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# NMR metabolomics of plant and yeast-based hydrolysates for cell culture media applications — A comprehensive assessment

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ABSTRACT

Cultivated meat products, generated by growing isolated skeletal muscle and fat tissue, offer the promise of a more sustainable and ethical alternative to traditional meat production. However, with cell culture media used to grow the cells accounting for 55–95% of the overall production cost, achieving true sustainability requires significant media optimization. One means of dealing with these high costs is the use of low-cost complex additives such as hydrolysates to provide a wide range of nutrients, from small molecules (metabolites) to growth factors and peptides. Despite their potential, most hydrolysate products remain poorly characterized and many are thought to suffer from persistent issues of high batch-to-batch variability. Although there have been a number of isolated efforts to determine metabolic profiles for a handful of hydrolysate products, we present the first attempt at a more comprehensive metabolomic characterization of nine different products (four plant and five yeast-based) from two to four different lots each.

NMR analysis identified 90 unique metabolites, with only 15 metabolites common to all hydrolysate products (including eight of the nine essential amino acids), and 16 metabolites found in only a single hydrolysate product. The different hydrolysate products were found to have substantial differences in metabolite concentrations (as a fraction of overall mass), ranging from a high of 43% in yeast extract to a low of 14% in soy hydrolysates. The proportion of various metabolites also varied between products, with carbohydrate concentrations particularly high in soy hydrolysates and nucleosides more prominent in two of the yeast products. Overall, yeast extract generally had higher metabolite concentrations than all the other products, whereas both yeast extract and cotton had the largest variety of metabolites. A direct calculation of batch-to-batch variability revealed although there are significant differences between lots, these are largely driven by a relatively small fraction of compounds. This report will hopefully serve as a useful starting point for a more nuanced consideration of hydrolysate products in cell culture media optimization, both in the context of cultivated meat and beyond.

## 1. Introduction

Since the first cultivated meat burger was produced in 2013 (Stephens et al., 2018), cultivated meat has been increasingly touted as a potentially sustainable solution to the demand for protein sources among a rising population. However, culturing mammalian cells at sufficient scale for direct human consumption requires extensive process optimization to be cost-efficient. With cell culture media accounting for 55–95% of production costs, media optimization stands at the forefront of cultivated meat research (Hubalek et al., 2022), and optimization is expected to remain important as media formulations are tailored to specific cell lines (O'Neill et al., 2022). Whereas the use of bovine serum has been largely abandoned in the biopharmaceutical context (Grosvenor, 2008), new rounds of serum elimination studies are still ongoing for promising cell lines in the cultivated meat context (Kim et al., 2023; Stout et al., 2022). Beyond the ethical concerns related to its production, serum is expensive (Ho et al., 2021; Hubalek et al., 2022), carries risk of contamination (Ho et al., 2021), and its batch-to-batch variability can effect muscle phenotype (O'Neill et al., 2020). Whereas many successful serum replacement strategies rely on the use of fully

*Abbreviations:* NMR, Nuclear Magnetic Resonance; DSS, sodium trimethylsilylpropanesulfonate; PCA, principal component analysis; PC, principal component.

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defined media formulations, this approach may not be cost effective for cultivated meat.

Protein hydrolysates are generally lauded as a cost-effective and popular alternative to serum (Ho et al., 2021; Hubalek et al., 2022; Zhang et al., 2024), with non-animal derived hydrolysates preferred over animal-based products as a means of reducing contamination risks (Girón-Calle et al., 2008; Ho et al., 2021; Lobo-Alfonso et al., 2010). While the use of hydrolysates is still limited within cultivated meat cultures (Hubalek et al., 2022), their promise has been demonstrated within other mammalian cell cultures when used in the 3–5 g/L range (Kim et al., 2006; Kim and Lee, 2009; Obaidi et al., 2021; Sung et al., 2004). As cell culture additives, hydrolysates are known to provide a mixture of amino acids, carbohydrates, trace elements, peptides, and lipids (Ho et al., 2021). However, there is likely to be considerable variation in the specific composition of any particular hydrolysate product. In addition to overarching product differences, batch-to-batch variability is also touted as a frequent concern (Gilbert et al., 2014; Richardson et al., 2015). Although complex additives like hydrolysates cannot achieve the status of a "defined" medium formulation, a better understanding of hydrolysate composition may nonetheless serve to improve reproducibility in cell cultures and reduce unpredictable effects of unknown constituents (Weiskirchen et al., 2023).

Although the complexity of hydrolysate composition prevents complete characterization via any single method, metabolomic profiling can be used to establish the concentration of all small molecules including amino acids, carbohydrates, short chain fatty acids, and vitamins, among others. Relatively few studies have applied metabolomic analysis to the characterization of hydrolysate products, and these efforts have generally focused on only one or two products at a time or without addressing the issue of batch-to-batch variability. For example, Trunfio et al. (2017) used multiple spectroscopic methods to characterize wheat hydrolysates for predicting product quality, while Quattrociocchi et al. (2021) used Nuclear Magnetic Resonance (NMR) to analyse yeast autolysate. In addition, there have been several comparisons of batch-to-batch variability within soy hydrolysates, where the primary focus was determining the effect of composition differences on cell culture responses (Gupta et al., 2014; Richardson et al., 2015) or predicting the cell culture response based on the metabolomic data (Luo and Chen, 2007). This study attempts to build on previous efforts by expanding the scope of metabolomic analysis across multiple hydrolysate products (four plant and five yeast-based) and multiple lots (between two to four per product). Using NMR, we aim to determine hydrolysate batch-to-batch variance, and the differences between hydrolysates of various sources.

## 2. Materials and methods

### 2.1. Hydrolysate products

This study analyses half of the available non-animal derived hydrolysates — encompassing four of the five available hydrolysate sources — in addition to a variety of ultrafiltered and Baker's yeast extracts available through Kerry Group. Four plant-based hydrolysates (Hy-Pea™ 7404, HyPep™ 4601N, HyPep™ 7504, and HyPep™ 1510) and five yeast hydrolysates (HyPep™ YE, Hy-Yest™ 412, Hy-Yest™ 466, Hy-Yest™ 503, and Hy-Yest™ 555) were provided by Kerry Group (Beloit, WI, USA). Hy-Pea™ 7404, HyPep™ 4601N, HyPep™ 7504, and HyPep™ 1510 will be referred to as pea, wheat, cotton, and soy (respectively) throughout this manuscript based on their source material. Similarly, HyPep™ YE will be referred to as yeast extract. Three separate lots were provided for each hydrolysate product with the exception of HyPep™ 1510 (four lots) and Hy-Yest™ 466 (two lots).

### 2.2. NMR

Directly prior to NMR analysis, 4 mg of each hydrolysate powder was dissolved in DI water at 4 g/L and passed through a 0.22 μM filter. NMR samples were prepared by combining 630 μL of the resulting hydrolysate solution with 70 μL of internal standard, 5 mM DSS (sodium trimethylsilylpropanesulfonate) dissolved in 99.9% $D_2O$ (Sigma-Aldrich). The samples were vortexed and pipetted into 5 mm glass tubes (Bruker). The samples were scanned on a 700 MHz Bruker Avance III spectrometer with a 1D-NOESY pulse sequence with 1 s of presaturation, 100 ms mixing time, and 4 s acquisition time (Sokolenko and Aucoin, 2015). Since NMR uses minimal and non-destructive sample preparation, this technique detects the distinct signals produced by free metabolites, whereas the bound metabolites remain intact within larger structures, such as peptides, thereby producing broader and less identifiable peaks. The NMR spectra were analysed with Chenomx NMR Suite — a software with comprehensive spectral libraries (covering over 300 metabolites available for reference at www.chenomx.com/libraries) used for metabolite identification and quantification (Ellinger et al., 2013). Baseline and phase corrections were performed automatically with the software and adjusted manually where necessary. After this processing step, metabolite concentrations were estimated using "targeted profiling", wherein metabolites are quantified by overlaying resonance peaks from the built-in libraries and compared to the fit of the internal standard — see Weljie et al. (2006) for more details.

### 2.3. Data and analysis

Concentration data was exported from Chenomx and analysed in R (version 4.3.1). Batch-to-batch variability was analysed by scaling the concentration standard deviation by the average metabolite concentration (thereby calculating coefficients of variance). To account for the variability in the coefficients of variance, percentiles are used as a measure of the overall batch-to-batch variability. For example, 90% of the coefficients of variance will fall below the value for the 90th percentile, thereby accounting for outliers where a single metabolite of high variance may not be representative of the overall batch variability. For principal component analysis (PCA), missing concentrations for metabolites considered to be present (identified in two or more samples of a given product) were set to the average metabolite concentration in order to reduce their weight to 0 (upon mean scaling).

## 3. Results and discussion

NMR was able to identify a total of 90 unique metabolites within 27 hydrolysate samples. Of these, 70 metabolites could be identified with relatively high confidence due to the presence of multiple peaks with minimal overlap (or singular peaks with a unique chemical shift or shape), whereas the remaining 20 identities were deemed uncertain either due to overlapping peaks or low concentrations near the detection limit and were omitted from the analysis. The pea hydrolysate spectra were observed to have minimal distinguishable peaks, which may have been caused by interference from metals, salts, or larger molecules. As such, pea hydrolysate samples were excluded from further analysis. A complete list of the observed metabolites (including low confidence identities) along with their concentrations is provided in Supplementary Tables 1–2, with the following sections focusing on the most pertinent general trends.

### 3.1. Overall metabolite coverage

On average, metabolites observed via NMR correspond to approximately 17% of the overall sample composition by mass (i.e., 0.17 g/L of a 1 g/L solution is accounted for). The only exceptions to this are Hy-Yest™ 412 with 37% and yeast extract with 43% (see Table 1), which provide a greater percentage of small metabolites than the other hydrolysate products. Due to the nature of NMR analysis, these values can be taken as a reasonable representation of all observable bulk metabolites (even if trace concentrations of some molecular species fall below

**Table 1**
The average percentage of mass quantified by NMR.

| Hydrolysate | Mass Percent |
|---|---|
| HyPep™ 1510 | 14 ± 4% |
| HyPep™ 4601N | 18 ± 6% |
| HyPep™ 7504 | 18 ± 1% |
| Hy-Yest™ 466 | 18 ± 7% |
| Hy-Yest™ 503 | 18 ± 3% |
| Hy-Yest™ 555 | 18 ± 8% |
| Hy-Yest™ 412 | 37 ± 5% |
| HyPep™ YE | 43 ± 14% |

the limit of detection). These observations also fall in line with previous reports — Djemal et al. (2021) estimated 16% carbohydrate composition in soy hydrolysates whereas Quattrociocchi et al. (2021) observed 57% metabolite composition in yeast extract. It should be noted that while the total number of metabolites identified in each hydrolysate source are similar, it is higher concentrations of specific metabolites such as glutamate, alanine, and lactate in Hy-Yest™ 412 and yeast extract that are primarily responsible for the observed difference.

The quantified metabolites were broken down into nine groups to facilitate comparison: four categories of amino acids (aromatic, charged, polar uncharged, and non-polar aliphatic), carbohydrates, organic acids, nucleotides (which includes other nucleic material such as nucleosides), vitamins, and other. The mass of each group as a percentage of overall quantified mass is presented in Fig. 1. Despite similar overall metabolite concentrations, there appears to be relatively significant differences in the underlying composition of the different samples as well as evidence of batch-to-batch variability. On average, soy hydrolysates were found to contain a greater fraction of carbohydrates than all other hydrolysate types — with four different carbohydrates identified and a comparatively high concentration of sucrose. Similar results have been previously observed in soy hydrolysates, with sucrose found to account for almost 40% of the total carbohydrates (Djemal et al., 2021). Cotton stood out within the "other" category, owing to its high concentrations of glycerol. Meanwhile, both Hy-Yest™ 503 and Hy-Yest™ 555 were found to contain a greater amount of nucleotides than the other hydrolysates. Similar to yeast extract and Hy-Yest™ 412, nucleotides have previously been reported to account for approximately 2% of yeast extract (Quattrociocchi et al., 2021; Wasito et al., 2022); however, this value reached as high as 5.6% for Hy-Yest™ 555. These differences between yeast products may play an important role in product performance as several studies have shown improvements in growth rate and production from the addition of nucleosides to mammalian cell cultures (Morrison et al., 2019; Takagi et al., 2017).

### 3.2. Bulk metabolite content

Considering just the top three to five metabolites with the highest concentration for each of the products offers a convenient snapshot of


**Fig. 1.** Contribution of each metabolite category to the mass of metabolites as a percentage of the quantifiable hydrolysate sample mass. For visual clarity only the top error bars, representing standard deviation, are shown; note that error bars are symmetrical around each bar.

general product variability. Cotton is characterized by high concentrations of glycerol, betaine, and sucrose, as compared to sucrose, glutamate, and fructose for soy and leucine, pyroglutamate, and glutamine for wheat. Yeast products were found to be more similar in this regard, with lactate, alanine, and glutamate generally found in highest concentrations, with oxypurinol also appearing in notable amounts. However, the specific order of these metabolites still varied greatly, with Hy-Yest™ 412 entirely dominated by lactate, whereas yeast extract, for example, featured the highest concentration of alanine (as seen in Table 2). Indeed, several of the highest concentration metabolites in some samples are entirely lacking in others, with glutamine and ribose only appearing in wheat samples, and fructose only appearing in one out five yeast products.

The average highest concentration metabolite in plant hydrolysates is glycerol, which can be found at noticeably higher concentrations in cotton samples (~18% of quantified mass on average). Its prominence in plant, but not yeast samples, may be due to substantial glycerol contents in cotton suberin (Moire et al., 1999) or an indication of glycerol synthesis as an abiotic stress responses in the crops during growth (Albertyn et al., 1994; Bahieldin et al., 2014). With differences in both plant structure and growth conditions, it is unsurprising that not only are there differences between products, but high batch-to-batch variability of glycerol in cotton (see Fig. 2). On the other hand, the highest concentration metabolites in yeast hydrolysates include lactate, alanine, and glutamate (see Fig. 3). All three of these small molecules are byproducts of the yeast metabolism (Luo et al., 2023; Takagi, 2019), and their concentrations can be influenced by the culture conditions. For example, glutamate can be found in higher concentrations if the yeast is grown under aerobic conditions compared to those grown in anaerobic conditions, while alanine production follows the opposite trend (Sirisena et al., 2024).

### 3.3. Metabolite variety

Overall, yeast extract and cotton boast the largest variety of components, at an average of 42 and 39 metabolites identified, respectively, with yeast extract also found to have the highest overall metabolite concentrations (as can be observed in Figs. 2 and 3). Only five metabolites — phenyl-alanine, threonine, pyroglutamate, alanine, and valine — were identified in all samples. However, an additional ten metabolites, including several other amino acids such as isoleucine, serine, and tryptophan were identified in at least one sample of all hydrolysate products. Apart from histidine, all essential amino acids are provided by the eight different hydrolysates tested. And among carbohydrates in general, yeast extract contains all five carbohydrates identified in yeast products. The differences between plant and yeast samples are made more apparent by several metabolites that only appear in yeast hydrolysates, namely, inosine, ornithine, propylene glycol, and uracil (which was otherwise only found in a single wheat sample). It should be noted that these results may not be generalizable to all hydrolysate products —

**Table 2**
Average concentration (in mM/g hydrolysate) and standard deviation for the overall top ten concentration metabolites within hydrolysate products.

| Compound | Cotton | Soy | Wheat | Yeast Extract | Hy-Yest™ 412 | Hy-Yest™ 466 | Hy-Yest™ 503 | Hy-Yest™ 555 |
|---|---|---|---|---|---|---|---|---|
| Lactate | 0.03 ± 0.01 | NA | 0.03 ± 0.01 | 0.28 ± 0.09 | 0.64 ± 0.11 | 0.03 ± 0.00 | NA | 0.16 ± 0.10 |
| Alanine | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.40 ± 0.13 | 0.29 ± 0.03 | 0.15 ± 0.02 | 0.16 ± 0.02 | 0.23 ± 0.04 |
| Glycerol | 0.35 ± 0.14 | NA | 0.10 ± 0.03 | NA | NA | 0.03 ± 0.02 | NA | 0.03 ± 0.01 |
| Glutamate | 0.04 ± 0.00 | NA | NA | 0.35 ± 0.09 | 0.19 ± 0.06 | 0.14 ± 0.08 | 0.18 ± 0.03 | 0.32 ± 0.02 |
| Leucine | 0.05 ± 0.01 | 0.09 ± 0.02 | 0.14 ± 0.03 | 0.16 ± 0.04 | 0.20 ± 0.03 | 0.11 ± 0.01 | NA ± NA | 0.06 ± 0.05 |
| Sucrose | 0.08 ± 0.01 | 0.17 ± 0.07 | 0.03 ± 0.01 | 0.04 ± 0.02 | NA | NA | NA | NA |
| Valine | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.08 ± 0.02 | 0.15 ± 0.05 | 0.12 ± 0.02 | 0.08 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.04 |
| Pyroglutamate | 0.03 ± 0.00 | 0.03 ± 0.02 | 0.12 ± 0.04 | 0.12 ± 0.04 | 0.13 ± 0.02 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.07 ± 0.01 |
| Betaine | 0.12 ± 0.01 | NA | NA | 0.10 ± 0.01 | 0.09 ± 0.02 | NA | 0.13 ± 0.03 | NA |
| 4-Aminobutyrate | NA | NA | NA | 0.06 ± 0.02 | 0.08 ± 0.03 | 0.12 ± 0.03 | 0.02 ± 0.00 | NA |

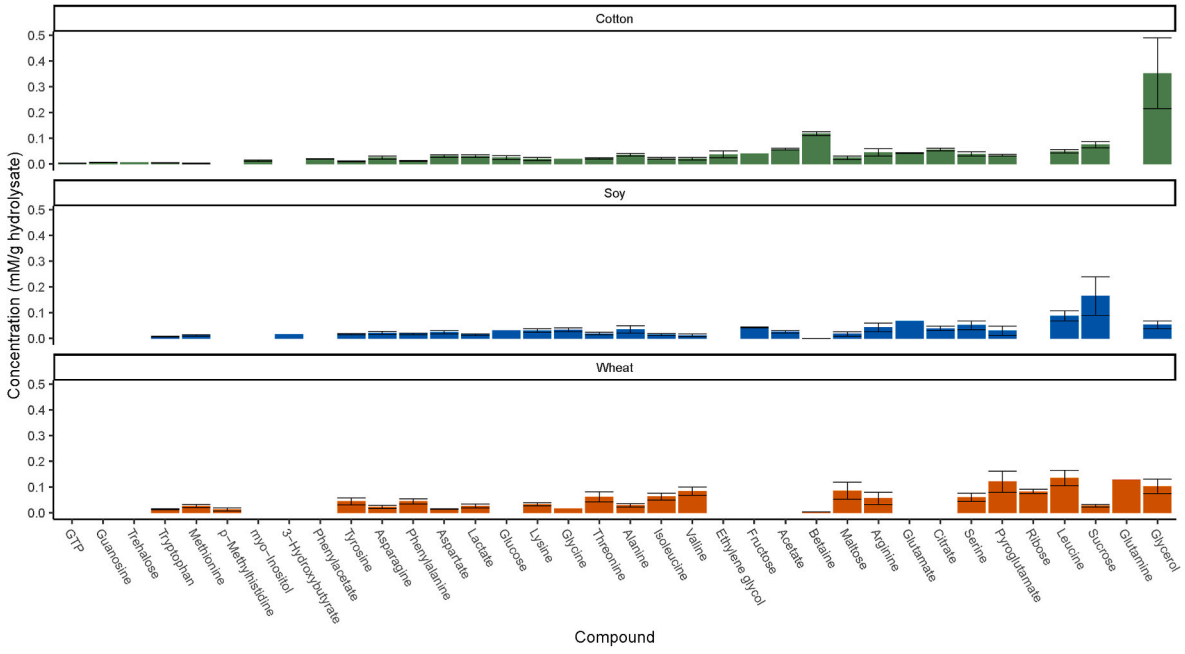


**Fig. 2.** Concentrations of the top 80% (by mass) metabolites identified in plant hydrolysates organized by the overall average metabolite concentration. Error bars correspond to the batch standard deviation.

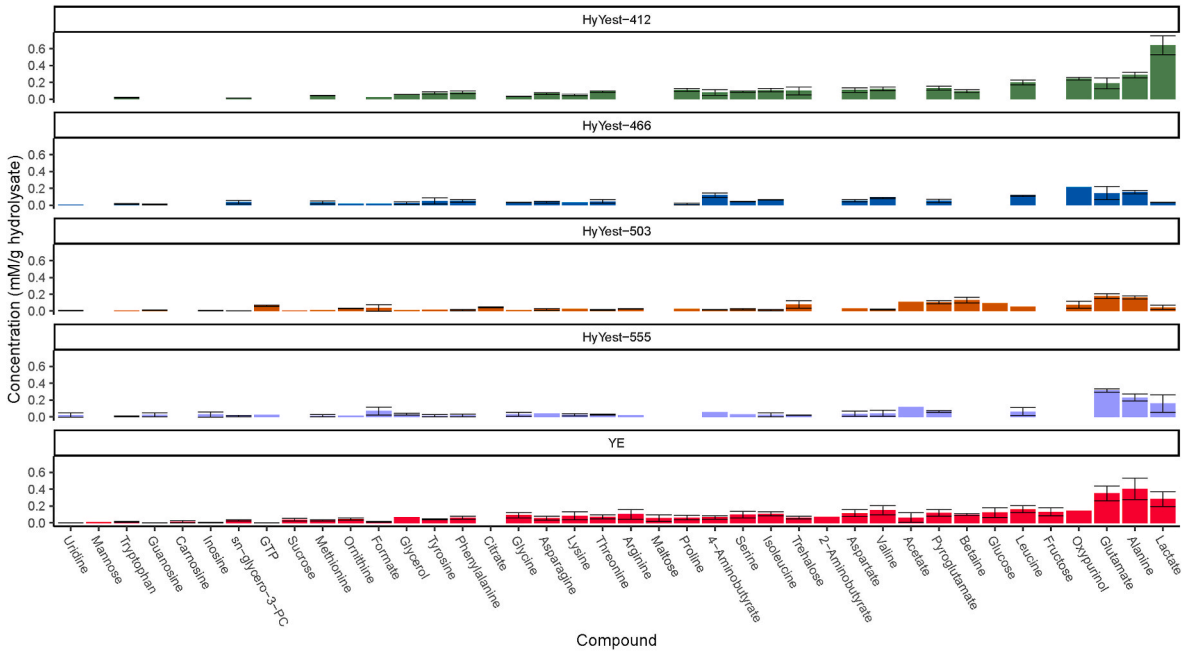


**Fig. 3.** Concentrations of the top 80% (by mass) metabolites identified in yeast hydrolysates, organized by the overall average metabolite concentration. Error bars correspond to the batch standard deviation.

ornithine, for example, has been previously observed in soy hydrolysates, where it was shown to have a positive correlation with titer in cell cultures (Richardson et al., 2015). Sixteen unique metabolites were identified consistently in only a single hydrolysate product (Table 3). Several of these metabolites (such as glutamine, adenine, IMP, and succinate) are likely to be infrequent as they are intermediates within the plant and yeast metabolism and are likely to be converted into other metabolites. For example, while glutamine was only identified in Hy-Yest™ 466, it can be converted to glutamate (Guillamön et al., 2001), which was present in all yeast samples.

Beyond patterns in general presence/absence, several metabolites were found to have substantial differences in concentration between products. Table 4 presents the top ten metabolites with the largest variance between products (with the complete list in Supplementary Table 3). Although concentration differences may be more apparent in metabolites that were found at high concentrations, the most significant between-product differences were found in several trace nucleotides/nucleosides when variability is measured as a coefficient of variance. Nucleotides have previously been found to vary from 1.5 to 7% of raw hydrolysate material, which has been attributed to differences in production processes (Mosser et al., 2013). Other notable differences in metabolite concentrations stemmed from alanine, leucine, lactate, glutamate, glycerol, and sucrose.

### 3.4. Batch-to-batch variance

Overall batch-to-batch variance was compared by calculating the coefficient of variance — a relative measure of standard deviation — for each metabolite (within each product), and comparing the percentiles of these values between products (Table 5). By this metric, either Hy-Yest™ 555 or yeast extract can be seen as the most variable products — with Hy-Yest™ 555 observed to have the highest 90th percentile coefficient of variance as well as the highest median value, whereas yeast extract was found to have the highest 25th and 75th percentile values. For both of these products, a median value of approximately 0.45 translates to 50% of metabolites having a coefficient of variance above 45% of their mean value. Although some of this variability could be attributed to the fact that yeast extract was observed to have the single largest variety of metabolites (42), cotton was not far behind (39) and found to have the lowest coefficients of variance of all the products (based on median values as well as 25th and 75th percentiles). It should be noted that the coefficient of variance from NMR measurement using Chenomx software tends to vary between 0.02 and 0.12, depending on the level of spectral overlap, which serves as a lower limit of observable variability in this study (Sokolenko et al., 2013, 2014). Indeed, the 25th and 75th percentile values for cotton, 0.054 and 0.157, are very likely to be running into this lower limit.

Comparing batch-to-batch variance values for specific metabolites revealed that high batch-to-batch variability was generally correlated with high between-batch variability, suggesting that high variability may be due to the intrinsic nature of specific metabolites. Metabolites with high variances include uridine, inosine, guanosine, uracil, glycerol, lysine. Of these, the nucleotides were found to be the most variable in Hy-Yest™ 555, glycerol in cotton, lysine in HyPep™-YE, and uracil in Hy-Yest™ 466. This indicates that overall batch-to-batch variability may be driven by a relatively small number of high-variance components, at least among the tested products. Previous studies have also shown relatively consistent gross composition between batches of soy hydrolysates (Djemal et al., 2021; Gupta et al., 2014) with potential variation in trace elements (Djemal et al., 2021). Taken together, whether or not batch-to-batch variability is a concern may depend largely on cellular response to a small set of specific high-variance metabolites.

**Table 3**
Metabolites identified in only one or two hydrolysate products (but at least half of a product's samples).

| Hydrolysate | Metabolite |
|---|---|
| HyPep™ 1510 | Ethanol, fructose, trigonelline |
| HyPep™ 4601N | Adenine, π-methylhistidine, ribose, hypoxanthine |
| HyPep™ 7504 | Ethylene glycol, isobutyrate, glucose, myo-inositol, succinate, phenylacetate, trigonelline |
| HyPep™ YE | Carnosine, glucose, succinate, fructose |
| Hy-Yest™ 412 | AMP |
| Hy-Yest™ 466 | Glutamine, ATP, thymol, tyramine, myo-inositol, ADP |
| Hy-Yest™ 503 | Indole-3-lactate, phenylacetate, N-Acetyltyrosine, ADP |
| Hy-Yest™ 555 | IMP, isopropanol, dCTP, hypoxanthine, N-Acetyltyrosine |

**Table 4**
Coefficients of variance depicting the overall metabolite variability and batch-to-batch variability for the top ten highest product variances. Variances are marked as NA for metabolites that were only identified within a single sample of a product.

| Metabolite | Overall | Cotton | Wheat | Soy | Yeast Extract | Hy-Yest™ 412 | Hy-Yest™ 466 | Hy-Yest™ 503 | Hy-Yest™ 555 |
|---|---|---|---|---|---|---|---|---|---|
| Uridine | 1.44 | 0.19 | NA | NA | 0.09 | NA | NA | 0.21 | 3.31 |
| Inosine | 1.38 | NA | NA | NA | 0.12 | NA | NA | 0.13 | 2.08 |
| GTP | 1.38 | 0.03 | NA | NA | 0.04 | NA | NA | 0.46 | NA |
| Cytidine | 1.31 | 0.3 | NA | NA | 0.07 | NA | NA | 0.21 | NA |
| Lactate | 1.31 | 0.03 | 0.04 | 0.02 | 0.52 | 0.65 | 0.02 | 0.15 | 0.61 |
| Glutamine | 1.28 | NA | NA | NA | NA | NA | NA | NA | NA |
| Glycerol | 1.23 | 1.28 | 0.26 | 0.14 | NA | 0 | 0.16 | NA | 0.09 |
| Guanosine | 1.14 | 0.13 | NA | NA | 0.07 | NA | 0.34 | 0.12 | 2.12 |
| Propylene glycol | 1.08 | NA | NA | NA | 0.23 | 0.15 | 0.09 | 0.63 | 0.09 |
| Formate | 0.95 | 0.11 | NA | 0.15 | 0.17 | NA | NA | 1.18 | 1.42 |

**Table 5**
The quantiles of metabolite coefficients of variance for each hydrolysate product.

| Hydrolysate | Q10 | Q25 | Q50 | Q75 | Q90 |
|---|---|---|---|---|---|
| Cotton | 0.032 | 0.054 | 0.093 | 0.157 | 0.289 |
| Soy | 0.057 | 0.091 | 0.153 | 0.201 | 0.474 |
| Wheat | 0.042 | 0.084 | 0.257 | 0.345 | 0.485 |
| Yeast Extract | 0.121 | 0.229 | 0.440 | 0.705 | 0.881 |
| Hy-Yest™ 412 | 0.029 | 0.156 | 0.266 | 0.405 | 0.528 |
| Hy-Yest™ 466 | 0.051 | 0.127 | 0.261 | 0.511 | 0.990 |
| Hy-Yest™ 503 | 0.062 | 0.123 | 0.148 | 0.234 | 0.510 |
| Hy-Yest™ 555 | 0.089 | 0.128 | 0.463 | 0.606 | 1.890 |

### 3.5. General relationships

An overall snapshot of both sample similarity and variability is presented via PCA scores and loadings plot in Fig. 4. Fig. 4A confirms that hydrolysate products tend to cluster together and are therefore most closely related to each other. Plant and yeast hydrolysates are mostly separated by principal component (PC) 2 whereas PC 1 accounts for the majority of the differences between yeast (and to a lesser extent plant) products. As expected based on previous analysis, Fig. 4B suggests that the differences between plant and yeast products are driven largely by nucleotides/nucleosides. Hy-Yest™ 555 and HyPep™-YE show the greatest spread between samples, serving as further confirmation that these hydrolysates have the largest batch-to-batch variability, while cotton and soy show tight clusters between batches.

## 4. Conclusion

Despite the fact that hydrolysate products are typically grouped together as a similar class of additives, NMR metabolomics of eight different products established significant differences in metabolite composition. Overall metabolite composition by mass trended around 18% for most products with a high of 43% for yeast extract. Carbohydrates played a prominent role in soy hydrolysates, while Hy-Yest™ 503 and Hy-Yest™ 555 were characterized by larger fractions of nucleotides. In addition, only fifteen metabolites were identified across all hydrolysate products, while sixteen metabolites were uniquely identified in a single hydrolysate. However, despite the differences, overall batch-to-batch metabolite variability was found to be lower than expected. Within any given product, a select few metabolites account for the majority of product variability — nucleotides in Hy-Yest™ 555, glycerol in cotton, lysine in HyPep™-YE, and uracil in Hy-Yest™ 466. Despite common concerns, this comprehensive metabolomic analysis has shown that batch-to-batch variability may be a more nuanced issue in hydrolysates than generally discussed. Although this analysis was performed with hydrolysates from a single manufacturer and manufacturing processes as well as raw material selection are likely to play a significant role in the results (including batch-to-batch variance), this study nonetheless establishes a useful baseline for continued characterization of hydrolysate products as a whole. By providing a complete breakdown of metabolite concentrations for several commonly available hydrolysate products, this work aims to help bridge the gap between the low cost hydrolysate additives and performance of fully "defined" media.

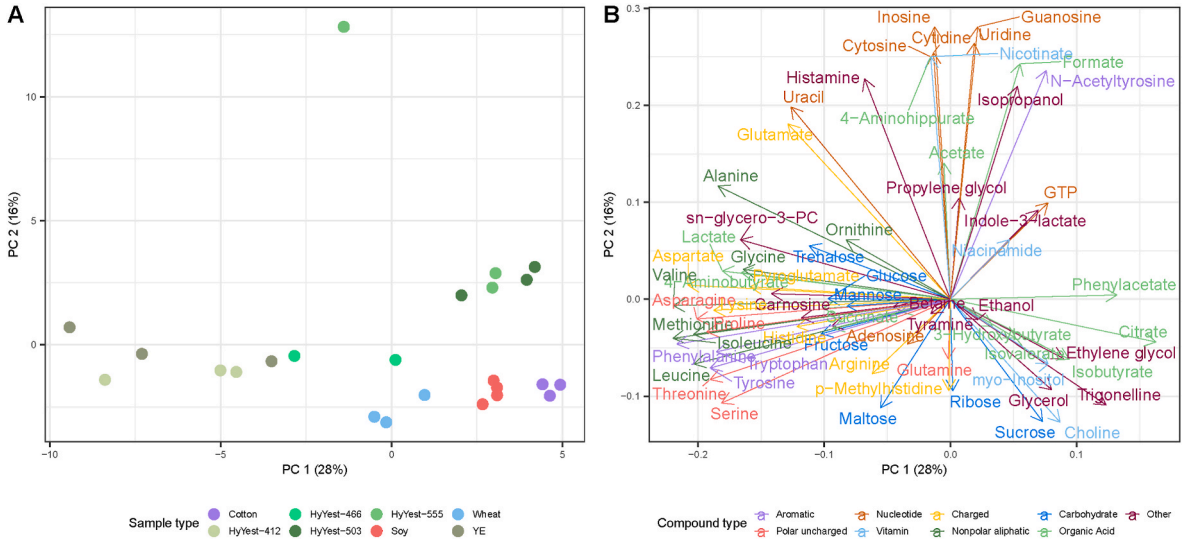


**Fig. 4.** The principal component analysis score (A) and loading (B) plots for plant and yeast hydrolysates using the first two principal components.

## CRediT authorship contribution statement

**Michelle Combe:** Data curation, Formal analysis, Data procurement and analysis, Writing – original draft. **Kathy Sharon Isaac:** Visualization. **Greg Potter:** Conceptualization, Helped conceptualize the work. **Stanislav Sokolenko:** Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stanislav Sokolenko reports equipment, drugs, or supplies was provided by Kerry Group. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data and code utilized in this work has been shared at the following link: https://doi.org/10.5281/zenodo.11357327.

NMR Metabolomics of Plant and Yeast-based Hydrolysates (Original data) (Zenodo)

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100855.

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