



Production and spray drying of protein hydrolyzate obtained from tilapia processing by-products

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ABSTRACT. In the last few decades, the offer of by-products obtained from the processing of tilapia (*Oreochromis niloticus*) has increased, and the need for developing products with high biological and nutritional values for use in animal nutrition motivated this study. Enzymatic hydrolysis of carcass, head and skin of tilapia was performed, as well as the separation of oil, residual solids and soluble proteins by centrifugation at high temperature and the spray drying of the protein fraction. Factorial designs were employed in the assays to evaluate the operating conditions of the spray dryer (inlet and outlet temperatures and flow rate) and the inclusion of drying aid agents (maltodextrin and calcium carbonate). The spray drying showed the best results with air inlet temperature of 190°C, outlet temperature of 90°C, flow rate of 30 L·h⁻¹ including 10% maltodextrin (mass) in the liquid feed as a drying aid. The final powder recovery was higher than 90% and the physical, chemical and microbiological analyses met the Brazilian legal standards.

Keywords: enzymatic hydrolysis, spray dryer, factorial design.

Produção e secagem por atomização de hidrolisado proteico, obtido de coprodutos do processamento de tilápia

RESUMO. Nas últimas décadas é crescente a oferta de coprodutos produzidos pelo processamento de tilápia (*Oreochromis niloticus*). A necessidade de desenvolvimento de produtos com alto valor biológico e nutricional para emprego na alimentação animal motivou o desenvolvimento deste trabalho. Foram realizadas a hidrólise enzimática de carcaça, cabeça e pele de tilápia, a separação do óleo, dos sólidos residuais e da proteína solúvel por centrifugação a quente e a secagem da fração proteica por atomização. Foram empregados delineamentos fatoriais na condução dos ensaios, para avaliar as condições de operação do equipamento de secagem (temperaturas de entrada e saída da câmara de secagem e vazão de alimentação de líquido) e inclusão de adjuvantes de secagem (maltodextrina e carbonato de cálcio). A secagem por *spray drier* apresentou os melhores resultados com temperatura de entrada do ar na câmara de 190°C, temperatura de saída de 90°C, vazão de alimentação de líquido de 30 L·h⁻¹ e inclusão de 10% de maltodextrina, em massa, no líquido, como adjuvante de secagem. A recuperação do produto final em pó foi superior a 90% e as análises físico-químicas e microbiológicas seguiram os padrões legais de comercialização do produto no Brasil.

Palavras chave: hidrólise enzimática, *spray drier*, planejamento fatorial.

Introduction

The amount of by-products from the fish industry has substantially increased in the last decades, in the case of tilapia, about 60% (considering head, skin, scales, spine and viscera) (Chalamaiah, Dineshkumar, Hemalatha, & Jyothirma, 2012). These by-products have high levels of high quality protein, which can be used in human and animal nutrition. However, they are usually employed in the production of meals of lower commercial value and quality, when

compared to a product obtained by enzymatic hydrolysis (Silva, Ribeiro, Silva, Cahú, & Bezerra, 2014). The production of meals from animal protein basically consists of conducting acid reactions under high pressure and temperature to obtain the final product. Enzymatic hydrolysis is carried out under mild conditions of temperature and pH, employing a biological catalyst with high affinity to the substrate, which provides high quality hydrolyzed protein. In an industrial process, storage and transport of liquid products is

expensive and requires large facilities for the operations. The drying of this product, when possible, extends shelf life, reduces microbiological contamination risks and facilitates its use in other processes (Rannou et al., 2015). The most effective way of drying products with high commercial values, such as hydrolyzed protein, at an industrial scale, is by means of spray drying. This operation is widely used in industries to obtain dried products, and consists of rapid evaporation of water from a sprayed liquid inside a drying chamber. Despite of the positive aspects, the operation can cause the loss of volatile materials and thermal degradation of heat sensitive compounds, due to the high inlet temperatures of the drying chamber (Bhandari, Patel, & Chen, 2008; Ishwarya, Anandharamkrishnan, & Stapley, 2015). Thus, the goal of this work was to produce protein hydrolyzate from the waste from tilapia processing (carcass, head and skin) using enzymatic catalysis and to evaluate the spray drying conditions of the product in an animal nutrition industry.

Material and methods

Material

For the production of protein hydrolyzate, it was used tilapia waste (carcass, head and skin) from a Brazilian Tilapia slaughterhouse from Toledo, State of Paraná, Brazil; the slaughterhouse is certified by the sanitary authorities. The proteolytic enzyme from *Bacillus licheniformis* (Alcalase® 2.5L), Novozymes, presented the optimal use conditions of 30-65°C, pH 7-10 and activity of 2.5AU·A·g⁻¹. For the drying step, a centrifuge Gratt, model GTA 3.0a, with operation capacity of 1,000 kg·h⁻¹ at 4,200 rpm was used to separate the liquid into three phases. The spray dryer used was constructed and provided by R.M. Máquinas Frigoríficas, model CTS-01, with evaporative capacity of 60 L·h⁻¹ and rotary atomizer operating at 12,000 rpm. The reagents used were of analytical grade and the industrial inputs used in the process were food grade.

Enzymatic hydrolysis of the raw material

Tilapia waste (carcass, head and skin) were ground to an average particle size of 5 mm in an industrial knife mill and hydrolyzed in a horizontal stainless steel reactor with capacity of 3,000 kg, enzyme at 60°C, for 2h. The reaction was conducted in aqueous medium at the ratio of 100:15 raw

material:water (w:v). The enzyme:substrate ratio was 1:200 (w:w). After hydrolysis, the enzyme was inactivated at 9°C for 15 min., and the pH was adjusted to 3.0 by adding 85% phosphoric acid to preserve the product (Dieterich et al., 2014).

Preparation of the hydrolyzate for drying

After the hydrolysis, the stabilized product was filtered through a double 6 mm mesh sieve for bone removal. Subsequently, the hydrolyzate was heated up to 95°C and pumped to the centrifuge to be separated into three phases (oil, soluble protein and suspended solids) at a mass flow of 1,000 kg·h⁻¹ and 1,000 rpm. The product was heated to favor the separation of the oil fraction. Operating data for the equipment were provided by the manufacturer.

Spray drying of the soluble protein

The drying study for the soluble protein was conducted in two different steps. The first evaluated the operating parameters of the drying equipment: inlet air temperature (IT), outlet air temperature (OT) and variation of flow (VF). Assays were performed according to a 2³ complete factorial design, with three replicates at the central point, and a total of eleven experiments. In the second step, starting with the best results obtained in the first, a new 2³ complete factorial design was adopted, with three replicates at the central point, in order to evaluate the inclusion of the drying aid agents in the process (maltodextrin and calcium carbonate) and the amount of solids present in the protein solution. The first experimental design was carried out with the levels of maltodextrin and calcium carbonate (10 and 1%, respectively, from the experimental data obtained at the laboratory) as fixed parameters, and the level of solids was 35% by controlling the moisture of the solution, according to instructions from the equipment manufacturer, for drying of products with protein features.

Analytical methods for characterization of the dried product

After the drying studies, the sample with the highest powder yield was evaluated for its physical, chemical and microbiological characteristics. Quantification of *Salmonella* sp. and total and fecal coliforms was done by an accredited laboratory of microbiological analysis (Allabor Laboratório de Alimentos LTDA, Toledo, Paraná State, Brasil). Determinations of moisture at 105°C, crude protein by micro Kjeldahl method, ether extract by Soxhlet method

and mineral matter in muffle at 600°C were performed according to the methodologies described by Association of Official Analytical Chemistry (AOAC, 1990).

Results and discussion

Evaluation of operating parameters of the drying equipment

Table 1 lists data of the experimental design with the specifications of the evaluated variables (IT, OT and VF) in coded and real forms (between parentheses), along with the responses obtained for the assays (protein content P and moisture M). Fixed parameters of the drying operation, obtained from the preliminary tests and indicated by the spray dryer manufacturer, were amounts of solids, maltodextrin and calcium carbonate (35, 10, and 1%, respectively). The results were similar for both responses in all assays, with higher values of protein and lower values of moisture obtained for the assay 2.

Table 1. Data and results of the 2³ complete factorial design: operating parameters of the dryer.

Assay	IT (°C)	OT (°C)	VF (L·h ⁻¹)	P (%)	M (%)
1	-1 (180)	-1 (80)	-1 (25)	42.37	3.32
2	1 (210)	-1 (80)	-1 (25)	44.14	2.67
3	-1 (180)	1 (110)	-1 (25)	42.04	3.13
4	1 (210)	1 (110)	-1 (25)	42.95	3.09
5	-1 (180)	-1 (80)	1 (40)	42.17	3.65
6	1 (210)	-1 (80)	1 (40)	42.84	3.59
7	-1 (180)	1 (110)	1 (40)	42.24	4.01
8	1 (210)	1 (110)	1 (40)	43.11	3.78
9	0 (195)	0 (95)	0 (32.5)	42.86	3.57
10	0 (195)	0 (95)	0 (32.5)	42.53	3.43
11	0 (195)	0 (95)	0 (32.5)	43.07	3.49

The effects for the variables, with the protein content as response, are shown in Table 2. For a confidence interval of 95%, only IT was significant (value in bold and italic). Considering the value of its coefficient, the variable positively influenced the process i.e., a higher inlet temperature of the drying chamber increased the amount of protein in the final product. The analysis of variance for the protein content provided the calculated F value of 9.60. The tabulated F value (Barros Neto, Scarminio, & Bruns, 2007) is 8.89. So, it is possible to affirm that the linear model proposed is valid, and allows us to define, empirically, the equation that is a function of the significant variables (Equation 1).

$$\text{Protein (\%)} = 42.76 + 0.53 \text{ IT} \quad (1)$$

The response surfaces of the variables evaluated for protein are shown in Figure 1. In order to achieve a higher amount of protein, inlet

temperatures at the drying chamber should be higher. With a higher value of IT, the amount of water removed will be greater, resulting in a mass balance favorable to the increase in protein content. Although the outlet temperature and mass flow showed no influence, in this case, some considerations can be made for these variables, based on the final product obtained after the drying process. Increase of liquid flow in the drying chamber, increase to yield, generates a final product with higher moisture, above the desired levels. To overcome this situation, the dryer was operated with higher IT and OT. However, at this point, the final product showed a darkened, less attractive visual aspect. So, it is recommend, for the tilapia hydrolyzate, dryer operation with lower flow and temperature, within the average range employed in this work. In a review conducted by Keshani, Daud, Nourouzi, Namvar, and Ghasemi (2015), several authors present some effects relating the operating temperature and the physical properties of the liquid to the drying chamber geometry and to the residence time of the liquid inside the chamber. Liquids with higher viscosity adhere more easily to the walls of the dryer. Drying of these particles produces amorphous powder with flexible surface structure. The temperature on the particle surface should not exceed the glass transition of the product at 20°C. When the feed rate in the chamber increases, larger droplets are formed. It reduces the evaporation rate of the liquid and provides extra adherence of the product to the dryer wall. To compensate for this effect and reach the desired moisture, if the temperatures in the chamber are raised, the surface temperature of the particles adhered to the wall can exceed the glass transition of the product, leading to lower quality powder (Roos & Karel, 1991; Bhandari, Datta, Crooks, Howes, & Rigby, 1997; Ozmen & Langrish, 2003; Aguilera & Lillford, 2007; Shrestha, Howes, Adhikari, & Bhandari, 2008; Roos, 2009; Woo, Daud, Tasirin, & Talib, 2009; Keshani et al., 2015).

Table 2. Effects estimative of variables for the protein content: operating parameters of the dryer.

Variable	Effect	Std. error	p-value	Coef.
Mean	42.76	0.07	0.00	42.76
IT (°C)	1.06	0.17	0.00	0.53
OT (°C)	-0.29	0.17	0.17	-0.14
VF (L·h ⁻¹)	-0.28	0.17	0.18	-0.14
IT x OT	-0.16	0.17	0.39	-0.08
IT x VF	-0.28	0.17	0.18	-0.14
OT x VF	0.46	0.17	0.06	0.23
IT x OT x VF	0.26	0.17	0.20	0.13

R² = 0.95.

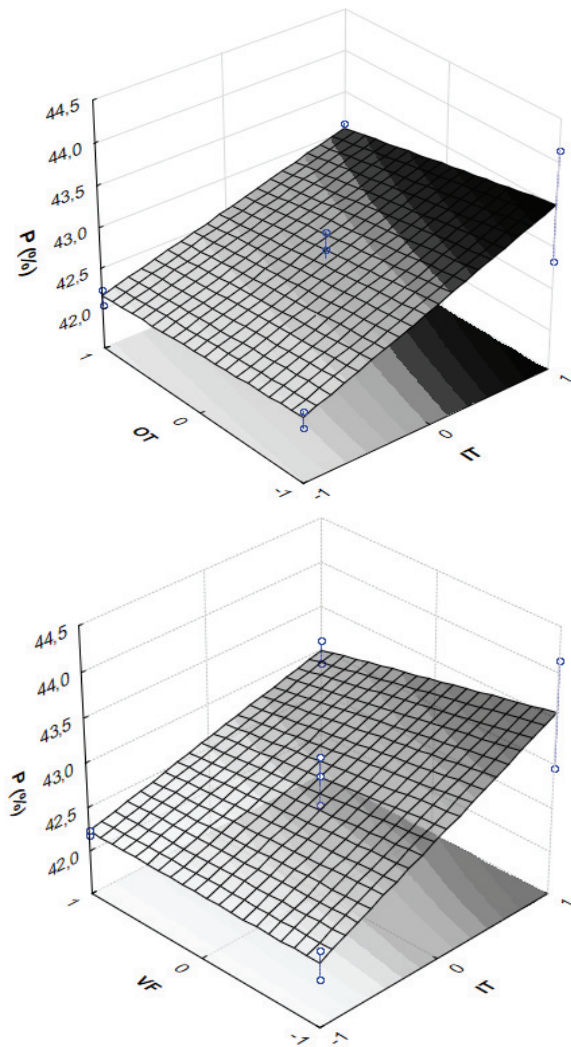


Figure 1. Response surfaces of the evaluated variables for protein content: operating parameters of the dryer.

The estimated effects for the evaluated variables, with the moisture as a response, are listed in Table 3. For a confidence interval of 95%, IT and VF were significant. Considering their coefficients, IT influenced the process with a negative magnitude, related to the final moisture of the powder. It means that the higher the IT, the lower the final moisture. On the other hand, the flow had a negative influence on the process, i.e., the higher the feeding flow, the higher the final moisture. The analysis of variance presented a calculated F value of 19.00. The tabulated F value (Barros Neto et al., 2007) is 8.89. It is possible to affirm that the linear model proposed is valid and the correlation coefficient showed a good fit of data to the model. It allows us to define, empirically, the equation that represents the model according to the significant variables (Equation 2).

$$M (\%) = 3.43 - 0.12 IT + 0.35 VF \quad (2)$$

The response surfaces for moisture content are shown in Figure 2, which indicated that a final product with lower moisture can be obtained if the drying system is operated using higher inlet temperatures and lower flow rate values.

Table 3. Effects estimation of the evaluated variables for the moisture: operation parameters of the dryer.

Variable	Effect	Std. error	p-value	Coef.
Mean	3.43	0.03	0.00	3.43
IT (°C)	-0.24	0.07	0.03	-0.12
OT (°C)	0.19	0.07	0.06	0.09
VF (L·h ⁻¹)	0.70	0.07	0.00	0.35
IT x OT	0.11	0.07	0.20	0.05
IT x VF	0.10	0.07	0.24	0.05
OT x VF	0.08	0.07	0.32	0.04
IT x OT x VF	-0.19	0.07	0.06	-0.09

R² = 0.98.

During the assays, an inverse relationship between inlet temperature and flow rate was found. For higher values of IT and lower values of VF, the final product showed a darkened aspect, which is not acceptable commercially. The lower the value of VF, the longer residence time of droplets inside the chamber and, with elevated IT, temperature on the surface of the particle tends to exceed the limit of glass transition of the product (Keshani et al., 2015). Although the statistic analysis and the response surfaces in Figure 2 have indicated that to obtain a product with lower moisture, the system should be operated with high IT and low VF, the protein concentrate in its powder form must follow specific laws that determine the maximum acceptable limits of moisture. In this study, the higher moisture obtained was 4%. Nevertheless, the Regulation of Industrial and Sanitary Inspection of Animal Products, Ministry of Agriculture (RIISPOA), Livestock and Supply, through the Decree 30691 of 29/03/1952 authorizes the commerce of fish flours of first quality with 10% of moisture. So, the equipment can be set to operate to obtain a product with higher moisture and quality for commerce, meeting legal parameters and regulations. The results obtained indicated that, for a higher protein content in the powder, the dryer must be operated at the higher range of IT (195-210°C). To obtain a product with lower moisture, the result indicated the higher range of inlet temperature (195-210°C) and lower flow rate (25-32 L·h⁻¹). However, given the operating conditions, the technical specifications of the dryer provided by the manufacturer, the desirable visual aspect of the powder for marketing and following the directions of the results obtained with the analysis previously presented and discussed, the second experimental design will be conducted with the following values of operating parameters: IT of 190°C, OT of 90°C and VF of 30 L·h⁻¹.

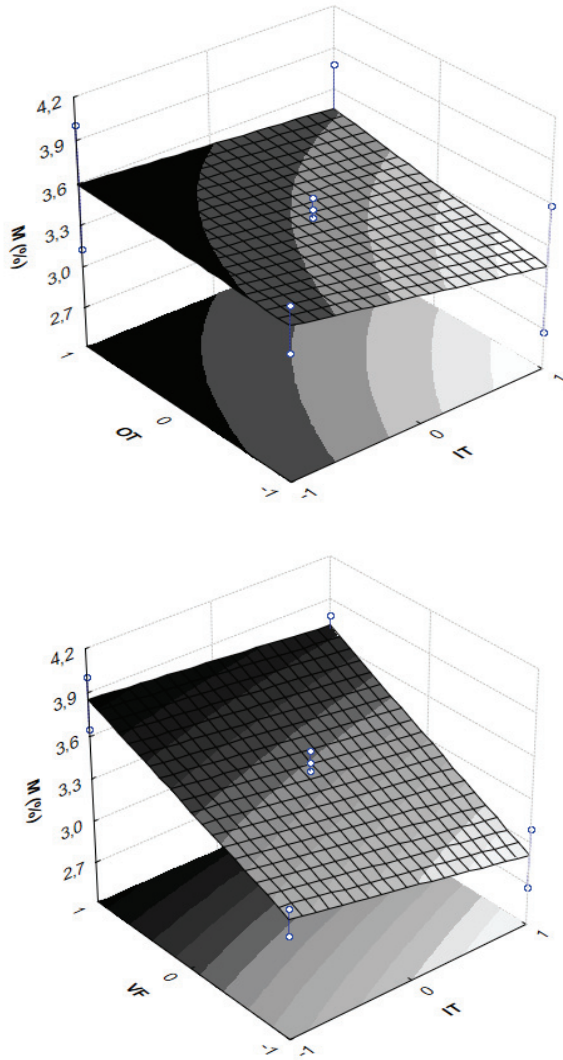


Figure 2. Response surfaces of the evaluated variables for moisture content: operating parameters of the dryer.

Evaluation of the inclusion of drying aid agents

The experimental design included the addition of drying aid agents to the liquid product, aiming to facilitate the removal of water and keeping the moisture levels acceptable after dehydration. The evaluated variables were the amount of solids in the sample (AS) and the inclusion of maltodextrin (MD) and calcium carbonate (CaCO_3). Data of the experimental design with the specified variables evaluated in its coded and real forms (between parentheses), along with the responses obtained for the assays proposed are presented on Table 4. Observe that the results for protein were similar for all assays. However, the moisture was lower at the central point (assays 9-11).

The estimate of the effects for the evaluated variables with the protein content as response is presented in Table 5. For a confidence interval of

95%, only the solid content was significant (value in bold and italic). Considering the value of its coefficient, the variable negatively influenced the process, that is, the more solids suspended in the liquid, the lower the protein content of the product. The interaction between the amount of solids and maltodextrin, although non-significant, presented a p-value very close to 0.05. This suggests that these variables can also influence the process to higher protein amount.

Table 4. Data and results of the 2^3 complete factorial design: inclusion of drying aid agents.

Assay	AS (%)	MD (%)	CaCO_3 (%)	P (%)	M (%)
1	-1 (30)	-1 (5)	-1 (1)	44.15	2.52
2	1 (40)	-1 (5)	-1 (1)	43.14	2.08
3	-1 (30)	1 (15)	-1 (1)	43.87	3.15
4	1 (40)	1 (15)	-1 (1)	42.98	2.39
5	-1 (30)	-1 (5)	1 (5)	44.48	2.79
6	1 (40)	-1 (5)	1 (5)	42.27	2.17
7	-1 (30)	1 (15)	1 (5)	43.79	3.07
8	1 (40)	1 (15)	1 (5)	44.09	2.44
9	0 (35)	0 (10)	0 (3)	43.31	1.59
10	0 (35)	0 (10)	0 (3)	43.44	1.78
11	0 (35)	0 (10)	0 (3)	44.07	1.96

Table 5. Effects estimative of variables for protein: inclusion of drying aid agents.

Variable	Effect	Std. error	p-value	Coef.
Mean	43.59	0.10	0.00	43.59
AS (%)	-0.95	0.23	0.03	-0.48
MD (%)	0.17	0.23	0.52	0.09
CaCO_3 (%)	0.12	0.23	0.64	0.06
AS x MD	0.66	0.23	0.06	0.33
AS x CaCO_3	-0.00	0.23	0.99	-0.00
MD x CaCO_3	0.39	0.23	0.19	0.19
AS x MD x CaCO_3	0.60	0.23	0.08	0.29

$R^2 = 0.92$.

ANOVA for amount of protein provided a calculated F value of 4.91. The tabulated F value is 8.89. It is not possible to affirm that the proposed empirical model of linear regression is valid. However, the response surfaces (Figure 3) can be used, with the objective of setting and indicating the direction that the experiments must follow to obtain a larger amount of protein. It is observed that a higher protein amount can be obtained using lower amounts of suspended solids, maltodextrin and larger amount of CaCO_3 .

Drying aid agents are essential to the recovery of the dehydrated protein, once they prevent the degradation of the protein material caused by IT in the drying chamber and its adherence to the walls of the equipment. Encapsulation and spray drying have, among other goals, to protect and stabilize the molecules on the final product. It can also improve aroma, flavor and appearance. This process depends on the operating conditions and the selection of the encapsulating material. In this

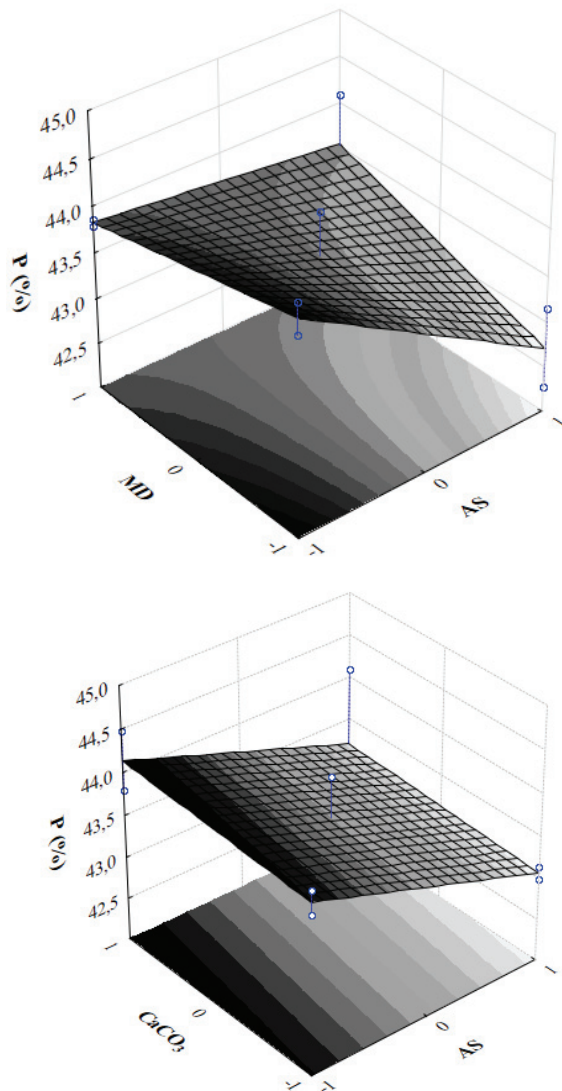


Figure 3. Response surfaces of the evaluated variables for protein: inclusion of drying aid agents.

case, it can be cited the use of gum arabic, maltodextrin, modified starch, among others (Krishnan, Bhosale, & Singhal, 2005; Baranauskienė, Rimantas, Dewettink, & Verdhé, 2006; Madene, Jacquot, Scher, & Stéphane, 2006; Gharsallaoui, Surel, Roudaut, Chambin, & Volley, 2007; Georgetti, Casagrande, Fernandes, Pereira, & Vieira, 2008; Kurozawa, Park, & Hubinger, 2009; Wang, Lu, & Lv, 2009; Kim, Chen, & Pearce, 2009; Cuq, Rondet, & Abecassis, 2011; Guadarrama-Lezama et al., 2012; Porrás-Saavedra et al., 2015). However, these materials must be used with caution as drying adjuvants. They are, in their majority, carbohydrates of high molecular weight, and if applied in excess to the liquid to be dehydrated can negatively interfere with the final protein. Results and graphs presented show that

the lowest amount of drying adjuvants to allow solid recovery and conducted a low final moisture of the product. The effects estimative for the evaluated variables, with the moisture as response, are listed in Table 6. For a confidence interval of 95%, none of the evaluated variables and interactions was significant in the range of the proposed study. All the variables presented p-values much greater than the confidence interval; which is reflected in the low correlation coefficient. The results of this analysis allow to affirm that the evaluated variables have no statistic relevance on the final moisture of the dried protein hydrolyzate obtained from tilapia processing by-products.

Table 6. Effects estimative of the evaluated variables for moisture: inclusion of drying aid agents.

Variable	Effect	Std. error	p-value	Coef.
Mean	2.36	0.21	0.00	2.35
AS (%)	-0.61	0.50	0.30	-0.30
MD (%)	0.37	0.50	0.50	0.18
CaCO ₃ (%)	0.08	0.50	0.87	0.04
AS x MD	-0.08	0.50	0.87	-0.04
AS x CaCO ₃	-0.01	0.50	0.98	-0.00
MD x CaCO ₃	-0.09	0.50	0.85	-0.04
AS x MD x CaCO ₃	0.07	0.50	0.88	0.03

$R^2 = 0.42$.

ANOVA provided the calculated F value of 0.33; lower than the tabulated value of 8.89. It is not possible to affirm that the empirical model of linear regression is valid. Nevertheless, response surfaces for the data set were generated (Figure 4) and indicated the probable direction the experiments must follow. It is observed that lower values of moisture can be obtained with larger amounts of suspended solids. The addition of maltodextrin and CaCO₃ not show way, in the response surfaces. This was expected, based on the principle that the operation of spray drying consists of removing water of a solution containing suspended solids at high temperatures. Regardless of the adjuvant employed in the process, water will be removed by the hot air flow, and the final moisture will more strongly depend on the operating parameters of the dryer than on the composition of the adjuvant used. In the specific case of maltodextrin, Painsi, Aliakbarian, Casazza, Lagazzo, Botter, & Perego (2015) studied the encapsulation of phenolic compounds extracted from olive pomace, and used maltodextrin as encapsulating agent, operating the spray dryer with inlet temperature between 150 and 160°C. According to the authors, the adjuvant was selected for its good features of solubility, thermal resistance and low viscosity at high concentrations of suspended solids (Gharsallaoui et al., 2007; Kha, Nguyen, & Roach, 2010; Robert, Gorena, Romero,

Sepulveda, Chavez, & Saenz, 2010). On the other hand, calcium carbonate has an anti-wetting property, preventing the product from absorbing water during storage.

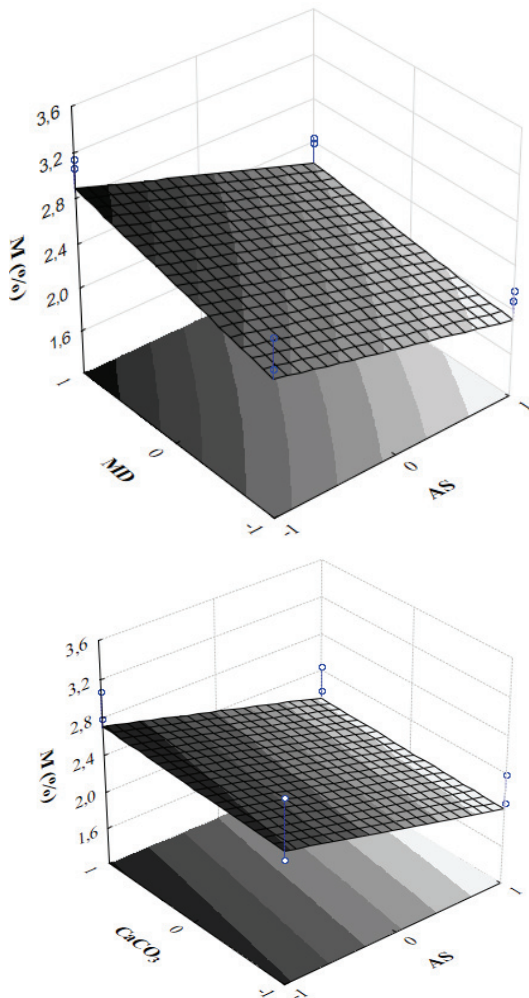


Figure 4. Response surfaces of the evaluated variables for moisture: inclusion of drying aid agents.

Observing the values in Table 4, the protein content was similar for all assays. On the other hand, lower values of moisture were obtained at the central point (10% MD and 3% CaCO_3). As previously mentioned, the inclusion of adjuvants to the spray drying process is necessary, so no liquid droplets adhere to the walls of the drying chamber (Keshani et al., 2015). To verify the better employment conditions of these adjuvants, with the results obtained from the experimental designs, two additional triplicate experiments were performed, adding 10% MD (experiment 1); 10% MD and 3% CaCO_3 (experiment 2). The dryer was operated with IT of 190°C , OT of 90°C and VF of $30 \text{ L}\cdot\text{h}^{-1}$. The criterion for choosing the best result was the amount of solids recovered at the end of each

experiment. In the experiment 1, 91.3% of the suspended solids were recovered, in the experiment 2, recovery was 74.5%. In both experiments, final moisture was around 2%. It indicates that, with the best operating conditions, the most advantageous process, economically, is the one that uses 10% MD, mass, in relation to the amount of hydrolyzate obtained from tilapia by-products. For physicochemical and biological characterization of the dehydrated hydrolyzate, samples of the experiment 1 were used. Results are presented in Table 7. Protein and moisture values are in accordance to that determined by law regulating marketing (RIISPOA of 29/03/1952). As it is a product obtained from raw material of animal origin (protein and bones), the amount of mineral matter is high, according to the results, and the low content of ether extract evidences the efficiency of oil separation in the centrifuging process, carried out before the drying. Some works, including Simões, Ribeiro, Ribeiro, Park, and Murr (2007), show the level of fatty material in tilapia fillets at approximately 2.6%. Muscle of this fish can contain until 36% lipids (Ogawa & Maia, 1999). The microbiological analysis allows to affirm that the dehydrated product is suitable for commerce and application in animal nutrition. These analyses follow the adequacy of parameters employed for foods intended for human consumption, determined by the Resolution of the Board of Directors, from the National Health Surveillance Agency, of 02/01/2001.

Table 7. Physicochemical and microbiological analysis of powder tilapia hydrolyzate.

Physicochemical	Results (%)	Microbiological	Results (CFU·25 g ⁻¹)
Moisture	2.14	Total Coliforms	< 1.0×10^1
Protein	44.80	<i>Salmonella</i> sp.	undetected
Lipid	0.32	Total <i>E. coli</i>	< 1.0×10^1
Total mineral	21.06		

Conclusion

The production of protein concentrate powder, using by-products of tilapia processing, for use in animal feed, represents an industrial technological process capable of generating good nutritional quality products with high added value. And, the use of statistical tools to study and optimize the parameters of these processes may increase the operating efficiency and decrease the production costs. When these systems are worked within the appropriate range, as the operating parameters evaluated in this paper, final product yield may be maximized, reducing costs with energy and equipment downtime. And, with the

drying aid agents, the choice of appropriate levels of inclusion leads to higher product recoveries, maintaining the final and desired characteristics of the powder.

Acknowledgements

To CNPq (process 453854/2013-3) and to Fundação Araucária/CAPES (processes 1032/2013 e 43234/2014) for financial support, to R.M. Máquinas Frigoríficas (Chapecó-SC-Brazil) for helping with the drying process and to Falbom Agroindustrial (Toledo, Paraná State, Brazil) for supporting the execution of the work.

References

- Aguilera, J., & Lillford, P. (2007). *Food materials science: principles and practice*. New York, USA: Springer Verlag.
- Association of Official Analytical Chemistry (AOAC). (1990). *Official methods of analysis*. Arlington, USA: AOAC International.
- Baranauskienė, R., Rimantas, V. P., Dewettink, K., & Verdhé, R. (2006). Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices. *Food Research International*, 39(4), 413-425.
- Barros Neto, B., Scarminio, I. S., & Bruns, R. E. (2007). *Como fazer experimentos*. Campinas, SP: Editora Unicamp.
- Bhandari, B. R., Datta, N., Crooks, R., Howes, T., & Rigby, S. (1997). A semi-empirical approach to optimise the quantity of drying aids required to spray dry sugar-rich food. *Drying Technology*, 15(10), 2509-2525.
- Bhandari, B. R., Patel, K. C., & Chen, X. D. (2008). Spray drying of food materials – process and product characteristics. In X. D. Chen, & S. A. Mujumdar (Eds.), *Drying technologies in food processing* (p. 113-157). Oxford, UK: Blackwell Publishing Ltd.
- Chalamaiah, M., Dineshkumar, B., Hemalatha, R., & Jyothirma, Y. I. T. (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chemistry*, 135(4), 3020-3038.
- Cuq, B., Rondet, E., & Abecassis, J. (2011). Food powders engineering, between know-how and science: constraints, stakes and opportunities. *Powder Technology*, 208(2), 244-251.
- Dieterich, F., Boscolo, W. R., Pacheco, M. T. B., Silva, V. S. N., Gonçalves, G. S., & Vidotti, R. M. (2014). Development and characterization of protein hydrolysates originated from animal agro industrial byproducts. *Journal of Dairy, Veterinary and Animal Research*, 1(2), 1-7.
- Georgetti, S. R., Casagrande, R., Fernandes, S. C., Pereira, O. W., & Vieira, F. M. (2008). Spray drying of the soy bean extracts: effects on chemical properties and antioxidant activity. *Food Science Technology*, 41(8), 1521-1527.
- Gharsallaoui, A., Surel, L., Roudaut, G., Chambin, O., & Volley, A. (2007). Applications of spray drying in microencapsulation of food ingredients: an overview. *Food Research International*, 40(9), 1107-1121.
- Guadarrama-Lezama, A. Y., Dorantes-Alvarez, L., Jaramillo-Flores, M. E., Pérez-Alonso, C., Nirajan, K., Gutiérrez-López, G. F., & Alamilla-Beltrán, L. (2012). Preparation and characterization of non-aqueous extracts from chilli (*Capsicum annum* L.) and their microencapsulates obtained by spray-drying. *Journal of Food Engineering*, 112(1-2), 29-37.
- Ishwarya, S. P., Anandharamkrishnan, C., & Stapley, A. G. F. (2015). Spray-freeze-drying: a novel process for the drying of foods and bioproducts. *Trends in Food Science and Technology*, 41(2), 161-181.
- Kha, T. C., Nguyen, N. H., & Roach, P. D. (2010). Effects of spray dryer conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. *Journal of Food Engineering*, 98(3), 385-392.
- Keshani, S., Daud, W. R. W., Nourouzi, M. M., Namvar, F., & Ghasemi, M. (2015). Spray drying: an overview on wall deposition, process and modeling. *Journal of Food Engineering*, 146(1), 152-162.
- Kim, E. H. J., Chen, D. X., & Pearce, D. (2009). Surface composition of industrial spray-dried milk powders. *Journal of Food Engineering*, 94(2), 169-181.
- Krishnan, S., Bhosale, R., & Singhal, R. S. (2005). Microencapsulation of *Cardamom oleoresin*: evaluations of blends of gum arabic, maltodextrin and a modified starch as wall materials. *Carbohydrate Polymers*, 61(1), 95-102.
- Kurozawa, L. E., Park, K. J., & Hubinger, D. M. (2009). Effect of carriers agent on the physicochemical properties of a spray dried chicken meat protein hydrolysate. *Journal of Food Engineering*, 94(3-4), 326-333.
- Madene, J., Jacquot, M., Scher, J., & Stéphane, D. (2006). Flavour encapsulation and controlled release – a review. *International Journal Food Science Technology*, 41(1), 1-21.
- Ogawa, M., & Maia, E. L. (1999). *Manual de pesca. Ciência e tecnologia do pescado*. São Paulo, SP: Editora Varela.
- Ozmen, L., & Langrish, T. (2003). An experimental investigation of the wall deposition of milk powder in a pilot-scale spray dryer. *Drying Technology*, 21(7), 1253-1272.
- Paini, M., Aliakbarian, B., Casazza, A. A., Lagazzo, A., Botter, R., & Perego, P. (2015). Microencapsulation of phenolic compounds from olive pomace using spray drying: a study of operative parameters. *Food Science and Technology*, 62(1), 177-185.
- Porras-Saavedra, J., Palacios-González, E., Lartundo-Rojas, L., Garibay-Febles, V., Yáñez-Fernández, J., Hernández-Sánchez, H., ... Alamilla-Beltrán, L.

- (2015). Microestrutural properties and distribution of components in microparticles obtained by spray-drying. *Journal of Food Engineering*, 152(1), 105-112.
- Rannou, C., Queveau, D., Beaumal, V., David-Briand, E., Le Borgne, C., Meynier, A., ... Loisel, C. (2015). Effect of spray-drying and storage conditions on the physical and functional properties on standard and n-3 enriched egg yolk powders. *Journal of Food Engineering*, 154(1), 58-68.
- Robert, P., Gorena, T., Romero, N., Sepulveda, E., Chavez, J., & Saenz, C. (2010). Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. *International Journal of Food Science & Technology*, 45(7), 1386-1394.
- Roos, Y. Solid and liquid states of lactose. (2009). *Advanced Dairy Chemistry, In: Advanced Dairy Chemistry*. Springer: New York, pp. 17-33.
- Roos, Y., & Karel, M. (1991). Applying states diagrams to food processing and development. *Food Technology*, 45(12), 66-71.
- Shrestha, A. K., Howes, T., Adhikari, B. P., & Bhandari, B. R. (2008). Spray drying of skim milk mixed with milk permeate: effect on drying behavior, physicochemical properties and storage stability of powder. *Drying Technology*, 26(2), 239-247.
- Silva, J. F. X., Ribeiro, K., Silva, J. F., Cahú, T. B., & Bezerra, R. S. (2014). Utilization of tilapia processing waste for the production of fish protein hydrolysate. *Animal Feed Science and Technology*, 196(1), 96-106.
- Simões, M. R., Ribeiro, C. F. A., Ribeiro, S. C. A., Park, K. J., & Murr, F. E. X. (2007). Composição físico-química, microbiológica e rendimento do filé de tilápia tailandesa (*Oreochromis niloticus*). *Ciência e Tecnologia de Alimentos*, 27(3), 608-613.
- Wang, Y., Lu, Z., & Lv, F. (2009). Study on microencapsulation of curcumin pigments by spray drying. *European Food Research and Technology*, 229(3), 391-396.
- Woo, M. W., Daud, W. R. W., Tasirin, S. M., & Talib, M. Z. M. (2009). Controlling food powder deposition in spray driers: wall surface energy manipulation as an alternative. *Journal of Food Engineering*, 94(2), 192-198.

Received on May 4, 2015.

Accepted on October 7, 2015.

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