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Modelling of inactivation of microorganisms in the process of sterilization using high pressure supercritical fluids

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Abstract

High hydrostatic pressure technology is a relatively new method for the food industry and is considered more as an alternative to traditional storage methods such as thermal processes. Inactivation of spores, models, yeasts, and viruses has been demonstrated by this method. Although issues related to the safety and longevity of food, as well as their legal permits, require extensive case studies, the available experimental findings can be useful in expanding the potential applications of high pressure in the food industry. In this paper, CO_2 is used as a fluid. Increasing the pressure in Weibull and log-logistic models from 2.5 MPa to 10 MPa has reduced the processing time from 700 minutes to 70 and 60 minutes, respectively. The log-logistic model in predicting the process of inactivation of microbes compared to the Weibull model has been the lowest, and also the log-logistic model has a suitable ability to predict the shoulder of the chart if the Weibull model does not have this ability and its error is almost high. Increasing the increase in pressure has increased the level of inactivation of Salmonella typhimurium and Listeria monocytogenes, except Listeria monocytogenes at a pressure of 6.05 MPa, which reduced inactivation.

Keywords: food industry; high-pressure supercritical fluids; CO_2; weibull; log-logistic.

Practical Application: Increased pressure in high hydrostatic pressure technology has reduced processing time.

1 Introduction

High hydrostatic pressure (HHP) is a method of food preservation, or sterilization in which the product is exposed to very high pressure and some harmful microorganisms and enzymes are inactivated (Plazzotta & Manzocco, 2019). High-pressure technology stops the chemical activity of microorganisms. In the last decade, the technology of its use in the food industry has also expanded (Erkmen, 2021). The effect of high pressures on the inactivation of microorganisms has been known since the beginning of the twentieth century. Unlike the thermal method, this method is not time and mass-dependent, so the time required to perform the process is short (Costa et al., 2021). Today, although food hygiene is very important for consumers, most consumers prefer foods that have the right appearance, aroma, and taste and are free of preservatives. These two goals can be achieved by using high-pressure process technology (Jabeen et al., 2021). High-pressure processing can be used to increase food storage time, defrost frozen food, and preserve food without the use of freezing. With proper pressure, undesirable microorganisms, spores, and enzymes are inactivated, resulting in increased food storage time. This technique is currently used to preserve fish, meat, buttermilk, salad dressings, fruits, and vegetables (Zhang et al., 2021). This new method does not change the sensory properties and texture of food and increases the material's shelf life. In this technology, the protein in the food is denatured, and the non-covalent bonds are weakened, but the original structure of the food is preserved because high pressure is a non-thermal method that does not affect the covalent bonds (Solichah et al., 2021). The high-pressure process is very fast and uniform and is not affected by the size of the food container and its thickness. In general, this technology is considered a natural preservation method in which no chemical preservatives are used (Carvalho, 2017). High-pressure food processing involves a standard

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process profile. The pressure increases at a certain rate until it reaches the target pressure, the target pressure is maintained for a certain period of time, and then the pressure is released at a certain rate. Common pressures applied to food are between 300 and 800 MPa. The use of high hydrostatic pressure has been considered one of the non-thermal food processing methods in recent years (Ciocca et al., 2017). This process is performed at medium temperature but at high pressure (up to 900 MPa), which as a result, the industrial implementation of this process faces economic problems. Economizing the high-pressure process requires the use of techniques and other effective factors in inactivating microbes (Fan et al., 2019). In this regard, the effect of high-pressure fluid has been identified in recent years. At moderate temperatures and pressures, fluid can inactivate germ cells, bacteria, yeasts, and models. In this method, the applied pressure can be less than 20 MPa, which is much more suitable compared to the applied pressure in the high-pressure process (Flynn et al., 2019).

In this study, it was tried to evaluate microbial growth over time. An effective design of processing treatments requires an exact perception of the heat resistance of this microorganism. Considering that industrial treatments are dynamic, this perception must include how the heat resistance of the microorganism is affected by the heating rate during the heating and cooling phases (Huertas et al., 2021). Such models configuration the engineering basis for designing, assessing, and optimizing high hydrostatic pressure processes as a new protection technique (Buzrul et al., 2005). Kahraman et al. (2017) treated with an MTS mixture of apple and carrot juice to inactivate Escherichia coli. They concluded that the Weibull and log-logistic models provided the foremost fitting of the inactivation data for the MTS treatments. Kingsley et al. (2007) examined the inactivation of norovirus by high-pressure processing. Characterizing inactivation of the norovirus successor FCV by HPP as a function of treatment time indicated that while increased treatment time yielded greater inactivation, there was a diminishing increase in inactivation stable with nonlinear Weibull or log-logistic inactivation kinetics.

2 Material and methods

2.1 Process time and kinetic deactivation

In this study, expect that inactivation is directly related to the time of the process, but the time required depends on the type of microbe (bacterium or fungus), secondly on the form of the microbe, and thirdly on the conditions of the process. If the inactivation process consists of two stages. The first stage of inactivation is due to the slow penetration of fluid into the cell wall, and the controlling stage is inactivation. In the second stage, fluid extracts vital compounds from the cytoplasm or membrane and causes cell death (Jagadeesan et al., 2019; Seyyedi & Ayati, 2021). A number of researchers has observed this two-stage kinetics. Some studies show only the second stage, which is linear, which can be just one type of two-stage kinetics. As the pressure and temperature increase, the first stage becomes shorter. Only the second stage is observed at some temperatures, but in general, two-stage curves are more common in studies. Some curves also show a high initial deactivation rate and then a slow deactivation (Kah et al., 2019).

2.2 The effect of temperature, pressure, and state

In general, inactivation increases with increasing temperature because the fluidity of cell membranes increases and makes it easier for CO_2 to penetrate, and on the other hand, with increasing temperature, the phenomenon of CO_2 penetration increases. Higher pressures facilitate the process of dissolving in water and penetrating the cell wall, and increasing the density and consequently the extraction power (Marvin et al., 2017). All of which increase the inactivation process, the supercritical state has the property of penetration similar to gas and density similar to the liquid phase.

The use of cyclic pressure is an effective way to increase process efficiency. Cyclic pressure includes the steps of increasing and decreasing pressure repeatedly. There are two theories to justify the effect of cyclic pressure on increasing microbial killing: A) Cell burst theory (Nogales et al., 2020), b) Increased mass transfer theory, the use of cyclic pressure also increases the destruction of spores.

2.3 Process modeling

Laboratory data are available as a correlation between the number of living organisms in the form of [$S_{(t)} = N_{(t)}/N_o$] and process time, which is usually shown in semi-logarithmic diagrams. In some processes, especially in the thermal inactivation of spores, the connection (log S), t is in the form of a straight line with a reduction of about 4-6 logarithmic cycles, in which case the kinetic calculations of the first degree are true (Figure 1a). Nonlinear semi-logarithmic survival diagrams include three different shapes, which are: ı) Curve with shoulder according to Figure 1b; ii) Tailing curve according to Figure 1dc; iii) S-shaped curves according to Figure 1ef.

In this paper, we seek to find a suitable mathematical model to predict the process of microbial inactivation or the same microbial survival chart so that the model has the ability to predict different types of microbial survival charts.

Commonly, control and optimization of food industry processes require the use of mathematical models. Among these, the Weibull model has been successfully used to define the kinetics of chemical, enzymatic and microbiological demotion processes (Issis et al., 2019). The Weibull distribution function

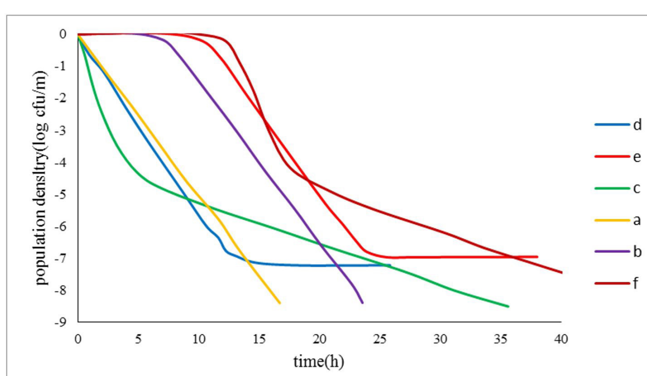

Figure 1. Types of microbial survival charts (Xiong et al., 1999).

can be used to describe the cleaning kinetics of high-pressure supercritical fluids (Gerhards et al., 2019). A hypothesis that the Weibull distribution could be used to approximate the experimental data was verified for estimated parameters of distribution (Kurek et al., 2020). Brodowska et al. (2017) modeled ozone-based therapies to inactivate microorganisms and take into account various microorganism sufficiency to ozone; it was of great importance to develop a susceptibility effective ozone dose to retain food products using various strains based on the microbial model. The kinetic rate constant can be modeled using Arrhenius and log-logistic models with satisfactory evaluation (Kaczmarek & Muzolf-Panek, 2021).

3 Results and discussion

3.1 Investigation of Weibull model

By passing the best curve using the Weibull model on the laboratory results of inactivation of three types of microorganisms in laboratory environments and different pressures. To solve the differential equations, the parameters of the models must be in the form of pressure functions. The results of the solution can be found in Figures 2, 3 and 4 saw. As can be seen from the diagrams in Figure 2, the effect of the amount of process pressure on the rate of inactivation and process time can be understood. Increasing the pressure from 2.5 MPa to 10 MPa has reduced the processing time from 700 minutes to 70 minutes, and this indicates that the appropriate pressure for inactivation of Saccharomyces cerevisiae in broth medium is higher than the high pressure of the supercritical fluid. As the pressure increases, the amount of comb in the graph decreases, meaning that more pressure can break down the microorganism resistance and inactivate them. Comparing the results of the model with the laboratory results, it can be concluded that the Weibull model is not very accurate in predicting the flat part at the beginning of the shoulder chart.

The diagrams in Figure 3a also show the fact that increasing the pressure from 1.51 MPa to 6 MPa, reduces the time required to complete the process from 160 minutes to 35 minutes and shows that whatever the process pressure to pressure. The closer the supercritical fluid is, the greater the success of the process, which can be due to the special properties of the fluid in the supercritical state, and in this case, if the process temperature also increases, it will have a greater effect and reduce the processing time. The process of inactivation of Listeria monocytogenes

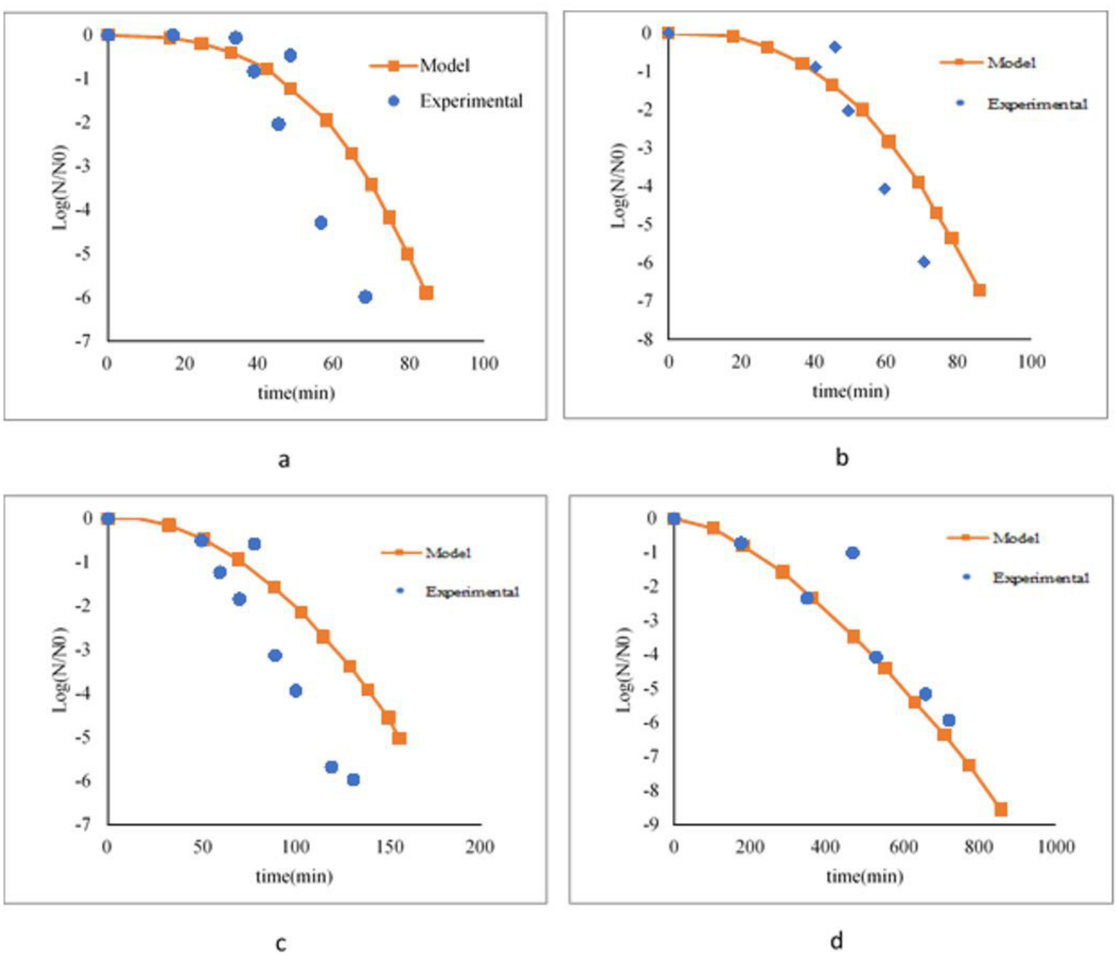

Figure 2. Saccharomyces cerevisiae inactivation curve using a high-pressure supercritical fluid with Weibull model at 40 °C and pressures (a) 10 MPa, (b) 7.5 MPa, (c) 5 MPa, (d) 2.5 MPa.

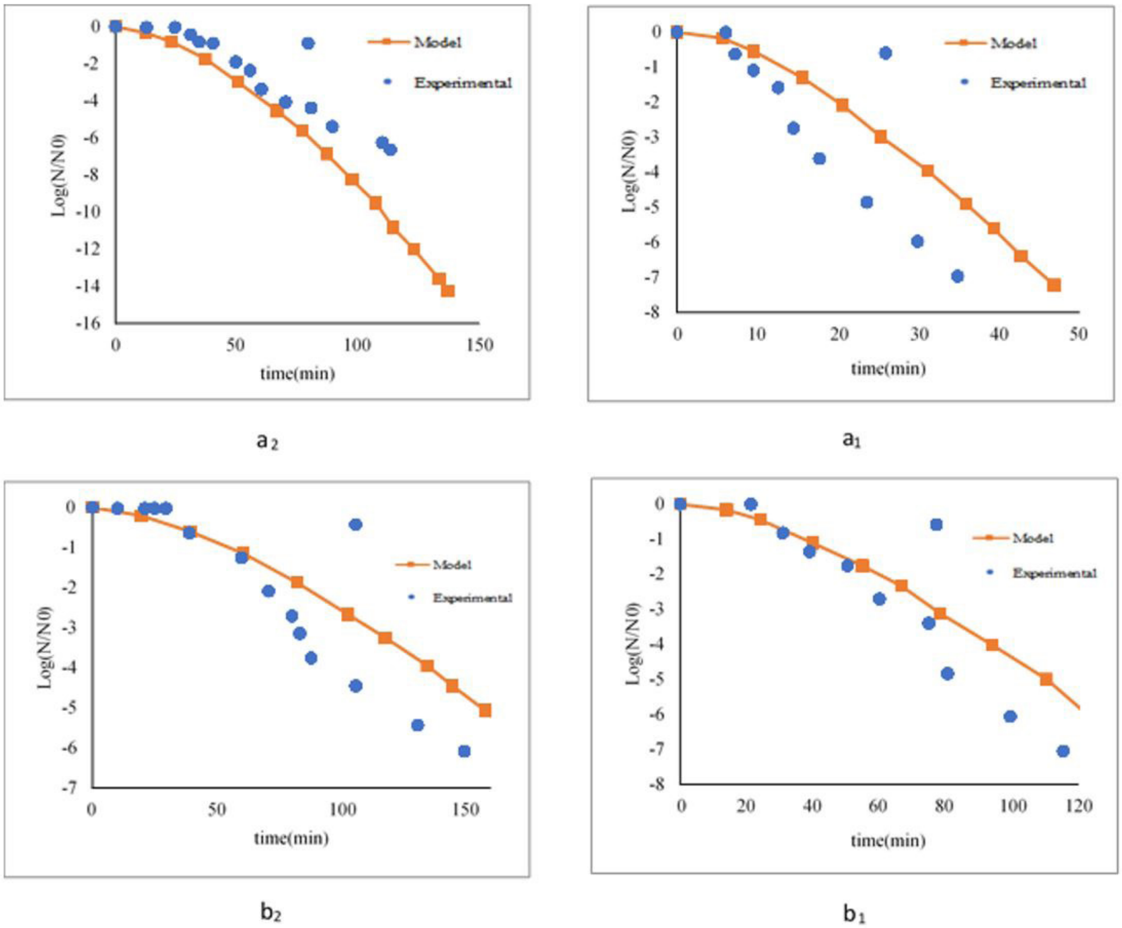

Figure 3. Prediction of Listeria monocytogenes inactivation curve in (a) saltwater, (b) broth, with the high-pressure supercritical fluid process at 25 °C by Weibull model and pressures (1) 6.05 MPa, (2) 3.02 MPa.

follows two-step kinetics, the first of which involves the resistance of the microbes to the lethal agent and forms the comb of the chart. As we have seen in the case of Saccharomyces cerevisiae, the Weibull model does not have the ability to predict the first stage of the graph, and its error is almost high.

In this case, the Weibull model had little accuracy in predicting the deactivation process and the total process time, so that at 3.02 MPa, according to laboratory results, the time required to reach seven logarithmic cycles is about 120 minutes, while the Weibull model only this time. It estimates 70 minutes and vice versa at 1.51 MPa the time predicted by the Weibull model for the process is more than the laboratory value, which reduces the confidence in the Weibull model. In general, the error of the Weibull model in predicting the inactivation of Listeria monocytogenes in a saline environment is high. It is clear that the broth environment is much more nutritious than the brine environment, thus protecting the microbes against the deadly agent and increasing their resistance, so to inactivate the microbes in a nutritious environment, have to increase the pressure. We will be the temperature to reduce the processing time; of course, other factors such as the use of cyclic pressure and additives can also be used. In this case, the adaptation of the model to the laboratory results is more appropriate than the saltwater environment, but the Weibull model still does not have the ability to predict the shoulder part of the chart well, and this has caused a lot of model error. As can be seen from the microbial inactivation process in Figures 4, in this case, both the temperature and the pressure are higher than those of Ester or monocytogenes, which reduces the microbial resistance to the lethal agent and the amount of time in the first stage of the ratio diagram. Listeria monocytogenes are reduced, but the gram-positive Salmonella bacterium can also cause this decrease in the shoulder of the graph because gram-negative bacteria are usually inactivated earlier than gram-positive. In this case, there is not much difference between the time of the first phase of the graph in the laboratory data and the time predicted by the Weibull model, but the model error is not appropriate in predicting the total deactivation process, and the model error is high especially at 7.56 MPa. Table 1 shows the Weibull model error value for predicting the survival curves of various microorganisms.

3.2 Investigation of log-logistics model

The best curve using the rubber model on the laboratory results of inactivation of three types of microorganisms in laboratory environments and different pressures. An important advantage of the log-logistic model compared to the Weibull model is its very

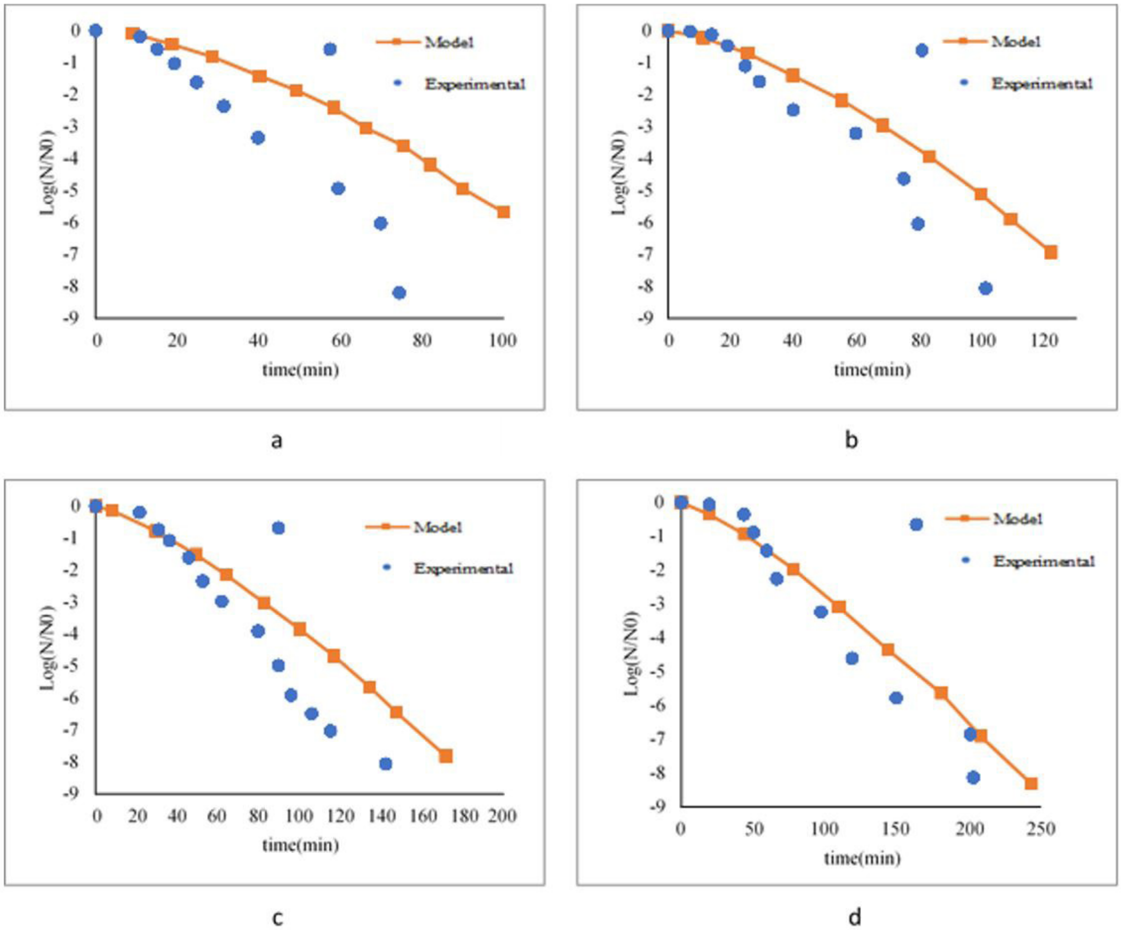

Figure 4. Prediction of Salmonella typhimurium inactivation curve in broth medium with the supercritical fluid process at 35 °C by Weibull model at pressures of (a) 7.56 MPa, (b) 6.05 MPa, (c) 3.02 MPa, (d) 1.51 MPa.

Table 1. Weibull model error value in predicting microbial survival curves for different microorganisms.

Microorganism	Pressure (MPa)	Error	R^2
Saccharomyces cerevisiae	10	0.1706	0.9455
In the broth environment	7.5	0.4959	0.9331
	5	0.6781	0.8883
	2.5	2.59	0.62
Listeria monocytogenes	6.05	0.7556	0.8904
In a saltwater environment	3.02	9.3138	0.517
	1.51	1.0476	0.8533
Listeria monocytogenes	6.05	0.1045	0.9858
In the environment of broth	3.02	0.2221	0.9556
	1.51	0.1415	0.819
Salmonella typhimurium	7.56	2.2547	0.7297
Broth environment	6.05	0.5168	0.9448
	3.02	0.4722	0.9458
	1.51	0.3106	0.9883

high flexibility in adapting to laboratory data, and it is much better than the Weibull model in predicting results such as the shoulder length of the graph. Although still the duration of resistance of microbes to the pressure factor (shoulder length chart).

Figure 5 shows the prediction of the Saccharomyces deactivation inertia curve using supercritical fluid at 40 °C for different pressures. Figure 5 demonstrates that the processing time has increased as the pressure has decreased, with the minimum time occurring at a pressure of 10 MPa and the maximum time occurring at a pressure of 2.5 MPa.

Figure 6 shows the log-logistic model prediction of the Listeria monocytogenes inactivation curve using high-pressure

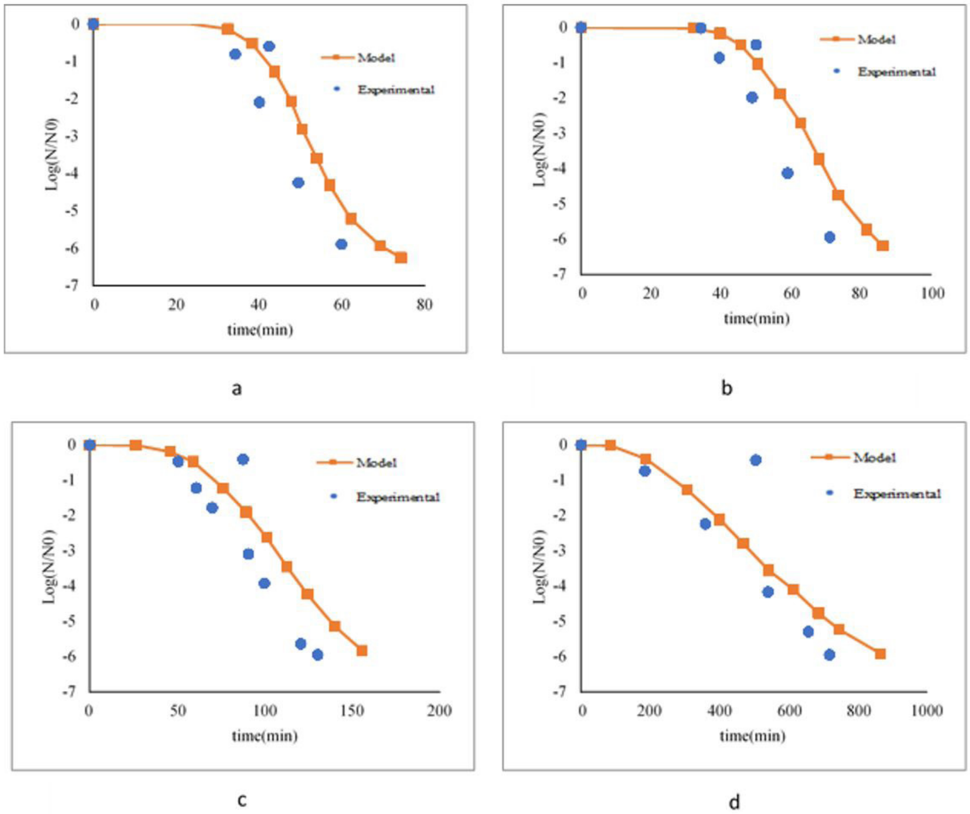

Figure 5. Prediction of Saccharomyces deactivation inertia curve using supercritical fluid at 40 °C and pressures of (a) 10 MPa, (b) 7.5 MPa, (c) 5 MPa, (d) 2.5 MPa.

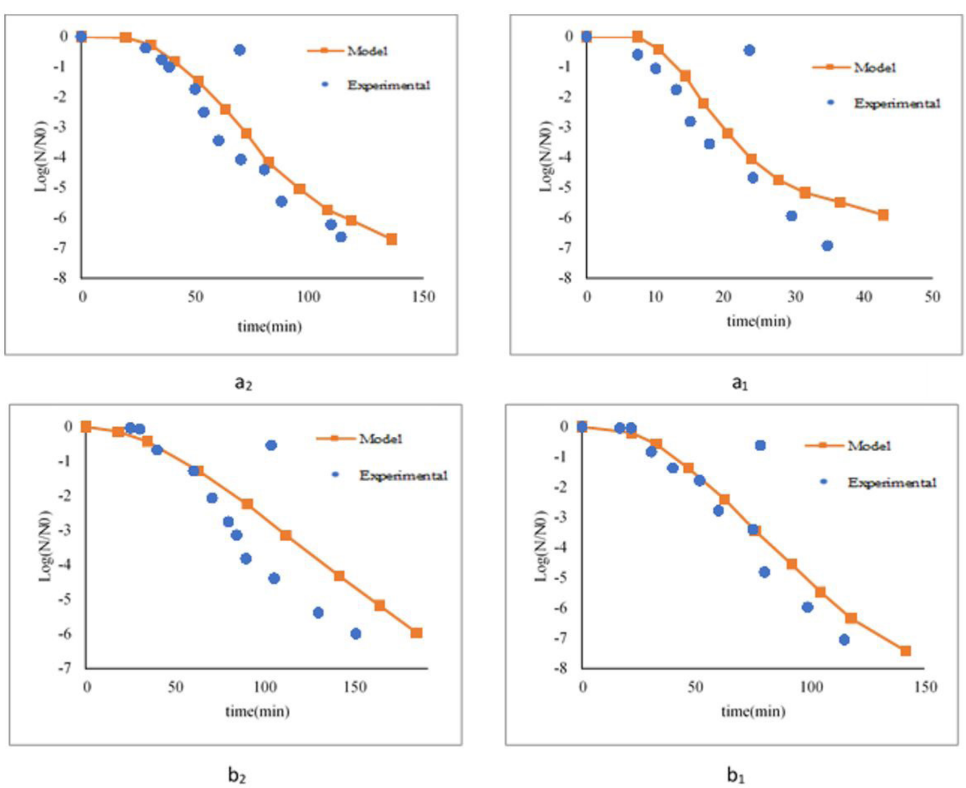

Figure 6. Prediction of Listeria monocytogenes inactivation curve using high-pressure supercritical fluid by the log-logistic model at 25 °C in (a) brine, (b) broth and pressures (1) 6.05 MPa, (2) 3.02 MPa.

supercritical fluid at 25 °C for two materials, brine and broth. In addition, each of the two materials has been evaluated at 3.02 and 6.05 MPa. Increasing the pressure decreased the processing time for brine but did not affect the processing time for broth, according to the results.

Figure 7 depicts the prediction of the inactivation curve of Salmonella typhimurium in the broth medium with a supercritical fluid process at high pressure and 45 °C by the log-logistic model at different pressures. According to Figure 7, it is evident that the processing time has increased as the pressure has decreased; thus, the shortest processing time occurred at a pressure of 7.56 MPa and the longest at 1.51 MPa.

The predictions made by the model are not entirely consistent with the experiments but are much better than the Weibull model. This factor, as well as the good flexibility of the log-logistic model, one of which may be the three-parameter nature of the model, has significantly reduced its error in predicting the deactivation process. As mentioned, the logistic model can better predict microbial survival curves and is more suitable with less error. The error values of the logistic model can be seen in Table 2.

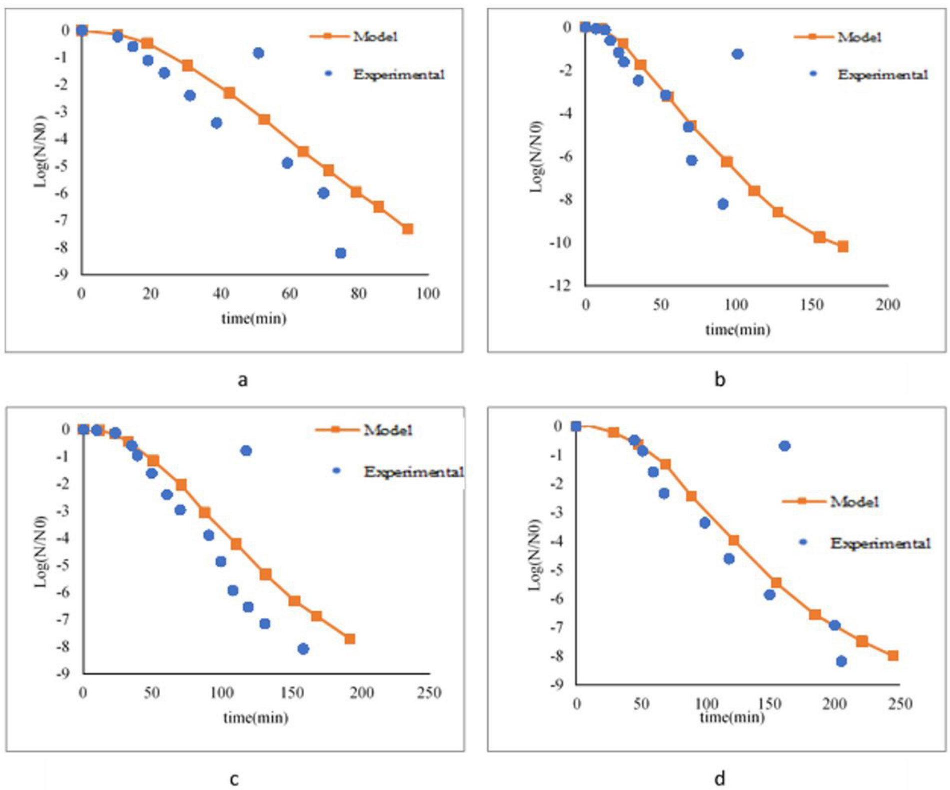

Figure 7. Prediction of Salmonella typhimurium inactivation curve in broth medium with the supercritical fluid process at high pressure at 45 °C by the log-logistic model at pressures of (a) 7.56 MPa, (b) 6.05 MPa, (c) 3.02 MPa, (d) 1.51 MPa.

Table 2. Log-logistic model error value in predicting microbial survival curves for different microorganisms.

Microorganism	Pressure (MPa)	Error	R^2
Saccharomyces cerevisiae	10	0.1017	0.9863
In the broth environment	7.5	0.0433	0.9956
	5	0.0464	0.9934
	2.5	0.0391	0.9959
Listeria monocytogenes	6.05	0.2591	0.9676
In a saltwater environment	3.02	0.0807	0.9882
	1.51	0.2111	0.9726
Listeria monocytogenes	6.05	0.1716	0.9767
In the environment of broth	3.02	0.2205	0.9605
	1.51	0.3401	0.9399
Salmonella typhimurium	7.56	0.2978	0.9883
Broth environment	6.05	0.2293	0.9879
	3.02	0.141	0.9973
	1.51	0.1997	0.9943

4 Conclusion

High hydrostatic pressure is a process that can inactivate microorganisms, spores, and viruses at low and medium temperatures while maintaining the sensory and nutritional properties of food. This new non-thermal technology has the potential to be used in the development of a new generation of value-added foods. High hydrostatic pressure is unlikely to replace all traditional processing methods but may be used as a complement to these methods. In addition, the new physicochemical and sensory properties obtained from this process provide new and exciting opportunities in the industry. Both Weibull and log-logistic models have suitable results in initial adaptation to laboratory data to find model parameters, and of course, the log-logistic model has much better results and very high flexibility in adapting to laboratory results.

The log-logistic model in predicting the process of inactivation of microbes compared to the Weibull model has been the lowest, and also the log-logistic model has a suitable ability to predict the shoulder of the chart if the Weibull model does not have this ability and its error is almost high. Increasing the increase in pressure has increased the level of inactivation of Salmonella typhimurium and Listeria monocytogenes, except Listeria monocytogenes at a pressure of 6.05 MPa, which reduced inactivation.

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