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SUPERCRITICAL CARBON DIOXIDE DRYING PROCESS OF FOOD MATRICES: MICROBIAL AND NUTRITIONAL ASPECTS

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Abstract

Dehydration is a common method to preserve food from spoilage and deterioration, thereby reducing its volume. Yet, every drying method presents some limitations, of which one of the most significant is the poor retention of nutrients after the process. In order to solve this issue, new drying techniques have been developed: one in particular is the treatment with supercritical fluids. Actually, in the last few decades, supercritical carbon dioxide (SC-CO_2 or HPCD) has been exploited as a low-temperature pasteurization method, resulting in a microbiologically safe product that maintains its qualities. The target of this Thesis is to characterize the bactericidal property and the capability of drying by means of this fluid, for the purpose of obtaining in a single process the drying and the pasteurization of the treated sample. The low temperatures involved in a process of this sort, suggest a better retention of nutritional attributes, as well as make the process an economical alternative to conventional techniques.

The work explores the inactivation of the naturally present microflora in coriander leaves (*Coriandrum Sativum L.*), achieved by optimizing the operative parameters, such as the drying time and the temperature. The results showed that at a temperature of 40°C and a pressure of 100 bar, the treatment was efficient with respect to mesophilic bacteria (about 4 log cycles of reduction), but not towards spores (only 0.5 log cycles of reduction). Instead, the total inactivation of yeast and molds was achieved, differently from the air drying treatment (static oven). Furthermore, from the nutritional analysis, a good retention of attributes like vitamin C, polyphenols and flavonoids could be deduced. Yet, color analysis demonstrated a substantial change of this property with respect to the fresh and the air dried sample, a condition that makes the SC-CO_2 dried coriander less attractive by the consumer.

In the view of the limitations exhibited from this process, especially related to the spore inactivation, the combination of the SC-CO_2 treatment with ultrasound was studied. The most impactful operative parameters on the microbial reduction were the ultrasound power and the temperature. An increasing of 10°C in the operative temperature and the highest ultrasound powers (80W) were, in fact, effective on the inactivation of spores: after the majority of the experimental trials their value was under the quantification limit. Still, the considerable color difference between the fresh and the treated sample increased, representing a potential obstacle in selling these products.

In the final analysis, in the light of the obtained results, the treatment with SC-CO_2 is a promising technique to obtain a dried and pasteurized product at the same time, at relatively mild operative conditions.

Riassunto

La disidratazione è un metodo diffuso nell'industria alimentare per prevenire il deterioramento dell'alimento e allo stesso tempo per ridurre il suo volume. Tuttavia, i metodi tradizionali di essiccamento presentano molteplici limitazioni, di cui una delle più importanti è lo scarso mantenimento dei nutrienti dopo il processo. Per ovviare a tale problema, nuove tecniche di essiccamento sono state sviluppate: una fra tutte è il trattamento con fluidi supercritici. In realtà, l'anidride carbonica supercritica (SC-CO_2) è da decenni sfruttata come metodo di pastorizzazione a basse temperature, consentendo di ottenere un prodotto microbiologicamente sano che mantiene le sue caratteristiche qualitative. Lo scopo della Tesi è quello di caratterizzare l'azione battericida e la capacità di essiccare del fluido, allo scopo di ottenere in un unico processo la pastorizzazione e l'essiccamento dell'alimento. Le basse temperature del trattamento suggeriscono una migliore ritenzione degli attributi nutrizionali, così come fanno di questo una alternativa più economica rispetto ai metodi tradizionali.

Il presente lavoro esplora l'inattivazione della flora batterica naturale su foglie di coriandolo (*Coriandrum Sativum L.*), ottenuta studiando l'influenza dei parametri operativi (tempo di essiccamento e temperatura). I risultati dimostrano che ad una temperatura di 40°C e pressione di 100 bar, il trattamento è efficace nei confronti dei mesofili (ridotti di circa 4 cicli logaritmici), meno nei confronti delle spore (circa 0.5 cicli logaritmici). Inattivazione totale si osserva invece nei confronti di lieviti e muffe, mentre ciò non avviene dopo il trattamento con metodi tradizionali (stufa). Inoltre, mentre si evince una buona ritenzione dei nutrienti, l'analisi del colore dimostra una sostanziale variazione di questo rispetto al prodotto fresco e al prodotto essiccato in stufa, proprietà che rende le matrici alimentari trattate con SC-CO_2 meno attraenti per il consumatore.

Allo scopo di superare le limitazioni esibite dal processo, in particolare nella riduzione delle spore, si è studiato il processo combinato di SC-CO_2 con ultrasuoni. I parametri che maggiormente hanno influenzato la riduzione microbica sono la potenza degli ultrasuoni e la temperatura. Un aumento di 10°C della temperatura operativa e le più alte potenze di ultrasuoni (80W) infatti si sono dimostrati efficaci nei confronti delle spore, le quali, dopo la maggior parte delle prove sperimentali, sono risultate sotto i limiti di quantificazione. La notevole differenza di colore tra campione fresco e trattato è però aumentata, costituendo quindi un potenziale ostacolo nella vendita di tali prodotti.

Alla luce di quanto ottenuto si può comunque concludere che il processo con SC-CO_2 consente di ottenere un prodotto alimentare secco e allo stesso tempo pastorizzato, in condizioni operative relativamente blande.

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Introduction

Dehydration of food has been widely studied and analysed through years, not only as a method to preserve food matrices from spoilage and deterioration, but also as a way to reduce a food weight and volume. Up to the present time, new methodologies have been developed, from the traditional sun drying, to the most advanced treatment with microwaves, ultrasounds and the use of supercritical fluids.

One of the most important limitation of conventional processes concerns the deterioration of the food sample after drying, in terms of nutritional attributes, functional properties and sensorial aspects. The high temperatures required from processes of this sort, in particular in air drying, are the principal reason of it. Modern techniques of drying, such as freeze-drying, partially offer a solution for the retention of nutrients, resulting in a very high-quality product. Yet, the long drying time makes the entire process energy-expensive and inconvenient under an industrial point of view. Elevated drying times, combined with high temperatures, in fact, inevitably contribute to raise the cost of processes, as well as the cost of the treated product and the energy consumption.

With this in mind, new methods of drying have to be developed to overcome these issues. In view of the bactericidal properties offered by supercritical carbon dioxide, in the present Thesis this capability is combined with the possibility of drying food matrices with this fluid, a feature that has been proved in other fields, different from the food sphere. The main target of this work is, in fact, to demonstrate the feasibility of drying and pasteurizing food samples after only one process. Since the critical temperature of supercritical carbon dioxide is low (31°C), the process takes place at slightly higher temperatures with respect to ambient temperature. This fact suggests a better retention of nutrients in the treated food, resulting in a better quality product with respect to the others drying methods. In addition, the drying time is considerably reduced. In the light of mild temperatures and shorter drying times involved in supercritical carbon dioxide drying, this technique seems to be a promising economic method to reduce the water content of a food product, and, at the same time, to pasteurise it.

The outstanding possibilities that a process of this sort appears to have, have pushed the research activity at European level. With this intention, Horizon 2020 Research and Innovation Program is supporting a project called Future Food, which strives for demonstrate the feasibility of supercritical carbon dioxide drying as an economic method to produce a safe and stable product, that maintains its qualitative and quantitative characteristics. University of Padua, as well as other European academic and industrial

partners, are involved in the project. Thus, all the experimental trials explained in this Thesis are performed on this background.

At present, no scientific publications concern the combined pasteurizing and drying properties of supercritical carbon dioxide. The Thesis is the first work to address this topic and widely analyse it.

The present work is organized as follows. The first Chapter presents the state of the art of conventional drying techniques, as well as the new technologies that have been developed in order to overcome the limitations of the previous ones. The description of the traditional methods of drying is founded on the microbial inactivation and on the preservation of nutritional attributes after the process. Supercritical carbon dioxide drying is then explained, highlighting the bactericidal properties of this fluid and illustrating the quality changes after a process of this sort. The final part of the Chapter presents the new advances in dehydration of food, that are combined processes, or “hurdle approach”, which enable to accelerate drying processes. Methodologies, procedures and the description of the reactors are explained in Chapter 2. Chapter 3 contains the obtained results and the discussion after HPCD process. First, the operative parameters (drying time and temperature) are analysed, in order to find the optimal ones, leading to the maximum microbial inactivation. Nutritional and color analysis on the treated sample complete the Chapter. Eventually, the last Chapter summarizes the results obtained after the combined HPCD+HPU process. Ultrasound power, drying time and temperature are analysed to improve the microbial reduction and reduce the drying time. Then, results of the color analysis are reported.

Chapter 1
Food drying: State of the art

In this Chapter, the principles of conventional drying process are illustrated, concerning both microbial aspects and quality changes in the product after the treatment. Then, a novel promising technique, supercritical carbon dioxide drying, is presented. This new process is attracting researchers worldwide since it promotes process sustainability in terms of sensorial quality of the dried product and microbial inactivation of it. In other words, the process seems to produce a dried and pasteurized product after only one step. Eventually, the last paragraph deals with combined processes, developed to overcome the disadvantages of supercritical CO_2 pasteurization and improve the process. In particular, the combination of supercritical carbon dioxide with ultrasound technology is explained in detail.

1.1 Water activity

It is an indisputable fact that some microorganisms are essential for the production of many familiar foods, including *Saccharomyces cerevisiae*, a specific kind of yeast employed in the majority of the industrial fermentations, and *Lactobacillus*, a genus of bacteria naturally present in the human body's microbiota and involved in the production of cheese and yogurt. Nevertheless, some of them can spoil and disrupt the organoleptic characteristics of the product in which they can get in, thereby representing a danger for the human body. In that case, microbial inactivation is the fundamental target to reach in food processing, in order to produce a completely safe product.

The primary sources of microorganisms in food include soil and water, plant and plant products, food utensils, intestinal tract of man and animals, animal hides and skin, air and dust. In production and preservation, factors that influence the microbial growth can be intrinsic (conditions naturally present in food) or extrinsic (environmental conditions).

As regard as the intrinsic factors, the most important one is the water availability. The amount of water available in food products is quantified by the water activity (a_w), a parameter defined as the ratio of the partial vapour pressure of water in a food substance (p) to the standard state vapour pressure of pure water at the same temperature (p_0), or, to put it differently, the equilibrium relative humidity of the product divided by 100:

$$a_w = \frac{p}{p_0} \tag{1.1}$$

Water activity can have a strong impact on color, taste and aroma of the product but, above all, it is a critical factor in determining the activity of enzymes and vitamins in foods. With this in mind, most pathogens require a value of the water activity greater than 0.90 to proliferate: for instance, *Clostridium botulinum* requires a value of 0.97, *Escherichia Coli* survives in environments with a value of 0.95 and *Salmonella* 0.93. The majority of fungi and molds can live in environments with a water activity of 0.80. Under a value of 0.5, no microbial proliferation is observed.

Environmental conditions, or extrinsic factors, can influence the microbial growth, too. In particular, the relative humidity, i.e. the amount of moisture in the atmosphere, can be a crucial factor in the deterioration of even dried products. Frequently, foods with low water activity placed in a highly humid environment can take up water and spoil.

Food preservation is a process in which physical and chemical agents are added to the product to prevent microbial spoilage of food and prolong its storage life. Two general principles are employed in food preservation: i) the inhibition principle and ii) the killing principle. According to the first principle, food preservation is achieved by inhibiting the growth and the multiplication of microorganisms: the reduction of water activity, as well as the reduction of the pH and the use of preservatives, represent important strategies to realise it. Unit operations like drying can reduce the water activity of a food product, so no microorganisms will allow growing in. Additionally, moisture removal reduces the weight and the bulk of food product to facilitate transport and storage. Conversely, pasteurization is a process that, in general, employed high temperatures in order to kill, or at least reduce, pathogenic bacteria so they are unlikely to cause diseases. For this reason, unit operations like those exploit the killing principle.

1.2 Conventional drying

Conventional drying techniques are based on high operative temperatures in order to decrease the water activity of various perishable materials, thus allowing their storage at ambient temperature. The processes used are numerous (Table 1.1), and they are chosen on the basis of the type and the quantity of product to dry, the final desired quality and the functionality of the treated sample.

Table 1.1. *Diversity of drying equipment and products in the food industry. (Bonazzi and Dumoulin, 2011)*

Equipment	Products
Drum dryer	thick liquids, pulps, pastes or slurries: mashed potatoes, carrots, baby cereals, soups, starch
Rotary dryer	particulate solid foods: alfalfa, pasta, sugar beet pulp, pomace, starch, whey, pectin residues, lemon pulps, and so on.
Deep bed/dihedral dryer	cereals: malt, maize, sunflower grain, rice, wheat
Spray dryer	liquids: milk, whey, coffee, tea, yeast
Pneumatic dryer	for the finish drying of powders or granulated materials: starch, flour, proteins, distillery residues, aspartame, guar gum, methionine
Tunnel conveyor, belt or band dryer	pasta, vegetables, gelatin, fruits, nuts, breakfast flakes, extruded pet foods, soy proteins, tobacco, seeds, and so on.
Fluidized bed	powders and small particles: milk powder, whey, yeast, casein, extruded food, coffee beans, soy beans, sunflower beans, chocolate granules, lactose, L-lysine, nutmeg, tomato powder, soy proteins, salt, sugar, and so on.
Freeze-dryer	high added-value, heat-sensitive products: coffee extracts, fruits, vegetables, mushrooms, aromatic plants
Ventilated cabinets	sausage, ham

The oldest type of dryer involves hot air flowing over an extensive area of the product to remove water by vaporization from the surface: chamber, tray or cabinet and fluid bed type dryers fall into this category. Air heating not only does it increase the driving force for heat transfer, accelerating drying, but it also reduces air relative humidity. They are particularly suitable for solid materials such as grains, sliced fruits and vegetables, and typical drying times range from a few hours to a day (Vega-Mercado *et al.*, 2001).

Another generation of driers are the so-called spray dryers, which are used for food dissolved in water, and are based on the formation of droplets that, when later dried, yield dry food particles. Initially, the feed is transformed from a fluid state into droplets and then into dried particles by spraying it continuously into a hot drying medium. The drying air is heated using a dry medium and, after the process, is cleaned using cyclones before releasing it to the atmosphere. A second configuration provides for the recirculation of the heating medium, in order to improve the energy efficiency. The main differences between spray and fluidized bed or other first generation method of drying, are in the feed characteristics (fluid in spray drying versus solid), residence time (5-100 s for spray and 1-300 min for fluidized bed) and particle size (10-500 μm for spray and 10-3000 μm for fluidized bed) (Vega-Mercado *et al.*, 2001).

A more advanced technique is freeze-drying, which was introduced on large scale in the 1940s for the production of dry plasma and blood products, antibiotics and biological materials. It works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. The four stages of the freeze-drying process are: pre-treatment, freezing, primary drying, and secondary drying.

Pre-treatment may include for example concentrating the product, addition of components to improve processing, decreasing a high-vapor-pressure solvent, or increasing the surface area. Then, the freezing stage begins: the food product is placed in a freeze-drying machine and begins to rotate in a bath, which is cooled by mechanical refrigeration, dry ice and methanol, or liquid nitrogen. In this step, it is important to cool the material below its triple point, to ensure that sublimation rather than evaporation will occur in the following steps. In the case of food, large ice crystals will break the cell walls, which can result in increasingly poor texture and nutritive content. Therefore, freezing is done rapidly, in order to lower the temperature of the material below its eutectic point quickly, thus avoiding the formation of large ice crystals. Usually, the freezing temperatures are between −50 °C and −80 °C.

In primary drying, the pressure is lowered (to the range of a few milli-bars), and heat is supplied to the material for the ice to sublime. In this initial drying phase, about 95% of the water in the material is sublimated. Pressure is controlled through the application of partial vacuum, which speeds up the sublimation. The pressure difference between the water vapour pressure in the ice interface and the partial water vapour pressure in the drying chamber is the driving force of sublimation (Ibarz and Barbosa-Cànovas, 2003). Condenser temperatures are typically below −50 °C. In the secondary drying phase, the ice in the product is removed and moisture comes from water partially bound to the material being dried. In this phase, the temperature is raised higher than in the primary drying phase, even above 0 °C, and the pressure is also lowered to encourage desorption (typically in the range of microbars).

Table 1.2. *Advantages and disadvantages of the principal drying techniques.*

Drying method	Advantages	Disadvantages
Air-drying	Cheap equipment	High temperatues
	Easy to clean	High drying times (few hous-a day)
	Easy scale-up	Bad retention of nutrients
	Simple operation	Risk of agglomeration
		Poor temperature uniformity
Fluid bed drier	Good homogeneity	Unsuitable for pasty or liquid materials
	Lower risk of agglomeration	Product recovery from exhaust
	Short drying times	High consumption of inert gas
Spray drying	Short drying time (5-30s)	Expensive equipment
	Continuous	Liquid or slurry feed
	Good product uniformity	Material recovery <100%
	Suitable for heat sensitive materials	
Freeze-drying	Excellent nutrients retention	High drying times (up to 30 h)
	High structural properties of the treated sample	Low process efficiency
	Suitable for heat-sensitive materials	Lack of evolution in equipment design
		Heterogeneity in drying
		Energy intensive

As soon as the freeze-drying process is completed, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed. The final residual water content in the product is extremely low (around 1% to 4%).
In Table 1.2. advantages and disadvantages of the principal drying techniques are summarized. The latest advance in food drying includes high-vacuum, the use of microwaves or ultrasound waves, radio frequency, supercritical fluids and the hurdle approach. Each of these technologies has a specific application, which depends on the final quality attributes of the intended product, as well as the physical/chemical characteristics of the raw materials being processed.

1.2.1 Microbial inactivation after conventional processes

Nowadays, drying techniques are evaluated for their performance to reduce water activity at the lowest cost possible, and at the same time to ensure good sensorial quality of the processed sample. However, as Bourdoux *et al.*, 2016, has pointed out, microbial inactivation on these products has not yet been assessed even for traditional methods of drying. In Table 1.3 the main studies related to this issue are described.
On the other hand, freeze drying is generally considered as the most successful method of preserving bacteria, thereby producing a so-called probiotic product that benefits to human health.
The work by Barbosa *et al.*, 2015, has described the survival of two types of lactic acid bacteria in orange powders obtained after spray-drying, freeze-drying and convective drying. Only in sample treated by convective drying a 2 log cycle reduction was observed. In the other samples no decrease in the microbial load was achieved. For this reason, spray and freeze drying appeared to be a good method to produce a new functional probiotic orange juice powder.
Microbial reduction after freeze-drying processes was also tested by Myiamoto-Shinohara and co-workers, 2005, which studied the survival of different microorganisms immediately after the process and for a storage time of up to 20 years. They discovered that 97.7% of *Saccharomyces Cerevisiae* that survived after freeze-drying, went on surviving each year of storage.

Table 1.3. *Studies related to the inactivation of microorganisms during and after conventional processes, for different food matrices (Bourdoux et al., 2016).*

Product	Drying process	Conditions	Microorganisms	Log reduction (CFU/g)	Reference
Apple slices	Convective air drying	62.8 °C for 6 h	*E. coli* O157:H7	3.3–3.5	Burnham and others 2001
Apple slices (Gala)	Convective air drying	60 °C for 6 h	*S. typhimurium*	4–4.2	DiPersio and others 2003
Apple slices (Gala)	Convective air drying	62.8 °C for 6 h	*E. coli* O157:H7	2.5–3.1	Derrickson-Tharrington, 2005
Apricot (Hacihalilioglu)	Open-air solar drying	38 °C (d), 29 °C (night) for 182 h	Mesophilic bacteria Yeasts and molds	Increase in microbial contamination	Karabulut and others 2007
	Convective air drying	70 °C for 20 h	Mesophilic bacteria Yeasts and molds	>3 >1	
Cowpea	Open-air solar drying	33 °C (average) for 1 h/day during 3 d; 40% RH	*S. typhimurium* *Bacillus* spp *Enterobacter* spp *Staphylococcus* spp	2.5 No reduction 2.2 Complete inactivation (>3)	Wachuku and others 2003
Cabbage slices	MW freeze-drying (2450 MHz)	700 W at 100 Pa for 6 h	Mesophilic bacteria	0.9	Duan and others 2007
	Freeze-drying	60 °C at 100 Pa for 15 h		No reduction	
Cabbage slices	Convective air drying	60 °C for 3 h	*S. enatum*	>4	Chiewchan and others 2009
Cabbage slices	Convective air drying	70 °C for 2,5 h	*S. enatum*	>4	Hawaree and others 2009
Cabbage slices	Convective air drying Vacuum drying LPSS drying	70 °C for 4 h 70 °C at 10 kPa for 1.5 h 70 °C at 10 kPa for 1.75 h	*S. enatum*	6.33 3.59 6.75	Phungamngoen and others 2011b
Cabbage slices	Convective air drying	60 °C for 6 h	*S. enatum*	5	Phungamngoen and others 2013
	Vacuum drying LPSS drying	60 °C at 10 kPa for 2,1 h 60 °C at 10 kPa for 3 h		3.4 5.2	
Carrot slices (Nantes)	Convective air drying	60 °C for 6 h	*S. typhimurium*	1.7–2.4	DiPersio and others 2005
Carrot slices (Nantes)	Convective air drying	60 °C for 6 h	*S. typhimurium*	1.6–1.7	DiPersio and others 2007
Carrot slices (Ithaca)	MW vacuum-drying (2450 MHz)	1800 W at 8 to 15 kPa for 58 min	Total aerobic count Yeasts and molds	1.7 1.5–2.2	Yaghmaee and Durance, 2007
	MW static air drying followed by MW vacuum drying (2450 MHz)	1800 W for 12 min then for 46 min at 8 to 15 kPa	Total aerobic count Yeasts and molds	4 1.3–3.9	
Carrot sticks (*Daucus carota* var. Sativa)	Convective air drying	60 °C for 10 to 12 h	*S. enatum*	3.3–4.2	Phungamngoen and others 2011a
Unpeeled ginger	Open-air solar drying Direct solar drying	30.8 °C (average) for 11 d 43.2 °C (average) for 11 d	Total aerobic count	No reduction <1	Eze and others 2011
Onion (Southport White Globe)	Catalytic infrared drying (average 2226 W/m²)	80 °C for 40 min	Mesophilic bacteria Coliforms Yeasts and molds	2 log CFU/10 g 3 log CFU/10 g <1 log CFU/10 g	Gabel and others 2006
Green onion (*Allium fistolosum*)	Convective air drying	70 °C for 3.8 h	Mesophilic bacteria Coliforms Yeasts and molds	2–3 2–3 1	Garcia and others 2010
Green onion	Convective air drying	47.8 °C for 20 h 55.1 °C for 20 h 62.4 °C for 20 h	Hepatitis A	1 2 3	Laird and others 2011
Parsley	MW vacuum-drying (2450 MHz)	1500 W at 8 to 15 kPa for 20 min	Total aerobic count Yeasts and molds	2.5 2.7	Yaghmaee and Durance, 2007
Potato	Osmotic dehydration	40 °C for 3 h in sucrose 50 °Brix (ratio solution 13:1)	Mesophilic bacteria	1.67 log CFU/cm²	Mitrakas and others 2008
Tomato (Roma)	Convective air drying	60 °C for 14 h	*S. typhimurium*	3.2–4.5	Yoon and others 2004
Tomatoes	Indirect solar drying	55 °C for 5 d	Mesophilic bacteria Yeasts and molds	>2 >3	Sohail and others 2011
Culinary herbs (11 sorts)	Convective air drying MW static air drying (2450 MHz)	38 °C for 48 h 650 W for 7.5 min	Mesophilic bacteria Mesophilic bacteria	0–2 1–4.5	Deans and others 1991

In order to improve the poor bactericidal property of this technique, Duan *et al.*, 2007, combined freeze-drying with microwave drying, demonstrating a 0.9 log reduction in the natural contamination of white cabbage slices. These results suggested that MW-assisted freeze drying could combine drying and pasteurization. Actually, pasteurization properties offered by microwave heating alone were studied by Candido da Silva *et al.*, 2016, in the fungal contamination of Brazilian nut seed. Not only microwave heating appeared to reduce the moisture content (46.4%) and water activity, but also showed a disinfectant property: colonization inside the nut shell decreased by 61.67%, without damaging the organoleptic properties of the product.

Microbial inactivation is achieved after the majority of drying processes, not only because of the decreasing of the water activity. Depending on the processes, in fact, microorganisms are exposed to several stresses at the same time (temperature or pressure variations, atmosphere changes or electromagnetic waves), which make the interpretation of inactivation results

difficult. Furthermore, bacteria species differ in sensitivity to heating and drying. In other words, different bacteria may present different survival curves, or the food itself may influence the viability of them.

1.2.2 Quality changes after conventional processes

In the current economic context, consumers ask for a product that maintain the original quality, as well as its flavour, texture and functionality, increasing their nutritional content and reducing toxins (Bonazzi and Doumulin, *Modern Drying Technology Volume 3: Product quality and Formulation*, 2011). This is why food industries are focused on maintaining the bioactivity and structural property of the product, an important issue that imposes important industrial constraints. Actually, processing food at the high temperatures employed in thermal drying, inevitably results in higher nutritional loss and in poorer quality, whereas it increases food safety. The most important modifications in the treated food include biochemical reactions induced by the process and physical transformations (i.e. glass transitions, melting of fat, evaporation or migration of volatile components).

Quality changes depend both on treatment time and on temperature. The operative temperature, as it is expected, is a key parameter in the design of a chemical process: temperature can degrade vitamins, denature proteins and enzymes, resulting in undesirable nutritional modifications in the sample, including changes in color, taste or aroma, formation of toxic compounds. In particular, vitamin C is an indicator of the nutrient quality of processes. Its retention after conventional drying methods (air-drying) is usually very low, whereas freeze-drying improves it. Treatment time in conventional drying is connected to the shrinkage of the sample. The shorter exposure time, the less substantial shrinkage is observed, which is directly associated with nice texture and crispiness of the product. Color is one of the most relevant characteristic with respect the quality of dried food, as it is determined by the presence of pigments such as carotenoids, chlorophylls, anthocyans, betalains. The color of the treated sample then, may change during processes, as a consequence of the thermal degradation of the pigments. The optimal trade-off between drying time and the level of severity of processing must, therefore, be designed in order to obtain the desired food characteristics.

More specifically, conventional air-drying is the most commonly used dehydration process in food industry, producing products that are characterised by low porosity and high apparent density. The high temperatures used during the process (typically 65°-85°C) cause damage to the microstructure and may also have a negative influence on the color, texture, taste, aroma and nutritional value of the fresh starting material thereby influencing the quality of both the dried and the rehydrated product. In particular, physical alterations of the treated product include shrinkage, increased or decreased of porosity, and decreased ability to imbibe water and damage to microscopic structure (Witrowa-Rajchert *et al.*, 2005).

Above all the conventional drying techniques, freeze-drying can produce products of excellent quality, allowing significant retention of most of physical, chemical, biological and sensorial properties of the fresh product (Ibarz and Barbosa-Cànovas, 2003). De Escalada Pla *et al.*, 2012, claims that better functional properties of dried peaches in terms of higher water holding capacity, water retention capacity and lower values of bulk density are obtained after freeze-drying process. Similarly, Borchani *et al.*, 2011, confirmed the same results in his studies on dried date fibre concentrates, while Ahmed and Attar, 2005, proved the advantage of this technique since freeze dried sample of chestnut flour dough had the highest maximum viscosity, heat stability and viscoelastic properties.

An interesting study by Chang *et al.*, 2005, demonstrated that, although the total amount of ascorbic acid in tomatoes depends highly on varieties and cultivation conditions, the amount of this compound after freeze-drying slightly decreases (10%) with respect to the fresh value. High temperatures, involved in air drying, appear to have a tremendous effect on the oxidation of ascorbic acid: after that process, the amount of ascorbic acid significantly decreases respecting to the fresh sample (around 60% of loss).

Regarding the color of the sample after the treatment, a quantitative evaluation was performed by Caparino *et al.*, 2012, on mango powders obtained after different drying processes. It has been proved that the color of freeze-dried mango was significantly different from drum dried (darker) and spray dried (lighter) counterparts. Furthermore, freeze-dried particles were more porous compared to the others.

In spite of these great advantages over the other drying methods, freeze-drying is extremely time consuming (up to 30 h of residence time) and highly energy-intensive. As a result, it is the most expensive drying method.

Table 1.4. *Effect of different drying methods on functional properties for different food matrices (Dehnad et al., 2016)*

Number	Protein type	Drying method	Drying conditions	Functional properties							Superior drying method/condition	Reference
				Protein solubility (%)	Water/Oil holding capacity		Emulsification stability/ Activity Index		Foaming stability & capacity			
					WHC[a] (g 100 g^{-1})	OHC[a] (g 100 g^{-1})	EAI[a] (m^2 g^{-1})	ESI[a] (min)	FC[a] (%)	FS[a] (%)		
1	Chickpea protein concentrates	Convective	40 °C for 60 h	48	213	191	410	164	55	5	Freeze drying	Ghribi et al. (2015)
			50 °C for 48 h	53	206	217	316	126	42	13		
		Freeze	−18 °C for 24 h	58	271	277	313	125	58	32		
2	Succinic acid deamidated wheat gluten	Spray	170 °C (inlet) & 95 °C (outlet)				21	42	290	60	Freeze drying	Liao et al. (2013)
		Freeze	−80 °C for 3 h, Then 0.07 m Pa for 24 h.				23	55	330	65		
3	Soy protein isolate	Freeze	−18 °C for 24 h, then −54 °C for 48 h	41			EA: 0.59 (ABS500)	65	5.5 mL	150 min	Spray drying	Hu et al. (2008)
		Spray	180 °C (inlet) & 85 °C (outlet)	70			EA: 0.63 (ABS500)	85	6.1 mL	200 min		
		Vacuum	60 °C and −0.098 MPa for 48 h	38			EA: 0.61 (ABS500)	58	5.8 mL	175 min		
4	Algae protein	Spray		Nitrogen solubility: 50%	292	65	Emulsification capacity: 60 ml g^{-1}	1760 s	40	After$_{90\ min}$: 20	Defatted (over raw) algae protein	Nirmala, Prakash, and Venkataraman (1992)
5	Faba Bean Protein	Spray	180 °C (inlet) & 100 °C (outlet)	42	Hygroscopicity: 20%		60%	42%			Spray drying	Cepeda, Villaran, and Aranguiz (1998)
		Freeze	80 Pa and 40 °C	28	Hygroscopicity: 0.78%		27%	22%				
6	Egg albumen powder	Fluidized bed	90 and 130 °C		WHC: 60−72 [72][b] % Protein in drain: 65−75 [60] (g L^{-1})				120−160 [170]	After$_{30\ min}$: 27−37 [25]	Dried (over raw) samples	Hammershøj, Rasmussen, Carstens, and Pedersen (2006)
7	Peanut protein concentrate	Vacuum oven	70 °C		110	90	87 (mL g^{-1})			2	Spray drying	Yu, Ahmedna, and Goktepe (2007)
		Spray			160	160	137 (mL g^{-1})			10		
8	Cowpea protein concentrate	Freeze	30 °C for 2 h, then −50 °C for 48 h	35	245	207					Convective drying	Mune Mune & Sogi (2015)
		Vacuum	50 °C and 3.33 kPa for 24 h	70	224	200						
		Convective	50 °C for 24 h	50	321	207						
9	Bambara bean protein concentrate	Freeze	30 °C for 2 h, then −50 °C for 48 h	20	377	232					Freeze drying	Mune Mune & Sogi (2015)
		Vacuum	50 °C and 3.33 kPa for 24 h	75	261	202						
		Convective drying	50 °C for 24 h	58	339	201						
10	Lentil protein isolate	Freeze	−18 °C for 24 h, then 48 °C and 0.5 mm vacuum pressure	78	48	Least gel concentration: 11% (w/v) Bulk density: 276 (kg m^{-3})					Freeze and spray drying	Joshi, Adhikari, Aldred, Panozzo, and Kasapis (2011)
		Spray	180 °C (inlet) & 85 °C (outlet)	81	43	Least gel concentration: 11% (w/v) Bulk density: 308 (kg m^{-3})						
		Vacuum	60 °C and 85 kPa for 48 h	50	47	Least gel concentration: 14% (w/v) Bulk density: 596 (kg m^{-3})						
11	Whey protein concentrate	Spray		100	Yield stress: 15 Pa				Overrun: 60%	Drainage time: 15 min	Spray drying	Luck et al. (2013)
		Freeze		100	Yield stress: 0 Pa				Overrun: 0%	Drainage time: 0 min		
12	Milk serum protein concentrates	Spray		98	Yield stress: 40 Pa				Overrun: 110%	Drainage time: 40 min	The same performance	Luck et al. (2013)
		Freeze		98	Yield stress: 40 Pa				Overrun: 110%	Drainage time: 40 min		

[a] WHC: Water holding capacity; OHC: Oil holding capacity; EAI: Emulsification activity index; ESI: Emulsification stability index; FC: Foaming capacity; FS: Foaming stability.
[b] The values in brackets show control amounts.

A clear summary of qualitative properties of some food matrices is contained in Table 1.4.
In order to overcome the strong limitations in the retention of qualities exhibited by conventional methods of drying, new drying technologies have been developed during the last decades, which involve lower temperatures and lower drying times.

1.3 Supercritical CO_2 drying

When a gas, contained under high pressure, is heated, changes occur in its physical properties and it becomes a supercritical fluid (SCF). Under these conditions, the gas possesses the solvating power of a liquid and the diffusivity of a gas; its density is similar to the density of a liquid and its very low surface tension is characteristic of a gas. For this reason, supercritical fluids work extremely well as a processing media for a wide variety of chemical, biological, and polymer matrices, and represent an effective alternative to traditional solvents. Figure 1.1 shows the supercritical region in a phase diagram.
The most commonly used SCF is carbon dioxide. It has a critical temperature of 31°C and a critical pressure of 73 atmospheres, as illustrated in Figure 1.2.

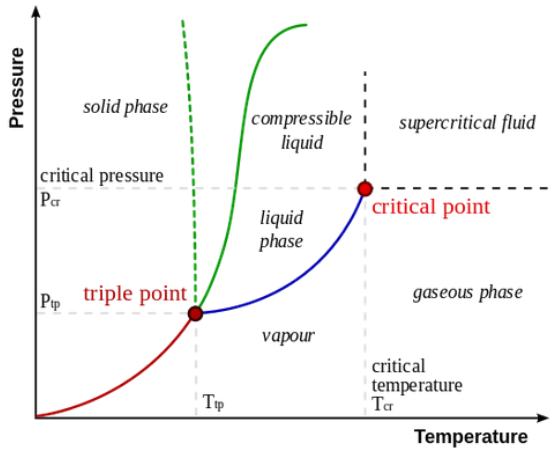

Figure 1.1. Phase diagram of a supercritical fluid. (www.physics.stackexchange.com)

Supercritical carbon dioxide has several advantages:

- It is natural, cheap and plentiful;
- It is a by-product in many industrial processes (brewing, ammonia synthesis, combustion);
- It is not toxic or flammable;
- It is defined as GRAS (Generally Regarded as safe for food processing);
- It has a high compressibility, which is exploited to tune the solvent to extract those components of interest while leaving others behind;
- With a small amount of cosolvents, the properties of the fluid could be modified: cosolvents are in general organic solvents, including for example ethanol, added to increase the solubility of polar substrates in SC-CO_2;
- It is inert to radical species, thus it is a useful medium in radical reactions.

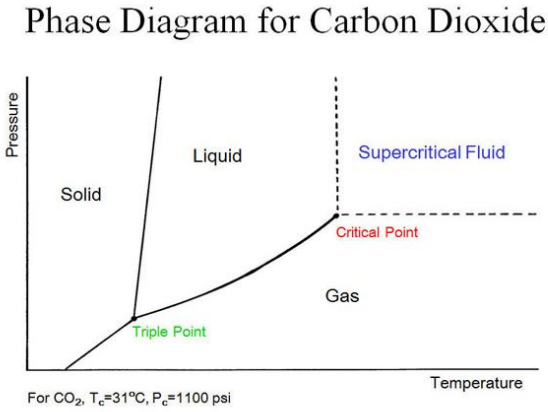

Figure 1.2. Phase diagram for carbon dioxide. (www.supercriticalfluids.com)

On the other hand, the moderate pressures required (over 70 bar) could make the process expensive on a large scale, because of the impossibility to make the reactor in standard material. Moreover, since it is non-polar molecule, carbon dioxide can effectively improve solubility of small non-polar molecules but the solubility of water in this fluid is modest.

Recently, supercritical CO_2 has been involved in drying processes. Surely, supercritical CO_2 drying presents a promising alternative with respect to conventional food drying techniques, in order to combine economic of the process, quality and microbial inactivation of the dried product. The process can be seen as an extraction where CO_2 is the solvent and water is the solute. So far, very few publications dealing with SC-CO_2 as a mean of food drier are available in literature.

Concerning the use of supercritical carbon dioxide as a water removal agent in food, one of the first work was published by Brown *et al.* (2007), where the removal of moisture from pieces of carrot has been investigated. Supercritical drying results on drying kinetic, rehydration properties of the product, texture and color of it (p=20 MPa, temperature between 40° and 60°C) were compared with air-drying results. It was noticed that drying using supercritical fluids proceeds via a different mechanism to that occurring in air-drying: the internal structure of the carrot does not control the rate of moisture removal, but it is instead controlled by the rate of mass transfer from the surface. Indeed, water is not removed by vaporization or sublimation, but it is dissolved in the SC-CO_2. For that reason, an important drawback of this technique is the low solubility of water in SC-CO_2. In addition, the drying time in case of supercritical drying is longer than in air-drying.

In the described procedure, the carrot sample was treated with air, pure CO_2 and supercritical CO_2 modified with 6 mol% of ethanol. Over half of the original moisture content of the carrot was lost in 90 minutes, indicating that drying in supercritical CO_2 alone is possible. Adding ethanol, as it was expected, the extent of moisture removal increased. Therefore, it increased the overall rate of drying in the supercritical systems.

Few years later, in 2014, Busic *et al.*, performed some experiments of supercritical CO_2 drying on basil (*Ocimum basilicum L.*), with the purpose to evaluate the sensory and nutritional properties of the food matrix after the treatment, and to compare them with the results obtained with conventional drying methods (air-drying and freeze-drying). Supercritical carbon dioxide drying technique was performed in a pilot plant with recirculation of CO_2 and the operative conditions were 40°C, 100 bar and a CO_2 flowrate of 1000 l/h. A dried product was obtained after 3 hours of treatment. Air-drying took place in an oven with circulation of air at a mild temperature (40°C) for 26 hours, whereas freeze-drying was performed at 0.005 bar and -20°C for four days. The indication of how much the food product is dried was determined gravimetrically calculating the dry matter, as the residue remaining after drying, according to the official A.O.A.C. method (1995). The weight difference between the final and the initial sample expressed the dry matter content. The results revealed that supercritical CO_2 drying and

freeze-drying can reduce the moisture content <10%, while air-drying provided basil with around 23% remaining water. In addition, no differences were observed in dried sample by supercritical carbon dioxide varying the operative pressure (80 or 100 bar) or drying time (2, 3 or 4 hours). Even though differences were not significant under a statistical point of view, CO_2 dried basil appeared to have a lower moisture content (5.41-5.69%) than the freeze-dried sample (6.05%).

Industrially, FeyeCon Development & Implementation B.V. owns the most important application of this novel technology. A patent (Agterof *et al.*, US 2010/0260891 A1, Oct. 14, 2010) covers the entire procedure, and it provides a method to dehydrate pieces of plant or animal tissue reducing the water content of at least 50%. The process occurs when a gas, with a pressure of at least $0.5*P_c$ and a temperature of at least T_c-60°C (where the subscript "c" stands for critical), is in contact with the matrix. Then, the patent describes a method to separate the pressurised gas from the dehydrated product, wherein around 90% in weight of the matter removed by the gas from the sample is water. Several examples support this invention: drying of broccoli with and without recirculation of the gas, drying of other vegetables and fruit such as apple parts, leek, red pepper (paprika), drying of herbs (parsley and chives) and drying of meat. In all experimental trials, the dried sample had a water activity under 0.5 and a final water content under 20%.

According to literature, HPCD drying technology is exploited in other fields, different from food processing: pharmaceutical (Walters *et al.*, 2014), tissue engineering (Zambon *et al.*, 2016, Reverchon and Cardea, 2012), production of highly porous aerogel (Brown *et al.*, 2010, Ozbakir *et al.*, 2014, Baldino *et al.*, 2015) and drying of coal (Iwai *et al.*, 2002).

1.3.1 Supercritical CO_2 pasteurization

In the last decades, HPCD technology has been exploited for its bactericidal and microbial inactivation properties, which are exhibited at mild condition of temperature (under 50°C), as an alternative method with respect to thermal processes. Several publications report supercritical carbon dioxide power of pasteurization in both solid and liquid food products, as well as on their natural microflora and on properly spiked microorganisms on them (Table 1.5). However, the real bactericidal effect still remains unknown. Many different authors, in particular Spilimbergo and Bertucco, 2003 and Damar and Balaban, 2006, have resumed Daniels' initial ideas (1985) and they agree on the explanation of the lethal action of supercritical carbon dioxide. Seven different steps, summarized by Garcia-Gonzales *et al.*, 2007, take place simultaneously and work in concert in order to kill microorganisms:

- CO_2 solubilizes in the external liquid phase to form carbonic acid. As a consequence, the media in contact with the supercritical fluid becomes acidic, thereby inhibiting microbial growth;
- CO_2 may diffuse and penetrate into cells' lipophilic layer (Spilimbergo *et al.*, 2002). The accumulation of this amount of gas, causes the membrane to be structurally and functionally modified;
- CO_2 starts to accumulate into the cell, due to the modification in the cell's membrane. As a result, pH decreases and the cell may be unable to restore its original pH;
- The catalytic activity of enzymes declines after the decreasing in pH;

Table 1.5. *Effect of physical state of CO_2 on the microbial inactivation, for different bacteria (Garcia-Gonzales et al., 2007).*

Target microorganism	Solution	State of CO_2	Process conditions	Reduction	References
Escherichia coli	PS[a] or distilled water	Gaseous	4 MPa, 20 °C, 120 min	3.9D	Kamihira et al. (1987)
			4 MPa, 35 °C, 120 min	4.0D	
		Liquid	10 MPa, 20 °C, 120 min	4.5D	
			20 MPa, 20 °C, 120 min	4.4D	
		Supercritical	10 MPa, 35 °C, 120 min	4.2D	
			20 MPa, 35 °C, 120 min (water content=65–75%)	5.1D	
Saccharomyces cerevisiae	PS or distilled water	Gaseous	4 MPa, 20 °C, 120 min	0.1D	
			4 MPa, 35 °C, 120 min	0.1D	
		Liquid	10 MPa, 20 °C, 120 min	0.3D	
			20 MPa, 20 °C, 120 min	0.9D	
		Supercritical	10 MPa, 35 °C, 120 min	3.9D	
			20 MPa, 35 °C, 120 min (water content=65–75%)	6.3D	
S. cerevisiae	Growth medium	Gaseous	6.9 MPa, 35 °C, 15 min	7D	Lin et al. (1992a)
		Liquid	6.9 MPa, 25 °C, 45 min	4D	
			13.8 MPa, 25 °C, 35 min	4D	
			20.7 MPa, 25 °C, 60 min	7D	
		Supercritical	13.8 MPa, 35 °C, 10 min	7D	
			20.7 MPa, 35 °C, 7 min	7D	
Leuconostoc dextranicum	Growth medium	Gaseous	6.9 MPa, 35 °C, 20 min	9D (*)[b]	Lin et al. (1993)
		Liquid	6.9 MPa, 25 °C, 40 min	9D (*)	
			20.7 MPa, 25 °C, 35 min	9D (*)	
		Supercritical	20.7 MPa, 35 °C, 15 min	9D (*)	
Lactobacillus brevis	PS	Gaseous	5 MPa, 35 °C, 15 min	2D	Ishikawa et al. (1995)
		Liquid	7 MPa, 25 °C, 15 min	2D	
		Supercritical	25 MPa, 35 °C, 15 min (treatment carried out with a 10-µm pore size filter)	6D (*)	
S. cerevisiae	PS	Gaseous	5 MPa, 35 °C, 15 min	3D	
		Liquid	7 MPa, 25 °C, 15 min	2.5D	
		Supercritical	25 MPa, 35 °C, 15 min (treatment carried out with a 10-µm pore size filter)	5D	

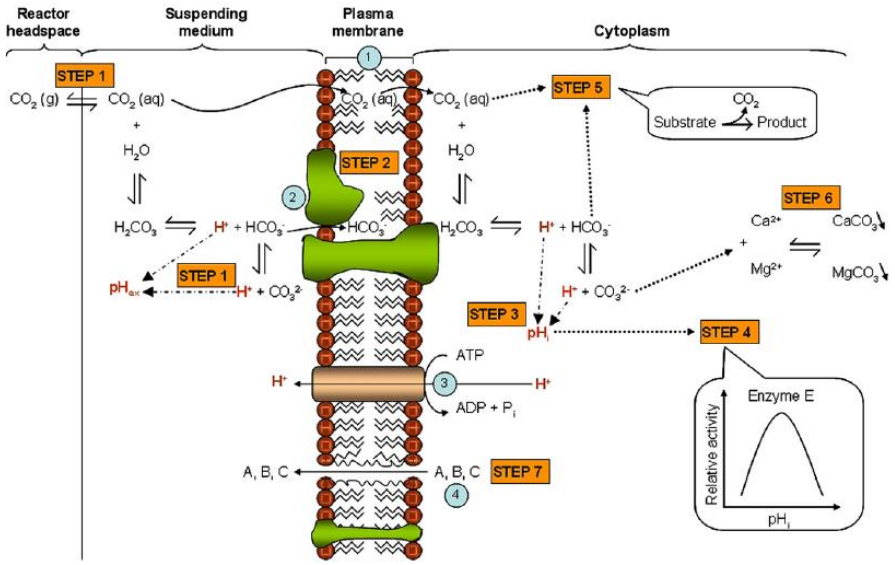

Figure 1.3. *Bactericidal effect of supercritical carbon dioxide (Garcia-Gonzales et al., 2007).*

- High concentration in carbonic acid and carbon dioxide may have an effect on microbial metabolism, in particular in the important reaction of carboxylation and decarboxylation;
- Carbonic acid and carbon dioxide may precipitate intracellular inorganic electrolytes, such as Ca^{2+} and Mg^{2+}, resulting in deleterious effects on the volume of cells;
- The high solvating power of CO_2 suggests it extracts vital constituents from the cells, like phospholipids and hydrophobic compounds, necessary to the cell's structure and balance of biological system. This removal process appeared to be caused by the sudden release of the applied pressure, thereby transferring intracellular material out of the cell into the extracellular environment.

A picture summarizes the entire mechanism of bactericidal inactivation (Figure 1.3).
The optimization of the HPCD pasteurization process consists in determine the most efficient operative conditions in order to determine the shortest treatment time. Actually, inactivation of microorganisms depends on several factors. The most important ones are pressure and temperature. In general, microbial inactivation is accelerated with increasing CO_2 pressure. In other words, at higher pressure, shorter exposure time is needed to reach the same degree of inactivation. Increasing pressure means that CO_2 is more solubilized in the medium, thereby facilitating the acidification of it. The microbial inactivation is also sensitive to the operative temperature. As it is expected, the inactivation rate increases with increasing temperature, because this condition stimulates the diffusivity of CO_2 and enhance the fluidity of the cell membrane, resulting in a membrane that is easier penetrable. Other factors influencing the inactivation of vegetative cell included the physical state of CO_2, the agitation, the water content, the properties and pH of the suspending medium.

Pasteurization by means of supercritical carbon dioxide fascinates numerous researchers: therefore, a wealthy literature could be found on this technique.

Spilimbergo *et al.*, 2012, performed some experiments on fresh-cut carrot. The results showed that increasing the operative temperature from 22° to 45°C, at a constant pressure of 12 MPa, induced an increase of inactivation rate: after 15 minutes of treatment, 3,5 log reductions of mesophilic bacteria were achieved at 45°C, where only 2,5 log at 22°C. In addition, the effect of pressure was investigated. Microbial inactivation was observed in 5 minutes at 8 MPa, while the process took only 3 minutes in case of 12 MPa, both at 35°C.

Few months later, Ferrentino *et al.*, 2012, performed HPCD process on cubed cooked ham, in order to optimize the process and the natural microbial flora inactivation. Ham was treated at different conditions of temperature (40°, 45° and 50°C), pressure (8, 10 and 12 MPa) and treatment time (5, 10 and 15 minutes). Results revealed that 12 MPa, 50°C and 5 minutes were the optimal conditions to obtain a mesophilic bacteria inactivation of 3 log-cycle and a high quality product. Therefore, it was confirmed that increasing operative pressure and temperature leads to a higher inactivation rate. As specified in literature, high temperatures stimulate the diffusion of CO_2 and increase the fluidity of cell membranes to facilitate the gas penetration, causing metabolic alteration responsible for cellular death.

Ferrentino *et al.*, 2012, went on carrying experimental trials on pieces of coconut, replicating the same results. At a constant temperature of 35°C and after 60 minutes of treatment, 2.5 log reductions were achieved at 8 MPa, whereas 3.5 log reduction were obtained at 12 MPa. At 25°C, no pasteurization was observed in the food product, due to the CO_2 phase: over the critical temperature in fact, the solvating power of the gas incredibly increases with respect to sub-critical temperatures.

Next, Cappelletti *et al.*, 2015, proved the efficacy of the pasteurization technique of porcine raw meat, where a reduction of 2 Log (CFU/cm^2) in the total mesophilic load was reached. The inactivation kinetics of bacteria were determined at 25°, 35° and 40°C, from 6 to 16 MPa and from 5 to 60 minutes. An increasing in temperature, led to a decreasing in the treatment time: 2 Log CFU/cm^2 was achieved in 60 minutes at 25°C or in 45 minutes at 35°C, or in 20 minutes at 40°C. Again, the optimal conditions were the most severe ones. On the other hand, higher pressures permit an increasing in density and solvation power of CO_2, but no significant results were obtained by modify the operative pressure from 6 to 16 MPa.

Conversely, towards latent forms of microorganisms, literature is scarce and conflicting, as highlighted by Spilimbergo and Bertucco, 2003. However, the majority of the research studies agree with the statement that they are less attachable by SC-CO_2 alone, since spores are the most resistant form of life. Recently, the so-called “hurdle approach” (or combination of more than one process) has been investigated, representing a valid method to inactivate spores at low temperatures.

Table 1.6. *Main advantages and drawbacks exhibited by supercritical carbon dioxide pasteurization (Garcia-Gonzales et al., 2007).*

Opportunities	Threats
Natural image	Inactivation mechanism not entirely clear
High fresh-like organoleptic quality	Extraction of/interaction with food ingredients
Spores can be inactivated when combined with e.g. heat, pressure pulsing and acid environment	Occurrence of HPCD-resistant mutants still needs to be investigated
Applicable to acid foods	Inactivation conditions required dependent on type of food and type of microorganism
Upscaling of equipment has been developed for other applications (e.g., extraction)	Large investment costs
Continuous processing for liquid foods is possible	Processing-related problems for solid foods (discontinuous in nature, limited diffusion of CO_2, packaging after treatment, ...)

Other food matrices have been treated so far, both liquid and solid (coconut water, Cappelletti *et al.*, 2014; liquid whole egg, Garcia-Gonzales, 2009; strawberry juice, Marszalek *et al.*, 2015; pieces of pear, Valverde *et al.*, 2010; etc.). The results of all the experiments appear to confirm the mechanisms of microbial inactivation of mesophilic bacteria and, therefore, the efficacy of pasteurizing by means of supercritical carbon dioxide.

1.3.2 Quality changes after SC-CO_2 drying

Many academic publications describe modifications in organoleptic properties of pasteurized products by means of supercritical carbon dioxide (Table 1.7). Yet, little is known about

Table 1.7. *Description of the effect of HPCD on some quality attributes for different food matrices (Ferrentino and Spilimbergo, 2012).*

Food	Observations	Reference
Strawberries, honeydew melon, cucumber	Tissue destruction	(Haas *et al.*, 1989)
Chives, oregano	Enhanced aroma	
Parsley	Similar taste of the untreated sample Slight off aroma	
Thyme	Worst taste compared to the untreated sample	
Mint	Better taste compared to the untreated sample Enhanced aroma	
Chicken meat and shrimp	Color change to whitish Cooked appearance Loss of liquid	(Wei *et al.*, 1991)
Ground beef	Color change to dark Cooked appearance	(Sirisee *et al.*, 1998)
Kimchi	Higher pH Lower titratable Better sensory properties No significant color, flavor and texture changes	(Hong & Park, 1999)
Alfaalfa seeds	No detrimental effect on viability of the seeds No detrimental effect on germination rate of the seeds	(Mazzoni *et al.*, 2001)
Ground beef	Higher tenderness No significant changes in juiciness, flavor intensity and off flavor intensity	(Meurehg, 2006)
Cocoa powder	Decreased water content No effect on physical aspect	(Calvo *et al.*, 2007)

changes of these features as a consequence of supercritical CO_2 drying, due to the lack of literature on this issues.

The first publication reporting the possibility of drying using supercritical carbon dioxide (Brown *et al.*, 2007), took on the modification of the microstructural characteristics of carrot pieces. Samples dried using SC-CO_2 retained the original volume and shape, presented less shrinkage and lower density. An even greater retention of these properties is observed in the sample dried with modified supercritical carbon dioxide (6 mol% of ethanol added). The preservation of the structure and the less shrinkage experienced by the product can be explained with the particular advantage of supercritical fluid drying of eliminate the vapour-liquid interface and capillary stresses.

They also have a faster instant rehydration rate than those samples dried using traditional techniques. Puncture test suggests that the lower density dried samples (dried with supercritical carbon dioxide) have a softer texture when rehydrated than those with low porosity and high density (obtained after air-drying).

About color, carrots that have been dried with supercritical carbon dioxide appeared to be paler than raw carrots. However, this is a reversible condition probably due to the decrease in the transparency of the cell walls during drying, because the color return significantly when the product is rehydrated. In contrast, air-dried carrots are darker than the untreated ones.

The supercritical carbon dioxide drying effect on bioactive compounds and sensory properties of a food product is investigated by Busic *et al.*, 2014, on basil samples. According to the publication, SC-CO_2 dried basil was compared to air-dried and freeze-dried in terms of content of chlorophyll, ascorbic acid, essential oil, antioxidant, polyphenolic and flavonoid compounds. The results pointed out that freeze-drying was the most suitable technique for the preservation of color, essential oil content and antioxidant capability of basil. However, drying with scCO_2 could be an alternative technique to conventional methods when shorter drying times are employed. The research was completed with a panel test, where trained judges evaluated basil tastes and aromas: SC-CO_2 dried basil appeared to be the most appreciated product. In fact, SC-CO_2 dried basil obtained higher scores in terms of green color intensity and uniformity, as well as in the taste. However, further optimization of the process should be performed to improve the aroma of the treated sample.

At present, literature lacks of publications focused on food quality aspects after supercritical carbon dioxide drying. Further studies are necessary to assess the organoleptic features and the visual characteristics (like the color) of the product.

1.4 Combined processes

The hurdle approach, or the combination of existing and/or novel techniques for food preservation, applied in series or in parallel, appears to partially overcome the limitations

related to the poor effect of supercritical carbon dioxide on spores (§1.3.1) and to provide an additional effect of microbial reduction on the sample, thereby accelerating the entire process.
According to Pataro *et al.*, 2013, the combination of PEF (Pulsed Electric Field) and HPCD treatment resulted in a significant microbial inactivation of *E.Coli* in a buffer solution. A decrease in the microbial load was observed with increasing the field strength, energy input, holding time and operative pressure. In another work by Liu *et al.*, 2011, the combined effect of HPCD process and mild heat treatment (55°C maximum) on the overall quality of watermelon juice has been investigated. Data showed a decreasing in the total amount of the enzymes responsible for the degradation of the sample (polyphenoloxidase, peroxidase and pectine methyesterase) at a lower operative pressure and lower treatment time with respect to the two treatments alone. Moreover, ultra-high pressure in combination with HPCD appeared to enhance the damaging of the structure of the enzymes and microbial cells. The work by Qianlin *et al.*, 2016, has concerned the effect of this combination on PPO (polyphenol oxidase) from *Litopanaeus vannamei*. Results has showed that the combined treatment not only shortened the inactivation time, but it also led to the irreversible denaturation of PPO.
Above all, the use of high power ultrasound (HPU), sound waves with a particular frequency (frequency>20 kHz) represents an efficient way to enhance and accelerate food processing.
The mechanism of acoustic-assisted drying has not been complete explained, but it is connected to a combination of different effects involving both internal and external mass transfer. When ultrasound waves encounter any deviation of particles, they pass some amount of energy to the adjacent particle, in any media, both in air and in water. As a result, disturbances spread in the medium in a cyclic manner, in the form of longitudinal waves, thereby creating rapid compressions and rarefactions, cavitation and bubble formation (Mulet *et al.*, 2003). In a liquid media, in particular, small bubbles are formed when cavitation occurs, and, if they are of a critical size, they explode releasing energy in the form of impulses, which can have a local temperature of 1000 K and pressure of 1000 atm. However, bubbles explosion do not raise the overall temperature of the media. This phenomenon results in structural disintegration and energy dissipation to the medium, as specified by Mason *et al.*, 1998, thereby leading to cell permeability. Consequently, mass transfer during drying or dehydration process increase, without introducing a high amount of thermal energy during drying.
Therefore, it is clear that the use of ultrasound has a great potential in the treatment of heat-sensitive matrices. The main drawback, however, is the transmission of the wave from the emitter's surface to a solid sample: air absorbs the acoustic energy preventing its transfer to the solid food.
Nevertheless, regarding pasteurization, only ultrasounds are not sufficiently severe to reduce the product microbial content, resulting in a non-pasteurized product. The hot zones generated in the medium are so localized, that bacteria do not died (Chemat *et al.*, 2011). An effective microbial inactivation can be achieved by combining ultrasound with supercritical carbon

dioxide pasteurization technology. Besides, the use of the supercritical fluid as a medium seems to overcome the HPU drawback in the application in solid products, as confirmed by Gao *et al.*, 2009.

Numerous evidences that could be found in literature underpin the efficiency of the combination of HPU with HPCD.

Ortuno *et al.*, 2012, combined ultrasound properties with HPCD bactericidal effect on *E. Coli*. A HPU unit was designed, embedded in the HPCD plant, and it was turned on when the desired pressure in the vessel was reached. The applied power during the experiments was 40W and it was maintained constant. Remarkable differences in the microbial inactivation rate were evident between the results of HPCD only treatment with the HPCD+HPU one. At a constant temperature of 36°C, 1.5 minutes of HPCD+HPU treatment were necessary to reach a reduction of 8 log-cycle, at 100, 225 and 290 bar. On the contrary, in HPCD treatment at 36°C, the survival curve showed an initial lag phase followed by a log-linear section. As the pressure increased from 225 to 290 and 350 bar, the lag phase decreased from 30 to 20 and 15 minutes, respectively. The lag phase probably represented the rate of penetration of CO_2 into the cell, which, in fact, depends on the pressure. At a constant pressure of 225 bar, the results of the two treatment were compared for different temperatures. In the HPCD treatment, a reduction of 8 log-cycles was achieved in 75 minutes at 31°C, in 55 minutes at 36°C, and in 40 minutes at 41°C: higher temperature enhanced diffusivity of CO_2 into the cell thereby accelerating the process. Thanks to the application of HPU, the treatment time decreased of 96% with respect to the time required in the HPCD process. In fact, a complete microbial inactivation (8 log-cycle) was achieved in only 2 minutes.

Spilimbergo *et al.*, 2014, have widely investigated the combination of HPU+HPCD in processing of dry cured ham, both on naturally present microorganisms and on specially inoculated bacteria. In the experimental procedure, the applied power was maintained constant (10±2 W) and ultrasounds were generated in cycle of 2 minutes, in order to allow the product to cool down. At 35°C and different values of operative pressure, the synergistic effect of HPCD+HPU treatment appeared to be more efficient than HPCD alone: HPCD alone induced in 30 minutes 3.4, 4.3 and 7 Log reductions at 6, 8 and 12 MPa respectively, whereas HPCD+HPU about 7.5 Log reductions after only 8, 6 and 5 minutes at 6, 8 and 12 MPa respectively. The ultrasound effect probably caused a better contact of the microorganisms and the pressurized CO_2, resulting in the acceleration of the diffusion of CO_2 through the cell membrane and a decreasing in the intracellular pH. These phenomena led to the bacteria's death.

Regarding the application of HPU in combination with supercritical carbon dioxide drying process of food, no examples can be found in literature so far.

In conclusion, HPCD+HPU is a novel promising technology to enhance pasteurization of food matrices. Thanks to its agitation effect, it allows the supercritical carbon dioxide to penetrate easier into cells, thereby improving the mass transfer and the microbial death process.

1.6 Motivation and aim

University of Padua, University of Ghent and University of Belgrade, with the support of two companies (FeyeCon and VNK Herbs B.V., specialized in production and processing of vegetables) and the Swedish Institute for Food and Biotechnologies (SIK), are involved in the European project called Future Food (Faster Upcoming Technology Uptake Relevant for the Environment in FOOds Drying). The project is supported within the Horizon 2020 Research and Innovation Program and its aim is to determine the feasibility of the supercritical carbon dioxide process as a technique after which the product is stable and microbiologically safe, and maintains its qualitative and quantitative attributes.

Traditional thermal treatments, like hot air drying and freeze-drying, present some limitations in the quality of the treated product, as well as in the overall cost of the process. While hot air drying is a relatively inexpensive technique, it provides a very poor quality product, in terms of retained amount of nutrients, due to the high temperatures involved in the process. On the other hand, freeze-drying maintains the color and the shape of the dried sample, but its energy consumption and its drying times are extremely high, resulting in a deeply expensive process. Supercritical carbon dioxide drying seems to combine the answer to the increasing demand of the consumer for high quality minimally processed food, food safety, strictly connected to the bactericidal property of it, and the economy of the process.

In the last decades, University of Padua's research group, led by Professor Sara Spilimbergo, has been involved in an intense research activity regarding the possibility of pasteurizing food matrices by means of supercritical carbon dioxide. Under these circumstances, her lab's specific task within the European project is to perform lab-scale experiments with vegetable matrices to both optimize the microbial inactivation efficiency during CO_2 drying and determine the preservation of nutrients after the process.

In particular, the present Thesis gathers all the preliminary experimental trials performed on this background. As a case study, coriander leaves were chosen as the vegetable matrix. Coriander is used in cuisines throughout the world, both in food preparations and as ready-to-eat food. Since it grows on the ground, it naturally contains a high amount of microbial load and its leaves spoil quickly when removed from the plant.

Both pasteurization and drying of coriander leaves were performed, following the procedure schematized in Figure 1.4. The optimum parameters of the process, such as the drying time and the operative temperature, were defined in order to improve the microbial inactivation.

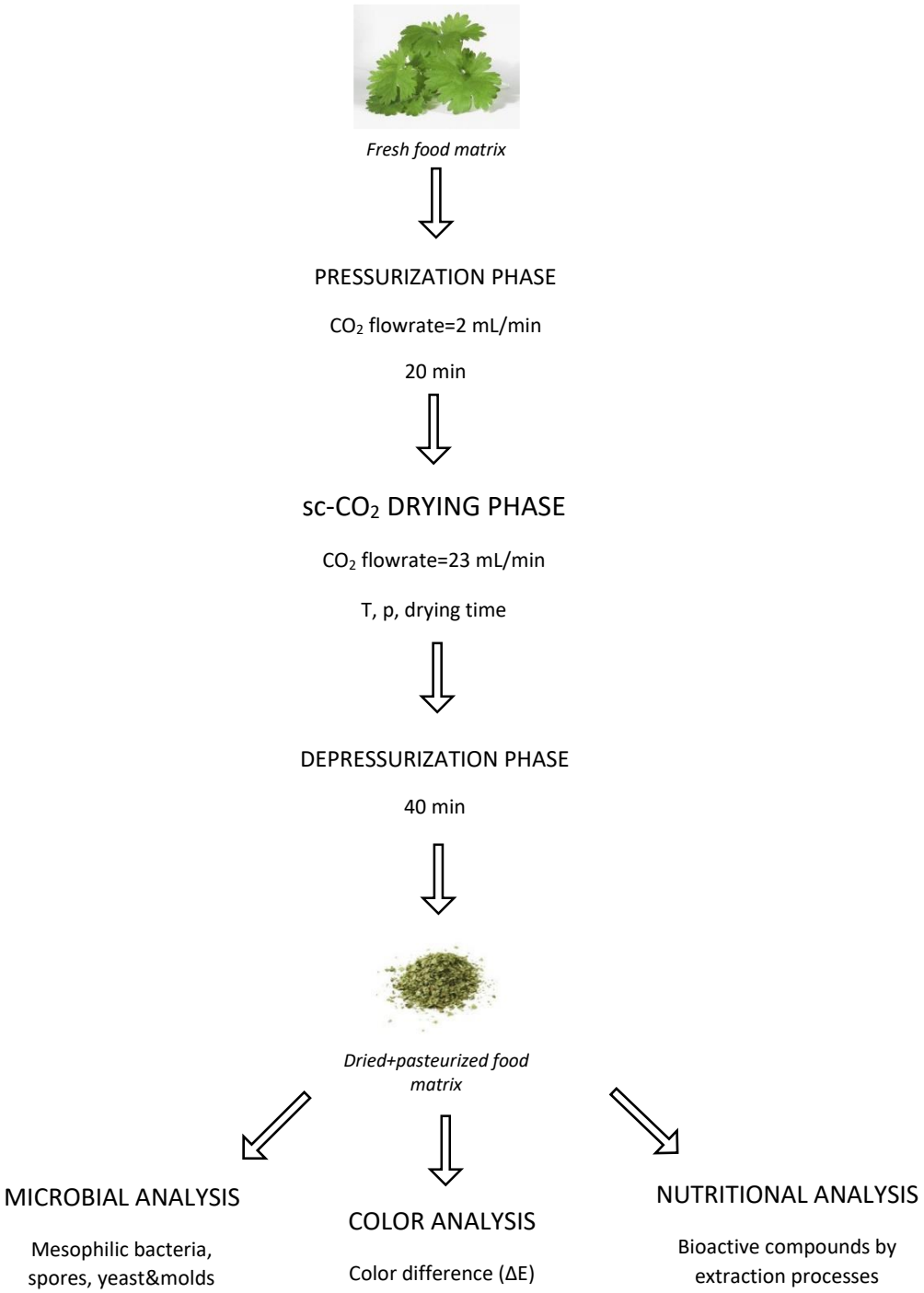

Figure 1.4. *Scheme of the process.*

In order to overcome one of the major issue exhibited by food pasteurization with SC-CO_2, that is the poor bactericidal property towards spores, hurdle approaches have been developed in the last few years. With this intention, the combined HPCD treatment with ultrasound technology (HPU) was applied on both pasteurization and drying of coriander samples. In particular, the

Thesis addresses some preliminary studies of the combination of supercritical CO_2 drying with ultrasounds, which is a completely novel technique.

In addition to the microbial analysis, a quantitative assessment of quality changes after the process was performed. The quality of the treated sample was evaluated in terms of color variation and retention of nutritional attributes, with respect to the fresh sample.

Chapter 2
Material and methods

In the present section, the plants exploited for the experimental runs are described: the HPCD lab-scale semi-continuous reactor is used for the pasteurization and the drying experimental trials. A conveniently designed sonotrode can be inserted in the HPCD reactor in order to perform combined HPCD+HPU experiments. The procedures used to run these plants are described in detail. Then, the air drying procedure will follow. Besides, the explanation of the analysis performed on the treated food matrix will be reported. In particular, the microbial analysis, in order to assess the microbial load before and after the process; the nutritional analysis, executed to estimate the retention of nutrients; and the color analysis, in order to quantify the color differences between the fresh and the treated sample.
All the experimental trials were performed on coriander leaves (*Coriandrum Sativum L.*).

2.1 HPCD equipment

The simplified PFD of the plant used for HPCD experimental trials is reported in Figure 2.1. The plant included the CO_2 tank, a chiller reservoir (WLK 1200 Lauda), a HPLC volumetric pump (SC-type piston pump, Model 307, Gilson), and the vessel (Separex S.a.S.), which was kept at the desired pressure by means of a thermostatic bath (ME, Julabo).
The liquid CO_2 is contained in the cylinder and stored at 60 bar. During the experiment, it was refrigerated in a cryostatic bath (M9000-TI, MPM Instruments, Bernareggio, Italy; M418-BC) and pressurized to the operative pressure by the HPLC volumetric pump, which has a maximum flowrate of 23 mL/min. The chiller maintained the head of the pump at a temperature of around 0°C, to keep the CO_2 liquid and avoid the cavitation of the pump. The fluid entered into the reactor from the top and exited from the bottom. A micrometric valve (Swagelok) mounted on the exit line of the reactor allowed the whole apparatus to depressurize. Rapid valve opening

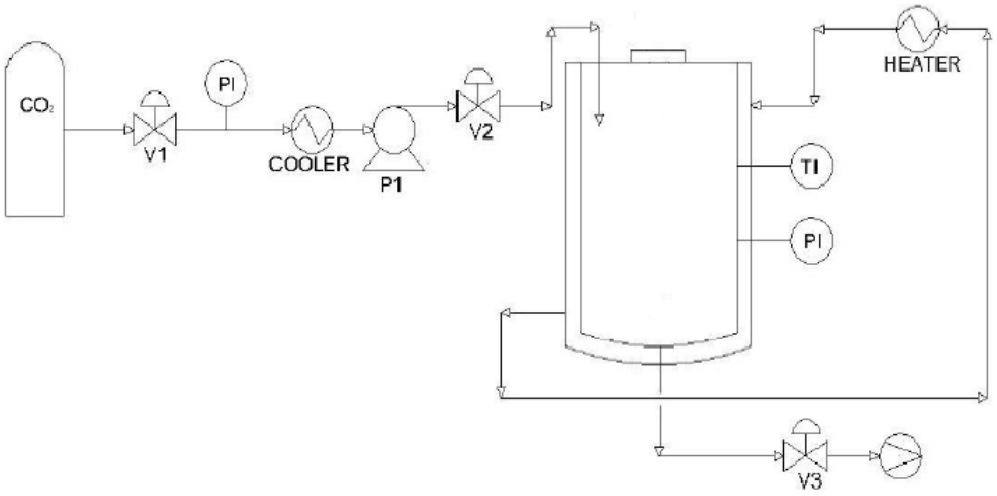

Figure 2.1. *Schematic representation of the HPCD process (taken by Calliari N., 2013).*

lead to the freezing of it, for this reason it was maintained at a constant temperature (around 40°C) by means of a thermostatic bath (ME, Julabo). In the case of batch operations, the valve was maintained closed during the experiment. In order to run the reactor in a semi-continuous way, the valve was manually opened to maintain a constant pressure inside the vessel. This kind of operation is exploited during the drying step of the experiments.

As far as the reactor is concerned, it consisted in a sapphire high pressure visualization cell (Separex S.A.S., France) with an internal volume of 50 ml (2.5 cm of diameter, 100 mm of height) designed to withstand up to 400 bar and 100°C. The entire reactor chamber was surrounded by a 100-ml jacket, which, when filled with water, was exploited to keep it at the operative temperature, by means of a thermostatic bath. The cell was also equipped with a safety device that consisted of a rupture disk calibrated to 400 bar. A thermocouple and a manometer were installed in order to measure and show the temperature and the pressure inside the vessel, respectively (Figure 2.2).

In Figure 2.3 the real lab-scale plant is illustrated.

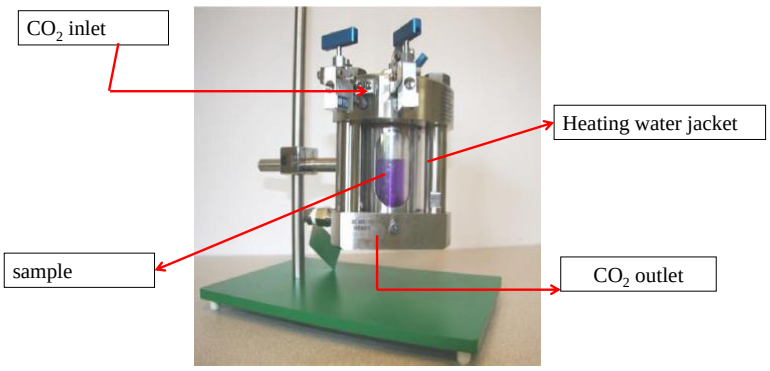

Figure 2.2. *HPCD high pressure sapphire cell.*

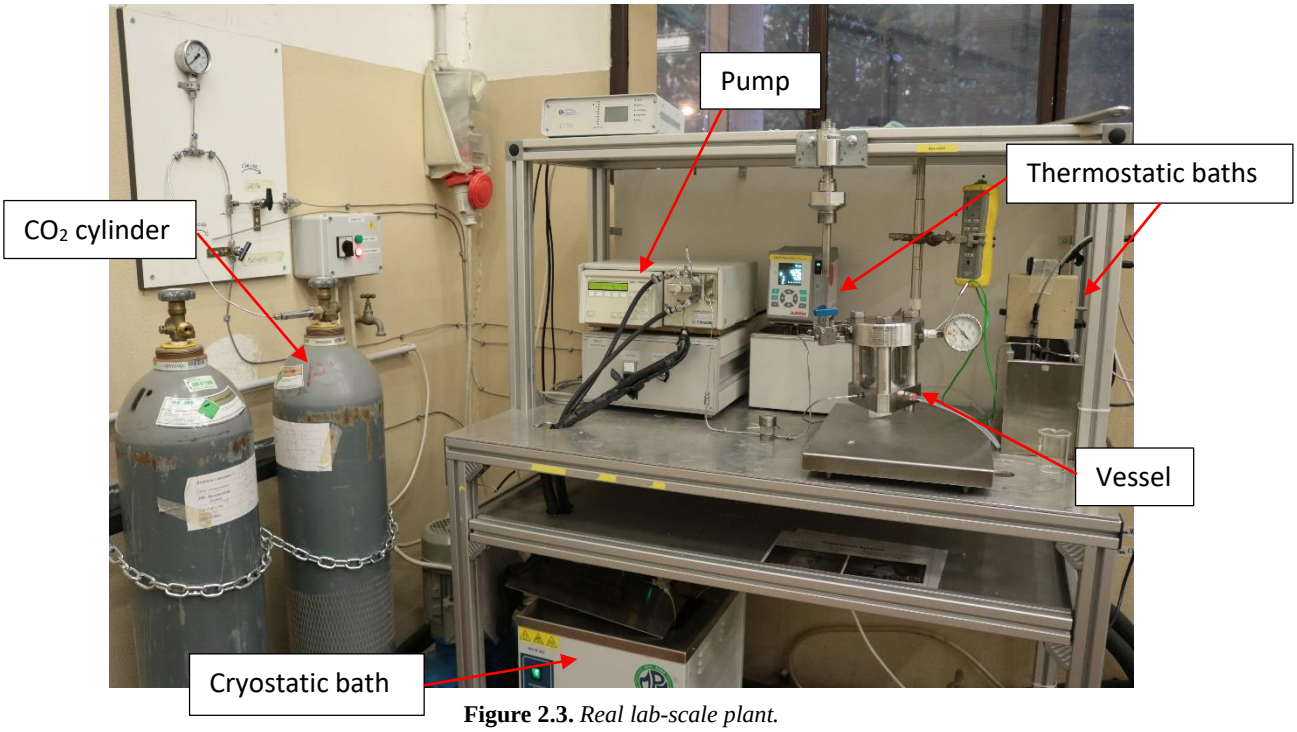

Figure 2.3. *Real lab-scale plant.*

2.2 HPCD experimental procedure

The coriander (*Coriandrum sativum L.*) was purchased from a local market in Padova, stored at 4°C and treated within 3 days after the purchase. Only the leaves were used for the experiments, selected by similar dimension and color. They were not washed in order to have a high initial contamination. For each test 1g (±0.1g) of product was used.

Before each treatment, the vessel was cleaned with ethanol filled up to the top, washed with sterile deionized water and flushed with CO_2 for few minutes in order to remove the water residues. The coriander samples were weighted inside a metallic basket that was previously cleaned with absolute ethanol (Sigma Aldrich, 99.8%) and burned with a Bunsen flame. The basket was then inserted inside the vessel by means of a tweezer, previously washed with ethanol and burned. Sterilizing tweezers that get in contact with the untreated sample and the basket before the treatment was important to reduce the contamination coming from these tools. In other words, only microorganisms naturally present in the untreated coriander leaves were present in the vessel before each treatment. The complete sterilization procedure is described in detail in Appendix A.

The HPCD process consisted in three main phases i) pressurization; ii) drying and iii) depressurization. At the beginning, the outlet valve is closed and the pressurization step starts when the CO_2 cylinder is opened and the pressure in the system increases up to 60 bar (which corresponds to the pressure value of CO_2 inside the tank). Then the pump is turned on to reach the operative set-up pressure of 100 bar. Obviously, the pressurization time depends on CO_2 flow rate (FR), as shown in Figure 2.4.

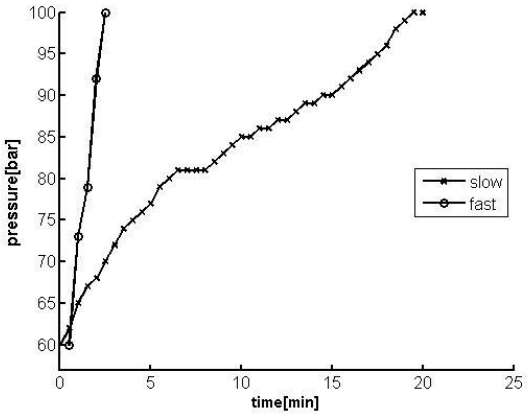

Figure 2.4 *Pressurization rate as a function of flow rate. Slow pressurization occurs in 20 minutes (FR= 2 ml/min), while fast pressurization occurs in 2 minutes (FR=23 ml/min).*

The pressurization rate depended on the CO_2 flowrate: 2 ml/min corresponded to a total pressurization time of about 20 minutes, and 23 ml/min, the maximum flow rate offered by the pump, led to a pressurization time of about 2 minutes.

As soon as the pressure reached 100 bar, the drying treatment started, at a constant CO_2 flow rate (23 ml/min), slightly opening the outlet valve to maintain the pressure constant. The drying time was set at 2,5 h.

After the treatment, the vessel was depressurized following the pattern described in Table 2.1.

Table 2.1. *Depressurization rate.*

time (min)	Pressure (bar)
0	100
5	70
10	40
15	30
20	22
25	15
30	10
35	6
40	1

The depressurization step was maintained constant in all the experimental runs performed.
When the pressure inside the reactor reached the atmospheric value, the vessel was opened and the basket was retrieved from the reactor close to a Bunsen flame with sterile tweezers, previously washed with ethanol, collected in a sterile tube and weighed to calculate the mass loss during the process. The treated coriander leaves were extracted from the basket under a laminar flow hood with sterile tweezers, in order to not contaminate the sample after the treatment.
The coriander mass loss was calculated as

$$mass\ loss = \left(1 - \frac{m_{dry}}{m_{fresh}}\right) * 100\% \tag{2.1}$$

Where m_{dry} and m_{fresh} indicate the mass of the sample after and before the process, respectively. The mass loss represents the mass of water extracted during drying.
Later, microbiological assessment on the dried sample has been done, as well as color analysis. Coriander leaves were treated by HPCD at different drying times and temperatures. A list of all the parameters involved in the experiments is reported in Table 2.2.

Table 2.2. *Value of parameters tested in the HPCD treatment.*

Parameter	Values tested
Pressure	100bar
Temperature	40°C, 50°C
Pressurization flow rate	2 mL/min
Drying flow rate	23mL/min
Drying time	0 min, 150 min
Depressurization time	40 min

All the experiments, at every process conditions, were performed in triplicate.

2.3 Medium-scale HPCD plant

A medium HPCD plant has been used to run experimental trials on other food matrices, different from the coriander. It is located in Weesp, The Netherlands, and owned by FeyeCon Development & Implementation B.V.. A schematic representation of it is reported in Figure 2.5.

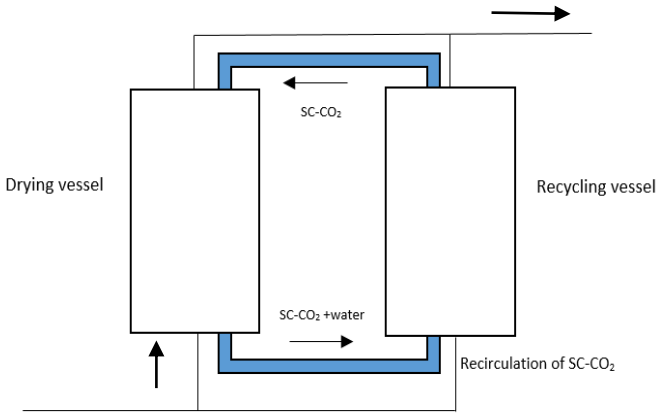

Figure 2.5. *Scheme of FeyeCon's medium-scale reactor of supercritical carbon dioxide drying.*

Basically, the medium-scale plant was similar to the one used to perform the experiments explained in the Thesis, except for the configuration of the reactor. In fact, its volume was 2 liters. Moreover, the supercritical fluid stream was recirculated. Actually, during the process, supercritical carbon dioxide extracted water, which was carried in a second vessel. In the latter, zeolites (solid desiccants) removed water from the supercritical gas flow and, consequently, it was recycled back to the first reactor, where a new cycle begins. This configuration represents a great advantage with respect to the cost of the entire process, since the supercritical carbon dioxide is fed only before the first cycle. In addition, the recirculation of the fluid enables the food matrices to retain their nutritional attributes. In fact, unlike the lab-scale configuration, where the CO_2 is released in the atmosphere, in this case the un-polar compounds extracted by the fluid were recirculated back to the food.
In Figure 2.6, two examples of supercritical carbon dioxide dried foods are represented.

Figure 2.6. *Examples of supercritical carbon dioxide dried food matrices (basil and red pepper).*

2.4 Combined HPCD+HPU equipment

The HPCD apparatus, described in §2.1, was equipped with an ultrasound system (Aktive Arc Sarl, Switzerland) designed on purpose and embedded in the plant. The system consisted of a transducer (40 KHz), a buster, a special retainer (M36x1.5), a sonotrode and a power generator unit, in which menu it was possible to control the amplitude, directly related to the output power, the treatment time and the energy supplied during the experiment. The ultrasonic sonotrode assembly and the power generator unit are reported in Figure 2.7 and 2.8.

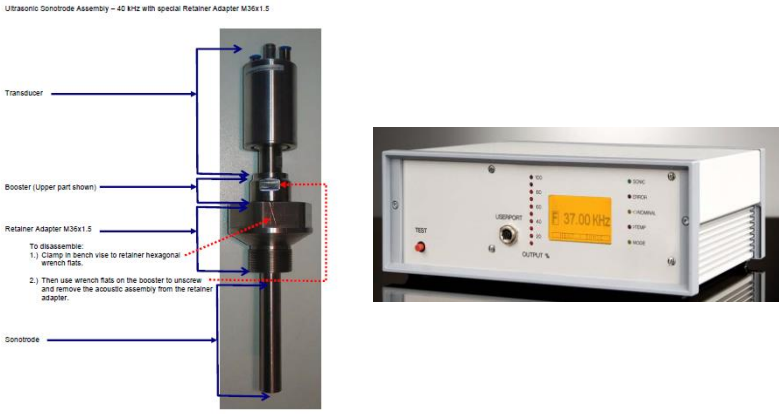

Figure 2.7. *Ultrasound system and the power generator unit designed for the high pressure sapphire cell.*

Figure 2.8 *Modification of the HPCD reactor when the sonotrode is inserted.*

The ultrasonic system was designed to give consistent amplitude (peak-to-peak displacement) to the sonotrode tip. When the sonotrode was used in various medium (solid or liquid) and pressure conditions, the effective loading imposed on the sonotrode body caused the change of the output power in order to maintain a constant amplitude. However, in these experimental trials, the output power, which linearly increased with the amplitude, was kept constant acting on the modification of the value of the amplitude.

2.5 Combined HPCD+HPU experimental procedure

In the experimental trials with HPU, the operative procedure was quite the same as the HPDC only process. At first, the reactor and the sonotrode, which served as a closing for it, were washed with ethanol (Sigma Aldrich, 99,8%) and sterile distilled water. The entire apparatus was flushed with CO_2 to eliminate any ethanol and water residues. In the meantime, the metallic basket was washed with ethanol and coriander leaves were initially weighted and inserted inside. The sample contained in the basket was put in the sterilized reactor.

Also in the HPCD+HPU treatment there were three main phases: pressurization, drying and depressurization. HPU started with the opening of the CO_2 cylinder that was the beginning of the pressurization step. During the pressurization step, the power provided by the ultrasonic system was maintained at a constant value, modifying the amplitude of the generator given to the sonotrode. To avoid an overheating of the sample, experiments were conducted in cycle of 10 seconds of HPCD+HPU and 10 seconds of HPCD alone, for the entire duration of the pressurization.

When the pressure reached 100 bar, the drying step started, at a constant CO_2 flow rate (23 ml/min). The drying time was modified in the experimental trials. HPU were working during the entire drying time, in cycles of 10 seconds.

After the treatment, the system was depressurized following the pattern showed in Table 2.1 and it remained constant during the experiments. Then the basket inside the vessel was extracted with sterile tweezers and weighted. The coriander leaves were then collected from the basket in a laminar flow hood and weighted, thereby calculating the mass loss with (2.1). The mass loss indicates the extracted mass of water during the process.

The coriander leaves were treated by HPCD+HPU at different drying times, powers and temperatures. A list of all the parameters involved in the experiments is reported in Table 2.3.

Similarly to HPCD only experiments, also the HPCD+HPU experimental trials were performed at least in triplicate.

Table 2.3. *Values of the parameters tested in the combined HPCD+HPU treatment.*

Parameter	Values tested
Pressure	100bar
Temperature	40°C, 50°C
Pressurization flow rate	2 mL/min
Drying flow rate	23mL/min
Drying time	0 min, 75 min, 150 min
Power	0W, 10W, 40W, 80W
HPU cycle	10s ON 10s OFF
Depressurization time	40 min

2.6 Air drying

In order to compare the results obtained during drying by means of SC-CO_2, experimental trials were performed using conventional drying methods, in particular air-drying.

Initially, the coriander leaves were chosen for similar color and freshness. In these experiments the stem was not considered, too. Then, coriander was put on a small piece of aluminum foil, which served as a small box to contain it, and weighted (1±0,1 g). The leaves were collected using a sterile tweezer, previously washed with absolute ethanol (Sigma Aldrich, 99.8%) and burnt with a Bunsen flame. This shrewdness was necessary to ensure that all the microorganisms in the leaves were naturally present and not brought by the tools used during the manipulation of them.

The drying took place in a static heater (Memmert), where no air was allowed to enter, at 80°C for 5 hours. The operative conditions were fixed during all the experimental trials, which were performed in triplicate.

At the end of the process, the residual coriander was extracted from the heater and was closed in the foil, in order to prevent an external contamination after drying. Then, it was weighted thereby calculating the mass reduction (with (2.1)), which corresponded to the water loss of the sample. In a laminar flow hood, the foil was opened and the sample was extracted aseptically.

2.7 Microbial characterization

In all the experimental trials, both drying with SC-CO_2 and conventional drying, the effects of the treatment were observed on the inactivation of the microflora naturally present in the food matrix (mesophilic bacteria, mesophilic spores, yeast and molds).

The microorganisms in the fresh untreated coriander were initially enumerated. After the process, the natural flora was assessed on the same day for all the treated samples with the

standard plate count procedure (ISO 6887-1, 1999; ISO 6887-2, 2003; ISO 7218, 2007) using a specific media (ISO/TS 11133).

The media were prepared dissolving a certain amount of powder (indicated on the packaging and specific for the media) in one liter of distilled water. Then, the bottle was stirred to the complete dissolution and sterilized in a retort (Sterilsteam 2) for 15 minutes at 121°C in order to eliminate any form of external contamination. Every different microorganism grows on a specific media, as indicated by Rinaldi Lazzerini and Tinti (2012).

The procedure initially consisted in diluting the sample in 10 ml of mq-sterilized water and shook it for one minute. Next, dilutions series were prepared adding 1 ml, taken from the 10-ml solution, to 9 ml of pure mq-sterilized water. For example, 1 ml of the initial solution (at zero dilution) was transferred to another tube that contained 9 ml of mq-sterilized water in order to constitute a 10-time diluted new solution. Successive dilutions were prepared following this procedure.

Then, 1 ml of all prepared solutions was pour-plated onto PCA medium (ISO 4833, Liofilchem, Teramo, Italy) in order to detect mesophilic bacteria.

Yeasts and molds were spread-plated with a glass stick, previously sterilized with ethanol (Sigma Aldrich, 99.8%) and burned with a Bunsen flame, onto the selective medium DBRC (ISO 21527, Liofilchem, Teramo, Italy). 100 µl of the diluted solutions were spread.

For the enumeration of mesophilic spores, the dilution tubes were inserted in a thermostatic bath (Memmert) at 80°C for 10 minutes. Then 1 ml of each solution was pour-plated onto PCA medium.

All the plates were prepared in duplicate and under a laminar flow hood (Asalair vertical 700 laminar flow), that prevented microbial agents present in the air to contaminate the plates.

Table 2.4 shows the analyzed population, the media and the conditions used.

Table 2.4. *Media and incubation conditions for the different microbiota.*

microorganisms	medium	incubation time (h)	incubation temperature (°C)	pour or spread plate
mesophilic bacteria	PCA	72	30	pour
mesophilic spores	PCA	72	30	pour
yeasts and molds	DBRC	72-120	22	spread

After the incubation time, the microorganisms in the plates were enumerated, compared with the microorganisms naturally present in the untreated coriander, detected with the same method, and referred to the weight of the initial fresh sample.

The enumeration was executed in plates that contained from 20 to 300 colonies. This upper limit corresponds to the maximum number of microorganisms detectable in a plate. In other words, if the number of colony counted in a plate is greater than this value, the plate is

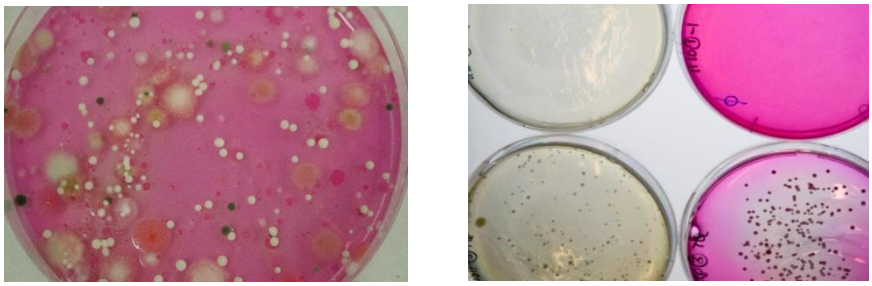

Figure 2.9. *Examples of "over counting" plate and "under detection limit" plate (clear).*

considered as "over counting" and do not produce a significant result. Microbiological count has to be done in the lower dilution plate.

The limit of quantification, the lower limit necessary to produce a quantitative result, was set at 20 CFU/ml for the pour plates and 200 CFU/ml for the spread plates.

The other limit to take into account is the limit of detection, set to 10 CFU/ml for the pour plate and to 100 CFU/ml for the spread plates. Under this value the plate is clear, i. e. no colony is present (Figure 2.9).

The results of the count were expressed in CFU/ml (colony forming unit per milliliters) and obtained multiplying number of microorganisms directly counted on the plate (NC) for the dilution in which it was counted and divided by the exact mass of coriander used for the zero dilution (m_{sample}):

$$N = \frac{NC * d * 10}{m_{sample}} \tag{2.4}$$

Where N is expressed in CFU/ml and represents the number of microorganisms, d is the dilution in which colonies were counted (for example the third dilution is diluted a thousand times from the zero solution, so d=10^3) and 10 is necessary to refer the measure to the initial 10 ml of solution (because a plate contains 1 ml of solution). In the case of yeast and molds, the number of microorganisms has to be multiplied for 100, since in the plate there are 100 µl of solution.

As the number of microorganisms was determined, the degree of inactivation was calculated and expressed as Log(N/N_0), where N_0 (in CFU/ml) was the number of microorganisms initially present in the untreated sample and N (in CFU/ml) was the number of survivors after the treatment.

For each treated sample, one experimental trial was performed but, since two plates were prepared from the same diluted solution, the result represented the mean value of two measurements, with their standard deviations.

2.8 Nutritional analysis

To investigate if supercritical treatments represent a valid alternative to conventional pasteurization and drying methods, not only the microbial inactivation is considered, but also the possible loss of bioactive compounds and sensory properties in the treated product. The mild temperatures involved in a process of this sort suggest a better retention of nutrients after the process, with respect to conventional thermal methods, which has to be confirmed by a nutritional analysis.

In this regard, this section is dedicated to the description of the procedure for the detection of carotenoids, polyphenols and volatile components of both the fresh and the treated coriander. The combination of these compounds gives the characteristic aroma to the plant. Moreover, it is well known for its antioxidant, antidiabetic, antimutagenic, antianxiety and antimicrobial activity along with analgesic and hormone balancing effect, and a good source of vitamins A, C, zinc and fibers (Duduzile Buthelezi *et al.*, 2016).

The nutritional analysis was performed in collaboration with the Dipartimento di Scienze del Farmaco of the University of Padua. The fresh and the dried coriander were provided by FeyeCon, Weesp (The Netherlands), where they were treated with the medium-scale reactor previously described (§2.3). The samples arrived about one month before the experimental trials. The fresh sample was stored frozen at -4°C, while the dried product was kept in its composite packaging made of aluminum at ambient temperature. FeyeCon also provided with other dried food samples, analyzed by University of Padua with the same procedures used for the coriander.

2.8.1 Carotenoids detection

The procedure to detect the amount of carotenoids in the coriander sample consisted in an extraction by means of a solution of 50% cyclohexane (Merck) in methyl acetate (Carlo Erba). The sample was weighted (200 mg) and put in a flask. Here, with a serological pipette, 10 ml of cyclohexane solution were poured and the flask was sonicated (Falc) for 10 minutes. When the sonication finished, the supernatant was collected in another flask (25-ml flask) with a Pasteur. In the original flask, other extraction solution was inserted (10 ml) in order to push the extraction, and sonicated for ten minutes. The supernatant was collected with a Pasteur and added to the other. The extraction procedure can be repeated many times if a complete separation of the supernatant from the solid sample is not achieved: in the case of coriander, three extractions were necessary. When the extraction was completed, other cyclohexane solution was added to the supernatant flask to reach 25 ml of volume. The flask containing the supernatant and the extraction solution was shook and after, with a Pasteur, around 2 ml were moved to an Eppendorf and centrifuged for five minutes. The liquid phase was transferred to a vial to constitute the sample to analyze. The analysis was performed in triplicate.

The analysis was implemented by means of HPLC-DAD with YMC carotenoid columns. For the quantification analysis, solutions of lycopene and tocopherol were used as standards.

2.8.2 Polyphenols detection

Coriander sample were extracted using a solution of 50% methanol (Carlo Erba, Italy) and 50% mq-water. The product was initially weighted (200 mg) and was grounded using a mortar and pestle. Then, few drops of the extraction solution were added to the sample. Above a 10 ml flask, a small, filter-paper funnel was assembled. With a Pasteur, the content of the mortar was moved in the funnel, where, after it was filtered from the paper, it formed the solution to analyze. At the end of the process, the total volume of 10 ml was reached adding other methanol-in-water solution. In an Eppendorf the liquid sample was centrifuged for five minutes and later about 1 ml of the supernatant was moved to a vial in order to proceed with the measurement. Qualitative analysis was performed in a Eclipse XDB C18 2.1x150mm column. The ion trap mass spectrometer was used in TDDS mode. Instead, quantitative analysis was executed with the same method, but HPLC-DAD was used.

2.8.3 Volatile components detection

For the detection of volatile compounds, 300 mg of dried product were put in a vial and analyzed. With a micropipette, 10 µl of a solution of internal standard 3-caren was added to the vial, in order to constitute the internal standard. The vial was then closed with Teflon and aluminum by means of a clamp. Then, the vial was put in a thermostatic bath (Julabo) for around 30 minutes at 65°C. As sampling mode, a SPME fiber (Carboxen/DVB/PDMS, Stableflex, Agilent technologies) was inserted in the vial. The fiber absorbs the volatile components in about 5-10 minutes, then it was inserted in the experimental machine. The analysis occurred in a GC-MS (Varian 3800, with ion trap mass detector model Saturn 2100), and a HP-5 (30 meters) as stationary phase.

Figure 2.10. *Some photos taken during the extraction process.*

2.9 Color analysis

Color analysis on the dried coriander was performed, in order to detect and quantify color differences with the fresh sample. The procedure follows the steps described by Ferrentino *et al.*, 2012.

The equipment was composed by a tungsten halogen lamp (HL-2000-FHSA) and a high resolution miniature spectrometer (HR2000+, Ocean Optics Inc., Dunedin, FL) which was connected to a fiber optic reflection probe (Ocean Optics Inc., Dunedin, FL). The fiber optic probe consisted of a tight bundle of seven optic fibers in a stainless steel ferule, six illumination fibers and one reading fiber in the middle, all with a diameter of 600 μm. The light from the tungsten halogen lamp was transmitted to the sample by the illuminating fibers, and the reflected light from the sample was acquired by the reading fiber and brought to the spectrometer. The illumination used a D65 standard illuminant and a 2-standard observer. The optical probe was fixed vertically on the sample, by means of a nut, and maintained in this configuration in all the experiments.

The detector output, integrated in the spectrometer, worked from 200 to 1100 nm providing an optical resolution of 0.035 nm and a sensitivity of 75 and 41 photons/count at 400 and 600 nm, respectively. A specific software (Spectra Suite, Ocean Optics, Inc.) computed the reflectance spectrum.

The spectrometer needed a calibration before each experiment for zero and 100% signal reflection. Calibration for zero signal reflection was taken by turning off the halogen lamp, or closing the spectrometer's shutter, while calibration for 100% signal reflection was acquired by inserting the probe in a white plastic cup. After the calibration, the spectrometer was ready to carry out the color analysis on the sample. Spectra Suite mathematically transformed the spectrum according to conventions established by the Commission International de l'Eclairage (CIE), into the three dimensional CIELab color coordinates: L^* (lightness), a^* (redness) and b^* (yellowness). The coordinates were interpreted on the basis of the work by Riva.

The lightness could be seen as the brightness of a surface compared to another one, which under the same light appears white. This parameter quantifies the achromatic component of a color. Redness and yellowness describe the chromatic component, which is responsible for the hue of a color. Therefore, three parameters are needed to identify a color. The lightness parameter has a range of 0-100 (100 equals to 100% whiteness, 0 means 100% darkness), both redness (a*) and yellowness (b*) can be positive or negative: a* measures red and green when positive and negative respectively, b* measures yellow and blue when positive and negative respectively.

The quantity of a chromatic part with respect to the achromatic one is called saturation, or chroma (C), and it is expressed as:

$$C = \sqrt{a^{*2} + b^{*2}} \tag{2.5}$$

Where a* and b* are the redness and the yellowness respectively.

Besides, CIELab coordinates allow quantifying visual differences between two colors, by means of the ΔE value. It was evaluated with the Hunter–Scotfield equation (Hunter and Harold, 1975):

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (2.6)$$

Where L*, a* and b* with subscript numbers represent lightness, redness and yellowness of the samples measure before (1) and after (2) treatments. Basically, ΔE value represent a method to express the difference among colors, if:

- $\Delta E < 0{,}2$: no difference is perceivable;
- $0{,}2 < \Delta E < 1{,}5$: there is a small difference;
- $2 < \Delta E < 6$: the two colors are distinguishable;
- $6 < \Delta E < 12$: there is a strong difference in color;
- $\Delta E > 12$: the colors are different.

The measurements both on the fresh coriander leaves and on the dried product were performed ten times, due to the high variability of the color of the fresh sample, and mean values and standard deviations were evaluated.

2.10 Dehydration kinetic

The purpose of these experiments is to find a correlation between the mass of the sample inserted in the reactor, the drying time and the water activity of the dried product, that represent an indication of the amount of water extracted in the process.

For these experimental trials, both the plant in the HPCD only configuration and HPCD+HPU was used. Since no microbiological assessment was performed on the treated sample, sterility of the equipment was not an important feature, so the reactor was not washed with ethanol before the experiments.

Coriander leaves used in the experiments were chosen by similarity in color and initially weighted. In each experimental trial 1±0,1 g of coriander was treated. Then, in HPCD experimental trials, an equal amount of the total mass of the coriander was inserted in two metallic baskets (0,5±0,05 g) and then put in the reactor together. In this way two simultaneous measurements were performed, one in the upper basket and the other one in the lower. The procedure was the same as the previously descripted one (§2.1, §2.5), as well as the operative pressure and temperature. Instead, the drying time was modified in each experiment: in particular, it lasted 15, 30, 45, 60, 90 and 150 minutes. The mass loss, as a result of the process, was calculated with (2.1).

Moreover, the water activity of all the dried samples was determined using a portable humidity and temperature instrument. The ROTRONIC probe type HC-AW-(USB) measured the humidity and the temperature of product samples and materials in bulk such as powders, seeds and leaves. The whole equipment consisted in a probe, a sample cup (item no. WP-14-S/WP-40) and a disposable sample container (PS-14/PS-40). The digital probe signal was processed by the indicator HP23-AW-A. When measuring water activity, the HP-AW-A waited for the full equilibration of the measured product and probe, it detected equilibrium conditions (humidity and temperature) and ended the measurement after typically 30-60 minutes.
Since two baskets were used to perform the experiments, two independent measure of weight loss and water activity were produced, so the results were calculated as the mean value of two replications, with their standard deviation and illustrated as weight reduction and water activity versus time.
Besides, in the experiments that combined HPCD with HPU, the sonotrode was used as a closing for the vessel. Because the sonotrode obstructed the superior part of the reactor, in this configuration it was impossible to use two baskets simultaneously. That means that each experimental trial corresponded to only one measurement performed, since coriander leaves were allocated in only one basket. As previously described, 1±0,1 g of sample was weighted and then inserted in the vessel. The complete HPCD+HPU was performed, but the drying time, occurring at a constant CO_2 flowrate (23 ml/min), was modified in each experiments: in particular, 15, 30, 45 and 75 minutes of drying time were analysed. The mass reduction of the sample indicated the water loss during the process.
One independent measurement was performed for each drying time, and the results are reported in graphs that show the weight loss and the water activity versus time.

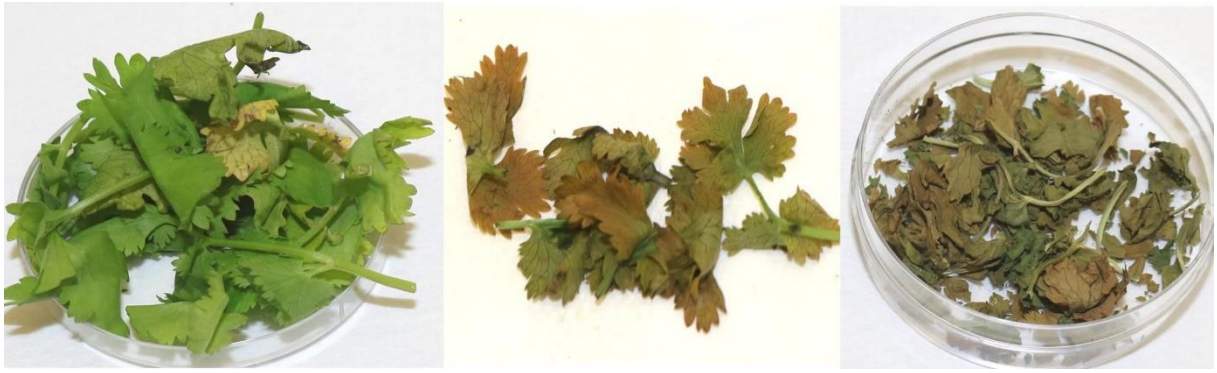

Figure 2.11. *Comparison of the fresh coriander and treated coriander at different drying times (0 min and 150 min).*

In Figure 2.11 examples of the treated coriander was reported, for different drying times.

Chapter 3
HPCD results and discussion

This chapter regards the obtained results of the analyzed food matrix, coriander leaves (*Coriandrum Sativum L.*), treated with HPCD and dried by conventional methods (air-drying). In order to find the optimum operative parameters to maximize the microbial inactivation, different operative conditions are analyzed.
After the microbial assessment and the definition of the dehydration kinetic, the Chapter presents the results of the sensorial analysis. First, preliminary studies of the nutritional analysis are reported, performed in collaboration with Dipartimento di Scienze del Farmaco, University of Padua. Second, the color of coriander leaves is analysed after each experimental trials, in order to detect important changes in color with respect to the untreated, fresh sample.

3.1 Microbial characterization

This section describes the microbial inactivation results on coriander leaves treated with supercritical carbon dioxide. Table 3.1 summarizes all the tested conditions, performed at the operative pressure of 100 bar.

Table 3.1. *Summary of the experimental conditions used during experiments. During the pressurization, the flow rate was set at 2 mL/min, while during drying it was fixed at 23 mL/min.*

Drying time (DT) (min)	T (°C)
0	40
	50
150	40
	50

All the experimental trials were performed in triplicate, for each condition tested. After each treatment, inactivation data of specific microorganisms naturally present on coriander leaves (mesophilic bacteria and spores, yeast and mold) are reported and compared with the initial microbial counts (Table 3.2).

Table 3.2. *Natural microflora present in the untreated fresh coriander.*

Mesophilic count	Spores	Yeasts and Molds
Log (CFU/g)		
7.99±0.55	3.64±0.22	5.42±0.56

The initial load is pretty high and quite variable, depending on the starting batch of coriander.

3.1.1 Drying time effect

In these first set of experiments, the bactericidal effect of supercritical CO_2 was investigated comparing the coriander's natural flora inactivation at different drying times, 0 and 150 minutes, in order to study if the time of the treatment has an effect on it.

Figure 3.1 shows this effect on the mesophilic bacteria and spores inactivation. The pressurization step lasted 20 minutes in both cases and the two conditions were performed at the same temperature (40°C). In the second condition, the drying time was 150 minutes, while there was no drying in the first. Data obtained show that the inactivation of mesophilic bacteria was quite similar (about 3.6 log cycles), meaning that the drying step did not lead to an improvement in the microbial reduction. This result suggested that the bacterial inactivation might occurs in the first step of the HPCD procedure, the pressurization step, or during the depressurization. Similarly, in the case of spores, the drying step seemed not to increase the inactivation.

The inactivation of yeast and molds was total: the value of the microbial reduction was equal to the value of the initial load of these microorganisms (about 5 log cycles).

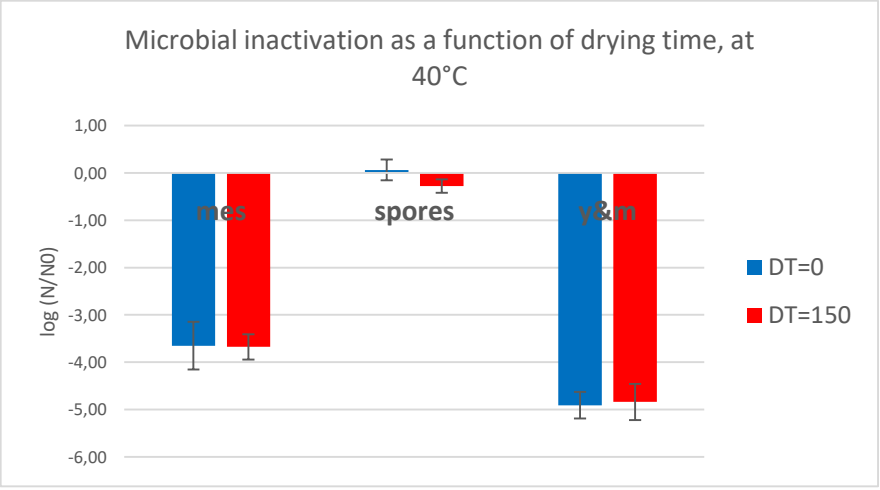

Figure 3. 1. *Inactivation of natural microflora as a function of the drying time (no drying time or 150-minute long drying time), at 40°C. Every point represents the mean value of three independent measurements and the standard deviation of the mean.*

3.1.2 Temperature effect

In this section, the effect of the temperature of the system was investigated, to verify if an increase of temperature improves the inactivation. According to literature, an increasing of the operative temperature has a negative effect on microorganisms' viability, due to the increasing in the fluidity of the cell membrane, resulting more sensitive to CO_2 penetration. The operative temperature was modified in the experimental trails with and without the drying time.

First, the modification on temperature was performed where no increase of inactivation was observed, that is in the conditions with a drying time different from zero, to verify if this operative parameter has an effect during the drying phase. Figure 3.2 shows the results of the inactivation performed with a drying time of 150 minutes, as a function of the temperature (40°C and 50°C). The results show that an increase in the operative temperature did not improve the inactivation of mesophilic microorganisms: at 40°C the inactivation is about 3.6 log (CFU/ml), at 50°C the inactivation is slightly over 4 log (CFU/ml). Moreover, the inactivation of the spores is quite similar for the two different temperatures (around 0,5 log CFU/ml).

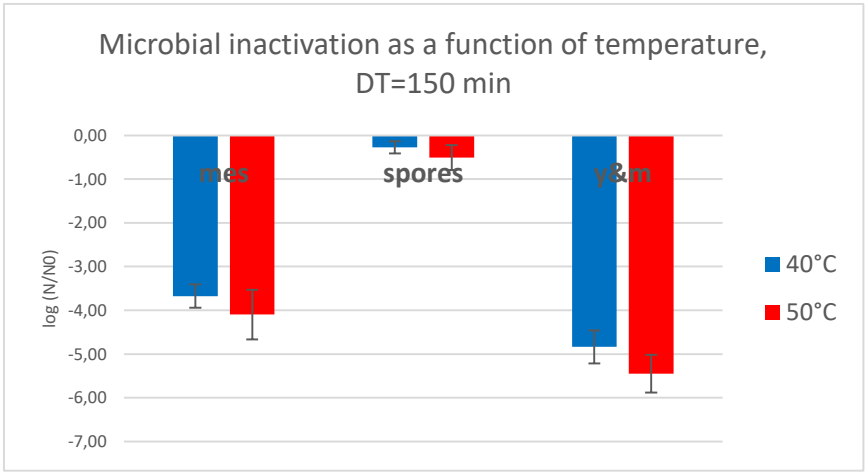

Figure 3.2. *Inactivation of natural microflora as a function of temperature, in case of DT=150 min. Every point represents the mean value of three independent measurements and the standard deviation of the mean.*

In the final analysis, considering the standard deviation of the mean, represented by the error bars, no significant difference was observed between the two temperatures in the microbial inactivation.

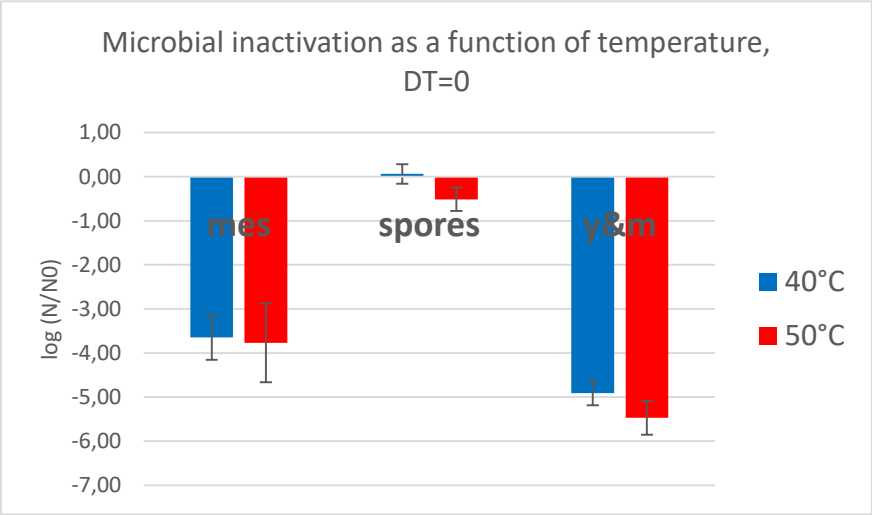

Figure 3.3. *Inactivation of natural microflora as a function of temperature, in case of DT=0. Every point represents the mean value of three independent measurements and the standard deviation of the mean.*

Similarly, Figure 3.3 shows the inactivation data for the microbial reduction at 40°C and 50 °C when the drying time was zero. The results were similar to those obtained when the drying time was 150 minutes: both with a temperature of 50°C and 40°C a mesophilic reduction of about 3.6 log (CFU/ml) was obtained. With respect to the spores, both of the treatments seem not to have a significant effect on the inactivation of them (about 0,5 log CFU/ml). Instead, a total inactivation of yeast and molds was achieved (equal to the initial load), regardless the operative temperature involved in the process.

3.2 Air-drying results

This section is intended to compare the results of the microbial inactivation as a consequence of the HPCD treatment with conventional methods of drying. The results of the inactivation of the natural microflora on air-dried coriander leaves are illustrated. The experimental trials were

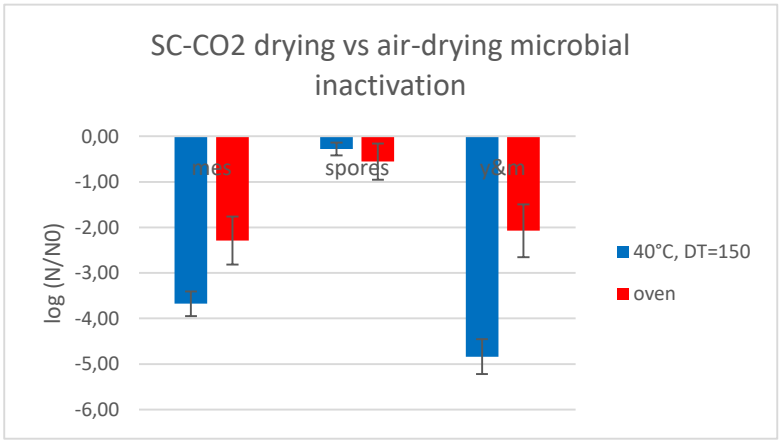

Figure 3.4. *Inactivation of natural microflora after SC-CO_2 drying (40°C, DT=150 min) and after air-drying (80°C, 5 h).*

performed in triplicate, and compared to the microbial inactivation reached after the HPCD treatment (40°C, 150 minutes). Figure 3.4 shows the comparison in the inactivation of these two processes, expressed in terms of the mean value and the standard deviation of the mean.
In this case, the inactivation of yeast and molds was not total. Actually, the inactivation of these microorganisms after air-drying was about 2 log cycles. Instead, after the treatment with SC-CO_2, the microbial reduction was total, meaning that yeast and molds were not detected on the treated sample.
This result again confirmed the efficiency of the supercritical carbon dioxide treatment as an unconventional method that combines pasteurization and drying of the sample. Actually, yeast and molds were totally eliminated after the only pasteurization with supercritical CO_2 process, according to literature.
The efficiency of supercritical carbon dioxide drying was also confirmed by the inactivation of mesophilic bacteria. The average value after the treatment was, in fact, about 3.6 log cycles, higher than the microbial inactivation achieved after air-drying (2 log cycles). About spores, the same inactivation was achieved after both of the processes.

3.3 HPCD dehydration kinetic

The results about the dehydration kinetic of the coriander leaves are here reported, after 15, 30, 45, 60, 90 and 150 minutes, at 40°C. In particular, 150 minutes is the drying time of the microbiological experiments performed with the HPCD technology. In the following Table 3.3 and Figure 3.5 the weight reduction of the coriander leaves is reported.

Table 3.3. *Weight reduction % of the sample.*

time (min)	weight reduction %	std dev
15	0,5665	0,1069
30	0,3253	0,0993
45	0,2254	0,0371
60	0,1626	0,0256
90	0,1594	0,0417
150	0,1790	0,1152

Every experimental trials, except for the ones performed at 60 and 150 minutes, were conducted in duplicate. Drying time of 60 minutes was investigated in four experiments, 150 minutes in three experiments.

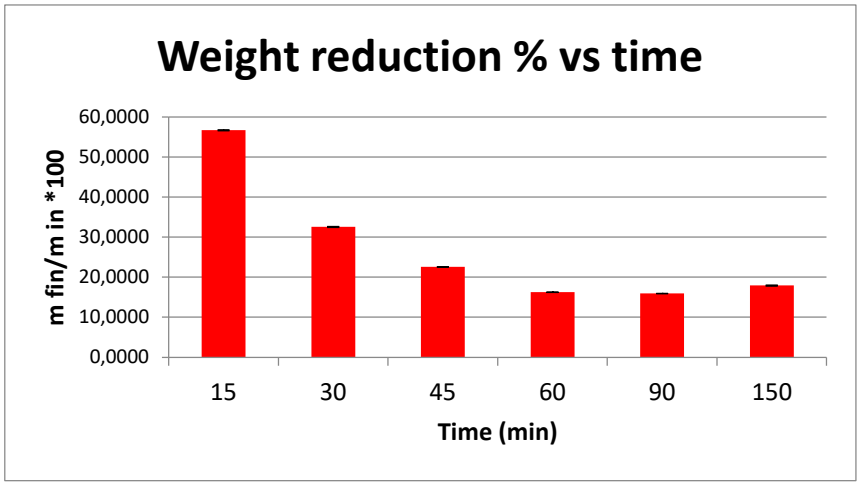

Figure 3.5. *Weight reduction, expressed as a percentage, as a function of the drying time. Each point represents the overall mean from two, three or four independent measurements and the standard deviation of the mean.*

The Figure shows that after 60 minutes, no further weight reduction on the sample was observed, i. e. a plateau in the weight reduction was reached. After only 15 minutes, the weight reduction is 56%, an indication that the drying process was not completed after this period of time. Increasing the drying time, an increase in the weight reduction is observed, until the plateau was reached. The maximum value to which the weight reduction settles is 80±0,3%, a measure that corresponded to the maximum quantity of water extracted from the coriander.
As far as water activity of the dried sample is concerned, Table 3.4 and Figure 3.6 illustrate the obtained results.

Table 3.4. *Water activity of the product.*

time (min)	aw	std dev
15	1	0
30	0,7965	0,1520
45	0,4150	0,0000
60	0,3785	0,0120
90	0,4045	0,0219
150	0,4185	0,0327

Each experiment was conducted in duplicate, except for the one performed at 150 minutes: in this case the result is the mean of three independent measurements.

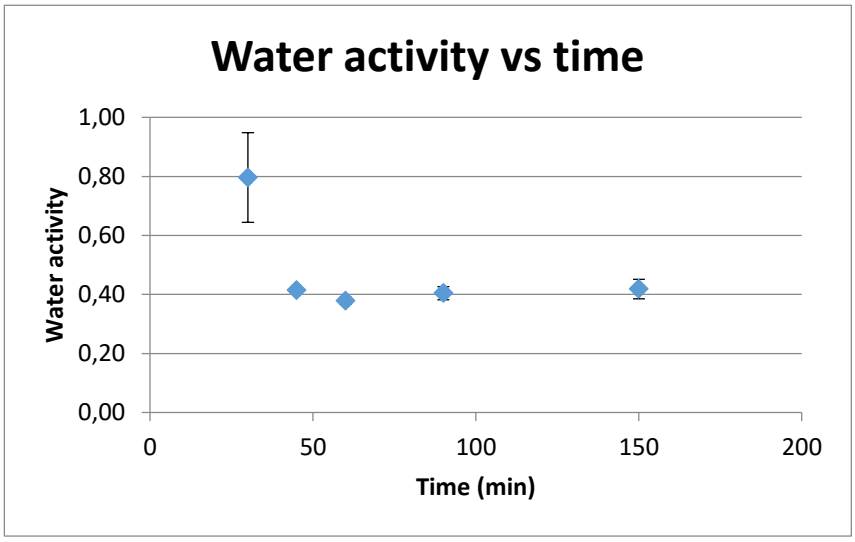

Figure 3.6. *Water activity as a function of the drying time. Each point represents the overall mean two or three independent measures, with the standard deviation of the mean.*

Figure 3.6 represents the water activity as a function of the drying time. The parameter decreased when the drying time increased, until it reached a plateau (that corresponded to a water activity of 0,4), similarly to what happened in the weight reduction experiments. In the trials with the drying times equal to 30 minutes and 45 minutes, high standard deviations are observed between the two measures. After 30 minutes of treatment, the coriander leaves of the upper basket was more hit by the CO_2 than the coriander in the lower basket. As a consequence, the upper sample was more dried than the other one and had a lower water activity.
The water activity is equal to one (sample not dried) after 15 minutes of drying time (56% of weight reduction).

3.4 Nutritional analysis

In the present section, the results of the quality of the dried coriander were reported. The dried coriander sample were obtained in the medium-scale reactor in FeyeCon B.V., The Netherlands (§2.3), and sent to University of Padua few days later. The main limitation related to the Padua's lab-scale reactor was not only its small volume, but also the configuration of the plant. In order to achieve a better characterization of nutrient retention, in fact, drying process has to be conducted in a plant with supercritical carbon dioxide recirculation. This fluid can extract some quality attributes from coriander leaves during the process, especially if they are non-polar compounds. In Padua's plant the supercritical fluid was released in the atmosphere after the drying process, causing the loss of these extracted compounds. For that reason, no nutrient analysis was performed on the samples treated with this configuration of the plant.

As explained in the previous Chapter (§ 2.8), the estimation of both flavonoid compounds and ascorbic acid (vitamin C) were performed by means of HPLC. Figure 3.7 shows the explicative chromatogram of the analyzed sample by HPLC-DAD.

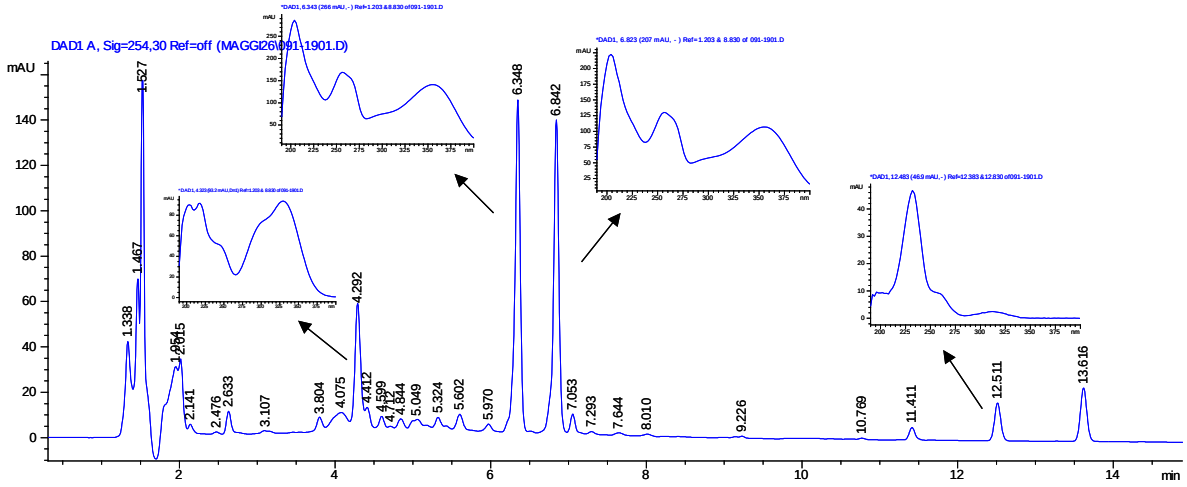

Figure 3.7. *Chromatogram of the SC-CO_2 dried analyzed sample (HPLC-DAD at 254 nm).*

In the X axis the retention time is reported. Every peak is univocally associated to a specific compound, and the area under the curve quantifies the amount of that substance.
From the analysis of the chromatogram, Table 3.5 reports the results of the quantification of the total amount of flavonoids in coriander leaves.

Table 3.5. *Quantitative analysis for different compounds. Each value considers the mean of three independent measurements and its standard deviation.*

Sample	Flavonoids as rutin (mg/g)	Cholorgenic acid derivatives (mg/g)	Gallic acid derivatives (mg/g)
Air dried	2.281 ± 0.044	2.945 ±0.001	2.816 ±0.016
CO_2 dried	2.313 ± 0.01	1.551 ±0.006	2.034 ±0.016
Fresh	0.18 ± 0.002	-	-

The total amount of flavonoids after both the processes appears to be greater than the amount in the fresh sample because it is more concentrated. In particular, SC-CO_2 dried coriander retains more flavonoids than the air dried sample (2.313 mg/g instead of 2.281 mg/g of the air dried sample).
Other polyphenolic compounds, detected by HPLC-MS, are reported in Table 3.6.

Table 3.6. *Qualitative assessment of the principal compounds in SC-CO_2 dried coriander.*

Tr	Compound	$[M-H]^-$	Fragments
02.04	Quinic Acid	191	111- 67
11.08	Clorogenic acid ione	535	191
12.02	Dimethoxycinnamoyl hexoside	369	189-127
13.08	Rutin	609	301
14.04	Quercetin 3-*O*-glucuronide	477	301
14.03	Quercetina esoside	463	301
14.06	Keamferolo rutinoside	593	285
14.08	Quercetina acetil esoside	505	301
15.03	Keamferolo glucuronide	461	285

Regarding the vitamin C investigation, the HPLC-MS analysis revealed only traces of this compound in the analyzed samples, probably due to the small amount of it in the fresh sample. Moreover, no significant modification in the quantity of vitamin C was detected before and after each treatment. Therefore, results are not reported.

The nutritional analysis performed on these samples was clearly a preliminary study, intended to evaluate the feasibility of the extraction as a method to detect bioactive compounds on the treated food. The same procedures were applied on other food matrices, such as red pepper and basil, dried by FeyeCon using the medium scale reactor (§2.3). The complete analysis was reported in Appendix B.

In the light of the obtained results, these techniques were effective and could represents a starting point for future investigations in this field.

3.5 Color analysis

Initially, the color analysis of fresh, untreated coriander was executed. Every measurement was performed on ten different locations of the coriander leaves, on both its sides, in order to take into account the color variations of the food matrix. In Table 3.7 the mean values of L*, a*, b* of the fresh sample are reported.

Table 3.7. *Mean values of L*, a*, b* of the fresh samples.*

	mean	std dev
L*	73,99	9,07172
a*	-3,42	0,81476
b*	8,55	3,581534

The mean considered seven independent measurements performed on seven different fresh samples, and the standard deviation of the mean.

3.5.1 HPCD results

Figure 3.8 shows the comparison of the value of ΔE for different temperatures and drying times.

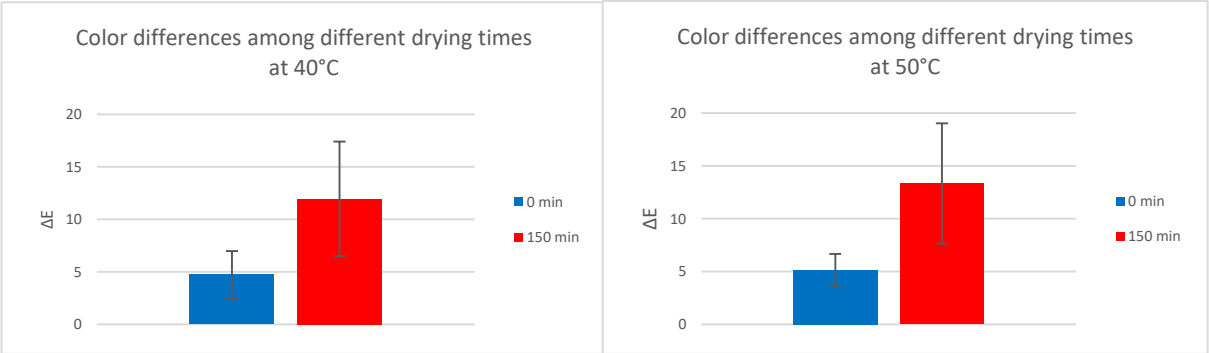

Figure 3.8. *Color differences in terms of ΔE among different drying times and temperatures (40°C and 50°C). Each point represents at least three independent measurements with the standard deviation of the mean.*

The clearest evidence is the different ΔE values between the only pasteurization (drying time=0 minutes) and the drying (drying time=150 minutes), with respect to the untreated sample. While in the first case the color difference was around 5, meaning that the two colors were distinguishable, in case of drying that difference is much higher, reaching a value of 12. According to the theory, this condition means that the two colors are completely different, suggesting that the consumer can detect visible color differences between the untreated and SC-CO_2 treated coriander. Moreover, the high standard deviations indicate a higher variability in the color of the dried sample with respect to the pasteurized one. On the other hand, the operative temperature appears to have a non-significant effect on the color of the sample. In fact, only a slightly increasing in the ΔE value in case of drying at 50°C was observed.

The main difference that led to a high ΔE value concerns the a* parameter. Actually, the treatment acted especially on the redness of the sample. With respect to the fresh coriander, which possessed a negative value of a* (-3.42, the sample is “greener”), the treated one had a positive value of this parameter (about 1) that indicated that its color tended to red. About b*, no significant differences existed between the fresh sample and the dried one. The value of the yellowness of all the treated coriander, in fact, was similar to the fresh sample (around 5-15).

Identically, the L* value of the sample did not experience changes before and after the process (70-80).

Some literature deals with color changes after pasteurization (drying time=0) with supercritical carbon dioxide. Ferrentino *et al.*, 2013, widely studied the quality attributes of fresh-cut coconut. Results showed that, while a* and b* did not significantly change after the process, L* decreased just after few minutes of treatment. In 2012, Ferrentino *et al.* studied the supercritical carbon dioxide treatment in cubed cooked ham. Even though they did not detect significant differences in the L*, a* and b* values, ΔE was equal to 1.3, suggesting that the overall color difference could not be experience through direct visual comparison. Other publications, instead, seems to confirm the obtained results with the coriander. According to Marszalek *et al.*, 2015, strawberry juice treated with SC-CO_2 showed in slightly decrease in L* and b* values, while a* increased a little. Moreover, no significant difference was detected at different values of operative pressure. In the coriander color analysis, no differences, in fact, were observed for different values of operative temperature. It must be remember, however, that ΔE values often depend on the food matrix, so a direct comparison of the obtained results on coriander leaves with different foods could not be possible.

3.5.2 Air-drying results

An important comparison to make is between air-drying and supercritical carbon dioxide drying. In the following treatise, only SC-CO_2 drying at 40°C and DT=150 minutes was considered. In Figure 3.10a,b the results related to the color difference and the L*, a* and b* parameters are illustrated.

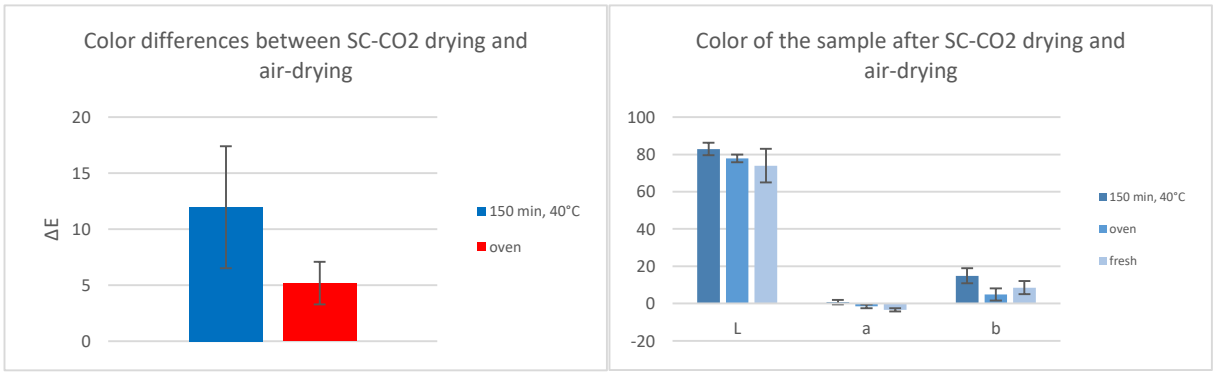

Figure 3.9. *Color differences in terms of ΔE (a) and in terms of coordinates L*, a*, b* (b) between SC-CO_2 drying and air-drying. Every point represents the mean value of three independent measurements with the standard deviation of the mean.*

The most important difference between the two treatments is the ΔE value. After SC-CO_2 drying, this value is higher (12) than in case of air-drying (5). Although color differences can be detected also in the air dried sample, they appeared to be much limited, suggesting that an air dried sample of coriander will be more accepted by the consumer. Regarding the L*, a* and

b* values, no significant differences were noted. The air dried products were slightly darker, according to literature (Brown *et al.*, 2007, detected a browning of air dried carrots with respect SC-CO_2 dried samples). On the other hand, they maintained the redness of the coriander, more than the other samples did. Also the b* value seemed to be closer to the fresh one. Globally speaking, the color of the air dried coriander appeared to be better, suggesting that air dried coriander will be more appreciated by the consumer.

Some publications can be found on the color changes before and after a drying process. An important work was performed by Busic *et al.*, 2014, on air dried, freeze dried and SC-CO_2 dried basil leaves. Regardless of the employed drying technique, L* decreased after each treatment, meaning that a decomposition of chlorophylls pigment occurred, in contrast with coriander results. However, dried basil also reported an increasing in the value of a*. About the total color difference, the least ΔE resulted in freeze dried basil, while the most pronounced was experienced by the SC-CO_2 dried samples, confirming coriander results.

The only publication focused on the drying of coriander is the one by Sarimeseli, 2010, who studied the quality properties of microwave dried coriander leaves. No significant differences in color values were observed, and they also were not dependent on the microwave power, even if some browning occurred compared to that of fresh coriander leaves. Again, microwave drying seems to produce a more attractive food, in terms of color, with respect the SC-CO_2 drying process.

3.6 Conclusions

To summarize, two operative parameters were modified in order to improve the process under a microbial point of view. A comparison of the counts for mesophilic spores and mesophilic bacteria after the treatment suggested that most of the vegetative bacteria were killed during the process even at the milder conditions: the process allows reaching an inactivation of around 3.6 log cycles in mesophilic count and around 0.5 log cycles for spores. Therefore, a longer drying time or an increased temperature did not induce a higher inactivation of microorganisms, since the remaining count was mostly accounting for mesophilic spores. Indeed, the spores and the vegetative bacteria counts were similar after the treatment, meaning that most of the detected colonies during microbiological analysis came from spores. In other words, with milder operative conditions, the maximal reduction for mesophilic vegetative bacteria was reached.

The complete inactivation of yeast and molds is another key point. This result justifies the effectiveness of supercritical carbon dioxide drying with respect to conventional drying methods (air-drying), where molds are detected after the process.

As far as nutritional analysis is concern, further investigation will be necessary in order to characterize all the present compounds completely. Additionally, other food matrices and

samples made by different batches should be analyzed. In general, the extraction method was effective in the detection of bioactive compounds.

Although the indisputable advantages of supercritical carbon dioxide treatments, the color of the sample is quite different from the untreated coriander and, above all, it is more pronounced with respect to the color difference exhibited by the air-dried samples. In other words, air dried coriander retains a better visual characteristic: its color is closer to what the consumer expects from a dried product. For that reason, supercritical carbon dioxide method is a promising technique of drying, but further improvements should be performed to ensure the acceptability of these products by the common opinion.

Chapter 4
HPCD+HPU results and discussion

In the final stage of this research project, a method to enhance the mass transfer during the drying process was analyzed, in terms of microbial inactivation (first paragraph) and color properties (second paragraph). In this last section, HPCD+HPU results are reported and commented. According to literature, in fact, the combination of the two technologies leads to an improvement in the inactivation of the natural microbiota in a food matrix. Nevertheless, no evidences in literature have been found regarding the combination of ultrasounds with supercritical carbon dioxide drying.

4.1 Microbial characterization

In this part the microbial inactivation results after the HPCD+HPU process are discussed. Prior to execute any experimental trial, microbial assessment on the untreated fresh coriander was performed. The initial microbial load, in terms of mesophilic bacteria, spores and yeast and molds is presented in Table 4.1, while Table 4.2 reports the operative conditions of the experimental trials.

Table 4.1. *Natural microflora present in the untreated fresh coriander.*

Mesophilic count	Spores	Yeasts and Molds
Log (CFU/g)		
8.01±0.49	3.61±0.18	5.60±0.35

Identically to the HPCD only process, the inactivation of yeast and molds was total after each experiments. For that reason, in the next sections only the results related to mesophilic bacteria and spores will be reported.

Table 4.2. *Summary of the experimental conditions used during experiments. During drying the CO_2 flow rate was fixed at 23 mL/min.*

US power (W)	Drying time (min)	T (°C)
0	0	40
		50
	150	40
10	0	40
		50
40	0	40
		50
	75	40
80	0	40
		50

All the experimental trials were performed at least in triplicate, so the results are expressed as the mean value of independent measurements with the standard deviation of the mean.

4.1.1 Ultrasound power effect

At first, studies on the microbial inactivation were performed for different ultrasound powers. Literature reported a beneficial effect of ultrasound with a power of 40W (Ortuno *et al.*, 2012; Ortuno *et al.*, 2013), in reducing the total drying time and lead to a better microbial inactivation. Then, Cappelletti *et al.*, 2014, and Spilimbergo *et al.*, 2014, demonstrated the efficacy of the combined techniques for ultrasound powers of 10 W on other food matrices. In this Thesis, both these ultrasound powers were tested. Moreover, coriander leaves were subjected to 80W in order to make the process more powerful.

Initially, experiments were executed only for zero drying time. Figure 4.1 reports the obtained results on the inactivation of mesophilic bacteria and spores.

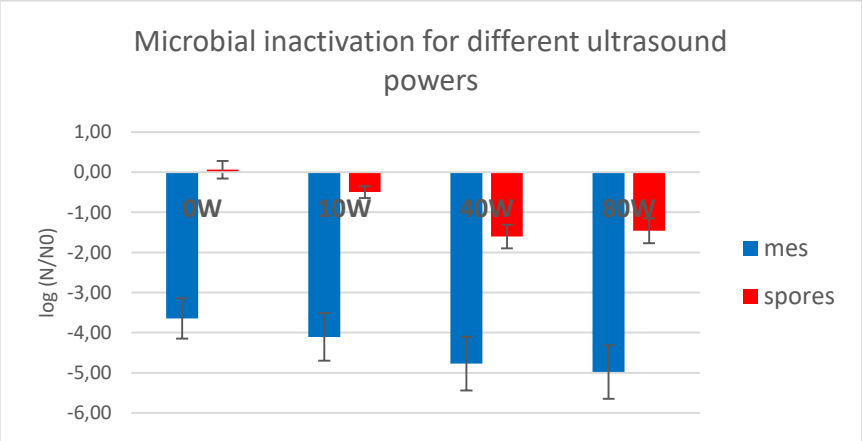

Figure 4.1 *Inactivation of mesophilic bacteria and spore as a function of different ultrasound powers (0W, 10W, 40W and 80W), at 40°C and for DT=0. Each point represents the mean value of at least three independent measurements and the standard deviation of the mean.*

Both mesophilic bacteria and spores are more prone to die when the aggressiveness of the process increases. In fact, a better inactivation was reached at ultrasound powers of 40W and 80W (around 5 log cycles for mesophilic bacteria and around 1.5 for spores) with respect to the process without ultrasounds (3.6 log cycles for mesophilic bacteria and no inactivation of spores). However, the increasing in the microbial inactivation was significant only for spores, as the error bars showed.

For an ultrasound power of 10W, an increasing in the microbial inactivation was not observed. This result appeared to be in contrast with the results claimed by Spilimbergo *et al.*, 2014, demonstrated on cured ham spiked with *Listeria monocytogenes*. In that case, 10W were sufficient to ensure the complete inactivation of this bacterium on the surface of the food matrix. Nevertheless, the microbial reduction was reached on a properly inoculated microorganism on the food matrix, so probably natural microflora is more resistant to process of this kind. However, a direct comparison could not be possible since the food matrix, as well as the treatment time, were different.

4.1.2 Drying time effect

Since the value of the microbial inactivation in case of 40W and 80W is quite similar, the investigation on how the drying time affects the inactivation was performed with the lowest power, 40W. Experimental trials were performed at 40°C and a drying time of 75 minutes. It was decided to compare these experiments with the condition of 0W, 150 minutes, 40°C, in order to have a comparison with FeyeCon's conditions.

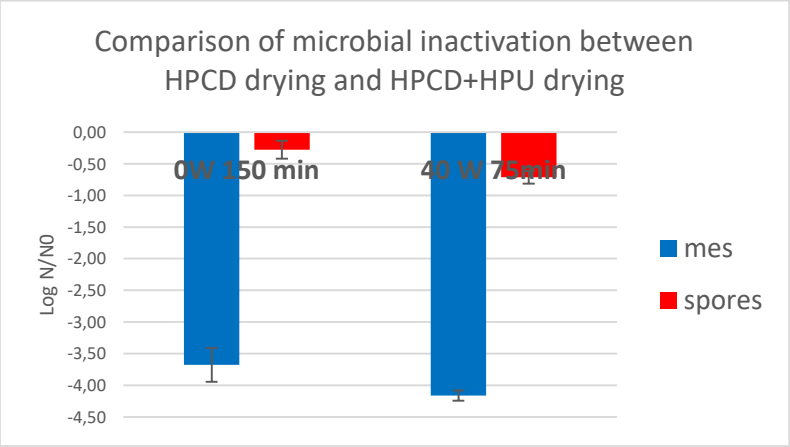

Figure 4.2. *Inactivation of mesophilic bacteria and spores at 40°C with and without ultrasound. Each point represents the mean value of three independent measurements and the standard deviation of the mean.*

As Figure 4.2 shows, no substantial increase in the microbial inactivation of mesophilic bacteria and spores was reached adding ultrasounds. In fact, the inactivation of mesophilic increased of

about 0.5 log cycles with the application of ultrasounds, while for spores the inactivation rate increased of about 0.25 log cycle. This result confirms that the drying step did not affect the microbial inactivation. On the other hand, testing the condition 10W, 150 minutes of drying time, 40°C, appeared not increase the microbial inactivation rate. In addition, greater drying times (120 and 150 minutes) led to the disintegration of the coriander sample, for an ultrasound power of 40W.

4.1.3 Temperature effect

At the end of the research study, two operative temperatures were tested, 40°C and 50°C, for different ultrasound powers and for no drying time. Figure 4.3 shows the obtained results.

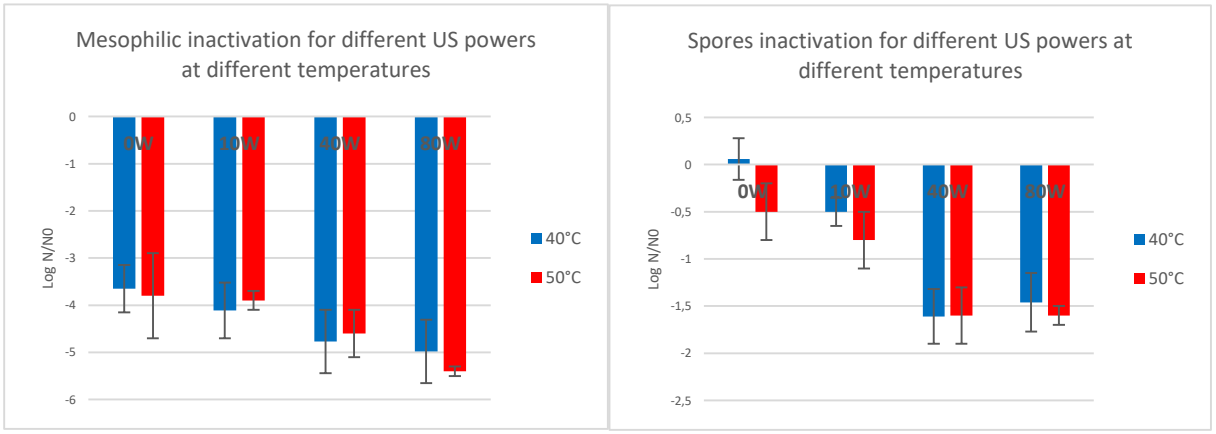

Figure 4.3. *Inactivation of mesophilic bacteria and spores for different ultrasound powers at different temperatures (40°C and 50°C). Each point represents the mean value of at least three independent measurements and the standard deviation of the mean.*

As far as mesophilic inactivation is concerned, no significant changes were noted increasing the operative temperature. The most important result was related to the spore inactivation. First, in case of 0W, the increasing in temperature led to an increasing of 0.5 log cycles. In other words, while at 40°C no difference in the number of spores before and after the treatment was detected, at 50°C spores were reduced after the process. Intensifying the ultrasound power, the spore inactivation gradually increased, until a value of around 1.5 log cycles at 40 and 80W. In those conditions, the number of the spores after the treatment was, in the majority of the cases, under the quantification limit.

4.2 Color analysis

The color analysis on the treated sample was performed, and color differences were evaluated with respect to the fresh sample. Initially, color measurements were executed on ten different locations of the fresh coriander leaves, in order to take into account the variability of this

property. The mean values and the standard deviations of L*, a* and b* are reported in Table 4.3, as a results of twelve independent measurements, performed in twelve different days.

Table 4.3. *Mean values of L*, a* and b* of the fresh samples.*

	mean	std dev
L*	75,42	8,104299
a*	-3,35	1,466004
b*	9,42	2,948942

The color of a natural product, as well as its quality properties, strongly depends on the area and the type of cultivation, and on following treatments, which coriander is subjected after the harvest. Under these circumstances, high variability in the color of the fresh sample was observed.

In the first place, color differences were evaluated for different temperatures (40°C and 50°C) and at different ultrasound powers (10W, 40W and 80W), with the same drying time (0 minutes). Figure 4.4 reported the obtained results, in terms of ΔE.

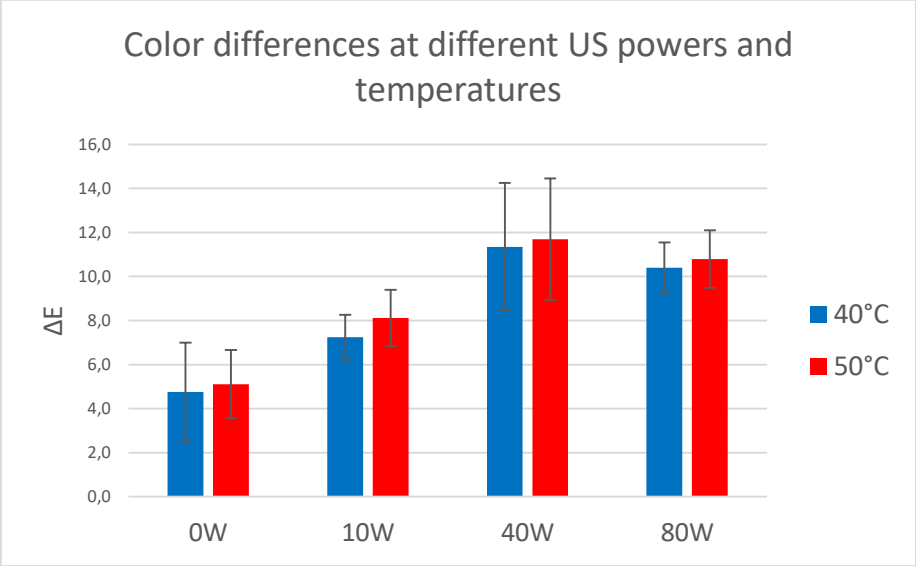

Figure 4.4. *Color differences in terms of ΔE among the fresh coriander and the coriander treated at different ultrasound powers and temperatures. Every point represents the mean value of three independent measurements with the standard deviation of the mean.*

As mentioned in the previous Chapter (§3.5.1), the treatment with no ultrasounds (0 W) generate a value of ΔE around 5. As one could expect, color modifications increase with an increasing in the ultrasound power, due to an increasing in the strength of the treatment. Experimental trials confirmed this supposition. The maximum gap was observed in the sample treated at 40W, with a value of ΔE of almost 12. The coriander sample appeared extremely different in color with respect to the fresh sample, being L* significantly decreased. The sample,

in fact, was darker than before. For that reason, for each applied ultrasound power, ΔE values suggested that a potential consumer could detect a color difference at a visual inspection. In other words, all the treatments negatively affect the sensory properties of the pasteurized coriander. Eventually, the operative temperature did not influence the color variation, as confirmed by the error bars.

Color analysis was performed on drying experiments, too. Figure 4.6 reports the color analysis for four different drying times (15, 30, 45 and 75 minutes) at 40°C and 40W. Ultrasounds were generated during all the drying phase, in cycle of 10 seconds.

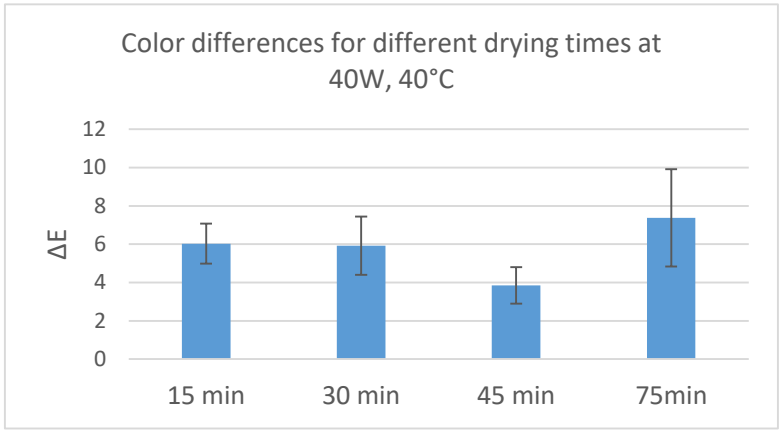

Figure 4.6. *Color differences in terms of ΔE for experimental trials at different drying times, at 40°C and 40W. Every point represents the mean value of ten independent measurements performed on the same sample, with the standard deviation of the mean.*

The greater drying time, the greater value of ΔE, meaning the greater color variation between the treated and the untreated sample. Visually, in fact, the treated sample appeared lighter than the fresh one. In any case, ΔE was greater than 4, so a visible color difference could be noted on the samples. A consequent comparison concerns the application of ultrasounds in drying at 40°C for 75 minutes. It was demonstrated (data not shown) that in case of 75-minute-long drying at 40°C, if no ultrasounds were applied (0 W), ΔE was equal to 4±0.96, that is a lower value than the one obtained at 40W (7.4±0.5). This results reaffirm the aggressiveness of the process when ultrasounds are exploited.

Results on coriander leaves could be compared with results generated on dry cured ham treated with both supercritical carbon dioxide and ultrasounds, as described by Spilimbergo *et al.*,

Figure 4.5. *Examples of coriander leaves dried by means of HPCD+HPU. The drying times are 15 min, 30 min and 45 min.*

2014. As far as color changes are concerned, the value of ΔE was equal to 4.4 after the treatment, a value that is similar to the one obtained for the coriander, meaning that a color difference is detected also in other food matrix. However, this coriander results are only preliminary. Further experimental trials should be executed of the treated sample to have a more reliable quantification, also considering the naturally high variability of this property.

4.3 Conclusions

The motivation that has conducted to the study of the combination of HPCD technology with HPU was the improvement in the microbial reduction. Under a microbial point of view, the total inactivation of yeast and molds was confirmed. Augmenting the ultrasound power, the microbial inactivation increased with respect to the same experiments when no ultrasounds were applied, showing a maximum at 40W (5 log cycles for mesophilic bacteria and 1.5 log cycles for spores). That power was tested in drying experiments, but the inactivation was not significant (an increase of only 0.5 log cycles). An increasing of 10°C in the operative temperature caused a slightly increase in the inactivation rate of spores, in spite of the great increment of this value with respect to the one obtained without ultrasound.

On the other hand, color differences were significant, especially in coriander samples treated at the highest powers, with a maximum value of ΔE of 12. Those samples were extremely darker with respect to the fresh coriander, showing a completely different color. Hence, those kind of products are not attractive and not desired from consumers.

Given these points, ultrasound technology in combination with HPCD appears to be beneficial especially in the spore inactivation, if during the process high powers are employed.

Nevertheless, this aggressive treatment causes undesirable color changes, a characteristic that may make the product unappealing from a visual point of view. Eventually, a quality analysis should be executed, in order to evaluate the retention of bioactive compounds after the treatment and estimate how ultrasound affects these nutrients.

Conclusions and future outlooks

The present Thesis was intended to investigate the possibility of pasteurizing food matrices during drying by means of supercritical carbon dioxide. In other words, the intention of this work was to study the feasibility of SC-CO_2 drying as a new method to obtain in a single process a dried and pasteurized product, which is microbiologically safe while maintaining its nutritional characteristics.

In the first place, the contribution of the operative parameters, such as the drying time and the temperature, was tested in the HPCD process, on the microbial inactivation of the naturally present microflora of coriander leaves. It was demonstrated that the microbial inactivation could be reached at the mildest operative conditions, resulting in a higher quality treated product. In fact, no differences on the reduction are detected if the drying time increases (from 0 to 150 minutes), or if the temperature increases (from 40°C to 50°C). Overall, the maximum mesophilic inactivation is about 4 log cycles, while 0.5 log cycles of reduction are achieved with respect to spores. Instead, the total inactivation of yeast and molds was observed, after all the experimental trials. With respect to air-drying, the microbial inactivation in case of HPCD treatment was greater, especially on the inactivation of yeast and molds. In fact, only 2 log cycles of reduction were observed on these microorganisms after air-drying. Yet, the color of the HPCD treated sample is considerably different from the air-dried coriander and from the fresh sample ($\Delta E=12$), suggesting that the consumer may find this product unattractive. In the final analysis, the preliminary study on the extraction process, as a method to detect bioactive compounds before and after the treatments, was positive.

In the last part of the research activity, the HPCD was combined with HPU, in order to improve the microbial inactivation especially towards spores. The procedure considered the variation of the operative parameters (drying time, temperature, ultrasound power) on the process. The temperature and the ultrasound power mainly affected the microbial inactivation. Actually, the greater ultrasound power, the greater the inactivation of microorganisms, reaching values of about 5 log cycle for mesophilic bacteria and 1.5 log cycles for spores, while yeast and molds were totally eliminated. In view of these results, the combined HPCD+HPU process represents an improvement with respect to the spore inactivation observed in the HPCD only process. Conversely, the combined process causes a marked browning of the treated samples, which is exhibited especially at high power (40W and 80W), resulting in a modified food product under a visual point of view ($\Delta E=12$).

Since it is one of the first work dealing with this new method of food drying, no direct comparison with academic literature could be made. This fact suggests that this novel technique needs further development and investigation both on a lab-scale and on a pilot-scale, before applying it to an industrial level. As one may be expected, it is important to test other food products, different from the coriander, in order to demonstrate that SC-CO_2 properties are valid for a great variety of matrices and, possibly, to infer a common microbial inactivation rate. Moreover, the microbial reduction values, obtained in the lab-scale, should be confirmed by medium and large-scale studies.

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Infine ringrazio tutti gli amici che ormai mi conoscono da una vita.

Appendix A

In this section, the detailed procedure to ensure the sterility of the reactor and of the glassware, before and after the treatment, is illustrated.

The parts of the HPCD+HPU equipment in contact with the sample were the vessel and the sonotrode, which needed to be completely sterile.

At first, the vessel was filled to the top with absolute ethanol (Sigma Aldrich, 99.8%) and was closed. In the HPCD+HPU configuration the sonotrode was the closing element of the vessel. The ethanol was allowed to react for about 10 minutes at ambient temperature. Then, a Bunsen flame was put in proximity of the vessel and it was maintained burning during the entire washing process. The reactor was opened and, with a sterile serological pipette, the ethanol was extracted. Ethanol residues had to be eliminated because they constitute an antimicrobial agent. In other words, it had to be ensured that microorganisms die because of the HPCD process and not because of the ethanol inside the vessel. Sterilized distilled water was poured inside the reactor in order to wash ethanol and, with a serological pipette, it was removed after few seconds. The sonotrode was rinsed with sterilized water, too. Washing with water is a quite delicate operation, because it is important not to wet the area of the vessel in contact with the opening. If so, microorganisms in this area could enter in the sterilized zone because dragged by the water.

Successively, the sonotrode was posted on a specific support, in order to easily dry the internal wall of the vessel. Small pieces of paper were previously sterilized and contained in sterile plastic bags. They were opened close to the Bunsen flame to assure the sterility of the paper. Then, tweezers were washed with absolute ethanol and burnt on the flame: with them a first piece of paper was extracted and used to dry the internal part of the vessel. This operation was repeated two times, otherwise humidity residue will affect the drying rate and time. With another piece of sterile paper, the sonotrode was dried. As soon as the washing procedure was completed, the sonotrode was put on the top of the vessel as a closing.

Similar to the drying procedure, the CO_2 cylinder was opened. The leak of CO_2 between the sonotrode and the opening of the vessel enabled the gas to flow and to dry the water or ethanol residues. When this area was totally dried, the cylinder was closed and the system was depressurized. Then, the reactor was closed firmly with a wrench. Again, the CO_2 cylinder was opened and, at that point, the pump was set to the maximum flow rate and turned on. When CO_2 became supercritical, the micrometric valve mounted on the exit line was slightly opened, to allow supercritical carbon dioxide to flush thereby eliminating ethanol and water residues inside the vessel. After few minutes, the pump was switched off and the cylinder was closed. A fast depressurization occurred, by opening the exit valve, until ambient pressure was achieved.

In the meantime, a metallic basket, which represented the casing of the sample, was washed with absolute ethanol (Sigma Aldrich, 99.8%), burnt on a Bunsen flame and inserted in a sterile tube by means of tweezers previously washed with ethanol and burnt. The tube with the basket was weighted in order to determine the final mass of the product after the process. Then, coriander leaves were separated from the stems and chose for similar color and freshness. With sterile tweezers, they were weighted and put in the basket inside the tube. Washing with ethanol and burn the tweezers is important to avoid adding microbial load to the naturally present one. The weighting procedure was done close to a Bunsen flame always maintained lit.

In order to insert the metallic basket with the sample, the reactor was opened with a wrench next to a Bunsen flame. Tweezers were dipped in absolute ethanol and burnt on the Bunsen flame. Then, the tube that contained the basket with the coriander was opened and the basket was put inside the reactor with the sterile tweezers. The vessel was closed with a wrench and the HPCD+HPU equipment was ready for the experimental trial. In the HPCD procedure the conventional closing was used, so the sonotrode was not washed.

At the end of the process, maintaining the sterility of every tool in contact with the dried sample is extremely important, in order to not contaminate the pasteurised product. Then, the Bunsen flame was lit and put close to the opening of the reactor and tweezers were sterilised with absolute ethanol and burnt on the flame. The reactor was opened with a wrench and the basket was extracted with the tweezers and quickly put in a new, previously weighted tube. That tube was again weighted in order to determine the final mass of the product, connected with the amount of water loss.

The dried sample was collected under a laminar hood, to be as aseptic as possible. This procedure was extremely delicate. Tweezers were dipped in absolute ethanol, burnt on a Bunsen flame and were left to cool for few seconds under the hood. Then, the tube was opened: the sample in the basket was extracted with the tweezers and put in another sterile tube previously weighted. The weight difference between this tube with the treated coriander and the tube allow to calculate the weight of the sample used in the microbial characterization.

The coriander sample was now ready for the microbial characterization.

Appendix B

This section reports the results of the nutritional analysis (§2.8) on basil and red pepper treated by the FeyeCon's medium-scale reactor (§2.3). The treated samples arrived at University of Padua few days after drying, stored in a composite bag made by aluminium. The procedures were the same to the ones used for the coriander analysis.

Basil underwent to the following analysis: analysis of the total polyphenol content, by HPLC-DAD, volatile analysis, and qualitative characterization by means of HPLC-MS (IT).

Table B.1. *Characterization of the nutrient compounds via HPLCD-MS/MS in SC-CO_2 dried basil.*

Tr	Compound	$[M-H]^-$	Fragments
2.4	Quinic Acid	191	111- 67
15.7	Caftaric acid	473	311-149
2.5	Tartaric acid	149	87-113
16.5	Rosmarinic acid	359	161
15.6	Chicoric acid	473	311-179
13.9	Rutin (quercetin-3-O-rhamnolglucosyde)	609	301
14.2	Lithospermic acid	537	349-295

The qualitative profile, illustrated in Figure B.1 indicates the presence of phenylpropanoids, typical basil compounds. Figure B.2 represents the results of the volatile compounds analysis.

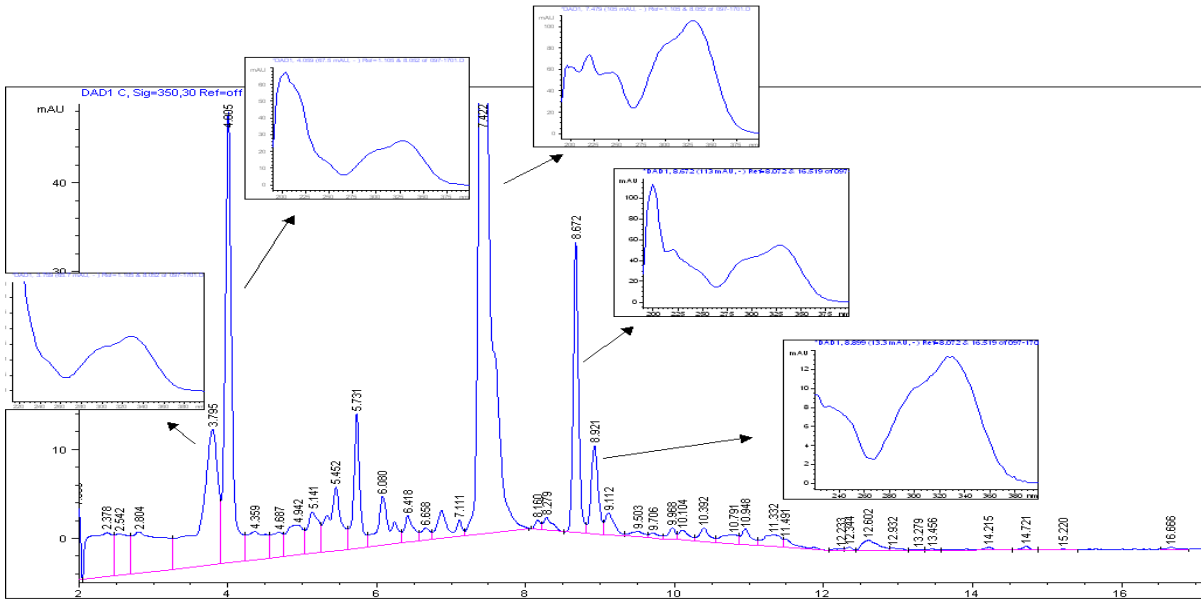

Figure B.1. *Basil chromatrogram at 350 nm with UV spectra of the main peaks.*

Considering this analysis, the extraction method appeared to be suitable for the detection. However, the overall amount of these compounds was quite poor compared to literature, meaning that a higher amount of dried sample was necessary to perform the investigation.

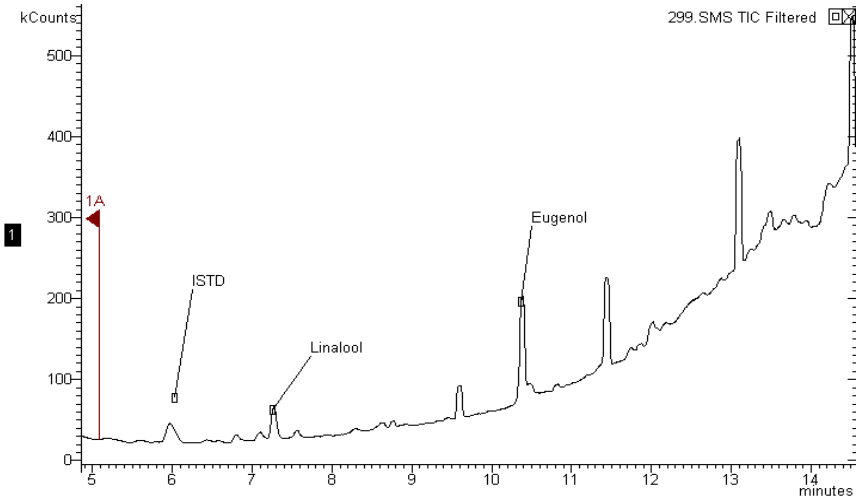

Figure B.2. *SPME GC-MS chromatogram of SC-CO_2 dried basil.*

For each sample of dried red pepper, the following analysis was performed: quantification (Figure B.3) and qualitative characterization of flavonoid content via HPLC-MS (Table B.2), quantification of ascorbic acid via HPLC-TQD.

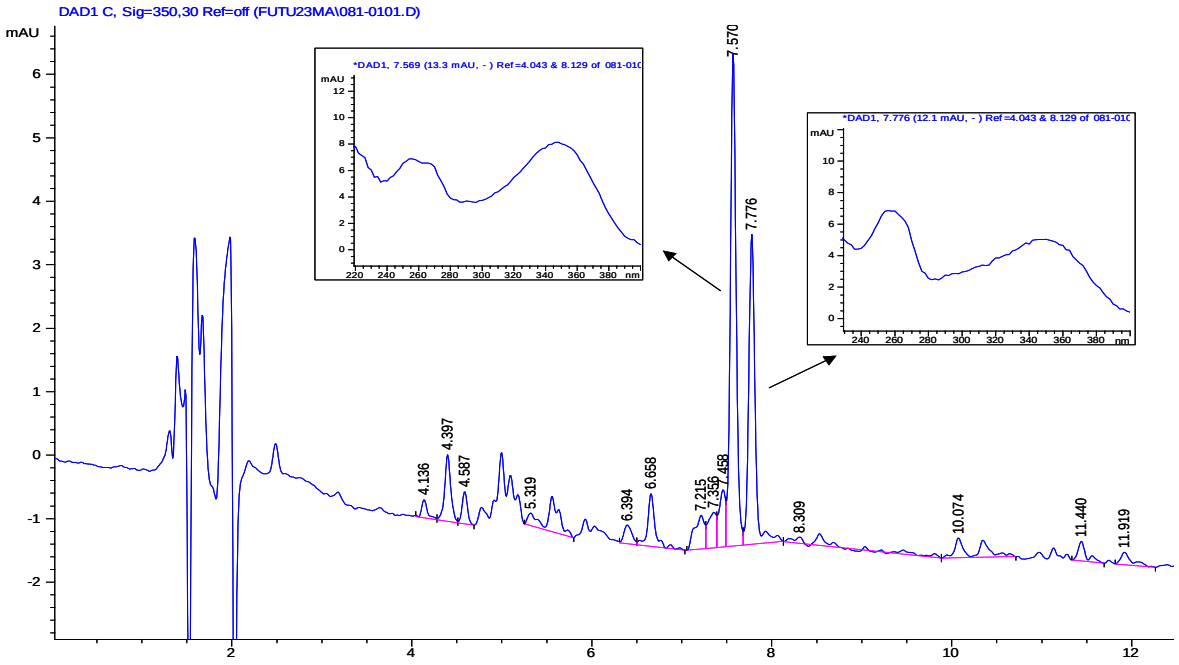

Figure B.3. *Red pepper chromatogram with UV spectra of the main peaks.*

Table B.2. *Characterization of the nutrient compounds via HPLCD-MS/MS in SC-CO_2 dried red pepper.*

Tr	Compound	$[M-H]^-$	Fragments
2.4	Quinic Acid	191	111- 67
11.9	Quercetin rhamnosil glucoside	609	463-447-301-273-151
12.32	Luteolin C-pentosyl-C-hexoside	579	489-459-441-399-369
14	luteolin-O-apiosil-hexoside	579	447-285
15.2	Quercetin-rhamnoside	447	301-271-179-248
14.95	Luteolin O-(apiosylacetyl)glucoside	621	579-447-285
15.00	Luteolin O-(apiosyl malonyl)glucoside	665	621-579-489-285

Eventually, the total ascorbic acid in the dried red pepper was determined (Table B.3). According to literature, the average content of ascorbic acid in the fresh food is 100 mg/100 g. Dried samples present an increase in this value because the removal of water causes an apparent concentration of this compound.

Table B.3. *Ascorbic acid content in different batches of SC-CO_2 dried red pepper.*

Sample	Ascorbic acid content (mg/100g)
b0(C) PAP 12-02-2016	273± 16
b1 (A) PAP 12-02-2016	278± 7
b2 (A) PAP 12-02-2016	254± 7
b3 (B) PAP 12-02-2016	260± 6

Appendix C

This section reports a preliminary qualitative study intended to estimate a correlation between the initial weight of the sample, its weight reduction (connected to the water loss) and the ultrasound power. The experimental trials were run in the HPCD+HPU configuration of the plant, and the tested power was 40W. Different drying times (15, 30, 45 and 75 minutes) were analyzed and after the process the sample was weighted and the water activity was measured. Table C.1 summarizes the obtained results, while Figure C.1 show the weight loss of the coriander, compared to the profile obtained without ultrasounds, for an equal amount of coriander inserted in the vessel (1±0.1 g). Every experimental condition was tested one time only, except for the experiment at 75 minutes, which was tested three times.

Table C.1. *Weight reduction and water activity of coriander after different drying times.*

	weight red%	std dev (weigh red)	aw	std dev (aw)
15	57,3709	0	0,842	0
30	42,8195	0	0,891	0
45	27,2945	0	0,665	0
75	21,7767	0,1113	0,518	0,1470

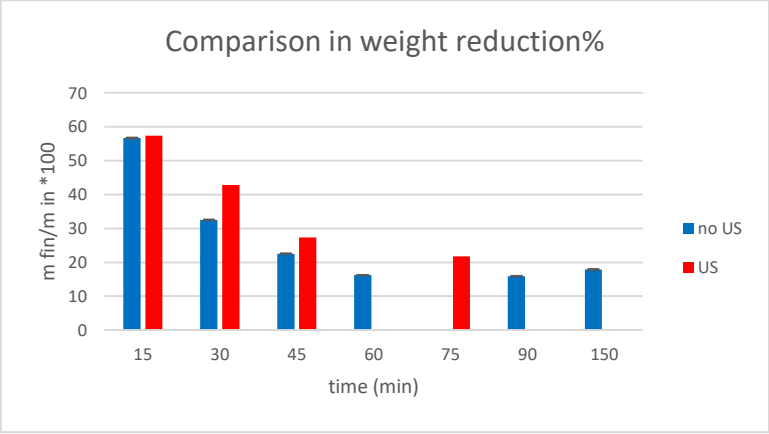

Figure C.1. *Comparison of the weight reduction of the sample for different drying times. In the experimental trials without US, each point represents the mean value of two independent measurements and the standard deviation of the mean.*

From Figure C.1, it appeared that when the plant is used in the HPCD only configuration, the weight reduction was greater than in the other case. To put it differently, adding ultrasounds did not result in a quicker dehydration kinetic of the product. For instance, after a drying time of 30 minutes, a reduction of only 42% is achieved, whereas without ultrasounds the weight

loss was around 30%. However, after a certain drying time (60 minutes), the dehydration kinetic of HPCD+HPU also showed a plateau in the weight reduction of the sample, i.e. a further contraction was not observed.

As far as water activity is concerned, Table C.1 and Figure C.2 report the values for the different analyzed drying times.

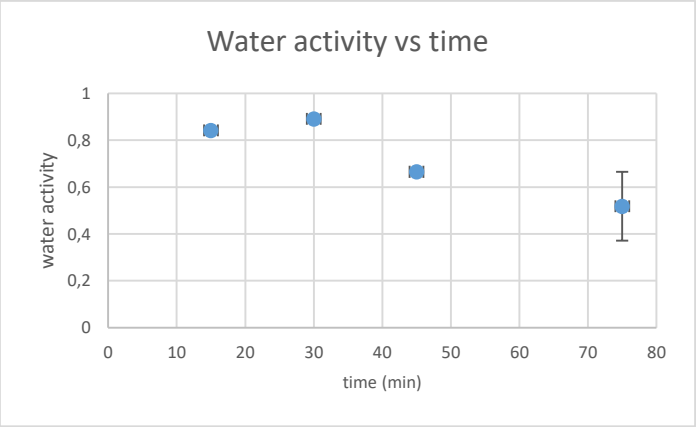

Figure C.2. *Water activity vs time for different drying times. The point at 75 minutes represents three independent measurements with the standard deviation of the mean.*

As illustrated in the Figure C.2, the water activity presented an unexpected behavior. Although after 30 minutes of drying a weight reduction of 30% is achieved, the water activity of the sample increased. Besides, after 60 minutes of treatment, the sample seemed not to be completely dried (water activity of 0.6). The value after 75 minutes of drying time is the mean of three independent measurements with the standard deviation of the mean. The error bar on that point is large, because high differences in the value of the water activity were reported after each trial (0.516, 0.666, 0.372).

The difference in the reduction values with and without ultrasounds, as well as the strange behavior of the water activity, may be due to the plant configuration. In fact, when the sonotrode is inserted in the vessel, coriander leaves are extremely close to each other and packed. For that reason, the flow of SC-CO_2 may be obstructed and it does not hit the sample properly, resulting in a poor dehydration of it. Inserting about 1 g of coriander in the vessel probably is not suitable, so a better analysis will be obtained with a lower amount of initial coriander. However, only a measurement was performed for each drying time, so no standard deviation can be calculated in order to validate the results. In the final analysis, it is clear that a different method should be used in order to completely characterize the dehydration curve.

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