TECHNO-ECONOMIC ANALYSIS OF LACTASE PRODUCTION: TÜRKİYE CASE

by
Eda Kapar

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TÜRKİYE CASE

APPROVED BY:

Assoc. Prof. Dr. Ali Özhan Aytekin ……
(Thesis Supervisor)
(Yeditepe University)

Assoc. Prof. Dr. İlkem Emrah Nikerel ……
(Yeditepe University)

Assist. Prof. Dr. Işık Çoban ……
(Medeniyet University)

DATE OF APPROVAL: ..../..../20..

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ABSTRACT

TECHNO-ECONOMIC ANALYSIS OF LACTASE PRODUCTION: TÜRKİYE CASE

Lactose is a carbohydrate which is found in milk and known as milk sugar. In addition, it is frequently used in the bakery industry and baby foods due to its fermentability. Depending on its consumption, the digestive system disease which is called lactose intolerance occurs as primary and secondary. As a result of the inability to produce the lactase, also known as a beta-galactosidase enzyme in the intestines or insufficient production. Therefore, in order to reduce the side effects of lactose intolerance, lactase enzyme is used in or externally consumed foods. The aim of this thesis is to carry out fermentation, separation, and purification of beta-galactosidase, an enzyme from *Saccharomyces fragilis* which was proceeded simulation with SuperPro Designer software, in working volumes as 1000 L, 2500 L, 5000 L, 7500 L and 10000 L under optimum production conditions for Türkiye, The United States of America and China. Since efficient production can be determined by simulation with SuperPro Designer, beta-galactosidase production has been investigated by techno-economic analysis. As a result, it has been concluded that the ß-gal production with *Saccharomyces fragilis* in Türkiye has US$ 11,753,414 total investment and US$ 2,564,766 operating cost, 76.61 percent gross margin, 62.64 percent years return on investment rates provide payback time in 1.60 years even in small working volume (2500 L). In volumes more than 2500 L, profitability will increase further, and profit will be made in a shorter time. Additionally, production of beta galactosidase in America and China are less profitable than in Türkiye. Finally, it has been determined that it is more profitable to produce beta galactosidase in Türkiye as an investor or an operator.

ÖZET

LAKTAZ ÜRETİMİNİN TEKNO-EKONOMİK ANALİZİ: TÜRKİYE ÖRNEĞİ

Laktoz, sütte bulunan ve süt şekeri olarak bilinen bir karbonhidrattır. Ayrıca fermente edilebilirliği nedeniyle fırıncılık sektöründe ve bebek mamalarında sıklıkla kullanılmaktadır. Laktoz intoleransı adı verilen sindirim sistemi hastalığı, tüketimine bağlı olarak, bağırsaklarda beta-galaktosidaz enzimi olarak da bilinen laktazın üretilememesi veya yetersiz üretim sonucunda birincil ve ikincil olarak ortaya çıkar. Laktoz intoleransının yan etkilerini azaltmak için laktaz enzimi haricen tüketilen gıdalarda kullanılmaktadır. Bu tezin amacı, Saccharomyces fragilis'ten bir enzim olan beta-galaktosidazın SuperPro Designer yazılımı ile simülasyonu yapılarak 1000 L, 2500 L, 5000 L, 7500 L ve 10000 çalışma hacimlerinde fermantasyonu, ayrılması ve saflaştırılmasını gerçekleştirmektir. SuperPro Designer ile simülasyon ile verimli üretim belirlenebildiğinden, beta-galaktosidaz üretimi tekno-ekonomik analiz ile araştırılmıştır. Sonuç olarak, Türkiye'de *Saccharomyces fragilis* ile ß-gal üretiminin 11.753.414 US$ toplam yatırım ve 2.564.766 US$ işletme maliyeti, yüzde 76,61 brüt kâr marjı, yüzde 62,64 yıl yatırım getirisi oranlarının küçük çalışma hacimlerinde bile 1,60 yılda geri ödeme süresi sağladığı sonucuna ulaşılmıştır (2500 L). Sonuç olarak Amerika ve Çin Türkiye'deki üretime göre daha az kârlıydı, bu yüzden Türkiye'de yatırımcı veya işletmeci olarak üretim yapmanın daha karlı olduğu belirlenmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

β-gal	β-galactosidase
CN	China
DOC	Dissolved oxygen concentration
IPTG	Isopropyl-β-D-1-Thiogalactopyranoside
LRT	Labor cost rate
NPV	Net present value
ONPG	O-Nitrophenyl-β-D-Galactopyranoside
TR	Türkiye
USA	The United States of America

1. INTRODUCTION

Lactose (4-O-ß-D-galactopyranosyl-D-glucose) is essential for the fermented dairy products which include milk and milk products synthesized in the Golgi apparatus; therefore, lactose is also called milk sugar [1].

When milk is obtained from mammalian sources for commercial production, the milk is pasteurized for use in dairy products. For example, milk is first processed to make for producing cheese and then, cheese whey, a byproduct of cheese making, is produced as whey protein and whey permeate [2]. Therefore, whey protein is produced to provide lactose main component for fermentation of enzyme production, as shown in Figure 1.1. Accordingly, whey protein could be considered a economic carbon source to provide the main component of lactose for fermentation of enzyme production. Moreover, this cheese whey permeate is mainly composed of 4-5 percentage of lactose and 0.5 percentage (w/v) of mineral salts [3].

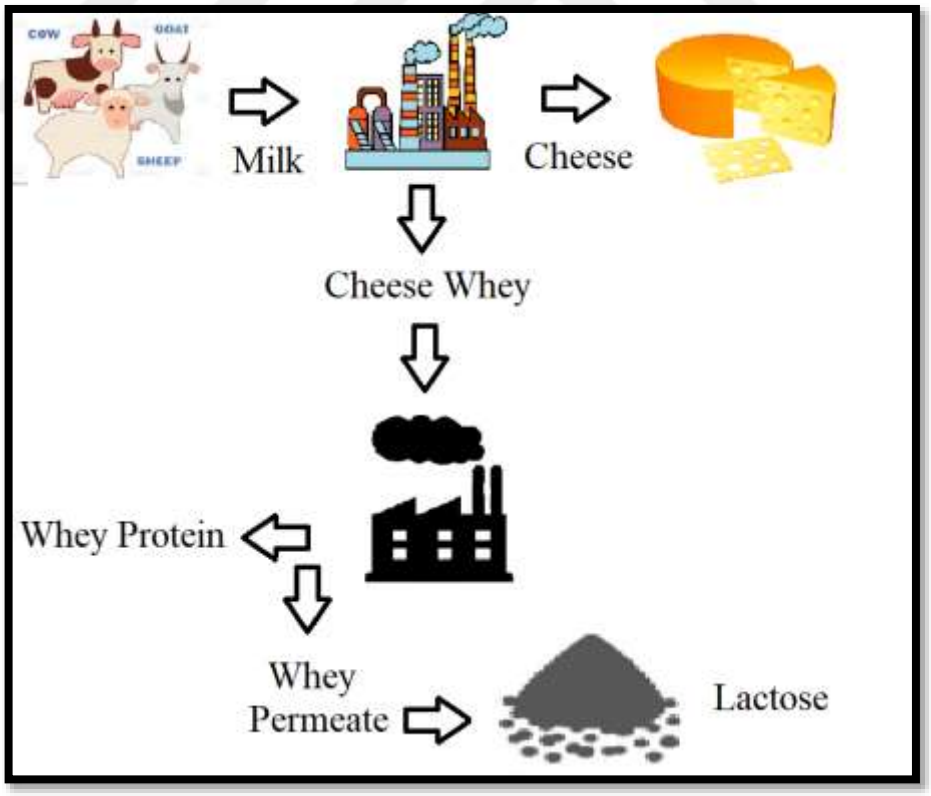

Figure 1.1. Lactose production from milk sources

Lactose is a carbohydrate that composed of its monosaccharides of glucose and galactose. It has molecular formula, $C_{12}H_{22}O_{11}$ with an average mass of 342.297 Da [4]. Among these glucose and galactose monosaccharides, the ß 1-4 glycosidic bond links to each other. And β galactosidase (β-gal) or lactase enzyme hydrolyzes the lactose by breaking the β 1-4 glycosidic bond between the glucose and galactose monomers [5].

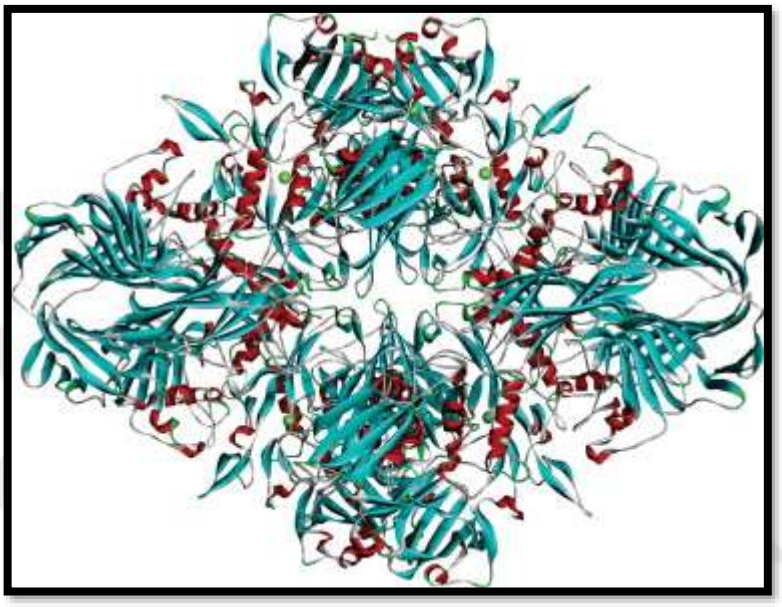

Figure 1.2. B-gal has four identical subunits and is shown as a biological model

As seen in Figure 1.2., β-gal has a molecular weight of 540 kDa and consists of four identical subunits of MW 135 kDa each with an independent active site [6,7]. In addition, lactose as a reducing sugar has an aldehyde group [8]. Most reducing sugars exist as ring structures fromed α or β enantiomers with an asymmetric carbon. Therefore, the enantiomers can be interchanged among each other due to the formation of the ring structure [9,10].

The ring structure of lactose contributes to the formation of flavour compounds and brown polymers, which is also known as Maillard reaction or non-enzymatic browning especially in foods [11]. Accordingly, monosaccharides are more reactive to Maillard browning and lactose is usually hydrolyzed in foods to glucose and galactose [12]. Lactose not only contributes to foods, but also has an adverse effect on humans. Therefore, the enzyme lactase is essential for humans to hydrolyze lactose in the intestine because mammalian infants use most of their energy sources from the lactose in milk during the first year of life [13].

Although young mammals can absorb lactose by these lactic acid bacteria secreting the corresponding β galactosidase enzyme in the small intestine and lactose is a type of carbohydrate that serves as an energy source in milk, older mammals could not absorb lactose from their small intestine as much as their juvenile counterparts because the activity to secrete the lactase enzyme is reduced or insufficient [14–16]. As a result of the lack of secreted lactase enzyme, unhydrolyzed lactose accumulates in the colon. For this reason, accumulated lactose causes the discomfort, despite that the intestinal track tries to balance osmotic pressures by drawing secretion of water into the intestinal lumen, accumulation of water causes dehydration that results watery stools called diarrhea [17]. Subsequently, the remaining monosaccharides are fermented by bacterial metabolism, producing gaseous components such as carbon dioxide, methane, and hydrogen. Depending on the ingestion of lactose, the resulting generated gaseous components cause pain, constipation, abdominal bloating, or diarrhoea with different symptoms [18,19]. This accumulation of lactose in large intestine generally called as lactose intolerance and lactose intolerance is studied into three types to classify it.

Primary lactose intolerance occurs when the infant is fed without breast milk, so, the β-gal activity is lower than when fed with breast milk [20]. In addition, the primary lactose intolerance may also be genetic if it occurs at an older age. This intolerance is also called adult type hypolactasia, lactase nonpersistence or hereditary lactase deficiency. Secondary lactose intolerance can occur at any age. Some diseases, surgeries, medications, or radiation can cause damage to the intestinal mucosa which is referred to as secondary lactose intolerance [21]. Finally, after feeding with mother’s milk is over, intestine could not rarely thrive, and β-gal is rarely absent, therefore, what the infantile diarrhea has occurred, this situation is called congenital lactose intolerance and since congenital lactose intolerance could not be treated, it continues to be lifelong [22].

Since the lactose intolerance is common, a group of researchers studied the prevalence of lactose intolerance in 18-60 year old patients. 59 males, and 85 females were referred from gastroenterology department of a Turkish hospital. The result was that all patients had lactose intolerance with different symptoms and different outcomes in their lives, but there are no control groups because there were no practical and inexpensive tests to determine lactose intolerance. Therefore, researchers have concluded that the patients belonging to the

control group almost all have lactose intolerance without showing symptoms [23]. There are two options to reduce the symptoms and consequences of lactose intolerance: first, the consumption of the lactose must be reduced or completely stopped in lactose intolerance patients. Lactose-containing foods contain 9 – 12 grams of lactose in a cup of milk, 4 – 17 grams of lactose in a cup of yoghurt, 0.04 – 0.05 grams of lactose in 9 grams of butter, and 2 – 6 grams of lactose in 15 grams of ice cream according to Salam A., Ibrahim *et al.*, 2021 [24]. Second, consumption of these foods containing lactose could be taken with pharmaceutical lactase products or chosen as lactose-free options [24].

In Türkiye, lactose free products are available for milk, yoghurt, cheese, and ice cream in commercial Turkish brands such as SÜTAŞ Süt Ürünleri A.Ş, PINAR Süt Mamülleri Sanayii A.Ş., Tat Gıda A.Ş. SEK Süt İşletmesi, AK GIDA Sanayi ve Ticaret A.Ş. İÇİM Süt İşletmesi for one of the solutions to lactose intolerance. In addition, lactose free products in Türkiye from world- renowned private labels such as Danone. Apart from the packaged products, there are some biotechnology companies for production of the β-gal such as MAYASAN Gıda San. Tic. A.Ş or DOĞADAN BİZİM GIDA Ve Süt Ürünleri Sanayi Ve Tic. Limited Şirketi. In pharmaceutical companies, these products are presented as food additives in Tablet and drop forms. These food supplements in drop form are LAKTAZM – Havan Pharmaceuticals, RELACTAZ – Assos Pharmaceuticals, and OSICOL – MCG Pharma, whereas the Tablet forms are LACDIGEST – Gensenta, LACTASE ENZYME – Nature’s Supreme.

1.1. LACTASE (OR B-GALACTOSIDASE (B-GAL)) PRODUCTION

The production of lactose from milk and whey mainly consists of liming, heat treatment and filtration to remove the protein, concentrated whey, refiltration, further concentration, crystallization, and basket centrifuging. Then, the whey is used as substrate for production of β-gal; powdered whey, concentrated whey and whey permeate could be processed by obtaining natural products [25]. For this reason, bacteria, yeast, and fungi can be used in β-gal fermentation processes [26–28].

Bosso *et al* found that cheese whey was the most economical as a carbon source for β-gal production of fifteen different fermentation media which were included sucrose, lactose, and glucose [29]. Therefore, the scientists studied microfiltered cheese whey to separate the

protein and lactose compounds and produce milk derivatives. The yeast *Saccharomyces fragilis* IZ 275, which was able to metabolize lactose by producing the intracellular β-gal enzyme, was cultured, and fermented. In addition, cell concentration and growth analyses, and analytical methods such as lactose content ability, specific enzymatic activity via o–nitrophenyl–β–D-thiogalactopyranoside substrate (ONPG), and enzymatic activity via lactose hydrolysis were studied. Accordingly, lactose was consumed within 12 hours of cultivation and substrate was left at a 11-percentage rate in the culture medium. This shows that the lactose consumed over fermentation time has an average intake rate of 2.63 g/Lh. As the cultivation time increased, the biomass production increasingly reached 11.06 mg/mL and specific growth rate reached 0.317 1/h in 20 hours of cultivation. The volumetric and specific β-gal activities were reached 14.028 U/mL and 0.039 U/mg, respectively, by the microfiltration process of cheese whey [29].

Moreover, recombinant technology is a biotechnological tool that is a solution to increase the native production of proteins and enable the production of useful commercial products [30]. This biotechnological tool is used for manipulating genes with desired properties to obtain high amounts of heterologous proteins, such as their productivity and improved enzymatic productions [31]. Recombinant techniques require produced out of the host microorganisms, then most used is *Escherichia coli* because of having well known characteristics such as growth rates, and fermentation capacities [32]. In addition, recombinant proteins must be optimized by ligands or tags with high affinity for the targets. These tags can be maltose binding proteins, cellulose binding domain and chitin binding proteins [33–35].

In a recombinant production study, heterologous proteins expressed in *E. coli* were used as operon lac, and then, lactose was used as an operon lac inducer, to induce the gene of interest. Therefore, two different *E. coli* cells, BL21 and Rosetta, were transformed from *Kluyveromyces sp.* and selected on agar containing the antibiotic kanamycin for each strain [36]. Powdered cheese whey and powdered cheese whey permeate, isopropyl- β-D-1-tiogalactoside (IPTG) and lactose induction solutions were used for the expression of β-gal. As a result, the expression was highest with IPTG specific activities of the recombinant *E. coli* strains were 22 U/mg and 33 U/mg proteins, respectively. In addition, the unit of enzymatic activity was represented per milligram of cell mass was expressed as U/mg protein to verify the effect of cell concentration on enzymatic activity [36]. Furthermore, the

highest specific activity was observed for the recombinant BL21 strain when induced with lactose and whey permeate at concentrations of 10 g/L and 20 g/L, respectively. Maximum specific lactase activity was reached after nine hours remained constant during the 24 hours. In the Rosetta strain, the highest specific activities were obtained with lactose and whey permeate at concentration of 20 g/L after nine hours, and the highest specific activity was 45 U/mg protein. In conclusion, the highest specific activity for each *E.coli* strain was obtained with lactose and whey permeate at concentration of 10 g/L and 20 g/L. Therefore, the solutions could be effective in industrial bioprocesses for the production of β-gal [36]. Another example is a recombinant *Kluyveromyces* sp. fused with cellulose binding domain to use in industrial applications. Because of its low costs, structural properties and abundance, cellulose binding domain fuses with β-gal that will be used in producing lactose derivatives [37]. Moreover, in similar study β-gal was produced in bioreactors from *Kluyveromyces* sp. by fed batch culture using the DO-stat feeding strategy and linear control. Therefore, *E. coli* was used as a host microorganism for the production of recombinant β-gal [38]. Production in a bioreactor allows the factors affecting the microbial growth to be adjusted for the temperature, dissolved oxygen concentration (DOC), medium pH and speed of stirrer [39]. Besides, fed batch fermentation is used to adjust the feedback control of microbial growth factors to increase productivity in such processes [40]. As a result, DO stat feeding strategy was increased the β-gal enzymatic activity to 42.367 U/L and specific β-gal enzymatic activity to 40 U/mg protein after 12 hours [39].

According to evolutionary relationships, β-gal enzymes are classified into glycoside hydrolase families, referred to as GH [41]. To improve the substrate solubility and reaction rate, GH42 enzymes from microorganisms can adapt the extreme conditions such as thermophilic, halophilic, and psychrophilic conditions. Using a genome *B. coagulans* NL01 as a template, the genes of BcBga and $BcBga^{m}$ were amplified in one study [42]. Therefore, the products were digested with restriction endonucleases and cloned into an expression vector digested with the same enzymes to obtain recombinant plasmids. Transformation of the recombinant plasmids into *E.coli* cells was supported. Protein expression, purification and concentration were then performed. Finally, the beta galactosidase enzymes from B. coagulant NL01 was cloned to GH42, therefore, the hydrolysis activity of the cloned enzyme was observed towards its specific enzymatic activity. The importance of this study was that the previous GH42 beta galactosidase had a weak lactose hydrolysis activity, and the

application was carried out with chromogenic substrates. In this study, higher specific enzyme activity for lactose hydrolysis in whey was observed as 27.18 U/mg after 3 hours than in previous studies in which beta galactosidase was isolated from *Thermus thermophilus* and *Geobacillus strearothermophilus* [43]. In addition, a Mig1 gene complex plays critical role in glucose repressions, so, the promoters of a variety of genes responsible for glucose repression may be bound via the Mig1 gene a zinc finger protein. On the other hand, Snf1 complex is a protein kinase encoded by SNF1 gene. Snf1 complex is phosphorylated by another kinase when the glucose concentration in the medium is absent or very low. Then, the Snf1 gene catalyzes the phosphorylation of Mig1 gene, which results in the Mig1 gene not being repressed by glucose. Therefore, the Mig1 gene can repress glucose with promoter binding when the Snf1 complex is inactivated at high glucose concentration in medium [44]. In a study, a gene similar to Mig1 was cloned in *Kluyveromyces marxianus.* To have a gene similar to Mig1, gene some mutants has been isolated from. Therefore, the glucose repression of invertase gene is decreased and the gluconeogenic carbon sources are repressed. Therefore, the transcriptional repressor Mig1 regulates gene expression and extracellular enzymes production in *Saccharomycopsis fibuligera,* resulting in, *K. marxianus* producing 4140 U/grams of lactase [45]. Because the Mig1 gene can express lactase and produce lactase enzymes, the Mig1 gene in *K. marxianus* was purified and examined. Finally, a Mig1 mutant was obtained that produced more lactase than native *K. marxianus,* the enzymatic activity of the mutant *K. marxianus* was 121 U/mL within 60 hours [46].

1.2. LACTASE (OR B-GALACTOSIDASE (B-GAL)) PRODUCTION ACCEPTANCE IN TÜRKİYE

In Türkiye, information about pharmaceutical companies sold the products containing lactase were obtained from individual conversations. Therefore, Osicol food supplement containing lactase enzyme is produced in 15 mL includes 35 percent lactase enzyme, and both the Relactase and Osicol food supplements are sold 83,320 boxes per year. One box of these supplements in Türkiye is almost 7 US$ shown in Table 1.1.

Table 1.1. Supplementary food market prices in Türkiye as of June 2022

Product Name	Market Price	Net Amount	Company Name
Osicol Drops	80 TL	15 mL	MCG Pharmaceuticals
Relactaz Drops	168 TL	15 mL	Asos Pharmaceuticals
Relactaz Tablets	9.67 TL	30 Tablets	Asos Pharmaceuticals

According to 2022 wages, While the gross minimum wage was 375 US$ in Türkiye; in China, it was 383 US$ (1 US$ is equivalent to 18 ₺.).

To build a plant, an industrial production process must first be evaluated based on the bioprocess which consists of upstream and downstream stages. The upstream stages are the operations to produce the desired product from cell cultivation, and the downstream stages are the operations to recover and purify the desired product. Thus, upstream, and downstream stages are based on the synthesis and analysis. The operating units are selected by synthesis step and required raw materials, resources, and total costs are determined by analysis step. In this study, economic performances of the processes are analyzed with the techno economic analysis method, according to capital costs, operating costs and revenues for technical and financial input parameters using SuperPro Designer. Then, in the sensitivity analysis evaluates the main assumptions that determine the profitability for a better economic performance are raw material costs, labor-related costs, facility dependents costs, consumable costs and utility costs, and the results are analyzed. Therefore, assumptions of economic model affect its predictions, sensitivity analysis is used to build the profiTable production by the most correct assumptions [47].

The calculation for the SuperPro designer workforce shown in equation in Equation (1.1) and Table 1.2, labor cost rate is equal to basic labor rate multiplied by the summary of benefits, supervision, supplies, administration and 1 as shown in Equation (1.1). Then, the labor cost is determined by basic rate multiplying with labor hours and summary of 1,

benefits, supervision, supplies and administration as seen in Equation (1.2). And the estimated labor time is a percentage of direct time use.

$$\text{LRT (labor cost rate)} = \text{Basic labor rate x (1+benefits + supervision +supplies +administration)} \quad (1.1)$$

Table 1.2. SuperPro Designer employee fee calculations

	Basic rate ($/hr)	Fringe Benefits factor	Supervision cost	Operator supplies cost	Administration
Operator	26.00	0.4	0.2	0.1	0.6
QC analyst	30.00	0.4	0.2	0.1	0.6

$$\text{Labor cost} = \text{Basic rate x } (1 + \text{benefits} + \text{Supervision} + \text{supplies} + \text{administration}) \text{ x Labor hours} \quad (1.2)$$

The production areas, provinces in Türkiye, were selected according to the state support program in order to keep the development level the same for each city in Türkiye and to support production activities are as follows. The cities where lactase enzymes can be produced were selected from the cities divided into regions by state in order to be close to the qualified employees in factories and large enterprises and to have easy access to technical support from outside. Factories and enterprises related to lactase enzyme production in nearby cities were considered in the evaluation. It is aimed that qualified employees from nearby cities can come to work without changing city. Considering the general salary system in the country, salaries and opportunities within the scope of the govenrment support to reach qualified workers are kept attractive.

For the provinces located in 6 different regions in Türkiye depending on their level of socio-economic development, incentive application is applied, VAT Exemption, Customs Duty Exemption, Tax Reduction, Insurance Premium Employer's Share Support, Interest or Profit Share Support, Income Tax Withholding Support, Insurance Incentive application in which support elements are offered by the state, in case of investments in certain conditions and sectors according to Republic of Türkiye. The state supports also include incentives for young entrepreneurs, value added tax exemption, customs duty exemption, tax reduction,

insurance premium, investment place allocation, interest support. These supports are increasing in the eastern provinces due to the lack of demand in the eastern provinces according to Ministry of Industry and Technology of the Republic of Türkiye.

It is necessary to control capital management throughout the beta galactosidase enzyme production and development process in order to correctly calculate the capital required to start the business and avoid excessive costs. Thanks to the equation for estimating the cost of capital, it is possible to perform fast and reliable calculations depending on the equipment.

$$C = Co \left(\frac{Q}{Qo}\right)^{a} \tag{1.3}$$

This calculation is nonlinear and is based on the capacity of the equipment, the capacity of similar equipment and pricing. The method for estimating the cost of capital is given in Equation 1.3. This equation is used to calculate as accurately as possible the cost required to increase capacity. The nonlinear increase in a value that should be used as an exponent and expected size Q are calculated according to the type of equipment [48]. The sixth tenth rule is used to easily calculate unknown equipment. Each calculation is calculated separately for the equipment and similar equipment should be purchased as base capacity and base cost [49].

1.3. AIM OF THE STUDY

The purpose of the thesis is that to evaluate and compare techno-economic analysis of native intracellular lactase (beta galactosidase) enzyme production from *Saccharomyces fragilis* at 1000 L, 2500 L, 5000 L, 7500 L and 10000 L working volume for Türkiye, China, and the United States of America by using SuperPro Designer software.

2. MATERIALS

Intracellular lactase enzyme was assumed producing from native *Saccharomyces fragilis* IZ 275 organisms. Parameters that altering with manufacturing capacity between at 1000 L, 2500 L, 5000 L, 7500 L and 10000 L working volume scenarios in Türkiye, in China and in The United States of America were analyzed at SuperPro Designer V11 Software (Intelligen, USA). The form of intracellular enzyme was analyzed with same key assumptions for every country according to altered production capacity and compared the operational costs and capital costs according to countries.

3. METHODS

Methods were set through the key assumptions for SuperPro Designer software, then the scenarios were detailed in followed parts. Beta galactosidase enzyme productions that was determined with the literature research through specific beta galactosidase activity (14.28 $U.mL^{-1}$) and volumetric beta galactosidase activity (0.039 $U.mg^{-1}$) were processed with optimized media ingredients via same B-gal production strategy as seen in Figure 3.1 [50]. Strategy was based on inoculum preparation, media preparation, fermentation, permeabilization and purification.

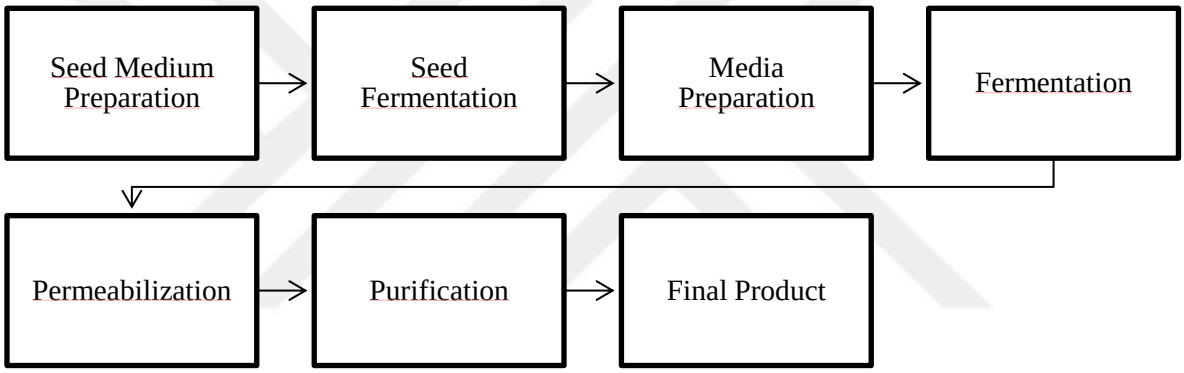

Figure 3.1. Beta galactosidase production strategy

3.1. KEY MODEL ASSUMPTIONS

Native enzyme production process and assumptions was simulated according to technical and economic parameters with necessary input and output stream data by SuperPro Designer. Based on baseline scenario shown in Table 3.1, working volume of seed fermenter alters between 100 L to 1000 L while working volume of the main fermenter were altering 1000 L to 10000 L. Both working volume for seed fermentation and main fermentation were eighty percent and temperature 30^{o}C, and fermentation time were 48 hours and 72 hours, respectively.

Table 3.1. In baseline scenario, assumptions for native enzyme production

Parameter	**Assumption**
Nominal volume of the seed fermenter	100 L – 1000 L
Working volume of seed fermenter	80 percent
Temperature for seed fermentation	30 ^{o}C
Seed fermentation time	48 h
Nominal volume of the main fermenter	1000 - 10000 L
Working volume of main fermenter	80 percent
Temperature for main fermentation	30 ^{o}C
Main fermentation time	72 h

Native beta galactosidase production from *Saccharomyces fragilis* was based on four steps which were media preparation and seed fermentation, fermentation, permeabilization and the recovery of B-gal enzyme as seen in Figure 3.1. While green section was representing production step, orange section represents the purification step in Figure 3.1. *S.fragilis* was inoculated from stock tube containing PDA medium (Potato Dextrose Agar) (data not shown). Then, the microorganism was inoculated with media containing 2 percent (w/v) of malt extract and 0.5 percent (w/v) of yeast extract then, the 10 percent (v/v) of *Saccharomyces fragilis* from seed fermenter was transferred to main fermenter. Main fermentation medium produced as native B-gal enzyme were prepared with 10 g/L lactose and glucose, 14 g/L sucrose and 17.7 g/L cheese whey for carbon sources, 5.14 g/L yeast extract and 7 g/L peptone for nitrogen source, 7 g/L magnesium sulfate for medium as a medium salt and 5 g/L dipotassium phosphate as a medium buffer as shown in Table 3.2 [29]. Based on *Yadav et al*, cheese whey composition for fermentation media was set as 5.8900 percent of ash, 0.5000 percent of fats, 79.6800 percent of lactose, 12.0300 percent of proteins, 1.9000 percent of water [51].

Table 3.2. Cheese whey compositions

Ingredient Name	Mass (percent)
Ash	5.8900
Fats	0.5000
Lactose	79.6800
Proteins	12.0300
Water	1.9000

Table 3.3. In baseline scenario, contents of main fermentation medium for β -gal enzyme production

Fermentation Medium	Carbon Sources	Carbon Support	Nitrogen Source	Salt	Buffer
Materials of Native Enzyme	10 g/L Lactose, 10 g/L Glucose, 14 g/L Sucrose	17.7 g/L Cheese Whey	5.14 g/L Yeast Extract, 7 g/L Peptone	7 g/L MgSO4	5 g/L K2HPO4

Stoichiometric equation was created according to carbon, nitrogen, oxygen, and hydrogen balances for model of the cell growth and batch fermentation. To produce native B-gal enzyme, stoichiometric coefficient of the biomass molar basis is equal to sum of the mass coefficient of carbon sources such as glucose, lactose, and sucrose. Accordingly, molecular biomass composition of *Saccharomyces fragilis* was modelled from the empirical formula of *Saccharomyces cerevisiae* as $CH_{1.748}O_{0.596}N_{0.148}$ [53]. Additionally, yeast extract and malt extract which were nitrogen sources were modelled as ammonia. Then, fermentation equations were determined by Gauss-Jordan elimination as followed below for glucose and sucrose or lactose, respectively [54].

$$0.268\ C6H12O6 + 0.624\ O2 + 0.065\ \text{Yeast Extract} + 0.085\ \text{Peptone} \rightarrow CH\ 1.748\ O\ 0.596\ N\ 0.148 + 0.802\ CO2 + H2O \quad (3.1)$$

$$0.150\ C12H22O11 + 0.774\ O2 + 0.148\ \text{Yeast Extract} + 0.0850\ \text{Peptone} \rightarrow CH\ 1.748\ O\ 0.596\ N\ 0.148 + 0.802\ CO2 + H2O\ S \quad (3.2)$$

For native B-gal enzyme production in Figure 3.2, given data in Table 3.3 for batch medium into seed fermenter was prepared with 2 percent (w/v) of malt extract and 0.5 percent (w/v) of yeast extract in storage (V-104) and autoclaved at 121^{o}C for 15 minutes in heating (HX-101). Then, autoclaved medium was transferred to seed fermenter (SFR-101), the yeast was inoculated with 10 percent (v/v) of ratio to autoclaved medium and cooled 30^{o}C in 15 minutes in seed fermenter. Thus, yeast has been grown at 30^{o}C for 48 h with 1 vvm aeration rate for all carbon sources while salt and buffer were continuing reacted. While the yeast was growing in seed fermenter (SFR-101), fermentation medium was prepared as Table 3.2 in a blending tank (V-101). Prepared medium was and autoclaved 121^{o}C for 15 minutes in heating (HX-102) and the autoclaved medium was transferred to main fermenter (FR-101) and cooled 30^{o}C in 15 minutes. While the reaction of salt and buffer were continuing during the fermentation, products were occurring at 30^{o}C for 72 h with 1 vvm aeration rate for all carbon sources. After the fermentation (FR-101), recovery section was begun with filtration at MF-101 with rejection coefficient of biomass was adjusted as 1 to obtain intracellular B-gal enzyme from produced biomass. After the biomass was concentrated, B-gal enzyme was extracted with high pressure homogenization with 800 bar. Homogenization material balance was assumed that the biomass was separated to lactase, cell debris, nucleic acids, and the proteins in homogenizer (HG-101) and stated with followed Equation (3.3).

$$10\ \text{Biomass} \rightarrow 1.2\ \text{Bgal} + 2.3\ \text{Cell Debris} + 1.5\ \text{Nucleic Acid} + 5\ \text{Proteins} \qquad (3.3)$$

After the biomass was homogenized, ethanol precipitation was achieved with 70 percent (v/v) of ethyl alcohol at storage tank (V-102). Then, the biomass and cell debris were thrown out the 98 percent of biomass and 9 percent of debris at centrifuge (DS-101) and solution gathered at tank (V-103). Gathered solution was ultrafiltered with 9 concentration factors and the rejection coefficient of B-gal was adjusted as 1, then, transferred again to same tank (V-101). Finally, purified B-gal enzyme was dried with 99.5 percent of water evaporation and 90 percent ethyl alcohol evaporation at freeze drying to obtain final product for industry.

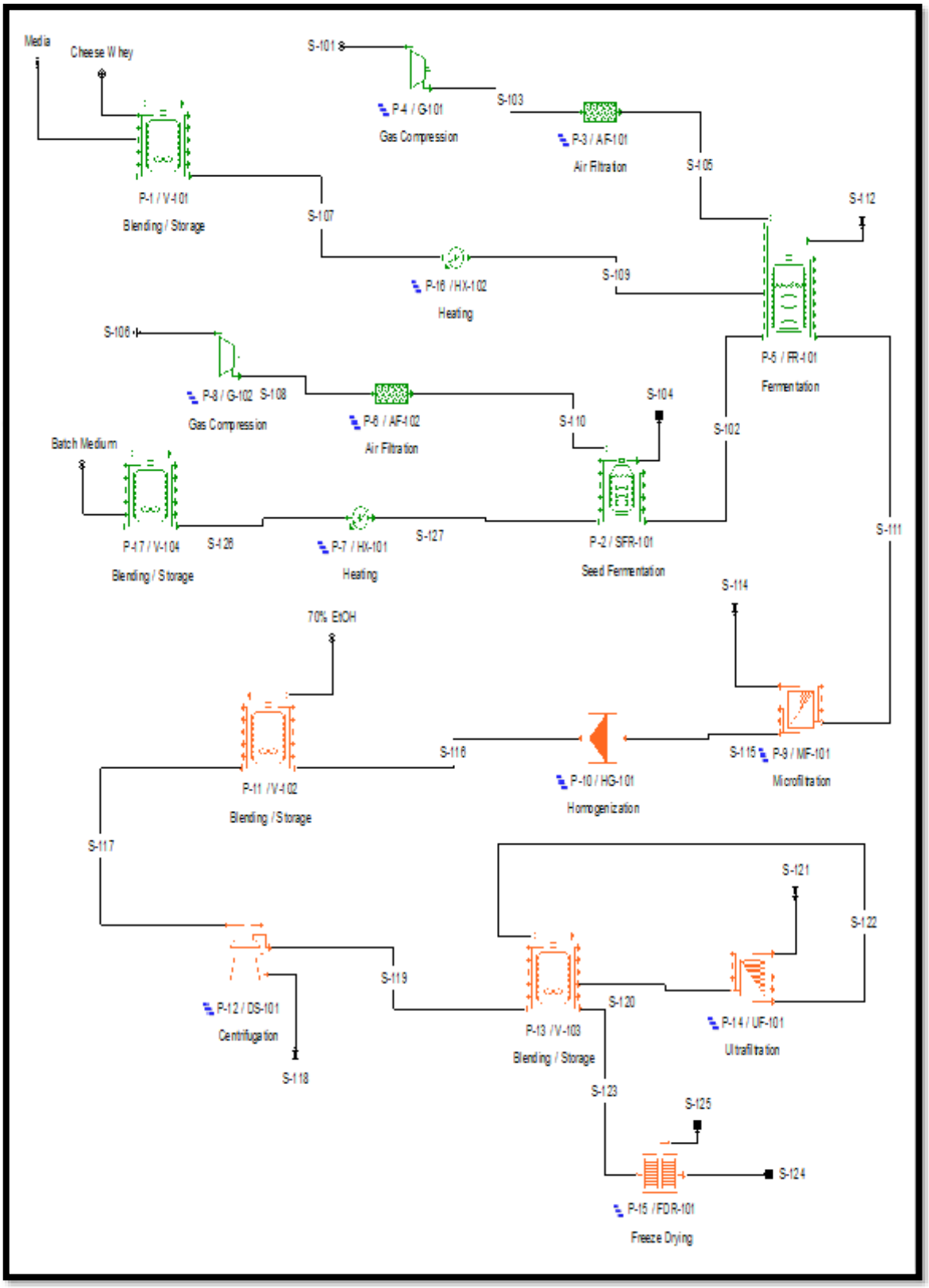

Figure 3.2. Simulation of native enzyme production with SuperPro Designer

Table 3.4. Materials entering the native enzyme production for the process

Ingredient	Unit Price ($)
Batch Medium	0.145
Cheese Whey	2.78
Dipotassium Phosphate	1.433
Ethyl Alcohol	0.750
Glucose	0.551
Lactose	3.600
Magnesium Sulfate	0.121
Peptone	7.496
Sucrose	5.000
Yeast Extract	5.000

* Basis of costs based on kg.

Scenarios for each country were adjusted as Table 3.4 which contains input materials for B-gal production process, raw material costs, equipment capacity and costs, and different fermenter volume. Therefore, unit prices of media and ethyl alcohol precipitation seen in Table 3.5 were set through the kg basis as 0.55 US$ glucose, 5 US$ sucrose, 3.6 US$ lactose, 7.5 US$ peptone, 5 US$ yeast extract, 2.78 US$ cheese whey, 1.43 US$ dipotassium phosphate, 0.12 US$ magnesium sulfate and 0.75 US$ ethyl alcohol, while unit price of batch medium was set 2.78 US$ which was calculated the unit price of the entering materials into inoculum medium. Labor cost estimations for dryer operator, filter operator, operator, reactor operator and supervisor were adjusted as 0.4 benefit factor, 0.1 operating supplies factor, 0.2 supervision factor and 0.6 administration factor in each study.

In labor section, there were dryer operator, filter operator, reactor operator, operator, supervisor, and the labor hours accepted same as correspond to per year and per batch production for every scenario. Also, the labor costs for all scenarios were estimated with parameters in Table 3.5 through the equation (1.2). While the benefit factor, operating supplies factor, supervision factor and administration factor were 0.40, 0.10, 0.20 and 0.6, respectively.

Table 3.5. Equipment capacities based on different working volume studies

Working Volumes		1000 L	2500 L	5000 L	7500 L	10000 L
Type	Air Filter (AF-101)	68,879 L/h	17,2197 L/h	40,035 L/h	516,592 L/h	68,8791 L/h
	Air Filter (AF-102)	7,033 L/h	17,582 L/h	34,4395 L/h	52,744 L/h	7,0326 L/h
	Disk-Stack Centrifuge (DS-101)	146 L/h	365 L/h	730 L/h	1,094 L/h	1,459L/h
	Freeze Dryer (FDR-101)	64 kg	160 kg	320 kg	8400 kg	641 kg
	Fermenter (FR-101)	1,150 L	2,800 L	5,600 L	8,400 L	1,1200 L
	Centrifugal Compressor (G-101)	10 kW	25 kW	50 kW	75 kW	100 kW
	Centrifugal Compressor (G-102)	1.5 kW	3 kW	6 kW	8 kW	10.5 kW
	Homogenizer (HG-101)	84 L/h	210 L/h	420 L/h	628 L/h	838 L/h
	Heat Exchanger (HX-101)	25 m2	7 m2	14 m2	25 m2	3 m2
	Heat Exchanger (HX-102)	3 m2	65 m2	125 m2	190 m2	250 m2
	Microfilter (MF-101)	5 m2	5 m2	5 m2	5 m2	5 m2
	Seed Fermenter (SFR-101)	120 L	290 L	580 L	865 L	1200 L
	Ultrafilter (UF-101)	0.3 m2	0.3 m2	0.3 m2	0.3 m2	0.3 m2
	Blending Tank (V-101)	1,050 L	2,250 L	5,050 L	7,550 L	10,050 L
	Blending Tank (V-102)	750 L	1,900 L	3,750 L	5,600 L	7,450 L
	Blending Tank (V-103)	1,650 L	1,650 L	3,250 L	4,850 L	6,450 L
	Blending Tank (V-104)	115 L	285 L	570 L	747 L	1,150 L

Determined equipment capacities were seen in Table 3.5 based on different working volume studies at 1000 L, 2500 L, 5000 L, 7500 L and 10000 L. Determined equipment capacities were also used in capital cost estimations.

Table 3.6. Heat transfer agents and unit costs

Heat Transfer Agent	Unit Cost ($)
Cooling Water	0.05
Freon	0.15
Hot Water	0.05
Steam	12.00

In order to calculate the costs of the heat transfer agents required for the equipment where the operations occur, the values over the unit price are entered as in Table 3.6.

Table 3.7. Total flowrates on revenue stream

		Unit Production Cost (US$/Kg MP)				
Scenario	**Working Volume**	1000 L	2500 L	5000 L	7500 L	10000 L
	TR	3,887	2,357	1,699	1,412	1,288
	USA	24,600	11,182	6,527	4,803	3,897
	CN	6,184	3,365	2,275	1,856	1,603
Unit Production Referance Rate (Kg MP/yr)		435	1088	2157	3235	4152

*Unit production costs includes depreciation.

In order to determine total revenue, output on FDR-101, S-124 stream, were determined as revenue stream, therefore, the selling prices were calculated as 1000 times of S-124 stream for each working volumes in each scenario at Table 3.7. Additionally, total power consumption costs are given in data at Table 3.8.

Table 3.8. Total power consumption cost

Scenario		USA	TR	CN
		Total Cost (us$)		
Working Volume	1000 L	20,247	10,731	19,032
	2500 L	50,445	26,727	47,418
	5000 L	99,787	52,888	93,800
	7500 L	148,205	79,290	139,313
	10000 L	193,824	101,739	180,442

* Unit cost of USA, TR and CN are based on 0.1 US$/kW-h, 0.053 US$/kW-h and 0.09 US$/kW-h, respectively.

3.2. SCENARIOS BASED ON KEY ASSUMPTIONS

Processes based on the key assumptions were evaluated through the different scenarios for different countries and different production volumes in B-gal production. Evaluations were analyzed through the key assumptions containing input material costs, equipment capacity and costs and labor costs for every country.

3.2.1. Scenario A: B-gal Production for The United States of America

Scenario A evaluated through the key assumptions and customized capital costs and operating costs for The United States of America. Therefore, the stated input material prices in Table 3.5, total prices for raw materials were calculated for media, cheese whey, batch medium and 70 percent of EtOH via the ingredient unit prices for B-gal production for scenario A. Determined working volumes for B-gal production for fermenter were 1000 L, 2500 L, 5000 L, 7500 L and 10000 L, therefore, the seed fermenter working volumes were determined the 10 percent of inoculum ratio. Therefore, different fermenter working volume

which were stated via B-gal enzyme demand for products, based on working volumes for fermenters, fermenter sizes and unit prices were stated in Table 3.6. Operation times were determined for the batch number and the operation orders for each volume. Therefore, equipment capacities, equipment unit prices, labor types and labor costs determined for 1000 L, 2500 L, 5000 L, 7500 L and 10000 L.

Table 3.9. Scenario A: Fermenter capacities and prices for different working volume for β-gal production

Working Volume	**Type**	**Unit Price ($/Unit)**
1000 L	Fermenter	735,000
100 L	Seed Fermenter	552,000
2500 L	Fermenter	833,000
250 L	Seed Fermenter	606,000
5000 L	Fermenter	919,000
500 L	Seed Fermenter	668,000
7500 L	Fermenter	973,000
750 L	Seed Fermenter	706,000
10000 L	Fermenter	1,017,000
1000 L	Seed Fermenter	740,000

Apart from fermenter and seed fermenter, equipment capacities and costs were stated as the highest production volume. According to equipment type, unit prices were determined through the equipment size with build in cost model at SuperPro Designer. Fermenter and seed fermenter capacities were calculated with 90 percent of volume vessel ratios as seen in Table 3.9. Therefore, the unit prices were determined in SuperPro Designer for each volume.

Table 3.10. Scenario A: Calculated unit prices in SuperPro Designer based on equipment capacity

Equipment Name	Working Volume	Unit Price (US$/Unit)				
		1000 L	**2500 L**	**5000 L**	**7500 L**	**10000 L**
	Air Filter (AF-101)	8,000	8,000	8,000	8,000	8,000
	Air Filter (AF-102)	8,000	8,000	8,000	8,000	8,000
	Disk-Stack Centrifuge (DS-101)	123,000	123,000	123,000	123,000	123,000
	Freeze Dryer (FDR-101)	564,000	1,263,000	2,163,000	2,780,000	3,170,000
	Centrifugal Compressor (G-101)	81,000	81,000	81,000	81,000	81,000
	Centrifugal Compressor (G-102)	81,000	81,000	81,000	81,000	81,000
	Homogenizer (HG-101)	25,000	25,000	25,000	25,000	25,000
	Heat Exchanger (HX-101)	17,000	28,000	42,000	59,000	63,000
	Heat Exchanger (HX-102)	59,000	105,000	156,000	200,000	236,000
	Microfilter (MF-101)	28,000	28,000	28,000	28,000	28,000
	Ultrafilter (UF-101)	31,000	31,000	31,000	31,000	31,000
	Blending Tank (V-101)	215,000	244,000	268,000	284,000	280,000
	Blending Tank (V-102)	205,000	234,000	257,000	272,000	284,000
	Blending Tank (V-103)	201,000	229,000	252,000	267,000	278,000
	Blending Tank (V-104)	164,000	179,000	198,000	209,000	218,000
TOTAL		**3,097,000**	**4,106,000**	**5,308,000**	**6,135,000**	**6,671,000**

Unit price of equipments seen in Table 3.10 were adjusted at SuperPro Designer with build in cost model by the capacity of each equipment. Through the capacities, unit prices (US$/unit) were basically determined as 8.000 for both air filters, 123.000 for disk stack centrifuge, 547.000 for freeze dryer, 81.000 for both centrifugal compressors, 25.000 for homogenizer. Total costs for each volume in Table 3.10 were include the costs of fermenter and seed fermenter for each working volume include. According to equipments and the operations, necessary heat transfer agents and the power demand were adjusted for the USA as Table 3.6 and 0.1 US$ for 110 V, respectively.

Table 3.11. Scenario A: Labor type and basic rate

Labor Type	Basic Rate (US$/h)
Dryer Operator	30.66
Filter Operator	30.66
Operator	30
Reactor Operator	30.66
Supervisor	50

These assumptions were evaluated at SuperPro Designer Tool, which is used in different industries such as biotechnology, food and pharmaceutical. It is a process simulator that is based on continuous and batch processes towards the process engineering techniques. Material and energy balances, equipment sizing and rating, cost of good analysis, through process economics, process scheduling and cycle time analysis and scale up and down process could be studied by users (Intelligen, USA). Based on the equipments, labor types and basic labor rate seen in Table 3.10 were adjusted for The U.S.A. scenario at the software as 30.66 (US$/h) for dryer, filter, and reactor operator, 30 (US$/h) for operator, and 50 (US$/h) supervisor at Table 3.11.

3.2.2. Scenario B: Bgal Production for Türkiye

Scenario B for Türkiye was adjusted as key assumption Table which contains input materials for B-gal production process, raw material costs, equipment capacity and costs, and different fermenter volume values. Therefore, unit prices of media and ethyl alcohol precipitation seen in Table 3.4 were set through the kg basis as 0.55 US$, glucose, 5 US$ sucrose, 3.6 US$ lactose, 7.5 US$ peptone, 5 US$ yeast extract, 2.78 US$ cheese whey, 1.43 US$ dipotassium phosphate, 0.12 US$ magnesium sulfate and 0.75 US$ ethyl alcohol, while unit price of batch medium was set 2.78 US$ which was calculated the unit price of the entering materials into inoculum medium. Through the stated input material prices in Table 3.4, total prices for raw materials were calculated for media, cheese whey, batch medium and 70 percent of EtOH via the ingredient unit prices in Table 3.4 for B-gal production for scenario B.

Equipment prices were calculated with the base capacity from marketing talks by capital cost estimation with sixth tenth rule rapid cost estimation method in equation 1.3. The basic capacities of the equipments which are obtained from marketing talks were determined according to 592.06 L and 118.90 L, 7.342,81 L/h, 73.212,25 L/h, 134.69 L/h, 61.9 kg, 10.64 kW, 1.07 kW, 88.84 L/h, 2.85 m2, 26.22 m2, 6.66 m2, 5.83 m2, 1.066,99 L, 603.84 L, 592.06 L and 118.90 L values, respectively. Through the capacities seen in Table 3.12, unit prices (US$/unit) were determined in Table 3.13 and 3.14.

Table 3.12. Scenario B: Equipment capacities and unit costs

Equipment Name	**Base Size (Capacity)**	**Exponent**	**Base Unit Costs (US$)**
Air Filter (AF-101)	3323.8 L/h	0.6	1,666
Air Filter (AF-102)	3323.8 L/h	0.6	1,666
Disk-Stack Centrifuge (DS-101)	7.34 L/h	0.6	33,333
Freeze Dryer (FDR-101)	59.02 L	0.6	27,777
Centrifugal Compressor (G-101)	61.9 kW	0.6	22,222
Centrifugal Compressor (G-102)	10.64 kW	0.6	16,666
Homogenizer (HG-101)	1.07 kW	0.6	16,666
Heat Exchanger (HX-101)	88.84 L/h	0.6	11,111
Heat Exchanger (HX-102)	2.85 L/h	0.6	3,333
Microfilter (MF-101)	26.22 m2	0.6	3,333
Ultrafilter (UF-101)	6.66 m2	0.6	13,888
Blending Tank (V-101)	5.92 L	0.6	8,333
Blending Tank (V-102)	5.83 L	0.6	16,666
Blending Tank (V-103)	1,066.99 L	0.6	2,777
Blending Tank (V-104)	603.84 L	0.6	2,777
Air Filter (AF-101)	592.06 L/h	0.6	2,777
Air Filter (AF-102)	118.90 L/h	0.6	555

Determined working volumes for B-gal production for fermenter were 1000 L, 2500 L, 5000 L, 7500 L and 10000 L, therefore, the seed fermenter working volumes were determined the 10 percent of inoculum ratio. Furthermore, different fermenter working volume which were stated via B-gal enzyme demand for products, based on working volumes for fermenters,

fermenter sizes and unit prices were stated in Table 3.9 by capital cost estimation method in equation 1.3. Base capacities at Table 3.12 and sixth tenth rule were used to calculate equipment unit price seen in Table 3.13.

Table 3.13. Scenario B: Equipment capacities and costs

Working Volume	**Type**	**Unit Price (US$/Unit)**
1000 L	Fermenter	132,000
100 L	Seed Fermenter	51,000
2500 L	Fermenter	225,000
250 L	Seed Fermenter	86,000
5000 L	Fermenter	341,000
500 L	Seed Fermenter	130,000
7500 L	Fermenter	435,000
750 L	Seed Fermenter	166,000
10000 L	Fermenter	517,000
1000 L	Seed Fermenter	202,000

Unit price of equipment's were calculated by capital cost estimation method from equipment capacities, base size, and exponent through the values at Table 3.10. According to equipment's and the operations, necessary heat transfer agents and the power demand were adjusted for the Türkiye as Table 3.6 and 0.053 US$ for 220 V, respectively. In Türkiye, information's which based on individual conversations, for Turkish employees, operators as seen in Table 3.15 were set minimum wage, and the supervisor was set 1.5 times of minimum wage per month.

Table 3.14. Scenario B: Calculated unit prices in SuperPro Designer based on equipment capacity

Equipment Name	Working Volume	Unit Price (US$/Unit)				
		1000 L	**2500 L**	**5000 L**	**7500 L**	**10000 L**
	Air Filter (AF-101)	10,000	18,000	27,000	34,000	41,000
	Air Filter (AF-102)	2,500	4,500	7,500	8,500	10,000
	Disk-Stack Centrifuge (DS-101)	200,000	347,000	527,000	671,000	798,000
	Freeze Dryer (FDR-101)	121,000	210,000	318,000	406,000	483,000
	Centrifugal Compressor (G-101)	104,000	181,000	274,000	350,000	416,000
	Centrifugal Compressor (G-102)	33,000	51,000	77,000	91,000	107,000
	Homogenizer (HG-101)	70,000	121,000	184,000	234,000	278,000
	Heat Exchanger (HX-101)	6,000	10,000	15,000	21,000	23,000
	Heat Exchanger (HX-102)	21,000	37,000	55,000	71,000	84,000
	Microfilter (MF-101)	77,000	77,000	77,000	77,000	77,000
	Ultrafilter (UF-101)	17,000	17,000	17,000	17,000	17,000
	Blending Tank (V-101)	18,000	30,000	46,000	58,000	69,000
	Blending Tank (V-102)	15,000	26,000	38,000	49,000	58,000
	Blending Tank (V-103)	13,000	23,000	35,000	45,000	53,000
	Blending Tank (V-104)	5,000	8,000	12,000	16,000	19,000
TOTAL		**895,500**	**1,471,500**	**2,180,500**	**2,749,500**	**3,252,000**

Capital cost estimation method eq 1.3 and sixth tenth rule were used in equipment capacities seen in Table 3.14. Total costs for each volume in Table 3.14 were include the costs of fermenter and seed fermenter for each working volume include.

Table 3.15. Scenario B: Labor type and basic rate

Labor Type	**Adj. Basic Rate ($/h)**
Dryer Operator	1.96
Filter Operator	1.96
Operator	1.91
Reactor Operator	1.98
Supervisor	2.52

Based on the equipment's, labor types and basic labor rate seen in Table 3.15 were adjusted for The U.S.A. scenario at the software as 1.96 (US$/h) for dryer and filter operator, 1.98 (US$/h) reactor operator, 1.91 (US$/h) for operator, and 2.52 (US$/h) supervisor.

3.2.3. Scenario C: B-gal Production for China

Scenario C for China was adjusted as key assumption Table which contains input materials for B-gal production process, raw material costs, equipment capacity and costs, and different fermenter volume values. Therefore, unit prices of media and ethyl alcohol precipitation seen in Table 3.4. were set through the kg basis as 0.55 US$ glucose, 5 US$ sucrose, 3.6 US$ lactose, 7.5 $ peptone, 5 US$ yeast extract, 2.78 US$ cheese whey, 1.43 US$ dipotassium phosphate, 0.12 US$ magnesium sulfate and 0.75 US$ ethyl alcohol, while unit price of batch medium was set 2.78 US$ which was calculated the unit price of the entering materials into inoculum medium. Through the stated input material prices in Table 3.4, total prices for raw materials were calculated for media, cheese whey, batch medium and 70 percent of EtOH via the ingredient unit prices in Table 3.4. for B-gal production for scenario B. Equipment prices were calculated with the base capacity from marketing talks by capital cost estimation with sixth tenth rule rapid cost estimation method in equation 1.3. Unit prices according to base sizes seen in Table 3.16. were 500 US$ for each air filters, 14000 US$ for disk stack centrifuge, 60000 US$ for freeze dryer, 5000 US$ for each centrifugal compressors, 3500 US$ for homogenizer, 1500 US$ for each heat exchangers, 3500 US$ for microfilter, 4000 US$ for ultrafilter, 3000 US$ for each blending tanks from marketing talks, respectively. Therefore, equipment total prices were calculated using base sizes, exponents, and the equipment capacities in Table 3.11. at result section.

Table 3.16. Scenario C: Equipment capacities and costs

Equipment Name	Base Size (Capacity)	Exponent	Base Unit Price (US$/Unit)
Air Filter (AF-101)	3323.8 L/h	0.6	500
Air Filter (AF-102)	3323.8 L/h	0.6	500
Disk-Stack Centrifuge (DS-101)	7.34 L/h	0.6	14,000
Freeze Dryer (FDR-101)	59.02 L	0.6	15,000
Centrifugal Compressor (G-101)	61.9 kW	0.6	60,000
Centrifugal Compressor (G-102)	10.64 kW	0.6	5,000
Homogenizer (HG-101)	1.07 kW	0.6	5,000
Heat Exchanger (HX-101)	88.84 L/h	0.6	3,500
Heat Exchanger (HX-102)	2.85 L/h	0.6	1,500
Microfilter (MF-101)	26.22 m2	0.6	1,500
Ultrafilter (UF-101)	6.66 m2	0.6	3,500
Blending Tank (V-101)	5.92 L	0.6	40,000
Blending Tank (V-102)	5.83 L	0.6	4,000
Blending Tank (V-103)	1,066.99 L	0.6	3,000
Blending Tank (V-104)	603.84 L	0.6	3,000
Air Filter (AF-101)	592.06 L/h	0.6	3,000
Air Filter (AF-102)	118.90 L/h	0.6	3,000

Based on capital cost estimation method, equipment prices were calculated with base size from marketing talks. High ends and exponent ratio were same as Scenario B for the variable in equation. In Table 3.16., base sizes, exponents, and base unit prices from marketing talks were stated for every type of equipment for design of B-gal production at SuperPro Designer.

Table 3.17. Scenario C: Fermenter and seed fermenter capacities and costs

Working Volume	Type	Unit Price (US$/Unit)
1000 L	Fermenter	356,000
100 L	Seed Fermenter	243,000
2500 L	Fermenter	608,000
250 L	Seed Fermenter	413,000
5000 L	Fermenter	921,000
500 L	Seed Fermenter	626,000
7500 L	Fermenter	1,175,000
750 L	Seed Fermenter	796,000
10000 L	Fermenter	1,397,000
1000 L	Seed Fermenter	969,000

Addition to Table 3.17, calculated capacities for fermenters and seed fermenters used in B-gal production were stated with equipment sizes and the unit prices for China scenario at 1000 L, 2500 L, 5000 L, 7500 L and 1000 L working volumes.

Cost of the fermenters and seed fermenters were calculated as the capital estimation rule as the equipment capacity calculations. The determined capacities were used in determining the unit prices of each equipment at scenario C for B-gal production.

In China, minimum gross wages were 383 US$, therefore, dryer and filter operators' basic rate was 7.13, operator basic rate was 6.90, reactor operator was 7.27, and the supervisor was 9.07. Also, unit cost for power demand in Scenario C was adjusted as 0.55 US$ for 220 V.

Table 3.18. Scenario C: Calculated unit prices in SuperPro Designer based on equipment capacity

Equipment Name	Working Volume	Unit Price (US$/Unit)				
		1000 L	2500 L	5000 L	7500 L	10000 L
	Air Filter (AF-101)	3,000	5,500	8,000	10,000	12,000
	Air Filter (AF-102)	1,000	1,500	2,000	2,500	3,000
	Disk-Stack Centrifuge (DS-101)	84,000	146,000	221,000	282,000	335,000
	Fermenter (FR-101)	356,000	608,000	921,000	1,175,000	1,397,000
	Freeze Dryer (FDR-101)	65,000	113,000	172,000	219,000	261,000
	Centrifugal Compressor (G-101)	31,000	54,000	82,000	105,000	125,000
	Centrifugal Compressor (G-102)	10,000	15,000	23,000	27,000	8,000
	Homogenizer (HG-101)	22,000	38,000	58,000	74,000	3,500
	Heat Exchanger (HX-101)	2,500	4,500	6,500	9,500	10,000
	Heat Exchanger (HX-102)	9,500	17,000	25,000	32,000	38,000
	Microfilter (MF-101)	19,000	19,000	19,000	19,000	19,000
	Seed Fermenter (SFR-101)	234,000	413,000	626,000	796,000	969,000
	Ultrafilter (UF-101)	4,000	4,000	4,000	4,000	4,000
	Blending Tank (V-101)	19,000	33,000	50,000	63,000	69,000
	Blending Tank (V-102)	16,000	28,000	41,000	53,000	63,000
	Blending Tank (V-103)	14,000	25,000	38,000	48,000	57,000
	Blending Tank (V-104)	19,000	33,000	50,000	64,000	76,000
TOTAL		909,000	1,557,500	2,405,000	2,983,000	3,449,500

Table 3.19. Scenario C: Labor types and basic rates

Labor Type	Adj. Basic Rate (US$/h)
Dryer Operator	7.13
Filter Operator	7.13
Operator	6.90
Reactor Operator	7.27
Supervisor	9.07

4. RESULTS AND DISCUSSIONS

4.1. KEY MODEL ASSUMPTIONS

In this thesis, beta galactosidase production from *Saccharomyces fragilis* was modeled to determine economic model through different production volumes and different countries. Based on literature, this production has more than one carbon sources because the economic reasons.

Since cheese whey contains valuable nutrients such as lactose carbohydrates, fatty acids, cell wall components, nucleic acids, and vitamins, it could be used in various mode of fermentation processes such as wastewater treatment or the biomass production at pilot scale and laboratory scale [54–56]. In addition, cheese whey has been used to produce probiotics, biomass, and enzymes such as β-galactosidase and lipase, peptides and proteins, and oligosaccharides [57,58]. The process was carried out according to the researchers of Bosso *et al.* who produced 366 U β-Gal enzyme from *Saccharomyces fragilis* using cheese whey as an economic media component in media [50,59]. Similarly, according to Lappa et al, cheese whey was used as a carbon source for the production of 2220 U β-Gal and 5.6-7 g/L bacterial cellulose by *Aspergillus awamori* during solid-state fermentation and submerged fermentation, respectively [60]. In addition, β-gal production by *Aspergillus oryzae* was performed in solid-state fermentation with glucose as the carbon source and sodium nitrate as the nitrogen source at a pH of 5 and a temperature of 30°C with unspecified enzyme activity [61]. In addition, *Aspergillus oryzae* grown in cheese whey were studied for ethanol and chloroform cell permeabilization, then β-gal extraction was performed. Accordingly, 25 percent of ethanol at 30^{o}C for 60 min produced β-gal with 0.44 U.mL^{-1} enzymatic activity, whereas 5.3 percent of chloroform at 48^{o}C produced β-gal with 0.17 U.mL^{-1} enzymatic activity [62]. Similarly, 0.116 U.mL^{-1} of β-gal enzymatic activity was produced by natural thermophilic lactic acid bacteria in skim milk, but this can be considered waste if the remaining medium is not converted into usable products of β-gal production [63]. In addition, β-gal production from recombinant or mutant organisms has been used to improve productivity. For example, β-gal production from *Aspergillus oryzae* was optimized for efficient lactose hydrolysis by adjusting the optimal pH of production [64]. In addition, *Lactobacillus helveticus* was cloned to produce β-galactosidase that was overexpressed in *E.*

coli and *Lactobacillus plantarum*. Therefore, the recombinant β-galactosidase with 26 kU of enzymatic activity was performed in promoter *E.coli,* but the synthesized enzyme was used in the formation of galactooligosaccharides [65]. Consequently, Bosso *et al* prepared media for the β-galactosidase production as 14 g/L of sucrose, 5.14 g/L of yeast extract, 7 g/L peptone and MgSO4, 10 g/L lactose and glucose and 5 g/L K2HPO4 with the 17.7 7 g/L cheese whey in optimized conditions for *Saccharomyces fragilis*, then, the enzymatic activity was obtained

After fermentation in the modeled production process, purification was performed according to the amount of impurities and difficult to remove material. First, purification was performed in the following order: Microfiltration (MF -101), where the most common impurities such as biomass were removed, Centrifugation (DS -101), where the simplest impurities such as biomass were removed after homogenization, Ultrafiltration (UF-101) of β-galactosidase, where difficult and expensive compositions were removed. While the purification of β-galactosidase was performed by microfiltration, centrifugation, homogenization, precipitation, centrifugation, and ultrafiltration for this intracellular enzyme production process, the extracellular β-galactosidase production process was performed by centrifugation, precipitation, and purification with chromatography for the collection of β-galactosidase [66]. In addition, purified enzyme through the ammonium sulfate precipitation, ultracentrifuge and gel permeation chromatography was immobilized to produce lactose reduced milk based on Anbalagan *et al*. Then, immobilization efficiency which detected the glucose content from 2.771 mg/mL glucose of first cycle to 4.891 mg/mL of forth cycle of immobilized β-galactosidase in milk was recorded by Glucose oxidase-peroxidase method. Consequently, *L. plantarum* was recorded to use as probiotic microorganism [67]. In addition, there are several methods for purification, namely ZnCl2 precipitation and chromatographic separation for enzymes with a molecular weight of 484 kg.mol-1; ammonium sulfate precipitation and chromatographic separation for heterodimeric enzymes with 105 $kg.mol^{-1}$ molecular weight; sonication, ammonium sulfate precipitation, and column chromatographic separation for 118 kg.mol-1 enzyme; and ammonium sulfate precipitation and gel permeation chromatography for intracellular enzyme with 530 $kg.mol^{-1}$ molecular weight [66,68–70]. Finally, the biomass composition after homogenization was carried out as the β-galactosidase production by Ferreira *et al* [72].

4.2. SCENARIOS FOR THE LACTASE PRODUCTIONS

Although the same ingredients were fed into the same B-gal production process and the same process was used, the unit prices and raw material costs change depending on the country. The fact that the cost of construction land, equipment, costs, and labor depends on each country and location leads to different techno-economic evaluations. For this reason, when a manufacturing company is established, priority is given to the region where construction will take place. A study on a hybrid energy system based on optimizing the configuration using an effective method for hydrogen production has investigated the characteristics of the plants in Iran [73]. In addition, the environmental impact of energy recovery from solid waste in Brazil has been studied based on the emissions of the biogas combustion system and the capture efficiency by the scenarios of the inhabitants [73].

Lactase enzyme production using Saccharomyces fragilis was carried out in 1000 L, 2500 L, 5000 L, 7500 L and 10000 L working volumes for Türkiye, China, and America in order to emphasize the importance of the impact of the economic factors of the countries on the production cost in the scenarios created. Unlike similar studies, the goal is to show the income and expenses of the investment and how much is gained or lost with this investment when the volume is increased by working in different volumes, rather than by looking at a single volume. The input material costs to be used by the countries are kept constant and the total amount and annual cost for 1000 L, 2500 L, 5000 L, 7500 L and 10000 L working volumes are given in Table 4.1. In calculating the annual cost, the prices of feedstocks given in the basic assumptions were calculated according to the needs of the study. Since air consumption is not charged, it is not included in the calculation of annual costs.

The unit price of the power supply that helps the company in production varies by country and is calculated according to the annual needs of each work. Considering the unit prices provided by the countries to the enterprises, it is observed that the highest total cost is in Türkiye. This is due to the fact that the unit price is approximately twice that of the other two countries.

Table 4.1. Input material costs for each scenario at different volumes

	Working Volume (L)	**Kg/yr**				
		1000	**2500**	**5000**	**7500**	**10000**
Input Material	Air	4,775,056	9,461,685	14,059887	18,392,808	17,685.39
	Batch Medium	27,842	55,167	81,978	107,241	103,12
	Cheese Whey	1,136	2,251	3,345	4,375	4,21
	Dipotassium phosphate	899	1,782	2,648	3,464	3,33
	Ethyl Alcohol	26,567	52,693	78,226	102,333	98,40
	Glucose	1,799	3,564	5,296	6,928	6,66
	Lactose	1,799	3,564	5,296	6,928	6,66
	Magnesium Sulfate	1,259	2,495	3,707	4,849	4,66
	Peptone	1,259	2,495	3,707	4,849	4,66
	Sucrose	2,518	4,989	7,414	9,699	9,33
	Water	243,819	483,145	717,912	939,155	903,03
	Yeast extract	924	1,832	2,722	3,561	3.42
	TOTAL	**5,084,876**	**10,075,660**	**14,972,136**	**19,586,191**	**18,832,88**
	Annual Cost (US$)	**48,342**	**95,827**	**142,342**	**186,208**	**19,337**

As can be seen in Table 4.2, 10000 L working volume has the highest cost of power, namely 1,055,777 US$. Contrary to this cost, China and America costs are very close to each other and have been calculated as approximately 193,000 US$. Another cost that will ensure production is heat transfer agents, these agents are kept constant in three countries and are priced according to unit volume at varying working volumes seen in Table 4.3. In order for this pricing to have the lowest possible cost, freon with the lowest unit price (0.05 US$) was preferred as a cooling agent in operations that can be used in the temperature ranges between -4^{o}C and -3^{o}C. Similarly, hot water, which can heat between 40^{o}C and 30^{o}C, is preferred in operations where heating is required. Thus, instead of agents with a unit price of around 20 US$ such as chilled water, hot water is preferred in order not to cause an increase in the operation cost in the utility cost.

Table 4.2. Total power cost for scenarios

Scenario		USA	TR	CN
		Total Cost (US$)		
Working Volume	1000 L	20,247	10,731	19,032
	2500 L	50,445	26,727	47,418
	5000 L	99,787	52,888	93,800
	7500 L	148,205	79,290	139,313
	10000 L	193,824	101,739	180,442

* Unit cost of USA, TR and CN are based on 0.1 US$/kW-h, 0.053 US$/kW-h and 0.09 US$/kW-h, respectively.

As seen in Figure 4.1., the number of workers required to continue the work is considered in the method section, when the adjusted gross wages are taken into account, the fact that the gross salary determined in Türkiye is lower than in other countries increases the profit from production, unlike in countries such as the USA.

Table 4.3. Heat transfer agent costs per working volume

Heat Transfer Agent	Working Volume (L)				
	1000	2500	5000	7500	10000
Cooling Water	8,628	1,079	2,137	3,206	4,114,70
Freon	4,082	1,531	3,033	4,550	5,839,94
Hot Water	50	5	12	19	23,95
Steam	129	4,261	7,673	11,510	14,772,56
Total Cost (US$/yr)	2,595	6,875	12,856	19,284	24,751

* Prices are based on US$.

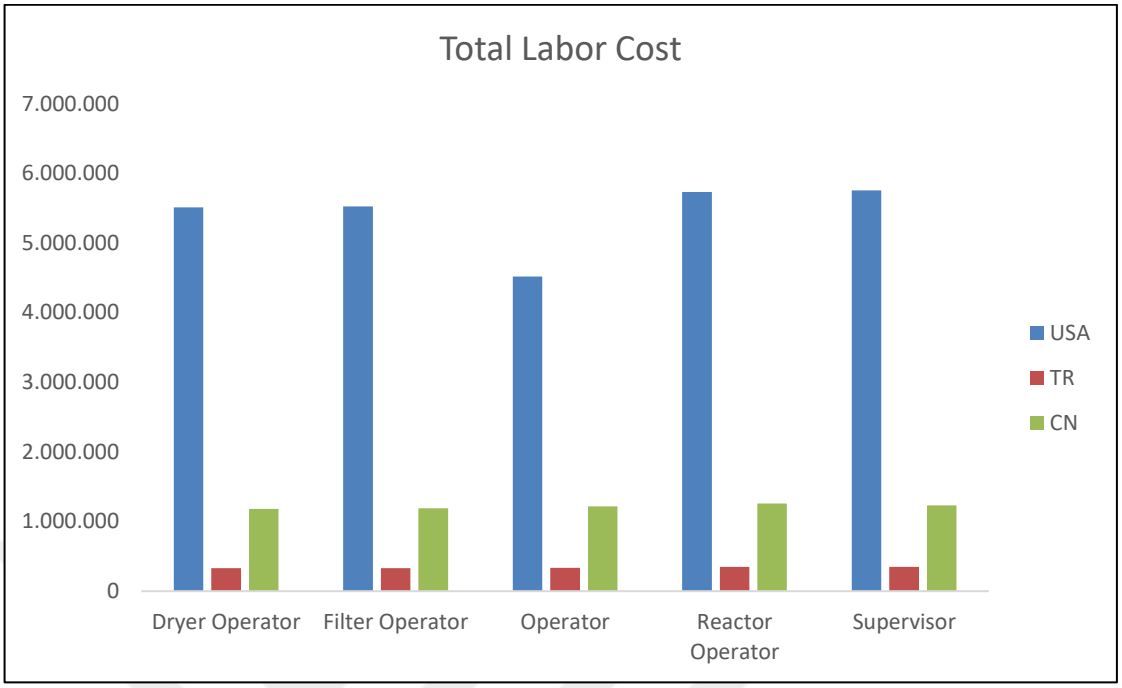

Figure 4.1. Total labor costs based on labor type for scenarios

* Prices are based on US$/yr.

An operator operating in Türkiye costs less labor cost even than in countries with low unit prices, as it can be clearly seen in Figure 4.1. When the costs of the equipment calculated in the Method section are included, the profitability rates calculated at SuperPro Designer and the payback time are examined, while beta galactosidase production starts to gain in 0.48 years in the Türkiye scenario for a 1000 L working volume, it is not expected to provide a gain in more than 20 years in the American and Chinese scenarios (not shown in Figure 4.2). In addition, even in the 2500 L volume, excluding the America scenario, profits begin to be made after about 2 years. Moreover, as seen in Figure 4.2, with the 5000 L beta galactosidase production volume study, the payback period decreases below 0.5 years for Türkiye in picture A, while it decreases to 0.63 and 1.68 years for China and America in picture B and C, respectively.

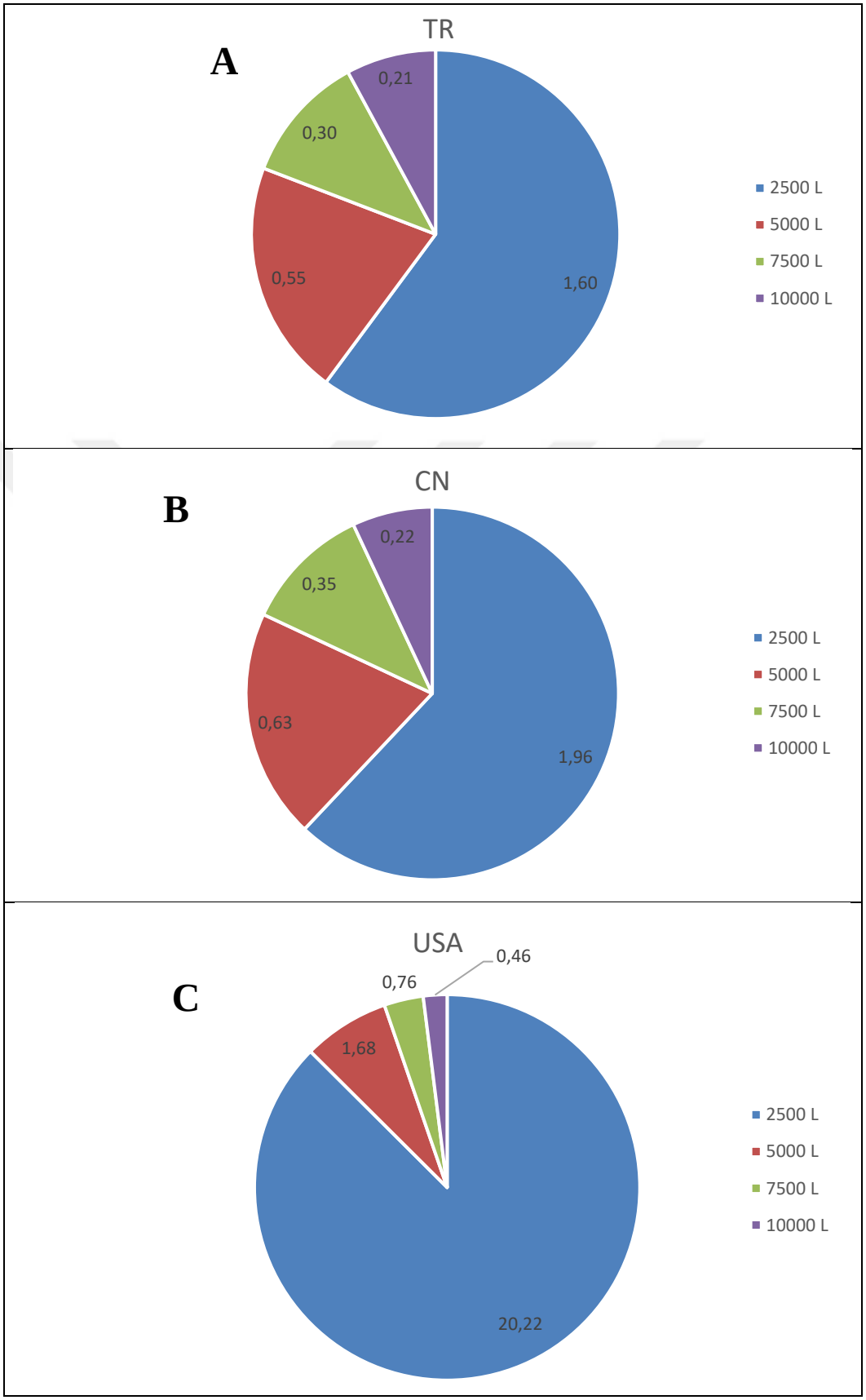

Figure 4.2. Payback time for scenarios

The total investment and operating cost values for beta galactosidase production at different volumes of Türkiye, USA, and China scenarios as a result of the calculation of the calculated input costs, utility costs (power and heat transfer agent), labor costs, equipment costs are indicated at Figure 4.3.

The organic wastes that come out of the centrifuge in the S-118 flow and are not included in the main flow can be sold as a by-product, thus reducing the cost. This flow has the characteristic of organic waste with high nutritional value, especially in animal feeds. In addition, it can be considered as having sufficient processing steps for lactose intolerance that may occur in animals. In addition, since the flow separated in ultrafiltration is of higher purity than the S-118 flow, it can be considered as a higher quality by-product. In addition, ethanol cost can be reduced by evaporation and collection of ethanol by recycling it into the flow. Another method that can be used to reduce the cost is the option of removing freeze drying and offering the product for sale in liquid form. However, since the storage conditions of the liquid product may be more sensitive than the powder product, the costs of transportation and storage conditions should be evaluated and taken into account according to these costs.

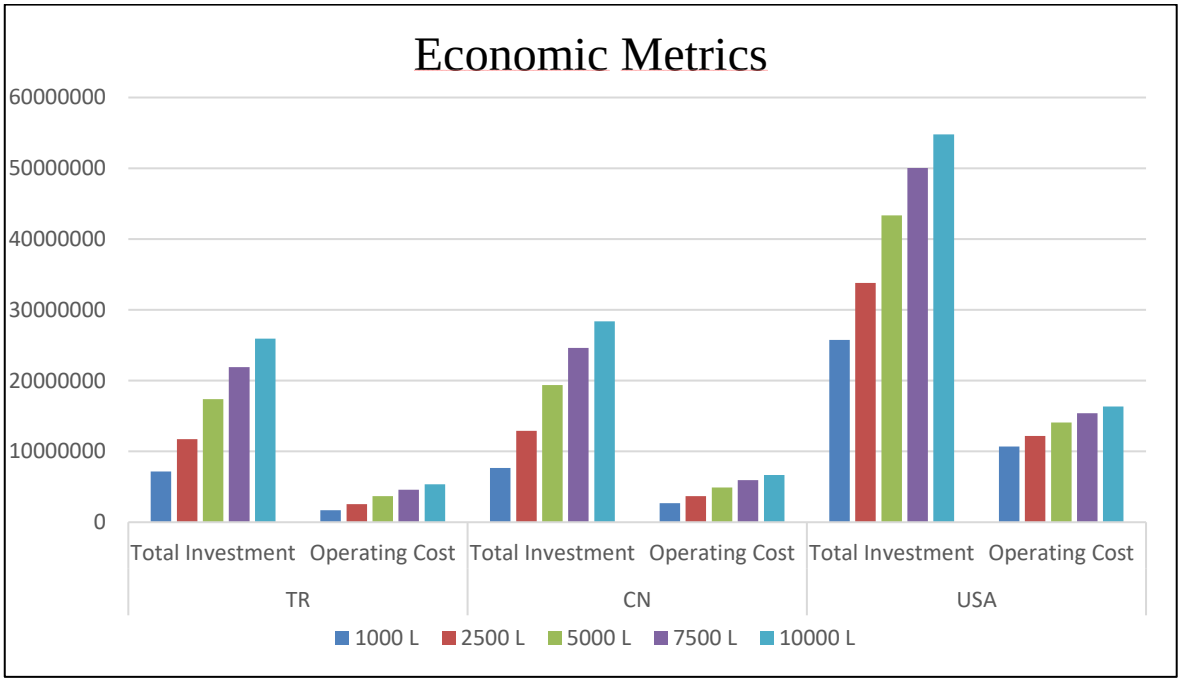

Figure 4.3. Economic metrics for scenarios

* Prices are based on US$.

As seen in the Figure 4.3, the investment in America requires high investments for beta galactosidase production for each working volume, on the contrary, China and Türkiye can provide lower cost productions. Although production in China scenario for 1000 L and 2500 L seems to be costly compared to Türkiye, 5000 L beta galactosidase needs a noticeably higher investment cost in production volume. In addition, when the operating costs for the stages after the establishment of the enterprise are examined, the values increase after the 2500 L working volume, although the values for the 1000 L and 2500 L beta galactosidase production volume are close between China and Türkiye.

Table 4.4. NPV and gross margin metrics for scenarios

	Scenario	Gross Margin (percent)			Return On Investment (percent)			NPV at (7.00 percent)		
		TR	CN	USA	TR	CN	USA	TR	CN	USA
Working Volume	1000 L	3.58	-53.42	-510.28	9.66	-3.75	-26.30	-2,225,618	-9,688,212	-74,835,190
	2500 L	76.61	66.62	-10.97	62.64	51.05	4.95	40,877,178	34,193,672	-22,742,148
	5000 L	91.57	88.71	67.62	180.61	157.84	59.36	206,157,963	198,739,179	139,516,831
	7500 L	95.33	93.86	84.11	327.91	285.74	130.72	488,632,117	475,473,813	415,674,598
	10000 L	96.80	96.02	90.33	477.72	433.02	217.47	852,846,903	845,050,558	792,328,415

* NPVs are based on US $.

Net present value (NPV), which is the difference between the present value of its inputs and the present value of cash inflows and outflows over a period, is analyzed in Table 4.4. for SuperPro Designer beta galactosidase scenarios to be used in capital budgeting and

investment planning to analyze the profitability of the project. Accordingly, in the scenarios of Türkiye, China and America, a profiTable operation is not achieved at a working volume of 1000 L in terms of NPV. On the other hand, when evaluated at the purchase price, it has been calculated that the gross margin value for Türkiye is advantageous in 1000 L volume. While the cash flow for the USA was not sufficient up to 5000 L, the gross margin values for China and Türkiye increased.

As a conclusion, it is possible to achieve high profits for Türkiye and China in the production working volume of 5000 L, 7500 L and 10000 L beta galactosidase. On the other hand, the costs arising from the equipment and labor costs of the costs discussed in the studies conducted in China cause this advantage to be lower than in Türkiye.

5. CONCLUSIONS

In this thesis, beta galactosidase produced in 1000 L, 2500 L, 5000 L, 7500 L and 10000 L working volumes were produced by choosing an efficient and *Saccharomyces fragilis*, low-cost organism, considering the economic exchange rates, material, and equipment prices of Türkiye, The United States of America and China. As a result, although the costs are close to each other between China and Türkiye scenarios, it has been observed that production in Türkiye is more profiTable in the long run as the capacity increases. In this way, it has been concluded that the sale of enzymes to countries where no production is made by exporting will increase the profit margin. The low cost of equipment greatly increased profit margins, while equipment fees impacting the capital charge helped to reduce payback time and generate short-term profits. Profitability and production capacity were examined from 1000 liters production volume to 10000 liters volume, and even when the smallest volume, 1000 L, is produced, it has been observed that the profit begins in the Türkiye scenario, and the payback time is approximately 10 years. As a result, even in small working volume (2500 L) in Türkiye with ß-gal production from *Saccharomyces fragilis* US$ 11,753,414 total investment and US$ 2,564,766 operating cost, 76.61 percent gross margin, 62.64 percent years return on investment rates provide payback time in 1.60 years for Türkiye. In volumes more than 2500 L, profitability will increase further, and profit will be made in a shorter time.

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