Supplementary Material

Chitosan-genipin film, a sustainable methodology for wine preservation

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1. *Chitosan analysis*

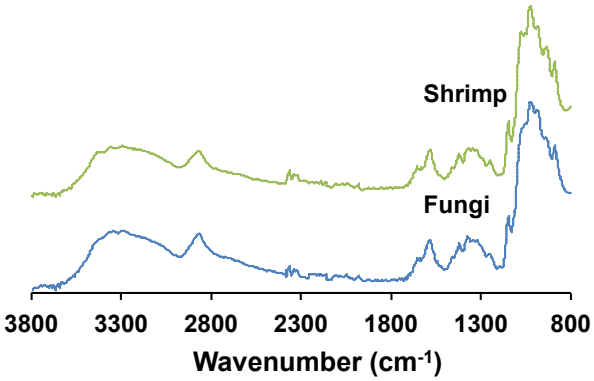

Figure S1. FTIR spectra of chitosan from shrimp and fungi

2. *Oenological parameters*

The oenological parameters of the wine samples were determined by the winemaking company (Dão Sul SA). The ethanol content, titratable acidity, volatile acidity, pH, dry extract, and reducing sugars were determined for each wine according to the methods described by the International Organisation of Vine and Wine[30]. All analyses were carried out in triplicate.

Table S1 - Oenological parameters of the white wine samples at the beginning and after 12 months of storage for 2010 and 2011 vintages.

	2010					2011				
	0 months		12 months			0 months		12 months		
	Untreated/ Chitosan film	Sulphur dioxide	Untreated	Sulphur dioxide	Chitosan film	Untreated/ Chitosan film	Sulphur dioxide	Untreated	Sulphur dioxide	Chitosan film
Alchoolic degree (%)	$13.2^{a} \pm 0.1$	$13.2^{a} \pm 0.1$	$11.7^{b} \pm 0.1$	$11.9^{b} \pm 0.1$	$12.0^{b} \pm 0.1$	$13.1^{a} \pm 0.1$	$13.1^{a} \pm 0.1$	$13.1^{a} \pm 0.0$	$13.2^{a} \pm 0.1$	$13.1^{a} \pm 0.1$
Volatile acidity (g/L acetic acid)	$0.38^{a} \pm 0.05$	$0.39^{a} \pm 0.06$	$0.60^{b,c} \pm 0.06$	$0.54^{b} \pm 0.05$	$0.67^{c} \pm 0.02$	$0.31^{a} \pm 0.04$	$0.36^{a} \pm 0.03$	$0.41^{d} \pm 0.01$	$0.41^{d} \pm 0.01$	$0.42^{d} \pm 0.01$
Total Acidity (g/L tartaric acid)	$6.5^{a} \pm 0.1$	$6.6^{a} \pm 0.3$	$6.2^{a,b} \pm 0.2$	$6.0^{b} \pm 0.1$	$6.0^{b} \pm 0.1$	$7.8^{c} \pm 0.3$	$8.1^{c} \pm 0.1$	$6.4^{a} \pm 0.0$	$6.5^{a} \pm 0.1$	$6.4^{a} \pm 0.1$
pH	3.4	3.4	3.5	3.4	3.5	3.1	3.2	3.1	3.1	3.1
Dry extract (g/L)	$27.2^{a} \pm 0.8$	$26.4^{a} \pm 0.6$	$25.4^{a} \pm 0.7$	$25.3^{a} \pm 0.5$	$25.1^{a} \pm 0.1$	$26.9^{a} \pm 0.8$	$25.7^{a} \pm 0.2$	$18.2^{b} \pm 0.6$	$18.1^{b} \pm 0.2$	$18.1^{b} \pm 0.2$
Density (g/cm³)	0.9862	0.9855	0.9859	0.9856	0.9855	0.9828	0.9832	0.9882	0.9881	0.9880
Sugars (g/L)	$1.4^{a} \pm 0.5$	$2.0^{b} \pm 0.2$	$2.1^{b} \pm 0.2$	$2.3^{bc} \pm 0.1$	$2.7^{c} \pm 0.4$	$3.1^{c} \pm 0.2$	$3.0^{c} \pm 0.1$	$2.3^{b} \pm 0.1$	$2.5^{b} \pm 0.1$	$2.3^{b} \pm 0.1$

In each line, different letters indicate statistically ($p>0.05$) different values.

3. Microbiological analysis

Microorganism load was determined following inoculation of serially diluted wine samples on Petri dishes containing specific culture media for bacteria (Wallerstein differential agar) and yeast (Rose Bengal Chloramphenicol agar). The incubation conditions were 48 h at 37 ºC for bacteria and 5 days at 25 ºC for yeasts. At least three replicates of each condition were performed. The cell concentration was expressed as colony-forming units per millilitre (CFU/mL).

Table S2 – Microbiological analysis of wine (CFU/mL) without treatment, with sulphur dioxide addition, and chitosan-based film treatment along 12 months of storage for 2010 and 2011 vintages

		2010				2011				
		0 months	**2 months**	**8 months**	**12 months**	**0 months**	**3 months**	**6 months**	**9 months**	**12 months**
Untreated	*Yeast*	12 ± 16	2100 ± 130	NG[1]	NG	NG	240 ± 55	NG	NG	NG
	Bacteria	NG	NG	250 ± 21	NG	1000 ± 140	60 ± 10	NG	NG	NG
Sulphur dioxide	*Yeast*	NG	3400 ± 780	8800 ± 910	NG	7.8 ± 0.3	NG	NG	NG	NG
	Bacteria	NG	NG	NG	NG	3.1 ± 0.0	NG	NG	NG	NG
Chitosan film	*Yeast*	NG	NG	NG	NG	26.9 ± 0.8	NG	NG	NG	NG
	Bacteria	NG	NG	NG	NG	3.1 ± 0.2	NG	NG	NG	NG

[1]NG – No Growth of CFU

4. Sensorial analysis

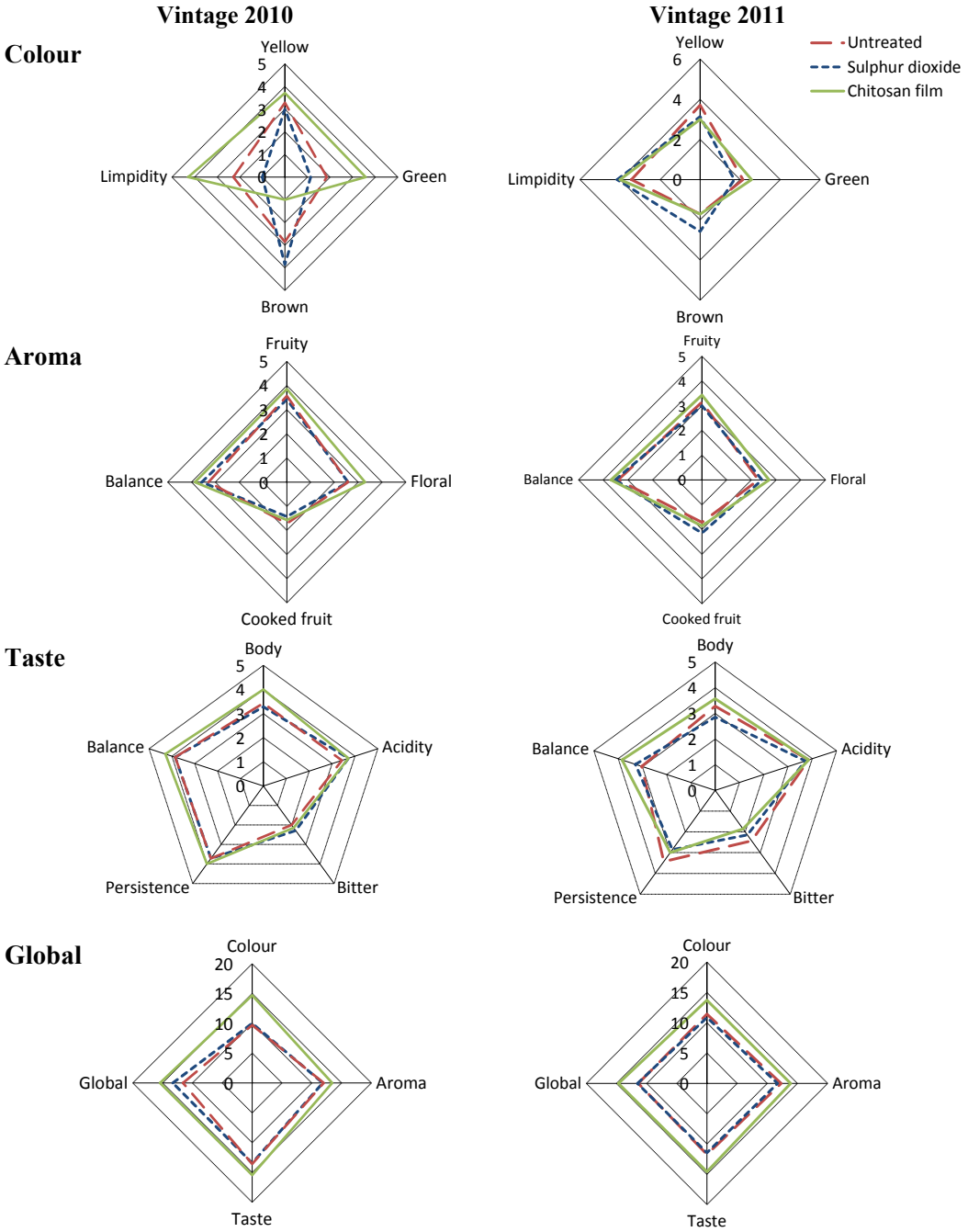

Figure S2. Descriptive sensory analysis of the colour, aroma, taste, and global attributes of white wine without treatment, with sulphur dioxide addition and chitosan-based film treatment from two vintages (2010 and 2011) after 12 months of storage.