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RECOVERY OF VALUABLE COMPOUNDS FROM BREWERY SPENT YEAST THROUGH A BIOREFINERY APPROACH FOR ECO-FRIENDLY PACKAGING APPLICATIONS

D. Pizzichini[1], M. Ciccone[2], E. Zamboni[3], G. P. Leone[1], I.La Rosa[1], L. Siroli[2], F. Patrignani[2], A. Celli[3].

1-ENEA *Italian National Agency for New Technologies, Energy and Sustainable Economic Development- Department for Sustainability.*
2- University of Bologna*-Department of Agricultural and Food Sciences,*
3-University of Bologna*- Department of Civil, Chemical, Environmental and Materials Engineering*

Italian National Agency for New Technologies, Energy and Sustainable Economic Development

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

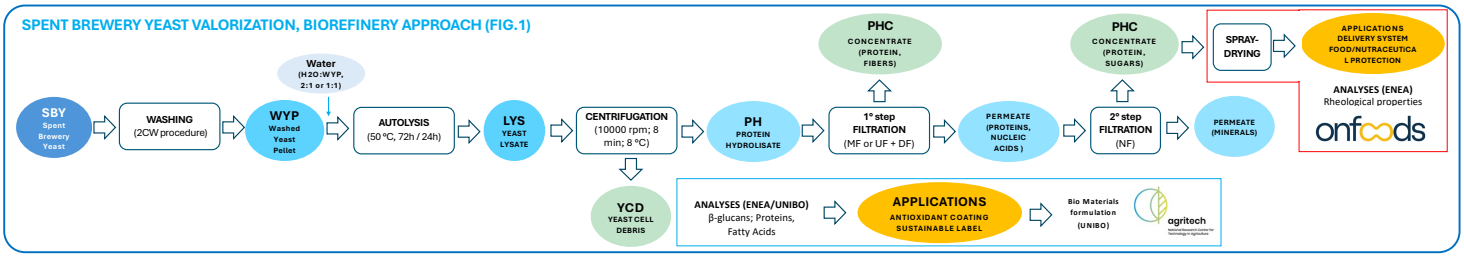
daniele.pizzichini@enea.it, francesca.patrignani@unibo.it, annamaria.celli@unibo.it

SPOKE 8
TASK 8.1.3

INTRODUCTION - Beer stands as the most widely consumed alcoholic beverage globally, **398,2 milion hectoliters of beer were produced in Europe in 2022** (Eurostat, 2023). Beer production involves significant use of water, energy, and raw materials, resulting in by-products like wastewaters, brewers' spent grain, and yeast residues (Pasquet et al., 2024). Spent Brewer's Yeast **(SBY) alone accounts for about 15% of total by-products.** It is estimated that 0,15 – 0,30 kg of SBY is produced for million of hectoliter of beer (Zeko-Pivač et al., 2023). In 2022 in Europe from 53,8 to 107,6 tons of SBY were produced. SBY is a prevalent by-product of the brewing industry, created when the yeast used in fermentations is no longer useful and must be disposed of (Olajire, 2020). It is estimated that 15 to 18 tons of surplus yeast are produced per 10,000 hL of finished beer(Łukaszewicz et al., 2024). From the chemical point of view, **SBY has high protein content, which can be between 45 and 60%.** It is also packed with a high content of essential amino acids. **SBY contains cca. 32% carbohydrates and 6% fat and is a good source of B vitamins and minerals** such as phosphorus, calcium, magnesium, and iron. Cell walls of **SBY make up 15–30% of the dry matter and are composed of high-molecular-weight molecule**s (β-glucan, mannoprotein, chitin, and glycogen) (Zeko-Pivač et al., 2023).

Its composition highlights its potential for **sustainable valorization** in the production of **valuable chemicals, including bioactive peptides, antioxidants, and nutraceuticals** (Jaeger et al., 2020). These constituents make it suitable to both **food and non-food products**, However, the high level of nucleic acids in SBY limits its use as a protein supplement in humans due to the negative health effects caused by an excess of uric acid. Therefore, to date, SBY is mainly utilized in animal feed formulations as a low-cost source of protein (Jaeger et al., 2020). In any case SBY is still largely employed as a reservoir of food functional ingredients, but various non-food applications are also reported, such as in the production of **biofuels, biodegradable plastics, and cosmetics** (Zeko-Pivač et al., 2023). For instance, the proteins and polysaccharides in spent yeast can be processed into as **fillers in composites materials** (Hejna et al., 2024) while its lipids are used in the formulation of cosmetics and as feedstock for biodiesel production. This work outlines the experimental approach adopted within **PNRR AGRITECH Spoke 8 (Task 8.1.3)** in collaboration with **PNRR ONFOODS spoke 2 (task 2.2.1)** to extract valuable compounds from SBY using a bio-refinery method. SBY was harvested, washed, and subjected to autolysis to break down the yeast cellular structure. The autolysate was further fractionated using filtration techniques (micro-, ultra-, and nano-filtration) to obtain a purified protein fraction. Proteins, the main molecular target, were blended and spray-dried into different powders. These powders were tested for flowability and their capacity to encapsulate fatty substances (e.g., vegetable oils) to serve as intermediates for specific applications, such as carriers of active molecules. Additionally, beta-glucans, sugars, and fatty acids were analyzed in specific fractions for their potential use as components of bio-materials. Finally, the cell debris obtained from autolyzed yeast centrifugation was tested as an **adhesive for green packaging applications**. This pathway suggests a sustainable approach to **exploit SBY's chemical potential by extracting valuable compounds and finding new applications for this by-product**

SPENT BREWERY YEAST VALORIZATION, BIOREFINERY APPROACH (FIG.1)



MATERIALS AND METHODS process layout is depicted in fig. 1 where are also reported the acronyms of each process fraction. **Washing and autolysis of SBY** - SBY has been taken from the Brewery (Ritual Lab Rome) and treated according with (Jacob et al., 2019; Oliveira et al., 2022; Fundo et al., 2023). Pretreatment includes two washing phases (2CW), adding distilled water and centrifuging (5,000 rpm, 8 minutes, 8°C) to remove hop residues and sugars from the washed yeast pellet (WYP). Demineralized water is added to the WYP in a 1:1 or 2:1 ratio (w/w), and it undergoes autolysis at 50°C with stirring (300 rpm) for 72h or 24h. Finally, yeasts are inactivated at 95°C for 5 minutes. The lysate (LYS) is centrifuged (10,000 rpm, 8 minutes, 8°C) protein hydrolysate (PH) is separated from the yeast cell debris (YCD). **Protein hydrolysate concentration by membrane processes** – The PH was concentrated using tangential flow filtration (TFF) in two different trials : 1) UF PES MWCO 10 kDa on a Vivaflow system (Sartorius, Göttingen, Germany), 2) a microfiltration (MF, MWCO: 300 kDa) followed by a diafiltration phase (MF/DF) to purify the feed; the MF/DF permeate is the feed of a nanofiltration (NF, MWCO: 800 Da). All trials have been performed on bench scale TFF systems: the MF with in-house assembled plant; the NF with a SEPACELL apparatus (fig. 2) with a flat membrane (filtering surface: 0.0266 m^2), **Microencapsulation of sunflower by spray-drying** – The LYS, PH, and YCD fractions from batches SC6 and SC7 are used according to different recipes to obtain the carrier solution to encapsulate sunflower oil (fig. 3) . The oil is added to obtain a core (oil)/wall material ratio equal to 1:3 (w/w).

SBY sampling date	Autolysis trial	Water:WYP ratio (w/w)	Autolysis temperature (°C)	time of autolysis (h)
14.09.2023	SC6	1:1	50	72
	SC7	1:1	50	24
	SC8	2:1	50	24
28.05.2024	SC9	2:1	50	24

Table 1: Autolysis conditions in different trials

Fig.2 : TFF lab scale plant (SEPACELL)

Fig.3: lab scale Spray Dryer

Fig 4. SC powders from Spray Drying

The emulsion is obtained with a T18 Basic Ultra-Turrax (IKA Staufen, Germany) at 14,000 rpm for 5 minutes. The encapsulation process is carried out with a lab-scale spray-dryer ICF-Industrie Cibec S.p.A. Mod. DF/500/B (fig. 3) with an evaporative capacity of up to 500 cc/h (RC ENEA Casaccia). **Evaluation of rheological and physical properties of powders Fig.4** – Bulk density is determined according to the method in (Sarabandi et al., 2017). The values of densities allow the measurement of flowability indexes (CI, Carr index; HR, Hausner ratio) and the classification of the flowability and cohesiveness according to (Jinapong et al., 2008). The moisture content/total solids of the powders is evaluated using a Sartorius MA 160 moisture analyzer (Sartorius, Göttingen, Germany) with 2 g of powder as the sample to be dried. **Total protein Analyses** : Total nitrogen was analysed by using the Kjeldahl method. Protein was estimated by multiplying total nitrogen by 6.25 (Tanguler and Erten, 2008) **Analyses on . β-glucans -Fatty acids, and dry matter** The dry matter content was determined gravimetrically on all samples of brewer's spent yeast. Samples were dryed at 105 °C and weighed in an analytical balance ± 0.001 to constant weight. Analyses were conducted in triplicate after four repetitions. β-Glucans content The β-glucans content of lyophilised spent yeast samples and spent yeast biomass was determined using a K-EBHLG enzyme kit (Magazyme, Bray, Ireland) **Analysis of fatty acids**: for the determination of fatty acids Hara and Radin method was applied (Hara and Radin, 1978). **Adhesive formulation** :1 g of material (SC8-YCD-X extract) was dissolved in 10 g of distilled water: a solution of about 3% by mass was obtained, measured with a thermobalance. The solution was homogenized 10 minutes with the Ultra Turrax yellow, IKA at 14000 rpm. A small amount of solution was applied to a polymer film (e.g., PET, PLA) using a laminator (Zehntner ZAA 2300) with a 20.57 µm rod: after application, the film was dried. The application was repeated 3 times. A film of bacterial cellulose in hydrogel form (thus containing water) was applied to the polymer film covered with adhesive.

The final material, consisting of 3 layers: polymer film, adhesive, and bacterial cellulose, was dried in an oven (the temperature and time are directly related to the polymer matrix used and the size of the cellulose label). **Antioxidant coating preparation (Figg. 5,6)** : To obtain a polymer film with antioxidant properties, a surface coating was made using the yeast residue SC8-YCD-X, which was found to have antioxidant properties (analysis results provided by UNIBO, Drs. Tassoni-Ferri.

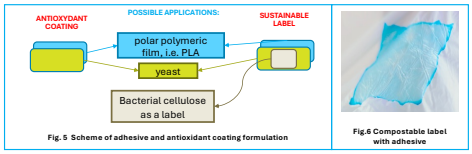
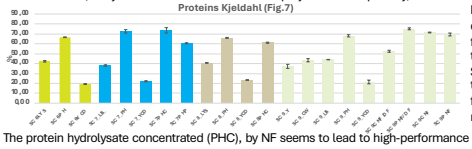


Fig. 5 Scheme of adhesive and antioxidant coating formulation

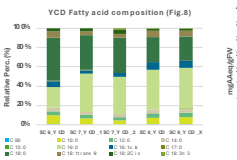
Fig.6 Compostable label with adhesive

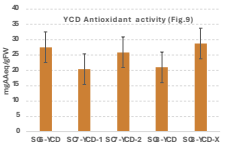
RESULTS: As described in fig.1, aliquots of SBY have been collected at brewery then washed. From Washed Yeast Pellet (WYP), **9 sample sets were obtained** SC1 to SC9 (SC: *Saccharomyces cerevisiae*). **Four sets (SC 6-9)** have been subjected to autolysis according with the protocols indicated in table 1. **Lysate (LYS)** constitutes the first fraction where yeast cell structure is disassembled. Some fractions of SC6-SC7-SC8 sample sets were analyzed in terms of beta Glucans, fatty acids and antioxidant power, aiming to investigate and to quantify the other **non protein bio-polymers who could be employed as plasticizers or even as crosslinkers molecules** (Noè et al., 2020). Analyses revealed that β-glucans seems more present in the 72 Hr PH (SC6 samples) rather than in the PH fractions at 24 hrs (SC7-SC8 samples- data not shown). LYS samples of each set were afterwards centrifugated in order to separate water soluble proteins from cellular debris; so pellet and surnatant were retrieved from LYS and labeled as **Protein Hydrolysate (PH) and Yeast Cell Debris (YCD)** fractions. **Fatty acid analyses were conducted on the fractions derived from the cell pellet (YCD),** including the sample that was later used for the formulation of the adhesive and antioxidant coating (SC8 YCD-X). **The analysis of fatty acids revealed a predominance of C16 and C18 acids.** The SC8-YCD-X sample appears to have the lowest value of fatty acids compared to the other samples (1.17% data not shown). These data needs to be confirmed, but they seem to indicate that the **saturated and monounsaturated C16 and C18 acids represent about 80% of the fatty acid composition (fig.8)**, particularly in similar samples such as SC8-YCD and SC8-YCD-X. The antioxidant capacity analysis also indicates that **YCD-X has the highest values, with 28.78 mgAAeq/gFW (Fig.9)**. In SC6 samples a vacuum concentration was performed on PH obtaining a Protein Hydrolysate Concentrate (PHC) and also **the concentration of PH through Membrane filtration was conducted on SC7 and SC9** sample sets. Batch of 200 ml and 3900 ml were respectively treated in two different filtration trials. UF have been adopted on SC-6 PH aliquots while and MF/DF+NF fractionation was applied to SC9 PH. The goal of this step is focused on **the recovery of a protein concentrate reducing at the same time the concentration of sugars and polysaccharides** who are partially removed through the permeate fraction. Other two fractions were so obtained: SC6-PHC and SC9- PHC. Protein analyses through Kjeldahl method were conducted to verify their distribution across the process intermediates. The chart of figure 7 shows the protein content of the various samples sets. **PH samples as PHC, PHP and also SC9 samples after fractionation through membranes show values over 60%.** While the samples of **YCD** show **levels below the 25 %,** LYS samples indicates intermediate values (40 %). In samples obtained from UF and NF was not observed an evident separation of between the concentrate and the permeate, so protein concentration are very similar in both filtration samples. LYS, PH, YCD, PHC, fractions were used to formulate the powders in the pool described in table 2, where the parameters of blending, yielding and spray drying are summarized. Batch SC9 was processed without oil addition to verify the rheological properties of the powder due to the concentration of PH with nanofiltration (CNF) In some cases, dextrins (DE) or whey proteins concentrates by UF have been added to increase the total solids until the optimal range for powder formation (Mohammed et al., 2020). Moreover the table reports some **evaluation about cohesiveness, flowability** and capacity to encapsulate fats (sunflower oil) of powders. Finally the **SC8 YCD sample has been used as adhesive** of bacterial cellulose labels and for **sustainable food packaging (antioxidant coating)**. It has been observed that: the yeast pellet SC8-YCD-X varies its properties according to the water content. The wet yeast tends to create adhesion with a polymeric film during the drying process; the interactions between yeast surface and the film requires polar groups in both surfaces. Then, the yeast is not able to interact with polymers at low polarity, PLA and cellulose, instead, are good candidates to interact with yeast.



DISCUSSION: **Use of SBY as Encapsulation agent**: fractions deriving from the autolysis process (LYS, PH) leaded to formulation of powders capable to carry oil successfully in terms also of rheological properties as flowability, cohesiveness SC7-SD4 and SC7-SD2 samples showed excellent results with the additional use of dextrins and, particularly, whey proteins from UF. these findings pave the way to include other agrifood residues as constituents of these intermediates;

The protein hydrolysate concentrated (PHC), by NF seems to lead to high-performance rheological powders (SC9-SC2), with high protein concentration (70%); the use of an **UF process does not allow protein concentration, maybe because autolysis generates peptides too short to be retained by a 10 KDa UF.** The use of PH and their derivatives as an encapsulant unlock a double opportunity to valorize spent yeast biomass, by the use of the yeast cell debris (YCD) as a biomaterial. **YCD samples were used for Adhesive and antioxidant coating formulation:** Experimental observations showed an improvement in adhesion **when a polar polymer film is used, while no adhesion is observed when the polymer film is non-polar.** Additionally, it was observed that the **yeast residue must be used in an aqueous solution**; otherwise, it does not have adhesive properties. Therefore, we can imagine a **complex mechanism where interactions between the polar groups of the yeast residue, water, and the polar groups of the polymer are important**. It is also important to emphasize that the water is eliminated by high-temperature treatment, leaving only the polar interactions between the yeast residue and the polymer. The sample used for the adhesive and antioxidant coating formulation tests conducted by UNIBO highlights a relatively low fatty acid content and a significant antioxidant capacity. These observations seem to support both the importance of polar bonds observed in the the label-adhesive interaction, as well as the antioxidant action detected in the coating formulation.





NEXT STEPS:

1) **Use of the protein NF concentrate for the encapsulation of high-value-added bioactive molecules or oil; and also use it for film casting using potential fat-based-plasticizers obtained from YCD fraction.**
2) **Maximizing the properties of the YCD pellet as a biomaterial confirming and deepening fatty acid composition**
3) **extending the approach to others not-protein/not-fat bio-polymers (e.g. β-glucans).**
4) **Enlightening the chemical behaviour of polar interactions between the adhesive and the polymer and clarifying which molecules are responsible of antioxidant power .**

Powder samples	Carrier Solution	Sunflower Oil added (g)	Spray-dryer inlet Temp. (°C)	Outlet Air Temp. (°C)	Powder Yield (%)	Moisture content (%)	Flowability	Cohesiveness
SC6-SD1	SC6-PH (200 ml) SC8-YCD (50 g)	8,5	112,1	66,9	61,8	4,5	Good	Low/Intermediate
SC6-SD2	SC6-PH (200 ml) MD (10 g)	7,5	119,9	74,9	60,5	8,9	Fair	Intermediate/high
SC6-SD3	SC6-PHC (130 ml)	8,9	119,0	70,0	61,4	7,1	Good/fai	Intermediate
SC7-SD1	SC7-PH (200 ml) SC7-YCD (50 g) DE (2 g)	9,6	106,4	67,1	58,6	7,0	Very good	Low
SC7-SD2	SC7-LIS(200 ml) DE (2 g)	8,2	103,7	61,5	68,4	6,3	Very good / good	Low
SC7-SD3	SC7-PH (80 ml) SC7-YCD (20 g) DE (0,8 g)	4,0	97,6	58,8	57,7	6,9	Good	Low/Intermediate
SC7-SD4	SC7-LIS(142 ml) CUR (5 g) DE (2 g)	8,0	102,1	59,7	58,2	5,2	Very good	Low
SC9-SD1	SC9-LIS (225 ml)	Not used	105,1	59,9	60,9	8,0	Very good	Low
SC9-SD2	SC9-CNF (220 ml)	Not used	107,9	62,2	53,8	10,4	Very good	Low

Table 2: powder characterization

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