Kaunas University of Technology

Faculty of Mechanical Engineering and Design

Research on Extraction Process of Proteins from Yeast

Master's Final Degree Project

Gurudath Thimmanayakanahally Vijayshankar

Project author

Assc. prof. Kazimieras Juzenas

Supervisor

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Industrial Engineering and Management (6211EX018)

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Supervisor

Assoc. prof. Vaida Jonaitienė

Reviewer

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Gurudath Thimmanayakanahally Vijayshankar

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Declaration of Academic Integrity

I confirm that the final project of mine, Gurudath Thimmanayakanahally Vijayshankar, on the topic "Research on Extraction Process of Proteins from Yeast" is written completely by myself; all the provided data and research results are correct and have been obtained honestly. None of the parts of this thesis have been plagiarised from any printed, Internet-based or otherwise recorded sources. All direct and indirect quotations from external resources are indicated in the list of references. No monetary funds (unless required by Law) have been paid to anyone for any contribution to this project.

I fully and completely understand that any discovery of any manifestations/case/facts of dishonesty inevitably results in me incurring a penalty according to the procedure(s) effective at Kaunas University of Technology.

(name and surname filled in by hand)	(signature)

Kaunas University of Technology

Faculty of Mechanical Engineering and Design

Task of the Master's final degree project

Given to the student – Gurudath Thimmanayakanahally Vijayshankar

1. Title of the project –

Research on Extraction Process of Proteins from Yeast

(In English)

Baltymų gavybos iš mielių proceso tyrimas

(In Lithuanian)

2. Aim and tasks of the project –

Aim-
To develop a process for the extraction of proteins from yeast.
Tasks-

1. To analyse the need for protein extraction from brewers spent yeast and the existing methods and select a suitable one.
2. To develop an experimental process for the extraction of proteins.
3. To propose the practical applications for industrial use of the extracted proteins.

3. Initial data of the project –

To extract the proteins from yeast effectively not less than 30%.

4. Main requirements and conditions –

Methods should assure that extracted proteins from yeast are suitable for food production.

Project author	Gurudath Thimmanayakanahally Vijayshankar		
	(Name, Surname)	*(Signature)*	*(Date)*
Supervisor	Kazimieras Juzėnas		
	(Name, Surname)	*(Signature)*	*(Date)*
Head of study field programs	Regita Bendikiene		
	(Name, Surname)	*(Signature)*	*(Date)*

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Study field and area (study field group): Production and Manufacturing Engineering (E10), Engineering Sciences (E).

Keywords: Hydrolysis, Brewers spent yeast, Protein extraction, Filtration

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Summary

In the current scenario, the human race is increasing every day and to fulfil the need of food for expanding population it is difficult through the traditional methods of food from fields. There are many studies going on to reuse the food or to extract nutrients from the residue of industrial waste. The brewers spent yeast is one among them which contains a high nutrient value and is used as mainly animal feed or waste. This research will help in the reuse of yeast for the extraction of proteins from it and to use in food industries. The yeast was mixed with lactic acid of lower concentrations and hydrolysed at different temperatures to breakdown the bonds between them and filter the mixture obtained. The yeast with lactic acid of 2% concentration hydrolysed at 140 °C gives the effective protein extraction from yeast sample. By this method, 80% of the protein was been extracted from the total amount of protein present in the sample. So, these extracted proteins can be used for manufacturing consumable products for human beings. The approximate cost analysis of setting up an industrial plant was analysed and Return on investment was calculated in this research which will be helpful for the buyers to choose this process for protein extraction from brewers spent yeast.

Thimmanayakanahally Vijayshankar, Gurudath. Baltymų gavybos iš mielių proceso tyrimas. Magistro baigiamasis projektas, doc. Kazimieras Juzėnas; Kauno technologijos universitetas, Mechanikos inžinerijos ir dizaino fakultetas.

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Reikšminiai žodžiai: hidrolizė, alaus daryklų mielių atliekos, baltymų išavimas, filtravimas

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Santrauka

Žemės gyventojų skaičius nuolatos didėja, o patenkinti maisto poreikį gausėjančiai populiacijai yra sudėtinga naudojant vien tradicinius maisto gavybos metodus. Yra atlikta daugybė tyrimų, kaip pakartotinai naudoti maistą arba išgauti maisto medžiagas iš pramoninių atliekų. Tokių alaus gamybos atliekų pavyzdys yra mielės, kurių maistinė vertė yra didelė ir kurios šiuo metu daugiausia naudojamos kaip gyvūnų pašarai arba utilizuojamos kaip atliekos. Atliktas tyrimas susijęs su pakartotinių mielių panaudojimu iš jų išgaunant baltymus, tinkamus naudoti maisto pramonėje. Mielės buvo sumaišytos su mažesnės koncentracijos pieno rūgštimi ir hidrolizuotos skirtingose temperatūrose, kad suskaidytų cheminius ryšius ir vėliau gautas mišinys filtruotas. Buvo nustatyta, kad mielės su 2% koncentracijos pieno rūgštimi, hidrolizuotos 140 ° C temperatūroje, yra tinkamos veiksmingai baltymų gavybai. Šiuo metodu iš viso mėginyje esančių baltymų kiekio buvo išgauta 80% baltymų. Vėliau šie išgauti baltymai gali būti naudojami gaminant maisto produktus žmonėms. Buvo atlikta apytikslė pramonės gavybos kaštų analizė ir apskaičiuota investicijų grąža. Tai gali būti naudinga informacija siekiant diegti šį baltymų išgavimo iš alaus gamyboje panaudotų mielių procesą.

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Abbreviations:

HD- Hydrolysis.
VF- Vacuum Filtration.
PF- Pressure Filtration.
VF1- Vacuum filtration of Hydrolysis 01 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 100 °C.
PF1- Pressure filtration of Hydrolysis 01 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 100 °C.
VF2- Vacuum filtration of Hydrolysis 02 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 100 °C.
PF2- Pressure filtration of Hydrolysis 02 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 100 °C
VF3- Vacuum filtration of Hydrolysis 03 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 120 °C.
PF3- Pressure filtration of Hydrolysis 03 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 120 °C
VF4- Vacuum filtration of Hydrolysis 04 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 120 °C.
PF4- Pressure filtration of Hydrolysis 04 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 120 °C
VF5- Vacuum filtration of Hydrolysis 05 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 140 °C.
PF5- Pressure filtration of Hydrolysis 05 i.e. yeast with a lactic acid concentration of 2% hydrolysed at 140 °C
VF6- Vacuum filtration of Hydrolysis 06 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 140 °C.
PF6- Pressure filtration of Hydrolysis 06 i.e. yeast with a lactic acid concentration of 4% hydrolysed at 140 °C
VF1dry – Weight of dried filtrate from vacuum filtration.
VFK1dry – Weight of dried filter cake obtained from vacuum filtration.
PF1dry– Weight of dried filtrate from pressure filtration.
PFK1dry– Weight of dried filtrate from pressure filtration.
BSY- Brewers spent yeast.

Introduction

The population of the world is increasing at a steady rate, and it is predicted that vast quantities and diverse nature of food will be required to cater to the dietary needs of the population. Assuming the rate of protein consumption will remain the same, it will expect an ever-increasing demand of proteins and will use large quantities of water, land and add to the greenhouse gases, contributing to global warming. Therefore, humans need to look for alternative sources of proteins to cope up with high demand in a sustainable manner.

Brewer's spent yeast is an underutilized resource which is mainly used for animal feed or as a waste can be used for protein extraction. This waste can play a resource if proteins from the yeast are harvested and put for the use of manufacturing consumables for humans. The yeast produced in the brewing process is an in trusting byproduct as it contains a high level of nutrients and there are various methods that can turn this yeast into a valuable resource.

In 2017 the European commission accepted for co-financing of "Life Yeast" Project to reuse the Brewers spent yeast and to create a better world. When the project becomes operational, it expects to reuse 11750 tons of brewers spent yeast per year. The approximate fund which was granted by EU-LIFE is 1.6 million euros[1]. So, this upcoming research will increase the production of sustainable proteins from the yeast using lactic acid hydrolysis, which will be helpful in obtaining higher and economical production of proteins, that can be introduced into the food chain as a reliable and sustainable source of proteins.

Aim-

To develop a process for the extraction of proteins from yeast.

Tasks-

1. To analyse the need for protein extraction from brewers spent yeast and the existing methods for the extraction and selection of a suitable one.
2. To develop an experimental process for the extraction of proteins.
3. To propose the practical applications for industrial use of the extracted proteins.

1. Analysis of existing processes for protein extraction from yeast

1.1. Importance of proteins and their recycling from BSY

Proteins are fundamental and functional components in every cell of the body and are included in the metabolic interactions in the body. As protein is a second abundant compound within the body after water and seen in every tissues and cell which gives the body with almost 10 to 15% of its dietary energy so it becomes important for the development and maintenance for good health. The huge amount of the proteins is in muscles (43%) with some proportions in the skin (15%) and in the blood (16%). Long chains of amino acids constitute proteins. The bio-chemical movement of the proteins is recognized by their singular structure, its shape and size and these factors are determined by the constituent amino acids.

There are around 20 diverse amino acids regularly found in plant and animal proteins. Leucine, Methionine, Phenylalanine, Tryptophan, Isoleucine, Valine, Threonine, Lysine are the eight of them must be given to the adults in the diet, so they are characterized as 'indispensable' or 'essential' amino acids. Arginine, glycine, tyrosine, histidine, cysteine, glutamine and proline are moreover thought to be fundamental (vital) amino acids for children's because children are incapable to create sufficient to meet their needs, they are specified as 'conditionally' fundamental. Amid grown-up life, there might also be characterized disease states when an amino acid becomes conditionally significant. Some other amino acids are not necessarily provided by the diet. This can be since, through the method called transamination, the amino group can be exchanged to another amino acid with a distinctive amino group and these are recognized as dispensable/non-essential amino acids [1].

The Dietary Reference Values for protein relies on the estimation of need. The estimated requirement of 0.6 g of protein per kilogram body weight per day is an average for adults. The Reference Nutrient Intake (RNI) is set at 0.75g of protein per kilogram body weight per day in adults. This equals to nearly 56 g/day and 45 g/day for men and women aged 19-50 years respectively. There are additional requirements for the development of children, pregnant and breastfeeding women. 17 kJ or 4 kcal of energy is generated by 1 g of protein, to the lesser extent fat must be the primary source of dietary energy [2].

Huang, M. et al. found that many cellular processes have to be adjusted to support efficient protein secretion. Mainly, changes in energy metabolism resulted in reduced respiration, and increased fermentation, as well as balancing of amino-acid biosynthesis, and brought down thiamine biosynthesis. These findings were consolidated by reverse engineering, and physiological characterisation, and showed that by tuning metabolism, cells could secrete recombinant proteins efficiently [3]. These findings provide analytical support to the question, which cellular regulations and pathways are connected with efficient protein secretion.

Need for Protein extraction from yeast

The population of the world is increasing at a steady rate, and this increasing population will have an expanding demand for food. Moreover, the scarcity of food in different parts of the world, and effect of political crises and forces of nature over food supply chain has created awareness of the poverty and shortage of food, especially the proteins. Therefore, it is vital for the sustenance of humans to search for alternate sources of proteins like microbial proteins and invest in their steady production and supply. Brewer's yeast is produced as a byproduct of ethanol manufacture at an industrial scale

and is considered as a source of protein. It is found that the brewer's yeast in dried form contains approximately 47 to 55 per cent protein which if successful and economically retrieved, can become a part of the food supply chain. So, for each hectoliter of the finished beer, around 1-2 kg of yeast is used. After the primary fermentation, the spent yeast is used in the next batch in a minimum quantity in order to initiate the fermentation process. The significant portion of the active yeast which ferments at a rapid rate is produced in the propagation plant. The state of yeast, when cold lagering process is complete, is called lagering cellular yeast, which amounts to 0.5 to 0.9 kilograms of condensed yeast per hectoliter of finished beer together. It is also accompanied by accelerated turbidity material and froth beer [4]. An enzyme from the brewery industry can be processed to create an extract of yeast which can further be refined to obtain proteins and various other chemicals. Yeast extract is determined as the dissolvable component of yeast cell after yeast cell wall is eliminated. A vast range of physiologically essential chemicals in present in the yeast cells make them a perfect yeast extract to be added to various kinds of food. When beer worts or high gravity worts containing large amounts of unmalted grains are fermented with these yeast extract, it increases their free α-amino nitrogen, resulting in enhanced yeast's supply of nutrients and fermentation efficiency. Free proteinogenic amino acids are the primary source most of the α-amino nitrogen. Yeast extracts are produced from used yeast stocks a large number of required amino acids for human consumption. Moreover, they are rich in B vitamins, in which the folate vitamers in the form of 5-methyltetrahydrofolate are a crucial part of the human diet and performs predominant metabolic exercises in body cells [4]. Moreover, the bioaction of this extract, that in turn is a measure of the reduction and antioxidative potential, makes these extracts very valuable to food the industry. Most of the beer around the world is produced from high gravity worts. For increasing the wort's extract, content sugar syrup is added to the mixture to change the balance of nutrients considering all physically effective constituents. This improves the metabolism of the yeast, which in term increases the quality of beer at the expense of the chemical composition of the yeast. Moreover, by using various types of non-Saccharomyces strains for the starter culture, the biodiversity of spent yeast increases, making it a compendium of yeast varieties. In addition to the biodiversity, the spent yeast takes up different organisms from the environment during storage and handling, which contaminates the yeast with various microorganisms. This diversity and contamination of the spent yeast affect its extraction process. The composition of yeast extracts which are commercially available differs widely from each other. This might be attributed to the different yeast extraction process followed, and the biodiversity of the starting material of the beer fermentation.

The spent yeast from the brewery industry is underutilized, and the production of high-value products lack mainly due to technological reasons. The spent yeast is squeezed squeeze out the entrained beer, and the yeast cake is sold wet or after drying. The profit margin in selling spent yeast are very low, which is driving the scientific community to find alternative disposal methods. If the waste yeast containing some beer with is discharged to the sewer or combined with spent grain, it tremendously increases the degradation load to the environment. When mixed with spent grain, it makes the dewatering process very difficult and increases the biochemical oxygen demand of the liquor pressed out of the spent grain [5].

Yeast is usually added to the diet in order to supply specific vitamins. Yeast protein is the good source of some of the nutritionally vital amino acids, and its addition to the experimental diet in particular cases initiates errors by supplying amino acids, in which the protein may be lacking. The addition of yeast to the experimental diet causes errors, whose magnitude could be estimated from the amino acid

composition of the yeast proteins, but information on this point is very inadequate. It is supposed that the protein in the yeast cell is present in combination with the nucleic acid. At least, no one has obtained a preparation other than nucleoprotein or a derivative thereof. The amount of nucleic acid in protein differs widely in these preparations, judging from the amount of phosphorus found. Usually, yeast is generally added in experimental diets, but not a yeast protein preparation. However, it has been found that direct acid hydrolysis of the whole yeast is not always acceptable for amino acid determination [6]. For example, cystine and histidine were found for some reason not yet explained to be mostly decomposed. Thus, the best way to use yeast is to first extract the proteins from it and use them as dietary supplement or food.

Applications of proteins extracted from yeast and Recycling

Brewers yeast (spent) which contains a high volume of nutrient value and there are various methods that can convert this waste into a valuable resource. Currently, AB InBev has extensively dried the brewers spent yeast which can be used for human foods. The Brewers spent yeast is produced around 125,000 tones per year in dry and wet form the company in Europe. Which is sold for animal feed for a lesser price for farmers. The objectives of this project developing a new methodology to process BSY into valuable constituents that can be used as raw materials in a wide range of industrial applications, and developing new applications for BSY, with an emphasis on technologies that are at or close to market readiness [1].

Spent brewer ‘s yeast extracts were obtained by hydrolysis method and higher contents of essential amino acids were derived. The tested yeast extracts obtained from the spent brewer’s yeast by the addition of proteolytic enzymes using the autolysis method are distinguished by a high content of essential amino acids that surpasses the protein reference developed by WHO/FAO. The lower molecular weight and high content of amino acids with peptides of high levels showed that extracts tested are not only valuable as flavourings but also it is advantages in the applications in the design of dietary supplements and functional foods as a source where their additive levels are defined by the level and tangible quality of nucleic acids. The yeast extracts tested are described by a comparable bouillon taste profile with a notable bitter after taste inferred from bitter substances, which is basically due to the high degree of proteins hydrolysis and the higher content of free glutamic acid, which can come from beer. These extracts can be especially valuable in planning a modern combination of functional foods of the bouillon taste profile that's basically missing in the market [7].

For the production of pharmaceutical proteins, the biotech industry depends on cell factories (few are top-selling medicines). Because of this, there is serious interest in increasing the production efficiency of protein by cell factories. Protein secretion incorporates numerous intracellular processes with numerous principal components remaining uncertain. To study mutant yeast strains genome-wide transcriptional response to secretion of proteins RNA seq is used.

With a growing population for keeping the health better in life dietary plans are needed. Mostly, this could be accomplished by keeping up muscle mass and strength as humans age. Common dietary proposals for protein intake may be inadequate to attain this objective as suggested by modern evidence which people might advantage by expanding their intake and frequency of utilization of high-quality protein. As the natural impacts of expanding animal-protein generation are a concern and elective, more worthy protein sources must be considered. Protein than any other macronutrients

is known to be more fulfilling t, and it isn't clear that diets in plant proteins is higher and influence the craving of grown-up people as they ought to be prescribed for people at malnutrition risk [8].

The production of particularly animal-based protein will have a detrimental impact on the environment by producing emissions of greenhouse gas, resulting in increased demand for water and land to cope up with the protein demand. Therefore, the human race needs to find alternative sources of proteins. Henchion. M. et al. (2017) studied different sources of proteins and their potential to provide protein in future in a sustainable manner, keeping in view all the forces like technological and environment domain and nutrition domain also consumer market domains. They found that various aspects affect the ability of the proteins current and novel sources. Current protein sources have detrimental environmental impacts and health concerns. Nevertheless, proteins provide social advantages including economic benefits and has a convictive consumer attachment. Proteins production requires the advancement of fresh value chains, easy manufacturing, low costs, food safety, scalability of the process developed, and consumer acceptance. Therefore, using an alternative protein source does not certainly says that environmental impacts of the source are acceptable and both the novel and existing source of proteins should be evaluated for better environmental impact. Thus, the study proposed that a confluence of political forces, and with an inclusive model of stakeholders in a governance role, and the rules of development and commercialisation, is required to ensure food security better.

Moreover, they are found to enhance the cell response of skin to protect from free radicals and work as an antiaging agent. Saccharomyces β-glucans were allowed by European Food Safety Authority (EFSA) as a novel dietary element has prescribed its use between fifty to two hundred milligrams in one serving. β-glucans found in spent yeast have high demand in food industry thickening agents, replacement of fats, source of fibres, viscosity increasing chemicals, films and emulsifiers. As an example, as its calories count is meagre, for an inexpensive source of fibre spent yeast can be utilized which is abundantly available, having probiotic characteristics, so it may make its way in cake and pastry world and help in developing specialised products.

Spent yeast as a source of protein for human use has a limitation as it contains high amounts of nucleic acids (six to fifteen per cent), which may result in an increase of uric acid in the tissue and blood in the form of precipitates and give rise to soaring and joint pains. This trait has limited spent yeast to the position of dietary supplements like powders, flakes, tablets, liquids, all of which are rich in bioactive components like minerals and various vitamins. For making it useful as an additive in dietary supplements, the spent yeast must pass through a debittering process to taste better. The debittering process can be carried out by treating with an aqueous alkaline solution, or by the process of distillation (water vapour) treatment by using or not using an organic solvent.

Julius, O. et al. evaluated protein single-cell of the brewer’s yeast (Saccharomyces cerevisiae), as feeding material to determine their feasibility to be used in poultry feed formulations. They made the spent yeast inactive by boiling in hot distilled water and then carried out its machine dried analysis to excellent out nutritional contents present. They found that the raw protein was 40.52% on a dry basis, and the quantity of raw crude fibre was found to be 4.31% (which was also calculated on a dry basis). The team also find the metabolizable energy of the additive yeast and found it to be 2606.07 kcal/kg [9].

Moreover, in amino acids (non-essential), they found the presence of glutamic and aspartic acid, alanine, serine, proline, and glycine in decreasing percentage order, respectively. No traces of cystic acid, methionine, and tryptophan were found. Therefore, they found that the brewers' yeast (spent) had high nutrition value, and contained vast variety of amino acids which make it a very suitable additive in the formulation of animal feeds (i.e. poultry feed, fish and birds) as a source protein, with a minor addition of methionine and tryptophan supplements (as they were not found in the spent yeast), and the soya bean can use as a source of protein could be substitued by brewer's yeast (spent) protein. This replacement of soya bean protein with spent yeast protein has also been substantiated by Carias, D and Millan, N et al. They analysed the possibility of replacement of soy-derived protein by a spent brewer's waste protein, by formulating an equal content mix of soy protein with brewer's yeast to be utilized in poultry fed as a protein source and observed it effects. It is found that a brewery waste protein can be replaced with twenty per cent soy protein by in the chicken feed resulted in no serious change in food intake and the mass of chickens, comparing with the chicken fed with soy protein. The Net Protein Ratio (NPR) and Protein Efficiency Ratio (PER) of the chicken feed were found to be comparable, and the plasma and liver lipids concentration remained almost similar in both cases. Moreover, it was found that higher quantities of spent yeast decreased the growth of chicken, whereas the total fats, triacylglycerols, and cholesterol in plasma, also cholesterol and total lipids in liver were not affected [10]. Hence, they found that spent yeast can be utilized in animal feed as a complementary protein source.

1.2. Existing methods of protein extraction from brewers spent yeast

The different method has been used in literature to extract proteins from yeast. Ge Wang (2010) studied the direct extraction of proteins from the yeast with the help of ionic liquids [11]. They studied twenty-one types of ionic liquids on the extraction efficiency of yeast proteins. For the preparation of ionic liquid, equal molar moieties of cationic and anionic liquids were mixed. The ionic liquid solutions pH values were measured and found to be in the range of 8.5 and 9.5. 3-(dimethylamino)-1-propylamine formate (ionic liquid type) showed the best extraction performance of yeast proteins. One of the best characteristics of this ionic liquid is that it can be removed effectively from the extract by using a vacuum, so it introduces the least amount of chemical impurity to the excerpt. Other ionic liquids employed to extract did not show any uptake of protein from yeast as the target concentration of the proteins were not changed after the process. In addition, the proteins that were extracted with suitable ionic liquid were tested for Western blotting by a standard method. The results tells that proteins maintained immunoreactivity and functions.

Kliss F.M. et al. worked on the extraction of surface-corelated proteins from active fungal cells. They found that lowering agents like β-mercaptoethanol and dithiothreitol which are taken to extract this protein are moderately lipophilic in nature and has a tendency to disturb plasma membrane, thereby facilitating the release of cytosolic proteins, primarily when the extraction process is carried out at high temperatures [12].

Cells were collected in the exponential phase by centrifugation technique and cleaned them with sodium phosphate buffer (cold 0.1 M, pH 8.0) and used in the further extraction process. Extraction of protiens was analysed in three different modes. Cleaned cells were incubated in either one of the three solutions. First, one being 1% v/v β-mercaptoethanol (∼0.143 M in 0.1 M sodium phosphate buffer, having pH of 8.0 at a temperature of 37 °C and for a time of 30 minutes), second solution studied was dithiothreitol (2 mM in 25 mM Tris–HCl with pH of 8.5, at a temperature of 4 ◦C and for

a time of 120 min), and the third solution studied was SDS (0.1% in 0.1 M sodium phosphate buffer with pH 8.0 at a temperature of 37 ◦C for a time of 30 min). The cells were shaken gently by a vertical turntable at 2 rpm to avoid fracture and damage caused by shrinkage of cells. The authors recommend to use less combination of reducing agent with less contact time and to perform extraction at 4 °C in order to secure the sanctity of the plasma membrane. Moreover, the authors also recommend that the Biotinylation of cell surface proteins are better to perform at low temperatures, without the use of dimethylsulphoxide.

The methods employed for extraction of proteins from yeast are labour-intensive or unreliable. Kushnirov, V et al. reported a secure and decisive method for extraction of protein for electrophoretic analysis. The yeast cells were treated with an alkali solution (mild) and is boiled in a standard electrophoresis loading buffer. Distinctive strains of Sacharomyces Cerevisiae and yeast Hansenula polymorpa DL-1 were processed by this method [13]. He found that the approach he proposed resulted in incomplete extraction of protein irrespective of the nature of the strain, conditions in which they were grown, and the molecular weight of the protein being extracted. Moreover, this method offers the benefit of functioning with less quantities of yeast cells produced over the agar plates [13].

Methias, (2017) evaluated the three kinds of wastes (solid brewery) namely spent grain, hot rub, and residual brewer's yeast, as alternative media to culture lactic acid bacteria, so that they could, in turn, evaluate the efficiency of these cultures for production of proteolytic enzyme. At first, a blend test plan was utilized to survey the impact of each residue, as well as diverse blends (with the protein substance set at 4 %) in the enzyme production. After some time, interval, the solid and liquid phases of the mix were differentiated with checking an extracellular proteolytic activity. Optimal experimental conditions were found, and after that, in a second experiment, factorial research designed was employed to find protein concentration in the solution (1 to 7 %), and fermentable sugar (glucose, 1 to 7 %). In all three wastes studied, the residual yeast was found to yield the highest concentration of enzymes by creating 2.6 U/mL of proteolytic extract in three hours. As the fermentable sugars concentration was low in the medium, the inclusion of the glucose had a boosting reaction over the yield and increased upto 4.9 U/mL of proteolytic activity. The ideal conditions of each experimenting plan were replicated, for comparing, and the enzyme mass was isolated by utilizing ethanol precipitation. The ideal medium brought about in a accelerated protein with the proteolytic action of 145.5 U/g [14].

Zhang, T. et al. proposed a new method to extract proteins from the yeast Saccharomyces cerevisiae cells. This innovative method uses lithium acetate and sodium hydroxide to increase the absorbent property of yeast cell wall before extraction of protein by using sample buffer SDS-PAGE [15]. This method was distinguished with available methods in literature and was found to be the safest method so far for protein extraction from yeast. The proteins extracted by this novel method had immunoreactive properties and were suitable for various biological applications.

Ganeva, V. et al. (2002) reported that larger output of intracellular enzymes could be achieved from the yeast in flow process by using set of electric field pulses. They found that eighty to ninety per cent of the whole task can be relieved without pre or post-treatment of cells. Their proposed approach rests on electron-induced modifies in the cell cover, resulting in drainage of a portion of intracellular proteins not development of any loose particles, and offers process flexibility in scale up to process large volumes. Moreover, the field variables needed a limited supply of electrical power. A minimum of 20% wet weight suspensions can be handled by this process. The optimal field parameters should

be tuned to the concentration of the suspension. A plentitude of yield enzymes is observed within four hours and at 30°C from Saccharomyces cerevisiae. β-D-galactosidase extraction from Kluyveromyces lactic continues for ten hours but can be expedited by the addition of dithiothreitol in the post-pulse environment [16]. The specific activities of the electro-extracted enzymes were found to be greater than those observed by enzymatic lysis or mechanical extraction.

Chae, H.J. et al. produced yeast extract from brewer's yeast by uniting enzymatic treatments utilizing the enzymes exoprotease, endoprotease, adenosine monophosphate (AMP)-deaminase, and 5'-phosphodiesterase. The overall impact of the enzyme mix, quantity, and steps followed during the treatment on the restoration of solids and protein, flavour and structural characteristics were inspected.

From the beer factory to carry on experiments dried brewer's yeast was collected. Two varieties of protease were employed in the hydrolysis of protein, namely from Bacillus licheniformis ProtamexTM (398.7 mAU/g, Endoprotease), and Flavourzyme TM (which is a complex of an enzymes from aspergillus oryzae with greater exoprotease (1046.1 LAPU/g), and lesser endoprotease activity (92.3 mAU/g)). Protein FNTM was used as it is having both endoprotease action and exoprotease action. Enzyme RP-1 was asscessed from the supplier. As an AMP-deaminase, Deamizyme was used which actually originated from Aspergillus melleus. In 120 ml of deionized water dried yeast was introduced to make 20 % (w/w) concentration. The mix was kept in a temperature-controlled environment. The initial pH of the mix was tuned for 6.5 by the addition of 10 N sodium hydroxide and at 95°C, this combination was heated for 5 minutes. Solution was cooled after the heating, and Protamex with Flavourzyme (0.6±2.0%) both were put to the mix combined. Every time, the amount of enzyme fed was determined on the basis of content of the protein. Reaction mixture was shaken, and hydrolysis of the mix took place at 50°C for about 12 hours. To find out the solids and contents of protein in hydrolysate, aliquots of reaction mixture of 2 ml were extracted and heated at 95°C for about 5 min and centrifuged for a time of 20 min (10,000 multiplied by the mass in grams of the sample (rpm) at 4°C). Enzyme RP-1 was put up into the mixture with various concentrations (0.015, 0.03, 0.04, 0.05%) to hydrolyze RNA after the protein hydrolysis with Protomex and flavourzyme for 12 hours continued by heat treatment to stop residual protease activity. Enzyme quantity is estimated from reliable content of the hydrolysate. The pH value (initial) of the mix was set to pH 5.5 (which is the best pH value for RP-1 enzyme) with the help of a 5N NaOH solution. Nuclease treatment was carried out at 60°C about 3 hours. In addition to this, at pH 5.5 by Deamizyme (0.03%) the mixture was treated, and at a temperature of 45°C for 2 hours. Once all of the enzymatic treatments are done, at 95°C for 5 min the hydrolysate was heated, centrifuged, the top layer of yeast extract was separated and used for further experimentation. It was found that the amount of Exoprotease greatly influenced the protein recovery, sensory characteristics and the degree of hydrolysis.When yeast cells were treated with the best-determined mixture of exoprotease and endoprotease (0.6% Flavourzyme and 0.6% Protamex), it results in high recovery of solids (48.3-53.1%), and the best flavour. In this way, in different sorts of treatment sequences utilizing different enzymes the treatment with protease (taken after by nuclease) gave the highest content of 5'-guanosine monophosphate (5'-GMP). 0.03% was found out to be the best concentrations of both AMP-deaminase and 5'-phosphodiesterase Therefore, it was observed that after treating the solution with the ideal combination of enzymes with its dosages, and the sequence of treatment for four proteins, a big stable yield of 55.1%, and 3.67% content of 5'-nucleotides were obtained [17].

In another study, Tangular, H. et al. studied induced autolysis by incubating the spent brewer's yeast cell suspensions at increasing temperatures of 45°, 50°, 55° and 60 °C, with varying a reaction time from 8 - 72 hours. Spent brewer's yeast was used in the experiments and it got as a by-product of a lager production. Spent brewer's yeast was bought to the laboratory for experimenting which was collected from the fermentation vessel after the end of 4th fermentation. In order to remove the beer liquor the spent brewer's yeast slurry was centrifuged for 10 min at 11,000 g, and 4 °C. To yeast paste, distilled water (sterilized) was added, and the pH level was balanced to 9 with help of NaOH solution. For 30 minutes at 4°C, the suspension was mixed and with sterilized distilled water for 2-3 times it was washed till the pH of the mix became neutral. Once the pH is neutralized, to attain brewer's yeast cells debittered, the suspension was mixed and centrifuged 11,000 g for 10 minutes at 4 °C. Yields and contents of stable protein, α-amino nitrogen, and carbohydrate were found. As a result, it was observed that the most favourable time and temperature for the yeast extract production were 50 °C for 24 hours based on the contents of protein and α -amino nitrogen. With powder of yeast extract sensory analysis was carried out and 0.5% and 1.0% yeast extract added samples showed overall high acceptance [18].

Eunice, A et al. compared the compositional, nutritional, and functional aspects of the whole yeast (WY) cells from a brewery with those of a phosphorylated protein concentrate (PPC), made from the identical cells. Saccharomyces cerevisiae yeast is a leading source of minerals and proteins also soluble fibres. The yeast biomass (S. cereVisiae) was collected as 20% cells (w/v) suspension from a brewery. This suspension was modified with an equal volume of water and by plate centrifuge it was centrifuged, where a slurry of biomass and lean solution was obtained. This slurry was spray-dried at 180°C. The separation of a PPC had mechanically broken cell walls, and to remove loose particulates centrifugation was followed, next with STMP the phosphorylation at alkaline pH, followed by phosphorylated protein precipitation at acidic pH. The cell wall of the solution containing the 10 per cent (w/v) cell fraction with a pH of 9.5 was smashed with a 2N NaOH solution, which was powered through a mill at the flow rate of 4.8L h-1 with 2400 rpm at a by a peristaltic pump. For the operation, 0.6mm to 0.9mm glass spheres were filled in the mill chamber for 70%. During operation 25°C cell suspension was kept and by double jacket chamber water/ethylene glycol mixture was circulated. Centrifugation was done to cracked suspension of cell, pH of soluble fraction was adjusted to 11 with 2 N NaOH solution and is treated with the food grade STMP at 35°C for 3 hours with concentration of 4% (w/w/ total solids). Then for pH 3.2 phosphorylated protein was acidified (3 N HCl) and then centrifuged. Resulting precipitate obtained was washed with distilled water (acidified) twice, and is diluted with distilled water, modified with NaOH to pH 7.0, and then freeze-dried. By not effecting the properties of emulsion of meat products the PPC and soy products replaced 20% or 40% chunk roll protein [19].

Podpora, B. et al. worked on obtaining autolysates which were extracted from brewer's yeast (spent), and showed their characteristics and properties as natural and outstanding ingredient, for the production of supplements (dietary) for functional foods. They worked on the yeast Saccharomyces cerevisiae. In the autolysates produced, they studied the analyses of proteins, dry matter, and quantity of amino acids, molecular weight evaluation of proteins, anti-oxidant characteristics, and sensory traits. They found that the analysed autolysates contained more amount of amino acids that are essential, that were more than an amount present in the reference proteins, which have been developed by United Nations Food and Agriculture Organization or WHO (World Health Organization). The sensory characteristics were the analysed autolysates was found to be a function of the extent of the

yeast protein autolysis process, that sets their use in the foood production. They controlled the time precisely during the process of autolysis, which helped in reaching the designed functional traits for a product [20]. Therefore, they concluded that the post-fermentation yeast or spent yeast might serve as an excellent raw material for production of yeast autolysates.

Nitrogen analysis method

The traditional method followed to find the protein contents of a particular food is to find out the amount of total nitrogen present. in the For past some years, foods protein content is determined based on complete nitrogen content as in Kjeldahl (or similar) method. The complete content of nitrogen is multiplied by a factor to determine the content of protein in the food. For this calculation to be accurate, two assumptions are hidden in the method, i.e. that fats and carbohydrates they do not possess any nitrogen, and all amount of nitrogen present in food is in the form of amino acids in proteins. According to early studies, the nitrogen (N) average fraction of proteins was determined about 16 %, that introduced the factor of 6.25 by calculating 6.25*N (1/0.16 = 6.25) for the conversion of nitrogen content into protein.

This factor of 6.25 has two limitations. First is that not all the nitrogen is present in the proteins and there is a substantial amount of nitrogen present in other forms such as non-protein nitrogen (NPN), and for synthesis (non-essential) amino acids only a minute fraction of NPN is available. Secondly, the nitrogen fraction (weight basis) of a given amino acids changes with the molecular weight and number of nitrogen atoms in it (which varies from 1 to 4 with different amino acids). Therefore, these facts led to the fact that proteins nitrogen content varies roughly between 13% to 19 %, thus proving that the conversion factors (N) range from 5.26 (1/0.19) to 7.69 (1/0.13) for different amino acids. The factor N x 6.25 was later replaced by Nxa; which changed with every food under analysis. It is now mentioned as “Jones factors”, and is broadly accepted for protein calculations. Proteins are composed of amino acids long chains attached by peptide linkages. They could be converted back to amino acids component by hydrolysis, and the concentration of constituent amino acids can be calculated by analytical techniques like ion-exchange, liquid chromatography etc. The sum of these amino acids shows the content of proteins (by weight) of the food. Sometimes it is mentioned as a “true protein”. The benefit of correct protein method is that it does not require Jones factor for determination of protein content as no information of NPN content or relative proportion of specific amino acids. The downside of this strategy is that it required more advanced equipment than the Kjeldahl way, so it may be past the capacity of numerous research facilities, particularly those that carry out only seasonal anaylses. Additionally, it is necessary to involve in this; some amino acids like tryptophan sulphur-containing amino acids are more challenging to find out as compared to others. In spite of the problems of analysis of amino acid, generally, there has been sensibly great understanding among the research facilities and strategies [21].

Filtration process analysis

The application of a pressure difference has an impact on filtration, which can be formed by pressurised fluid, centrifugal force or by a vacuum and gravity. Commonly, in the process of pressure filtration to deliver suspension to the filter pump is needed. Same way vacuum pump is needed for vacuum filtration. The pump is utilized to empty the gas from a filtrate recipient, where the filtrate is separated from the gas. At that point this filtrate is drained either with the assistance of a barometric leg of at slightest 8 to 10 m or by a pump that can run on wheeze. At times, the liquid is permitted to

stream through the filter medium only by gravity (gravity filtration). Perforated centrifuge rotors are used for the process of centrifugal filtration. Cake is openly approachable in vaccum filters it assists in automatic handling of cake. But with high vapour pressure hot liquids cannot be handled by vaccum filters. The distinction in pressure over vacuum filters is exceptionally limited, and the remaining moisture of the filter cake is greater than pressure filters. High-pressure variations is permitted by ressure filters. Pressure filters are thought preferable when the product is kept in a closed framework for security reasons, or in case the remaining moisture content is noteworthy.It is more challenging to handle the filter cake in a pressure filter. With centrifugal force, filtration process needs more technical equipments, but it gives solids with lesser residual moisture [22].

2. Methodology

After analysing the existing methods for protein extraction from yeast such as electrophoretic analysis, autolysis, yeast with ionic liquids and hydrolysis. The hydrolysis at different temperatures was selected for this research as this method extract proteins effectively from the yeast.

Hydrolysis is a decomposition process where one of the reactants is water, i.e., used to break the chemical bonds in the other reactant. Hydrolysis is the reverse reaction of condensation in which two molecules combine to form another product [19]. By analysing the existing processes for protein extraction from yeast, Hydrolysis is selected for the protein extraction from yeast. The yeast is hydrolysed in the reactor with lactic acid as a catalyst for hydrolysis to breakdown the chemical bonds and to extract the maximum value of proteins. The lactic acid plays an important role in fermentation and preserving of food consumables which is also inexpensive and often little or no heat required in their preparations. In this research Lactic acid of 2% and 4% concentrations are used for hydrolysis process with yeast.

2.1. Sample preparation

The yeast vivo sample which is bakers yeast with a model feedstock that has similar properties of brewers yeast was selected for conducting the hydrolysis experiment; the sample was taken and weighed for 62 grams (± 1%). The lactic acid with 2% and 4% concentration was made by mixing with demineralized water, which weighs about 336 grams (±2%) in the beaker. The dry matter and nitrogen analysis were conducted for the yeast sample to know the percentage of moisture, dry matter, and nitrogen present. The values obtained from dry matter tests are shown below (Table 1), and the nitrogen present in yeast was determined which was 7.94%. The weighed yeast sample was added to the lactic acid of 2% and 4%, respectively, and this mixture was set up for the hydrolysis process.

Table 1. Dry matter values of yeast sample

Sample	Weight (gms)	Moisture %	Dry matter %
Sample-1	2.52	68.05	31.94
Sample-2	1.94	67.33	32.66

2.2. Hydrolysis

The solution of yeast and lactic acid was correctly mixed and placed in the hydrolysis reactor, and the temperature sensor was placed to know the temperature change in the reactor, and the heating system was switched on. The hydrolysis was done for different temperatures (100,120 & 140° C) with different samples. The reactor was set to the required temperature with gradually increasing orders, at one necessary temperature, the motor was switched on, the water supply provides the cooling to the adhesive in the reactor magnetic drive. The hydrolysis was carried out for 5 hours, and change in temperature values was noted in different time intervals, then the system was turned off. After the completion of hydrolysis, the reactor was cooled to 65° C by the fan for decreasing the pressure inside the reactor. Then the reactor was opened, and the reaction mixture was transferred to a beaker with proper mixing, after which the mixture was weighed. This process was repeated for three samples of yeast with 2% lactic acid, and three samples of yeast with 4% lactic acid at 100,120,140 °C as each experiment was carried out for 5 hours, respectively.

The Hydrolysis pressure reactor shown is shown below (Fig. 1) which has the pressure meter (1) on the top to show the change in pressure inside the reactor and a heating coil (2) below to provide heating supply to the reactor (3) and Thermocouple (4) which gives the value of temperature inside the reactor, this total setup was held up vertically with a stand.

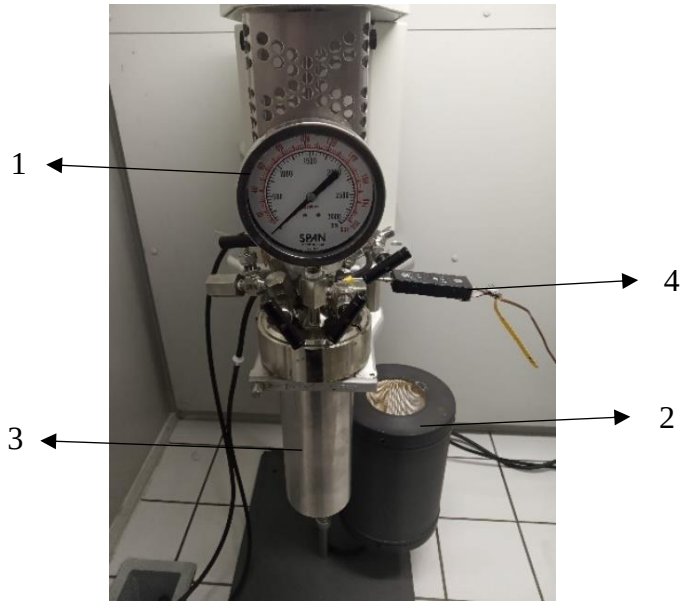

Fig. 1. Hydrolysis equipment

2.3. Vacuum and Pressure Filtration

As there are many types of filtration processes like Gravity, Mechanical, Vacuum and Pressure filtration to filtrate the obtained reaction mixture. In this research for obtained mixture two types of filtrations were conducted i.e. Vacuum and Pressure filtration to increase the rate of filtration by the vacuum and extra pressure in the pressure filtration. The reaction mixture obtained after the hydrolysis process was weighed and added 2.5 % Diatomaceous earth powder (K70) to the total value obtained to fasten the filtration, then the mixture was cooled to 65 °C for the filtration process, i.e., both the filtration was carried out at 65°C.

For the vacuum filtration (Fig. 2), a beaker with the rubber cork was fixed and connected to the filtration equipment, which in turn was connected to the vacuum cylinders. The vacuum filtration was carried out at -100 mbar pressure, and that helped create the pressure inside the beaker for the more easy filtration process. The mixture was poured in the top ceramic holding, which has the filter paper in it through which the mixture was filtered into the beaker. After completion of the filtration, the filter cake remains in the ceramic holding and filtrate in the glass cup.

For the pressure filtration (Fig. 2), the mixture was placed in the holder above the cylindrical reactor, which in turn was connected to the nitrogen outlet pipe. The pressure of +0.5 bars was maintained inside the cylinder. The mixture which was filtered was collected in the conical flask placed below. The time taken to manage different volumes of the filtrate was noted using a stopwatch for both filtration processes.

Fig. 2. Vacuum (1) and Pressure (2) filtration setup

2.4. Nitrogen Analysis

The amount of protein present in the sample can be detected using different methods such as Bradford assay and Lowry assay. In this research, the Dumas method has been used to detect the amount of protein percentage in the yeast sample. The protein percentage present in the filtrate of the different samples was calculated using the values obtained from nitrogen test for the filtrate samples i.e. Protein percentage = 6.25× Percentage of Nitrogen.

After the filtration was completed, the obtained filtrate and the filter cake from pressure filtration was weighed, and values were noted. The dry matter test was conducted for different samples of filtrate, and filter cake obtained. Then the Filtrate and filter cake was placed in the oven for drying and after all the moisture present was evaporated nitrogen tests were conducted using below equipment (Fig.3) for different samples. The remaining examples after all test were laminated and stored for future investigations. This process is repeated for all the six samples of filtrate and filter cake which was obtained from six different hydrolysis processes.

Fig. 3. Nitrogen testing equipment (flash 2000)

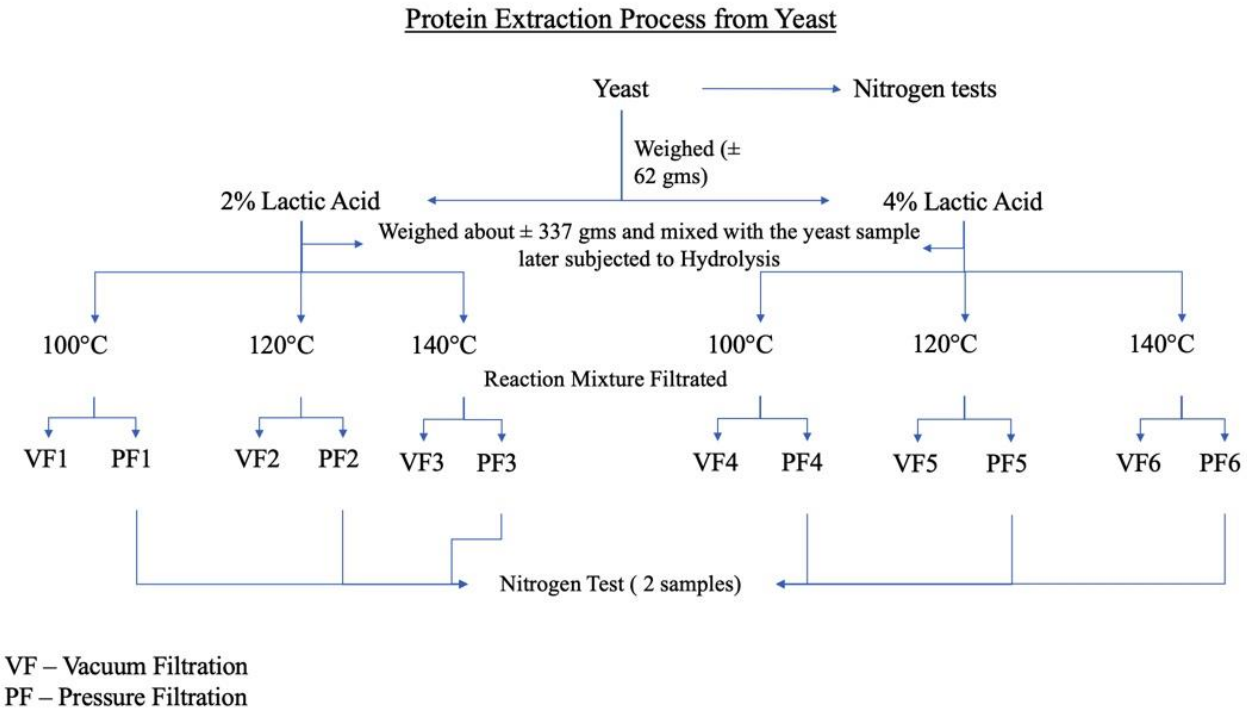

Fig. 4. Flow chart of experiments carried out

The above figure shows the series of experiments conducted in this research i.e. yeast with lactic acid of 2 and 4% which is hydrolysed at 100,120,140°C for 6 samples of yeast and the filtration process was conducted. The filtrate obtained from the filtration process was analysed for nitrogen test and through which the protein percentage was been calculated.

3. Results and Analysis

3.1. Yeast Hydrolysis 01

In this Experiment, Yeast was hydrolysed at 100 °C. The 61.33 gms of Yeast was mixed with a Lactic acid of ~2% concentration weights about 336.32gms. The mixed mixture is then placed in the reactor, and Hydrolysed at 100 °C and values are noted.

Table 2. Hydrolysis of 2% lactic acid with yeast at 100 ° C

Time	Temperature [°C]	Note
7:20	22 °C	The mixture placed in the reactor
7:37	75 °C	Start heating Set 90 ° C
7:45	95 °C	Set 100 ° C
8:15	102 °C	
8:40	100 °C	Motor is ON
10:07	100 °C	
11:02	100 °C	
12:50	100 °C	The heater is off and Colling by a fan
13:20	65 °C	Cooled to 65 °C

The reaction mixture obtained m_{rs} = 695.12g – 302.62g (beaker) = 392.5g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture was cooled to 65 °C, and for faster filtration process, 2.5% of Diatomaceous earth has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (VF1)

The weight of the reaction mixture m_{rs}= 100.06g

Kremelina 2.5 % = 2.51g

Mass of filter paper m_{fp} = 0.81g

Weight of total mixture = 103.38g

Filtration was carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper is used. The volume of the filtrate obtained is noted with the timings below.

Table 3. Vacuum filtration of hydrolysis 01

Filtrate [ml]	Time [min:sec]
10	1:01
20	2:24
30	3:52
40	6:02
50	8:23
60	12:32
70	17:03
80	24:37

The mass of Filtrate obtained, m_{VF1} = 192.90g – 105.03g (beaker) = 87.87g

The mass of Filter cake, m_{VFK1} = 55.34g – 43.96g (plate) = 11.38g

Pressure Filtration (PF1)

The weight of the reaction mixture = 292.12 g

Kremelina 2.5 % = 7.30 g

Total mixture = 299.42 g

Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained is noted with the timings below.

Table 4. Pressure filtration of hydrolysis 01

Filtrate [ml]	Time [min:sec]
25	2:42
30	4:07
35	6:13
40	9:08
55	21:21
65	31:22
75	41:38

The mass of Filtrate obtained, m_{PF1} = 191.79g – 115.48g (beaker)= 76.31g

The mass of Filter cake, m_{PFK1} = 50.16g – 40.05g (plate) = 10.11g

Dried Filter cake and Filtrate.

The filtrate and filter cake obtained from the filtration process is dried in the oven till all the water evaporates, then the Nitrogen test has been conducted for the detection of Nitrogen content in that

and compared with the standard Yeast taken, and Protein content will be calculated from the Nitrogen percentage.

The dried filtrate and filter cakes are shown in the below picture, and their weights are noted.

Weight of the filter cake from VF_{1dry} = 4.99g, PF_{1dry} = 4.09g

The dry matter is conducted for the filtrate obtained from both the filtrations of hydrolysis 01.

Dry matter present in the filtrate of VF_1= 3.66%

Dry matter present in the filtrate of PF_2= 3.52%

Fig. 5. Dried Filtrate and Filtercake of Hydrolysis 01

3.2. Yeast Hydrolysis 02

In this Experiment, Yeast was hydrolysed at 100 °C. The 61.76 gms of Yeast were mixed with a Lactic acid of ~4% concentration weights about 337.55 gms. The mixed mixture will be placed in the reactor, and Hydrolysed at 100 °C and values are noted.

Table 5. Hydrolysis of 4% lactic acid with yeast at 100 ° C

Time	**Temperature [°C]**	**Note**
6:32	20 °C	The mixture placed in the reactor
6:40	20 °C	Start heating Set 90 ° C
7:06	103 °C	Set 100 ° C
7:25	100 °C	
7:36	100 °C	Motor is ON
11:00	100 °C	
12:08	100 °C	The heater is off and Colling by a fan
12:28	68 °C	

The reaction mixture obtained m_{rs} = 701.93g + 9.87g (K70) – 305.63g (beaker weight) = 406.11g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture was cooled to 65 °C, and for faster filtration process, 2.5% of Kremelina has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (VF2)

Weight of total mixture m_{rs} = 86.57g

Filtration is carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper was used. The volume of the filtrate obtained is noted with the timings below.

Table 6. Vacuum filtration of hydrolysis 02

Filtrate [ml]	**Time [min:sec]**
10	0:52
20	2:50
30	4:27
40	7:49
50	10:50
60	13:34
70	19:44
80	23:56

The mass of Filtrate obtained, m_{VF2} = 179.58g – 106.05g (beaker) = 73.53g

The mass of Filter cake, m_{VFK2} = 55.43g – 45.93g (plate) = 9.5g

Pressure Filtration (PF2)

The total weight of the reaction mixture m_{rs} = 84.37g

Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained is noted with the timings below.

Table 7. Pressure filtration of hydrolysis 02

Filtrate [ml]	**Time [hr:min:sec]**
25	5:05
30	8:15
35	11:45
40	16:30

55	36:24
65	49:40
70	1:00:37

The mass of Filtrate obtained, m_{PF2}= 175.13g – 106.84g (beaker)= 68.29g

The mass of Filter cake, m_{PFK2} = 54.08g – 44.11g (plate) = 9.97g

Dried Filter cake and Filtrate.

The dried filtrate and filter cakes are shown in the below picture, and their weights are noted.

Weight of the filter cake from VF_{2dry} = 4.32g, PF_{2dry} = 3.84g

The dry matter test was conducted for the filtrate obtained from both the filtrations of hydrolysis 02.

Dry matter present in the filtrate of VF_2 = 4.21%

Dry matter present in the filtrate of PF_2 = 4.27%

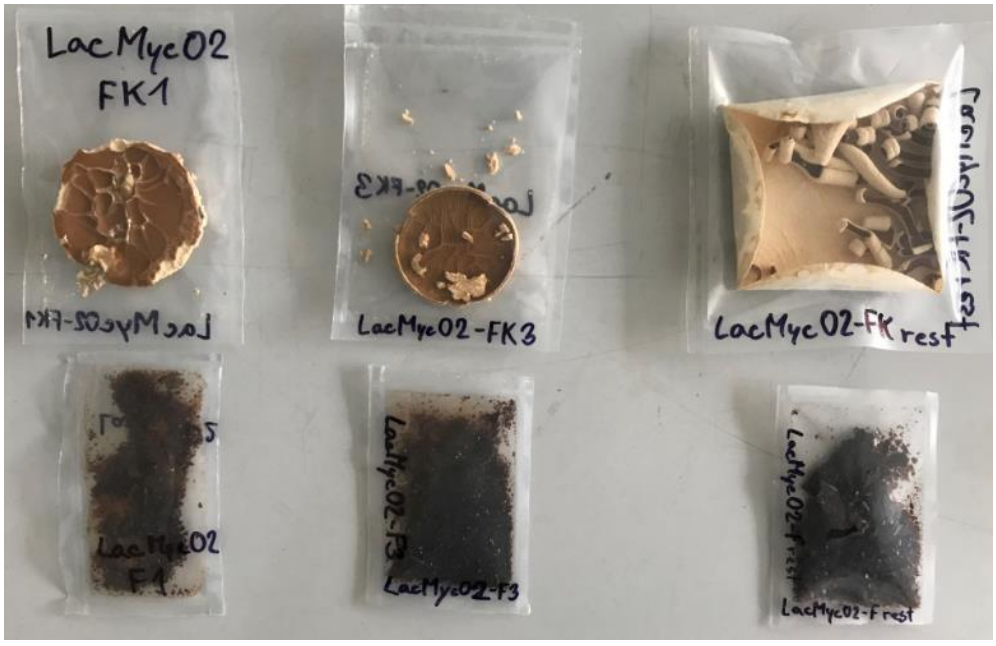

Fig. 6. Dried Filtrate and Filtercake of Hydrolysis 02

3.3. Yeast Hydrolysis 03

In this Experiment, Yeast hydrolysed at 120 °C. The 62.06 gms of Yeast were mixed with a Lactic acid of ~2% concentration weights about 336.85 gms. The mixed mixture will be placed in the reactor, and Hydrolysed at 120 °C and values are noted.

Table 8. Hydrolysis of 2% lactic acid with yeast at 120°C

Time	Temperature [°C]	Note
7:15	20 °C	The mixture placed in the reactor
7:20	20 °C	Start heating Set 90 ° C
7:45	103 °C	Set 110 ° C
7:51	111 °C	Set 118 °C
8:02	120 °C	Set 120 °C
8:32	121 °C	Motor is ON
10:01	120 °C	
12:22	120 °C	
13:02	120 °C	The heater is off and Colling by a fan
13:26	70 °C	

The reaction mixture obtained m_{rs} = 701.14g + 9.8g (K70)-395.51g (beaker) = 405.35g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture is cooled to 65 °C, and for faster filtration process, 2.5% of Kremelina has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (VF3)

The total weight of the reaction mixture m_{rs}= 86.02g

Mass of Filter paper m_{fp}= 0.81g

Filtration is carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper is used. The volume of the filtrate obtained is noted with the timings below.

Table 9. Vacuum filtration of hydrolysis 03

Filtrate [ml]	Time [hr:min:sec]
10	5:50
20	19:30
30	36:18
40	52:15
50	1:11:10
80	3:00:00

The mass of Filtrate obtained, m_{VF3} = 180.51g – 105.96g (beaker) = 74.55g

The mass of Filter cake, m_{VFK3} = 53.55g – 43.95g (plate) = 9.6g

Pressure Filtration (PF3)

The total weight of the reaction mixture = 72.19g

Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained is noted with the timings below.

Table 10. Pressure filtration of hydrolysis 03

Filtrate [ml]	**Time [hr:min:sec]**
25	31:19
30	44:11
35	59:52
40	1:19:38
50	1:52:19

The mass of Filtrate obtained, m_{PF3} = 163.22g – 102.54g (beaker weight)= 60.68g

The mass of Filter cake, m_{PFK3} = 49.45g – 43.92g (plate weight) = 5.53g

Dried Filter cake and Filtrate

The dried filtrate and filter cakes are shown in below picture, and their weights are noted.

Weight of the filter cake from VF_{3dry} = 3.66g, PF_{3dry} = 2.65g

The dry test matter was conducted for the filtrate obtained from both the filtrations of hydrolysis 03.

Dry matter present in the filtrate of VF_3= 4.95%

Dry matter present in the filtrate of PF_3= 4.27%

Fig. 7. Dried Filtrate and Filtercake of Hydrolysis 03

3.4. Yeast Hydrolysis 04

In this Experiment, Yeast was hydrolysed at 120 °C. The 62.02 gms of Yeast were mixed with a Lactic acid of ~4% concentration weights about 338.02 gms. The mixed mixture will be placed in the reactor, and Hydrolysed at 120 °C and values are noted.

Table 11. Hydrolysis of 4% lactic acid with yeast at 120 ° C

Time	Temperature [°C]	Note
6:25	18 °C	The mixture placed in the reactor
6:33	20 °C	Start heating Set 100 ° C
7:03	118 °C	Set 120 ° C
7:17	120 °C	
7:50	120 °C	Motor is ON
11:50	120 °C	
12:20	120 °C	The heater is off and Colling by a fan

The reaction mixture obtained m_{rs} = 701.36g + 9.89g (K70) – 305.63g (beaker) = 405.62g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture was cooled to 65 °C, and for faster filtration process, 2.5% of Kremelina has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (VF4)

The total weight of the reaction mixture m_{rs}= 86.90 g
Mass of filter paper m_{fp} = 0.81g
Filtration is carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper was used. The volume of the filtrate obtained is noted with the timings below.

Table 12. Vacuum filtration of hydrolysis 04

Filtrate [ml]	Time [hr:min:sec]
10	5:50
20	18:30
30	42:40
40	1:14:01
50	1:25:42
70	6:54:42

The mass of Filtrate obtained, m_{VF4} = 7.63g
The mass of Filter cake, m_{VFK4} = 9.17g

Pressure Filtration (PF4)

The total weight of the reaction mixture = 84.18g
Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained is noted with the timings below.

Table 13. Pressure filtration of hydrolysis 04

Filtrate [ml]	Time [hr:min:sec]
25	31:19
30	44:11
35	59:52
40	1:19:38
75	6:56:48

The mass of Filtrate obtained, m_{PF4} = 76.93g

The mass of Filter cake, m_{PFK4} = 5.80g

Dried Filter cake and Filtrate

The dried filtrate and filter cakes are shown in the below picture, and their weights were noted.

Weight of the filter cake from VF_{4dry} = 3.48g, PF_{4dry} = 3.16g

The dry matter was conducted for the filtrate obtained from both the filtrations of hydrolysis 04.

Dry matter present in the filtrate of VF_4= 5.99%

Dry matter present in the filtrate of PF_4= 5.95%

Fig. 8. Dried Filtrate and Filtercake of Hydrolysis 04

3.5. Yeast Hydrolysis 05

In this Experiment, Yeast was hydrolysed at 140 °C. The 62.01 gms of Yeast were mixed with a Lactic acid of ~2% concentration weights about 337.71 gms. The mixed mixture will be placed in the reactor and Hydrolysed at 140 °C (~+2.6 bar), and values are noted.

Table 14. Hydrolysis of 2% lactic acid with yeast at 140 ° C

Time	Temperature [°C]	Note
7.30	18 °C	The mixture placed in the reactor
7:40	20 °C	Start heating Set 130 ° C
8:16	129 °C	Set 138 ° C
8:26	140 °C	Set 140
9:00	140 °C	Motor is ON
10:55	140 °C	
13:03	140 °C	
13:30	140 °C	The heater is off and Colling by a fan

The reaction mixture obtained m_{rs} = 700.5g + 9.87g (K70) – 305.62g (beaker) = 404.82g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture was cooled to 65 °C, and for faster filtration process, 2.5% of Kremelina has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (V F5)

The total weight of the reaction mixture m_{rs}= 91.43g

Mass of filter paper m_{fp} = 0.17g, Filtration is carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper was used. The volume of the filtrate obtained is noted with the timings below.

Table 15. Vacuum filtration of hydrolysis 05

Filtrate [ml]	Time [min:sec]
10	00:06
20	00:11
30	00:16
40	00:18
50	00:23
60	00:34
70	00:41
80	00:45

The mass of Filtrate obtained, m_{VF5}= 185.03 – 102.54g (beaker) = 82.49g

The mass of Filter cake, m_{VFK5} = 59.70g – 52.70g (plate) = 7.0g

Pressure Filtration (PF5)

The total weight of the reaction mixture = 108.14g

Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained is noted with the timings below.

Table 16. Pressure filtration of hydrolysis 05

Filtrate [ml]	**Time [min:]**
25	00:10
50	00:19
75	00:29
90	00:45

The mass of Filtrate obtained, m_{PF5} = 191.42g – 100.42g (beaker)= 91.00g

The mass of Filter cake, m_{PFK5} = 52.40g – 46.10g (plate) = 6.30g

Dried Filter cake and Filtrate

The dried filtrate and filter cakes are shown in the below picture, and their weights are noted.

Weight of the filter cake from VF_{5dry} = 3.1g, PF_{5dry} = 2.98g

The dry matter is conducted for the filtrate obtained from both the filtrations of hydrolysis 05.

Dry matter present in the filtrate of VF_5= 5.34%

Dry matter present in the filtrate of PF_5= 5.07%

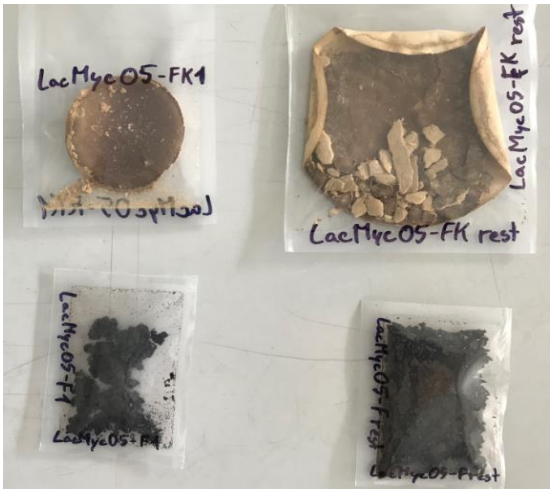

Fig. 9. Dried Filtrate and Filtercake of Hydrolysis 05

3.6. Yeast Hydrolysis 06

In this Experiment, Yeast was hydrolysed at 140 °C. The 61.99 gms of Yeast are mixed with a Lactic acid of ~4% concentration weights about 337.94 gms. The mixed mixture will be placed in the reactor and Hydrolysed at 140 °C (~+2.6 bar), and values are noted.

Table 17. Hydrolysis of 4% lactic acid with yeast at 140 ° C

Time	Temperature [°C]	Note
7:28		The mixture placed in the reactor
7:33	21 °C	Start heating Set 135 ° C
8:13	133 °C	Set 139 ° C
8:25	139 °C	Set 140 ° C
8:57	140 °C	Motor is ON
10:00	140 °C	
12:00	140 °C	
13:25	140 °C	The heater is off and Colling by a fan

The reaction mixture obtained m_{rs} = 701.82g + 9.90g (K70) – 305.60g (beaker weight) = 406.12g

Filtering the reaction mixture

The reaction obtained was filtrated using Vaccum and Pressure Filtration, and the timing was noted. The filtration processes were carried out once the reaction mixture is cooled to 65 °C, and for faster filtration process, 2.5% of Kremelina has been added according to the weight of the reaction mixture taken for the filtration process.

Vacuum Filtration (VF6)

The total weight of the reaction mixture m_{rs}= 94.6g

Mass of filter paper m_{fp}= 0.17g

Filtration is carried at 65 °C and -100 mbar excess pressure on the filter device. For the filtration process, KA1of 55mm diameter filter paper was used. The volume of the filtrate obtained is noted with the timings below.

Table 18. Vacuum filtration of hydrolysis 06

Filtrate [ml]	Time [min:sec]
20	00:10
30	00:15
50	00:22
70	00:46
80	00:58

The mass of Filtrate obtained, m_{VF6} = 192.75 – 107.04g (beaker) = 85.71g

The mass of Filter cake, m_{VFK6} = 50.54g – 44.11g (plate) = 6.43g

Pressure Filtration (PF6)

The total weight of the reaction mixture = 86.97g

Filtration is carried out at 65 °C and 1/2 bar excess pressure on the filter device. The volume of the filtrate obtained was noted with the timings below.

Table 19. Pressure filtration of hydrolysis 06

Filtrate [ml]	**Time [min:sec]**
25	00:04
50	00:09
70	00:17
90	00:22

The mass of Filtrate obtained, m_{PF6} = 179.00g – 105.60g (beaker)= 73.40g

The mass of Filter cake, m_{PFK6} = 47.58g – 42.78g (plate) = 4.80g

Dried Filter cake and Filtrate

The dried filtrate and filter cakes are shown in the below picture, and their weights are noted.

Weight of the filter cake fromVF_{6dry} = 3.15g, PF_{6dry} = 2.36g

The dry matter was conducted for the filtrate obtained from both the filtrations of hydrolysis 06.

Dry matter present in the filtrate of VF_6= 6.17%

Dry matter present in the filtrate of PF_6= 5.83%, F3= 6.23%

Fig. 10. Dried Filtrate and Filtercake of Hydrolysis 06

4. Filtration Analysis

4.1. Vacuum Filtration analysis

The vacuum filtration was conducted for the reaction mixture obtained from the hydrolysis process. The reaction mixture was maintained at 65°C before starting of the filtration process and the filtration process was conducted in -100 mbar pressure, the time taken for filtration processes was calculated using a stopwatch. The below graph is plotted by the values obtained from the Vacuum filtration process of 2% and 4% lactic acid with yeast hydrolysed at 100°C,120°C, and 140°C. In this logarithmic graph, time taken for filtration is plotted against the volume of the filtrate collected. The hydrolysis experiments are shown in different coloured bars for better understanding.

In comparison with all the Vacuum filtration, the time taken by VF 1,2,3& 4 is too high compared to VF 5&6. The filtration time taken by VF 1&2 was 24 minutes in collecting 80 ml of filtrate whereas VF 3 took 3 hours in collecting the 80 ml of filtrate, VF 4 took almost 7 hours to obtain the same amount of filtrate. The time taken by VF 5&6 was too low compared to all others. It took less than a minute to filtrate 80ml of the mixture. When VF 5&6 are compared, VF 5 took 45 seconds, whereas VF 6 took 58 seconds to filtrate 80ml.

The time to collect 80 ml of the filtrate through the vacuum filtration was minimum for VF-05, i.e., Yeast hydrolysed with lactic acid at 140 °C in the reactor for 5 hours. Therefore VF 5 has taken the minimum amount of time, and VF 4 took the maximum time for the filtration of the filtrate of the same volume.

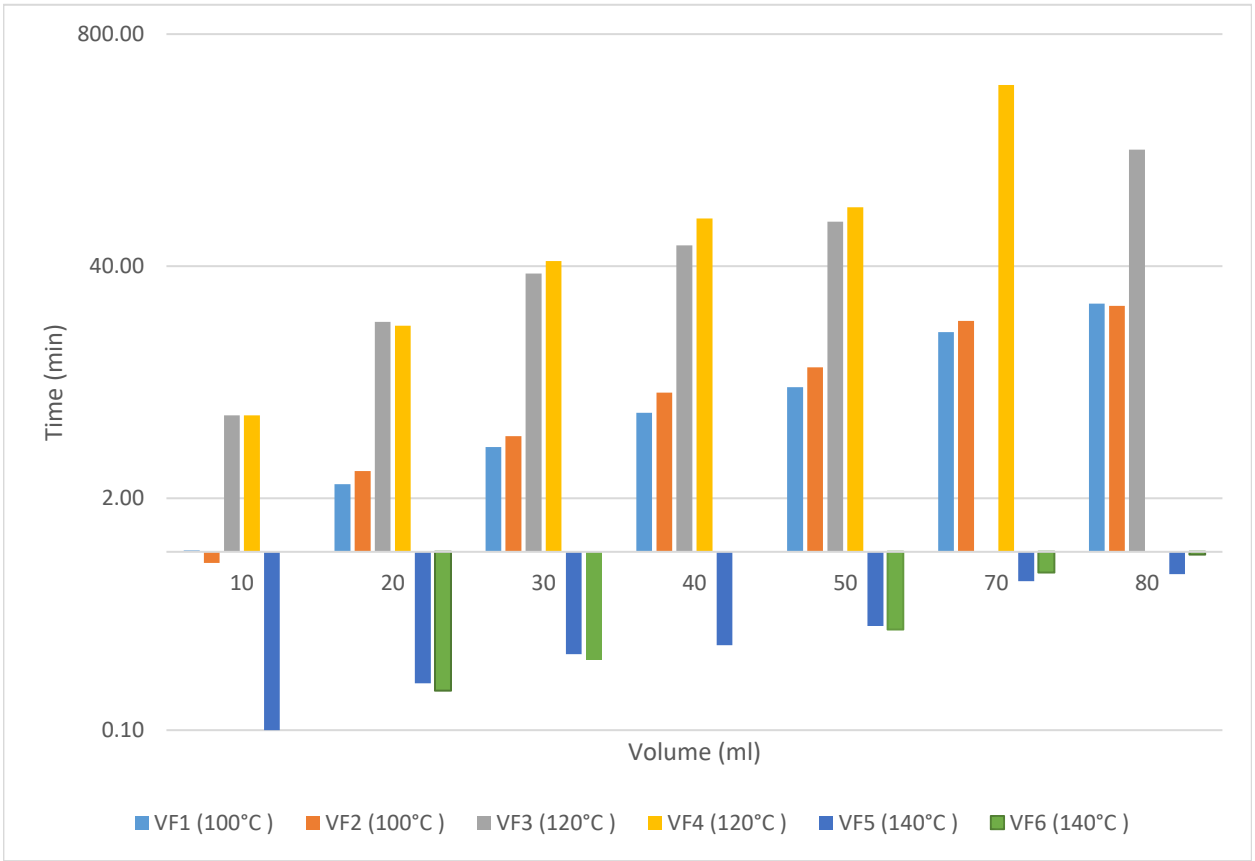

Fig. 11. Time is taken by vacuum filtration of all hydrolysis processes

Table 20. Vacuum filtration times for different volume of filtrate‘s obtained

Volume (ml)	VF1 (100°C) min	VF2 (100°C) min	VF3 (120°C) min	VF4 (120°C) min	VF5(140°C) min	VF6 (140°C) min
10	1.02	087	5.83	5.83	0.10	-
20	2.40	2.83	19.50	18.50	0.18	-
30	3.87	4.45	36.30	42.67	0.27	0.25
40	6.03	7.82	52.25	74.02	0.30	-
50	8.38	10.83	71.17	85.70	0.38	0.37
70	17.05	19.73	-	414.70	0.68	0.77
80	24.62	23.93	180.0	-	0.75	0.97

4.2. Pressure Filtration of Hydrolysis 01 & 02

The below graph is plotted for the values obtained from the Pressure filtration of 2% & 4% Lactic acid with Yeast, which was Hydrolysed at 100 °C. The filtration of PF1 was a bit faster in the starting stage of the filtration but gradually, the time increased as the volume of the filtrate obtained. It took almost 38 minutes to collect 70ml of filtrate from the reaction mixture placed. The filtration of PF2 was a bit slow from the starting stage but compared to PF1. It almost took 20 minutes more to collect the same volume of the filtrate.

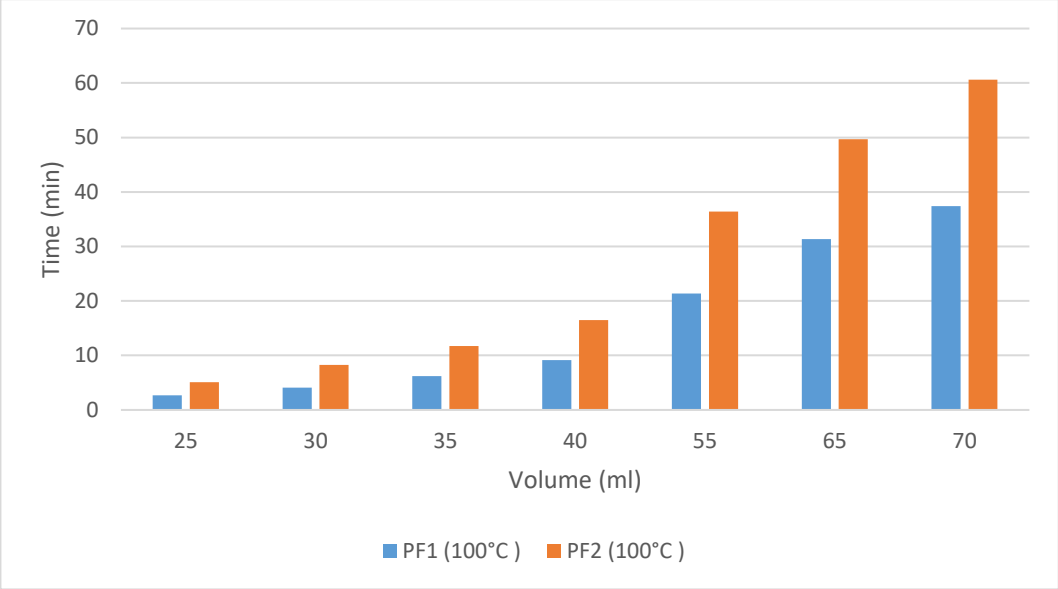

Fig. 12. Results of pressure filtration for hydrolysis 01 & 02

4.3. Pressure Filtration of Hydrolysis 03 & 04

The below graph is plotted for the values obtained from the Pressure filtration of 2% & 4% Lactic acid with Yeast, which was Hydrolysed at 120°C. Both the filtrations were too slow from start to end of the process. Even though the hydrolysis temperature was increased, the filtration time was more. Only for collecting 50 ml of the filtrate PF3 took almost 2 hours. Whereas the PF4 took nearly 7 hours in collecting the 75 ml of the filtrate through the filtration process. Therefore this filtration was time consuming compared with PF 1&2; even the temperature of the process was higher.

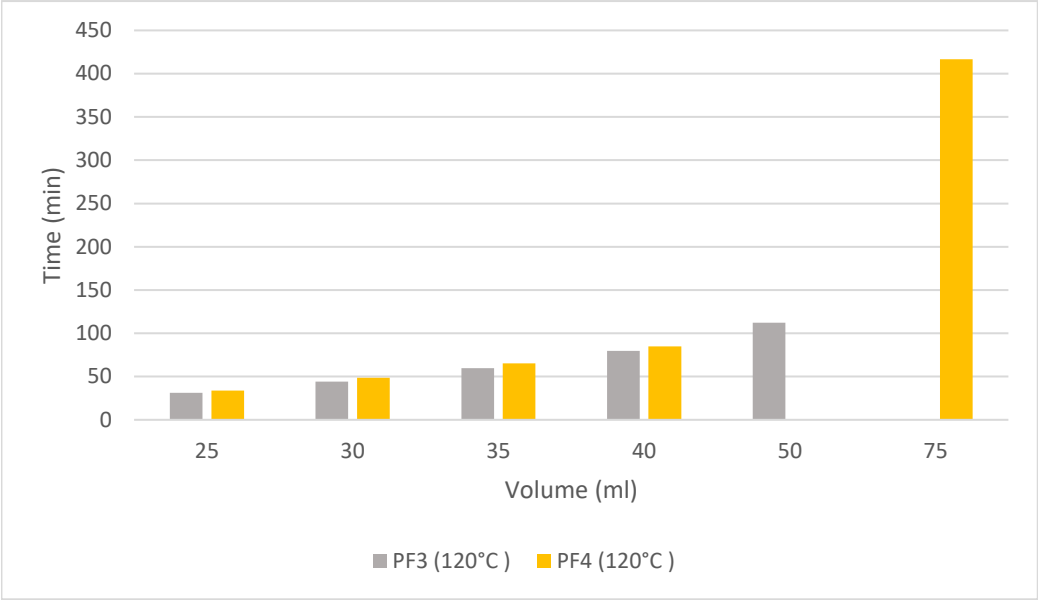

Fig. 13. Results of pressure filtration for hydrolysis 03 & 04

4.4. Pressure Filtration of Hydrolysis 05 & 06

The below graph is plotted for the values obtained from the Pressure filtration of 2% & 4% Lactic acid with Yeast, which was Hydrolysed at 140°C, which has the pressure of around ~+2.6 bars. The filtration process was much faster compared to the 100 and 120°C hydrolysis filtration process. The total time taken to collect 90 ml of filtrate was less than a minute, but the filtration of PF6 was 20 seconds faster than the PF5 to receive the same volume of the filtrate. Whereas both PF 5&6 was too quick, the intermediate values could not be noted at different intervals like previous ones.

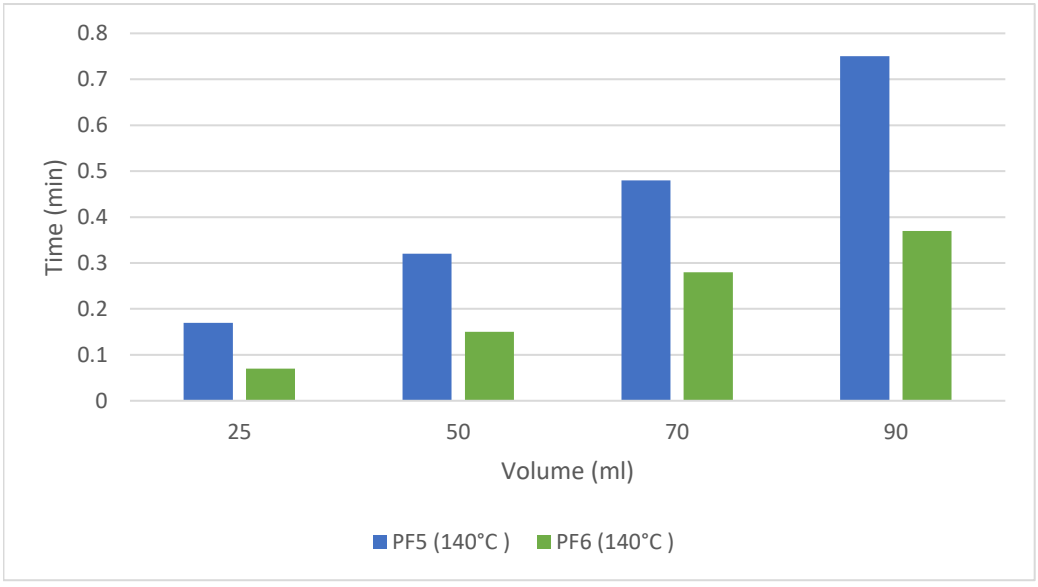

Fig. 14. Results of pressure filtration for Hydrolysis 05 & 06

4.5. Nitrogen test Analysis

The Nitrogen test was conducted for the dried filtrate and filter cake which was obtained from the filtration process. The values obtained from the nitrogen test will be used for protein percentage calculations.

Protein calculations from the amount of Nitrogen present in the dry matter are done and compared in the table below. The amount of nitrogen present in the yeast taken for investigations is 7.94% (got from the dry matter test). Therefore protein percentage was calculated by using 6.25×Nitrogen percentage, i.e. Proteins present in the Yeast sample taken = 6.25*7.94= 49.62%

From the above calculation, we can compare the proteins which were present in the yeast sample, and the proteins present in the extracted filtrate, and those calculated values are shown in the table below (**Error! Reference source not found.**).

Table 21. Calculated Protein percentage values using nitrogen percentage

Sample	Number of Analysis	Nitrogen in the dry matter %	Standard deviation	Relative deviation	Proteins percentage %
PF1 (~2% & 100°C)	2	4.6	0.1	1.86	28.75
PFK1 (~2% & 100°C)	2	3.24	0.04	1.16	20.25
PF2 (~4% & 100°C)	2	4.29	0.16	3.76	26.81
PFK2 (~4% & 100°C)	2	3.56	0.17	4.72	22.25
PF3 (~2% & 120°C)	2	5.8	0.04	0.63	36.25
PFK3 (~2% & 120°C)	2	2.69	0.01	0.32	16.81
PF4 (~4% & 120°C)	2	5.26	0.02	0.45	32.88
PFK4 (~4% & 120°C)	2	2.03	0.01	0.72	12.69
PF5 (~2% & 140°C)	**2**	**6.35**	**0.05**	**0.84**	**39.69**
PFK (~25% & 140°C)	2	1.91	0.04	2.09	11.94
PF6 (~4% & 140°C)	2	5.46	0.01	0.25	34.13
PFK6 (~4% & 140°C)	2	1.32	0.02	1.56	8.25

The protein which was present the actual sample for testing was 49.62%, and the amount of protein percentage in the filtrate obtained from filtration process gradually increased with increase in hydrolysis temperature for both 2% & 4% lactic acid concentration and also the protein percentage in the filter cake kept decreasing for elevated temperatures.

From the above table, it can be seen that the amount of protein percentage present in 2% lactic acid with yeast hydrolysed at 140°C has almost 40% and 4% lactic acid with yeast hydrolysed at 140°C has 34%. It can also see that yeast hydrolysed with 2% lactic acid at 120 °C contains 36.25% of protein but the filtration time was too high compared to PF 5&6. The minimum amount of protein present in the filtrate was for the filtrate obtained from the pressure filtration of hydrolysis 01& 02, which is around 28%, but the rest of the other four hydrolyses have more than the 30% of the protein content.

The below graph shows the comparison between the Protein present infiltrate and filter cake of all the pressure filtration processes. The protein percentage in the filter cake was high in PKF1 and minimum in PFK6, whereas the protein percentage in pressure filtrate was high in PF5 and minimum in PF2. Therefore filtrate and filter cake from pressure filtration of hydrolysis at the higher temperature has the maximum amount of protein infiltrate and minimum in the filter cake.

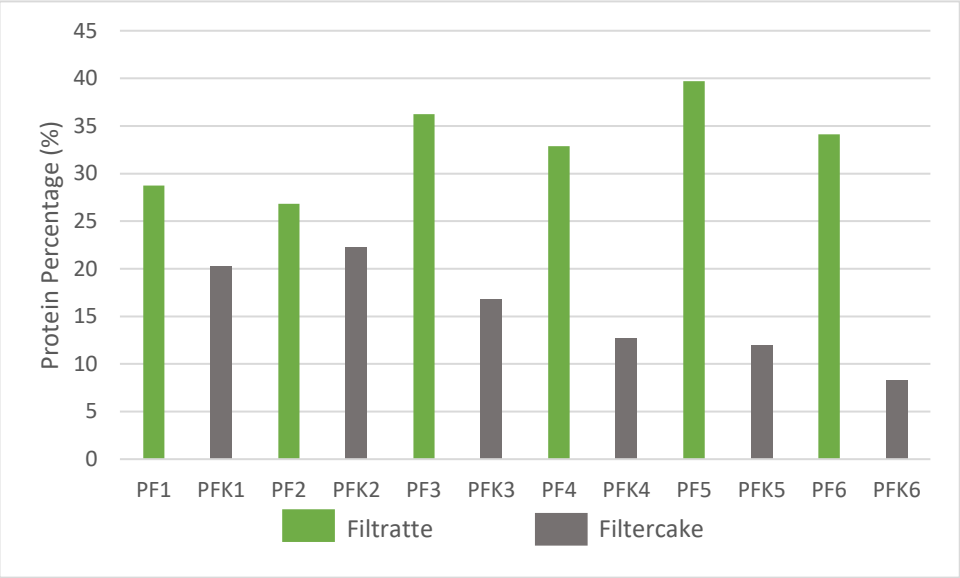

Fig. 15. Comparison between the protein percentage in the filtrate and filter cake

By comparing all the above, vacuum, and pressure filtration graphs of all hydrolysis, we can know that the time taken by hydrolysis-5&6 is minimum, i.e., less than a minute to complete both the filtration process whereas hydrolysis -3&4 took maximum time for both the filtration process. The hydrolysis-01&02 took a steady time for filtrations, which is less than 3&4. Therefore yeast with lactic acid of 2% and 4% concentration hydrolysis at 120 °C taken the maximum time for both the filtration and yeast hydrolysed with lactic acid of 2% and 4% concentration has made the minimum time for both the filtrations, i.e., vacuum and pressure filtrations.

And from the above nitrogen test values, the protein percentage was calculated. The filtrate obtained from pressure filtration of hydrolysis 01&02 has the minimum amount of protein, and hydrolysis-05 has the maximum value of protein in the filtrate, i.e. 2% lactic acid with yeast hydrolysed at 140°C Therefore this was more effective compared to all others, the protein present in the filtrate was 39.69% which was almost 80% of the yeast sample taken and also had the minimum amount of protein left in the filter cake, i.e. 11.94%. As the time is made for both vacuum and pressure filtration of hydrolysis, 05 was also very fast, which 0.68 min for vacuum filtration and 0.48 min for pressure filtration to collect 70 ml of the filtrate from the reaction mixture obtained from the hydrolysis.

So, yeast with lactic acid of 2% concentration hydrolysed at 140°C method was more effective in the extraction of the maximum amount of protein and faster filtration, too, and this can be recommended for the industrial purpose.

5. Managerial Recommendations on the Proposed Process

5.1. The Amount of Yeast Spent in Europe

In 2017, around 9,449 breweries were working in the European Union, including microbreweries, and they produced a total of 396 million hectolitres of beer. In 2010 production was estimated to be a total of 383 million hectolitres. Europe maintains an established position as one of the world's best and essential beer producers, as compared with Russia, Brazil, the United States, and China. In 2008, China overseeded the European Union as the biggest beer producer in the world. To produce every 100 ltr of beer it was evaluated that 1.5 to 3 kilograms of residual brewer yeast along with 14 to 20 kilograms of spent brewers grain, and 0.2 to 0.4 kilograms of hot trub is generated [23]. Therefore, an estimated amount of at least 6 million tons of yeast was produced in Europe in 2017. The exact distribution of the spent yeast resue for Europe is not available in literature, however, Kerby, C. et al. (2017) gave a distribution of spent yeast reuse for rural and urban craft brewers of United Kindom, which gives an idea of how spent yeast is reused in different applications in Europe [24].

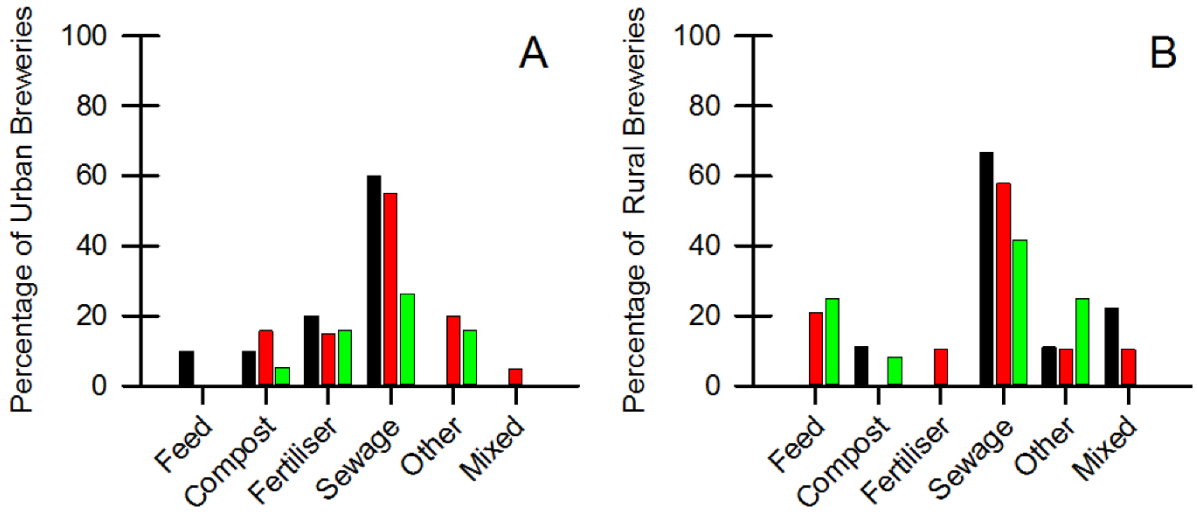

Fig. 16. Spent yeast disposal methods in urban and rural-based craft breweries [24]

Fig 16 is the graph of disposal method of spent yeast in (A) urban breweries (B) rural breweries in Europe. The black, red and green bars indicate the batch capacity of breweries. Black bar indicated capacity lesser than the 1000L, green greater than 2000L, and red less than 2000L and more than 1000L.

The most part is disposed to sewage in both urban and rural breweries whereas in rural breweries little amount is used as an animal feed compared to the urban region. But more than 50% is disposed of as waste and rest are used for agricultural and others. This waste which contains nutrient value can be reused by extracting the proteins present in them and that can be helpful for human consumption.

5.2. SWOT analysis

The SWOT analysis was done to analyse the strengths, weakness, opportunities and threats for the process of proteins extraction from yeast. The swot analysis of brewer‘s spent yeast feasibility for extraction of proteins through hydrolysis as shown in (Table 22) below. The left column shows the potential of the technology, and the right column shows the limitation and retardants for protein extract from spent yeast.

Table 22. SWOT analysis of spent yeast for the extraction of proteins

STRENGTHS	**WEAKNESS**
• Rich in proteins and carbohydrates • Can be used via for other applications to handle supply surges • Environmental friendly disposal possible • Pretreatment is not difficult for the residue obtained from breweries • Protein cost can be reduced due to the high percentage protein recovery • At large scale, it can be applied • The process is not affected by the minor presence of different types of yeast	• Time-consuming process • Very least studied • Optimised routes of hyrolysis are not developed for high protein recovery • Uncertainty exists because of lack of data. • Cost is unknown for initial industrial setup
OPPORTUNITIES	**THREATS**
• Application in numerous ways is feasible i.e. for medical, food and cosmetic industries • With the primary production process of breweries, it can be integrated • Abundantly available yeast from the breweries	• The uncertain future roadmap of extracted proteins from yeast • Cultural hindrance in accepting proteins derived from spent yeast • Competition with meat and other soy-based protein products

5.3. Segmentation and Analysis

The spent brewer‘s yeast is rich in proteins and can be used and disposed of by various environmental friendly processes, yet protein extraction by hydrolysis is a value addition process, which has tremendous economic potential in the food industry. The process is simple and is easy to be applied industrially for protein production. The process can be integrated with an existing beer production facility, and the excess yeast may be utilised instead of being disposed of. On the other hand, the process is a bit time consuming and not optimised yet for industrial production. Moreover, the proteins extracted from the yeast has a cultural barrier in its widespread use and will compete with the meat and soy protein markets.

Table 23. Segmentation and analysis

	Segment 1	**Segment 2**
Client Profile Characteristics: Understanding Your client	Demographic: Pharmaceutical formulation from a lab or firm.	Demographic: Food industry product.
	Social: Protein extraction firm for medicinal use	Social: Protein additive for the food industry as replacement of soy proteins Large protein-based product manufacturing firm.
	Economical: Small startup A novel method for delivering a quality product and a new way of extraction of protein from brewer's yeast	Economical: Voluminous production with less labour with medium induction price initially A novel method to extract proteins from yeast and to develop the human consumable product.
	Physiographic: Health and diet-conscious customers.	Physiographic: People who are interested in and have knowledge of the importance of recycling and their products.
Gain Creators and Pain Relievers Needs and desires concerning the product	Researched based extraction of a specific protein(s) from brewer's yeast. The research firm can create a wide range of medicines.	With a small workplace, companies or startups they gain money by either leasing or outsourcing yeast for manufacturing.
Sacrifices Costs and inconveniences for the customer that has your product	Product extracted from a spent brewer's yeast, which was discarded to the sewer before by the brewers and was considered a waste. As it's not a high-value product in people's views, they might show hesitation in accepting protein products originating from spent yeast.	Customers who are not interested in the consumption of proteins extracted from brewers spent yeast.
Value Proposition What does my process contribute to the chosen market segment?	Adds a variety in choice of therapeutic proteins or protein-based diet. Adds variety over the shelf and provides an alternative and sustainable source of food proteins other than meat or soy proteins.	New to the market, demand for the customisation is more from the consumer analysis. It can be marketed to brand fewer value sellers and increase the cost of products in the future.

5.4. Benefits and Applications

Spent brewer‘s yeast extracts were obtained by hydrolysis method and higher contents of essential amino acids was derived. The lower molecular weight and high content of amino acids with peptides of high levels showed that extracts tested are not only valuable as flavourings but also it is advantages in the applications in the design of dietary supplements and functional foods as a source where their additive levels is defined by the level and tangible quality of nucleic acids. Extracts which were tested have polyphenols higher content and higher antioxidant activities comparing to that of tea, which might be affected by the wort composition utilized within the production process of beer [25].

5.5. Environmental and Social Impacts of the Process

Brewer spent yeast is an underutilised resource, and most of the yeast is disposed to the sewage system (Fig. 16) as there is no widely applied process available to handle the tremendous amounts of yeast produced in during beer production and convert it into valuable products. The methods for its reuse and extraction are in the development and research phase, and a breakthrough with a good yield of relevant and commercially viable product can make a big difference in the resue of yeast. When the yeast is disposed to the sewage system, it is decayed into carbon dioxide and water in the presence of other bacteria, thus increasing the waste load and nutrient-rich environment, which may lead to eutrification of water bodies.

If the yeast is used for extraction of proteins, as demonstrated by this research, it will have a tremendous economic and environmental benefits, as yeast will be used to extract valuable proteins instead of being disposed off to the sewer, which will decrease the decay load and over the water bodies, and the nutrients will be value-added to protein which a lucrative price tag in the market.

A similar bouillon taste profile characterises the tested yeast extracts with a noticeable bitter after taste derived from the bitter substances, which is mainly due to the high degree of hydrolysis of proteins and the high content of free glutamic acid, which can come from beer. These extracts can be particularly useful in designing a new assortment of functional foods of the bouillon taste profile that is critically absent on the market. Moreover, as a natural hesitation of in accepting yeast-derived protein is expected from people, which can be covered by marketing and educating strategies.

The proteins that will be extracted from the spent yeast of the breweries will play an important role in producing consumable products and cosmetics. This recycled protein can be sold in the market for a lesser price than the traditional one and make easily available in the market. This proteins from waste are profitable and by recycling process, the waste dumped into the environment can be reduced.

5.6. Cost Estimation

The cost estimation was done based on the types of equipment needed for the proposed process and approximate cost has been stated below.

Hydrolysis equipment

The main process in this research carried out was hydrolysis for which the hydrolysis equipment is needed and the equipment is based on implementation for industrial purpose.

Capacity – 200L

Working temperature - ~60 to 200 °C

Rotating speed – 0~680 rpm

Power supply – 220V/50 Hz

Weight - ~350 kgs

Material – Stainless steel

Approximate cost - ~12250 Euros (The cost is based on the similar kind of equipment found in the market and the approximate cost is quoted) [26].

Filtration equipment

As the filtration should be done after the hydrolysis process and this equipment should have the capacity to filter the total amount of reaction mixture obtained from the hydrolysis process.

Filter funnel capacity – 100L

Receiving flask capacity – 70 or 100 L

Funnel material – Stainless steel

Approximate Cost – ~3600 Euros (The cost is based on the similar kind of equipment found in the market and the approximate cost is quoted) [27].

Storage vessels and others

The process should be done with clean vessels and the final mixture obtained from filtration must be stored in uncontaminated place. The approximate cost for this was taken as 2,500 Euros.

Therefore the total cost for setting up an industrial-scale production plant will be 17,850 Euros approximately which has the capacity of hydrolysing the 15kgs of yeast at one time. If that process is done for 5 hours and it is repeated twice a day the total amount of yeast hydrolysed will be 30kgs. Once the hydrolysis is completed the obtained mixture can be placed for filtration and second-time hydrolysis can be conducted in the filtering time.

This plant will have the capacity to recycle around 8.5 tons of yeast per year. This can be started as a separate industry where the spent yeast fro the breweries are collected and the process will be carried out or to avoid transportation and storage cost it can be set up in the brewery itself.

If we calculate return on investment for the proposed plant, 80% of the protein can be extracted from the yeast using this process i.e. if approximately 30% of protein present in the yeast from the breweries, 24% of which can be extracted successfully. For 8.5 tons of yeast hydrolysed and filtrated 2,040kgs of proteins can be extracted per year. The average cost of raw materials i.e brewers spent yeast from the breweries is bought for 0.50 euros per 1 kilogram it will be 4,250 euros for 8.5 tons of raw material. If the average cost of protein per kg is 20 to 25 Euros than the total savings will be around 40,800-51,000 euros annually. If we deduct all the initial investment and the raw materials price total approximate income would be 18,700-28,900 euros annually from the plant of 8.5 tons recycled per year. If the maintenance and labour cost is taken as 5% of the equipment cost per month it will around 892.5 Euros per month and 10,710 Euros annually. If the protein percentage in the yeast will be 50% like the sample taken then 40% of it can be extracted which is 3,400 kgs from which annual profit will of approximately 60,800-85,000 euros annually. As there is no much mechanical forces are involved labour required for maintanance is also minimum. The high cost involved is in setting up of the plant and once the plant is completely operational the profit will be less for the first year and it will be more in upcoming days as the equipment are already set up. This plant will also help in reuse of brewers spent yeast for producing consumable proteins instead of wasting for animal feed and composite wastes. The payback for this project will be around 6-7 months of time by considering the initial investment and average cash flow per year.

Table 24. Investment costs involved in setting up a plant

Investment	**Cost (Euros)**
Hydrolysis Equipment	12,250
Filtration Equipment	3,600
Other costs (storage vessels)	2,000
Maintenance costs/year (5% of total costs)	10,710
Raw materials	4,250
Total (Euros)	32,810

Table 25. Approximate return on investment from the proposed plan

Case	**1**	**2**
Yeast with Protein percentage	30%	50%
80% of Protein extracted	24%	40%
Total amount of proteins extracted (kgs) from 8.5 tons	2,040	3,400
The market price for 1 kg of Protein (Euros)	20-25	20-25
Total value of proteins extracted (Euros)	40,800-51,000	68,000-85,000
Pay back period (months)	6-7	3-5

Proposed industrial equipment setup is shown in the below diagram. This setup can be fit in a room which contains two major equipment i.e. hydrolysis reactor and filtration equipment. The final proteins extracted can be stored and sent to the food industry for manufacturing of consumable products for humans from the extracted proteins. As the waste is minimum it can be disposed of easily.

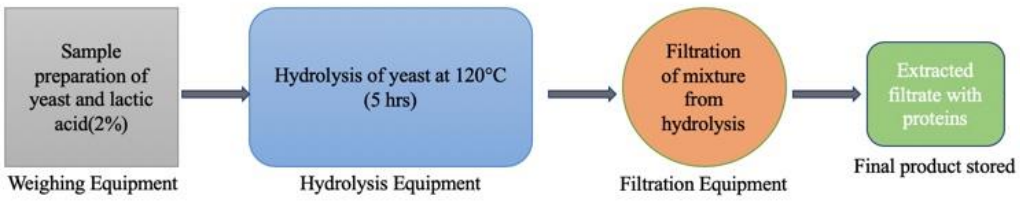

Fig. 17. Proposed industrial setup

5.7. Recommendations for the protein extraction process

It was found that high temperature and lactic acid concentration facilitates the yield of proteins from yeast. Thus these parameters should be further optimised and refined to be implemented as the main process variables for industrial-scale production of proteins. The hydrolysis should be done for yeast with lactic acid of higher percentage concentration at 140 °C and test for the presence of proteins in the filtrate obtained. The brewers spent yeast should be taken as a sample for conducting hydrolysis and detect the amount of protein present and select the best optimum temperature for hydrolysis. It would recommend completing the research by using different protein detecting methods instead of the nitrogen test to know the exact amount of protein percentage in the sample and extracted residue.

Conclusions

1. The yeast from the breweries is mainly used as an animal feed or waste which contains a high nutrient value. So, it is necessary to know about the effective utilization of Brewers spent yeast for the extraction of proteins. After analysing the existing methods such as autolysis, ionic liquids and hydrolysis, the hydrolysis process is selected for the research. In this research, the hydrolysis process is used for the breakdown of chemical bonds between the yeast at different temperatures and extract proteins through the filtration process. As the filtration processes were also fast at higher temperatures it is easy to filter a large amount of mixture obtained from hydrolysis. The proteins extracted from the yeast can be used for manufacturing consumable products for humans.
2. The yeast sample with lactic acid of 2% concentration hydrolysed at 140°C method gives the highest yield of the current study when compared to all other hydrolysis processes of yeast with lactic acid of 2% and 4% concentrations, and this parameters describe the optimum for extraction of proteins from yeast i.e. 80% of the protein was extracted from the total amount of protein present in the taken yeast sample. The filtration processes of this hydrolysis were also rapid compared to all other hydrolysis conducted. Therefore from this research, this method can be proposed for the implementation in industrial scale for the extraction of proteins from yeast.
3. This process could be applied as a practical approach to nutrient regeneration/production, which can be used as supplements with biological properties in food. They can be used in the food industry for technological purposes due to their mineral and amino acids content. Approximate cost analysis for setting up of an industrial plant is also analysed considering worse and best scenarios. The proposed plant is capable of recycling around 8.5 tons of brewers spent yeast per year once it is fully operational. The return on investment is made to satisfy the need of the buyer in order to enjoy the benefits from this proposed process of protein extraction from yeast. The payback is calculated approximately as 6-7 months which is less than a year for the proposed scale project.

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