THE POTENTIAL USE OF YEAST LEES (1-3, 1-6)-B-GLUCANS AS FUNCTIONAL FOOD INGREDIENTS

Francesca CECCHINI*, Noemi BEVILACQUA, Barbara GIANNINI, Massimo MORASSUT

CRA (Council for Agricultural Research and Economics) - Unit for Oenology in Central Italy (ENC).

Via Cantina Sperimentale, 1, 00049, Velletri (Rm),Italy

*Corresponding author-mail: francesca.cecchini@entecra.it

Introduction

Soluble fiber such as β-glucans are widely distributed in nature, especially in algae, fungi and yeast. They form the major structural components of cell walls, and act as storage carbohydrates. Yeast β-glucans serve a variety of biological functions, substantially enhance the functions of the immune system by activating macrophages, one of the primary defenses of the immune system (Chen et al. 1993; Rop et al 2009). They sometimes play a protective role by forming, at specific sites, in response to particular stimuli such as wounding (Kogan and Kocher 2007). Researches indicate that the potent anti-tumor properties of polysaccharides fractions extracted of certain strains of mushrooms, can be attributed to linear 6-branched (1-3)-β-glucan (Ohno et al. 1985). Similar properties have been assigned to (1-3)(1-4)-β-glucans from cereal grains (e.g. oats and barley). Soluble fiber sources, including oats, have consistently been shown to lower serum cholesterol concentrations independently of alterations in fat intake (Othman et al 2011). Several studies have indicated that consumption of oat bran lowers blood cholesterol and this effect has been attributed specifically to oat bran's soluble fiber (β-glucan) (Braaten et al. 1994; Othman et al 2011). The main component of the soluble fiber of oats, β-glucan, significantly reduced the total and LDL cholesterol levels of hypercholesterolemic adults without changing HDL cholesterol (Kerckhoffs et al. 2003; Langella et al. 2015).

Winemaking is a biochemical process of transformation of the grape must in wine, carried out by yeasts. The cell wall of the yeast, contains β-glucan, chitin and mannoproteins. The yeast cell of *Saccaromyces cerevisiae*, the principal yeast used in winemaking, is known to consist of three layers: an inner layer of alkali-insoluble β-glucan (30-35%); a middle layer of alkali-soluble β-glucan (20-22%); an outer layer of glycoprotein (30%) in which the carbohydrate is composed of phosphorylated mannan. The main difference in chemical structure between soluble and insoluble glucan is the number of β-(1→6)-linked glucose residues which are present in the long sequences of β-(1→3)-linked glucose chains (Lipke and Ovalle 1998). At the end winemaking process, between the products so-called “discarded products” of winemaking besides seeds , skin, and pomace, that represent a important source of nutraceutical compounds with high antioxidant power, there are the dead yeast cells. After the yeasts have exhausted their life cycle, they fall to

the bottom of the fermentation tank as sediment known as yeast lees. The yeast lees, therefore, can be considered a waste product of winemaking. The yeast lees isolated from wine after fermentation represent a product rich of β-glucans that can be extracts and utilized as functional food ingredient.

The aim of this work was to extract and to determine the β-glucans isolate from yeast lees obtained at the end alcoholic fermentation from white and red grape must.

Material and Methods

Winemaking. The vinifications were made using *Vitis vinifera*, L. white Malvasia del Lazio (MLV) cultivar and red Cabernet Souvignon (CS) cultivar. For every trial, three independent replications were made for each of the two grape cultivars used.

MLV grape was crushed, destemmed and pressed using a basket press. 50mg/L SO_2 was added and placed into 100 L stainless steel wine vat. Then gelatine (8 g/hL) and silica gel (25 mL/hL) was added to the must and kept at 7°C for 24 hr, followed by filtration. CBS grape was crushed, destemmed and both pulp and the solid parts (skins and seeds) were placed together into 100 L stainless steel wine vat and 50 mg/L SO_2 was added. Before fermentation the musts were inoculated with pure cultures of *Saccaromyces cerevisiae* S6u (Lallemand, Canada) yeast strain (20g/hL). The fermentations of the inoculated white must was carried out at a controlled temperature (18°C), while the fermentations of the inoculated red must was carried out at a controlled temperature (25°C). After the start of the alcoholic fermentation of CBS must, fermenting wine was punched down twice a day, until the cap remained submerged. At the end of the alcoholic fermentation the red wines were pressed and both red and white wines racked to eliminate the gross suspended solid, and then stabilized for 1 month at 4°C before of the analysis. After this period the lees were separated from wine by centrifugation at 4000 rps for 20 min. The supernatant was discarded and the pellet contenting the yeast lees was resuspended with 50% (v/v) aqueous ethanol solution and mix well by vortex, and then centrifuged at 4000 g. for 20 min. The supernatant was discarded and the pellet contenting the yeast lees was dried at 80°C up to constant weight. 20 mg of the dried yeast lees was treated for extraction and determination of the β-glucans.

Enological parameters. Reducing sugars of the musts and alcoholic content of the wines were determined according to standard methods (O.I.V. 1991).

Determination of (1-3)(1-6)-β-glucans. The determination of β-glucans was carried out using the enzymatic yeast β-glucans assay (Megazyme) following the manufactory instructions. Briefly: at 20

mg of dried yeast lees was added 2 N potassium hydroxide with stirring and the solution was subsequently adjusted to pH 4.0 -4.5 with 1.2 M sodium acetate buffer (pH 3.8) mixed well and then 40µL of enzymatic suspension (exo-1,3-β-glucanase, endo-1,3- β-glucanase, β-glucosidase and chitinase) was added and left for 16 h. at 40°C. After dilution with 10mL of water and centrifugation, an aliquot of 0.1 mL was added with 4 mL of reagent enzymes (Glucose oxidase, peroxidase, and 4-aminoantipyrine) for 20 min. at 40°C. Finally, the absorbance of solution was read at 510 nm against a reagent blank (consisting in 0.1 mL of sodium acetate buffer + 4 mL of glucose oxidase/peroxidase reagent). The results was expressed as β-glucan mg/g of dried matter.

Statistical analysis. The statistical analysis of the β- glucans content was carried out with analysis of variance (ANOVA), and the means were compared with the least significant difference (LSD) test. For data analysis, the Statistic package (Version 7.1, StatSoft Inc., Tulsa, OK) was used.

Results and Discussion

In table 1 are reported the sugar content in must and the ethanol content in wine, from white and red cultivars respectively.

Table 1. Reducing sugars and ethanol %(v/v) in white and red musts and wines

Wine	Reducing sugars g/L	Ethanol %(v/v)
MVL must	195 ± 1.8	-
MVL wine	-	11.50 ± 0.03
CBS must	200 ± 2.2	-
CBS wine	-	11.92 ± 0.01

Values represent the mean of three replications ± standard deviation

In figure 1 are reported the β-glucans content obtained from yeast lees isolated from white and red wines at the end fermentation. The results show that all the yeast lees obtained both red and white wines, contain β-glucans. However the β-glucans content was significantly higher ($p \leq 0.01$) in yeast lees obtained from red wine.

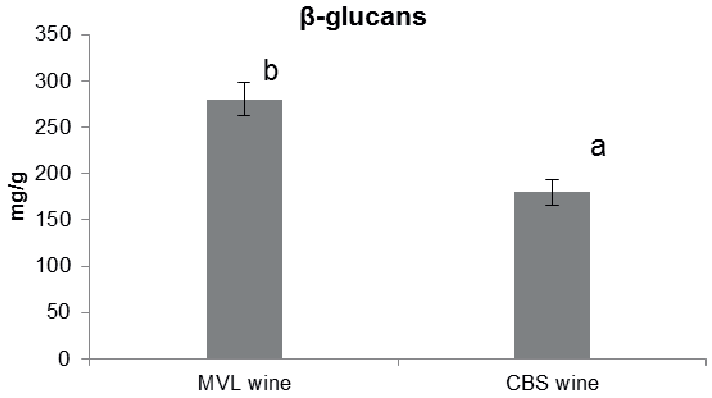

Figure 1. β-glucans content in the dried yeast lees extract from white MVL and red CBS wines. Values represent the mean of three replications. Bars indicate ± standard deviation. Different letters indicate significant differences (p ≤ 0.01).

The differences observed between lees from yeast obtained from white and red wines can be ascribable to the different alcoholic fermentation process. During fermentation, there are several factors to take into consideration, with the most influential to ethanol production being sugar content in the must, the yeast strain used, and the fermentation temperature. In this work, white wine was fermented 18°C, while the red wine was fermented at higher temperature of 25°C. Fermentation at higher temperatures might have adverse effect on the hydrolysis of the cell wall of the yeast. The macromolecular components of the yeast cell wall, are partially released during alcoholic fermentation. The release of these macromolecules is the result of an enzymatic autolysis of the lees. The activity of endogenous β-glucanases present in the yeast cell wall increase with temperature. Therefore, during red alcoholic fermentation, due to the activity of β-Glucanases, the yeast could release a higher content of β-glucans into wine with respect to a white alcoholic fermentation. Probably, for this reason the yeast lees from red fermentation contain a lower content of β-glucans.

In addiction the glucans obtained as a by-products of the alcoholic fermentation can be used as a integrator food for celiac food because gluten-free at differences of the glucans extract from cereals.

Conclusion

The interest on developing product and processes for winery residues is increasing and this is evident from the number of scientific publications. The yeast lees represent a by-product of winery industry with a sufficiently high content of β-glucans. Therefore, since the β-glucans have showed a beneficial properties for humans, the yeast lees represent a waste product ideal as a raw material for the manufacture of β-glucans.

References

Braaten J.T., Wood P.J., Scott F.W., Wolynetz M.S., Lowe M.K., Bradley-White P., Collins M.W. (1994). Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J Clin Nutr. 48(7):465-74.

Chen, J.T., Hasumi, K. (1993). Activation of peritoneal macrophages in patients with ginecological malignancies by sizofiran and recombinant interferon-gamma. J. Biotherapy, (6): 189-194.

Kerckhoffs D.A.J.M. , Hornstra G., Mensink R.P. (2003). Cholesterol-lowering effect of β-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am. J. Clin. Nutr. (78): 221-227.

Kogan G., and Kocher A. (2007). Role of yeast cell wall polysaccharides in pig nutrition and healt protection. Livestock Science (109), 161-165.

Langella C., Naviglio D., Marino M., Gallo M. (2015). Study of the effects of a diet supplemented with active components on lipid and glycemic profiles. Nutrition., 31(1):180-186.

Lipke P.N., And Ovalle R. (1998).Cell Wall Architecture in Yeast: New Structure and New.Challenges. J. of Bacteriology. 180, (15): 3735–3740.

Ohno, N., Lino, K., Takahiro, T. et al. (1985). Structural Characterisation and Antitumour Activity of the Extracts from Matted Mycelium of Cultured *Grifola frondosa*. Chem. Pharm. Bull., 33, 3395-3401.

O.I.V. (Organisation Internationale de la Vigne et du Vin) (1991). Recueil des méthodes internationales d'analyse des vins.

Othman R.A., Moghadasian, M.H., Jones P.J. (2011). Cholesterol-lowering effects of oat β-glucan. Nut. Rev. 69 (6): 299-309.

Rop, O., Mlcek, J., Jurikova, T. (2009). Beta-glucans in higher fungi and their health effects. Nutr. Rev. 67 (11): 624-631.

Abstract

The interest on developing product and processes for winery residues is increasing and this is evident from the number of scientific publications. The yeast lees represent a by-product of winery industry with a sufficiently high content of β-glucans.

β-glucans one of the major yeast cell wall components of yeast, play multiple healthy functions, between theme the reduction of blood cholesterol. The aim of this work was to extract and to determine the β-glucans isolate from yeast lees obtained at the end alcoholic fermentation from white and red grape must. The determination of β-glucans was carried out using the enzymatic yeast β-glucans spectrophotometric assay. The results show that all the yeast lees obtained both red and white wines, contain β-glucans. However, the higher β-glucans content was found in yeast lees obtained from white wines.

Keywords: β-glucans, yeast lees, winery by-product.