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Ultrasound-assisted supercritical carbon dioxide drying of cod fish (*Gadus morhua*)

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A R T I C L E I N F O

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A B S T R A C T

Supercritical CO_2 ($scCO_2$) drying has emerged as a promising alternative to reduce water content at low temperatures and simultaneously inactivate microorganisms, thus increasing product safety while preserving product quality. In this study, $scCO_2$ drying was applied to cod fish samples at 40 °C and 10 MPa for 360 min alone and in combination with high power ultrasound (HPU). Results were compared with traditional air drying at 40 and 60 °C. The drying rate for the $scCO_2$ was similar to that of air drying at 40 °C while adding HPU induced a higher water loss comparable to air drying at 60 °C, reaching a final water activity of 0.565 ± 0.051. The inactivation of *E. coli* and *L. innocua* was possible for both $scCO_2$ treatments, achieving a complete inactivation (>5-log reduction) after 60 min of treatment. No significant inactivation was achieved with air drying, even at 60 °C. $ScCO_2$ and $scCO_2$+ HPU-dried samples exhibited higher specific volume, lightness, porosity, wettability, rehydration capacity, and improved texture compared to air-dried samples. Overall, these findings demonstrate the feasibility of using $scCO_2$ to produce a microbiological safer dried fish product with higher quality compared with traditional air drying and that the combination with HPU can accelerate the process without side effects on the product. Future work shall focus on the evaluation of sensory quality, consumer acceptance, and the preservation of key nutritional attributes during storage to further support industrial applications.

1. Introduction

Fish consumption provides essential nutrients to a large portion of the global population, making a significant contribution to human nutrition. Fish is highly palatable and represents a vital source of high-quality animal protein, vitamins, minerals, micronutrients, and essential fatty acids. As the health benefits of fish are increasingly recognized, consumer demand continues to grow (Chen et al., 2022; FAO, 2024).

Dried fish is a valuable alternative to fresh consumption, as it extends shelf life, reduces waste, and facilitates distribution, making fish more accessible and affordable, particularly in less developed regions (Siddhnath et al., 2022). However, maintaining the nutritional value and overall quality of dried fish is crucial to ensuring its benefits for consumers. Conventional drying methods involving simultaneous heat

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and mass transfer, such as sun and hot-air drying, induce numerous physicochemical and nutritional changes that affect the overall quality of dried products (Vidinamo et al., 2021; Zhang et al., 2025). Over the past decades, several low-temperature drying methods have been developed to reduce moisture content while better preserving product quality and nutritional value. Among these, freeze drying is the most widely used technique. While it ensures excellent preservation of the quality (Ma et al., 2023), the process is typically costly and time-consuming (Liu et al., 2022). Additionally, neither freeze drying nor air drying significantly reduces the microbial load of the products (Alp and Bulantekin, 2021; Bourdoux et al., 2018).

In recent years, the use of supercritical carbon dioxide ($scCO_2$) has been explored for drying various food products, including both plant-based (Bourdoux et al., 2018; Zambon et al., 2021, 2022b) and protein-rich matrices (Bernardo et al., 2024; Morbiato et al., 2019). This technique offers potential advantages in terms of reducing drying time compared to freeze drying while still preserving the structural and nutritional integrity of food. Preliminary studies on tuna (Bernardo et al., 2025) and cod fish (Bernardo et al., 2024), have demonstrated that $scCO_2$ drying can effectively reduce moisture, however, aspects related to product safety and quality remain largely unexplored. Dehydrated fish products, particularly those stored at ambient conditions, are susceptible to microbiological risks, such as the persistence of pathogenic or spoilage microorganisms, including *Listeria monocytogenes*, *Salmonella* spp., or *Vibrio* spp. (Bahrndorff et al., 2022; Han et al., 2025). Furthermore, their quality is strongly influenced by textural properties, color, rehydration ability, and the retention of bioactive compounds, all of which are affected by the drying process and must be carefully assessed (Fitri et al., 2022; Rasul et al., 2022).

To further enhance the efficiency of $scCO_2$ drying, high-power ultrasounds (HPU) have been proposed as a complementary technology (Cardin et al., 2024; Michelino et al., 2018; Morbiato et al., 2019). HPUs facilitate moisture removal by enhancing mass transfer, which leads to improved drying kinetics (Astráin-Redín et al., 2019) achieving lower water activity (Cardin et al., 2024; Michelino et al., 2018).

Therefore, this study aimed to investigate the application of $scCO_2$ in combination with HPU as a novel low-temperature drying method for fish, evaluating process efficiency as well as product safety and quality aspects that have so far received limited attention. Cod fish (*Gadus morhua*) was selected as an abundant and widely consumed species worldwide; results were compared with traditional air drying performed at two different temperatures. The study focused on evaluating the water removal efficacy, the inactivation of two pathogen-surrogate microorganisms (*Escherichia coli* and *Listeria innocua*), followed by the assessment of key quality attributes, including color, wettability, microstructure, and texture. By integrating HPU with $scCO_2$, this work aims to demonstrate the feasibility and advantages of this innovative process for producing high-quality, microbiologically safe dried fish products.

2. Material and methods

2.1. Sample preparation

Atlantic cod (*Gadus morhua*) fillets were purchased from a local fish market in Padova, Italy. The fillets were transported under refrigerated conditions (6 ± 1 °C) to the laboratory at the University of Padova, Italy, and subsequently stored at −80 °C. Before each drying treatment or analysis, the samples were thawed at 4 °C for 12 h. This strategy allowed to work on the same product for the whole duration of the experimental campaign. For the experiments, sample pieces of 1.0 ± 0.1 g were prepared in the form of cubes (1 cm × 1 cm x 1 cm). The sample dimensions were selected to ensure uniform heat and mass transfer and to comply with the geometrical constraints of the supercritical CO_2 drying reactor.

2.2. Drying with supercritical carbon dioxide

Drying with supercritical carbon dioxide ($scCO_2$), both alone and in combination with high-power ultrasound ($scCO_2$+ HPU), was conducted using a lab-scale plant (Separex S.A.S., Champigneulles, France) where CO_2 is continuously recycled and regenerated, minimizing the fluid consumption (Bernardo et al., 2024; Bourdoux et al., 2018; Zambon et al., 2022a). A simplified representation of the system is shown in Fig. 1.

Briefly, the setup included two vessels: the drying one (DV, 150 mL, 2.5-cm diameter), where the food samples are placed, and a regeneration one (RV, 600 mL, 6.0-cm diameter), where the humidity present in the CO_2 flow is adsorbed onto molecular sieves (zeolites MS-562, D&F Techniek B.V., Rijen, Netherlands). The system also comprises a CO_2-tank (purity 4.0, Siad Spa, Bergamo, Italy), a cooling reservoir (H1, M418-BC, MPM Instruments Srl, Italy), pressurization (P1, EK01, Lewa GmbH, Leonberg, Germany) and recirculation pumps (P2, P300, Separex S.A.S., Champigneulles, France), a heat exchanger (H2), and an automated control system managed by LabVIEW software (National Instruments Corp., Austin, TX, USA). This software facilitates monitoring pressure, temperature, and flow rate.

Drying experiments were conducted at 40 °C with a pressure of 10 MPa. These operating conditions were selected based on previous studies reporting efficient water removal while limiting thermal degradation of fish proteins and lipids (Bernardo et al., 2024). A low CO_2 flow rate, 10 kg/h, corresponding to a velocity in the drying vessel of 0.0091 m/s, was chosen for both $scCO_2$ drying and $scCO_2$+ HPU treatments due to the significant influence of fluid velocity on HPU efficiency during the drying process, as highlighted by Cárcel et al. (2007). Pressurization and depressurization phases proceeded at rates of 0.4 MPa/min and 0.667 MPa/min, respectively. The total drying time was set at 360 min, with a target water activity level below 0.700 to prevent the growth of pathogens and non-halophilic microbes. A piezoelectric sonotrode connected to an ultrasonic generator (AA-WG1-Special, Aktive Arc Sarl, La Vue-des-Alpes, Switzerland) was used to generate HPU at 40 kHz, following the methodology of Morbiato et al. (2019). The sonotrode is composed of a hollow cylinder extending for the entire length of the drying vessel, with an internal diameter of 1.5 cm.

During the drying process, HPU was consistently operated at 10 ± 3 W, with manual adjustments to the sonotrode amplitude to maintain constant power. This power level was selected based on preliminary tests (results not shown) and on previous studies demonstrating that low-intensity ultrasound (≤15 W) is sufficient to enhance mass transfer during $scCO_2$-assisted processes without inducing significant heating or structural damage to the food matrix (Cardin et al., 2024; Morbiato et al., 2019). HPU was not utilized during the pressurization and depressurization phases. To ensure consistent experimental conditions across treatments, the sonotrode was also inserted into the drying chamber during $scCO_2$ drying tests without HPU, but it was kept switched off throughout the process.

For each experimental batch, 7 cod fish samples were placed inside individual stainless steel mesh baskets and stacked vertically within the sonotrode internal cavity. Preliminary tests were conducted to assess ultrasound propagation, power variation, and potential thermal effects. The results indicated no significant differences in ultrasound efficiency along the reactor length ($p < 0.05$), except for the first sample at the top of the reactor, which underwent a higher degree of drying. This behavior was also observed in the absence of HPU and is likely attributed to its proximity to the fluid inlet (less than 3 cm), where localized higher velocity may enhance moisture removal. For this reason, this sample was excluded from all subsequent analyses. Additionally, the results showed that the selected ultrasound frequency and power did not lead to significant energy transfer to the system, maintaining the target temperature within a ±2 °C range.

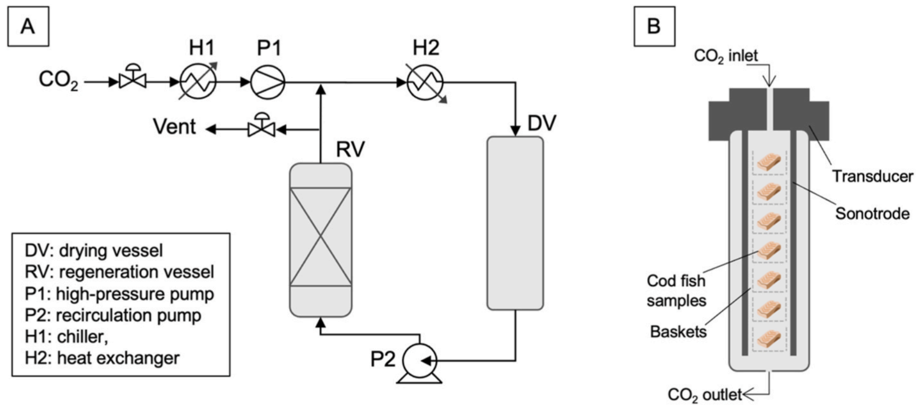

Fig. 1. (A) Simplified scheme of the scCO2-based drying plant with recirculation and (B) Schematic representation of the drying vessel.

2.3. Air drying

Air drying was employed as a conventional method for comparison with the innovative drying technique. A commercial convective dryer (Biosec Domus B5, Tauroessicatori, Vicenza, Italy) was used at two temperatures, 40 °C and 60 °C, for time intervals matching those of the innovative drying methods.

2.4. Evaluation of drying efficiency

The efficiency of the drying processes was assessed by measuring moisture content and water activity in the dried products, following the methodology outlined by Zambon et al. (2022a). The weight of each sample was precisely measured before and after treatment using a precision scale (PS 6000 R2, Radwag, Radom, Poland). To determine moisture content, samples were dried in an incubator (G-Cell 035, Fratelli Galli, Milano, Italy) at 70 °C until a constant weight was achieved, as previously reported (Bernardo et al., 2025; de Bruijn and Bórquez, 2014; Zambon et al., 2022b). The moisture content (wet basis), expressed as a percentage, was then calculated using Equation (1):

$$MC\,(\%) = \frac{W - W_{sm}}{W} \cdot 100\% \tag{1}$$

where W is the weight of the sample and W_{sm} represents the weight of the solid matter after complete dehydration.

Water activity of the samples, both before and after processing, were measured at room temperature (20 °C) using a HygroPalm HP23-AW-A device (Rotronic AG, Switzerland).

2.5. Microbial inactivation

For microbial inactivation studies, two reference strains were used: *Escherichia coli* NCTC 9001 (human urine isolate) and *Listeria innocua* NCTC 11288 (bovine brain isolate), were used to inoculate the samples, as described by Santi et al. (2023). Briefly, *E. coli* and *L. innocua* cultures were incubated overnight in Luria–Bertani (LB) medium broth (Sacco Srl, Como, Italy) and in BHI (Brain Heart Infusion) broth (Microbiol Srl, Cagliari, Italy) at 37 °C, respectively. The microbial suspensions were centrifuged at 6000 rpm for 8 min (Rotina 380 R, Hettich GmbH, Tuttlingen, Germany), the supernatant was discarded, and the pellet was resuspended in 10 mL of Ringer's solution (Merck KGaA, Darmstadt, Germany) to achieve a final concentration of 10^8 CFU/mL (Colony Forming Units per mL). Subsequently, 100 μL of the suspension was applied to the cod samples and allowed to sit for 15 min at room temperature before drying.

Fresh and dried cod samples were placed in a sterile 50 mL falcon tube containing 10 mL of sterile Ringer's solution. The tube was vortexed (ZX3 Advanced Vortex Mixer, Velp Scientifica Srl, Usmate, Italy) at 2200 rpm for 90 s. The solution was then serially diluted (1:10) in Ringer's solution. For the enumeration of *E. coli* and *L. innocua*, 100 μL of the appropriate dilutions were spread-plated on MacConkey agar with crystal violet (Microbiol Srl, Cagliari, Italy) and BHI agar (Microbiol Srl, Cagliari, Italy), respectively. Plates were incubated for 24 h at 37 °C in an incubator (Memmert GmbH, Schwabach, Germany) before enumeration.

2.6. Product quality evaluation

2.6.1. Color parameter measurement

The surface color of the samples was also assessed using a Tristimulus colorimeter (NR100, 3nh, Guangzhou, China) within the CIE 1976 (L*, a*, b*) color space, where L* represents lightness, a* redness, and b* yellowness, with scales ranging from white to black, red to green, and yellow to blue, respectively.

2.6.2. Specific volume measurement

Sample specific volume ($cm^3\ g^{-1}$) was obtained by rapeseed displacement according to AOAC methods (AOAC, 2000). Briefly, a container of known volume was filled with rapeseeds and gently levelled without compression. The rapeseeds were then partially removed, and the sample was placed inside the container. Rapeseeds were added again and the excess was levelled off. The volume of the sample was calculated from the difference between the volume of rapeseeds required to fill the container with and without the sample. Sample weight was recorded using an analytical balance (±0.001 g), and specific volume was calculated as the ratio between sample volume and weight ($cm^3\ g^{-1}$).

2.6.3. Scanning electron microscopy (SEM)

Field Emission Gun Scanning Electron Microscopy (FEG-SEM, Tescan Mira3, CZ, working distance = 10 mm, voltage = 10 kV) was used to evaluate the sample microstructure. Before observation, samples were made conductive by sputtering with a thin gold layer. Micrographs were acquired at different magnitudes (x100, x400, x800, x2,000, x5,000, and x10,000).

2.6.4. Instrumental texture

Firmness was measured by uniaxial compression test using an Instron 34TM-5 (Instron Ltd, High Wycombe, UK). Samples were tested by a 12.7-mm-diameter cylindrical probe (5 kN compression head) at a 5-mm min^{-1} crosshead speed.

2.6.5. Drop stability evaluation (wettability)

Sample wettability was evaluated as reported by Pagliarini et al. (2024) and by using a drop shape analyzer (DSA30S, Kruss, Hamburg, Germany). A 4-μL distilled water drop was measured out from the syringe and dropped on the surface of the samples at 25 °C. Videos were recorded and contact angles were analyzed using Drop Shape Analysis Software (Version 1.92.1.1). The drop was measured overtime, and the final value of contact angle calculated as the mean of at least 3 independent drops.

2.6.6. Rehydration capacity

Rehydration was performed following the method described by Morbiato et al. (2019), with slight modifications. After the drying process, the fish sample was placed in a beaker containing distilled water at a constant temperature of 30 °C for 15, 45, 90, 210 and 300 min. A solid-to-liquid ratio of 1:50 was employed to ensure excess water conditions. After each time, the samples were removed, drained for 15 min on a fine metal mesh to remove surface water, and then weighed. Rehydration ability was evaluated by calculating the rehydration factor (RF), which is the ratio between the rehydrated sample weight and the fresh one, as described by Wang et al. (2023).

2.7. Statistical analysis

Experiments were carried out with a minimum of three independent biological replicates, and data were expressed as the mean and corresponding standard deviations. A two-way analysis of variance (ANOVA) was conducted to assess the effect of treatment and time. When significant interactions or main effects were detected, Šídák's multiple comparison post-hoc test was applied to determine significant differences between specific groups. Statistical significance was set at p-value <0.05. All analyses were performed using GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Drying performance evaluation

The cod fish samples exhibited initial moisture content (wet basis) and water activity (a_w) values equal to 81.22 ± 0.43% and 0.974 ± 0.016, respectively, which are consistent with previous studies in literature (Boeri et al., 2013; Santacatalina et al., 2016).

These parameters were monitored during drying time, and the results are shown in Fig. 2. In particular, the kinetics of $scCO_2$ drying, with and without HPU, were compared to conventional air drying at two different temperatures, 40 and 60 °C. The values and the statistical analysis are showed in the Supplementary information (Table S1 and Table S2).

As expected, moisture content (Fig. 2A) progressively decreased during the drying process under all the tested conditions. In conventional air drying, moisture content followed a similar trend at both 40 and 60 °C for the first 200 min, a phase typically dominated by the evaporation of surface water. At longer treatment times, the process enters the falling rate period, where internal diffusion becomes the rate-limiting step. In this stage, the 60 °C became more effective due to higher thermal energy, which increases the water vapor pressure and enhances the effective water diffusion coefficient within the muscle matrix (Mujumdar, 2020). The $scCO_2$ drying resulted in a slower moisture reduction compared to air drying at the same temperature (40 °C), reaching a slightly higher final moisture content of 33.04 ± 2.88%. This difference can be attributed to different mass transfer mechanisms: despite the gas-like diffusivity of $scCO_2$ facilitating pore penetration, the limited solubility of water in the supercritical phase could acts as a bottleneck compared to air (Wang et al., 2018).

In the previous work by Bernardo et al. (2024), $scCO_2$ drying at 45 °C and 10 MPa applied on the same product reached of treatment a final moisture content of 62.64 ± 5.47% after 360 min. The higher drying efficiency observed in the present work is primarily due to the smaller sample size, which was reduced to permit the allocation of the sonotrode. The smaller size favored a faster water removal due to the shorter diffusion path, underlying the significant role of this factor in the drying processes. Notably, when the flow rate was increased from 15 kg/h to 25 kg/h under the same pressure and temperature conditions, a comparable final moisture content (35.19 ± 4.52%) was obtained (Bernardo et al., 2024). These results confirm the importance of flow rate in enhancing mass transfer during $scCO_2$ as previously observed also in red pepper (Zambon et al., 2020) and strawberry (Zambon et al., 2022a).

The influence of HPU was evident from early stages, accelerating moisture removal to reach a final moisture content comparable to air drying at 60 °C and approximately 11% lower than $scCO_2$ alone. This enhancement can be attributed to the “sponge effect” (repeated cycles of compression and expansion) and acoustic streaming induced by HPU waves, which mechanically facilitate the migration of water from the internal pores to the surface and enhance the turbulence at the solid-fluid interface (Meroni et al., 2022; Vallespir et al., 2019).

A similar behavior can be observed for a_w (Fig. 2B). All drying processes resulted in a gradual decrease in a_w over time, with significant differences in the final values. Air drying showed a progressive reduction in a_w, reaching final values of 0.763 ± 0.014 at 40 °C and 0.583 ± 0.030 at 60 °C. These results reflect the enhanced water removal capacity at higher temperatures and confirm that conventional drying can effectively lower water activity, particularly when high temperature is used. $ScCO_2$ drying exhibited a final a_w value of 0.763 ± 0.036, similar to that observed in air drying at 40 °C. However, when HPU assisted the $scCO_2$ drying, the water removal was enhanced, leading to a final a_w of 0.565 ± 0.049. Thus, the addition of HPU enabled the production of samples with a_w lower than 0.7, a threshold commonly associated with microbial stability for bacteria, yeasts, molds and mycotoxins (Peleg, 2022), which was not achieved with $scCO_2$ drying alone after 6 h. These findings are consistent with previous studies which reported the drying performance of $scCO_2$+ HPU in coriander (Michelino et al., 2018), chicken breast (Morbiato et al., 2019) apples and peas (Cardin et al., 2024). It is worth to highlight that the final a_w values achieved by $scCO_2$+ HPU is similar to the one achieved with air drying at 60 °C. This outcome supports the potential of the assisted HPU technology for

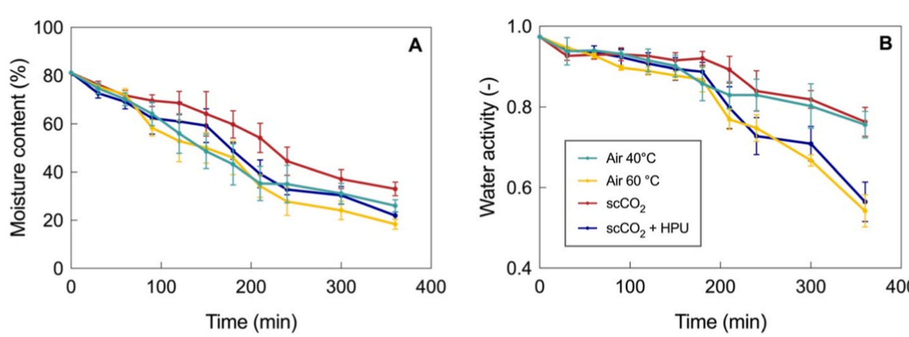

Fig. 2. (A) Moisture content (wet basis, %) and (B) water activity (−) of cod fish samples dried using air, at 40 and 60 °C, supercritical CO_2 ($scCO_2$), and supercritical CO_2 combined with high-power ultrasounds ($scCO_2$+ HPU). Statistical analysis is shown in Tables S1 and S2.

developing faster low-temperature drying strategies.

3.2. Microbial inactivation performance

The microbial inactivation performance of the four analyzed treatments was assessed by enumerating two pathogen-surrogate bacteria: *E. coli* and *L. innocua*. The results are presented in Fig. 3.

The initial microbial load of the inoculated cod fish samples was 6.80 ± 0.11 Log CFU/g for *E. coli* and 7.27 ± 0.13 Log CFU/g for *L. innocua*, respectively. As a negative control, non-inoculated samples showed microbial counts below the detection limit for both *E. coli* and *L. innocua*, confirming the absence of background contamination (data not shown).

No significant microbial inactivation was achieved with air drying, even at 60 °C up to 60 min for both strains. These findings confirmed that traditional drying methods are not effective in reducing microbial load (Majumdar et al., 2023).

On the contrary, both microbial strains were reduced after using $scCO_2$ drying alone and in combination with HPU. Specifically, about 1-log reduction was achieved after the pressurization and depressurization cycle (t = 0 min). Increasing the drying time, both *E. coli* and *L. innocua* were completely inactivated after 60 min of treatment, exhibiting a similar inactivation trend between $scCO_2$ and $scCO_2$ +HPU. Comparable studies have been reported in literature which are summarized in Table 1, together with this current data.

The results suggest that microbial inactivation kinetics are strongly influenced by the specific characteristics of the matrix, rather than a simple distinction based on biological origin (animal vs. plant). While apples (Zambon et al., 2021) and coriander (Bourdoux et al., 2018) showed a complete inactivation immediately after the pressurization/depressurization cycle, strawberries (Zambon et al., 2022b) presented an inactivation similar to chicken breast (Morbiato et al., 2019) and cod fish (this study). In animal muscle (cod and chicken), the presence of lipids and complex proteins might exert a protective effect on the microbial membrane against the scCO2 action (Zhou et al., 2009). The slower microbial inactivation observed in strawberries compared to apples may be partly attributed to the compositional characteristics of the fruit matrix. Strawberries contain significant amounts of organic acids (mainly citric and malic acids), polyphenols (such as ellagic acid and flavanols), and anthocyanins (predominantly pelargonidin derivatives), which can influence microbial survival and the interaction between microorganisms and the food matrix during non-thermal processing. These compositional features may contribute to differences in microbial inactivation kinetics during supercritical CO_2 treatments (Giampieri et al., 2012; Koyama et al., 2022).

Moreover, similar to this study, there is no evidence of a significant difference between the inactivation capacity against Gram-negative bacteria, such as *E. Coli* and *Salmonella* spp., and Gram-positive bacteria, such as *Listeria* spp.

Table 1
Published research studies reporting the inactivation of inoculated microorganisms (pathogens or pathogen surrogates) during $scCO_2$ drying. Process conditions were the same for all the studies: 40 °C and 10 MPa. Initial microbial load was always between 5.5 and 7 Log CFU/g.

Product	Bacterial strain	Inactivation at 0 min[a] (Log CFU/g)	Total inactivation time	Ref.
Cod fish	*E. coli* *L. innocua*	0.98 ± 0.18 1.28 ± 0.44	<60 min	This work
Strawberry	*E. coli* O157:H7 *Salmonella* spp. *L. monocytogenes*	» 1.04[b] » 1.66[b] 1.96 ± 0.41	<6 h	Zambon et al. (2022b)
Strawberry	*E. coli* O157:H7 *Salmonella* spp. *L. monocytogenes*	n.d.	<7 h	Zambon et al. (2022a)
Apple	*E. coli* O157:H7 *Salmonella* spp. *L. monocytogenes*	Complete inactivation	0 min[a]	Zambon et al. (2021)
Chicken breast	*Salmonella* spp.	1.52 ± 0.14	<45 min	Morbiato et al. (2019)
Coriander	*E. coli* O157:H7 *Salmonella* spp. *L. monocytogenes*	Complete inactivation	0 min[a]	Bourdoux et al. (2018)

n.d. no data.
[a] 0 min: only pressurization and depressurization of the drying vessel.
[b] calculated as the average of the different strains.

The application of HPU during the $scCO_2$ drying did not lead to an improvement of microbial inactivation on cod fish samples at any of the tested drying time. In contrast, results on chicken breast (Morbiato et al., 2019) showed and higher inactivation when the $scCO_2$ was assisted with HPU, but only after 15 min of process while the synergism was neglectable at longer drying time. Since in the work with chicken breast only 2 drying times were reported for the inactivation, a comprehensive comparison is difficult to achieve. Moreover, a different design and sonotrode probe was adopted with chicken meat, making a direct comparison mostly impossible.

3.3. Effect of the drying technique on product quality

The quality of dried fish products is a key determinant of their acceptance and application in the food industry. Different drying methods can lead to different modifications in the chemico-physical and structural properties, affecting parameters such as color, texture, rehydration, and wettability (Fitri et al., 2022). In this section, the impact of the four different drying techniques on cod fish samples is examined.

3.3.1. Color analysis

Color is a critical parameter in food quality assessment, influencing

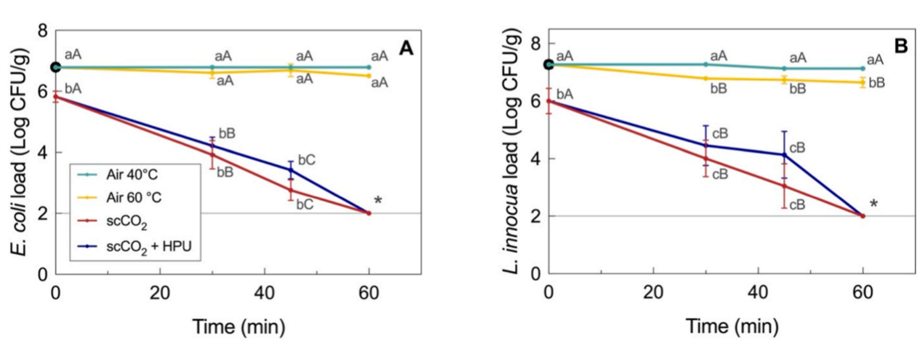

Fig. 3. Microbial load (Log CFU/g) of (A) *E. coli* and (B) *L. innocua* in cod fish samples dried using air (40 and 60 °C), supercritical CO_2 ($scCO_2$), and supercritical CO_2 combined with high-power ultrasounds ($scCO_2$+ HPU). The black dot in the plots represents the initial microbial load of the samples. Different letters indicate significant differences at $p < 0.05$. Capital letters denote differences among time points within the same treatment, while lowercase letters denote differences among treatments at the same time point. *under detection (<2 Log CFU/g).

consumer perception and market acceptance (Li et al., 2020). The color of the fresh and the dried samples, expressed as L*, a*, and b* parameters, is shown in Table 2.

A significant effect of the drying method was observed for the L* parameter, with $scCO_2$ and $scCO_2$+ HPU-treated samples showing significantly higher lightness values compared to air-dried samples. Fresh samples exhibited L* values closer to those of air-dried cod. Despite comparable L* values, air-dried samples displayed a more translucent and glassier surface, while scCO2-trated samples appeared more similar to fresh material, albeit with a lighter and more opaque aspect, as documented in the photographic images reported in the Supplementary Material (Fig. S1). The increase in lightness (L*) observed in $scCO_2$-treated samples may instead reflect a change in surface properties and light scattering behavior, likely linked to structural modifications such as increased porosity (Oikonomopoulou et al., 2011; Petikirige et al., 2022). These differences in optical properties are commonly reported in dried porous materials and suggest that $scCO_2$ drying, with or without the aid of HPU, may result in a more open microstructure. This hypothesis is further supported by the subsequent analysis of specific volume and microstructure (see §3.3.2).

In contrast, the a* and b* parameters exhibited only limited variations among treatments, indicating that the drying processes did not induce pronounced chromatic changes in redness or yellowness; the observed shifts from slightly negative values in fresh samples to positive values in dried products are likely attributable to dehydration-induced changes in light scattering rather than to intrinsic pigment alterations, consistent with the naturally whitish color of cod fish muscle.

No significant differences in L*, a*, or b* were detected between the $scCO_2$ and $scCO_2$+ HPU treatments, indicating that the application of HPU at the selected power level enhanced mass transfer without altering the visual appearance of the final product. To the best of the authors' knowledge, there are no studies on the application of HPU and $scCO_2$ on fishery products. However, HPU was adopted on other drying techniques such as ultrasonic-assisted vacuum drying, often leading to an increase in color variation (İsmail and Kocabay, 2020), but such effects were not observed under the present conditions.

To verify that the application of ultrasounds did not induce other changes in product quality, further evaluations were conducted on specific volume and microstructure.

3.3.2. Specific volume and microstructure

The specific volume of the dried samples is reported in Fig. S2. The $scCO_2$-dried samples showed significantly higher values compared to those dried with air, indicating a more expanded and porous internal structure. Specifically, specific volume values were 2.176 ± 0.104 cm^3/g for air drying at 40 °C, 2.044 ± 0.060 cm^3/g at 60 °C, while they increased to 3.331 ± 0.289 cm^3/g with $scCO_2$ drying and further to 3.537 ± 0.083 cm^3/g when using $scCO_2$ combined with high-power ultrasounds. This higher porosity of $scCO_2$-treated samples can be also confirmed by the SEM images shown in Fig. 4. Additional pictures at different magnitudes are reported in the Supplementary Materials (Figure S3–5 and Figure S6).

SEM pictures revealed clear structural differences between the drying methods. Samples dried with $scCO_2$ and $scCO_2$+ HPU exhibited a more porous structure, whereas those dried with air appeared denser and more compact.

This higher porosity of $scCO_2$-dried samples may be attributed to the low surface tension of CO_2 and the mild depressurization, which reduce matrix collapse during drying (Basak and Singhal, 2023; Manzocco et al., 2024). By contrast, during air drying, intense capillary forces are generated inside the fish tissue, due to the water-vapor interfaces, leading to significant structural collapse (shrinkage) (Khan et al., 2021; Marbade et al., 2024).

Interestingly, this greater porosity is consistent with the higher lightness (L*) values observed in $scCO_2$-treated samples. A more porous microstructure can enhance surface light scattering, thus increasing sample brightness without altering chromatic parameters (a* and b*) (Petikirige et al., 2022). Therefore, the optical and structural analyses converge in supporting the ability of $scCO_2$, particularly when assisted by HPU, to promote internal expansion and preserve microstructural integrity while achieving effective drying.

3.3.3. Texture and mechanical properties

Compression tests were performed to evaluate how the drying methods affected the mechanical response of the cop fish samples. The compressive strain (%) – stress (MPa) curves for the four treatments are shown in Fig. 5.

The results clearly reflect the structural differences previously observed in terms of specific volume and porosity. Samples dried with $scCO_2$, both with and without HPU, exhibited a linear elastic behavior over the tested strain range, suggesting a more flexible and resilient structure. In contrast, samples air-dried at 60 and 40 °C displayed a more brittle behavior, characterized by a noticeable yield point at force values of around 60 N (59.1 ± 10.4 N and 63.3 ± 6.7 N, respectively). Although the yield force did not differ significantly between the two air-drying temperatures ($p > 0.05$, Student's t-test), the yield point occurred at lower compressive displacement values (1.1 mm) and more sharply in the 60 °C air-dried samples, as compared to the 40 °C air-dried samples (1.5 mm), indicating an increased susceptibility to structural failure under compression.

The major structural factors affecting texture are indeed associated with the connective tissues and myofibrillar protein (myosin and actin) (Bernardo et al., 2022; Ortiz et al., 2013). The use of extreme temperatures may generate excessive protein denaturation and collagen degradation at higher temperatures, leading to reduced elasticity and increased brittleness.

3.3.4. Wettability and rehydration

The structural differences previously discussed are expected to influence the behavior of dried samples upon contact with water. This is a critical characteristic of dried samples for their commercial uses, such as in ready-to-eat or reconstituted food products. Thus, wettability and rehydration tests were conducted to assess how different drying techniques can affect water absorption dynamics.

Thus, a water droplet was placed on the dried sample surface, and its behavior was recorded over time. Selected frames right after the water drop are shown in Fig. 6, while contact angle and remaining water volumes are reported in Table 3.

After only 2 s from the contact, no detectable water volume remained on the surface of the samples dried using $scCO_2$, either with or without the application of HPU. In contrast, a measurable volume of water was still present even after 30 s on the air-dried samples, particularly for those treated at 60 °C. These results clearly prove the superior wettability of $scCO_2$-treated samples, likely due to the increased porosity and surface roughness induced by $scCO_2$ drying, which enhances surface roughness and promotes capillary-driven water adsorption.

Interestingly, no substantial differences in wettability were detected with and without the addition of HPU, suggesting that ultrasound treatment did not significantly alter the surface water absorption behavior. However, increasing the air-drying temperature from 40 to

Table 2
Lightness (L*), redness (a*), and yellowness (b*) of fresh cod fish and samples dried using air, at 40 and 60 °C, supercritical CO_2 ($scCO_2$), and supercritical CO_2 combined with high-power ultrasounds ($scCO_2$+ HPU). Different small letters (a, b, c) for the same color parameter represent statistically different values.

Sample	L*	a*	b*
Fresh	32.19 ± 1.79^{b}	-1.53 ± 0.33^{d}	-2.52 ± 0.39^{b}
Air 40 °C	24.96 ± 4.10^{bc}	0.32 ± 0.60^{ab}	5.53 ± 0.97^{a}
Air 60 °C	23.73 ± 1.65^{c}	0.75 ± 0.14^{a}	8.04 ± 0.75^{a}
$scCO_2$	46.10 ± 8.91^{a}	0.08 ± 0.05^{bc}	8.89 ± 1.16^{a}
$scCO_2$ + HPU	44.45 ± 3.44^{a}	0.33 ± 0.38^{ab}	7.96 ± 1.57^{a}

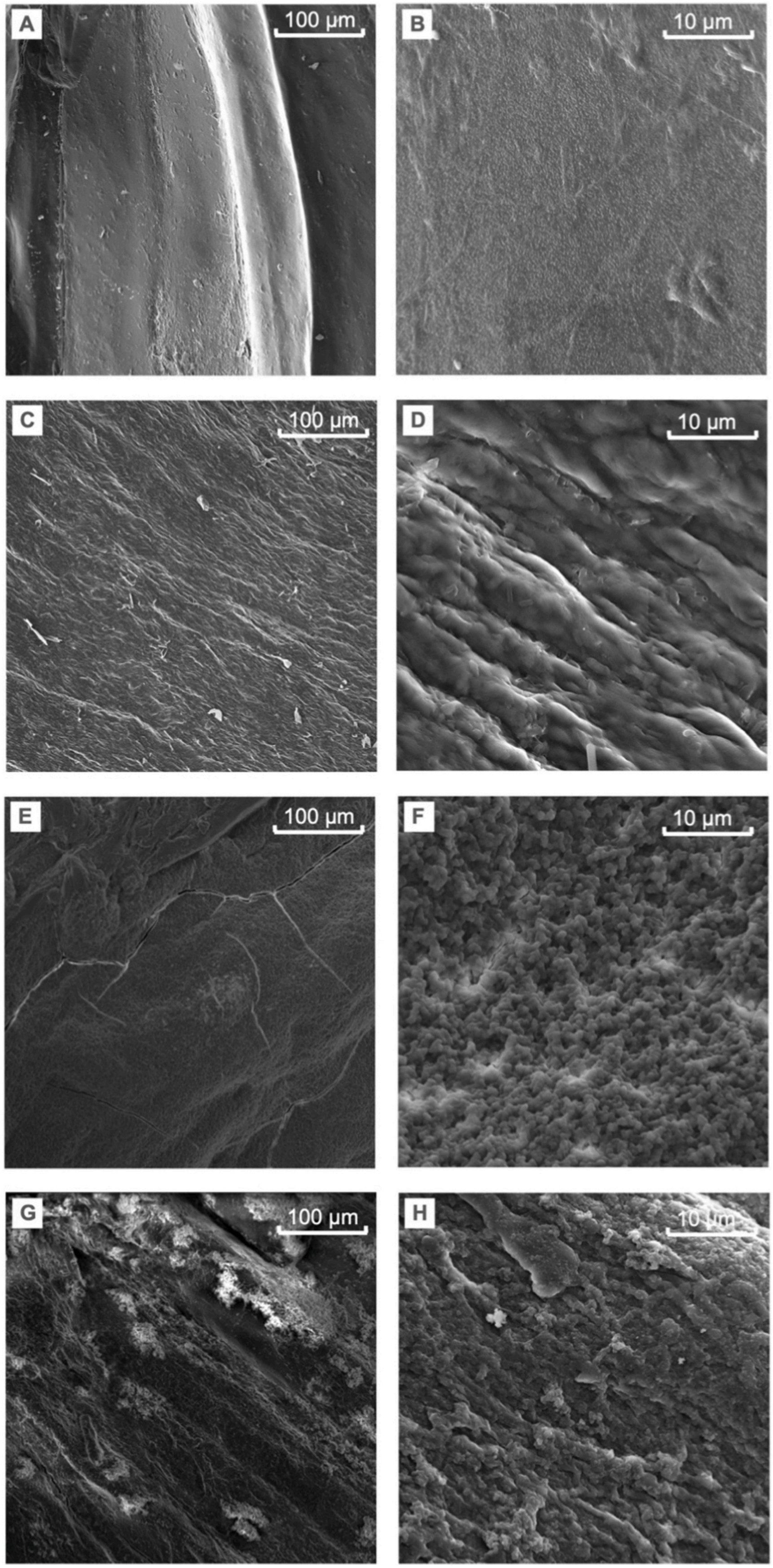

Fig. 4. SEM images of cod fish samples dried using air, at 40 °C (A and B), and 60 °C (C and D), $scCO_2$ (E and F), and $scCO_2$+ HPU (G and H) at two different magnitudes, ×400 (A, C, E, and, G) and x5,000 (B, D, F, and H).

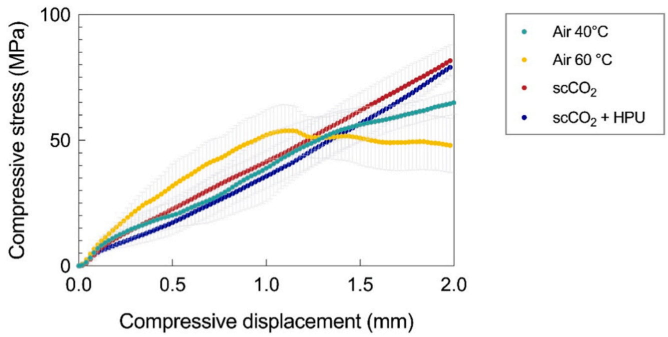

Fig. 5. Force (N) in function of compressive strain (mm) recorded by a compression test of cod fish samples dried using air, at 40 and 60 °C, supercritical CO_2 ($scCO_2$), and supercritical CO_2 combined with high-power ultrasounds ($scCO_2$+ HPU). Determinations were carried out in triplicate on three replicated samples (n = 3).

60 °C led to a marked decrease in wettability, in line with previous observations on increased compactness, reduced specific volume, and greater brittleness caused by thermal degradation of the tissue matrix (Calín-Sánchez et al., 2020; Khan et al., 2021).

On the other hand, the rehydration factor was slightly higher in air-dried samples, particularly at 40 °C, compared to those dried with $scCO_2$ (Fig. S7). This result may seem contradictory to the improved wettability of $scCO_2$-treated samples. However, it could be explained by the higher surface tension presented by air-dried samples which retained external free water over the dried sample. Future studies using larger sample sizes could help clarify these findings by minimizing surface water effects and providing a more comprehensive understanding of rehydration behavior.

4. Conclusion

The $scCO_2$ drying alone and assisted with HPU, was compared to air drying at 40 and 60 °C for cod fish dehydration. Drying with $scCO_2$ achieved similar drying times to air drying, while adding HPU further enhanced water removal, matching the kinetics of air drying at 60 °C. Microbiologically, air drying had no significant effect on the inactivation of *E. coli* and *L. innocua*, whereas both $scCO_2$ treatments completely inactivated the microbial load within 60 min, with no additional effect from the assisted HPU drying. From a quality perspective, $scCO_2$ drying alone and in combination with HPU produced lighter, more porous structures with higher specific volume and improved wettability. These features are translated into enhanced mechanical elasticity. In contrast, air drying, especially at 60 °C, caused significant structural damage, leading to brittle textures and reduced water interaction. Overall, these findings highlight the potential of HPU assisted $scCO_2$ drying as a valuable technique for a faster drying kinetic and high-quality dried fish

Table 3
Contact angle and remaining water volume after 2, 6, and 10 s on cod fish samples dried using different techniques.

Treatment	Contact angle (°)			Volume (*μL*)		
	2 s	6 s	10 s	2 s	6 s	10 s
Air 40 °C	47.5 ± 0.82	43.0 ± 0.71	43.0 ± 0.71	0.152	0.066	0.066
Air 60 °C	77.4 ± 3.95	69.2 ± 5.13	64.7 ± 5.81	0.445	0.283	0.240
$scCO_2$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$scCO_2$+ HPU	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. non detected.

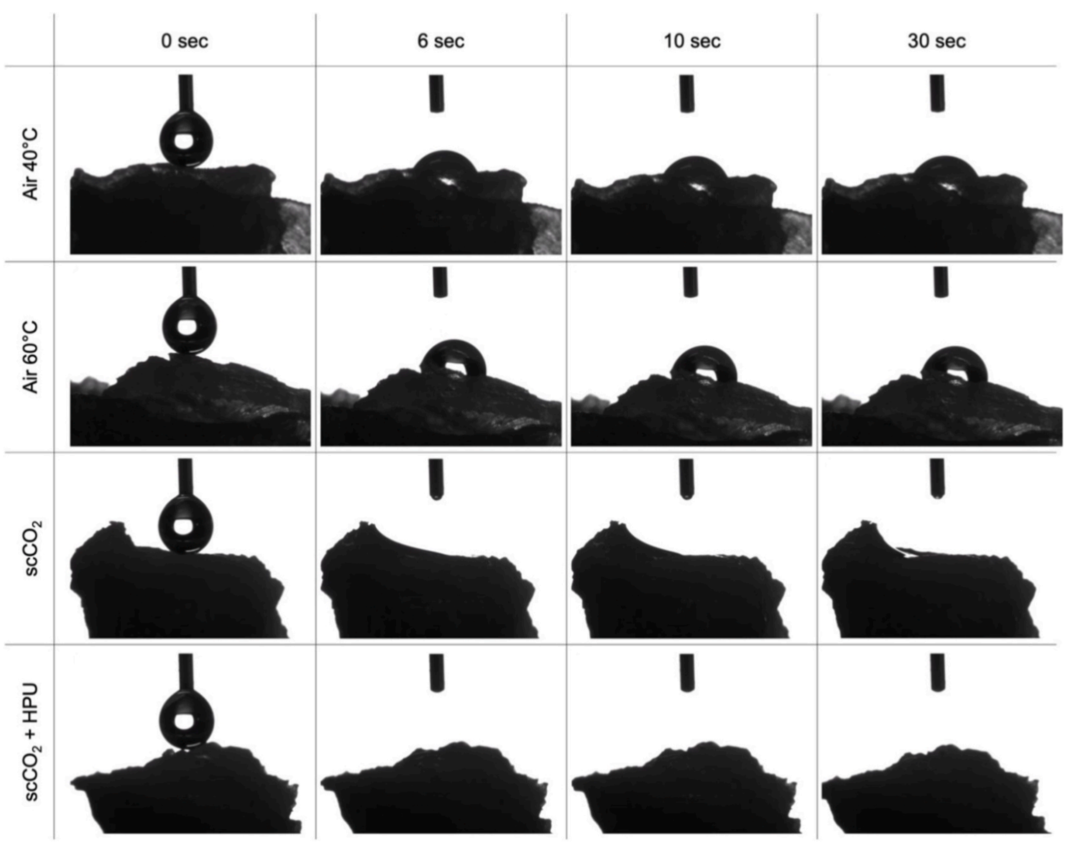

Fig. 6. Frames showing the evolution of a water droplet at 0, 6, 10 and 30 s after deposition on cod fish samples dried using air at 40 and 60 °C, $scCO_2$, and $scCO_2$+ HPU.

while effectively eliminating microbial contaminants. Future investigations should assess its effects on other pathogenic strains such as *Salmonella* and *Vibrio*, sensory attributes, oxidative stability, and shelf life to further validate its industrial applicability. Moreover, the scalability of the process and the influence of larger and more complex product dimensions on drying response and quality attributes need to be fully evaluated.

CRediT authorship contribution statement

Riccardo Zulli: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Yago A.A. Bernardo:** Writing – review & editing, Validation, Methodology, Investigation. **Marco Cardin:** Validation, Methodology, Investigation. **Stella Plazzotta:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Carlos A. Conte-Junior:** Writing – review & editing, Supervision, Resources. **Sara Spilimbergo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Alessandro Zambon:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfoodeng.2026.113150.

Data availability

Data will be made available on request.

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