Ohly Application Note

MOS Products: Not every Yeast Cell Wall is created equal

Article Date: 18/01/2019

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Abstract: Yeast cell walls, commonly referred to as MOS, are widely used by the animal feed industry as antibiotic replacers. However, there are an increasing number of reports from farmers that their performance under farming conditions is not consistent. This is because yeast cell walls can be highly variable in composition and structure, depending on the growth conditions of the yeast, the yeast strain, and the yeast cell wall production process.

Therefore, a shift in mind-set is required, where much more attention is paid to the robust production of the yeast cell walls. Only then can the future use of yeast cell walls by farmers as trusted ingredients to improve animal health and performance be secured.

Keywords: Yeast Cell Wall, MOS, β-glucan, Composition, Consistency, Structure, Variability, Antibiotic replacement

Introduction

Yeast cell walls, commonly referred to as MOS, are rich in mannan oligosaccharides (MOS) and (1,3)(1,6)-β-D-glucan, two natural functional polysaccharides with known health improving properties. Some 1000 scientific papers have proven their benefits and have shown that yeast cell walls can improve animal performance with respect to average daily gain and feed conversion ratio, as well as the health status of animals (Krüger and van der Werf, 2019a; Krüger and van der Werf, 2019b).

With the increasing trend to ban the sub-therapeutic use of antibiotics in husbandry and aquaculture, the feed industry is looking for alternatives that are able to limit or reduce the impact of bacterial infections in animal production. Given the health benefits of yeast cell walls, they are increasingly used in the animal feed industry (Spring, P. et al., 2015; Credence Research, 2018).

However, despite the well-accepted health benefits of yeast cell walls/MOS and all the scientific evidence to that effect, there are an increasing number of reports from farmers that their performance under farming conditions is not consistent. This is because yeast cell walls can actually be highly different in composition.

What are Yeast Cell Walls?

Traditionally, yeast cell walls are a by-product from the yeast extract production industry (www.yeastextract.info). When producing yeast extracts, yeast cells are lysed by either yeast own enzymes (autolysis) or by added enzymes (hydrolysis). After cell lysis is completed, the soluble intracellular fraction (the yeast extract) is separated from the insoluble yeast cell wall fraction by centrifugation (Fig. 1).

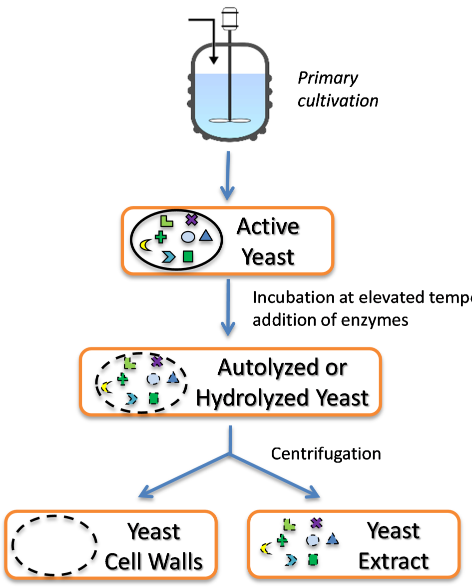

Figure 1: Production of Yeast Cell Walls

The production of 1 kg of yeast extract results in ≈ 0.5 kg of yeast cell wall by-product. Traditionally, yeast cell walls were sold at (very) low prices to farmers close to the yeast extract factory in a liquid form (≈12-15% dry matter), because of its protein content (20-30%) and because it is a palatant, especially for pigs.

With the increasing interest from the animal feed industry in yeast cell walls as antibiotic replacers, companies emerged that collected the liquid yeast cell walls from different yeast extract producers. They dried the yeast cell walls, increasing their shelf life from some 7 days to >2 years, allowing the global distribution of these products.

Upon even further growth of the demand for yeast cell walls-based products and/or because of cost reasons, more recently there are companies that use spent yeast from breweries, distilleries or bioethanol production plants as the raw material for the production of yeast cell walls for animal feed.

Yeast Cell Wall Composition is highly Variable

Yeast cell walls contain three main sugar polymers: β-glucan, mannan oligosaccharides (in the form of mannosylated proteins) and chitin (Table 1).

Table 1: Macromolecular composition of Saccharomyces cerevisiae cell walls

Macromolecule	Content (% DM)	DP*
Mannoproteins	25-70	200
Glucan	30-60	1500
Chitin	1-8	190

*Degree of Polymerisation
** Aguilar-Uscanga and François, 2003; Klis, F.M., et al., 2002; Orlean, P., 2012.

It was long thought, that the cell wall of yeasts is a relatively static structure with limited changes in composition. However, in the last decades it has been demonstrated undisputedly that the opposite is the case: yeast cell walls are highly variable in composition as well as in structure (Klis, F.M., et al., 2002, Orlean, P., 2012).

There are several factors that influence the yeast cell wall composition:

i. Growth conditions of the yeast

The composition of the yeast cell wall is highly dependent on the fermentation conditions of the yeast. It is dependent on the carbon source, nutrient availability, oxygen availability, external pH, temperature, stress, etc. (Aguilar-Uscanga and François, 2003; Ganner and Schatzmayr, 2012; Klis, F.M., et al., 2002; Kwiatkowski and Kwiatkowski, 2012; Lesage and Bussey, 2006). In Table 2 it is shown that depending on the growth conditions, the glucan and mannan content of yeast cell walls can be up to 100% higher or lower, and an even higher variation in chitin content was observed.

Table 2: Effect of Growth conditions on Cell Wall Composition of Saccharomyces cerevisiae* *(% w/w)*

Growth Condition	β-Glucan	Mannan	Chitin
Glucose	34	51	5.2
Sucrose	41	35	4.8
Ethanol	36	54	6.4
pH=3	34	35	6.9
pH=6	41	38	6.4
22 °C	48	34	5.2
30 °C	37	52	5.5
37 °C	57	31	7.9
$pO_2 \approx 0$	42	67	1.4
$pO_2 > 50\%$	34	50	5.2

*from Aguilar-Uscanga and François, 2003

Moreover, not only the total amount of cell wall sugars change, but also the structure of these polymers (i.e. DP, degree of branching, length of the branches, etc) are affected by the growth conditions, as well as the yeast cell wall mass as a percentage of total cell mass (Aguilar-Uscanga and François, 2003; de Groot, P.W.J., et al., 2005; Ganner and Schatzmayr, 2012).

ii. Yeast species and strain

Not only the growth conditions determine the yeast cell wall composition, also the yeast species, and even the *Saccharomyces cerevisiae* yeast strain used affects the yeast cell composition (Kwiatkowski and Kwiatkowski, 2012). As yeast extract producers, breweries and bioethanol producer all use their own, mostly proprietary, production strains this will undoubtfully result in variations in the yeast cell wall composition. In the case of yeast cell walls derived from cane-molasses based bioethanol production, the situation is even more complex, as (different) wild yeast strains take over in the course of the production campaign (Basso et al., 2008).

iii. Production process

At large, there are two different production processes by which yeast cell walls are produced: autolysis and hydrolysis (Noordam, B. and J.G. Kortes, 2003). Although it is commonly thought that yeast cell walls are produced by autolysis, as this is the cheaper process, in fact more and more yeast cell walls are being produced by hydrolysis. The hydrolysis process is used when producing high nucleotide yeast extracts (a class of yeast extracts in much higher demand than 'conventional' yeast extracts). Moreover, the increasing use of bioethanol spent yeast as the raw material for yeast cell wall production requires in many instances the addition of exogenous proteases to lyse the yeast cell walls. This because the yeast own enzymes have already been inactivated during the downstream processing of the bioethanol.

The addition of exogenous proteases does not only result in the lysis of the yeast, it also results in the hydrolysis of the mannoproteins present on the outside of the yeast. Hydrolyzed mannoproteins are soluble, and as a consequence they no longer end up in the insoluble yeast cell wall fraction after centrifugation (see also Fig. 1). Therefore, the MOS content of hydrolyzed yeast cell walls is some 50% lower than that of autolyzed yeast cell walls.

Moreover, yeast cell walls are spheres (Fig. 2), and therefore the molecules present on its outside determine its functionality. Autolyzed yeast cell walls contain MOS on the outside, while hydrolyzed yeast cell walls contain a mixture of MOS and β-glucans on the outside (Fig. 2).

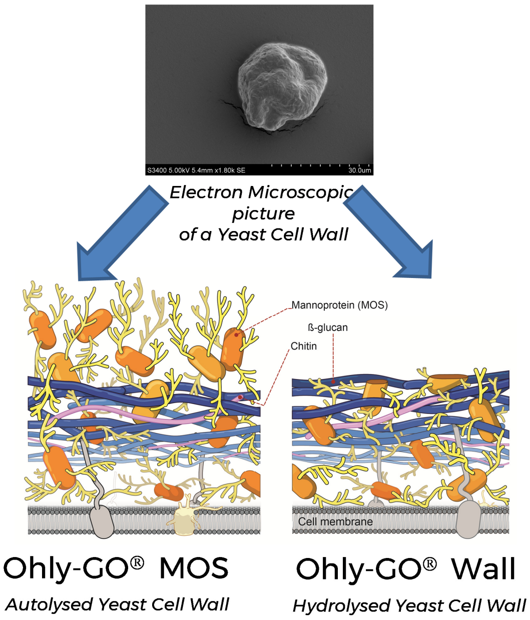

Figure 2: Difference in yeast cell wall structure between autolyzed and hydrolyzed yeast cell walls (EM Picture by SGS Institute Fresenius).

Also the purity of the yeast cell walls varies greatly. Yeast cell walls produced as a co-product during yeast extract production, are generally washed intensively (some 3-4 consecutive centrifugation steps resulting in a washing efficiency of ≥ 99%) generating a relatively pure yeast cell wall product. In contrast, yeast cell walls produced from biofuel spent yeast by-product are generally only centrifuged once and not washed, therefore lowering the MOS and β-glucan contents of such cell walls.

From by-product to co-product

Traditionally, yeast cell walls have been looked upon as by-products from the yeast extract industry, a not planned product of little or no economic value. As a consequence no, or very little, attention was paid to the composition of these products. Other companies took these by-products off the hands of the yeast extract producers at very low prices. Something similar holds when spent yeast is used as the raw material for yeast cell wall production. Consequently, depending on the production location and, given the different yeast cell wall production processes used at one location, even the day that the by-product is collected, the composition and structure of the yeast cell walls of different batches will vary.

Yeast cell walls play a crucial role in controlling microbial infections in animal husbandry and aquaculture. The importance of these products is increasing, since the ban of the sub-therapeutic use of antibiotics in animal feed. Given their importance, it is crucial that farmers can rely on their robust performance. Therefore, a mind-shift is required, in which yeast cell walls are no longer looked upon as by-products but as co-products: products that are produced along the main product and that carry equal importance as the main product. Only in this way, a focus on yeast cell wall product quality with respect to product composition and structure will be implemented that secures also the future use of yeast cell walls as trusted ingredients to improve animal health and performance.

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