An Integrative Model of Beet Juice-Based Productions of Amino Acids Using Ion Chromatography Technique and High-Amino Acid Beet Pulp with Sugar Manufacturing

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ABSTRACT*: To investigate the impact of the different geometry and physical conditions parameters on the industrial process of amino acids recovery from sugar beet thin juice (TJ), several techniques such as computational fluid dynamics and scale-down approaches have been applied. Process modeling can support the design and optimization of all these processes. The Model, considering the process operative parameters and process stoichiometry, using the data from the Egypt Beet Sugar Industry, give an estimation of the process efficiency, product quality, selectivity as well and the condition for an optimal yield. Combining the results obtained by the model with the data obtained by the scale-down devices, the optimal process configuration and all requirements of the fluid sugar were simultaneously identified in an early phase of development for the subsequent processes of sugar manufacturing. In this case, the choice of an ion exchanger is associated with the development of a method for extracting an amino acid based on the study of the dynamic patterns of sorption and desorption, depending on several factors. These include the shape of the ion exchanger, the degree of its granulation and cross-linking; parameters of ion-exchange columns; flow rate and temperature of working solutions; and efficiency of the eluent. Furthermore, to maximize the utilization of the amino acids extract was added to the Sugar Beet Pulp (SBP) to produce High Amino acid Beet Pulp (HABP) with a high nutritional value and subsequently high marketing value. The results of this paper provide useful information for the design and modeling of beet juice-based production of amino acids integrated with beet processing for sugar production.*

KEYWORDS: *Beet juice; Amino acids; Automation; Anion exchange bed chromatography*.

INTRODUCTION

Beet thin juice is an intermediate product obtained from the processing of sugar beet and its amino acids content is the worst offender that impedes the crystallization of sucrose, which significantly reduces the amount of sugar produced and this because of their abundance, and their charge effects [1]. Amino acids dissociate in the aqueous solution of TJ, forming characteristic ionic species depending on the solution pH value. These properties make

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amino acids to be hydrophilic at any pH-value. For this reason, their physical extraction is practically impossible [2-5]. Consequently, in the classical process of sugar manufacturing, the TJ passes through different industrial treatment stages down to the final sugar product without removing the amino acids and eventually graduated with molasses [6-8].

Therefore, the utilization of TJ stream as a raw material for obtaining a valuable product – amino acids, will allow to reap economic benefits as well as reduce the negative impact of their undesirable consequences on the sugar processing.

Amino acids are commercially produced through three different routes, namely, microbial processes (enzymatic synthesis and fermentation), protein hydrolysis and chemical synthesis. The most efficient methods are the first two, but the results indicated that the separation of amino acids from fermentation broths or protein hydrolysates is rather difficult. In general, the main bottleneck of these methods is that they are highly dependent on the availability of natural protein rich resources, so that it may be difficult to satisfy the increasing demand for amino acids [9-11].

The microbial processes such as enzymatically catalyzed synthesis and fermentation. However, the enzymes are usually expensive and their limited stability is one of the main drawbacks of this process [9]. Most of the current industrial processes for amino acids production are based on fermentation route. However, it requires sterility and high energy consumption for oxygen transfer (for the aerobic fermentations) and mixing as well as water addition that impact on capital and operation costs. Moreover, the requirement of bigger reactors, compared to the other amino acids production methods, leads to a high capital investment [12] but, due to its economic and environmental advantages, fermentation is the most used process at industrial scale [9].

The last route to produce amino acids is through the chemical synthesis has been the classical pathway to produce achiral amino acids [13, 14]. However, the main drawbacks of the chemical synthesis are associated with the price of the catalyst as well as with the use of hazardous cyanide sources [15, 16]. The Bucherer-Berg method is the most common industrial chemical process for the manufacture of racemic amino [17]. However, the drawbacks of this method are the long reaction times and the elevated temperatures [18].

The separation techniques currently applied for removal and purification of amino acids from dilute aqueous solutions consist generally of ionic exchange, crystallization at the isoelectric point or chromatography [19]. However, these techniques are rather difficult to be transposed at larger scale, thus affecting the production of amino acids, and increasing the cost of the applied technology. Consequently, ion exchangers are still used for analytical purposes and fermented broth purification step in the manufacture of amino acids by fermentation but have not been yet used on an industrial scale to manufacture amino acids. Whereas the operation mode of continuous production of amino acids can provide productivity and process output 2.5-fold higher than the fed-batch technology [20-22].

Therefore, the industrial processes to produce amino acids still need to be optimized. For this reason, many companies and academic institutions have started research in this field with the aim of finding more cost-effective and sustainable routes to produce amino acids [23-26].

Although the intermediate sugar TJ considered to be quite promising and cheap starting material to produce amino acids, it practically did not be separately used as a source for their production, because of the significant economic cost. The way to solve this economic obstacle is to produce amino acids from the stream TJ by ion exchange chromatography with simultaneous production of the beet sugar and obtaining environmentally friendly integrative manufacture.

Therefore, the objective of this innovative work is to establish the TJ-based production of amino acids by ion exchange chromatography integrated with beet processing for sugar production on an industrial scale-up. It will have great economic advantages. This is associated with the selection of a selective ion exchange, which is achieved by experimental methods, and with control of the process in automatic mode. To do this, it is necessary to organize a timely sequence of effective processes that ensure extracting the target components from the TJ medium at the lowest cost, as well as to provide precise control of equipment operating parameters and their regulation according to the operation conditions selected. Process modeling can support the design and the optimization of all these processes.

EXPERIMENTAL SECTION

Raw material

The thin juice (TJ) of 16% dry substance (DS) was collected through the regular manufacture procedures after the decalcification process at a pH 10 and a temperature 70-80°C (a factory produces 1.15 kg of juice per each kg of beets). The composition of the TJ varies according to quality of beet, factory processes and fertilization. It can be simply summarized that 90% of its solid substance is sugar and 10% consists of non-sugar substances. The non-sugar substances consist of approximately 60% amino acids (0.960 g/100 mL) [7].

Apparatus

Strongly basic anion exchange resins (SBA) in the Cl^- form type 1 with functional group trimethyl ammonium (Amberjet 4200) and a uniform particle size (0.6-0.8 mm) were purchased from Rohm and Hass Company. The uniformity and mean particle size of resins have been optimized for use in industrial equipment with a total exchange capacity ≥ 1.3 eq/L. Laboratory-scale studies of amino acid separation from TJ were performed with a glass column of 4.4 cm internal diameter (Vantage® L44x500), from Merck Millipore, Darmstadt, Germany. The Äkta explorer™ 100 chromatography system (GE Healthcare, Uppsala, Sweden) was used to supply flow to the column.

Analysis

Analysis of the amino acids in the extract was performed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS-MS) system: Agilent 1100 series HPLC (Santa Clara, CA, USA) equipped with a binary pump, a vacuum degasser, and a thermostatic column oven. HTC Pal autosampler (CTC Analytics, Zwingen, Switzerland) was equipped with a 20-µl sample loop. Thermo Electron (Waltham, MA, USA) TSQ Quantum triple quadrupole mass spectrometer. Separation was achieved on a strong cation exchange (SAX) column (Luna 5 µm SAX 100 Å) with 30 mM ammonium acetate in water (A) and 5% acetic acid in water (B). Quantification was accomplished by the use of d_5-phenylalanine (d_5-Phe) as an internal standard achieving limits of detection of 0.1–3.0 µM.

Amino acids extract contents and standard amino acids profile in HABP were determined using Waters 600E Multisolvent Delivery System, Pico-Tag Analysis Column (column H 125×4 mm, pre-column H 60×4 mm), Waters 484 Detector and Workstation with 815 Baseline Program H1 (U.S.A).

Chromatographic peak areas were identified and quantified using a data analysis system that is attached to the amino acid analyzer system. A calibration file that was prepared from the average values of the retention times and areas of the amino acids was used in 10 standard runs.

Proximate analysis of feed samples was done to find the average value of dry substance (DS), crude protein (CP), ash percentage, and crude fiber (CF). Feed samples were oven-dried at 105°C for 4 h to find DM content. CP was calculated as N × 6.25 and nitrogen (N) was determined by the Kjeldhal method. All the analysis was carried out according to the procedure described by the Official Methods of Analysis of AOAC International [27].

Procedures for amino acids extraction on a lab scale

The TJ obtained from decalcification under operation conditions of temperature 70-80°C and pH 10 was tested for its amino acid content. 5 L of this TJ is passed continuously through a small column (50 cm high × 4.4 cm dia.) containing 500 mL (33 cm bed height) of SBA bed at flowrate 16.6 mL/min until the resin is nearly exhausted (saturated with the amino acids).

An Äkta explorer 100 chromatography system was used to supply flow to the column. When amino acids breakthrough from the SBA, the percolation is stopped. The amino acid is used as the lead substance, even a small number of amino acids can be reliably detected with ninhydrin reagent (2%). The elution and regeneration sequence includes first an air–scouring step of the resin, followed by a backwash with the treated juice to loosen the resin mass, reclassify the resin particles, and remove foreign particulates.

One of the main challenges of this work is that the SBA because of their swelling and shrinkage, require substantial amounts of water to de-sweeten them, which makes the fluid sugar greatly diluted as reported by *Asadi* [7]. To solve this problem, the final treated juice drawn from the product surge tank was used for de-sweetening in the technical operation of the backwash, regeneration, and rinse of the resins. Consequently, the fluid sugar obtained satisfies all requirements for the subsequent processes of sugar manufacturing.

The backwashing cycle brings the resin to its original order because, during the service cycle, resin particles become classified (the largest particles remain at the bottom, while the smaller ones are distributed on a higher level). A backwash is provided by applying a uniform flow of treated juice from the bottom of the bed to fluidize the resin. Typically, the volume of treated juice required is about 2 bed volumes. The backwash stream is collected in a buffer tank, from which it is continuously pumped to the filters after the second carbonation in the regular sugar processing line.

All eluting and regenerating steps are done in an up-flow mode. The resin is cooled to 40°C with cold-treated juice and then it is cleaned from the juice with cold water. The loaded resin is then eluted up-flow with ammonium hydroxide 5%. The amino acids content in the eluate was determined by using amino acid analyzer and amounted to the amino acids originally contained in the TJ. The fixation capacity of the resins was calculated using the inlet and outlet acids concentration in the solutions.

The eluate from column is concentrated in a hot plate to a DS content of 70%. The resin is then regenerated up-flow with 35℃ cold treated juice, drawn from the product surge tank, containing 40 g/L NaOH (as 100%) to return the resin to its original capacity. The resin is rinsed with 75°C hot treated thin juice to wash away the sodium hydroxide and heat up the resin bed.

Engineering data of the ion exchanger used for amino acid extraction

For the up-scaling calculations and adjusting the model of amino acids production from TJ according to the actual operating data of sugar manufacture, values common in the Egypt Sugar Industry Plant were applied. Mass flow of sugar beet 10,000 tons per day (417 t/h). At a draft equal to 115% of processed beets, a factory produces 1.15 kg of juice per kg of beets, the mass flow of TJ is equal to 478 t/h ≈ 446 m^3/h (juice density ≈ 1.07 kg/L). From this and other boundary conditions, the system incorporates three ion exchangers in parallel design of a capacity of 223 m^3/h, two ion exchangers being exhausted on thin juice simultaneously. These are staggered with respect to exhaustion so that both do not require regeneration at the same time. The third exchanger is being regenerated or is on standby.

The exhaustion cycle length of eight hours (breakthrough time) is necessary to properly turn around an ion exchanger and preserve the continuous operation. The cycle is so dimensioned that the ion exchanger is removed from service before a high leakage amino acid will appear compared to that designed, at this time the next ion exchanger is placed online. The cut-off point is determined by the volume totalizing of treated thin juice.

To design ion exchangers there are some aspects that should be taken into consideration, such as the volume of resin, the surface area of resin, the height of columns, the number of exchangers, and the pressure drop. The data obtained from small-column (4.4 cm inside diameter) were scaled up directly to the full-scale design provided that the surface loading rate and empty bed contact time are the same. To get the above-described parameters, the optimum volumetric flow rate was determined from the experiments at which a maximum breakthrough capacity was achieved. According to the values of the Egypt Sugar Industry Plant, the maximum bed height used in the industry is 3 m. The maximum bed height is established to prevent the big pressure drop that would affect the stability of the resin. A bed height is preferred but not exceed the maximum and based on manufacturer's production information, the minimum bed depth for the selected resin type is 800mm.

Ion exchanger design calculation

In this calculation a flow rate of 100 L/h, the amino acid originally contained in 1L of thin juice was 9.60 g, the concentration of extracted amino acids was 9.34 g/L and the residual concentration of amino acids was 0.26 g/L was taken from the experiment results. The required bed height in the ion exchanger has been assumed as 1.7 m based on sugar manufacturers' information on juice softeners.

Breakthrough capacity

The concentration of amino acids extracted by the resin = 9.34 g/L

$$\text{The breakthrough capacity} = 9.34\ \text{g/L} \times 5\ \text{L (juice)} = 46.7\ \text{g} \quad (1)$$

$$= \frac{46.7\ \text{g}}{0.5\ \text{L (resin)}} = 93.4\ \text{g/L} \quad (2)$$

Pressure drops

Based on the manufacture's data, the maximum pressure drop for the resin bed is 200 kPa. Both of surface loading and bed height have impact on pressure drop

through the resin. The relevant information can be found in pressure drop formula provided by manufacture. The pressure drop of ion exchange resin beds is calculated according the following formula:

$$\Delta p\ (\text{kPa}) = h\ (\text{m}) \times v\ (\text{m/h}) \times FV \times FT \times FR \quad (3)$$

h = Resin bed depth (m), v = Linear velocity (m/h), FR = Resin factor (specific pressure drop($\text{kPa/m}^2.\text{h}$) in this case, FR = 0.8, FT = 1.1, FV = 1.3, Resin bed height (m) h = 1.7 m

$$\text{Linear velocity/surface loading m/h} = \frac{100}{1000 \times \pi \times (0.022)^2} = 65.8\ \text{m/h} \quad (4)$$

$$\Delta p = 1.7\ (\text{m}) \times 65.8\ (\text{m/h}) \times 1.3 \times 1.1 \times 0.8 = 127\ \text{kPa} \quad (5)$$

Service flow rate (SFR)

The volume of the bed occupied by the resin:

$$BV = \text{area} \times \text{depth} = \pi \times (0.22)^2(\text{dm}) \times 17(\text{dm}) = 2.6\ \text{L} \quad (6)$$

$$SFR = \frac{100(\text{L/h}) \times 1BV}{2.6\ (\text{L})} = 38\ \text{BV/h} \quad (7)$$

Volume of resin

Treated juice flow rate = 223 m^3/h

$$\text{Total required resin volume} = \frac{\text{Treated juice flow rate}}{SFR} = \frac{223\ \text{m}^3/\text{h}}{38\ \text{BV/h}} = 6\ \text{m}^3 \quad (8)$$

Surface area of resin required

$$\text{Total required surface area} = \frac{\text{Resin volume}}{\text{Resin depth}} = \frac{6\ (\text{m}^3)}{1.7\ (\text{m})} = 3.5\ \text{m}^2\ \text{Diameter} = \left(\frac{3.5\ \text{m}^2}{\pi}\right)^{0.5} \times 2 = 2\ \text{m} \quad (9)\ (10)$$

Breakthrough time

$$\text{Breakthrough time} = \frac{6.0\ \text{m}^3 \times 1000\ \text{L/m}^3 \times 93.4\ \text{g/L}}{0.26\ \text{g/L} \times 223000\ \text{L/h}} = 9\ \text{h} \quad (11)$$

Column height

An extra space is needed for the bed expansion during backwashing which is considerable risk of resin loss, of about 70%.

$$\text{The required height for backwashing} = 1.7\ \text{m} \times 70\% = 1.19\ \text{m} \quad (12)$$

$$\text{Column height} = 1.7\ \text{m} + 1.19\ \text{m} = 2.89\ \text{m} \quad (13)$$

(Without considering of the height for resin support and inlet distributor).

Empty bed contact time (EBCT)

$$EBCT = \frac{H}{v} \qquad v = \text{surface loading } \left(\frac{\text{m}}{\text{h}}\right),$$

H = bed height (m)

$$EBCT = \frac{1.7\ \text{m}}{65.8\ \text{m/h}} = 0.026 = 1.55\ \text{min} \quad (14)$$

Based on the experiment results and calculations, the engineering data of the ion exchanger are summarized as follows: Number of ion exchangers: 3-each with a capacity of 223 m^3/h. Bed height: 1.7 m. Column height: 2.9 m (without considering the height for resin support and inlet distributor). Pressure drop: 1.7 KPa. Surface loading: 65.8 m/h. EBCT: 1.6 minutes. Breakthrough time: 9 h.

Technical process description of the automated model for amino acids production

The previously optimized lab-processes are able to produce a profitable direct separation of amino acids using SAE from TJ on a scale-up by modeling of these procedures based on engineering data as readily evident from Fig. 1. The TJ obtained from decalcification is the step of combining the conventional method of sugar production from beet with the innovative method for further processing to separate the amino acids that have been preserved unaltered in the purification and decalcification processes.

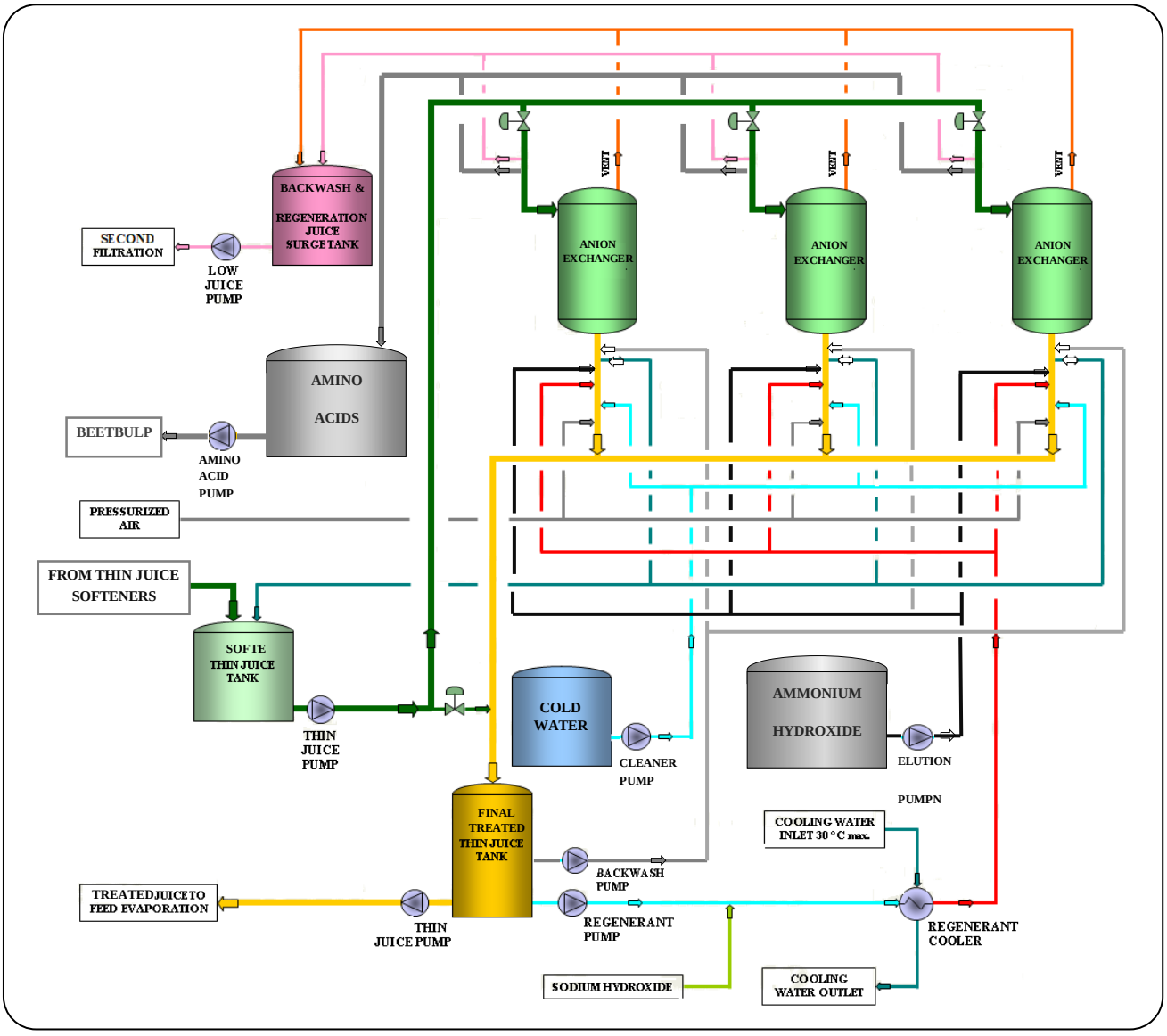


Fig. 1: Scale-up model procedures for amino acids production from TJ by SBA, consisting of charging, elution, and regeneration processes for the AE.

The suggested model for production of amino acids from TJ was fully automated for computer controlling as shown in Fig. 2. On each process of the model production, regulates all requirements of flow rate, temperature, pressure, and dry substance to increase the accuracy of control and to minimize material cost, also to satisfy the ion exchange conditions for achieving the best results and the possibility of integrating this operation with fully automatic factories in simple manure.

Ion exchanger charging

The TJ from the soft juice tank is pumped to the two service exchanges each with flow rate 223 m^3/h, the quantity is controlled by (FT21030) which is controlled by valve (FCV21030), when the volume totalizer of any of the ion exchangers (VT21030) is reached to 1784 m^3 which corresponds to a "breakthrough" point (one cycle of 8 hours), the valve (FCV 21030) will be closed, then the feed is shifted to the available second or third exchanger, and then the first primary exchanger is purified and eluted or regenerated.

The ion exchanger has (PT21029) and (PVC210129) which control the pressure of the ion exchanger to be (127 kPa), also has (LT 21028) and (LVC21028) which control

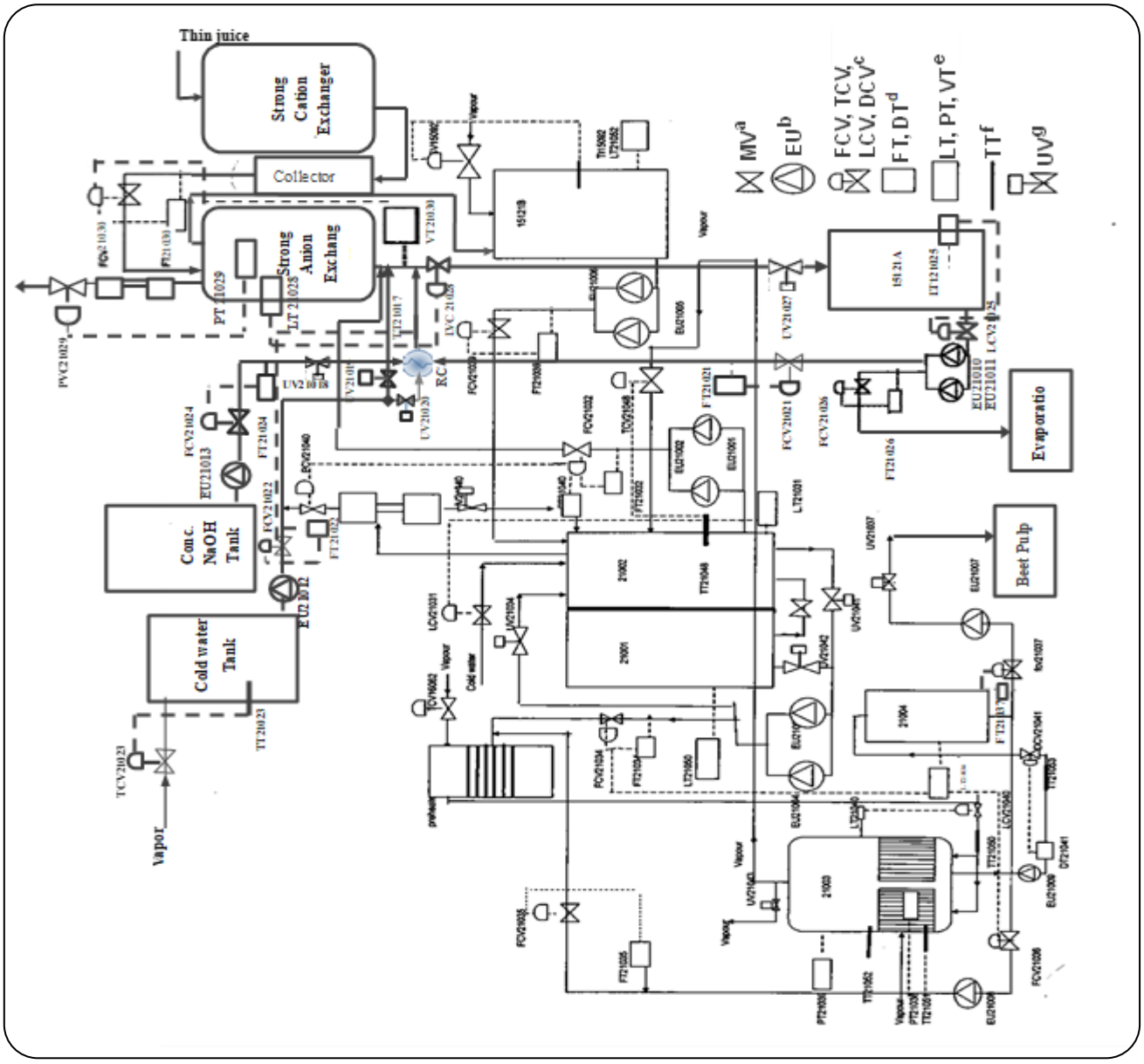


Fig. 2: Automated scheme installation for amino acids production from TJ by ion-exchange.
a) MV: manual valve; b) EU: electric actuators; c) FCV: Flowrate Control Valve, TCV: Temperature Control Valve, LCV: Level Control Valve, DCV: Dry Control Valve; d) FT: flowrate transmitter, DT: Dry substance transmitter; e) LT: Level transmitter, PT: Pressure transmitter, VT: volume totalizer transmitter; f) TT: Temperature transmitter; g) UV: Butterfly

the resin level to expand within 0.3 m of the distributor at the top of the ion exchanger.

The fluid sugar solution then is directed to tank (15121A) which is equipped with (LT15025) and (LCV15025) to control the solution level in tank to be not less than 30 cm. The solution is pumped by (EU21011) to the evaporation station, the quantity is controlled by (FT21026) and the valve (FCV21026) with flowrate 446m^3/h.

The ion exchanger is switched to the subsequent stages of the elution and concentration the eluate.

Ion exchanger eluting

The valve (UV21019) is checked to be open, then the water from tank (21006) is pumped by (EU21012) to ion exchanger at up-flowrate 25 m^3/h, the quantity is controlled by (FT21022) which controls the valve (FCV21022) for cleaning the resins from juice residue. The tank (21006)

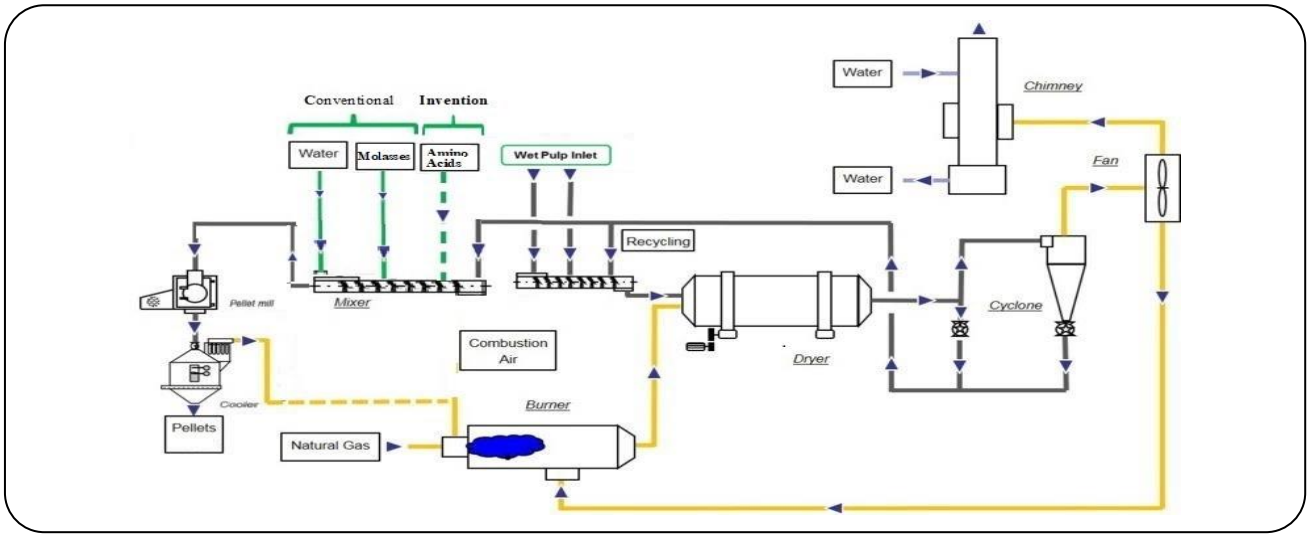


Fig. 3: Innovative flow diagram for Amino Acid-Pressed SBP Pellets production.

is equipped with (TT21023) and (TCV21023) to control the temperature of water in the tank to 40°C.

The concentrated ammonium hydroxide in tank (21001) with level (LT21050) is pumped by (Eu21003) and (Eu21004) to dilution tank (21002) where cold water is added (for reaching to concentration 5%) via control valve (LCV 21031) when the tank (21002) is reached to 3 m, the valve (LCV 21031) will be closed, the tank (21002) has (PT 21040) and (PCV 21040) which control the pressure of the tank to be (-0.8 bar/m), also equipped with (TT 21048) and (TCV 21048) to control the temperature of the fluid to be 40°C.

The solution in the tank (21002) is pumped by (EU 21032) and (EU 21002) to SBA with flowrate 25 m^3/h, the quantity is controlled by (FT 21032) which controls the valve (FCV 21032), when the level (LT 21031) of the tank (21002) becomes 5 cm the valve (FCV 21032) closes and the pump (EU 21001) and (EU 21002) stop.

The eluate of the ion exchanger is directed to tank (15121 B) which is equipped with (TT 15092) and (FCV 15092) to control the eluate temperature to 40°C. This eluate is pumped by (EU 21005) and (EU 21002) into the tank (21002), the quantity is controlled by (FT 21039) which controls the valve (FCV 21039) with flow rate (0: 25 m^3/h).

The solution in tank (21002) is pumped by (EU 21003) and (EU 21004) controlled by (FT 21034) which controls the valve (FCV 21034) to preheater, in plate type heat exchangers using different, vapors with flow rate from 5-15 m^3/h, then to the Robert-evaporator (21003) is directly heated with exhaust steam from the turbines.

The concentrated solution from evaporator (21003) is pumped by (EU 21009) to tank (21004). The evaporator (21003) is equipped with (DT 21041) which controls the valve (DCV 21041) to control the Brix (DS) to be 70°. Also, the evaporator is equipped with (PT 21038), (PT 21039), (TT 21051) and (TT 21052) to control the difference in pressure and temperature between the steam chest and the juice in calandria and equipped with level (LT 21040) which controls the valve (LCV 21040). When the level (LT 21036) in tank (21004) reaches 3.5 m, the valve (FCV 21034) is closed and the valve (FCV 21036) opens.

The concentrated solution in tank (21004) is pumped by (EU 21007) to SBP station for mixing with the dried pulp before pelletizing with a ratio (7% w/w) instead of water or molasses, which is added in the regular process, to adjust the final protein content and its nutritional value to satisfy all requirements for animal fodder as shown in Fig. 3.

Ion exchanger regeneration

The system incorporates a three-ion exchanger design with two exchangers being exhausted on TJ simultaneously. These are staggered with respect to exhaustion so that both do not require regeneration at the same time. The third exchanger is being regenerated or is on standby. All regenerating steps are done in an up-flow mode and use TJ drawn from the product surge tank.

The regeneration sequence includes first an air from air compressor – scouring step of the resin. The valves (UV21018) and (UV21020) is checked to be closed and the treated juice from tank (15121A) is pumped

by (EU21010) to ion exchanger at flowrate 12m^3/h, the quantity is controlled by (FT21021) which controls the valve (FCV 21021) for loosening the resin mass and freeing it from foreign matter.

The backwash stream is collected in tank (15121B), from which it is continuously pumped to the filters after the second carbonation. The valve (UV21020) is opened and the cold water from tank (21006) is pumped by (EU21012) to the regenerant cooler (RC), the quantity is controlled by (TT 21017) and (FT21022) which control the valve (FCV21022) to control the temperature of the outlet TJ from (RC) to 35°C for cooling the resins to 35°C.

Then the concentrated sodium hydroxide in tank (21005) is pumped by (EU21013) at flowrate 0.385m^3/h to RC, the quantity is controlled by (FT21024) which controls the valve (FCV21024) to get together with the cold treated juice and then the regenerant with 35°C cold treated juice is subjected in up-flow to ion exchanger. The regeneration effluent is collected in a buffer tank (15121B) and from there it is continuously pumped to the filters after carbonation system. When the level in the tank (21005) is reached 5cm, the valves (UV21018) and (UV21020) close.

The up-flow fluid of hot treated juice at 70°C is continuously pumped by (EU21010) at a flowrate 12 m^3/h to ion exchange for washing away the sodium hydroxide and heating up the resin bed. The summary of the sequence processes of the innovative technical operation steps of TJ ion exchanging are summarized in Table 1.

RESULT AND DISCUSSIONS

In this study, the ion exchanger was selected considering of the operating parameters conditions of the sugar manufacturing pH 10 and temperature 70-80°C to obtain fluid sugar that satisfies all requirements for the subsequent processes of sugar manufacturing. Accordingly, it must be of industrial grade and has a longer lifetime, high capacity for amino acids at high pH, more tolerable to high temperature, cost-efficient operation, and suitable for effective separation of ionizable soluble amino acids via the reversal of the types of net charges on the adsorbed amino acids. Therefore, a proper SBA of positive charge which had an especially high capacity for amino acids, a high mechanical stability of small particles, and a short diffusion path between the active groups SBA was selected.

The extraction of amino acids from TJ with SBA column chromatography relies primarily on electrostatic attractions for adsorptions and electrostatic repulsions for elution. To achieve the maximum of adsorption capacity and elution efficacy, the amino acid should have a higher net ionic charge under column conditions and the high difference between the pH of the column and the isoelectric point of amino acid is needed.

Therefore, the column pH was adjusted to be closer to 10 of a low enough for the isoelectric points (pI) of the main amino acids (pH < pI) to bear negative net charges and are attracted to the ion exchanger. Whereas, the non-electrolyte such as sugar and non-ampholytic aids group are carried zero net charges at the given pH and are passed through the column. This matches with the data from literature, which indicates that weak cation exchangers are negatively charged at pH higher than the pKa, while the opposite occurs for strong and weak SBA, which are positively charged at pH lower than the pKa. Strong cation exchangers are practically always charged at any pH. At pH values approaching the pka (pI ~11), the net charge approaches zero and the electrostatic binding strength becomes weaker, resulting in a lower binding capacity [28, 29].

The separation of various amino acids is significantly affected by the pH of the solution. The cysteine is the most sensitive for the pH and temperature of the solution under separation. Cysteine must be eluted and completely separated directly after alanine. At the selected ion exchanger conditions of pH 10 and temperature 70-80°C, cysteine was just be positioned between alanine and valine as shown in Fig. 4. Which confirms that the selected column conditions were the most suitable for the extraction of amino acids from TJ.

The use of ammonium hydroxide for elution was the reason for the high amino acids content in the eluting solution by providing the increase in the ionic strength and pH to reach the pI of the amino acids to change the electrostatic attractions to electrostatic repulsions resulted in the all trapped amino acids in the resins being eluted. This finding supports past literature on the separation of some amino acids from their mixture obtained either by fermentation or protein hydrolysis by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) indicated the possibility of the amino acids selective separation as a function of the pH value of aqueous solution and the acidic or basic character of each amino acid [30].

Table 1: Summary of the innovative technical process steps of TJ ion exchange.

NO	Step	Ion exchange medium in	From tank	Ion exchange medium out	To tank
1	Charging	TJ	TJ tank	Treated juice	Treated juice tank
2	Lowering			Treated juice	Treated juice tank
3	Lifting	TJ	TJ tank		
4	Lowering			Backwash effluent	Backwash effluent tank
5	Aeration	Air	Air tank	Air	
6	Backwashing	Treated	Treated juice tank	Backwash effluent	Backwash effluent tank
7	Lowering			Backwash effluent	Backwash effluent tank
8	Cooling	Cold treated juice	Treated juice tank	Backwash effluent	Backwash effluent tank
9	Cleaning	Cold water	Cold water tank	Backwash effluent	Backwash effluent tank
9	Elution	NH_4OH (5%)	NH_4OH tank	Amino acids effluent	Amino acids effluent Tank
10	Regeneration 1	Treated juice + NaOH	Treated juice tank	Backwash effluent	Backwash effluent tank
11	Regeneration 1	Treated juice + NaOH	Treated juice tank	Regeneration effluent	Regeneration effluent tank
12	Washing 1	Treated juice-cold	Treated juice tank	Regeneration effluent	Regeneration effluent tank
13	Washing 2	Treated juice -hot	Treated juice tank	Regeneration effluent	Regeneration effluent tank
14	Washing 2	Treated juice -hot	Treated juice tank	Regeneration effluent	Regeneration effluent tank
15	Lowering			Treated juice	Treated juice tank
16	Pause				

The flow rate of the eluting buffer is important, as it determines the time of the extraction process. If the flowrate through the column is faster than the optimal, the fractions departing the column become unsymmetrical, leading to tailing, in addition the amino acid peaks can overlap, while slower flowrate leads to degradation of sucrose in the fluid sugar with time of extraction. The results indicate that at the flow rate of 100 L/h, an acceptable extraction yield was achieved as well as the fluid sugar obtained satisfies all requirements for the subsequent processes of sugar manufacturing.

The desired results from the scaled-up study for amino acids production were achieved by the carefully selection of the ion exchange resin having an especially high capacity for amino acids and adequate retention time. The automation of the production of amino acids in the innovative work system has been instrumental in ensuring that the correct temperature, pH, and addition of ammonium hydroxide were maintained. Therefore, the control of material flows and ratios and other processes variables through the fully automated system has gone a long way toward obtaining the best results.

16 amino acids were satisfactory separated, identified, and quantified as shown in the typical chromatogram of amino acids eluate in Fig. 4. The elute of the exchanger was not discolored, apparently because of the use of the TJ after decalcification. Consequently, a direct crystallization of the amino acids' eluate is possible or concentrated through the evaporator to be used as nutrient additive for SBP pellets to increase its nutritional value.

The confirmation of chemical identity and quantity for amino acids molecules in the extract were further apprised with the use of a MS detector equipped with an ESI source, by applying LC-ESI-MS-MS method. MS spectra were examined in positive ion modes. Amino acids in the positive ion mode had higher sensitivity and clearer mass spectra, which made it easier to confirm molecular ions or quasi-molecular ions in the identification of each peak. The chemical structures of 16 components were characterized based on their retention behavior and MS information, such as quasi-molecular ions $[M+H]^+$ and fragment ions. The ion pairs of precursor → product ion for MRM detection were generated by the XCalibur Version 1.3 software. The signal of each compound was optimized by altering Cone Voltage (CV) qnd Collision Energies (CE). Under the optimized LC and MS/MS conditions, all 16 compounds in TJ were identified and quantified. Retention Time (RT) and MS information for each analyte including Molecular Weight (MW), MRM transitions (precursor → product ion), and CE are shown

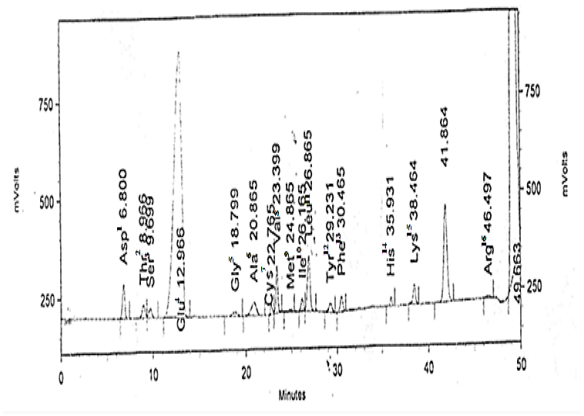


Fig. 4: Elution profile chromatogram of amino acids extracted from beet TJ on SBA at pH 10 and temperature 75°C. 1) Asp: aspartic acid; 2) Thr: threonine; 3) Ser: serine; 4) Glu: Glutamic acid; 5) Gly: glycine; 6) Ala: alanine; 7) Cys: cysteine; 8) Val: valine; 9) Met: methionine; 10) Ile: isoleucine; 11) Leu: leucine; 12) Tyr: tyrosine; 13) Phe: phenylalanine; 14) His: Histidine; 15) Lys: lysine; 16) Arg: arginine Phe: phenylalanine.

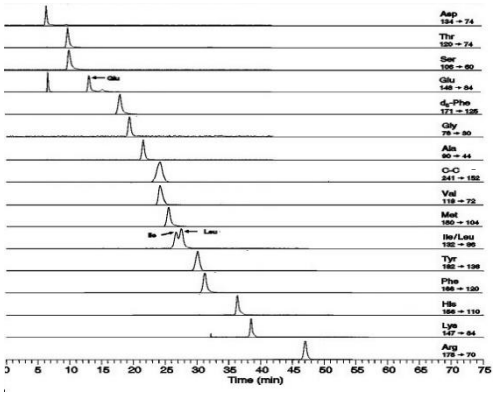


Fig. 5: Selected ion chromatograms of standard amino acids and d_5-Phe obtained in the multiple reaction monitoring (MRM) mode. Separation was achieved isocratically at pH 2.5 during the first 42 min and in a second isocratic step at pH 6.0 for 19 min using a SCX column. The numbers in the right corner are m/z ions of precursor ions and product ions in MRM for each analyte. a) d_5-phenylalanine (d5-Phe) as internal standard; b) cysteine.

in Table 2, and representative MRM chromatography of 16 markers in Fig. 5. The areas of those peaks with the same retention times as the calibration standard as well as concurrent MS spectra were integrated.

The mean amino acids content in the fraction of the eluate of ion exchanger are presented in Table 3, concentrations are given in percent based on g/100 mL. The obtained scaled-up study results revealed that the extract contained 16 amino acids, among them 9 essential amino acids were found, with different ranges of concentration, in which more than 50% is glutamic acid. The total amino acid (TAA) content of the extract was 0.87% of TJ (w/w) and as to sugar beet was 1% (w/w). Based on the actual data of the sugar factory operating with a production capacity of 10,000 tons per day, the quantity of amino acids produced per day was 100 tons. The total essential amino acid (EAA) content was 0.36%. In addition, the ratio of EAA/TAA was 68.90% and ETAA/total non-essential amino acid (NEAA) was 40.79% of the ratios recommended by the WHO/FAO [31], indicating that the protein contents in the sugar beet are ideal and consistent with those reported in a previous study [32]. Moreover, these results are in accordance with literature Dutton and *Hu et al.* [33], who stated the TAA contents of beet roots ranged from 0.30 to 0.62%. The ETAA/TAA (40%) and ETAA/NETAA (60%).

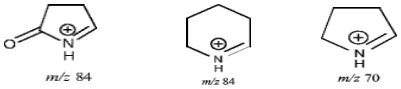
Regarding the results which were obtained from other authors with different methods, this is in accordance with the results of *Ghada et al.* [34] who indicated that wastes of agriculture like tomatoes or sugar beet straw can be used through hydrolyzing it by sulfuric acid (H_2SO_4, 6M), with the ratio of 1:3 (tomato or sugar beet straw: hydrolytic agent) under 105°C for 24 hr. into an air oven, as a source of amino acids, wastes are somewhat enriched in their contents of amino acids than those from sugar beet wastes, and both of them contain 17 amino acids. Of the highest amino acids concentration was aspartic, glycine and glutamic acids. Indeed, the extracts from the aerial parts of plants investigated have a higher amino acid content and more diverse composition than the underground organs as reported by *Mykhailenko et al.* [35]. Also, when *Varaee et al.* [36] used Supercritical Fluid Extraction (SFE) to extract free amino acids from sugar beet and sugar cane molasses, the extraction recoveries for SGB and SGC molasses were 42% and 31% for aspartic acid, 63% and 37%, for glutamic acid, 46% and 48% for alanine, and 31% and 20% for lysine sequentially.

High-amino acids beet pulp pellets (HABP)

SBP has been recognized worldwide as a major energy feed ingredient in the diets of livestock. Its major nutritional limitation has been the low protein content and

Table 2: Precursor and product ions for LC-ESI-MS analysis of 20 underivatized amino acids and d_5-Phe (internal standard).

Compound	Molecular weight (g/mol)	MRM transitions		Collision energy (eV)
		Precursor ion $[M+H]^+$ (m/z)	Product ion (m/z)	
Aspartic acid (Asp)	133.10	134	74 $[HO_2C–CH=NH_2]^+$	22
Threonine (Thr)	119.12	120	74 $[R–CH=NH_2]^+$	16
Serine (Ser)	105.09	106	60 $[R–CH=NH_2]^+$	20
Glutamic acid (Glu)	147.13	148	84 $[C_4H_6NO]^{+a}$	22
d_5-phenylalanine (d5-Phe)	170.22	171	125 $[R–CH=NH_2]^+$	18
Glycine (Gly)	75.07	76	30 $[R–CH=NH_2]^+$	16
Alanine (Ala)	89.09	90	44 $[R–CH=NH_2]^+$	14
Cysteine (C-C)	240.30	241	152 $[M+H – 89]^+$	20
Valine (Val)	117.15	118	72 $[R–CH=NH_2]^+$	16
Methionine (Met)	149.21	150	104 $[R–CH=NH_2]^+$	16
Isoleucine (Ile)	131.17	132	86 $[R–CH=NH_2]^+$	14
Leucine (leu)	131.17	132	86 $[R–CH=NH_2]^+$	14
Tyrosine (Tyr)	181.19	182	136 $[R–CH=NH_2]^+$	20
Phenylalanine (Phe)	165.19	166	120 $[R–CH=NH_2]^+$	20
Histidine (His)	155.16	156	110 $[R–CH=NH_2]^+$	20
Lysine (Ly)	146.19	147	84 $[C_5H_{10}N]^{+b}$	24
Arginine (Arg)	174.20	175	70 $[C4H8N]^{+c}$	28



a) b) c)

Table 3: Composition of amino acids (essential and non-essential) extracted from TJ by SBA.

Essential Amino Acids	Concentration, g/100 mL	Non-Essential Amino Acids	Concentration, g/100 mL
Threonine	0.036	Aspartic acid	0.068
Valine	0.030	Serine	0.024
Methionine	0.021	Glutamic acid	0.315
Isoleucine	0.014	Glycine	0.013
Leucine	0.104	Alanine	0.069
Phenylalanine	0.041	Cysteine	0.032
Histidine	0.023	Tyrosine	0.032
Lysine	0.078		
Arginine	0.034		
Total amino acids	0.934	Extraction recovery	98.29%
Originally amino acids	0.960		5.84% DS*

**DS Dry substance*

Table 4: Chemical composition of SBP (%) DS.

Components	Result (%)	Components	Result (%)
Dry matter	93.50	Minerals	2.50
Moisture	6.50	Fat	0.72
Crude protein	14.61	Sugar	2.50
Crude fiber	18.40	Nitrogen free extract	49.30
Ash	5.47	Cross energy	392 cal/g

Table 5: Amino acids profile of SBP and HABP (%) DS.

Amino acid	SBP (%)	HABP (%)	Amino acid	SBP (%)	HABP (%)
Aspartic	0.65	1.18	Methionine	0.25	0.33
Threonine	0.45	0.82	Leucine	0.71	1.27
Serine	0.57	1.04	Tyrosine	0.43	0.78
Glutamic	1.1	2.00	Phenylalanine	0.34	0.62
Glycine	0.47	0.85	Histidine	0.34	0.62
Alanine	0.53	0.96	Lysine	0.6	1.09
Cysteine	0.19	0.24	Arginine	0.42	0.76
Valine	0.69	1.25			
Total amino acids	7.60	13.81	Crud protein	8.40	14.61

poor protein quality because it consists mainly of non-essential amino acids which necessitates the use of expensive high-protein supplements or synthetic amino acids in diets containing a proportion of SBP. Generally, the low protein content of the SBP limits its nutritive value as a source of food for livestock [37]. The protein content and all chemical analysis of the SBP are shown in Table 4.

In this innovative work the quality of SBP protein was improved by the addition of concentrated amino acids extract to the dried pulp before pelletizing. The amount of amino acids added was calculated to be about 7% (w/w) of the beet pulp. Results obtained from the scaled-up study indicated that the addition of concentrated amino acids extract to SBP increased the amino acids content by 81.7% from 7.60 % for BP alone to 13.81 % DS, resulting in the CP increasing by 73.9% from about 8.40 % for BP alone to 14.61 % as shown in Table 5. Therefore, improvement in the protein quality of normal SBP through the development of a new class of SBP pellets known as "high-amino acids beet pulp pellets (HABP)" has been a major boost for livestock production, particularly in developing countries, where dietary supplemental protein is either expensive or imported. In this regard, the obtained results showed that, based on the percentage increase in CP level, the daily feed intake of HABP can be reduced to about 50 % for those who rely on normal SBP as animal feed.

Emam [38] reported from several studies that SBP protein is considered low (6.6 to 10.3% with an average of 9.9%), while it contains high level of crude fiber (14.6 to 24.8% with an average of 19.7%) and amino acids content 7.74% DS. According to *Foster et al.* [39], the CP contents of SBP ranged from 7 to 8%, but, high fiber content, it is mainly composed of hemicellulose fraction (45–61%), 20–24% cellulose and 1–2% lignin. In another study, *Bąk et al.* [40] postulated that SBP is relatively low in CP (8-10%). A recent study by *Minarovicova et al.* [41] reported that the protein, fat, and ash contents of SBP, 10.31, 0.42 and 3.56%, respectively. He also established that SBP contains a higher amount of total dietary fiber; insoluble dietary fiber and soluble dietary fiber (69.84%, 49.91% and 19.93%, respectively). The SBP raw proteins have a digestibility of 75% with enclose indispensable amino acids, particular lysine; methionine; cysteine and threonine.

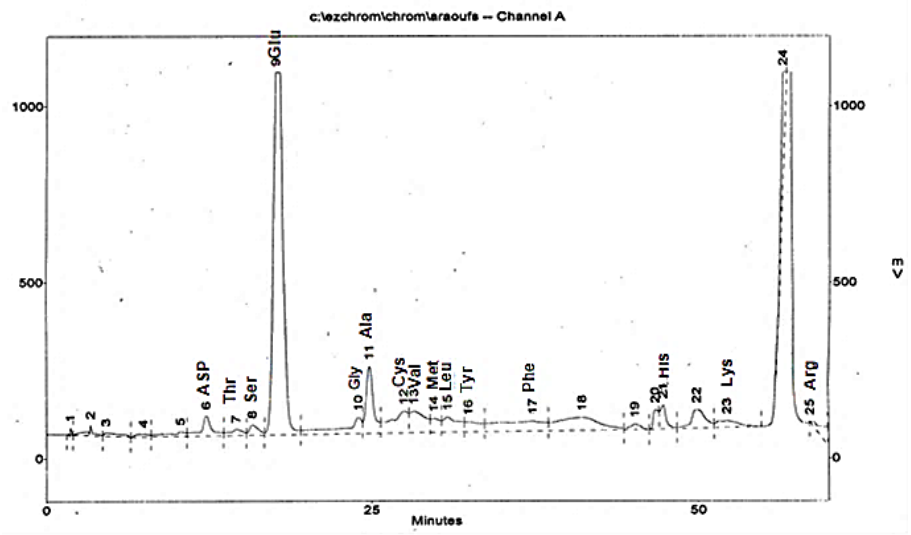


Fig. 6: The chromatogram of amino acid in a real sample of HABP.
6) Asp: aspartic acid; 7) Thr: threonine; 8) Ser: serine; 9) Glu: Glutamic acid; 10) Gly: glycine; 11) Ala: alanine; 12) Cys: cysteine; 13) Val: valine; 14) Met: methionine; 15) Leu: leucine; 16) Tyr: tyrosine; 17) Phe: phenylalanine; 21) His: Histidine; 23) Lys: lysine; 24) Arg: arginine.

Hence this work was focused on maximizing the nutritional value of feed additives by putting the essential building blocks of high-quality protein through the production of HABP rich in important amino acids for modern animal nutrition.

Mahn et al. [42] mentioned that the α-amino nitrogen content in the upper part of the beet was three times higher than in the root. The average content of α-amino nitrogen in root and tail amounted to 33 and 38 mg/100 g beet. However, Bąk et al. [40] found that the average content of α-amino nitrogen was more than two times higher in the crown than in the root and tail. The content of α-amino nitrogen in crown fluctuated in a wide range, from 68 to 87 mg/100 g of beet.

The amino acids profile of HABP showed that it is enriched with all essential amino acids as shown in Fig. 6. The HABP contained 15 amino acids, 8 of which are essential amino acids, and has sufficient lysine and threonine contents. Therefore, the development of these nutritionally improved HABP is of particular significance to those who rely on SBP as animal feed, and can thereby improve such diets nutritionally at no added cost. It has the potential to replace high portions of cereals in concentrate mixtures for dairy cattle.

On the basis of the comparison between the retention times for the peaks of the chromatogram of amino acid in a real sample of HABP with those of a standard amino acid profile, which determined using Waters 600E Multisolvent Delivery System, Pico-Tag Analysis Column (column H 125×4 mm, pre-column H 60×4 mm), Waters 484 Detector and Workstation with 815 Baseline Program H1 (U.S.A) as shown in Fig. 7, the following amino acids were identified in almost all the analyzed extracts: aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), cysteine (Cys) valine (Val), methionine (Met), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), arginine (Arg).

Results obtained by *Boisen et al.* [43] indicated that modern diets should solely be based on digestible amino acids, it generates more cost-efficient diets and can lower the environmental impact in comparison to a diet based on CP. In any case, the feed ration shall consider the need to satisfy all the requirements in essential amino acids, notably in methionine, cysteine, lysine, threonine, tryptophan, isoleucine, and valine. This study identified that the content of amino acid in HABP amounted to 10.24% DS (w/w). However, *Corzo et al.* [44] observed that in livestock diet, methionine is one of the most limiting amino acids. Methionine is required for the growth performance i.e., maximum body weight gain of birds. For maximum mass production, amino acids, i.e., lysine and methionine, are commonly replaced with natural protein. Moreover, *Labadan et al.* [45] reported

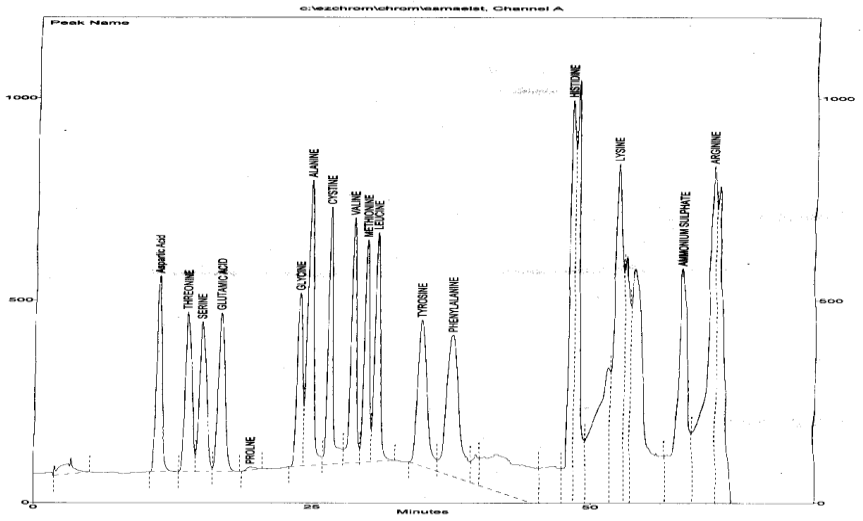


Fig. 7: The chromatogram of a standard amino acid mixture, standard program H1 on pre-column type H 60 × 4 mm

that replacement of CP with synthetic amino acids is not only important for economical but also for nutritional and environmental aspects. Synthetic amino acids can be used in low protein diet for the maximum weight gain with best feed conversion ratio (FCR).

CONCLUSIONS

In this work, methodology for extracting a mixture of plant-based amino acids from TJ stream with successful automation according to the operation conditions of sugar processing to ensure that the fluid sugar obtained satisfies all requirements for the subsequent processes of sugar manufacturing has been innovated. The control of material flows, ratios and other variables of the effective processes were recognized through the process modeling with fully automated system that ensure extracting the amino acids from the TJ at the lowest cost, as well as to provide precise control of equipment operating parameters and their regulation according to the selected operation conditions for obtaining the best results. As a result of this work, the total content of 16 amino acids extracted from TJ was 1% of beet (w/w) among them 9 essential amino acids were found. The ratio of EAA/TAA was 68.90% and ETAA/ NEAA was 40.79% of the ratios recommended by the WHO/FAO.

From a technical point of view, as the method innovated in this work is easy to scale-up purpose and it has provided good solubilization yields of amino acids from the TJ. Based on the actual data of the sugar factory operating with a production capacity of 10.000 tons beet per day (478 tons juice/h) the engineering data of the ion exchanger showed that the number of ion exchangers required to produce 100 tons of amino acids per day are 3-each with a capacity of 223 m^3/h, bed height 1.7 m, column height (without considering of the height for resin support and inlet distributor) 2.9 m, pressure drop 1.7 KPa, surface loading 65.8 m/h. EBCT 1.6 minutes and breakthrough time: 9 h.

For subsequent application, amino acids are used as nutrient additives for the by-product of sugar beet processing, SBP pellets, to increase its nutritional value and then can be used as integrated animal feed. The addition of amino acids to SBP improved the essential amino acids content and CP value from 8.40 to 14.61. As the scale up procedures are now established, the use of stream TJ as the alternative raw materials to produce the natural amino acids at industrial scale appears to be an interesting opportunity to maximize the benefit from the intermediates in the process of sugar beet and to optimize the beet sugar manufacturing.

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