009	1.94	6	0.32	2.10E-04
	92	10	0.20	0.018	3.60	6	0.60	4.00E-04
	101	11	0.20	0.013	2.56	5	0.51	2.80E-04
	109	12	0.23	0.012	2.69	7	0.38	3.00E-04
	118	13	0.18	0.012	2.20	6	0.37	2.40E-04
	131	14	0.20	0.011	2.20	6	0.37	2.40E-04
	142	15	0.19	0.012	2.32	7	0.33	2.60E-04
	153	16	0.23	0.011	2.53	6	0.42	2.80E-04
	164	17	0.17	0.013	2.18	6	0.36	2.40E-04
	174	18	0.20	0.012	2.30	6	0.38	2.50E-04
	181	19	0.15	0.011	1.67	6	0.28	1.80E-04
	192	20	0.16	0.012	1.84	6	0.31	2.00E-04
Total/Average			**4.12**	**0.012**	**49.16**	**121**	**0.41**	**5.40E-03**

Calculations for all tables

- Concentration in ppm: from HPLC analysis
- Concentration of quercetin per volume of ethanol:

$$\mu g / \mu l = ppm / 1000 \qquad \text{Eqn. A1}$$

- Total quercetin:

$$\mu g = ethanol(ml) * conc.(\mu g / \mu l) * 1000 \qquad \text{Eqn. A2}$$

- Total ratio:

$$\mu g / L = quercetin(\mu g) / CO_2(L) \qquad \text{Eqn. A3}$$

- Quercetin per weight of onion peel:

$$g / kg = \left[\left(quercetin(\mu g) / (1000 * g\ of\ onion\ peel\ used\right)\right] \qquad \text{Eqn. A4}$$

Table A2. Experimental data collected during dynamic mode

Extraction #	Time (min.)	Vial #	Volume per extract (ml)	Conc.[a] (μg/μl)	Total quercetin (μg)	Flow rate (L/min)	Volume of CO_2 (L)	Extraction ratio (μg of quercetin/ L of CO_2)	g of quercetin/ kg of onion skins
1	0	1	0.10	0.006	0.64	1.25	16	0.0402	6.37E-05
	14	2	0.10	0.005	0.51	1.00	14	0.0363	5.04E-05
	28	3	0.10	0.005	0.50	1.00	16	0.0315	5.00E-05
	42	4	0.08	0.007	0.54	1.00	13	0.0415	5.35E-05
	55	5	0.10	0.007	0.72	1.00	16	0.0448	7.10E-05
	69	6	0.14	0.006	0.84	1.13	15	0.0563	8.37E-05
	83	7	0.15	0.005	0.81	1.15	15	0.0538	7.98E-05
	96	8	0.17	0.006	1.09	1.13	15	0.0728	1.08E-04
	110	9	0.19	0.003	0.48	1.14	16	0.0301	4.76E-05
	124	10	0.18	0.005	0.82	1.18	14	0.0589	8.16E-05
	137	11	0.23	0.003	0.75	1.13	13	0.0580	7.47E-05
	149	12	0.17	0.004	0.62	1.16	15	0.0415	6.16E-05
	162	13	0.22	0.003	0.68	1.15	14	0.0489	6.78E-05
	174	14	0.28	0.003	0.87	1.13	15	0.0581	8.64E-05
Total/Average			**0.16**	**0.005**	**9.90**	**1.11**	**207**	**0.0481**	**9.80E-04**

[a] Concentration of quercetin per ethanol volume per extract

Table A2. Continuation

2	0	1	1.100	0.000	0.000	1.580	43	0.0000	0.00E+00
	24	2	0.300	0.001	0.260	2.050	27	0.0095	2.06E-05
	38	3	0.530	0.007	3.960	2.220	30	0.1322	3.20E-04
	50	4	0.500	0.004	2.060	2.400	27	0.0762	1.66E-04
	61	5	0.090	0.007	0.660	1.760	24	0.0273	5.29E-05
	72	6	0.100	0.009	0.900	3.000	27	0.0333	7.26E-05
	85	7	0.350	0.005	1.580	1.870	42	0.0376	1.27E-04
	107	8	0.200	0.011	2.140	1.870	45	0.0475	1.73E-04
	129	9	0.100	0.010	1.020	1.870	30	0.0341	8.24E-05
	148	10	0.090	0.001	0.120	1.870	53	0.0023	9.96E-06
	169	11	0.070	0.002	0.110	1.870	55	0.0019	8.64E-06
Total/Average			**0.310**	**0.005**	**12.810**	**2.030**	**403**	**0.0365**	**1.03E-03**

HPLC analysis

Table A3. Data for calibration curve

Area	**Standard concentration (ppm)**
2135.08	178.72
1097.10	89.36
573.55	44.68
188.55	26.81

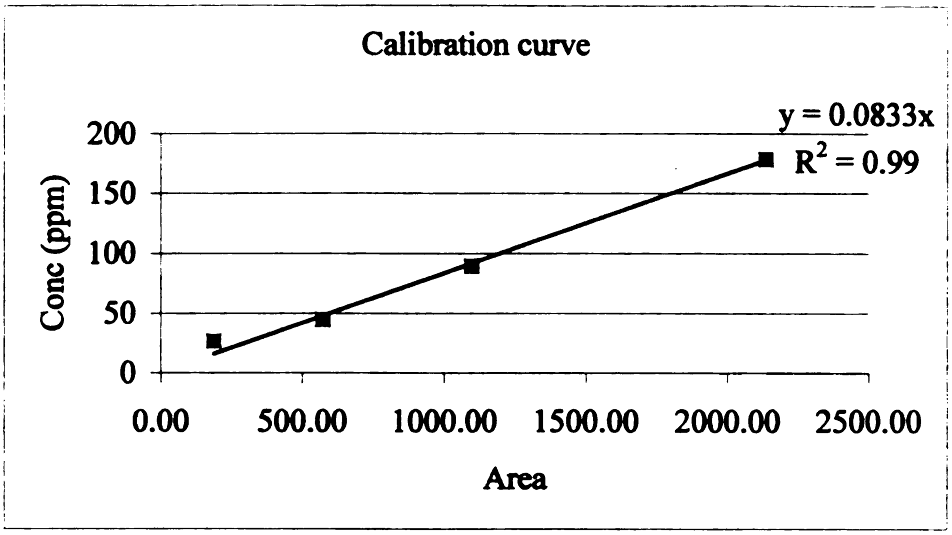

Figure A1. Calibration curve

Calculation of quercetin concentration

$$y = 0.0833 * x \qquad \text{Eqn. A5}$$

Where:

y = concentration of quercetin in ppm

x = area under the peak

Primary results

Red variety

Table A4. Experimental data and results

Extraction #, (Date)	Time (min.)	Vial #	Volume per extract (ml)	Conc. (μg/μl) [a]	Total quercetin (μg)	Volume CO_2 (L)	Extraction ratio (μg of quercetin/L of CO_2)	g of quercetin /kg of onion skins
1, (11/08/01)	0	1	0.92	0.057	52.376	15	3.49	0.0044
	20	2	0.91	0.008	6.857	15	0.46	0.0006
	38	3	0.91	0.064	58.283	15	3.89	0.0049
	53	4	0.78	0.056	44.004	15	2.93	0.0037
	70	5	1.08	0.066	71.324	15	4.75	0.0060
	89	6	0.59	0.026	15.053	15	1.00	0.0013
	104	7	0.89	0.051	45.772	15	3.05	0.0039
	115	8	0.49	0.022	10.863	15	0.72	0.0009
	130	9	0.43	0.015	6.497	15	0.43	0.0006
	145	10	0.32	0.007	2.187	15	0.15	0.0002
Total /Average			**7.32**	**0.037**	**313.216**	**150**	**2.09**	**0.0265**
2, (11/12/01)	0	1	1.10	0.078	85.383	15	5.69	0.0061
	17	2	0.79	0.045	35.378	15	2.36	0.0025
	35	3	1.21	0.019	22.796	15	1.52	0.0016
	46	4	0.89	0.021	18.827	15	1.26	0.0014
	63	5	1.02	0.040	41.153	15	2.74	0.0030
	82	6	1.00	0.033	33.078	15	2.21	0.0024
	96	7	0.78	0.028	21.505	15	1.43	0.0015
	112	8	0.90	0.028	25.374	15	1.69	0.0018
	127	9	0.87	0.026	22.193	15	1.48	0.0016
	143	10	0.83	0.020	16.947	15	1.13	0.0012
Total /Average			**9.39**	**0.034**	**322.636**	**150**	**2.15**	**0.0232**

[a] Concentration of quercetin per ethanol volume per extract

Table A4. Continuation

3, (11/26/01)	0	1	0.85	0.053	44.938	15	3.00	0.0035
	17	2	0.94	0.049	45.715	15	3.05	0.0036
	35	3	0.92	0.050	46.431	15	3.10	0.0036
	51	4	0.90	0.043	38.469	15	2.56	0.0030
	70	5	0.84	0.039	32.740	15	2.18	0.0026
	89	6	0.76	0.034	25.953	15	1.73	0.0020
	103	7	0.68	0.025	17.155	15	1.14	0.0013
	120	8	0.74	0.020	15.109	15	1.01	0.0012
	136	9	0.71	0.020	14.260	15	0.95	0.0011
	152	10	0.60	0.017	10.140	15	0.68	0.0008
Total/Average			**7.94**	**0.035**	**290.910**	**150**	**1.94**	**0.0227**
4, (01/22/02)	0	1	1.04	0.053	54.845	15	3.66	0.0046
	16	2	1.20	0.069	82.386	15	5.49	0.0069
	35	3	1.10	0.031	33.965	15	2.26	0.0029
	50	4	0.94	0.005	5.161	15	0.34	0.0004
	72	5	1.09	0.045	48.513	15	3.23	0.0041
	90	6	0.61	0.019	11.785	15	0.79	0.0010
	103	7	0.68	0.014	9.493	15	0.63	0.0008
	120	8	0.69	0.025	17.352	15	1.16	0.0015
	137	9	0.64	0.016	10.110	15	0.67	0.0008
	152	10	0.74	0.018	12.959	15	0.86	0.0011
Total/Average			**8.73**	**0.029**	**286.569**	**150**	**1.91**	**0.0241**
5, (01/25/02)	0	1	0.76	0.039	29.695	15	1.98	0.0025
	17	2	0.75	0.032	23.920	15	1.59	0.0020
	39	3	0.91	0.042	38.045	15	2.54	0.0032
	55	4	0.87	0.020	17.667	15	1.18	0.0015
	74	5	0.90	0.040	35.556	15	2.37	0.0030
	98	6	0.73	0.029	21.138	15	1.41	0.0018
	109	7	0.67	0.027	17.845	15	1.19	0.0015
	125	8	0.77	0.020	15.530	15	1.04	0.0013
	142	9	0.68	0.013	9.099	15	0.61	0.0008
	157	10	0.69	0.025	17.479	15	1.17	0.0015
Total/Average			**7.73**	**0.029**	**225.976**	**150**	**1.51**	**0.0188**

Yellow variety

Table A5. Experimental data collected and results

Extraction #, (date)	**Time (min.)**	**Vial #**	**Volume per extract (ml)**	**Conc. (µg/µl)** [a]	**Total quercetin (µg)**	**Volume (CO_2)**	**Extraction ratio (µg of quercetin/L of CO_2)**	**g of quercetin/ kg of onion peels**
1, (02/07/02)	0	1	3.10	0.023	70.085	15	4.67	0.0080
	18	2	1.10	0.018	20.277	15	1.35	0.0020
	37	3	1.20	0.020	23.886	15	1.59	0.0030
	52	4	1.15	0.017	19.251	17	1.13	0.0020
	68	5	0.78	0.031	24.166	15	1.61	0.0030
	92	6	0.87	0.013	11.011	15	0.73	0.0010
	106	7	0.83	0.010	8.207	15	0.55	0.0010
	123	8	0.72	0.009	6.502	15	0.43	0.0010
	140	9	0.63	0.007	4.516	15	0.30	0.0010
	156	10	0.69	0.007	4.622	15	0.31	0.0010
Total/ Average			**11.07**	**0.015**	**192.523**	**152**	**1.27**	**0.0220**
2, (02/13/02)	0	1	1.60	0.025	40.647	15	2.71	0.0050
	17	2	1.60	0.022	34.671	15	2.31	0.0040
	35	3	1.30	0.015	19.688	15	1.31	0.0020
	63	4	1.30	0.019	24.162	17	1.42	0.0030
	71	5	1.10	0.017	18.273	15	1.22	0.0020
	90	6	1.00	0.019	18.663	15	1.24	0.0020
	103	7	1.10	0.014	15.582	15	1.04	0.0020
	119	8	1.00	0.013	13.356	15	0.89	0.0020
	135	9	0.90	0.010	9.203	15	0.61	0.0010
	151	10	0.90	0.008	7.203	15	0.48	0.0010
Total /Average			**11.80**	**0.016**	**201.448**	**152**	**1.32**	**0.0250**

[a] Concentration of quercetin per ethanol volume per extract

Table A5. Continuation

3, (02/20/02)	0	1	1.55	0.024	37.899	16	2.37	0.004
	18	2	1.40	0.024	33.806	15	2.25	0.004
	36	3	1.25	0.022	28.020	15	1.87	0.003
	52	4	1.20	0.026	30.754	17	1.81	0.003
	70	5	1.10	0.020	21.621	15	1.44	0.002
	89	6	1.00	0.021	21.336	15	1.42	0.002
	108	7	0.90	0.016	14.344	15	0.96	0.001
	127	8	0.80	0.014	11.473	15	0.76	0.001
	143	9	0.90	0.018	16.643	15	1.11	0.002
	159	10	0.80	0.011	9.180	15	0.61	0.001
Total/Average			**10.90**	**0.020**	**225.076**	**153**	**1.46**	**0.023**
4, (02/28/02)	0	1	1.00	0.020	20.084	15	1.34	0.002
	20	2	0.77	0.009	6.657	15	0.44	0.001
	41	3	0.68	0.026	17.382	15	1.16	0.002
	57	4	0.77	0.010	7.338	17	0.43	0.001
	81	5	0.61	0.018	11.118	15	0.74	0.001
	99	6	0.73	0.043	31.367	15	2.09	0.004
	114	7	0.72	0.030	21.841	15	1.46	0.003
	132	8	0.68	0.023	15.735	15	1.05	0.002
	148	9	0.68	0.018	12.084	15	0.81	0.001
	164	10	0.69	0.008	5.586	15	0.37	0.001
Total/Average			**7.33**	**0.020**	**149.193**	**152**	**0.99**	**0.018**
5, (03/05/02)	0	1	1.00	0.018	17.772	15	1.18	0.002
	18	2	0.61	0.007	4.258	15	0.28	0.001
	35	3	0.44	0.010	4.620	15	0.31	0.001
	51	4	0.64	0.012	7.617	17	0.45	0.001
	69	5	0.69	0.036	24.828	15	1.66	0.003
	87	6	0.73	0.013	9.701	15	0.65	0.001
	101	7	0.84	0.014	11.362	15	0.76	0.001
	118	8	0.78	0.010	7.426	15	0.50	0.001
	134	9	0.60	0.010	5.910	15	0.39	0.001
	150	10	0.73	0.010	7.606	15	0.51	0.001
Total/Average			**7.06**	**0.014**	**101.098**	**152**	**0.67**	**0.012**

Pure quercetin

Table A6. Data collected and results from static mode extraction of pure quercetin

Extraction #, (date)	**Time (min.)**	**Vial #**	**Volume per extract (ml)**	**Conc.[a] (μg/μl)**	**Total quercetin (μg)**	**Volume CO_2 (L)**	**Extraction ratio (μg of quercetin/L of CO_2)**
1, (03/26/02)	0	1	0.50	0.015	7.37	15	0.49
	17	2	0.90	0.025	22.79	15	1.52
	35	3	1.10	0.019	21.05	18	1.17
	50	4	0.75	0.020	14.95	15	1.00
	64	5	0.48	0.020	9.68	15	0.65
	82	6	0.67	0.013	8.49	15	0.57
	96	7	0.57	0.012	6.85	15	0.46
	110	8	0.62	0.011	6.64	15	0.44
	125	9	0.68	0.009	5.87	15	0.39
	139	10	0.60	0.007	4.11	15	0.27
Total/Average			**0.69**	**0.015**	**107.79**	**153**	**0.70**
2, (04/01/02)	0	1	0.50	0.046	22.92	15	1.53
	16	2	0.76	0.093	70.85	15	4.72
	35	3	0.56	0.077	43.05	15	2.87
	51	4	0.60	0.122	73.14	15	4.88
	70	5	0.71	0.110	78.41	15	5.23
	88	6	0.58	0.114	65.96	15	4.40
	102	7	0.65	0.118	76.92	15	5.13
	118	8	0.60	0.139	83.16	15	5.54
	135	9	0.60	0.101	60.64	15	4.04
	152	10	0.62	0.122	75.38	15	5.03
Total/Average			**0.62**	**0.104**	**650.43**	**150**	**4.34**

[a] Concentration of quercetin per ethanol volume per extract

Table A6. Continuation

3, (04/03/02)	0	1	0.86	0.028	24.35	15	1.62
	19	2	0.95	0.031	29.55	15	1.97
	37	3	0.44	0.034	14.99	15	1.00
	53	4	0.44	0.035	15.20	15	1.01
	72	5	0.70	0.054	37.84	15	2.52
	90	6	0.63	0.040	24.90	15	1.66
	104	7	0.62	0.073	45.37	15	3.02
	121	8	0.50	0.036	17.92	15	1.19
	136	9	0.59	0.027	16.17	15	1.08
	151	10	0.53	0.039	20.75	15	1.38
Total/Average			**0.63**	**0.04**	**247.06**	**150**	**1.65**
4, (04/10/02)	0	1	0.75	0.133	99.72	15	6.65
	17	2	1.50	0.394	591.68	15	39.45
	33	3	1.90	0.253	481.64	16	30.10
	48	4	1.00	0.166	166.15	15	11.08
	67	5	1.10	0.138	151.96	15	10.13
	85	6	0.69	0.111	76.76	15	5.12
	99	7	0.75	0.202	151.39	15	10.09
	120	8	0.65	0.087	56.48	15	3.77
	130	9	0.57	0.05	28.68	15	1.91
	146	10	0.65	0.043	28.03	15	1.87
Total/Average			**0.96**	**0.158**	**1832.50**	**151**	**12.02**
5, (04/17/02)	0	1	0.53	0.018	9.66	15	0.64
	17	2	0.61	0.037	22.76	15	1.52
	36	3	0.57	0.084	47.81	16	2.99
	48	4	0.65	0.096	62.44	15	4.16
	67	5	0.79	0.104	81.95	15	5.46
	85	6	0.90	0.135	121.35	15	8.09
	98	7	1.00	0.112	112.33	15	7.49
	115	8	0.76	0.117	88.77	15	5.92
	131	9	1.00	0.083	83.17	15	5.54
	146	10	0.93	0.082	75.80	15	5.05
Total/Average			**0.77**	**0.087**	**706.02**	**151**	**4.69**

APPENDIX B. HPLC Chromatograms and others

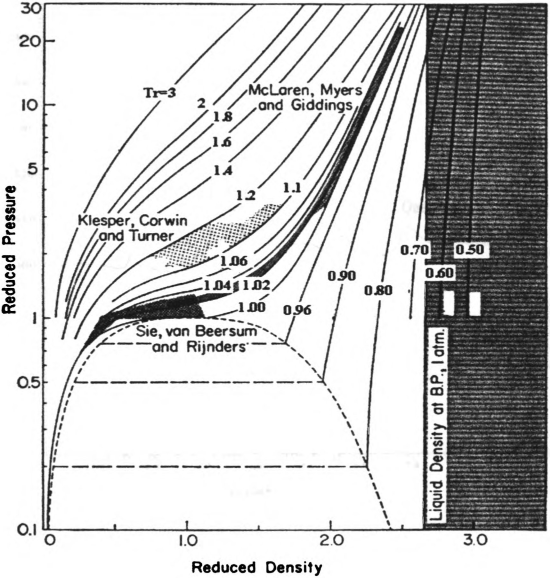

Figure B1. Giddings state curves. Giddings J. C. et al., 1968

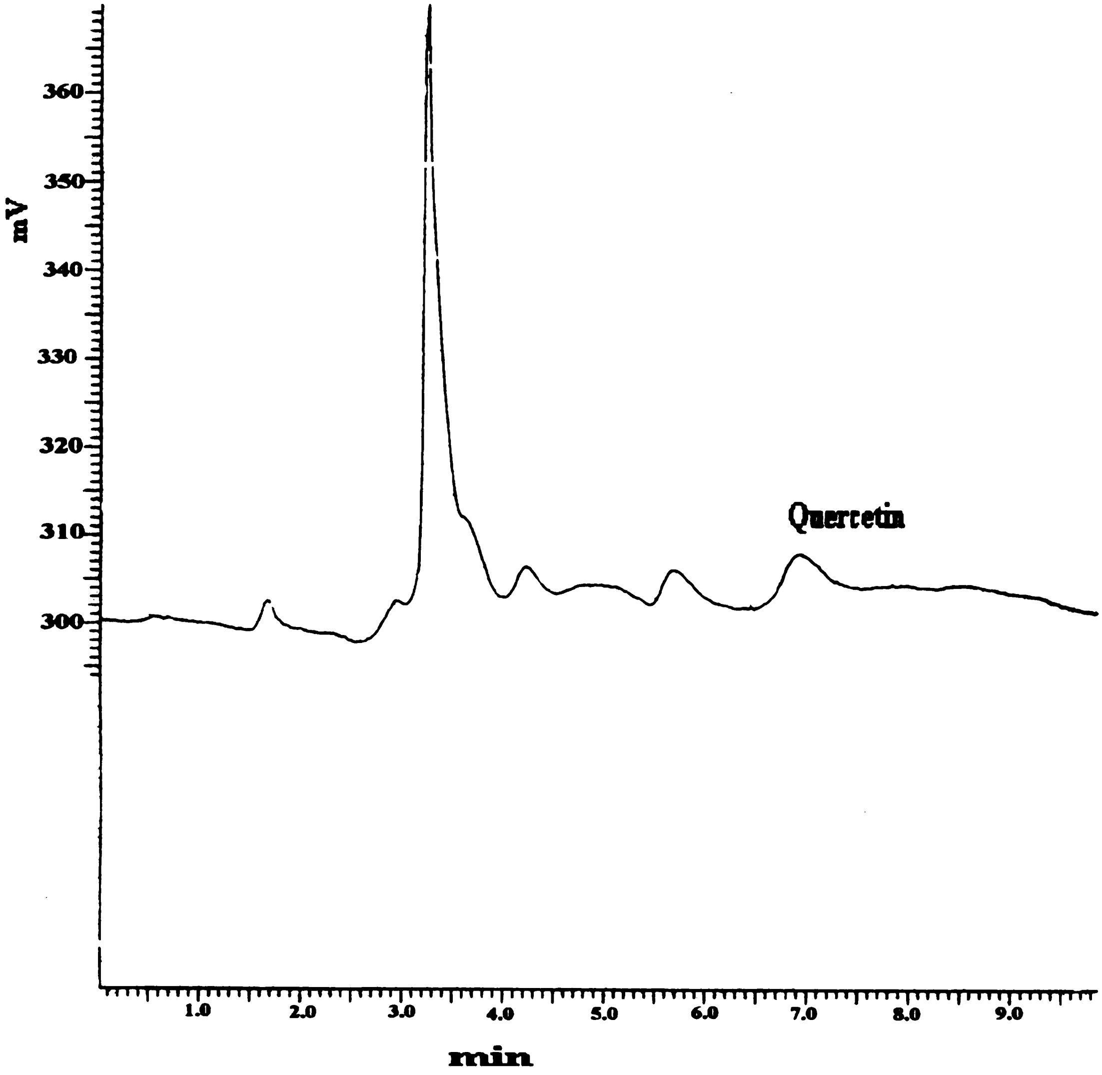

Figure B2. Static mode method. Example of the chromatograms

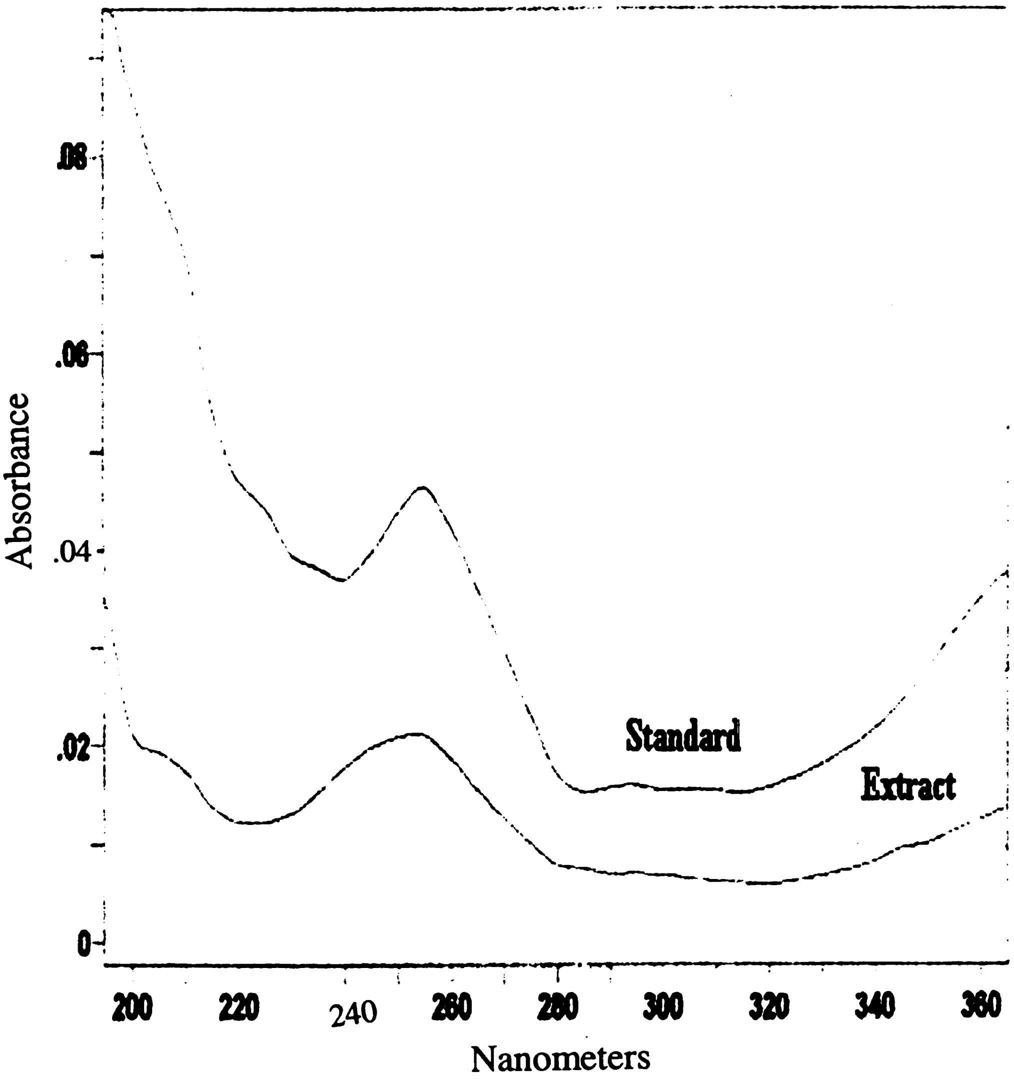

Figure B3. UV spectrum of quercetin standard and extract from SFE

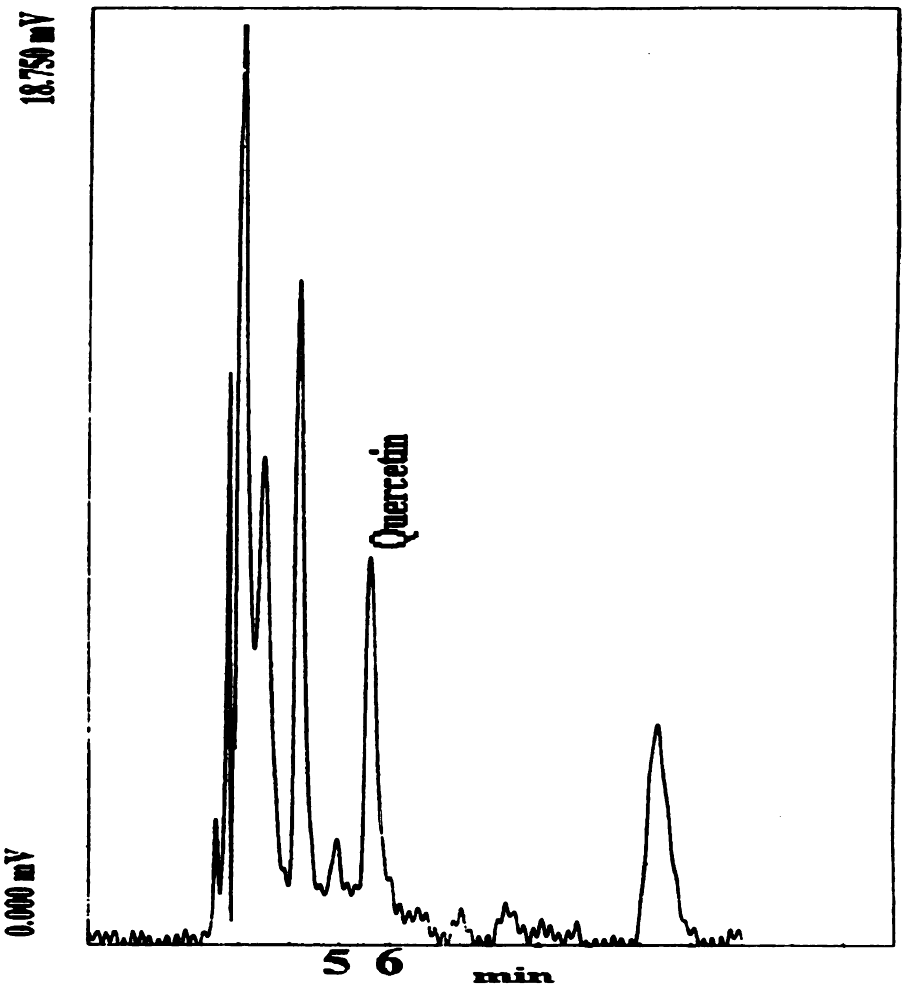

Figure B4. Dynamic mode method. Example of the chromatograms

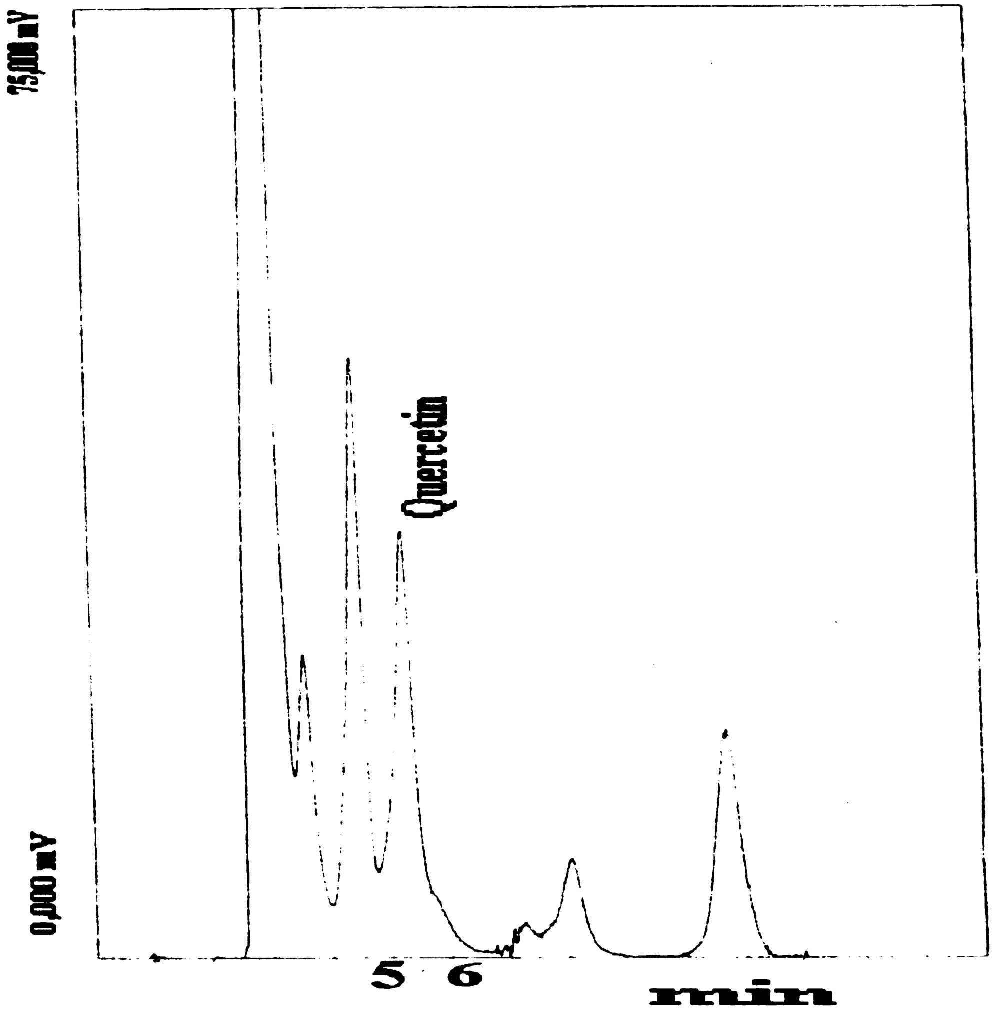

Figure B5. Static mode for red onion variety. Example of the chromatograms

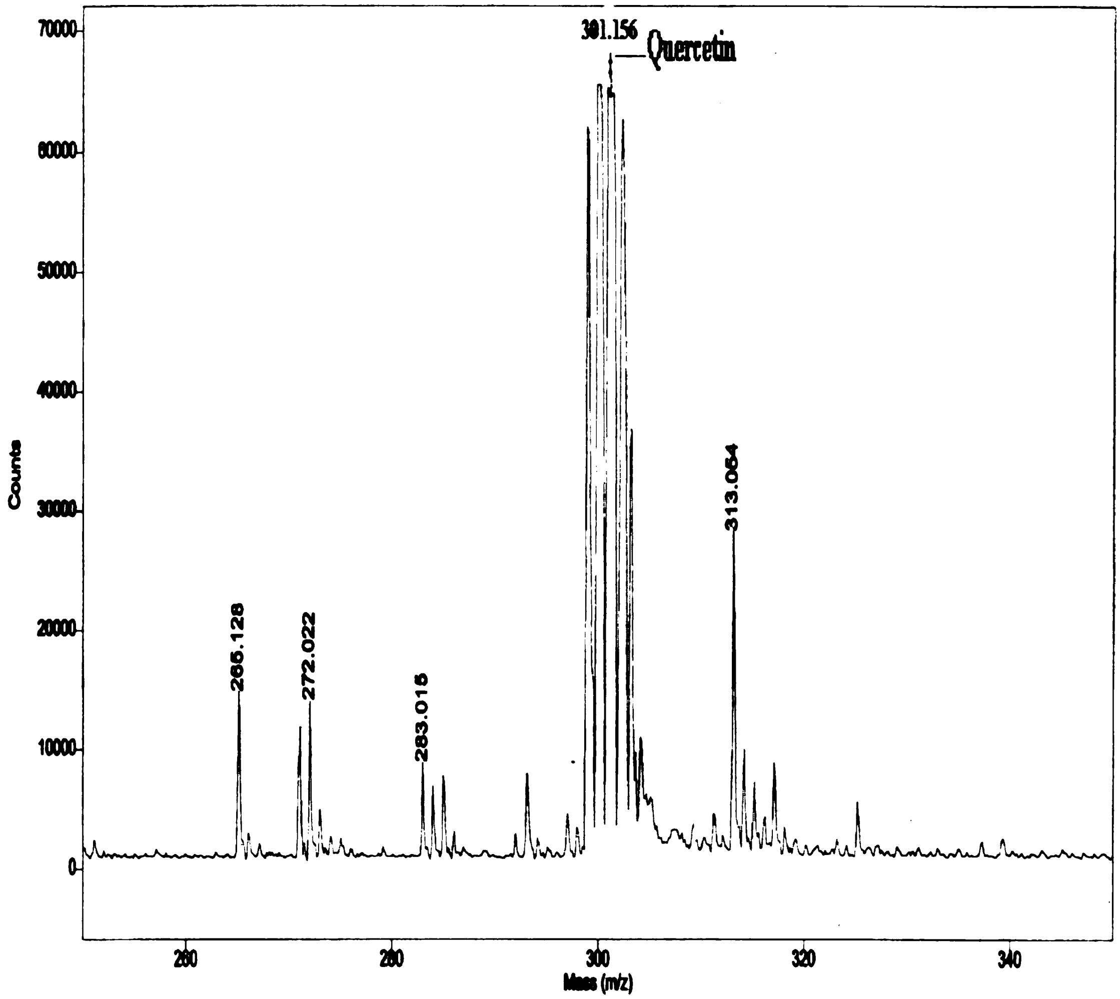

Figure B6. Mass spectrometry of the standard sample

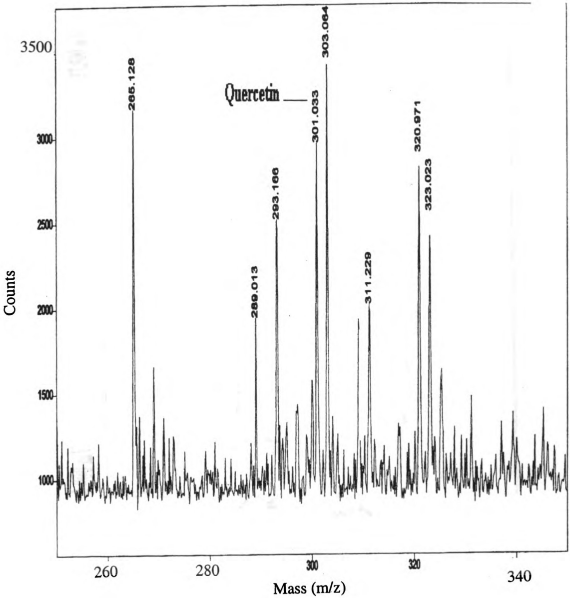

Figure B7. Mass spectrometry of the SFE extract

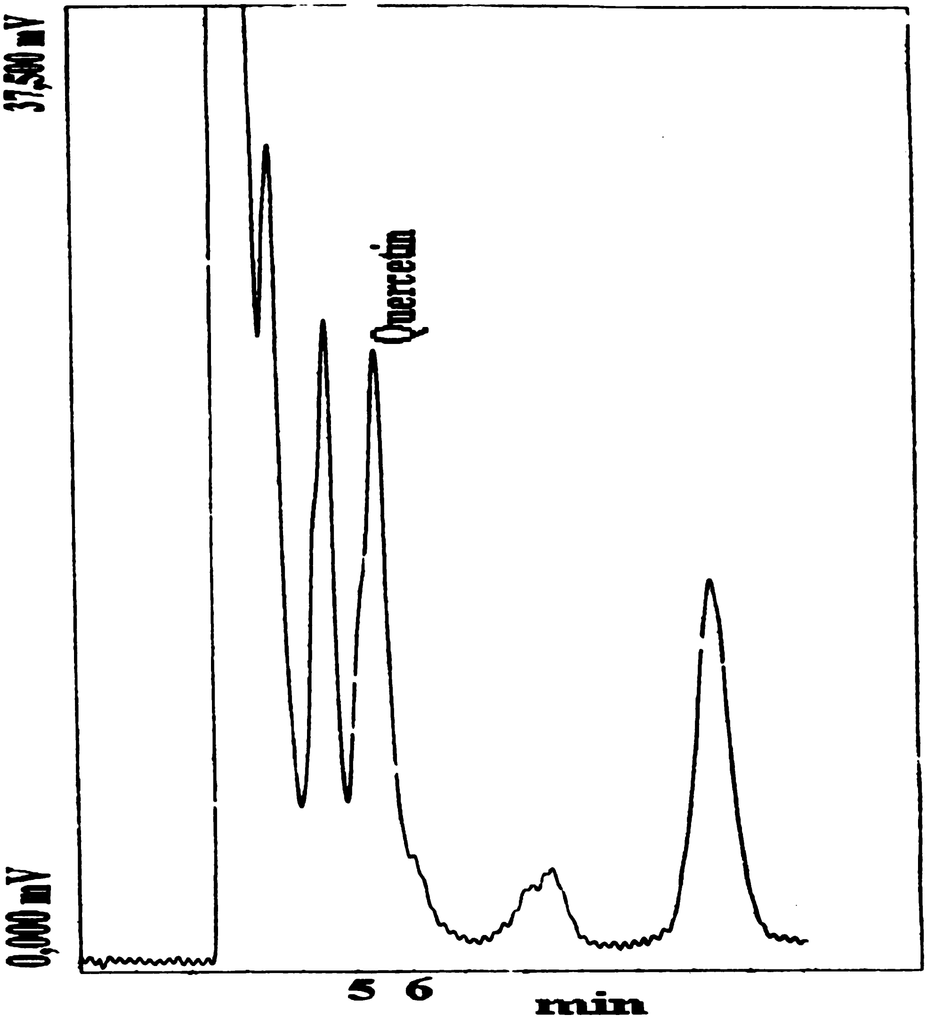

Figure B8. Static mode for the yellow variety. Example of the chromatograms

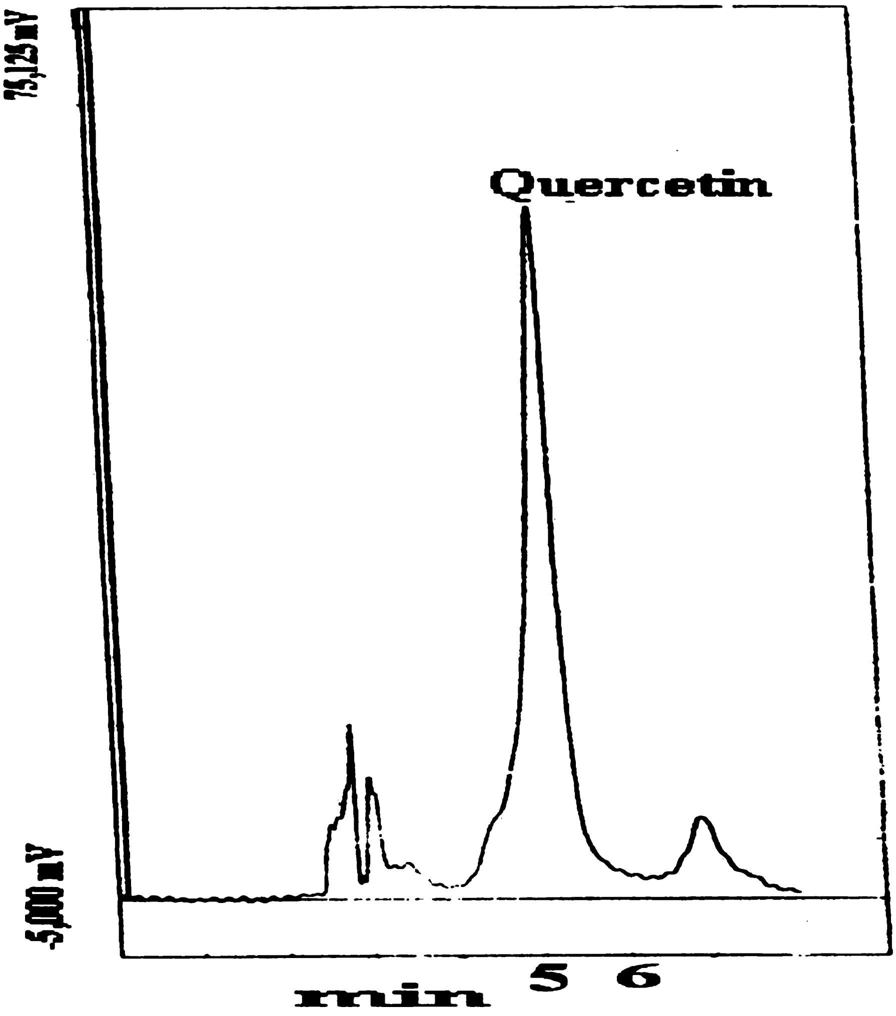

Figure B9. Example of the chromatograms of the pure quercetin extract

APPENDIX C. Statistical Analyses

RED VARIETY

ANALYSIS C1: quercetin (Y) versus volume of ethanol (X) collected

Volume (ml)	Total quercetin (μg)
0.934	53.447
0.918	38.851
1.010	39.904
0.876	24.826
0.986	45.857
0.738	21.401
0.740	22.354
0.718	16.846
0.666	12.432
0.636	11.942

<u>FROM MINITAB</u>

```
The regression equation is
Y = - 50.7 + 96.7 X

Predictor      Coef   SE Coef       T      P
Constant     -50.70     13.09   -3.87  0.005
X             96.67     15.72    6.15  0.000

S = 6.490     R-Sq = 82.5%    R-Sq(adj) = 80.4%

Analysis of Variance

Source          DF       SS        MS       F      P
Regression       1   1592.9    1592.9   37.82  0.000
Residual Error   8    336.9      42.1
Total            9   1929.9
```

ANALYSIS C2: ethanol collected (Y) versus time (X)

Time (min)	Volume (ml)
0.00	0.93
17.40	0.92
36.40	1.01
51.00	0.88
69.80	0.99
89.60	0.74
103.00	0.74
118.40	0.72
134.40	0.67
149.80	0.64

```
FROM MINITAB
The regression equation is
Y = 1.00 - 0.00237 X

Predictor      Coef       SE Coef       T      P
Constant     1.00479     0.04284     23.46   0.000
X         -0.0023719   0.0004723     -5.02   0.001

S = 0.07163    R-Sq = 75.9%    R-Sq(adj) = 72.9%

Analysis of Variance

Source          DF       SS          MS         F       P
Regression       1    0.12940    0.12940    25.22   0.001
Residual Error   8    0.04105    0.00513
Total            9    0.17044
```

ANALYSIS C3: extraction ratio (Y) versus time (X)

Time (min)	Extraction ratio (µg of quercetin/L of CO2)
0.00	3.56
17.40	2.59
36.40	2.66
51.00	1.65
69.80	3.05
89.60	1.43
103.00	1.49
118.40	1.12
134.40	0.83
149.80	0.80

FROM MINITAB

```
The regression equation is
Y = 3.23 - 0.0171 X

Predictor       Coef    SE Coef       T      P
Constant      3.2331     0.2891   11.18  0.000
X          -0.017071   0.003188   -5.36  0.001

S = 0.4835    R-Sq = 78.2%    R-Sq(adj) = 75.5%

Analysis of Variance

Source          DF      SS       MS       F      P
Regression       1  6.7028   6.7028   28.68  0.001
Residual Error   8  1.8698   0.2337
Total            9  8.5726
```

YELLOW VARIETY

ANALYSIS C4: quercetin (Y) versus ethanol volume collected (X)

Volume (ml)	Total quercetin (µg)
1.650	37.297
1.096	19.934
0.974	18.719
1.012	17.824
0.856	20.001
0.866	18.416
0.878	14.267
0.796	10.898
0.742	9.671
0.762	6.839

FROM MINITAB

```
The regression equation is
Y = - 11.1 + 29.6 X

Predictor       Coef   SE Coef       T      P
Constant     -11.095     3.896   -2.85  0.022
X             29.570     3.913    7.56  0.000

S = 3.124     R-Sq = 87.7%    R-Sq(adj) = 86.2%

Analysis of Variance

Source            DF        SS        MS       F      P
Regression         1    557.32    557.32   57.12  0.000
Residual Error     8     78.06      9.76
Total              9    635.37
```

ANALYSIS C5: ethanol collected (Y) versus time (X)

Time (min.)	**Volume (ml)**
0.00	1.65
18.20	1.10
36.80	0.97
55.00	1.01
71.80	0.86
91.40	0.87
106.40	0.88
123.80	0.80
140.00	0.74
156.00	0.76

FROM MINITAB

```
The regression equation is
Y = 1.29 - 0.00414 X

Predictor        Coef     SE Coef       T      P
Constant      1.29454     0.09667   13.39  0.000
X           -0.004145    0.001026   -4.04  0.004

S = 0.1619     R-Sq = 67.1%     R-Sq(adj) = 63.0%

Analysis of Variance

Source           DF        SS        MS       F      P
Regression        1   0.42776   0.42776   16.32  0.004
Residual Error    8   0.20964   0.02620
Total             9   0.63739
```

ANALYSIS C6: extraction ratio (Y) versus time (X)

Time (min.)	**Total ratio (µg of querc./L of CO_2)**
0.0	2.454
18.2	1.326
36.8	1.248
55.0	1.048
71.8	1.334
91.4	1.226
106.4	0.954
123.8	0.726
140.0	0.644
156.0	0.456

```
FROM MINITAB
The regression equation is
Y = 1.86 - 0.00901 X

Predictor       Coef     SE Coef       T       P
Constant      1.8620      0.1797   10.36   0.000
X          -0.009011    0.001907   -4.73   0.001

S = 0.3009     R-Sq = 73.6%     R-Sq(adj) = 70.3%

Analysis of Variance

Source          DF        SS        MS       F       P
Regression       1    2.0220    2.0220   22.34   0.001
Residual Error   8    0.7242    0.0905
Total            9    2.7462
```

COMPARISON BETWEEN VARIETIES

ANALYSIS C7: g of quercetin extracted/ kg of onion skins for both varieties

g of quercetin/ kg of onion skins (Red)	g of quercetin/ kg of onion skins (Yellow)
0.027	0.022
0.027	0.025
0.023	0.023
0.024	0.018
0.019	0.012

FROM MINITAB

```
Analysis of Variance
Source  DF      SS          MS          F     P
C2       1  0.0000400  0.0000400  2.13  0.182
Error    8  0.0001500  0.0000188
Total    9  0.0001900


Level    N    Mean      StDev
0        5  0.024000  0.003317
1        5  0.020000  0.005148
```

0 = red
1 = yellow

COMPARISON BETWEEN PRELIMINARY AND PRIMARY EXPERIMENTS

ANALYSIS C8: total quercetin obtained

Total quercetin (μg)	
Preliminary	**Primary**
71.57	192.52
11.45	201.45
49.16	225.08
	149.19
	101.1

FROM MINITAB

```
Analysis of Variance
Source    DF     SS      MS       F      P
C5         1   31594   31594   16.51  0.007
Error      6   11482    1914
Total      7   43076


               N    Mean    StDev
               0      3   44.06    30.38
               1      5  173.87    49.08

               0 = Preliminary
               1 = Primary
```

ANALYSIS C9: g of quercetin per kg of onion skins obtained

g of quercetin/kg of onion skins	
Preliminary	**Primary**
0.006	0.022
0.001	0.025
0.005	0.023
	0.018
	0.012

FROM MINITAB

```
Analysis of Variance
Source    DF     SS        MS         F       P
C8         1  0.00048  0.00048   24.00   0.003
Error      6  0.00012  0.00002
Total      7  0.0006


               Level    N    Mean     StDev
               0        3    0.004    0.002646
               1        5    0.02     0.005148

               0 = Preliminary
               1 = Primary
```

ANALYSIS C10: volume of ethanol collected

Volume (ml)	
Preliminary	**Primary**
0.468	1.107
0.234	1.180
0.206	1.090
	0.733
	0.706

FROM MINITAB

```
Analysis of Variance
Source   DF      SS       MS       F      P
C11      1     0.8181   0.8181   20.10  0.004
Error    6     0.2442   0.0407
Total    7     1.0623


Level      N    Mean    StDev
0          3   0.3026  0.1437
1          5   0.9632  0.2252
```

0 = Preliminary experiments

1 = Experiments

ANALYSIS C11: extraction ratio

Extraction ratio (µg of quercetin/L of CO_2)	
Preliminary	**Primary**
0.57	1.27
0.19	1.32
0.41	1.46
	0.99
	0.67

FROM MINITAB

```
Analysis of Variance
Source   DF      SS       MS       F      P
C2        1    1.0603   1.0603   13.60  0.010
Error     6    0.4679   0.0780
Total     7    1.5282


Level      N    Mean    StDev
0          3   0.3900  0.1908
1          5   1.1420  0.3143
```

0 = Preliminary

1 = Primary

COMPARISON BETWEEN SF AND SOLID-LIQUID EXTRACTION

ANALYSIS C12: SFE and methanol for red variety

g of quercetin/kg of onion skins	
SFE	**Methanol**
0.027	10.14
0.027	15.90
0.023	8.73
0.024	10.82
0.019	8.33

FROM MINITAB

```
Level     N    Mean    StDev
0         5   0.024    0.003
1         5  10.784    3.034
```

0 = SFE
1 = Methanol

ANALYSIS C13: SFE and methanol for yellow variety

g of quercetin/kg of onion skins	
SFE	**Methanol**
0.022	3.80
0.025	10.41
0.023	3.07
0.018	3.48
0.012	7.62

FROM MINITAB

```
Level     N    Mean    StDev
0         5   0.020    0.005
1         5   5.676    3.214
```

0 = SFE
1 = Methanol

PURE QUERCETIN

ANALYSIS C14: quercetin extracted(Y) versus ethanol collected (X)

Volume (ml)	Total quercetin (µg)
0.628	32.804
0.944	147.526
0.914	121.708
0.688	66.376
0.756	71.968
0.694	59.492
0.718	78.572
0.626	50.594
0.688	38.906
0.666	40.814

```
        FROM MINITAB
        The regression equation is
        Y = - 165 + 322 X

Predictor       Coef   SE Coef       T       P
Constant     -164.55     25.18   -6.53   0.000
X             321.54     34.04    9.44   0.000

S = 11.32     R-Sq = 91.8%    R-Sq(adj) = 90.7%

Analysis of Variance

Source          DF       SS       MS       F       P
Regression       1    11431    11431   89.20   0.000
Residual Error   8     1025      128
Total            9    12456
```

ANALYSIS C15: ethanol collected (Y) versus time (X)

Time (min.)	Volume (ml)
0.0	0.628
17.2	0.944
35.2	0.914
50.0	0.688
68.0	0.756
86.0	0.694
99.8	0.718
116.8	0.626
131.4	0.688
146.8	0.666

<u>FROM MINITAB</u>

```
The regression equation is
Y = 0.811 - 0.00105 X

Predictor       Coef     SE Coef        T      P
Constant     0.81120     0.06193    13.10  0.000
X         -0.0010517   0.0006991    -1.50  0.171

S = 0.1038    R-Sq = 22.1%    R-Sq(adj) = 12.3%

Analysis of Variance

Source          DF       SS        MS       F      P
Regression       1  0.02438   0.02438    2.26  0.171
Residual Error   8  0.08618   0.01077
Total            9  0.11056
```

ANALYSIS C16: extraction ratio (Y) versus time (X)

Time (min.)	**Extraction ratio (µg of quercetin/L of CO$_2$)**
0.0	2.186
17.2	9.836
35.2	7.626
50.0	4.426
68.0	4.798
86.0	3.968
99.8	5.238
116.8	3.372
131.4	2.592
146.8	2.720

FROM MINITAB

```
The regression equation is
Y = 6.48 - 0.0240 X

Predictor      Coef     SE Coef      T      P
Constant      6.482     1.327      4.89  0.001
X          -0.02404     0.01498   -1.60  0.147

S = 2.224     R-Sq = 24.4%     R-Sq(adj) = 14.9%

Analysis of Variance

Source          DF      SS        MS       F      P
Regression       1    12.736    12.736   2.58  0.147
Residual Error   8    39.556     4.945
Total            9    52.292
```

ANALYSIS C17: comparison of extraction ratios from inert and natural matrices

Extraction ratio (µg of quercetin/L of CO$_2$)	
Pure quercetin	*Yellow variety*
0.7	1.27
4.34	1.32
1.65	1.46
12.02	0.99
4.69	0.67

FROM MINITAB

```
Analysis of Variance
Source   DF     SS      MS      F      P
C14       1   31.29   31.29   3.15   0.114
Error     8   79.41    9.93
Total     9  110.70

Level    N    Mean    StDev
0        5   4.680    4.444
1        5   1.142    0.314
0 = Pure quercetin
1 = Yellow variety
```

ANALYSIS C18: quercetin collected from the natural matrix versus inert matrix

g of quercetin/kg of matrix	
Natural	**Inert**
0.022	0.002
0.025	0.013
0.023	0.005
0.018	0.036
0.012	0.014

FROM MINITAB

```
Analysis of Variance
Source   DF    SS        MS        F     P
C2        1  0.000090  0.000090  0.88  0.375
Error     8  0.000816  0.000102
Total     9  0.000906


Level    N    Mean     StDev
0        5   0.02000  0.00515
1        5   0.01400  0.01332
```

0 = natural
1 = inert

REFERENCES

Bilyk A., Cooper P. L., Sapers G. M. (1984). *Varietal Differences in Distribution of Quercetin and Kaempferol in Onion (Allium cepa L.)* Tissue. J. Agric. Food Chem., 32, 274-276.

Caltagirone S. (2000). *Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential.* Alternative Medicine Review. http://www.findarticles.com/cf_0/m0FDN/6_5/68727260/p1/article.jhtml?term=quercetin

Giddings J. C., Myers M. N., McLaren L., Keller R. A. (1968). *High Pressure Gas Chromatography on Nonvolatile Species.* Science, 162 (67), 67-73.

Hawthorne S. B. (1990). *Analytical-Scale Supercritical Fluid Extraction.* Analytical Chemistry, 62 (11), 633-642.

Hayashi A. (2000). *Effects of Daily Oral Administration of Quercetin Chalcone and Modified Citrus Pectin on Implanted Colon-25 Tumor Growth in Balb-c Mice.* Alternative Medicine Review. http://www.findarticles.com/cf_0/m0FDN/6_5/68727252/print.jhtml

Herrmann K. (1976). *Flavonols and flavones in food plants: a review.* J. Food Technology, 11, 433-448.

Karger B. L., Snyder L. R., Horvath C. (1973). *Separation Equilibria.* An Introduction to Separation Science. John Wiley & Sons. New York, NY.

King J. W. (1989). *Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science.* Journal of Chromatographic Science, 27, 355-364.

King J. W. (1990). *Quantitative Correlations Between Solute Molecular Structure and Solubility in Supercritical Fluids*. Journal of Chromatography, 517, 449-458.

Mukhopadhyay M. (2000). *Natural Extracts using Supercritical Carbon Dioxide*. CRC Press LLC. Boca Raton, Florida.

Merken H. M. (2000). *Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review*. J. Agric. Food Chem, 48 (3), 577-599.

Palma M., Taylor L. T. (1999). *Statistical Design for Optimization of Extraction of Polyphenols from an Inert Matrix using Carbon Dioxide-based Fluids*. Analytica Chimica Acta, 391, 321-329.

Palma M., Taylor L. T., Zoecklein B. W., Douglas L. S. (2000). *Supercritical Fluid Extraction of Grape Glycosides*. J. Agric. Food Chem, 48, 775-779.

Palmer M. V., Ting S. S. T. (1995). *Applications of supercritical fluid technology in food processing*. Food Chemistry, 52, 345-352.

Pascual-Marti M. C., Salvador A., Chafer A., Berna A. (2001). *Supercritical Fluid Extraction of Resveratrol from Grape Skin of Vitis Vinifiera and Determination by HPLC*. Talanta, 54, 735-740.

Price K. R., Rhodes M. J. C. (1997). *Analysis of the Major Flavonol Glycosides Present in Four Varieties of Onion (Allium cepa) and Changes in Composition Resulting from Autolysis*. J. Sci. Food Agric., 74, 331-339.

Rozzi N. L., Singh R. K. (2002). *Supercritical Fluids and the Food Industry*. Comprehensive Reviews in Food Science and Food Safety, 1, 33-44.

Schoenmakers P. J., Billiet H. A. H., Galan L. de. (1982). *The Solubility Parameter as a Tool in Understanding Liquid Chromatography*. Chromatographia, 15, 205-214.

Taylor L. T. (1995). *Strategies for Analytical SFE*. Analytical Chemistry, A, 364-370.

Tena M. T., Rios A., Valcarcel M. (1998). *Supercritical Fluid Extraction of T-Resveratrol and Other Phenolics from a Spiked Solid*. Frenesius J. Anal. Chem, 361, 143-148.

Xing N., Chen Y., Mitchell S. H., Young C. Y. F. (2001). *Quercetin Inhibits the Expression and Function of the Androgen Receptor in LNCaP Prostate Cancer Cells*. Carcinogenesis, 22 (3), 409-414.

General references

Barton A. F. M. (1975). *Solubility Parameters*. Chemical Reviews, 75 (6), 731-753.

Dron A., Guyer D. E., Gage D. A., Lira C. T. (1997). *Yield and Quality of Onion Flavor Oil Obtained by Supercritical Fluid Extraction and Other Methods*. Journal of Food Process Engineering, 20, 107-124.

Fedors R. F. (1974). *A Method for Estimating Both the Solubility Parameters and Molar Volumes of Liquids*. Polymer Engineering and Science, 14 (2), 147-154.

Giddings J. C., Myers M. N., King J. W. (1969). *Dense Gas Chromatography at Pressures to 2000 Atmospheres*. Journal of Chromatographic Science, 7, 276-283.

Häkkinen S. H., Kärenlampi S. O., Heinonen I. M., Mykkänen H. M., Törrönen A. R. (1998). *HPLC Method for Screening of Flavonoids and Phenolic Acids in Berries.* J. Sci. Food Agric., 77, 543-551.

Hirota S., Shimoda T., Takahama U. (1998). *Tissue and Spatial Distribution of Flavonol and Peroxidase in Onion Bulbs and Stability of Flavonol Glucosides during Boiling of the Scales.* J. Agric. Food Chem., 46, 3497-3502.

Karastogiannidou C. (1999). *Effects of Onion Quercetin on Oxidative Stability of Cook-chill Chicken in Vacuum-sealed Containers.* Journal of Food Science, 64 (6), 978-981.

McDonald M. S., Hughes M., Burns J., Lean M. E. J., Matthews D., Crozier A. (1998). *Survey of the Free and Conjugated Myricetin and Quercetin Content of Red Wines of Different Geographical Origins.* J. Agric. Food Chem., 46, 368-375.

McHugh M., Krukovis V. (1994). *Supercritical Fluid Extraction: Principles and Pratices.* Second Edition. Butterworth-Heinemann. Stoneham, MA.

Nuss J., Guyer D. E., Gage D. A. (1997). *Concentration of Onion Juice Volatiles by Reverse Osmosis and its Effects on Supercritical CO_2 Extraction.* Journal of Food Process Engineering, 20, 125-139.

Ollanketo M., Hartonen K., Riekkola M-L., Holm Y., Hiltunen R. (2001). *Supercritical Carbon Dioxide Extraction of Lycopene in tomato skins.* Eur. Food Res. Technol., 212, 561-565.

Reid R. C., Prausnitz J. M., Poling B. E. (1987). *The Properties of Gases and Liquids.* Fourth Edition. McGraw-Hill. New York, NY.

Saengcharoenrat C. (1998). *Optimization of Onion Flavor Recovery in the Process of Supercritical Carbon Dioxide Extraction and Desorption.* Doctor of Philosophy's thesis. Michigan State Universtiy. East Lansing. MI.

Simándi B., Sass-Kiss A., Czukor B., Deák A., Prechl A., Csordás A., Sawinsky J. (2000). *Pilot-scale Extraction and Fractional Separation of Onion Oleoresin using Supercritical Carbon Dioxide*. Journal of Food Engineering, 46, 183-188.

Taylor L. T. (1996). *Supercritical Fluid Extraction.* John Wiley & Sons, Inc. New York, NY.

Westwood S. A. (1993). *Supercritical Fluid Extraction and its use in Chromatographic Sample Preparation.* CRC Press, Inc. Boca Raton, FL.