Effets de la formulation et des technologies de séchage sur les propriétés des poudres probiotiques

Tahir FIROOZ[1], Claudia COGNE[1], Claire BORDES[1], Zahra ZEGGANE[1], Abdelkader SELMI[2], Alexandra CLAYER MONTEMBALT[3], Laurent DAVID[3], Jacqueline RESENDE DE AZEVEDO[1]

1 : Universite Claude Bernard Lyon 1, LAGEPP UMR 5007 CNRS, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France
2 : Biofactory, CPE, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France
3 : Universite Claude Bernard Lyon 1, IMP UMR 5223 CNRS, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France

Résumé

Ce travail porte sur l'étude des procédés de séchage par atomisation et par lyophilisation pour la production de poudres de probiotique *Saccharomyces cerevisiae* à partir de différentes formulations liquides. L'influence des ingrédients de la formulation sur les propriétés des poudres a été quantifiée en combinant des agents protecteurs couramment utilisés (tréhalose, maltodextrine, gomme arabique) à un chitosane modifié (chitosane@DOTAGA). Un plan d'expérience et des surfaces de réponse triangulaire ont été utilisés pour évaluer les effets des mélanges sur les réponses en rendement de procédé de séchage, en taux de survie des levures et en teneur en eau des poudres formulées. En ce qui concerne la comparaison des procédés, la lyophilisation donne de meilleurs résultats que l'atomisation en terme de matière sèche des poudres et en rendement, mais la viabilité cellulaire de la poudre lyophilisée est plus faible après 2 mois de stockage. En termes de formulation, l'ajout de chitosane réduit le rendement du procédé mais améliore significativement la stabilité de la poudre.

Effects of Formulation and Drying Technologies on the Properties of Probiotic Powders

Tahir FIROOZ[1], Claudia COGNE[1], Claire BORDES[1], Zahra ZEGGANE[1], Abdelkader SELMI[2], Alexandra CLAYER MONTEMBALT[3], Laurent DAVID[3], Jacqueline RESENDE DE AZEVEDO[1]

1 : Universite Claude Bernard Lyon 1, LAGEPP UMR 5007 CNRS, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France
2 : Biofactory, CPE, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France
3 : Universite Claude Bernard Lyon 1, IMP UMR 5223 CNRS, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France

Abstract

Spray and freeze-drying processes for production of the probiotic Saccharomyces cerevisiae from different formulations were investigated. The impact of formulation ingredients on the properties of the powders was studied by adding protective agents currently used (trehalose, maltodextrin, gum arabic) combined with a modified chitosan (chitosan@DOTAGA). An experimental design and triangular response surface were used to evaluate the effects of the mixtures on the responses in term of yield of process, survival ratio and moisture content for powders formulated. Concerning process comparison, freeze-drying leads to better results in terms of dry matter and yield than atomization, but cell viability of freeze-dried powder is lower after 2 months of storage. In terms of formulation, the addition of chitosan reduces process yield but improves powder stability.

1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate quantities (10^6–10^7 CFU/g or mL of the final product), confer health benefits to the host (FAO/WHO, 2006). Their effects are primarily exerted in the gastrointestinal tract, where they can influence the intestinal microflora. However, the viability of microorganisms can be compromised by acidic gastric conditions, thermal stress during heating, interactions with complex food matrices, and environmental stress during storage. To achieve beneficial health effects, it is crucial to ensure their metabolic activity and a sufficient level of viability throughout the manufacturing, transport, and storage stages, up to the point they reach the site of action.
Dry forms of probiotics are generally preferred over liquid forms due to their better stability and ease of transport. Freeze-drying and spray-drying processes are widely employed in the dry probiotics industry (Chandralekha et al., 2017). Freeze-drying, although the most commonly used due to its ability to preserve microorganism viability, is a time-consuming and energy-intensive process. Spray-drying, on the other hand, generates greater thermal stress and leads to lower yields (Verlhac et al., 2020). Nevertheless, it has been shown to be more effective than freeze-drying in

specific conditions for probiotic *Bacillus amyloliquefaciens* drying (Luangthongkam et al., 2021). In freeze-drying, freezing and drying are combined into a single process to produce high-quality finished products. However, the freezing step is particularly critical, as it can negatively impact the viability and physiological state of the yeast (Brashears and Gilliland, 1995; Tymczyszyn et al., 2005). In spray-drying, heat and mass transfer occur simultaneously between the air and the atomized droplets to form and dry the particles (Riveros, Ferrer, and Borquez, 2000). Despite its advantages, microorganisms are exposed to various stress factors during spray-drying (osmotic, thermal, oxidative), which can cause membrane damage and inactivation. The success of both processes to probiotics drying relies on careful control of process parameters and the appropriate selection of protective agents in the formulation.

In lyophilization, factors such as freezing temperature and duration, heating rate, pressure, and overall lyophilization time have a significant impact on the physicochemical properties and functionality of the resulting powders (Dong et al., 2024; Faustino et al., 2023; Öztürk, 2022).Similarly, in spray drying, key parameters like air inlet and outlet temperatures, feed rate, nozzle size, and air flow play a crucial role in determining the powders' characteristics and functionality(Vanden Braber et al., 2020; Vorländer et al., 2023).

Proteins, polysaccharides, sugars and their combinaison can be used as protection agents and carrier for probiotics, including alginate, gum arabic, maltodextrin, modified starch and whey protein (Aksoylu Özbek and Günç Ergönül, 2020; Arslan et al., 2015; Cui et al., 2021; Perrechil et al., 2021; Ruengdech and Siripatrawan, 2022). A range of cryoprotectants, including sucrose, trehalose, skimmed milk, sorbitol, and amino acids, has been used to minimize damage to bacterial cells during the freeze-drying process (Kanimozhi and Sukumar, 2023). In general, the choice of protective agent depends on various criteria, such as the type of active ingredient to be protected, its compatibility with the formulation, its solubility and viscosity in the solvent used, as well as its cost, availability, and impact on the properties of the final product. In spray drying, the choice particularly depends on the protective agent's ability to encapsulate the active ingredient and maintain its stability and effectiveness. In freeze-drying, the protective agent must stabilize the product during the freezing and to avoid collapse during sublimation. When drying probiotics, the choice is also based on the protective agents' ability to reduce cellular damage, maintain a good viability rate, and preserve the function of the microorganisms' cells.

Chitosan has also been used for the microencapsulation of several bioactive compounds, such as enzymes, antioxidants, antibiotics and vitamins(Budinčić et al., 2021; Estevinho et al., 2013). For probiotics, chitosan is rarely used as a carrier due to its antimicrobial activity. Chitosan's antimicrobial activity has been attributed to its ability to bind to anionic cell wall macromolecules and its interaction with the cell membrane. This antimicrobial activity in turn depends on the molecular weight of chitosan and the availability of amino groups (linked to the degree of acetylation). Some strategies are used to include chitosan in the microencapsulation of probiotics. These include the coating of microparticles formed by other encapsulation agents (Parsana et al., 2023; Thinkohkaew et al., 2024) and the use of modified chitosan(Díaz Vergara et al., 2023; Vanden Braber et al., 2020). Vanden Braber et al. (2020) studied the effect of the microencapsulation of the probiotic yeast *Kluyveromyces marxianus* VM004 by spray drying using a binary mixtures formed

to whey protein concentrate and water soluble chitosan. Yeasts microencapsulated in water soluble chitosan showed a significantly improved tolerance to simulated gastrointestinal conditions in comparison to free yeasts and yeasts microencapsulated in whey protein concentrate. More recently, the same research group encapsulated this yeast using a similar water-soluble chitosan derivative with a lower degree of deacetylation and molecular weight than those used previously. The results showed an encapsulation rate of over 57%, with improved resistance to gastrointestinal conditions and enhanced storage stability (Díaz Vergara et al., 2023). These studies demonstrated the feasibility of using modified chitosan as a sole wall material or in combination with other protective agents.
Based in this context, we decided to investigate a new chitosan derivative (Chitosan@DOTAGA synthetized by (Grange et al., 2023) in combination with wall materials (trehalose, maltodextrin, gum arabic) for the production of dry forms containing probiotics. *Saccharomyces cerevisiae* (*S.c*) yeasts was used as a model organism to develop dry formulations through freeze-drying and spray-drying. The impact of formulation ingredients and drying process on powders properties was studied using an experimental plan and triangular response surface, focusing on yield of process, survival rate and moisture content.

2. Materials and methods

2.1 Materials

Saccharomyces cerevisiae (S.c) yeast (batch number DSM1333-0522-001) was supplied by DSMZ (Germany). Trehalose (TH) was purchased from DFE, France (batch number X20022603), maltodextrin (MD) from Roquette, France (Glucidex 12D, batch number E542K), gum arabic (GA) from COOPER, France (batch number 07070121/B) and glycerol (batch number 2346033) in the UK. Yeast-Peptone-Dextrose liquid culture medium (YPD) was supplied by Becton Dickinson, France (batch number 242810) and YPD powder with Agar (Yeast-Peptone-Dextrose-Agar) from Roth, Germany (batch number 183337580). Chitosan@DOTAGA (noted $CHI_{@DOTAGA}$), with a molecular weight of 227 kDa and a degree of acetylation of 5%, was produced by a French academic laboratory (Ingénierie des Matériaux Polymères, Universite Claude Bernard Lyon 1, France).

2.2 Culture of *Saccharomyces cerevisiae*

The yeast *Saccharomyces cerevisiae* (S.c) was selected as a model organism to study the impact of formulation and type of drying process on the properties of the powders obtained. A culture medium containing 5% w/v YPD or 6% w/v YPD-agar in water, sterilized at 121°C for 15 min by autoclaving (Certoclav Multicontrol), was used to incubate S.c. yeast at 30°C for 48 h (the time required to ensure optimal yeast growth).

2.3 Formulation production by experimental design

The influence of the formulation on the final properties of probiotic powders was studied from an experimental design and triangular response surface with an unstudied zone, following an experiment plan NemrodW® (LPRAI, Marseille, France)(Table 1). The independent variables evaluated included TH, MD and GA concentrations, in the absence and presence of CHI_{DA} with max. 15% w/w dry matter. The experimental plan was developed to ensure acceptable formulation viscosities in particular for spray drying (maximum 300 mPa.s), CHI_{DA} concentration was limited to 1% and that of the GA to 4%. Before the drying process, the wall materials were solubilized in water to obtained pure, binary or ternary combinations containing S.c suspension (ratio S.c suspension: wall material solution 1:1 w/w). Chitosan was solubilized in water with a few drops of acetic acid 5%. The formulations are then sterilized at 121°C for 15 minutes using a Certoclav Multicontrol autoclave. The response variables were the process yield, moisture content and survival rate. The 19 formulations were immediately dried by spray and freeze-drying methods. Powders collected were stored in glass flasks and placed at room temperature and at 4°C for subsequent analysis.

2.4 Drying processes

Preliminary drying tests were used to select the operating parameters for the two processes, as presented in the two following paragraphs.

2.4.1 Spray drying

The atomization process was performed using a Mini-Spray dryer B-290 (Büchi, Switzerland) equipped with a two fluid nozzle atomiser (diameter 1.5 mm). Approximately 50 ± 4 g of the formulations was pumped into the drying chamber using a peristaltic pump rate of 12% ± 2 (4 ± 1 g/min). Drying process was carried out at an air inlet air temperature of 120°C, corresponding to an outlet air temperature of 60 ± 2°C, aspirator rate of 100% and spraying flow rate 600 L/h. The micro particles were separated by a cyclone and collected in a collection vessel. These processing conditions were maintained for all experiments.

2.4.2 Freeze-drying

Freeze-drying was realized using a Cryotec device (France). 19 solutions were dried in a single cycle. Each solution was weighted (approximatively 1g) and placed in a vial. The solutions were frozen at -40°C for 3 hours, then sublimed at a pressure of 0.251 mbar with a gradual temperature rise from -40°C to -10°C for 30 hours. Finally, desiccation takes place at a pressure of 0.1 mbar for 9 hours at 15°C. After drying, powders in vials were weighted.

2.5 Physical-chemical, microbiological and process analyses

2.5.1 *Enumeration of S.c colonies (CFU/g)*

To estimate the initial concentration of *S.c* (noted N_0 in eq. 1), the suspensions were diluted (10^{-4}; $10^{-4}/2$; $10^{-4}/4$). For each formulation, 100 µL of the three dilutions were spread on Petri plates containing gelled YPD-Agar medium. The plates were incubated for 48 hours at 30°C to enumerate colony forming units per gram (CFU/g). Each test was conducted in triplicate.
To estimate *S.c* concentration in powders after drying (noted N in eq. 2), 0.1 g of powder were rehydrated with 0.9 g of YPD solution. Then, following the identical method used to determine the CFU/g in the initial suspensions, the CFU/g in the dry powders was counted.

2.5.2 *Drying Yield* (Y)

The drying yield (Y) is defined by Equation 1:

$$Y\,(\%) = \frac{\text{Masse of dry powder recovered after drying}}{\text{Mass of dry material in the formulation before drying}} \text{x}100 \quad (1)$$

2.5.3 *Moisture content*

Approximately 20 mg of each powder sample was analysed with a Karl Fischer titrator (860 KF Thermoprep. Metrohm. Herisau. Switzerland). An air flow rate of 100 ml/min was used at a temperature of 120°C to avoid sample degradation. Moisture content (MC) was expressed as a percentage of initial weight.

2.5.4 *Survival rate*

The survival rate of *S.c* is calculated by Equation 2:

$$\text{SR}(\%) = 100 \text{ x} \frac{N}{N_0} \quad (2)$$

where N is the number of viable cells (CFU/g) in the recovered powder after drying and N_0 is the number of viable cells (CFU/g dry matter) in the solution before drying. Viability tests on *S.c* were assessed immediately after drying.

2.5.5 *Logarithmic reduction of viable cells*

The logarithmic reduction of viable cells (LRVC) is calculated according to the Equation 3:

$$\text{LRVC} = log \frac{N_{60}}{N} \quad (3)$$

Where N and N_{60} refers to the number of cells respectively before storage and after 60 days of storage at 4°C in a hermetic vial.

3. Results and discussions

The experimental design responses for the 19 trials in terms of process yield (Y), survival rate (SR), and powder moisture content (MC) are presented in Table 1, for both spray drying and freeze drying.

3.1 Responses surface analysis

3.1.1 *Process yield*

Figure 1 presents the response surface plot for process yield, mainly determined by the powder collection efficiency. As seen in table 1, yield is more important in freeze-drying (between 93% and 100%) because material loss is less important in vial. Formulation has no significant effect on freeze-drying yield, so response surface results are not shown in Figure 1. In spray-drying, material loss is due to the attachment of powder in the apparatus wall and to poor efficiency of cyclone to collect fine particles. Response surfaces show that without $CHI_{@DOTAGA}$ (Figure 1a), spray-drying yields are higher, reaching up to 75% at high trehalose (TH) concentrations and at high maltodextrin (MD) concentrations. Incorporation of $CHI_{@DOTAGA}$ or GA reduces these values, with the highest $CHI_{@DOTAGA}$ concentration (1%) resulting in the lowest yields (Figure 1b) due to the high viscosity of the formulations. In summary, trehalose has a beneficial effect, while maltodextrin has a beneficial effect in the absence of $CHI_{@DOTAGA}$, although this effect is less pronounced than that of TH alone, and $CHI_{@DOTAGA}$ and GA have a deleterious impact on the spray-drying yield.

Table 1. Experimental design of the formulations and responses obtained by spray-drying and freeze-drying.

N°	Experimental name	Input variables				Response variables					
						Spray-drying			Freeze-drying		
		TH (%)	$CHI_{@DOTAGA}$ (%)	MD (%)	GA (%)	Y (%)	SR (%)	MC (%)	Y (%)	SR (%)	MC (%)
1	TH15	15	0	0	0	82.0	70.0	5.8	98.6	95.2	1.8
2	TH14:$CHI_{@DOTAGA}$1	14	1	0	0	68.9	69.8	3.6	97.8	90.9	1.9
3	TH11:GA4	11	0	0	4	57.3	80.3	4.1	96.5	95.4	1.7
4	TH10:$CHI_{@DOTAGA}$1:GA4	10	1	0	4	57.7	82.8	5.8	97.0	86.7	1.9
5	MD15	0	0	15	0	72.2	67	6.7	97.2	88.3	1.8
6	$CHI_{@DOTAGA}$1: MD14	0	1	14	0	55.0	49.8	3.9	98.7	80.9	1.9
7	MD11:GA4	0	0	11	4	72.7	60.1	9.6	98.3	84.6	1.6
8	$CHI_{@DOTAGA}$1:MD10:GA4	0	1	10	4	48.0	63.8	4.2	> 100	79.3	1.9
9	TH14.5: $CHI_{@DOTAGA}$0.5	14.5	0.5	0	0	70.3	77.5	1.9	99.0	79.9	1.9
10	TH13:GA2	13	0	0	2	79.5	71.8	6.2	98.4	94.7	2
11	TH7.5:MD7.5	7.5	0	7.5	0	78.9	76.5	6.7	97.3	90.5	1.9
12	TH12: $CHI_{@DOTAGA}$1:GA2	12	1	0	2	58.7	63.6	5.2	98.2	83.8	1.9
13	TH7: $CHI_{@DOTAGA}$1:MD7	7	1	7	0	36.5	63.7	4.3	100	78.3	1.9
14	TH5.5:MD5.5:GA4	5.5	0	5.5	4	75.2	70.2	7.1	97.7	87.2	1.8
15	$CHI_{@DOTAGA}$0.5:MD12.5:GA2	0	0.5	12.5	2	39.3	65.8	8.6	98.2	88.5	2
16	TH6.5:MD6.5:GA2	6.5	0	6.5	2	71.0	66.4	7.7	96.2	88.5	1.9
17	TH6:$CHI_{@DOTAGA}$1:MD6:GA2	6	1	6	2	51.8	60.6	5.7	93.5	89.4	1.7
18	TH5.25:$CHI_{@DOTAGA}$0.5:MD5.25:GA4	5.25	0.5	5.25	4	72.4	46.6	5.9	98.3	82.7	1.6
19	TH6.25:$CHI_{@DOTAGA}$0.5:MD6.25:GA2	6.25	0.5	6.25	2	53.8	49.2	5.3	> 100	89.7	1.5

TH: Trehalose, $CHI_{@DOTAGA}$*: Chitosan@DOTAGA, MD: Maltodextrin, GA: Gum Arabic, Y: drying yield, SR: survival rate, MC: moisture content.*

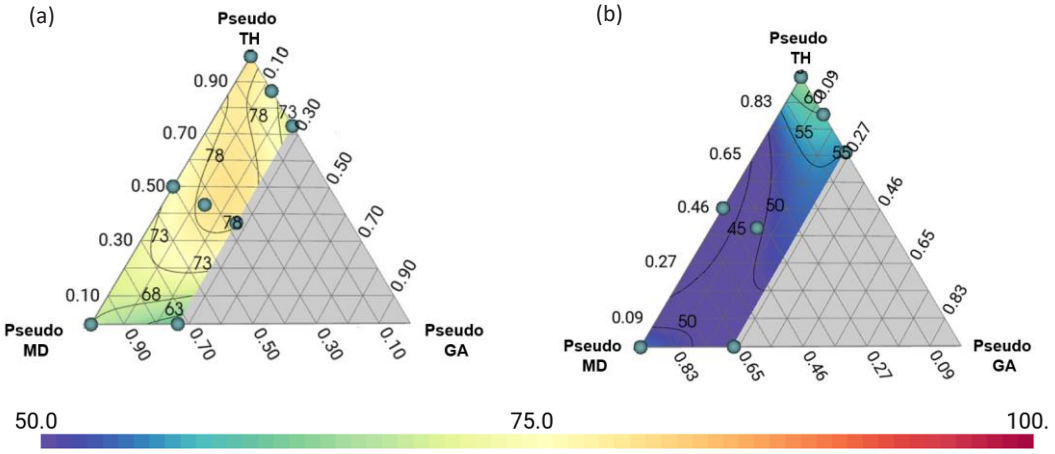

Figure 1. Response surface plot for spray-drying process yield: (a) formulations without CHI$_{@DOTAGA}$, (b) formulations with CHI$_{@DOTAGA}$ 1%w/w.

3.1.2 *Moisture content*

As presented in Table 1, the 19 freeze-dried samples have a moisture content of less than 2%, meaning that no impact of formulation composition on powder humidity was observed. Concerning spray-drying, results show that the moisture content varies considerably according to their composition, ranging from 1.9% to 9.6%. Figure 2 shows that formulations with CHI$_{@DOTAGA}$ lead to the lowest moisture content. Samples containing TH show intermediate moisture values (between 4% and 5%), while formulations with high MD concentrations show the highest levels (up to 9% without CHI$_{@DOTAGA}$).

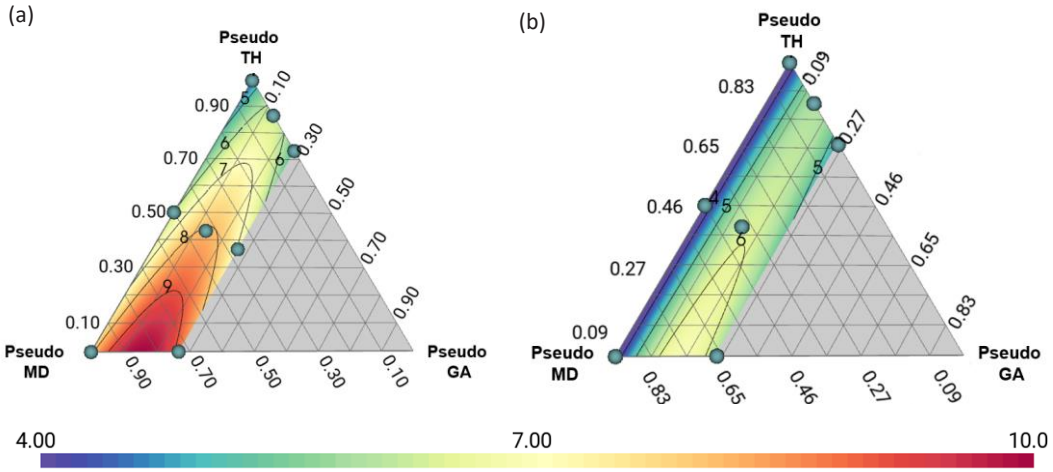

Figure 2. Response surface plot for powder moisture content: (a) formulations without CHI$_{@DOTAGA}$, (b) formulations with CHI$_{@DOTAGA}$ 1%w/w.

3.1.3 *Survival rate*

Figure 3 presents the response surface plot for the *S.c* survival rates after drying. In general, the values obtained are higher for freeze-drying, which may be explained by the deleterious effect of high temperatures used in spray-drying. In freeze-drying as in spray-drying, the addition of chitosan brings no benefits. Nevertheless, the survival ratio after spray-drying is significantly improved at around 80% by formulations with high concentrations of TH combined with a small amount of GA. On the other hand, formulations containing high concentrations of MD show lower survival rates, close to 60%. These survival rates are lower than those reported by Arslan *et al.* [6], where microencapsulation of *S.c* with various wall materials resulted in survival rates of between 84% and 92%, particularly with MD. This discrepancy could result from variations in drying parameters, such as the higher inlet temperature used in our study, which is known to significantly reduce the

cell viability of encapsulated microorganisms. Nevertheless, all formulations maintained a permissible yeast concentration for probiotics, greater than or equal to 10^6 CFU/g.

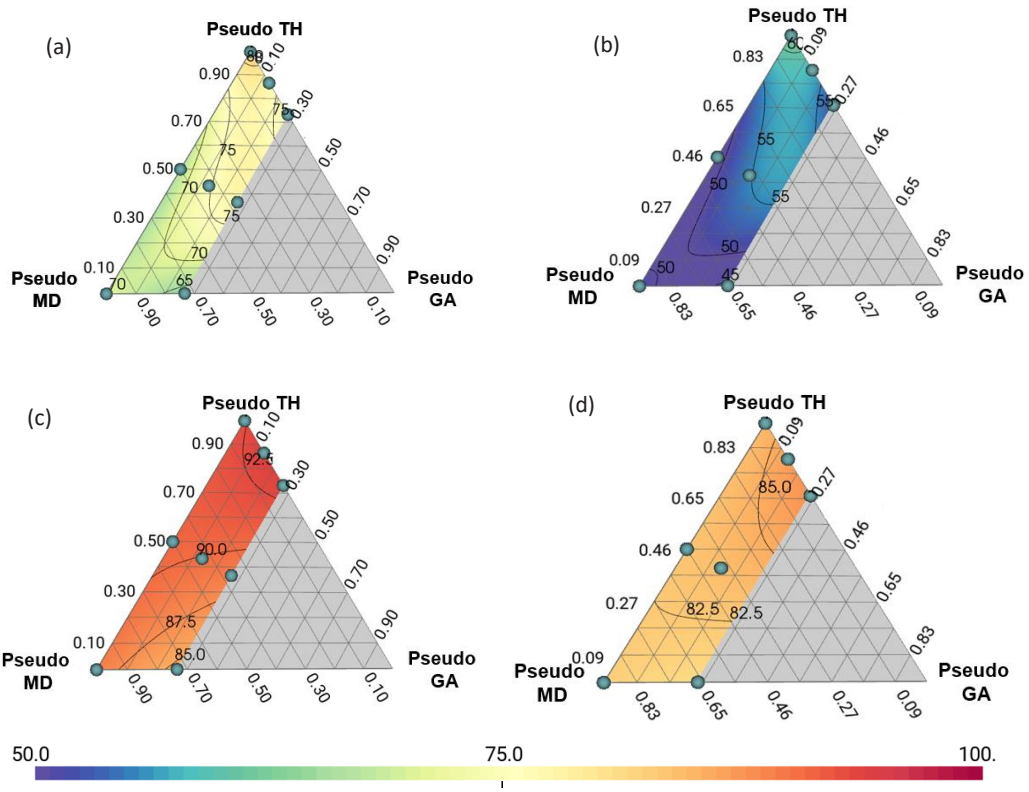

Figure 3. Response surface plot for survival rate: (a) and (b) for spray-drying, (c) and (d) for freeze-drying; (a) and (c) formulations without $CHI_{@DOTAGA}$, (b) and (d) for formulations with $CHI_{@DOTAGA}$ 1%w/w.

3.2 Stability during storage

The logarithmic reduction of viable cells after 60 days of storage at 4°C is presented on Figure 4 for spray-drying (red histogram) and freeze-drying (blue histogram). Loss of viability is generally low for spray-dried powders. For freeze-dried samples, powder stability is highly dependent on formulation. In particular, maltodextrin appears to have a negative effect on *S.c* viability after 2 months of storage. Formulations MD15 and MD11:GA4 had such high viability losses that they cannot be considered as probiotics (CFU/g is less than 10^6).
As regards spray-dried formulations, a sufficient quantity of chitosan (1%) seems to lead to good stability, with a decrease in logarithm of less than 0.5log. Other authors have reported this protective effect of chitosan during storage of probiotic-containing powders (Díaz Vergara et al., 2023; Trabelsi et al., 2014; Vanden Braber et al., 2020).

This can be attributed to the film-forming properties of chitosan which provide the microcapsules with a smoother appearance, reduce their oxygen and water vapor permeability, decrease the molecular mobility of water in the powder, and enhance their stability during storage (Cazón and Vázquez, 2020). In our case, the differences in formulation stability observed in relation to the type of drying process may be linked to the formation of micro particles by atomization (containing microorganisms encapsulated in the particle “cores”), which does not occur after freeze-drying (microorganisms dispersed in the matrix) (confirmed by microscopy images, results not shown).

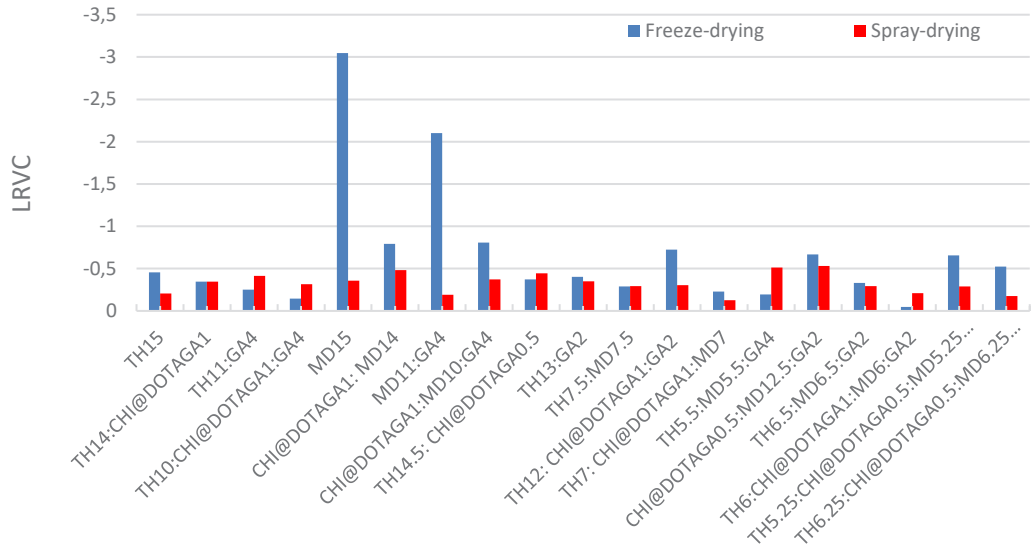

Figure 4. Logarithmic reduction of viable cells (LRVC) after 60 days of storage at 4°C. Blue: freeze-drying; red: spray-drying

4. Conclusions

In this work, 19 yeast-based formulations containg *S.c* were dried either by spray drying or freeze-drying. Concerning drying processes comparison, freeze-drying in flasks naturally leads to higher yields (> 93%) than those obtained by spray-drying (between 52 and 82%) due to matter content losses on the atomizer walls. *S.c* survival rates are better for freeze-dried powders (between 78% and 96%), due to lower drying temperatures than for spray-dried powders (survival rates between 36% and 82%). Moisture contents are also better in freeze-drying than in spray-drying. But powders stability after 60 days of storage at 4°C is generally better for atomized powders. The addition of modified chitosan to the formulations had a negative effect on atomization yield, due to increased viscosity but has a positive effect on the powder stability during storage: combined with optimal quantities of trehalose and maltodextrin, it helped to promote cell viability over time. In perspectives, the most promising formulations in terms of process yield and powder qualities will be used to optimize drying conditions, i.e freezing rates and sublimation time for freeze-drying as well as air temperature and flow rates in spray-drying.

Acknowledgment

LAGEPP would like to thank the InnoBioVir Platform, the Auvergne-Rhône-Alpes Region and the European Regional Development Fund (ERDF) for their support.
Authors are grateful to the Campus France program for the financial support received during this work.

References

Aksoylu Özbek, Z., & Günç Ergönül, P. (2020). Optimisation of wall material composition of freeze–dried pumpkin seed oil microcapsules: Interaction effects of whey protein, maltodextrin, and gum Arabic by D–optimal mixture design approach. *Food Hydrocolloids*, *107*. https://doi.org/10.1016/j.foodhyd.2020.105909

Arslan, S., Erbas, M., Tontul, I., & Topuz, A. (2015). Microencapsulation of probiotic Saccharomyces cerevisiae var: Boulardii with different wall materials by spray drying. *LWT*, *63*(1), 685–690. https://doi.org/10.1016/j.lwt.2015.03.034

Brashears, M. M., & Gilliland, S. E. (1995). Survival During Frozen and Subsequent Refrigerated Storage of Lactobacillus acidophilus Cells as Influenced by the Growth Phase. *Journal of Dairy Science*, *78*(11), 2326–2335. https://doi.org/10.3168/jds.S0022-0302(95)76859-X

Budinčić, J. M., Petrović, L., Đekić, L., Fraj, J., Bučko, S., Katona, J., & Spasojević, L. (2021). Study of vitamin E microencapsulation and controlled release from chitosan/sodium lauryl ether sulfate microcapsules. *Carbohydrate Polymers*, *251*. https://doi.org/10.1016/j.carbpol.2020.116988Cazón, P., Vázquez, M. Mechanical and barrier properties of chitosan combined with other components as food packaging film. Environ Chem Lett 18, 257–267 (2020). https://doi.org/10.1007/s10311-019-00936-3

Chandralekha, A., Rani, A., Tavanandi, H. A., Amrutha, N., Hebbar, U., & Raghavarao, K. S. M. S. (2017). Role of carrier material in encapsulation of yeast (Saccharomyces cerevisiae) by spray drying. *Drying Technology*, *35*(8), 1029–1042. https://doi.org/10.1080/07373937.2016.1230626

Cui, T., Chen, C., Jia, A., Li, D., Shi, Y., Zhang, M., Bai, X., Liu, X., & Liu, C. (2021). Characterization and human microfold cell assay of fish oil microcapsules: Effect of spray drying and freeze-drying using konjac glucomannan (KGM)-soybean protein isolate (SPI) as wall materials. *Journal of Functional Foods*, *83*. https://doi.org/10.1016/j.jff.2021.104542

Díaz Vergara, L. I., Arata Badano, J., Aminahuel, C. A., Vanden Braber, N. L., Rossi, Y. E., Pereyra, C. M., Cavaglieri, L. R., & Montenegro, M. A. (2023). Chitosan-glucose derivative as effective wall material for probiotic yeasts microencapsulation. *International Journal of Biological Macromolecules*, *253*. https://doi.org/10.1016/j.ijbiomac.2023.127167

Dong, X., Woo, M. W., & Quek, S. Y. (2024). The physicochemical properties, functionality, and digestibility of hempseed protein isolate as impacted by spray drying and freeze drying. *Food Chemistry*, *433*. https://doi.org/10.1016/j.foodchem.2023.137310

Estevinho, B. N., Rocha, F., Santos, L., & Alves, A. (2013). Microencapsulation with chitosan by spray drying for industry applications - A review. In *Trends in Food Science and Technology* (Vol. 31, Issue 2, pp. 138–155). https://doi.org/10.1016/j.tifs.2013.04.001

Faustino, M., Pereira, C. F., Durão, J., Oliveira, A. S., Pereira, J. O., Ferreira, C., Pintado, M. E., & Carvalho, A. P. (2023). Effect of drying technology in Saccharomyces cerevisiae mannans: Structural, physicochemical, and functional properties. *Food Chemistry*, *412*. https://doi.org/10.1016/j.foodchem.2023.135545

Grange, C., Aigle, A., Ehrlich, V., Salazar Ariza, J. F., Brichart, T., Da Cruz-Boisson, F., David, L., Lux, F., & Tillement, O. (2023). Design of a water-soluble chitosan-based polymer with antioxidant and chelating properties for labile iron extraction. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-34251-3

Kanimozhi, N. V., & Sukumar, M. (2023). Effect of different cryoprotectants on the stability and survivability of freeze dried probiotics. *Food Chemistry Advances*, *3*. https://doi.org/10.1016/j.focha.2023.100428

Luangthongkam, P., Blinco, J. A., Dart, P., Callaghan, M., & Speight, R. (2021). Comparison of spray-drying and freeze-drying for inoculum production of the probiotic Bacillus amyloliquefaciens strain H57. *Food and Bioproducts Processing*, *130*, 121–131. https://doi.org/10.1016/j.fbp.2021.09.010

Öztürk, H. İ. (2022). The effect of different lyophilisation pressures on the microbiological stability, physicochemical, microstructural, and sensorial properties of yoghurt powders. *International Dairy Journal*, *129*. https://doi.org/10.1016/j.idairyj.2022.105347

Parsana, Y., Yadav, M., & Kumar, S. (2023). Microencapsulation in the chitosan-coated alginate-inulin matrix of Limosilactobacillus reuteri SW23 and Lactobacillus salivarius RBL50 and their

characterization. *Carbohydrate Polymer Technologies and Applications*, *5*. https://doi.org/10.1016/j.carpta.2023.100285

Perrechil, F., Louzi, V. C., Alves da Silva Paiva, L., Valentin Natal, G. S., & Braga, M. B. (2021). Evaluation of modified starch and rice protein concentrate as wall materials on the microencapsulation of flaxseed oil by freeze-drying. *LWT*, *140*. https://doi.org/10.1016/j.lwt.2020.110760

Ruengdech, A., & Siripatrawan, U. (2022). Improving encapsulating efficiency, stability, and antioxidant activity of catechin nanoemulsion using foam mat freeze-drying: The effect of wall material types and concentrations. *LWT*, *162*. https://doi.org/10.1016/j.lwt.2022.113478

Thinkohkaew, K., Jonjaroen, V., Niamsiri, N., Panya, A., Suppavorasatit, I., & Potiyaraj, P. (2024). Microencapsulation of probiotics in chitosan-coated alginate/gellan gum: Optimization for viability and stability enhancement. *Food Hydrocolloids*, *151*. https://doi.org/10.1016/j.foodhyd.2024.109788

Trabelsi, I., Ayadi, D., Bejar, W., Bejar, S., Chouayekh, H., & Ben Salah, R. (2014). Effects of Lactobacillus plantarum immobilization in alginate coated with chitosan and gelatin on antibacterial activity. *International Journal of Biological Macromolecules*, *64*, 84–89. https://doi.org/10.1016/j.ijbiomac.2013.11.031

Vanden Braber, N. L., Díaz Vergara, L. I., Rossi, Y. E., Aminahuel, C. A., Mauri, A. N., Cavaglieri, L. R., & Montenegro, M. A. (2020). Effect of microencapsulation in whey protein and water-soluble chitosan derivative on the viability of the probiotic Kluyveromyces marxianus VM004 during storage and in simulated gastrointestinal conditions. *LWT*, *118*. https://doi.org/10.1016/j.lwt.2019.108844

Verlhac, P., Vessot-Crastes, S., Degobert, G., Cogné, C., Andrieu, J., Beney, L., Gervais, P., & Moundanga, S. (2020). Experimental study and optimization of freeze-drying cycles of a model Casei type probiotic bacteria. *Drying Technology*, *38*(16), 2120–2133. https://doi.org/10.1080/07373937.2019.1683859

Vorländer, K., Pramann, P., Kwade, A., Finke, J. H., & Kampen, I. (2023). Process and formulation parameters influencing the survival of Saccharomyces cerevisiae during spray drying and tableting. *International Journal of Pharmaceutics*, *642*. https://doi.org/10.1016/j.ijpharm.2023.123100