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*Systematic Review*

# The Yeast-Based Probiotic Encapsulation Scenario: A Systematic Review and Meta-Analysis

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**Abstract:** One of the biggest challenges in the food industry is the incorporation of probiotics into food products while maintaining their properties, both in the processing phases and in the gastrointestinal tract. The production of this type of functional food, which has been used to prevent and/or help in the treatment of some diseases, needs improvements at the technological and economic levels. This review provides a comprehensive view of the main techniques used to encapsulate probiotic yeasts and analyzes the main variables involved in the industrial process. A systematic review and meta-analysis were carried out, considering the most current technical recommendations for this type of study, as well as the standardized criteria for the eligibility of articles. From a total of 1269 initial articles, only 14 complete articles, published in high-impact journals over the years 2013 to 2019 and focused on in vitro assays with probiotic yeasts, were considered in the analysis performed. In general, microencapsulation was efficient in maintaining yeast survival after gastrointestinal tests, viability studies, and thermal resistance in distilled water and food. Many variables can affect microencapsulation, but they are not always described or properly elucidated, leading to the conclusion that better delineated research is needed. Examples of these challenges include selecting appropriate encapsulating materials, optimizing encapsulation techniques, and ensuring the stability and viability of probiotics during processing and storage. Due to these challenges, the industrial application of probiotic microencapsulation is not yet well established; however, it holds promising potential.

**Keywords:** yeast; probiotic; microencapsulation; encapsulation; *Saccharomyces*; food industry



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## 1. Introduction

The food industry's development, awareness of the importance of diet in disease prevention, and improvements in nutritional science have led to a better understanding of the beneficial effects of foods and food ingredients on overall human health and

well-being [1–4]. Thus, probiotics have gained prominence in food research. The World Gastroenterology Organization (2020) defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". For a microorganism to be considered a probiotic, it must be safe for the host, resistant to gastric acidity and pancreatic secretions, able to adhere to the epithelium, and it must present antimicrobial activity, resistance to antibiotics, tolerance to food additives, and stability in the food matrix.

Several studies have already shown that probiotics can reduce the development of cancer cells, decrease the occurrence of dysentery in patients with lactose intolerance, and promote immune improvement and nutrient absorption [5–9]. Recognized as promoters of health and well-being, probiotics are also able to stimulate the digestive system, increasing digestion capacity and helping in weight loss, as well as presenting beneficial effects on the cardiovascular systems, the bones, the brain, and the nervous system by restoring the intestinal flora [4,8,10–13].

The most studied probiotics so far have been *Lactobacillus* spp. (9000 articles, 1265 of which from clinical trials), *Bifidobacterium* sp. (625 clinical trials), Streptococcus (180 clinical trials), *Bacillus* (110 clinical trials), and *Saccharomyces* spp. (100 clinical trials) [4,14]. The referred data show that the literature on probiotics of bacterial origin is extensive. However, studies on yeasts as probiotics are still scarce.

The genus *Saccharomyces* (yeast) is the most studied, with the two most used being *S. boulardii* in humans and *S. cerevisiae* in veterinary medicine [15–19]. One of the biggest challenges for the food industry is the incorporation of these probiotics into food products, maintaining their properties both in the processing stages and in the gastrointestinal tract [8,20–22]. The beneficial effects of probiotics are directly dependent on a minimum number of living cells remaining viable when exposed to different types of pH in the stomach and intestine, to digestive enzymes, and to possible metabolic degradation during the digestive process [23–28].

To avoid the loss of probiotic viability, the development of methods that allow the survival of these cells to increase, especially when exposed to an adverse environment, was encouraged. Faced with this challenge, the encapsulation process has emerged as one of the most efficient technologies for protecting living cells [28–31]. There are several techniques that can be used in the probiotic encapsulation process, and the most used methods are spray drying, spray chilling, spray coating, gelation, and extrusion [30,32,33].

Given the importance of probiotic encapsulation methods, this work seeks to review yeast encapsulation techniques, as well as the main components used for encapsulation and their advantages. In addition, the effect of the encapsulated probiotic will also be analyzed.

## 2. Materials and Methods

This systematic review of the literature was recorded in PROSPERO (ID No. CRD42020180732) and was conducted according to PRISMA (Preferred Reporting Items for systematic reviews and meta-analyses) (http://www.prisma-statement.org, accessed on 18 October 2021) [34]. The questions in this review were as follows: (a) What is the global scenario for studies on the encapsulation of yeast-based probiotics? (b) What is the encapsulation efficiency of yeast in different types of processes? (c) What are the most common techniques related to yeast encapsulation? (d) What are the physical–chemical factors related to the yeast encapsulation process? and (e) What are the best conditions for yeast encapsulation?

### 2.1. Eligibility Criteria

Papers eligible for inclusion in the review were original research studies performed with microencapsulated probiotic yeasts which evaluated their ability to survive when exposed to simulated gastric conditions, food production, and/or storage, and which were published in English, Spanish, or Portuguese from 2010 to 2020. Non-original articles (reviews, editorials, letters, comments, and book chapters) were excluded.

### 2.2. Search Strategy

A systematic search to identify evidence on the effectiveness of the microencapsulation of exposed probiotic yeast during simulated gastric conditions, food production or storage, or animal models was performed. To optimize data collection and more accurately determine the inclusion or exclusion of studies, the research question was structured according to the PICO acronym. In this context, P (Population) refers to probiotic yeasts; I (Intervention) denotes probiotic yeasts subjected to the encapsulation process; C (Control) includes non-encapsulated probiotic yeasts; and R (Outcome) examines the survival viability of encapsulated probiotic yeasts after testing [34,35].

Electronic databases, including PubMed, Science Direct, LILACS, Scielo, Tripdatabase, and Cochrane, were used. The search keywords used were as follows: "probiotic", "yeast", "microencapsulation", "encapsulation" and "*Saccharomyces*". The keywords were crossed with each other to obtain greater coverage and relevance in the results.

### 2.3. Study Selection and Assessment of Methodological Quality

The articles identified through the search strategy were evaluated by two independent reviewers (E.D.C and W.d.C.O) and the number of citations of keywords searched to obtain data on the online platforms is described in Table 1. The initial phase of article selection consisted of analyzing the titles, followed by the abstracts, and, finally, reading the studies in full to verify the eligibility criteria. Any disagreements that arose between the two reviewers were resolved through discussion or with a third reviewer. However, the studies selected for the systematic review were assessed for quality using the Critical Assessment Skills Program principles tool (CASP) [36,37]. This instrument is designed to judge study quality in a systematic and transparent way. The quality judgment is therefore derived from a set of standardized methodological questions applied to all studies in question. For the purposes of this study, questions for quality assessment were centered on the following themes: research question; recruitment; control group characteristics; rigor in investigative measures; and confounding factors. Figure 1 shows the flow diagram for the identification and selection of articles for the study.

**Table 1.** Number of citations of the researched keywords to obtain data on online platforms.

| Keyword | Platforms—No. of Articles | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Science Direct | | PubMed | | Scielo | | Cochrane | | TripDatabase | | Lilacs | |
| | Total | Included | Total | Included | Total | Included | Total | Included | Total | Included | Total | Included |
| Yeast + Probiotic + Microencapsulation | 131 | 11 | 12 | 6 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Probiotic + Microencapsulation | 537 | 11 | 11 | 0 | 14 | 1 | 15 | 0 | 26 | 0 | 7 | 0 |
| Probiotic + Encapsulation | 1180 | 12 | 5 | 0 | 12 | 2 | 17 | 0 | 37 | 0 | 9 | 0 |
| *Saccharomyces* + Probiotic + Microencapsulation | 40 | 9 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| **TOTAL** | 1888 | 42 | 28 | 6 | 27 | 3 | 33 | 1 | 66 | 0 | 16 | 0 |

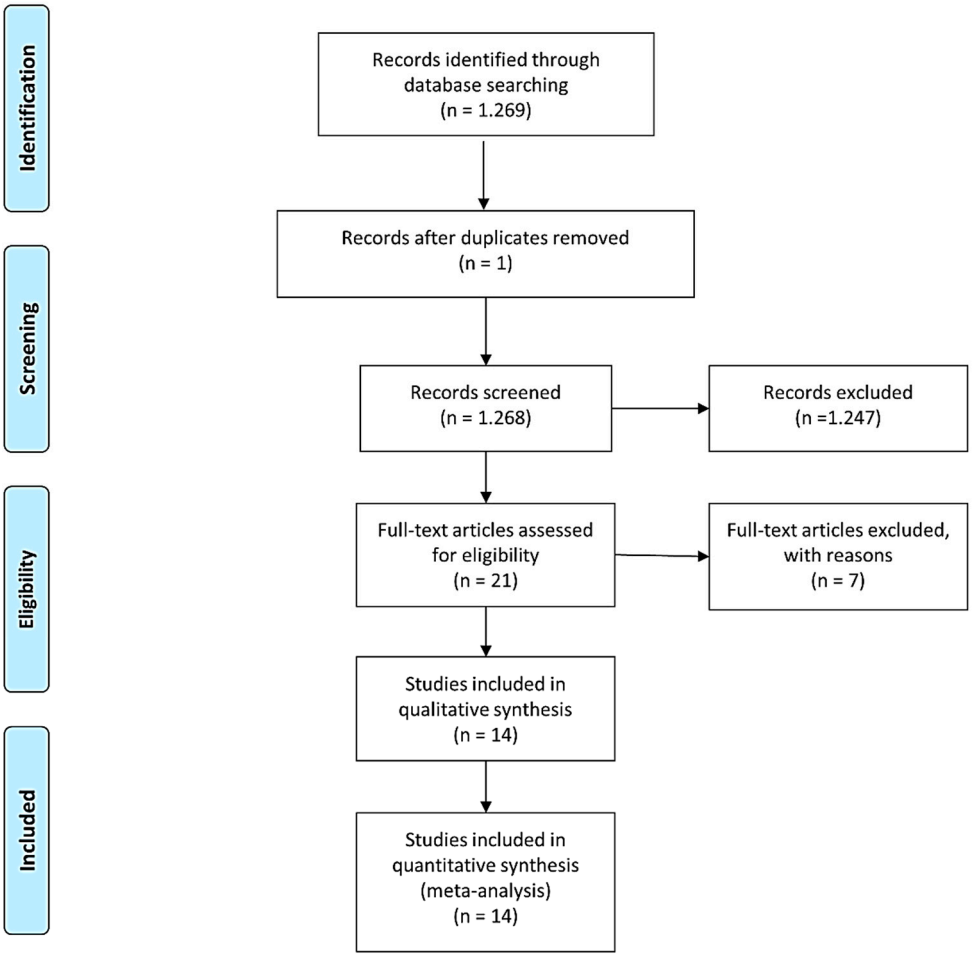


**Figure 1.** Flow diagram for the identification and selection of articles for the study.

### *2.4. Statistical Analysis*

For data analysis, the ANOVA variance test with a significance level of $p < 0.05$, followed by the Tukey and Duncan test ($p < 0.05$), was used. All analyses were conducted using the Minitab statistical software (Minitab for Windows, version 19). The outcomes were presented through the Optimization Plot model. Previously, the data were treated by multiple linear regression, as the analyses encompassed more than one variable. The main evaluation parameters for this type of graph are as follows: (D) composite desirability, which evaluates how the configurations optimize a set of responses in general; (d) individual desirability, which evaluates how the settings optimize a single response, and (y), which is the response variable or the dependent variable. In other words, (y) is the quantity being observed, measured, or optimized in the study. The vertical red lines in the optimization graph represent the current factor settings, indicating the specific combinations at the time of analysis. Conversely, dashed blue lines suggest composite responses or desirability for the current factor level, providing a visual view of the relationships between factor configurations and desired responses. These lines guide the understanding of how different adjustments in factor levels can impact optimization goals, allowing an intuitive analysis of the relationships between variables and responses in the context of the studied process. The statistical investigation in this study was guided by the selection of optimal indicators, whether categorical or continuous, capable of eliciting a more favorable response in the keywords of the microorganism's survival percentage following successful encapsulation. The investigation of the percentage of survival was grounded in the examination of three variables: pH, temperature, and the materials employed in executing the encapsulation process.

## 3. Results and Discussion

*Study Inclusion*

The article search was carried out between June 2020 and February 2021, chronologically, both for surveying articles and for data collection and analysis. Initially, a survey was carried out for data collection on the Cochrane, LILACS, PubMed, Scielo, ScienceDirect, and TripDatabase platforms (Table 1). A total of 2058 citations were obtained considering the following keywords: "Yeast + Probiotic + Microencapsulation", "Probiotic + Microencapsulation", "Probiotic + Encapsulation", and "*Saccharomyces* + Probiotic + Microencapsulation". ScienceDirect had the highest number of citations (n = 1888), followed by TripDatabase (n = 66). In both databases, the term "Probiotic + encapsulation" had the highest number of citations at 1180 and 37, respectively. The second term with the highest number of citations was "Probiotic + microencapsulation" with 537 and 26 observations in ScienceDirect and TripDatabase, respectively.

In the articles survey and data collection, four stages were considered: identification; sorting; eligibility; and articles included in the analysis (Figure 1). The records identified through the search in the databases considered a total of 1269 articles with the researched theme. In the screening stage, one article was excluded because it was duplicated—that is, it was found on more than one platform—and 1247 were excluded because they were outside the theme and objective of the study. Subsequently, in the eligibility stage, the criteria for which were determined at the beginning of the research, seven articles were excluded for the following reasons: they addressed the use of lactic acid bacteria (LAB); yeasts were not microencapsulated/encapsulated; they used of some cell wall components as LAB encapsulation material; they evaluated the encapsulated yeast in relation to the effects on the organism's health, without describing the viability of the encapsulated probiotic itself; they conducted studies on animal models; their data centered around morphology, but additional media data were not provided to investigate microencapsulation efficiency; and the data obtained were related to the growth of fish, but did not present data related to its action, nor how its viability could be measured.

Therefore, 14 complete articles, published in high-impact journals throughout the years 2013 to 2019 were selected for analysis, focused on in vitro assays with probiotic yeasts. In the referenced works, the spray drying (four times) and extrusion (four times) techniques were the most recurrent. Spray chilling and gelation techniques were reported only once each.

The encapsulation technique was not directly related to the size of the microcapsule; that is, different patterns were observed in the same technique, with the smallest reported microcapsule measuring 3.31 μm and the largest measuring 2960 μm. However, as observed in Table 2, the largest capsule size values (1500 and 1700–2960 μm) were obtained in the EX-technique. This pattern was not maintained in all studies, since, in two studies, the size ranged from 50 to 90 μm.

The microcapsule composition varied among the analyzed articles, with emphasis on seven ingredients (milk protein, starch, maltodextrin, gum arabic, xanthan gum, alginate, and chitosan) which appeared in more than one formulation. In some studies, more than one formulation of ingredients was used, reaching a total of five ingredients in the same formulation. The most common compounds among the formulations were as follows: alginate (sodium and calcium) and maltodextrin, which appear in eight and five formulations, respectively, alone or in combination. Alginate was cited as a component of the microcapsule in 6 studies and gum arabic in 4 of the 14 articles analyzed. In [38], four of the six most used ingredients in other studies (milk protein, starch, maltodextrin, and gum arabic) were used individually. In contrast, ref. [39] used three ingredients, both individually and in combination (sodium alginate; sodium alginate/chitosan; sodium alginate/starch/vegetable oil).

The encapsulation efficiency (EE) is a very important category of data in studying the encapsulation process of a probiotic. However, only few articles (n = 4) reported these data in their results. The EE varied between 35 and 98.1%. The highest EE were

observed in studies using gum arabic (91.4–98.1%) and sodium alginate (90–94%) in their composition. The authors of [39] reported an EE ranging from 35% to 45%, indicating the lowest observed efficiency. A total of eight yeast genera/species were used as probiotics in the tests, with *Saccharomyces boulardii* reported in seven studies, *Saccharomyces cerevisiae* reported in five studies, and *Issatchenkia occidentali*, *Kluyveromyces marxianus*, *Pichia barkeri*, *Yarrowia lipolytica*, *Wickerharomyces anomalus*, *Lactobacillus acidophilus*, and *Sacchoromyces* sp. reported only once. The yeast survival after the different treatments ranged from 1.7 to 100%. These values were obtained from 9 works that described these data in their results.

Conclusively, in the chosen articles, yeast microencapsulation was efficient in maintaining survival after gastrointestinal tests, viability studies, and thermal resistance in distilled water and food.

The determined survival percentage varied greatly among and within the articles themselves due to the high number of treatments and variables considered. The path taken by probiotic microorganisms from encapsulation to the colon presents many challenges. Temperature and storage time can present concerns, namely viability through the pH and temperature of the gastrointestinal tract, among other factors [40,41]. In addition, there is the possibility that microorganisms do not establish themselves as part of the intestinal microbiota. All these factors influence the effectiveness of the delivery of probiotics by commercial formulations or through food. The more influential are humidity (RH), temperature, pH, osmotic stress, and the amount of oxygen available in the environment [42–44]. Therefore, it is necessary to develop technologies that allow for achieving greater probiotic stability [43,44].

Microencapsulation emerges as a viable and efficient alternative to systems commonly used to encapsulate small molecules, such as vitamins, minerals, and some drugs, and systems that do not contain bacteria and yeasts [45–47]. The strategies employed in microencapsulation to improve the viability of probiotics are diverse and must be considered: the presence of a physical barrier blocking the adversities from the environment; joining the probiotics with nutrients or additives that will help in their survival and in maintaining the environmental conditions inside the capsule; and capturing compounds from the external environment that help the survival of probiotic microorganisms [48–50].

From the point of view of human health, it is worth discussing that the cell viability of the probiotic is not the only variable to be considered. The term postbiotic has been used to refer to inanimate microorganisms and/or their components that confer health benefits. Therefore, the beneficial effects of probiotics are not only due to the action of the living cell in the intestine, but also due to the immunomodulatory potential of the constituent molecules [51–53].

The study carried out differs from the method commonly used in keywords of statistical investigation, since the interest in it is the choice of the best results, meaning the indicators (categorical or continuous) that were able to generate a better response in keywords of efficiency. The tool chosen for analysis was the “Response Optimizer”, which determines, based on the amount of data provided, which indicators should be used to produce the best response. It previously required a simple regression analysis (which could be linear, quadratic, or cubic) or multiple linear to verify the values of the indicators that produced the best response and, consequently, the optimal value produced by the regression models for such a response.

Obviously, the more curve-fitted the data are, the better the level of analysis. However, as this is a problem involving more than one variable, only multiple linear regression offers a solution to the problem and the results will be based on this. Initially, the factor chosen as a response was the efficiency percentage, since most of the articles provided such data or enabled the calculation of the final and initial population. Another justification is that, in the case portrayed, in general, the estimates related to efficiency must be continuous variables. To choose the indicators, we used those that were most frequently reported.

**Table 2.** Overview of recent studies (2010–2020) on yeast-based probiotic encapsulation.

| Reference | pH | Experiment Temperature (°C) | Experiment Encapsulation (°C) | Encapsulation Method | Microcapsule Size (µm) | Microcapsule Composition | Encapsulation Efficiency (%) | M.O | Test Type | % Subservience |
|---|---|---|---|---|---|---|---|---|---|---|
| [38] | 1.0; 1.5; 2.0 | 37 | nr | Spray drying | 3.31–4.08 | Gelatin; milk protein; starch; maltodextrin; pea protein; gum arabic | nr | *S. boulardii* | Gastric | 13.8–78.6 |
| [54] | 2.0; 8.0 | 37; 50; 60; 70; 80 | entry: 120 output: 50 | Spray chilling; spray drying | 24.1–612.5 | Gum Arabic/ß-cyclodextrin; hydrogenated palm oil | 91.4–98.1 | *S. boulardii*; *L. acidophilus* | Viability; gastric; thermal resistance | 28.5–99.3 |
| [55] | nr | 102 | nr | Pulverization | nr | Arabic gum; B-cyclodextrin | nr | *S. boulardii* | Food | 67.4 |
| [56] | nr | 18; 49 | nr | Pulverization | nr | Skimmed milk/sucrose/ carboxymethylcellulose/ xanthan gum; skimmed milk/maltodextrin/sucrose/ carboxymethylcellulose/ xanthan gum; maltodextrin/ sucrose/carboxymethylcellulose/ xanthan gum | nr | *Saccharomyces* sp. | Viability | 96.6–97.9 |
| [57] | 4.0; 5.0; 6.0; 7.0 | 70; 80; 90 | nr | Pulverization | 8–15 | whey protein | nr | *S. boulardii* | Viability | 0–40 |
| [58] | 2.0 | nr | nr | Extrusion | 1500 | Ca_alginate/potato dextrose/glycerol/xanthan/inulin | nr | *S. boulardii* ATCC 74068 | Gastrointestinal | nr |
| [59] | 2.0; 6.5; 8.0 | 28 | nr | Extrusion | 50–90 | Na_alginate/calcium chloride | nr | *S. cerevisiae* ATCC 9763 | Gastric; distilled water | nr |
| [60] | 2.0; 8.0 | 28 | nr | Extrusion | 50–90 | Na_alginate/calcium chloride | nr | *S. boulardii* ATCC 74068 | Gastrointestinal | 20–100 |
| [61] | 1.5; 5.6; 7.5 | 37 | nr | nr | nr | Agar-agar; Arabic gum; Iota-carrageenan; linseed mucilage; taro mucilage; yam mucilage; okra mucilage | nr | *S. cerevisiae* | Gastrointestinal | nr |
| [62] | 1.0; 7.4 | 37 | nr | Gelation/ emulsification | nr | Na_alginate/NaCl solution/paraffin/chitosan | nr | *S. cerevisiae* Y235 | Gastrointestinal | nr |
| [63] | 2.0; 6.5 | nr | nr | Emulsion | 9.2 | Na_alginate/inulin/mucilage from Opuntia ficus-indica | nr | *S. boulardii* | Food | 1.7–81.2 |

nr: not reported.

In this case, three indicator variables were chosen: pH, temperature, and materials used for encapsulation. In the evaluated case, two variables were initially used to obtain the desired response. This is because, when checking the distribution of points in relation to a single variable, no curve was adequately adjusted and, therefore, analyses focused on simple regressions were discarded. Therefore, four analyses were obtained, focusing on the use of the response optimizer from the following variables: pH and temperature; pH and encapsulation materials; temperature and encapsulation materials; and, finally, pH, temperature, and encapsulation materials. The analysis is based on some numerical results and graphs generated (Figures 2–4). For the basis that must be taken into account in this research, only three factors will be analyzed to prove a given result.

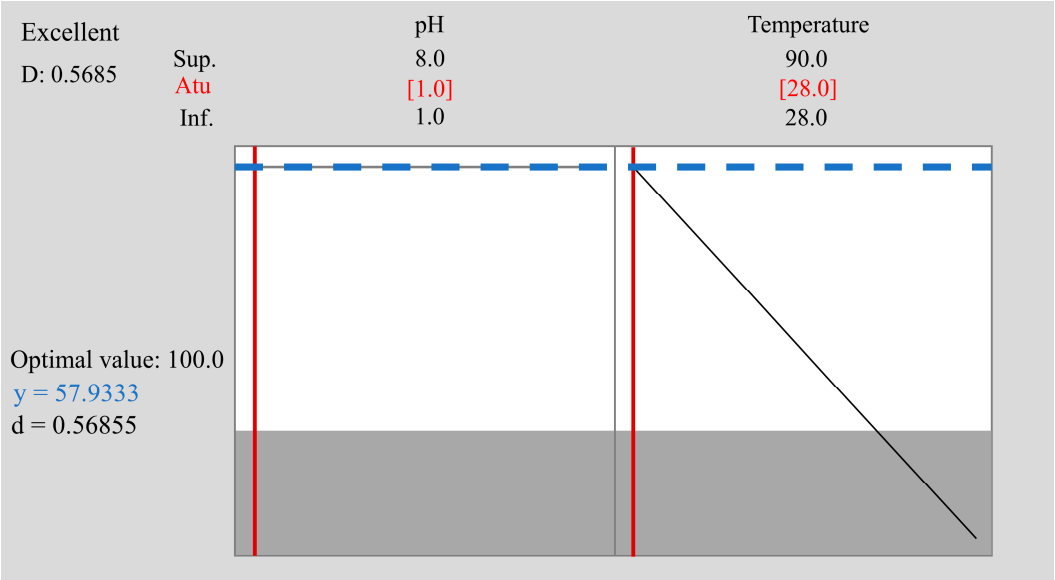


**Figure 2.** Response optimization: percent survival—pH and temperature. (D) Composite desirability, which evaluates how the configurations optimize a set of responses in general; (d) individual desirability, which evaluates how the settings optimize a single response, and (y) the response variable or dependent variable. The optimizer sets a goal, which is denoted by "optimal value" and represents the best value that the optimizer can achieve. The vertical red lines in the optimization graph represent the current factor settings, indicating the specific combinations at the time of analysis. Dashed blue lines suggest composite responses or desirability for the current factor level, providing a visual view of the relationships between factor configurations and desired responses.

The first group is the multi-response prediction group, which shows the configuration of variables that optimizes the outcome of interest. Such values may be intermediate values, in the case of continuous values, or values that necessarily belong to the data obtained. The second group is the adjusted survival percentage, which represents the maximum value obtained for the variable of interest (survival percentage). The third and most relevant group for judging the distribution of data and the efficiency of the optimization model is the composite desirability, which measures the assertiveness of the combination to obtain the optimal result. Values for this parameter are between 0 and 1, where values close to 0 indicate that one or more responses are within the acceptable limits. In the case of values close to 0, we can justify that the data are not compatible or even represent non-consonant levels to be interpreted in a grouped way.

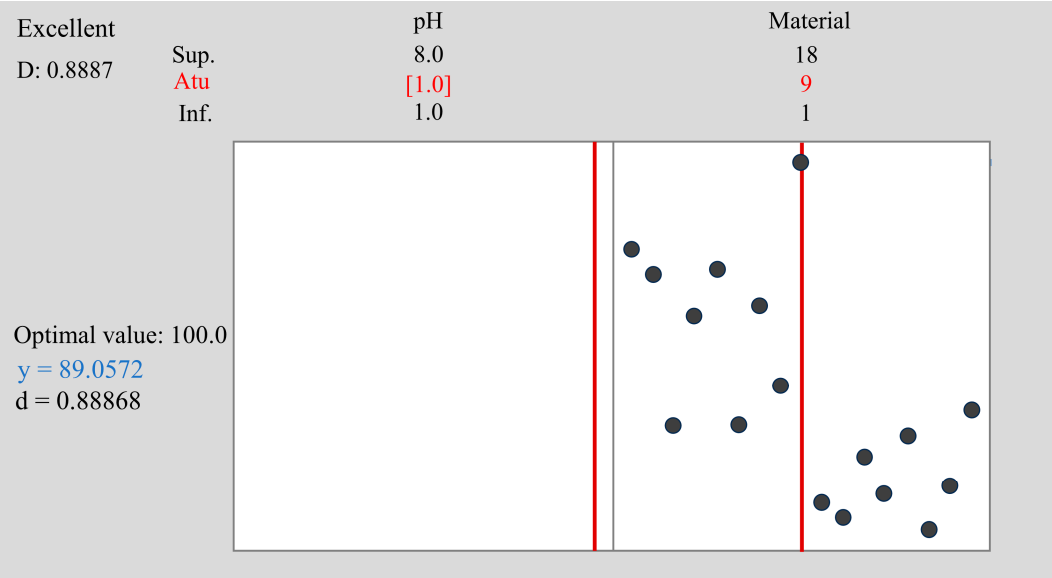


**Figure 3.** Response optimization: survival percentage—pH and materials. (D) Composite desirability, which evaluates how the configurations optimize a set of responses in general; (d) individual desirability, which evaluates how the settings optimize a single response, and (y) the response variable or the dependent variable. The optimizer sets a goal, which is denoted by "optimal value" and represents the best value that the optimizer can achieve. The vertical red lines in the optimization graph represent the current factor settings, indicating the specific combinations at the time of analysis.

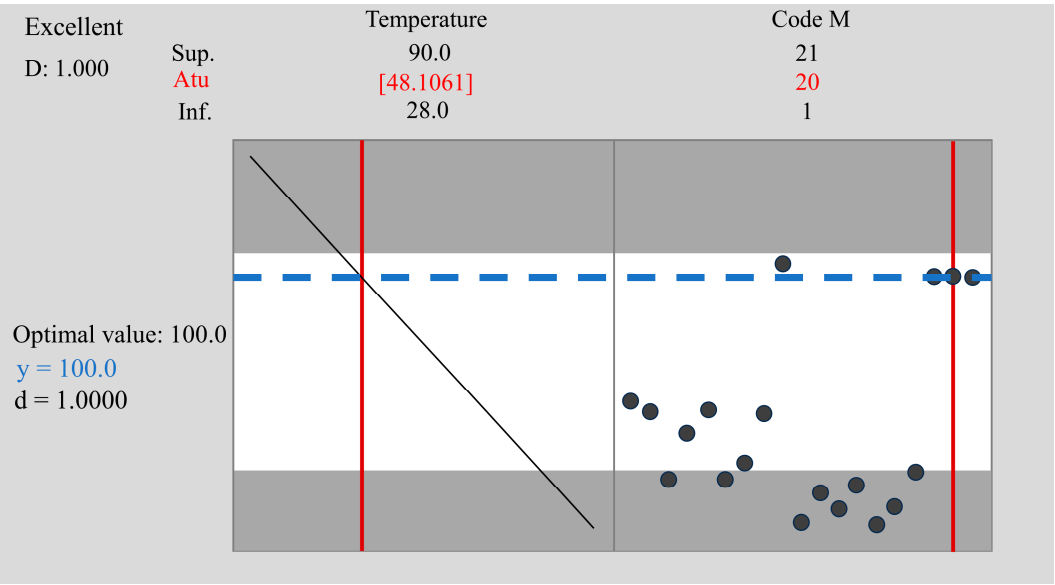


**Figure 4.** Response optimization: survival percentage—temperature and materials. (D) Composite desirability, which evaluates how the configurations optimize a set of responses in general; (d) individual desirability, which evaluates how the settings optimize a single response; and (y) the response variable or the dependent variable. The optimizer sets a goal, which is denoted by "optimal value" and represents the best value that the optimizer can achieve. The vertical red lines in the optimization graph represent the current factor settings, indicating the specific combinations at the time of analysis. Dashed blue lines suggest composite responses or desirability for the current factor level, providing a visual view of the relationships between factor configurations and desired responses.

In Figure 2, the impact of temperature and pH variables on the percentage of survival in encapsulated probiotic yeasts in gastric simulation tests was evaluated. Figure 2 shows a

composite desirability (D) value of 0.5685. Additionally, it shows an individual desirability (d) value of 0.5685 and a predicted response (y) value of 57.933. There is a red line for pH 1 and a red line for temperature 28. According to the statistical model used, it can be stated that the expected survival percentage, considering the temperature and pH variables, is approximately 57.93%. Therefore, the analysis suggests that, although the optimized configurations have achieved a moderate level of desirability for the general set of three variables involved, there is still room for improvement, especially when considering the variables individually. The predicted response provides an estimate of the expected performance under the optimized conditions, indicating that further adjustments may lead to more favorable results in keywords for percentage survival in encapsulated probiotic yeasts in gastrointestinal simulation tests. The vertical red lines represent the current factor settings. Each vertical red line indicates a specific set of settings for the different factors under analysis. The red lines serve as visual markers to indicate the conditions under which experiments are being conducted or optimized. The horizontal blue line indicates how the composite response or desirability varies in relation to different factor configurations.

Figure 2 also depicts the relevant elements that describe the optimal choices given a set of options as outlined in our initial list of variables. As it can be seen, the optimizer establishes a target, which is denoted by "optimal value" and represents the best value that the optimizer can achieve. Evidently, attaining such a level indicates either that the empirical conditions are equivalent to the theoretical ones or that no interaction is expected between two or more factors. Therefore, a variable "y", which corresponds to the predictor, is given. This variable is our compass in order to validate how the interaction between two factors can induce a survival level that is different from the target. Each combination of optimal elements produces different values for "y" and the best combination (highlighted in red) is given by the maximum value that the predictor can yield.

The data obtained also show that there is a strong negative correlation between the survival response and the temperature factor, since, in the analysis, a straight line with a negative angular coefficient was obtained (black solid line). On the other hand, the pH factor, according to the collected articles, does not seem to play such a significant role. Initially, one must consider that the articles considered in this review mostly carried out gastric simulation tests with narrower pH ranges (an acidic pH value and a basic pH value). As for temperature, a wider range of conditions can be verified. This fact may justify the lower impact of pH as an individual variable. Furthermore, the resistance of probiotics to the effect of pH may lie in the natural tolerance of some strains to acidic or alkaline variations. The presence of a protective layer resulting from the microencapsulation process may also play a crucial role, offering an effective defense against pH fluctuations during gastrointestinal simulation. Moreover, our analysis was restricted to two factors because the sole investigation of variables does not account for the effect of the interaction of several variables simultaneously. For that reason, graphs for single-variable regressions were not taken into account.

In Figure 3, the impact of pH and the type of encapsulation material on the percentage survival of encapsulated probiotic yeasts was evaluated. Figure 3 shows a composite desirability value (D) of 0.8887. Moreover, it shows an individual desirability value (d) of 0.88868 and a predicted response value (y) of 89.0572. There is a red line for pH 1 and a red line for material 9. The set of variables analyzed at the same time suggests that optimized configurations have the potential to reach a percentage of approximately 90% encapsulated probiotic yeasts. These results indicate a successful optimization for the variable of interest, promoting an environment which is conducive to the survival of the analyzed elements. The stability of the process, the effectiveness of the statistical model, and the possible positive sensitivity of probiotics to pH and materials are also factors that can contribute to the robustness of these results. The data therefore indicate a successful optimization for the variable of interest, promoting an environment which is conducive to the survival of the analyzed elements. It can be seen, again, that the pH value exerts little influence on the survival analysis, while the type of material used is capable of drastically modifying

this value. A possible explanation for this is the different resistance of certain types of materials in physicochemical keywords. Characteristics such as porosity, resistance to thermodynamic stress, and chemical and mechanical changes may be able to be supported more easily, leading to yeast conservation.

As yeast cells can also be used for the encapsulation of other compounds, new studies have helped to elucidate their resistance to adverse conditions. Compared to the cell wall of baker's yeast (*Saccharomyces cerevisiae*), brewer's yeast has a more complex network of polysaccharides, which are rich in (β1 → 3)-glucans and covalently linked to (α1 → 4)- and (β1 → 4)-glucans, in addition to residual mannoproteins. Furthermore, molecular differences in the cell wall may also have a direct implication in the immunostimulant potential, conferred by the presence of (β1 → 3)-glucans, as explored, for example, in [64–66].

Faced with consumer demand for food of better quality and the limited consumption of compounds with low nutritional value, combined with the search for more sustainable production that reduces waste emissions, it is becoming important to prioritize encapsulation strategies with coatings that offer health benefits and are environmentally friendly [67–69]. New studies on encapsulation should improve the use of compounds such as fiber, amino acids, vitamins, natural antioxidants, essential fatty acids, and probiotics, instead of ingredients that are not healthy such as simple sugars, cholesterol, or allergenic compounds such as dairy products, saturated fats, and trans fatty acids [69].

New works may also prioritize inputs recovered from seeds, bark, bones, and scales, among others, reducing the accumulation of by-products and agro-industrial residues. Further studies on co-microencapsulation techniques combining two or more active compounds in a single system are also suggested. The synergy between the compounds could enhance their beneficial effects, offer more possibilities for the application of bioactive compounds, favor increased shelf lives, and generate lower production costs. The main application of co-microencapsulation is the combination of probiotics with other active compounds such as prebiotics, lipids, and, more recently, polyphenols [68].

In Figure 4, the impact of temperature and the type of encapsulation material on the percentage survival of encapsulated probiotic yeasts was evaluated. Figure 4 shows a composite desirability value (D) of 1.0. Additionally, it has an individual desirability value (d) of 1.0 and a predicted response value (y) of 100. There is a red line for a temperature of 48.1061 and a red line for a material of 20. In this case, a D of 1.0 suggests that the independent variables, represented by temperature and material type, were chosen optimally. The predicted response value (y) of 100 indicates that, under these specific conditions, the survival percentage of the encapsulated probiotic yeasts reached its maximum potential. These results are promising, as they indicate that, under the established conditions, the encapsulation method used is highly efficient in preserving probiotic yeasts. However, it is essential to highlight the need for additional analysis, considering factors such as long-term stability, associated costs, and the possible practical applications of these encapsulated yeasts. Optimized data for the two variables considered seem to be crucial for the success of the survival percentage. The data achieved can be explained based on considerations related to thermodynamic compatibility, the characteristics of the encapsulation material, and the kinetics of biological reactions. The stability of the encapsulation matrix, influenced by chemical and physical interactions, can also play a significant role.

The role of probiotics in promoting intestinal health has been widely recognized. In this direction, probiotic yeasts emerge as promising alternatives to traditional lactobacilli and bifidobacteria. To improve the benefits of this type of probiotic, it is essential to develop advanced encapsulation strategies and optimize the conditions that guarantee its survival in the gastrointestinal tract.

New encapsulation materials are being used to protect probiotic yeasts against gastrointestinal tract conditions. Nanoparticles, liposomes, and microspheres are some of the technologies that have been shown to improve the viability of yeast during its passage through the digestive tract, allowing for controlled release in the intestine. In addition,

microencapsulation offers significant advantages in the stability and shelf life of probiotics, enabling the formulation of more convenient and affordable foods.

Finally, improving the survival conditions for probiotic yeasts in the gastrointestinal tract is crucial for probiotics to be used as an effective and safe therapeutic strategy.

## 4. Conclusions

This study has allowed the verification of the main components and methods of the microencapsulation of probiotics that have been used in recent years. We verified the importance and diversity of probiotics and probiotic foods, which have shown improvements in their effectiveness through the application of microencapsulation technology, increasing cell viability. However, many factors can affect the microencapsulation process, requiring extensive research to develop a well-accepted and correctly used technique in the food industry. The industrial application of probiotic microencapsulation is still far from widespread, since many details still need to be standardized, as interfered from the final evaluation of the process.

Several questions have not yet been answered by the scientific community regarding this topic, such as the following: Which materials are more efficient or which can be improved?; Do the current research results confirm previous ones?; What type of correlations exist between process factors and the effectiveness of microencapsulation in different products?; Is it possible to optimize the process to improve the viability, decrease the costs, and maintain the sensory properties of the products? Although many questions and details still need to be answered and revealed, in general, the microencapsulation of probiotics seems promising for the future. This type of reasoning can open new horizons for extensive research in this field.

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