# CAROB POD AS A FEEDSTOCK FOR THE PRODUCTION OF BIOETHANOL IN MEDITERRANEAN AREAS

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**Abstract**

There is a growing interest worldwide to find out new and cheap carbohydrate sources for production of bioethanol. In this context, carob pod (ceratonia siliqua) is proposed as an economical source for bioethanol production, especially, in arid regions. The carob tree is an evergreen shrub native to the Mediterranean region, cultivated for its edible seed pods and it is currently being reemphasised as an alternative in dryland areas, because no carbon-enriched lands are necessary. In this work, the global process of ethanol production from carob pod was studied. In a first stage, aqueous extraction of sugars from the pod was conducted, achieving very high yields (>99 %) in a short period of time. The process was followed by acid or alkaline hydrolysis of washed pod at different operating conditions, the best results ( R = 38.20%) being reached with sulphuric acid (2% v/v) at 90ºC, using a L/S (liquid/solid) ratio of 7.5 and shaking at 700 rpm for 420 min. After that, fermentation of hydrolysates were tested at 30ºC, 125 rpm, 200 g/L of sugars and 15 g/L of yeast with three different kinds of yeasts. In these conditions a maximum of 95 g/L of ethanol was obtained after 24h. Finally, the distillation and dehydration of water-bioethanol mixtures was analysed using the chemical process simulation software CHEMCAD with the aim of estimate the energy requirements of the process.

Keywords: bioethanol, agricultural crops, carob pod, sugars, acid hydrolysis,

## 1. Introduction

The development of cost-effective technologies for fuel ethanol production is a priority for many public and private institutions since this biofuel is one of the most important resources contributing to the growing use of renewable energy sources. The use of biofuels can contribute to improve the air quality and to decrease green house gas emissions [1], Other advantages derived from bio-fuels are security of energy supply and development of rural areas [2-3]. The main types of feedstocks for the production of ethanol are: (i) raw materials containing fermentable sugars (sugar cane, beet and sweet shorgum), (ii) polysaccharides that can be hydrolyzed for obtaining fermentable sugars (starch contained in several grains, like maize and wheat) and (iii) lignocellulosic biomass. However, several technical difficulties have been identified in the use of biofuels associated with the production costs that are uncertain and vary with the feedstock, moreover enzymatic hydrolysis involves a high cost of enzyme production and the biochemistry and mechanistic fundamentals are not still well-known [4]. The large amount of existing and not completely developed technologies for the production of ethanol and the intrinsic biological difficulties of the process, require continuous efforts for the diversification and adaptation to new biomass sources [5].

The present work deals with the idea of bioethanol production from carob pods. The Carob tree (Ceratonia siliqua) is an evergreen shrub or tree native to the Mediterranean region, cultivated for its edible seed pods with an average production of 2000 – 3500 Kg/ha. It is currently being reemphasised as an alternative in dryland areas with Mediterranean climates for diversification and revitalisation of coastal agriculture [6].Carob has drought resistance, requires little maintenance and produces a range of products from the seed and the pod. From the seed, the endosperm is extracted to produce a galactomannan, which forms locust bean gum (LBG), a valuable natural food additive for its strong gel characteristics, which are useful in products such as canned pet food, since they are maintained after heating. The carob pod is used actually as animal feed or is grinded to obtain carob powder, which can be used for human consumption although high tannin content limits this application. The production

of ethanol from nonsterilized carob pod extracts using Saccharomyces Cerevisiae have been investigated [8-10]. However, no data for the global process of ethanol production have been reported. Therefore, the aim of the present investigation was to analyze the global process of bioethanol production from carob pod different by *Saccharomyces Cerevisiae* yeast cells, including physical pretreatment, sugar extraction, hydrolysis of washed carob pod, fermentation of aqueous extracts, distillation and dehydration simulation of water-bioethanol mixtures.

## 2. Materials and methods

### *2.1.* Materials

The study was carried out using grinded carob pod (without seeds) from various locations supplied by Mondial Carob Group (Cartagena, Spain). Chemical characterization of carob pod samples were carried out following the European Community (EC) directives for the official control of feeding stuffs [11-14]. Particle size distribution of carob pod samples was determined using a vibrating screen. *Saccharomyces Cerevisiae* yeast A were supplied by S.I. Lessafre, B and C were supplied by local market.

### 2.2. Aqueous extraction test

Sugar extraction from carob pods (S) were carried out with water (L) at different L/S ratio. 50 g of grinded carob pod were immersed in the adequate amount of water and mechanically shook in open flasks at ambient temperature (20-25ºC) until attain extraction equilibrium. Then the mixture was filtered and the extract was analysed for its content of total sugars by the Luff - Schoorl method [11-14]. The slurry was used for hydrolysis tests after extensive washing with water. Eq. (1) was used to calculate the yield of total sugar in the extract

$$T.S.(\%\ w/w) = \frac{total\ sugars\ in\ the\ solution}{total\ sugars\ amount\ of\ grinded\ carob\ pod} \cdot 100 \quad (1)$$

## 2.3. Hydrolysis of washed carob pod

Hydrolysis of washed carob pod (free of sugars) were carried out with the necessary amount of acid (phosphoric and sulphuric) or alkaline (sodium hydroxide) agents in a batch reactor at 90ºC with total reflux and mechanical shaking at a fixed L/S ratio of 7.5 for 420 minutes. Samples (10 mL) were periodically withdrawn, to follow the time course of hydrolysis. Total sugars and reducing sugars were quantified in the supernatant using the Luff -Schoorl method [11-14] after removal of the insoluble material by filtration.

The term hydrolysis yield is defined as follows:

$$H.Y.(\%\ w/w) = \frac{total\ sugars\ in\ hydrolizate\ solution}{mass\ of\ washed\ carob\ pod} \cdot 100 \quad (2)$$

## 2.4. Simultaneous hydrolysis and extraction tests

Simultaneous sulphuric acid hydrolysis and extraction of carob pods were carried out in a batch reactor with total reflux and mechanical shaking at a fixed L/S ratio of 3 for 300 min. These experiments were carried at different temperatures, ranging from 66 to 95ºC, and sulphuric acid concentrations, from 0.6 to 3.4% v/v. To quantitatively determine the effect of each parameter on sugar extraction, a response-surface factorial design was used. The experiments were designed using a central composite design (CCD). Independent variables selected were temperature (T) and acid concentration (C) (Table 5). Fourteen experiments were performed according to Table 5. Six replications were at centre points (80ºC, 2.0%v/v), and the axial points were determined to be $\sqrt{2}$. Total ($R_t$) and reducing ($R_r$) sugars were chosen as dependent variables and were determined by analysis of the samples periodically withdrawn using the Luff-Schoorl method [11-14]. Statistical analysis was done using MINITAB 13 software.

The coefficients of the polynomial model were calculated using the following equation [15]:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{i,j} X_i^2 + \sum_{i_{i<j}}^{k} \sum_{j}^{k} b_{i,j} X_i X_j \qquad (1)$$

Where *Y* was the predicted response, *i,j* were linear and quadratic coefficients, respectively, *b* was the regression coefficients and *k* was the number of factors studied in the experiment.

### *2.5. Fermentation of aqueous solutions*

The anaerobic fermentation stage was carried out in a 3 L fermentation tank with several sample taking facilities. For optimal operating conditions, the fermentation tank has temperature controls and rpm-regulated agitator. As feedstock, the aqueous extract from the extraction test were used in each batch. Prior to the addition of this aqueous extract to the fermentation tank, solid residues were removed using a vibrating screen with a mesh size of 0.5 mm. After that, ammonium phosphate (3.2 g/L), potassium sulphate (1 g/L) and magnesium sulphate (1.8 g/L) were added as inorganic nutrients over the previous aqueous solution. Then, the pH was adjusted to 3.5-4, using diluted sulphuric acid. The resulting solution was sterilized by heating until its boiling point and then cooled at 35ºC. This solution was fed to the fermentation reactor thermostatized at 35ºC and the mixture of reaction was stirred 125 r.p.m. Free cells of *Saccharomyces Cerevisiae* (15 g/L) were used as yeast for the sugar to ethanol conversion

The evolution of fermentation process was determined by measurement of density of hydro-alcoholic solutions obtained and by gas chromatography using a HP-INNOWAX column (30m × 0.53mm × 0.25μm, Agilent), in the following conditions: temperature program: 28 ºC, 6min; 15 ºC/min, 200 ºC; 200 ºC, 2 min; split ratio: 50/1; inyector: 200 ºC; detector: 260 ºC. The monosaccharides and oligosaccharides in the fermentation broth were semiquantitatively analyzed by HPLC using a CarboPac PAI-PG1 column, a PED, Dionex 2010I, 6.0 g/l NaOH**.** Microbial growth using population measurements with a

Neubauer chamber or $CO_2$ analysis was used as complementary analytical methods.

*2.6. Distillation and dehydration of water-ethanol mixtures*

Due to the well-known characteristics of the distillation process in bioethanol industry, this stage was analyzed using the chemical process simulation software CHEMCAD.

## 3. Results and discussion.

### *3.1. Characterization of carob pod*

Table 1 shows the chemical characterization of carob pod samples from various locations determined following the European Community (EC) [11-14] directives. The results are expressed as weight percent for each parameter.

**[Insert Table 1 about here]**

As can be seen in this Table, the sugar fractions represent a high percentage of the total weight of the carob pod. Furthermore, there are a significant percentage of fiber in the waste, which are rich sources for the production of sugars by hydrolysis. These results are according to those claimed by Avallone et al. [16].

The carob pod chosen for this study was the one from Murcia-Alicante (Spain) 2005, which has the following composition (expressed in g/100 g dry weight basis): moisture, 10–12; starch, 0.94–0.95; total sugar (glucose, fructose, sucrose and maltose), 46–48; crude protein, 6.0–6.5; crude fibre, 10.0–11.5; total ash, 1.2–1.8; pH 4.5–4.8.

The monosaccharides and oligosaccharides content in this sample were 70.60% of sucrose, 8.93% of glucose and 18.30% of fructose, with respect to

261 the total amount of sugars in the pod. The mean size of the fraction selected
262 was 0.57 mm.
263
264 **3.2. *Aqueous extraction of sugars from carob pods***
265
266 Since the price of the feedstock contributes up to 70% to the production cost of
267 bio-ethanol in the case of molasses [17], sugar content of carob pod and
268 extraction conditions need to be clearly established to ensure later evaluation of
269 the overall carob to ethanol process.
270
271 In order to analyze the effect of the ratio of carob pod (S) to water (L) on the
272 efficiency of the extraction of sugars, the extraction process were carried with
273 five different ratios S/L ranging from 4.67 to 38.5 at room temperature.
274
275 **[Insert Figure 1 about here]**
276
277 As can be seen in Figure 1, almost complete aqueous extraction of sugars from
278 carob pods was achieved in a short period of time (less than 30 min.), so this
279 process can be considered easy for industrial application. It was also found that
280 higher total sugar extraction yields were achieved using higher L/S ratios. Since
281 solutions with a sugar content of 20% w/w are needed for practical industrial
282 application, the following conditions were established for preparing aqueous
283 extractors for the fermentation process: L/S ratio of 2.5 for 20 minutes.
284
285 Table 2 shows parameters for the regression of extraction data using a
286 Langmuir type model, where *a* parameter represents the maximum theoretical
287 yield that it could be achieve in that conditions.
288
289 **[Insert Table 2 about here]**
290
291 ***3.3. Acid and alkaline hydrolysis.***
292
293 Several authors have reported [18-20] the use of dilute acid and/or modified
294 steam-explosion processes as pretreatment steps prior to enzymatic hydrolysis

295 in order to raise sugar conversion yield through the breakdown of lignocellulosic
296 biomass. The high energy requirement of these processes, the elevated costs
297 of enzymes and the low content of fiber and starch in this crop lead us to
298 discard this alternative as economically feasible. However, with the aim of
299 maximizing the process yield, direct acid hydrolysis of washed carob pod were
300 tested.
301
302 Direct acid and basic hydrolysis of washed carob pod were tested at 90ºC with
303 total reflux and mechanical shaking at a fixed L/S ratio of 7.5 for 420 minutes.
304
305 Figure 2 shows the acid hydrolysis of carob pod with sulphuric acid at different
306 acid concentrations.
307
308 **[Insert Figure 2 about here]**
309
310
311 As can be seen in this Figure, transformation yields higher than 30% were
312 reached in all cases with sulphuric acid hydrolysis, except for acid concentration
313 0.5% and lower. The best results (yield = 38.20%) were achieved with a 2% v/v
314 acid concentration. It is worthy of note that for acid concentrations higher than
315 3.0%, a decrease in the sugar extraction yield was observed, which can be
316 attributed to degradation reactions of sugars, that convert some sugars in
317 furfural, acetic acid and other undesirable compounds [18-23]. A similar
318 behaviour was observed by Saha et al. [24] in the hydrolysis of wheat straw at
319 121ºC for 1h with sulphuric acid at 2%v/v and 4% v/v, who also found yield
320 losses (5.5%) after enzymatic saccharification.
321
322 Figure 3, shows the evolution with time of sugar extraction yield in the acid
323 hydrolysis of carob pod with phosphoric acid at different acid concentrations.
324 The use of phosphoric acid has the added advantage that, after neutralization of
325 hydrolysates with NaOH, sodium phosphate is formed. This salt can remain in
326 the hydrolyzates, because it is used as nutrient by microorganisms, improving
327 process profitability and having a positive impact on the environment [25].
328

**[Insert Figure 3 about here]**

The results obtained show a qualitative behaviour similar than those obtained for sulphuric acid hydrolysis. In this case, the maximum yield obtained was slightly higher than 20% w/w (30.57 g/l). Similar yields were found by Gamez et al. [26], who obtained 23.2 g/L of total sugars from phosphoric hydrolysis of sugar cane bagasse at a sulphuric acid concentration of 4% v/v, L/S ratio of 8, 122ºC and 300 min.

Other minerals acids like $HNO_3$ or HCl were not used due to their inhibitory effects in alcoholic fermentation, corrosive properties and enviromental impact, which limits its application. [25,27].

The use of an alkaline reagent (sodium hydroxide) at low concentrations for the hydrolysis process showed similar yields than that obtained with acids (see Figure 4). However, for higher alkaline concentrations, yield losses were observed due to peeling of the end groups and hydrolytic reactions [20, 28- 30].

**[Insert Figure 4 about here]**

The experimental extraction data for the hydrolysis tests were adjusted using a Langmuir type model. Table 3 shows parameters for this regression:

**[Insert Table 3 about here]**

To sum up, the best operation results were obtained for sulphuric acid at a 2% v/v of acid concentration.

### 3.4. Simultaneous extraction and hydrolysis process

In order to test the feasibility of a unique stage for the sugar extraction and mild hydrolysis of the carob pod, experiments for the combined process were performed using a central composite design (CCD). Values for independent variables, temperature and acid concentration, are showed in Table 4. Fourteen

363 experiments were performed according to Table 5. Six replications were at
364 centre points (80ºC, 2.0%v/v).
365
366 **[Insert Table 4 about here]**
367
368 **[Insert Table 5 about here]**
369
370 Results obtained for total and reducing sugars in each run are showed in Table
371 6. Statistical analysis of the results is summarized in Table 6. A second order
372 dependence between temperature and process yield is confirmed, reaching a
373 maximum for process temperature between 75-80 ºC. The influence of acid
374 concentration is negative for the overall process and total yield is lower than the
375 sugar content of the carob pod.
376
377 **[Insert Table 6 about here]**
378
379 **[Insert Figure 5 and 6 about here]**
380
381 These results confirm partial hydrolysis of dissolved sucrose to obtain glucose
382 and fructose (reducing sugars), so the following fermentation process can be
383 easily carried out with higher sugar to ethanol yield conversion. However,
384 simultaneous sugar extraction and mild acid hydrolysis is not feasible from a
385 techno-economic point of view because total sugar extraction yields are lower
386 than those obtained with water extraction. This fact is due to degradation
387 reactions of sugars in acid media [19, 22-23].
388
389
390
391
392 **3.5. Fermentation of carob pod extracts**
393
394 Since aerobic fermentation of glucose and sucrose extracted from carob pod by
395 *xanthomonas campestris* bacterium may produce xanthan gum [31] (a
396 polysaccharide used as a food additive and rheology modifier), anaerobic
397 fermentation tests were carried out with sterilized carob pod extracts. These

extracts were analized with the following average composition: 197.5 g/l for total sugars and 61.36 g/l for reducing sugars. Three different kinds of *Saccharomyces Cerevisiae* yeast cells from several commercial suppliers were tested. Figure 7 shows the time evolution of ethanol in the fermentation process of aqueous extracts according to the method described in 2.2.

**[Insert Figure 7 about here]**

The ethanol concentration increased rapidly during the first hours of fermentation until reach a maximum ethanol level (95 g/L) after 30 h of incubation using yeast A and B. With yeast C a maximum of 70 g/L was achieved after 60 h**.** These results were similar than those reported by for sugarcane-based processes [17] and better than those reported for carob pods processes [9] with the same initial sugar concentrations (200 g/L) in the aqueous extracts. This value was the maximum concentration that ensures correct metabolization of the sugars in the culture. Possible reasons for these different ethanol levels are the strain of organisms used, the sterilization pretreatment of the solution and the removal of dissolved solids prior to the fermentation stage. Measurement of residual sugars confirms a decrease during fermentation.

It is worthy of noting that when incubation times were greater than 30 h., concentration of ethanol was kept constant and no degradation products were observed in the solution.

### *3.6. Distillation of fermentation products.*

In order to evaluate energy requirements for the carob to ethanol process, steady-state simulation of the distillation stage was carried out using the software ChemCad. Feed streams coming from fermentation step contains usually water, ethanol and up to 5% of several compounds like methanol, acetaldehyde and fusel alcohols (1-propanol, 2-propanol, 1-butanol, 3-methyl-1-butanol and 2-pentanol). Exact composition depends on the raw material used, and saccharification and fermentation process conditions. The conventional

schedule for distillation columns layout in grain to ethanol plants includes one or two columns for the separation of vinasses. The removal of dissolved solids prior to the fermentation step in the process presented in this work let us to discard these equipments. Since gas chromatography analysis shows the absence of significant amounts of methanol, acetaldehyde and fusel alcohols in the solution coming from distillation step, a second distillation column was included in the flow diagram chosen for the simulation and showed in figure 8.

**[Insert Figure 8 about here]**

Azeotropic, vacuum or extractive distillation procedures were not considered as viable options for the production of fuel grade ethanol, since these distillation routes have significant impact on the environment, are energy intensive and more expensive than the recent pressure swing adsorption (PSA) with molecular sieve trays. This technology is based on the selective adsorption of ethanol molecules with a sized molecular sieve sorbent [32]. UNIFAC method (UNIQUAC Functional-group Activity Coefficient) was used to estimate equilibrium conditions. A theoretical feed stream including methanol, acetaldehyde and fusel alcohols were used for simulation purposes. The results are summarized in tables 7a and 7b.

**[Insert Table 7 about here]**

The minimum number of ideal equilibrium stages for the first column (water recovery) was founded to be 20 and calculated energy consumption for the reboiler was 1165.8 kcal/kg ethanol. The use of a partial condenser does not contribute to the removal of significant quantities of methanol and acetaldehyde and some losses of ethanol are observed. Referring to the second column, it must be pointed out that the energy consumed in the reboiler is related directly to the distribution of fusel oils between top and bottom products. If the energy input is high, only C4 fusel oils appears in the bottom stream and losses of ethanol and water are not observed. For low energy inputs, all fusel oils and a considerable fraction of water and ethanol are withdrawn from the base of the column. Results depicted in table 7b were obtained for 10 theoretical

equilibrium stages and energy consumption was fixed to 285 kcal/kg. The requirements of refrigeration utilities for the second column were minimal because the main fraction of the bottom product remains in vapour phase previous to the adsorption of ethanol in the PSA unit. According to these results, a precise calculation of total energy requirements for the process depends on the exact composition of the solutions coming from distillation stage.

### *3.7. Overall Process Discussion.*

Figure 9 presents the block diagram for the overall carob to ethanol process proposed and assessed in this work. For normal harvesting conditions, annual carob pod production in Spain is 60000 to 65000 Tn/year. Taking into account average total sugar contents, the global “carob to ethanol” process yield (extraction, fermentation, distillation and dehydration stages) ranges between 19200 and 20800 cubic meters of fuel ethanol (>99.95%) per year. Comparison of ethanol productivity from different feedstocks is showed in table 8. According to these results, carob pod can be presented as a viable alternative for the production of fuel ethanol.

**[Insert Table 8 and Figure 9 about here]**

## 4. Conclusions.

The results showed that carob pod is a suitable feedstock to produce fuel – grade ethanol because its high sugar content round 50%. The recovery of these sugars is easy using water as a solvent with agitation times less than 30 min.

The solid waste of the extraction process was hydrolyzated with sulphuric acid, phosphoric acid and sodium hydroxide. The best results were obtained with sulphuric acid at 2% v/v. Simultaneous acid and extraction processes were also tested, however, total sugar extraction yields in this case were lower than those obtained with water extraction due to degradation reactions of sugars in acid media.

The fermentation of the aqueous extracts were done achieving yields of 47.5% in ethanol. The distillation process were simulated with CHEMCAD 6.0 with the aim of estimate the energy requirements for the process, 1450.8 Kcal/Kg ethanol were estimated.

In addition, several major advantages derived from the carob tree cultivation can be highlighted: (i) carob pod does not compete with food consumption, (ii) the expected production costs are similar to those from sugar cane processes, (iii) the use of perennial crops, achieves substancial benefits due to the reduction of green house gas emissions associated to fertilizers production and fixation of carbon in mineral soils and (iv) mild acid hydrolysis could improve etanol yields. These advantages, together with the above results suggest that carob pod could be a potential feedstock for bioethanol production in arid regions.

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612

# LIST OF TABLES

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683 Table 1. Carob pod characterization (wet basis) of samples studied.

684

| Sample origin | Sicily (Italy) ([a]) | Murcia (Spain) 2004 | Valencia (Spain) 2004 | Murcia Alicante (Spain) 2005 | Morocco |
|---|---|---|---|---|---|
| Moisture (%) | 7.0 | 14.2 | 16.4 | 13.6 | 12.6 |
| Ash (%) | 3.0 | 3.0 | 3.0 | --- | 3.8 |
| Fiber (%) | --- | 11.6 | 10.1 | --- | 14.9 |
| Protein (%) | 3.0 | 4.8 | 6.2 | --- | 3.1 |
| Fat (%) | 0.6 | 0.0 | 0.1 | --- | 0.0 |
| Starch (%) | 0.8 | 4.7 | 6.2 | 0.2 | --- |
| Total sugar (%) | 44.0 | 52.5 | 53.8 | 49.9 | 40.4 |
| Reducing sugar (%) | 10.0 | 30.6 | 25.0 | 17.5 | 29.5 |

685

686 (a) Results reported by Avallone et al. [16] in dry basis.

687

688 Table 2.Results for the regression of data from water extraction of sugars
689 contained in carob pod samples.

690

| Langmuir isotherm model | $y = \frac{a \cdot b \cdot t}{1 + b \cdot t}$ | | |
|---|---|---|---|
| S/L ratio | a | b | $r^2$ |
| 38.50 | 101.2 | 1.53 | 0.990 |
| 17.85 | 102.6 | 2.37 | 0.999 |
| 9.35 | 100.3 | 1.59 | 0.995 |
| 5.85 | 98.8 | 5.53 | 0.999 |
| 4.67 | 99.4 | 1.80 | 0.999 |

691

692 Table 3. Results for the regression of hydrolysis experiments in washed carob
693 pod samples

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| Langmuir isotherm model | $y = \frac{a \cdot b \cdot t}{1 + b \cdot t}$ | | |
|---|---|---|---|
| Sulphuric acid | | | |
| C% (v/v) | a | b | $r^2$ |
| 0.5 | 30.97 | 0.013 | 0.999 |
| 1.0 | 40.87 | 0.013 | 0.999 |
| 2.0 | 41.22 | 0.029 | 0.999 |
| 3.0 | 41.93 | 0.015 | 0.998 |
| Phosphoric acid | | | |
| C % (v/v) | a | b | $r^2$ |
| 0.5 | 15.38 | 0.023 | 0.996 |
| 2.0 | 22.46 | 0.045 | 0.998 |
| 4.0 | 23.56 | 0.054 | 0.999 |
| Sodium hydroxide | | | |

| C % (w/v) | a | b | $r^2$ |
|---|---|---|---|
| 0.9 | 21.55 | 0.241 | 0.999 |
| 1.78 | 24.54 | 0.110 | 0.998 |

Table 4. Variables in the experimental design of simultaneous extraction and hydrolysis processes.

| | Coded levels | | | | |
|---|---|---|---|---|---|
| Dimensionless Variable | -1.414 | -1 | 0 | 1 | 1.414 |
| Temperature (ºC) | 66 | 70 | 80 | 90 | 95 |
| Acid concentration (%v/v) | 0.6 | 1 | 2 | 3 | 3.4 |

Table 5. Central composite design matrix for simultaneous extraction and hydrolysis experiments.

| | **C (%v/v)** | **T (ºC)** | **$R_r$ (%w/w)** | **$R_t$ (%w/w)** |
|---|---|---|---|---|
| 1 | 3.0 | 70 | 36.60 | 36.89 |
| 2 | 1.0 | 90 | 40.31 | 39.05 |
| 3 | 2.0 | 80 | 38.15 | 38.03 |
| 4 | 1.0 | 70 | 38.28 | 38.99 |
| 5 | 2.0 | 80 | 36.96 | 36.90 |
| 6 | 2.0 | 80 | 37.82 | 38.37 |
| 7 | 3.0 | 90 | 35.08 | 35.55 |
| 8 | 2.0 | 95 | 35.32 | 35.02 |
| 9 | 3.4 | 80 | 37.44 | 37.81 |
| 10 | 2.0 | 80 | 38.78 | 38.38 |
| 11 | 2.0 | 66 | 37.04 | 37.87 |
| 12 | 2.0 | 80 | 37.89 | 40.07 |
| 13 | 2.0 | 80 | 38.00 | 39.96 |
| 14 | 0.6 | 80 | 37.96 | 37.96 |

**C: Sulphuric acid concentration, T: Temperature**

Table 6. Statistical results of carob pod simultaneous hydrolysis and extraction model for reducing sugars ($R^2$ = 0,900) and total sugars ($R^2$ = 0,854).

| Reducing Sugars | | | | |
|---|---|---|---|---|
| | Regression coefficient | Standard error | t | P |
| Constant | - 17.91 | 10.6964 | - 1.674 | 0.138 |
| C | - 0.78 | 0.2208 | - 3.525 | 0.010 |
| T | 1.50 | 0.2689 | 5.569 | 0.001 |
| T x T | - 0.01 | 0.0017 | - 5.820 | 0.001 |
| **Total Sugars** | | | | |
| | Regression coefficient | Standard error | t | P |
| Constant | - 30.50 | 17.4495 | - 1.748 | 0.124 |
| C | - 0.93 | 0.3602 | - 2.570 | 0.037 |
| T | 1.86 | 0.4386 | 4.244 | 0.004 |
| T x T | - 0.01 | 0.0027 | - 4.457 | 0.003 |

Table 7a. Results obtained for the steady-state simulation of distillation column 1 according to the process showed in figure 8 using CHEMCAD software.

| Stream | Water/ethanol feed mixture | Head product | Vent Gas | Bottom product |
|---|---|---|---|---|
| Temperature (ºC) | 50 | 80.38 | 80.38 | 102.3 |
| Pressure (atm) | 1.1 | 1.1 | 1.1 | 1.1 |
| Total flow rate (kg/h) | 23375 | 2522.5 | 26.6 | 20825.9 |
| Ethanol (kg/h) | 2100 | 2076.8 | 22.6 | 0.6 |
| Water (kg/h) | 21230 | 403 | 3.1 | 20823.9 |
| Methanol (kg/h) | 10 | 8.47 | 0.08 | 1.45 |
| Acetaldehyde (kg/h) | 10 | 9.33 | 0.68 | - |
| Fusel oils (kg/h) | 25 | 24.9 | 0.1 | - |

Table 7b. Results obtained for the steady-state simulation of distillation column 2 according to the process showed in figure 8 using CHEMCAD software.

| Stream | Head product (azeotrope) | Bottom product |
|---|---|---|
| Temperature (ºC) | 80.77 | 85.92 |
| Pressure (atm) | 1.1 | 1.1 |
| Total flow rate (kg/h) | 2301.7 | 220.7 |
| Ethanol (kg/h) | 2000 | 76.8 |
| Water (kg/h) | 272.1 | 130.9 |
| Methanol (kg/h) | 7.50 | 0.97 |
| Acetaldehyde (kg/h) | 9.33 | - |
| Fusel oils (kg/h) | 12.71 | 12.15 |

Table 8. Comparison of ethanol productivity from different feedstocks.

| Feedstock | Fuel ethanol productivity (L/ Kg) |
|---|---|
| Corn | 0.409 |
| Wheat | 0.360 |
| **Carob Pod** | **0.320** |
| Sugar Beet | 0.200 |
| Cassava | 0.182 |
| Sweet Sorghum | 0.140 |
| Sugar Cane | 0.085 |

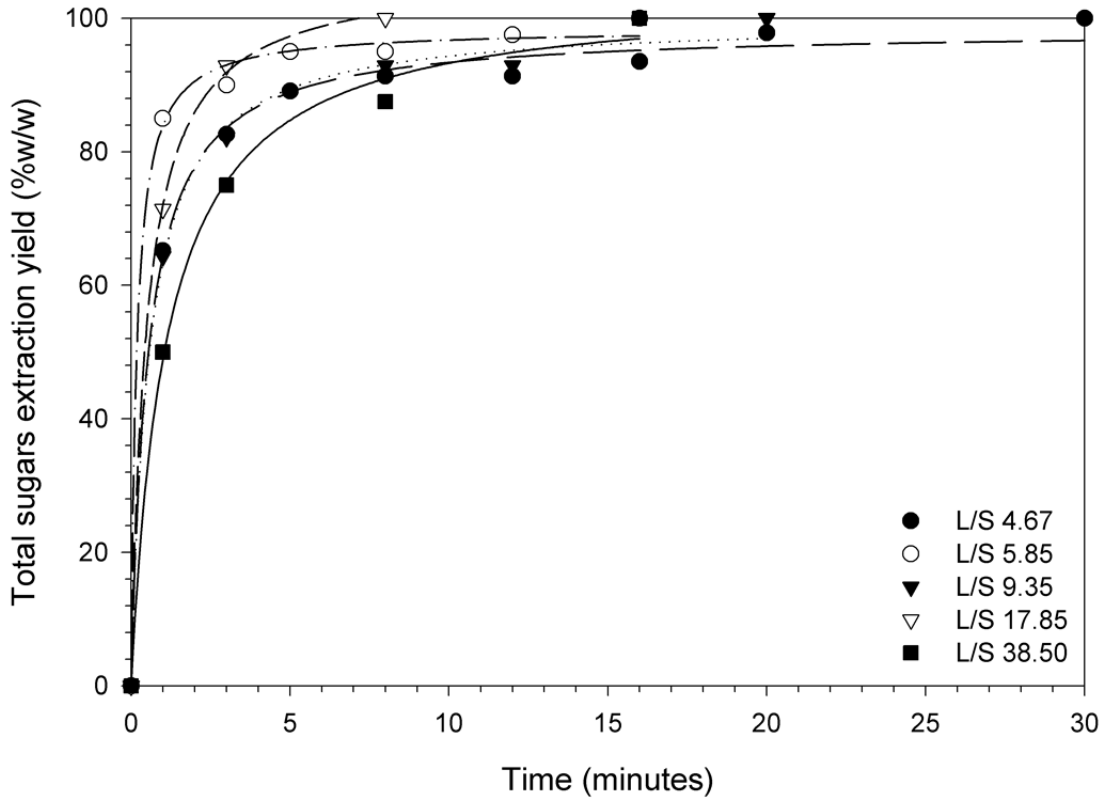


Figure 1. Sugar extraction yield of sugars contained in carob pod samples using water at room temperature.

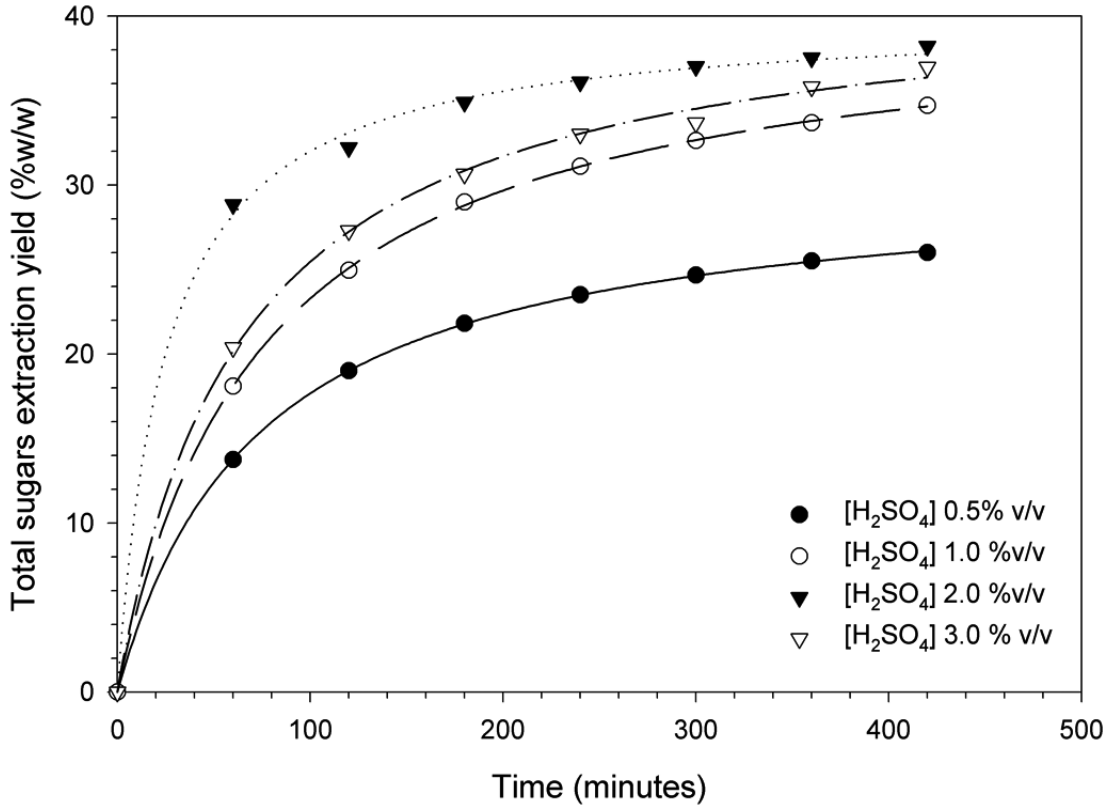


Figure 2. Hydrolysis yield in washed carob pod samples using sulphuric acid at 90ºC and L/S ratio of 7.5.

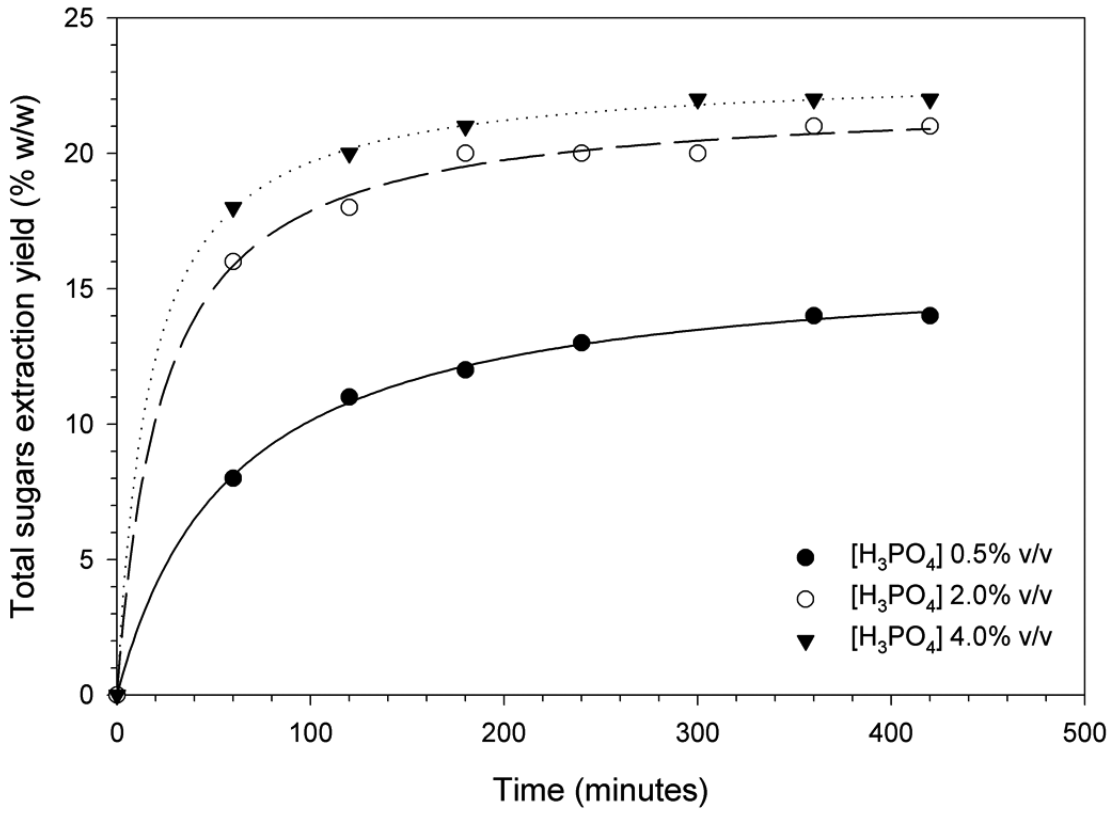


Figure 3. Hydrolysis yield in washed carob pod samples using phosphoric acid at 90ºC and L/S ratio of 7.5.

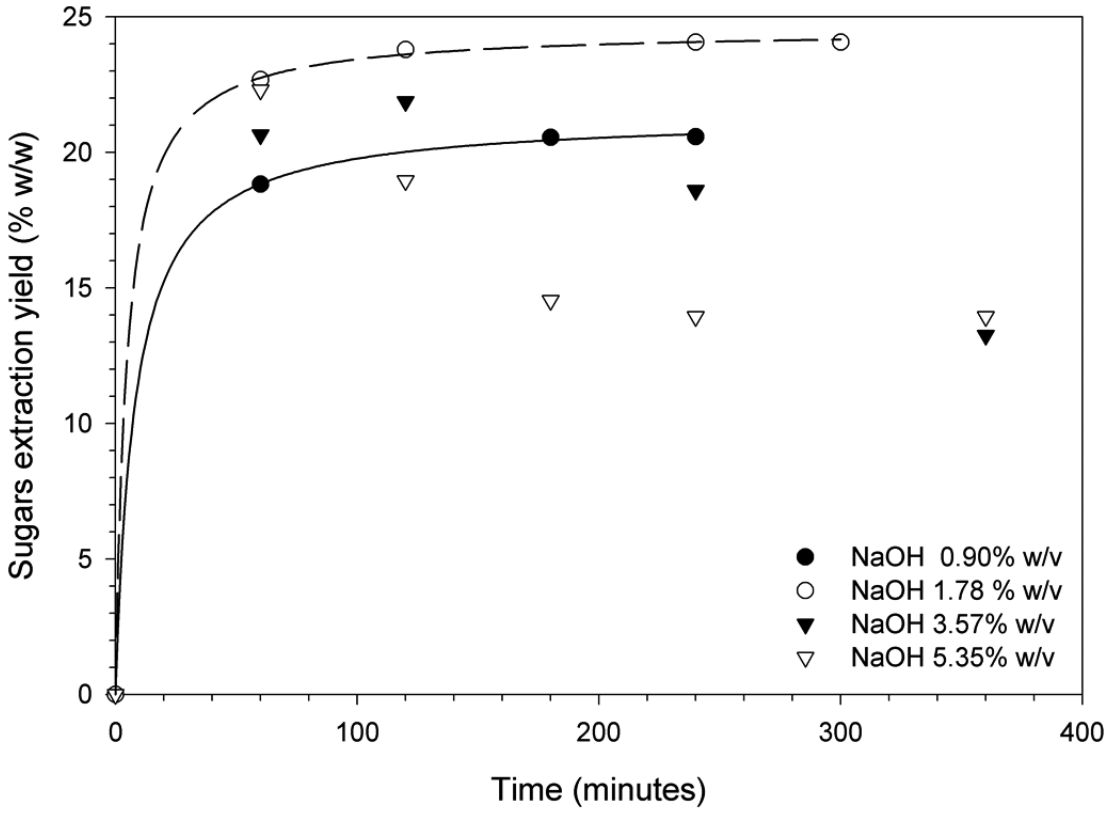


Figure 4. Hydrolysis yield in washed carob pod samples using sodium hydroxide at 90ºC and L/S ratio of 7.5.

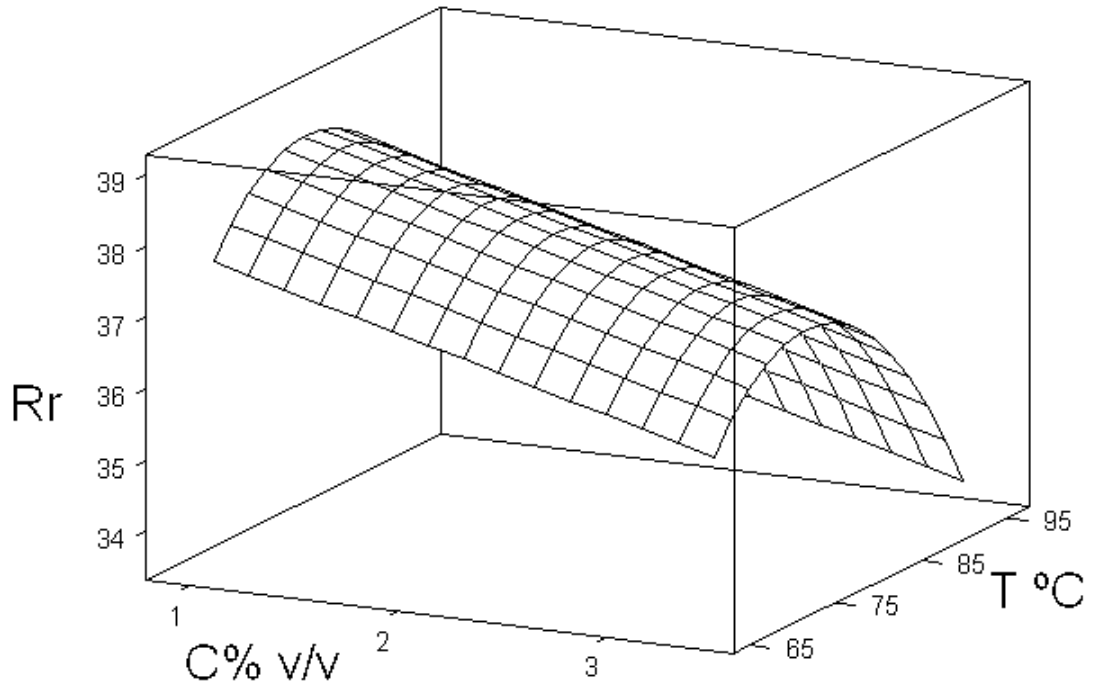


Figure 5. Response surface plot of T (ºC) vs C (%v/v) on reducing sugars extraction yield

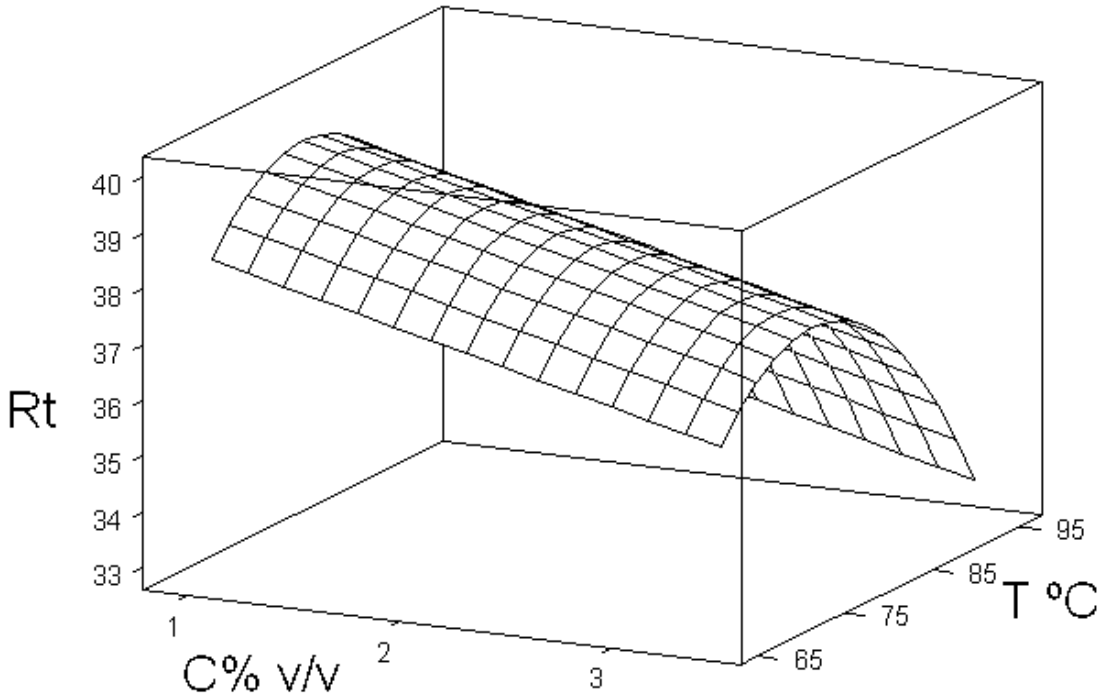


Figure 6. Response surface plot of T (ºC) vs C (%v/v) on total sugars extraction yield

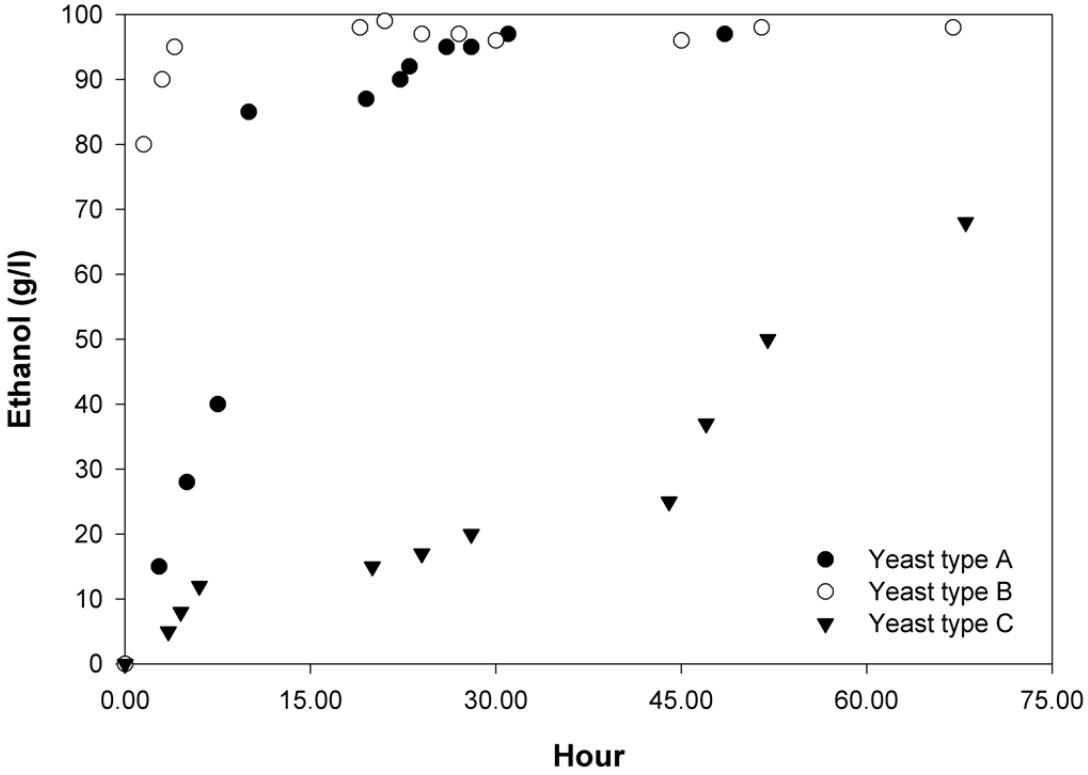


Figure 7. Kinetics of fermentation of aqueous extracts with free cells of Saccharomyces Cerevisiae. Experimental conditions: pH 3.5-4, 35ºC, 125 r.p.m. and yeast concentration of 15 g/L.

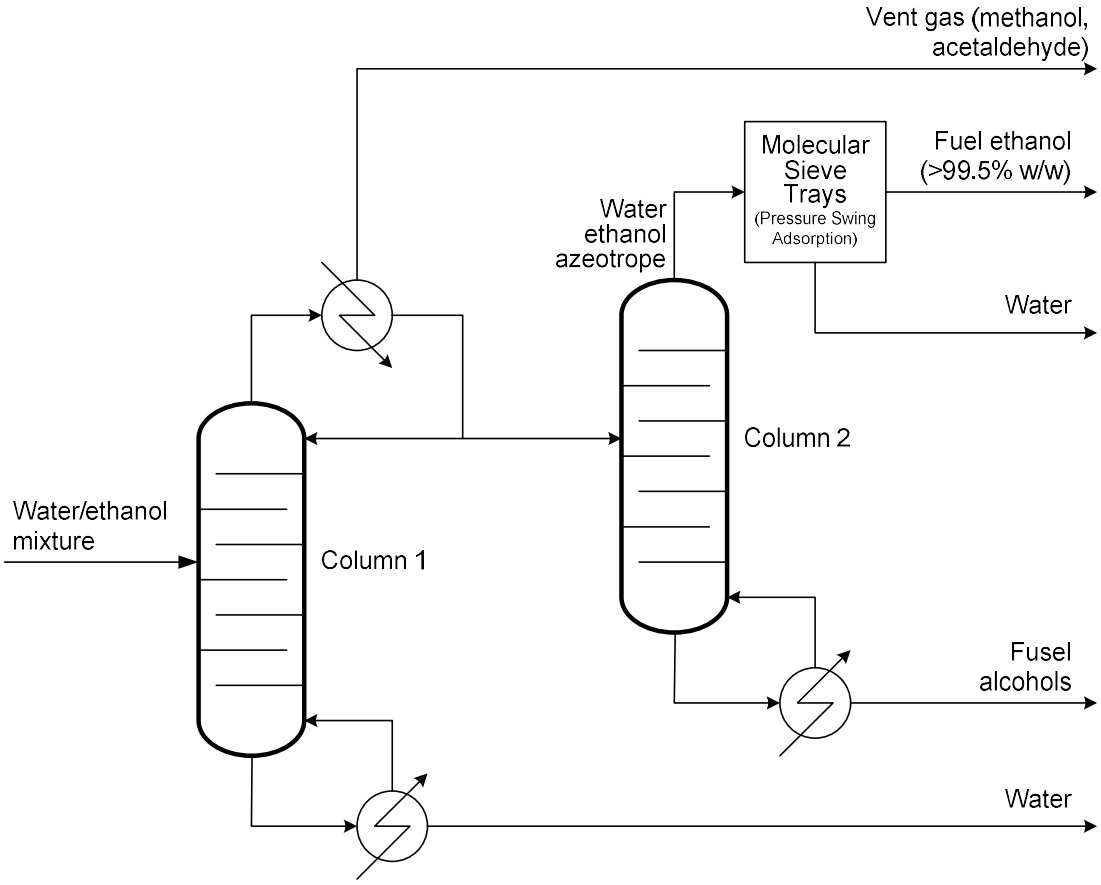


Figure 8. Flow diagram for the distillation and dehydration stages of carob pod to ethanol process.

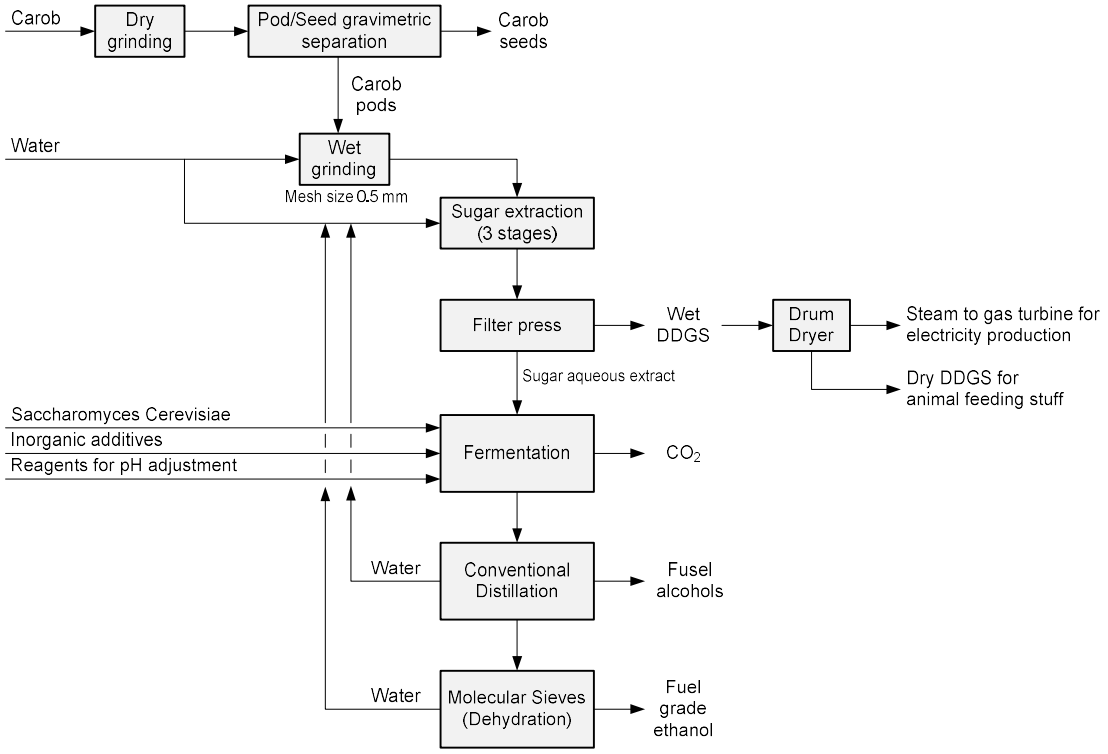


Figure 9. Block diagram for the overall carob to ethanol process.