Application to amend the Food Standard relating to food additives: mannoproteins

Contents

PART 1 GENERAL INFORMATION

1.1 Applicant

(a) Company name: Laffort Services (ABN 18 102 154 530)

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Laffort Oenologie
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(d) Nature of business: Manufacturer of additive.

1.2 Nature of application

This application is:

(a) To vary existing standards (4.5.1 & 1.3.1).

(b) Being made on behalf of a single company.

PART 2 SPECIFIC INFORMATION

2.1 Details of the additive

(a) Common name of additive = mannoprotein extracted from yeast cell walls.
(b) The application relates to wine.
(c) Proposed minimum level of application = 10 g/hL.
Proposed maximum level of application = 30 g/hL.

Function and method of action of yeast mannoproteins

For a general discussion on the use of yeast mannoproteins in wine, see Ribéreau-Gayon et al, 2006a.

Mannostab™ is a purified yeast (*Saccharomyces cerevisiae*) cell wall preparation (Ribéreau-Gayon et al, 2006a), consisting of low molecular weight mannoproteins. It is prepared by the enzymatic digestion of the yeast using β-glucanase, an enzyme specified as a permitted food processing aid in Standard 1.3.3, clause 17.

Mannoproteins in the mass range of around 40 kDa inhibit the crystallisation of potassium bitartrate, a salt that is wine in a super-saturated state at the completion of alcoholic fermentation. Thus, Mannostab™ falls into a class of colloids termed "protective colloids", other examples of which include Gum Arabic (Standard 4.5.1 clause 3) and β-glucane, derived from *Botrytis cinerea* infection of grapes (Ribéreau-Gayon et al, 2006b). Mannoproteins of lower molecular weight can also stabilise wine with respect to protein instability, potentially reducing or eliminating the requirement for bentonite.

Protective colloids function by coating the site of crystallisation or aggregation. Mechanisms for this process have been postulated but not yet elucidated. Current thinking intimates that the colloidal molecule adsorbs onto the surface being protected whilst also maintaining a separation zone with the immediate environment, thus hindering access to approaching molecules or particles (Figure 1).

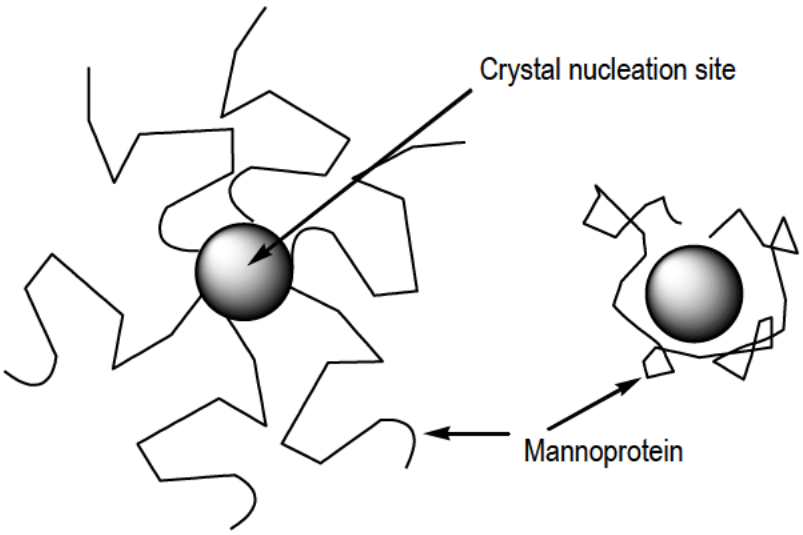


Figure 1: Functional methodology attributed to protective colloids such as mannoproteins.

2.2 Purpose and efficacy of the additive

The additive is for use only in wine. Continuous experimentation since 1997 has indicated that Mannostab™ is highly efficient at inhibiting potassium bitartrate crystallisation. As the removal of potassium bitartrate is a common time-consuming and expensive winemaking process, a simple additive that will remove this requirement represents a significant advantage in terms of production efficiency, logistics and overall expenditure.

Outlines of some experiments are provided below. These experiments formed the basis of doctoral candidacy for Laffort Oenologie's Product Manager for Fining Agents, Dr Virginie Moine-Ledoux.

Experiment 1

Aim: To determine the dosage of Mannostab™ in a white wine required to effect potassium bitartrate stability and compare its effectiveness with cold stabilisation.

Method: (a) A total of 126 hL of 2000 AOC Bordeaux white wine was treated with Mannostab™ thusly: 0, 10, 15, 20, 25 and 30 g/hL. Dosage was performed using a venturi in a closed tank system, with mixing for 1 hour, followed by bottling. The change in potassium ion concentration was measured before and after cooling of the wine (Table 1). When no change was observed in the potassium ion concentration with increasing dosages of Mannostab™ the wine was declared cold stable, which provided the dosage rate for experiment 1 part b. (b) Visual measurements were also made by examining the time required for potassium bitartrate crystal formation when the samples were held at -4 °C (Table 2). Wines that produced no precipitation after 6 days at –4 °C were declared stable. Wines were analysed by standard wine chemical measurements after completion of the experiments (Table 3).

Results:

Table 1

[Mannostab] g/hL	0	10	15	20	25	30
Δ [K^+] mg/L	224	207	113	37	37	37

Treatment level for potassium bitartrate stability: **25 g/hL**.

Table 2

Stabilisation method	Duration before crystallisation
Control (no treatment)	3 days
Cold stabilisation	> 95 months
Mannostab™ 25 g/hL	> 95 months

Table 3

Treatment	Control	Cold stabilisation	Mannostab™
Alcohol %(v/v)	11.05	11.05	11.05
Residual sugar (g/L)	1.3	1.3	1.3
Total acidity (g/L H_2SO_4)	3.55	3.40	3.40
pH	3.48	3.45	3.48
Volatile acidity (g/L H_2SO_4)	0.33	0.33	0.33
Free SO_2 (mg/L)	22	35*	27
Total SO_2 (mg/L)	114	123*	114

* Elevations in SO_2 levels due to additions of SO_2 made prior to bottling.

Conclusion: Treatment of white wines with Mannostab™ at a sufficient dosage inhibits the precipitation of potassium bitartrate in a similar manner to cold stabilisation and does not alter the physiochemical characteristics of the wine.

Experiment 2

Aim: To determine the dosage of Mannostab™ in two white wines of differing vintage required to effect potassium bitartrate stability and compare its effectiveness with metatartaric acid addition.

Method: (a)Two wines, 26 hL of 2000 AOC Jurançon dry white and 60 hL of 1999 AOC Jurançon sweet white, were treated with Mannostab™ thusly: 0, 10, 15, 20, 25 and 30 g/hL. Dosage was performed by addition to the top of the tank followed by agitation, followed by filtration and bottling. Comparisons were made with metatartaric acid additions. The change in potassium ion concentration was measured before and after cooling of the wine (Table 4). When no change was observed in the potassium ion concentration with increasing dosages of Mannostab™ the wine was declared cold stable, which provided the dosage rate for experiment 1 part b. (b) Visual measurements were also made by examining the time required for potassium bitartrate crystal formation when the samples were held at –4 °C (Table 5). Wines that produced no precipitation after 6 days at –4 °C were declared stable. Wines were analysed by standard wine chemical measurements after completion of the experiments (Table 6a & b).

Results:

Table 4

[Mannostab] g/hL	0	10	15	20	25	30
Δ [K^+] mg/L 2000 AOC Jurançon white	226	144	42	20	20	42
Δ [K^+] mg/L 1999 AOC Jurançon white	186	103	42	20	20	20

Treatment level for potassium bitartrate stability: **20 g/hL**.

Table 5

Stabilisation method	2000 AOC Jurançon dry white	1999 AOC Jurançon sweet white
Control	4 days	5 days
Metatartaric acid	> 8 months	> 8 months
Mannostab™	> 95 months	> 95 months

Table 6a 2000 AOC Jurançon dry white

Treatment	Control	Metatartaric acid	Mannostab™
Alcohol %(v/v)	13.70	13.70	13.70
Residual sugar (g/L)	1.5	1.6	1.6
Total acidity (g/L H_2SO_4)	5.70	5.70	5.70
pH	3.07	3.07	3.07
Volatile acidity (g/L H_2SO_4)	0.34	0.35	0.34
Free SO_2 (mg/L)	22	22	22
Total SO_2 (mg/L)	72	73	72
OD 420	0.105	0.106	0.105
OD 520	0.023	0.023	0.024

Table 6b 1999 AOC Jurançon sweet white

Treatment	Control	Metatartaric acid	Mannostab™
Alcohol %(v/v)	13.65	13.65	13.65
Residual sugar (g/L)	72	72	72
Total acidity (g/L H_2SO_4)	5.15	5.20	5.20
pH	3.14	3.12	3.13
Volatile acidity (g/L H_2SO_4)	0.064	0.064	0.064
Free SO_2 (mg/L)	32	34	33
Total SO_2 (mg/L)	132	136	133
OD 420	0.173	0.168	0.165
OD 520	0.033	0.029	0.029

Conclusion: Treatment of white wines with Mannostab™ at a sufficient dosage inhibits the precipitation of potassium bitartrate in a superior manner to metatartaric acid and does not alter the physiochemical characteristics of the wine. Residual sugar in the wine does not affect the performance of Mannostab™.

Experiment 3

Aim: To determine the dosage of Mannostab™ in a rosé wine and a red wine required to effect potassium bitartrate stability and to compare its effectiveness with cold stabilisation and metatartaric acid addition.

Method: (a) Two wines, 150 hL of 2000 AOC Irouléguy rosé and 160 hL of 2000 AOC Irouléguy red, were treated with Mannostab™ thusly: 0, 10, 15, 20, 25 and 30 g/hL. Dosage was performed by addition to the top of the tank followed by agitation, followed by filtration and bottling. Comparisons were made with metatartaric acid additions and cold stabilisation. The change in potassium ion concentration was measured before and after cooling of the wine (Table 7). When no change was observed in the potassium ion concentration with increasing dosages of Mannostab™ the wine was declared cold stable, which provided the dosage rate for experiment 1 part b. (b) Visual measurements were also made by examining the time required for potassium bitartrate crystal formation when the samples were held at –4 °C (Table 8). Wines that produced no precipitation after 6 days at –4 °C were declared stable. Wines were analysed by standard wine chemical measurements after completion of the experiments (Table 9a & b).

Results:

Table 7

[Mannostab] g/hL	0	10	15	20	25	30
Δ $[K^+]$ mg/L 2000 AOC Irouléguy rosé	226	207	188	132	19	37
Δ $[K^+]$ mg/L 2000 AOC Irouléguy red	103	-	62	41	20	20

Treatment levels for potassium bitartrate stability: **30 g/hL** for the Irouléguy rosé and **25 g/hL** for the Irouléguy red.

Table 8

Stabilisation method	2000 AOC Irouléguy red	2000 AOC Irouléguy red
Control	4 days	5 days
Cold stabilisation	> 3 months	-
Metatartaric acid	-	> 4 months
Mannostab™	> 95 months	> 95 months

Table 9a 2000 AOC Irouléguy rosé

Treatment	Control	Cold stabilisation	Mannostab™
Alcohol %(v/v)	12.45	12.45	12.35
Residual sugar (g/L)	3.0	3.1	3.1
Total acidity (g/L H_2SO_4)	5.00	4.85	5.00
pH	3.45	3.44	3.43
Volatile acidity (g/L H_2SO_4)	0.26	0.23	0.21
Free SO_2 (mg/L)	17	23	27*
Total SO_2 (mg/L)	85	97	107
Colour intensity	1.10	1.01	1.03

*Difference due to SO_2 addition prior to bottling

Table 9b 2000 AOC Irouléguy red

Treatment	Control	Metatartaric acid	Mannostab™
Alcohol %(v/v)	12.40	12.25	12.25
Residual sugar (g/L)	1.0	1.1	1.1
Total acidity (g/L H_2SO_4)	3.65	3.75	3.65
pH	3.85	3.81	3.85
Volatile acidity (g/L H_2SO_4)	0.50	0.46	0.52
Free SO_2 (mg/L)	10	15	39*
Total SO_2 (mg/L)	31	46	90*

*Difference due to SO_2 addition prior to bottling

Conclusion: Treatment of rosé and red wines with Mannostab™ at sufficient dosages inhibits the precipitation of potassium bitartrate and does not alter the physiochemical characteristics of the wine.

Sensory analysis

Aim: To examine wine preference when treated with Mannostab™ as compared with control experiments and traditional treatments.

Method: Sensory analyses were completed on control, traditional treatment and Mannostab™-treated wines for each of the experimental wines using 28 people, who were asked to rank the wines in order of preference from 1 to 3 in each case.

Results: Figure 2 indicates the results of the analyses. The columns represent the sums of the ranks, hence **a lower sum indicates a higher preference**.

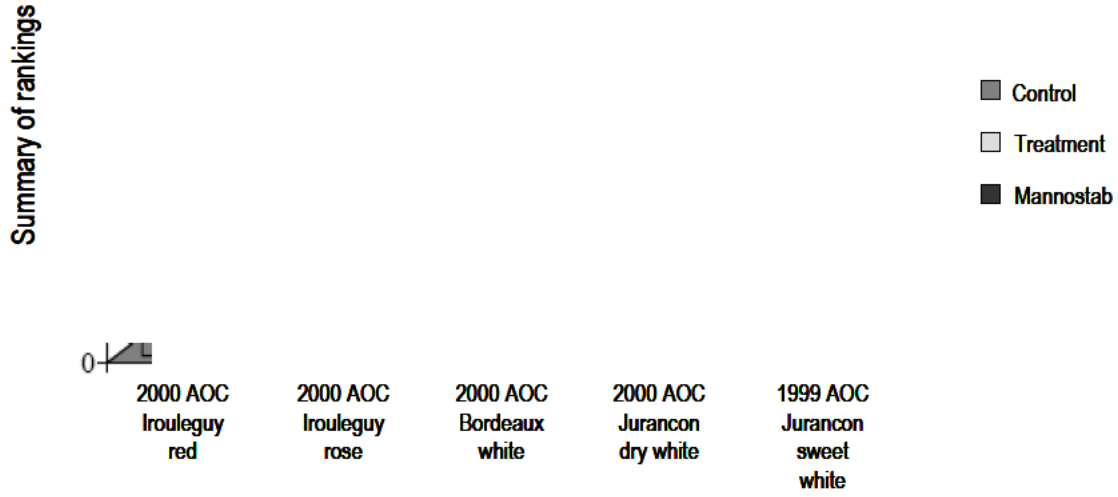


Figure 2: Summations of the wine preferences according to the various treatments. A lower score represents a higher preference.

Conclusion: Treatment with Mannostab™ does not reduce consumer preference in any significant way for white wines, and for rose and red wines can lead to significantly improved preference over standard stabilisation methods.

Summary of experimental results

The experiments confirm that Mannostab™ is effective in preventing potassium bitartrate precipitation. A bench trial is initially required to determine the appropriate Mannostab™ treatment level required for each wine. The duration of Mannostab™ effectiveness is equal to (cold stabilisation) or better than (metatartaric acid) that of existing treatments. The usage of Mannostab™ does not alter the physiochemical characteristics of treated wines. Sensory analysis of treated wines indicated either no significant difference from controls in the case of whites, and an improvement in the wines in the case of treated reds.

2.3 Justification for the use of the additive

Wine bitartrate stabilisation is an integral part of the production process. If a wine is not stabilised, crystallisation produces crystals that resemble glass shards, leading to consumer rejection. Good manufacturing process (GMP) does not alter the saturation level of tartrate salts in a wine, thus GMP is not an applicable methodology.

Other methods used in Australia to effect potassium bitartrate stability are **cold stabilisation** and **metatartaric acid addition**.

Cold stabilisation involves cooling the wine to approximately –4 °C and holding the wine at this temperature from days to weeks. After this time precipitation may have occurred, in which case the wine can be racked or filtered, then re-assessed for stability and re-treated if necessary. A variant on this procedure is called the **contact process**, where the cooled solution is seeded with potassium bitartrate crystals to expedite the crystallisation. In either case production efficiency is poor, logistics

costs are high, infrastructure costs are high and energy consumption is high. In summary, this is an environmentally unsound yet (at this point in time) necessary practice.

Metatartaric acid is a condensation polymer of tartaric acid. The polymer inhibits the crystallisation of potassium bitartrate, but the effect is not permanent due to the instability of the polymer itself in the wine medium. It has been estimated that at 20 ºC metatartaric acid is effective at inhibiting potassium bitartrate crystallisation for approximately 3 months only (Ribéreau-Gayon et al, 2006a). Moreover, the eventual decomposition of metatartaric acid increases the tartaric acid concentration of the wine and thus leads to increased instability.

In summary, although two effective treatments are currently available for ensuring wine potassium bitartrate stability, both have disadvantages in terms of either cost, environmental impact, efficiency and logistics (particularly cold stabilisation) or duration of effectiveness (metatartaric acid addition). Mannostab™ is not subject to these constraints, and is moreover a natural product of yeast cell walls.

2.4 Establish need for the additive

Since Mannostab™ is currently not available on the Australian market, nor is there an equivalent product, few requests have been made for relevant information. Nevertheless, the largest wine production group in Australia, Fosters Wines, has contacted Laffort Oenologie Australia specifically to obtain information on Mannostab™ (Fosters information request).

During initial enquiries FSANZ indicated that Laffort Oenologie should seek endorsement of Mannostab™ by the Winemaker's Federation of Australia (WFA). Endorsement for Mannostab™ by the WFA is attached (pages 1 and 2).

2.5 Nutritional implications of the use of the proposed additive

Analyses were performed on four batches of Mannostab™ (IEEB, Bordeaux, full report accessible here).

Carbohydrates

The carbohydrate content of Mannostab™ is low, mainly in the forms of glucose and mannose (Table 10).

Table 10: The carbohydrate composition of Mannostab™.

Analysis	Unit	Results Batch number / production date			
		2 02 0516 16.05.02	1 34 8100 Déc. 2000	1 00 1220 20.12.00	1 00 1219 19.12.00
fructose	g/100g	<0.005	<0.005	0.040	<0.005
glucose	g/100g	0.230	0.230	0.910	0.780
mannose	g/100g	0.135	3.50	0.030	0.400
raffinose	g/100g	<0.005	<0.005	<0.005	<0.005
turanose	g/100g	0.080	0.170	0.155	0.245

Proteins and amino acids

The protein and amino acid contents of Mannostab™ are low, with three amino acids (alanine, aspartic acid and valine) dominating (Table 11).

Table 11: Protein and amino acid contents of Mannostab™ in different batches.

Analysis	Unit	Results			
		Batch number / production date			
		2 02 0516 16.05.02	1 34 8100 Déc. 2000	1 00 1220 20.12.00	1 00 1219 19.12.00
Total proteins	g/100g	6.70	7.10	8.20	8.30
aspartic acid	g/100g	<0.020	<0.020	0.02	<0.020
threonine	g/100g	<0.008	0.01	0.01	<0.008
sérine	g/100g	<0.009	0.01	<0.009	<0.009
glutamic acid	g/100g	<0.033	<0.033	<0.033	<0.033
proline	g/100g	<0.018	<0.018	<0.018	<0.018
glycine	g/100g	<0.007	<0.007	0.01	<0.007
alanine	g/100g	0.01	0.02	0.04	0.02
cystine	g/100g	<0.005	<0.005	<0.005	<0.005
valine	g/100g	0.01	0.02	0.03	0.02
methionine	g/100g	<0.004	<0.004	0.01	<0.004
isoleucine	g/100g	0.01	<0.007	0.02	0.01
leucine	g/100g	0.01	0.01	0.03	0.02
tyrosine	g/100g	<0.008	0.01	<0.008	<0.008
phénylalanine	g/100g	0.01	0.01	0.02	0.02
lysine	g/100g	<0.008	0.03	0.01	0.01
histidine	g/100g	<0.004	0.04	<0.004	<0.004
arginine	g/100g	<0.009	0.01	<0.009	<0.009

Lipids and sterols

Raw fat contents are consistently low (<1 %), with good stability observed (Table 12).

Table 12: Fat contents of Mannostab™ in different batches.

Analysis	Unit	Results			
		Batch number / production date			
		2 02 0516 16.05.02	1 34 8100 Déc. 2000	1 00 1220 20.12.00	1 00 1219 19.12.00
Total fat content	g/100g	0.47	0.37	0.31	0.33

Minerals and trace elements

Metal element content is low, with the dominant metallic element being iron (Table 13).

Table 13: Mineral contents of Mannostab™ in different batches.

Element	Unit	Results Batch number / production date			
		1 34 8100 Déc. 2000	1 00 1220 20.12.00	1 00 1219 19.12.00	2 02 0516 16.05.02
Iron	mg/kg	1.7	44	20	7.2
Selenium	mg/kg	0.060	0.030	0.050	0.040
Copper	mg/kg	-	-	-	-
Zinc	mg/kg	-	-	-	-
Calcium	g/100g	0.23	0.059	0.030	0.010
Phosphorus	g/100g	0.50	0.74	0.58	0.29
Magnesium	g/100g	0.055	0.069	0.036	0.0057

Vitamins

Vitamin content of Mannostab™ is low and close to detection limits (Table 14). Storage does not appear to alter the vitamin content.

Table 14: Vitamin contents of Mannostab™ in different batches.

Vitamins	Unit	Results Batch number / production date			
		2 02 0516 16.05.02	1 34 8100 Déc. 2000	1 00 1220 20.12.00	1 00 1219 19.12.00
Ergosterol	mg/kg	0.43	0.18	0.15	0.79
vitamin B1 (thiamine)	mg/kg	<0.1	<0.1	<0.1	<0.1
vitamin B2 (riboflavine)	mg/kg	0.4	0.4	0.8	0.3
Vitamin B3-PP (niacine)	mg/kg	<5	<5	<5	<5
vitamin B6 (pyridoxine)	mg/kg	<0.2	<0.2	<0.2	<0.1
Vitamin B9 (folic acid)	mg/100g	<0.0005	0.0015	0.0007	<0.0005
Vitamin D2 (ergocalciferol)	UI/kg	<200	<200	<200	<200

Metabolism and digestibility

Yeast cell walls are essentially comprised of β-glucans and mannoproteins, with the latter accounting for 25-50 % by weight in *S. cerevisiae*. An *in vivo* study by Adrian et al (1996) examined the digestibility of yeast cell walls, in which a control diet was compared with one containing 20 % dried yeast cell walls. The results indicated that the digestibility of the supplemented diet is high (92 %), being close to the control diet, and that the nitrogenous digestibility was particularly high indicating that cell wall proteins are available to the proteases of the digestive tract.

An *in vitro* study (Moine, 2003) compared the effect of reconstituted intestinal fluid on dry active yeasts, yeast cell walls and mannoproteins. The study found that active dry yeasts and yeast cell walls are hydrolysable into hydrocarbon compounds, and that the mannoprotein content analysis of the hydrolisate shows that the digestion of active dry yeasts and yeast cell walls results in the formation of mannoproteins. Thus, in terms of digestibility, mannoproteins behave like proteins.

Competition with digestive flora

During the Mannostab™ production process any trace of *S. cerevisiae* is eliminated, even though this strain is widely used in human and animal nutrition. A literature analysis indicates no competition mechanisms towards digestive flora.

2.6 Dietary implications of intake of the additive

Yeasts of the gender *Saccharomyces* are very widely used. Considering that Mannostab™ is extracted from yeast cell walls, and in order to characterise the risks linked to the ingestion of these mannoproteins, we focused on assessing exposures. We first enumerated the forms under which these yeasts are ingested. We then performed an *in vitro* study to quantify the amounts of mannoproteins potentially absorbed from the ingestion of *Saccharomyces'* living cells or cell walls. From these data and according to the results of consumption surveys, we were able to estimate the level of ingestion of mannoproteins and to assess the contribution of Mannostab™ to total exposure. Since French people are considered globally as over-consumers of alcoholic beverages and bread, we consider this to be representative of a worst-case scenario**.**

As Mannostab™ is a yeast-derived product it has no separate MSDS.

Human exposures to Saccharomyces cerevisiae

The present uses of yeasts of the gender *Saccharomyces* are manifold. From a literature search identified uses are:

1. Animal nutrition
    i. Protein or vitamin contribution
    ii. Nutritional supplements
2. Human nutrition
    i. Yeasts used in bakery, breakfast breads, brewery and vinification
    ii. Yeasts used as food supplements
3. In human medicine, in the treatment of different pathologies.

Since we were unable to find data on a possible transfer of mannoproteins from the consumption of animals having ingested yeasts, we did not explore this exposure path. The use of yeasts or yeast cell walls as food supplements is widespread. However, even if the doses are indicated on the packaging the ingestion of these products is not controlled. We are thus unable to estimate this contribution in a realistic manner. According to the BIAM databank (www.biam2.org), the species of yeast that is the most used in human medicine is *Saccharomyces cerevisiae*. These medical preparations have all been subject to a request for a full market approval, meaning they went through an evaluation of the AFSSAPS (French Drug Agency). The doses used show that the daily absorption through this path can be up to 360 mg of *S. cerevisiae* a day for 3month chronic treatments. Dosages for the uses of *Saccharomyces cerevisiae* in human nutrition are difficult to quantify. The only information we were able to find was provided by the Bakery Yeast Manufacturers Committee of the European Union (www.Cofalec.com), which indicated that, in a bakery, the dosage of yeast used ranges from 2-5 % (2-5 kg yeast/100 kg flour) and that 1g of baker's yeast contains about 1 billion *Saccharomyces cerevisiae* living cells.

Consumption levels in goods and beverages intended for human nutrition which may contain yeasts

The mean consumption of foodstuffs and beverages that may contain yeasts like *Saccharomyces cerevisiae* are listed in Table 15. These data were provided by the French consumption inquiry: INCA (Volatier 2000, in French). In this inquiry, the consumption was not calculated for snacks alone but for a mix of snacks, walnuts and almonds. Applying a precautionary approach, we considered that the

exposures should be calculated with the consumption value of the mix and not on a fraction extrapolated from non-scientific bases.

Table 15: Mean daily consumption of products that may contain yeasts.

Food Class	Consumer	Consumers rate (%)	Ingested amounts (g/day/person)
Bread, rusks, cereals	Adults	98	129
	Children	97	81
Pastries, viennoiseries	Adults	83	55
	Children	91	59
Wines and Champagnes	Adults	Ns	111
Beers	Adults	Ns	28

Ns: not specified

Search for mannoproteins in goods and beverages intended for human nutrition

In order to estimate the "normal" exposures to mannoproteins, Moine-Ledoux (2003) determined their concentration in commercial products that may contain them. The results of this study showed that if industrial bread contains no mannoproteins, organic bread or leaven bread contain between 750 and 1100 mg/kg of mannoproteins. The analyses of 11 commercial beers show mean contents of 192 ± 35 mg/L, ranging from 83 to 507 mg/L. These data show that, ingested volumes being the same, the contribution of mannoproteins in beer is higher than in wines (100 to 150 mg/L). In conclusion, this study confirms that fermentation products contain mannoproteins and that we are thus already exposed to them.

Assessment of exposure to mannoproteins from *Saccharomyces*

In order to calculate the contribution of Mannostab™ to the general exposure to yeast mannoproteins, we made the following calculations. The first calculation is based on the amounts of yeasts used in human nutrition and on the amounts of mannoproteins that may be released during digestion (34% by mass) and secondly on the results of the search for mannoproteins in the goods or beverages intended for human nutrition. In the second calculation, we used a worst-case scenario where the consumer would also be exposed to mannoproteins through medical treatment. Note that according to the INCA inquiry, children do not drink alcoholic beverages. As a consequence, risk will not be assessed for these consumers since Mannostab™ is intended for wine products consumed by adults.

Case 1

An adult consumes all foods containing yeasts within the amounts indicated in Table 11:

Mean consumption = 129 + 55 = 184 g/day/person

If every food contains yeast at the maximum rate of 5 % found in bread, and if 34 % by mass of mannoproteins are released from cell walls during digestion and in consideration that the cell wall represents 50 % of the weight of a cell:

Exposure = 184 x 0.05 x 0.34 x 0.50 = 1.56 g mannoproteins/day/person

The adults are also exposed by the consumption of alcoholic beverages, with mean natural mannoprotein contents of:

Wine: 100-150 mg/L (mean = 125 mg/kg)

Beer: 192 mg/L

Considering consumption levels, adult exposure to mannoproteins through beverages is:

Wine: 125 x 10^{-3} x 111 = 13.87 mg mannoprotein/day/person
Beer: 192 x 10^{-3} x 28 = 5.38 mg mannoproteins/day/person

Total exposure to mannoproteins is thus:

1.56 + 13.87 x 10^{-3} + 5.38 x 10^{-3} = **1.63 g mannoproteins/day/person**

The maximum use requested for Mannostab™ is 300 mg/L (300 ppm), hence the consumption of mannoproteins from Mannostab™ alone will be:

111 x 300 x 10^{-3} = 33.3 mg mannoproteins/day/person

Thus, total consumption of mannoproteins through diet will be:

1.63 + 33.3 x 10^{-3} = **1.66 g mannoproteins/day/person**

The contribution of Mannostab™ to the total exposure to mannoproteins thus equals **2 %.**

Case 2
The consumer is exposed to additional levels of mannoprotein through medical treatment.

Yeast intake can be up to 360 mg/day/person (section 9.1). If 34 % by mass mannoprotein is released from cell walls, which are 50 % by weight of the cell, then exposure is given by:

360 x 0.34 x 0.50 = 61.2 mg mannoproteins/day/person

Taking into account the contribution of food, including wine treated with Mannostab™, the total intake becomes:

1.66 + 0.061 = **1.72 g mannoproteins/day/person**

Thus, the contribution of Mannostab™ is **2 %**. By way of comparison, the contribution from medical treatments is 3.6 %.

Uncertainties

This risk assessment did not take into account the mannoprotein contributions of:

1. Animal nutrition
2. Food supplements
3. Special diets (for example dietetic food)
4. Other unlisted nutritional uses.

2.7 Advantage to the consumer of the additive

The consumer will have no direct dietary advantage by choosing products made with Mannostab™, however it is possible that the cost of said goods may be lowered due to increased production efficiency. Since such a decision would be up to the producer, we cannot speculate further.

PART 3 REGULATORY/LEGISLATIVE IMPLICATIONS

3.1 International standards

The current specification for yeast mannoproteins are included with this application for:

- The European Union (OIV).

3.2 International legislation

Currently, mannoproteins are authorised for usage in wines in:

- The European Union;
- Argentina.

"Preparations of yeast cell wall" are also permitted additives in the EU.

Mannostab™ has not been rejected or withdrawn by any regulatory bodies.

The "**Australian Treaty Series 1994 No. 6**" is relevant to this application. The treaty covers the agreement between Australia and the European Community of trade in wine. Therein is listed, for wines originating in Australia, under:

- "ANNEX 1" section 1. under the sub-heading of;
- "(a) authorized without any time limit" under clause;
- "(22) use of preparations of yeast cell wall, up to a maximum of 40 grams per hectolitre".

The same agreement exists for wines originating in the EU under section 2 clause (6) of ANNEX 1.

3.3 Regulatory impact statement

Cost implications

Since the use of Mannostab™ would offset current production costs associated with cold stabilisation and the contact process (i.e. refrigeration costs, purchase of potassium bitartrate, infrastructure and maintenance of refrigeration units, ethanol for coolant, personnel etc) for potassium bitartrate stabilising a wine, it is anticipated that there will be no increase in the cost of wine to the consumer. In the European market we have not observed any wine price increases in concert with usage of the product.

Importantly, Mannostab™ is a natural wine additive, which does not incur the costs, both economic and environmental, of traditional stabilisation treatments.

Profit implications

Advised by FSANZ that this section is not relevant.

Market share implications

Since Laffort Oenologie holds international patents for the production of Mannostab™, and no such equivalent product exists in the market, we anticipate 100% market share in terms of this type of additive. In terms of overall market share as pertaining to potassium bitartrate stabilisation of wine, we anticipate market share in the region of 5%. We anticipate that the acceptance of this product will be

greatest by those wineries interested in increasing production efficiency and lowering production costs, which we believe translates to the larger wine producers.

Price implications

Currently Mannostab™ sells in the EU between €180-220/kg. We anticipate a similar price on the Australian market. In terms of usage of the product, we do not anticipate any cost increase that will be passed on to the consumer, based on observations in the EU market since the release of Mannostab™.

Trade implications

Since an agreement already exists between the EU and Australia that covers the addition of mannoproteins to wine, no trade implications between these two bodies are anticipated.

The representative of Laffort Oenologie in the United States of America is currently initiating the process of including mannoproteins in the food standard. Mannostab™ has not undergone application for inclusion in the USA & Australian Food Standards until now because production capacity was limited, hence only certain markets could be serviced. The commissioning of a new production facility in Bordeaux in mid-2007 will allow production increases, hence this application and that in the USA.

The UK is our biggest wine export market. Since mannoproteins are a permissible wine additive in the EU, no trade implications are pertinent. On completion of this submission to FSANZ an application will be made to the relevant body in the USA to seek compliance.

Mannoproteins are currently permitted wine additives in Argentina.

Employment implications

We anticipate no negative employment implications through the use of Mannostab™, since existing refrigeration systems are usually fully automated. Additionally, since we anticipate only a small percentage of the market will use Mannostab™, effects on employment are likely to be low.

PART 4 ANALYTICAL PROCEDURES

4.1 Analytical method for additive

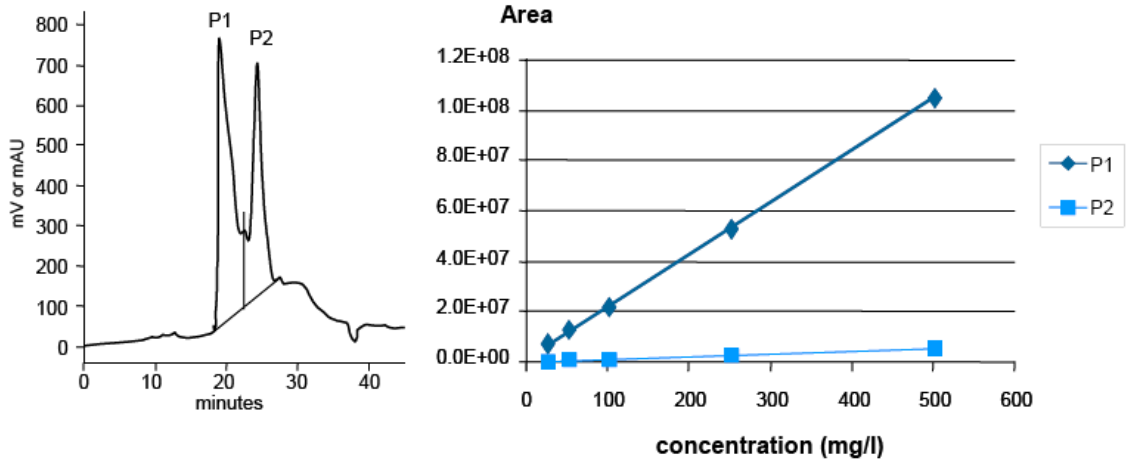
Mannoprotein concentrations are determined by Molecular Screening High Performance Liquid Chromatography (HPLC). An example of the results of such a determination is given in the Mannostab™ technical data sheet.

The mannoproteins are separated by High Performance Liquid Chromatography with a molecular screening on two serial set steel columns. The first column (0.75 x 7.5 cm), conditioned with trisacryl GF05 gel (IBF), separates the molecules by chromatographic exclusion. This 3,000 Da molecular exclusion weight gel is usually used for desalting in low pressure chromatography, but its good mechanical resistance allows supporting pressures of about 10 bar. The second column (0.75 x 60 cm), containing TSK G2000 SW (LKB) gel, is a molecular screening analytical column. The molecular exclusion weight of the column is 70,000 Da for globular proteins. The macromolecules are thus separated from the other components by exclusion chromatography on the first column and molecular screening chromatography on the second column. The analysis conditions are:

- Injected volume: 200µL
- Eluant: NaCl 0.1 M
- Flow: 0.6 mL/h (2150 HPLC pump)
- Pressure: 10 bar
- Recording speed: 0.5 mm/min (2210 recorder).

The mannoproteins are detected by spectrophotometry at 220 nm (2158 Uvicrod Sd). Calibration and identification – Figure 3) are realised by comparing the retention times to reference samples obtained from purified mannoproteins extracted according to the method described by Moine-Ledoux et *al.*, 1997.

Figure 3: Example of chromatogram and calibration curves for the mannoprotein (P1 & P2) dosage by



molecular screening HPLC. P1: y = 209209x + 742118; **r^2 = 0.999**. P2: y = 10665x + 0.5251; **r^2 = 0.999**.

4.2 Analytical method for by-products

No known or reasonably expected substances are formed in wine as a result of the use of Mannostab™. Since mannoproteins occur naturally in wine through yeast autolysis, any substances formed through the breakdown of Mannostab™ in wine will be in concert with those present due to the breakdown of the mannoproteins already present from yeast autolysis.

PART 5 DETAILS OF THE PROPOSED ADDITIVE

All publications in the international literature pertinent to this application are presented in "Publications part 1" (in French) and "Publications part 2" (some French, some English).

5.1 Identity of the proposed additive

5.1.1 Chemical name

Mannostab™ is not a pure chemical. It is biologically derived and is comprised of mannoproteins derived from the cell walls of *Saccharomyces cerevisiae* by enzymatic digestion.

5.1.2 Other names

Mannoprotein; yeast cell wall extract; Mannostab™.

5.1.3 Marketing name of additive

Mannostab™.

5.1.4 CAS registry number

As Mannostab™ is biologically derived and is not a discrete chemical, it has no CAS registry number.

5.1.5 Molecular and structural formula

As Mannostab™ is biologically derived and is not a single chemical, it has no discrete molecular structure that can be presented.

5.1.6 Molecular weight

The molecular weight range of the extracted mannoproteins is 30 - 40 kDa.

5.2 Chemical and physical properties

Relevant data for Mannostab™ are provided in Table 16.

Table 16: Chemical and physical properties of Mannostab™.

Colour	White or beige
Melting range	Decomposes upon heating
Odour	Nil
Oxidation stability	Stable for two years in a sealed container < 12 °C
Photolysis	Stable
Physical state	Powder
Solubility in organic solvents	Insoluble in ethanol
Solubility in water	Soluble
Thermal stability	Decomposes on excessive heating. Storage to be 4 – 12 °C

5.3 Impurity profile

A Technical Data Sheet for Mannostab™ is provided.

5.4 Standard for identity

The full OIV codex is supplied, which contains a specification for yeast mannoproteins.

Specification for Mannostab™

The OIV mannoprotein specification is provided. Selected specification limits are indicated in Table 17.

Mannostab™ is a mannoprotein extracted from the cell wall of the yeast *Saccharomyces cerevisiae* by enzymatic digestion using β-glucanase. The crude extract is purified by ultrafiltration with the mannoprotein concentrate being commercialised in solid form.

Table 17: Selected OIV specification limits for mannoproteins derived from *Saccharomyces cerevisiae*.

Parameter	OIV specification
Assay	> 600 g/kg as mannose
Ash	< 8 %
Appearance	White or beige powder; odourless.
Solubility	Soluble in water; insoluble in ethanol.
Optical rotation	$[\alpha]_D^{20}$ = 80-150° (c = 0.01 g/mL; l= dm)
Moisture content	< 4 %
Preparation of solution for trials	Prepare a 10 g/L solution in water
Heavy metals (other than lead)	< 30 mg/kg
Lead	< 5 mg/kg
Mercury	< 0.15 mg/kg
Arsenic	< 1 mg/kg
Cadmium	< 0.5 mg/kg
Total nitrogen	5 – 75 g/kg
Total aerobic mesophile flora	< 10,000/g
Coliforms	< 10 CFU/g
Staphylococcus aureus	None in a 1 g sample
Salmonella	None in a 25 g sample
Escherichia coli	None in a 25 g sample
Lactic bacteria	< 10^4 CFU/g in a 25 g sample
Mould	< 50 CFU/g
Yeasts	< 10^2 CFU/g

PART 6 MANUFACTURE AND TOXICOLOGY

6.1 Manufacturing process

(a) Comprehensive outline of the manufacturing process; and

(b) Full details of the analytical controls and quality assurance procedures used during manufacturing, processing and packaging of the additive

The mannoproteins of the yeast *Saccharomyces cerevisiae* are extracted by the enzymatic treatment of the yeast cell walls with a β-glucanase enzyme, specified as a permitted food processing aid in Standard 1.3.3, clause 17. This process (Figure 3) mimics the natural yeast lysis during fermentation or digestion releases mannoproteins, which are subsequently absorbed by humans.

Mannoproteins and glucans are the main components of the cell walls of the yeast *Saccharomyces cerevisiae*. Mannoproteins have different structures depending on their molecular weights and the degree and type of glycosylation. The β-glucanase enzyme used in the production of Mannostab™ hydrolyses the cell wall of the yeast that then allows the mannoproteins to be solubilized. All media involved in the production of Mannostab™ are of food grade. The product is obtained as a colourless, odourless powder or as a yellow translucent colloidal solution. The yeast and enzyme are both approved for use in Australia as food processing aids (Standard 1.3.3 clause 17).

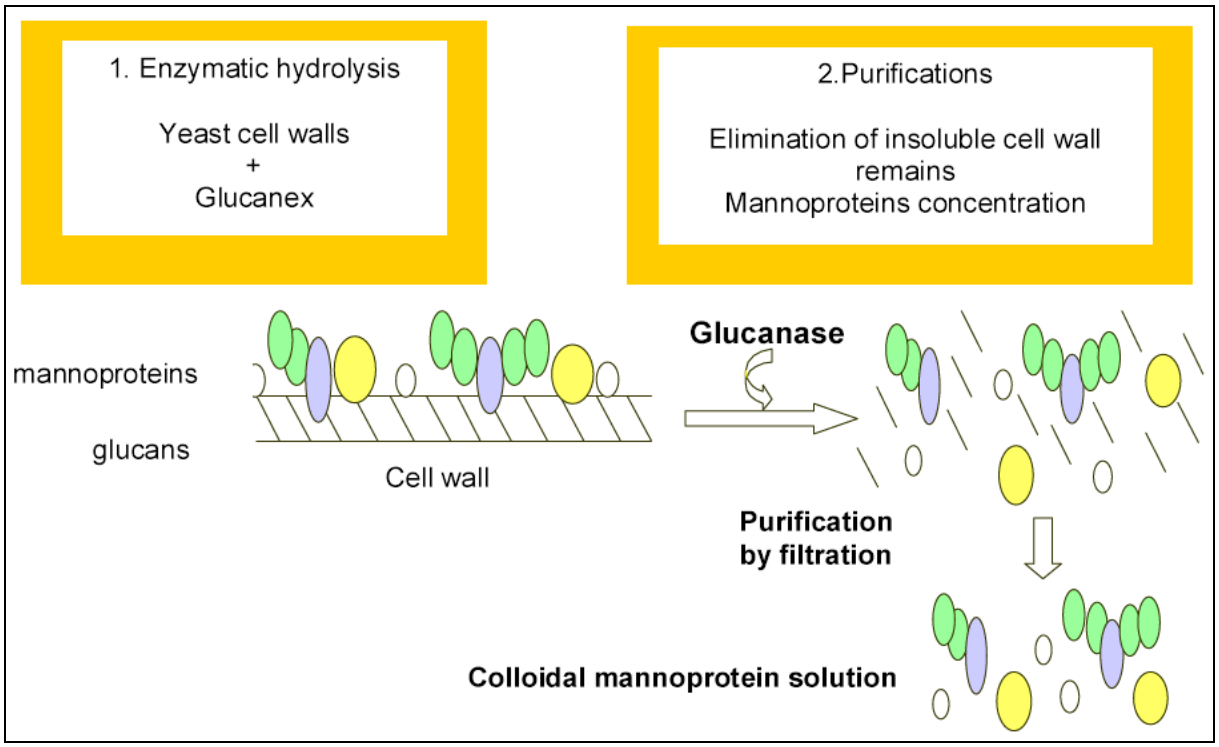


Figure 3: Process for the extraction of mannoproteins from *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae cell walls

The yeast cell walls used in the production of Mannostab™ are from a microorganism identified and classified as *Saccharomyces cerevisiae*. This strain is not genetically modified. The parameters of the hydrolysis conditions (ie pH, °Brix, odour etc) are monitored throughout the enzymatic digestion.

Safety and historical data

S. cerevisiae has been used by humans since at least 9000 BC, most notably in the production of bread and beer. The American Type Culture Collection (ATCC) classifies the biosafety of *S. cerevisiae* as Level 1 – "not known to cause disease in healthy adult humans". In the European classification for risks associated with biological agents this corresponds to Group 1: "A biological agent that is most unlikely to cause human disease".

Glucanex®

Glucanex® is a product of the Swiss company Novozyme (Glucanex® 200 from Novozyme, CH). It is a preparation of exo-1,3-β-glucanase obtained from non-genetically modified organisms.

Manufacturing process

Mannostab™ is produced by the hydrolysis of *S. cerevisiae* cell walls using a β-glucanase enzyme, as described in "6.1 b" above. All ingredients and devices used in the production procedure are of food grade.

Hygiene

The production for Mannostab™ unit is certified in accordance with ISO 9001 and HACCP. The yeast cell walls are controlled before use. Glucanex® 200 is quality-assured before sale, including the determination of heavy metal and microbiological contamination levels. Appropriate cleaning and washing procedures are implemented to ensure appropriate standards of hygiene are maintained. Each batch of Mannostab™ is sterilised by filtration and analysed to certify the levels of chemical and microbiological contaminants.

Preservation

If kept sealed in a dry location at 20 °C Mannostab™ has a very long lifespan. Under these conditions Mannostab™ is stable for a minimum of 22 months. If in a colloidal solution, the product must be stored in a hermetically sealed container prior to use.

6.2 Toxicology

6.2.1 Summary of toxicology data

For a **full report** on the toxological studies of Mannostab™, including original reports, see the accompanying document "Sensitization studies of Mannostab".

Preamble

In order to evaluate the safety of the mannoproteins issued from *Saccharomyces cerevisiae*, we made several literature searches in 2003. Our purpose was to identify works on the toxinogenic or pathogenic potential of the strain *Saccharomyces cerevisiae*, and possible mutagenic, acute or after repeated administration toxicity effects of cell wall mannoproteins.
The mannoproteins belonging to the glycoprotein family, we also looked for works on immune or allergising effects of these products.
These searches were conducted cross-using the following keywords:

- *Saccharomyces*
- *Saccharomyces cerevisiae*
- Yeast*
- Mannoprot*
- Glycoprot*
- Adverse effect*

- Toxic*
- Muta*
- Allerg*
- Immun*
- Antigen*
- Use*

The databanks we interrogated were:

- Chemical Abstract Series (1967-2003)
- Life Science Collection (1978-1995)
- Biosis Previews (1969-1995)
- Medline (1966-2003))
- Toxline (1965-2003)

To increase our hits, our search was completed with a search on the "world wide web" using Copernic®.

Background

Yeasts of the gender *Saccharomyces* are largely used ever since man discovered fermentation. As early as the Stone Age (about 9 000 years BC), countries in the Middle East did grow cereals and some authors think that the making of bear and bread started at that period. In Egypt, about 5 000 BC, salaries were paid in beer which was manufactured according to the "barley breads" technique. Egyptians and Babylonians knew, 3 000 BC, how to enhance the fermentation activity of wild yeasts, by mean of leaven, to make dimpled bread instead of the traditional compact pancake. The first century BC, the Celtics knew the malting phase and, except the technological means, bear mashing was surprisingly nearly the same as it is nowadays. But it was only between 1857 and 1863 that Louis Pasteur demonstrated the role played by yeasts, as the micro-organism responsible for fermentation. He noted at that time that "*All yeasts* that ferment bread, bear, wine, cider are corresponding to a population of living cells of a microscopic fungus, *Saccharomyces cerevisiae*. A number of varieties of *Saccharomyces cerevisiae* exist in nature and are more or less adapted to these different fermentations" (www.cofalec.com ; www.inox.qc.ca/origines.asp).

Nowadays, the use of yeasts is not limited to the production of bread or fermented beverages. Indeed, because of their nutritional characteristics (protein, vitamin, mineral and amino acid content), preparations of living cells or yeast cell walls are commercialised as food supplements or medicine. During the second world war, such preparations were recommended by the WHO as a protein substitute in animal nutrition (Annex 3). Still today, *Saccharomyces* supplements for animal nutrition can be found on the market (www.anima-strath.com ; www.pubnix.net …).

Animal toxicity

An exhaustive literature search identified toxicological studies on the effects of *Saccharomyces cerevisae* or yeast preparations (lysates, walls, extracts,…) on animals. In a communication at the FAO's technical committee, Schmidt (1953) indicated that during the first half of the twentieth century, Germany had great problems in the provisioning in protein matters intended in animal nutrition. A number of works were undertaken to replace these proteins by yeasts like *Saccharomyces cerevisaie* and *Torula utilis*. Trials were performed on poultry, trout, bovine and pig. They resulted in the proposition of a diet containing 5% yeasts (dry matter). In the nutrition of chick this content could rise up to 25% of dry matter. More recently, a study on chicken (Poo et Millan, 1990) showed that a 50% substitution of the protein ratio with *Saccharomyces carlsbergensis* cells induced no metabolic effect. These works demonstrated the innocuousness of the yeasts or yeast lysates for adult or growing animals and this for different species. In the context of Community Regulation, the Council authorises living *Saccharomyces cerevisiae* yeasts or as lysates in animal nutrition on any animal species and with no restriction on the amounts to be used (Directive 82/471/EEC and amendments).

In Northern America, animal nutrition supplements made of *Saccharomyces cerevisiae* lysates are commercialised. The amounts to use vary considering a small (cat, rabbit, guinea pig, hamster, chicken, bird, fish), a medium sized (dog, goat, sheep) or a big animal (horse, cow, beef, calf, pig). There is no limit on the duration of the treatment. On the contrary, the manufacturer recommends a continuous daily administration (www.anima-strath.com).

Human toxicity

An exhaustive literature search shows that even if the oral absorption of yeasts is important because their numerous nutritional and medical uses, **no toxicological data are available on these microorganisms or preparations issued from them (lysates, walls, extracts etc.)**
The only relevant information is that *Saccharomyces cerevisiae* is not listed as a pathogenic or toxinogenic agent by the European Community.

In human nutrition, we identified multiple and various uses of yeasts. For example:

- Yeasts as are in oenological treatment, bakery and brewery,
- Cell walls in oenological treatment,
- Enzymatic preparations issued from yeasts as technological aids,
- Food supplements

Surprisingly, and to our knowledge, only few of these uses fall into a National or European Regulation.

1. Concerning the invertase issued from *Saccharomyces cerevisiae* :
   - arrêté du 5 septembre 1989 relatif à l'emploi de préparations enzymatiques dans la fabrication de certaines denrées et boissons destinées à l'alimentation humaine (OJ du 1.10.89).
   - Commission Directive 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners (JO L 339 of 30.12.1996).
2. Concerning the use of yeasts as are or in the form of leaven in bread manufacturing:
   - European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners (OJ n° L 61 of 18. 3. 1995).
   - arrêté du 2 octobre 1997 (J.O. du 08-11-1997) relatif aux additifs pouvant être employés dans la fabrication des denrées destinées à l'alimentation humaine.
3. Concerning the yeasts as are in oenological processes:
   - Council Regulation (EC) No 1493/1999 of 17 May 1999 on the common organisation of the market in wine (OJ L 179 of 14.7.1999) annex IV- list of authorised oenological practices and processes.
4. Concerning yeast extracts (crusts, lees) in oenological processes:
   - Council Regulation (EC) No 1493/1999 of 17 May 1999 on the common organisation of the market in wine (OJ L 179 of 14.7.1999) annex IV- list of authorised oenological practices and processes.
   - Commission Regulation (EC) No 1622/2000 of 24 July 2000 laying down certain detailed rules for implementing Regulation (EC) No 1493/1999 on the common organisation of the market in wine and establishing a Community code of oenological practices and processes (OJ of 31.7.2000, L 194/1).

For most of these authorizations, they were granted on criteria concerning their uses (oenological processing, baking etc). However, the authorization of the invertase as a food processing aid was given subsequently to an authorization to use request at the *Direction Générale de la Consommation, de la Concurrence et de la Répression des Fraudes* (DGCCRF). This request was examined by the French Superior Council of Public Health on the basis of a scientific dossier demonstrating the innocuousness of the strain, the process to obtain the enzymatic preparation and the enzymatic preparation itself.

The uses of yeasts or yeast cell walls as food complements are numerous. For deontological reasons, no trademarks will be cited here, but a quick search on the internet returns many results. Concerning *Saccharomyces cerevisiae*, and without taking into account any unusual "therapeutic" uses, the generally used doses range from 4 to 6 capsules of 500 mg a day for a 3 to 4 months treatment. For some of these products, containing living cells within the recommended doses, the ingestion is corresponding to 40 to 60 billion ingested living cells a day.

To our knowledge, no adverse effect has been recorded following the ingestion of these food complements. Yeasts are also used in human medicine into different preparations. The yeast species which is the most used in medicine is *Saccharomyces cerevisiae*. The medical preparations in these tables have all been subject to a request for a full market approval meaning they went through an evaluation of the AFSSAPS (French Drug Agency). The posologies show that the daily absorption can rise up to 360 mg of *S. cerevisiae* a day for 3 months chronic treatments.

6.2.2-6.2.10 Toxicological profile

(i) Oral toxicity

An exhaustive literature search shows that even if the oral absorption of yeasts is important because of their numerous nutritional and medical uses, no toxicological data are available on these microorganisms or preparations issued from them (lysates, walls, extracts etc.). The only relevant information is that *Saccharomyces cerevisiae* is not listed as a pathogenic or toxinogenic agent by the European Community.

(ii) Dermal toxicity

A cutaneous irritation study was conducted according to Table 18.

Table 18: Cutaneous irritation protocol adopted for the testing of Mannostab™

Reference:	Richeux F. (2002) Assessment of acute irritant/corrosive effect on the skin, Phycher Bio Développement, report n°IC-OCDE-PH-02/0051
Protocol:	OECD 404 (1992) and Directive 92/69/EEC (1992), method B4
Species/strain:	Albinos rabbit, New Zeeland strain
Number of animals:	3 males,
Tested Substance:	Mannostab™
Batch n°:	10064/06-2000
Dose:	0.5 g ou 0.5 ml
Certification:	GLP and QA signed

The substance was applied (0.5 g) by means of a semi-occlusive dressing on a healthy skin part of the right flank of each animal. On the left flank and under the same conditions 0.5 mL of distilled water were applied on an equivalent zone of healthy skin. The cutaneous reactions were evaluated 1, 24, 48 and 72 hours after dressing removal. No macroscopic cutaneous reaction (erythema, oedema) was observed, in any of the animals and regardless of the exposure time.

(iii) Inhalation toxicity

See (i) above.

(iv) Eye irritation

An ocular irritation study was conducted according to Table 19

Table 19: Ocular irritation protocol adopted for the testing of Mannostab™

Reference:	Richeux F. (2002) Assessment of acute irritant/corrosive effect on the eyes, Phycher Bio Développement, report n°IO-OCDE-PH-02/0051
Protocol:	OECD 405 (1987) and Directive 92/69/EEC (1992), method B5
Species / strain:	Albinos rabbit, New Zeeland strain
Number of animals :	3 females
Tested substance:	Mannostab™
Batch n°:	10064/06-2000
Dose:	100 mg
Certification:	GLP and QA signed

The substance was applied neat (0.1 g) in one eye, the other serving as a control. Ocular reactions were evaluated 1, 24, 48 and 72 hours after instillation. Ocular reactions stayed very low and were limited to the conjunctiva: lacrimation and enanthema of very low-intensity were observed one hour after application. These effects were totally reversible after 3 days. Mannostab™ is considered as "weakly irritating for the eyes" according to the scale described in the Official Journal of the French Republic, dated 10th of July 1992.

(v) Skin irritation

See (ii) above.

(vi) Skin sensitisation

A sensitisation study was conducted in 2003 using Mannostab™ on Albino guinea pigs as indicated in Table 20.

Table 20: Sensitisation protocol adopted for the testing of Mannostab™.

Tested Substance:	Mannostab™
Batch n°:	10064/06-2000
Concentrations:	
1st induction:	2 intradermal injections (0.1 ml) of Freund adjuvant at 50% in physiological serum, 2 intradermal injections (0.1 ml) of the substance at 40% in physiological serum, 2 intradermal injections (0.1 ml) of a mixture of (V/V) Freund adjuvant at 50% and substance at 80% in physiological serum,
2nd induction:	topical application of the substance (pure), 18 days rest,
1st release:	topical application under occlusive dressing at concentrations of 50% and 25% for 48h, 11 days rest,
2nd release:	topical application under occlusive dressing at concentrations of 10% and 5% for 48h.
Certification:	GLP and QA signed
Reference:	Richeux F. (2002) Assessment of sensitising properties on albino guinea pig, maximisation test according to Magnusson and Kligman. Phycher Bio Développement, report n° SMK-PH-02/0051
Protocol:	OECD 406 (1992) and Directive 96/54/EEC, method B6
Species/strain:	Albinos guinae pig, strain Dunkin-Hartley
Number of animals:	37 females, 7 for the preliminary tests, 10 for the controls and 20 for the treated

The induction phase was sequenced into three periods. It commenced with the intradermal injection of Freund adjuvant, the substance and a mixture of the two. Eight days later, it was continued by the application of a 10% solution of sodium lauryl sulphide and the day after by a topical application of the pure substance. The release phase took place after 18 days of rest by a topical application under occlusive dressing of the substance during 24 hours. The evaluation of cutaneous reactions took place after 24 and 48 hours. A second release phase was performed after 11 days of rest by a topical application under occlusive dressing of the substance during 24 hours. The evaluation of cutaneous reactions took place after 24 and 48 hours.

The preliminary tests showed the absence of necroses by intradermal injection at the highest dose (40%). The topical application under occlusive dressing during 24 hours at the maximum dose of 100% induced no cutaneous reaction. The topical application under occlusive dressing during 24 hours at the maximum dose of 100% after intradermal induction with physiological serum and topical application of distilled water showed a slight erythema in two animals treated with the highest dose. After the first release phase, a macroscopic cutaneous reaction was noted (moderate erythema) in 5% of the animals of the treated batch (1/20), 24 and 48 hours after removal of the occlusive dressing, at the 50% treated site. No reaction of cutaneous intolerance was observed either in the negative control batch or the 25% treated batch. A second release phase was performed to confirm or invalidate these results after 11 days of rest. No macroscopic cutaneous reaction from an allergenic reaction was noticed during the readings that followed the removal of the occlusive dressings.

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P. Ribéreau-Gayon, Y. Glories, A. Maujean and D. Dubourdieu (2006a) Handbook of enology – the chemistry of wine stabilization and treatment, volume 2 (2nd ed.). Wiley & Sons. Ltd (Chichester), pp 43-46.

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INDEX OF RELEVANT SUPPORTING DOCUMENTS

1. Handbook of Enology section on yeast mannoproteins 2006.
2. Argentina legislation.
3. Australian treaty series (relevant section).
4. European Union (EU) oenological codex 2006 (relevant section).
5. EU regulation permitting “preparations of yeast cell wall”.
6. EU regulation permitting yeast mannoproteins for tartrate and protein stabilisation of wine.
7. EU list of authorised oenological practices and processes (relevant section).
8. Fosters Mannostab™ information request.
9. Laffort Oenologie GMO certificate.
10. IEEB Bordeaux nutritional studies on 4 batches of Mannostab™.
11. Laffort Oenologie ISO9001 certificate.
12. Laffort Oenologie HACCP certificate.
13. Mannostab™ technical data sheet.
14. OIV mannoprotein specification.
15. Publications relating to mannoproteins part 1: Moine (1997) publication on method for the identification and quantification of mannoproteins.
16. Publications relating to mannoproteins part 2.
17. Sensitizing and irradiation studies of Mannostab™.
18. Volatier (2000): Data on the consumption of food containing yeasts or parts of yeasts.
19. Winemakers Federation of Australia endorsement for mannoproteins.