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Evaluation of process parameters in the industrial scale production of fish nuggets

Avaliação de parâmetros de processo na produção de empanados de peixe em escala industrial

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Abstract

This work reports the use of experimental design for the assessment of the effects of process parameters on the production of fish nuggets in an industrial scale environment. The effect of independent factors on the physicochemical and microbiological parameters was investigated through a full 24 experimental design. The studied factors included the temperature of fish fillet and pulp in the mixer, the temperature of the added fat, the temperature of water and the ratio of protein extraction time to emulsion time. The physicochemical analyses showed that the higher temperature of the pulp and fillet of fish, the lower the protein in the final product. Microbiological analyses revealed that the counting of *Staphylococcus coagulase positive*, total and thermo-tolerant coliforms were in accordance with the current legislation.

Keywords: fish nuggets; experimental design; process parameters.

Resumo

Este trabalho teve como objetivo o uso da metodologia de planejamento de experimentos para avaliação dos efeitos de parâmetros de processo na produção de empanados de peixe em escala industrial. Os efeitos destes parâmetros nas características físico-químicas e microbiológicas do produto final foram avaliados através de um planejamento completo 24. As variáveis independentes estudadas foram temperaturas do filé e da polpa, temperatura da gordura e da água adicionadas na formulação do produto, bem como razão entre tempo de extração da proteína e tempo de emulsão. As análises físico-químicas mostraram que maiores temperaturas de adição de polpa e filé de peixe resultaram em uma menor concentração de proteína no produto final. As análises microbiológicas realizadas resultaram em contagens de *Staphylococcus coagulase positiva*, totais e termotolerantes de acordo com a legislação vigente.

Palavras-chave: empanados de peixe; planejamento de experimentos; parâmetros de processo.

1 Introduction

Fish meat has excellent nutritional value, being rich in proteins, vitamins and unsaturated (w-3) fatty acids (GERMANO; OLIVEIRA; GERMANO, 1993; FRANCO; LANDGRAF, 1996). Fish and mechanically separated meat (MSM) are important sources of proteins; minerals, mainly calcium and phosphorus; and vitamins A, D and B, which make them products of high nutritional value (HALL, 1992; SU et al., 2003; CASTRO et al., 2004).

Tilapia (*Oreochromis niloticus*) is the second fish species in terms of production in the whole world (JORY; ALCESTE; CABRERA, 2000). The main exporters of tilapia are South American countries, such as Costa Rica, Ecuador and Honduras (JORY; ALCESTE; CABRERA, 2000; REDMAYNE, 2000).

Considering the tendency of increasing demand for food products, mainly those having proteins of high nutritional values, the use of fish proteins, especially from species of low commercial value or from by-products, seems to be a promising and interesting alternative for both consumers and food market.

In this context, the main objective of this work was to evaluate some process parameters, in the industrial scale

environment, for the production of a fish industrialized derivative. For this purpose, it was adopted an experimental design towards assessing physicochemical and microbiological characteristics of the final product.

2 Materials and methods

Fillets of tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*), and mechanically separated meat (MSM) of both fish species were kindly donated by 'Cardume Indústria e Comércio de Peixes Ltda' (Chapecó, SC, Brazil). The ingredients used for product formulation were the following: soy hydrogenated lipid (COAMO), soy texturized protein (DUPLOGEL MF MARSUL), soy concentrated protein (PROTIMARTI GV AP MARSUL), carrageen (GENU IBRACK), salt and spices (COMPACTO 2172; IBRACK), water, whole milk and thickener for nuggets (C-B 420; BREMIL).

2.1 Nuggets production

The fillets and MSM samples were stored at -14 °C (± 1 °C). Before nuggets production, the raw materials were removed

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from the freezing chambers and kept at 6 °C until reaching the temperatures established for the experimental design. The fillets, MSM, additives and other ingredients were weighed taking into account the product formulation previously established by the industry.

The raw materials were ground in industrial mill (3 mm diameter) twice. Before grinding, the soy texturized protein (flakes) was hydrated for 12 hours in filtered water at 8.5 °C (mass ratio of 1.3:4). The raw materials were mixed following a pre-established order defined for nuggets production, as shown in Table 1. All nugget samples were collected after mixing the raw materials.

The effect of some process variables were evaluated through a full 2⁴ experimental design with triplicate measurements at the central point, thus resulting in a total of 19 experiments. All experiments were performed in the industrial unit. The independent variables evaluated were temperature of fillet and MSM (-6 °C to -2 °C); temperature of fat (10 °C to 14 °C); water temperature (6 °C to 10 °C); and ratio of extraction time/emulsion time (4/12 to 8/14). After processing, all nugget samples were evaluated in terms of physicochemical and microbiological analyses.

2.2 Analytical methods

Protein, lipids, ash and pH determinations

The protein content was determined by Kjeldhal method (VELP Scientifica, model VDK126A). The lipid concentration was determined by organic solvent extraction (Soxhlet). The ash content was evaluated using an oven at 550 °C (Brasimet, model K, 1,300 °C). The pH values were determined using a pH-meter (Analyser, model pH300). All the methodologies employed followed the standards established by the Brazilian Health Minister (BRASIL, 2005).

Water activity (a_w) determination

The a_w values were determined using the Aqualab CX-2 Water Activity-System, calibrated with deionized water and NaCl solution (18.5%).

Table 1. Sequence of ingredients addition in the fish nuggets production.

Ingredients	Mixing time (minutes)
Fillet and MSM	1
Salt	1
Water	1
Additives	1
1/3 of total fat	2
Soy protein (flakes)	3
Soy protein (powder)	2
2/3 of total fat	3
Powdered milk	2

Moisture content

The moisture content was determined by the method of desiccation in oven with air circulation (model FANEM 320-SE) at 105 °C, as described by IAL (INSTITUTO..., 1985).

Color evaluation

The CIE coordinates L^* (lightness), a^* (red-green), and b^* (yellow-blue) of fish nuggets were determined using a Colorimeter Minolta CR400 (Konica Minolta). Three readings were performed for each replicate to obtain uniform color measurements.

2.3 Microbiological methods

About 25 g of the product were transferred to a sterile stomacher bag and 225 mL of sterile 0.1% peptone water (Oxoid) were added and mixed for 1 minute in a Stomacher machine (ITR mod. MK 1204). Serial decimal dilutions in 0.1% peptone water were prepared and 1 mL or 0.1 mL samples of appropriate dilutions were poured or spread in duplicate. Analyses were carried out according to APHA (AMERICAN..., 1992): (a) *Staphylococcus* coagulase positive on Baird-Parker medium (Oxoid) with egg yolk tellurite emulsion (Oxoid) incubated at 37 °C for 24-48 hours; (b) total coliforms on Brilliant Green Bile (2%) Broth (Oxoid) after 48 hours at 37 °C (MPN method); and (c) thermo-tolerant coliforms on EC broth (Oxoid) incubated at 42 °C for 24 hours (MPN method).

2.4 Statistical analysis

The experimental data were analyzed through the experimental design methodology and analysis of variance followed by Tukey's test ($p < 0.05$), using the software Statistica 5.0 (MONTGOMERY, 1991).

3 Results and discussion

3.1 Physicochemical analyses

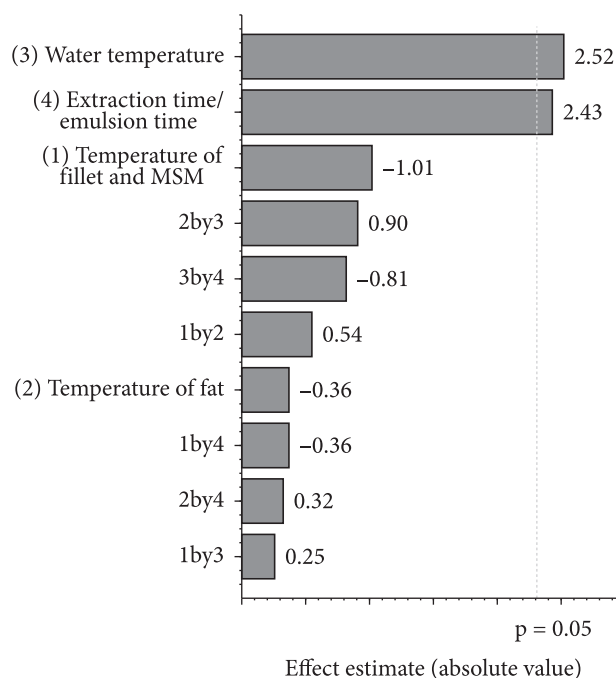
Table 2 presents the results obtained from the physicochemical analyses. As it can be seen in this table, fat content values varied from 4.0 to 14.0% for experiments 1 and 4, respectively. The lower value (experiment 1) corresponds to the lowest ratio of protein extraction time to emulsion time and also to the lowest water temperature (6 °C). These two factors hampered the extraction of myofibrillar proteins, leading to poor emulsion formation. The highest fat content (14.0%) may be associated with the higher levels of temperature of the raw material (fillet, MSM) and fat. The high viscosity of fat can favor the formation of lower particles that require longer times to be reduced to very small particles.

Figure 1 presents the Pareto chart showing the effect of the studied variables on the fat content of the produced fish nuggets. One can observe that the water temperature and the ratio of protein extraction time to emulsion time had a significant effect on the fat content of fish nuggets. The effect of these variables

Table 2. Physicochemical analyses in the fish nuggets product in terms of experimental conditions established in the experimental design.

Run	Fat (%)	Protein (%)	Ash (%)	pH	a _w	Moisture (%)	L*	a*	b*
1	6.1	18.7	2.2	6.1	0.99	71.3	69.7	7.5	15.8
2	7.1	16.0	3.2	6.5	0.99	67.1	69.0	7.1	16.3
3	6.9	18.3	3.0	6.1	0.99	64.3	63.6	8.4	17.9
4	4.0	17.3	3.2	6.4	0.98	63.9	68.5	8.4	15.4
5	9.8	17.2	1.9	6.9	0.99	65.6	62.6	8.3	13.0
6	10.1	14.7	3.1	6.9	0.99	65.6	64.8	5.8	12.9
7	10.2	15.8	2.1	6.6	1.00	66.5	61.0	7.0	13.3
8	8.9	15.4	3.7	6.8	0.99	63.2	63.9	6.8	13.0
9	13.1	16.0	3.1	6.4	0.99	65.1	67.5	8.9	17.3
10	7.9	15.3	3.2	6.4	0.99	63.8	68.2	8.1	16.8
11	8.1	17.5	2.6	6.6	0.99	65.9	59.9	6.1	12.9
12	9.5	16.6	2.3	6.8	0.99	66.3	62.3	6.1	11.8
13	12.3	18.1	2.6	6.6	1.00	65.5	62.9	5.8	13.1
14	9.2	16.9	2.1	6.3	0.99	66.1	52.6	7.4	13.0
15	12.0	17.0	3.0	6.3	0.99	65.3	68.5	7.5	16.5
16	12.7	17.7	3.1	6.3	0.99	66.3	67.7	7.1	17.7
17	6.4	17.4	2.9	6.2	0.98	70.7	70.0	5.0	17.5
18	5.4	17.1	2.6	6.2	0.98	67.9	68.2	5.6	17.9
19	9.5	17.5	1.9	6.4	0.99	66.9	51.8	8.7	12.4

L*: lightness; a*: red color; b*: yellow color.

**Figure 1.** Pareto chart with the estimated effect (absolute value) of variables evaluated in the full 2⁴ experimental design in terms of fat content.

was positive ($p < 0.05$), indicating that an increase in these variables lead to higher fat content.

Peixoto, Sousa and Mota (2002) evaluated the influence of fat content on fish sausages and fish nuggets and showed that values of 1.7% and from 6.1% to 14.0% were observed for sausages and nuggets, respectively. Flores et al. (2002)

reported a fat content of 6.1% for tuna fillets cooked at 68 °C and stored at 5 °C. Echarte et al. (2003) evaluated the nutritional composition of fish pate and reported values of 26.4%, 16.1% and 13.7%, for salmon, anchovy and tuna fillets, respectively. Also, Aquerreta et al. (2002) found fat contents of 10.0%, 28.9% and 26.2%, respectively for tuna, salmon and anchovy. The differences obtained may be explained in terms of the different amounts of raw materials (fish and pulp) and other additives used in the formulation.

Concerning the determination of protein content, the values varied from 14.7% to 18.7% in experiments 6 and 17, respectively. From Table 2, one can observe that higher protein contents (18.1% to 18.7%) were obtained when lower temperatures were employed. Experiments 6, 8 and 10 presented the lowest protein contents (14.7% to 15.4%), probably caused by protein denaturation that occurred during protein extraction and emulsion time, favored by the high temperatures used in these experiments.

Figure 2 presents the Pareto chart depicting the effect of the investigated variables on protein content. From this figure, it can be observed that temperature exerted a negative effect on protein content. This result is in agreement with the literature, which reports a reduction in protein solubility in carps with increasing temperature (SAEKI; INOUE, 1997). This fact seems to be related to protein denaturation caused by physical agents (heat, irradiation, ultraviolet) and chemicals (guanidine chloride, urea, organic solvents, detergents and extreme pH conditions) (SAEKI; INOUE, 1997). The myofibrillars proteins of some fish species are thermal and chemically unstable compared to proteins obtained from other vertebrate animals (JONES, 1982). It should be mentioned that the presence of solubilized proteins during the sample preparation step, mainly after salt addition, is important to the formation of the interfacial proteic

film on drop surface of lipid, so as to stabilize the emulsion (KINSELLA, 1976).

The ash content varied from 1.9% to 3.7% and these values are related to the curing salts, NaCl and other additives, ingredients of the nuggets formulation. On the other hand, lower ash contents (~1.5%) were observed in fresh fish, hence corroborating the influence of the ingredients on the ash content (SURH et al., 2005).

Table 2 shows that the experiments that presented lower protein contents led to higher ash contents in the fish nugget (experiments 6, 8, and 10). Conversely, experiments resulting in lower protein contents presented higher ash content (experiments 6, 8 and 10).

The pH values were also evaluated and varied from 6.1 to 6.9 for all experimental conditions. It was observed that higher temperature of water, fillets and pulp led to higher pH values. This result is in agreement with the work of Scherer et al. (2004), where the *post mortem* was showed to be characterized by the action of endogenous enzymes and microorganisms that change the nitrogen compounds present in fish meat, enhancing the pH at high temperatures. Possibly, this is the reason why the temperature specified for conservation of fish and its products is about 5 °C (± 1 °C).

a_w values from 0.98 to 1.0 were obtained in all experiments. Pate made from tilapia (*O. niloticus*) presented a_w values between 0.989 and 0.995 (MINOZZO, 2005). a_w values are related to the

interactions between fish compounds and water (CHEFTEL; CHEFTEL, 1992). Raw materials temperature had a negative effect on a_w values.

It can be noted that experiments 1 and 17, which presented the highest protein contents, also presented the highest moisture contents (Table 2). The high moisture values occurred as a consequence of high protein contents present in fish nuggets. This parameter is directly related to the water retention in the product. Statistical analysis showed that the interaction between protein extraction and time/emulsion time and fat temperature had a positive effect on moisture content. Some authors referenced that the fat content can favor the formation of small fat particles, which allow more efficient protein extraction and emulsion formation (SAEKI; INOUE, 1997; SATO et al., 2003).

Lightness (L^*) values varied from 51.8 to 70.0. Experiments 1 and 17 presented the highest lightness (L^*) values (69.7 and 70.0, respectively). From Table 2, one can learn that there is a straight relationship between lightness values and protein content. The values of red color (a^*) varied from 8.9 and 5.0, for experiments 9 and 17, respectively.

3.2 Microbiological analysis

Table 3 presents the results obtained from the application of the experimental design related to the microbiological analysis. In all experiments, the total coliforms counting varied from 2.24 UFC.g⁻¹ to 3.62 UFC.g⁻¹. The lowest value was obtained for experiment 7 and the highest for run 8. Experiments number 10, 3.30 UFC.g⁻¹ and number 2, 3.41 UFC.g⁻¹, also presented

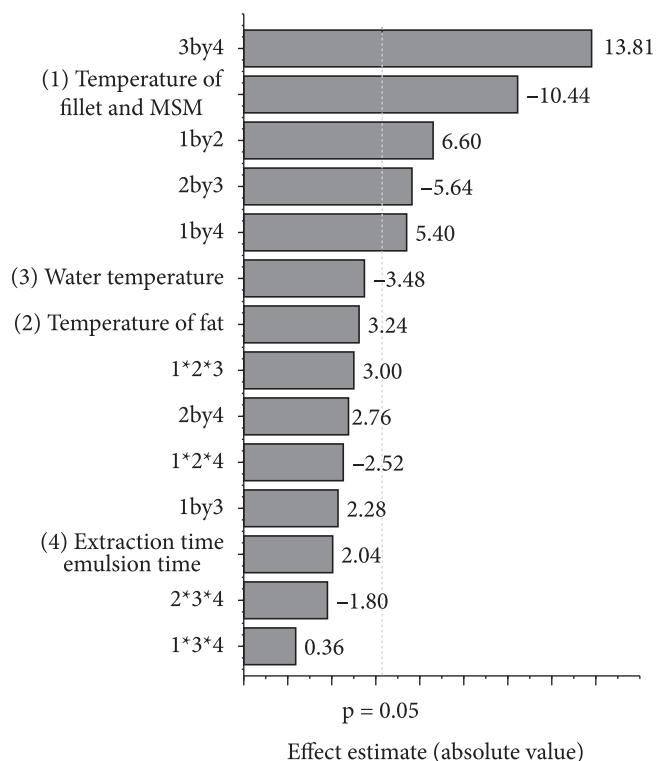


Figure 2. Pareto chart with the estimated effect (absolute value) of variables evaluated in the full 2^4 experimental design in terms of protein content.

Table 3. Microbiological analyses in the fish nuggets product in terms of experimental conditions established in the experimental design.

Run	Total coliforms* (UFC.g ⁻¹)	Fecal coliforms* (UFC.g ⁻¹)	<i>Staphylococcus coagulase</i> * (UFC.g ⁻¹)
1	2.88 ± 0.60 ^{a,b,c}	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a
2	3.41 ± 0.20 ^b	1.59 ± 0.13 ^{a,c}	1.00 ± 0.01 ^a
3	2.77 ± 0.59 ^{a,b,c}	1.00 ± 0.1 ^a	1.00 ± 0.01 ^a
4	3.29 ± 0.45 ^{a,d}	1.30 ± 0.35 ^{a,c}	1.00 ± 0.01 ^a
5	2.36 ± 0.21 ^c	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a
6	2.48 ± 0.02 ^{c,d}	1.00 ± 0.01 ^a	1.45 ± 0.10 ^b
7	2.24 ± 0.07 ^c	1.00 ± 0.01 ^a	1.50 ± 0.10 ^b
8	3.62 ± 0.41 ^a	2.39 ± 0.32 ^b	1.35 ± 0.01 ^b
9	2.30 ± 0.15 ^c	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a
10	3.30 ± 0.51 ^{a,b,d}	2.49 ± 0.34 ^b	1.39 ± 0.01 ^b
11	3.06 ± 0.13 ^{a,b,c}	1.00 ± 0.01 ^a	1.60 ± 0.01 ^b
12	2.89 ± 0.09 ^{a,b,c}	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a
13	3.13 ± 0.11 ^{a,b,c}	1.88 ± 0.46 ^{b,c}	1.39 ± 0.01 ^b
14	3.27 ± 0.22 ^a	1.94 ± 0.64 ^{b,c}	1.00 ± 0.01 ^a
15	3.48 ± 0.01 ^a	1.59 ± 0.13 ^{a,b}	1.30 ± 0.01 ^b
16	2.99 ± 0.78 ^{a,c,d}	1.18 ± 0.35 ^a	1.00 ± 0.01 ^a
17	3.51 ± 0.02 ^a	2.54 ± 0.28 ^b	1.00 ± 0.01 ^a
18	3.09 ± 0.16 ^{a,c,d}	1.00 ± 0.01 ^a	1.30 ± 0.01 ^b
19	3.23 ± 0.32 ^{a,d}	2.77 ± 0.27 ^b	1.45 ± 0.01 ^b

*UFC.g⁻¹ Unidade formadora de colônia g⁻¹; mean of 3 replications, equal letters do not differ statistically at 5% (Tukey test). Total coliforms in the raw material: fillet 2.21 and pulp 2.10.

high counting, probably due to the higher temperatures of raw materials (pulp and fillet) during product formulation. Other experiments that merit inspection, since high counting was obtained, are runs 13 and 14. These results are due to high water temperature and extraction time and emulsion adopted in these experiments. It may be interesting to highlight the fact that these results are beyond the limit proposed by the French legislation – 3.00 UFC.g⁻¹ (DANTAS; SILVA, 1982). The microbiological quality of catfish produced in the USA presented total counting from 1.30 UFC.g⁻¹ to 3.20 UFC.g⁻¹ (FERNANDES et al., 1997).

The fecal coliforms counting varied from 1.00 UFC.g⁻¹ to 2.77 UFC.g⁻¹ for all experiments performed. The differences observed may have resulted from manipulation, since the raw materials used in the processing step were obtained from fish caught in weirs, far away from any sewage (GONZÁLEZ-RODRIGUES et al., 2002). In a study performed by Peixoto, Sousa and Mota (2002), lower amounts of coliforms were found in fish sausages.

Table 3 reveals that the fecal coliforms counting in raw material (fillet and pulp) and in the final product (fish nugget) varied from 2.00 UFC.g⁻¹ to 2.77 UFC.g⁻¹. The statistical analysis of experimental data showed that no variable presented a significant effect on thermo-tolerant coliforms (45 °C), with their presence directly related to the processing. One should notice that the values obtained here are in agreement with the legislation. The best results in terms of thermo-tolerant coliforms were achieved in experiments 1, 3, 5, 7 and 9, corresponding to lower temperatures of raw materials. It can also be observed that better results were reached for lower values of water temperature (experiments 1, 3 and 9).

The *Staphylococcus* coagulase positive counting varied from 1.00 UFC.g⁻¹ to 1.60 UFC.g⁻¹ in all performed experiments. Experiments 7, 1.50 UFC.g⁻¹, and 11, 1.60 UFC.g⁻¹, presented higher *Staphylococcus* coagulase positive counting, such counting figures were favored by water temperature and fat used for nuggets formulation.

The ideal value of a_w for *S. aureus* is 0.86 and the optimum pH for its growth and toxin production is between 6.0 and 7.0; and the enterotoxin production is detectable at 4.00 UFC.g⁻¹ (GONZÁLEZ-RODRIGUES et al., 2002). The a_w and pH values in all experiments varied from 0.98 to 0.99 (Table 2) and 6.1 to 6.9, respectively. These values show that the nuggets presented ideal conditions for *Staphylococcus* coagulase positive growth. However, the highest counting obtained (1.60 UFC.g⁻¹) is lower than that necessary to enterotoxin production. Minozzo (2005) found *Staphylococcus* coagulase positive counting in tilapia fillets and pates lower than 2.00 UFC.g⁻¹.

4 Conclusions

The production of fish nuggets was carried out in an industrial unit allowing the identification of manufacturing parameters that affect the final product. Conventionally, the method adopted for determining optimal conditions in industrial processes the variation of one parameter while keeping others at a constant level. This is a time and cost ineffective method that also presents the disadvantage of not

including the interaction effects among its variables. Factorial design technique can overcome such drawbacks, but it has been seldom used to evaluate the effects of process parameters in the industrial environment. The physicochemical analyses performed in the final product showed that the higher the temperature of fish pulp and fillet, the lower the protein level in the final product. The microbiological analyses also led to *Staphylococcus* coagulase positive, total coliforms and thermo-tolerant coliforms counting in agreement with the legislation.

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