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Use of FT-NIR and XPS techniques to distinguish cell hull fractions prepared by autolysis or HPH from *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* strains

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Summary Yeast hulls can be used to adsorb undesirable compounds such as volatile phenols that may be present in wine. To understand this adsorption process, the properties of the cell walls and their chemical composition need to be better understood. A study was conducted using four different yeast fractions of either autolysed or high-pressure homogeniser (HPH)-crushed yeast biomasses of *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis*. Near-infrared spectroscopy (FT-NIR) coupled with X-ray photoelectron spectroscopy (XPS) was used and analysed by principal component analysis (PCA). The FT-NIR spectral region of *Saccharomyces* and *Brettanomyces* statistically analysed by PCA showed a clear discrimination accounting for 76% of the variation in the data for PC1; moreover, yeast hulls prepared from the same strain and subjected to two different treatments were also separated. These methods classify yeast cell hulls (YCH) according to strain, composition and treatment applied. Our results indicate that yeast hulls obtained by autolysis are less rich in proteins than those resulting from HPH treatment due to the high pressure that releases more proteins and exposes them on the surface of the cell wall. The composition of YCH at the extreme surface is similar to that found deeper in the wall.

Keywords Beverages, high pressure homogeniser, mannoproteins, multivariate analysis, surface analysis, XPS, yeast hulls.

Introduction

Yeast hulls from *Saccharomyces* species can be used during the winemaking process to prevent and control stuck fermentations due to their ability to adsorb unwanted compounds. In fact, the addition of yeast hulls acts as an adsorbent by lowering the concentration of toxic substances in the fermentation medium and preventing the accumulation of medium-chain fatty acids in the must. These substances are considered to be the main cause of stuck fermentation as they prevent yeast growth (Munoz & Ingledew, 1990).

Some other studies have highlighted their potential as curative treatments to remove volatile phenols, such as 4-ethylphenol, which are associated with the unpleasant aroma described as 'horse sweat' that causes wine spoilage (Petrozziello *et al.*, 2014; Malfeito-Ferreira, 2018). It is known that the yeast cell hull (YCH) is mainly composed of polysaccharides, mannans and glucans (β -1,3 and β -1,6-linked), connected by covalent bonds (Jiménez-Moreno & Ancín-Azpilicueta, 2009) and chitin (Li & Karboune, 2018). The advantage of using YCH as a biosorbent is mainly due to the presence of macromolecules such as mannoproteins, which are located on the cell wall surface and can adsorb volatile compounds. However, cell wall

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composition varies depending on the yeast strain and culture conditions (Aguilar-Uscanga & François, 2003), which could explain the observed differences in sorption capacities of different strains (Pradelles *et al.*, 2008). YCH are of rigid structure, however, mannoproteins can be released by processing methods such as autolysis, bead milling, high pressure thermolysis and enzymatic hydrolysis (Alexandre & Guilloux-Benatier, 2006; Bzducha-Wróbel *et al.*, 2014; Martínez *et al.*, 2016). Structural and biochemical changes in the cell wall after these treatments can be evaluated by microscopy and biochemical analysis, which are time-consuming, and also by non-destructive techniques such as XPS and FT-NIR.

FT-NIR is a fast (Jiang *et al.*, 2019), effective and non-destructive method (Plata *et al.*, 2013) that has already been used in food and beverage analysis (Marsico *et al.*, 2018) and to study the response of yeast to various stresses (Corte *et al.*, 2010; Shi *et al.*, 2010; Saharan & Sharma, 2011). These tools allow us to explore global cell wall surface chemistry, including mannoproteins and glucans, and assign vibrational band regions to known compounds, without any sample preparation or significant pre-processing (Moore *et al.*, 2015).

While XPS is more commonly used to analyse inorganic compounds and metals, it can also be used to study the cell surface of microorganisms (Rouxhet *et al.*, 2008). This technique uses an X-ray source that irradiates the sample and results in the emission of photoelectrons, leading to the analysis of the outermost molecular layers of the surface (2–5 nm) (Dufréne *et al.*, 1997; Tougaard, 2018). XPS provides elemental and functional group analysis of the surface related to species, strain and physiological state. These methods can help to explain the relationship between the adsorption capacity of phenolic compounds present in the wine medium and the major components of YCH.

In this work, we used FT-NIR coupled with XPS to analyse four different yeast-derived fractions consisting of the insoluble fraction of either autolysed or HPH-destroyed yeast biomasses. The YCH studied included two different strains of *S. cerevisiae* subjected to the two different procedures, as well as YCH obtained from a *B. bruxellensis* strain subjected to the two treatments as described above. It is known that the precursor of 4-ethylphenol is hydrocinnamic acids, such as p-coumaric acid, and that *B. bruxellensis* is responsible for this bio-conversion, but some studies clearly show that the cell wall of *B. bruxellensis* is able to adsorb p-coumaric acid found in the medium (Salameh *et al.*, 2008), so it may be interesting to know its composition.

Chemical and enzymatic methods are traditional techniques commonly used to determine the composition of YCH (Aguilar-Uscanga & François, 2003; Schiavone *et al.*, 2014), but there are many drawbacks

to using these methods. Apart from the environmental impact, these methods are considered expensive, tedious, require a long reaction time and have low yields (Ribeiro *et al.*, 2014). The combined use of FT-NIR and XPS offers a significant advantage to study YCH from different aspects, determines the important components and discriminates between YCH preparations to determine which could be the best for the removal of volatile phenols. The main objective of the study is to explore and compare FT-NIR and XPS methods as two complementary and rapid approaches to discriminate yeasts depending on the combination of preparation type and strain and to determine the chemical composition of YCH. Two yeast genera were selected based on studies that *B. bruxellensis* preferentially adsorbs p-coumaric acid, while *S. cerevisiae* preferentially adsorbs 4-ethylphenol (Salameh, 2008).

Material and methods

Yeast hulls strains and growth conditions

After screening different YCH strains for their adsorption capacity of p-coumaric acid and 4-ethylphenol, we selected YCH prepared from two *S. cerevisiae* strains (Lallemand, Montreal, Canada) and one *B. bruxellensis* strain (LGC, Toulouse, France). The *S. cerevisiae* strains were cultured aerobically at 32 °C using a fed batch method and a specific growth rate of 0.18 h⁻¹ for a total of 17 h. The strains were grown in a controlled environment. Beet molasses was used as sugar substrate and ammonia hydroxide as nitrogen source. Autolysis fractions (S1-TA) were obtained by maintaining yeast biomass at 55 °C and pH of 5.5 for 20 h. S2-HPH was subjected to twenty cycles of HPH treatment, the pressure was between 1000 and 2000 bar and the temperature was 4 °C. It is based on the disruption of yeast cells by high pressure together with the recovery of intracellular components such as proteins (Comuzzo *et al.*, 2015). *B. bruxellensis* was cultured in YPD medium (10 g L⁻¹ yeast extract, 10 g L⁻¹ soy peptone and 20 g L⁻¹ glucose) at 30 °C until the exponential growth phase was reached and then subjected to either autolysis (B-TA) or HPH treatment (B-HPH). A centrifugation step at 15 000 g for 15 min was performed to separate soluble and insoluble fractions and obtain yeast derivatives rich in cell walls. The insoluble fraction of the *S. cerevisiae* derivatives was spray-dried using a Gea Niro CDP-R500 with an evaporation rate of 2780 kg h⁻¹ and T_{IN}/T_{OUT} of 210/52 °C and an air flow of 50.000 kg h⁻¹, while the *B. bruxellensis* -derived products were freeze-dried at -80 °C. Two different drying procedures were used due to the differences between industrial (*Saccharomyces*) and laboratory (*Brettanomyces*) production.

FT-NIR spectroscopy

FT-NIR analyses were recorded using the spectrometer 'Antaris FT-NIR Analyzer' equipped with an integrating sphere module in combination with a sample cup spinner adapter to measure powders by diffuse reflectance and to obtain bulk or average information quickly and reliably. The spectra acquisition range was 12 000–3800 cm^{-1} with a resolution of 8.0 cm^{-1} over sixteen scans. The operating temperature varied between 15 and 35 °C and the humidity was constant. FT-NIR spectrometer was placed in a thermocontrolled room and the temperature was fixed at 25 °C during the experiments. The detector used was an InGaAs/Germanium. OMNIC™ for Antaris 9 software (Thermo Scientific, Madison, WI, USA) was used for spectra acquisition. Chemometric analysis was performed using Chemflow/Galaxy. A blank was measured before recording each spectrum to eliminate background noise. For each strain, fifteen repetitions were performed at different times of the day and over a period of 1 week to compensate for changes due to temperature and humidity.

Pre-treatments of acquired FT-NIR spectral data

The raw FT-NIR spectral data acquired may not be in a form suitable for chemometric treatments. These spectra may have errors related to background noise or a distorted baseline. Pre-processing was performed to correct for these errors. Two pre-processing methods were used: detrend (DT) and standard normal variate (SNV). DT is performed for smoothing, derivation and baseline correction, while SNV is used to normalise the spectra. SNV pre-processing is very common because it can be applied to a single spectrum and does not require adjustment of settings. It removes the mean of a spectrum x at each point and is then divided by the standard deviation of x . DT pre-processing is used to correct spectra affected by an increasing trend with wavelengths. It consists of revoking the global trend from the spectrum and obtaining a polynomial of different degree (Sila *et al.*, 2016). In this study, a combination of second degree DT and SNV was used as pre-processing.

Chemometric analysis

Principal component analysis (PCA) is an exploration tool usually applied to large spectral data to reduce dimensionality. It was used in this research to distinguish YCH based on their chemical composition. Note that PCA was performed using the singular vector decomposition (SVD) algorithm. Two principal components were identified, representing 99% of the total explained variance. For each one of them, the

percentage of explained variance is represented on the PC1 (x) and PC2 (y) axis. The Chemflow project developed on Galaxy (<https://vm-chemflow-francegrille.eu>) was used for the chemometric analysis.

XPS analysis

Photoelectron emission spectra were recorded with a monochromatised Al K α source ($h\nu = 1486.6$ eV) on a ThermoScientific K-Alpha system. The X-ray spot size was approximately 400 μm . The pass energy was set to 30 eV with a step of 0.1 eV for core levels and 150 eV for surveys (step 1 eV). Energy calibration of the spectrometer was done using Au 4f $_{7/2}$ (83.9 ± 0.1 eV) and Cu 2p $_{3/2}$ (932.8 ± 0.1 eV) photoelectron lines. XPS spectra were recorded in direct mode N (Ec) and the background signal was removed using the Shirley method (Repoux, 1992). The flood gun was used to neutralise charge effects on the top surface.

Results and discussion

FT-NIR applied to the four samples

The aim of this study is to compare the composition of YCH and identify the chemical functions responsible for the adsorption of p-coumaric acid and 4-ethylphenol on YCH. The FT-NIR spectra of two types of YCH from *B. bruxellensis* (B-TA and B-HPH) and two strains of *S. cerevisiae* (S1-TA and S2-HPH) are shown in Figure S1. Both the yeast genus and the YCH preparation method induced changes shown in the FT-NIR spectra.

The multivariate analysis method PCA was applied to the combined spectra to identify groups and hidden structures. A preliminary PCA analysis was performed on the four samples with fifteen replicates for each sample (Fig. 1). Two distinct groups and separation between *Saccharomyces* and *Brettanomyces* were observed. PC1 appears to be important in separating the different yeast species and explains 76% of the variation in the data, while PC2 accounts for a further 23% of the variation. The statistical model can be almost completely described by means of the first two PCs, which accounted for 99% of the total variance. The PCA clearly shows that YCH can be classified based on genus, which is consistent with previous studies showing that FTIR spectra of yeast cells can be used to discriminate yeasts strains and species (Kümmerle *et al.*, 1998; Oelofse *et al.*, 2010; Niu *et al.*, 2018).

In Fig. 1, the two-dimensional score plots in the quadrants defined by PC1 and PC2 show different clusters with good separation. The vibrations mainly responsible for the clustering of the sample can be seen

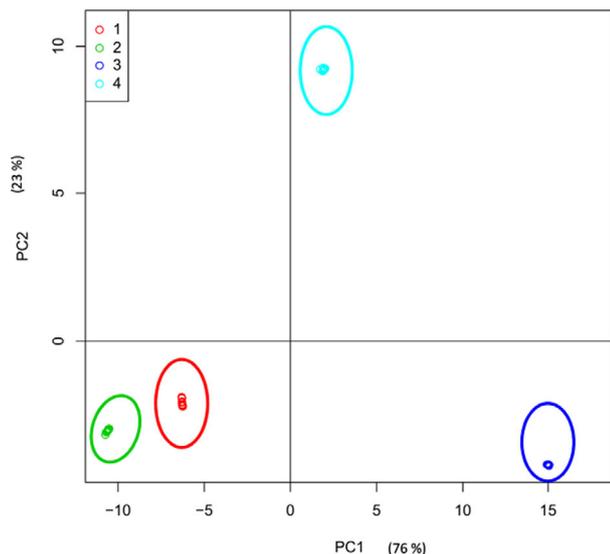


Figure 1 Analysis of yeast cell hull prepared from *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* with two different methods, autolysis or HPH. PCA of yeast cell hulls preparations using FT NIR spectral data. (●) B HPH; (●) B TA; (●) S1 TA and (●) S2 HPH. B HPH and B TA are issued from one strain of *Brettanomyces* that were treated in two different ways: HPH and autolysis, respectively; while S1 TA and S2 HPH are two strain of *Saccharomyces* that were treated as above.

in the loading plots for each variable for PC1 and PC2 (Figure S2).

The influence of genus is reflected in PC1, with a clear separation between *Brettanomyces* and *Saccharomyces* YCH (Fig. 1).

PC2 separates the preparation method of *S. cerevisiae* YCH; S1-TA: negative score value and S2-HPH: positive score value (Fig. 1). Moreover, PC2 score values locate S2-HPH far from S1-TA, while both B-TA and B-HPH have similar score value. Perhaps it is because the *S. cerevisiae* strains were spray-dried while *B. bruxellensis* was freeze-dried. Indeed, each drying process has different effects on yeast cell surface properties, considering hydrophobicity, electron donor character and zeta-potential. Drying can alter the physicochemical properties of the cell surface (Pradelles *et al.*, 2009).

The main contributions to PC1 (Figure S2A) can be attributed to lipids, while 4740 cm^{-1} corresponds to proteins. The assignment of the NIR bands is summarised in Table 1. These chemical functions are more likely to be attributed to *Saccharomyces* than *Brettanomyces*, as they are located on the positive axis of the PC1 loading plot.

It can be concluded that S1-TA and S2-HPH YCH, both derived from *S. cerevisiae* but subjected to two

different processes, have the highest amount of lipids and proteins compared to B-TA and B-HPH produced from a single strain of *B. bruxellensis*.

Instead, in PC2 (Figure S2B), the main contributions are associated with *Saccharomyces* YCH, the bands at 4810 , 5340 and 6950 cm^{-1} are more pronounced in S1-TA, B-TA and B-HPH than in S2-HPH.

Moreover, in the near-infrared region of the PC2-loading diagram (Figure S2B), the vibrations at 4450 cm^{-1} correspond to the CH_2 combination in the methyl group; 4610 cm^{-1} to the combination modes between the N-H stretches, which are probably due to amide functions; 5040 cm^{-1} to O-H str. + O-H def. of H_2O ; 5940 cm^{-1} to first OT Car-H str. can be observed. The latter bands are specific to S2-HPH when PCA is consulted, which locates S2-HPH alone in the positive score values.

In summary, second-derivative PCA FT-NIR allows us to separate samples by yeast genus, and within *S. cerevisiae* we detect separation by the combination of both strain and process. The first two PCs highlight that the data corresponding to *Brettanomyces* and *Saccharomyces* have similar chemical functions, but more pronounced depending on the genus. At this stage, it can be concluded that FT-NIR allows discrimination between the genera *Saccharomyces* and *Brettanomyces* YCH in addition to the effect of both strain and process.

FT-NIR applied to yeast genus data group

S. cerevisiae yeast cell hull data group

To investigate the differences observed above in more depth, the data were analysed within the yeast genus. This step is somewhat more complex due to the very similar chemical functions between the strains.

First, the PCA of the two strains of *S. cerevisiae* hulls are shown in Figure S3. As can be seen, PC1 clearly separates S1-TA and S2-HPH, with S1-TA on the negative side of the axis and S2-HPH on the positive. The statistical model related to *S. cerevisiae* can be described by PC1, which accounts for 99% of the total variance. Figure S4 shows the loading plot of PC1.

Table 1 FTNIR band assignment for *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* yeast cell hulls

Wavelength (cm ⁻¹)	Functional groups	Assignment
4327	$\nu_{\text{as}}(\text{CH}_2) + \delta(\text{CH}_2)$ (Lénárt <i>et al.</i> , 2012)	Lipids
5793	$2 \times \nu(\text{CH}_2)$; $2 \times (\text{CH})$ (Troshchynska <i>et al.</i> , 2019)	
8262	$3 \times \nu(\text{CH}_2)$ (Troshchynska <i>et al.</i> , 2019)	
4740	$\nu(\text{NH}) + \delta(\text{NH})/\text{NH}$ stretch with amide III (Krikorian & Mahpour, 1973)	Proteins

It has been previously shown that spectral vibrations are able to characterise and discriminate YCH among *S. cerevisiae* strains (Moore *et al.*, 2015), while in our study two different processes were used: autolysis and HPH. This distinction combines both strain and process.

The chemical functions detected at 4725, 5787, 6930 and 8230 cm^{-1} resulted in a negative contribution. In other words, these chemical functions are more pronounced in S1-TA than in S2-HPH.

In contrast, the chemical functions detected at 4590, 5130, 6580, 7500–8110 and 8330–8810 cm^{-1} gave a positive contribution, so these functions are more expressed in S2-HPH rather than S1-TA. From the NIR band assignment, it can be inferred that the amount of lipids and proteins is higher in S2-HPH than in S1-TA, but the amount of polysaccharides is higher in S1-TA than in S2-HPH (Table 2).

Brettanomyces YCH data group

Second, the same analysis was performed with *Brettanomyces* YCH. The separation between the two preparations is reflected in PC1, which separates B-TA from B-HPH (Figure S5). PC1 accounts for 99% of the total variance. The most intense bands of B-HPH observed are at 4340, 4610, 4840, 5828 and 6622 cm^{-1} (Figure S6). Conversely, the positive contribution bands are located at 5155 cm^{-1} . Each band corresponds to a particular chemical function (Table 3). This function is more pronounced in B-TA than in B-HPH. It can be concluded that B-HPH has the highest content of lipids and proteins, while B-TA has the highest content of polysaccharides.

Table 2 FT NIR band assignment for *Saccharomyces cerevisiae* yeast cell hulls

Wavelength (cm^{-1})	Functional groups	Assignment
4590	N H/C N/C O combination band from secondary amides (Workman & Weyer, 2012)	Proteins
6580	N H secondary amine (Workman & Weyer, 2012)	
6930	N H primary aromatic amine (Workman & Weyer, 2012)	
8330 8810	CH_2 symmetric stretching, 2nd overtone (Lénárt <i>et al.</i> , 2012)	Lipids and proteins
7500 8110	Hydroxyl stretching (Lénárt <i>et al.</i> , 2012)	Lipids hydrocarbons
8230	C H methylene ($-\text{CH}_2$) (Workman & Weyer, 2012)	aliphatic
4725	ν (OH) + δ (OH) (Troshchynska <i>et al.</i> , 2019)	Polysaccharides carbohydrates
5787	C H stretching (first overtone) of CH_2 (Ma <i>et al.</i> , 2018)	

After assigning each band to the corresponding functional group, it was found that YCH from autolysis have a higher amount of polysaccharides, while those from HPH have a higher amount of lipids and proteins. Indeed, the autolysis process leads to the hydrolysis of proteins, which could explain the lower content in S1-TA and B-TA samples.

FT-NIR allows us to classify YCH depending on many factors: composition, strain and applied treatment.

XPS analysis of YCH

X-ray photoelectron spectroscopy analysis of YCH in conjunction with FTNIR allowed us to better understand the chemical environment that composes YCH at the extreme surface. The information obtained by XPS on the four YCH is summarised in Table 4. The FWHM value, which ranges from 1.2 to 2, is an indicator of the chemical state changes and physical influences. It is the result of several factors, such as a change in the number of chemical bonds contributing to the peak shape, a change in sample condition and cell surface charge.

Carbon is the most abundant element. The C1s peak was decomposed into four component functions in the range 282.0–291 eV (Figure S7D). The C1s component at 284.6 eV is attributed to carbon participating in C-C and C-H bonds. The C1s component at 286.2 eV can be attributed to the C-O and -C-N- units. It is known that YCH is rich in polysaccharides, which explains the presence of C-O units. The third component can be attributed to C=O units, which appear at 287.5 eV. The signal at 289.0 eV is caused by carboxyl groups. From these carbon data, it can be observed

Table 3 FT NIR band assignment for *Brettanomyces bruxelensis* yeast cell hulls

Wavelength (cm^{-1})	Functional groups	Assignment
4610	Amide B amide II combination (Miyazawa & Sonoyama, 1998)	Proteins (and polypeptides)
4840	N H associated stretch band (Workman & Weyer, 2012)	
8330 8810	CH_2 symmetric stretching, second overtone (Lénárt <i>et al.</i> , 2012)	Lipids and proteins
4330	Combination O H stretch/HOH deformation; O H band second overtone (Lénárt <i>et al.</i> , 2012)	Lipids
5828	$2 \times \nu$ (CH) (Troshchynska <i>et al.</i> , 2019)	
6622	$2 \times \nu$ (OH) (Troshchynska <i>et al.</i> , 2019)	Polysaccharides
9608	$3 \times \nu$ (OH) (Troshchynska <i>et al.</i> , 2019)	

Table 4 Summary of the information obtained by XPS on the four yeast cell hull preparations; B TA, B HPH, S1 TA and S2 HPH

Element	Component functions	Peak BE (eV)	FWHM (eV)	Atomic %			
				B-TA	B-HPH	S1-TA	S2-HPH
P (2p)	PO ₄	133.4	1.8	0.4	1.4	0.8	0.7
C (1s)	C C or C H	284.6	1.4	23.4	42.9	33.9	43.2
	C O and C N	286.2	1.2	29.4	15.8	21.6	12.8
	C O	287.5	1.5	11.1	6.6	8.8	8.6
	O C O	289.0	1.3	1.0	2.9	3.1	2.7
N (1s)	N C O	399.8	1.6	1.1	2.8	4.1	6.1
	NH ₄ ⁺	401.7	2.0	0.2	0.6	0.8	2.0
O (1s)	O C, O C, OP,	532.6	1.6	33.6	20.9	26.2	21.4
K (2p)	K ⁺	292.5	1.4	0.4	1.4	0.8	0.7

that B-TA has the highest content of polysaccharides, followed by S1-TA, B-HPH and S2-HPH and that S1-TA has the highest content of lipids followed by B-HPH, S2-HPH and B-TA.

Nitrogen was found in a much lower concentration in the four YCHs. The N1s peak has been decomposed into two components: amide and alkylammonium (Figure S7B). The first component is detected at 399.8 eV and is attributed to the amide function found in the peptidic linkage of proteins (Rouxhet & Genet, 2011) and in N-acetylglucosamine. They are present in small amounts in YCH and are particularly characterised in the junction of mannans with proteins of the YCH.

The second component is characterised at 401.7 eV and it is attributed to ammonium ions. These ions are found in very small proportions in the four YCHs. The origin of the ammonium ions is most likely the growth medium. The negative sites of the YCH may retain these ammonium ions, which are positively charged by ion exchange, and a small amount may remain even though the cells have been washed, which explains the small amount found. Another possibility is the presence of terminal amine functions that do not participate in peptide bonding to form amide functions, but remain as amines and may take some form of protonation depending on pH conditions. (Amory *et al.*, 1988). Looking at the atomic percentages for each of the four YCHs (Table 5), it can be seen that S2-HPH has the highest percentage of proteins, followed by S1-TA, B-HPH and B-TA.

Phosphorus is detected at 133.4 eV, which corresponds to phosphate (Figure S7A, Table 4). This element is found as phosphodiester in phosphomannans and as phosphorylated mannose groups in the manno-proteins necessary for YCH (Amory *et al.*, 1988). Potassium is also found in very small amounts at 292.5 eV and forms 50% of the total ionic content. These results confirm that XPS analysis of YCH is possible and reliable as observed in other studies (Amory *et al.*, 1988).

Comparison of FT-NIR and XPS

The chemometric study revealed that XPS and FT-NIR can be used to classify YCH according to their abundance of polysaccharides, lipids and proteins. By comparing the two methods, reproducible results were obtained for B-TA and B-HPH with the two methods used. On the other hand, when the S2-HPH strain was considered, S2-HPH was found to be less lipid rich than S1-TA using XPS, but more lipid rich than S1-TA using FT-NIR. This could be due to the preparation method associated with the spray-drying process that can alter the surface structures of yeast and the physicochemical properties of the cell wall, which is the first barrier against environmental stress (Pradelles *et al.*, 2009). In addition, when HPH is used as a method to disrupt yeast cells, the released products are not selective and YCH contains a large amount of cell debris, which can make purification difficult (Martínez *et al.*, 2016).

It can be concluded that the composition of YCH at the extreme surface (of a few nm) obtained by XPS is similar to that found deeper in the wall, knowing that the near-infrared penetration depth measurement is a few millimetres.

Yeasts subjected to autolysis should be less rich in soluble proteins than those subjected to HPH. Yeasts are more likely to release soluble proteins and amino acids into the wine-like medium upon increasing

Table 5 Comparison of atomic percentage found in the four yeast cell hulls preparations from *S. cerevisiae* (S) and *B. bruxellensis* (B) either by autolysis (TA) or high pressure homogenisation (HPH) by XPS analysis

	S1-TA	S2-HPH	B-TA	B-HPH
Proteins	4.1	6.1	1.1	2.8
Lipids	8.8	8.6	11.1	6.6
Polysaccharides	21.6	12.8	29.4	15.8

pressure and autolysis (Dimopoulos *et al.*, 2020). Indeed, autolysis produces proteolytic enzymes such as proteases, nucleases and glucanases that cleave proteins into amino acids and peptides or degrade mannoproteins, break down RNA and DNA into nucleosides and polynucleotides, and degrade glucans in the cell wall respectively (Schiavone, 2014). Thus, by releasing proteins into the wine-like environment, the yeast will be less rich in proteins. In our study, XPS and FT-NIR show that S2-HPH and B-HPH are richer in proteins than S1-TA and B-TA, confirming the results of Comuzzo (Comuzzo *et al.*, 2015). To confirm this hypothesis, biochemical analysis was performed in Tallinn Competence Centre on the four YCHs and, as we can see in Table S1, S2-HPH has a total protein content of 32.12% compared to 13.94% for S1-TA. Moreover, for the same *Brettanomyces* species cultured under the same conditions but treated differently, we can see that B-HPH has a protein content of 3.92% compared to 2.05% for B-TA. According to our observations, high pressure could release more proteins and expose them on the surface of the cell wall and affect the adsorption capacity of certain compounds such as p-coumaric acid and 4-ethylphenol. It is known that HPH is able to affect the physicochemical properties of a number of proteins (Comuzzo & Calligaris, 2019; Voce *et al.*, 2020) and can lead to a certain degree of protein unfolding with increased surface hydrophobicity (Pradelles *et al.*, 2008). Further studies in the field of thermodynamic equilibrium need to be performed to confirm this hypothesis. On the other hand, it is necessary to consider the composition and conditions of the culture medium, which can significantly affect the composition of YCHs (Amory *et al.*, 1988).

It is known that the YCH (which consists mainly of the cell wall and lipid membrane) is composed of 60–85% polysaccharides, 8% proteins and 8% lipids. Studies have shown that the polysaccharide content in the cell wall can vary by more than 50% depending on the carbon source, nitrogen limitation, pH, temperature, oxygenation and the mode of culture (Aguilar-Uscanga & François, 2003). Looking at the biochemical analysis of the four YCHs (Table S1), on average, the YCHs are composed of 3.76% lipids. However, the percentage of proteins varies considerably among the four YCHs, depending on the strain, the culture conditions and the treatment applied.

Conclusion

The combination of FT-NIR and XPS shows compatible results, implying that the composition of the YCH at the outermost surface is similar to that deeper in the wall. They can be attributed to polysaccharides, lipids and proteins, which are the main

components of YCH. These two techniques allow the differences in YCH composition between the two yeast genera to be studied as a function of how they are produced and can be used as a simple, non-destructive and rapid method for classifying yeast products. FT-NIR classifies YCHs depending on strain, composition and treatment applied. YCHs prepared from HPH show a higher amount of proteins than those prepared from autolysis, which is due to the high pressure that releases more proteins and exposes them on the surface of the cell wall, and the results were confirmed by biochemical analysis. The adsorption kinetics of p-coumaric acid and 4-ethylphenol on YCHs will be studied and the chemical functions responsible for this adsorption will be determined.

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Author contribution

Elena Bakhos: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Writing-original draft (equal). **Wadih Skaff:** Software (equal); Supervision (equal); Validation (equal). **Jerome Esvan:** Software (equal); Validation (equal). **Alexandre Monnier:** Supervision (equal); Validation (equal). **Nathalie Sieczkowski:** Funding acquisition (equal); Project administration (equal); Supervision (equal); Validation (equal); Visualization (equal). **Roger Lteif:** Supervision. **Cedric Brandam:** Project administration; Supervision; Validation. **Dominique Salameh:** Project administration; Supervision; Validation.

Conflict of interest

The authors declare no competing financial interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

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Data availability statement

The data that support the findings of this study are available in the supplementary material of this article.

References

In this study, the authors show that the variation in the composition of the yeast cell walls depends strongly on different factors and could vary by more than 50% if the carbon source, nitrogen limitation, pH, temperature, aeration and mode of culture change. This can explain the differences found in terms of protein and polysaccharides content in our study; it is dependant not only on the treatment applied and the way of drying but also on the strain and conditions of culture.

The authors applied HPH treatment on wine yeast strains and observed the release of macromolecules. They explain how the high pressure induced on yeast strains is able to generate autolysis and release soluble proteins and other glucidic compounds in the wine medium. It helped us to understand the results obtained in our study considering the differences in the composition between autolysis and HPH treated yeast cells.

In this study, Pradelles is showing the importance of drying process on the physicochemical properties of yeast cells. The characteristics of the surface such as surface hydrophobicity, electron donor character and zeta potential change drastically depending on the drying process applied and, thus, have an important effect on the adsorption capacity of 4 ethylphenol. In our research, the treatment applied (autolysis or HPH) and the drying process are key factors to consider later on in the study of adsorption capacity of the four yeast cell hulls.

This publication considers XPS analysis made on bioorganic systems such as microorganisms. It gives some recommendations about the methodology and sample preparation that should be used to apply XPS on microorganisms. It shows how to treat peak decomposition in microorganisms and insists on the fact of attributing peaks in terms of classes of biochemical compounds.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Analysis of yeast cell hull prepared from *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* with two different methods, autolysis or HPH. FT-NIR spectra of yeast cell hull of *S. cerevisiae* and *B. bruxellensis* in the spectral region 4000–10 000 cm^{-1} combining detrend and standard normal variate pretreatments. Yeast cell hulls from autolysis of *S. cerevisiae* (S1-TA in cyan line) and *B. bruxellensis* (B-TA in yellow). Yeasts cell hulls from HPH are represented in blue and purple for *S. cerevisiae* and *B. bruxellensis*, respectively.

Figure S2. Loading plots of PC1 (a) and PC2 (b) of four yeasts cell hull preparations analyzed by FT-NIR. Red = peaks corresponding to negative score value; Green = peaks corresponding to positive score values.

Figure S3. PCA of *Saccharomyces cerevisiae* yeast cell hull preparations analyzed by FT-NIR.

Figure S4. Loading plots of PC1 of *Saccharomyces cerevisiae* yeast cell hull preparations analyzed by FT-NIR. Red = peaks corresponding to negative score values; Green = peaks corresponding to positive score values.

Figure S5. PCA of *Brettanomyces bruxellensis* yeast cell hull preparations analyzed by FT-NIR.

Figure S6. Loading plots of PC1 of *Brettanomyces bruxellensis* yeast cell hull preparations analyzed by FT-NIR. Red = peaks corresponding to negative score values; Green = peaks corresponding to positive score values.

Figure S7. Representative XPS spectra of the *Saccharomyces cerevisiae* cell hull prepared by autolysis (S1-TA) and illustration of the decomposition of P2p (a), N1s (b), O1s (c) and C1s (d) peaks.

Table S1. Analysis of total content of carbohydrates, proteins and lipids for the four YCH by biochemical method.