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Probiotic yeast *Saccharomyces cerevisiae var. boulardii*: properties and peculiarities of use in functional foods development

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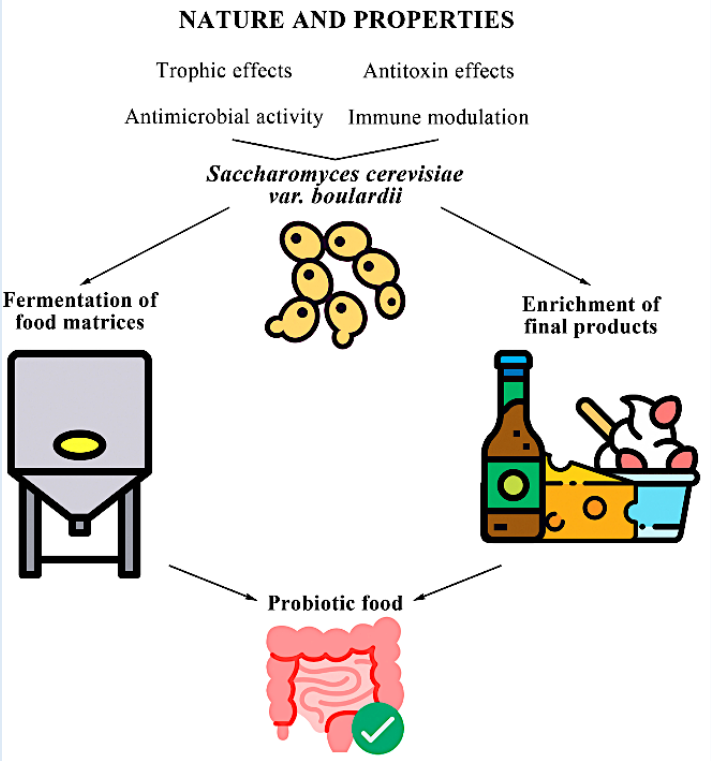
ABSTRACT:

The unconscious human consumption of probiotic microorganisms due to fermented foods in the daily diet has lasted for many centuries. Meanwhile, the transition to the conscious use of probiotic biopotential in a healthy diet dates back only to the beginning of the previous century. It is associated with the development of the concept of functional food. The yeast *Saccharomyces cerevisiae var. boulardii*, discovered in the 1920s, is the only eukaryotic probiotic microorganism registered. The probiotic activity of *Saccharomyces cerevisiae var. boulardii* is determined by antimicrobial, antitoxin, immune modulation, and trophic effects. These different types of activities, in their turn, have provided a wide range of applications of this yeast culture to treat various types of diarrheas; clostridium difficile infection; inflammatory bowel diseases; irritable bowel syndrome; ulcerative colitis; Crohn's disease; sepsis; acne; and vaginal candidiasis. In addition to the application of *Saccharomyces cerevisiae var. boulardii* in medical practice, it becomes possible to incorporate the microorganism into food products, which can impart them functional, namely, probiotic properties, thus enhancing their biological activity and contributing unique sensory characteristics to final products in the case of fermented ones. This review aims to systematize the data concerning the properties of the yeast *Saccharomyces cerevisiae var. boulardii* and its application in the functional food development.

Keywords: *Saccharomyces cerevisiae var. boulardii*, probiotics, functional food, fermented foods, yeast



Graphical Abstract: Probiotic yeast *Saccharomyces cerevisiae var. boulardii*: properties and peculiarities of use in functional foods development

INTRODUCTION

The concept of "functional food", which first emerged in Japan in the 1980s [1], has become an integral trend of the modern food industry, forming an independent segment with a rapidly growing market.

The concept of functional food has been defined several times, but this term has no universally accepted international definition [2]. Functional food products are usually considered to contain ingredients that offer health benefits beyond the fundamental nutritional value of the food itself [3,4]. One group of such ingredients is probiotics, which, according to the actual definition, are «live microorganisms that, when administered in appropriate doses, confer a health benefit to the host» [5]. The minimum concentration of probiotics in functional food to achieve the intended health benefits should be 10^6 CFU/g (or mL) at the time of consumption [6]. Lactic acid-producing bacteria (LAB), mainly the *Lactobacillus* and *Bifidobacterium genera*, are the most commonly used probiotics [7,8]. Moreover, they are natural inhabitants of the human intestine and have a long history of safe use in the food industry [6,9]. The yeast *Saccharomyces cerevisiae var. boulardii* (*S.c.b.*) is also a probiotic, recognized along with LAB [7]. This yeast strain was first registered as a drug in 1953, and it is the only registered eukaryotic probiotic microorganism to date [10]. These days, *S.c.b.* is used in over 80 European countries, North and South America, the Middle East, and Asia. Numerous commercial probiotic products, including Florastor® (Biocodex), S.c.b, are reportedly available. PLUS MOS (Jarrow Formulas), Flora (Institute Rosell-Lallemand), Nexabiotic (DrFormulas), and others [11,12].

Although *S. cerevisiae is traditionally used in capsule or powder form rather than in food preparation [6] as*

opposed to probiotic LAB found in milk fermented products, there has been recent interest in the food science community to incorporate this yeast culture as a functional ingredient into various food matrices. For example, it has been reported that S.c.b. should be incorporated into dairy foods, grain beverages, fruit juices, chocolate, coffee, and tea [13]. It is worth noting that this yeast culture can be used in food technology as an enriching functional ingredient and as a starter culture in fermentation processes. *S.c.b*. is also considered a source of many bioactive metabolites, which generally demonstrate antioxidant, antibacterial, antitumor, anti-inflammatory, and other properties [14].

This review presents the data systematization on both *S.c.b*. properties and the application of this yeast in functional food development. Moreover, particular attention is paid to classifying the main directions of *S.c.b*. use in food processing.

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Retrieval of published studies: A literature review of published research concerning the properties of *S.c.b.* and its application in developing functional food products was conducted electronically using PubMed®, ScienceDirect, and relevant journals from the Functional Food Center's database. These databases were chosen based on the large number of items housed within them that apply to our literature review. A total of 90 review and research articles published mainly in the last 25 years were included, which reflects the active development in the study of *S.c.b.* and also proves the rather large biopotential of this yeast culture. Articles were selected for their objective, scientific insights on *S.c.b.* nature and properties, and the processes that occur when *S.c.b.* is used as a starter culture or fortifying ingredient in functional food technologies. Keywords for the search included "*Saccharomyces cerevisiae var. boulardii*", "functional food", "probiotic food products", and "fermented foods".

Nature and properties: The French microbiologist Henri Boulard first identified *S.c.b.* in 1920 during his visit to Indo-China, looking for novel yeast strains for fermentation processes. At the time, there was an active cholera outbreak, and Boulard noted that the people not affected by this illness drank some special brew. This beverage was obtained by brewing the rinds of tropical fruits such as lychee and mangosteen. Boulard isolated the active agent that produced this effect (a unique yeast strain that he later named *Saccharomyces boulardii*). Returning to France in 1947, he patented the strain and sold the rights to the Biocodex company, which further initiated research and production processes [10,15]. In 1953, the strain was officially registered as a drug, and these days it remains the only registered eukaryotic probiotic microorganism [10].

S.c.b. has been a topic of great debate regarding its taxonomic classification [12]. According to the results of the study by Aa Kühle et al. [16] *S.c.b.* was classified outside the *Saccharomyces cerevisiae* (*S.c.*) group using electrophoretic karyotyping and multifactorial polymorphism analysis. Nevertheless, the comparative genomic hybridization has shown that *S.c.* and *S.c.b.* are the same species [17]. Moreover, molecular phylogenetics and species-specific polymerase chain reaction (PCR) have confirmed *S.c.b.* to be a strain of *S.c.* [17]. However, *S.c.b.* exhibits genetic differences from other *S.c.* strains, particularly the presence of chromosome IX trisomy [18]. Compared with *S.c.* (S288c and 1171T strains), 100% similarity was found in the sequence of the D1/D2 26S rDNA domain of *S.c.b.,* and more than 95% similarity with the sequences of the mitochondrial cytochrome c oxidase II (COX2) gene, which also confirms their affinity [19,20]. The most significant difference between *S.c.b.* and other *S.c.* strains is that no Ty elements, including Ty1, Ty3, Ty4 or their long terminal repeats, are present in *S.c.b.* Furthermore, *S. cerevisiae possesses* neither genes

encoding the hexose transporters HXT11 and HXT9 nor genes required for asparagine utilization [21].

The taxonomy based on the nucleus-proteome puts *S.c.b.* along with the wine *S.c.* strains. It seems interesting to note that some genes involved in encoding the probiotic properties of *S. c.b. are present in wine strains S. c.* (YJM339, RM11-1a, L1528, and YS9). Moreover, probiotic yeast S.c.b. are homothallic diploids, whose genome has both types of mating loci [22].

At first sight, the cell morphology of *S.c.b*. does not differ significantly from industrial strains of *S.c*. Thus, the cells of this strain are oval or spherical with 2–3 μm width and 2.5–10.5 μm length [23]. However, Hudson et. al. [24] found that the total thickness of the *S.c.b.* cell wall is greater than that of *S.c.* strains. The increased thickness is not due to any one layer of the cell wall structure, including the inner chitin, the internal β-glucan and the outer mannoprotein layers.

Several studies [9–12] have reported that a higher optimal growth temperature for *S.c.b. is* 37°C versus 30°C for *S.c*. This has become one of the defining traits of this yeast strain. However, the research data reflecting the growth optimum occurring at 37℃ rather than at higher or lower temperatures is unavailable. In its turn, some research [25–27] only confirmed the ability of a *S.c.b*. to grow at a temperature of 37℃. Thus, the precise determination of the optimal growth temperature for *S.c.b*. remains an urgent problem to be solved due to the significance of this parameter from both scientific and practice-oriented technological viewpoints.

An essential characteristic of *S.c.b*. is its complex resistance to aggressive environmental factors such as high acidity, temperature, and the presence of ethanol. Edwards-Ingram et al. [28] showed that a decrease in pH down to 2 and holding this value for an hour caused no changes in the concentration of living cells in the case of *S.c.b*., while the viability of *S.c.* strains dropped to less than 4%. At the same time, Graff S. et al. [29] reported viability retention (about 50%) of *S.c.b*. even at pH = 1.0 for six hours; observation under scanning and transmission electron microscopy showed morphological damage and rupture of its cell wall.

According to Fietto J. et al. [26], *S.c.b*. is more resistant to relatively high temperatures than *S.c*. Thus, the viability of *S.c.b*. and *S.c.* W303 at 52°C for one hour was 65% and 45%, respectively. The resistance of *S.c.b*. to high temperatures is also supported by Du L. et al. [25], who determined its thermal death temperature (55-56°C). Moreover, *S.c.b*. can be characterized as a well ethanol-tolerant strain, with a limiting concentration of about 20% of ethanol in the medium [25,30].

It has long been considered a distinctive feature of *S.c.b.* that it cannot metabolize galactose [9,10]. This, however, was challenged by Liu J. et al. [31], who showed that *S.c.b.* can utilize galactose, albeit at a much-reduced rate compared to *S.c*. In addition, the effect of aeration on galactose uptake by *S. c.b. and S. c.* was studied. Under aerobic conditions, galactose utilization rates were higher for both strains. The galactose uptake rate by *S. c.b under oxygen-limited conditions was much lower than that of S. c.* These results indicate that galactose metabolism of *S. c.b. may be more dependent on aeration than that of S. c*.

The scientific community has paid considerable attention to the metabolism of *S.c.b*. Fu J. et al. [14] comprehensively analyzed the metabolism of *S.c.b.* compared to *S.c.* The study showed that *S.c.b.* produces many primary and secondary metabolites, including organic acids, amino acids and their derivatives, phenolic acids, alkaloids, nucleosides, and vitamins. Therewith, organic acids and heterocyclic compounds accounted for 60% of all metabolites, followed by phenolic and nucleoside compounds. The metabolite profiles of *S.c.b.* and *S.c.* were qualitatively similar but quantitatively different, which was shown by comparative analysis of extracellular metabolites. *S.c.b.* produced more indole compounds and purine derivatives (indoleacetaldehyde, indolepyruvate, hypoxanthine, thymine) than *S.c.*,

whereas *S.c.* produced more organic and amino acid derivatives (citric acid, malic acid, γ-aminobutyric acid). Furthermore, *S.c.b.* produced higher phenolic acids and nucleosides, including p-aminobenzoic acid and inosine, whereas *S.c.* metabolized choline and benzenoid compounds, including benzoic acid, and tyrosol.

Datta S. et al. [32] investigated the relationship between S.c.b.'s metabolism and antioxidant properties. They found that S.c.b. demonstrated a six-to ten-fold greater antioxidant potential than *S.c.* This was associated with a high total phenolic and flavonoid content in the extracellular fraction: 70 and 20 times more.

The successful medical application of *S.c.b*. worldwide for more than half a century is obviously due to its probiotic activity, which can be rightly called the key complex property of this yeast. According to the current available data, *S.c.b*. has shown efficacy in the treatment of acute and chronic diseases, namely, antibiotic-associated, acute, persistent, enteral nutrition-related and Traveler's diarrheas; clostridium difficile infection; inflammatory bowel diseases; irritable bowel syndrome; ulcerative colitis; Crohn's disease; sepsis; acne; and vaginal candidiasis [11,12]. Therewith, most of the postulated mechanisms of *S.c.b.* are antimicrobial activity, antitoxin effects, immune modulation, and trophic effects [9–12,23].

How S.c.b. exerts its effect on the gastrointestinal microbiota has been thoroughly studied. In the human gut, several mechanisms by which *S. c.b. may exhibit antibacterial and antiviral effects include (1) direct suppression of pathogenic intestinal microbes and normalization of the gastrointestinal pH; (2) indirect modulation of the gut microenvironment,* and (3) immunomodulatory effects on the host organism [11,33]. *S.c.b* has been documented to be antibacterial against a wide range of Gram-positive and Gram-negative bacterial and viral pathogens, including Bacillus anthracis, Shigella, E. coli, Vibrio cholerae, Helicobacter pylori, C. difficile, Salmonella, and Rotavirus [34].

S.c.b. may compete with pathogen toxin receptor sites, acting as a decoy for pathogenic toxins, or directly break down the pathogenic toxin [12]. *S.c.b.* was found by Castigliuolo et al. [17] to produce a 54 kDa serine protease that degrades *C. difficile* toxin A and prevents it from binding to the BB receptor. Buts et al. [18] showed that *S.c.b.* produces an alkaline phosphatase (63 kDa protein) that neutralizes the endotoxin formed by pathogenic *E. coli*. It has also been reported that *S.c.b.* synthesizes a 120 kDa protein which decreases the production of chloride secretions induced by cholera toxin by lowering cAMP levels [19]. Furthermore, *S.c.b.* can bind cholera toxin through its cell wall, thus attenuating its toxicity [35].

S.c.b. may regulate the immune responses by enhancing the immune system or inhibiting the proinflammatory reactions [9,12]. *S.c.b.* has been shown to stimulate IgA secretion in the intestine [36], and to increase serum IgG to *C. difficile* toxins A and B [37]. Moreover, *S.c.b* may inhibit NF-kappa B-mediated signal transduction pathways, which produce proinflammatory cytokines [38,39]. It has been demonstrated that *S.c.b.* binds to both the surface of dendritic cells and contributes to the alteration of their expression of toll-like receptors (TLRs) and cytokines [40–42]. Besides, *S.c.b.* retards the retention of T helper cells in mesenteric lymph nodes and reduces inflammation [43].

In addition to its antimicrobial activity, antitoxin effects, and immune modulation, *S.c.b.* exhibits a significant trophic effect. Thus, several studies have shown that *S.c.b.* stimulates a broad range of trophic effects, including stimulation of brush border sucrase, lactase, and maltase activities [44], glucoamylase and N-aminopeptidase activities [45], and leucine-aminopeptidase activities [46]. Polyamines secreted by *S.c.b.* may promote the activity of these digestive

enzymes by RNA binding and stabilization, which supports the synthesis of the enzymes [47].

Practice of *s.c.b.* Application in functional foods development: The first attempts to include *S.c.b.* in food matrices date back to the 2000s. Since then, much experience has been accumulated, which allows us to classify the key areas of *S.c.b.* use in food technology, including functional ones. Thus, *S.c.b.* can be applied as a starter culture in fermented products and as an enriching functional ingredient. On the other hand, classification can be made according to the type of food matrix in which the probiotic yeast is incorporated. This section systematizes the data on the use of *S.c.b.* in developing dairy products and plant-based beverages because these topics have been mainly investigated. At the same time, other food matrices are considered separately.

Dairy products: Dairy products, primarily fermented ones, are traditionally considered as a source of probiotic microorganisms in our daily diet. This reasonable fact is due to the indispensable use of LAB for producing dairy foods, some of which are indeed microorganisms with proven probiotic activity. Furthermore, there is an opinion that dairy food is a suitable vehicle for the delivery of probiotics into the gastrointestinal tract due to its supportive environment for the growth and survival of these microorganisms [23,48].

Although yeasts are not specific microorganisms for the dairy industry, some species of the genus Saccharomyces (S. cerevisiae, S. boulardii, S. rosinii, S. kluyveri*, S. burnetii)* are often found in dairy foods and considered as secondary flora or contaminants [48,49]. Despite the inability of some yeast (e.g., S. cerevisiae) to utilize lactose, it can survive and develop in dairy foods due to the metabolic products of LAB, namely, glucose and galactose (as hydrolysis derivatives of lactose), lactic and other organic acids [49–51]. Moreover, fruit additives, which are often included in dairy products, may be a direct source of simple sugars assimilated by yeast, such as fructose, glucose, and sucrose. In any case, the application of lactose-negative yeasts in the technology of fermented dairy foods is worthwhile mainly in combination with LAB as a part of mixed starter culture or as a functional ingredient added to the final product made with LAB. The practice of *S.c.b.* application in the development of dairy products is reflected in Table 1.

The first attempt to incorporate *S.c.b.* into a dairy matrix was performed in a study by Lourens-Hattingh and Viljoen [48] aimed to investigate the *S.c.b.* growth into various dairy foods such as plain and fruit yogurts, as well as ultra-high temperature treated (UHT) yogurt and milk. Product samples were inoculated with *S.c.b.* at 2.5% m/v to reach an initial yeast concentration >7 log10 CFU/mL after which the samples were stored at 5℃ for 29 days. It was found that the general cell population of *S.c.b.* in plain and UHT yogurt remained nearly identical (~7.6 log10 CFU/mL) during storage. Meanwhile, in the case of fruit yogurt and UHT milk, there was an increase in yeast cells from 7.7 log10 CFU/mL to 8.1 log10 CFU/mL and from 8.15 log10 CFU/mL to 8.5 log10 CFU/mL, respectively. *S.c.b.* growth in fruit yogurt was explained by the presence of fermentable simple sugars from fruits in the food matrix, whereas in UHT milk, it had no obvious explanation. It was presumed that, due to the inability of *S.c.b.* to assimilate milk lactose, protein, and fat [52], it utilized other milk constituents: free amino and fatty acids, galactose, and glucose, presented in small amounts in milk.

Parrella et al. [51] fermented milk with mixed starter cultures containing different Lactobacillus species and S. c.b. in a 1:1 ratio (10^6-10^7 cells/mL each). It was found that fermentation performed with yeast resulted in lower acidity of the final product, caused by the consumption of organic acids by yeast. Moreover, the food samples obtained with *S.c.b.* were characterized by higher antioxidant activity, which was presumably associated with improved scavenging activity of casein

caused by the increased pH [53]. Another interesting fact was the favorable effect of yeast on the LAB viability during cold storage at 4℃ for 21 days, which seems to be also related to the lower acidity of the final product.

Niamah also found the beneficial effect of S.c.b. on the growth and survival of LAB (Lactobacillus bulgaricus and Streptococcus thermophilus) [54]. Thus, in samples of yogurt obtained with *S.c.b.* and stored at 4℃ for 21 days, the concentration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cells was higher (by ~1.53 and ~1.05 log10 CFU/g, respectively) than in samples made without yeast. In contrast to the previous results, the pH of yogurts prepared with *S. c.b.* was lower than those prepared with LAB. The authors hypothesized that the positive effect of yeast on LAB leads to greater lactic acid biosynthesis and, consequently, to decreased pH. In addition, it was found that the use of *S.c.b.* resulted in an increase in total and water-soluble nitrogen in the final product due to the proteolytic activity of the yeast, and this increase was positively correlated with the yeast inoculum rate.

The effect of incorporating *S.c.b.* in the fermentation process on the organoleptic properties of a fermented dairy product (goat yogurt) was assessed by Karaolis et al. [55]. The final product's sensory characteristics were not negatively affected by the yeast. The panel tasters obtained stable organoleptic properties and good acceptability when the yogurt was prepared with a combined addition of LAB starter culture (YC-380; Chr Hansens, Denmark) and probiotic yeast. Nevertheless, it was observed that the total organoleptic score decreased in storage at 6°C for 28 days due to the development of an ethanol aroma and the appearance of syneresis. As with previous studies, the presence of yeast promoted LAB survival.

To increase the biological value, food products can be enriched with both probiotics and prebiotics, and the obtained product is classified as a synbiotic. Therewith, besides improving sensorial properties, adding prebiotics into the food products containing probiotics could improve the viability and efficacy of these probiotics. Sarvar et al. [56] synbiotic yogurt enriched with *S.c.b.* and inulin. Although the use of yeast alone resulted in reduced hardness, it was observed that simultaneous *S.c.b.* and inulin application had a positive effect on product textural properties such as hardness, cohesiveness, and adhesiveness. Including both ingredients in the yogurt formulation led to the increased content of flavor-contributing volatile compounds (acids, esters, alcohols, and others) in the final product. The latter can presumably be explained by the effect of the inulin presence on the microorganism's metabolism, namely, *S.c.b.* and those included in the commercial yogurt starter culture.

Cheese is suggested to have some advantages as a carrier of probiotic microorganisms compared to other, generally more acidic, dairy products. Thus, this food product can act as a buffer against the gastric environment, thereby reducing the wounding effect of gastric juice on probiotic microorganisms. At the same time, due to its dense matrix, cheese can function as an additional mechanical protective barrier [57,58]. Rafael et al. [59] developed and characterized the symbiotic cheese with *S.c.b.* and inulin. Probiotic yeast was included in the product in encapsulated form with sodium alginate and cactus mucilage in such an amount that the curd had a final yeast concentration of 9 log10 CFU/g. It was reported that the chemical composition of the cheese was not affected by the inclusion of synbiotic composition (*S.c.b* and inulin). Still, the organoleptic properties were improved, and consumer acceptance increased. Furthermore, the minimum level of microorganisms' viability during the storage period (at 4°C for 30 days) was above the recommended by FAO (6 log10 CFU/g).

Ice cream, which is classified as a frozen dessert food product, can also be considered as a carrier of both live probiotic microorganisms and substances with

prebiotic activity [23]. The synbiotic ice cream with *S.c.b.* and inulin was developed by Sarwar et al. [60]. The authors evaluated the effects of this combination on the probiotic viability, physicochemical properties, and stability of the final product. Results showed that the viability of *S.c.b.* was enhanced by adding inulin, with a viable count of 6.16 log10 CFU/g after storage at -18°C for 120 days. The firmness of ice cream samples was significantly reduced by fermentation with *S.c.b.* compared to the control ones (510.2 g vs 545.9 g). Nevertheless, the inclusion of 1% and 2% inulin restored the firmness of the synbiotic ice cream up to 535.3 g and 548.3 g, respectively. Moreover, particle size, zeta potential, and light scattering measurements revealed the melting resistance and stability. As expected, including a fermentation step with S. c.b. in ice cream production resulted in higher concentrations of volatile compounds in the final product compared to the control (1479 µg/L vs 616.4 µg/L). However, adding inulin together with probiotic yeast resulted in an even higher concentration of volatile compounds up to 3983 µg/L, the reason for which may be the inulin effect on the *S.c.b*. metabolism.

Goktas et al. [61] showed that probiotic strain, timing of inoculation (before or after the aging step), and culture type (single or co-culture) can affect the formation of different ice cream aroma profiles. All product samples contained ethanol, L-limonene, n-hexadecane, and propanoic acid. Meanwhile, ice cream samples inoculated with *S.c.b.* as a single culture also contained 3-methyl-1-butanol, isoamyl alcohol, and acetaldehyde. In contrast, action was found only in the ice cream samples inoculated with *L. rhamnosus*. The food product samples inoculated with both microbial cultures before the aging step contained acetic acid ethyl ester, hexanoic acid, and propanoic acid 2-hydroxy ethyl ester.

Table 1: Application of *S.c.b.* in dairy product development

Products	Functional ingredients	Main findings	References
Bio-yogurts (plain and fruit)	*S.c.b.*	*S.c.b.* had the ability to grow in bio-yogurt, reaching maximum counts exceeding 10^7 CFU/g (the highest concentration of cells was observed in fruit yogurt). Excessive gas and alcohol production by the yeast is the major constraint to its use in yogurt formulations.	[48]
Fermented milk	*S.c.b.* and LAB	The co-incubation of LAB with *S.c.b* determined a stronger product antioxidant activity. The inclusion of *S.c.b.* in the fermentation process increased LAB viability during storage.	[51]
Yogurt	*S.c.b.* and LAB	The beneficial effect of *S.c.b.* on the growth and survival of LAB was found. *S.c.b.* use resulted in the increase in total and water-soluble nitrogen in the final product.	[54]
Goat yogurt	*S.c.b.* and LAB	Stable organoleptic properties and good acceptability were obtained when the yogurt was prepared with a combined addition of LAB starter culture and *S.c.b.* *S.c.b.* presence promoted LAB survival.	[55]
Yogurt	*S.c.b.*, LAB, and inulin	Simultaneous *S.c.b.* and inulin application had a positive effect on product textural properties and increased the content of flavor-contributing volatile compounds in the final product.	[56]
Cheese	*S.c.b.* and inulin	The chemical composition of the cheese was not affected by the inclusion of synbiotic composition (*S.c.b.* and inulin), but the organoleptic properties were improved and consumer acceptance increased.	[59]
Ice cream	*S.c.b.*, LAB, and inulin	The use of inulin increased the viability of *S.c.b.* during storage. The addition of inulin together with *S.c.b.* resulted in hight concentration of volatile compounds in the product.	[60]
Ice cream	*S.c.b.* and LAB	The rheological parameters of ice cream improved with the co-inoculation of *S.c.b.* and LAB. The aroma profile of ice cream samples altered with probiotic inoculation and ethanol was the major volatile compound in ice cream produced with *S.c.b.*	[61]

Non-dairy beverages: The idea of using *S.c.b.* as a functional ingredient in beverage technology has aroused the greatest interest among researchers in the field of brewing science, which is confirmed by a large number of works in this area published in recent years. This fact is probably associated with the indispensable application of

yeast for beer production and the high degree of S. cerevisiae relatedness to top-fermenting brewing yeast brewingevisiae strains). It is worth noting that ethanol, which is traditionally contained in beer, wine, and cider, is the only problem in introducing the concept of functional food into brewing and wine- and cidermaking. Despite the controversial issue of the benefits and harms of ethanol for human health, including scientifically based data on the benefits of moderate consumption of alcoholic beverages [62], this issue has been resolved unequivocally in functional food. For example, in the EU, no beverages containing more than 1.2% v/v ethanol can bear health claims [63]. For example, beer with a probiotic microorganism can only be associated with alcohol-free or low-alcohol beers containing no more than 0.5% v/v and 1.2% v/v of ethanol, respectively [64]. The practice of *S.c.b.* application in developing non-dairy beverages is reflected in Table 2.

Beer with potential probiotic activity through the use of *S.c.b.* as a starter culture was developed by Mulero-Cerezo et al. [65]. It was observed that the beer produced with the probiotic yeast (in comparison with beer obtained with brewing yeast strain Safale S-04), possessed higher antioxidant activity, lower alcohol content, much higher yeast viability, and more acidification, which is very desirable to reduce contamination risks at large-scale production. The concentration of *S.c.b.* cells in the final product was indeed relatively high, even after the storage at 4℃ for 45 days, and amounted to $8.3\pm1.4\times10^6$ CFU/mL (the initial pitching rate was 10^6 cells/mL). Sensory evaluation results showed that using *S.c.b.* had no adverse effect on beer aroma and produced beer with acceptable sensory attributes. Moreover, it is notable that the high antioxidant activity of beer was not associated with polyphenols and, apparently, could be attributed to the antioxidant compounds not belonging to polyphenols, secreted by the probiotic yeast.

The aromas and flavors of alcohol-free and low-alcohol beers are well-known to be defective in many ways. Thus, beers with low ethanol content produced by interrupted fermentation generally have worty off-flavors and a lack of aroma and flavor compounds [66]. Nevertheless, flavor development can be enhanced with optimization of the fermentation process [67]. Senkarcinova et al. [68] used response surface methodology to determine the most critical parameters affecting the production of esters (ES) and higher alcohols (HA) by *S.c.b.* during beer wort fermentation. The results showed that temperature and pitching rate significantly affected ES and HA biosynthesis. At the same time, the maximum HA and ES formation was observed in the range of original wort extract from 9 to 12% w/v, which indicates the need to dilute wort or finished beer to produce non-alcoholic beer, for which original wort extract should be 4–7.5% w/v [69]. In addition, Senkarcinova et al. studied the effect of ethanol and iso-α-bitter acids on *S.c.b.* growth kinetics on glucose medium at 30°C. It was found that ethanol (5% v/v) and iso-α-bitter acids (50 IBU) decreased the specific growth rate on glucose (30°C) by 20% and 23%, respectively.

Pereira de Paula et al. [70] developed a potential probiotic wheat beer using S. cerevisiae and investigated the technological features of this yeast. It was found that *S.c.b.* prefers to use glucose as the primary carbon source and that maltose consumption is inhibited when glucose concentrations exceed 8 g/L. Additionally, it was observed that *S.c.b.* tends to produce acetic acid, which can impart a bitter and sour taste to the beer [71]. Additionally, it was shown that *S.c.b.* viability is reduced by stress induced by the beer matrix or gastrointestinal transit. However, the yeast population was greater than 6 log10 CFU/mL, and, as a result, a 12-oz glass of potential

probiotic wheat beer contained more than eight billion viable cells.

The use of mixed cultures and consequent cofermentation is a promising direction in brewing due to the complexity of the organoleptic profile of the final product and the achievement of its uniqueness. The effect of integrating *S.c.b.* in mixed cultures with *S.c.* Strains for beer production were investigated by Capece et al. [72]. The results showed that the probiotic yeast predominated over the *S. c.* strains in almost all cases in the mixed fermentations by the end of the process. The final products had high concentrations of viable *S.c.b.* cells (from 8×10^6 to 7.0×10^7 CFU/mL). The main volatile compounds of the beers were analyzed, and it was found that the *S.c.b.* as part of mixed starter cultures did not harm the beer's aroma. Furthermore, beers produced with mixed starter cultures had an increased antioxidant activity and more polyphenols than those fermented with single-strain cultures, indicating that *S.c.b.* has a positive effect on these characteristics.

The probiotic yeast *S.c.b.* has also found successful applications in other beverage technologies. Thus, *S.c.b*. was used by Mulero-Cerezo et al. [73] to elaborate both alcoholic and non-alcoholic rosé wines. It was demonstrated that the sensory characteristics of wines produced with *S. cerevisiae bai were close to controls (wines fermented by S. cerevisiae* EC-1118). Additionally, it was found that experimental rosé wines retained potential probiotic properties for six months of storage.

Fermented beverages made from diluted honey and spices remain popular with consumers. A potentially probiotic mead was successfully developed by Souza et al. [74]. It was shown that an initial wort concentration of 30 °Brix and an S.c.b. pitching rate of 0.030 g/L allow obtaining a final product with a viable yeast cell count of 6.53 log10 CFU/mL and an alcohol content of 5.05%. The beverage also contained phenolic compounds (17.72 mg GAE/100 mL) and antioxidants (62.79 and 1.37 µmol TE/100 mL detected by ABTS and FRAP methods, respectively). In their subsequent research, Souza et al. [75] produced the potentially probiotic mead with a mixed starter culture consisting of *S.c.b.* and water kefir, a symbiotic community of bacteria and yeasts. The beverage had *S.c.b.* and LAB counts greater than 8 log10 CFU/mL. Beyond that, both *S.c.b.* and LAB survived more than 70% (> 6 log10 CFU/mL) after simulated gastrointestinal transit in vitro. The potential probiotic mead also had good brightness, a yellowish hue, the presence of phenolic compounds, and antioxidants.

As it is known, malted barley can be used not only for beer production, but also for malt beverages, which can be formulated without hops. However, in its turn, the application of various flavoring plant raw materials and food additives is possible, although in brewing, this is limited due to the adherence of some beer producers to the German Beer Purity Law. Gutiérrez-Nava et al. [76] developed the probiotic barley malt beverage with *S.c.b.*, where strawberry and nut flavorings were included in the formulation to improve consumer acceptability. The authors made four formulations, differing in the type of flavoring (strawberry or nutty) and inoculum size (10^3 or 10^4 CFU/mL). As a result of sensory analysis, the sample obtained using nutty flavoring (0.4 % v/v) and 10^4 CFU/mL pitching rate was the most optimal (it had greater preference for taste, color, and odor attributes). Likewise, the beverage sample had high final yeast concentration (9 log10 CFU/mL). Moreover, the study determined the effect of inoculum concentration on the *S.c.b.* growth kinetics. Thus, it was shown that the inoculum size had no effect on specific growth rate, but it affected the duration of the lag phase, which decreased from 13.22 to 2.46 h when the inoculum size increased from 10^3 to 10^4 CFU/mL. In addition, it was discovered that the fermentation process with *S.c.b.* increased the

content of prebiotic oligosaccharides (maltotetraose, maltopentose, maltohexose, and maltoheptosis) in fermented barley wort, which could be beneficial for the survival and viability of *S.c.b.* as well as human microbiota.

Boza was another cereal fermented beverage produced using *S.c.b.* in the research of Arslan et al. [77]. It is a traditional fermented cereal beverage consumed widely in Turkey and Balkan countries. The boza production usually involves boiling individual cereals (millet, maize, oats, wheat, or rice) or their blend for approximately two hours. After that, sugar and water are added to the cooled porridge, which is homogenized. The fermentation is performed at 25–30°C for 24 hours with LAB and yeast. Maize and wheat semolina in a 3:1 ratio were the grain base in the Arslan et al. study. Probiotics such as *S.c.b.* (4×10^3 CFU/mL), *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 (each ranging from 6×10^7 to 12×10^7 CFU/mL) were used as a mixed starter culture. The authors performed microbiological analysis during fermentation, at 25℃ for 24 hours, and sensory analysis during the storage period at 4℃ for fifteen days. It was observed that *S.c.b.* and *L. acidophilus* cell concentrations increased by nearly 0.5 log10, while *B. bifidum* counts decreased by 0.6 log10 units, but increased by almost 0.5 log10 during the first twelve hours of fermentation. Whereby *S.c.b.* counts continued to grow during storage, whereas the viability of other probiotics decreased. Nevertheless, after fifteen days of storage, the total probiotic concentration in the beverage was above 6 log10 CFU/mL. The researchers also determined that the produced beverage shelf life amounted to twelve days at 4℃, which was associated with a significant decrease in consumer properties such as odor, mouthfeel, acidity, and acceptability after this period. According to the authors, this decrease might be caused by sugar degradation and producing microorganism metabolites such as organic acids and alcohols.

Probiotic vegetable juices (radish, black carrots and beets) fermented with *S.c.b.* were produced by Degirmencioglu et al. [78]. Using *S.c.b.* in these food matrices yielded a final product with high antioxidant activity correlated with increased phenolic compounds content. However, the type of vegetable used for juice production was shown to affect the profile of the phenolic compounds. For example, a cyanidin-3-O-glycoside chloride was present only in black carrot juice at high concentrations (1549.86–1774.86 mg/L) while vanillic acid was found in all fermented juices. The study also determined the positive effect of *S.c.b.* on the sensory profile of beverages; thus, the preferred sample was the red carrot juice fermented by *S.c.b* but not by *S.c*.

Trung et al. [79] obtained the beverage from the algae *Hydropuntia eucheumatoide* fermented with probiotic microorganism monocultures (*S.c.b* and *Lactobacillus casei*). The macroalgae hydrolysate was fermented at pH 5.2 and 32°C with agitation for 120 hours. The initial concentration of *L. casei* and *S.c.b.* was 6.3 and 6.2 log10 CFU/mL, respectively. It was found that the produced beverages were characterized not only by the presence of a sufficient number of probiotic cells (more than 6 log10 CFU/mL), but also by prebiotic oligosaccharides, antioxidants, and enzymatic activities due to the microorganisms' metabolic activity. The researchers also noted the effect of the microbial culture type on the final product's organoleptic properties. The alcoholic beverage obtained with L. casei had an odor and sour taste typical of lactic acid fermentation. In contrast, fermentation with *S.c.b.* provided a honey-like aroma with a note of isoamyl acetate and a slightly tart and sweet taste.

To increase the survivability of probiotic cells in functional products, especially during their storage and gastrointestinal transit, it is possible to use various protection methods, and encapsulation is one of these methods [80]. Fratianni et al. [81] developed functional berry juice fermented with *S.c.b.* microencapsulated in alginate, inulin, and xanthan gum. It was demonstrated that the encapsulation technique significantly increased the viability of probiotic yeast cells in the beverage after both storage at 4°C for four weeks, and treatment with gastric juice compared to the control (free cells). Thus, in the case of encapsulated cells, viability loss was reduced by less than 2 log10 CFU/mL, while in the case of free cells, it was more than 4 log10 CFU/mL.

Tea is among the most popular beverages worldwide [82], with global annual consumption of 6.4 million tons (in 2021) [83]. This beverage was also enriched with *S.c.b.* by fermentation. In the works of Wang et al. [84,85] fermented green tea was obtained using monocultures of *S.c.b.* and *Lactiplantibacillus plantarum* 299V, as well as their co-culture. To stimulate probiotic growth and survival, the tea was supplemented with 0.5% glucose and 0.06% yeast extract, and after inoculation of probiotic cultures (the initial cell concentration of about 5-7 log10 CFU/mL), fermented at 30°C for two days, followed by storage at 25°C for 87 days. It was observed that application of *S.c.b*., both as mono- and mixed-culture, provided enhanced aroma component formation during the fermentation, such as ethyl esters, which imparted fruity notes to the beverage sensory profile. Compared to the mono-fermentation product, the tea obtained by co-fermentation contained higher amounts of methyl salicylate, geraniol, 2-phenylethyl alcohol, and less lactic acid.

As mentioned above, *S.c.b.* can be used in beverage technology as a fermenting agent and as a probiotic additive in a final product, as Santana et al. demonstrated [86]. The researchers produced clarified "Cerrado" cashew juice fortified with *S.c.b.* and containing different sweeteners, with further evaluation of its properties during storage (at 7°C, for 28 days). In the study, the authors used sugars such as sucrose, fructose, and xylitol and sugar substitutes such as stevia, aspartame, and sucralose. It was shown that developed beverage formulations ensured a sufficient amount of live S.c.b. cells during storage, more than 7 log10 CFU/mL with the initial concentration of 8 log10 CFU/mL. Interestingly, the cell concentration was not higher in the samples containing sugars compared to those comprising sugar substitutes (except sucralose) and the juice without any sweeteners. This fact suggests that reducing the caloric content of the functional food products is possible without losing their probiotic properties. Moreover, samples of probiotic cashew juice were characterized by total phenolic and flavonoid content (75–105 and average ~71 mg/100 mL, respectively), antioxidant activity (86–88% DPPH reduction), and high vitamin C content (54–71 mg/100 mL).

Table 2: Application of *S.c.b.* in non-dairy beverage development

Products	Functional ingredients	Main findings	References
Beer	*S.c.b.*	The beer produced with *S.c.b.* possessed higher antioxidant activity, lower alcohol content, much higher yeast viability, and more acidification than beer obtained with a brewing yeast strain.	[65]
Beer	*S.c.b.*	The inhibitory effect of ethanol (5% v/v) and iso-α-bitter acids (50 IBU) on *S.c.b.* growth was established.	[68]

Products	Functional ingredients	Main findings	References
		Optimal parameters for biosynthesis of esters and higher alcohols during wort fermentation with *S.c.b.* have been determined.	
Beer	*S.c.b.*	The inhibitory effect of glucose on maltose uptake by *S.c.b.* was found. *S.c.b.* tended to produce more acetic acid than the brewing yeast strain.	[71]
Beer	*S.c.b.*	*S.c.b.* predominated over *S.c.* strains at the end of the cofermentation processes. Beers produced with *S.c.b.* and *S.c.* strains in mixed culture form had an increased antioxidant activity and more polyphenols compared to beers fermented with *S.c.* monocultures.	[72]
Mead	*S.c.b.* and kefir grains	Combining kefir grains and *S.c.b.* produced a potentially probiotic mead with a viable cell count of more than 8 log10 CFU/mL of *S.c.b.* and LAB.	[75]
Malt beverage	*S.c.b.*	The effect of the inoculum concentration on reducing sugar consumption, the concentration of soluble solids, and pH was evaluated. The fermentation process with *S.c.b.* increased the content of prebiotic oligosaccharides in barley wort.	[76]
Boza	*S.c.b.* and LAB	The probiotic boza with a probiotic microorganism cell concentration greater than 6 log10 CFU/mL has been developed. The effect of cold storage on the consumer properties of the product has been established.	[77]
Fermented vegetable juices	*S.c.b.*	S.c.b. fermented radish, black carrot, and beet juices to yield the final product with high antioxidant activity. The positive effect of *S.c.b.* on the sensory profile of beverages was found.	[78]
Fermented beverage from the macroalgae	*S.c.b.* and LAB	The beverage was characterized by sufficient probiotic cells (more than 6 log10 CFU/mL) and prebiotic oligosaccharides, antioxidant and enzymatic activities.	[79]
Fermented green tea	*S.c.b.* and LAB	Applying S.c.b. both as mono- and mixed-culture with LAB enhanced aroma component formation during the fermentation.	[84,85]

Other food products: The probiotic yeast culture *S.c.b.* has also been used to develop other food products (Table 3).

Healthy snacks and sweet products are an emerging segment of the food industry. Cielecka-Piontek et al. [87] developed the extremely complex functional snack with a white chocolate core supplemented with lyophilized raspberry fruit and inulin and an outer shell of probiotic-enriched dark chocolate. The following lyophilized microbial cultures were used separately to impart probiotic properties to the final product: *L. rhamnosus* GG; *B. coagulans* GBI-30, 6086; *B. breve* DSM 16604; *Bifidobacterium animalis subsp. lactis* as well as *S.c.b.* (in quantities ensuring a concentration of 8 log10 CFU/g). Along with the survival of probiotic microorganisms (during the storage and in vitro digestion model), the researchers determined the final product's physicochemical and sensory properties and phenolic compounds' content. It was determined that the storage of snack samples at the temperatures of 4 and 20 ℃ for six months in all cases except *L. rhamnosus* GG did not significantly reduce the concentration of live probiotic cells, which remained close to the initial concentration (8 log10 CFU/g). Under GI-transit conditions (*in vitro*), *S.c.b.* showed the highest survival rate compared to lactic acid bacteria, with a concentration of 7.3×10^8 CFU/g after the complete digestion process. It was also shown that adding probiotics to the product did not significantly affect its physicochemical (pH and water activity) and organoleptic properties, as well as the content of phenolic compounds.

Fermentation of vegetables with yeast is one of the ways to preserve them for a longer period. Chun et al. [88] investigated the effect of *S.c.b.* (and seven other yeast cultures) on the functional properties of fermented cabbage. Fermentation of cabbage with *S.c.b.* and *S.c.*

was found to increase the content of sulforaphane by 6.4 and 5.6 times, respectively. As it is known, sulforaphane has antioxidant, anti-inflammatory, and anti-cancer activities. It was also demonstrated that yeast-fermented cabbages exhibited ABTS radical-scavenging activity equivalent to 1 µg/ml of ascorbic acid. Moreover, the researchers found that cabbage fermented with *S.c.b.* had an anti-inflammatory effect, which was confirmed by significant suppression of lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells.

Singu et al. [89] suggested technological approaches to preserving microorganisms' viability and increasing probiotic product shelf life. This study aimed to develop heat-stable probiotic cornflakes using hydrocolloids as a coating agent. Being treated with hot milk, *S.c.b*. protection by 6% acacia gum coating was highest, 7.3 ± 0.1 log10 CFU/g, compared to 2.3 ± 0.1 log10 CFU/g in the control. Probiotic-coated corn flakes demonstrated considerable reductions in the water absorption index and capacity, milk absorption capacity, and surface area. Resistance of *S.c.b.* to acidic conditions and pepsin, as well as to pancreatic juice, was shown in a simulated gastrointestinal model. In addition, the coated cornflakes maintained 88.3% *S.c.b*. viability during storage at 30 ± 2°C for 90 days.

Silva Farinazzo et al. [90] evaluated the influence of *S.c.b.* on the fermentation kinetics of apple pulp (organic and conventional), phenolic compound content, and antioxidant activity. It was shown that in terms of growth kinetics, there were no differences between organic and traditional apple pulps; thus, the specific growth rate was 0,020±0,003 and 0,021 ± 0,001 h^{-1}, respectively. However, organic apple pulp contained more phenolic compounds and had a higher antioxidant activity than conventional pulp. It is worth noting that during the fermentation process, both of these indicators increased, which was suggested to be related to yeast metabolism.

Table 3: Application of *S.c.b.* in the development of other food products

Products	Functional ingredients	Main findings	References
Chocolate snack	*S.c.b.* and LAB	*S.c.b.* showed the highest survival rate compared to LAB, with a concentration of 7.3 × 10^8 CFU/g after the complete digestion process. The addition of probiotics to the product did not significantly affect its physicochemical and organoleptic properties.	[87]
Fermented cabbage	*S.c.b.*	Fermentation of cabbage with *S.c.b.* was found to increase the content of sulforaphane by 6.4 times. The anti-inflammatory effect of cabbage fermented with *S.c.b.* was determined.	[88]
Cornflakes	*S.c.b.*	Probiotic-coated cornflakes with acacia gum showed maximum protection of *S.c.b.* (7.3 ± 0.1 log CFU/g) when treated with pre-heated milk. Probiotic-coated cornflakes showed an 88.3% survival rate of *S.c.b.* when stored at 30±2 °C for 90 days.	[89]

CONCLUSION

To date, yeast *S.c.b.* is the only registered eukaryotic probiotic microorganism. Despite the successful application of this probiotic yeast culture in medical practice, the researchers in functional food development have demonstrated that its introduction into food formulations is a direct way of imparting probiotic properties to foods. There are two ways of *S.c.b.* incorporation into food products, namely, simple enrichment of final products and fermentation of food matrices with *S.c.b* as a starter culture, with the subsequent obtaining of fermented products. Simple enrichment of final products allows obtaining a functional product only with probiotic activity, provided that the concentration of live cells at the end of the shelf life of the food product is greater than 10^6 CFU/mL (g).

Meanwhile, the fermentation of food matrices with S.c.b. is a broader direction due to the presence of metabolic activity during manufacturing, which may have a positive effect on the functional and sensory characteristics of the products.

List of Abbreviations: *Saccharomyces cerevisiae var. boulardii*, *S.c.b.; Saccharomyces cerevisiae*, *S.c.;* cytochrome c oxidase II, COX2; cyclic adenosine monophosphate, cAMP; immunoglobulin A, IgA; immunoglobulin G, IgG; lactic acid bacteria, LAB; ultra-high temperature treated, UHT; esters, ES; higher alcohols, HA; iso-α-bitter acids, IBU.

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