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EXTRACTION OF GARLIC WITH SUPERCRITICAL CO_2 AND CONVENTIONAL ORGANIC SOLVENTS

J. M. del Valle[1*], C. Mena[1] and M. Budinich[1]

[1]Departamento de Ingeniería Química y Bioprocesos, Pontificia Universidad Católica de Chile,
Phone: (56-2) 354 4418, Fax: (56-2) 354 5803, Avda,
Vicuña Mackenna 4860, Macul, Santiago, Chile.
E-mail: delvalle@ing.puc.cl

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Abstract - Garlic (*Allium sativum* L.) and garlic extracts have therapeutical properties that stem from their sulfur-containing compounds, mainly allicin. The main objective of this work was to compare conventional and "premium" garlic extracts in terms of yield and quality, with the latter being obtained using supercritical carbon dioxide (SC-CO_2) as the solvent. Yield ranged between 0.65 and 1.0% and increased with extraction pressure (150-400 bar) at a constant temperature of 50°C. Extraction temperature (35-60°C), on the other hand, had little effect at a constant pressure of 300 bar. Based on yield and quality considerations, the best extraction conditions using SC-CO_2 were 35-50°C and 300-400 bar. A yield of 5.5% was obtained by conventional extraction using ethanol as the solvent, but ethanol appeared to be less selective for valuable components than SC-CO_2. The use of fresh garlic resulted in extracts that more closely resembled commercial products, possibly because of thermal and oxidative degradation of valuable microconstituents during drying.
Keywords: Ethanol extraction; Garlic; Supercritical CO_2; Supercritical extraction; Thiosulfinates.

INTRODUCTION

Garlic (*Allium sativum* L.) and garlic extracts are used as food ingredients, but also as nutraceuticals (food supplements that help maintaining human health) or phytopharmaceuticals for the prevention and treatment of various illnesses, including cardiovascular disease and cancer (Carson, 1987). These therapeutical properties of garlic extracts stem from their sulfur-containing compounds (thiosulfinates), mainly allicin. Allicin derives from the precursor alliin in fresh garlic tissue by the enzymatic action of alliinase. Allicin and other degradation products from alliin also give garlic its characteristic flavor.

Supercritical fluids (SCFs) often prove to be efficient solvents with better transport properties (diffusivity, mass transfer coefficient, penetration ability) than most common organic solvents in the liquid state (Brunner, 1994). When using carbon dioxide (CO_2) in particular, a high selectivity for valuable microconstituents in natural products and a complete separation of solvent traces from the extract and treated matrix can be achieved (del Valle and Aguilera, 1999). A further quality advantage for oxidation-prone substances is that these are exposed to neither oxygen nor high temperatures during treatment with supercritical CO_2 (SC-CO_2), in contrast to conventional separation methods. Thus, "premium" quality products can be obtained by SCF extraction with CO_2.

There have been some reports in the scientific literature on the SC-CO_2 extraction of allium species, and although many of these correspond to analytical applications for garlic (Calvey et al., 1994, 1997; Rybak et al., 2004), there are also reports on process development for industrial applications for other allium species. There were no compositional differences between extracts of an homogenate of fresh garlic in water using CO_2 and organic solvents (Calvey et al., 1994), but SC-CO_2 extraction

*To whom correspondence should be addressed

produced fresh onion-like flavor components from aqueous *Allium cepa* homogenates, unlike the cooked flavors obtained by steam distillation or solvent extraction (Dron et al., 1997). Sinha et al. (1992) reported that there were 28 sulfur-containing compounds found exclusively in an SC-CO_2 onion extract, including diallyl thiosulfinate and an isomer, propyl methanethiosulfonate, dithiin derivatives, diallyl sulfide, diallyl trisulfide and other compounds; three compounds (methyl propyl trisulfide, dipropyl trisulfide, dipropyl tetrasulfide) that were only found in a commercial sample of steam-distilled onion oil and at high concentration levels; and just 13 compounds that were shared by the two products. Extraction conditions for onion powder have been optimized in laboratory and pilot plant studies (Sass-Kiss et al., 1998a, b). Process temperature (35-65°C) and pressure (100-450 bar) have a significant and positive effect on yield, but sulfur content was higher in extracts obtained at 100 bar than at 300 bar. Sulfur content also increased with process temperature in experiments carried out at 300-400 bar. Process conditions had no effect on volatile oil content. Multistage precipitation in a pilot plant allowed the selective recovery of sulfur-containing and aroma compounds in onion from an SC-CO_2 extract obtained at 65°C and 450 bar in the second (low-pressure) stage (Simandi et al., 2000).

The objectives of this work were to study the effect of process temperature and pressure on yield and quality of garlic with SC-CO_2, and to compare these "premium" extracts with conventional products.

MATERIALS AND METHODS

Substrate for Extraction

Dehydrated garlic flakes from Inversiones Rauco Ltda. (Paine, Chile) with *ca* 9% moisture were used as the main substrate, which was kept at room temperature (20°C) in a dry environment. Flakes were coarsely ground in a hammer mill (M2 Assa, Assa Tecnología S.A., Santiago, Chile) with 5mm openings, and classified by size in a Ro-Tap test sieve shaker (W.S. Tyler, Mentor, OH). Three fractions were separated for further analysis: –4 mesh (particle diameter (D_p) > 4.75 mm); –4/+14 mesh (1.18 mm < D_p < 4.75 mm); and –14 mesh Tyler (D_p < 1.18 mm). Fresh garlic purchased in a local market was also used as a substrate. Finally, commercial garlic capsules from Centrum (Whitehall-Robins Healthcare, Madison, WI), which were claimed to contain 300mg garlic powder and 1300μg alliin, were used as the reference product. Extraction solvents included technical quality azeotropic ethanol (Idapquim, Santiago, Chile) and food-grade liquid CO_2 in high-pressure cylinders (AGA S.A., Santiago, Chile).

Extraction Experiments

SC-CO_2 extraction was carried out in an SFE-1L process development unit from Thar Technologies (Pittsburgh, PA) similar to the one described by del Valle et al. (2003). In this case, however, the 200mL extraction vessel was equipped with a heating jacket and temperature was controlled by recirculation of heated water through a thermally equilibrated bath, instead of by placing the extractor in a thermally equilibrated forced-air oven. All experiments were carried out in the dynamic mode using 100g samples and 20g/min of CO_2. Extract was sampled at 1h intervals in 25mL cyclone separators and dissolved with <15mL ethanol aliquots. Some preliminary experiments were carried out to select the extraction time and the particle size. The effect of extraction temperature (35, 50, or 60°C) was studied at a constant pressure of 300 bar, whereas that of extraction pressure (150, 225, 300, or 400 bar) was studied at a constant temperature of 50°C. Most experiments were not replicated.

Conventional extraction of a 2kg sample was carried out with 12L of azeotropic ethanol in a large, Soxhlet-type pilot plant apparatus (Quickfit and Quarz Ltd., London, UK) for 48 h. The extract was concentrated at 70°C and 170 mbar in a Labor Rota pilot plant rotary evaporator (Heindolph Elektro GmbH & Co., Kelheim, Germany) which was equipped with a Gast vacuum pump (Gast Manufacturing Inc., Carlstadt, NJ).

Analysis

Moisture contents of garlic samples and the amount of soluble solids in ethanol dissolutions were determined gravimetrically by drying in a convection oven (Binder WTC, Tutlingen, Germany) set at 105°C until constant final weight was reached (drying times of 1 day and 2 h, respectively).

Extract samples were analyzed by HPLC chromatography using the method proposed by Block et al. (1992). For this purpose, the four fractions corresponding to each SC-CO_2 extraction experiment were pooled together and filtered through Durapore PVDF microfiltration membranes with 0.45μm openings (Millipore Corp., Bedford, MA)

prior to analysis. The HPLC device included a multisolvent delivery system (Waters 600E, Milford, MA), a tunable absorbance detector (Waters 484 UV/Vis), and an integrator (Shimadzu Chromatopac C-R6A, Kyoto, Japan). Separation was carried out in a Symmetry RP18 column (4.6×250 mm) using 0.8 mL/min of a 1:1 (v/v) mixture of HPLC quality methanol and water, both from Fisher Scientific (New Jersey, NJ) as the mobile phase. Detection was at a wavelength of λ = 254 nm with a sensitivity of AUFS = 0.01.

Reference samples for HPLC analysis were prepared according to the procedure of Calvey et al. (1994), consisting of homogenization of 1g samples with 10mL distilled water, continuous stirring for 30 min at room temperature on a stirring plate, filtration through Whatman N° 4 paper (Whatman International Ltd., Maidstone, UK), saturation with technical quality NaCl, second stage filtration through paper, and fractional extraction with three parts (v/v) technical dichloromethane in a decantation funnel. The organic phase was dried using analytic-grade anhydride sodium sulphate, filtered, rotary-evaporated to dryness at room temperature under vacuum, and redissolved with the mobile phase for HPLC analysis.

RESULTS AND DISCUSSION

Yield

The extraction yield of garlic flakes was 5.5% (w/w) when using azeotropic ethanol. Sass-Kiss et al. (1998b) reported a 35% yield for onion powder treated with ethanol. Although onion and garlic both belong to the genus allium, they are not of the same species. Furthermore, variety differences within species are common, which may justify the large differences in yield. Another factor that may have affected differences in yield between garlic and onion is sample pretreatment. In our work there was a problem of agglomeration of milled garlic flakes during large-scale extraction, which were not reported for the extraction of onion powder by Sass-Kiss et al. (1998b) and which may have limited the yield of the process. As a result of agglomeration in a packed bed, the specific surface for extraction diminishes and the diffusion path within the solid matrix increases, creating mass transfer limitations to yield.

The extraction yield of garlic flakes with SC-CO_2 changed with sample particle size and process temperature, pressure, and time. Since the milled substrate showed a tendency to agglomerate during extraction, optimal particle size was determined in a preliminary set of experiments as the smallest that did not cause perceptible agglomeration problems; this size was the –4/+14 mesh fraction (1.18-4.75 mm). FLAVEX Naturextrakte offers a product named Garlic CO_2-to, which corresponds to an extract that is extended with olive oil (Type N° 005-001) (www.flavex.com). The extract is obtained by mixing fine grated fresh garlic with kieselguhr (probably to avoid agglomeration problems) prior to extraction with SC-CO_2 (undisclosed extraction conditions) (www.flavex.com).

Another preliminary experiment indicated a limited increase of 9.3% in extraction yield with SC-CO_2 at 50ºC and 300 bar when treatment time increased from 4 to 8 h, so all subsequent experiments were carried out for a total extraction time of 4 h. The four replicates of the four-hour experiment at 50°C and 300 bar without extract sampling resulted in a yield of 0.86 ± 0.08 g oleoresin/100 g substrate (variance=9%).

Figure 1 shows the effect of extraction temperature on integral extraction yield of garlic oleoresin as a function of process time. It was concluded that treatment temperatures in the 35-50ºC range do not affect the extraction rate or yield of garlic treated with SC-CO_2 at 300 bar. Yield was *ca* 0.8% (w/w) in these experiments following a four-hour treatment. The solubility of a solute in SC-CO_2 may decrease (at near-critical pressure), remain constant (at the cross-over pressure), or increase (at pressures above the cross-over level) as the temperature increases due to the conflicting effect of temperature changes on the density of the SCF and the vapor pressure of the solute. However, cross-over pressures rarely exceed 150-200 bar for SC-CO_2 (although they are solute dependent) (Foster et al., 1991), so in these experiments extraction temperature was expected to have a positive effect, which should have been compounded by the positive effect of temperature on transport properties and mass transfer rates.

In Figure 2 the effect of extraction pressure on integral extraction yield of garlic oleoresin as a function of process time is shown. It can be observed that the extraction rate and yield of garlic following a four-hour treatment with SC-CO_2 at 50ºC increases as process pressure increases. Yield increased from 0.7% (w/w) at 150 bar to 1.0% (w/w) at 400 bar. This may have been due to the positive effect of pressure on the density and solvent power of SC-CO_2. Sass-Kiss et al. (1998a) reported a top yield of 0.9% for onion oleoresin with SC-CO_2 at 57°C and 350 bar. NATex Prozesstechnologie reports a yield of 0.3-0.5% total aqueous extract from garlic using SC-CO_2 at 20-50°C and 100-450 bar (www.natex.at).

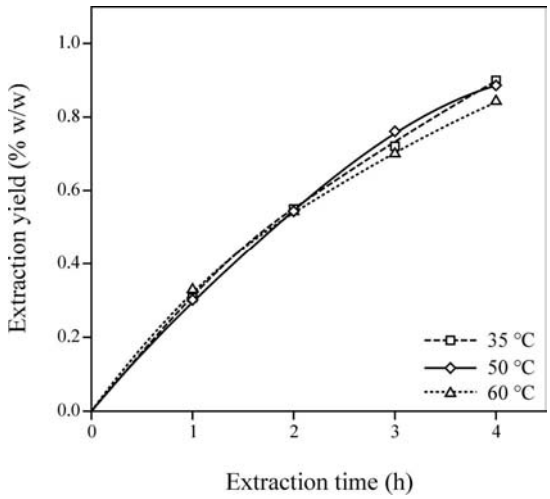

Figure 1: Effect of extraction temperature on integral extraction yield of garlic with SC-CO_2 as a function of process time at a constant process pressure of 300 bar.

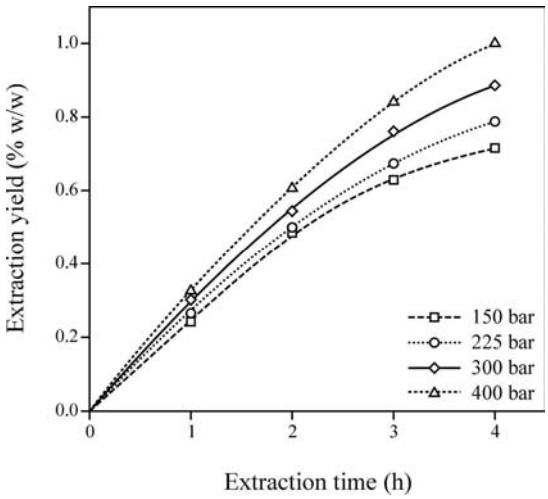

Figure 2: Effect of extraction pressure on integral extraction yield of garlic with SC-CO_2 as a function of process time at a constant process temperature of 50ºC.

Quality of Extracts

Fingerprints of extract samples were obtained by HPLC analysis (Fig. 3). The quantification of allicin and other thiosulfinates of nutraceutical significance is complicated by the chemical instability and resulting expense of HPLC standards, which were not purchased in this work. Instead, the fingerprints of the various extracts were compared to those of a commercial product with specific compositional claims. Fresh garlic (crushed) and commercial garlic capsules (Centrum) were used as reference samples. Figure 3 includes selected HPLC chromatographs of Centrum capsules, fresh garlic, dehydrated garlic flakes, and ethanol and SC-CO_2 extracts of garlic flakes. To make quantitative comparisons between the samples, four peaks that were especially large in Centrum capsules and that are marked 1-4 in Figure 3a were selected. For each sample, the area of these four peaks was divided by the amount of soluble solids that was injected into the HPLC column, so as to obtain a standardized peak area representing the concentration of the compound in the sample or extract. The standardized peak areas were divided by the corresponding values in the reference product and expressed as percent concentration of the compound in the sample or extract as compared with their concentration in Centrum capsules. These values were finally plotted along four radial axes in Figure 4. The size and shape of each polygon in relation to those of the regular rhombus corresponding to Centrum capsules indicate how enriched or concentrated the sample/extract is and its degree of similarity with the reference product.

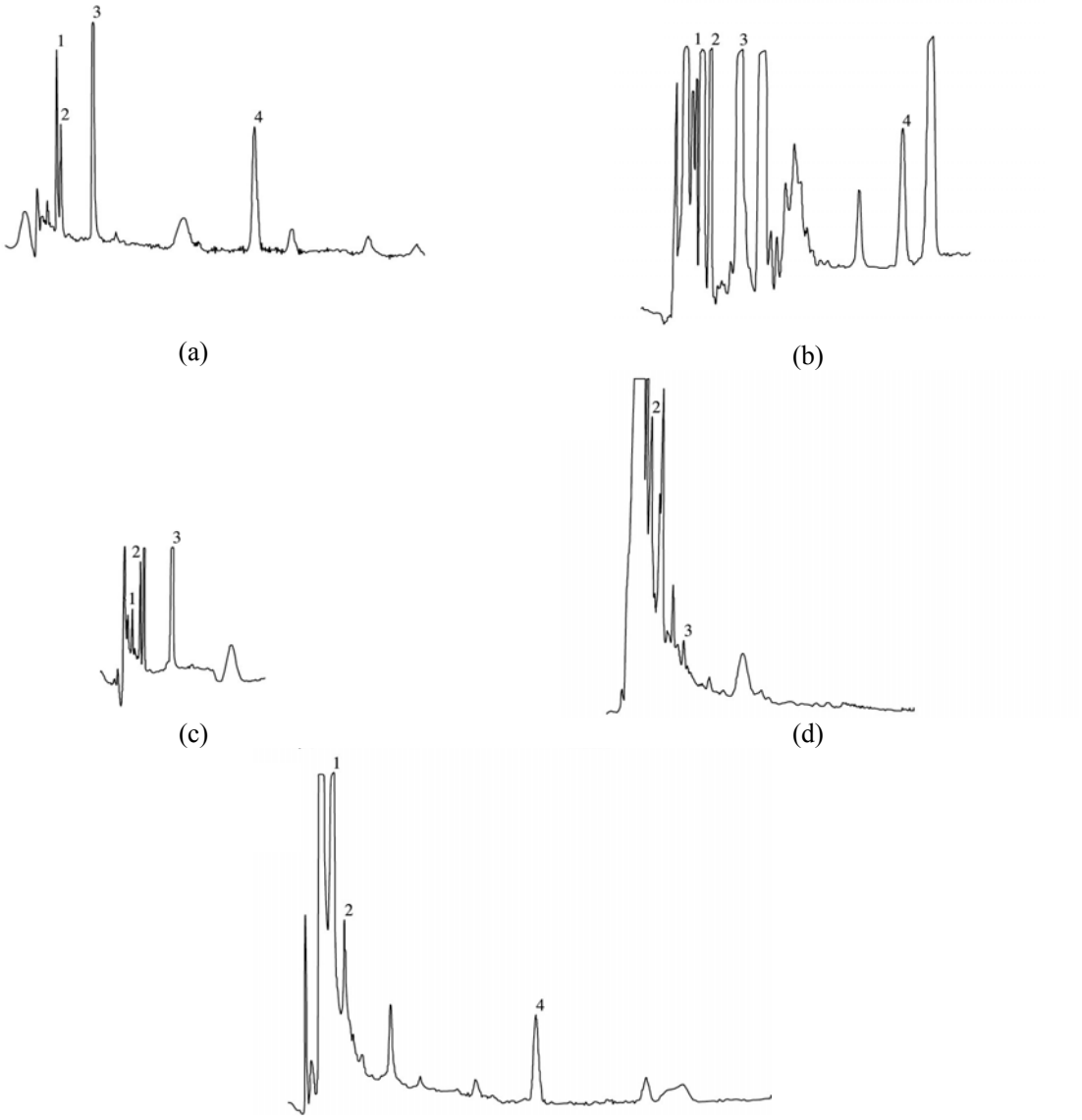

Figure 3: HPLC chromatographs of (a) Centrum capsules, (b) fresh garlic, (c) dehydrated garlic flakes, (d) ethanol extract of dehydrated garlic flakes, and (e) SC-CO_2 extract of dehydrated garlic flakes treated at 50ºC and 300 bar. (The selected compounds 1-4 are labeled in the pertinent chromatographs.)

Figure 4a shows the effect of the raw material. The fingerprint of Centrum appeared to be very similar to that of fresh garlic, as expected. The manufacturing process of Whitehall-Robins Healthcare is very benign with some microconstituents in garlic, but a comparison of chromatograms in Figures 3a and 3b indicates that Centrum is enriched with compounds 1-4. On the other hand, flaking and drying caused a major change in that there was a decrease in the concentration of compounds 1 and 3 and the disappearance of compound 4. These changes may have been due to thermal and/or oxidative degradation of precursors, intermediates, and/or end products during hot air drying.

Figures 4b and 4c show the effects of extraction temperature and pressure, respectively, on the quality of SC-CO_2 extracts. It is apparent that SC-CO_2 could not extract compound 3. The relative contents of most other compounds increased as the temperature increased up to 50ºC (Fig. 4b) or as the pressure increased (Fig. 4c). Thus, most SC-CO_2 extracts

were contained in the triangle of that obtained under the optimal extraction conditions of 50ºC and 300 bar. These differences have been related to increased solubility of desirable components with process temperature and pressure up to these levels. Further increases in process temperature and pressure may have caused thermal damage and/or co-extraction of undesirable compounds with the end result of degradation and/or dilution of desirable components.

In Figure 4d the ethanol extract and optimal SC-CO_2 extract (obtained at 50ºC and 300 bar) are compared with the commercial product. The least desirable extract seems to be the one obtained with ethanol, which is limited to two desirable compounds (2 and 4). However, close examination of the chromatogram in Figure 3d suggests the very poor selectivity of ethanol for the extraction of valuable microconstituents from garlic. Although the yield of sulfur compounds increased from 0.21 g/kg for SC-CO_2 extraction to 3.79 g/kg for Soxhlet extraction with ethanol in the work of Sass-Kiss et al. (1998a), the concentration decreased from 2.3% of the SC-CO_2 extract to 1.1% of the conventional oleoresin.

It is also possible that the best garlic product to use as a nutraceutical may be obtained by combining extracts obtained with different solvents. Thus, New Chapter sells Garlicforce, which is obtained using a proprietary patent-pending process (www.new-chapter.com). Galicforce is a final product for direct consumption, corresponding to soft gel capsules containing 80 mg of sulphur-rich (alliin-derivative) supercritical extract, 160 mg of cisteine-derivative-rich ethanolic extract from the residual product of supercritical extraction, and 22 mg of a mixture of supercritical extracts from several species that act synergically with garlic.

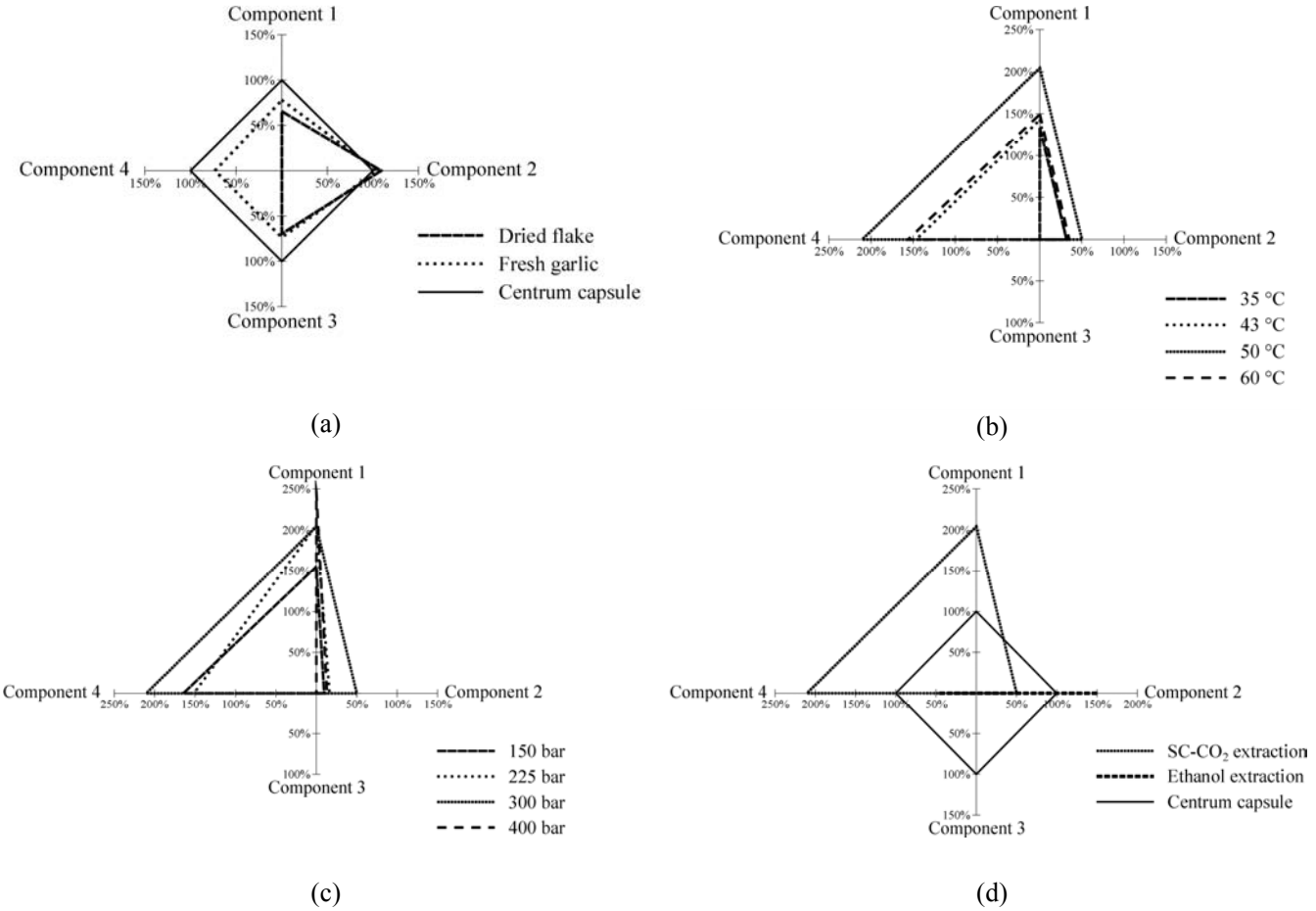

Figure 4: Comparison of radial plots of the four selected compounds for (a) different substrates, (b) different process temperatures for SC-CO_2 extraction experiments at 300 bar, (c) different process pressures for SC-CO_2 extraction experiments at 50ºC, and (d) different extraction processes with optimal SC-CO_2 extraction conditions of 50ºC and 300 bar.

CONCLUDING REMARKS

This work has demonstrated that it is possible to obtain widely different extracts from garlic by changing the extraction conditions, which affect both the yield and selectivity of the extraction process. The methods used in this work to characterize extract samples should be improved. Standards of relevant thiosulfinate compounds should be isolated, synthesized, or purchased for HPLC quantification purposes. Alternatively, extract samples should be analyzed by GC/MS.

Dehydrated garlic flakes are extremely hygroscopic and tend to agglomerate during extraction. This tendency towards agglomeration depends not only on particle size, but also on moisture content (Eggers et al., 2000) and possibly process temperature. This tendency to agglomeration has been explained as being due to the glass-rubber transition of amorphous food powders, where caking is being caused by the difference between process temperature and the glass transition temperature (T_g) of the material (T_g decreases as the moisture content increases) (Aguilera et al., 1995). According to this hypothesis, any increase in sample moisture and/or process temperature would favor flow-dependent caking phenomena. Thus a combination of a limited reduction in particle size and a water removal step combined with processing at a moderate temperature should be optimized to allow fast extractions with no solvent channeling in the packed bed. An alternative would be to extract water homogenates as attempted by Dron et al. (1997) with onion juice. Since the initial concentration of the aqueous solution has a positive effect on the yield of the SCF extraction process without negative effects on extract quality, preconcentration of the juice by reverse osmosis from 10 to 18 °Brix was evaluated by Nuss et al. (1997). Alternative mild concentration processes could be attempted.

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