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Characterization of different techniques for obtaining minced fish from tilapia waste

Matheus Hernandes LEIRA[1]*, Ariane Flávia do NASCIMENTO[2], Felipe Ribeiro ALVES[3], Laura ORFAO[2], Yasmim Gonçalves LACERDA[2], Hortência Aparecida BOTELHO[4], Lucas REGHIM[5], Aline de Assis LAGO[6]

Abstract

The majority of tilapia farming is used for the production of fillets, resulting in the generation of waste that can be used to increase food stocks by producing minced fish. The aim of this study was to characterize and evaluate the viability of boiling and mechanical techniques for obtaining minced fish in relation to performance, physical-chemical and microbiological parameters and to reduce the waste generated in the process of filleting Nile tilapia. Based on performance outcomes, the mechanical technique proved to be 12% more efficient than the boiling one. The cost of the boiling technique was higher due to performance and operating costs. In both techniques microbiological alterations were not observed. Centesimal analysis results showed a difference in humidity between the techniques, which was expected due to loss of water during the boiling process, which possibly also affected protein levels. Over all, the use of a deboning machine allied lower operating cost, better performance, quality and practicality. The debone machine technique it's feasible considering the large volume of waste generated daily on fish farms, which requires a speedy and effective disposal. Further, makes possible obtaining a nutritional raw material of high added-value and contributing to the reduction of environmental impacts.

Keywords: chemical composition; environmental impact; mechanically deboned meat (MDM); microbiological evaluation; percentage yield.

Pratica Application: Waste control and reuse.

1 Introduction

The increase in waste production has been causing environmental impacts due to the inability of the environment to degrade it. However, due to the implementation of more stringent environmental laws, which prioritize environmental management, there is growing awareness of the harmful effects caused by the continued dumping of solid and liquid waste into the environment (Fiori et al., 2008). Thus, the use of fish processing residues to obtain new products can be effectively carried out to enable an increase in revenue and contribute to environmental conservation. Waste can basically be divided into two groups, one which can be exploited in animal / plant production and the other for use in human consumption. The first group consists of waste unsuitable for the development of value-added products intended for human consumption (entrails, scales, and skeleton including the head). These residues are usually discarded or used in the production of flour, oils, silage, and fish composts intended for animal feed and / or fertilizer.

Waste generated by fish-processing industry have greater added value when destined to human consumption. Thus, some alternatives like boiling and mechanically separating techniques are nowadays frequently used.

Boiling process occurs by moist heat. Mechanically separated meat from bones and wheels, known as "mechanically deboned meat" (MDM), is immediately frozen by quick processes (Pessatti, 2001). Also known as pulp, it is a product of high nutritional value, a nutritious ingredient of high calorific value, and also an excellent source of vitamins and minerals, mainly calcium, directed to the other prosthetic foods available in the market (Simões et al., 2004). The fish processing industries are already in operation in the new product based on a mechanically separated technique, being more usual in the separation of the filleting operation. A greater amount of money can be recovered this way, thus avoiding waste and providing greater profitability in production.

Therefore, the aim of this study was to characterize and evaluate the feasibility of boiling and mechanical techniques to obtain minced fish from filleting process residues of tilapia of the Nile in relation to percentage yield, physical, chemical, and microbiological parameters while seeking to reduce the environmental impact at the same time.

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[1] *Departamento de Medicina Veterinária, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil*
[2] *Programa de Pós-graduação em Ciência Animal, Departamento de Agronomia, Universidade José do Rosário Vellano – UNIFENAS, Alfenas, MG, Brasil*
[3] *Programa de Pós-graduação em Reprodução, Bem-Estar e Sanidade Animal, Departamento de Medicina Veterinária, Universidade José do Rosário Vellano – UNIFENAS, Alfenas, MG, Brasil*
[4] *Departamento de Zootecnia, Escola de Medicina Veterinária e Zootecnia – UFG, Goiânia, GO, Brasil*
[5] *Departamento de Medicina Veterinária, Centro Universitário do Sul de Minas – UNIS, Varginha, MG, Brasil*
[6] *Departamento de Zootecnia, Instituto Federal de Educação, Ciência e Tecnologia – IF Baiano, Santa Inês, BA, Brasil*
*Corresponding author: matheushernandes@uol.com.br

2 Material and methods

2.1 Obtaining minced fish from tilapia carcasses (Oreochromis niloticus)

The tilapia residues used in boiling technique in order to obtain minced fish were evaluated and processed in the Fish Farm Pontal, located in the city of Bom Sucesso - Minas Gerais - Brazil (21°01'59"S; 44°45'29"W).

The residues used in the deboning machine technique were acquired from the Fisheries Station of the Federal University of Lavras (UFLA) and transported for processing in the Research Laboratory of the Food Science Department.

2.2 Boiling method

Tilapias were taken from net cage, weighed on scales (C&F – Model C15 – n° 8273/99). The weight of 100 kg was obtained and fractioned in four replicates of 25 kg. After weighing, the fillets were removed without "V" cutting, and were once again weighed. With the removal of the fillets the weighing of the carcasses with head was carried out. After weighing, the carcasses were returned to the filleting room, where the head, tail, and dorsal fins were removed, leaving only the housing rib. The rib was removed with pliers and subsequently, went through a manual, internal cleansing process. With the removal and cleaning of the rib, the boiling process began. They remained for five minutes, which facilitated the removal of fishbone. After boiling, the whole fishbone was removed.

The residue processing was performed by two trained technicians. The process duration was recorded from the beginning of the rib removal process to the attainment of the final minced fish product.

2.3 Deboning machine method

During the processing of minced tilapia fish, 100 kg of carcass (four replications of 25 kg) without the fillet, head, fins and viscera, were measured out on a precision BL-3200 scale and cooled on ice to a temperature of about 4 °C. The carcasses were added to a *High Tech* HT 100C model deboning machine by means of an endless thread they were pressed with a tube of circular blades with 1mm microfissures, where the fishbones were separated from the meat. The yield of MDM was obtained according to the following formula (1):

$$R = \frac{(\text{minced fish weight/kg})}{(100 \text{ kg of carcass})} \times 100 \quad (1)$$

After processing, the minced fish was washed in 10% saline solution and pressed to remove excess water and salt, for preservation (Zaitsev et al., 1969). After the removal of the minced fish, the yield differences between the boiling and mechanical techniques as well as the centesimal and microbiological composition of the final product were analyzed.

2.4 Procedures for the analysis of the centesimal composition of the minced fish residues from tilapia processing

The centesimal analysis (humidity, crude protein, ethereal extract and mineral matter / ashes) of the minced fish obtained from tilapia residues using boiling and mechanical deboning techniques were performed in triplicate, according to the Association of Official Analytical Chemists (1997) recommendations.

2.5 Determination of humidity

The gravimetric method was used, where approximately 10 g of sample were added in porcelain capsules, whose weight was already known, and weighed on analytical scales. This method is based on the evaporation of the water present in the food in a temperature controlled oven at 105 °C for approximately 72 hours until consistent weight is achieved. After this, the capsules were removed from the oven, stored in desiccators until they reached room temperature, and weighed once again.

2.6 Determination of ethereal extract

The Soxhlet method was used, where approximately 2 g of each dried sample was weighed into analytical balance using filter paper cartridges and added to the ethyl ether within reboilers whose weight had already been ascertained. The reboilers were placed in the extractor tube for 3 hours. After this period, the cartridges containing the dry and defatted material were suspended for 30 minutes to drain excess solvent and the reboilers were oven dried at 105 °C for 12 hours where the ether was removed and then stored in desiccators, with only ethereal extract of the sample remaining at the end. For determination of lipids, the reboilers were weighed out of the sample containing kiln and compared to the initial weight of the empty reboilers.

2.7 Determination of mineral material (ash)

The gravimetric method was used, which relies on the determination of the weight loss of material subjected to heating at 550 °C, where approximately 1.5 g of dry and defatted matter was weighed in calcined crucibles. The material was incinerated on a stove over asbestos screens. After charring, the crucibles were transferred to the muffle at 550 °C, leaving them for about 12 hours, until a constant weight was obtained. After 1 hour in desiccators the crucibles were weighed again.

2.8 Determination of crude protein

The Microkejeldhal method was used for the determination of crude protein. Approximately 50 mg of dried and degreased sample was weighed on butter paper using an analytical balance and transferred (sample and paper) to Microkejeldhal tubes, to which 600 mg of potassium sulphate, 300 mg of copper sulfate, and 5 mL of sulfuric acid were added. The tubes containing the samples and the previously mentioned reagents were subjected to digestion in digestion blocks placed inside a hood for a period of 4 hours. The initial temperature of the digester block of 100 °C was gradually increased to 360 °C. The samples, after returning to room temperature, were coupled with the Microkejeldhal distillation apparatus. 25 mL of sodium hydroxide (50%) was

subsequently added. The distillate was received in 250 ml erlenmeyers containing 10 ml of saturated boric acid solution (containing 1% indicator solution: methyl bromo - red green). Distillation was carried out at up to 100 mL of distillate. The distillate was titrated using 0.02N sulfuric acid until it reached a red stain, obtaining the total nitrogen content in the dry and degreased sample. For transformation into crude protein content, the nitrogen content was multiplied by a factor of 6.25.

2.9 Microbiological analyzes

To verify the processing conditions, and hygiene in manipulation of tilapia minced fish, microbiological analyzes of the samples were carried out. The tests were undertaken at the Laboratory of Microbiology of the Federal University of Lavras - UFLA. After the production of the minced fish obtained from the tilapia filleting residue, 25 g of the product was aseptically removed for analysis. A method for total counting of heterocyclic aerobic mesophilic bacteria, detection and quantification of filamentous fungi and yeasts, as well as detection and quantification of total coliforms (35 °C) and thermotolerant coliforms (45 °C), coagulase positive *Staphylococcus* count and *Salmonella* spp was carried out according to the technique recommended by Silva et al. (2001).

2.10 Preparation of samples and serial dilutions

From the samples collected, aliquots of 25 g were removed and added to 225 mL of 0.85% saline solution, constituting a 10^{-1} dilution followed by successive decimal dilutions (10^{-2} to 10^{-5}).

2.11 Total aerobic count of mesophilic microorganisms on plaques

For the determination of aerobic mesophilic bacteria, the samples were homogenized and 25 mL were diluted in 225 mL of sterile physiological solution and decimal dilutions of 10^{-1} to 10^{-5} were performed. The Pour Plate technique was used, where a 1.0 mL aliquot was added to a 90mmm Petri dishe, zrht, and then 25 mL of Standard Agar for Plate Counting (PCA) was added. Plates were incubated at 35.5 °C for 24-48 hours.

2.12 Total count of filamentous fungi and yeasts

For the total count of filamentous fungi and yeasts, the surface scattering technique was used, where 0.1 mL of the dilutions were inoculated in Petri dishes containing Potato Dextrose Agar (PDA) and, with the help of Drigalsky's handle, were scattered on the surface of Hagar. Subsequently, the plates were incubated at 25 °C for 48 hours and a Colony Forming Units (CFU) count was carried out.

2.13 Coliform counts at 35 °C and 45 °C using the most probable number method

1.0 ml aliquots were extracted from each dilution and inoculated into tubes. The culture medium used was lauryl sulfate tryptase (LST) in series of 3 tubes for each sample in dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} containing inverted *Durhan's* tube.

All samples were incubated at 35.5 °C for 48 hours. After 48 hours it was observed if gas was present in the *Durhan* tube (presumptive test).

2.14 Experimental design

The experimental design was completely randomized, with 2 processes (boiling and mechanical techniques) with 4 replications. Statistical analyses were conducted with the R software program version 2.9.0 (R Development Core Team, 2010). Means were tested for significant differences using Student's t-test. The level of significance was $p < 0.05$.

3 Results and discussions

In this study, two techniques to obtaining minced fish from tilapia waste (head carcass, fins and viscera) were compared. When the boiling technique was used 42% yield of minced fish was obtained compared to 54% yield generated through the mechanical technique using the *High Tech HT* 100C deboning machine (Table 1).

MDM is the most frequent means of separating the meat from the fillet residues, whereby a greater amount of meat can be recovered, resulting in a higher fish yield.

During the filleting process of Nile Tilapia, approximately 65% of residues are generated, and parts of the carcasses can be used to produce MDM. However, the discards originated by wastes or by-products, if not handled properly, can turn into health and environmental issues. The MDM product is more economically feasible than filleting, since it presents an additional meat recovery between 10 and 20% (Neiva, 2006).

Based on this outcome, there was a 12% difference between the two techniques. Taking into account the value of a kg of the minced fish sold for US$ 3.10, every 100 kg processed had a positive difference of US$ 37.25 when the mechanical technique using the deboning machine was used.

According to Galan et al. (2006), the MDM yield after washing, calculated on the basis of carcass weight was 56.80%, consistent with the present study.

In the boiling technique to obtain minced fish, the time taken to process 100 kg of residue was approximately 24 hours, counting on the assistance of two trained technicians, where the whole process for the removal of the rib took 13 minutes and 3 seconds. Thus, a total of 26 minutes and 6 seconds to obtain 1,845 g of carcass rib, that is, 4,241.37 g/h was necessary.

The removal of the fishbone lasted 29 minutes and 12 seconds to obtain 775 g, that is, 1,552.58 g/h.

Table 1. Yield (kg of carcass x kg of residues) obtained by the two techniques for removal of minced fish from *Oreochromis Niloticus* (expressed in mean ± standard deviation).

Technique	Processed Volume/kg	Yield%*
Boiling	100	42 ± 2.2
Mechanical	100	54 ± 1.8

*Means are significantly different by Student's t-test; t = 0.00007.

However, by means of the mechanical technique (deboning machine) on average one hour was spent for the processing of 100 kg of residue, with the aid of a trained technician (Table 2).

In this case, the operational costs, taking into account time spent and the number of technicians required, becomes higher due the labor costs, where:

Labor time with expenses = salary × 0.56 / 65.72

Labor time with charges = US 262.87 × 0.56 / 65.72

Labor time with charges = US 2.25

With the boiling technique, processing 100 kg costs US$ 107.83, (24 hours × 2 technicians), which differs from the mechanical technique, where processing 100 kg costs US$ 2.25 (1 hour × 1 technician).

In terms of operating costs, evaluating the time spent and the number of technicians, the cost using the boiling technique becomes higher, with an additional cost in this methodology for each 100 kg processed of US 62.76, in other words, 21 kg of minced fish.

If it is considered the value of the yield minus operating costs, the overall cost for a yield of 33 kg of minced fish is US$ 96.60.

As for the result of the operational cost considering the time spent and the number of employees, the value when using the boiling method becomes 21% higher. However, considering the value minus operating costs, the expenses rise to 33%.

Table 3 shows the mean values obtained from the centesimal analysis (Humidity%, Crude Protein%, Fat%, Ash%) of the minced fish obtained using the two techniques (boiling and mechanical), in the total product. The results of the centesimal analysis of the meat showed a difference in humidity that can be understood as resulting from the loss of water during the boiling process, that can be the result of the high temperature, causing the loss of water, as was observed by Ferreira et al. (2007).

The temperature can modifying the levels and structure of the proteins of the product (Ferreira et al., 2007; Badiani et al., 2002; Potter & Hotchkiss, 1995; Rosa, 2003), as was observed in this study.

According to Galan et al. (2006), humidity, lipid, protein and ash contents were, respectively, 75.71%, 12.97%, 10.60% and 0.83%, results that mirrored those of the present study.

The hygienic-sanitary conditions of the tilapia minced fish obtained using the two techniques showed an absence of contamination according to the microbiological analyzes (Table 4).

Absence of Coagulase-positive *Staphylococcus* and *Salmonella* confirm that sanitary and hygienic procedures were correctly followed, from capture to raw material preparation. *Staphylococcus* is the microorganism responsible for approximately 45% of the world's toxinfections. *Salmonella* habitat is the intestinal tract and its presence indicates probable fecal contamination from human or animal sources. Fish caught in unpolluted waters are *Salmonella* free, because it is not part of the fish's natural microbiota. Their presence in this food usually comes from handling or contact with improperly sanitized surfaces. The presence of *Salmonella* is a sufficient reason for the sample to be discarded (Leitão, 1977).

The RDC 12/2001 of the Agency of Sanitary Surveillance establishes the value of 103 $CFUg^{-1}$ as limit of tolerance for coagulase positive *Staphylococcus aureus*, in cooled fresh fish, which indicates that the fillets of tilapia evaluated in the present study were presented within the legal standards for said parameter.

Although the Brazilian legislation does not establish a specific standard for counting thermotolerant coliforms in fresh fish, these can be considered quality indicators microorganisms,

Table 2. Analysis of the time spent in processing to obtain the minced fish from *Oreochromis Niloticus* using the two techniques (boiling and mechanical) in relation to the number of technicians used in the process.

Technique	Processed volume/kg	Processing time/hour	Number of professionals
Boil	100	24	2
Mechanical	100	1	1

Table 3. Mean values obtained from the centesimal analysis (Humidity%, Protein%, Fat%, Ash%) of minced fish from *Oreochromis Niloticus* obtained by two techniques (boiling and mechanical), in the whole matter.

Technique	Humidity	Ethereal extract	Protein	Ash
Boiling	64.00	17.22	11.94	3.67
Mechanical	72.64	12.73	14.49	1.00
t value	0.00024	0.00010	0.00100	0.00062

Table 4. Results of the microbiological analyzes of minced fish from *Oreochromis Niloticus* obtained by two techniques (boiling and mechanical).

Technique	Salmonella	Staphylococcus coagulase positiva	Total coliforms 35 °C	Thermotolerants coliforms 45 °C	Mesophilic aerobic
		($CFU.g^{-1}$)	($MPN.g^{-1}$)	($MPN.g^{-1}$)	($CFU.g^{-1}$)
Boiling	Absent	Absent	< 0.2	< 0.3	Absent
Mechanical	Absent	Absent	<0.3	< 0.3	Absent

CFU: Colony forming units; MPN: most probable number.

due to the presence of pathogenic bacteria (Rall et al., 2008), considered as a risk to consummers' health.

According to Agnese et al. (2001), values above 100 MPN / g for total coliforms in fish meat are related to poor hygiene in processing and sale of the product, which require quality control. This indicates that the products were processed, with good hygienic-sanitary standards, as all samples analyzed presented low levels.

Regarding the total mesophilic aerobe count, they were absent in all analyzed samples. In fresh products, the counting of these microorganisms indicates the effectiveness of the sanitary procedures used during their processing, handling and storage. The Brazilian legislation does not stipulate limits for this group of microorganisms; however, it is known that values above 10^6 CFU/g in freshwater fish are considered critical.

4 Conclusion

Based on the yield results, the use of mechanical technique to obtain the minced demonstrated a better performance in relation to the boiling technique. Taking into account operational costs, yield, and benefits in terms of quality control and practicality, the use of the deboning machine is more viable due to the large volume of waste generated daily in fish farms, whose owners must achieve a rapid and effective waste disposal and thus have the opportunity to obtain a nutritional raw material with high added value as well as aid in the reduction of environmental impacts.

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