Physics Research and Technology

Handbook on Supercritical Fluids

Fundamentals, Properties and Applications

Jane Osborne
Editor

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EDITOR

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PREFACE

Supercritical fluid carbon dioxide (sc-CO_2) possesses both gas-like and liquid-like properties. It is capable of depositing nanoparticles in small structures and poorly wettable substrates. Deposition and array formation of metal and metal sulfide nanoparticles on various substrates using sc-CO_2 as a medium has been a subject of considerable interest for researchers in nanomaterials area in recent years. This handbook begins by exploring nanoparticle deposition using supercritical fluid carbon dioxide. Further topics in this handbook include separation of oils using supercritical carbon dioxide; the application of an integrated supercritical extraction and impregnation process for incorporation of thyme extracts into different carriers; supercritical fluid extraction application on dairy products and by-products; and supercritical fluid technology applications in pharmaceutical drug formulations.

Chapter 1 – Supercritical fluid carbon dioxide (sc-CO_2) possesses both gas-like and liquid-like properties. It is capable of depositing nanoparticles in small structures and poorly wettable substrates. Deposition and array formation of metal and metal sulfide nanoparticles on various substrates using sc-CO_2 as a medium has been a subject of considerable interest for researchers in nanomaterials area in recent years. The deposition processes, taking advantages of unique properties of sc-CO_2 including its gas-like diffusivity, liquid-like solubility, near zero surface tension, and tunable solvation strength, offer enormous potential for developing nanoparticles-based new materials. This paper presents a review of sc-CO_2 techniques for deposition of metal and metal sulfide nanoparticles on different substrates including carbon nanotubes, silicon wafer, glass, and others. The paper starts with an introduction of supercritical fluid properties which is followed by nanoparticles synthesis, nanoparticles deposition techniques, and examples of applications. Supercritical fluid preparation of metal nanoparticle-carbon nanotube composite materials and their applications in chemical catalysis and fuel cell developments is reviewed. Deposition of metal nanoparticles in plastic beads and their applications as reusable catalysts for hydrogenation and hydrodechlorination reactions in sc-CO_2 are described. Supercritical fluid deposition of nanoparticles in nanostructures of silicon wafer and its implications in nano-devices fabrication is discussed. Recent reports on formation of uniform metal sulfide nanoparticle films in sc-CO_2 for energy transfer studies are summarized. Comparisons of sc-CO_2 deposition and conventional solvent deposition techniques for filling nanostructures and formation of uniform long-range nanoparticle films are also given in this review.

Chapter 2 – The electrospray process has been intensely studied for the effects of voltage applied, needle-target distance, polymer concentration, flow rate and so forth in order to prepare polymer samples with different morphologies from polymer solution. To the authors' best knowledge, these studies were conducted by electrospraying into air at ambient pressure. This study first reports the electrospray of poly(lactic-*co*-glycolic acid) (PLGA)-acetone solution into high-pressure carbon dioxide (CO_2) and CO_2-organic solvent mixture at different pressures. The morphologies of the collected samples are compared under different electrospray conditions, including ambient air, hexane, high-pressure CO_2, and CO_2-hexane mixture at the pressure of 400, 600, and 800 psi and the morphologies are in the form of films, mirocroporous particles, and solid particles. The formation of either polymer films or solid particles can be controlled by changing the system pressure when electrospraying PLGA solution into the CO_2-hexane mixture.

Chapter 3 – The main features of the separation of essential and fish-derived oils using supercritical carbon dioxide are described, with reference to the deterpenation of citrus essential oils and the production of omega-3 oils from fish oil ethyl esters. The state of the art of these two processes with high potential for industrial applications is reviewed, through a selection of experimental research works. A critical analysis of the experimental data available in the literature is carried out, with the aim of summarising the main findings and providing guidelines for process design. General aspects related to the process design of the separation of these oils using supercritical carbon dioxide are reported, with special focus dedicated to countercurrent multi-stage processes. In this regard, the methodologies for the selection of the operating parameters of the process are described, together with the procedures for determining the relationships between the number of theoretical stages, the reflux ratio and the solvent to feed ratio of the process. Besides isothermal columns with the enriching section fed by an external reflux of extract, some examples of separation processes with internal reflux generated by a temperature gradient inside the column are also discussed.

Chapter 4 – The main objective of this chapter is to review and highlight the main applications of Supercritical Carbon Dioxide (SCO_2) techniques in several fields such as food science, pharmaceuticals, chemical residues, biofuel, and polymers. In particular supercritical processes such as particle design, impregnation and extraction processes will be investigated. Supercritical Fluid Particle Design (SCFPD), through different processes, allows to increase the solubility and dissolution rate of poorly water-soluble drugs and to improve the molecular properties of Active Pharmaceutical Ingredients (API) and Drug Delivery System (DDS).

The chapter will be focused on the most recent developments of these processes covering the fundamental concept of SCO_2 and the principle of SCFPD processes that are typically used to control both particle size and polymorphic form of the treated substances. Extraction with supercritical fluids arises in the last three decades as a promising alternative to conventional analytical methods for lipid extraction, and currently has consolidated its application in extraction processes of raw materials of plant and animal origin. Its advantages, over conventional techniques, are many and well known, but this technology stands out mainly by environmental factors and products quality. The aspects related to the use of SCO_2 for extraction and fractionation of lipids, with special emphasis in triglycerols and fatty acids (in particular Polyunsaturated Fatty Acids – PUFA) will be investigated. A brief overview on the separation procedures involving supercritical technology, as supercritical fluid chromatography, will be also presented.

Chapter 5 – Polypropylene (PP) nanocomposites were prepared by melt intercalation in an intermeshing co-rotating twin-screw extruder with the assistance of supercritical CO_2 injection. The effect of molecular weight of PP-MA (maleic anhydride modified polypropylene) on clay dispersion and mechanical properties of nanocomposites were investigated. After injection molding, the tensile properties and impact strength were measured. The best overall mechanical properties were found for composites containing PP-MA with the highest molecular weight. The basal spacing of clay in the composites was measured by X-ray diffraction (XRD). Nano-scale morphology of samples was observed by transmission electron microscopy (TEM). The crystallization kinetics was measured by differential scanning calorimetry (DSC) and by optical microscopy at a fixed crystallization temperature. For well dispersed two-component system, PP-MA330k/clay, the crystallization kinetics and the spherulite size remained almost unchanged and the impact strength decreased with increasing the clay content. On the other hand, the intercalated three component system, PP/PP-MA330k/clay, containing some dispersed clay as well as the clay tactoids, showed much smaller size of spherulites and a slight increase in impact strength with increasing the clay content. The influence of supercritical CO_2 on mixing was evaluated together with the effect of initial melting temperature. Increasing initial melting temperature causes gradual decrease in bulk crystallization kinetics with exception of the 240-260°C temperature range for system without CO_2. Optical microscopy revealed large number of small spherulites for system without CO_2 after initial melting at 250°C. After 28 min of initial induction period of crystallization many small spherulites appeared in the vicinity of large spherulites for the system with CO_2 indicating beginning of homogenous nucleation. X-ray diffraction (XRD) and direct observation of the samples after tensile testing helped in comparison of dispersion of nanoclay for the systems with and without CO_2. Practical importance of this topic is demonstrated by the interest from Toyota Motor Corporation in this area for the applications in the automotive industry. When properly mixed, only small amount (1-3%) of nanoclay improves tremendously mechanical properties.

Chapter 6 – Near and supercritical fluids offer very interesting alternatives for separation processes, reaction medium and in the synthesis of new materials. Particularly, the applications of supercritical fluids (SCF) in the polymer industry are growing up in the last decades. These applications include polymerizations, properties modifications, processing, recycling, etc. In this sense, the use of SCF extraction is an interesting method to improve the separation of thermoplastic polymer blends. Some authors have studied the phase equilibria for polypropylene-hydrocarbon systems in terms of the effects of polymer solubility, solvent quality and polymer molecular weight. Also, the study of phase equilibria of a combination of solvents (system type: polymer-solvent A-solvent B) was carried out. The adding of a new solvent can improve or diminish the polymer solubility acting as co-solvent or antisolvent, respectively depending on the affinity.

In this chapter, the use of high pressure-high temperature n-alkanes for high molecular weight Polypropylene/Polystyrene (PP/PS) blends separation is presented. The selectivity of two solvents (n-pentane and n-heptane) on pure polymers, at high pressure and over a wide range of temperatures, is evaluated. Based on this study, a method for polymer blend demixing, physical and reactive ones, is proposed and the influence of the blend morphology and composition on the separation efficiency for the physical blend (PP/PS) it is discussed. Additionally, a diffusion model is proposed to describe the selective dissolution of a high molecular weight polymer, from an immiscible polymer blend. Also, a discussion of polymer mechanical degradation by the use of high pressure process is performed, analyzing the effects of temperature and concentration on chain scission. Finally, the knowledge of polyolefin solubilization in high pressure – high temperature n-alkanes, allows to design a new method to graft styrene onto commercial polyethylene (PE) by using near critical n-heptane as reaction medium and $AlCl_3$ as catalyst, with very good results.

Chapter 7 – Food industry is always looking for processes that can minimize the environmental impact, decrease toxic residues, use by-products more efficiently and also obtain high-quality products with good nutritional and organoleptic properties and preserved original ingredients.

Supercritical fluid extraction (SFE) has attracted considerable attention in recent years as a promising alternative to the conventional solvent extraction and mechanical pressing in food processing as it offers a number of advantages, including the absence of solvent residue and better retention of aromatic compounds. In the last few decades this powerful separation process has drawn an increasing interest in commercial application, particularly due to its technical and environmental advantages compared to the current classical extraction methods by organic solvents. Carbon dioxide (CO_2) as an environmentally-friendly solvent is mainly used as the extraction agent in SFE. Extracts obtained using CO_2 as the extraction solvent are solvent-free / without any trace of toxic extraction solvents, and are thereby highly valued.

SFE is still relatively new and is not widely used on the commercial scale for the extraction of edible oils. This is mainly due to very high investment costs of SFE equipment. But nowadays, according to global trends, "green" products and technologies are needed to replace conventional ones. When considering industrial application, it is essential to provide research on the fundamentals of the supercritical processes and to test the applicability of the appropriate model used for the scale-up of laboratory data to industrial design purposes.

This chapter will highlight the application of supercritical fluid technology in the extraction of edible oil from different seeds. A number of SFE applications in edible oil production will be reviewed. The different processes for production of edible oils (solvent extraction, supercritical CO_2 extraction and mechanical pressing) will be compared and evaluated in terms of obtained oil yield and from the environmental and economic points of view.

Chapter 8 – Fruits contain important molecules, which have pharmaceutical, cosmetic, chemical and food applications due to its valuable biologically active compounds, such as vitamins, antioxidants, carotenoids, flavonoids and minerals that can positively impact human health and well-being. Antioxidants in particular, have much interest because of its contribution for preventing diseases and for helping to prevent the damaging effects of oxidation on cells. Therefore, the extraction of fruit extracts containing such compounds is studied to improve the yield and quality of the extracts. According to this, the supercritical extraction processes are very attractive to obtain antioxidant-rich extracts with high yields and free of solvents. This chapter presents the design of supercritical processes to extract antioxidants including the microencapsulation to stabilize the antioxidant-rich extracts from fruits. All these processes are simulated to obtain the mass and energy balances. Then, a techno-economic assessment in the Colombian context is analyzed to predict the energy consumption and economic parameters such as total production cost per kg of antioxidant-rich extract, raw material cost, energy requirements and total cost of the extraction process.

Chapter 9 – Supercritical carbon dioxide ($scCO_2$) is known as a good solvent for a wide range of bioactive compounds from natural sources. Extraction of bioactive compounds from plant material using $scCO_2$ is a favorable technique for producing solvent-free extracts suitable for a wide range of applications in pharmaceutical, biomedical, cosmetic and food industries. Incorporation of drugs or other bioactive substances into polymeric matrices by conventional techniques is connected to some significant drawbacks (use of organic solvents, undesired substances reactions and/or degradation, low incorporation yields and heterogeneous dispersion). Supercritical solvent impregnation (SSI), and namely impregnation using $scCO_2$, has been proposed as an alternative methodology to overcome most of these problems. The main advantages of this technique are avoidance of organic solvents and possibility to work at relatively low operational temperatures and with hydrophobic drugs/substances which cannot be impregnated by aqueous solution/suspension soaking methods. Additionally, unique physical and transport properties of $scCO_2$ can be used simultaneously for impregnation of biopolymers and tailoring the chemistry and morphology thereof to obtain the desired microstructure of the final product.

The present chapter is particularly aimed to demonstrate feasibility of incorporating of thyme extracts into different solid matrices by using a methodology that combines the supercritical fluid extraction (SFE) and SSI. Compared to both processes to be conducted separately, the advantage of using one single integrated process is basically to save energy and time by avoiding an intermediate decompression procedure. Representative commercial carriers with relevance for pharmaceutical, biomedical and food applications (cotton gauze, polypropylene non-woven fabrics, chitosan and starch gels, cellulose acetate and polycaprolactone) were used to test feasibility of thyme extract incorporation by the coupled SFE-SSI process. By setting the relevant parameters of each process separately, the challenge of this methodology aims to harmonize these parameters in order to increase the extraction and impregnation efficiencies as well as to obtain an even distribution of the solute within the solid matrix.

Chapter 10 – The consumer trend and preferences towards healthier food choices increased the interest in new functional dairy products with a long shelf-life and in particular those with health benefits. There are a number of possible methods to meet these demands in search of better economies or enhanced functionality, as is the case in many dairy products with nutraceutical emphasis. Supercritical Fluid Extraction (SFE) is one such process, and it consists of separating one component (the extract) from another (the matrix) using supercritical fluids, most commonly carbon dioxide. This process uses the fluid dissolving power, which under specific conditions above its critical temperature and pressure results in different dissolving properties. Supercritical fluids have been used extensively during the last decades to modify different food products obtaining new ones. In this sense, some studies have been developed using SFE in dairy products and by-products. This chapter is a review to discuss what was done of applications of this technology in dairy foods and mainly in butter, cheese, cheese whey, whey cream and buttermilk. In cheese, the results can be as surprising as cheese lower in triglycerides and cholesterol and richer in phospholipids. Furthermore, this technology is potential for obtaining a high self-life product decreasing the microbial population. Applying SFE on buttermilk also is possible for obtaining concentrates of polar lipids, as phospholipids, from milk fat globule membrane (MFGM). Supercritical fluid extraction with CO_2 has great potential in the dairy industry and in commercial applications for offering functional food products and ingredients with a higher economic and bioactive value.

Chapter 11 – Biodegradable or partially biodegradable polymers have received ever-increasing attentions in the pharmaceutical and medical fields since these biomaterials can be utilized in the formation of composite microparticles tuned as coating agents in drug delivery devices, and the fabrication of porous scaffolds as temporary supports for cells that grow into new tissue before it is transplanted back to the host tissue. However, through current fabrication techniques, organic solvent's residues may remain in these polymers and then damage medical applications. To eliminate the involvement of organic solvent from the overall fabrication process, supercritical CO_2 may be used as the carrier solvent and swelling agent for processing polymers as it is free of residual organic solvent. In this study, the authors reported their work on supercritical carbon dioxide treatment of poly (lactic-co-glycolic acid) (PLGA) through with the rapid expansion of supercritical solution (RESS) process integrated with the supercritical pressure quench (SPQ) method. The samples thus obtained were characterized by using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). The particle formation through the RESS technique has been investigated under different extraction temperatures and pressures. Submicron spherical particles (0.1~0.3 micrometer) of PLGA were obtained and the morphology and size of the precipitated particles were significantly affected by extraction pressure and temperature. Simultaneously, porous scaffold sponges were successfully fabricated from fractionalized parent PLGA matrices by means of the SPQ method and the porous morphologies and structural features of the sponge samples in terms of the porosity, relative density, expansion factor and average pore size are observed to heavily depend on processing temperature and pressure. These porous sponges may be suitable for potential applications in bone tissue engineering.

Chapter 12 – Supercritical Fluid Technology has been documented as a successful process for the formation of submicron particles for the pharmaceutical drug delivery applications because it offers the possibility of controlling particle size, allowing them to be smaller, with a narrow size distribution. In addition, this process avoids thermal and chemical degradation of the product, allowing better particle stability. For instance, conventional processes for particle formation suffer limitations in producing a desirable end product. The milling reduces particle size between 10 to 50 μm with a wide particle size distribution, moreover this process generates high local temperatures that are likely to modify or even damage the bioactive molecules in the formulation. This approach reduces product development times, lowering manufacturing costs while using green chemistry technology. In this chapter a variety of supercritical fluid techniques relevant to drug formulation and delivery will be reviewed, with recent advances and novel applications being discussed, including progress of their industrialization in the pharmaceutical manufacturing environment with process scaled-up under current good manufacturing practices specification.

Chapter 13 – Supercritical CO_2 microemulsions have attracted much interest with the development of green chemistry in recent years and have a wide range of applications in areas such as chemical reaction, material synthesis, and extraction. This chapter reviews recent advances in the supercritical CO_2 microemulsions, and pays more attention to phase behavior of supercritical CO_2 microemulsions with different surfactants. The applications of these microemulsions in selective solubilizing polyols from its dilute aqueous solution in fermentation broth are also discussed. The results may provide useful thermodynamics data for industrial design and a feasible basis and practical guidance for the further research on the selective extraction of polyalcohol in biotechnology and helpful for industrialization.

Chapter 14 – Solid polymer electrolytes (SPEs) have attracted much interests recently as next-generation battery materials because they are safe, flexible and light-weight when compared with current liquid solution-based electrolytes. To achieve sufficient ionic conductivity of SPEs, the authors have been focusing on supercritical carbon dioxide ($scCO_2$) as a treatment medium for them. They have found that $scCO_2$ is able to decrease the glass-transition temperature (T_g) of the polymer matrices of SPEs by its plasticizing effect and significantly improve the ionic conductivity. Raman spectrometry has revealed that $scCO_2$ treatment reduces the number of aggregated ionic species and increase the number of carrier ion. In addition, it was demonstrated that the amount of dissolved CO_2 and ionic conductivity increased with increasing CO_2 pressure and showed high value particularly in supercritical condition, and anionic species with F atom seemed suitable due to their good compatibility with CO_2 molecules. Furthermore, the authors tried to improve the dispersibility of clays as inorganic fillers in the polyether-based electrolytes by using $scCO_2$ treatment and succeeded in improving the ionic conductivity.

Chapter 15 – Supercritical carbon dioxide (SC-CO_2) jet fracturing is expected to be an efficient technique for developing the unconventional oil and gas. In this chapter, a computational fluid dynamics (CFD) model was formulated based on Span-Wagner equation of state to simulate the flow field in the perforation cavity during SC-CO_2 jet fracturing.

The results indicated that in comparison with water jet, SC-CO_2 jet had higher velocity and longer high-speed jet region under the same conditions; the pressure boosting effect of SC-CO_2 jet fracturing was stronger than that of water jet fracturing. SC-CO_2 jet would generate noticeable Joule-Thomson effect which would decrease the fluid temperature rapidly. During SC-CO_2 jet fracturing, SC-CO_2 density decreased firstly, and then increased with the growth of jet, and SC-CO_2 viscosity was much less than water viscosity. The characteristics of flow field in perforation cavity during SC-CO_2 jet fracturing were obtained in this study, which provided theoretical basis for the research and applications of this technique.

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Chapter 10

SUPERCRITICAL FLUID EXTRACTION APPLICATION ON DAIRY PRODUCTS AND BY-PRODUCTS

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ABSTRACT

The consumer trend and preferences towards healthier food choices increased the interest in new functional dairy products with a long shelf-life and in particular those with health benefits. There are a number of possible methods to meet these demands in search of better economies or enhanced functionality, as is the case in many dairy products with nutraceutical emphasis. Supercritical Fluid Extraction (SFE) is one such process, and it consists of separating one component (the extract) from another (the matrix) using supercritical fluids, most commonly carbon dioxide. This process uses the fluid dissolving power, which under specific conditions above its critical temperature and pressure results in different dissolving properties. Supercritical fluids have been used extensively during the last decades to modify different food products obtaining new ones. In this sense, some studies have been developed using SFE in dairy products and by-products. This chapter is a review to discuss what was done of applications of this technology in dairy foods and mainly in butter, cheese, cheese whey, whey cream and buttermilk. In cheese, the results can be as surprising as cheese lower in triglycerides and cholesterol and richer in phospholipids. Furthermore, this technology is potential for obtaining a high self-life product decreasing the microbial population. Applying SFE on buttermilk also is possible for obtaining concentrates of polar lipids, as phospholipids, from milk fat globule membrane (MFGM). Supercritical fluid extraction with CO_2 has

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great potential in the dairy industry and in commercial applications for offering functional food products and ingredients with a higher economic and bioactive value.

INTRODUCTION

The application of supercritical CO_2 (SC-CO_2) extraction in dairy products ranges from flavor compound extraction from cheese [1, 2], lactones [3] and cholesterol extraction from milk fat [4] to fractionation of milk fat [5], concentration of polar MFGM lipids from buttermilk, whey cream, and whey buttermilk [6-8] and determination of fat soluble vitamins in milk powder [9] and in cheese [10]. Most interesting for the food and dairy industries is the ability of SC-CO2 to dissolve triglycerides and non-polar components, and through its flow, to remove them from the food matrix. However, this lipid extraction is very different in its results to the more common solvent extraction.

Lipids are considered to be hydrophobic compounds. A high diversity of lipids exists due to the different combinations of hydrophobic acyl chains, and the combination with phosphoric acid or carbohydrates, which in turn can vary their polarity. Based on this high diversity, lipids can be classified into: 1) neutral lipids without polar groups, as tri-, di- and mono-glycerides, fatty acids, and sterols, and 2) polar or complex lipids containing polar groups, as in the case of phospholipids, sphingolipids, ceramides, glycolipids and others.

Carbon dioxide used as supercritical fluid is considered as a "green solvent" and most used substance in supercritical fluid extraction. It is well known that CO_2 is a selective polar component, non-corrosive, non-flammable, non-toxic, and gaseous at ambient pressure and temperature. For this reason, and because its critical temperature and pressure are mild (31°C and 7.3 MPa), this makes it very suitable to be used in thermo sensitive foods.

Supercritical fluid extraction methods with CO_2 is preferred over conventional extraction procedures because of the resulting products are free from organic solvent residues, and there is a minimal risk of thermal oxidation. SFE can also offer many benefits achieved by using other high pressure and carbon dioxide processing practices. Moreover, SC-CO_2 has been shown to successfully kill a wide range of microbes, vegetative cells, and spores, as well as inactivating deleterious enzymes in certain food products [11]. Carbon dioxide processing is also known to displace oxygen, reduce pH, and penetrate cells rapidly.

The solvent power of supercritical fluids is dependent on density and can be finely adjusted by changing the temperature or pressure. A small increase in pressure or decrease in temperature will increase fluid density [12, 13]. This is the attraction of using supercritical fluids as a solvent. Supercritical fluids take advantage of the solvent power near the critical point. At the critical point, gas and liquid phases merge to form a supercritical phase [13]. No phase boundary is crossed as both the critical temperature and critical pressure is exceeded [14]. This phase has both, liquid and gas like properties but is neither a gas nor a liquid. In addition supercritical fluids have a higher diffusing coefficient, lower viscosity and surface tension than a liquid solvent which contributes to a more favorable mass transfer [12]. The density of supercritical fluids is 100-1000 times greater than the density of gases. Therefore, molecular interactions are much stronger [15]. However, an alternative phenomenon has been discovered close to the critical point that does not follow these general observations. At low pressures it has been noted that an increase in temperature actually lowers solubility.

This is due to the fact that an increase in solute vapor pressure is not enough to compensate for the reduction in solvent density [16].

SC-CO_2 cannot extract complex lipids unless an organic co-solvent or "modifier" is also used, and ethanol is the most commonly used as a modifier to CO_2. Among the other near- and supercritical fluids that could be considered, dimethyl ether (DME) is a strong solvent for both, neutral and polar lipids and has a wide range of potential use in food, pharmaceutical, and cosmetic applications. DME is non-toxic, non-reactive, and does not cause a pH change in the aqueous solution. A polar lipid extract could also be produced from spray dried powder by extracting first with DME to obtain a mixed neutral/complex lipid extract, then re-extracting the lipid extract with CO_2 to remove neutral lipids [17].

There are additional gases that can be used for supercritical extraction, listed in the table below. However, carbon dioxide offers a practical advantage over other gasses in industrialized applications [18] (Table 1).

Numerous studies have evaluated the impact of extraction variables on the yield, recovery, and composition of the extracted oils from various sources [19]. The extraction variables are temperature, pressure, particle size and moisture content of feed material, extraction time, CO_2 flow rate and solvent-to-feed ratio.

In general, extraction yield increases with pressure due to a rise in lipid solubility in SC-CO_2 based on an increase in CO_2 density.

The impact of temperature is dependent on competing parameters; the CO_2 density decreases with temperature while the vapor pressure of solutes increases.

This review chapter is centered on the application of SFE with CO_2 with a major focus on dairy products and by-products. Many works exist in the literature about this specific theme, however in this chapter review, the most relevant or important studies of supercritical fluid CO_2 applied on milk, cheese, butter and whey from different origins are detailed. Also, as mentioned in the previous section, the potential microbial inactivation due to de SC-CO_2 on dairy products will be also addressed.

MICROBIAL REDUCTION USING SC-CO_2

Much attention has been paid to supercritical fluid pasteurization of food products, with a special consideration dedicated to inactivation of highly heat, radiation and chemical agents resistant spores [20].

Table 1. Potential gases used for near-critical fluid extraction [18]

Name	Formula	T_c (°C)	P_c (Mpa)
Carbon dioxide	CO_2	31.1	7.38
Nitrous oxide	N_2O	36.4	7.15
Ammonia	NH_3	132.4	111.3
Ethane	$C2H_6$	32.2	48.2
Propane	C_3H_8	96.6	41.9
Ethylene	C_2H_4	9.2	49.7
Freon 13	$CCOF_3$	28.9	38.7

As clearly shown by several authors [21-23], cell decay is considerably increased when CO_2 pressure is raised beyond the critical pressure, boosting both fluid dissolution inside cell and membrane lipids interaction.

Carbon dioxide can be used to achieve microbial reduction in foods without use of high temperatures, being an attractive processing alternative over currently used thermal processes. Hong and Pyun [24] and Isenschmid et al. [23] observed that *Lactobacillus plantarum* and yeast cells exposed to subcritical CO_2 (30°C) released their intracellular ions and as well cells were nonviable but remained intact.

Werner and Hotchkiss [25] compared the antimicrobial effectiveness of subcritical and supercritical CO_2 applied in a continuous flow system on indigenous psychrotrophic vegetative cells. They evaluated pressures between 10.3 and 48.3 MPa; temperatures of 15, 30, 35 and 40°C; and CO_2 concentrations of 0, 3, 66 and 132 g/Kg of milk. When comparing the subcritical and supercritical CO_2 treatments with control samples, a number of total viable cells were reduced significantly. For both aged and inoculated milks, CO_2 had a greater overall lethal effect at 132 g/kg than at 66 g/kg and a greater effect at 35 than at 30°C. Ishikawa et al. [26] found no real effect of pressure increase (increasing pressure did enhance lethality) on viability of yeast and lactic acid bacteria treated at 25°C with CO_2 at pressures of 8, 15, 20, or 25 MPa; at 35°C, above the critical temperature of CO_2.

The most important mechanisms proposed for this lethality are related to alteration of cell membrane and to its internal metabolism [27]; cell wall rupture or perforation because of the interaction of supercritical fluid with the lipids [27, 28]; and enzyme inactivation due to the pH decrease inside the cell or lipid extraction [28-30].

Spilimbergo et al. [11] reported a progressive permeability of the cell during treatment at 10 MPa and 36°C. Their data evidenced a correlation between cellular death and CO_2 permeabilization inside the cell. They reported that the initial damage to the cellular envelope was not lethal for the cell. After approximately 4 min of treatment, the fraction of surviving cells was still nearly 100%, but after 10 min, the treatment induced irreversible damage that ultimately caused cell death. The amount of CO_2 accumulated in the lipid phase may then structurally and functionally disrupt the cell membrane because of an order loss of the lipid chain, which may increase the fluidity [23].

Werner and Hotchkiss [25] also analyzed the influence of CO_2-to-product ratio and pressure (Figure 1). They observed that milk carbonated to 132 g/kg of CO_2 resulted in a higher cfu/g reduction at 48.3 MPa than at 24.1 MPa. At the same time, they reported that milk treated with 3g/Kg of CO_2 resulted in a lower reduction than those milks treated with 132 g/Kg of CO_2; this result was very similar to the milk achieved in similar temperature and pressure without CO_2 addition.

These results suggest that CO_2 was present below threshold lethal levels and that effects were due to combined influences of temperature and pressures [25].

After the intensive review made by Perrut [20], it is emphasized that pressure, temperature and treatment duration are the basic parameters controlling microorganism survival rate. The author concluded that:

- Temperature below 40ºC decreases bacterial reduction.
- A medium-pressure range (8-15 MPa) seems adequate, because a very high pressure (> 20 MPa) does not lead to significant improvement.

- Survival rate versus exposure time: the final efficacy ranges between few minutes to few hours, depending on strains, matrixes, temperatures and pressures.
- Presence of water drastically increases the bactericidal effect of CO_2, with a strong and irreversible effect on the most enzymes.

FLUID MILK

By definition, casein protein in milk is the protein that is precipitated out at the isoelectric point of pH 4. When CO_2 dissolves in aqueous solution, it reacts with water to form carbonic acid. If enough CO_2 is incorporated into the milk to reduce the pH to 4.6, the casein will precipitate out. Cheeses made from CO_2 treated milk have been shown improved renneting characteristics and slightly higher cheese yields [31].

However, if milk with partially precipitated protein is used, probably curd would not be satisfactorily firm, and consequently higher loss of the curd in whey will exist.

However whey proteins are more resistant to the effects of high pressure CO_2 than caseins. Experiments on skim milk found that at all processing parameters investigated, whey proteins were not precipitated [32], on contrary, caseins were readily precipitated under the same conditions. The mildest treatment (2 MPa, 30 min, 40ºC) precipitated 35% of the caseins, while the most severe treatment (5 MPa, 180 min, 40ºC) precipitated 87% by weight.

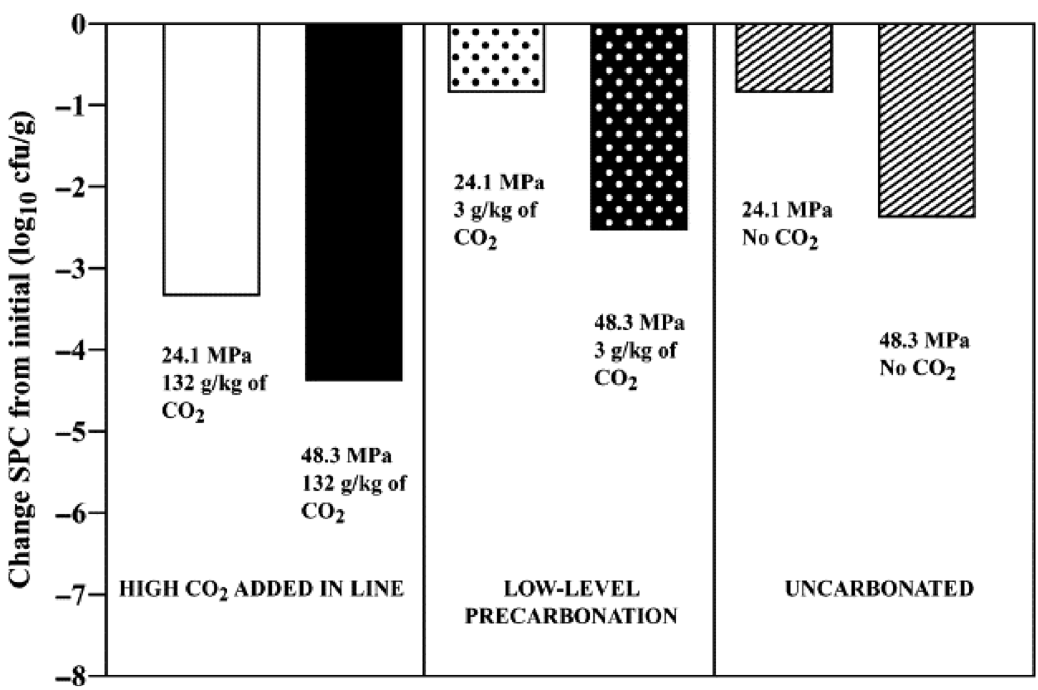


Figure 1. Change in standard plate count (SPC) from initial count ($\log_{10}$ cfu/g) of aged raw skim milk, carbonated inline to 132 g/kg, precarbonated to 3 g/kg, or uncarbonated; and treated at 40°C and 24.1 or 48.3 MPa [25].

Jordan et al. [33] have studied the ability of CO_2 to precipitate caseins from skim milk. The authors found that at 3.5 MPa and 50ºC, CO_2 was able to precipitate 99% of the caseins. When a range of pressures was used, these authors found that the pressures required for casein precipitation decreased as the temperature increased.

Although the lowest temperature investigated was 40°C, the trend of the data implies that processing at lower temperatures may avoid precipitation. If under the previous conditions (subcritical CO_2) caseins are precipitated, it is considered that when SC-CO_2 is used (above 7.1 MPa) caseins will also precipitate.

Aldo Tisi [34] evaluated the application of dense phase of CO_2 (subcritical and supercritical conditions) between 7 and 62 MPa and 15º to 40ºC on fluid milk. This author observed that the pH of milk samples dropped to approximately 5.9, regardless of treatment. After degassing, pH of all samples raised again to its original value in the control milk sample (Figure 2).

The pH values are, in this case, sufficiently high to avoid the caseins precipitation. However, in this study, the author described that when whole milk was used, at the lower temperatures, big particles (> 100 µm) very similar to the butter grains appeared, probably due to milk fat globules coalescence. On the other hand, when skim milk was used, a different particle size distribution occurred, which could be due to a partial casein aggregation.

COW AND GOAT CHEESE

Lipids are vital components of our diet; however, consumption of low fat products is positively correlated with a better cardiovascular health [35]. On the other hand, consumers desire to purchase delicious, convenient, and nutritious food. There are numerous studies in the literature about the drawbacks of low-fat cheese.

As described by Sánchez-Macías et al. [36], low-fat cheeses characteristics are: too compact matrix, abnormal instrumental texture and color characteristics, different proteolysis and lipolysis rate, and unnatural and unappealing perception by consumers.

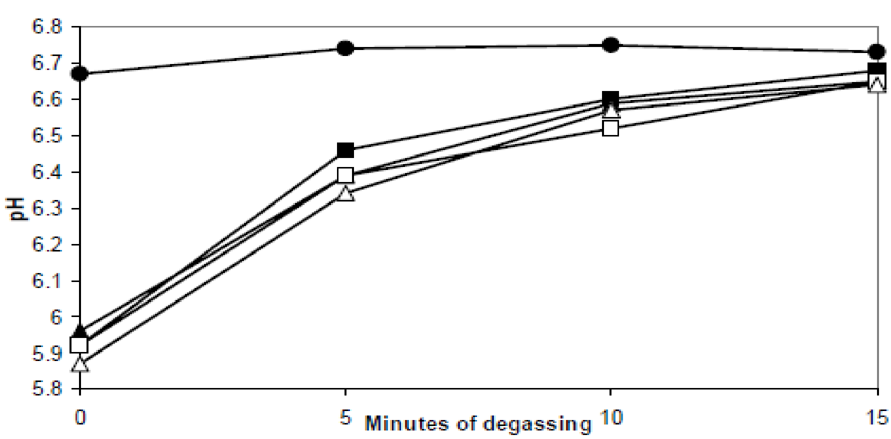


Figure 2. Mean pH of whole milk after in-line treatment at 7 MPa 15ºC (empty square), 7 MPa 40ºC (empty triangle), 62 MPa 15ºC (solid square), 62 MPa 40ºC (solid triangle) and untreated control (solid circle) after 15 minutes of degassing [34].

Many works described in the literature have tried to modify or improve low-fat cheese using several methods such as those resumed by Sánchez-Macías et al. [36]: modification of cheese-making procedures, inclusion of additives and fat replacers, and the use of starter strains that produce exopolysaccharides.

One other approach is to manufacture a full-fat cheese and then remove the fat from the product. In 2007, Yee et al. [37] firstly used the SFE technology as an alternative method in low-fat cheese processing. They developed lower fat Cheddar and Parmesan grated cheeses using SFE and characterized their flavor profile in comparison to their full-fat counterparts. Trials were carried out combining pressures (20 or 35 MPa), temperatures (35 or 40ºC), and CO_2 levels (500 or 1000 g). They observed that the lipid removal was efficient for Cheddar cheese at 20 MPa and 35ºC, while it was better at 35 MPa and 40ºC for Parmensan cheese, both with 1000 g/Kg of CO_2.

Recently, in 2013, Sánchez-Macías et al. [36] first applied the SFE with CO_2 on artisanal goat cheese (Majorero) and on Gouda-type goat cheese. They used a total of 1000 g of CO_2 at 35ºC in each trial, and pressure was the independent variable: 10, 20, 30 or 40 MPa. An increased fat reduction in cheese was observed as pressure increased for the artisan cheese (50-57%), but no differences were found for the fat extraction in Gouda-type goat cheese among the programmed pressures (48-55%). Yee et al. [38] observed that under the same SFE conditions, the efficiency of fat extraction depends on the cheese matrix.

It has been reported by different authors [36, 38] that the moisture content was reduced around 8-12% after SC-CO_2 treatment during 55 min at 35ºC. This moisture loss is independent of the pressure, and perhaps depends on the exposure time to the dynamic flow of SC-CO_2. The lower moisture content seems to give to the cheese the sensation of a more mature cheese, but with the same or higher flavor. Yee et al. [37] also found that SFE treated cheese retain flavor compounds that may not exist typically when cheeses are elaborated with low-fat milk.

Yee et al. [37] and Sánchez-Macías et al. [36], after thin layer chromatography analyses, found only nonpolar lipids (triacylglycerides, free fatty acids and cholesterol) in the recovered lipids extracted from cheeses by SFE; indicating that polar lipids such as phospholipids are retained in the cheese matrix. Sánchez-Macías et al. [36] observed that artisanal goat cheese had higher polar lipid content than the Gouda-type goat cheese (Figure 3).

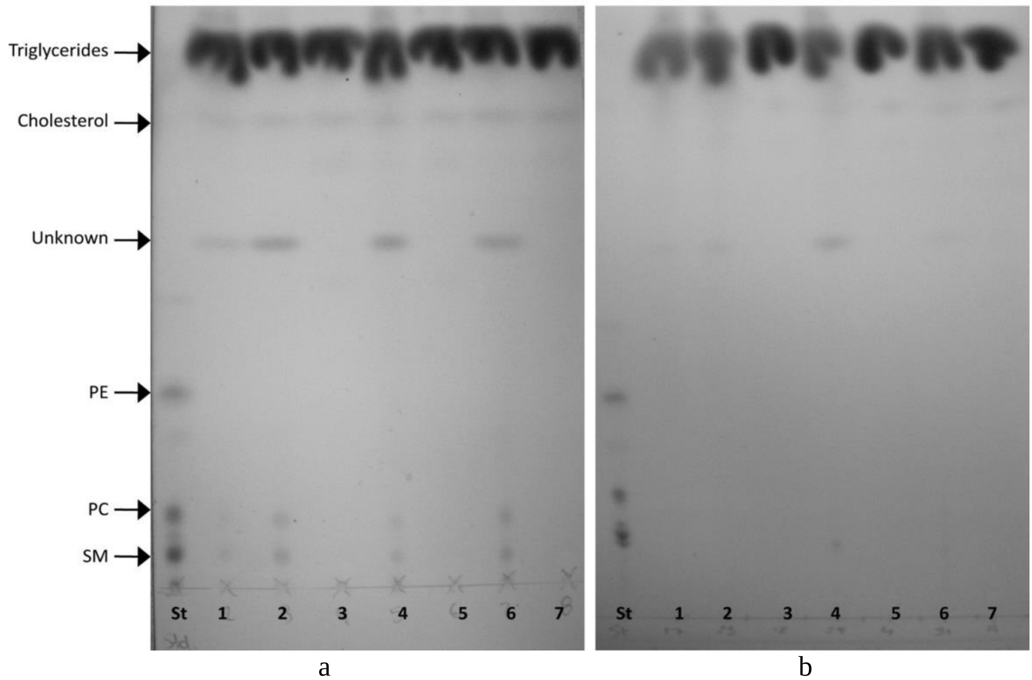
The obtained results could be explained by the Gouda cheese-making procedure. After curd cutting, temperature is increased for heating the grains, which induces the formation of fat globules aggregates and then, pressing of the curd results in a greater disruption of their membranes, including the coalescence and formation of nonglobular fat [39]. This fact was confirmed by microstructure imaging by confocal laser scanning microscope (Figure 4) of the cheese matrix of artisan and Gouda-type cheese [36].

In these micrographs, fat in artisanal goat cheese (Majorero) seems to be surrounded and limited, probably by the fat globule membrane, rich in polar lipids.

On the other hand, in Gouda-type cheese, fat appears as nonglobular or free, maybe because of the membrane losses during cheese-making.

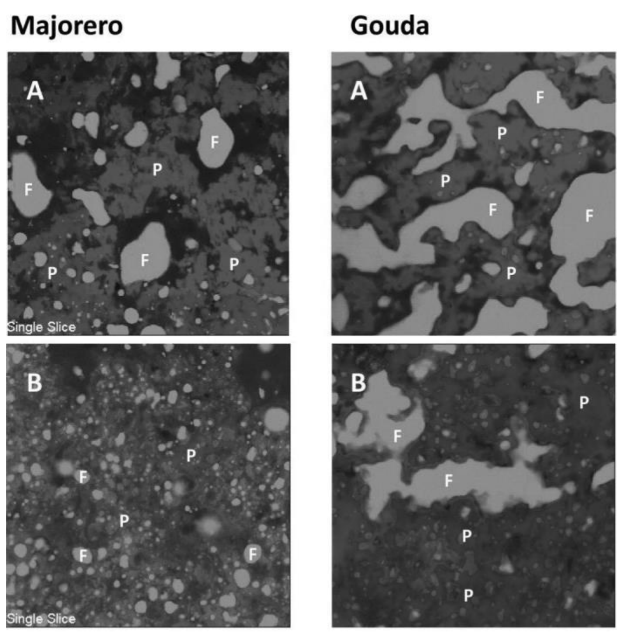
The more open matrix and whey pockets found in the artisanal goat cheese could result from the greater amount of fat extracted when comparing with the Gouda-type goat cheese.

After SFE with CO_2, and because of the selective extraction of nonpolar lipids, it was found that phospholipids were concentrated in cheese [36, 38], as shown in the Figure 5.



St = standard; PE = phosphatidylethanolamine; PC = phosphatidylcholine; SM = sphingomyelin [36].

Figure 3. Thin layer chromatography plate showing the polar lipid profile of (a) Majorero and (b) Gouda-type cheeses. Control cheese (lane 1) and cheese treated with supercritical CO_2 at 10 (lane 2), 30 (lane 4), and 40 × 106 Pa of pressure (lane 6). Residual extracted fat from cheese treated with supercritical CO_2 at 10 (lane 3), 30 (lane 5), and 40×10^6 Pa of pressure (lane 7).

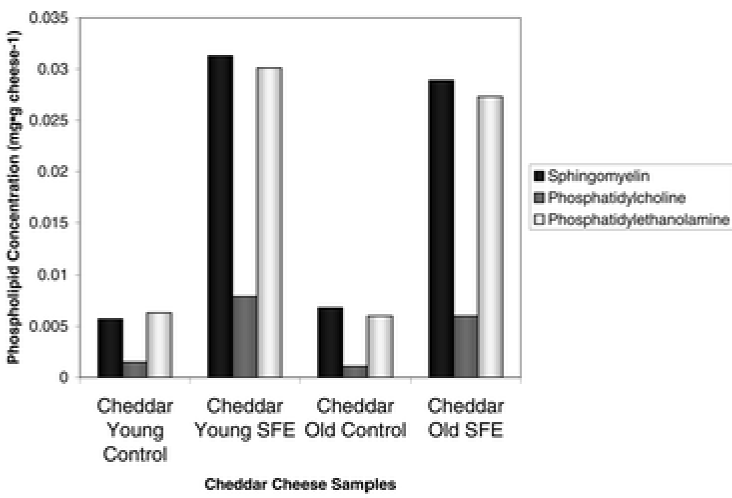


F = Fat; P = Protein; the serum phase is black [36].

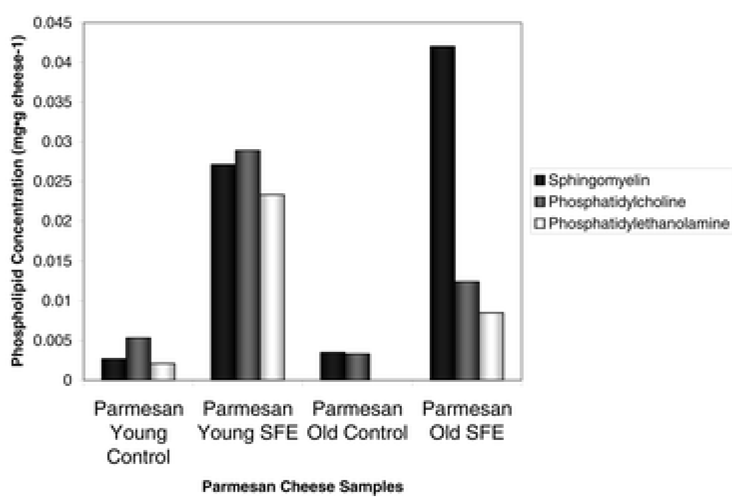
Figure 4. Confocal laser scanning micrographs of artisanal goat cheese (Majorero) and goat Gouda-type control cheese (A), and cheese treated with supercritical fluid at 10 MPa (B).

However, because in Gouda-type goat cheese a membrane globule disruption occurs, the level of phospholipids is lower in control cheese, but higher after SFE with CO_2.

Supercritical fluids are shown to have the ability to kill the most of microorganisms and to inactivate viruses. They have been used as an alternative method for treating heat-sensitive products, compounds reacting with sterilizing chemicals or radiolysis of biomolecules. Only one study exists in the literature about using supercritical fluid extraction in cheese and its effects on cheese microbiology [36]. The lethality found for the artisanal goat cheese was higher (from 1.5 to 5 logs of reduction) than in gouda-type cheese (from 2 to 3 logs), probably because of the more open matrix and the higher counts in the first cheese (Table 2). Moreover, in the artisanal goat cheese, the reduction of *Lactobacillus* and *Lactococcus* counts was higher as pressure increased, while there were no differences in microbial counts in Gouda-type cheese due to differing pressure.



a



b

Figure 5. Average phospholipid concentration of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine in (a) Cheddar cheese samples and (b) Parmesan cheese samples determined by thin layer chromatography polar plates [38].

These results are disagreement with the conclusion of Perrut [20], who explains that over 20 MPa of pressure does not improve the microbial reduction. Cheese matrix plays an important role in this case.

As conclusion of this section, the level of fat extraction is dependent on cheese type and SFE parameters for different cheese varieties. The parameters would need to be optimized and further researched to determine fat extraction efficiencies.

It has been shown that the SFE process is a relatively quick and easy method to reduce nonpolar lipids and further concentrate polar lipids.

Butter and Anhydrous Milk Fat

The wide melting range of milk fat makes it unsuitable for a number of food applications. Fractionation of milk fat into oil, plastic and solid fat fractions with different chemical and physical characteristics provide more specified milk fat products [40].

Currently, the fractionation of milk fat is more feasible as a method for improving technological functionality of milk fat than for improving its nutritional properties [40]. For example, high melting fat fractions are usually used for ice cream, while low melting fat fractions are suitable for spreadable butter, bakery products or reconstitutable milk powder. During the dry-melt fractionation process, unsaturated fatty acids and short-chain saturated fatty acids are concentrated in liquid olein fractions however, flavoring compounds, pigments, cholesterol, vitamin A, and long-chain saturated fatty acids are enriched in solid stearin fractions [41].

Nevertheless, not only products functionality is important when fractionate milk fat, but also nutritional values. The use of milk fat fractions with decreased concentrations of saturated fatty acids may improve the nutritional properties of dairy products.

For example, Abd El-Aziz [42] showed that the higher content of unsaturated fatty acids in the low-melting milk fat fractions was related directly to the oxidative instability.

Table 2. Effects of different SFE treatments on the total aerobic, lactococci and lactobacilli counts (log cfu) [36]

	Treatment[1]						
Item	Control	CI	P10	P20	P30	P40	SEM
Majorero cheese							
Aerobic bacteria	5.38^{a}	5.06^{ab}	3.93^{b}	3.24^{b}	2.66^{b}	3.28^{b}	0.48
Lactococci	9.28^{a}	8.78^{a}	7.43^{b}	7.22^{b}	6.97^{b}	4.27^{c}	0.55
Lactobacilli	8.70^{a}	8.53^{a}	6.19^{b}	5.65^{bc}	5.19^{c}	4.39^{d}	0.22
Goat Gouda-type cheese							
Aerobic bacteria	6.15^{a}	6.09^{a}	2.96^{c}	3.47^{bc}	3.60^{b}	3.55^{b}	0.19
Lactococci	5.53^{a}	5.46^{a}	2.73^{c}	3.17^{bc}	3.27^{b}	3.52^{b}	0.18
Lactobacilli	—	—	—	—	—	—	—

$^{a\text{-}d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Control = cheese at 4°C; CI = cheese in incubator at 35°C for 50 min; P10, P20, P30, and P40 = cheese treated with 1,000 g of supercritical CO2 at 35°C for 50 min at 10, 20, 30, and 40 × 106 Pa of pressure, respectively.

Romero et al. [43] used SC-CO_2 extraction to concentrate conjugated linoleic acid from anhydrous milk fat. They proposed that the high melting fraction of milk fat obtained by supercritical fluid extraction might be beneficial for application in dairy products improving the nutritional value, which may be attributed to its high concentrations of CLA, β-carotene and unsaturated fatty acids.

Fatouh et al. [5] used a multi-step supercritical carbon dioxide extraction procedure for fractionation of buffalo butter oil. Four fractions were obtained by sequentially increasing the pressure and temperature: pressure of 10.9 and 15 MPa at 50ºC and pressure of 22.3 and 40.1 MPa at 70ºC. The authors observed that cholesterol, short-chain fatty acids and saturated fatty acids were enriched in the first fractions obtained at lower pressures, while long-chain fatty acids and unsaturated fatty acids were enriched in the later fractions.

Moreover, fractions obtained in the initial stages of the fractionation exhibited lower melting behavior than that of those obtained in the late stages (Table 3).

In this way, the problem of lipid oxidation in butter described by Romero et al. [43] is resolved with SFE with CO_2, because the low melting fraction is now lower in unsaturated fatty acids.

WHEY PROTEINS

Whey proteins are commonly used in the food industry as an ingredient [44] because of their functionalities. They enhanced nutritional value and physical functionality. Whey proteins are also used as a protective barrier coating to increase the shelf-life and quality of different products [45, 46].

Table 3. Solid fat content (g/100g) of buffalo butter oil (BO) and its fractions (F1-F4)[1] [5]

Temperature (°C)	BO	F_1	F_2	F_3	F_4
0	41.7±1.4^c	21.5±1.5^e	34.0±1.9^d	68.8±3.3^b	87.7±3.1^a
5	34.6±0.6^c	11.6±0.9^e	27.9±0.8^d	61.2±1.5^b	83.6±2.6^a
10	28.0±1.4^c	3.8±0.4^e	21.0±1.4^d	55.7±2.2^b	76.1±1.9^a
15	18.6±2.4^c	0.0	14.7±0.3^d	48.9±1.4^b	69.0±1.8^a
20	11.9±3.6^c	–	6.9±0.5^d	37.6±2.3^b	62.3±0.6^a
25	9.6±1.3^c	–	2.4±0.1^d	31.9±2.5^b	58.7±2.1^a
30	3.3±1.3^c	–	0.0	25.8±1.3^b	46.8±1.5^a
35	1.4±0.7^c	–	–	11.2±0.9^b	39.8±0.7^a
40	0.0	–	–	6.3±0.2^b	31.7±2.3^a
45	–	–	–	4.2±0.1^b	26.8±1.9^a
50	–	–	–	0.0	12.5±0.4
55	–	–	–	–	7.4±0.3
60	–	–	–	–	0.0

[1]Fractions were obtained at different temperature and pressure conditions of supercritical carbon dioxide. F_1 at 50°C/10.9 MPa; F_2 at 50°C/15.0 MPa; F_3 at 70°C/22.3 MPa; F_4 at 70 ºC/40.1 MPa.

[a-e]Different letters within the same row are significantly different ($P < 0.05$).

Whey protein functional properties are as follow: solubility, water holding capacity, structure formation in dairy and meat products, the ability to form and stabilize oil-water (in emulsions) and air-water (in foams) interfaces, and the ability to remain in suspension during thermal processing [47]. The functional properties of food proteins are closely related to their structure [48]. Zhong and Jin [49] described the effect of SC-CO_2 treatment on the rheological properties of whey protein, and inferred that conformational, structure and functionalities of whey protein isolate were modified after SC-CO_2 processing. They reported that when whey protein isolate or whey protein concentrate was treated with SC-CO_2, moisture and lipid content of the protein powder granules decreased. In the late product, lipid content decreased when a treatment above 30 MPa of pressure was applied. These authors found that after the SC-CO_2 treatment at 65ºC and 30 MPa of pressure, the whey protein isolate and concentrate appeared as fine powder compared with the untreated whey proteins. The panelist reported that the treatments that lowered lipid content might also reduce the chance of sticking between powder granules.

The effects of SC-CO_2 treatment on whey protein isolate have been investigated by Xu et al. [50]. They evaluated the effects of temperature (30-60ºC) at 20 MPa for 1 hour with SC-CO_2 or N_2 on a whey protein isolate solution (10% w/v). These authors revealed that the structure and conformation of these proteins were modified with SC-CO_2 treatment, depending on the temperature. The treatment with SC-CO_2 increased the turbidity, being significantly higher at 50-60ºC. However, temperature in control and N_2 treated samples did not increase the turbidity of whey protein isolate (Figure 6), suggesting a protein denaturation when the SC-CO_2 treatment is applied.

The particle size was increased after treatment with CO_2 at 30-50ºC, indicating a partial aggregation of the proteins. The authors noted an increase in the mean particle size and a decrease in the polydispersity when whey protein isolate were treated with SC-CO_2 at 60ºC, which indicates unfolding in protein structure (Table 4).

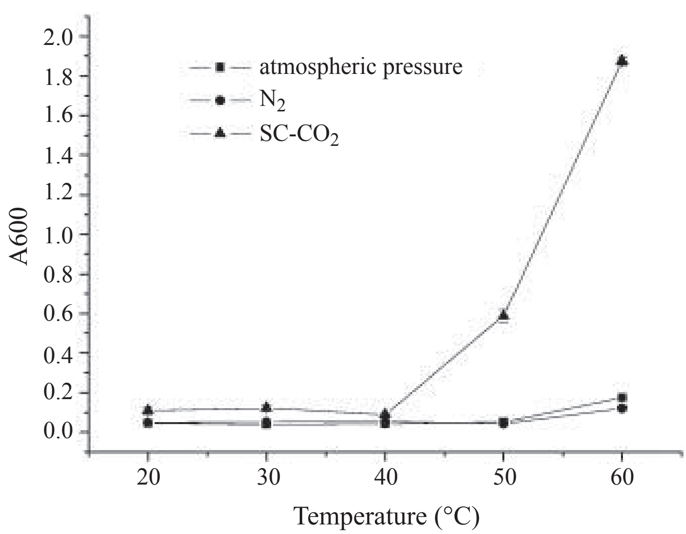


Figure 6. Turbidity of whey protein isolate solution treated with SC-CO_2 and N_2 at 20 MPa and atmospheric thermal treatment for 1 h as a function of temperature [50].

Table 4. Particle size and PdI (polydispersity index, size distribution) of whey protein isolate processed by SC-CO_2 at 20 MPa and atmospheric thermal treatment for 1 h at different temperatures [50]

Temperature	Thermal-processed		SC-CO_2-processed	
(°C)	PdI	Size (nm)	PdI	Size (nm)
30	0.460 ± 0.057	153.8 ± 4.1	0.711 ± 0.079	242.6 ± 14.3
40	0.478 ± 0.026	162.9 ± 7.7	0.701 ± 0.181	236.3 ± 32.1
50	0.456 ± 0.074	174.4 ± 27.7	0.979 ± 0.020	554.8 ± 22.0
60	0.351 ± 0.042	108.2 ± 26.5	0.391 ± 0.216	4764 ± 392.1

The SC-CO_2 treatment at 60ºC produced a partial denaturation and lead to the exposure of more hydrophobic regions, decreasing the α-helix content and hydrogen bonds, while the amount of β-sheet was increased. Whey protein isolate treated at lower temperatures of SC-CO_2 might recover almost completely to their original secondary structure [51].

In summary by modifying the structure and conformation of whey proteins by SC-CO_2 processing, it could obtain new or different functionalities of these proteins, more suitable for food or medical industry.

CHEESE WHEY, BUTTERMILK AND WHEY BUTTERMILK

The buttermilk is a by-product from cream processing to butter, rich in milk fat globule membrane (MFGM). These membranes surround each of the milk fat globules allowing them to remain dispersed in milk. MFGM is a polar lipid bilayer, which contains proteins, enzymes, and neutral lipids [52]. MFGM components, especially the phospholipids, have an important role as emulsifiers in food systems, which can be used to improve the features of bread, chocolate, margarine and dairy products, even used as a carrier.

Moreover, these complexes biological lipids are of interest because they define the structural properties of membranes and lipoproteins, as well they also work in a variety of biological processes and they are associated with metabolic and age-related diseases, stress responses and apoptosis.

In 2003, Astaire et al. [6] developed a two-step method to produce buttermilk derivative ingredients containing increased concentrations of the polar MFGM lipid by microfiltration and supercritical fluid extraction. Buttermilk contains a three-fold percentage of phospholipids compared to regular milk [53].

In a first step, these authors treated the buttermilk through microfiltration using a ceramic membrane with a pore size of 0.8µm. Buttermilk was treated using two temperatures of the process (4ºC and 50ºC) using two buttermilk sources (fresh or reconstituted from powder). After spray-drying, buttermilk and microfiltration retentates from buttermilk were extracted with SC-CO_2 at 27.5 MPa, 77ºC and three cycles of 75 min at 20g/min of flow rate. Runs of SFE on retentates samples reduced total fat by 38%, maintaining the polar lipids and extracting the nonpolar lipids, as shown in Figure 7 but on the other hand, fat is reduced by 30% in buttermilk, exclusively also nonpolar lipids.

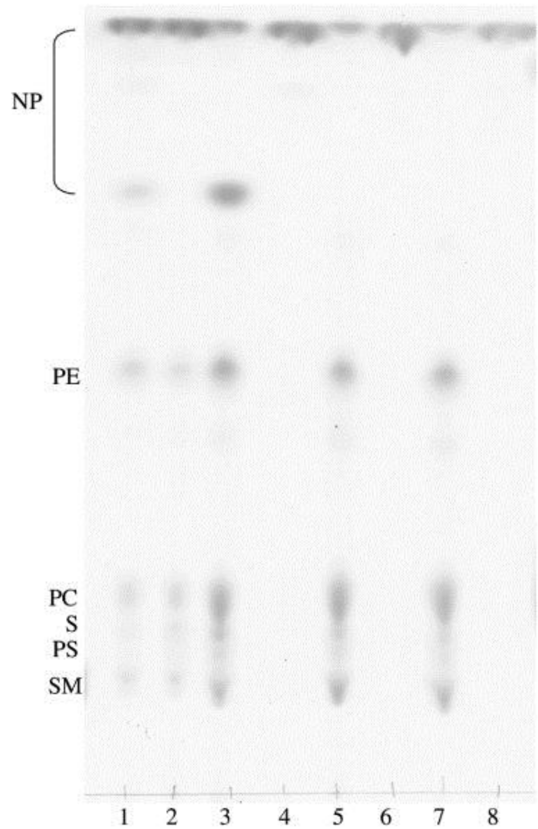
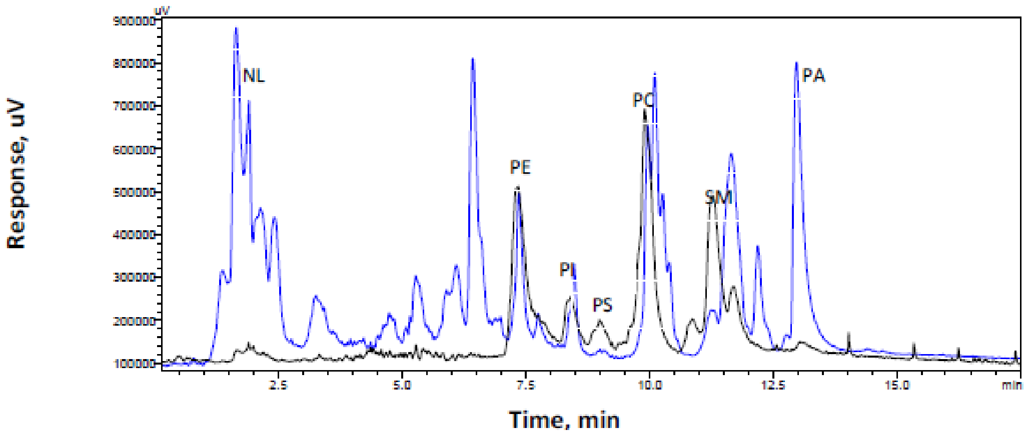


Figure 7. Thin layer chromatograph showing polar lipid profiles from SFE trials with microfiltration-enriched powder. Sample order is as follows: lane 1, buttermilk; 2, microfiltration-enriched powder; 3, trial (A) microfiltration-enriched powder after SFE; 4, trial (A) removed fat; 5, trial (B) microfiltration-enriched powder after SFE; 6, trial (B) removed fat; 7, trial (C) microfiltration-enriched powder after SFE; 8, trial (C) removed fat. SM = sphingomyelin, PS = phosphatidylserine, S = sphingosine, PC = phosphatidylcholine, PE = phosphatidylethanolamine, NP = nonpolar [6].

Astaire et al. [6] also reported by using gas chromatography that after SFE, the microfiltrated fraction powder showed a significant decrease in triglycerides (five-fold) and an increase in phospholipids concentration (two-fold), resulting in a concentration of polar lipids in samples after supercritical fluid extraction. Moreover, they observed that the lipid in samples were richer in fatty acids of long chain length (in the range from C18:2 to C24) however fatty acids of short and medium length were decreased in samples. These results are in accordance with the previous information given for application of SFE on buffalo butter oil [5], where the first fraction of lipid obtained from butter oil was richer in short and medium length fatty acids. It has been demonstrated [54] that triglycerides rich in short and medium length fatty acids and in saturated fatty acids with lower molecular weights (C12:0, C14:0, and C16:0) were more soluble in SC-CO_2 than that of triglycerides rich in unsaturated fats with higher molecular weights (C18:1, C18:3, C20:0).

Figure 8 depicts real HPLC data of a sample extraction of whey cream. The peaks shown in the early chromatograms (1 to 7 minutes) are nonpolar lipids that are almost completely extracted by SFE, while the phospholipids remain in the sample.



After extraction (Black) the remaining phospholipids are shown; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; NL, neutral lipids; PA, phosphatidic acid. The arrow points to the major difference peaks before and after extraction (original data from Jiménez-Flores laboratory, California Polytechnic State University).

Figure 8. Normal-phase liquid chromatography (LC) coupled with charged aerosol detector (CAD) chromatograms of Whey Cream sample (Blue) where clearly the neutral lipids (NL) are shown.

The major difficulty in isolation and concentration of the MFGM components from buttermilk by microfiltration is the presence of skim milk solids, especially casein micelles, which restricts the concentration of MFGM. A previous microfiltration of milk, buttermilk, or whey buttermilk can be also suitable to reduce lactose and casein content, since it has been demonstrated that lactose presence could interfere with the SFE process [9], and casein micelles may influence the manner in which SFE carries the lipids away from the powders.

Spence et al. [7] used the same two-step procedure previously described by Astaire et al. [6], but on buttermilk and whey buttermilk, a by-product from whey cream processing to butter (whey cream is obtained from cheese whey). Ceramic membranes for microfiltration were 0.45 µm-pore size and the temperature of the procedure was 8-10ºC. Final retentates were spray-dried and carried out to SFE system at the same operating conditions as described Astaire et al. [6]. As a conclusion, the authors were able to concentrate MFGM components in both, whey cream and regular cream buttermilk powder.

Costa et al. [8] applied the SFE with CO_2 (35 MPa, 50ºC) on whey buttermilk after, in this case, ultrafiltration/diafiltration. In the two latter studies, whey buttermilk and whey cream were fat reduced but concentrated in phospholipids.

The ingredients obtained from buttermilk, whey cream and whey buttermilk after applying microfiltration or ultrafiltration/diafiltration prior to SFE with CO_2 allows the production of a dairy powder enriched in MFGM phospholipids and proteins. Besides the use of these ingredients in the food industry, new challenges are arising in the field of medicine. Fuller et al. [55] probed the anti-rotavirus activity of natural and whey buttermilk powders containing bovine MFGM enriched in polar lipids after microfiltration and supercritical fluid extractions. They observed that anti-infective activity was doses dependent and with only 10 mg/ml of treated buttermilk or 13 mg/ml cheese whey, the inhibition of rotavirus infectivity was 94% and 81%, respectively (Table 5).

Table 5. Effect of exposure to whole milk fat globule membrane (MFGM) isolated from either buttermilk (BM) or cheese whey (CW) on the infectivity of the neuraminidase-sensitive rotavirus strain (OSY-RV)[1] [55]

Item	% of MEM control
MEM control	100 ± 2.3[a]
BM MFGM dose	
0.1 mg/mL	69 ± 3.0[b]
0.7 mg/mL	38 ± 4.0[c]
1.4 mg/mL	30 ± 7.8[c]
3.4 mg/mL	11 ± 4.8[d]
10.0 mg/mL	6 ± 2.1[d]
17.0 mg/mL	5 ± 0.5[d]
CW MFGM dose	
0.1 mg/mL	63 ± 5.0[b]
0.5 mg/mL	42 ± 4.0[c]
1.2 mg/mL	38 ± 3.5[cd]
2.6 mg/mL	30 ± 5.9[cde]
7.8 mg/mL	25 ± 3.2[de]
13.0 mg/mL	19 ± 0.5[e]

[a-e]Means with different superscripts differ at $P < 0.05$.

[1]Percentage infectivity relative to that of minimal essential medium (MEM) control measured using focus-forming unit assay; means ± SEM; n = 4 wells per dose.

After isolation of MFGM lipids by a single-phase extraction with organic solvent, the data indicated that the organic solvent extract retained the inhibitory activity, which may suggest that MFGM lipids are the responsible for the inhibitory activity.

Conclusion

In this chapter we have attempted to depict some of the most salient aspects of SFE applied to dairy products. Admittedly this review is not exhaustive nor depicts any commercial products. Supercritical treatment of foods is a nascent area of research that has shown great potential in foods given its suitability to thermally liable substances and increasing concern with natural products and functionalities. At the same time, SFE has shown great promise in food safety and microbiological control. We hope that this chapter makes justice to the many researchers that are working to shed some light and optimize this process for the benefit of food technology.

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