Improving and Optimizing Protein Concentration Yield from Homogenized Baker's Yeast at Different Ratios of Buffer Solution

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ABSTRACT

Baker's yeast (*Saccharomyces cerevisiae*) and other lignocellulosic materials use as energy producing biomass have improved over time. These to date have continued to play a massive and quality role in the developmental needs of energy for sustainable future. Baker's yeast dominance in this energy trends has shown to be very productive as part realization of the set goal ahead. This through current research has shown to be positively supportive in the tackling of the rising greenhouse gas emissions (GHG) and global warming.

In this study, the mechanical pre-treatment of yeast substrate was conducted through its homogenization in the high-pressure homogenizer (HPH). With the operating conditions of temperature, pressure and number of passes (cycles) ranging from (30 - 50 °C), (30 – 90 MPa) and (1-5) respectively, this was ensured that the HPH machine maximum pressure of 100MPa was not exceeded. The homogenized yeast was diluted in three levels of dilutions with buffer solutions; denoted as solution C in the ratio of; 10:90, 20:80; and 30:70 respectively. The resultant protein concentration yield so obtained was analyzed and optimized through using the *Design Expert V.8* in the comparison of the three samples in the ratios given with the 3 input factors of pressure, temperature and number of cycles estimated within the considered limits.

Keywords: Buffer Solution; Protein; Optimization; GHG; Biomass

1. INTRODUCTION

Comparatively, baker's yeast has now been seen as a great source of energy production just as in other microbial products whose cell produces high protein contents required for bioenergy production. As energy has become the prime mover of economic growth in this 21st Century due to the growing demand for the rising population worldwide, it is, therefore, necessary to develop an alternative energy source whose production are affordable and will not in any way interferes with society negatively. This when developed, will compensate present energy need through the reduction of environmental concerns owed to pollution and global warming [1]. All biomasses are produced by green plants converting sunlight into plant material through photosynthesis [2] and baker's yeast as a biomass is an organic material that has stored sunlight in the form of chemical energy. This as well known, biomass does not add carbon dioxide to the atmosphere as it absorbs the same amount of carbon in growing during its releases when consumed as fuel. As part contribution of biomass to energy development worldwide, via renewable energy source (RES) this [3], pointed out that only in the last 10 years, the energy demands have doubled, as the current energy resources as known would not meet the market request. Energy from biomass conversion will, therefore, be considered as the most important fuel worldwide after coal, oil, and natural gas and is expected to become one of the key energy resources for global sustainable development [4].

Baker's yeast as a biomass substrate and its consideration in this work was based on its inexpensiveness and easily availability along with the protein contents stored in the substrate high enough to be compared to in the 3 levels of dilutions. Literature survey has also shown this substrate to be in the top group with higher protein content. Energy development overall has shown to be of greater improvement and this has included the use of baker's yeast for energy efficiency and improvement through mechanical disruption technique of high-pressure homogenizer (HPH). Ref [5] have concluded in his editorial paper on, "The 3rd international conference on sustainable energy and environmental protection (SEEP) 2009" that more research and developments will be needed to tackle the energy problem so as to continually reduce the emission, for a reasonable standard of living for our world. Fernandez et al. [6] have explained and highlighted yeast and yeast derivatives being widely used in the formulation of food systems. This also has now been extended widely to the production of energy in addition to producing food. Accordingly, Vasallo et al. [7] stemmed this interest in yeast and polysaccharides increasing as a result of the continuously growing fermentation industry which produces yeast biomass as a by-product. While Nielsen et al. [8] discussed extensively on the metabolic engineering of yeast for production of fuels and chemicals, they considered it offering many advantages as a platform cell factory for such production. Ref [9] evaluated yeast and considered it as the workhouse in the current biofuel industry due to its use in the production of ethanol. S. cerevisiae has been metabolically engineered to produce several advanced biofuels: 1-butanol [10] and isobutanol

[11]. Baker's yeast usefulness for energy production can never be ascertained without the disruption of the cell wall for the release of the innermost contents. Protein concentration which is stored in the cell wall of the baker's yeast will need to be fully disrupted for its release. This is a key step towards the isolation and purification of many biotechnological products that are present in the interior of cells of the cell walls of micro-organisms [12]. These are liberated using the high-pressure homogenizer (HPH). White and Marcus [13] and Keshavarz et al. [14] have suggested factors such as the type of microorganisms, growth and storage conditions of the cells as the important choices in considering disruption method required. This, however, when chosen must consider coupling of a high disintegration efficiency with a short disruption time [15]. Cell wall breakage of baker's yeast *(Saccharomyces cerevisiae)* for the release of intracellular contents of protein as analyzed using the high-pressure homogenizer (HPH) shown in Figure 1 satisfies the condition for the disruption process. Authors [16-20] and many others have exhibited in their research works through using high-pressure homogenizer (HPH) for biomass substrates disruptions for energy yield improvements.

This paper, therefore, considered the improvement of protein concentration yield at the different rate of buffer solution when added to the baker's yeast after homogenization and through optimization, the yield was eventually determined. In essence, the objective basically was to determine the highest yield of protein in the considered ratio of buffer against the homogenized yeast. The considered ratio of homogenized yeast against buffer solution were 10:90, 20:80, and 30:70

2. BACKGROUND

The use of baker's yeast in our everyday lives cannot be underestimated. This has remarkably been considered very useful in all human endeavours. Reed and Nagodawithana [21] found yeast to be important as a raw material for food, pharmaceutical and cosmetic industries, in addition, to being an excellent source of nutrients, mainly protein, vitamins of the B complex and essential minerals. As a source of protein, it has been regarded as a class of nitrogenous organic compounds which have large molecules composed of one or more long chains of amino acids and as an essential part of all living organisms, especially as structural components of body tissues. Of great importance in this study, is the ability of the protein to be fully liberated when homogenized under high pressure. Most widely used amongst microorganisms for ethanol fermentation is Saccharomyces cerevisiae a form of baker's yeast; this is due to its ability to hydrolyze cane sucrose into fermentable sugars. Ref [22] explained that cell biologists often use the term 'yeast' as against *Saccharomyces cerevisiae* and that this is due to it being by far most studied along with being best characterized unicellular eukaryote. They are considered to be eukaryotic unicellular microorganisms because of their wide distribution in the environment naturally [23]. Fukuda et al. [24] examined their ability to grow under anaerobic conditions. They

suggested a certain amount of oxygen will be necessary for synthesizing some essential fatty acids and other compounds contained in it to form energy. Its use currently in the production of energy has been proven beyond doubts as an eye opener in the world of science with a great source of vitality and as a multipurpose resource. While at the same time in the editorial paper published on the "developments of sustainable energy and environmental protection" in 2011, the author stressed the need for simulation and modelling as an important aspect in improving energy efficiency [25]. A scope considered within the limit of the field of sustainable energy development, and environmental protection and therefore seen as synonymous with the disruption of baker's yeast via HPH and its optimization using the *design of experiment.*

This paper, therefore, showcases the protein concentration yields from the different rate of buffer solution dilution with the homogenized yeast. Through optimization using the design of experiment (DOE) the mathematical model are represented and the developed mathematical models were tested for adequacy via the analysis of variance (ANOVA) and other adequacy measures. The relationships between the yeast homogenizing parameters (pressure, number of cycle and temperature) and the response; protein concentration were also identified.

3. HOMOGENIZATION PROCESS

As this paper focuses on the extraction and emulsification of protein from baker's yeast cell wall through constricting prepared soluble baker's yeast via the exit point of the High-pressure homogenizer. In fact, cell rupture is considered as one step in the downstream processing required in the recovering of biological products that are located inside cells [26]. In an energy producing terms, Wenger *et al.* [27] studies showed that intracellular proteins expressed in yeast, through small-scale cell breakage methods are capable of disrupting the rigid cell wall. This needed to match the protein release and contaminant profile of full-scale methods like homogenization thereby enabling representative studies of subsequent downstream operations being performed. In order to achieve this, a pre-treatment phase is generally necessary to the breakdown of the cell wall for the liberation of protein within the biomass. Literature has considered many types for different kinds of fully homogenized substrates and yeast, subjected to high-pressure homogenization as the mechanical means for breaking down the product and this method is also used at an industrial scale due to its effectiveness [28-31]. This has been employed in the recovery of intracellular products and has been a subject of continuing research interest over the last two decades [31]. Other methods also employed in the recovery of intracellular products are considered non-mechanical. This can either be the physical way or the enzymatic way. But the mechanical methods are shown to be non-specific as their efficiency is higher and application broader in comparison to other methods [32]. Ahmad Raus et al.

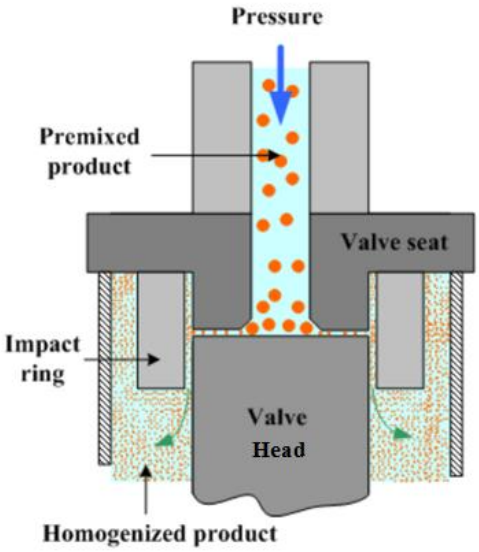
[26] emphasized that this is highly dependent on the nature of the product of interest, the cell or tissue itself, like the extent of the cell's fragility. For baker's yeast and other microbial to be fully liberated of their inner contents (intracellular products), homogenizing parameters of the working substrates needs to be considered and be fully understood so as to be able to critically analyzed the process of homogenization. Though, this is dependent on the type of high-pressure homogenizer in use. For this study, the high-pressure homogenizer series GYB40-10S 2 stages Homogenizing valves is employed as in (Figure 1) and the working parameters; Temperature (°C), Pressure (MPa), Number of Cycles (Passes) and Ratio as a categorical factor are all considered.

- **Figure 1**: GYB40-10S 2 stage Homogenizing Valves

Technological processes in high-pressure homogenizer are based on the principles of the working parts of the machine which are considered as the main functionalities of the HPH. These are known as the valve seat, impact ring and the valve head in operation as represented in (Figure 2).

In essence, just as applicable in other techniques such as that with mechanical action (for example; Hollander beater, bead mills, spray drying, ultrasounds, and autoclave) or the non-mechanical action (for example; freezing, organic solvents, base and enzyme reactions, and osmotic shock and acid). The overall aim is to achieve the hope of 100% renewable energy by 2050 not only in the developed countries but also with most of the developing countries as well [33]. In its shorter proposal of achieving 50% energy system for 2030, it is viewed that this would enable the transport sector to attain its target of longer term goal of substantial economic benefits of 100% in 2050 [33].

In contrast to this development, offsetting this target would mean that a farsighted diversified yearly mix of energies as suggested to different countries, aimed at increasing security of supply and efficiency not being met as a wide and contemporary view of energy interchanges between states was not available [34].



- **Figure 2:** HPH parts during cell disruption [35]

3.1 GYB40-10S 2 stage Homogenizing Valves HPH Principle of Operation

The machine is made of a reciprocating plunger pump and a homogenizing valve, with its homogenizing portion made up of a double stage homogenizing system which includes a 1st stage homogenizing valve and a 2nd stage homogenizing valve. The two stage homogenizing valve pressures are adjustable under the scope of nominal pressure, and at the same time can also be used separately due to the high-low of homogenizing pressure which directly relates to the speed of materials through the homogenizing valve. The material particle size is considered to be less than 2 μm. The GYB series HPH, therefore, makes the incompatibility in liquid-liquid or liquid-solid materials to be compatible, superfine, and homogenous. The stability in the liquid-liquid or solid-liquid under the multiple actions of cavitations effect can result in high-speed impact and shearing through the adjustment of pressure homogenizing valve of the HPH [36]. The operating units depict the 2-stage homogenizing valve HPH with the serial name as GYB40-10S /GYB60-6S in Figure 1, and this has been employed in this work based on the technical specification as indicated in the table (Table 1). The high-pressure homogenizer (HPH) has the same features as others, but its unique feature is in its operation during homogenization in 2-stages with the different hand wheels for raising

pressure and stabilizing it at that point. This enables the homogenization pressure to be read at any point during cell disruption.

- **Table 1:** GYB40-10S / GYB 60-6S High-Pressure Homogenizer technical specification [36]

Technical Data (Item)	**Specification**	
	GYB40-10S	GYB 60-6S
Pressure (MPa) **Maximum Pressure (MPa)** **Working Pressure (MPa)**	One grade 0 ~ 100 Two grade 0 ~ 20 100 90	One grade 0 ~ 60 Two grade 0 ~ 20 100 90
Flow Rate (L/h)	40	60
Volume efficiency (%)	≥ 85	≥ 85
Motor Power (Kw)	3 (380V, 50 HZ, 3 Phase)	3 (380V, 50 HZ, 3 Phase)
Material Temperature (°C)	0 ~ 120	0 ~ 120
Noise (Db)	≤80	≤80
Corrosive-proof (PH)	2 ~ 10	2 ~ 10
Dimension (mm)	920 x 445 x 1220	920 x 445 x 1220
Weight (kg)	265	265

4. MATERIALS AND METHODS

4.1 Micro-Organism

Commercially pressed baker's yeast *(Saccharomyces cerevisiae)* in block form was used in this experiment. This was supplied by Dublin Food Sales and each block was weighed 1 kilogram. For sufficient soluble baker's yeast, 3 - 4 blocks were required for experimental work conducted each day. This was subsequently broken down into the large beaker to be used from the block form weighing 950g. 725 ml of solution C added and then ran under the stirrer until it was completely mixed. Using the following composition; the solutions A, B, and C as below where prepared. The notation for solutions A, B, and C are indicated in 4.3 (Buffer Solution) below.

4.2 Buffer Content and Preparation

The buffer solution contents and preparation were conducted as detailed below. This was needed in the preparation of the baker's yeast substrates before being homogenized using the HPH.

Solution A (0.1M KH_2PO_4 + 0.15M NaCl), this is equivalent of 1litre 13.6g of KH_2PO_4 weighed into a beaker and dissolved using the deionized water, also; 8.8g of NaCl weighed into a beaker and

dissolved using the deionized water, both mixed together and filled to the 1-litre mark. This was repeated thrice for 3 litres of the solution to be obtained.

Solution B (0.1M K_2HPO4 + 0.15M NaCl), 4.6g of K_2HPO_4 weighed into a beaker and dissolved using the deionized water, also; 1.8g of NaCl weighed into a beaker and dissolved using the deionized water, both mixed together and filled to the 200 ml mark.

Solution C obtained through gradually adding Solution B to Solution A until the pH scale of 5.3 was attained.725 ml of solution C added to the 950g broken yeast and mixed using the electric mixer.

4.3 Cell Disruption Using HPH

The experiments were conducted using the high-pressure homogenizer (HPH) shown in (Figure 1) and the materials and methods are as detailed in the previous paper [37] including the buffer solution which was prepared accordingly with the same rates and volumes. The soluble baker's yeast cell was disrupted using the high-pressure homogenizer and samples of the homogenate taken at different pressures varied at (30, 60 and 90 MPa), temperature at (30, 40 and 50 °C) and number of cycles (1, 3 and 5) as determined by the design expert and are indicated in the results and discussion section (Table 3). The significant change is the constituents of the filtrate after centrifugation the homogenized for 60 minutes and at 13,000 rev/mins wherein the samples have been diluted in the ratio of the homogenized solution against the buffer solution as 10:90, 20:80 and 30:70 respectively. The diluted ratios in solutions were treated with protein reagent and then tested for protein concentration yields using the spectrophotometer. The procedures are as detailed in the previous paper [37].

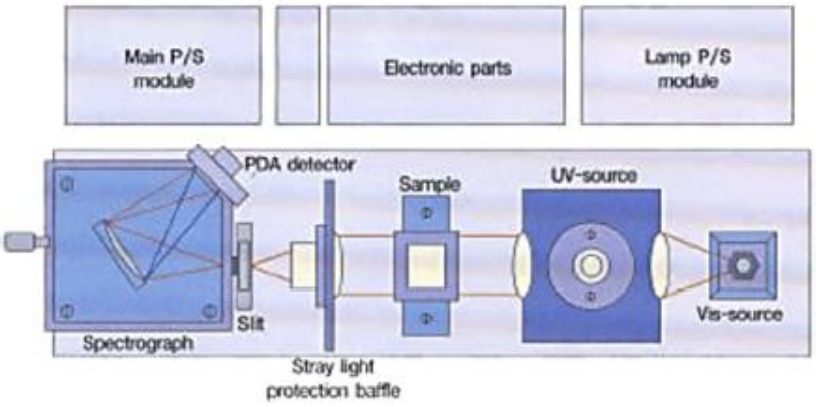
4.4 Homogenized Baker's Yeast Substrate to Buffer Ratios

As part of the analysis in determining the effects and improvement of protein concentration yield for the different ratios of homogenized baker's yeast against buffer solution ratio. The homogenized baker's yeast was further diluted with solution C as 10:90, 20:80 and 30:70. This means that for the 10:90 ratio; 10 ml of homogenized baker's yeast is mixed 90 ml of solution C. The essence of this was to determine the effect of buffer rate on homogenized yeast and to further determine the protein concentration yield when optimized using the *Design Expert V.8.*

4.5 Protein Measurement

Protein concentration yield and its measurement were of utmost concern in the research work. This was measured in (mg/mL). Determining the amount of protein concentration yield was considered as the quantity of cell wall broken down during the homogenization process. The more cell walls are broken in the soluble baker's yeast, the more the liberation of the protein content. After the

homogenization, this will require first, the centrifugation of the sample using the centrifuge machine and for a better understanding of the principle of operation see [37] which covered the process in the previous paper. Further characterization was conducted to measure the protein concentration yield through spectrophotometer and prepared protein curve. As the optical density (OD) was set at 550 nm, the clear liquid of filtered homogenate could then be determined to ascertain the quantity of cells broken down within the baker's yeast cell wall. The wavelength (absorbance) was measured through mixing the clear liquid of filtered homogenate with Total reagent (Blue Protein Reagent) and placed within the spectrophotometer. The quantifications are as detailed in [37] and was based on the absorbance of the resulting solution being measured using the spectrophotometer (Figure 3) and the measured absorbance of the protein versus the known concentration of BSA (bovine serum albumin) was plotted [38]. The resulting graph will then be the protein standard curve for determining the unknown protein concentration.



- **Figure 3:** Spectrophotometer S-3100 PDA schematic view (with modification from Ref. [39])

4.6 Experimental Investigation

The experiment aimed at investigating and determining protein concentration yield in the different ratio of substrates to buffer solution quantification as well as optimizing the improvement of these yields after homogenization. The experiment considered the variance of these parameters; Pressure, Temperature, Number of Cycles and Ratio considered as the categorical factor and all these are as determined by the *Design Expert* (See Table 2). The workability of the HPH has already been discussed and of concern is the working principle of the main functionalities of the valve head, valve seat and impact ring within the HPH. These were taken into account during the homogenization process as the hand wheel were being turned to compress the soluble baker's yeast through the outlet for yeast cell disruption. During the homogenization operation, the viscous soluble baker's yeast passes through the constricted gap to break down the cell wall within the yeast to release the protein content.

This clarifies the mechanism of cell rupture in terms of the rapid release of pressure as cells pass through the high-pressure homogenizer. The collected homogenized yeast at the different pressures at (30, 60 and 90 MPa), against temperatures (30, 40 and 50 °C) and number of cycles recorded (1, 3 and 5) corresponding to the design matrix as shown below.

4.7 Design Expert Software Application

The Design of Experiment (DOE) V.8 was considered as a software needed in the creation of the experimental run order and statistical analysis which provides extensive graphs that showcase the relationship between the input variables and the output responses in this paper [40]. Based on this procedure, the response surface methodology adoption, therefore, follows the Box-Behnken Design (BBD) which is in contrast to the Central Composite Design (CCD) due to their levels of factorial design. The BBD has been considered in this work based on the design of 3 levels for each variable and this is as indicated in the table (Table 2).

Determining protein concentration during the homogenization process tends to improve the yields at the different ratios of buffer solution against the homogenized yeast. For this purpose, a design of experiments through using the Response Surface Methodology (RSM) for improving and optimizing the yield was applied.

$$Y = b_o + \sum b_i \chi_i + \sum b_{ii} \chi_{ii}^2 + \sum b_{ij} \chi_i \chi_j \quad (1)$$

The generated equation as shown in equation (1) is aimed at optimizing single or multiple responses and for this work, it is considered for optimizing a single response of 'Protein Concentration Yield' and the values of the coefficients b_0, *bi*, *bii* and *bij* can be calculated using regression analysis. The 2nd order polynomial model as indicated by Equation (1) through stepwise regression was fitted and then applied on the response (Protein Concentration) measured in mg/mL. This was also used in generating ANOVA (Analysis of Variance) in Table 4 and response plot. While the categorical factor of ratio (factor 4) determines these yield of protein concentration.

The probability > F otherwise known as the p-value of the model and of each term as regards the model can be computed through the ANOVA. If the Prob. > F of the model and of each term in the model does not exceed the level of significance which as known is $\alpha = 0.1$ then the model may be considered adequate within the confidence interval of (1- α).

4.7.1 Comparing Box-Behnken Design (BBD) to Central Composite Design (CCD)

As indicated in Section 4.7, the type of RSM design selected is dependent on the matrix and CCD and BBD design matrices are represented in coded values. The matrix for each experiment are developed using the statistical software. For three factors, the experimental runs for BBD and CCD are 17 and 20 respectively [41, 42]. Comparison between BBD and CCD may become absolutely necessary as researcher will be unsure of why choosing one design over the other and the listed points below will eventually determine that.

4.7.2 Box-Behnken Design (BBD)

- Has specific positioning of design points.
- This design has 3 levels for each factor.
- Created for estimating a quadratic model.
- Provides strong coefficient estimates near the centre of the design space, but weaker at the corners of the cube, due to the absence of design points.
- Sensitive to missing data and a bad run.
- Region of interest and region of operability are nearly the same.

4.7.3 Central Composite Design (CCD)

The main features of this RSM design are:

- Created from a 2-level factorial design, improved with centre points and axial points.
- Normally has 5 levels for each factor, this can be modified to a face centred CCD by choosing $\alpha = 1.0$. The face-centred design has only three levels for each factor.
- Created for estimating a quadratic model.
- Rather insensitive to missing data, making them more robust to problems.
- Replicated centre points provide excellent prediction capability near the centre of the design space.
- Region of operability must be greater than region of interest to accommodate axial runs.

5. RESULTS AND DISCUSSIONS

Table 2: RSM showing design level and coded values against process variables

Variables	Code	Unit	Limits Coded/actual		
			-1	0	+1
Pressure	A	MPa	30	60	90
No of Cycles	B	-	1	3	5
Temp.	C	°C	30	40	50
Ratio	D	-	10:90	20:80	30:70

Table 3: Design matrix with actual values

		Factor 1	Factor 2	Factor 3	Factor 4	Response 1
Exp. No.	Run Order	A: Pressure	B: No. of cycles	C: Temp	D: Ratio	Protein Conc.
		MPa	-	(°C)	-	[mg/mL]
1	13	30	1	40	10:90	0.0161
2	18	90	1	40	10:90	0.6290
3	23	30	5	40	10:90	0.0968
4	10	90	5	40	10:90	0.9145
5	5	30	3	30	10:90	0.0484
6	48	90	3	30	10:90	0.1290
7	45	30	3	50	10:90	0.0645
8	36	90	3	50	10:90	0.2258
9	11	60	1	30	10:90	0.2661
10	42	60	5	30	10:90	0.3581
11	49	60	1	50	10:90	0.3177
12	12	60	5	50	10:90	0.2903
13	9	60	3	40	10:90	0.4597
14	25	60	3	40	10:90	0.5194
15	47	60	3	40	10:90	0.4726
16	37	60	3	40	10:90	0.6726
17	27	60	3	40	10:90	0.6258
18	6	30	1	40	20:80	0.0645
19	32	90	1	40	20:80	1.1306
20	21	30	5	40	20:80	0.1097
21	28	90	5	40	20:80	1.1210
22	8	30	3	30	20:80	0.0806
23	29	90	3	30	20:80	0.1855
24	43	30	3	50	20:80	0.1742

25	16	90	3	50	20:80	0.2710
26	51	60	1	30	20:80	0.2871
27	24	60	5	30	20:80	0.6403
28	1	60	1	50	20:80	0.4145
29	2	60	5	50	20:80	0.7871
30	20	60	3	40	20:80	0.5968
31	31	60	3	40	20:80	0.5758
32	34	60	3	40	20:80	0.5565
33	30	60	3	40	20:80	0.8677
34	44	60	3	40	20:80	0.7935
35	4	30	1	40	30:70	0.0919
36	35	90	1	40	30:70	0.9500
37	40	30	5	40	30:70	0.1452
38	3	90	5	40	30:70	1.3387
39	38	30	3	30	30:70	0.1129
40	22	90	3	30	30:70	0.2371
41	15	30	3	50	30:70	0.1968
42	19	90	3	50	30:70	0.3226
43	41	60	1	30	30:70	0.3097
44	26	60	5	30	30:70	0.9435
45	7	60	1	50	30:70	0.5855
46	39	60	5	50	30:70	0.9919
47	14	60	3	40	30:70	0.6371
48	46	60	3	40	30:70	0.6097
49	33	60	3	40	30:70	0.7306
50	50	60	3	40	30:70	0.9629
51	17	60	3	40	30:70	0.9016

The design of experiment with four factors has been applied here using the Box-Behnken Design (BBD). These 4 factors are; temperature, pressure, the number of cycles, and ratio (as a categorical factor), as well as a response of a protein concentration, were analyzed with 51 runs of experiments as seen in Table 3. While Table 2, shows the Response Surface Methodology (RSM) which is an experimental technique invented to find the optimal response within specified ranges of the factors.

5.1 Developed Mathematical Model for Yeast Cell Wall Disruption

The fit summary output indicates that for the response, the quadratic model is statistically recommended for further analysis as they have the maximum predicted and adjusted R^2 as 0.567 and 0.666 respectively [43, 44]. The RSM provided the optimum combinations to be tested in order to capture the biggest variability in y_s with minimum amount of runs. Table 3 shows the result of response of protein concentration yield based on the RSM coded design matrix sorted by standard order. As previously considered, the summary of fit output shows that the quadratic model being

statistically significant for the response. A reduce quadratic model analysis was adopted for the response resulting in the model terms of R^2 = 0.719, adjusted-R^2 = 0.666, and predicted-R^2 = 0.567. As the values are over 0.5, technically they are therefore assumed tends to 1 as analysed by the *design expert* [41- 44] and as a result, indicate that the adopted model is adequate. Also from the developed model and statistical analysis it is proven that the adjusted and predicted R^2 should be within 20% of each other which in the case here is valid as the adjusted and predicted R^2 is only 9.9% of each other. This therefore enable the model to be adequate and further analysis of the ANOVA Table is discussed below for the reduced quadratic model of (30 - 50 °C)

Table 4: ANOVA Table for Protein Concentration reduced Quadratic Model (30 – 50 °C)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	4.184	8	0.523	13.435	< 0.0001	Significant
A-Pressure	1.629	1	1.629	41.855	< 0.0001	
B-No. of cycles	0.298	1	0.298	7.655	0.0084	
C-Temp	0.045	1	0.045	1.166	0.2865	
D-Ratio	0.474	2	0.237	6.091	0.0048	
A^2	0.669	1	0.669	17.174	0.0002	
B^2	0.168	1	0.168	4.309	0.0441	
C^2	0.885	1	0.885	22.741	< 0.0001	
Residual	1.635	42	0.039			
Lack of Fit	1.418	30	0.047	2.621	0.0400	Not Significant
Pure Error	0.216	12	0.018			
Cor Total	5.819	50				
R^2 = 0.719			Pred R^2 = 0.567			
Adj R^2 = 0.666			Adeq Precision = 15.552			

The test of significance of the regression models, the test for significance of individual coefficients and the lack of fit test performed using the same statistical package for the response. Through selecting the step-wise regression method, the insignificant model terms can be automatically be eliminated. The resulting ANOVA table for the quadratic model outline the analysis of variance for the response and illustrate the significant model terms (Table 4). This also shows the other adequacy measures R^2, adjusted R^2 and predicted R^2. The entire adequacy measures are close to 1, which are in reasonable agreement and therefore indicate adequate models [43, 44]. The adequate precision (15.552) compares the range of the predicted value at the design points to the average prediction error. In this case, the values of adequate precision ratios are dramatically greater than 4. An adequate precision ratio above 4 indicates that the model is adequate [44]. An adequate model means that the reduced model has successfully passed all the required statistical tests and can be used to predict the responses or to optimize the process and so on. The final mathematical model linked to the response

with regards to the coded factors and actual factors as determined by the software are equations (2) , (3), (4) and (5) respectively. The actual factor equations are (3), (4) and (5) are representing the 10:90, 20:80 and 30:70 dilution ratios of homogenized yeast to buffer solution so achieved. The categoric factor (Ratio) has no implied order which means settings of a categorical factor are discrete and have no intrinsic order [45].

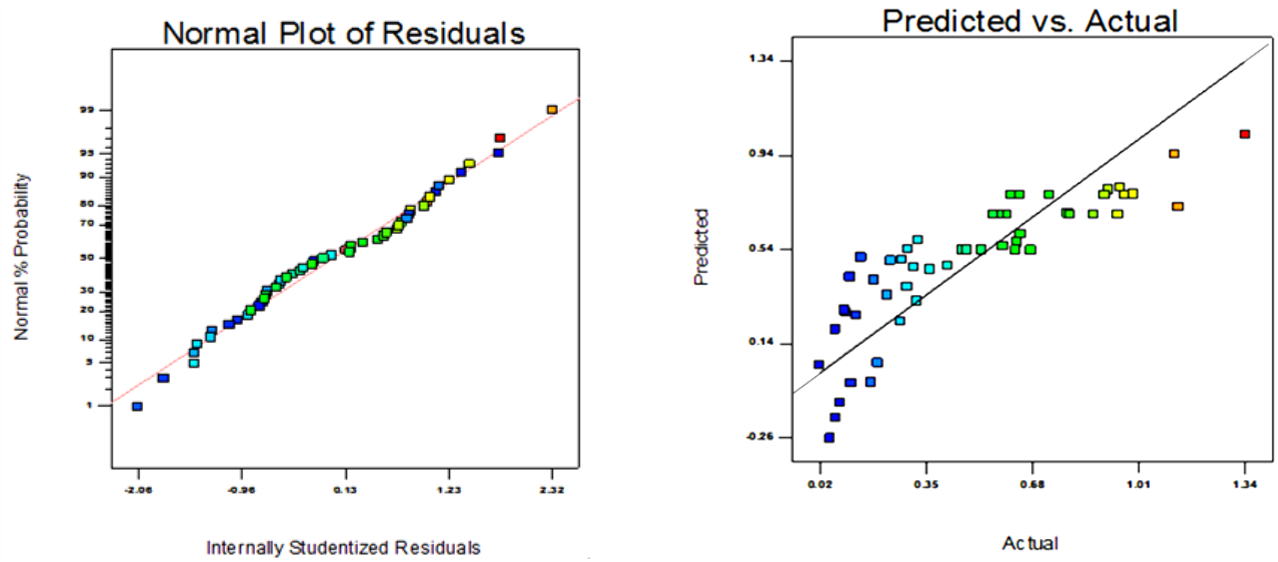


Figure 4: Scatter diagrams of normal plot of residuals (a) and predicted vs. actual (b)

Final Equation in Terms of Coded Factors:

*Protein Concentration = 0.665483871 + 0.260551075 * A + 0.111424731 * B*

*+ 0.043481183 * C - 0.127672359 * D[1] + 0.022327641 * D[2]*

*- 0.230053763 * A^2 + 0.115241935 * B^2 - 0.264731183 * C^2* (2)

Final Equation in Terms of Actual Factors:

Ratio 10:90;

*Protein Concentration = -5.07097209 + 0.0039358871 * Pressure - 0.117150538**

*No. of cycles + 0.216133065 * Temperature - 0.000255615*

**Pressure^2 + 0.028810484* No. of cycles^2 – 0.002647312 **

Temperature^2 (3)

Ratio 20:80;

$$\textit{Protein Concentration} = -5.07097209 + 0.0039358871 * \textit{Pressure} - 0.117150538 * \textit{No. of cycles} + 0.216133065 * \textit{Temperature} - 0.000255615 * \textit{Pressure}^2 + 0.028810484 * \textit{No. of cycles}^2 - 0.002647312 * \textit{Temperature}^2 \quad (4)$$

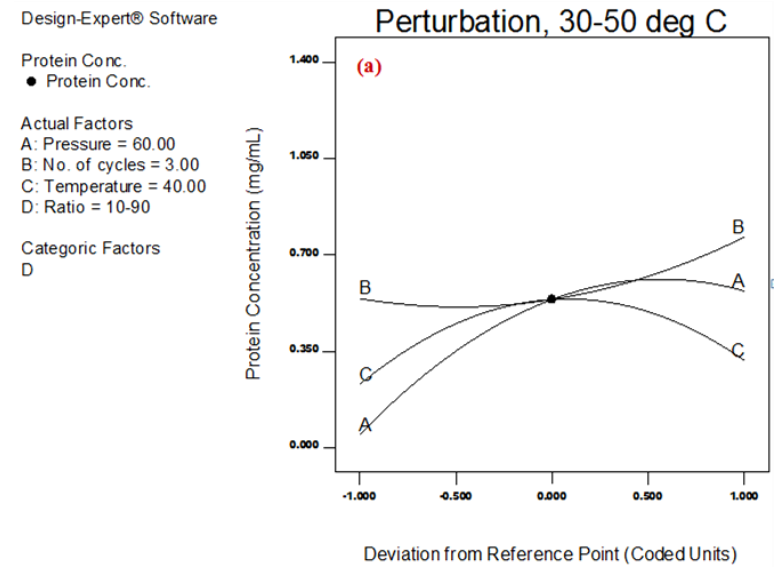
Ratio 30:70;

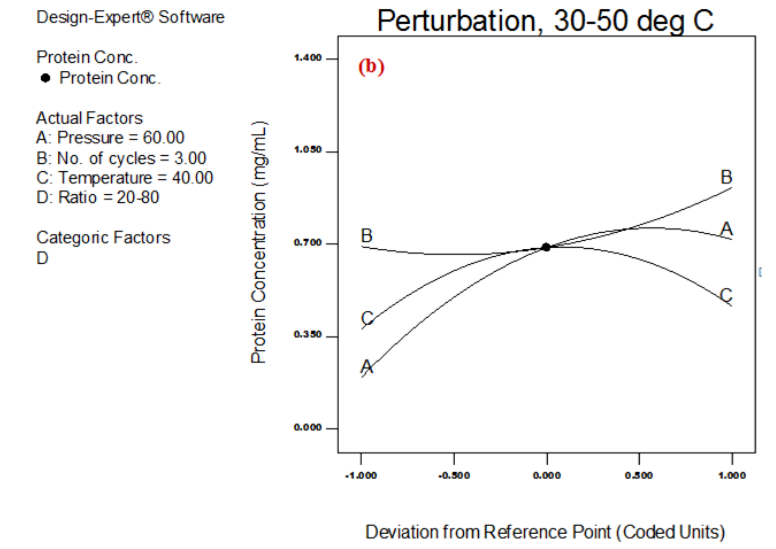
$$\textit{Protein Concentration} = -4.987955013 + 0.0039358871 * \textit{Pressure} - 0.117150538 * \textit{No. of cycles} + 0.216133065 * \textit{Temperature} - 0.000255615 * \textit{Pressure}^2 + 0.028810484 * \textit{No. of cycles}^2 - 0.002647312 * \textit{Temperature}^2 \quad (5)$$

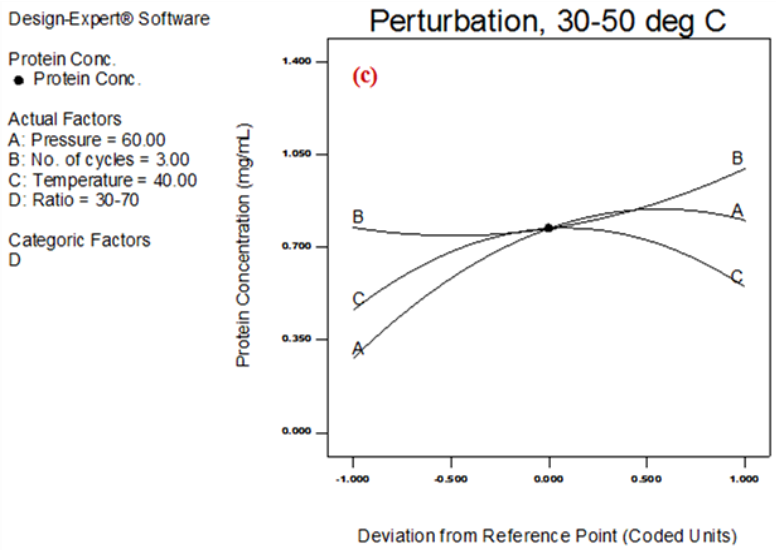
5.2 Effect of process parameters on the responses - Protein Concentration

5.2.1 Perturbation Plots

The perturbation plots Figure 5 (a, b and c) for the protein concentration yield from the 30 - 50 degrees temperature range is illustrated. The perturbation plot helped in comparing the effect of all the factors at a particular point in the design space. Temperature has shown to be effective in protein concentration yield in the three plots. The reason for this change is seen as a result of the effect of temperature on the biomass substrate before being homogenized. Pressure effect was negligible as well as that of temperature and because the substrate has been treated under heat to raise the temperature, passing it through the HPH over the number of cycles say, 3 would have entirely broken down the cell wall of the yeast substrate. Considering the categorical factor of ratio in this study, protein concentration yield has been seen to be of highest yield at the ratio 30:70 when compared to other ratios of 10:90 and 20:80. Invariably, it, therefore, means that as the ratio increase, the yield in protein concentration also increases at the same rate as pressure rises but in this case here, the only number of cycles has taken the incremental rate in the protein concentration yield. This can be seen in Figure 5(c) showing the highest of yield in protein concentration as against the other two.



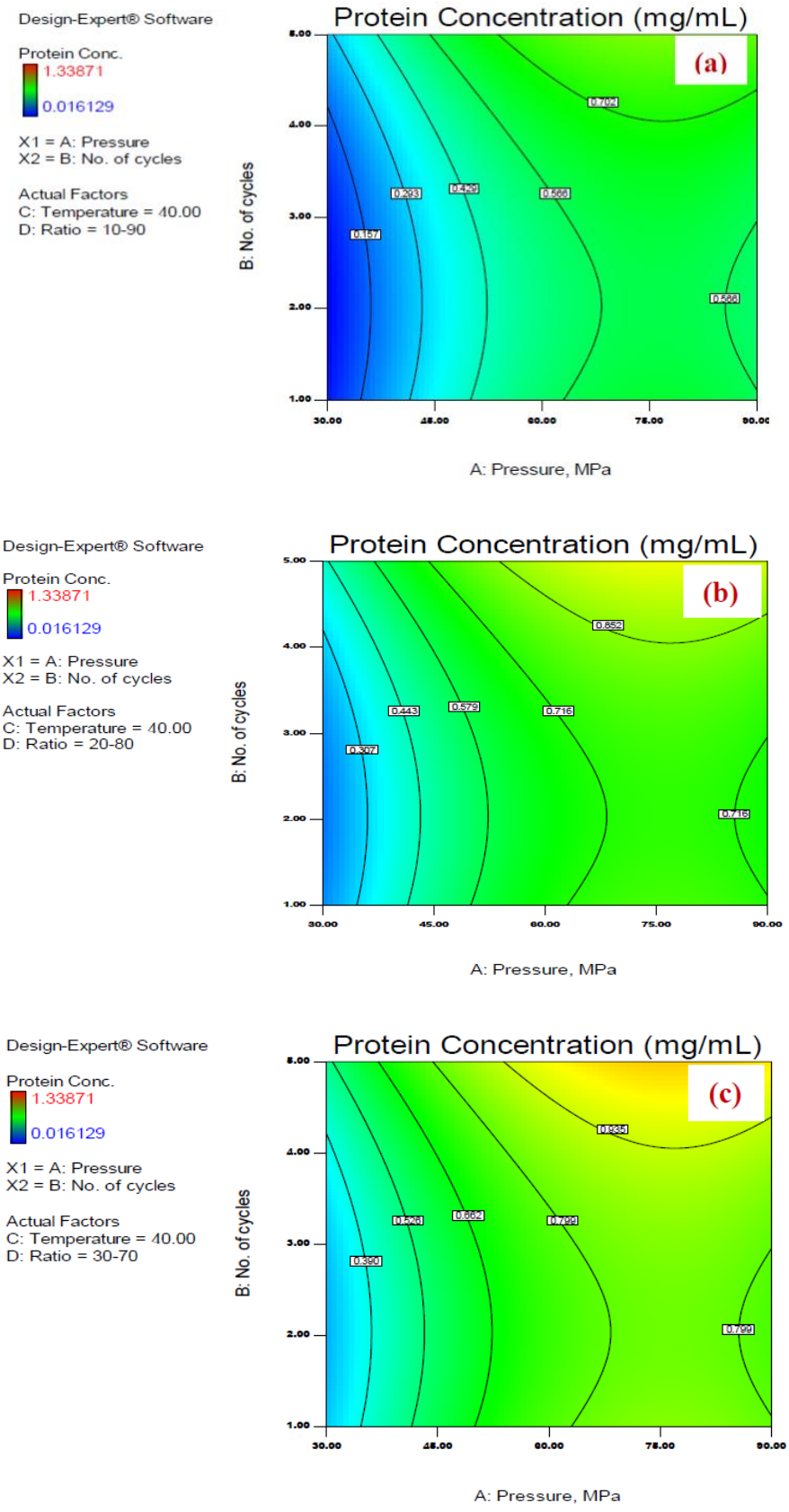




- **Figure 5:** Perturbation plot showing pressure increasing with the number of cycles as yeast is homogenized

5.2.2 Contour Plots

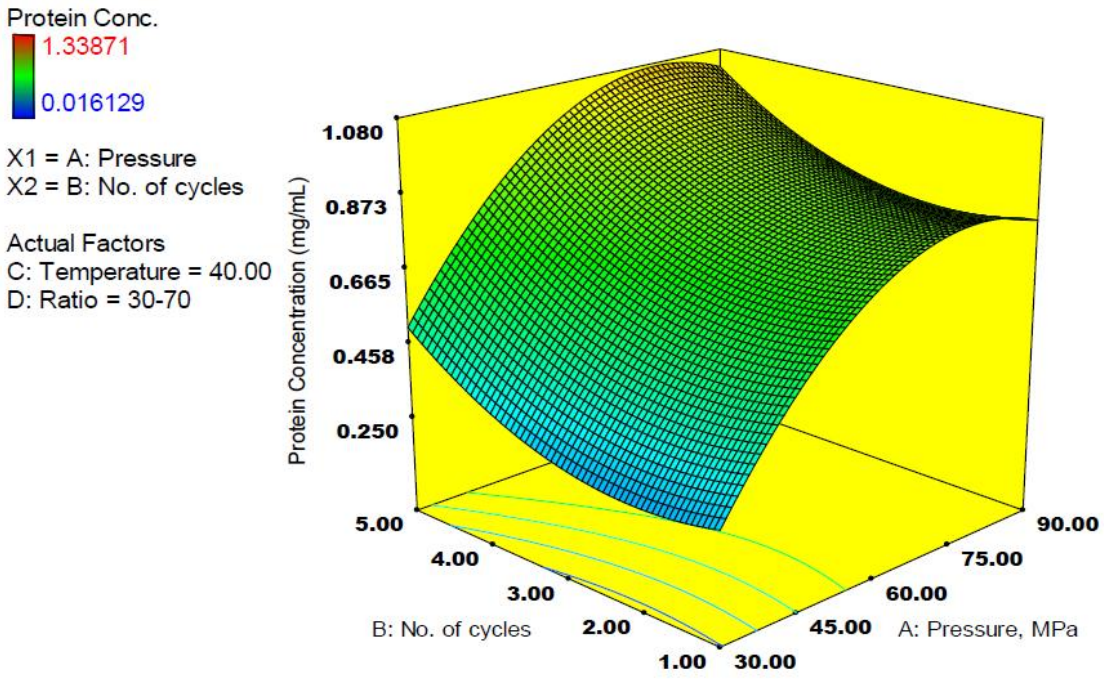
Figure 6 shows the contour graphs for the effect of temperature, pressure and number of the cycle on protein yield. This is presented in the categorical factors (D) as a, b, and c, representing 10:90, 20:80, and 30:70, respectively. This plot provides a 2-dimensional view where all points that have the same response are connected to produce contour lines of constant responses, and thus, illustrate the optimum level of each variable on protein concentration yield. From the values provided, the blue areas contained 0.016129 of protein yields while the red zone contains the highest software-estimated protein concentration yield. With the number of cycles and pressure considered as parameters, more protein yield tends to be produced from the ratio of 30:70. The yellow area in Figure 6 (c) yield of protein is more pronounced. This is shown with the value of the contour plots as 0.935 while that of ratios 10:90, and 20:80 are considered as, 0.702 and 0.852 respectively. As the same actual factors of the temperature of (40°C) given for the 3 ratios, 10:90, 20:80, and 30:70, protein yield was estimated highest in the ratio of 30:70 dilution.



- **Figure 6:** Contours plots (a), (b), and (c) showing the effect of the number of cycles, temperature, and pressure on the response – protein concentration yield

5.2.3 Response Surface Plot

Figure 7 is the response surface plot of protein concentration yield in (mg/mL) (with actual factors temperature at 40°C, and the ratio, at 30:70). This shows the effect of pressure and number of cycles on the yield. In the plot, the red zone shows areas of higher concentration of protein yield, while the blue zones are zones with the lowest concentration of protein yield. It is evident from the Figure 7 that as the pressure increases the protein yield also increases while the relationship between the number of cycles and the operating pressure is opposite.



- **Figure 7: Response surface plot of protein concentration yield in (mg/mL) (with actual factors temperature considered at 40 °C and the ratio at 30:70)**

5.3 Optimization of Homogenization Process and Parameter

Optimizing the homogenization process for the substrate for optimal yield of protein concentration has been necessitated as to determine the different yields at the different dilution ratios of the buffer. The research through *Design Expert software* application centres on obtaining results with the maximum yield at minimum expense. The desirability function is one of the most widely used methods in industry for the optimization of multiple response processes. This was based on the quality of a product or process which has multiple characteristics, and such a process is unacceptable if outside some of the desired limits. The method, therefore, found operating conditions that would provide the most desirable value for the response and for this purpose, only the numerical optimization will be considered. The criterion found quality which was here referred to as higher

protein concentration yield and has two conditions as (Restricted and Not Restricted) considered as a basis for determining the higher yield and whose target was to maximize protein concentration. In the optimization, the first 5 optimal solutions for combinations of categorical factor levels were considered as to determine the yields in protein concentration. Further analysis indicated that through 'Restriction' the parameters were required to be maximized except for the categorical factor of ratio and for the 'Not Restricted', all parameters were required to be in range so as to determine the yield for protein concentration in both cases (Tables 6 and 8).

Table 5: Quality for Protein Concentration yield - Restricted

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Pressure	maximize	30.00	90.00	1.00	1.00	3.00
No. of cycles	maximize	1.00	5.00	1.00	1.00	3.00
Temperature	maximize	30.00	50.00	1.00	1.00	3.00
Ratio	is in range	10:90	30:70	1.00	1.00	3.00
Protein Conc.	maximize	0.02	1.34	1.00	1.00	5.00

From Table 5, the numerical optimization for quality in the yield of protein concentration was analyzed for homogenizing Baker's yeast over a temperature range of 30 – 50 °C. Quality was used here to describe the economic effectiveness of protein concentration in terms of higher yield. The emphasis was to maximize protein concentration yield at minimal expense.

Table 6: The Optimal solution for 3 combinations of categorical factor levels – Quality (Restricted)

No.	Pressure	No. of cycles	Temp	Ratio	Protein Conc.	Cost	Desirability	
1	90.00	5.00	47.00	30:70	0.93	0.45	0.85	Selected
2	86.00	5.00	47.20	30:70	0.95	0.45	0.84	
3	90.00	5.00	46.60	20:80	0.86	0.45	0.82	
4	90.00	5.00	45.50	20:80	0.88	0.45	0.81	
5	90.00	5.00	45.90	10:90	0.73	0.44	0.76	

Table 6 shows the optimal desired solution. A desirability of 0.85 was achieved, when homogenized at 90 MPa, over 5 cycles, at 47 °C temperature and a categorical factor of 30:70 ratio yielding 0.93 mg/mL when optimized numerically. Whereas in the real sense of it, the second on the list at Table 6 showed a better result in terms of the protein concentration yield when homogenized at even lower pressure of 86 MPa and a slightly higher temperature of 47.2 °C resulted in 0.95 mg/mL of protein

concentration. This was an increment of 2.2% of the yield when compared to the desired one rated by the *Design Expert* as 85. One reason to consider here, for the desirability selection would probably be as a result of the lower operating temperature that was applicable.

Table 7: Quality for Protein Concentration yield – Not Restricted (Over the 30 – 50 °C Temperature Range)

		Lower	Upper	Lower	Upper	
Name	**Goal**	**Limit**	**Limit**	**Weight**	**Weight**	**Importance**
Pressure	is in range	30.00	90.00	1	1.00	3.00
No. of cycles	is in range	1.00	5.00	1	1.00	3.00
Temperature	is in range	30.00	50.00	1	1.00	3.00
Ratio	is in range	10:90	30:70	1	1.00	3.00
Protein Conc.	maximize	0.02	1.34	1	1.00	5.00

Table 7 shows the quality for protein concentration yield for no restrictions attached. A desirability of 0.80 was achieved at a 30:70 ratios as the categorical factor's choice yielding 1.07 mg/mL of protein concentration, through the homogenization at 77 MPa, 5 cycles, and at a temperature of 40.8 °C. Based on the criteria above, the 'Not Restricted' scenario was favorable compared to the 'Restricted'. It resulted in higher protein concentration yield in the desirability selections (see Tables 6 and 8). From the scenario above, the 'Not Restricted' has been favoured as against the 'Restricted' resulting in 13.1% increment in protein concentration yield. (See the desirability selections of Tables 6 and 8 of both 'Restricted' and 'Not Restricted' to compare results).

Table 8: The Optimal solution for 2 Combinations of Categorical Factor Level – Quality (Not Restricted)

No.	Pressure	No. of cycles	Temp.	Ratio	Protein Conc.	Cost	Desirability	
1	77.00	5.00	40.80	30:70	1.07	0.43	0.80	Selected
2	80.00	5.00	40.90	30:70	1.07	0.43	0.80	
3	77.00	5.00	42.00	30:70	1.07	0.44	0.80	
4	77.00	5.00	41.00	20:80	0.99	0.43	0.74	
5	76.00	5.00	42.00	20:80	0.99	0.44	0.73	

6. CONCLUSION

The outcome of the experimental results shows a great potential of the novel protein improvement through yeast use and cell wall breakage in HPH and as analyzed using the design expert (DOE). The following points have therefore been achieved.

- Conducted the homogenization process using the 2-Stage HPH GYB40-10S to obtain set results of the four factors (Temperature, Pressure, Number of cycles as well as ratio as categorical factor) and one response (Protein Concentration) using the *Design Expert*
- Protein yielded shows an increment rate as the ratio increases from 10:90 to 30:70.
- The four factors temperature, pressure, number of cycles and ratio as categorical factors considered showed an effect in the resultant protein yield.
- Ratio 30:70 showed the highest yield of protein concentration
- Pressure rise during the experimental work indicates that the viscosity of yeast will be distorted and then increase the protein yield.
- Due to the roughness of the surface, at a certain velocity high enough, the streamline in the flow will no longer maintain the same shape and thereby deviates from the orderliness in the pattern of movement.
- The high-pressure homogenizer has been seen as one of the best ways of disrupting microbial product for high protein yield particularly the used GYB40-10S series with two homogenizing valves.
- Finally, this as in other energy equipment are developed always by researchers and scientists so as to continually create a platform in the provision of a lasting solution to the current energy and emission crises. Similarly, Achour & Olabi 2016 [46] have explained in their recent paper of the need to develop portable reliable equipment at low cost which will give the ability in the monitoring of local emissions of the transport sector. This is thought to provide local government access to reliable tools that can accurately estimate the contribution of traffic related pollutant levels to local emissions Inventories.

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