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Comparison of pilot- and laboratory-scale centrifuge recovery and dewatering performances on beta-glucan production

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ABSTRACT

Beta-glucan production involves separation of yeast cell wall from soluble cellular components and centrifugation is commonly employed. Poor liquid-solid separation and solid recovery, however, are common obstacles during process scale-up. In this study, the recovery and dewatering performance of floor-type centrifuge (Beckman J-6M/E) and pilot scale disc-stack centrifuge (Westfalia separator SA1-01-175 with Double Centripetal Pump) were compared. Centrifuges at both scales were used to separate cell wall from yeast autolysate as well as to wash cell wall. Centrifugation of yeast autolysate and homogenate (500 mL) in a 1-L bucket at 3500 rpm for 40 min with 15% w/w, the floor-type centrifuge could achieve 30% w/w sediment. The disc-stack centrifuge for pilot scale centrifugation, on the other hand, could achieve up to 20% w/w sediment when feeding samples with 1-5% w/w at 1 L/min, 9,000 rpm. Wetter sediment at a pilot-scale did not lead to higher contaminants in the finished product, allowing the process scale up. Before spray drying, the beta-glucan recovery yield in laboratory- and pilot-scale were 21% and 10%, respectively. The major loss during pilot-scale production was, indeed, due to disc-stack centrifuge. It is important that the flow rate used has to be compromised between the processing time required and % solid recovery. Flow duration, however, did not influence the recovery until the point that the bowl was completely full.

Keywords: beta-glucan, scale-up, centrifugation

INTRODUCTION

Beta-glucan is carbohydrate found in the cell wall of microorganism, fungi, yeast, plants (Rahar, Swami, Nagpal, Nagpal, & Singh, 2011). Approximately 50-60% of dry yeast cell wall consists of beta-glucan and chitin which are essential for the strength of inner cell wall (Klis, Mol, Hellingwerf, & Brul, 2002). β-(1→3)-D-glucan was reported to be the major type of beta-glucan in yeast cell wall while β-(1→6)-D-glucan was a minor component (Manners, Masson, Patterson, Björndal, & Lindberg, 1973). This inner layer of cell wall is covered by the outer layer cell wall which comprises of mannoproteins (Klis et al., 2002). The clinical applications of yeast beta-glucan has been reviewed elsewhere and these included but not limited to antitumor, immunomodulating effects, anti-diabetics, and lowering blood pressure and cholesterol (Bashir & Choi, 2017). Besides medical applications, yeast beta-glucan also showed potential in food applications and can be used as prebiotics. For example, it can be used as fat replacer in mayonnaise (Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006). Due to its wide range of applications, beta-glucan has gained much interests in food supplement industry.

Yeast autolysis was an initial step in producing beta-glucan. Intracellular components are digested and released into the extracellular liquid. In order to obtain pure beta-glucan, proteins, other carbohydrates, and fat need to be solubilised and removed. The previous research at Department of Biotechnology, Mahidol University showed that hot alkaline extraction and acid extraction could be used to produce beta-glucan that stimulated shrimp immunity (Suphantharika, Khunrae, Thanardkit, & Verduyn, 2003, Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). Other extraction methods were reviewed elsewhere and they involved centrifugation (Varelas, Liouni, Calokerinos, & Nerantzis, 2015).

Centrifugation is a common unit operation in pilot- and industrial-scale process of solid-liquid separation. Various types of large-scale centrifugation are available, providing options for scaling up. The ability of centrifuge to separate solid and liquid was based on Stokes' law and the factors influencing clarification include flow rate, density difference, centrifugal force provided (rotational speed and effective radius), fluid viscosity, particle size, and settling distance (Ambler, 1959). Based on Stokes' law, it is possible to compare the performance between different centrifuges. However, the clarification and recovery when scaling up centrifugation could be difficult to predict. This is

due to the differences in design and operation of large-scale centrifuge which can disrupt solid sedimentation.

Disc-stack centrifuge is commonly used in downstream processing and food processing for solid recovery or removal. It can generate high centrifugal force and can be operated continuously. However, it tends to have poor dewatering capacity and should be used with low-solid-content feed. It also tends to have a poor cooling system and heat is removed as the feed flows through the centrifuge (Boychyn et al., 2004). In addition, shear generated within the disc-stack centrifuge has been shown to cause reduce particle size distribution and reduced clarification (Byrne, Fitzpatrick, Pampel, & Titchener-Hooker, 2002). Such effects could occur during beta-glucan extraction and these could affect both yield and purity of beta-glucan finished product. Insufficient centrifugation would lead to lower recovery of insoluble solid or beta-glucan. Although lower flow rate may improve solid recovery, excessively long processing time would be required. Poor dewatering capacity leads to wet sediment and potential carry-over of contaminants. The aim of this research was to compare the performance of floor-type centrifuge with the pilot scale disc-stack centrifuge and highlighted potential bottleneck for an industrial-scale beta-glucan production process.

MATERIAL AND METHODS

Material and reagent

Spent brewer's yeast with the solid content between 15-20% w/w was kindly provided by Pathumthani Brewery Company Limited, Bangkok, Thailand. All chemicals used were of reagent-grade. During laboratory-scale production, distilled water was used throughout the experiment. During pilot-scale production, tap-water was used for alkaline and acid extraction. Potable water was used in the final step for removing remaining acid from beta-glucan.

Yeast homogenate autolysate preparation

At both laboratory and pilot scales, 45 kg of yeast slurry was autolysed in a temperature control mixer for 24 h at 50-55°C and the initial pH was adjusted to 5. For laboratory scale, 500 ml of yeast autolysate was transferred into 1-L buckets and centrifuged at 3500 rpm for 40 min using Beckman J-6M/E (USA) centrifuge. The sediment was resuspended in distilled water until the solid content reached 15%. Then, cells were disrupted by passing through high pressure homogeniser (Panda, Niro Soavi, Italy) at 600 bar for 6 rounds. For pilot-scale, cells were disrupted using pilot-scale high pressure

homogeniser (GEA Niro Soavi Panther NS3006L) at 600 bar for 3 rounds. These conditions were previously shown to achieve the same degree of protein released to that of the laboratory-scale high pressure homogeniser.

Pre-assessment on pilot-scale centrifugation capacity and performance

The use of floor-type centrifugation for beta-glucan extraction was done according to the previous report (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). Due to the design limitation, the rotational speed of the disc-stack centrifuge used in this study (Westfalia separator with Double Centripetal Pump, (model) SA1-01-175) could not be adjusted and can only be run at 9,000 rpm. Therefore, the clarification at different flow rates were studied. To assess the effect of solid deposition on clarification, yeast slurry was diluted to reach 1-2% solid content. The mixture was fed into the disc-stack centrifuge (Westfalia separator with Double Centripetal Pump, (model) SA1-01-175) at a constant flow rate of 1 L/min using peristaltic pump and the bowl was rotating at 9,000 rpm. The supernatants were collected at a 4-min interval to measure the solid content. The bowl was empty as the supernatant appeared cloudy. To investigate the effect of flow rate on the recovery of yeast cell wall debris, the flow rate of homogenate autolysate was varied between 0.5 – 1 L/min. The supernatant was analysed for an optical density at 600 nm (OD_{600}) and solid content.

Beta-glucan preparation

The debris was resuspened in distilled water and 1 N NaOH was added until the pH reached 13. The solid content was approximately 20% and the final volume was 50 L. The mixture was stirred at 72°C for 2 h. The suspension was centrifuged at 3500 rpm for 30 min. The pellet was collected and resuspened in 3-volume of distilled water and centrifuged once again. The wash was repeated 3 times. Then, the sediment was resuspened in distilled water and 0.5 M acetic acid was added until the pH reached 3.0. The final volume was 50 L with approximately 20% solid content. The suspension was centrifuged and the sediment was washed 3 times. Finally, the sediment was resuspended in distilled water to reach 10% w/w before being spray-dried using NIRO ATOMIZER Mobile MINOR Spray Dryer with an inlet temperature of 180°C. The outlet temperature was maintained between 85-90°C by adjusting the flow rate. For pilot-scale production, similar processes were performed except that the pilot-scale centrifuge was used instead. The flow rate used was 1 L/min and the solid content was approximately 5% w/w.

Analytical assay

All samples including yeast slurry, yeast autolysate, clarified supernatant, yeast cell wall after autolysis, and beta-glucan were sent to the Institution of Nutrition, Mahidol University for a proximate analysis using standard AOAC methods. Based on the methodology of the Institution of Nutrition, all the measurements were repeated twice. In brief, moisture content was analysed by following AOAC (2016) 952.08 method. Protein content was quantified based on AOAC (2016) 992.23 using a conversion factor of 5.7 for yeast-based samples and 6.25 for beta-glucan. Ash content was analysed based on AOAC (2016) 930.30, 945.46 protocol. Total fat was quantified according to AOAC (2016) 948.15 method. Due to such excessively long extraction time, each scale was conducted once. Protein, carbohydrate, fat, ash, moisture in each step were measured twice according to the procedure of Institution of Nutrition, Mahidol University. For routine moisture content, solid content was analysed by drying sample in an oven at 105°C for 3 days. After the sample was cooled to a room temperature in the desiccator, the dry weight was measured.

Statistical Analysis

Two-tail t was used to compare the components of raw material and finished product beta-glucan powder from both scales.

RESULTS AND DISCUSSIONS

The performance of floor-type centrifugation

Yeast autolysate with approximately 15% w/w solid content could be clarified by a floor-type centrifuge at 3,500 rpm in 40 min based on the previous study (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). The sediment had 31% (w/w) dry solid while the supernatant contained 7.22% (w/w) solid (Table 1). This solid content was similar to the previous reported by Verduyn, Suksomcheep, & Suphantharika (1999) where 6.8% w/w was obtained in the supernatant upon autolysis. The dry solid in the sediment was 3.81 kg resulting in 67% solid recovery. This was higher than the previous report under the same autolysis protocol where 57% of an initial solid was in the insoluble fraction (Thanardkit, Khunrae, Suphantharika, & Verduyn, 2002). The supernatant consisted of 1.32 kg of dry solid. Therefore, 23% of total solid was in the supernatant while 38% of solid yield was previously reported (Thanardkit et al., 2002). Such discrepancy between both experiments may be due to the raw material itself. For example, longer storage of spent brewer's yeast tends to reduce the degree of autolysis.

The clear supernatant fraction contained certain soluble solids or solutes such as vitamins, minerals, amino acids. Since the liquid part of the yeast autolysate would also contain the same concentration of soluble solid as in the supernatant (7.22% w/w), there would be approximately 2.48 kg of soluble solid in the initial autolysate. Consequently, 53% of soluble solid was recovered in the supernatant. Due to the fact that the sediment contained 68% water, the soluble solid would be present in the sediment as well. Therefore, the total dry solid quantified in the sediment would comprise of both soluble and insoluble solids. The actual insoluble solid such as cell wall would be slightly less than the quantified dry solid. The liquid in the sediment would contain the same concentration of soluble solid as the supernatant at 7.22% w/w. By that rational, the mass of soluble solid in 12.2-kg wet sediment would contain 0.64 kg of soluble solid while the insoluble solid (cell wall, insoluble protein, lipid) would be 3.17 kg. This made nearly 100% the recovery yield of insoluble solid from yeast autolysate by floor-type centrifuge.

Table 1. The performance of floor-type centrifuge in separating yeast autolysate

	Moisture g/100g	Mass (kg)	Solid content g/100g	Dry solid (kg)
Yeast slurry	87.42	45.5	12.58	5.72
Yeast autolysate	85.84	40.0	14.16	5.66
Water		34.3		
Soluble solid				2.48
Insoluble solid				3.18
Centrifugation				
Supernatant 24 h	92.78	18.3	7.22	1.32
Sediment after 24 h autolysis	68.25	12.2	31.75	3.81
Water		8.33		
Soluble solid				0.64
Insoluble solid				3.17
% Solid recovery in sediment				67%
% Soluble solid recovery in supernatant				53%

The performance of pilot-scale disc-stack centrifuge

Prior to performing yeast autolysate separation, the capacity and performance of disc-stack centrifuge was assessed with a 1-2% w/w yeast slurry. The solid content in the supernatant was monitored every 4 min to see the separation capability upon solid accumulation as shown in Table 2. Upon continuous feeding, the cloudy supernatant was observed at approximately 8 min indicating that discharge was required. At this point, 114 g of solid was deposited in the centrifuge. Upon a desludge or solid removal, the volume of sediment was approximately 600 ml and the solid content was roughly 20% w/w. At 16 min, the bowl was desludged 3 times in order to remove most of the sediment in the bowl. The gross volume of discharged solid was 2.0 L with the solid content of 11% w/w. Lower solid content was observed because the solid-liquid separation was not complete during the last few discharges resulting in wetter sediment. The same effect would be expected for an early discharge. This could also lead to high carry-over of soluble contaminant. The total dry solid in the sediment was 220 g, corresponding to 76.9% solid recovery.

Table 2. The solid content in the supernatant after feeding of yeast slurry (at 1 L/min)

Sample	% Solid w/w	Total volume (L)	Total dry solid (g)
Feed	1.43	20	286
Supernatant		17	41
T_0	0.20		
T_4	0.30		
T_8 (after discharge)	0.20		
T_{12}	0.21		
T_{16} before discharge	0.30		
Sediment	11	2	220
% Solid recovery in sediment			76.9%

The flow rate at 1L/ min was used to separate cell wall from yeast autolysate. Although lower flow rate could result in better separation due to longer sedimentation time, the processing time would be far too long and become inapplicable at larger scale. Higher flow rate would lead to cloudy supernatant and lower yield. In order to compromise between processing time and recovery yield, the yeast autolysate was diluted to 5% w/w. Due to a very large processing volume involved, all the supernatant could not be collected and pooled to homogeneity and only the sediment was pooled and analysed. With these operating conditions, the recovery yield of solid in the sediment was 65% as shown in Table 3. This was similar to the performance of the floor-type centrifuge shown in Table 1. However, the solid recovered in the sediment was lower than when separating yeast slurry with 1.43 % w/w solid content (Table 2). This may be due to the fact that the intracellular contents were released into the liquid upon autolysis and they were removed during centrifugation. These components dissolved in the water and became the solute in the supernatant and did not settle.

Table 3. The performance of disc-stack centrifuge in separating cell wall from yeast hydrolysate

Samples	Moisture g/100g	Mass (kg)	solid content (%)	Dry solid (kg)
Yeast slurry	82.8	53.9	17.2	9.29
Yeast autolysate	83.0	51.7	17	8.79
Centrifugation				
Feed	94.9	172	5.1	8.78
Sediment	78.71	26.82	21.29	5.71
% Solid recovery in sediment				65.0%

Using mass balance, the solid in the supernatant would be 3.07 kg which contributed to 35% of the total solid before centrifugation. On the other hand, the supernatant obtained from floor-type centrifuge contained 1.32 kg of solid which was 28.8% of the total solid. Here, it might seem that the recovery of soluble solid in the supernatant using disc-stack centrifuge was better. It should be taken into consideration that the solid in the supernatant consisted of both soluble and insoluble solids and high solid content may also be due to a poor solid separation. Indeed, floor-type centrifuge can recover 83% of solid in the sediment while disc-stack centrifuge could only achieve 65.0 % recovery. Therefore, high solid content in the supernatant obtained from disc-stack centrifuge was due to poorer solid-liquid separation. At this stage, the solid content of feed was 5.1% w/w which was slightly lower than the feed for floor-type centrifuge (7.22% w/w). Since the glucan was not released into the feed at this stage, it was unlikely to affect the viscosity of the sample. Incomplete solid discharge could also contribute to the losses but minimisation was attempted. At the end of the centrifugation, water was fed into the centrifuge and desludge was carried out at least 3 times in order to remove most sediment. Yet, the recovery was still lower than yeast slurry (Table 2). The lower recovery yield could be a combination of insufficient deslude or shear stress generated within the centrifuge. The latter factor will be discussed later. The multiple rounds of centrifugation would be the step that contribute to the major loss during the whole pilot scale process.

After cell disruption, the presence of glucan in the feed of both processing scales may influence fluid viscosity and impede the sedimentation of cell wall debris. However, during all washing steps, the solid content of all samples to be separated by floor-type centrifuge was adjusted to approximately

8% w/w while 5% w/w solid-content feed was prepared for disc-stack centrifuge. It can be seen that the solid content for floor-type centrifuge was slightly higher than the solid content for disc-stack centrifuge which could contribute to marginally higher viscosity fluid. However, the solid recovery was still higher by floor-type centrifuge after multiple washing steps. Therefore, the difference in viscosity was unlikely to cause poor solid-liquid separation in disc-stack centrifuge. The poorer clarification ability of pilot scale centrifuge was likely to be due to the design of disc-stack centrifuge. The work of Maybury, Hoare, & Dunnill, (2000) illustrated that high shear stress was generated during desludge or feed zone which led to smaller particulate formation and poorer sedimentation of shear sensitive materials. This level of shear was significant to cause damage on *E. coli* cells (Chatel, Kumpalume, & Hoare, 2014). Since yeast cell was autolyzed, the cell wall was also digested by endogenous enzyme and resulted in weakening of cell wall structure and could be shear sensitive. Multiple rounds of centrifugations by pilot scale centrifuge could accumulatively reduce the particle size. Indeed, the total solid in the beta-glucan slurry obtained from pilot-scale process was approximately 0.9 kg (10% yield) while the laboratory-scale achieved at least 1.2 kg of solid (21% yield) even though less solid was used as raw material. The effect of shear within the disc-stack centrifuge will need to be further investigated in order to minimise the losses. If this is the case, addition of flocculating agent may improve the recovery but this may increase process cost and also lowering product purity.

Disc-stack centrifuge produced wetter sediment than the floor-type centrifuge. The poor dewatering ability of disc-stack centrifuge could result in higher contaminants in the beta-glucan finished product. The compositions of beta-glucan obtained from both production scales were compared in Table 4 and 5. According to Table 5, the dry basis compositions of the raw materials (yeast slurry) at both scale were different (P value < 0.02). This was to be expected because yeast slurry was the waste from brewing process and no quality control of such material was not required. The protein content in yeast slurry used in both scale was significantly different while ash and fat contents were rather similar (P value < 0.02). In the process of beta-glucan extraction, proteins, fat, and certain carbohydrates were solubilised with the aid of NaOH and acetic acid. The combination of these chemicals and heat allowed greater than 90% removal of total protein, fat, and ash from yeast cells. During the process, both NaOH and acetic acid must be removed by multiple washing and centrifugation of sediment before spray drying. Poor dewatering could result in carryover of NaOH and higher ash content. It can be seen that in the finished

product, however, the ash content of beta-glucan from pilot-scale became higher than beta-glucan obtained from lab-scale process. Other contaminants were found at a similar level in both scales (P value ≥ 0.02). This implied that the extraction efficiency of protein and fat was comparable in both scales and pilot scale centrifugation can probably wash solubilised protein and fat effectively. By that rational, the removal of soluble minerals or ash, especially water-soluble NaOH should also be comparable. Consequently, the higher ash content was highly likely to be due to the difference in the quality of water used. Although the ash content was higher, such level of ash contaminant was still acceptable because it did not contribute to the significant difference in the total carbohydrate content. Consideration in the quality of water used during the industrial scale process must be carefully considered. Although using distilled water in the extraction process resulted in less ash content, it would lead to uneconomical running cost.

Table 4. The composition of yeast slurry and beta-glucan from both production scales (g/100g wet basis)

	Moisture	Protein	Total fat	Ash	Carbohydrate
Yeast slurry Pilot scale	82.93 ± 0.325	6.76 ± 0.02	0.37 ± 0	0.97 ± 0	
Yeast slurry lab scale	87.42 ± 0.04	6.95 ± 0.005	0.36 ± 0	0.98 ± 0.015	9.19 ± 0.06
beta glucan pilot scale	7.44 ± 0.165	2.28 ± 0.035	2.68 ± 0.135	0.44 ± 0.005	87.16 ± 0.27
beta glucan lab scale	5.34 ± 0.095	2.51 ± 0	3.69 ± 0.125	0.28 ± 0	88.46 ± 0.03

The mean values ± standard deviations were shown in the table.

Table 5. The composition of yeast slurry and beta-glucan in pilot- and laboratory-scale production (g/g dry basis)

	Protein (g/g)	Total fat (g/g)	Ash (g/g)	Carbohydrate (g/g)
Yeast slurry (pilot-scale)	0.40	0.022	0.057	?*
Yeast slurry (lab-scale)	0.55	0.029	0.078	0.731
P Value	0.0001	N.D.	0.0028	N.D.
Beta-glucan (pilot-scale)	0.0246	0.0290	0.0048	0.942
Beta-glucan (lab-scale)	0.0265	0.0390	0.0030	0.935
P Value	0.040	0.037	0.0010	0.076

*Note: carbohydrate in yeast slurry at pilot scale was not quantified.

Process scale up has always posed a challenge in delivering product from laboratory research to the industrial sector. The laboratory scale was not likely to illustrate the actual processing time and purity at industrial scale process. The downstream processing tends to contribute to high cost as well as complication. The previous studies of beta-glucan extraction in our department used floor-type centrifuge throughout the process (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). Based on dry basis, the protein and ash contents in beta-glucan in the previous study based were in a good agreement with the protein and ash contents in beta-glucan obtained from pilot-scale process in this study. The protein content in the previous study was 4.43% (w/w) which was slightly higher than this study (2.46% w/w). When using pilot scale centrifuge, the ash content was 0.48% (w/w) which was slightly higher than the previous study (0.41% w/w). Fat, on the other hand, was much higher in this study but this was likely to be due to extraction conditions rather than centrifugation.

When looking at the overall process, batch centrifugation by floor-type centrifuge can achieve high clarification and recovery yield. However, it is not compatible with industrial scale production. Disc-stack centrifuge offered continuous solid-liquid separation and larger sedimentation sedimentation area can be achieved if more discs are used. Unfortunately, this type of centrifuge can only work with low solid content and has poor dewatering capacity. The fine tune in operating condition including solid content and flow rate was highly essential for minimising processing time and maximising recovery yield.

CONCLUSION

Disc-stack centrifuge could be operated in a semi-continuous mode which provided advantages over batch-mode centrifuges. However, during process scale-up for beta-glucan production, centrifugation was the major bottleneck with 2 primary limitations. Firstly, poor solid recovery of disc-stack centrifuge contributed to the major loss of product. The second limitation was due to the fact that applicable with only feed with low solid content. Therefore, longer processing time was required. Dewatering, however, did not appear to influence the level of protein, and fat contaminant. The increase in ash content was likely to be due to the quality of water used during the production process.

REFERENCES

Ambler, C. (1959). The theory of scaling up laboratory data for the sedimentation type centrifuge. *J Biochem Microbiol Technol Eng*, *1*(2), 185–205.

Bashir, K. M. I., & Choi, J. S. (2017). Clinical and physiological perspectives of β-glucans: The past, present, and future. *International Journal of Molecular Sciences*, *18*(9).

Boychyn, M., Yim, S. S. S., Bulmer, M., More, J., Bracewell, D. G., & Hoare, M. (2004).

Performance prediction of industrial centrifuges using scale- down models. *Bioprocess and Biosystems Engineering*, *26*(6), 385–391.

Byrne, E. P., Fitzpatrick, J. J., Pampel, L. W., & Titchener-Hooker, N. J. (2002).

Influence of shear on particle size and fractal dimension of whey protein precipitates: Implications for scale-up and centrifugal clarification efficiency. *Chemical Engineering Science*, *57*(18), 3767–3779.

Chatel, A., Kumpalume, P., & Hoare, M. (2014). Ultra scale-down characterization of the impact of conditioning methods for harvested cell broths on clarification by continuous centrifugation-Recovery of domain antibodies from rec *E. coli*. *Biotechnology and Bioengineering,* 111(5), 913–924.

Klis, F. M., Mol, P., Hellingwerf, K., & Brul, S. (2002). Dynamics of cell wall ructure in Saccharomyces cerevisiae. *FEMS Microbiology Reviews*, *26*(3), 239–256.

Manners, D. J., Masson, A. J., Patterson, J. C., Björndal, H., & Lindberg, B. (1973).

The structure of a β-(1→6)-d-glucan from yeast cell walls. *Biochemical Journal*, *135*(1), 31–36.

Maybury, J. P., Hoare, M., & Dunnill, P. (2000). The use of laboratory centrifugation studies to predict performance of industrial machines: studies of shear-insensitive and shear-sensitive materials. Biotechnology and Bioengineering, 67(3), 265–273.

Rahar, S., Swami, G., Nagpal, N., Nagpal, M., & Singh, G. (2011). Preparation, characterization, and biological properties of β-glucans. *Journal of Advanced Pharmaceutical Technology & Research*, *2*(2), 94.

Suphantharika, M., Khunrae, P., Thanardkit, P., & Verduyn, C. (2003). Preparation of spent brewer's yeast beta-glucans with a potential

application as an immunostimulant for black tiger shrimp, Penaeus monodon. *Bioresource Technology*, *88*(1), 55–60.

Thammakiti, S., Suphantharika, M., Phaesuwan, T., & Verduyn, C. (2004). Preparation of spent brewer' s yeast b -glucans for potential applications in the food industry. *International Journal of Food Science and Technology*, *39*, 21–29.

Thanardkit, P., Khunrae, P., Suphantharika, M., & Verduyn, C. (2002). Glucan from spent brewer's yeast: preparation, analysis and use as a potential immunostimulants in shrimp feed. *World J. Microbiol. Biotechnol*, *18*, 527–539.

Varelas, V., Liouni, M., Calokerinos, A. C., & Nerantzis, E. T. (2015). An evaluation study of different methods for the production of β-D-glucan from yeast biomass. *Drug Testing and Analysis*, (May), 46–55. https://doi.org/10.1002/dta.1833

Vieira, E. F., Carvalho, J., Pinto, E., Cunha, S., Almeida, A. A., & Ferreira, I. M. P. L. V. O. (2016). Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract. *Journal of Food Composition and Analysis*, 52, 44–51.

Worrasinchai, S., Suphantharika, M., Pinjai, S., & Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocolloids*, *20*(1), 68-78. https://doi.org/10.1016/j.foodhyd.2005.03.005