



Oxidative stability of tilapia feeds containing *Saccharomyces cerevisiae* and *Spirulina platensis*

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ABSTRACT: *The aim of this study was to evaluate the effect of the inclusion of microbial biomass on the oxidative rancidity of tilapia rations stored for 12 months. Treatments included a control diet and diets supplemented with either 0.01% vitamin E, 0.25 and 0.5% of *Saccharomyces cerevisiae* and 0.25 and 0.5% of *Spirulina platensis*. Experimental diets were stored in the dark inside plastic bags at room temperature (25 °C) for 12 months. The oxidative rancidity was measured as thiobarbituric acid reactive substances (TBARS). It was concluded that the inclusions of *Spirulina platensis* at 0.25% (1.734 ± 0.206) and 0.5% (1.629 ± 0.181) and *Saccharomyces cerevisiae* at 0.5% (1.459 ± 0.305) minimized the oxidative rancidity in comparison to control diet (2.843 ± 0.109) of Nile tilapia until 12 months of storage.*

Key words: biomass, lipid oxidation, ratio, TBARS.

Estabilidade oxidativa de rações de tilápia contendo *Saccharomyces cerevisiae* e *Spirulina platensis*

RESUMO: *O objetivo deste estudo foi avaliar o efeito da inclusão de biomassa microbiana sobre a rancidez oxidativa de rações de tilápia armazenadas por 12 meses. Os tratamentos incluíram uma dieta controle e dietas suplementadas com 0,01% de vitamina E, 0,01%, com 0,25 e 0,5% de *S. cerevisiae* e com 0,25 e 0,5% de *S. platensis*. As dietas experimentais foram armazenadas em sacos plásticos escuros, em temperatura ambiente (25 °C), durante 12 meses. A rancidez oxidativa foi mensurada pelas substâncias reativas ao ácido tiobarbitúrico (TBARS). Concluiu-se que as inclusões de 0,25% (1,734 ± 0,206) e 0,5% (1,629 ± 0,181) de *Spirulina platensis* e 0,5% (1,459 ± 0,305) de *Saccharomyces cerevisiae* reduziram a rancidez das dietas em comparação a dieta controle de tilápias-do-Nilo após 12 meses de armazenamento.*

Palavras-chave: biomassa, oxidação lipídica, ração, TBARS.

Oxidative rancidity or lipid oxidation refers to the lipid deterioration featured by the formation of chemicals that change food aroma and taste, decreasing palatability and nutritional value of diets (LAGUERRE et al., 2007). The process consists of a complex chain of reactions induced by oxygen in the presence of catalysts such as heat, free radicals, light, pigments and metallic ions (LAGUERRE et al., 2007). The first stage of the chain (initiation phase) occurs with the formation of free radicals under conditions favored by light and heat and is followed by the formation of primary products (propagation phase), especially peroxides and hydroperoxides. The latter, when exposed to prolonged oxidation conditions, give rise to side products like aldehydes, ketones, epoxides, hydroxy compounds, oligomers and polymers (termination phase). Malonaldehyde (MDA) is one of the main aldehydes originated in the process and

commonly used as a marker to quantify the reaction (BARRIUSO et al., 2013).

The stability of animal feed during storage is associated with lipid oxidation and so explains the use of antioxidants to slow down the process, increase the shelf life of the product, reduce the waste of raw materials and increase the range of fats that can be used in the formulation of diets, especially those of high energy (AKLAKUR, 2018).

Synthetic vitamin E may be used to prevent rancidity in fish feeds; although, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butyl hydroquinone (TBHQ), propyl gallate (PG) and ethoxyquinone (EQ) are the most widely used sources of additives for that purpose (AKLAKUR, 2018). However, these latter compounds may pose a risk to the health of animals and consumers since toxic and carcinogenic effects are assigned to them (PONCE-PALAFIX et al.,

2004), thus giving rise to a growing demand for antioxidants of natural origin. Two microbial biomasses (MB) with antioxidant potential stand out as fish feed ingredients. *Saccharomyces cerevisiae* yeast is used in the preparation of various products (e.g. ethanol) and marketed as a co-product at the end of processes. The yeast biomass contains proteins, vitamins such as E and complex B, enzymes, volatile fatty acids, chelated minerals, natural antibiotics and peptides (RAN et al., 2016). *Spirulina (Arthrospira) platensis* biomass contains proteins, vitamins, essential amino acids, minerals, essential fatty acids and pigments, such as carotenoids and phycocyanins, which impart it high nutritional value and antioxidant properties (MORIST et al., 2001; LI et al., 2003). The microalga is able to grow on different substrates, including industrial effluents and simple and low-cost culture media, making its production totally viable (ARRUDA et al., 2006).

The purpose of this study was to evaluate the effect of microbial biomasses on the oxidative rancidity of tilapia rations stored for 12 months.

The study adopted a completely randomized design with six treatments and three replicates. Treatments included a control basal diet (CD) and five diets supplemented with either 0.01% vitamin E (VE), or *S. cerevisiae* at 0.25% (SC25) and 0.5% (SC50) or *S. platensis* at 0.25% (SP25) and 0.5% (SP50). The calculated and analyzed values of the nutrients in the diets are presented in table 1. The proximate composition of the biomasses were: 4% moisture, 45% protein, 0.2% lipid and 5% ash for *S. cerevisiae*; and 6% moisture, 46% protein, 3% lipid and 8% ash for *S. platensis*. The biomasses were dissolved in soy oil and incorporated to the basal diet inside a Y-shaped mixer. The same amount of soy oil was added to the control group. The experimental diets were stored in the dark inside plastic bags (polypropylene) at room temperature (25 °C) for 12 months.

For the thiobarbituric acid reactive substances (TBARS) quantification, 1 g of feed was homogenized with 25 ml of 7.5% trichloroacetic acid using turrax for 1 min (TBARS extraction). Then, the mixture was filtered and 5 ml of the extract were transferred to a tube containing 5 ml 0.02 M thiobarbituric acid. The tubes were heated in boiling water bath (100 °C) for 40 min and cooled in running water for 10 min. The TBARS were measured at 538 nm and the values were expressed as mg malonaldehyde/kg (GRASSI et al., 2016, 2018).

All data collected were subjected to analysis of variance and the significance of differences between means was tested by Tukey's test, using Action version 3.1[®]. Broken line regression was used to estimate the biomass requirements to avoid the oxidative rancidity as a function of MB inclusion, using PPM (Practical Program for Modeling, <https://sites.google.com/site/programapratricodemodelagem/>). The significance level was set at 0.05.

The SC50, SP25 and SP50 had lower TBARS than the control group while SC25 and VE were not able to prevent the lipid oxidation during the storage for 12 months (Table 2). The antioxidant activity of biomass minimized the oxidative rancidity, increasing the shelflife of Nile tilapia feed. This effect may be assigned to carotenoids and phycocyanins in *Spirulina platensis* and to rutin, hesperidin, sakuranetin, quercetin, naringin, naringenin and vitamin E in *Saccharomyces cerevisiae* (SOARES et al., 2005). The inclusion of *S. cerevisiae* at 0.25% (SC25) had insufficient antioxidant activity to reduce the oxidative rancidity. The broken line regression indicated that 0.4392% *S. cerevisiae* (Figure. 1.A) and 0.2852% *S. platensis* (Figure. 1.B) biomasses in the rations are enough to provide the lowest TBARS in the diets.

GRASSI et al. (2016) also reported a delay in tilapia diets stored until 12 months when a bacterial biomass (*Rubrivivax gelatinosus*) was added up to 0.14% and assigned the effect to the presence of carotenoids in the biomass.

The need to seek alternatives to the synthetic antioxidant substances has increased the interest for the production of natural antioxidants through biotechnology. For instance, microbial biomasses of yeast *Phaffia rhodozyma* and microalgae *Haematococcus pluvialis* and *Dunaliella salina* have been used as sources of carotenoids in the feed of aquatic animals (PONCE-PALAFIX et al., 2004). Vitamin E produced by microorganisms also has been used in fish diets to prevent the oxidative rancidity and protect the tissue integrity of several fish species (PENG et al., 2009). Although, the antioxidant activity of vitamin E in feed is recognized, in this experiment, it was not able to prevent the lipid oxidation like the biomasses did.

More than benefits to the preservation of the ration, *S. platensis* and *S. cerevisiae* biomasses can improve fish health and meat quality. GRASSI et al. (2018) included microbial biomasses in tilapia diets and reported an improvement in the

Table 1 - Experimental diet for tilapias.

| Ingredients (%) | CD | VE | SC25 | SC50 | SP25 | SP50 |
|--|-------|-------|-------|-------|-------|-------|
| Feather meal | 2 | 2 | 2 | 2 | 2 | 2 |
| Ground corn | 33.06 | 33.05 | 33.06 | 33.06 | 33.06 | 33.06 |
| Poultry meal by-products | 6 | 6 | 6 | 6 | 6 | 6 |
| Soybean meal | 10.44 | 10.44 | 10.19 | 9.94 | 10.19 | 9.94 |
| Wheat meal | 15.88 | 15.88 | 15.88 | 15.88 | 15.88 | 15.88 |
| Meat meal | 17.14 | 17.14 | 17.14 | 17.14 | 17.14 | 17.14 |
| Blood meal | 12 | 12 | 12 | 12 | 12 | 12 |
| NaCl | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Chicken oil | 2 | 2 | 2 | 2 | 2 | 2 |
| Premix ^a | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Methionine | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Vitamin E | 0 | 0.01 | 0 | 0 | 0 | 0 |
| <i>S. cerevisiae</i> | 0 | 0 | 0.25 | 0.5 | 0 | 0 |
| <i>S. platensis</i> | 0 | 0 | 0 | 0 | 0.25 | 0.5 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| -----Nutrients/Energy – calculated values----- | | | | | | |
| Digestible energy (kcal/kg) | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| Digestible protein (%) | 27.63 | 27.63 | 27.63 | 27.63 | 27.63 | 27.63 |
| Ether extract (%) | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 |
| Crude fiber (%) | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Mineral composition (%) | 10.48 | 10.48 | 10.48 | 10.48 | 10.48 | 10.48 |
| Total calcium (%) | 2.74 | 2.74 | 2.74 | 2.74 | 2.74 | 2.74 |
| Total phosphorus (%) | 1.57 | 1.57 | 1.57 | 1.57 | 1.57 | 1.57 |
| Starch (%) | 27.03 | 27.03 | 27.03 | 27.03 | 27.03 | 27.03 |
| Available phosphorus (%) | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 |
| Arginine (%) | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 |
| Lysine (%) | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 |
| Threonine (%) | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Tryptophan (%) | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| Methionine (%) | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Vitamin C (mg/kg) | 700 | 700 | 700 | 700 | 700 | 700 |
| -----Nutrients – analyzed values (%)----- | | | | | | |
| Protein | 32.29 | 32.18 | 32.26 | 32.22 | 32.41 | 32.34 |
| Ether extract | 8.11 | 8.15 | 8.14 | 8.11 | 8.18 | 8.09 |
| Ash | 9.79 | 9.84 | 9.68 | 9.82 | 9.69 | 9.84 |
| Crude fiber | 3.62 | 3.65 | 3.57 | 3.72 | 3.62 | 3.59 |
| Moisture | 10.75 | 10.63 | 10.65 | 10.85 | 10.93 | 10.86 |

CD - control diet, VE - CD + 0.01% vitamin E, SC25 - CD + 0.25% *S. cerevisiae*, SC50 - CD + 0.5% *S. cerevisiae*, SP25 - CD + 0.25% *S. platensis* and SP50 - CD + 0.5% *S. platensis*. ^a Composition per kg of product - Choline 83,333 mg, vitamin A 2,083 UI, vitamin D3 500 UI, vitamin E 37,500 mg, vitamin K3 2,500 mg, vitamin B1 4,167 mg, vitamin B2 4,167 mg, vitamin B6 3,333 mg, vitamin B12 7,500 µg, niacin 12,500 mg, calcium pantothenate 8,152 mg, pantothenic acid 7,500 mg, folic acid 833 mg, biotin 125 mg, vitamin C 116,667 mg, inositol 12,500 mg, iron specific source 12,500 mg, Fe 12,500 mg, Cu 2,500 mg, Mn 7,500 mg, Zn 16,667 mg, Co 42 mg, I 125 mg, Se 67 mg.

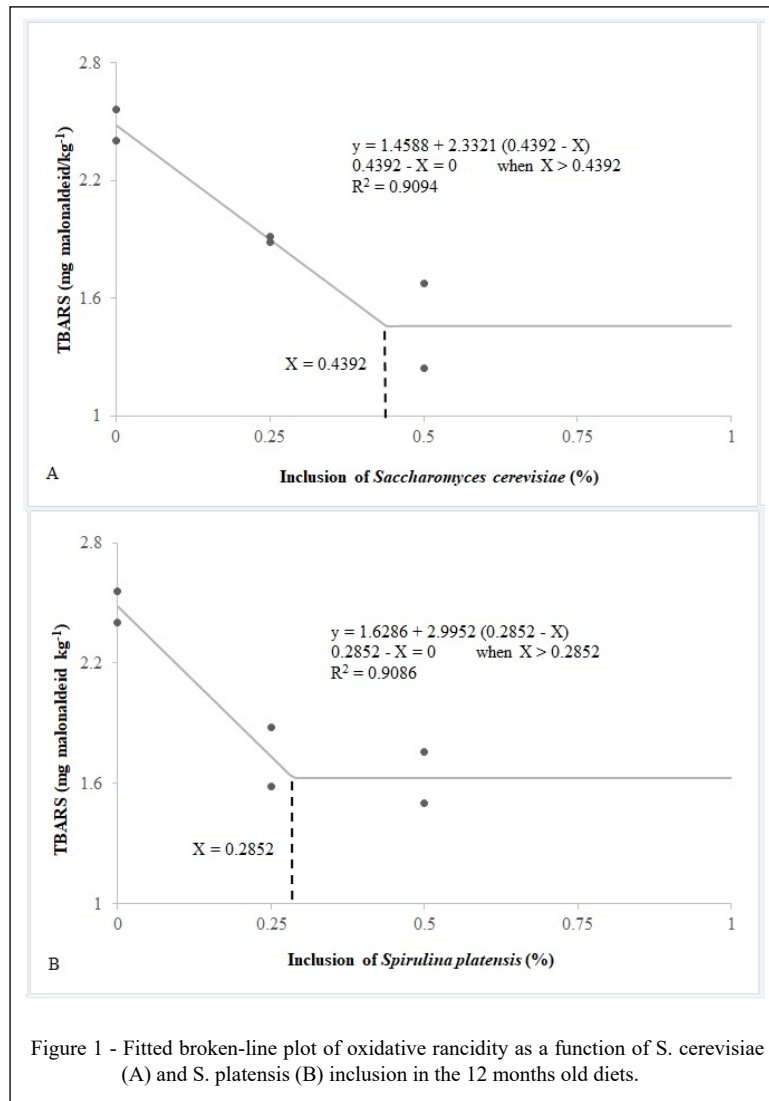
oxidative status of the animals and a delay in the fillets oxidation up to 60 days of storage. Moreover, *S. platensis* biomass increased redness and carotenoids in the tilapia fillets, so improving sensory and functional properties of the flesh (GRASSI et al., 2018).

Results presented herein indicated that *S. cerevisiae* and *S. platensis* biomasses can be considered as natural alternatives to replace the synthetic antioxidants currently in use. It was concluded that the inclusions of *Spirulina platensis* at 0.25 and 0.5% and *Saccharomyces cerevisiae* at 0.5%

Table 2 - Oxidative rancidity of feed containing microbial biomass.

| Treatment | TBARS* (mg malonaldehyde kg ⁻¹) |
|-----------|---|
| CD | 2.483 ± 0.109 ^a |
| VE | 1.941 ± 0.158 ^{ab} |
| SC25 | 1.900 ± 0.019 ^{ab} |
| SC50 | 1.459 ± 0.305 ^b |
| SP25 | 1.734 ± 0.206 ^b |
| SP50 | 1.629 ± 0.181 ^b |
| P | 0.0155 |
| CV (%) | 19.6 |

*Thiobarbituric acid reactive substances; CD - control diet, VE - CD + 0.01% vitamin E, SC25 - CD + 0.25% *S. cerevisiae*, SC50 - CD + 0.5% *S. cerevisiae*, SP25 - CD + 0.25% *S. platensis* and SP50 - CD + 0.5% *S. platensis*. CV - coefficient of variation; ^{ab} Means followed by different letters are significantly different (P<0.05).



minimized the oxidative rancidity in Nile tilapia diets until 12 months of storage.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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