High pressure carbon dioxide process conditions: comparisons and some disparities in food processing

Condições de processo de dióxido de carbono de alta pressão: comparações e algumas disparidades no processamento de alimentos

Condiciones del proceso de dióxido de carbono a alta presión: comparaciones y algunas disparidades en el procesamiento de alimentos

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Abstract
Due to the growing and intense demand of the consumer for high quality food, in the last years, an amount of research has been focused on minimizing nutritional losses and remaining the fresh-like feature, often affected by conventional thermal treatment. Among the emerging technologies, high-pressure carbon dioxide (HP-CO_2) has shown efficient results for food stabilization. Some disparities regarding the effect of process parameters in the treatment of foods with HP-CO_2 were observed in the literature. Thus, in this work, the data found in a bibliographic survey were organized into the five most discussed in topics the consulted works: the thermodynamic state, temperature, pressure, CO_2 ratio, and treatment time. In general, it was observed that enzymes and microorganisms have different resistance to process parameters, which can vary even longer with the change of environment components, the type of microorganism, or enzyme source. Therefore, results observed in a given food matrix may be different if the same treatment is applied to another different matrix.
Keywords: Dense phase CO_2; Stabilization food; Non-conventional; Fresh-like.

Resumo
Devido a demanda cada vez mais intensa do consumidor por alimentos de alta qualidade, nos últimos anos cada vez mais pesquisas têm sido desenvolvidas para amenizar perdas nutricionais e manter a característica de frescor que muitas vezes são comprometidas pelo tratamento térmico convencional. Dentre as tecnologias emergentes, o dióxido de carbono de alta pressão (HP-CO_2) tem mostrado resultados eficientes para estabilização de alimentos. Algumas disparidades a respeito do efeito dos parâmetros de processo no tratamento dos alimentos com HP-CO_2 foram observadas na literatura. Dessa forma, neste trabalho, os dados encontrados em um levantamento bibliográfico foram organizados nos cinco tópicos mais discutidos nas obras consultadas: a fase termodinâmica, temperatura, pressão, razão de CO_2 e tempo de tratamento. De modo geral, observou-se que enzimas e microrganismos apresentam resistências diferentes aos parâmetros do processo, podendo variar ainda mais com a mudança dos componentes da matriz, do tipo de microrganismo ou da fonte enzimática. Portanto, é possível que os resultados observados em uma determinada matriz alimentar sejam díspares caso o mesmo tratamento seja aplicado em outra matriz diferente.
Palavras-chave: CO_2 em fase densa; Estabilização de alimentos; Não-convencional; Frescor.

Resumen
La alta demanda de los consumidores por alimentos de excelente calidad ha llevado al desarrollo de investigaciones en la reducción de perdidas nutricionales y organolépticas causadas por los tratamientos térmicos convencionales. El uso de CO_2 a alta presión (HP-CO_2) es una de estas tecnologías emergentes que ha mostrado excelentes resultados en la conservación de alimentos. En la literatura se pueden encontrar divergencias en el efecto de los parámetros de proceso con HP-CO_2. Por esto, en este trabajo realizamos una revisión bibliográfica sobre estos parámetros los cuales son: fase termodinámica, presión, relación de CO_2 e tiempo de tratamiento. Se pudo observar, que las enzimas y microorganismos poseen diferente resistencia a los parámetros de proceso y la cuál puede ser aun mayor si se tiene en cuenta, el tipo de microorganismo, enzima y composición del alimento. Por lo tanto, es posible que los resultados

obtenidos en un alimento sean diferentes a otro así el tratamiento sea el mismo debido a la diferencia en la composición del alimento.

Palabras clave: CO_2 en fase densa; Conservación de alimentos; Tecnología no convencional; Frescor.

1. Introduction

Heat treatment for food preservation represents a set of well-established techniques used by the food industry to avoid changes in food and ensure its quality at the time of consumption (Zhang et al., 2018). However, although conventional heat treatments are effective in their role of inhibiting enzymatic and microbiological activity, they are often associated with changes in the nutritional and organoleptic quality of food (Smigic et al., 2019). Then, due to the increasingly intense consumer demand for high-quality foods with a satisfactory shelf-life, in the last fifty years, research has been developed to minimize nutritional losses and maintain the fresh-like characteristic (Wang et al., 2020; Silva et al., 2020a; Yu et al., 2020). In this way, non-thermal processing technologies, such as High-Pressure Carbon Dioxide (from now on HP-CO_2), High Hydrostatic Pressure (HHP), pulsed electric fields, UV-light, and ultrasound, have gained increasing credibility in the food sector (Zhang et al., 2018; Silva et al., 2020b; Roobab et al., 2021; Alvarenga et al., 2021).

In the 20's, the possible bactericidal effects of carbon dioxide (from now on CO_2) were already being discussed (Valley & Rettger, 1927) and later, in the 50's, Fraser (1951) observed the inactivation effect of pressurized CO_2 on *Escherichia coli*. Since then, the interest in these types of investigation as a possible alternative for microbiological inactivation to replace thermal pasteurization has grown considerably (Yu et al., 2020; Rao et al., 2015).

More recently, many authors have been devoted to understanding the mechanism of CO_2 inactivation and the real influence of treatment conditions (Fleury et al., 2018; Martín-Muñoz et al., 2022; Marszałek et al., 2019). In general, the bactericidal effect of the CO_2 can be attributed to some factors, such as damage to the phospholipid layer and, consequently, to the cell membrane; the fluidity and permeability of the membrane increased by hydrophobic interactions; the dissociation of carbonic acid in water and in the cytoplasm which reduces extracellular and intracellular pH (Yang et al., 2022), etc. In the case of enzymatic inactivation, it can also be attributed to a few reasons: in common with microorganisms, the acidification of the medium; the conformational changes of the enzyme caused by the process; and the inhibitory effect of molecular CO_2 on its activity (Hu et al., 2013).

In non-thermal pasteurization technology with HP-CO_2, the food is subjected to contact with pressurized CO_2, either in the subcritical or supercritical phase, for a sufficient time for the necessary inactivation. This process can occur in batch mode, pseudo-continuous mode, or a continuous flow, depending on its specificities (Garcia-Gonzalez et al., 2007).

Often, beyond being pressurized, CO_2 is elevated to conditions above its critical point and has characteristics typical of supercritical carbon dioxide (from now on SC-CO_2). In addition to SC-CO_2 having its conditions relatively accessible for the industrial context, with 7.38 MPa and 32.2 °C, it is inert, GRAS (generally recognized as safe), found naturally in the atmosphere, is not flammable, toxic, or corrosive and is easily removed by depressurization or degassing (Silva et al., 2020; Hu et al., 2013). Furthermore, when compared to other substances, it showed satisfactory performance as a pasteurizing agent (Enomoto et al., 1997; Gasperi et al., 2009). Due to the properties of CO_2, the SC-CO_2 treatment can be developed under mild conditions of temperature and pressure, so its effects tend to be less aggressive for substances of interest in the food (e.g. vitamins, antioxidants, flavonoids) (Silva et al., 2018). Due to these positive features, it has been investigated to produce higher sensory and nutritional quality foods (Porębska et al., 2017; Manzocco et al., 2017).

In the last years, the work by Garcia-Gonzalez et al. (2007) discussed some process conditions that still have no role and influence completely elucidated, such as: the influence of pressure and decompression ratio and the proportion of CO_2 in the treatment. After a bibliographic survey of recent literature, it was observed that some process conditions with HP-CO_2 in different studies exert different influences, sometimes not following a trend. Motivated by this scenario, this work aims to

discuss the current data made available regarding the most studied parameters with HP-CO$_2$, they are temperature; pressure and depressurization ratio; CO$_2$ ratio; exposure time. In this sense, the similarities and differences of the results obtained were shown in a discussion of potential interest to the industry and food researchers.

2. Methodology

This work was carried out on the systematic literature review context, as described by Donato & Donato (2019). Complementarily, the publication by Smigic et al. (2019), a review in the specific area of this research, which also served as a model. After an adaptation of the methodology described by both references, the methodology of this work consists of the following six steps:

1. Research question: Comparing studies of food treatment with HP-CO$_2$, what trends can be observed?

2. Research protocol: Scientific databases such as Science Direct, Web of science and Scielo were used for research and bibliography surveys.

3. Bibliographic research – find papers: Only research articles and literature reviews were included, preferably from the last five years (2017-2022). For the search, the following terms were used: "supercritical carbon dioxide", "high-pressure carbon dioxide", combined with the term's "pasteurization", "food" and "inactivation". From the results found, papers were selected which discussed the treatment with HP-CO$_2$ for food stabilization.

4. Inclusion and exclusion criteria: As exclusion criteria, works regarding the treatment of HP-CO$_2$ for drying or compound extraction were not considered.

5. Assessment of the quality of the studies and data extraction: Based on the criteria of steps so far, the papers were read to select only the works that discussed the treatment with HP-CO$_2$ for food stabilization. To discuss the results regarding trends and disparities, 22 recent articles dealing with process conditions were selected.

6. Data synthesis and quality of evidence assessment: The data found were grouped by similarities in relation to the four main parameters of the HP-CO$_2$ process (temperature; pressure and depressurization ratio; CO$_2$ ratio; treatment time) and gathered in two tables (Table 2 - enzymatic inactivation; Table 3 - microbiological inactivation) for discussion and more effective visualization of results.

3. Results and Discussion

After the initial search for the works, Table 1 presents the research corpus, which allows the visualization of the results found in total, by date, and after selection and exclusion.

Table 1 - Organization of results before and after exclusion criteria.

	Results
Papers found with search terms	70
Papers found published in 2017	4
Papers found published in 2018	11
Papers found published in 2019	9
Papers found published in 2020	7
Papers found published in 2021	11
Papers found published in 2022	6
Papers after the exclusion and inclusion criteria	**22**

Source: Authors (2022).

This section will be discussed the disparities found in the five selected parameters. From the data collected in this bibliographic survey, it was observed that about a third of the studies considered that the temperature parameter influenced the results of stabilization by HP-CO_2. This proportion was very similar for the pressure condition, followed by the treatment time, while among the four parameters discussed here, the CO_2 ratio was the least discussed in the literature (around 10 % of papers). The discussion regarding the data from the 22 works used for this summary will be started by the section that discusses the CO_2 thermodynamic state, which provides a brief discussion of the properties that can influence the effectiveness of the inactivation process. The following one will be discussed the temperature, pressure, CO_2 ratio, and time influence.

In order to provide an overview of the reviewed works, two tables were also prepared, which are presented below. Table 2 shows the main parameters and results of enzyme inactivation in different matrices. Table 3 presents the parameters and results of the treatment with HP-CO_2, in which SC-CO_2 is included, for microbial inactivation in different matrices.

Table 2 - Effect of HC-CO_2 treatment on endogenous enzyme inactivation in different environment.

	Environment	Associated treatment	P (MPa)	t (min)	T (°C)	CO_2 ratio	Maximum Inactivation	Summarized observations	Reference
Enzyme									
PPO	Quince crude	Non	20	20	55	N.D.	65.8%	Residual activities ↓ with T↑	Iqbal et al., 2019
		Non	20	20	65		Nearly total		
PPO	Apple juice	Non	20	20	55	70% *	Total	Enzymes have different stability at the same process	Murtaza et al., 2020
Peroxidase	Apple juice	Non	20	20	65	70% *	Total	parameters; POD stability > PPO	
Alkaline phosphatase	Raw bovine milk	Non	20	50	50	N.D.	Total	Inactivation was fast with T↑	Liao et al., 2019
PPO Peroxidase	Model solution	Non	65	30	50	90% *	88% 98.8%	Activities ↓ with P↑ PPO resistance > POD	Marszałek et al., 2019
PPO	Apple juice	Non	25	20	55	N.D.	Total	The critical and supercritical states, T and P had a strong inhibitory effect on the PPO activity.	Murtaza et al., 2019
PME	Tomato juice	Non	20	90	55	60% *	98.8%	T was most determinant than P to a faster inactivation rate.	Illera et al., 2018
PG	Tomato juice	Non	20	90	55	60% *	59%	PG was not very HP-CO_2 P or T sensitive	
PPO	PPO solutions	Non	>20	<9	25 - 45	≥3g/mL CO_2:solution	Almost total	CO_2 ratio, P and T are the statistically significant factors, while t not.	Benito-Román et al., 2019
PPO	Apple juice	Non	12	10	35	N.D.	80%	t ↓ with P and T ↑, but up to 12MPa and 35°C.	Manzocco et al., 2017

P = Pressure; t = Time; T = Temperature; V = Volume; PPO = Polyphenol oxidase; POD = Peroxidase; PME = Pectinmethylesterase; PG = Polygalacturonase; N.D. = Not Documented.
*The percentage of CO_2 volume was evaluated in relation to the total volume of the high-pressure reactor.
Source: The table was built using the articles that composed the bibliographic review, cited in the last column on the right.

Table 3 - Effect of HC-CO_2 treatment on microorganisms inactivation in different environment.

	Environment	Associated treatment	P (MPa)	t (min)	T (°C)	CO_2 ratio	Log reduction	Summarized observations	Reference
Microorganism									
E. coli	Mozzarella	Non	9.9	30	35	N.D.	6.5	Significant interaction between CO_2 and peracetic acid	Sikin et al., 2016
		Peracetic acid					7.9		
L. innocua			9.9	30	35	N.D.	4.6		
A. acidoterrestris spores	Apple juice 11.2 Brix	Non	60	40	75	N.D.	3.4	Soluble solids content was relevant to inactivation efficiency with SC-CO_2.	Porębska et al., 2017
	70.7 Brix		60	40	75	N.D.	0.5		
L. casei	Apple juice	Non	10	30	55	70% (v:v)	6.93	CO_2 volume ratio presents the most significant effect; while P did not influence.	Silva et al., 2018
E. coli; S. cerevisiae; L. innocua	Dietary supplement	Non	17	10	55	2.1 (m:m) CO_2:Sol.	Total	T had more effect than P.	Fleury et al., 2018
S. cerevisiae	Orange juice	Ultrasound	10	3.06	31	N.D.	2.60	T produce no significant differences	Paniagua-Martinez et al., 2018
E. coli	Orange juice	Ultrasound	10	3.06	41	N.D.	3.84		
Total aerobic mesophilic	Orange juice	Ultrasound	10	3.06	41	N.D.	3.95		
E. coli	Cured Ham	Ultrasound	35.0	5	51	N.D.	3.2	Only T had a significant effect; saline solution intensifies the treatment effect	Castillo-Zamudio et al., 2021
	Cured Ham	Ultrasound	24.33	12.2	48.4	N.D.	3.88		
S. aureus	Raw Salmon	Non	22	120	35	1:0.2 (m:m) Salmon:CO_2	5.3	P and depressurization rate were the most significantly factors; while the mass ratio was not significant.	Barbosa et al., 2020
E. coli	Pumpkin puree	Non	27.5	480	32	1:1 (v:v) CO_2:puree	3.17	*E. coli* decreases as the t increases.	Santos-Júnior et al., 2021

E. coli	Alfalfa	Non	12	20	45	N.D.	2.92	Type of seed, t, and P had a	Bourdoux et al., 2022
E. coli	Leek	Non	12	20	45	N.D.	4.96	significant effect on the	
L. monocytogenes	Leek	Non	12	20	45	N.D.	2.93	inactivation.	
Salmonella spp	Leek	Non	12	20	45	N.D.	3.18		
E. coli	Almonds	Thyme oil	10	30	70	N.D.	5.16	P did not further improve the reduction. t increase the reduction up to 45min.	H. Chen et al., 2022
Legionella	Aqueous solutions	Non	10	30	38	N.D.	Total	From 10 to 20 MPa produced a increase in the inactivation, while from 20 to 30MPa, did not.	Martins-Muñoz et al., 2022
		Non	10	1	28	1.0 (m:m)	Total	Efficiency increase with t and T and flow ratio.	
E. coli	Aqueous solutions	Non	5	30	25	N.D.	86.57%	Extracellular pH was more relevant than pressurization.	Yang et al., 2022
E. coli	Elderberry juice	Non	18	90	45	N.D.	Total	Increase in temperature decreased t and decrease in P decreased inactivation.	Torabian et al., 2018
Total viable count	Mongolian cheese	Non	20	60	60	N.D.	Total	P had a greatest influence than T	Feng et al., 2022

P = Pressure; t = Time; T = Temperature; V = Volume; (m:m) = mass ratio; (v:v) = volume ratio; N.D. = Not Documented; Sol. = Solution. Source: The table was built using the articles that composed the bibliographic review, cited in the last column on the right.

With the data presented in this way, it is possible to make comparisons and observe disparities. For example, while temperature possibly contributes to the inactivation of the researched enzymes and presented in Table 2, with the researched microorganisms (Table 3) the effects are more variable according to other process parameters, such as pressure, pH or treatment time.

It is important to note that the enzymes and investigated microorganism varied, despite the fact that polyphenol oxidase has been investigated in several works, just as *Escherichia coli* was a frequently researched microorganism. Although there are several works with the same objective, establishing comparisons is a challenge, because even in studies that used the same matrix, small variations were able to generate different results. In addition to the comparisons, the table systematizes the data to give an overview of the main results of works involving this topic.

3.1 CO₂ thermodynamic state

Herein will be discussed results that show that the properties (e.g., diffusivity and solubility) linked to the CO_2 thermodynamic state can influence the yields of the inactivation process. Although several results go towards the same trends, some disparities are found and will be pointed out below. Some authors realized that the properties of CO_2 physical, physical-chemistry and thermophysical), in addition to other parameters, also influence the effectiveness of the treatment (Marszałek et al., 2019; Gasperi et al., 2009).

The SC-CO_2, like other substances in the same state, behaves between liquid and gas. It is dense like a liquid and viscous like a gas, lacking surface tension and its diffusion coefficient is intermediate between the two phases (Amaral et al., 2017). In this sense, the combination of these properties has been widely explored for it provides good penetration into biological structures such as membranes and cell walls (Wang et al., 2020; Chen et al., 2022; Barbosa et al., 2020; Soares et al., 2020; Soares et al., 2019).

Based on this, Murtaza et al. (2019) investigated the effects of the CO_2 thermodynamic state on the inactivation of polyphenol oxidase in apple juice. It was observed that polyphenol oxidase had most of its activity maintained when treated for 20 min with CO_2 gas (40 °C, 5 MPa) and liquid (25 °C, 10 MPa). On the other hand, the critical state (32.2 °C, 7.38 MPa) caused an abrupt drop on enzymatic activity and in supercritical complete inactivation occurred (40 °C, 25 MPa). This decrease may have occurred due to the increase in the diffusivity and solubility of the fluid in the matrix, which may have induced severe conformational changes in the enzyme or the pH of the medium, making polyphenol oxidase activity unfeasible.

In the inactivation of species of the genus *Legionella* in an aqueous solution, although the treatment with liquid CO_2 provided good reduction results, complete inactivation was only achieved with the supercritical phase, possibly due to the greater ability to penetrate the cell (Martín-Muñoz et al., 2022). Furthermore, when *Saccharomyces cerevisiae* was treated with subcritical CO_2 for 60 min (5 MPa; room temperature), 1.5 log it was reduced (Debs-Louka et al., 1999), while when subjected to SC-CO_2 for 10 min (55 °C, 10 MPa), was completely destroyed (Fleury et al., 2018).

On the other hand, Kincal et al. (2006) compared the inactivation of *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* with subcritical and supercritical CO_2 in orange juice varying the temperature from 25 (below the critical point) to 34.5 °C (above the critical point) and found that the addition of heat (consequently the change to supercritical phase) was not the determining factor for microbial reduction in this system being, therefore, a disparity in relation the recent results mentioned.

3.2 Temperature

Temperature is another important factor and one of the most cited in HP-CO_2 and SC-CO_2 pasteurization processes literature. It´s worth the comment that the temperature of the process is one of the more cited parameters by literature. Was already been reported that higher temperatures can stimulate inactivation due to increased solution vapor pressure, increased CO_2 diffusivity that becomes easier the penetration and increased cell membrane fluidity (Buszewski et al., 2021; Fleury et al., 2018; Perrut, 2012; Spilimbergo & Bertucco, 2003). However, it is important to note that the magnitude of temperature in operations close to the critical pressure of CO_2 triggers the effect of increasing solubility, attributed to the drastic drop in fluid density (Soares et al, 2019; Illera et al., 2019).

Several authors emphasize the relevance of the temperature parameter in the CO_2 inactivation process and observed that, in general, the treatment effect increases with temperature (Martins-Muñoz et al., 2022; Feng et al., 2022; Murtaza et al., 2019; Manzocco et al., 2017; Benito-Román et al., 2019; Silva et al., 2018; Fleury et al., 2018; Castillo-Zamudio at al., 2021; Illera et al., 2018; Liao et al., 2019). As can be seen in Tables 2 and 3, the temperature range

generally used for the inactivation of enzymes and microorganisms is 25 to 55 °C, that in turn is below the temperature value that characterizes a heat treatment (<60 °C) (Illera et al., 2018) and it is lower than what often occurs in other treatments such as ultrasound or microwaves (Gallo et al., 2018; Kutlu et al., 2021).

For enzyme inactivation processes, an efficiency of more than 80% has often been achieved (Manzocco et al., 2017; Illera et al., 2018; Liao et al., 2019). One of the exceptions to this trend was the enzyme polygalacturonase, which presented resistance to treatment with HP-CO$_2$ in tomato juice. The enzyme was treated at 55 °C, 20 MPa for 90 min and had only 59% of its activity reduced (Illera et al., 2018). Likewise, the peroxidase enzyme from apple juice showed more resistance to treatment temperature than polyphenol oxidase (Marszałek et al., 2017). Other studies conducted with microorganisms (see Table 3), such as *Escherichia coli*, *Saccharomyces cerevisiae*, and *Lactobacillus casei*, achieved considerable inactivation - up to 6.93 log by Silva et al. (2018) or 6.89 log by Feng et al. (2022) - that were attributed to the optimization of synergistic effects of temperature (Silva et al., 2018; Fleury et al., 2018, Bourdoux et al., 2022).

There is a disparity found in temperature parameters about the ones mentioned so far. In the treatment of *Saccharomyces cerevisiae* in orange juice conducted by the ultrasound-assisted SC-CO$_2$ technique, the variation of 31, 36, and 41 °C did not produce significant differences in microbiological reduction (Paniagua-Martinez et al., 2018). However, Castillo-Zamudio et al. (2021), also conducted their ultrasound-assisted SC-CO$_2$ treatment in cured ham and observed that only temperature had a significant effect on *Escherichia coli* inactivation being, therefore a disparity between the results found by Paniagua-Martinez et al. (2018).

3.3 Pressure and depressurization ratio

The pressure parameter, as well as temperature, is one of the most cited parameters regarding the inactivation process as realized in Tables 2 and 3. High-pressure mechanisms, as in HHP processes, are able of inactivating vegetative cells by damaging the cell membrane or, from a molecular point of view can occur changes in the electrostatic, hydrophobic, and ionic interactions become the functioning of the structures infeasible (Sehrawat et al., 2020). However, the pressures adopted in the HP-CO$_2$ treatment (generally <25 MPa) are considerably lower than those of the processes with HHP (around 300 – 600 MPa) (Wang et al., 2020).

Due to the substance features the HP-CO$_2$ technology becomes a low implementation cost alternative when compared to systems operating with other substances in the supercritical phase (Amaral et al., 2017). However, beyond financial concerns, due to the lower intensity of pressures used in HP-CO$_2$ processes, these effects only will be achieved with the synergistic interaction between pressure and CO$_2$ (Fleury et al., 2018), in this sense, only the low pressures cited are not enough to food stabilization.

Regarding the inactivation mechanism, it has already been proposed that cellular infeasibility could be caused by the blast from the decompression and expansion of CO$_2$ in the intracellular environment. However, a higher depressurization rate did not always increase inactivation (Enomoto et al., 1997). Although HP-CO$_2$ can promote cell wall disruption (Fraser, 1951; Nakamura et al., 1994), these effects have not been considered determinants of microbial reduction efficiency (Garcia-Gonzalez et al., 2007).

Investigating the synergistic effects of this parameter, some authors classified pressure as the most relevant in their process (Barbosa et al., 2020; Feng et al., 2022; Berenhauser et al., 2017; Hossain et al., 2015). Several other authors have observed a strong synergistic effect between pressure and other process variables, although it has not always been considered the most influential parameter in the treatment with HP-CO$_2$ (Melo Silva et al., 2013; Soares et al., 2013; Berenhauser et al., 2017; Michelino et al., 2018). As shown in Tables 2 and 3, this happened for

treatments with pure HP-CO_2 or with additives (Rao et al., 2016; Torabian et al., 2018), in the treatment either enzymes (e.g., polifenol oxidase, pectinmethylesterase and polygalacturonase) or microorganisms (e.g., *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subitilis spores*), in solid (Bourdoux et al., 2022; Zhang et al., 2021; Barbosa et al., 2020; Feng et al., 2022) or non-solid matrices (Manzocco et al., 2017; Murtaza et al., 2019; Kincal et al., 2006; Benito-Román at al., 2019; Fleury et al., 2018; Illera et al., 2018; Marszałek et al., 2019; Yang et al., 2022).

Analyzing the synergistic effect of process parameters, Illera et al. (2018) noticed an increase in the rate of pectinmethylesterase inactivation with increasing treatment pressure that varying from 8.5 to 20 MPa at 45 °C in tomato juice, while polygalacturonase was more resistant. One similar behavior was also observed in apple juice peroxidase and polyphenol oxidase enzymes that demonstrated sensitivity to pressure, however, peroxidase was more resistant and, therefore, exhibited different inactivation kinetics (Murtaza et al., 2020).

As well as the juices mentioned, mushroom polyphenol oxidase and horseradish peroxidase also had their residual activities reduced with the pressure increased from 10 to 65 MPa (Marszałek et al., 2019). The authors observed that even working at considerably lower pressures, CO_2 treatment was much more effective than HHP in inactivating peroxidase (Marszałek et al., 2019). Moreover, pressure and depressurization ratio were the most relevant parameters in the inactivation of *Staphylococcus aureus* in salmon (Barbosa et al., 2020) and in the microbiological inactivation of Mongolian cheese, which went from 2.03 log at 10 MPa to 6.89 log at 20 MPa (Feng et al., 2022). However, a lower limit for its effect was observed, below 3.5 MPa, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Enterococcus faecalis* appeared insensitive to the HP-CO_2 process (Debs-Louka et al., 1999).

In this sense, the pressure proved to be a significant parameter and the processes commented on so far were sensible to its changes. On other hand, opposite trends were found that can be considered disparities. In the SC-CO_2 study conducted by Silva et al. (2018), no influence of pressure (10 – 20 MPa) was observed on hm the reduction of *Lactobacillus casei* in apple juice. It was argued that the effects of pressure may be more relevant in processes with HHP.

Similar results by Silva et al. (2018) were obtained by Chen et al. (2022) in their study with almonds. The authors found that increasing pressure from 15 to 20 MPa did not increase the *Escherichia coli K12* inactivation. Despite the increase in pressure from 10 to 20 MPa causing an increase in the destruction of species of the genus *Legionella* in aqueous solution, this behavior was not observed with the variation from 20 to 30 MPa (Martín-Muñoz et al., 2022).

Although pressure influences the increase in solubility, its effect may be limited due to the saturation capacity of CO_2 in the medium (Barbosa et al., 2020). It has been observed that the CO_2 solubility increases appreciably with increasing pressure up to 10 MPa. Above that, there is a trend towards stabilization in which a slight increase in solubility can mean considerable additional costs with increasing pressure (Illera et al., 2019; Spilimbergo et al., 2005). In addition to the interference of the saturation of the medium, microorganisms may have different susceptibilities to pressure. Of those studied by Debs-Louka et al. (1999), *Escherichia coli* was the most susceptible, followed by *Saccharomyces cerevisiae*, while *Enterococcus faecalis* suffered only slightly from this effect.

3.4 CO_2 ratio influence

The role of CO_2 as a pasteurizing agent is related to its diffusivity through the food cellular material (Silva et al., 2020). Knowing its solubility and diffusivity in the matrices and working conditions provides an assertive choice of the necessary amount to be used to guarantee an efficient process. Matrices with high lipid content may induce a low inactivation via HP- CO_2 due to their lipophilic behavior, which tends to reduce their action on the phospholipid bilayer in cells (Garcia-González et al., 2007).

In general, the CO_2 solubility showed a strong dependence on the sugar content of the matrix (Illera et al., 2019; Ferrentino et al., 2010) while occurred a slight variation with citric acid content as noticed by Illera et al. (2019). Thus, even in treatments with the same conditions in a matrix (apple juice) with different soluble solids content (see Table 3), the inactivation efficiency can be greatly impacted due to the CO_2 solubilization capacity (Porębska et al., 2017).

The majority of the works presented in Tables 2 and 3 do not include the CO_2 ratio (mass or volume) in their discussions. However, Silva et al. (2018) observed that the CO_2 volumetric ratio was the most significant parameter for the *Lactobacillus casei* inactivation in apple juice. The authors achieved inactivation of more than 6 log operating at 15 MPa, 55 °C at 70% CO_2 volume for 30 min. Due to the synergistic effect of the SC-CO_2 ratio, below 30% only a treatment above 55 °C would provide a 6 log reduction. Furthermore, in the enzymatic treatment of polyphenol oxidase conducted by Benito-Román et al. (2019), a significant effect attributed to the CO_2: volume ratio was observed, and the authors achieved almost total inactivation by treating the polyphenol oxidase solution with CO_2 ratios up to 3 g/mL.

For the treatment of *Escherichia coli* in pumpkin puree, Santos Júnior et al. (2021) observed that among the volumetric ratios of 1:0.5, 1:0.75, and 1:1 (puree: CO_2), the one with the highest CO_2 proportion favored the best *Escherichia coli* inactivation condition. In the study for inactivation of species of the genus *Legionella* in continuous flow water, total inactivation was achieved at a ratio of 1.0 CO_2/suspension flow rate, which was not observed at 0.5 (Martín-Muñoz et al., 2022). Likewise, among the mass ratios 1:0.2; 1:0.6, and 1:1 (human milk: CO_2) evaluated, the greatest results for reducing mesophilic aerobic and *Escherichia coli* amounts were achieved with the highest CO_2 content (1:1) (Michelino et al., 2018). In the same trend, Casas et al. (2012) reduced 4 logs of *Alicyclobacillus acidoterrestris* in apple cream with CO_2 at 30 °C and 10 MPa and it was noticed that an increase in inactivation was achieved by doubling the CO_2 flow rate to 4g/min. However, the treatment efficiency was only increased when the increase in flow was associated with the increase in turbulence.

Although the commented data so far for this parameter shows it sensible to CO_2 ratio, different trends were found in other works. For example, when the Salmon: CO_2 mass ratio varied from 1:0.2 to 1:1.0, Barbosa et al. (2020) did not observe a significant increase in the inactivation of *Staphylococcus aureus* in raw salmon. Likewise, Kincal et al. (2006) did not observe the influence of the CO_2 mass ratio: orange juice on the inactivation of *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. Ceni et al. (2016) observed only a slight difference, from 94.5 to 98.2%, in alkaline phosphatase inactivation with an increasing CO_2:milk mass ratio from 0.05 to 0.45 and besides that, was noticed by Ceni et al. (2016) that this same higher ratio caused the milk coagulation due to the higher concentration of carbonic acid in the medium. In this sense, this issue must be taken into account in the treatment of raw materials sensitive to acidic media.

3.5 Time treatment influence

Likewise that in the conventional pasteurization treatments, the contact time between CO_2 and the cellular material has shown a significant effect on the efficiency result achieved as well (Ceni et al., 2016; Barbosa et al., 2020). A longer process time provides a more efficient diffusion of CO_2 in the aqueous medium and, consequently, greater contact with the matrix components and microbiological reduction (Silva et al., 2018). Despite this, in general, it is desired to reduce the process time. As realized in Tables 2 and 3, approximately 26% of the works considered that the process time influenced the inactivation or directed the endeavor to achieve satisfactory inactivation in a possible shorter time. For this, some studies optimized other parameters so that their synergistic effect resulted in a decrease in the time required (Martín-Muñoz et al., 2022; Ceni et al., 2016; Silva, 2018; Paniagua-Martínez et al., 2018).

Was realized in the last paragraph that quite often it's expected that the achieved inactivation increases as much as the process time increases as well. On the other hand, in the results found in this review, process times for HP-CO_2 treatments vary

widely, from some minutes to a few hours (Benito-Román et al., 2019; Paniagua-Martinez et al., 2018; Castillo-Zamudio et al., 2021; Santos-Júnior et al., 2021; Barbosa et al., 2020). However, as seen in Tables 2 and 3, most of the works achieve their greatest results in around 20 and 30 min (Rao et al., 2016; Sikin et al., 2016; Silva et al., 2018; Marszałek et al., 2019; Murtaza et al., 2019; Bourdoux et al., 2022; Chen et al., 2022).

Even though the process larger time provides the greatest contact between the CO_2 and the matrix compounds, ultrasound-assisted HP-CO_2 studies achieved effective inactivation of *Escherichia coli* with treatment times of 3 and 5 minutes (Paniagua-Martinez et al., 2018; Castillo-Zamudio et al., 2021). Chen et al. (2022) conducted their study with SC-CO_2 in almonds and noticed an increase in *Escherichia coli* inactivation with processing time as well. However, the authors observed a peak behavior in the results and after that, at 45 min, no increase in log reduction was noticed. It has been suggested that, in this case, the CO_2 inactivation capacity was complete by that time (Chen et al., 2022). Similar behaviors were also noticed by Fleury et al. (2018) and Santos Júnior et al. (2021).

Manzocco et al. (2017) achieved to reduce time for polyphenol oxidase inactivation in apple juice with increasing temperature and pressure up to 35 °C and 12 MPa, from which, the trend of time reduction did not remain. Although not a food study, in some of the trials by Hossain et al. (2015), the relevance of the synergistic effect of process time was suppressed by pressure and temperature parameters in the treatment of *Enterococcus faecalis* and *Escherichia coli* in clinical solid waste. Therefore, some results showed that the action of time can be limited or even suppressed by the influence of other parameters.

4. Final Considerations

Based on this discussion, it is possible to understand that process parameters have different effects in different situations. The temperature, even if below the value that characterizes a heat treatment, in most of the studies presented in this research, this parameter played a meaningful role in food preservation. This is possibly due to the increase in fluid diffusivity and the stimulation of permeability of membranes and other structures, although there are a few cases where this parameter does not influence the process yields.

The pressure was also one of the parameters most cited as significant in this process and its effects were relevant in the stabilization of solid and non-solid foods. It induces the solubilization of CO_2 until its saturation in the medium and was a meaningful parameter in some works, although in some others with HP-CO_2, slight variations (up to 10 MPa) have not increased significantly in the treatment of microorganisms.

In most of the data discussed in this work, assays with a higher CO_2 ratio were better in terms of efficiency, when compared to the same treatments with smaller amounts. Despite this, around only 10 % of the studies mentioned its effects. It is possible that the amount (or ratio) of CO_2 used in the treatment is not directly related to its solubility in the matrix. In this case, to become the process more affordable, the choice of treatment ratio must also be evaluated in terms of its solubility. Despite large variations, most of the studies reported the variation from 20 to 30 min as the needed time for their greatest inactivation. It is possible that this time can be reduced if HP-CO_2 is combined with other technologies such as ultrasound.

In order to make clear comparisons and conclude HP-CO_2 treatment is still a challenge, as different equipment and process parameters have been used in different studies. As in other conventional heat treatments, in general, it was observed that enzymes and microorganisms present different resistances to the HP-CO_2 processes parameters which can vary even more with the change of the components of the matrix and the source of the microorganism or enzyme. Therefore, results observed in a given food matrix may be observed differently with the same treatment applied to another matrix. Furthermore, the HP-

CO_2 treatment has shown to be efficient for the inactivation of enzymes and microorganisms in a series of works, showing itself as a technology with potential applicability for the stabilization of foods without heat treatment.

In this sense, some disparities occurs in the main parameters of the inactivation process being more drastic or not that in turn must be assessed depending on the case. Therefore, further research is essential to demonstrate and explain the effects and the overall expected results for of each one of HP-CO_2 process parameters and variables to food stabilization.

Acknowledgments

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