Acta Scientiarum. Technology
ISSN: 1806-2563
ISSN: 1807-8664
actatech@uem.br
Universidade Estadual de Maringá
Brasil

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Acta Scientiarum. Technology, vol. 42, e48792, 2020
Universidade Estadual de Maringá, Brasil
Disponible en: https://www.redalyc.org/articulo.oa?id=303265671045
DOI: https://doi.org/10.4025/actascitechnol.v42i1.48792

Science, Food Technology and Food Engineering

Cellular disruption and its influence over the drying kinetics of brewer's spent yeast biomass

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DOI: https://doi.org/10.4025/actascitechnol.v42i1.48792
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Recepción: 17 Julio 2019
Aprobación: 04 Octubre 2019

Abstract:

Spent yeast biomass is one of the residues of brewing. It is specifically the second-largest residue from brewing industry. Most of the spent yeast is sold at low prices, or disposed as waste or used as animal feed. Spent yeast biomass is predominantly composed of proteins, and it has a high biological value, being an excellent source of high-quality protein, comparable in value with soy protein. Therefore, spent yeast biomass has great potential for use in foodstuffs for human consumption. The objective of this work was to evaluate the influence of cell rupture over the drying kinetics of spent yeast biomass using mathematical models (Lewis and Page). Also, to verify the influence of cell rupture method over the amount of protein. The cellular rupture was performed by two methods (chemical method and physical method: ultrasound). The drying process was performed by freeze-drying, and the parameters of the models were obtained using the non-linear regression (Generalized Reduced Gradient Nonlinear Optimization Code). Mathematical models of drying kinetics showed a strong correlation with the experimental data, $R^2 > 0.96$. The disruption process did not significantly affect the drying time and protein content. But the cellular autolysis improves the protein digestibility since the proteins will be totally available to the digestive enzymes and also increase the bioavailability of nutrients.

Keywords: autolysis, freeze-drying kinetics, mathematical models, yeast biomass.

Introduction

Global concern about natural resources, as well as the environment, directly reflects on the way in which industries manage their waste: some of them look for ways to minimize waste generation; other industries look for different methods to reuse the waste. In the food segment, the beverage industry is responsible for 26 % of the waste generated by this sector (Baiano, 2014). Specifically, the brewing industry generates several residues throughout its process: spent grains, hops, and yeast (Mussatto, 2009). The spent yeast is the second-largest residue from brewing industry (Podpora et al., 2015). Most of spent yeast is sold at low prices, or disposed as a waste (Amorim et al., 2016) or used as animal feed due to its high protein content (Ferreira et al., 2010).

Spent yeast is predominantly composed of proteins (35-60 % dry basis) that include all the essential amino acids, and it has high biological value (Chae, Joo & In, 2001), thus being an excellent source of high-quality protein, comparable in value with soy protein (Otero et al., 2000). Also, spent yeast is rich in B vitamins, and it is recognized as safe (GRAS) for human consumption (Chae, Joo, & In, 2001; Vieira, Brandao, & Ferreira, 2013; Podpora et al., 2015). In addition, it has been demonstrated that this waste has a huge potential as a

source of phenolic compounds (León-González et al., 2018), as a flavor enhancer (Vieira et al., 2016) and as a nutrient-enrichment agent in food (Pancrazio et al., 2016).

The most important nutritional compounds are inside the yeast cell. For these compounds to be released, the cell wall must be broken. To this end, mechanical methods, including shear force, and non-mechanical methods, involving chemical and biological agents, are employed (Liu et al., 2016). Moreover, the breakdown of the cell wall improves protein digestibility by making proteins fully exposed to the action of digestive enzymes (Yamada et al., 2003), and it increases bio-availability of nutrients (Pinto et al., 2013).

To facilitate its use in the food industry, the removal of water from spent yeast biomass is an essential step since it contributes to the increase of shelf-life, avoiding its degradation by microorganisms. Furthermore, drying reduces the volume resulting in lower packaging, storage, and transportation costs (Barati & Esfahani, 2011). Food drying is usually made using oven or freeze-dryers. Freeze-dryers are considered the best method to keep the organoleptic properties, also avoiding nutritional losses. However, the operational cost of freeze-drying is high when compared to other methods (Vieira, Nicoleti & Telis, 2012; Oliveira Júnior et al., 2018). Moreover, freeze dryers are expensive systems, both in capital investment and operational cost. Therefore, studying production capacity and drying time is of great importance (Berk, 2009).

Aiming to reduce the costs of drying via freeze-drying, it is crucial to understand the food matrix behavior during the dehydration process. Empirical and semi-empirical mathematical models are widely used to characterize this process. Semi-empirical models are based on Newton's Law of Cooling applied to mass transfer, such as the Page and Lewis models. Their parameters have physical meanings, and they are adjusted to the experimental data (Barati & Esfahani, 2011).

Given the problems described above, the objective of this work was to evaluate the influence of two different cell rupture methods over the drying kinetics of yeast biomass using mathematical models. Also, to verify this influence over the amount of protein.

Material and methods

Intact yeast biomass (IY)

The spent yeast biomass was supplied by a brewery located in Chapecó, SC (Brazil). The process for removing excess water was adapted from Sgarbieri et al. 1999: centrifuging the spent yeast (Centribium, Cienlab, Brazil) using 15 mL tubes, for 10 minutes, at 2500 rpm. After the supernatant was discarded, 2:1 distilled water (distilled water/yeast) was added to remove impurities and the remaining alcohol. The centrifugation process was performed twice, resulting in the clean yeast. Part of the clean yeast was placed in appropriate containers, capped, and identified for subsequent determination of initial moisture and drying kinetic tests. Another part of the clean yeast was placed in Petri dishes and taken to the ultrafreezer (IULT 335D, Indrel, Brazil) for 24 hours at a mean temperature of -86°C. Afterwards, the samples underwent freeze-drying for 24 hours in a benchtop freeze-dryer (TFD5503, Ilshin Lab. Co. Ltd., Korea), at -61°C and a pressure of 67 mbar. After freeze-drying, the yeast was crushed with a pistil and mortar and then sieved (mesh 32 – 500 mm/μm) to obtain a powder with uniform grain sizes.

Chemically autolysed yeast biomass (CAY)

To obtain the chemically autolysed yeast, the process adapted from Sgarbieri et al. (1999) was employed. The clean yeast obtained in the previous process was subjected to autolysis to rupture cell walls. This process consisted of resuspension of natural yeast into 1:1 (w v^{-1}) distilled water, addition of ethanol (7% w v^{-1}), and NaCl (2% w v^{-1}). The mixture was taken to the incubator (Luca-223, Lucadema, Brazil) for 24 hours at

55°C and 150 rpm, so as to stir and homogenize the sample. After 24 hours, autolysis was discontinued, with heat treatment at 85°C for 15 min. in a water bath (Dubnoff Luca 157/28, Lucadema, Brazil). Part of the autolysed yeast was placed in appropriate containers, capped, and identified for subsequent determination of initial moisture and drying kinetic tests. Another part of autolysed yeast was placed in Petri dishes and taken to the ultrafreezer (IULT 335 D, Indrel, Brazil) for 24 hours at a mean temperature of -86°C. Afterwards, the samples were lyophilized for 24 hours in a benchtop freeze-dryer (TFD5503, Ilshin Lab. Co. Ltd., Korea), at -61°C and a pressure of 67 mbar. After freeze-drying, the yeasts were crushed with a pistil and mortar, and then sieved (mesh 32 – 500 mm μm^{-1}) to obtain a powder with uniform particle sizes.

Physically autolysed yeast biomass (PAY)

To obtain the physically autolysed yeast, the clean yeast was put into a 250 mL Erlenmeyer, with 1:1 (w v^{-1}) distilled water. The Erlenmeyer was placed in an ultrasonic bath (Q335D, Quimis, Brazil) for one hour, frequency 40 kHz, ultrasonic power of 135 W, and 30ºC final temperature. After that, part of the physically autolysed yeast was placed in appropriate containers, capped, and identified for subsequent determination of initial moisture and drying kinetic tests. While another part of the physically autolysed yeast was placed in Petri dishes, frozen in the ultrafreezer, freeze-dried, and sieved, following the same parameters of the chemically autolysed yeast.

After sieving, all samples were placed in appropriate containers, capped, and identified for subsequent determination of nitrogen content.

Determination of nitrogen

Protein and initial moisture content

For the determination of the nitrogen content micro Kjeldahl (LUCA-341/02, Lucadema, Brazil) was used, and protein content was calculated using the conversion factor of 5.8. The initial moisture was determined by gravimetric method of direct drying in an oven (Cienlab, Brazil) at 105 ºC until reaching a constant weight. All analyses were performed in triplicate and according to the procedures of AOAC (2002).

Drying kinetics

To obtain the drying curves, 2 samples (50 g) of each yeast biomass (IY, CAY and PAY) were placed in tubes (part of the freeze-dryer equipment) and then in the ultrafreezer (IULT 335 D, Indrel, Brazil), being rotated every minute for the formation of a thin and even layer around the tubes. Afterwards, the tubes were quantified using an analytical balance (ATY224, Shimadzu, Japan) and coupled to the freeze-dryer (TFD5503, Ilshin Lab. Co. Ltd., Korea), -58°C initial temperature. The quantification of the samples occurred every half hour in the first 4 and a half hours of drying, and every hour for the other 7 hours of processing. The final mass determination was performed at the end of the 24 hours. At each quantification, system pressure and temperature and mass of each tube were recorded. The mathematical models of drying kinetics are shown in Table 1.

TABLE 1.
Mathematical models of drying kinetics.

Model¤	Equation¤	Reference¤	¤
Lewis¤	$MR = exp(-kt)$¤	Lewis·(1921)¤	(1)¤
Page¤	$MR = exp(-kt^n)$¤	Page·(1948)¤	(2)¤

The moisture ratio was determined using Equation 3,

$$MR = \frac{M_t - M_e}{M_o - M_e} \quad (3)$$

where MR represents the ratio between moistures, M_0 is the initial moisture content (dry basis), and M_t is the moisture content (dry basis) at any time, M_e is the equilibrium moisture content (dry basis).

Statistical analysis

The parameters "n" and "k" were obtained using the non-linear regression (Generalized Reduced Gradient Nonlinear Optimization Code), by means of the software Excel 2016. The coefficient of determination (R^2) and the root mean square error (RMSE) were used to determine the proper fit. All of them are described as equations (4) and (5), respectively (Ergün, Çahşkan & Dirin, 2016).

$$R^2 = \frac{N\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{\left(\sum_{i=1}^{N} MR_{pre,i}\right)^2} \quad (4)$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{1/2} \quad (5)$$

where the $MR_{exp,i}$ is the experimental moisture ratio, $MR_{pre,i}$ is the predicted moisture ratio, and N is the number of experimental data points.

It is important to emphasize that for R^2, the higher the coefficient, the better the model, and for RMSE, the smaller the result, the better the applicability of the mathematical model.

The statistical evaluation of the parameters "k" and "n" from the Page model obtained in duplicate and protein content were performed with the PAST 3.25 Software (Hammer®) by analysis of variance (ANOVA), and Tukey's test at 5 % of significance.

Results and discussion

The initial average moisture content of the samples (dry basis) was 3.91 ± 0.02 for the intact yeast biomass (IY), 6.94 ± 0.02 for the chemically autolysed yeast biomass (PAY), and 9.25 ± 0.52 for the physically autolysed biomass (CAY). These differences in the initial moisture values are due to the dilutions performed during the autolysis processes.

Figure 1 shows the moisture decrease of the samples during the freeze-drying process. The results are presented as the dimensionless moisture ratio, MR.

As it can be seen in Figure 1, the behavior of the three samples during the drying procedure is similar: much of the water is removed at the beginning of the process, in the first 330 minutes (at this point, the temperature reached – 62°C and stabilized). After that, the moisture gradually decreased until it reached the

equilibrium of moisture (before 500 minutes). This behavior was similar to a study carried out by Izli and Polat (2019), where quince was dried using freeze-drying.

Figure 2 shows the drying rates for intact yeast (IY), chemically autolysed yeast (CAY), and physically autolysed yeast (PAY) as a function of moisture content (dry basis), which was calculated using the following equation (Izli & Polat, 2019):

$$Drying\ rate = \frac{M_{t+dt} - M_t}{dt} \quad (6)$$

where M_t is the instantaneous moisture content (dry basis) at a given time, M_{t+dt} is the moisture content at $t + dt$, and t is the drying time (seconds).

After the initial period, where the drying rate reached the highest value (due to the large quantity of water ice on the surface of the sample, mainly on the surface of PAY), is possible to note two distinct periods: the stage where the drying rate remained almost constant - this period is called constant-rate drying period, and in this study it started nearly 0.8 of moisture ratio. And the second period, which is called falling-rate drying period (diffusion phase), where the drying rate decreased as moisture content also decreases (this period starts almost at 0.2 moisture ratio).

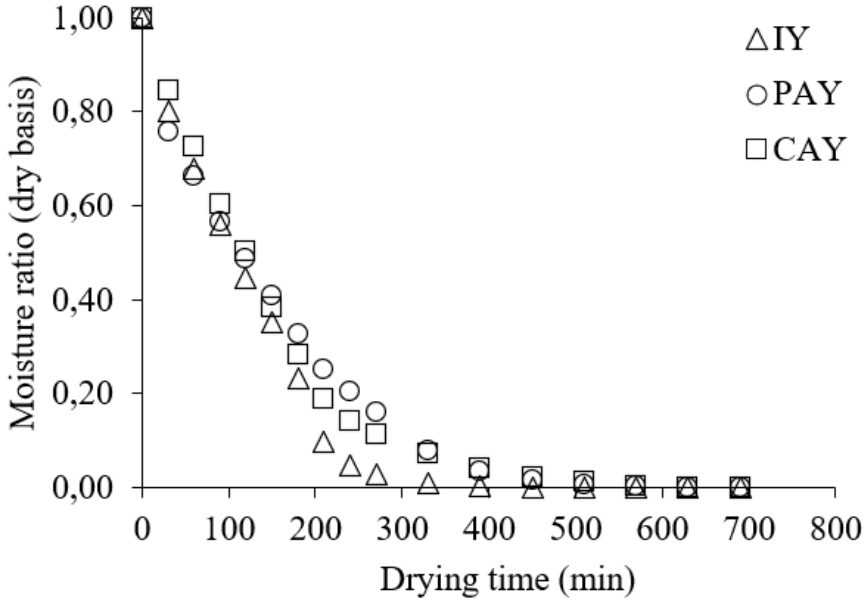

FIGURE 1.
Moisture ratio as a function of drying time for intact yeast (IY), chemically autolysed yeast (CAY) and physically autolysed yeast (PAY).

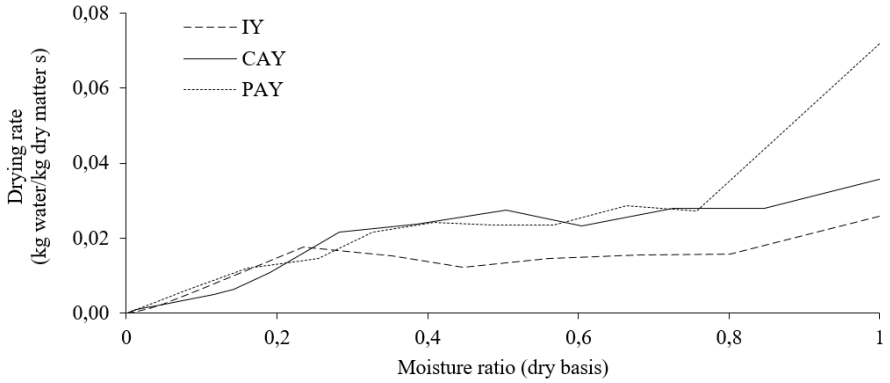

FIGURE 2.
Drying rates of intact (IY), chemically autolysed (CAY) and physically autolysed (PAY) yeast, as a function of moisture ratio.

In the constant rate drying period, there is still ice on the surface of the sample, and this ice evaporates easily by sublimation. And as the dried layer gets bigger it increased the resistance to ice vapor to leave the sample. Therefore, in the second period, the drying rate decreases due to the increasing mass transfer resistance with the increasing dry layer thickness. It means that in this period, vaporized water diffuses through the pores of the dried layer before it leaves the sample and goes to the atmosphere in the drying chamber (Toledo, 2007).

The statistical results of the mathematical drying models are shown in Table 2. In all cases, the R^2 values were higher than 0.96, and the RMSE values were lower than 0.05328, pointing to a very strong correlation between models and experimental data.

It is important to mention that these equations are widely used in drying of food and agro-industrial products getting satisfactory results even in different drying process (freeze-drying, convective drying, for example) (Onwude et al., 2016; Caliskan & Dirim, 2017; Keneni, Hvoslef-Eide & Marchetti, 2019).

According to Corrêa et al. (2007), the "k" constant represents the effect of external drying conditions, while the "n" constant reflects the internal resistance of the product to drying. The statistical evaluation of the parameters "k" and "n" from the Page model showed that there was no significant difference between the samples (significant differences ($p \leq 0.05$) by Tukey test). It means all the samples had the same behavior during the drying process. Also, the disruption process did not affect the drying time significantly.

Another way to verify the effect of the cellular rupture process in the samples is by quantifying the amount of protein released after the disruption. Protein is confined mostly within cytoplasm by cell membrane (Balasundaram, Harrison & Bracewell, 2009). Therefore, the release of protein can be used to evaluate the extent of cell membrane damage (Vieira, Brandão & Ferreira, 2013; Zhang et al., 2014).

From Table 3, it is possible to note that there is no significant difference between the intact yeast and the physically autolysed yeast. But the cellular autolysis can improve the protein digestibility since proteins will be totally available to digestive enzymes (Yamada et al., 2003). Besides, there is a better bioavailability of nutrients and lower nucleic acid content (Pinto et al., 2013).

The cellular disruption process by ultrasound resulted in greater protein content than the chemical autolysis process. It demonstrates that physically autolysis is an alternative to obtain significant values of protein. About the process, while chemical autolysis is carried out for 24 hours, the ultrasound process requires only one hour. Furthermore, in ultrasound autolysis process the yeast is not placed in an environment with extreme working conditions, such as the use of reagents or high temperatures for a long time. For example, research reports showed that cell disruption occurs efficiently at 25°C (Zhang et al., 2014).

A study carried out by Knorr et al. (1979) showed that chemical autolysis has the disadvantages of long reaction times, high cost and low protein yields. Moreover, the low yield of protein was related to the fact

that the protein can undergo hydrolysis, depolymerization, and denaturation during the process of chemical autolysis (Halász & Lásztity, 1991). Therefore, this could explain the values obtained for chemically autolysed yeast (Table 3).

The protein values found in this work are similar to those found in the literature (Podpora et al., 2015; Jacob, Hutzler & Methner, 2019). For example, the total protein content of spent yeast biomass samples, ranged between 45.8 and 49.4 (w w^{-1} dry yeast cell) in a study carried out by Vieira et al. (2013) and between 39.32 and 43.80 (g 100 g^{-1} of dry spent yeast biomass) in a study carried out by Bertolo et al. (2019).

Figure 3 shows the behaviors of the intact, chemically autolysed and physically autolysed yeast spent biomass, in comparison to the kinetic models' predictions: Lewis and Page.

TABLE 2.
Parameters of the mathematical models with their respective coefficients of determination (R^2) and the sum of root mean square error (RMSE) for yeast in its intact (IY), chemically autolysed (CAY) and physically autolysed (PAY) forms.

Models	Samples	Parameters			
		k (min^{-1})	n	R^2	RMSE
Lewis	IY	0.0083	-	0.96	0.05328
	CAY	0.0068	-	0.98	0.03729
	PAY	0.0066	-	0.98	0.02740
Page	IY	0.0012	1.3689	0.98	0.03273
	CAY	0.0016	1.2783	0.99	0.01519
	PAY	0.0054	1.0366	0.98	0.02688

TABLE 3.
Protein content for yeast in its intact (IY), chemically autolysed (CAY), and physically autolysed (PAY) forms.

Constituent (%)	Natural	Chemically autolysed	Physically autolysed
Protein ($N \times 5.8$)	50.58±0.63[a]	41.08±4.77[b]	51.95±2.26[a]

Values expressed as average ± standard deviation. Different letters in the same line indicate significant differences ($p \leq 0.05$) by Tukey test.

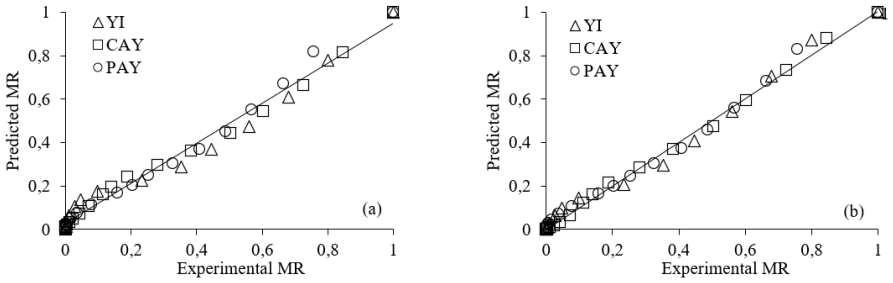

FIGURE 3.
Comparison between experimental and predicted moisture ratios using Lewis (a) and Page Models (b).

The predicted values using Lewis and Page models showed moisture ratio values distributed along a straight line, which demonstrate what is shown in Table 2, i.e., the excellent correlation of the Lewis and Page mathematical models to the actual drying process. These models were able to characterize the system efficiently.

Conclusion

To evaluate the influence of different cell disruption methods over the drying kinetics of spent yeast biomass, the change in moisture ratio with time was determined experimentally. Lewis and Page models showed an excellent correlation with the experimental data, $R^2 > 0.96$, RMSE < 0.05328. It demonstrated their significant potential in the characterization of the freeze-drying process. By the way, the different forms of autolysis used in this work did not significantly affect the drying process or the protein release. Given this, it is still necessary to improve autolysis processes to obtain expressive results.

Acknowledgements

The authors thank UDESC for all support and to Dalla brewery for their raw material donation. This research was supported by FAPESC (Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina – Grant: 2015TR295).

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