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Protein Enrichment of Apple Pomace and Use in Feed for Nile Tilapia

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Abstract The purpose of this paper is to investigate the protein enrichment of apple pomace by *Gongronella butleri* through solid-state cultivation and addition of this material as feed for tilapia fry (*Oreochromis niloticus*). Factorial experimental design was used for the assessment of culture conditions to determine the effects of the source of nitrogen, initial moisture, and granulometry on the protein enrichment of apple pomace. During culture, the consumption of reducing sugars and the production of soluble protein were determined. The best conditions obtained were with urea (5% *w*/*w*), initial moisture of 70% and granulometry in the range from 0.85 to 1.70 mm, producing 19.63% of soluble protein. The fry submitted to the diet containing treated apple pomace presented an increase of 44% in body mass, demonstrating that apple pomace biotransformed may represent an excellent food supplement.

Keywords Protein enrichment · Apple pomace · Solid-state cultivation · Tilapia fry · Agro-industrial residues

Introduction

Algae, fungi, and bacteria are the main sources of microbial protein used as single-cell protein (SCP) [1]. *Aspergillus niger* [2, 3], *Saccharomyces cerevisiae* [2, 4], *Fusarium graminearum* [2], *Penicillium cyclopium* [5], and white fungi [6] are examples of

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microorganisms used worldwide as SCP. *Mucor*, *Rhizopus*, *Aspergillus*, and *Penicillium* are the most widely used filamentous fungi in processes involving solid-state fermentation (SSF), lending themselves to this application by their ability to grow in the absence of free water and their versatility of application and manipulation [1, 7]. Residues such as sugar cane bagasse [8, 9], wheat bran [10–12], and rice bran [13] are the main agro-industrial subproducts used in processes involving SSF.

In Brazil, the state of Santa Catarina, as the largest national producer of apples, producing approximately 800,000 tons/year [14], generates large quantities of solid residues, among which the main one is apple pomace, consisting of a mixture of skin, pulp, and seeds. This residue is derived from the production of concentrated apple juice, jam, and sweets [15, 16]. According to the company Yakult S. A., based in the municipality of Lages in the state of Santa Catarina, apple pomace is sold to the farmers of the region at a price of approximately US $1.50/ton. Currently, this residue is used as an organic compost in crop fields and as animal feed, although its protein content is low, and when destined for animal feed without biological treatment, it can give rise to the phenomenon known as alcoholemia, the result of fermenting the apple pomace in the rumen of the animal, with consequent production of alcohol, causing intoxication [17, 18].

This material has high moisture content, possesses insoluble carbohydrates such as cellulose, hemicellulose and lignin, reducing sugars such as glucose, fructose and sucrose, and is low in protein, essential amino acids, salts, and vitamin C [16, 17, 19–23]. Due to its levels of sugars, apple pomace may be an alternative substrate for the production of SCP, besides reducing the quantity of fibrous and mineral material. This solid residue cultivated by a filamentous fungus is transformed into a material rich in proteins by the conversion of sugars, nitrogenous material, fibers, and ashes present in the apple pomace in SCP and could be added to animal feed. The culture of microorganisms by means of the process of solid-state cultivation also enables the use of the cultured material together with the SCP, eliminating the need for downstream processes [23].

Many researchers, looking for added-value products, have proposed the use of apple pomace for the production of enzymes [15, 21, 24, 25], organic acids [26], protein-enriched feeds [16, 23, 27, 28], edible mushrooms [29, 30], ethanol [31–33], aroma compounds [34–36], natural antioxidants [37, 38], edible fibers [33, 38, 39], among many others.

With the rapid expansion of fish production over recent years, there has been a rise in the demand for alternative sources of proteins in diets to reduce feed costs, which represent from 50% to 80% of the total production cost [40, 41]. Agro-industrial residues are regarded as rich raw material from the point of view of production of single-cell protein; however, they are often not made use of and are released directly into the environment, where they have a serious impact. The biotechnological employment of these residues, as well as reducing the level of pollutants, leads to the production of a food with an excellent nutritional profile, in this way increasing its value [42]. Various sources of proteins and carbohydrates such as coffee pulp [43], cotton seed [44], *Moringa oleífera* Lam. [45], the yeast *Torulla* sp. [46], shrimp residues [47], and starch [48] have been used in the feeding of tilapia.

The addition of apple pomace cultivated with a filamentous fungus to diets for fish may be a way of giving added value to this agro-industrial residue, reducing environmental problems and at the same time reducing feed costs. Consequently, the objective of the present study was the treatment of apple pomace through protein enrichment by the process of solid-state cultivation and its addition as a food supplement to the diet of Nile tilapia fry (*Oreochromis niloticus*).

Materials and Methods

Microorganism

The microorganism used was the filamentous fungus *Gongronella butleri* CCT 4274, obtained from the Tropical Cultures Collection of the Fundação "André Tosello" (Campinas-São Paulo), maintained in potato dextrose agar at 4°C. The fungus was maintained in slants containing agar and Roux bottle incubated at 30°C for 7 days and later maintained at 4°C.

Culture Medium

The apple pomace, supplied by Yakult S. A. located in the municipality of Lages-SC and maintained at −20°C, was thawed and dried in an oven at 50°C for 24 h, triturated, and passed through a series of Tyler sieves (6 to 10 mesh for 0.85 to 1.70 mm and 10 to 20 mesh for 1.70 to 3.35 mm). The moisture content of the dried apple pomace was adjusted by the addition of distilled water, containing the nitrogen source (5% *w*/*v*) in solution (Table 1), then autoclaved at 121°C/15 min. The addition of water to the solution led to slight increase in the particle size. After cooling, it was inoculated with 4×10^6 spores per gram dry weight and transferred for the Raimbault columns (35×120 mm) under aseptic conditions. The incubation temperature was 30°C, aeration was 0.4 l min^{-1} per column, and the culture time was 7 days. The system used for the SSF is illustrated in Fig. 1.

Experimental Design

The following factors were analyzed: nitrogen source, initial moisture, and granulometry in the protein enrichment of apple pomace, and the effects were estimated by means of Statistica 6.0 Software. The statistical significance of differences between means was determined by Student's *t* tests. *p* values <0.05 were considered significant. The nitrogen source and the initial moisture in the culture medium were analyzed by 3^2 factorial experimental design (completely randomized design, two variables in three levels), as shown in Table 1. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2.

According to the applied design, 11 combinations were executed, and their observations were fitted to the following second-order polynomial model:

$$Z = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

where Z is the dependent variable (soluble protein); X_1 (nitrogen source) and X_2 (moisture) are the independent variables as mentioned above; b_0 is the regression coefficient at center point; b_1 and b_2 are linear coefficients; and b_{11} and b_{22} are quadratic coefficients.

Table 1 Levels of 3^2 full factorial experimental design.

Levels	Nitrogen source[a]	Moisture (%)
−1	Ammonium sulfate	50
0	Sodium nitrate	60
+1	Urea	70

[a] 5% (*w*/*v*) selected in previous works (data not showed)

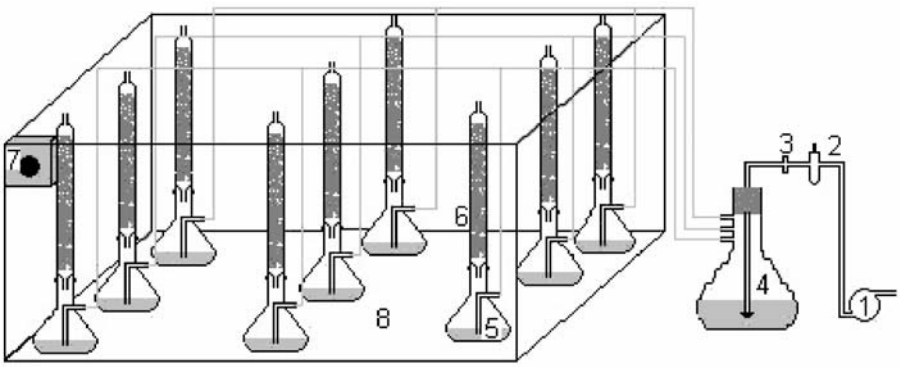

Fig. 1 System used for the solid-state cultivation. (*1*) air compressor, (*2*) pressure regulator, (*3*) air filter, (*4*) primary humidifier, (*5*) secondary humidifier, (*6*) Raimbault column, (*7*) thermostat, (*8*) water

After determining the best source of nitrogen for the protein enrichment of apple pomace, 2^2 factorial experimental design (two variables and two levels) was performed to determine the level of initial moisture and the granulometry of the apple pomace, which are given in Table 2.

After determining the best culture conditions (nitrogen source, initial moisture, and granulometry), the apple pomace was fermented by solid-state cultivation and added to diets for Nile tilapia fry in the proportion of 30% (*w*/*w*). All factorial experimental designs were accomplished in duplicate replications with two center points.

Analytical Measurements

Soluble Protein and Reducing Sugars

The concentration of soluble protein was measured according to the methodology proposed by Lowry et al. [49], and the content of reducing sugars was determined by the 3,5-dinitrosalicylic acid [50]. For the extraction of soluble protein and sugars, 5 g of the material was added to 100 ml volumetric flasks that had had their volume adjusted with distilled water. After incubation in a water bath at 60°C for 3 h, the material was filtered through qualitative filter papers, and the extract was submitted for the analyses for soluble protein and reducing sugars and compared to the standard curves of albumin and glucose, respectively.

Total Nitrogen

The total nitrogen in the apple pomace was quantified by the methodology of Kjeldahl [51].

Table 2 Levels of the 2^2 full factorial experimental design.

Levels	Moisture (%)	Granulometry (mm)
−1	60	0.86–1.70
+1	70	1.70–3.35

Ash and Moisture

The ashes and moisture content of the apple pomace was determined according to the methodology proposed by the AOAC [51].

Biometric Parameters

The biological tests were carried out in fry of tilapia (*O. niloticus*) supplied by the company MOGIVET, located in the municipality of Mogi das Cruzes, São Paulo. Four aquaria were used, each containing 30 fry. Two diets were analyzed, conventional feed (CF) as control and conventional feed supplemented with 30% (*w*/*w*) biologically treated apple pomace (CFP), with two aquaria being used for CF and two for CFP. The length, height, and mass of the fry were measured on the 1st, 15th, and 30th days, and the results compared by analysis of variance.

Results and Discussion

Composition of Apple Pomace

Table 3 presents the composition of the apple pomace used in the protein enrichment by solid-state cultivation.

From the analysis of the physico-chemical composition of the apple pomace (Table 3), it can be seen that the solid material had a moisture content of approximately 73%, was rich in reducing sugars (11.32%), and had low nitrogen and soluble protein contents and around 3% mineral material. As could be seen from Table 1, apple pomace contains high amounts of sugar and appears to be an excellent substrate for bioprocesses, being rich in different carbon sources. Besides, the pomace is very cheap and is abundantly available during the harvesting season. Thus, several microorganisms can use this apple residue as a substrate.

Effect of the Nitrogen Source

The results obtained for the 3^2 factorial design can be seen in Table 4. The responses examined—soluble protein and reducing sugars consumed—as well as the results obtained for the control (C) and for the apple pomace without culture (B) are also presented.

By examining Table 4, we can see that experiments 6 ($NaNO_3$) and 9 (urea), both with a moisture content of 70%, presented greater quantities of soluble protein, 14.88±0.19% and 15.22±0.51% respectively, increasing the protein content of the apple pomace 2.5 times when compared to the material without biological treatment (B). Experiments 5 ($NaNO_3$)

Table 3 Physico-chemical composition of apple pomace *in natura*.

Component	% (*w*/*w*)
Moisture	73.34±0.51
Total reducing sugars	11.32±0.34
Total nitrogen	0.62±0.02
Soluble protein	4.97±0.21
Ash	3.07±0.09

Table 4 Matrix containing the real variables and responses obtained for the 3^2 full factorial experimental design.

Experiment	Real variables		Responses	
	Nitrogen source	Moisture (%)	Soluble protein (%)	Reducing sugars consumed (%)
1	Sulfate	50	6.71±0.45	3.04±0.35
2	Sulfate	60	7.58±0.91	3.06±0.87
3	Sulfate	70	7.70±1.68	4.03±0.07
4	Nitrate	50	11.62±0.82	34.49±4.41
5	Nitrate	60	14.05±0.29	43.38±4.54
6	Nitrate	70	14.88±0.19	48.37±0.82
7	Urea	50	12.50±0.54	38.29±2.09
8	Urea	60	13.64±0.09	45.80±2.99
9	Urea	70	15.22±0.51	50.49±2.41
10	Nitrate	60	13.72±0.69	43.44±4.34
11	Nitrate	60	14.50±0.74	48.20±0.05
C	–	–	8.72±0.41	7.10±0.23
B	–	–	5.94±0.20	0.50±0.10

Experiments 10 and 11 are central points.

C Without addition of nitrogen and with initial moisture content of 73.3%; *B* without addition of nitrogen, without addition of inoculum and with initial moisture content of 73.3%.

and 8 (urea), employing a moisture content of 60%, returned similar results to each other at 14.05±0.29% and 13.64±0.09%. It can be seen that both urea and sodium nitrate markedly increased the quantity of soluble protein when initial moisture contents of 60% and 70% were used. The experiments employing ammonium sulfate (1, 2, and 3) resulted in low cell growth and consequently low soluble protein content and a low reduction in the sugar content.

The results presented in Table 4 show that the majority of the experiments presented significant decreases in the reducing sugars content, except the experiments that used ammonium sulfate as the nitrogen source. Experiments 6 and 9 reduced the quantity of reducing sugars by 48.37±0.82% and 50.49±2.41%, respectively. The reduction in the reducing sugar content, in addition to being an indicator of cell growth, is an important aspect due to the reduction in the organic matter, since when this material is used in fish food the content of sugars is directly related to the organic load added. The presence of a large quantity of organic material can lead to the complete extinction of the oxygen in the water, causing the disappearance of fish and other forms of aquatic life, increasing costs with the oxygenation system of lakes. A raised value for biochemical oxygen demand can indicate an increase in the microflora present and interfere in the balance of aquatic life, besides producing unpleasant tastes and odors [52].

Figure 2 presents the surface response for the 3^2 factorial experimental design for the soluble protein response.

From Fig. 2, it can be seen that there was a greater increase in the production of soluble protein when sodium nitrate (level 0) and urea (level +1) were used as the nitrogen source and the initial moisture content was 60% (level 0) and 70% (level +1).

The response surface analysis was conducted based on the response of soluble protein (SP). The model describing the response surface of soluble protein yield is as follows:

$$\mathrm{SP}(\%) = 13.929 + 3.229 \times X_1 - 3.076 \times X_1^2 + 1.161 \times X_2 - 0.438 \times X_2^2$$

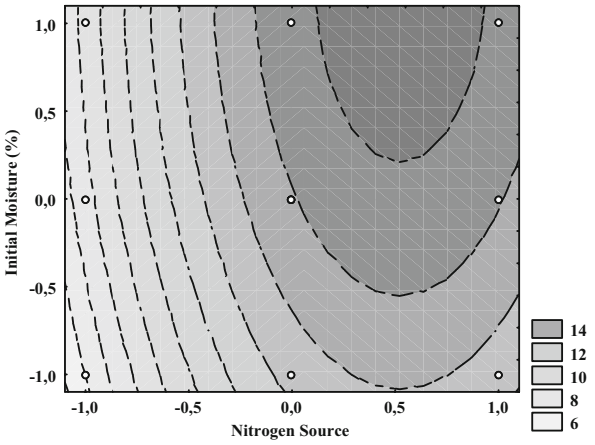

Fig. 2 Contour curves of 3^2 experimental factorial design for soluble protein (%) in the apple pomace

where SP is the soluble protein (%), X_1 is the nitrogen source, and X_2 is the initial moisture (%) The coefficient of determination R^2, which was found to be 0.92, indicates that 92% of the variability in the response can be explained by the model. This revealed that the equation is a suitable model to describe the response of the experiment.

It can be seen from Table 5 that the nitrogen source had a significant effect ($p<0.05$) on the soluble protein response, since when urea and sodium nitrate were used the level of soluble protein increased by an average of 6.45%. The same result was obtained for the initial moisture content of the medium ($p<0.05$), with the soluble protein content increasing by an average of 2.32% when the moisture level was 60% or 70%.

Zheng and Shetty [20] used apple pomace to produce a food rich in proteins by employing the fungus *Rhizopus oligosporus*. High moisture content results in an increase in mycelial production, consequently interfering in the transfer of oxygen. By contrast, low levels of moisture reduce fungal growth. Bisaria et al. [53] used the fungus *Pleurotus sajor-caju* in the bioconversion of rice straw and wheat straw. The authors observed that supplementation of the solid residue with urea and ammonium nitrate increased the bioconversion, the protein level being increased from 2.87% to 6.3% (*w*/*w*) with rice straw and from 3.1% to 7.5% (*w*/*w*) with wheat straw. Bhalla and Joshi [27] employed the fungi *Thrichoderma viride* and *A. niger* and the yeasts *S. cerevisiae* and *Candida utilis* combined for the protein enrichment of apple pomace in solid-state cultivation and submerged cultivation, with the yeasts using the sugars released by enzymatic action. Culture on a solid medium presented a 200% increase in the protein enrichment when the combination of *C. utilis* and *A. niger* was used.

Based on these results, urea was used as the nitrogen source due to its lower relative cost, and the study moved on to an assessment of moisture (60% and 70%) and granulometry (0.85 to 1.70 mm and 1.70 to 3.35 mm).

Table 5 Estimate of the effects and p values for 3^2 factorial experimental design for the soluble protein response.

	Effect	p value
Mean	11.5862	0.0000
Nitrogen source	6.4583	0.0000
Moisture	2.3233	0.0069
Nitrogen source × moisture	0.8625	0.1260

Table 6 Matrix containing the real variables and responses obtained for the 2^2 factorial experimental design.

Experiment	Real variables		Responses	
	Moisture (%)	Granulometry (mm)	Soluble Protein (%)	Reducing Sugars (%)
12	60	0.85–1.70	15.96±0.80	53.52±1.25
13	60	1.70–3.35	15.87±0.27	54.81±0.20
14	70	0.85–1.70	19.63±0.99	66.79±0.63
15	70	1.70–3.35	17.48±0.88	58.86±2.18

Effect of Moisture and Granulometry

Table 6 presents the matrix for the second 2^2 factorial experimental design with the real variables and the responses for soluble protein and reduction in the content of reducing sugars in the apple pomace.

From Table 6, it can be seen that assay 14 (70% and 0.85–1.70 mm) afforded a higher production of soluble protein, achieving a mean value of 19.63±0.99%, increasing the protein content of the apple pomace by a factor of 3.3 when compared to the untreated apple pomace (B). This increase in the quantity of soluble protein is related to the increase in the surface area of the cultivation bed. Zadrazil and Puniya [6] used white fungi to investigate the effect of particle size, employing sugar cane bagasse as the substrate through solid-state cultivation. The authors highlighted the fact that granulometry favors the conversion of the substrate to biomass, since the material is exposed with a greater surface area, a very important factor in cases that employ lingocellulosic materials. The estimate of the effects and p values for the factors initial moisture and granulometry of the culture medium can be seen in Table 7.

From Table 7, it can be seen that the variables present a significant effect ($p<0.05$) on soluble protein production. From the analysis of isolated effects, it can be seen that the moisture content of 70% increased the soluble protein content in the apple pomace by an average of 2.64%, while the variable granulometry increased on average 1.12% when granulometry in the range from 0.85 to 1.70 mm was used.

Del Bianchi et al. [7] report that the particle size of the substrate can be problematic because the substrates requires a granulometry that allows the circulation of air through the cultivation bed, as well as the dissipation of gases and heat produced during microbial growth. Albuquerque et al. [16] employed the filamentous fungus *R. oligosporus* in the protein enrichment of apple pomace, and their results showed that the soluble protein content increased fivefold in the best culture conditions. Other assays gave results in the range from 7% to 17% for the soluble protein content, with a maximum of 45% of reducing sugars consumed during 3 days of cultivation. Villas-Bôas et al. [18] cultured *C. utilis* and *Pleurotus ostreatus* on apple pomace. When the pomace was treated with *C. utilis* for 6 days, there was an increase of 100% in the level of crude protein, as well as a reduction of

Table 7 Estimate of the effects and p values for 2^2 factorial experimental design for the soluble protein response.

	Effect	p value
Mean	17.23	0.0000
Moisture	2.64	0.0019
Granulometry	−1.12	0.0374
Moisture × granulometry	−1.03	0.0531

up to 97% in the content of free sugars in the substrate. On the other hand, after treatment with *P. ostreatus*, the crude protein content was increased by 500% after 60 days of cultivation, although the material exhibited lower digestibility due to its high concentration of lignin.

The results obtained demonstrate that apple pomace is a substrate that could be used in the culture of *G. butleri* with a view to the protein enrichment of this material, considering the great potential for growth revealed by the total colonization of the substrate in 7 days. The increase of 3.2 times in the level of soluble proteins allows the use of this material as a food supplement in animal diets, partially replacing the feed with a view to reducing costs, reducing environmental problems, and adding value to this agro-industrial residue.

Biological Tests and Biometric Parameters

With the objective of testing the apple pomace treated biologically with *G. butleri* as a food supplement in animal feed, two diets were given to the fry of Nile tilapia. The diets used, over a period of 30 days, were conventional feed and conventional feed with the addition of 30% (*w*/*w*) treated apple pomace (Table 8). The indexes "0" (zero) and "30" (thirty) refer to the initial and final measurements for the responses length, height, and mass of the fry. Figure 3 presents the results obtained for the responses initial and final lengths of the fry submitted to the diets CF and CFP.

From Fig. 3, it can be seen that the fry submitted to the CF diet exhibited a length of 4.6 cm, while those submitted to the CFP diet reached 5.2 cm at the end of 30 days, increasing their length by 13%. Figure 4 illustrates the height of the fry fed over 30 days with feeds CF and CFP.

It can be seen that the fry submitted to the CF diet presented an average height of 1.30 cm, while those fry submitted to the CFP diet had a mean height of 1.45 cm, representing an increase of 11.5%. The results obtained for the length and height of the fry, in this case, are indicative of the main response, that is, the mass of the fry, since an increase in the responses length and height should result in an increase in mass. Figure 5 presents the responses for the mass of the fry submitted to diets CF and CFP.

Analyzing Fig. 5, it can be seen that the indicators of an increase in the length and height are confirmed when the results are reported as mass. The fry submitted to the CFP diet

Table 8 Composition of the conventional feed.

Material	Amount
Ingredients (% *w*/*w*)	
Fish flour	27.0
Wheat flour	13.0
Crushed maize	47.3
Soybean flour	11.0
Vegetable oil	1.7
Total	100.0
Composition	
Dry matter (% *w*/*w*)	87.9
Crude protein (% *w*/*w*)	28.0
Calcium (% *w*/*w*)	1.5
Phosphorus (% *w*/*w*)	1.5
Caloric value (kCal/kg)	2,933.0

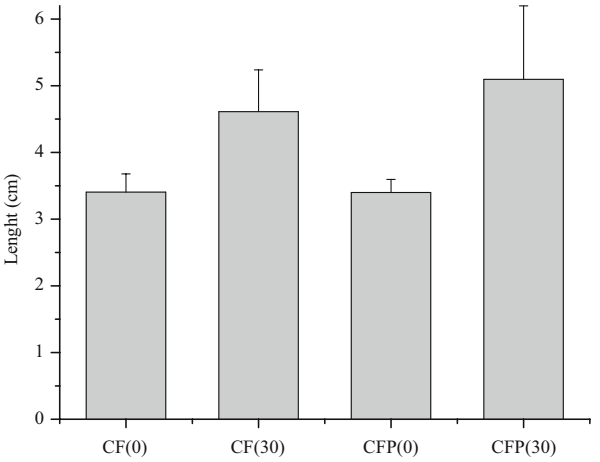

Fig. 3 Mean and standard deviation for the length of fry submitted to diets CF and CFP

reached on average 2.30 g, while the fry submitted to the CF diet reached on average 1.60 g. This difference of 0.70 g represents an increase of approximately 44%.

Table 9 presents the analysis of variance for the length, height, and mass of the fry in the two experiments. It can be seen that the comparison CF (0)–CFP (0) for each of the parameters did not reveal any significant differences ($p>0.05$), demonstrating that the fry used were homogeneous.

From an analysis of Table 9, it can be seen that the CF (30)–CFP (30) comparison, resulting in −0.149 cm, indicates that the CFP diet increased the height of the fry by an average of 0.149 cm, an increase of 11.5%, although this was not significant ($p>0.05$). It is also verified that the CF (30)–CFP (30) comparison presented significant differences ($p<0.05$), giving an increase of 0.506 cm in length, on average, indicating that the quantity of apple pomace added to diets has positive results on fry feeding. Analyzing the mass of fry can be seen that the comparison between CF (30) and CFP (30) returned a result of −0.705 ($p<0.001$), that is, using treatment CFP (30) resulted in an average increase of 0.705 g in the mass of the fry at the end of 30 days of feeding, which is suggested indirectly

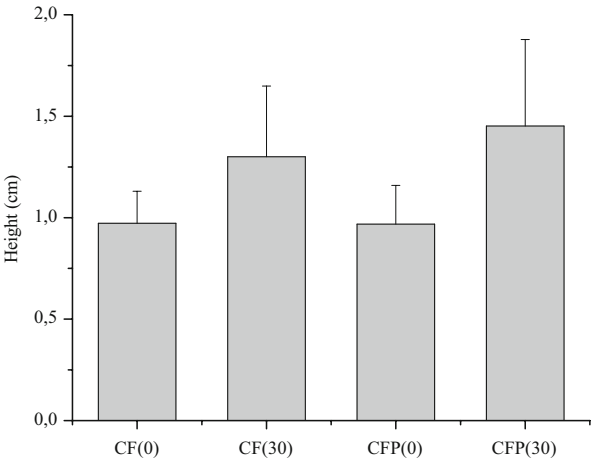

Fig. 4 Mean and standard deviation for the height of fry submitted to the CF and CFP diets

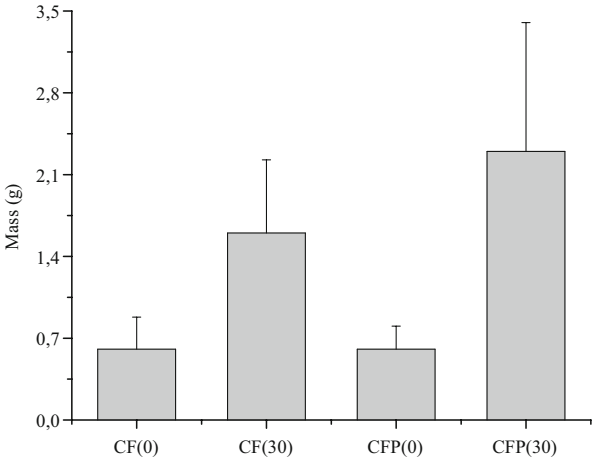

Fig. 5 Mean and standard deviation for the mass of fry submitted to the CF and CFP diets

by the increase in the length and the height of the fry, demonstrating that the apple pomace treated with *G. butleri* may be used as a partial feed substitute in diets for tilapia fry.

Baccarin and Pezzato [54] examined the effect of adding 10% of dried *S. cerevisiae* yeast as a vitamin supplement substitute in diets for Nile tilapia. The results did not show any significant influence on weight gain nor on the specific growth rate. Davies and Wareham [55] reported that in experiments carried out with *Tilapia mossambicus*, the inclusion of up to 10% of single-cell protein did not reduce production levels. In fact, higher levels result in a substantial reduction in the growth rate. Mahnken et al. [56] also did not find significant differences in weight gain using levels of up to 40% of alcohol yeast

Table 9 Difference between means and analysis of variance for the height, length, and mass of fry.

Comparison	Difference	*q*	*p* value
Height (cm)			
RC (0)–RC (30)	−0.332	6.302	<0.001
RC (0)–RCB (0)	0.001	0.062	>0.050
RC (0)–RCB (30)	−0.480	8.872	<0.001
RC (30)–RCB (0)	0.331	6.364	<0.001
RC (30)–RCB (30)	−0.148	2.647	>0.050
RCB (0)–RCB (30)	−0.479	8.932	<0.001
Length (cm)			
RC (0)–RC (30)	−1.196	9.299	<0.001
RC (0)–RCB (0)	0.021	0.392	>0.050
RC (0)–RCB (30)	−1.703	13.840	<0.001
RC (30)–RCB (0)	1.217	10.314	<0.001
RC (30)–RCB (30)	−0.507	4.036	<0.050
RCB (0)–RCB (30)	−1.724	14.218	<0.001
Mass (g)			
RC (0)–RC (30)	−1.000	8.314	<0.001
RC (0)–RCB (0)	0.014	0.145	>0.050
RC (0)–RCB (30)	−1.702	14.098	<0.001
RC (30)–RCB (0)	1.014	8.458	<0.001
RC (30)–RCB (30)	−0.702	5.848	<0.001
RCB (0)–RCB (30)	−1.716	14.239	<0.001

as a replacement for fish flour in the feed of rainbow trout. Dongmeza et al. [46], after alcohol production, also examined the addition of *M. oleífera* Lam. to diets for Nile tilapia fish with an initial mass of 4.9 to 5.2 g. From the fifth week of the experiment, a significant reduction ($p<0.05$) was found in the growth of the fish submitted to diets containing *M. oleífera*, while there were no apparent differences in the physico-chemical composition of the fish. Novoa et al. [47] examined the effects of the addition of *C. utilis* as a source of protein in the proportion of 25%, 30%, 35%, 40%, and 45% to tilapia diets. The diet containing 25% of *C. utilis* exhibited a higher conversion factor, and the diet containing 30% produced greater fish growth, although they did not present significant differences ($p>0.05$) when compared to the standard, showing that *C. utilis* at a proportion of up to 30% may be added to diets for tilapias.

These results show that the protein produced by the filamentous fungus *G. butleri* during protein enrichment of the apple pomace enables its use as a feed supplement in diets for fry, representing a useful destination for the apple pomace and other agro-industrial residues. Besides avoiding the dumping of this residue directly into the environment and thereby reducing the levels of environmental pollution, this application also reduces the costs of fish feed, which represent around 60% of the production cost. Another important factor is its applicability, that is, after its biological treatment, this material does not need to undergo numerous secondary operations, except for a reduction in the moisture content and particle size for storage and its addition to conventional feed.

Conclusion

The apple pomace was found to be a good substrate for the production of a protein food from the high content of soluble proteins and the rapid colonization of the substrate by the filamentous fungus *G. butleri*, suggesting a potential use of this agro-industrial residue. The highest concentration of soluble protein was obtained with an initial moisture content of 70%, urea at a concentration of 5% as the nitrogen source, and granulometry in the range of 0.85–1.70 mm, producing an increase of 3.3 times with respect to the soluble protein content. The apple pomace fermented with *G. butleri* incorporated into the diet of the fry showed itself to be a nutritive product and gave an increase of 13% in length, 11.5% in height, and 44% in body mass, besides being well accepted by the fry, all of which represented statistically significant differences at a level of 0.05 in relation to the control diet using conventional feed. These results demonstrate that the protein produced by the filamentous fungus *G. butleri* in the protein enrichment by solid-state cultivation of the apple pomace makes possible its use as an alimentary complement in diets for fry, also showing that even in the initial phases of development when the metabolic activity of the animals is more pronounced, due to the accelerated growth, the microbial protein is supplying the energy demand of the animals.

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