

CORRELATION OF HYDROLYTIC ENZYMES PRODUCED BY SOLID-STATE FERMENTATION ON THE RELEASE OF BIOACTIVE COMPOUNDS FROM GRAPE POMACE

Aline Soares Cascaes Teles¹, Davy William Hidalgo Chávez^{2,3}, Leda Maria Fortes Gottschalk⁴, Renata Valeriano Tonon⁴

1. Federal University of Rio de Janeiro, Institute of Chemistry – Rio de Janeiro, RJ – E-mail: aline_cascaes@yahoo.com.br.
2. Federal Rural University of Rio de Janeiro, Department of Food Science and Technology – Rio de Janeiro
3. Institute of Education, Science and Technology of Rio de Janeiro, Valença Campus, Department of Food Engineering, RJ – E-mail: davyhw76@gmail.com
4. Embrapa Food Agroindustry, Avenue of Americas, 29501, Zip code 23020-470 - Rio de Janeiro – RJ, E-mail: leda.fortes@embrapa.br, renata.tonon@embrapa.br.

INTRODUCTION

- ❖ **Grape pomace:** co-product of the wine industry and highly rich in phenolic compounds, mainly proanthocyanidins with antioxidant capacity.
- ❖ **Solid-state fermentation (SSF):** can produce many enzymes that can be used for hydrolysis of the plant cell wall to release the phenolic compounds of grape pomace.
- ❖ **Component principal analysis (PCA):** correlation between activity of enzymes and bioactive compounds released during hydrolysis.

OBJECTIVE

To evaluate the correlation between the activity of enzymes produced by SSF and the release of bioactive compounds from grape pomace during enzyme assisted extraction.

MATERIAL AND METHODS

- ❖ **Material:**
 - Alicante bouschet from red wine production - Rio Sol winery (Lagoa Grande, PE, Brazil);
 - Wheat bran - Bunge Alimentos S.A. (Rio de Janeiro, Brazil).
- ❖ **Fermentative processes:**
 - *Aspergillus niger* 3T5B8;
 - SSF - grape pomace and wheat bran as Mixed Medium (MD) and wheat bran as Standard Medium (SM);
 - Extraction of enzymes - (24, 48, 72 and 96 hours) during SSF.
- ❖ **Extraction of bioactive compounds:**
 - Grape pomace (0.5 g) in 4mL sodium acetate buffer (0.02 M, pH 5.0);
 - Enzymatic pool (0,555 mL) - 50°C, 2 hours (Xu et al. (2014)).

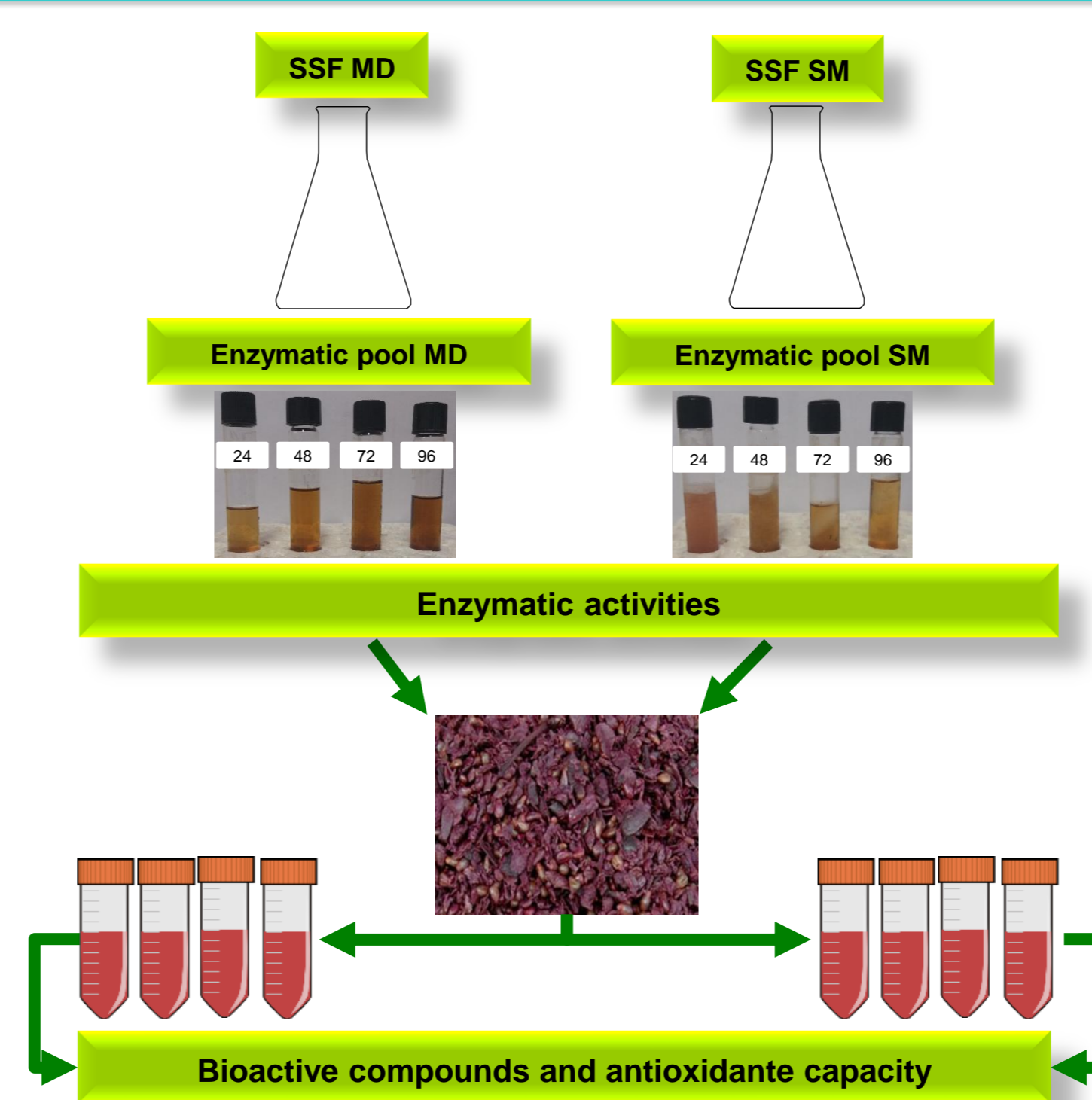


Diagram for obtaining extracts rich in bioactive compounds

Determination of enzymatic activities:

- ❖ Xylanase (Miller 1959).
- ❖ CMCase and β -glucosidase (IUPAC, Ghose et al., 1987).
- ❖ Polygalacturonase (Couri & Farias, 1995).
- ❖ Tannase (Sharma et al, 2000).

Bioactive compounds and antioxidant capacity dosages:

- ❖ Total phenolic compounds (Singleton & Rossi, 1965).
- ❖ Total anthocyanins (Giusti & Wrolstad 2001).
- ❖ Proanthocyanidins (Broadhurst & Jones 1978).
- ❖ Antioxidant capacity – ABTS⁺ (Re et al., 1999).

RESULTS AND DISCUSSION

Table 1. Enzymatic activities of the different pool used for release of bioactive compounds from grape pomace.

Time (hours)	Xylanase activity (U mL ⁻¹)	CMCase activity (U mL ⁻¹)	Polygalacturonase activity (U mL ⁻¹)	β -glucosidase activity (U mL ⁻¹)	Tannase activity (U mL ⁻¹)
24 MD	9	1	4	6	0.08
48 MD	11	3	5	24	0.09
72 MD	5	4	4	20	0.07
96 MD	6	5	13	25	0.21
24 SM	30	2	22	6	0.09
48 SM	31	3	17	15	0.02
72 SM	21	4	21	19	0.02
96 SM	22	4	19	23	0.01

Table 2. Bioactive compounds and antioxidant capacity in extracts obtained for extraction enzyme-assisted.

Sample	TP (mg GAE.100g ⁻¹)	PAAs (g CE.100g ⁻¹)	TA (mg Cyn.100g ⁻¹)	ABTS ⁺ (μ mol TE.g ⁻¹)	ORAC (μ mol TE.g ⁻¹)
Control	506.09 \pm 3.32 ^e	2.60 \pm 0.04 ^d	29.00 \pm 0.32 ^b	36.65 \pm 0.09 ^b	127.85 \pm 9.49 ^e
24 MD	719.90 \pm 3.35 ^c	3.27 \pm 0.04 ^c	34.93 \pm 0.36 ^a	39.09 \pm 0.71 ^b	163.09 \pm 3.28 ^c
48 MD	733.80 \pm 7.31 ^c	5.49 \pm 0.01 ^a	17.36 \pm 0.55 ^c	18.66 \pm 0.34 ^c	173.24 \pm 3.59 ^b
72 MD	670.96 \pm 3.33 ^d	5.30 \pm 0.02 ^a	12.61 \pm 0.75 ^d	13.68 \pm 0.25 ^c	192.33 \pm 12.80 ^a
96 MD	910.56 \pm 2.22 ^a	5.76 \pm 0.02 ^a	11.57 \pm 0.01 ^d	52.14 \pm 0.83 ^a	161.95 \pm 16.80 ^c
24 SM	847.02 \pm 7.84 ^b	2.27 \pm 0.01 ^d	34.45 \pm 1.21 ^a	36.75 \pm 0.18 ^b	175.84 \pm 3.11 ^b
48 SM	909.98 \pm 4.45 ^a	3.59 \pm 0.01 ^c	12.68 \pm 0.66 ^d	34.00 \pm 0.63 ^b	158.27 \pm 0.84 ^c
72 SM	747.57 \pm 5.58 ^c	4.35 \pm 0.01 ^b	8.47 \pm 0.36 ^d	32.86 \pm 0.16 ^b	140.73 \pm 5.78 ^d
96 SM	923.43 \pm 2.17 ^a	4.77 \pm 0.04 ^b	7.51 \pm 0.24 ^d	36.90 \pm 0.03 ^b	172.54 \pm 2.95 ^b

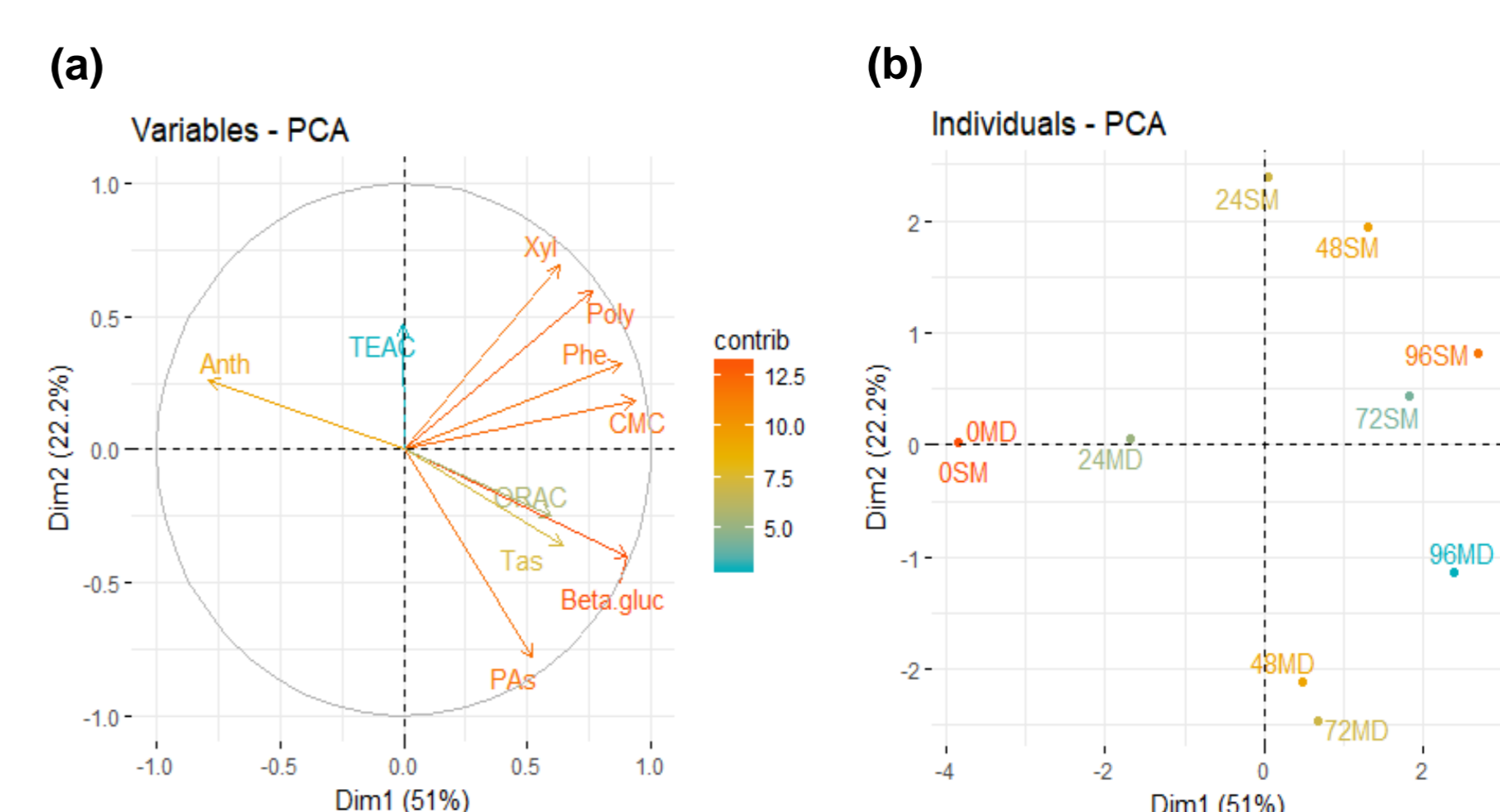


Figure 1. Two principal components analysis of enzymatic activity and bioactive compounds. MD: Mixed Medium and SM, Standard Medium (Fig.3a and b). Xyl: xylanase, CMC: carboxymethylcellulase, Poly: polygalacturonase, Beta.gluc: β -glucosidase, Tas: tannase, Phe: phenolics, PAs: proanthocyanidins, Anth: anthocyanins.

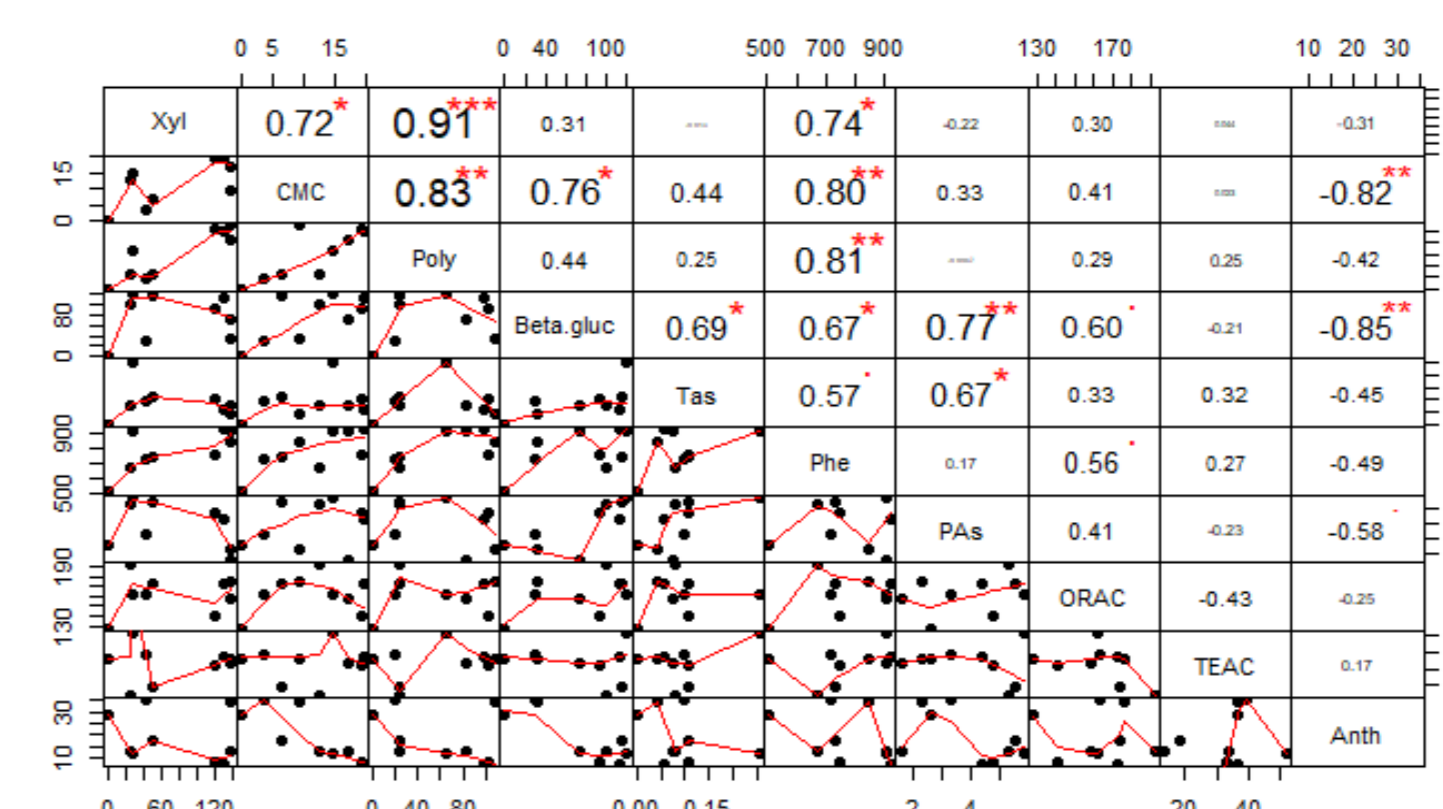


Figure 2. Correlogram between enzyme activity (MD and SM) and bioactive compounds from grape pomace extracts. Xyl: xylanase, CMC: carboxymethylcellulase, Poly: polygalacturonase, β -gluc: β -glucosidase, Tas: tannase, Phe: phenolics, PAs: proanthocyanidins, Anth: anthocyanins. *** $p < 0.0001$, ** $p < 0.001$, * $p < 0.01$, $p < 0.05$.

CONCLUSION

The substrates for SSF promoted the synthesis of enzymes with high activity, mainly the enzymatic pool produced after 96 hours of SSF. This result was confirmed by the strong correlation between the enzymatic activity and the compounds released from grape pomace.

REFERENCES

- Couri, S. et al. (2000). Hydrolytic enzyme production in solid-state fermentation by *Aspergillus niger* 3T5B8. *Proc. Biochem.* 36(3).
- Giusti, M. M. & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Cur. Prot. Food Anal. Chem.*
- Sharma, S., et al. (2000). A spectrophotometric method for assay of tannase using rhodanine. *Anal. Biochem.* 279(1).
- Xu, C. et al. (2014). Enzyme release of phenolics from muscadine grape (*Vitis rotundifolia* Michx.) skins and seeds. *Food Chem.* 157.
- Zulueta, A., et al. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry* 114(1).
- Georgé, S. Brat et al. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry*, 53(5).
- Re, R. Pellegrini et al. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10).

Acknowledgment