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## RESEARCH ARTICLE

### A New Ready-To-Bake Seafood Meal Based on Pacific White Shrimp: Product Development, Cost Evaluation, Consumer Acceptability, and Shelf Life Stability

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#### Abstract:

#### Background:

Considering that consumers are increasingly demanding and attentive to a healthy diet, while also looking for food that is easy to prepare, the objective of this work was to develop a new value-added product with Pacific white shrimp.

#### Methods:

Fresh shrimps were headless, eviscerated, cut, washed, immersed in brine (10% NaCl + 5% sodium tripolyphosphate – STPP, 30 min), drained, weighed, immersed in sweet-sour sauce (15 min), drained, stuffed with cream cheese, weighed, vacuum packed, frozen (-30°C / 24h), and stored (-18°C). Microbiological, centesimal composition and physico-chemical analyzes were performed for fresh and stuffed shrimp. Confirmed the microbiological safety, samples were baked (180°C, 20 min) and sensorially evaluated. The shelf life study for the frozen stuffed shrimp was carried out during 180 days of storage (-18°C) and at each 45 days, samples were withdrawn for microbiological and physico-chemical analyzes.

#### Results:

All parameters analyzed were within the limits of Brazilian legislation, which demonstrated the initial shrimp freshness, and hygienic health care. The overall acceptance test was 8.72, the acceptability index of 96% and purchase intention of 82%. The final cost per piece was lower and viable. The nutritional value was in accordance to the literature for this shrimp, and the physico-chemical results during the shelf life evaluation were stable.

#### Conclusion:

The vacuum packaging, and freezing storage, associated with hygienic-sanitary care promoted the stability of microbiological assessment, and physical-chemical properties, and the overall quality and safety product for the consumer, which can be a new alternative for the shrimp industry.

**Keywords:** New product, Value-added, *Litopenaeus vannamei*, Quality, Shelf life, Shrimp, Frozen food.

#### Article History

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## 1. INTRODUCTION

The seafood is an important source of food for mankind, especially as a source of high biological value proteins; so, it is not difficult to understand in the last decades, the increase in world seafood consumption, period marked by the awareness of human needs, the cult of well-being, health, and food safety [1, 2]. Among the sea food species, crustaceans are the most

valued products in the international food market (both in value and production) and much appreciated in several countries [3], especially the Pacific white shrimp (*L. vannamei*) which has grown in global demand, accounting for 80% of world shrimp production [4]. Due to its commercial production in many countries, and its industrialization in full growth, today, the shrimp consumers can have access to various forms of presentation, e.g. fresh, frozen, breaded, salted, smoked and canned. In addition, there are few choices of ready-to-cook products, in small portions and very varied preparations, available throughout the year, which facilitate the consumer's life under the aspect of practicality and availability [5].

Nowadays consumers are increasingly demanding and

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attentive to a healthy diet, at the same time as looking for food that is easy to prepare and convenient [5, 6]. The shrimp is appreciated by the unique flavor and texture and can be served as appetizers or snacks. More importantly, in terms of human health, the high cholesterol content in shrimp is compensated by the low total lipids content and the predominance of polyunsaturated fatty acids [7]. In addition, the final presentation, in a more elaborate way [8], associated to the use of some natural spices, adding value to the product, can improve the final quality and make it more appreciated by consumers [9]. So, alternative forms of seafood processing, in addition to the usual ones (*i.e.* fresh, frozen, breaded or canned) such as new formulations, preprepared foods, and practical products should be offered.

Value aggregation in shrimp does not require the acquisition of large equipment and investments, as it can be represented by diversification in the presentation of products from local raw materials, such as coconut, cassava flour, and corn flour, low-cost products [10]. It is fundamental that the food professional glimpse a wonderful opportunity to develop researches for new products, suggesting innovative and profitable products, recognizing that the planning and the sustainability of shrimp are challenges that cannot be overlooked [10, 11]. Thus, the present study proposes to develop a new valued added product (stuffed and ready to bake) with the Pacific white shrimp (*Litopenaeus vannamei*) using low cost ingredients and simple technology.

## 2. MATERIALS AND METHODS

### 2.1. Raw Material

The Pacific white shrimp (*L. vannamei*) were supplied by the shrimp farm Aquadelta Agroindustrial S.A. (Mossoró, RN, Brazil), slaughtered by thermal shock in water and ice (1: 1), immediately treated with 3% sodium metabisulphite, transported in isothermal boxes to the Laboratory of Seafood Technology and Quality Control (LAPESC / UFERSA) where they were immediately washed, weighed, counted (number of pieces per kilogram), and kept under refrigeration (4°C) until the beginning of product development (time between slaughter and processing <2h).

### 2.2. Product Development

The whole fresh shrimps (Fig. 1) were headless (leaving all shell from the first segment to the telson), washed (5 ppm chlorinated water), weighed, gutted, cut in the dorsal region (butterfly cut), and immersed in brine (10% NaCl + 5% sodium tripolyphosphate STPP) for 30 min, drained, immersed in the sweet-sour sauce (garlic, finger pepper, ginger, sugar, salt, rice vinegar and starch) for 15 min (for flavor absorption), drained, filled (manually) with cream cheese (Philadelphia Cream Cheese, Kraft Foods Brazil), weighed (yield calculation), vacuum packed, identified, frozen in ultrafreezer (-30°C) and stored under freezer (-18°C). The cost analysis of the formulation of the developed product was made based on the current market value of each ingredient used (shrimp, sweet-sour sauce and cream cheese).

### 2.3. Yield Calculation

For each step during the product development, samples were weighed, to calculate the partial and overall yield of the final product. The methodology adopted was described by Gonçalves [12] according to the following Equations 1 and 2:

$$\text{Partial Yield (\%)} = [(Pf - Pi) / Pi] \times 100 \quad (1)$$

$$\text{Overall Yield (\%)} = [(Pi - Pf) / Pi] \times 100 \quad (2)$$

where: Pi = Initial weight (g); Pf = Final weight (g)

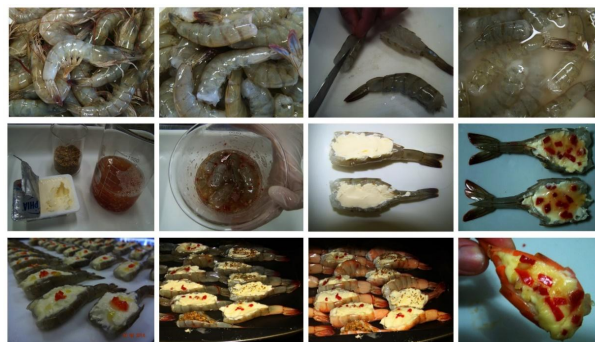


Fig. (1). Steps for the stuffed shrimp development.

### 2.4. Centesimal Composition and Physicochemical Evaluation

The centesimal composition (fresh shrimp and stuffed shrimp) was carried out through the analysis of moisture (Method 950.46), crude protein (Method 940.25), total lipids (Method 948.15), and ash (Method 938.08), according to AOAC's official methodology [13]. The pH, nitrogen of total volatile bases (TVBN) and trimethylamine (TMA) determination were performed according to the methodology recommended by Laboratório Nacional de Referência Animal (LANARA) [14]. All analyzes were done in triplicate.

### 2.5. Microbiological Analyzes

The microbiological analyzes were carried out in accordance with Agência Nacional de Vigilância Sanitária (ANVISA, Brazil) - Resolução da Diretoria Colegiada (RDC) 12/2001 [15] using the Brazilian official methodology (Instrução Normativa No. 62, de 26/08/2003) [16] The microorganisms analyzed for raw fresh shrimp were: total counts of mesophilic and psychrophilic bacteria, *Salmonella sp.*, and coagulase-positive staphylococci; and for stuffed shrimp: total count of mesophilic and psychrophilic bacteria, *Salmonella sp.*, coagulase positive staphylococci, and thermotolerant coliforms. All analyzes were performed at the Clinical and Immunological Analysis Center (CACIM, Mossoró, RN, Brazil).

### 2.6. Sensory Analysis

Confirmed the microbiological safety, samples baked in a preheated gas oven (180°C) for 20 min and submitted to the sensory panel evaluation. The sensory analysis was performed with 50 untrained panelists (32% men and 68% women,

ranging in age from 18 to 48 years) who received still warm stuffed shrimp samples along with the evaluation form (structured hedonic scale of 9 points ranging from “extremely dislike” to “I liked very much”), which consisted in the subjective manifestation of the product tasted, demonstrating whether such a product pleases or displeases, whether it is accepted or not [17, 18]. The acceptability index (IA) was calculated considering that 100% is the maximum score achieved by the product (stuffed shrimp) and the decision criterion for the IA to be well accepted is at least 70% [19, 20]. The consumer's buy intention was also evaluated using the Attitude Scale Test structured in five points that range from “I would certainly buy” to “I certainly would not buy” [17, 20].

### 2.7. Product Stability During Frozen Storage

The shelf life study of stuffed, frozen, vacuum-packed shrimp stored for 180 days at -18°C was monitored every 45 days by microbiological (total count of mesophilic and psychrophilic bacteria, thermotolerant coliforms, *Salmonella* sp., and coagulase positive *staphylococci*) and physico-chemical (pH, TVBN, TMA) analyzes.

### 2.8. Statistical Analysis

The means were compared using analysis of variance (ANOVA) and the effects were considered significant (by the Tukey test) when  $p \leq 0.05$ . All analyzes were performed by the XLSTAT Trial Version 2018.2 software (Addinsoft 1995-2018).

## 3. RESULTS AND DISCUSSION

### 3.1. Product Yield and Formulation Cost

The shrimp weighs at each step and the respective yields are shown in Table 1. For each 1,000g of shrimp, 43 units of shrimp were counted, which represents a shrimp with ~ 23g (per unit). After the headless shrimp step, the whole fresh shrimp lost 37.5% by weight, which represents a possibility of using this residue (head) for a new by-product development. After the brining step, 4.8% increase in weight was observed due to the incorporation of water by the presence of NaCl and

STPP (humectant food additive). The immersion in the sweet-sour sauce, promoted the increment of 17% in weight, representing besides the weight gain, the improvement of the sensorial quality (tasty) by the incorporation of ingredients which can increase consumer acceptance. The cream cheese addition promotes an increase in weight of 15.70%. The weight loss by the head removal step was not totally recovered by the increase in the final weight (resulting from filling with cream cheese), but to a certain extent, the losses (overall yield of 41.87% from headless shrimp) was minimized by the ingredients used. The same situation was also found during the developing a value-added product (e.g. butterfly breaded shrimp) with the same species [11].

The increase of 8.27% in the cost (comparing to the shrimp value) of the stuffed shrimp formulation (Table 2) was observed, and the final cost was R\$ 32.27 (~US\$ 7,70/kg raw fresh shrimp). However, when compared to ready-made dishes in restaurants and crustacean houses, this cost becomes lower than the average prices since dishes made with crustaceans reach the average value of R\$ 40.00 (US\$ 9.50) in local commerce. There are also no comparable products in the national market since most markets only sell shrimp in fresh/whole form or cooked or frozen.

Considering that this new product would be an ideal for sale per unit, and not per kilo, remembering the weight of shrimp of 23 g, the cost per piece would reach the value of R \$ 0.75 (or US \$ 0.18). The value added having the differential of being a “baked product” (less caloric than the breaded and fried ones) the stuffed shrimp becomes a viable and best option. When compared to fried, the grilled or baked shrimp is presented as the best nutritional option, because it contains fewer lipids, saturated fat, and can provide the desirable in polyunsaturated [21].

### 3.2. Nutritional and Physico-Chemical Analysis

The results of the centesimal composition (moisture, protein, lipid, and ash) and physico-chemical (pH, TVBN and TMA) analyzes of fresh and stuffed shrimp are presented in Table 3.

**Table 1. Partial yield (each step) and overall yield for the stuffed shrimp.**

| STEPS                              | Weight (g) | Yield (%) | Gain (+)   Loss (-) (%) |
|------------------------------------|------------|-----------|-------------------------|
| Whole fresh shrimp                 | 1,000      | -         | -                       |
| Headless shrimp                    | 625        | 62.50     | -37.50                  |
| Shrimp after brine step            | 655        | 104.80    | +4.80                   |
| Shrimp after sweet-sour sauce step | 766.33     | 117.00    | +17.00                  |
| Stuffed shrimp with cream cheese   | 886.66     | 115.70    | +15.70                  |
| Overall yield*                     | -          | 141.87    | +41.87                  |

\*from headless shrimp

**Table 2. Costs of the ingredients used for the stuffed shrimp development.**

| Ingredient         | Commercial quantity (g) | Price | Used quantity (g) | R\$ (US\$) |
|--------------------|-------------------------|-------|-------------------|------------|
| Fresh whole shrimp | 1,000                   | 24.00 | 1,000             | 24.00      |

(Table 2) contd....

| Ingredient         | Commercial quantity (g) | Price | Used quantity (g) | RS (US\$)               |
|--------------------|-------------------------|-------|-------------------|-------------------------|
| Cream cheese       | 150                     | 5.00  | 150               | 5.00                    |
| Saccharose (sugar) | 1,000                   | 1,60  | 200               | 0.32                    |
| Rice vinegar       | 750 mL                  | 3,00  | 150mL             | 0.60                    |
| Maize starch       | 200                     | 1,80  | 16                | 0.14                    |
| Peppers (10 units) | -                       | 1,50  | -                 | 1.50                    |
| Ginger             | 1,000                   | 13,00 | 16                | 0.21                    |
| Garlic             | 43                      | 0,50  | 43                | 0.50                    |
| <b>TOTAL</b>       |                         |       |                   | <b>32.27 (US\$ 7.7)</b> |

**Table 3. Results of centesimal and physico-chemical composition analysis of fresh raw shrimp and stuffed shrimp.**

| Parameters        | Fresh raw shrimp         |                          | Stuffed shrimp           |                        |
|-------------------|--------------------------|--------------------------|--------------------------|------------------------|
|                   | Wet basis (w.b.)         | Dry basis (d.b.)         | Wet basis (w.b.)         | Dry basis (d.b.)       |
| Moisture (%)      | 73 ± 1 <sup>a</sup>      | –                        | 66 ± 2 <sup>b</sup>      | –                      |
| Crude protein (%) | 6.7 ± 0.3 <sup>a</sup>   | 24 ± 1 <sup>*</sup>      | 5.2 ± 0.7 <sup>b</sup>   | 15 ± 2 <sup>†</sup>    |
| Total lipids (%)  | 0.20 ± 0.01 <sup>a</sup> | 0.70 ± 0.01 <sup>*</sup> | 4.7 ± 0.9 <sup>b</sup>   | 14 ± 3 <sup>†</sup>    |
| Ash (%)           | 0.60 ± 0.01 <sup>a</sup> | 2.30 ± 0.03 <sup>*</sup> | 0.40 ± 0.02 <sup>b</sup> | 1.2 ± 0.6 <sup>†</sup> |
| pH                | 7.7 ± 0.1 <sup>a</sup>   |                          | 6.40 ± 0.02 <sup>b</sup> |                        |
| TVBN (mg/100g)    | 9.6 ± 0.1 <sup>a</sup>   |                          | 9.7 ± 0.2 <sup>a</sup>   |                        |
| TMA (mg/100g)     | 1.8 ± 0.1 <sup>a</sup>   |                          | 1.9 ± 0.1 <sup>a</sup>   |                        |

The averages followed by the same letter (or symbol) on the line do not differ ( $p > 0.05$ ) from each other at a significance level of 5% (mean ± SD,  $n = 3$ ).

The moisture content for fresh shrimp (72.67%) corroborates with that found in the scientific literature for *L. vannamei* [22]: (72.8% organic farmed shrimp; 71.7% traditional farmed shrimp); however, lower than those found by other authors: 78% [11], 77.2% [23], 84.7% [24], 74.1% [25], and 77.9% [26]. The percentage of moisture content for stuffed shrimp (66.27%) was lower and statistically different ( $p < 0.05$ ) when compared with the fresh one due to the incorporation, mainly of cream cheese, which increased the lipid content. Unfortunately, it was not found in the literature products like the one developed in the present research for comparison.

The protein content observed for fresh (24%, d.b.) was higher statistically different ( $p < 0.05$ ) comparing to stuffed shrimp (15.4%, d.b.). Since cream cheese is more energetic than protein, this difference can be justified. The protein content was close to the white shrimp [22] from both organic (23.7%) and traditional farm (25.3%). Sriket *et al.* [23] evaluating the chemical composition of the *monodon* and *vannamei* species, observed lower protein content (19% and 22%, respectively). Gonçalves & Gomes [11] also found lower values for fresh shrimp (19%), and for breaded shrimp (13%).

The lipids content for fresh raw shrimp was 0.7% (d.b.), remaining between the range of values found in the literature, however, lower (0.2% w.b.) than those found in the literature 0.3% [25] and 0.33% [11]; while Chirol [22] found higher values for both *vannamei* in traditional (1.32%, w.b.) and organic farm (1.47%, w.b.). For the stuffed shrimp, the lipid content was higher (13.9%, d.b.) and statistically different ( $p < 0.05$ ) when comparing to the fresh shrimp. According to

Perry [27], dairy products, such as cream cheese, are rich in lipids, and according to the nutritional information table from the cream cheese manufacturer, the total fat (8.1 g a portion of 30 grams) is higher and can contribute to the increment of lipid content in stuffed shrimp.

The ash content found was 2.3% (d.b.) for fresh raw shrimp and in accordance to those found for pink shrimp *Parapenaeus longirostris* (2.3%) [24], and for *L. vannamei* from organic (2.1%, d.b.) [22], but higher than those from traditional farm (1.6%, d.b.) [22]. Araujo *et al.* [25] also found a lower ash content (1.5%, d.b.) than those found in the present study, however, it should be noted that these authors analyzed the meat without the exoskeleton. Gonçalves & Gomes [11] developing a value added product with *L. vannamei*, also found lower ash content (1.6% d.b. fresh shrimp and 1.2% d.b. breaded shrimp). This difference of percentages can be justified because of the presence of the exoskeleton (from the first segment follow up to the telson). In addition, the exoskeleton of crustaceans is composed basically of calcium carbonate, which can lead, therefore, to a higher ash content. For the stuffed shrimp, the ash content found was lower (1.24%, d.b.) and statistically different ( $p < 0.05$ ) compared to the fresh one, but closer to that found by Gonçalves & Gomes [11], for breaded shrimp.

The pH drops statistically ( $p < 0.05$ ) from raw fresh shrimp (7.7) to the stuffed shrimp (6.4) and can be associated to the increment of cream cheese, which according to Phadungath [28] states that the cream cheese pH is between 4.4 and 4.9. Despite the drop in pH, the results is in accordance with the pH limited as a parameter of freshness by Brazilian legislation [29] for raw fresh shrimp (7.85) and also, corroborated with the low values of TVBN and TMA (and without statically difference,  $p > 0.05$ , between fresh and stuffed shrimp), being also in accordance with the current legislation. It is emphasized that the shrimp was treated with sodium metabisulfite immediately after slaughter and with the food grade humectant additive (STPP) during the processing, which may result in a slight increase in pH value. In addition, the way in which shrimp is handled, from capture to consumer arrival, determines the intensity with which they present the enzymatic, oxidative and microbiological changes [2, 30]. The speed with which each of these changes develops depends on how the basic principles of conservation, hygiene, cold chain and capture methods were applied [2]. In the present study, all hygienic-sanitary care was done thinking mainly about the quality of the final product and the safety of the consumer.

### 3.3. Microbiological Analysis

No growth of microorganisms was observed in fresh raw and stuffed shrimp, demonstrating that all technological processes were carried out within the hygienic-sanitary standards since collection at the farm, guaranteeing the perfect cold chain along the transport, and handling, and the use of chlorinated water in each washing step. Honda [31] states that inadequate storage temperature during transportation, storage, and marketing contributes greatly to the multiplication of microorganisms present in shrimp. In addition, the use of gloves, masks by the manipulators, as well as the hygiene of utensils and workbench, and the use of sodium metabisulphite after the slaughter, and the brining process (NaCl + STPP) corroborated with the result and leave the product apt and safe for consumption (sensory analysis) and suitable for later storage (shelf life study).

### 3.4. Sensory Analysis

The average score in the Hedonic Scale for the stuffed shrimp was 8.72 which represents the scale between “I liked very much” and “I liked it extremely”. Eighty-six percent were attributed as “I liked extremely”, demonstrating an excellent acceptability, which corroborates with the Acceptability Index (AI) calculated of 96.9% (excellent acceptance of the product among the tasters). This result was higher when compared to those value-added products found in the literature: butterfly breaded shrimp (87.8%) [11], and smoked fish fillets (82.2%) [32]. On the other hand, Fernandes *et al.* [33] evaluating the cake made with shrimp cephalothorax meal showed medium acceptance, with average scores above 7.0 (“I liked moderately”). Despite the divergence between acceptance values found in the literature for several products, the product acceptance varies with living standards and cultural basis, and demonstrates the consumer's reaction to various aspects, such as price, age, hygiene, place of consumption, and not only if whether or not it pleased the product [33, 34]. In this sense, the purchase intention for stuffed shrimp corresponded to 82% (“Surely I would buy”), going to 14% (“Probably I would buy”) and 4% (“Maybe I would buy / maybe I would not buy”). The percentage of 82% (“surely I would buy”) indicated that the product was well accepted by consumers, suggesting that there is potential for marketing.

### 3.5. Product Stability (Shelf Life)

The microbiological analysis of food is a prerequisite for microbial safety management [35]. As expected, the results of the microbiological analyzes showed absence of the analyzed microorganisms (total mesophilic and psychophilic counts, *Salmonella sp.*, coagulase positive staphylococci and thermo-tolerant coliform) at the beginning of storage (Time Zero), and remained unchanged throughout the evaluated period, demonstrating that the hygienic-sanitary quality at the beginning of the study corroborated with its subsequent microbiological stability throughout the 180 days of storage.

The results of the physico-chemical analyze of the stuffed shrimp along the 180 days of storage (-18°C) are presented in Table 4. No significative difference ( $p > 0.05$ ) was observed in all parameters evaluated during the 180 days of storage. The

pH values for the stuffed shrimp remained stable throughout the storage period, and below the legal limits [29] for shrimp freshness (7.85), which corroborates with the results of the lower TVBN, TMA and microbiological analyzes; however, differed from the results of the shelf life of frozen shrimp (*Macrobrachium rosenbergii*) at different temperatures: the initial pH of 6.95 increased to 7.93 (-12°C) in 39 days and after to 7.85 (-15°C) in 74 days [36].

The results for TVBN and TMA remained stable over the storage period, and below the legal limits for freshness, *i.e.*,  $< 30 \text{ mg} / 100\text{g}$  and  $< 5 \text{ mg} / 100\text{g}$ , respectively. According to Soares & Gonçalves [30], the total volatile bases represent the set of nitrogenous bases, such as ammonia, trimethylamine, dimethylamine, monomethyl amine, putrescine, cadaverine and spermidine, normally present in deteriorated fish and, according to [37], the production of TBVN during seafood storage is a result of the action of tissue enzymes and microbiological activity. Hocaoglu [38] observed in frozen shrimp (-18°C) 12.33 mg/100g and Oliveira *et al.* [39] observed 14.57 mg/100g. Tsironi *et al.* [36] detected values of up to 25 mg/100g after eight months of frozen storage (-15°C), and Bono *et al.* [40] observed results up to 86 mg/100g after eight months of storage. As the initial microbiological quality of the stuffed shrimp was excellent (*i.e.* absent), no degradative chemical changes from microorganisms during frozen storage can be achieved, which corroborated the physicochemical stability of the product throughout the storage time.

**Table 4. Physico-chemical results of frozen stuffed shrimp stored at -18°C for 180 days.**

| Parameters   | Storage days |            |            |            |           |
|--|--------------|------------|------------|------------|-----------|
|  | 0            | 45         | 90         | 135        | 180       |
| pH   | 6.40 ± 0.02  | 6.3 ± 0.1  | 6.5 ± 0.2  | 6.5 ± 0.2  | 6.2 ± 0.1 |
| TVBN (mg/100g)   | 9.7 ± 0.2    | 10.1 ± 0.3 | 10.4 ± 0.3 | 10.0 ± 0.2 | 9.9 ± 0.2 |
| TMA (mg/100g)  | 1.9 ± 0.1    | 2 ± 0.1    | 1.8 ± 0.1  | 1.7 ± 0.1  | 1.8 ± 0.1 |
| T0 = time zero day; T45 = time 45 days; T90 = time 90 days; T135 = time 135 days; T180 = time 180 days; (mean ± SD, n = 3) |              |            |            |            |           |

## CONCLUSION

The incorporation of the sweet-sour sauce and the cream cheese gave the partial recovery (~70%) of the losses (yield) obtained during the heading process. The product developed in this study (stuffed shrimp ready-to-bake) is easy to prepare, quick and dispenses with the use of oil for frying. The product had a relatively low cost per unit (R\$ 0.75 or US\$ 0.18). The results of chemical composition demonstrated a good nutritional value for the product developed. The stuffed shrimp presented excellent acceptability (96.89%) and purchase (buy) intention (82%). The vacuum packaging, storage at -18°C for 180 days (6 months), associated with hygienic-sanitary care during processing promoted the physical-chemical and microbiological stability, and thus a safe and quality product for the consumer. The economic evaluation and a higher storage time for the shelf life are suggested to ensure the stability in more than 6 months, thus providing greater visibility and viability of this product for the industry.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

No animals/humans were used for studies that are the basis of this research.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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