Supercritical Fluid Extraction Technology for Fat Reduction

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Introduction

Awareness that consumers demand lean meat products has spurred a considerable amount of research in product development of low-fat products. With the interest in reducing fat in meat products sweeping the country, this session provides meat scientists with an opportunity to learn about many current technologies available. One of the more innovative techniques for fat reduction may be the use of supercritical fluid extraction (SFE). Researchers recently developed interest in the use of SFE as a method to alter the composition of meat (King et al., 1989; Chao et al., 1991a). This interest, coupled with available equipment and expertise, has led to the nucleus of a SFE research program at MU. This segment of the program allows me to introduce SFE technology and share some of the results of SFE work related to meat and other food products.

Background and Theory of SFE

Before embarking on a discussion of the applications of SFE for meat products, it would be helpful to review what supercritical fluids are and how the extraction process functions to remove fat. First, supercritical fluids are a phase that fluids enter as a result of elevated pressure and temperature conditions that exceed a critical point unique to the fluid. Above the critical temperature, no amount of pressure will force the gas into a liquid and therefore the fluid remains in a supercritical phase with properties somewhat like both a gas and a liquid. Phase diagrams, such as the one shown in Figure 1, are rather typical and reflect the relationship between supercritical fluids and the more commonly encountered gas, liquid and solid phases. The interest in supercritical fluids arises because they have unique density and diffusivity values that are usually between those of the gas and liquid phase (Rizvi et al., 1986a). One can observe from the phase diagram that the supercritical region encompasses a wide range of temperatures and pressures and this feature may be harnessed to enhance the selectivity of the supercritical solvent. Hyatt (1984) and Rizvi et al. (1986a) reported that knowledge of SFE can be traced back to the latter half of the 19th century. Brief summaries of SFE from this early period up to the intense activity of the 1970's can be found in both articles.

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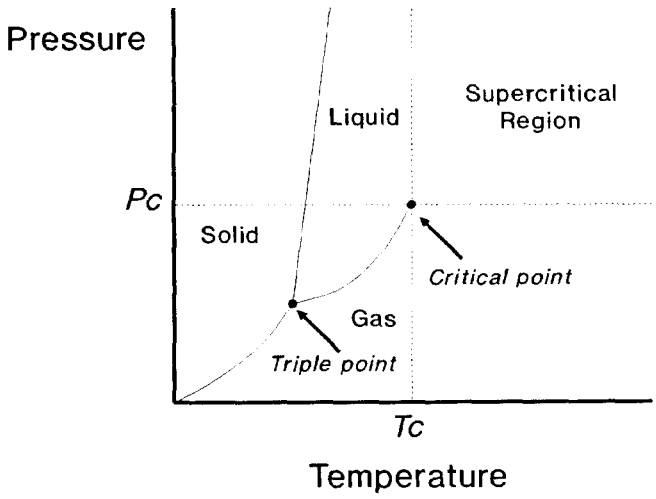
Reciprocal Meat Conference Proceedings, Volume 44, 1991.

The properties of supercritical carbon dioxide and other solvents are well documented (Brogle, 1982; Hyatt, 1984; Rizvi et al., 1986a). The critical temperature and pressure for the fluid may be important because these conditions influence the requirements for equipment to process materials. For example, the critical temperature and pressure of carbon dioxide is 31°C and 7.38 MPa (Brogle, 1982; Rizvi et al., 1986b). The critical conditions for water are approximately 374°C and 22 MPa (Hoyer, 1985; Rizvi et al., 1986a) and the temperature precludes use of supercritical water for food applications. In fact, the ability to use supercritical carbon dioxide at temperatures below 40°C is most fortunate for maintaining quality of raw materials (e.g. meats). A study by Zou et al. (1990) also provides insight that low (40°C) temperatures improve the solubility of fatty acids such as oleic and linoleic acid in the supercritical carbon dioxide. Extraction at pressures well above 22 MPa are practical; for example, processing of oilseeds at pressures of 65-70 MPa has been studied (Friedrich et al., 1982). Although it is evident that the critical pressure is attainable for many solvents, the critical temperature has a significant impact on the quality of foods and must be considered.

Selection of a solvent for SFE depends on the sample and the intended solute. In most cases, selection should be based on the solvent's ability to dissolve specific components, but in many cases, factors such as cost, toxicity,

Figure 1

Typical pressure/temperature phase diagram for a pure material.

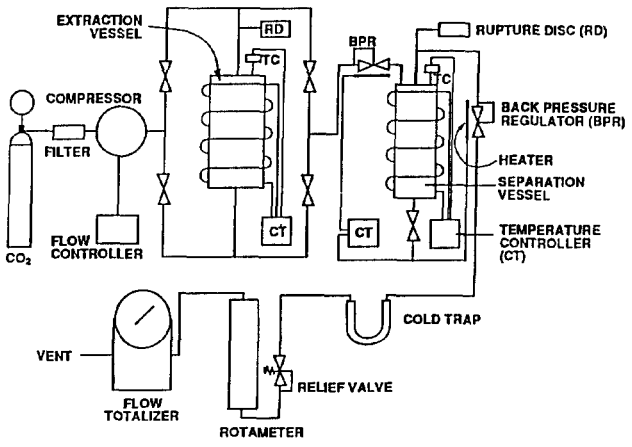


flammability and availability must be considered (Friedrich and Pryde, 1984; Hyatt, 1984; Rizvi et al., 1986b). Since any product that is processed by SFE is totally immersed in the fluid, safety and ease of solvent removal are also important issues for the selection of the appropriate solvent. Carbon dioxide is most commonly used because it is relatively safe and meets most other requirements for a supercritical solvent. In cases where the desired solute is insoluble in supercritical carbon dioxide, one could select another solvent. Considering the highly desirable safety and solvent characteristics of supercritical carbon dioxide, it is easy to understand why CO_2 is an easy choice for food industry applications.

Despite significant interest, the food industry has been slow to adopt SFE and this may be due to the difficulty with selection of appropriate SFE methods and equipment. Obviously, there must be a substantial capital investment for any commercial scale production in supercritical extraction processing. However, a complication in the decision process is that more than one design for a SFE system is possible. What makes the selection of an appropriate system particularly difficult is the general lack of engineering information to successfully scale-up laboratory (or even pilot) processes to the commercial level. A final obstacle is that most laboratory operations are designed for batch processing rather than continuous operation. Figure 2 is a schematic representation of the system in use at the University of Missouri with the essential components of the system and some modifications that are possible. The four principal parts of the system are the solvent compressor, the extraction vessel, the separation vessel and the temperature/pressure control system. Extraction systems may have additional pumps for the introduction of entrainers (cosolvents) or multiple separatory vessels (with independent temperature/pressure control) to enhance fractionation of mixtures. Other refinements may include using adsorbents (e.g. activated alumina) before the separation vessel or the recycling of solvent. Descriptions for these configurations may be found in various resources (Friedrich and Pryde, 1984; Rizvi et al., 1986b; Huang, 1990).

Figure 2

Supercritical fluid extraction unit with one separator.



Applications for SFE of Foods

Applications for SFE in the food industry encompass a tremendous variety of products. Decaffeination of coffee and extraction of flavor compounds from hops have been reported as the principal uses for SFE on a commercial scale (Gardner, 1982; Morris, 1982; Dziezak, 1986). Several techniques for extraction of various oilseeds are reported (Friedrich et al., 1982; Stahl et al., 1984; Taniguchi et al., 1985; Fattori et al., 1987). Examples of SFE being used for extraction of pigments such as annatto (Chao et al., 1991b) or carotene (Favati et al., 1988) can be found. Animal products such as dried egg yolk (Froning et al., 1990) and butter (Shishikura et al., 1986; Anonymous, 1989) have also been modified with SFE.

A few interesting applications for SFE include fractionation of fats, deodorization of oils and extraction of flavor compounds. Some studies on fractionation of tallow (Rizvi et al., 1986b; Huang, 1990) and lard (King et al., 1989) are available and another report on tallow fractionation by S.S.H. Rizvi of Cornell University may be obtained from the National Live Stock and Meat Board. Fujimoto et al. (1989) reported that sardine meat powder subjected to SFE had less fishy odor and this may increase the opportunities for using such material in sensitive products, such as surimi. Clearly, one of the most successful applications of SFE is the commercial processing of hops for flavor compounds (Gardner, 1982), but the extraction of citrus oils also has potential (Tamelli et al., 1988).

SFE Technology and Meats

Given the success that SFE has in the fractionation of tallow and butter fats and the ability to extract oils from various oilseeds, it seems inevitable that applications in meats would be studied. Already, articles related to the composition of meats (King et al., 1989; Chao et al., 1991a), krill (Yamaguchi et al., 1986) and fish (Hardardottir and Kinsella, 1988; Fujimoto et al., 1989) have been published. The National Live Stock and Meat Board has contributed to additional SFE research for modification or fractionation of beef, pork and tallow at the University of Nebraska, University of Missouri and Cornell University, respectively. Reports from these projects should be published soon; in the meantime, summaries are available from the Meat Board.

In 1989, the media projected that shoppers would soon be able to buy fat-free, cholesterol-free steaks in their local markets. Although SFE has been useful in many food applications, the production of cholesterol-free steaks is unlikely to ever be accomplished. However, the possibility that SFE can be used with dried muscle tissue to produce low-fat or low-cholesterol ingredients for further processed items is much closer to reality.

Fresh Meats

The application of SFE to fresh meat products has not been overly successful with respect to fat and cholesterol reduction. One of the few published reports dealt with the extraction of fresh ground beef (Chao et al., 1991a). At the University of Missouri, one of our projects was to thoroughly investigate extraction of fresh pork (Clarke et al., 1991b). Our

Table 1. Composition of Porcine Biceps Femoris Muscle Extracted with Supercritical Fluid CO_2 at 35° or 50°C and Various Pressures Compared with Non-Extracted Tissue.

Treatment	*Moisture* %	*Crude Fat* % *dbw*[a]	*Cholesterol* *mg/100 g dwb*[a]
Control (nonextracted)	71.3	23.0	198.43
17.3 MPa, 35°C	69.5	22.3	218.79
17.3 MPa, 50°C	70.1	19.7	209.77
24.2 MPa, 35°C	68.4	22.2	207.25
24.2 MPa, 50°C	60.3	22.9	191.28
31.1 MPa, 35°C	66.6	22.3	202.96
31.1 MPa, 50°C	66.3	23.4	201.04

[a]Dry weight basis.

objectives were to establish the efficiency of SFE conditions by determining the total lipid and cholesterol level of extracted muscle tissue and to measure selected chemical, physical and functional properties of SFE muscle tissue.

Samples of extracted pork were analyzed for composition and water-holding capacity and compared with nonextracted tissue. Changes in myofibrillar protein due to SFE was investigated in selected samples by SDS-PAGE electrophoresis. Results from a comparison of two extraction temperatures (35 and 50°C) and three extraction pressures (17.3, 24.2 and 31.1 MPa) did not result in a significant reduction in total lipid or cholesterol (Table 1). The fatty acid profile, however, indicated a slight reduction of fatty acids in meat residues which were extracted at 24.2 MPa and 35°C. The water-holding capacity of the non-extracted tissue was approximately 60% greater than the average of the extracted residues and demonstrated that this functional property was severely reduced by the SFE procedure. The electrophoresis of isolated myofibrillar proteins did not reveal differences between SFE-treated pork muscle and nonextracted tissue. Extractions of fresh ground pork with wide differences in composition were performed to determine if the initial concentration of fat and cholesterol affected the efficiency of SFE (Table 2). Under uniform extraction conditions, greater amounts of fat were removed when higher initial levels were present.

These results confirmed that SFE can indeed remove fat, but only materials with very high levels of fat (approximately 40%) had significant reductions (to 30%) in total lipid. The requirement that materials have high initial fat levels for significant fat reduction by SFE processing demonstrates the unlikely usefulness of SFE for fresh meats. Presumably other researchers have tried placing fresh product into an extraction vessel and empirically testing various temperature and pressure conditions, but the literature reviewed did not reveal successful extractions.

Dried Meats

Virtually all of the evidence that SFE can remove lipids from muscle foods is based on extractions of dried materials with moisture levels below 10% (Table 3). The studies by Hardardottir and Kinsella (1988) and King et al. (1989) were not designed for production of a usable meat residue but the intention was to maximize fat extraction for testing of pesticides and other residues. Objectives of the other studies (Table 3) were to generate low-fat food residues and to test extraction efficiency.

The research group at the University of Missouri has experimented on dried pork samples with an assortment of extraction conditions. An example of this work is presented in Table 4. Multiple replications of the SFE process under the same conditions were conducted to demonstrate the degree of reproducibility in the process. Relative to the non-extracted control, it is clear that the crude fat and cholesterol content was reduced by the SFE process. A high, but not perfect, degree of reproducibility was found in this phase of the project. Results for the crude fat content may be artificially low as evidenced by the fatty acid values at the lower portion of Table 4. Increased protein:lipid interactions resulting from the SFE process may have prevented the removal of some lipid when using the AOAC ether extraction method to determine crude fat content. It is suggested that a comparison between the fatty acid values is more accurate due to the higher degree of recovery in the gas chromatography technique. Clearly, there was a dramatic reduction in all types of

Table 2. Composition of Ground Pork Extracted with Supercritical Fluid CO_2 Under Constant Conditions[a].

Treatment	*Moisture* %	*Protein* % *dwb*[b]	*Crude Fat* % *dwb*[b]	*Cholesterol* *mg/100 g dwb*[b]
Low fat				
Nonextracted	65.5	56.5	41.7	245.5
Residue	62.8	58.9	39.9	242.5
Medium fat				
Nonextracted	59.5	43.5	55.3	185.7
Residue	59.0	50.2	48.7	188.8
High fat				
Nonextracted	46.5	25.2	75.0	159.9
Residue	51.8	37.8	61.2	166.8

[a]34.5 MPa, 40°C and 5 m^3 of CO_2.
[b]Dry weight basis.

Table 3. Comparison of SFE Efficiency for Fat Removal From Dried Muscle Foods and Egg Yolk.

Food	*Pressure, MPa*	*Temperature °C*	*% Fat Extracted*	*Moisture %*	*Sample Size, g*
Pork[a]	31.1	50	71.9	3.9	200
Beef powder[b]	30.4	45	66.9	2.5	
Beef chunks[b]	30.4	45	97.9	1.7	
Luncheon meat[c]	34.5	80	98.9	1.8	109.13
Imported ham[c]	34.5	80	97.3	2.3	241.19
Trout[d]	27.6	40	78.2	<2	5
Sardine meat powder[e]	25.4	40	57.4	33.0	—
Egg yolk[f]	31.0	45	33.9	6.7	115

[a]Clarke 1991.
[b]Froning 1991.
[c]King et al. 1989.
[d]Hardardottir & Kinsella 1988.
[e]Fujimoto et al. 1989.
[f] Froning et al. 1990.

fatty acids after SFE processing. Based on the average for SFE residue, the saturated fatty acids were reduced by 74.8% and the unsaturated fatty acids were reduced by 69.8% compared to non-extracted, freeze-dried samples. Overall, the fatty acids were reduced by over 71.9% when compared to the control sample. Cholesterol was similarly reduced by more than 78% to an average of 37.2 mg/100 g in the extracted meat residue. These results are most valuable for demonstrating the efficiency of SFE as a technique with potential to alter the composition of dried meats without the use of toxic organic solvents.

Using Entrainers or Cosolvents for SFE

Another University of Missouri experiment was used to test the feasibility for further fat or cholesterol reduction in freeze-dried pork tissue by using cosolvent extraction. A 3% level of ethanol (w/w) was incorporated into the carbon dioxide stream prior to supercritical pressurization by using a Milton Roy VS Minipump. Pressure of 31.1 MPa at 50°C and 116 g CO_2/g sample were evaluated as the extraction conditions. Table 5 has results for the effect of ethanol as a cosolvent during the SFE process. The extraction without cosolvent was most efficient for reducing the crude fat and cholesterol content relative to the initial sample. Although cosolvent extraction did reduce the fatty acid content of the dried pork when compared to the initial material, it resulted in a significantly higher fatty acid content than for samples extracted without the cosolvent. Cosolvent extraction favored removal of the cholesterol rather than the fatty acids, as shown by a 30% reduction of cholesterol compared to the samples extracted without ethanol.

Hardardottir and Kinsella (1988) also reported the effect of ethanol as a cosolvent with a 97.1% reduction of lipids (versus 78.2% shown in Table 3). The usefulness of cosolvent extraction is probably limited to analytical processing, since removal of cosolvent residues from biological materials is not easy and therefore defeats the idea for using carbon dioxide as a "safe" solvent.

Table 4. Proximate and Fatty Acid Composition of Freeze-Dried Pork Extracted[a] with Supercritical Carbon Dioxide.

	Control	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 4*
Crude protein, %	82.6	93.8	92.7	90.9	91.1
Crude fat, %	9.5	<0.1	<0.1	0.1	1.4
Ash, %	4.24	4.88	4.71	4.54	4.62
Moisture, %	4.5	3.4	3.4	4.6	4.2
Cholesterol, mg/100g	170	23.9	40.0	43.3	41.6
Fatty acids, mg/100g					
C14:0	83.1	10.5	11.3	7.1	13.6
C16:0	2402	308	629	608	720
C16:1	257	22.6	57.6	58.2	59.3
C18:0	1460	234	539	466	426
C18:1, oleic	4414	355	1693	1466	1340
C18:2	627	228	407	355	360
Total	**9243.1**	**1158.1**	**3336.9**	**2960.3**	**2918.9**

[a]31.1 MPa, 50°C.

Table 5. Effect of Ethanol as a Cosolvent for Supercritical Carbon Dioxide Extraction of Freeze-Dried Pork Longissimus Dorsi.

	Control	31.1 MPa No EtOH, 50°C	31.1 MPa 3% EtOH, 50°C
Crude protein, %	80.6	93.3	87.2
Crude fat, %	13.0	1.4	2.5
Moisture, %	3.7	3.3	8.0
Cholesterol, mg/100	208	33.4	22.6
Fatty acids, mg/100 g			
C14:0	136	n/d	n/d
C16:0	3245	275	1140
C16:1	397	n/d	n/d
C18:0	1815	244	839
C18:1	6105	372	1003
C18:2	1075	606	1890
C18:3	92	n/d	n/d

n/d = none detected.

Meat Products

One application where SFE processed muscle tissue is more likely to be accepted is in further processed products. If the extracted material has a desirable nutrient profile and the proteins remain highly functional, then the residue from SFE processing could find a role as an ingredient in processed meats. At present, the extraction process seems to be limited to dried residue and thus the vision of intact cholesterol-free steaks is certainly beyond practicality. Evaluations of products made with fresh pork subjected to SFE recently have been reported (Clarke et al., 1991a) and the gel strength of kamaboko with extracted sardine meat powder has been published (Fujimoto et al., 1989). To determine whether dried pork powder after SFE is of any value, the research group at the University of Missouri has begun testing the material in finely comminuted products modeled after a simple frankfurter formulation. Composition and textural qualities are the primary focus at this time.

In the Missouri experiments, the SFE pork (previously freeze-dried) was rehydrated with three parts of distilled water (RSFE) and used at 0%, 25%, 50% or 100% of the product formulation. The balance of the product was fresh ground lean pork. The mixture included appropriate levels of non-meat ingredients (salt, 2.6%; sodium erythorbate, 0.05%; and sodium nitrite, 0.015%) common to commercial frankfurters. The 100% RSFE product reflected a 76% reduction of both crude fat and cholesterol compared to 100% fresh pork. Each 400 g batch was chopped in a Cuisinart food processor for 2 min with 40 ml of ice water and the nonmeat ingredients. Polypropylene centrifuge tubes (O.D. 28.7X103 mm) were filled with approximately 50 g of each mixture (8 tubes/treatment) and then cooked in a 75°C water bath to an internal temperature of 70°C.

Results from the measurement of Warner-Bratzler shear force and hardness are presented in Table 6. Hardness was evaluated by penetration of 1 cm thick slices with an 8 mm cylindrical probe and by compression to 50% of original height. It was evident that shear force and product hardness declined as greater quantities of RSFE material were incorporated into the product, particularly when 50% or more was RSFE material. This suggested that the performance of the rehydrated meat powder in emulsion-type products was less than for unprocessed muscle tissue. An important point, however, is that the differences observed were not particularly large. Some modification of texture through the use of nonmeat binders or other ingredients might be tested to determine if RSFE products could compare more favorably to all meat items.

Summary

Awareness that SFE has potential as a new procedure in food and flavor processing has been heightened by recent articles in trade and research journals. The principle behind SFE as a process is that supercritical fluids have solvent properties that are between gases and liquids and therefore the supercritical fluids have solubility characteristics similar to liquids, but can penetrate material matrices with the ease of gases. By changing extracting conditions of temperature and/or pressure, selectivity of the solvent can be modified

Table 6. Means (g/cm^2) and Standard Deviations for Various Textural Measurements of Comminuted Pork Products Manufactured with Different Levels of Fresh and Extracted Pork.

	Trtmt. 1 100% Pork	Trtmt. 2 75:25 Pork:RSFE[a]	Trtmt. 3 50:50 Pork:RSFE	Trtmt. 4 100% RSFE
Warner Bratzler shear	172.3	159.4	126.7	121.8
	13.72	11.59	7.41	12.95
Penetration[b]	1183.5	1134.6	831.4	821.6
	92.28	87.49	62.63	101.65
Compression[c] (first peak)	1295.7	1205.3	1057.8	1128.6
	101.35	71.89	101.63	81.64
Compression[c] (second peak)	1014.5	951.6	800.2	849.4
	60.92	55.61	79.26	76.15

[a]RSFE = freeze-dried pork subjected to SFE at 31.1 MPa/50°C and rehydrated 3:1 water:pork.
[b]Cylindrical probe, 8mm diameter.
[c]Compression to 50% of sample height.

and thus extraction or fractionation of selected solutes may be controlled.

Research with SFE culminated with commercial applications for the decaffeination of coffee and the extraction of flavorings from hops over a decade ago. Several other foods have been tested in SFE systems, with the extraction of oils, flavors and pigments being the principal goals. Fractionation of oils or fats has also been investigated and results show some potential for this application. Recent studies with muscle foods indicate that extracted ingredients for further processed products with significantly lower fat and cholesterol levels may be produced by SFE.

In the future, more applications of SFE are expected to be developed. Commercialization of SFE for food processing may be somewhat slow due to absence of engineering data for scale-up or continuous processing. With further study and increasing knowledge, some of the limitations to commercial application of SFE may be overcome. Furthermore, concern over the use and disposal of organic solvents used in traditional extraction operations may accelerate interest in the use of supercritical carbon dioxide as an alternative solvent.

ACKNOWLEDGEMENTS

Research on supercritical fluid extraction at the University of Missouri has been supported by the National Live Stock and Meat Board, Missouri Beef Industry Council, Missouri Agricultural Experiment Station and the Missouri Research Assistance Act.

Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 11427.

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Discussion

D. Johnson: Can you address the effect on lipid stability of that product and also on its effect on microbial content?

A. Clarke: OK, Dwain. The question about the lipid stability, my particular testing, we have not looked at that. But speaking a little bit for Glenn's project, he looked at the beef chunks and beef powder. He took dehydrated meat powders and extracted those, and, if I recollect, his TBA values and sensory evaluations on those found a remarkably reasonable stability of the process. In terms of TBA values, at least, he was not finding a tremendous decrease in those over time, and from the sensory standpoint, they made up some broths with this meat powder and had an odor and flavor checking on that and found that they were not objectionable. The microbiological aspect—that's a good point. Again, we're holding this material at say 31° or 40° or 50°C for perhaps, again as I mentioned, 4 hours or so. That could perhaps be a problem. We'd like to look at that. The only key advantage I can see is that we're doing this under completely anaerobic conditions. Obviously, we've flooded the system with supercritical CO_2 and I wouldn't expect microbiological growth under those conditions.

Johnson: I guess I raise the question because there's some work at our university in the food science department where they're looking at supercritical fluid extraction of juices as a low-temperature sterilization technique. So I didn't know if that would have any application or not.

Clarke: It's certainly something worthy of looking at. We certainly would like to have a look at it because our Human Subjects Review Board would like us to be certain that the product is safe before we conduct sensory testing on the products. Those kind of results I don't have to present because we haven't been able to get that far.