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PROTEIN SOURCES FOR ANIMAL FEED: YEAST BIOMASS OF BEER AND/OR WINE - REVIEW

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Abstract

This paper relates to studies regarding the influence of cultivation's substrates (carbon source - molasses, glucose, methanol, malt extract, nitrogen source yeast extract, phosphorus source monobasic and dibasic phosphates), inoculum (inoculation rate and inoculum age - CFU / ml), bioprocess parameters (temperature, aeration, rate of stirring) on the development of wine and / or brewing yeast biomass. Biomasses of brewing and / or wine yeast obtained (active and inactive) have been proven to be a rich source of protein (SCP) and mono-oligosaccharides (MOS) in different feed recipes compared to other protein sources (soybean meal, corn) for animal feed. It has been demonstrated that yeast biomass obtained with use in feed recipe has beneficial effects on animal health (ruminants, pigs, horses, poultry).

Key words: *feed recipe, protein, yeast.*

INTRODUCTION

Yeast is a unicellular eukaryotic organism with many nutritional benefits. Because of their high nutritional value, they are part of animal nutrition supplements (Shurson G.C., 2018; Ingrid Marie Håkenåsen, 2017). In 1996 yeasts, bacteria, fungi and algae have been used to develop new feed recipes. The protein thus obtained from microbial sources was called "Single Cell Protein" - SCP (Anamika Malav et al., 2017). Although SCP's have been successfully marketed for decades in the UK, optimal fermentation conditions are still under scrutiny by many researchers. From the studies performed, it was found that the fermentation conditions and media used had a major effect on the yield (g/l) and the productivity ($g/l \cdot h^{-1}$) of SCP's (Fatemeh S., Reihani S., 2019; Hezarjaribi M., 2016).

The manufacturing process of many products are dependent on processes triggered and sustained by microorganisms. Wine, beer, sake, and bread are just some examples of yeast-dependent products, especially Saccharomyces cerevisiae species (Rocio Gomez-Pastor et al., 2011).

Saccharomyces cerevisiae yeasts have demonstrated beneficial health effects over the years due to their vitamin content (especially vitamin B) and their role in the production of microbial proteins, β-glucans and mannans (Jach M.E. et al., 2015).

Globally, it is estimated that approximately 0.4 million metric tons of yeast biomass is produced, of which 0.2 million are bakery yeast (Rocio Gomez-Pastor, 2011).

Due to the protein content and probiotic properties of yeasts of the species Saccharomyces cerevisiae, yeast biomass is an option to consider for animal feed. (Suarez & Guevara, 2018).

The major components of the yeast cell wall are polysaccharides. In *Saccharomyces cerevisiae*, up to 90% of the cell wall's dry weight are α-mannan and β-D-glucan polysaccharides with properties to interact with the host's immune system (Kogan G., Kocher A., 2007). Another growth promoter taken into consideration today is mannan oligosaccharides (MOS). MOS is derivate from yeast cell wall of *Saccharomyces cerevisiae* species (Brendemuhl & Harvey, 1999).

The cell wall of yeast, 26-32% of dry weight, is a structural component that gives yeast the form and specific rigidity. The literature specifies the immune cell stimulation property of beta-glucan in the cell wall of the yeast. In addition, the MOS, another yeast cell wall component, has been successfully used to

prevent diarrhea in weaning pigs (EURASYP, 2016).
The yeast cell wall typically contains 15-30% Beta-Glucan and 15-30% MOS, representing an effective alternative for antibiotic-growth promoters. (Ronel Jay & Conejos V., 2012).
The present review presents the importance of yeast biomass in the feed recipe as a beneficial effect on animal health.

Yeast used for biotechnological process in order to obtain active and inactive biomass yeast

In the market for fodder products, there are numerous yeast-based supplements marketed and used in animal feed for sources of nutrients, nutraceutical compounds, and probiotics or as participants in different nutritional functions (Middelbos, 2007).
Yeast has vegetative states that predominantly reproduce by budding or fission and do not form their sexual state within or on a fruit body. They can be defined as basidiomycetous, ascomycetous or as unicellular fungi (Kurtzman & Fell, 1998). Yeasts are widespread in nature, but they do not appear totally random, they form communities in specific habitats - especially areas where they are vineyards (Lachance & Starmer, 1998).
Currently, there are about 60 different types of yeast composed of about 500 different species. Species distribution is based on variation in cell morphology, metabolism of different substrates, and different reproduction processes (Stone, 2006).
Of all kinds of yeast, only a few species are used commercially. Typical commercial yeast applications include alcoholic beverages (beer, wine and spirits), soft drinks (root beer, kvass, kombucha, kefir, mauby), bread and food baking, bioremediation, (to generate carbon dioxide for plant growth in the aquarium), food additives and flavouring agents, scientific research, and genetic engineering prophecies (Shurson, 2018).
In order to obtain a source of SCP, fermentations with substrates as carbon source - molasses, glucose, methanol, malt extract and nitrogen source yeast extract, is used. The biomass resulted represents a protein source and a most part of the yeast is reach in MOS.

Cultivation methods

Yeast production uses fermentative processes (Chandrani-Wijeyaratne & Tayathilake, 2000). It was involving the incubation of selected strains of microorganisms in environments that meet the needs of these microorganisms, with the aim of growing the culture followed by separation processes (Gour Suman, 2015).
To maximize the final product, the literature describes the efforts made to evaluate the effects on fermentation of the various factors: pH, temperature, incubation period, dissolved oxygen, aeration rate, carbon and nitrogen sources (Chandrani-Wijeyaratne & Tayathilake, 2000).
One of the yeast strains that has proven useful over time in fermenting on various substrates is *Saccharomyces cerevisiae*.
This strain is considered very useful in animal feed considering its probiotic nature (Suarez, 2018).
An example of this process is given by G.G. Fonseca et al., 2007, with ATCC 26548 yeast (CBS 6556, NCYC 2597, NRRLy 7571). This yeast strain was cultivated in YPD medium (yeast extract, 10 g $\times l^{-1}$; peptone, 20 g $\times$ l^{-1}; glucose, 20 g $\times$ l^{-1}).

Inoculum preparation

Regarding the preparation of the inoculum, this was prepared in 250 ml Erlenmeyer flasks with 100 ml medium sterilized at 121ºC for 15 minutes. The composition of the medium was: 150 g $\times$ l^{-1} sucrose, 6 g $\times$ l^{-1} yeast extract, 5 g $\times$ l^{-1} monobasic potassium phosphate, 5 g $\times$ l^{-1} ammonium chloride, 1 g $\times$ l^{-1} magnesium sulphate (Erika Vieira, 2013).

Fermentation

The outcome of the fermentation is affected by the substrate used (Spalvins, 2017).

Molasses and glucose

Regarding the carbon source, molasses is the most used in the production of yeast (especially for the production of bakery yeast). Molasses is a by-product of the sugar industry that contains about 55% fermentable sugars. Its composition includes sucrose, glucose, fructose, raffinose, melibiose and galactose.

Methanol

In addition to conventional materials such as molasses, fruit and vegetable wastes and unconventional substrates such as petroleum by-products, natural gas, ethanol, and methanol have been used.

Malt extract and molasses

Due to the cost of cane molasses, different medium was used. The yeast biomass results achieved from malt medium and from molasses medium were not very different (Ragheb et al., 2015).

Nitrogen source yeast extract

As with the carbon source, the use of different sources of nitrogen can increase the yield of yeast production.

A positive effect on yeast production has yeast extract, peptone and soybean meal, whereas urea can have a negative effect (Zhao G., 2010).

Fermentation with yeast extract in 0.5% concentration yielded maximum yield. Significant increase in growth could be observed with the increase in nitrogen source and phosphorus source (monobasic and dibasic).

Effects of initial KH_2PO_4/K_2HPO_4 concentration on biomass production

In 2017, Nicolas Ouedraogo determined the effects of the initial concentration of KH_2PO_4/K_2HPO_4 on the fermentation medium on the growth of biomass production by experimenting with concentrations of KH_2PO_4 / K_2HPO_4. This concentration was changed between 0.1 and 0.5 g of l^{-1} inoculum (inoculum rate and inoculum age - CFU/ml).

In general, type of microorganism used, incubation temperature, incubation time, shake rate, chemical structure, and availability of carbon source from different substrates were considered and were monitored in various works. However, inoculum size, inoculum age, pH, and aeration rate are also important factors for which their effects on the SCP production need to be investigated (Fatemeh S. et al, 2019).

The optimal pH for most strains of *Saccharomyces cerevisiae* is 4.5 (Rose, 1987). The required amount of raw molasses, nitrogen and phosphorus salts was calculated based on the spreadsheet simulator. The molasses purity was 56% as determined by sugar HPLC (fructose, glucose and sucrose) and Brix analysis. (Erika Vieira, 2013).

The degree of SCP (Single Cell Protein) production depends on the type of substrate used and also on media composition (Spalvins, 2017).

After fermentation, the culture media is separated, purified and dried.

Depending on the drying process, the yeast obtained may be active or inactive.

Active yeast can be obtained by lyophilization or by fluidized bed dryer and inactive yeast results by spray drying or rolled drums process.

An investigation of the optimization of parameters of an industrial continuous fluidized bed dryer for the production of instant active dry yeast was made by Hamidreza Akbari et al. (2012).

Example for active yeast used for feed animal:

Active Yeast - A-YEAST™ Yeast Culture provides a boost in rumen fermentation, production and feed efficiency, while mitigating the effects of heat stress (https://ahanimalnutrition.com/species/dairy/products/a-yeast). Levucell® SC is active dry yeast for use as a direct fed microbial in ruminant feeds. It is a unique strain exclusively selected to improve rumen function. RumiSacc is a commercial live yeast culture. It contains live yeast and autolyzed yeast (Sakine Yalçin, 2011).

Saccharomyces cerevisiae, *Candida robusta* and *Hansenula polymorpha* were taken in study in batch fermentation in order to study profile of yeast growth and biomass accumulation. Maximum cell density was reached after 24 h and amounted to 178.22 g WCW per litre of medium, corresponding to 36.4 g/l CDW.

It was established the bioprocess flowsheet at micropilot level in order to obtain a viable proteic biomass with yeast mixed culture (*Saccharomyces cerevisiae*, *Candida robusta* and *Hansenula polymorpha*).

Yeast single-cell protein (SCP) is a high nutrient feed substitute (Burgents et al, 2004). Among these, most popular are yeast species *Candida* (Bozakouk, 2002), *Hansenula*, *Pitchia*, *Torulopsis* and *Saccharomyces*.

Active dry yeast is comprised of 15-25 billion live yeast cells (colony forming units; CFU's) per gram (Stone, 2006). The three most common processing methods include tunnel dried yeast (granular powder), fluid-bed dried yeast (quick rise yeast in oval shaped spheroids), and rotolouver dried yeast (produces small spheres or balls). The tunnel dried and fluid-bed drying methods are most common in the U.S., while the rotolouver drying method is more common in Europe and Latin America (NPCS Board of Consultants and Engineers, 2011). Of these drying processses, fluid-bed drying has become the most popular because it causes less damage to yeast cells, and thus, maintains their viability. In the 1960's, yeast biomass-producing plants contributed to the technology of producing large amounts of active dry yeast (ADY), and its use rapidly spread to European countries (Reed & Nagodawithana, 1988). Nowadays, modern industries require very large amounts of selected yeasts to obtain high quality reproducible products and to ensure fast, complete fermentations. (Rocio Gomez-Pastor, 2011).
Beside the content of proteins, yeast biomass is a good source of B-complex vitamins, nucleic acids and minerals.
Commercial brewer's yeast is inactive yeast remaining after the brewing process with an inexpensive nitrogen source with good nutritional characteristics and a very bitter taste (Bekatorou, Psarianos, & Koutinas, 2006).
Yeast single-cell protein (SCP) is a high-nutrient feed substitute (Burgents et al., 2004). Among these, most popular are yeast species *Candida* (Bozakouk, 2002), *Hansenula*, *Pitchia*, *Torulopsis* and *Saccharomyces* (Gour Suman, 2015).

ANALYSES

Determination of dry cell weight

According to Gaensly F. (2014), the dry cell weight can be determined by drying the yeast biomass at 60ºC after centrifuging 10 ml of sample at 6000 rpm until the biomass reach a constant weight.
Another method was used by De Sous et al. (2006), the method assumes that at the end of the fermentation yeast biomass was collected. The collected biomass was centrifuged at 6000x G/min at 4°C and washed two times with distilled water. The resulting precipitate was dried in oven at 105°C for 4 h and the yeast biomass was measured with an analytical balance.

Biomass determination

The biomass concentration was determined by turbidity measurements at 610 nm, correlated to dry weight from duplicate samples (5 ml, taken in the middle and at the end of cultivation) which were centrifuged (5000x G, 10 min), washed twice with distilled water, and dried for at least 24 h at 110ºC. The specific growth rate was calculated from the biomass measurements, as well as from the heat measurements, since during anaerobic exponential growth, the rate of heat production is directly correlated to the specific growth rate.

Total protein content was determined with samples of freeze-dried cells (prepared as described previously (Gurakan, 1990), resuspended in 3 ml of 1M NaOH, by a modified biuret method (Verduyn, 1991). The A555 was measured, and bovine serum albumin (Amersham Life Science, Little Chalfont, England) was used as a standard).
Sugar content was measured by a saccharometer (Dujardin-Salleron, France), phosphate concentration was measured according to the micro determination method (Chen P.S., 1956; Ehab El-Helow et al., 2015)

Total carbohydrates could be determined through the colorimetric method from Dubois et al. (1958).

Total lipids could be extracted through the procedure of Blight and Dyer (1959) and after that determined gravimetrically (Sideney Becker Onofre, 2017).
The results concerning the content of protein and lipid from different yeast biomass are shown in the Table 1.

MOS analyses

Yeast cell wall is a non-specific stimulator of the immune system in both human and animals which is generally composed of 30-60% polysaccharides (beta-glucan and mannan sugar polymers), 15-30% proteins, 5 - 20% lipids and a small amount of chitin. Most of the protein is

linked to the Mannan-OligoSaccharides (MOS) and is referred to as the Mannoprotein complex (Aguilar-Uscanga & Francois, 2003; Huang et al., 2005; Klis et al., 2006).
MOS is derived from the cell wall of the yeast Saccharomyces cerevisiae (Linn. Brendemuhl & Harvey, 1999).

Extraction of crude mannan oligosaccharides
For the extraction of the MOS, 5 g of dry yeast were used. Extraction with 1% NaOH (50 ml) was performed at 100°C for 2 h. After cooling, the solution was diluted with HCl solution until pH 7 reached.
After filtration, the mannan oligosaccharides were precipitated by adding 200 ml (4 volumes) of ethanol absolute. The precipitate was washed with ethanol absolute and diethyl ether dissolved in water, dialyzed against 2 changes of water and subsequent drying (Huang et al., 2010; Al-Manhel, 2017).

Application: The potential effect of mannanoligosaccharides on the intestinal immune system, combined with the high concentration of mannan components in dry yeast cell wall (YCW), may make YCW preparations useful functional dietary ingredients in pet foods by improving intestinal health and resistance against intestinal upset (Middelbos I.S., 2007). Benefit from adding yeast to the diets of monogastric animal can be illustrated based on that yeast cells containing mannan in the outer layer of their cell wall (Ofek et al., 1977). Mannan has anti-adhesive properties that can help in the adhesion of bacteria to epithelial of mucous membranes. Addition of yeast also can stimulate the immunity of animals. (Qamar et al., 2001). Yeasts are also known to contain other essential microelements that participate in the physiological and metabolic processes in human organism, such as Zn, Cu, Mn (Barbulescu et al., 2010), Fe (Pirman & Oresnik, 2012).

Yeast– ingredients for animal feed
MOS-fed animals had greater height compared with the control and the doxycycline-fed animals. The corresponding improvement in digestibility was correlated with the increase in height due to their high correlation coefficient (r = 0.70–0.87). MOS is therefore an effective alternative for antibiotic-growth promotants. (Jay Ronel V. Conejos, 2012). Mannan-Oligo-Saccharide (MOS), another constituent of Yeast Cell Wall, has been demonstrated to prevent diarrhoea in weaning pigs.
The quality of viable yeast biomass obtained by the protocol performed by Campeanu Gh. et al. (2002) is good and it could be used as probiotic product for animal nutrition. Saccharomyces cerevisiae known also as the brewer's yeasts or baker's yeasts is one of the well-known and commercially significant yeast species (Håkenåsen, 2017). In animals, Saccharomyces cerevisiae in their diets are known to play several vital roles including prevention of diarrhea and mortality, boosting of immune system, performance, milk production, fiber degradation and nutrient digestability, adsorption of toxic metal such as cadmium, stabilization of rumen pH and microorganisms (Sylvester Chibueze Izah, 2018).
In addition, yeast strain, production conditions and processing, could affect the chemical composition and the biological availability of the yeast. The yeast included in the feed in the present experiment was dried and hence inactivated as a probiotic.

CONCLUSIONS AND FUTURE PERSPECTIVE

The quality of yeast biomass produced in the mentioned conditions by Campeanu Gh. et al. (2002) is high and allow its use as additive in animal fodder.
The beneficial effects of *Saccharomyces cerevisiae* have been demonstrated by research in the field and are largely due to the vitamin B complex and the mineral content, but also due to its role in the production of microbial proteins, β-glucans and mannans (Jach M.E., 2015).
Saccharomyces cerevisiae yeast has been used in animal feed in different proportions as part of the diet, 10% in cattle, sheep and 5% in the birds (Caridad Suarez, 2018). Since it has a good composition of amino acids, e.g. high levels of lysine, threonine and leucine and, also, high levels of B-complex vitamins, *Saccharomyce cerevisae* may be a satisfactory

alternative to soybean meal in animal diets, provided it has notoxic or antinutrient properties (Winkler, 2011). 50 kg/tonne of soybean meal = 48.5 kg/tonne of corn + 1.5 kg/tonne of L-Lysine HCl (http://www.fao.org/3/y5019e/y5019e0a.htm)

Yeast can be a protein source *for animal feed (ruminants, pigs, horses, poultry).*

For future studies, the aim is to obtain new yeast biomass of beer and/or wine (Figure 1) as a potential source of protein for feed animals.

Table 1. Chemical composition of brewer's yeast biomass and wine yeast biomass

Yeast biomass	brewer's yeast biomass - *Saccharomyces cerevisiae*	*Candida utilis*	*K. marxianus* ATCC	Wine yeast
Protein (% N × 6.25)	49.63 ± 2.43	54.8 ± 0.12	54.6 ± 1.5	-
Lipids	4.64 ± 0.52	15.12 ± 0.98	5.2 ± 0.2	6.06 ± 0.2
Ashes	7.98 ± 0.76	8.1 ± 0.18	3.0 ± 0.2	-
Total Carbohydrates	31.55 ± 4.32	2.8 ± 0. 2	26.5 ± 0.8	28.42 ± 0.31
References	Sideney Becker Onofre, 2017	Ouedraogo et al., 2017	Gustavo Graciano Fonseca, 2007	Chiselita Oleg, et al., 2004),

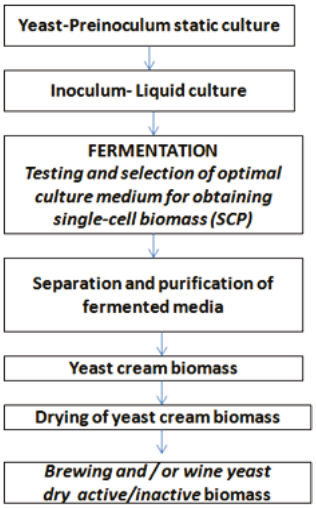

Figure 1. Fermentation process for obtaining dry active/inactive yeast biomass.

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