Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.101522

Effect of spray-drying conditions on the physical and antioxidant properties of a hydrolysate from red tilapia (*Oreochromis* spp.) viscera

Cindy SEPÚLVEDA RINCÓN[1]*, Priscilla VÁSQUEZ[1], José ZAPATA MONTOYA[1]

Abstract

Fish hydrolysates have become one of the most remarkable sources of bioactive peptides. However, the processing conditions for incorporating hydrolysates into food matrices can affect their bioactive performance. The effect of temperature and pH on the radical scavenging activity of tilapia hydrolysate was determined in the wet hydrolysate. Also, a central composite design was used to study the effect of the drying conditions on moisture, drying ratio, productivity, drying rate, and antioxidant activity in the tilapia hydrolysate. The results showed that the hydrolysate has high activity at acidic and neutral pH; but at pH 10, the activity decreases significantly. In the spray-drying process, the antioxidant activity was higher at 115 °C. Moreover, inlet air temperature and feed flow had a statistically significant effect ($p < 0.05$) on response variables. High inlet air temperature and fast feed flow decrease the moisture of the powder hydrolysate and increase the drying rate and antioxidant activity. Scanning electron microscopy showed liquid bridges between particles with irregular concavities or pores on the surface and the presence of particle agglomerations due to the hygroscopicity of the hydrolysate.

Keywords: fish hydrolysate; antioxidant activity; bioactive peptides; enzymatic hydrolysis.

Practical Application: Fish processing by-products are usually discarded as organic waste affecting the aquatic ecosystems, but it contains high-quality functional compounds. In this word, variables related to the thermal processes to which food is exposed were studied to understand their effect on the hydrolysate of fish viscera, whose information is helpful in scaling processes for a subsequent industrial application. As a result, we found that the powdered fish hydrolysate retains its bioactive properties and can be used in food matrices.

1 Introduction

Bioactive peptides resulting from the enzymatic hydrolysis of proteins are of scientific interest due to their biological potential in human health and use in the food industry (Villamil et al., 2017). Hydrolysates from fish proteins are a viable resource in nutritional and pharmaceutical applications because they have amino acids available for different physiological functions (Benhabiles et al., 2012; Rosa Zavareze et al., 2014). Fish protein hydrolysates are a source of bioactive compounds and have shown antioxidant activity (Bougatef et al., 2010, 2016; Jang et al., 2016; Je et al., 2015), antihypertensive activity (Aissaoui et al., 2017; Borges-Contreras et al., 2019; Korczek et al., 2018), and antiproliferative activity (Alemán et al., 2011; Gómez et al., 2019; Hsu et al., 2011). The enzymatic hydrolysis of fish proteins has also shown relevance in the food industry because it promotes functional properties such as emulsifying capacity, solubility, water and oil holding capacity, and foaming capacity compared to non-hydrolyzed protein (Alahmad et al., 2022; Vásquez et al., 2022).

Heat treatments are widely used in the food industry to remove moisture and eliminate microbial load. However, subjecting food to high temperatures can modify the structure of proteins and peptides, can generate denaturation, and also the loss of biological properties (Rivero-Pino, 2023). On the other hand, the pH has a strong influence on the stability of the hydrolysates, since it has an effect on the interactions between the amino acids of the peptides, which could lead to the loss of structure and biological properties (Félix-Medina et al., 2022).

The drying of the hydrolysates is necessary to simplify their storage and preserve their biological activity and functional properties over time. Furthermore, the drying process decreases the water activity of food, reducing the risk of microbiological and enzymatic reactions (Kurozawa et al., 2009). Spray-drying is widely used in the pharmaceutical and food industry due to its low cost, speed, and versatility in obtaining specific characteristics in the final product (Ingvarsson et al., 2011).

Despite the enormous advantages of using spray-drying, this process can promote the instability of the chemical structures and can damage the bioactive compounds due to thermal destruction and shear stresses (Sarabandi et al., 2020). Some temperature-induced effects in spray-drying are changes around the protein-protein hydrophobic interactions, electrostatic interactions, hydrogen bonds, and disulfide-sulfhydryl interactions (Chen et al., 2012).

Several works have focused on studying the effect of drying conditions on the particle properties of the hydrolysates (Abdul-Hamid et al., 2002; Favaro-Trindade et al., 2010; Hassan et al., 2019; Rodríguez-Díaz et al., 2014). However, those studies focused

Received 01 Oct., 2022
Accepted 09 Jan., 2023
[1] *Nutrition and Food Technology Group, Department of Food, Faculty of Pharmaceutical and Food Sciences, University of Antioquia – UDEA, Medellin, Antioquia, Colombia*
*Corresponding author: tatiana.sepulvedarincon@gmail.com; tatiana1827@gmail.com

on the effect of drying conditions on the antioxidant activity of hydrolysates are limited. This work aimed to investigate the effects of temperature and pH on the stability of the antioxidant activity of wet tilapia hydrolysate in addition to the effect of the drying conditions on moisture, drying ratio, productivity, drying rate, and the antioxidant activity of tilapia protein hydrolysate.

2 Materials and methods

2.1 The production of fish hydrolysate

Protein hydrolysate was obtained from tilapia (*Oreochromis* spp.) viscera following the procedure described previously (Sepúlveda & Zapata, 2020). The hydrolysis was carried out in a Bioflo 310® reactor (New Brunswick Scientific Co., Inc. USA) at 10 g of protein per liter using Alcalase® 2.4 L (Novozymes, Denmark) with an enzyme/substrate (E/S) ratio of 10% (w/w). The pH was maintained at 10 using the pH-stat technique. The hydrolysate had a notorious number of peptides, around 336 Da, with 48% of hydrophobic amino acids and a high quantity of glutamic, aspartic acid, and glycine (Sepúlveda et al., 2021).

2.2 Antioxidant activity

The free radical scavenging activity of hydrolysate against the ABTS radical and the ability of hydrolysate to reduce ferric iron (Fe3+) present in a complex with 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) up to the ferrous form (Fe2+) were used to determine the antioxidant activity of hydrolysates, which were dissolved in distilled water. ABTS and ferric reducing/antioxidant power (FRAP) methods were carried out following a protocol described previously (Sepúlveda & Zapata, 2020). The determination was made in triplicate, and the results were calculated by extrapolation in a calibration curve with Trolox as standard. The values obtained were expressed as equivalent micromoles of Trolox per gram of protein (μmol TE/g). All samples were analyzed in triplicate.

2.3 Effect of pH and temperature on the antioxidant activity of the wet hydrolysate

After hydrolysis the pH of the tilapia hydrolysate was adjusted to 4, 7, and 10 using NaOH or HCl 1 M. The samples were heated for 1 hour, and since there were no changes, they were finally left for 9 hours at 23, 85, 100, and 115 °C using a digital dry bath (Labnet International, Inc., New Jersey, USA). Three vials of each sample were extracted to determine the free radical scavenging activity of hydrolysate against the ABTS radical.

2.4 Experimental design and optimization of spray-drying process of tilapia hydrolysate

To evaluate the effect of the drying conditions of the hydrolysate a central composite design (CCD), face-centered with 5 center points and 13 runs, was conducted. The factors were inlet temperature (T) and feed flow (FF). The aqueous hydrolysate was adjusted to pH 7 and was spray-dried using a SD-06 Spray Dryer (LabPlant®, North Yorkshire, UK). Hydrolysate solution was sent into the dryer by a peristaltic volumetric pump at a feed flow rate, according to the experimental design (FF). The pressure nozzle with an internal diameter of 0.5 mm was used to spray the hydrolysates into the chamber in co-current flow with a compressed air flow rate of 30 m^3/h. The inlet air temperature was according to the experimental design (T).

The studied responses were moisture, drying ratio, productivity, drying rate, and the antioxidant activity of spray-dried hydrolysate. The moisture of hydrolysate was determined by thermogravimetric analysis using a moisture analyzer (Ohaus, New Jersey, USA). Drying ratio, productivity, and drying rate were determined to study the drying performance of tilapia hydrolysate according to Cai & Corke (2000) using the Equations 1-3:

$$Drying\ ratio = \left(M_0 + 1\right) / \left(M_f + 1\right) \quad (1)$$

Where M_0 is the feed moisture, and M_f is the powder moisture

$$Productivity\ \left(g/h\right) = Feed\ flow\ rate / Drying\ ratio \quad (2)$$

$$Drying\ rate\ \left(g/h\right) = Feed\ flow\ rate - Productivity \quad (3)$$

Table 1 shows the experimental runs randomized. The experiments were designed and analyzed using Design-Expert® software (Stat-Ease, Inc., Minneapolis, USA). The developed models from CCD and the statistical significance of the regression coefficients were

Table 1. Effect of inlet temperature (T) and feed flow (FF) on moisture, drying ratio, productivity, drying rate, and the antioxidant activity of spray-dried hydrolysate.

Run	T (°C)	FF (mL/h)	Moisture (%)	Drying ratio	Productivity (g/h)	Drying rate (g/h)	ABTS (μmol TE/g)	FRAP (μmol TE/g)
1	200	339.5	5.4	8.63	41.1	313.9	553.1	125.8
2	165	485.0	9.0	11.5	31.0	324.1	522.0	85.8
3	200	630.5	7.3	15.3	23.2	331.9	629.7	134.7
4	165	485.0	8.1	8.7	58.1	449.2	534.0	90.9
5	165	339.5	7.6	9.8	51.7	455.6	505.0	82.9
6	130	485.0	10.2	9.8	51.7	455.6	533.5	88.4
7	165	485.0	8.8	10.0	50.9	456.4	520.1	88.4
8	200	485.0	7.3	10.8	46.8	460.5	573.0	127.5
9	130	339.5	10.4	10.8	46.8	460.5	537.8	87.8
10	130	630.5	10.5	11.8	43.1	464.2	545.1	88.4
11	165	630.5	9.8	8.5	77.4	582.0	526.0	90.0
12	165	485.0	8.1	9.1	72.8	586.7	528.5	85.9
13	165	485.0	9.0	11.9	55.6	603.9	541.7	85.0

Note: Temperature (T), feed flow (FF).

tested using the analysis of variance (ANOVA). The models were optimized to determine the levels of the factors that provide the maximum value for drying ratio, productivity, drying rate, and antioxidant activity, and the minimum value for the moisture. The powder was collected at the bottom of the dryer's cyclone and stored in closed plastic bottles inside a silica desiccator prior to further analysis. Before spray drying, a sample of the liquid hydrolysate was taken separately and frozen at —20 °C overnight and then was freeze-dried at —51 °C at pressure 0.1 mBar for 48 h. The freeze-dried hydrolysate (FDH) was used to compare it with the hydrolysate dried by spray at the optimal conditions (SDH) in terms of moistures and antioxidant activity.

2.5 Morphological analysis by scanning electron microscopy

The morphology of the hydrolysate dried at the optimal conditions was examined using a scanning electron microscope (JEOL, JSM 6490 LV, Tokyo, Japan) at an accelerating voltage of 5 kV. The powder was attached to a double-sided graphite adhesive tape and coated with gold under vacuum using a coat sputter (Denton Vacuum, Desk IV, New Jersey, USA).

2.6 Statistical analysis

The method Fisher's minimum significant difference (LSD) was used to discriminate between the means (Statgraphics® Centurion XVI, Statgraphics Technologies Inc., USA). The statistically significant difference was established at $p < 0.05$.

3 Results and discussion

3.1 Effect of pH and temperature on the antioxidant activity of the liquid hydrolysate

The stability of the antioxidant activity of the hydrolysate concerning pH and temperature measured every 1 h for 9 h remained unchanged over time (data not shown). Figure 1 shows no statistical differences in the ABTS between 23 and 85 °C at the same pH. The samples at pH 4 and 7 did not show differences between subjecting the hydrolysate to 100 or 115 °C. The samples at pH 10 had the lowest antioxidant activity compared to the other

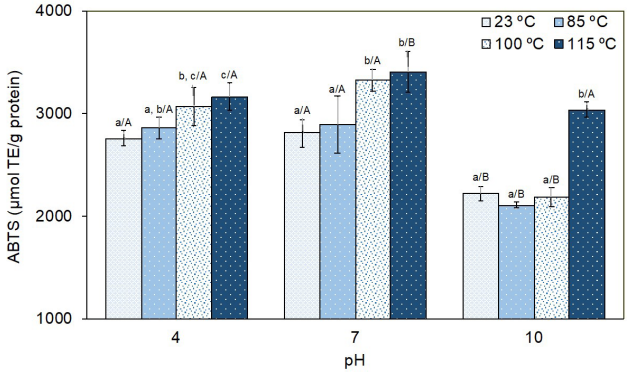

Figure 1. The effect of pH (4, 7, and 10) and temperature (23, 85, 100, and 115 °C) on the antioxidant activity of the tilapia hydrolysate. Different letters (a, b, c) indicate significant differences ($p < 0.05$) among the same pH. Different letters (A, B) indicate significant differences among the same temperature.

pHs, and there were no differences between subjecting the samples to 23, 85, and 100 °C. However, at 115 °C there was a significantly increased activity. It suggests that increasing the temperature up to 115 °C, can significantly increase the antioxidant activity of the hydrolysate. It could be due to the presence of products from the Maillard reaction, which is characterized by the reaction between side chains of amino acids and the carbonyl group of reducing sugars promoted by high temperatures. This reaction produces compounds with high antioxidant activity which is positively related to the development of a brown color (Djouab & Aïder, 2019; Morales & Jiménez-Pérez, 2001). Similarly, Rivero-Pino et al. (2020) obtained higher 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in a hydrolysate of sardine hydrolysates when the temperature was increased from 90 to 110 °C, and they attribute the rise in activity to the increase in Maillard compounds. On the other hand, Tang & Zhuang (2015) found a strong thermal stability in a peptide (Leu-Pro-Leu) since the radical scavenging remains after 5 h at 100 °C.

The low antioxidant activity at alkaline pH may be related to the racemization of proteins, the formation of L and D isomers with different structural stability, and also antioxidant activity (Zhang et al., 2016). Zhu et al. (2014) found that the antioxidant activity determined by the DPPH of a peptide extracted from Jinhua ham remained 90% at pH 3 (at neutral pH, it showed its highest activity); but the pH above 9 showed a significant decrease in activity. The authors explain that, in addition to racemization, pH promotes deamination reactions that produce structural and conformational changes that affects the biological activity of the peptide.

3.2 Experimental design and optimization of spray-drying process of tilapia hydrolysate

The central composite design was applied to study the effect of the inlet air temperature (T) and the feed flow (FF) on the moisture, drying ratio, productivity, drying rate, and antioxidant activity of tilapia hydrolysate in the spray-drying process. The ANOVA (Table 2) indicated that the inlet air temperature and the feed flow (FF) have a statistically significant effect on all responses studied. The two independent variables were significant in their linear term for all response variables. The interaction between the factors only had a significant effect on the antioxidant activity determined by ABTS. A significant quadratic effect of temperature ($p < 0.05$) is observed for the antioxidant activity measured by the two methods. The quadratic term indicates the existence of extreme points in the range of levels studied. The R-squares values and the lack of fit p-values indicate that the models adequately explain the experimental data. Figure 2 represents the change of response surfaces for moisture, drying ratio, productivity drying rate, and antioxidant activity with varying inlet air temperature and feed flow rate. At higher FF and lower temperatures, the final moisture of hydrolysate increases (Figure 2a). As might be expected, a higher inlet temperature promotes a higher temperature gradient between the drops and the air, which implies a higher heat transfer, and this effect is more visible at low FF. These results can be explained considering that high FF causes a less efficient heat transfer

Table 2. Analysis of variance (ANOVA) of the *p* -values for the moisture, drying ratio, productivity, drying rate, and antioxidant activity.

Source	Moisture (%)	Drying ratio	Productivity (g/h)	Drying rate (g/h)	ABTS (µmol TE/g)	FRAP (µmol TE/g)
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0001
T	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0009	< 0.0001
FF	0.0093	0.0090	< 0.0001	< 0.0001	0.0050	0.0176
T.FF	-	-	-	-	0.0145	-
T^2	-	-	-	-	0.0004	< 0.0001
Lack of fit	0.3927	0.2191	0.4972	0.4972	0.2340	0.5896
R-squared	0.89	0.89	0.97	0.99	0.91	0.99

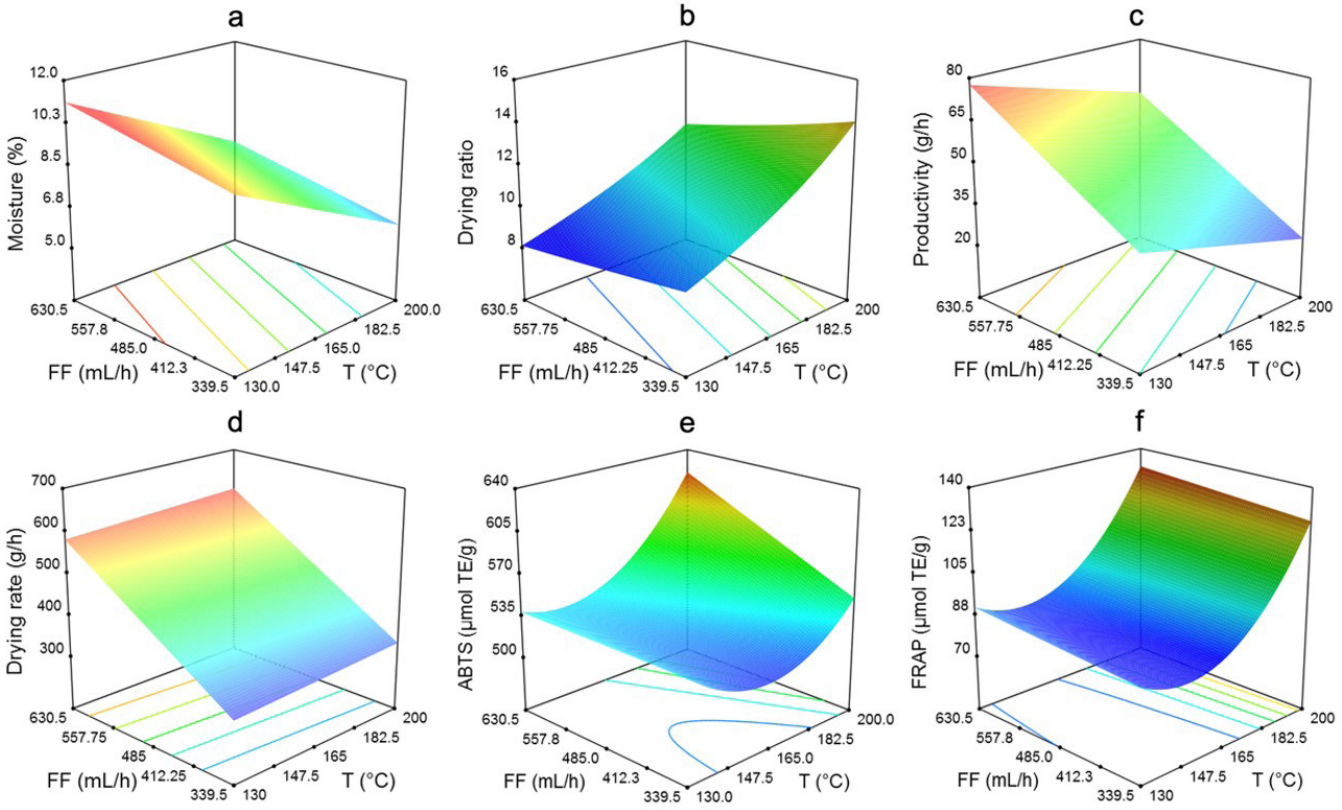

Figure 2. Response surface for moisture (a), drying ratio (b), productivity (c), drying rate (d), ABTS method (e), and FRAP method (f).

between droplets and the drying air, reducing the evaporation rate and, therefore, higher moisture.

When the concentration of the solids of the dry material increases with respect to the solids fed, the drying ratio is high. The drying ratio was higher at low FF and high temperature which may increase the mass transfer rate during spray-drying (Figure 2b). The results suggest that the higher the inlet temperature, the higher the moisture reduction, and the above behavior is stronger when the hydrolysate feed is slow.

Productivity is a critical parameter in drying because it relates to the cost of the process (Chong et al., 2014). Figure 2c shows that increasing FF at low temperatures significantly raised powder productivity, reducing production cost. However, considering that these conditions give rise to a hydrolysate with higher moisture, productivity is affected in the long term by the loss of stability of the powder. Chong et al. (2014) found that a low drying ratio caused higher productivity at higher FF in the spray-drying of *Amaranthus gangeticus* extract. According to Figure 2d, the drying rate was higher by increasing FF in all temperatures studied because this variable is strongly influenced by the rate of drying of fed hydrolysate though it is not affected by the final moisture content of the powder. As a conclusion of Figure 2a-d, a high FF improves the speed and productivity of the process; and combined with a high inlet temperature, the moisture of the final product is decreased making the process more efficient. Similarly, Ozdikicierler et al. (2014) found that decreasing the FF decreases productivity and drying rate, but increases the drying ratio, making it possible to obtain an extract of *Gypsophila* in powder with low moisture.

The response surface of the antioxidant activity determined by ABTS (Figure 2e) shows a curvature in the temperature given by the significance of this variable squared. The maximum activity in the range of the evaluated conditions is reached in the maximum levels of temperature and FF. These results agree with those found in the study of the stability of the antioxidant activity of the hydrolysate

previously shown. Considering the high presence of amino acids in the hydrolysate (Sepúlveda et al., 2021) and the drying temperature, the Maillard reaction could explain the increased activity during thermal processing which takes place between reducing the sugars and the available amino acids producing peptide degradation, peptide cross-linking, polymerization, and amino acid loss (Hwang et al., 2011).

According to the results found in anchovy protein hydrolysate after the Maillard reaction, the consumed and generated components indicated that molecular rearrangements and the production of new smaller molecules occurred simultaneously, propitiating an increase in the bioactivity of the hydrolysate (Zhao et al., 2018). In a study carried out in a peptide fraction of peanut hydrolysate, it was found that the smaller peptides (1–3 kDa) had a higher increase in antioxidant activity during Maillard reaction (Su et al., 2011; Zhao et al., 2018). According to the above, it is important to mention that the hydrolysate used in this study has a significant amount of low molecular weight peptides (<1 kDa) (Sepúlveda et al., 2021).

The antioxidant activity determined by FRAP (Figure 2f) showed a quadratic behavior in the temperature variable. The effects of a higher concentration of hydrolysate at a high temperature are reflected in higher antioxidant activity. The results suggest that peptides capable of reducing Fe^{3+} are stable at high temperatures.

In this study, the antioxidant activity of the hydrolysate by ABTS and FRAP increased as the inlet air temperature raised. It could be explained considering the formation of cross-linked amino acids side chains, the breaking and/or recombination of intramolecular disulfide bridges, the reaction between amino acids to promote the formation of iso-peptide, and Maillard reactions during heat treatment. All these possible reactions can change the structure of proteins and peptides and, as a result, an increasing or decreasing activity (Kurozawa et al., 2011; Rodríguez-Díaz et al., 2014). For example, it is well known that the Maillard reaction products have a high antioxidant capacity and this could be adding to the activity of the hydrolysate; furthermore, this reaction is used to improve the technological functionalities such as thermostability in proteins (Nooshkam et al., 2020).

The enzymatic hydrolysate was spray-dried at the optimal inlet air temperature (200 °C) and the optimal feed flow (630.5 mL/h) according to the response surface methodology. Table 3 shows the theoretical values of spray-dried hydrolysate (SDH) calculated by the software compared with the values of freeze-dried hydrolysate (FDH). Bearing in mind that drying conditions can alter the characteristics of protein hydrolysates (Liu et al., 2022), the results show there are no statistically significant differences between the moisture of SDH and FDH. The antioxidant activity by the ABTS method was higher in SHD than in FDH, and the antioxidant activity by the FRAP method had no difference between the two samples. In a study that evaluated the effects of freeze-drying and spray-drying on *Perinereis aibuhitensis* hydrolysates, they found that the spray-dried hydrolysate got more species of free amino acid and volatile organic compounds, and better antioxidant activities compared to the freeze-dried hydrolysate (Liu et al., 2022).

Although the predicted values were not as close to the experimental values, the experimental showed relatively high values compared to most of the experimental runs. Additionally, the proximity of the predicted and experimental values is consistent with the r squares of the resulting models.

3.3 Scanning electron microscopy (SEM)

The morphological analysis of the spray-dried hydrolysate shows non-spherical particles contrary to what would be expected in spray-dried products. The Figure 3 shows liquid bridges between particles that may be due to the hygroscopicity. During protein hydrolysis, the release of hydrophobic and hygroscopic amino acid residues occurs, which confers this characteristic to the hydrolysate (Ma et al., 2014). Furthermore, tilapia hydrolysate

Table 3. Optimal predicted values and experimental validation for the central composite design (CCD) and comparison with a freeze-dried hydrolysate.

Variable	Predicted value	Experimental SDH	FDH
Moisture (%)	7.4	8.8 ± 1.8[a]	11.5 ± 1.0[a]
ABTS (μmol TE/g)	620.1	580.6 ± 10.3[a]	535.9 ± 8.7[b]
FRAP (μmol TE/g)	132.1	120.1 ± 7.6[a]	114.8 ± 8.3[a]

Results are presented as the mean of triplicate ± standard deviation. Mean values within a row followed by different letters mean significant differences ($p < 0.05$). Note: Freeze-dried hydrolysate (FDH) has been included in this table for comparison purposes only.

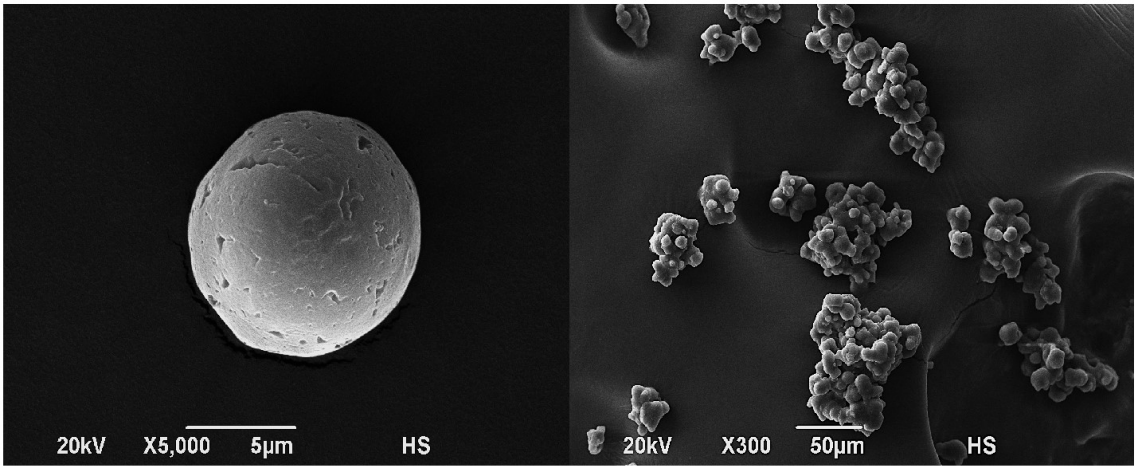

Figure 3. SEM micrographs of spray-dried tilapia hydrolysate (SDH) obtained at an inlet air temperature of 200 °C and feed flow of 630.5 mL/h.

contains low molecular weight peptides (around 336 Da) which could explain also a high hygroscopicity (Kurozawa et al., 2011) which is one of the most common problems in protein hydrolysates (Sarabandi et al., 2020). Similar microphotographs were obtained in the study of a pure Mussel protein hydrolysate after spray-drying, however, when the hydrolysate was dried using a carrier agent, spherical-shaped particles with a smooth surface were observed (Silva et al., 2012).

Irregular concavities or pores on the surface of the samples are associated with the fast evaporation of water during the spray-drying process, which leads to the formation of smooth surface particles (Sarabandi et al., 2020). The spray-drying process is made up of two stages. In the first one, the solvent evaporation shrinks the drop, and the increase of the solute concentration generates a crystallization in the surface. In the second stage, the internal steam flow restricted by the shell formed promotes the formation of bubbles within the particle, which are responsible for the formation of hollow structures (Rodríguez-Díaz et al., 2014). According to Chuaychan et al. (2017), a high inlet air temperature causes a harder shell of the particles. It prevents deflation, while at a low temperature, the shell remains moist, and the particle deflates as its temperature decreases.

4 Conclusions

According to the results, an acidic or neutral pH adequately preserves the antioxidant activity; however, an alkaline pH significantly reduces the antioxidant activity of the hydrolysate. In the spray-drying process, both inlet air temperature and feed flow had statistically significant effects on moisture, drying ratio, productivity, drying rate, and antioxidant activity. Optimization of the drying process indicated that higher temperatures decrease moisture and increase the drying rate and antioxidant activity of the hydrolysate. Optimum conditions allowed the obtention of hydrolysate with higher characteristics than a freeze-dried hydrolysate. Hence, it can be concluded that it is possible to obtain a spray-dried tilapia hydrolysate with low moisture, high productivity, and high antioxidant activity. However, future studies focused on determining the mechanism by which antioxidant activity increases with increasing temperature and on validating the presence of Maillard reaction compounds are necessary. Likewise, the study of carrier agents that reduce hygroscopicity and therefore increase the stability of the hydrolysate.

Acknowledgements

The authors are grateful for the financial support provided by Comité para el Desarrollo de la Investigación en la Universidad de Antioquia (CODI) through the sustainability program 2018-2019, and Estrategia de Formación de Alto Nivel del Ministerio de Ciencia, Tecnología e Innovación by Ministerio de Ciencia, Tecnología e Innovación through Call 647.

References

Abdul-Hamid, A., Bakar, J., & Bee, G. H. (2002). Nutritional quality of spray dried protein hydrolysate from Black Tilapia (Oreochromis mossambicus). *Food Chemistry*, 78(1), 69-74. http://dx.doi.org/10.1016/S0308-8146(01)00380-6.

Aissaoui, N., Abidi, F., Hardouin, J., Abdelkafi, Z., Marrakchi, N., Jouenne, T., & Marzouki, M. N. (2017). ACE inhibitory and antioxidant activities of novel peptides from Scorpaena notata By-product protein hydrolysate. *International Journal of Peptide Research and Therapeutics*, 23(1), 13-23. http://dx.doi.org/10.1007/s10989-016-9536-6.

Alahmad, K., Xia, W., Jiang, Q., & Xu, Y. (2022). Effect of the degree of hydrolysis on nutritional, functional, and morphological characteristics of protein hydrolysate produced from bighead carp (Hypophthalmichthys nobilis) using ficin enzyme. *Foods*, 11(9), 1320. http://dx.doi.org/10.3390/foods11091320. PMid:35564040.

Alemán, A., Pérez-Santín, E., Bordenave-Juchereau, S., Arnaudin, I., Gómez-Guillén, M. C., & Montero, P. (2011). Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity. *Food Research International*, 44(4), 1044-1051. http://dx.doi.org/10.1016/j.foodres.2011.03.010.

Benhabiles, M. S., Abdi, N., Drouiche, N., Lounici, H., Pauss, A., Goosen, M. F. A., & Mameri, N. (2012). Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. *Materials Science and Engineering C*, 32(4), 922-928. http://dx.doi.org/10.1016/j.msec.2012.02.013.

Borges-Contreras, B., Martínez-Sánchez, C. E., Herman-Lara, E., Rodríguez-Miranda, J., Hernández-Santos, B., Juárez-Barrientos, J. M., Guerra-Almonacid, C. M., Betancur-Ancona, D. A., & Torruco-Uco, J. G. (2019). Angiotensin-Converting enzyme inhibition in vitro by protein hydrolysates and peptide fractions from Mojarra of Nile Tilapia (Oreochromis niloticus) Skeleton. *Journal of Medicinal Food*, 22(3), 286-293. http://dx.doi.org/10.1089/jmf.2018.0163. PMid:30835154.

Bougatef, A., Nedjar-Arroume, N., Manni, L., Ravallec, R., Barkia, A., Guillochon, D., & Nasri, M. (2010). Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (Sardinella aurita) by-products proteins. *Food Chemistry*, 118(3), 559-565. http://dx.doi.org/10.1016/j.foodchem.2009.05.021.

Bougatef, A., Nedjar-Arroume, N., Manni, L., Ravallec, R., Barkia, A., Guillochon, D., & Nasri, M. (2016). Amino acid composition and in vitro antioxidant and cytoprotective activity of abalone viscera hydrolysate. *Food Chemistry*, 21(1), 1674-1682. http://dx.doi.org/10.1016/j.foodres.2013.03.034.

Cai, Y. Z., & Corke, H. (2000). Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science*, 65(6), 1248-1252. http://dx.doi.org/10.1111/j.1365-2621.2000.tb10273.x.

Chen, C., Chi, Y. J., & Xu, W. (2012). Comparisons on the functional properties and antioxidant activity of spray-dried and freeze-dried egg white protein hydrolysate. *Food and Bioprocess Technology*, 5(6), 2342-2352. http://dx.doi.org/10.1007/s11947-011-0606-7.

Chong, P. H., Yusof, Y. A., Aziz, M. G., Nazli, N. M., Chin, N. L., & Muhammad, S. K. S. (2014). Effects of spray drying conditions of microencapsulation of Amaranthus gangeticus extract on drying behaviour. *Agriculture and Agricultural Science Procedia*, 2, 33-42. http://dx.doi.org/10.1016/j.aaspro.2014.11.006.

Chuaychan, S., Benjakul, S., & Sae-Leaw, T. (2017). Gelatin hydrolysate powder from the scales of spotted golden goatfish: effect of drying conditions and juice fortification. *Drying Technology*, 35(10), 1195-1203. http://dx.doi.org/10.1080/07373937.2016.1236129.

Djouab, A., & Aïder, M. (2019). Effect of drying temperature on the antioxidant capacity of a cathodic electroactivated whey permeate. *ACS Sustainable Chemistry & Engineering*, 7(5), 5111-5121. http://dx.doi.org/10.1021/acssuschemeng.8b05962.

Favaro-Trindade, C. S., Santana, A. S., Monterrey-Quintero, E. S., Trindade, M. A., & Netto, F. M. (2010). The use of spray drying technology to

reduce bitter taste of casein hydrolysate. *Food Hydrocolloids*, 24(4), 336-340. http://dx.doi.org/10.1016/j.foodhyd.2009.10.012.

Félix-Medina, J. V., Sepúlveda-Haro, A. G., & Quintero-Soto, M. F. (2022). Stability of antioxidant and hypoglycemic activities of peptide fractions of Maize (Zea mays L.) under different processes. *Journal of Food Measurement and Characterization*. http://dx.doi.org/10.1007/s11694-022-01618-5.

Gómez, L. J., Gómez, N. A., Zapata, J. E., López-García, G., Cilla, A., & Alegría, A. (2019). In-vitro antioxidant capacity and cytoprotective/cytotoxic effects upon Caco-2 cells of red tilapia (Oreochromis spp.) viscera hydrolysates. *Food Research International*, 120, 52-61. https://doi.org/10.1016/j.foodres.2019.02.029.

Hassan, M. A., Deepitha, R. P., Xavier, K. A. M., Gupta, S., Nayak, B. B., & Balange, A. K. (2019). Evaluation of the properties of spray dried visceral protein hydrolysate from Pangasianodon hypophthalmus (Sauvage, 1978) extracted by enzymatic and chemical methods. *Waste and Biomass Valorization*, 10(9), 2547-2558. http://dx.doi.org/10.1007/s12649-018-0302-1.

Hsu, K.-C., Li-Chan, E. C. Y., & Jao, C.-L. (2011). Antiproliferative activity of peptides prepared from enzymatic hydrolysates of tuna dark muscle on human breast cancer cell line MCF-7. *Food Chemistry*, 126(2), 617-622. http://dx.doi.org/10.1016/j.foodchem.2010.11.066.

Hwang, I. G., Kim, H. Y., Woo, K. S., Lee, J., & Jeong, H. S. (2011). Biological activities of Maillard reaction products (MRPs) in a sugar–amino acid model system. *Food Chemistry*, 126(1), 221-227. http://dx.doi.org/10.1016/j.foodchem.2010.10.103.

Ingvarsson, P. T., Yang, M., Nielsen, H., Rantanen, J., & Foged, C. (2011). Stabilization of liposomes during drying. *Expert Opinion on Drug Delivery*, 8(3), 375-388. http://dx.doi.org/10.1517/17425247.2011.553219. PMid:21294603.

Jang, H. L., Liceaga, A. M., & Yoon, K. Y. (2016). Purification, characterisation and stability of an antioxidant peptide derived from sandfish (Arctoscopus japonicus) protein hydrolysates. *Journal of Functional Foods*, 20, 433-442. http://dx.doi.org/10.1016/j.jff.2015.11.020.

Je, J.-Y., Park, S. Y., Hwang, J.-Y., & Ahn, C.-B. (2015). Amino acid composition and in vitro antioxidant and cytoprotective activity of abalone viscera hydrolysate. *Journal of Functional Foods*, 16, 94-103. http://dx.doi.org/10.1016/j.jff.2015.04.023.

Korczek, K., Tkaczewska, J., & Migdał, W. (2018). Antioxidant and antihypertensive protein hydrolysates in fish products - A Review. *Czech Journal of Food Sciences*, 36(3), 195-207. http://dx.doi.org/10.17221/283/2017-CJFS.

Kurozawa, L. E., Park, K. J., & Hubinger, M. D. (2009). Effect of carrier agents on the physicochemical properties of a spray dried chicken meat protein hydrolysate. *Journal of Food Engineering*, 94(3-4), 326-333. http://dx.doi.org/10.1016/j.jfoodeng.2009.03.025.

Kurozawa, L. E., Park, K. J., & Hubinger, M. D. (2011). Spray drying of chicken meat protein hydrolysate: influence of process conditions on powder property and dryer performance. *Drying Technology*, 29(2), 163-173. http://dx.doi.org/10.1080/07373937.2010.482711.

Liu, T., Wang, Y., Yu, X., Li, H., Ji, L., Sun, Y., Jiang, X., Li, X., & Liu, H. (2022). Effects of freeze-drying and spray-drying on the physical and chemical properties of Perinereis aibuhitensis hydrolysates: sensory characteristics and antioxidant activities. *Food Chemistry*, 382, 132317. http://dx.doi.org/10.1016/j.foodchem.2022.132317. PMid:35149461.

Ma, J. J., Mao, X. Y., Wang, Q., Yang, S., Zhang, D., Chen, S. W., & Li, Y. H. (2014). Effect of spray drying and freeze drying on the immunomodulatory activity, bitter taste and hygroscopicity of hydrolysate derived from whey protein concentrate. *Lebensmittel-Wissenschaft + Technologie*, 56(2), 296-302. http://dx.doi.org/10.1016/j.lwt.2013.12.019.

Morales, F. J., & Jiménez-Pérez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chemistry*, 72(1), 119-125. http://dx.doi.org/10.1016/S0308-8146(00)00239-9.

Nooshkam, M., Varidi, M., & Verma, D. K. (2020). Functional and biological properties of Maillard conjugates and their potential application in medical and food: a review. *Food Research International*, 131, 109003. https://doi.org/10.1016/j.foodres.2020.109003.

Ozdikicierler, O., Dirim, S. N., & Pazir, F. (2014). The effects of spray drying process parameters on the characteristic process indices and rheological powder properties of microencapsulated plant (Gypsophila) extract powder. *Powder Technology*, 253, 474-480. http://dx.doi.org/10.1016/j.powtec.2013.12.004.

Rivero-Pino, F. (2023). Bioactive food-derived peptides for functional nutrition: effect of fortification, processing and storage on peptide stability and bioactivity within food matrices. *Food Chemistry*, 406, 135046. http://dx.doi.org/10.1016/j.foodchem.2022.135046. PMid:36446284.

Rivero-Pino, F., Espejo-Carpio, F. J., & Guadix, E. M. (2020). Bioactive fish hydrolysates resistance to food processing. *LWT,* 117, 108670. https://doi.org/10.1016/j.lwt.2019.108670.

Rodríguez-Díaz, J. C., Tonon, R. V., & Hubinger, M. D. (2014). Spray drying of blue shark skin protein hydrolysate: physical, morphological, and antioxidant properties. *Drying Technology*, 32(16), 1986-1996. http://dx.doi.org/10.1080/07373937.2014.928726.

Rosa Zavareze, E., Telles, A. C., Mello El Halal, S. L., da Rocha, M., Colussi, R., Marques de Assis, L., Suita de Castro, L. A., Guerra Dias, A. R., & Prentice-Hernández, C. (2014). Production and characterization of encapsulated antioxidative protein hydrolysates from Whitemouth croaker (*Micropogonias furnieri*) muscle and byproduct. *LWT*, 59(2 Pt 1), 841-848. https://doi.org/10.1016/j.lwt.2014.05.013.

Sarabandi, K., Gharehbeglou, P., & Jafari, S. M. (2020). Spray-drying encapsulation of protein hydrolysates and bioactive peptides: opportunities and challenges. *Drying Technology*, 38(5-6), 577-595. http://dx.doi.org/10.1080/07373937.2019.1689399.

Sepúlveda, C. T., & Zapata, J. E. (2020). Effects of enzymatic hydrolysis conditions on the antioxidant activity of red Tilapia (*Oreochromis* spp.) Viscera hydrolysates. *Current Pharmaceutical Biotechnology*, 21(12), 1249-1258. http://dx.doi.org/10.2174/1389201021666200506072526. PMid:32370711.

Sepúlveda, C. T., Zapata, J. E., Martínez-Álvarez, O., Alemán, A., Montero, M. P., & Gómez-Guillén, M. C. (2021). The preferential use of a soy-rapeseed lecithin blend for the liposomal encapsulation of a tilapia viscera hydrolysate. *LWT*, 139, 110530. http://dx.doi.org/10.1016/j.lwt.2020.110530.

Silva, V. M., Kurozawa, L. E., Park, K. J., & Hubinger, M. D. (2012). Influence of carrier agents on the physicochemical properties of mussel protein hydrolysate powder. *Drying Technology*, 30(6), 653-663. http://dx.doi.org/10.1080/07373937.2012.657727.

Su, G., Zheng, L., Cui, C., Yang, B., Ren, J., & Zhao, M. (2011). Characterization of antioxidant activity and volatile compounds of Maillard reaction products derived from different peptide fractions of peanut hydrolysate. *Food Research International*, 44(10), 3250-3258. http://dx.doi.org/10.1016/j.foodres.2011.09.009.

Tang, N., & Zhuang, H. (2015). Studies on the antioxidative stability of corn antioxidant peptide leu-pro-phe. *Journal of Chinese Institute of Food Science and Technology*, 15(2), 49-55. http://dx.doi.org/10.16429/j.1009-7848.2015.02.008.

Vásquez, P., Sepúlveda, C. T., & Zapata, J. E. (2022). Functional properties of rainbow trout (Oncorhynchus mykiss) viscera protein hydrolysates. *Biocatalysis and Agricultural Biotechnology*, 39, 102268. https://doi.org/10.1016/j.bcab.2021.102268.

Villamil, O., Váquiro, H., & Solanilla, J. F. (2017). Fish viscera protein hydrolysates: production, potential applications and functional and bioactive properties. *Food Chemistry*, 224, 160-171. http://dx.doi.org/10.1016/j.foodchem.2016.12.057. PMID: 28159251.

Zhang, C., Zhang, N., Li, Z., Tian, Y., Zhang, L., & Zheng, B. (2016). Stability of antioxidant peptides prepared from large yellow croaker (Pseudosciaena crocea). *Current Topics in Nutraceutical Research*, 14(1), 37-47.

Zhao, T., Zhang, Q., Wang, S., Qiu, C., Liu, Y., Su, G., & Zhao, M. (2018). Effects of Maillard reaction on bioactivities promotion of anchovy protein hydrolysate: the key role of MRPs and newly formed peptides with basic and aromatic amino acids. *LWT*, 97, 245-253. http://dx.doi.org/10.1016/j.lwt.2018.06.051.

Zhu, C. Z., Zhang, W. G., Kang, Z. L., Zhou, G. H., & Xu, X. L. (2014). Stability of an antioxidant peptide extracted from Jinhua ham. *Meat Science*, 96(2 Pt A), 783-789. http://dx.doi.org/10.1016/j.meatsci.2013.09.004. PMid:24200571.