PRODUCTION OF ENZYMATIC EXTRACTS FOR AQUAFEEDS BY SOLID-STATE FERMENTATION WITH *Aspergillus ibericus* OF WINERY AND OLIVE MILL WASTES

Filipe, D[1¥]; Salgado, J[2¥]; Fernandes, H[1,3].; Castro, C[1]; Belo, I[2]; Aires, T[4], Oliva-Teles, A[1,3*]; Moyano, FJ[5]; Peres, H[1,3]

[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre Ed. FC4, 4169-007 Porto, Portugal
[2] CEB – Centre of Biological Engineering of University of Minho, Campus de Gualtar 4710-057 Braga, Portugal
[3]CIMAR/CIIMAR-Centro Interdisciplinar Marinha e Ambiental,, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos 4450-208 Matosinhos, Portugal
[4]Aquasoja, Sorgal, SA; [5]Applied Biology Department, University of Almeria, Almería, Spain
[¥]Both authors contributed equally to this work
*Presenting author: aoteles@fc.up.pt

Introduction
The replacement of fishmeal by plant ingredients in aquafeeds imposed new formulation strategies to overcome some nutritional restrictions associated with these alternative ingredients. Thus, supplementation of plant-based diets with feed additives, as exogenous enzymes and antioxidants compounds, has recently attracted increasing interest to improve feed utilization and to promote animal health. Solid state fermentation (SSF) of agro-industrial wastes has a high potential for the production of these additives, being a practical, economical, and environmentally-friendly process. Olive mill and winery wastes have valuable compounds that may be valorized thought SSF and that may be used as additives for aquafeeds. This study was conducted to optimize the production of non-starch carbohydrases through the SSF of the olive mill and winery wastes and to test its efficacy to improve the release of pentoses during digestion of a plant-based diet in European seabass.

Materials & Methods
Olive mill wastes (crude and exhausted olive pomace; COP and EOP, respectively) and winery wastes (exhausted grape marc and vine trimming shoots; EGM and VTS, respectively) were fermented by *Aspergillus ibericus* MUM 03.49 (10g solid; 75% moisture; $2*10^6$ spores). A simplex-centroid design was performed to optimize the production of cellulases, xylanases, and β-glucosidases with the four solid wastes in 15 different mixtures (4 runs with single wastes; 6 runs with binary mixtures; 4 runs with ternary mixtures; and three central points with quaternary mixture of wastes). After SSF, the enzymes produced were extracted with distilled water. The recovered extract was lyophilized and added (0.4% diet) to a plant-based diet (15% fish meal + 60% plant feedstuffs), and an *in vitro* assay simulating European seabass sequential acidic and basic digestion was performed according to Morales & Moyano (2010).

Results & Discussion
Compared to the use of single wastes as substrate, mixtures olive mills and wineries wastes increased the production of lignocellulolytic enzymes by SSF. The mixture of solid waste that maximized xylanases, cellulases, and β-glucosidases production was 30% EGM + 36% VTS + 34% EOP (Fig.1a-c). The SSF of this mixture leads to an enzymatic activity of 78.2 U xylanase; 39 U cellulose; 21 U β-glucosidase per g of lyophilized extract.

In vitro digestibility trials confirmed that supplementation of a plant-based diet with 0.4% of this extract increased the release of pentoses during alkaline digestion (Table 1). During acid digestion, this effect was significant only when the fish enzymes were inactivated.

Conclusions
Solid-state fermentation by *A. ibericus* of olive mill and winery wastes mixtures allowed higher production of lignocellulolytic enzymes compared SSF of the wastes separately. Overall, the mixture of 30% EGM + 36% VTS + 34% EOP was the one that provided better results. Further, the enzymatic activity of the extract was more active during alkaline than acidic digestion.

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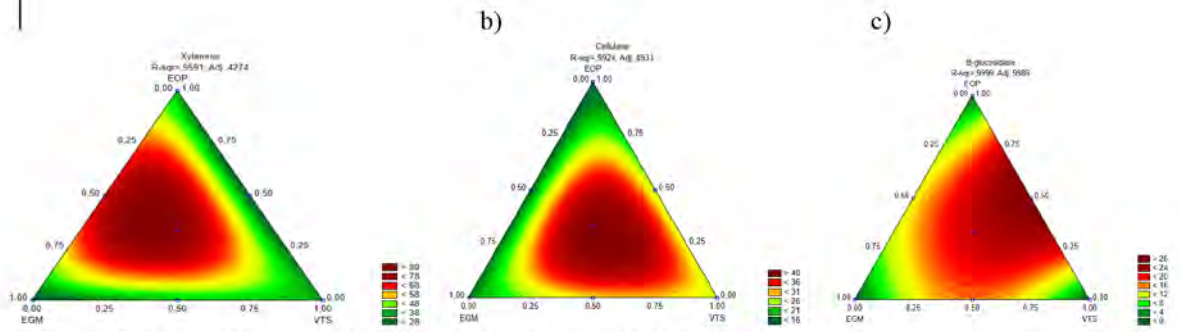

Figure I: Contour diagram for (a) xylanase, (b) cellulase, and (c) β-glucosidase.

Table 1. Pentoses release (µg g^{-1}) during acid and alkaline *in vitro* digestion of the experimental diets with active (A) and inactive (I) European seabass digestive enzymes.

Digestion	**Acid**				**Alkaline**			
Diet	Control		0.4% SSF-extract		Control		0.4% SSF-extract	
Fish enz.	A	I	A	I	A	I	A	I
	98.2± 2.8	43.1± 6.3	102.3± 10.5	80.2± 4.3	96.4± 5.6	38.7± 1.7	129± 4.1	57.8± 2.4
Two-way ANOVA					**Two-way ANOVA**			
Factor	diet	Fish extract	Interaction		diet	Fish extract	Interaction	
	≤0.001	≤0.001	≤0.001		≤0.001	≤0.001	n.s	