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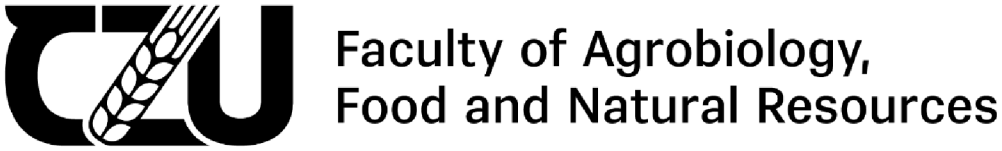


**Diploma thesis**

# Utilisation of brewers' spent yeast in protein powder product

**Bc. Lukáš Polanka**

Declaration

declare that the Diploma Thesis "Utilisation of Brewers' Spent Yeast as source of proteinaceous food additive " is my own work, and all the sources cited in it are listed in the Bibliography. AI was used for the translation of foreign papers and grammatical correction.

Prague, 19.4.2024 _____

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## Souhrn

Vedlejší průmyslové produkty, které jsou často vnímány jako odpad, vyvolávají obavy z kontaminace životního prostředí. V posledních letech se klade stále větší důraz na opětovné využití těchto vedlejších produktů k vytvoření výrobků s přidanou hodnotou kvůli podpoře udržitelnosti a snížení množství průmyslového odpadu. Tato práce zkoumá potenciál využití průmyslového odpadu z pivovarnictví, konkrétně se zaměřením na použité pivovarské kvasnice (SBY). Výsledky studie ukazují, že proteinový koncentrát získaný z SBY vykazuje vysoký obsah bílkovin s vysokou rozpustností a dobrými emulgačními vlastnosti. Tento proteinový koncentrát ze SBY proto může sloužit jako funkční potravina, nebo jako aditivum do potravinových produktů, kterým může napomoci ke zlepšení technologických vlastností.

Cílem této práce bylo získat bílkovinné koncentráty z použitých pivovarských kvasnic a otestovat jejich vlastnosti pro použití při vývoji potravinářských výrobků. Předem připravený materiál (kvasnicová mouka) byl podroben solubilizaci bílkovin alkalizací a izoelektrickou precipitací. Byla stanovena výtěžnost získaných lyofilizovaných bílkovinných koncentrátů a hodnoceny některé fyzikálně-chemické (barva, velikost částic, rozpustnost) a funkční vlastnosti (emulgační schopnost a stabilita, schopnost zadržovat vodu a olej, rozpustnost, želírovací vlastnosti) při různých hodnotách pH.

Tato práce se snaží prokázat, že existují možnosti využití SBY jakožto udržitelného zdroje pro výrobu proteinových koncentrátů. Využíváním tohoto vedlejšího produktu se najde nové využití pro odpadní materiály a dojde ke zvýšení udržitelnosti dosavadní výroby. Informace získané v tomto výzkumu vybízejí k dalším možnostem implementace těchto procesů a vybízejí začlenění bílkovinných koncentrátů SBY pro potenciál jejich širokého uplatnění v potravinářském průmyslu.



**Klíčová slova:** použité pivovarské kvasnice, vedlejší produkty pivovarnictví, proteinový koncentrát, zhodnocení odpadu, proteinový nápoj

## Abstract

Industrial residues, often perceived as waste, have raised concerns regarding environmental contamination. In recent years, there has been a growing emphasis on repurposing these byproducts to create products with added value, promoting sustainability and reducing industrial waste. This work explores the potential of utilizing industrial residues, specifically focusing on spent brewers' yeast (SBY) from the beer production industry. Results from the study indicate that the protein concentrate derived from SBY exhibits high solubility, yield and emulsifying properties. This suggests that SBY protein concentrate could serve as a functional additive in various products which could benefit from these properties.

This thesis aimed to obtain protein concentrates from spent brewer's yeast and test them as functional additives for being used on food products development. Pre-conditioned material (flour) was submitted to protein solubilisation by alkalinisation and isoelectric precipitation. The yield of the obtained freeze-dried protein concentrates was determined and some physicochemical (color, particle size, solubility) and functional properties (emulsifying capacity and stability, water and oil holding capacity, solubility, gelling properties) at different pH values were evaluated.

In conclusion, the thesis demonstrates the viability of using SBY as a sustainable source for producing protein concentrates. By valorising this by-product, the brewing industry can reduce waste and contribute to environmental sustainability. The findings encourage further research into the implementation of this process and the integration of SBY protein concentrates into the market, highlighting the potential for widespread application in food and nutrition sectors.

**Keywords**: spent brewers' yeast, brewery by-products, protein concentrate, waste valorisation, protein drink

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# Introduction

## 1.1 Brewer's spent yeast as source of nutritional compounds

Spent Brewer's Yeast (SBY), a by-product of the beer brewing process, has garnered significant attention in scientific and industrial fields for its potential in sustainable protein production. Predominantly comprising of *Saccharomyces cerevisiae* SBY is mainly constituted by proteins, carbohydrates, vitamins, minerals (Marson et al. 2020).

Despite its nutritional value, it often remains underutilized or discarded. The possibility of using this residue to develop an added-value product (in this case, a food additive) would contribute to sustainable production and reduction of solids. Even though it was not studied in this work, SBY is also a good source of functional polysaccharides such as β-glucans.

Historically, spent brewer's yeast was often considered waste or used in low-value applications despite its rich nutritional profile. Early uses were primarily in animal feed or as a soil conditioner, reflecting a limited understanding of its potential. However, in the 21st century there has been a growing demand for sustainable and efficient protein sources, leading to a re-evaluation of SBY. Recent scientific research has revealed the high-quality protein content of SBY, along with essential amino acids, vitamins, and minerals, positioning it as a viable ingredient for human nutrition. This shift is particularly important in the face of the global protein challenge, where finding sustainable alternatives to traditional animal-based proteins is crucial. Exploring SBY in protein production is not only a scientific pursuit but also an environmentally conscious response. It is in line with global efforts to decrease carbon footprint and promote circular economy principles in industrial practices.

## 1.2 Aim and potential benefits of the study.

This diploma thesis investigates the usage of brewer's yeast and its functional properties in the production of protein concentrate, including its impact on the quality of the final product. Additionally, the thesis investigates the impact of processing methods on the functional properties of brewer's yeast and the resulting protein concentrate. Potential benefits of the study include the utilization of a byproduct that is generated in large quantities by the brewing industry, thereby reducing waste and promoting sustainability.

This thesis aimed to obtain protein concentrates from an underutilized industrial residue in production of protein concentrate used as food additive for food products development applications. Besides its nutritional value, proteins are valuable molecules due to its techno functional properties such as emulsifying properties, water holding capacity, oil holding capacity and solubility. The use of alternative low-cost proteins such as soybean proteins has enabled the development of functional products with high protein content, low fat content, and enhanced textural properties.

The developed protein protein concentrate could be a cost-effective alternative to the commercially available protein concentrates. Brewer's yeast is a rich source of protein and essential amino acids, which are important for muscle building, tissue repair, and overall health. The developed protein concentrate could provide a nutritional supplement for individuals who are unable to consume sufficient protein through their diet or have specific dietary needs. The product could be also used as an ingredient in various food products, such as baked goods, snacks, and beverages, thereby increasing the nutritional value of such products.

# Literature Review

## 1.3 State of the art

The sustainability movement in the food and beverage industry has spurred interest in the innovative use of by-products. A prime example of this trend is the utilization of spent brewer's yeast, primarily *Saccharomyces cerevisiae*, in protein production. Recent advancements in the field highlight the potential of spent brewer's yeast as a valuable resource for functional food, and dietary supplements, and as a source of bioactive peptides.

Podpora et al. (2015) explores the potential of spent brewer's yeast autolysates as a component in functional food and dietary supplements. This research explores the rich protein content of spent brewer's yeast and demonstrates its capabilities as a natural and valuable ingredient intended to produce functional food. The study's focus on autolysates provides a unique perspective on converting brewery waste into a value-added product.

Marson et al. (2020) delve into the extraction of high-value molecules from spent brewer's yeast. This comprehensive review presents a detailed analysis of the characteristics, processing, and potential applications of spent brewer's yeast. It particularly focuses on the methods to disrupt the yeast cell wall to produce protein hydrolysates, which are valuable in various industries due to their bioactive properties.

Oliveira et al. (2022) provided an extensive overview of the potential of spent brewer's yeast as a source of bioactive peptides. The study highlights the recovery of this fermentation by-product to produce protein-rich extracts. These extracts, particularly rich in bioactive peptides, have immense potential in nutraceuticals and pharmaceuticals, offering a sustainable alternative to traditional protein sources.

In 2020, Jaeger et al. explored alternative, value-added applications for spent brewer’s yeast in their study. This review delves into the challenges and opportunities associated with the use of spent brewer’s yeast. It discusses the potential of yeast extract production from this by-product, focusing on overcoming challenges like cell wall disruption for effective protein extraction. The review underscores the value of spent brewer’s yeast as a rich protein source and its application in various industries, including the food and feed sectors.

The research on spent brewer's yeast showcases its remarkable versatility and potential as a sustainable resource in various industries. These studies collectively emphasize the value of this brewing by-product, not only as a protein source but also as a provider of bioactive compounds and vitamins. The innovative use of spent brewer's yeast aligns with the growing trend of sustainable practices in the food and beverage industry, representing a significant step towards a more circular economy. As research and technology in this area continue to evolve, the full potential of spent brewer's yeast in protein production and beyond is yet to be fully realized, promising exciting developments in the future.

## 1.4 Overview of brewing industry and brewer's yeast

### 1.4.1 Brewer's yeast

Brewer's yeast is classified into two species – *Saccharomyces cerevisiae* for the top-fermented beer and *Saccharomyces pastorianus* for bottom beer fermentation. Brewer's strains are typical by their polyploidy (three or more sets of chromosomes are present in the cell) and are mostly tetraploid or aneuploid (chromosomes may not be represented in equal numbers). *S. pastorianus* can grow at lower temperatures, are adapted to the conditions of fermentation at lower temperatures (8–14 °C) utilizing the sugar melibiose. They do not grow at temperatures above 37 °C. Strains of *S. pastorianus* are divided into two groups: Saaz and Frohberg. The Saaz group includes yeast used to produce Pilsner-type beer (Czech Republic). Frohberg includes a group of Dutch strains (Heineken). *S. cerevisiae*, used for top-fermented beer (Ale, Stout, Wheat beer) is adapted to fermentation at higher temperatures (18–24 °C), also it cannot utilize melibiose unlike their bottom fermenting counterparts (Krescanková, 2015).

*Saccharomyces cerevisiae*, commonly known as baker's yeast, is a unicellular fungus that is widely used in baking, brewing, and wine making. It is a eukaryotic microbe that belongs to the kingdom Fungi and the phylum Ascomycota. It is known for its ability to ferment sugar and produce carbon dioxide and ethanol as metabolic byproducts. *S. cerevisiae* is a facultative anaerobe, meaning it can switch between aerobic and anaerobic respiration depending on the availability of oxygen. Under aerobic conditions, it metabolizes glucose through the tricarboxylic acid cycle (TCA cycle) and oxidative phosphorylation to produce ATP. Under anaerobic conditions, it undergoes fermentation, producing ethanol, carbon dioxide, and a small amount of ATP (Stewart, 2014).

### 1.4.2 Brewing technology

During the first part of beer production, a so-called wort is created. In this production phase, water is mixed with crushed malt (mashing). Under constant stirring in stainless steel tanks and gradually increasing the temperature, the enzymatic degradation of starch (saccharification) occurs, which is contained in the malt, into lower fermentable sugars. This occurs because the malt has increased enzymatic activity. The water enriched with extractive

substances from the malt (wort) is filtered through stainless steel sieves – a process called straining. After straining the wort, the intermediate product is heated to 100-102 °C for 90 minutes while gradually adding hop products (hopping). After completion of the hopping, the wort is transferred to a tank where it is centrifugally mixed and the boiled hop residues settle out of the liquid (Novotný 2019).

Subsequently, the wort is aerated with sterile air and transferred through a plate cooler to fermenting tanks or cylindroconical tanks with the addition of yeast. The so-called pitching temperature is 8-10 °C. The intermediate product is already referred to as young beer. The main fermentation takes place under anaerobic conditions in open tanks or cylindroconical tanks for a period of 3-6 days at temperatures of 8-15 °C. After completion of the main fermentation, the young beer is separated from almost all the yeast. The phenomenon called flocculation helps to separate yeast from young beer. Flocculation is the clustering of yeast cells, which is facilitated by the specific cell wall of the yeast.

They either settle to the bottom of the fermentation vessels or remain on the surface of the liquid, depending on whether it is bottom or top fermentation (Vidgren & Londesborough 2011). The young beer is then transferred to lagering tanks, where it continues to ferment at reduced pressure for several weeks (Zarnkow 2014).

After the lagering process in tanks is completed, the beer is stabilized according to the available brewery technology. It is desirable to separate all remaining yeast and biomass from the finished product to increase the shelf life of the product. Membrane filtration with pores smaller than 0.2 μm can be used. Furthermore, thermal pasteurization is often used in large breweries, which can have a deteriorating effect on the organoleptic properties of beer, so efforts are being made to replace old methods with new ones, such as the use of ultrasound, high pressure, or pulsed magnetic field (Peña-Gómez et al. 2020).

### 1.4.3 Industrial production of yeast and additives

The production of microbial biomass of protein-rich yeasts is currently a highly debated topic. Thanks to the enzymatic activity of *S. cerevisiae* yeasts and advanced genetic engineering methods, yeasts can be applied in other industries beyond traditional biotechnology. One possibility is the production of single cell protein (SCP). The application of proteins obtained from yeasts in the form of biomass could be useful, for example, in animal feed, and potentially also in human nutrition in the future. The production of yeast protein involves obtaining the highest possible volume of biomass from the yeast culture and its subsequent processing. Since SCP should have a competitive advantage in the market compared to other protein-rich products in the future, the production technology must be as efficient as possible, because the quality and organoleptic properties still do not match conventionally obtained protein sources. The most expensive item in SCP production is the yeast cultivation medium, and therefore efforts are being made to use waste materials or byproducts of other food products. Molasses, raw materials rich in polysaccharides and other sugar components are used. The process of SCP production is divided into several steps and is usually carried out in continuous bioreactors.

The production process begins with the preparation and stabilization of the substrate for fermentation, sterilization of the input raw materials of the cultivation media, addition of additives, inoculation of the media with the yeast culture, followed by the actual fermentation, during which the volume of yeast biomass multiplies. This is followed by centrifugation to remove excess water, disruption of the yeast cell structures in the biomass, spray drying, packaging, and distribution. To properly maintain the continuous growth of yeast, all requirements for macrobiogenic substances and trace elements must also be met. Therefore, a source of nitrogen (ammonia or urea), phosphate salts, and other macroelementary elements (K, S, Mg, Ca etc.) are also added to the bioreactors at the beginning of the fermentation process. In the bioreactor, fermentation (aerobic respiration) is conducted, during which pH, pressure, oxygen, and CO2 are deliberately adjusted. All these factors have an impact on the amount of yeast biomass produced and the amount of SCP obtained. (García-Garibay et al. 2014).

## 1.5 Nutritional composition of spent brewer's yeast

Spent brewer's yeast is collected from brewery after several usages in brewing process in the form of slurry. The SBY slurry, a residue rich in organic matter, has a moisture content between 85% and 97% and exhibits a high chemical oxygen demand (COD) of 1308 mg g−1 The final pH value of SBY is approximately 5.9, which is higher than that of beer, typically ranging from 4.2 to 4.5 (Mathias et al. 2015).

The chemical and nutritional composition of spent brewer's yeast (SBY) is influenced by several factors including biodiversity, such as the yeast strain used, operational conditions during fermentation and beer processing, the frequency of yeast reuse (repitching), the timing of yeast collection, and the methods used for yeast extract production. The primary yeast strains employed in beer production are *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, though a variety of non-*Saccharomyces* strains like *Saccharomycodes ludwigii, Saccharomycopsis fibuligera, Brettanomyces bruxellensis, and Torulaspora delbrueckii* are also utilized. Recent findings by Jacob et al. (2019) highlighted that the composition of yeast extracts varies with different yeast strains, even under identical brewing conditions. The study also noted that antioxidant properties are influenced by the specific type of spent yeast used.

Other variables such as repitching, yeast strain, and the methods used for yeast cell rupture and extraction significantly impact the final product's composition, as reported in various studies (Marson et al. 2019; Mathias et al. 2015; Vieira et al. 2013). These variations underscore the need for a robust manufacturing process capable of accommodating the inherent diversity in brewing parameters (Jacob et al. 2019).

Additionally, spent yeasts from alcohol distilleries, predominantly *S. cerevisiae*, represent a viable by-product for processing, especially in Brazil and other major ethanol-producing

countries. These yeasts share a similar overall composition to those used in brewing but typically have higher contents of RNA, ash, and lipids (Steckelberg et al. 2013).

### 1.5.1 Protein Content

One of the most remarkable aspects of spent brewer's yeast is its high protein content. A study by Podpora et al. (2015), highlights that spent brewer's yeast is not only a rich source of proteins but also contains all the essential amino acids required by humans. Studies report that the percentage of proteins found for brewing yeast ranges between 32 and 62% on a dry weight basis, making it an excellent alternative to traditional protein sources (Sgarbieri et al. 1999; Yamada and Sgarbieri 2005; Yamada et al. 2003).

The digestibility of these proteins is also high, which enhances their nutritional value. Older study by Dwivedi et al. (1970) showed similar protein content 47,3 %, in dry debittered powder while also emphasizing long shelf life attributed to low fat and carbohydrate contents.

Bertolo et al. (2019) measured contents of protein in yeast cells while comparing different methods of cell disruption ranging from 39.32 to 43.80%.

| Component (g/100 g of DB) | Composition | | | |
|---|---|---|---|---|
| | NY | MRY | MAY | TSP |
| Protein | $42.83 \pm 0.11^{a}$ | $43.80 \pm 0.62^{a}$ | $39.32 \pm 0.87^{b}$ | $42.81 \pm 1.03^{a}$ |
| Total lipids | $1.45 \pm 0.40^{ab}$ | $2.04 \pm 0.41^{a}$ | $1.25 \pm 0.48^{b}$ | $0.14 \pm 0.00^{c}$ |
| Ashes | $1.74 \pm 0.17^{c}$ | $2.36 \pm 0.30^{c}$ | $13.14 \pm 0.67^{a}$ | $6.77 \pm 0.05^{b}$ |
| Moisture | $0.07 \pm 0.00^{d}$ | $0.10 \pm 0.00^{b}$ | $0.09 \pm 0.00^{c}$ | $0.14 \pm 0.00^{a}$ |
| Total carbohydrates | 53.91 | 51.70 | 46.48 | 48.64 |
| Water activity | $0.161 \pm 0.044^{a}$ | $0.172 \pm 0.008^{a}$ | $0.194 \pm 0.010^{a}$ | – |
| Parameters | Color analysis | | | |
| | NY | MRY | MAY | |
| L* | $59.13 \pm 0.55^{a}$ | $59.31 \pm 1.79^{a}$ | $54.77 \pm 2.76^{b}$ | |
| a* | $4.52 \pm 0.18^{b}$ | $4.45 \pm 0.10^{b}$ | $5.50 \pm 0.47^{a}$ | |
| b* | $16.42 \pm 1.60^{ab}$ | $16.78 \pm 1.41^{a}$ | $15.00 \pm 1.24^{b}$ | |
| ΔE | – | 1.77 | 5.26 | |

Values expressed as mean ± standard deviation. Different letters on the same row indicate a significant difference ($p \leq 0.05$) by the Tukey's test

*DB* dry basis

**Table 1**. Physicochemical characterization of natural yeast (NY), mechanically ruptured yeast (MRY), modified autolysis yeast (MAY), textured soy protein (TSP) and colorimetric parameters of the samples as measured by Bertolo et al. (2019)

Marson et al. (2020) did similar experiment and reports that SBY is rich in carbohydrates and proteins (40–50% dry weight, each) with lower amounts of ash, fibres and ribonucleic acids.

| Macronutrients (g 100 $g^{-1}$, d.w.) | SBY slurry/biomass non-treated[A] | SBY obtained by mechanical rupture (US and glass beads)[B] | SBY autolysate[C] | SBY enzymatic hydrolysate[D] |
|---|---|---|---|---|
| Total nitrogen | 7.3–10.5 | 7.0–14.2 | 3.1–6.8 | 1.5–12.0 |
| Protein nitrogen | 41–49 | 43–78 | 18–45 | 9.3–69.0 |
| Free amino nitrogen | 0.2–0.4 | 2.6–16.5 | 3.8–45.1 | 28- -35 |
| Ribonucleic acids | 1.9–7.5 | 2.2–7.5 | 4.0–8.0 | 5.6 |
| Total sugars | 22–54 | 8.3–51.7 | 12.3–48.0 | 3.0–48 |
| Fibers | 6.6–36.2 | 3.1–12.2 | nd | nd |
| Insoluble fibers | < 2.6 | 0.5–2.6 | nd | nd |
| Soluble fibers | < 9.6 | 2.7–9.6 | nd | nd |
| Lipids | < 3.9 | 0.02–6.5 | 0.5–1.3 | 0.2–1.0 |
| Ashes | 1.7–8.5 | 0.2–14.0 | 13 | 3.0–22.0 |

**Table 2.** Marson et al. (2020) - Macronutrient composition in non-treated and yeast extracts of spent brewer's yeast (SBY) produced by mechanical rupture [ultrasound (US) and glass bead milling], autolysis and enzymatic hydrolysis.

Spent yeast from brewing processes is recognized as an excellent source of high biological value proteins with a well-balanced amino acid profile that aligns with the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (Jacob et al. 2019). In spent brewer's yeast (SBY), acidic amino acids such as glutamic acid and aspartic acid, along with essential amino acids like leucine and lysine, are particularly abundant. In contrast, sulphur-containing amino acids, including methionine and cysteine, are found in lower quantities (Jung et al. 2012; Caballero-Córdoba et al. 1997; Halász and Lásztity 1991). It is important to note that the amino acid composition can vary significantly depending on the processing methods used.

| Amino Acids | Autolysate | Reference Protein According To Fao/Who | CS**) |
|---|---|---|---|
| | mg/g of protein | mg/g of protein | % |
| Isoleucine (EG.)*) | 23.7 | 28 | 84.6 |
| Leucine + Norleucine (EG.) | 65.1 | 66 | 98.7 |
| Lysine (EG.) | 47.2 | 58 | 81.4 |
| Methionine (EG.) + Cysteine | 23.8 | 25 | 92.0 |
| Phenylalanine (EG.) + Tyrosine | 90.6 | 63 | 143.8 |
| Threonine (EG.) | 61.3 | 34 | 180.3 |
| Tryptophan (EG.) | 11.9 | 11 | 108.2 |
| Valine (EG.) | 69.7 | 35 | 199.2 |
| Sum of exogenous amino acids | 393.3 | 320 | - |
| EAAI***) | 117 | 100 | - |

*) EG. - essential amino acids
**) CS - chemical score
***) EAAI - essential amino acids index

**Table 3**. Comparison of the content of essential amino acids in the yeast's autolysate after 48 h of autolysis in relation to the content of amino acids in protein standard established by the FAO/WHO according to Podpora et al. (2015)

### 1.5.2 Vitamins and Minerals

Spent brewer's yeast is a treasure trove of B vitamins, notably thiamine, riboflavin, niacin, pantothenic acid, biotin, and folic acid. A comprehensive study by Marson et al. (2020), elaborates on the presence of these vitamins, which play crucial roles in metabolic pathways. The yeast is also a good source of essential minerals such as potassium, magnesium, calcium, and trace elements like zinc and iron, making it a well-rounded nutritional supplement.

Jacob et al (2019) also states that brewing yeasts are widely recognized as a rich source of vitamin B, which plays crucial roles in human metabolism, including catalysing essential reactions in amino acid and protein metabolism, and possessing detoxifying and antioxidative properties. Detailed analysis regarding vitamin B contents in SBY was conducted in their research using different extraction methods.

| | Cell mill | Sonotrode | Autolysis | Autolysis - Industrial product |
|---|---|---|---|---|
| Vitamins (mg/100 g dw) | | | | |
| Thiamine (B1) | 6.88 ± 0.31 | 7.05 ± 0.43 | 5.18 ± 0.20 | 7.46 ± 0.5 |
| Riboflavin (B2) | 2.16 ± 0.25 | 2.41 ± 0.33 | 1.16 ± 0.19 | 10.55 ± 0.5 |
| Niacin (B3) | 94.19 ± 1.21 | 103.62 ± 2.15 | 68.34 ± 0.98 | 78.58 ± 2.0 |
| Pantothenic acid (B5) | 20.36 ± 0.54 | 18.56 ± 0.78 | 15.73 ± 0.28 | 43.18 ± 2.0 |
| Pyridoxine (B6) | 4.86 ± 0.28 | 5.10 ± 0.51 | 3.09 ± 0.19 | 5.90 ± 0.5 |
| Biotin (B7) | 113.92 ± 2.67 | 127.94 ± 3.69 | 138.55 ± 1.58 | 618.65 ± 2.0 |
| Total folate (B9) | 4.52 ± 0.26 | 4.94 ± 0.42 | 1.35 ± 0.18 | 5.29 ± 0.1 |
| Cobalamin (B12) | 0.18 ± 0.02 | 0.12 ± 0.04 | 1.12 ± 0.05 | 0.16 ± 0.1 |

**Table 4.** Vitamin B contents of the SBY extracts produced using different methods for vitamins B1, B2, B3,B5, B6, B7, B9, and B12 measured by Jacob et al (2019).

### 1.5.3 Dietary Fibers

The cell wall components of spent brewer's yeast, primarily composed of beta-glucans and mannan-oligosaccharides, constitute a valuable source of dietary fibres. Oliveira et al. (2022) highlight the prebiotic potential of these components, beneficial for gut health. Additionally, nucleotides present in yeast cells are crucial for various bodily functions, including the immune response and cellular metabolism.

The spent brewer's yeast (SBY) cell wall is structurally composed of an inner layer of β-glucans, constituting 8 % (w/w dry weight), and an external layer formed by mannoproteins. These compounds are notable for their immunomodulatory, antimutagenic, and anticarcinogenic properties, and have recently found applications in the cosmetic industry and food sector as natural emulsifiers. The physicochemical properties of β-glucans are influenced by their primary structure, which includes the type of linkage, degree of branching, molecular weight, and conformation. Specifically, yeast-derived β-glucans, with a (1,3)-β-linked backbone and minor (1,6)-β-linked side chains, are primarily recognized for their immune-modulating effects. In the food industry, β-glucans from SBY are leveraged for various applications such as food thickeners, fat replacers, dietary fibres, viscosity imparting agents, emulsifiers, and films. For instance, due to its low-calorie content, SBY serves as a cost-effective source of easily assimilable fibre, which has also demonstrated a prebiotic effect. This makes SBY particularly attractive to the pastry industry for developing value-added products (Vieira et al. 2016; Martins et al. 2015).

### 1.5.4 Lipids

Lipids make up less than 4% of the composition of yeast. Of these lipids, saturated fatty acids represent the majority, followed by monounsaturated fatty acids, and then polyunsaturated fatty acids (Caballero Córdoba et al. 1997).

Although the lipid content in spent brewer's yeast is relatively low, it includes essential fatty acids and ergosterol, a precursor of vitamin D. A review by Jaeger et al. (2020), published in Fermentation, discusses the presence of beta-glucans in the yeast cell wall. These beta-glucans have been recognized for their immunomodulatory effects and potential cholesterol-lowering properties, marking spent brewer's yeast as a candidate for functional food development.

Yamada et al. (2005) detailed the fatty acid profiles of WY (wine yeast) and PPC (protein precipitate complex), noting similarities and differences in their compositions. Both WY and PPC predominantly contained palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 ω9), and linoleic acid (C18:2 ω6). WY exhibited higher concentrations of oleic, linoleic, and arachidic acids, whereas PPC had greater levels of palmitic, palmitoleic, stearic, and docosahexaenoic acid (DHA). Overall, both WY and PPC were dominated by saturated fatty acids. WY presented a more balanced distribution of saturated, monounsaturated, and polyunsaturated fatty acids, whereas PPC had higher concentrations of saturated fatty acids and lower levels of polyunsaturated fatty acids.

According to Halász and Lásztity (1991), unsaturated fatty acids are predominant in yeast during the exponential growth phase. However, a shift toward saturated fatty acids occurs as the yeast cells age or are subjected to stress, such as that from high alcohol concentrations and repeated use in successive fermentation cycles, which are common in alcoholic fermentation processes.

| fatty acid (% of total) | WY | PPC |
|---|---|---|
| caprylic (C8.0) | 2.01 | nd |
| capric (C10:0) | 0.73 | 0.60 |
| hundecanoic (C11:0) | 0.33 | 0.20 |
| lauric (C12:0) | 2.03 | 2.00 |
| myristic (C14:0) | 0.97 | 0.60 |
| pentadecanoic (C15:0) | 0.33 | 0.20 |
| palmitic (C16:0) | 24.60 | 30.80 |
| not identified | nd | 0.60 |
| palmitoleic (C16:1 $\omega$7) | 5.77 | 14.40 |
| margaric (C17:0) | nd | 0.30 |
| *cis*-10-heptadecenoic | nd | 0.20 |
| stearic (C18:0) | 9.03 | 14.80 |
| elaidic (C18:1 $\omega$9T) | 1.57 | 0.20 |
| oleic (C18:1 $\omega$9) | 22.47 | 14.60 |
| *trans*-linoleic (C18:2 $\omega$6T) | nd | 0.20 |
| linoleic (C18:2 $\omega$6) | 29.90 | 12.20 |
| $\alpha$-linolenic (C18:3 $\omega$3$\alpha$) | 0.53 | 0.60 |
| arachidic (C20:0) | 5.03 | 0.80 |
| not identified | nd | 0.20 |
| behenic (C22:0) | nd | 0.70 |
| arachidonic (C20:4 $\omega$6) | nd | 0.40 |
| not identified | nd | 0.30 |
| eicosapentaenoic (C20:5 $\omega$3) | nd | 0.70 |
| docosahexaenoic (C20:5 $\omega$3) | nd | 4.90 |
| saturated | 42.71 | 52.00 |
| monounsaturated | 28.31 | 29.20 |
| polyunsaturated | 28.90 | 19.20 |

[a] T, trans; $\omega$, omega; nd, not detected.

**Table 5**. Fatty Acid Composition of Whole Yeast Cells (WY) and Phosphorylated Yeast Protein Concentrate (PPC) according to Yamada et al. (2005).

### 1.5.5 Phenolic compounds

A study by León-González et al. (2018) developed an efficient method to extract and quantify phenolic compounds from residual brewing yeast using aqueous extraction, reversed-phase high-performance capillary liquid chromatography (cLC-DAD), and chemometric tools. The study optimized extraction conditions to maximize the yield of total phenolic content (TPC), total flavonoids content (TFC), and antioxidant capacity. The findings revealed the presence of several key polyphenols, including flavanols such as rutin and kaempferol, flavonoids like naringin, and phenolic acids such as gallic acid and trans-ferulic acid, in brewing residues. Chemometric analysis, including multifactor ANOVA and principal component analysis (PCA), showed that lyophilization as a pretreatment adversely affected the content of individual polyphenols, suggesting significant losses during vacuum drying.

Additionally, the use of filtration for separating extracts was deemed unsuitable due to phenolic compound losses. The study confirmed that residual brewing yeast holds higher amounts of polyphenols than liquid beer waste, indicating its potential as a valuable source of bioactive polyphenols for the food, cosmetic, and pharmaceutical industries. The research not only demonstrated the feasibility of scaling the extraction protocol to pilot plant levels but also highlighted the substantial polyphenolic value that could be harvested from brewery by-products.

| Sample | Total polyphenols, mgGAE $g^{-1}$ | Flavonoids, mgQE $g^{-1}$ | Antioxidants, mgGAE $g^{-1}$ |
|---|---|---|---|
| RBY1 | 0.43±0.03 | 2.7±0.8 | 3.9±0.3 |
| RBY2 | 2.11±0.02 | 9.9±0.5 | 18±2 |
| LBW1 | 0.19±0.03 | 0.37±0.01 | 0.18±0.03 |
| LBW2 | 0.25±0.02 | 7.5±0.6 | 1.59±0.06 |

**Table 6**. Comparison of the total polyphenol content, total flavonoid content, and antioxidant capacity in water extract of residual brewing yeast and liquid brewing waste according to León-González et al. (2018).

## 1.6 Extraction methods of intracellular yeast compounds

To tap into the beneficial components of yeast for food and nutraceutical applications, yeast cells can be processed into yeast extract. Yeast extract is essentially the soluble content derived from yeast cells, obtained after the cell walls have been disrupted and removed. This process exposes the intracellular compounds, including proteins, vitamins, minerals, and flavour-enhancing compounds like amino acids and nucleotides, making them available for use in various applications (Lodolo 2008).

The choice of method to disrupt the yeast cell wall can significantly impact the composition of the resulting yeast extract. Mechanical methods, such as using a cell mill or sonotrode, and non-mechanical methods, like autolysis, are commonly employed for this purpose. However, on an industrial scale, non-mechanical methods are more frequently utilized. Manufacturing methods for yeast extracts typically fall into two categories: autolysis and hydrolysis. Yeast hydrolysates are prepared by adding hydrochloric acid or proteolytic enzymes to the yeast, whereas yeast autolysis involves the natural self-digestion of the cell by its endogenous enzymes, occurring at the end of the cell's life cycle. During autolysis, various cellular components, including proteins, glycogen, nucleic acids, and others, are degraded, ultimately influencing the composition of the resulting yeast extract (Liepins et al. 2015).

#### 1.6.1.1 Autolysis

In a study by Jacob et al. (2019), the impact of different yeast cell wall disruption methods on the composition of yeast extract was thoroughly investigated. The study utilized a cell mill for mechanical disruption and autolysis for non-mechanical disruption, showcasing how each method affects the release of key components within the yeast. For the autolysis process, the yeast material was heated to 50 °C and maintained for 24 hours. At the start of this process, additives such as sodium chloride and ethyl acetate were incorporated to facilitate the breakdown of the cell walls. In contrast, cell disruption via cavitation was achieved using an ultrasonic sonotrode, highlighting an alternative mechanical approach. Following the disruption of the cell walls, centrifugation was employed to separate the cellular contents from the cell wall fragments. The study found that autolysis was particularly effective in releasing amino acids critical to the aroma properties of beer, such as leucine, isoleucine, valine, histidine, proline, cysteine, and glutamine. These amino acids are essential for flavour development in products derived from yeast extracts. The research indicated that the method used to prepare yeast extracts significantly influences the levels of free amino acids and the protein content post-processing. Nonetheless, the variability in the starting material, such as different sources of spent brewer's yeast (SBY), complicates direct comparisons between the outcomes of various disruption techniques. This variability underscores the need for tailored approaches in yeast extract production to optimize the yield and functional properties of the final product.

#### 1.6.1.2 Ultrafiltration

Novel processing methods for yeast extract production continue to evolve, with some researchers exploring techniques that combine autolysis, enzymatic hydrolysis, and selective membrane filtration. One such method published in a study by Amorim et al. (2016), focuses on the ultrafiltration of spent brewer’s yeast autolysates. In this innovative approach, 100 Liters of spent brewer’s yeast autolysate was first filtered and then dehydrated using lyophilization, resulting in 1 kg of dry matter. The ultrafiltration process enabled the separation of this material into four distinct fractions, each differing in size, molecular weight, and nutritional

composition. This method shows promise in its ability to generate specific yeast fractions, each tailored for different nutritional and functional applications.

Despite the potential of this advanced processing method, the existing literature on the topic remains sparse. However, the ability to customize yeast extract fractions could open up new avenues in food science and other industries, allowing for the development of specialized products based on the unique properties of each fraction. This method's precision in targeting specific molecular profiles could significantly enhance the versatility and application of yeast extracts in various sectors.

### 1.6.2 Debittering process

Compared to fresh yeast, yeast resulting from fermentation processes requires pretreatment to achieve food-grade quality by removing bitter compounds. These bitter compounds, which originate from hop resins, are adsorbed onto the yeast during fermentation. Specifically, yeast absorbs about 9–27% of the total bitter substances present in the wort. This adsorption necessitates additional processing steps to eliminate the bitterness before the yeast can be used in food products (Dixon 1968).

To be utilized as a dietary supplement, spent brewer's yeast (SBY) must undergo a debittering process to remove undesirable bitter compounds. This debittering can be conventionally achieved through several methods. One common approach involves washing the yeast with an alkaline aqueous solution, which helps neutralize and remove the bitter compounds. Alternatively, water vapor distillation, either with or without the addition of an organic solvent, can also be employed. These processes are designed to ensure that the yeast retains its nutritional qualities while eliminating the bitterness that would otherwise make it unsuitable for consumption as a dietary supplement (Podpora et al 2016).

Simard and Bouksaim (1998) discovered method which effectively removes up to 98% of the bitter taste from brewer's yeast, focusing on the compounds located on the cell surface rather than those within the cytoplasm. This approach targets the bitter compounds that are bound to the yeast surface by hydrogen bonds formed through adsorption forces. To disrupt these bonds and maintain cellular integrity, the yeast suspension is treated with 2 N NaOH to achieve a pH of 10, at a temperature of 50°C. Additionally, incorporating 20% polysorbate into the treatment not only aids in bitterness removal but also plays a crucial role in reactivating the cells, ensuring that their functionality is fully restored for subsequent uses. This method achieves significant debittering in just 5 minutes, demonstrating both efficiency and effectiveness in preserving the yeast's viability.

## 1.7 Functional properties of spent brewer's yeast

### 1.7.1 Emulsifying properties

Recent research has explored the potential of SBY materials as sustainable and technologically viable alternatives to synthetic emulsifiers. The emulsifying properties of SBY are often linked to its mannoproteins and β-d-glucans, which are known for their functional capabilities, such as water retention, thickening, emulsifying, and stabilizing (Araújo et al. 2014; Kollar et al. 1992).

Mannoproteins extracted from SBY (*Saccharomyces uvarum*) have shown promise as stabilizers and emulsifiers in mayonnaise formulations, effectively replacing xanthan gum without adversely affecting the sensory qualities of the product (Araújo et al. 2014). These mannoproteins have also successfully emulsified and stabilized French salad dressings, enhancing their nutritional value and sensory acceptance (Melo et al. 2015).

Additionally, inactivated high-pressure homogenized baker's yeast has been used in low-fat dressings, demonstrating the potential of yeast biomass as an alternative emulsifier (Fernandez et al. 2012). Mechanical disruption of SBY using glass beads has produced gel stabilizing extracts that, when added to cooked hams, increased hardness, chewiness, sliceability, and water-holding capacity, also enriching them with amino acids and proteins of high biological value without affecting sensory properties (Pancrazio et al. 2016). The addition of up to 1.5% yeast extract in Frankfurt-type sausages also showed no sensory changes (Yamada et al. 2010).

However, challenges remain, as non-treated and ultrasound treated SBY cells have displayed good emulsifying properties in model emulsions, whereas autolysed samples have performed poorly. The foaming ability and stability were reduced in autolysed samples, and while ultrasound treatment significantly improved the oil-holding capacity, it reduced the water-holding properties (Bertolo et al. 2019). Studies have also indicated that processing disruptions and the addition of salts can enhance the solubility of yeast proteins (Bertolo et al. 2019).

Yeast materials are being investigated as novel carrier agents for microencapsulation due to their compositional and functional properties, such as gel formation and stabilization. Post-chemical treatment, *S. cerevisiae* cells demonstrated high encapsulation yields of chlorogenic acid, a natural hydrophilic antioxidant (Paramera et al. 2014; Shi et al. 2010).

### 1.7.1 Gelling properties

A study by López-García et al. (2021) explored the utilization of brewery by-products, specifically trub and spent brewer's yeast, as stabilizers in the production of high internal phase emulsion (HIPE) gels. Trub is an effluent mainly composed of hop particles, colloidal proteins, and residual beer liquor. The research assessed how varying the pH and the concentration of the by-products influenced the structure and stability of the gels. The results indicated that higher by-product concentrations and increased pH levels contributed to more uniform oil droplet distribution and improved structural integrity. Additionally, these conditions enhanced the viscoelastic properties of the gels, suggesting better stability under different stress conditions. While further investigation is necessary, these findings highlight the potential of brewery by-products as effective stabilizers in HIPE gel production, which could improve the textural properties of food products. This stability is attributed to the abundance of yeast cell debris and the cleavage of yeast cell walls, processes that release intracellular compounds. These released compounds increase the viscosity of the aqueous phase and enhance the solubility of mannoproteins (López-García et al. 2021).

A study conducted by Pancrazio et al. (2016) explored the application of spent brewer's yeast extract in cooked hams, focusing on its potential as a textural agent and flavour enhancer. The addition of 1% SBY extract to the hams resulted in a significant increase in both hardness and chewiness compared to control samples. Additionally, the levels of protein and free amino acids were higher in the hams that contained the yeast extract. These findings highlight the dual role of SBY extract not only in enhancing the textural properties of cooked hams but also in improving their nutritional value. The presence of SBY extract contributed to a notable improvement in texture parameters such as hardness and chewiness. While these preliminary results are promising, further research is needed to fully understand the mechanisms behind these improvements and to optimize the use of SBY extract in meat products. The study suggests that SBY extract has potential as a gel stabilizer, which could improve the sliceability and water-holding capacity of hams, making it a valuable ingredient in the meat processing industry.

### 1.7.2 Encapsulating agent

In a study conducted by Marson et al. (2020), spent brewer's yeast was explored for its potential as an encapsulating agent, particularly focusing on its application with ascorbic acid, which is widely used in the food industry but is noted for its instability. The SBY used in the study was derived from *Saccharomyces* species involved in lager beer production. The yeast material was prepared by enzymatically hydrolysing it to facilitate the encapsulation process, employing enzymes such as Alcalase, Protamex from Novozymes, and Brauzyn from Prozyn. This process highlighted the innovative use of SBY, traditionally a waste byproduct, as a valuable resource for food processing applications, specifically in enhancing the stability and usability of sensitive ingredients like ascorbic acid. To enhance the functional properties of the protein, such as solubility, a Maillard reaction was induced by heating the hydrolysed cells in a wet medium at 75 °C for 12 hours, followed by rapid cooling in an ice bath. Post Maillard

reaction, the yeast mixture was spray-dried using a mini spray drier equipped with a double-fluid-type atomizer nozzle. The microstructure and shape of the resulting particles were analysed using Scanning Electron Microscopy, which revealed that the particles maintain properties very similar to the control (particles produced without ascorbic acid), exhibiting high encapsulation yield, low water activity (Aw), and good hygroscopicity. Fourier transform infrared technology further analysed the particles, confirming the presence of yeast cell debris on the surface, indicating successful encapsulation. While these findings underscore the potential of hydrolysed SBY debris as a viable encapsulating agent, the study suggests the need for further research to assess particle viability, storage stability, and nutritional value before it can be widely applied in the food industry.

### 1.7.3 Reducing agent

Yeast cells are recognized as a rich source of glutathione, a tripeptide with potent antioxidant properties. Glutathione, along with cysteine, plays a crucial role as reducing agents in bread dough production. The function of reducing agents in dough formulations is multifaceted: they help to shorten the mixing time, enhance dough elasticity, and increase extensibility. In the context of bread making, the strength and structure of the dough are primarily dictated by the formation of disulfide (−SS−) bonds within the gluten matrix. Reducing agents such as glutathione and cysteine act by breaking these disulfide bonds. This disruption leads to a weakening of the dough structure, which is beneficial for modifying the dough's characteristics during processing, making it less dense and more workable (Wieser 2007).

Verheyen et al. (2015) conducted a study to investigate the impact of reducing agents on wheat doughs, specifically examining the effects of cysteine and reduced glutathione. The study's findings revealed that these reducing agents significantly altered the dough's properties. By introducing free thiol (−SH) groups into the dough, both cysteine and reduced glutathione effectively reduced the dough development time and increased the softness of the dough. These changes are due to the action of the thiol groups, which disrupt the disulfide bonds within the gluten network. These results highlight the practical applications of reducing agents like cysteine and glutathione in optimizing dough characteristics for better baking outcomes.

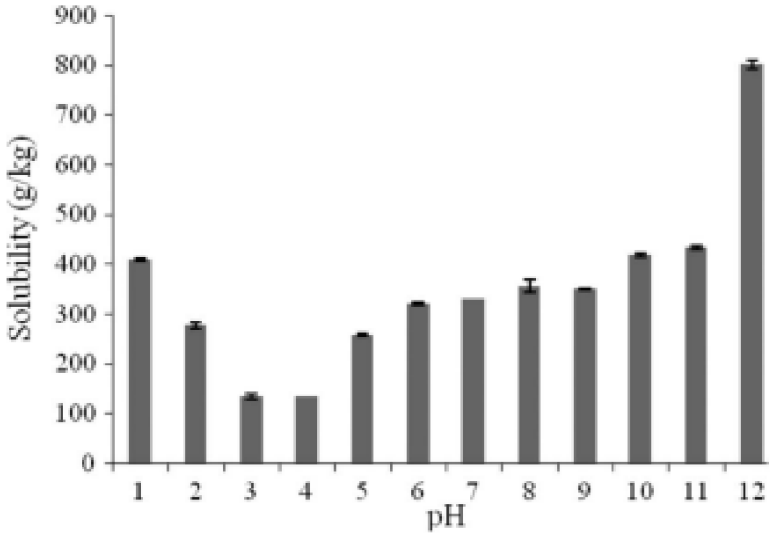
### 1.7.4 Solubility of yeast protein

Li and Karboune (2019) explored the functional properties of mannoproteins extracted from spent brewer’s yeast, observing that their solubility increased when the pH was adjusted from 3 to 9. This observation supports the hypothesis that mannoproteins become more soluble at higher pH levels. Additionally, it was noted that alkaline treatment aids in severing the glycosylphosphatidylinositol anchor that connects mannoproteins to β-glucan. As a result, while mannoproteins become more soluble, β-glucans remain insoluble, consequently increasing the viscosity of the aqueous phase.

The addition of more by-product to the mixture was found to increase the viscosity of the bulk phase. This enhancement in viscosity is likely attributable to the greater quantity of yeast cell debris present, which constitutes an insoluble fraction of the by-product composed of large proteins and polysaccharides. These components are generated during the preparation of the aqueous phase and contribute to the overall stability of the mixture. Additionally, the solubilization of proteins within the mixture plays a significant role, as these solubilized proteins can act as thickeners and emulsifiers, further improving the textural and stability properties of the bulk phase. This indicates a dual mechanism where both the insoluble fractions and the soluble proteins work synergistically to enhance the functional properties of the system Vélez-Erazo (2020).

Graph 1 illustrates how protein solubility in *Saccharomyces cerevisiae* varies across a pH range from 1 to 12. The findings demonstrate that protein solubility is lowest at pH values of 3 and 4, where solubility levels are recorded at 132.5 g/kg and 134.6 g/kg of total proteins, respectively. This low solubility correlates with the proteins' isoelectric point, estimated to be around pH 4. At the isoelectric point, electrostatic interactions between protein molecules are at their maximum, leading to a net charge close to zero, which reduces their solubility.

Conversely, the highest protein solubility was observed at pH 12, reaching up to 801.1 g/kg. At this highly alkaline pH, electrostatic interactions are minimized, enhancing protein solubility. This pattern of solubility, with increases towards both the acidic and alkaline ends of the pH spectrum, is crucial for food formulation. The ability of proteins to dissolve in both acidic and alkaline environments make them versatile for various applications in food processing and product development, where pH adjustments are commonly used to optimize the functional properties of food ingredients (Zidani et al. 2012).




**Graph 1.** Effect of pH on solubility of protein isolated from *S. cerevisiae* biomass (Zidani et al. 2012).

Zidani et al. 2012 also compared effects of three drying methods on the solubility of proteins from dried biomass and compares these results to the solubility of proteins in fresh biomass. The findings indicate that the solubility of proteins in dried biomass was lower across all drying methods compared to the fresh biomass. Among the drying techniques, protein solubility was highest with vacuum drying, closely followed by freeze drying, which showed similar results to vacuum drying. Consequently, the study concludes that the most effective methods for preserving the solubility of proteins in biomass are freeze drying and vacuum drying. These methods are preferred over air drying, as they better maintain the functional properties of proteins, making them more suitable for applications where high solubility is required. This comparison underscores the importance of selecting appropriate drying technologies to optimize the quality and functionality of dried protein products.

| | Air drying | Vacuum drying | Freeze drying | Fresh biomass |
|---|---|---|---|---|
| Parameters | 70°C | 25°C/30 cmHg | | |
| Soluble proteins (g/kg) | 763.7 ± 0.35$^{de}$ | 937.5 ± 0.132$^{a}$ | 937.5 ± 0.199$^{a}$ | 972.3 ± 0.12$^{a}$ |

**Table 7**. Comparison of three drying methods on S. cerevisiae protein solubility (Zidani et al. 2012).

Zidani et al. 2012 states that soluble protein content of the freeze-dried biomass from yeast, which is reported at a notably high level of 937.5 ± 1.9 g/kg of total proteins. This value aligns with existing literature, as referenced by Fennema (1996), underscoring the efficiency of freeze-drying in preserving protein integrity compared to other drying methods.

Liapi and Bruttini (2006) further elucidate that freeze-drying helps minimize various detrimental reactions that typically occur during air drying, such as nonenzymatic browning, protein denaturation, and enzymatic reactions. A key advantage of freeze-drying is that about 90% of water is removed as vapor, which prevents significant migration of salts and carbohydrates to the drying surface. This minimal migration helps in reducing interactions among components, thus leaving the solubility of proteins largely unaffected. This context highlights the unique effectiveness of freeze-drying for preserving the solubility and functionality of yeast-derived proteins, making them highly valuable for various applications.

## 1.8 Current usage of spent brewer's yeast

### 1.8.1 Animal feed

Spent brewer's yeast is primarily recognized for its role as a valuable component in animal feeds, predominantly due to its rich protein content, essential minerals, and B vitamins. It can be utilized either as a wet slurry or in a dried form, depending on the specific requirements of the feed production process. The incorporation of SBY in animal feeds has consistently demonstrated enhancements in feed quality and utilization efficiency. In the context of ruminant nutrition, SBY not only provides substantial nutritional benefits but also positively impacts animal health and productivity. Notably, in dairy cattle, the addition of yeast to the diet has shown promising results, such as increased milk production and improved digestive processes Wohlt et al. (1998).

A study by Wohlt et al. (1998) highlighted that supplementing bovine diets with 10–20 grams of yeast per day could significantly boost milk yield. This improvement is largely attributed to the active role of yeast metabolites in the rumen. These metabolites enhance the ruminal environment by interacting beneficially with other microbes present, thus improving the overall fermentation process and nutrient absorption.

Several studies have examined the impact of incorporating yeast into poultry diets, with notable research conducted by Line et al. (1998) focusing on the potential health benefits of adding *Saccharomyces boulardii* to chicken feed. This study aimed primarily at reducing the gut colonization of pathogenic bacteria, specifically *Salmonella* and *Campylobacter*, which are known to cause significant human foodborne infections, with poultry serving as a common reservoir for these pathogens. Interestingly, the study also noted that there was no significant change in the weight of the chickens, indicating that the yeast supplementation did not adversely affect the growth or general health of the poultry. This suggests that yeast, specifically *Saccharomyces boulardii*, could be a beneficial addition to poultry diets not only for enhancing animal health by reducing pathogen load but also without impacting their growth performance.

### 1.8.2 Functional food applications

Spent brewer's yeast is highly regarded as an excellent source of high-quality protein, comparable to soy protein, enriched with significant amounts of glutamic acid and glutamine. These components increase SBY's viability as a "hidden ingredient" in the food industry, providing a natural source of monosodium glutamate (MSG) to impart the distinctive "umami" flavour akin to that of meat aromas. In addition to its role as a flavour enhancer, SBY demonstrates strong antioxidant activity, on par with that of teas, largely due to phenolic compounds absorbed from malt and hops during the brewing process. These phenolics, including gallic acid, protocatechuic acid, (±) catechin, p-coumaric, ferulic, and cinnamic acids, are present in both free and bound forms, positioning SBY as a potential functional ingredient in health-focused products (Borchani et al. 2015; Vieira et al. 2016).

#### 1.8.2.1 Antimicrobial properties

SBY also contains α- and β-acids from hops, known for their robust antimicrobial properties. The concentration of these acids varies depending on the separation method used, with centrifugation generally resulting in higher hop acid content due to the affinity of SBY for these compounds. However, the use of SBY as a direct protein source in human nutrition is somewhat limited due to its high nucleic acid content (6–15%), which can elevate uric acid levels in the blood and tissues. Consequently, SBY is typically processed into dietary supplements available in forms such as powders, flakes, tablets, or liquids, which are rich in bioactive compounds like B vitamins and minerals (calcium, phosphorus, potassium, magnesium, copper, iron, zinc, manganese, selenium, and chromium) (Podpora et al 2016).

#### 1.8.2.2 Food flavouring

Spent brewer's yeast is recognized for its high RNA content, which typically constitutes about 8-11% of its dry mass, making it a rich source of nucleotides. These nucleotides, particularly 5′-monophosphates, are well-known for their role as flavour enhancers in the food industry. During the process of autolysis, the yeast's endogenous enzymes naturally break down nucleic acids into nucleotides, including 5′-nucleotides which are pivotal in enhancing flavours. Additionally, this breakdown can be augmented through the application of specific enzymes such as 5′-phosphodiesterase, which facilitates the partial hydrolysis of nucleic acids. This enzymatic treatment enhances the yield and specificity of 5′-nucleotide production, further capitalizing on the flavours-enhancing properties of SBY, and expanding its use in various culinary applications where flavour depth and enhancement are desired (Komorowska et al 2003).

Yeast extract from SBY serves as a potent flavour enhancer in numerous foods, due to nucleotides and nucleosides like 5'-guanosine monophosphate and 5'-inosine monophosphate, which enhance flavours—contributing the umami effect. Proteases in the yeast extract break down proteins into smaller polypeptides and sulphur amino acids that enrich flavours, offering a continuity, mouthfulness, and a robust taste profile. Key sulphur-containing compounds, such as S-allyl-cysteine sulphoxide (alliin) and glutathione (GSH, γ-Glu-Cys-Gly), are pivotal in achieving these sensory effects (Kuroda 2015).

#### 1.8.2.3 Meat substitutes

Gibson and Dwivedi (1970) investigated the potential of using debittered and dried SBY, prepared through a modified alkaline wash process, as a foundational ingredient in the production of meat substitutes. The study explored the integration of isolated soy protein with the SBY to improve textural attributes and boost the overall protein content of the final product. The process for creating these meat analogues involved a critical step where vegetable proteins were unfolded through heat treatment. This unfolding was pivotal as it exposed the protein's polypeptide chains, making them more reactive. Following the heat treatment, the production process manipulated the pH to encourage the formation of cation bridges between these polypeptides. This chemical interaction enhanced the structural integrity and texture of the meat substitute, making it more like real meat in terms of feel and functionality.

#### 1.8.2.4 Microorganism substrate

Mathias et al. (2017) conducted a study investigating the potential of various brewing by-products, including spent brewer’s yeast, as growth media for lactic acid bacteria, known for their crucial role in fermentation processes such as those used in dairy and probiotic production. Due to its high nutritional value, low cost, and particularly favourable low carbon-to-nitrogen ratio, SBY emerged as an effective substrate that could provide essential nitrogen for microorganism growth. The study evaluated the efficacy of these substrates by measuring the increase in acidity, indicative of lactic acid production by the lactic acid bacteria. Among the various by-products tested, SBY demonstrated the most potential, showing significant enhancement in the growth and metabolic activity of the bacteria. Notably, whether used alone or in combination with other brewery by-products, the addition of SBY extract significantly improved the fermentation process, leading to increased lactic acid production.

#### 1.8.2.5 Antioxidant properties

Marson et al. (2019) conducted a study to evaluate the impact of sequential enzymatic hydrolysis on the antioxidant properties of spent yeast, particularly focusing on its potential as a food or nutraceutical ingredient due to its antioxidant activity attributed to polyphenolic compounds. The research revealed that autolysis of spent brewer's yeast (SBY) enhanced the total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and ACE-inhibitory activity.

The enzymatic hydrolysis process effectively released internal cell components, including nutrients and enzymes, leading to a notable increase of 63% in antioxidant properties, as assessed by FRAP and 1,1-diphenyl-1-picrylhydrazyl (DPPH) assays. Furthermore, the study reported significant improvements in crude protein and total protein recovery, with enhancements of 50% and 83%, respectively. Additionally, the study investigated the influence of re-pitching on the hydrolysis process and found that repatched yeast required up to 3.5 times longer to achieve the same level of hydrolysis compared to non-re-pitched yeast. This finding underscores the importance of considering the yeast's lifecycle and usage history in enzymatic hydrolysis processes.

Comparatively, SBY extracts exhibited higher levels of polyphenols, antioxidants, and flavonoids compared to liquid beer waste, positioning SBY as a promising industrial source of polyphenols for applications in the food and nutraceutical industries. These findings highlight the potential of SBY as a valuable resource for enhancing antioxidant-rich formulations in various food and nutritional products (Vieira et al. 2016).

# Materials and methods

## 1.9 Material

Spent brewer's yeasts sp. *Saccharomyces cerevisiae* was kindly supplied from brewery Únětický Pivovar Unfiltered, semi-dark special beer "Únětice Christmas Special Beer, 13°" This residue was several times batched for beer production, fermented, and matured for 25 days. Original degree: 13,2°; Alcohol content: 5,2 %; Bitterness: 29 bU; Colour: 30 jEBC. Samples were collected in the form of SBY slurry. All reagents used for the analyses were analytical grade.

## 1.10 Material conditioning

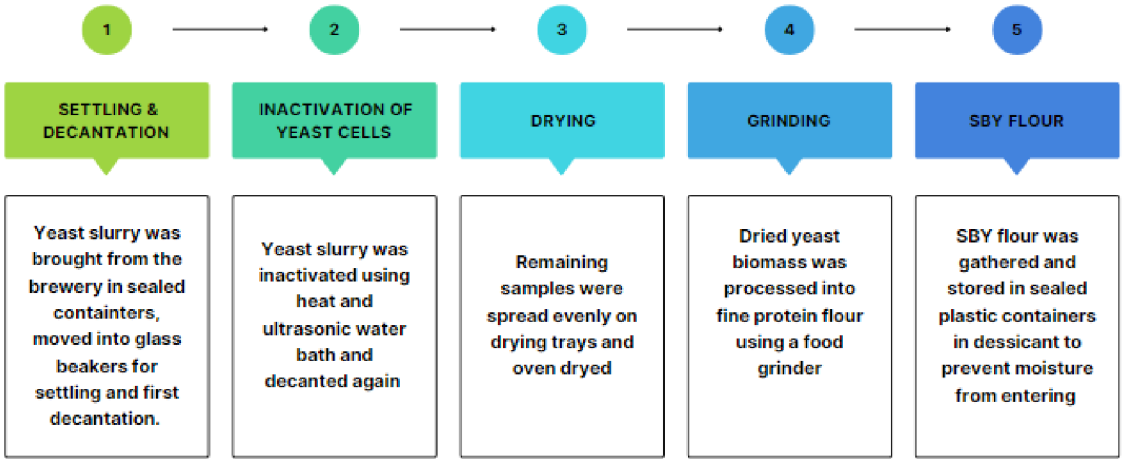



**Diagram 1.** SBY conditioning steps.

The material from the brewery was transported to the laboratory and pre-conditioned for protein concentrate extraction as seen in diagram 1. First the material was moved to glass beakers and allowed to settle for the first time, while removing the excess water by decanting. To inactivate the yeast, the slurry was heated to 60 degrees Celsius for 30 minutes using an ultrasonic water bath. The excess liquid was removed by decanting, and the remaining samples were poured into drying trays. They were evenly spread in a thin layer and dried at a temperature of 60°C to prevent degradation of yeast components. The drying process took about 6 to 8 hours, depending on the initial moisture content. The yeast spread was ensured to have a uniform thickness for consistent drying. The drying process was considered complete when the yeast became brittle. The trays were then removed from the oven, and the dried yeast was cooled to room temperature in a desiccator. The dry yeast was collected from the aluminium drying trays and processed into fine powder (flour) using a food processor. The powder was weighed for yield determination and stored in desiccator for later use.

**Image 1**. SBY slurry samples before and after settling (Step 1, Diagram 1.)

**Image 2**. SBY flour (Step 5, Diagram 1.).

## 1.11 Protein concentrates extraction

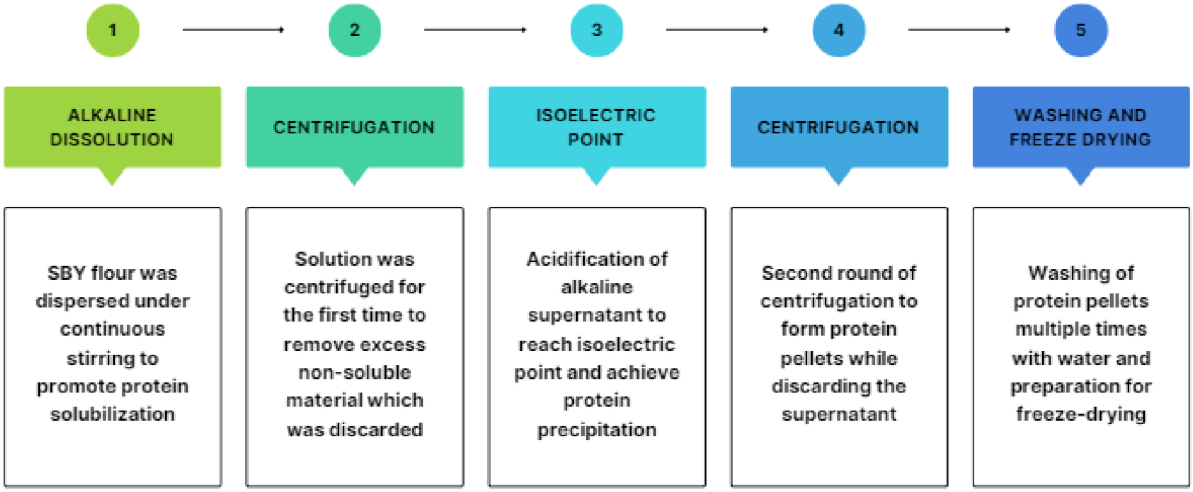



**Diagram 2**. Extraction procedure for protein concentrates from SBY

For protein extraction the starting material (pre-treated spent yeast) was dispersed in an alkaline solution of 0.05 N NaOH (pH 12) at 1:10 weight/volume ratio (SBY:alkaline solution). This mixture was then stirred continuously using magnetic stirrer at a room temperature, extending over a period of one hour. This step was crucial to ensure effective solubilization of proteins within the SBY biomass.

Following this solubilization phase, the dispersions were centrifuged at 4500 rpm at 4 °C for 10 minutes. The purpose of this centrifugation was to separate the unwanted non-soluble material. Afterwards, the supernatant was recovered and adjusted to 3.5 using 1 N HCl, for protein precipitation. This adjustment brought the protein solution close to the isoelectric point, at which the proteins precipitate.

The acidified supernatant was subjected to another round of centrifugation under the same conditions. This step facilitated the formation of a protein-rich pellet. The protein concentrates were washed with distilled water until neutralisation. This neutralization was not only crucial for the stability of the proteins but also prepared them for potential applications in various biochemical analyses or biotechnological processes. Through this protocol, the integrity and purity of the extracted proteins were maintained, ensuring their suitability for further scientific exploration. Finally, the obtained protein concentrates were freeze dried and stored.

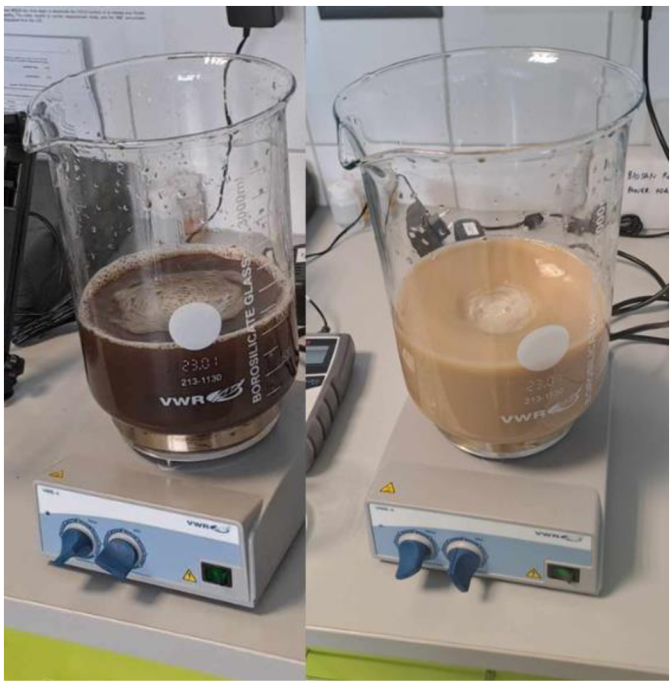



**Image 3**. Isoelectric point

Protein yield was calculated by using the equation 1 and expressed as percentage. Experiment was carried out in duplicate.

$$\textbf{Protein yield (\%)} = \frac{\textbf{Total protein extracted}}{\textbf{Initial sample weight}} \textbf{ x 100} \quad (1)$$

## 1.1 Colour

Colour values of powdered spent brewer's yeast and spent yeast protein concentrate were assessed using a colorimeter Minolta and defined using CIELAB colour space. Using the CIELAB coordinates chroma (saturation) and hue were calculated according to formulas 2 and 3. Measurements were recorded in triplicates.

$$\textbf{chroma (C*)} = \sqrt{(\mathbf{a}*)^2 + (\mathbf{b}*)^2} \quad (2)$$

$$\textbf{Hue Angle (h)} = \tan^{-1}\left(\frac{b^*}{a^*}\right)^\circ \quad (3)$$

To establish the colour difference of the two samples Metric Hue-Difference was calculated according to formula 4.

$$\textbf{Hue-Difference } (\Delta H^*) = (\Delta a^*)^2 - (\Delta b^*)^2 - (\Delta C^*)^2 \quad (4)$$

## 1.2 Particle size

The particle size distribution of protein concentration was measured by laser diffraction using a Mastersizer 3000 (Malvern Instruments, UK) and an Aero S unit (Malvern Instruments, UK). The refractive index of the particles was set to 1.46 and the absorption index to 0.01. The evaluation of the measurements was performed using the instrument software (Malvern Instruments, UK) and the particle size was expressed as Dx (50).

## 1.3 Protein solubility

The protein solubility was determined by following the method described by Wang et al., 2019) with some modifications. A 0.1M NaOH solution was prepared, and its pH measured to confirm that it was approximately 12. The solution was then portioned out, and the pH of each part was adjusted by adding 0.1 M HCl. This adjustment aimed to achieve a range of specific pH levels at 2, 4, 6, 8, 10, and 12, with the highest value being designated as the control.

In the next phase of the procedure, solutions of spent yeast protein (SYP) were prepared at a 1% concentration. This involved dissolving 0.1 gram of SYP in 10 ml of solvent. Once dissolved, these SYP solutions were left undisturbed overnight. This resting period was crucial for enhancing the solubilization of the proteins within the solutions.

Following the solubilization process, the SYP solutions were centrifugated at 4500 rpm for 10 minutes. The supernatant obtained from this centrifugation was then employed for the quantifying of the soluble protein content. For this purpose, the Lowry method was adapted, specifically for application in microplate analysis. Each sample was prepared in duplicate.

## 1.4 Lowry Protein Assay

In the realm of biochemical research and biotechnology, the quantification of protein concentration holds paramount importance. The Lowry Protein Assay emerges as a distinguished method, celebrated for its sensitivity and simplicity, along with its broad compatibility with diverse sample types. At its core, the Lowry Protein Assay capitalizes on the reduction of copper ions in an alkaline milieu in the presence of proteins and Folin-Ciocalteu reagent. This interaction begets a copper-protein complex with a distinct blue hue, the intensity of which directly correlates with the protein concentration. The assay encompasses a bifurcated approach: the reduction of proteins and subsequent colour development.

The preparation began with the creation of a stock solution, using Albumin Standard at a strength of 2.0 mg/mL, and Elution Buffer was utilized as the Diluent. Following this, BSA Standards were carefully prepared according to the required specifications.

| Vial (name) | Volume of Diluent | Volume and Source of BSA | Final BSA Concentration |
|---|---|---|---|
| A | 250 µL | 750 µL of stock | 1,500 µg/mL |
| B | 625 µL | 625 µL of stock | 1,000 µg/mL |
| C | 310 µL | 310 µL of vial A dilution | 750 µg/mL |
| D | 625 µL | 625 µL of vial B dilution | 500 µg/mL |
| E | 625 µL | 625 µL of vial D dilution | 250 µg/mL |
| F | 625 µL | 625 µL of vial E dilution | 125 µg/mL |
| G | 800 µL | 200 µL of vial F dilution | 25 µg/mL |
| H | 800 µL | 200 µL of vial G dilution | 5 µg/mL |
| I | 800 µL | 200 µL of vial H dilution | 1 µg/mL |
| J | 1000 µL | 0 | 0 µg/mL = blank |

**Table 8**. Standard preparation for Lowry protein Assay

In the next stage, the Folin-Ciocalteu Reagent was prepared by diluting the 2X concentration reagent with autoclaved water in equal parts, achieving a 1:1 ratio. For each assay in a 96-well plate, 20 µL of this freshly prepared 1X Folin-Ciocalteu Reagent was deemed

necessary. The preparation of this diluted reagent was done on the day of the experiment, owing to its inherent instability.

Thereafter, 40 µL of both the BSA Standards and the unknown samples were pipetted into the designated wells of the plate. Following this, each well received an addition of 200 µL of Modified Lowry reagent. This was followed by an immediate and thorough mixing of the microplate, for a period of 30 seconds.

The microplate was then securely covered with a plastic lid and left to incubate at room temperature, ensuring that this period lasted for exactly ten minutes. After this incubation, another 20 µL of the 1X Folin-Ciocalteu Reagent was introduced to each well. This was swiftly followed by a second round of mixing, lasting an additional 30 seconds, to ensure homogeneity of the mixture.

Subsequently, the plate was covered once again and subjected to a further incubation at room temperature, this time extending for 30 minutes. Upon completion of this incubation period, the crucial step of measuring absorbance was undertaken. Utilizing a plate reader, absorbance readings were captured at 750 nm. To refine these readings, the average absorbance value noted in the Blank standard replicates at 750 nm was subtracted from the values obtained from the other standards and unknown sample replicates, providing a clear measure of the samples' properties.

## 1.5 Emulsifying capacity (EC) and emulsion stability (ES)

Emulsifying properties, namely Emulsion Capacity (EC) and Emulsion Stability (ES), were evaluated in protein emulsions. These were prepared using protein solutions at 1% (w/v) concentration, adjusted to varying pH levels. Gradually, oil at a concentration of 30% was incorporated into each protein solution. This blending was performed under consistent homogenization, utilizing an Ultraturrax operating at 7000 rpm for a duration of 60 seconds.

The Emulsion Capacity (EC) was determined using Equation 5. This involved measuring the height of the emulsified layer and the total height of the system immediately after the homogenization process. The formula applied was:

$$\textbf{Emulsion capacity (EC)} = \frac{\textbf{Emulsified layer (cm)}}{\textbf{Total height (cm)}} \textbf{ x 100} \qquad (5)$$

Emulsion Stability (ES) was assessed by allowing the emulsion to stand at room temperature for one week. Post this duration, the extent of phase separation was observed and quantified. This measure was then compared with the initial emulsion layer using formula 6:

$$\textbf{Emulsion stability (ES)} = \frac{\textbf{Remaining emulsified layer (cm)}}{\textbf{Initial emulsified layer (cm)}} \textbf{ x 100} \quad (6)$$

## 1.6 Water holding capacity (WHC) and Oil holding capacity (OHC)

For WHC and OHC, 100mg of SYP were mixed with 1 mL of deionized water or oil and stirred for 10s every 5 minutes six times. Then the mixture was centrifuged at 1800 rpm for 20 minutes at room temperature. The floating solution on the surface was decanted. The pellet collected was weighed. WHC and OHC was calculated as the difference between sample's weight before and after absorptions, divided by initial weight, and expressed as percentage. The experiments were carried out in duplicate.

## 1.7 The least gelation concentration endpoint (LGE)

SYP samples were rehydrated at a concentration of 4, 6, 8, 10 and 12% weight to volume ratio with distilled water and stirred for 30 min at room temperature. The solutions were then heated in water bath at 90°C for 30 minutes and immediately cooled down in cold water bath for 5 minutes. The Samples were stored at 4°C and assessed at 0, 24, 72 h. LGE was determined as the concentration when the sample gel did not fall or slip out of beaker when inverted. The experiments were done in duplicate.

# Results

## 1.8 Protein concentrates yield

The protein concentrate obtained from SBY is shown in Image 4. After the extraction the Protein concentrate yield from SBY flour was 43,53 % ± 0,72%.

**Image 4.** Spent yeast protein concentrate after freeze drying.

## 1.9 Colour

Colour values show clear difference between the input material and processed samples. Metric Hue-Difference value was established -16,079 ± 0,626 which can be clearly on image 5. SYP concentrate show noticeably lighter colour profile than the original yeast sample.

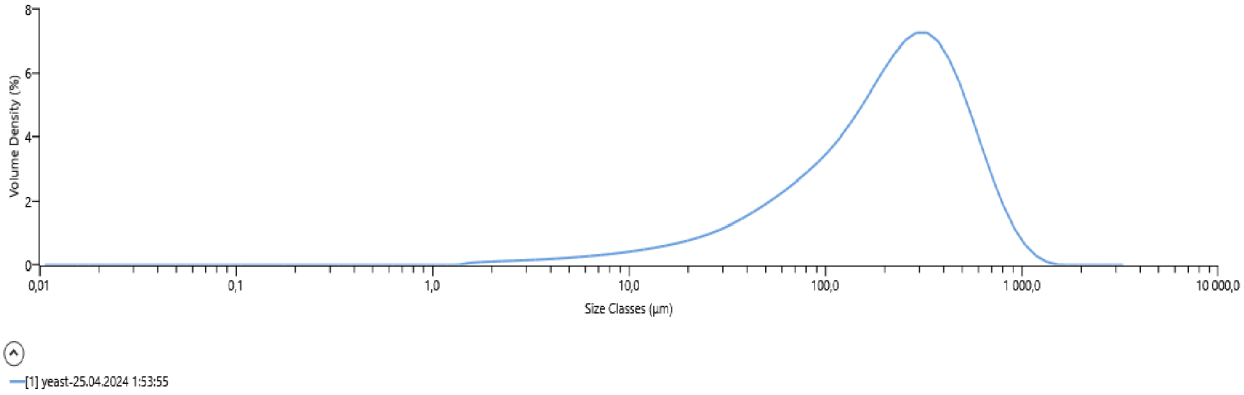
| | **SBY powder** | **SYP concentrate** |
|---|---|---|
| **L** | 40,013 ± 0,035 | 60,393 ± 0,177 |
| **a*** | 13,266 ± 0,031 | 7,226 ± 0,022 |
| **b*** | 25,683 ± 0,148 | 21,686 ± 0,091 |
| **chroma** | 28,907 ± 0,147 | 22,859 ± 1,094 |
| **Hue-Angle (°)** | 1,093 ± 0,001 | 1,249 ± 0,002 |

**Table 10**. CIELAB coordinates and parameters of powdered SBY and SYP concentrate.

**Image 5.** Comparison between SBY and SYP concentrate.

## 1.10 Particle size

The protein concentrates particle size distribution was expressed as Dx (50). Graph 2. shows a monomodal and normal behaviour with negatively skewed distribution.




**Graph 2**. Particle size distribution and density

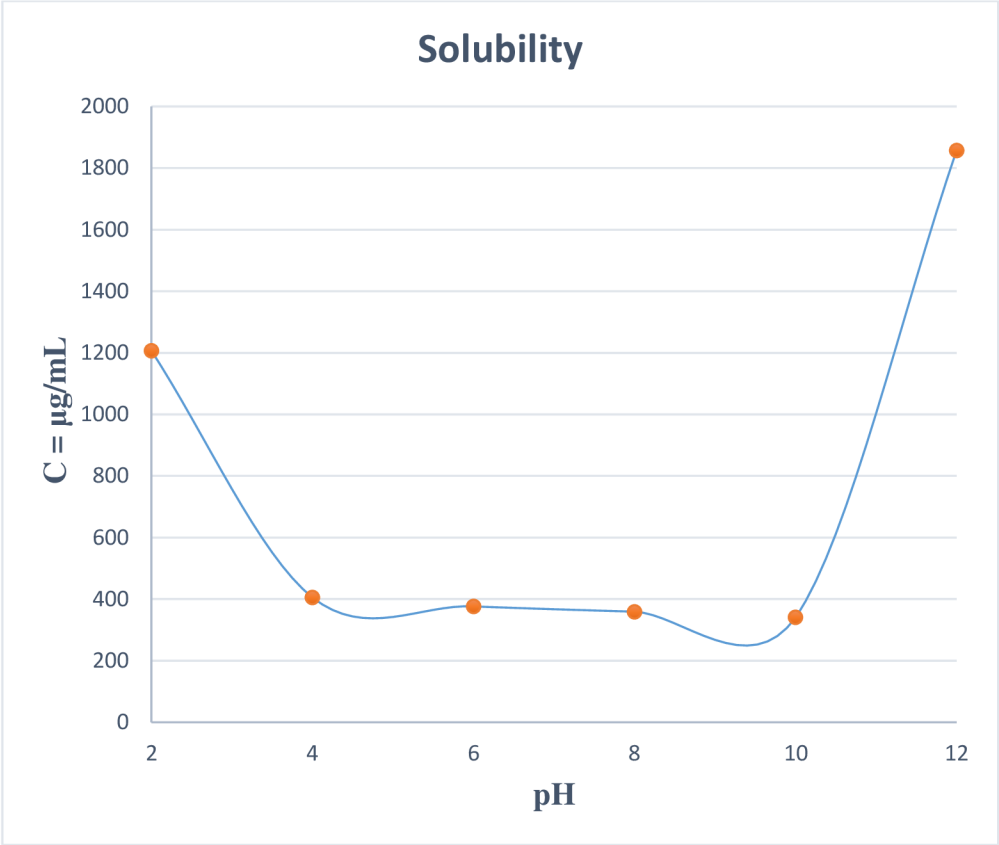
According to table 11. 10% of the particles is smaller than 8.95 3 0.353 µm. 50% of the particles is smaller than 224 ± 4.242 µm. 90% of particles is smaller than 560.5 ± 2.121 µm. Therefore, the volume-basis median value size of particles in the system is 224 ± 4.242 µm. The volume mean diameter is 268.5 ± 0.707 µm.

| | **Dx (10)** | **Dx (50)** | **Dx (90)** | **D [4;3]** |
|---|---|---|---|---|
| **SYP concentrate** | 38.95 ± 0.353 | 224 ± 4.242 | 560.5 ± 2.121 | 268.5 ± 0.707 |

**Table 11.** Values of particle size distribution.

## 1.11 Protein Solubility

Graph 3. illustrates how different pH values determine solubility of SYP concentrate. Protein solubility was highest in highly alkaline environment at pH 12 reaching concentrations of 1857,1 ± 3,5 µg/mL. Second highest concentration was at pH 2 reaching 1206,6 ± 41µg/mL. Lowest values were measured in between pH 8-10 and 4-6.

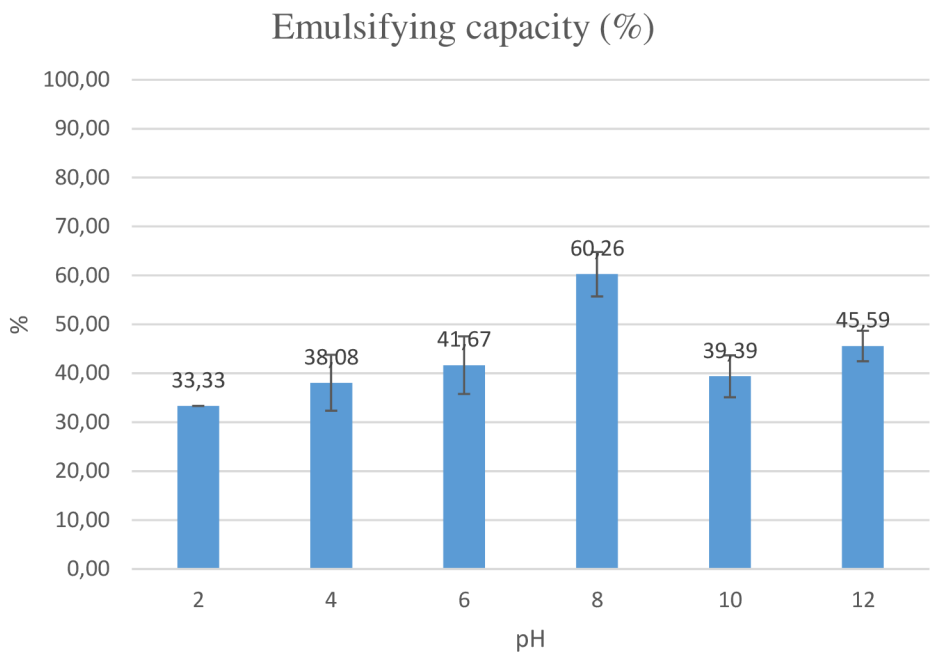



**Graph 3**. Solubility curve of SYP according to pH values

## 1.12 Functional properties

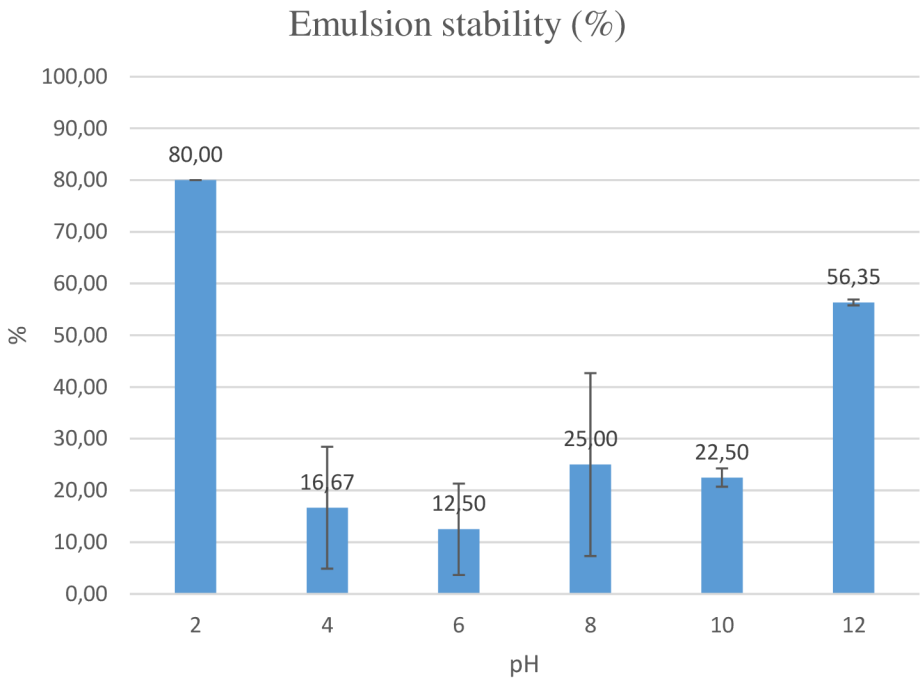
### 1.12.1 Emulsifying capacity (EC) and emulsion stability (ES)

Graph 4. shows emulsifying capacity measured immediately after the homogenization process. SYP shows consistent emulsion capacity across the whole pH range. Highest emulsifying capacity was at pH 8 and 12. Lowest at pH 2.




**Graph 4**. Emulsifying capacity in different pH values in percentage with standard deviation.

ES was assessed by allowing the emulsion to stand and measuring the extent of phase separation. This measure was then compared with the initial emulsion layer. Results are shown in graph 4. Highest emulsion stability was achieved at pH 2, followed by pH 12.




**Graph 5**. Emulsion stability in different pH values in percentage with standard deviation.

### 1.12.2 Water holding capacity (WHC) and oil holding capacity (OHC)

The measurements were performed at neutral pH. The average values of WHC and OHC for SYP samples were 20.57 % ± 1.12 % for WHC and 26.22 % ± 1.31 % for OHC.

### 1.12.3 The least gelation concentration endpoint (LGE)

Least gelation concentration endpoint experiment showed negative results, as the gelation of sample did not occur in both attempts at any given time or concentration according to table 9.

| C (%) | Sample 1 | | | Sample 2 | | |
|---|---|---|---|---|---|---|
| | 0h | 24h | 72h | 0h | 24h | 72h |
| **4** | x | x | x | x | x | x |
| **6** | x | x | x | x | x | x |
| **8** | x | x | x | x | x | x |
| **10** | x | x | x | x | x | x |
| **12** | x | x | x | x | x | x |

**Table 9**. Least gelation concentration endpoint results.

# Discussion

Research regarding spent yeast protein needs more attention as there are almost no studies focusing on properties of SYP concentrates. When comparing these findings with existing literature, it becomes evident that the functional properties of SBY and SYP concentrates are influenced significantly by the processing methods used in their production. Bertolo et al. (2019) has shown variations in emulsifying and gelling properties based on the treatment of yeast cells, suggesting that processing conditions can influence these functional properties. Most of the authors such as Jacob et al. (2019), Marson et al. (2019); Mathias et al. (2015); Vieira et al. (2013) reach consensus regarding variable nature of yeast products. They report different functional properties, nutritional compositions in yeast extracts depending on the yeast strain used even under identical brewing conditions. Production processes and processing methods also greatly influence properties of final products which makes comparisons between existing works hard.

Regarding the protein yield there are no reported values using the same methodology as this thesis. Higher yields have been reported by Li et al. (2022) who reported 73.94% using same starting material, however, their methodology combined alkaline extraction, ultrasound assisted extraction and ultrafiltration. These variations in yield highlight the importance of combining proper extraction and disruption techniques in maximizing protein yield. The method we chose, involving alkaline extraction, was a correct choice, however extra steps were necessary to achieve maximum efficiency. These results highlight the importance of extraction methods on the yield and probably the quality of the product for its use as potential source of protein. Nevertheless, the high protein content coupled with beneficial functional properties suggests that spent brewer's yeast can be a viable alternative to conventional protein sources in various industrial applications.

Colour results showed significant differences between the colour of SBY and processed protein concentrate. This can be attributed to the discarding of most non-soluble excess particles during the protein extraction process.

No studies measuring particle size of SYP concentrates were found for this research, however particle size mean diameter is 268.5 ± 0.707 μm is quite comparable to the particle sizes of conventional protein sources. Onwulata et al. (2004) while trying to minimize variations in functionality of whey protein concentrates by altering particle size compared six different whey protein products reporting mean diameter 238,38 ± 72,22 μm.

The protein solubility is an important factor for determining the potential application of this additive in food products. Its functionality is a key to quality parameters such as texture and physical stability. Zidani et al (2012) reported protein solubility over pH range from 1 to 12. The minimum solubility value was observed at a pH range of 3 and 4 with 132.5 g/kg and 134.6 g/kg of total proteins, respectively, with solubility increase at both ends of pH. The best solubility was observed in basic medium with a maximum value of 801.1 g/kg at pH 12. These

results show similar trends as our research. Mainly low solubility near the isoelectric point (pH 3,5-4) and highest solubility at pH 12.

Analysis of the water holding capacity (WHC) and oil holding capacity (OHC) revealed that SYP could be considered for use in food systems requiring moisture retention, such as baked goods or meat analogs.

Furthermore, the emulsifying capacity of SYP was evaluated, showing promising applications in emulsion-based food products. Although the stability of these emulsions was lower at some pH levels, the overall capacity for emulsification remains notable and consistent across the whole pH spectrum and suggests potential use in sauces, dressings, and spreads where emulsification is desired. The emulsifying capacity and stability, with results indicating good performance at a standard 30% oil concentration, further showcase the functional versatility of spent brewer's yeast protein. The emulsifying properties of SBY are often linked to its mannoproteins and β-d-glucans content.

Samples of SYP have shown no gelation capabilities according to boundary conditions we set however, this might be due to many factors such as concentrations used or unadjusted pH levels. These properties could also be enhanced by using another gelation methods rather than heat-induced gelation. No research papers using same samples were found to support these theories. Further research needs to be conducted regarding gelation properties of spent brewer's yeast protein concentrates. SYB can however be used as gel stabilizers. This utility is supported by the research from Pancrazio et al. (2016), where 1% SYB extracts added to hams improved the textural properties of foods by increasing hardness and chewiness, while enriching them with amino acids and proteins. Analysis of the water holding capacity (WHC) and oil holding capacity (OHC) revealed that SYP could be considered for use in food systems requiring moisture retention, such as baked goods or meat analogs.

The findings from this study contribute to a growing body of literature supporting the use of by-products in food systems, which is critical for enhancing sustainability in the food industry. Utilizing SBY not only helps in waste reduction but also offers a cost-effective source of protein rich supplement which has potential to enhance functional properties of other products. SBY might therefore show valuable potential for various food applications.

# Conclusion

This thesis has successfully demonstrated the innovative utilization of brewers' spent yeast (SBY) as a viable source for producing high-quality protein concentrates. By utilizing the rich nutritional profile of SBY, predominantly consisting of proteins, vitamins, and minerals, SBY shows potential as high potential food additive. The experiments conducted have shown that SBY can be efficiently processed to yield protein extracts with good solubility, emulsification stability, and nutritional qualities, suitable for both food and non-food applications.

Significantly, the findings indicate that the SYP concentrate derived from SBY can meet quality standards of conventional protein concentrates in terms of functional properties. The potential benefits of this approach include the valorisation of a by-product, reduction in industrial waste, and the creation alternative to traditional protein supplements and enhancers. This study proves possibility of integrating new uses for byproducts of the brewing industry, offering a template for similar sustainability efforts in other sectors.

Overall, the adoption of brewers' spent yeast for protein concentrate production could have far-reaching implications for environmental sustainability, economic viability, and nutritional security. Future studies could explore the introduction of this process on a bigger scale, potential market applications, and the long-term impacts of introducing SBY-based products or food enhancer components into the consumer market.

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