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USE OF PEA FRACTIONATION WASTES IN BIOETHANOL PRODUCTION

Ye.A. Vovk
S.P. Tsygankov

State Institution «Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine»

E-mail: vovkeu@gmail.com

The production of protein and purified protein isolates from legumes using wet fractionation technology generates liquid starch-containing waste, a significant disposal problem. Such wastes can be considered feedstocks for biofuels, particularly bioethanol. In this way can be achieved a double effect: the valorisation of starch liquid wastes (SLW) and the partial replacement of food raw materials for energy needs.

Aim. To investigate the possibility of preparing a complex nutrient medium using standardised SLW after pea fractionation with the addition of milled corn for further fermentation to produce bioethanol.

Methods. Standardised SLW was obtained by the wet fractionation laboratory method; starch content was determined according to GSTU 46.045-2003, and protein content was determined by the Kjeldahl method in accordance with AOAC 979.09. Bioethanol was obtained in a laboratory fermenter using a simultaneous saccharification and fermentation (SSF) process. The leading indicators in the fermented samples were determined using the methods specified in SOU 15.9-37-242:2005.

Results. For all samples of combined wort with a concentration range of 25–29% w/v dry matter (DM), the process can practically be completed in 72 hours. Ethanol content in the fermented wort was 10.6±0.05—12.1±0.05% v/v for wort samples of the mentioned DM concentration.

Conclusions. During the fermentation of combined wort, pea starch, which is also present in the wort, gives an increase in the ethanol concentration in the fermented wort. The final ethanol yield from combined wort starch is low (0,6±0,04 l/kg), lower than from corn starch (0.665 l/kg). For the development of Very High Gravity (VHG) fermentation technology, it is necessary to select appropriate enzymes and parameters for the hydrofermentative preparation of combined wort.

Keywords: pea, starch liquid waste, fractionation, fermentation, enzymes, mash, VHG fermentation, bioethanol.

The world continues to trend towards increasing biofuel production in order to prevent an increase in the concentration of greenhouse gases in the atmosphere. According to the latest estimates of the World Bioenergy Association (WBA), according to statistical reports and current reviews, global production, in particular, of bioethanol, will reach more than 116 billion liters by the end of 2025, which is about 70% of global liquid biofuel production [1].

This in turn requires the use of ever-increasing volumes of plant raw materials for energy needs, often at the expense of food or feed. This is especially true for the production of bioethanol. According to preliminary estimates, starch-containing sources are used as the main raw materials - about 60%, the share of production from sugar-containing raw materials (mainly sugar cane, sugar beet) will be approximately 40% [2].

Balancing the use of crops for food and feed production or biofuel production remains a challenge with both economic and ethical dimensions. To avoid criticism of using of food raw materials to meet energy needs, alternative feedstock sources are being considered for the latter [1–3].

For more than 30 years, lignocellulose (the so-called second-generation feedstock) has

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been considered as an alternative feedstock for bioethanol. However, the realization of hopes for lignocellulose feedstock has not yielded the expected results, but rather has led to disappointment in the economic attractiveness of lignocellulose-based bioethanol technologies. The immaturity of the proposed technological solutions, fluctuations in fossil fuel prices and logistical problems have led to a decrease in optimism regarding the replacement of traditional starch with cellulose for bioethanol production. The same problems exist with respect to bioethanol from third- and fourth-generation feedstocks – algae and the use of various genetically modified organisms (microalgae and cyanobacteria) that are capable of assimilating CO_2 to form biofuels [3–5].

Given the problems of scaling up new generation bioethanol technologies to the industrial level, the first-generation bioethanol technology is also being improved. In particular, the concept of "1.5 generation" has appeared quite recently. The technology aims to maximize the yield of the target product and obtain bioethanol from non-starch polysaccharides and other compounds contained in grain raw materials through the use of new technologies and a full range of enzymes that form fermentable sugars in the fermentation medium [6].

On the other hand, various starch-containing wastes are used in production and are not considered food or feed raw materials. Recently, the production of protein isolates from legumes has been increasing. Soybeans, peas and other crops are subjected to grinding and fractionation, obtaining protein-enriched fractions for further use in the food industry. There are a number of technologies for fractionation of yellow field peas, including the air classification process (dry fractionation) and wet fractionation [7, 8].

Both fractionation methods can partially separate the protein and starch parts. Comparing the dry and wet fractionation methods, it should be noted that the wet fractionation method yields higher-purity soluble and insoluble protein fractions. The prospects of wet pea fractionation technology are confirmed by plans to build production capacities of up to 35,000 t/year by Phyto Organix in Canada and Alfa Laval for Lantm nnen in Sweden by 2027.

However, it should be noted that during wet fractionation in industry, significant amounts of water are used (from 5–10 m^3 of water per ton of milled pea). A number of technological solutions used in fractionation are aimed at obtaining highly purified protein isolates, whose need is growing. Requirements for the purity of the target product result in the formation of starch-containing liquid waste, which is a significant problem [7–10].

Given their unsuitability for further processing into food or feed products, such liquids can be considered feedstocks for biofuels, particularly bioethanol [7, 9]. This will achieve a double effect — valorization of starch-containing liquid waste (SLW) and partial substitution of food raw materials for energy needs.

This work aims to investigate the feasibility of preparing a complex nutrient medium from standardised starch-containing liquid waste from pea fractionation, supplemented with corn milling, for further fermentation to produce bioethanol.

Materials and Methods

Obtaining standardised SLW

Waste from wet fractionation at different enterprises, depending on technological features, has specific differences in composition and concentration. Based on literature data, a wet fractionation method was simulated in laboratory conditions to obtain standardized SLW, which was subsequently used in experiments [8].

The raw material used was yellow pea beans (*Pisum sativum* L.), with a starch content of 45.3% DM according to GSTU 46.045-2003 and a protein content of 21% DM according to the Kjeldahl method (AOAC 979.09). The dry matter content was determined by drying on a Kern ADS-50 moisture balance.

Pea grinding was carried out using a grain mill LZM-1 (Ukraine) until a homogeneous grind was obtained (a sieve with a hole diameter of 1 mm used).

Milled yellow pea (50 g) was dissolved in distilled water in a ratio of 1:10. The pH of the solution was adjusted to 8.0–8.6 by adding a decinormal solution of NaOH, and the determination was performed using a laboratory analyzer.

The slurry was left to mix on a magnetic stirrer for 1 hour and 30 minutes. Centrifugation was performed on a laboratory centrifuge at 5000 rpm for 15 minutes. The precipitate separated from the supernatant contained a starch fraction with grain residues.

The supernatant was used for protein precipitation. Precipitation was carried out with a decinormal H_2SO_4 solution. The pH of the solution was adjusted to 4. Centrifugation was carried out at 5000 rpm for 15 min. The separated precipitate contained the protein fraction. The resulting supernatant contained starch and protein residues.

The precipitate was added to the separated liquid after the first stage of fractionation to obtain 10% of the DM at pH 4.6. The content of the main components of the standardised SLW: conditional starch content — 62.9% of the DM (the primary method of GSTU 46.045-2003), protein (Kjeldahl method) — 5.8% of the DM. The scheme for obtaining standardised SLW and the main indicators are presented in Fig. 1.

Raw materials for medium preparation

The obtained standardised SLW, with additional milled corn, was used as a feedstock for fermentation medium preparation. The content of the main components of corn grain: starch — 63% DM, protein — 5.5% DM, moisture content — 14%.

Corn was milled until a homogeneous grind was obtained (passage through a sieve with a diameter of 1 mm hole ≥ 95%). The finished grind was added to the standardised SLW to 25, 27, and 29% dry matter for different samples.

Preparation of fermentation medium

Bioethanol production was carried out in a laboratory fermenter for wort with a volume of 1.5 l for each of the samples, according to the scheme of simultaneous saccharification and fermentation.

The main enzymes (manufactured by International Flavors & Fragrances, Health & Biosciences division) included: thermostable α-amylase (starch hydrolysis) — Amylex® 6T, glucoamylase (formation of fermentable sugars during the fermentation stage) — Diazyme® TGA, acid protease (hydrolysis of peptides and proteins) — Alphalase® AFP, phytase (formation of free phosphorus and inositol for additional yeast nutrition) — Optimash® Ptytase, fungal α-amylase (reduction of insoluble starch due to hydrolysis during the fermentation stage) — Optimash® SA. Test samples provided by LLC Agrotechnology. The required amount of enzyme solution (1:100) for each sample was calculated according to the manufacturer's recommendations, accounting for the DM amount.

The required amount of α-amylase was added to the previously prepared samples at pH 6.0±0.2. The medium was heated to 75–80 °C, with periodic stirring to prevent dry matter from sticking. After reaching 80 °C,

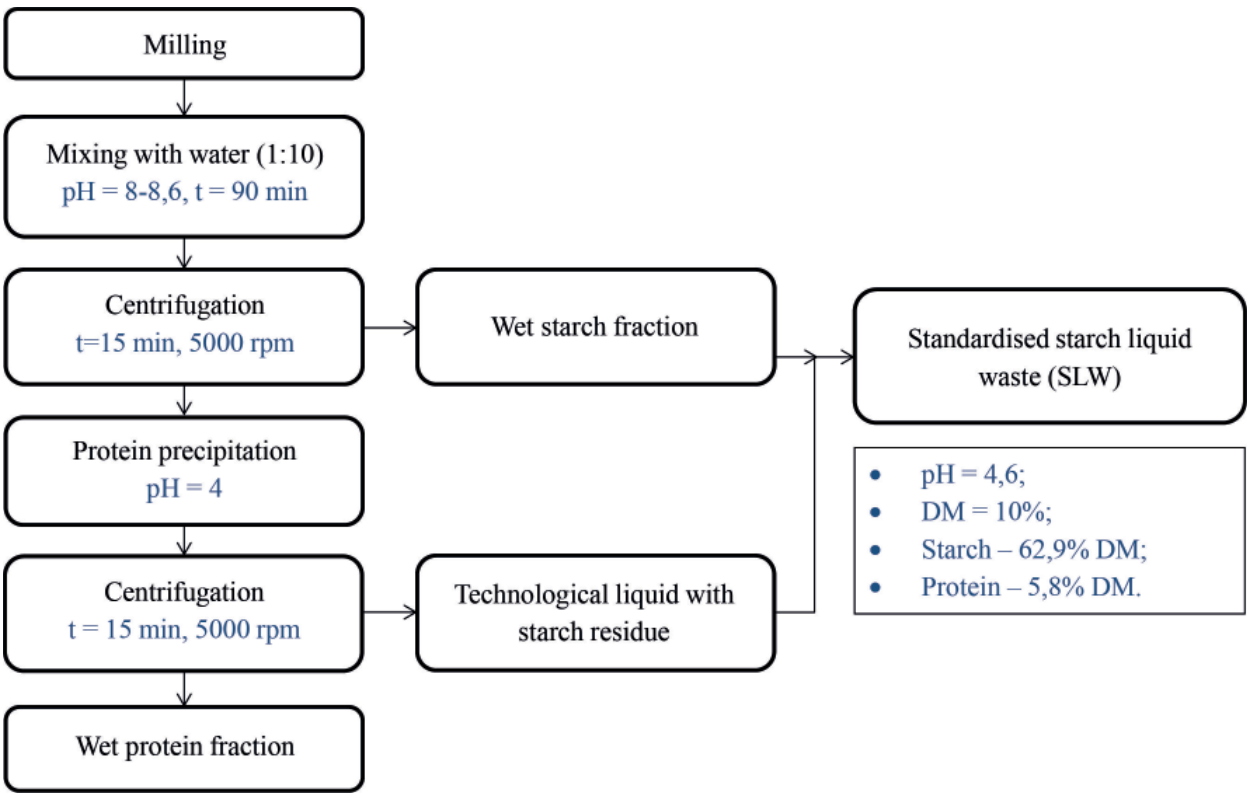

Fig. 1. **Scheme of obtaining standardised starch liquid waste after pea fractionation in laboratory conditions**

gelatinization of the starch in the medium and its hydrolysis were observed, and the medium was observed to be liquefied. The temperature was increased to 88–90 °C and maintained for 3 h under stirring. The saccharification process was monitored by reaction with iodine solution. The absence of a color change indicated the efficiency of the starch hydrolysis process catalyzed by α-amylase. After the hydroenzymatic treatment, the medium was cooled to 33±2 °C.

To 1.5 liters of cooled wort, 85% concentrated orthophosphoric acid (0.3 ml per sample), 25% urea solution (22.5 ml per sample), enzymes solutions (1:100) in the required amount, and 0.1% antiseptic solution (4.5 ml per sample) were added.

Process of fermentation of the medium with the production of bioethanol

Fermentation was carried out on a MicroFerm Laboratory Fermentor fermentation stand (USA). An industrial strain of dry yeast was introduced into the finished, cooled wort. A test sample of *Saccharomyces cerevisiae* Ethanol E yeast, manufactured by Angel (China), was provided by LLC Agrotechnology. The choice of culture was justified by its high resistance to temperature, pH, ethanol concentration in the medium, and other stress factors that arise during fermentation. Fermentation of each sample was carried out for 72 h, with mixing, cooling, and temperature stabilisation maintained throughout. Sampling for analysis was carried out every 24 h.

Research on the fermented medium

In the fermented samples, the main indicators were determined according to the methods of SOU 15.9-37-242:2005. The volumetric ethanol content was determined using a hydrometer at 20°C and an Anton Paar density meter DMA 4101.

Statistical analysis

After conducting experiments in triplicate, the arithmetic mean minus the standard deviation was determined and taken as the result. Statistical analysis of experimental data was performed using Microsoft Excel (Microsoft Corporation, USA) and Origin (OriginLab Corporation, USA). The reliability of the difference between indicators was assessed using a Student's t-test at the 5% significance level.

Results and Discussion

Since the preparation of a complex nutrient medium from starch-containing raw materials has certain features, for the implementation of the experimental part, we proposed the use of standardised SLW. For this, the wet starch residue, with the residual protein and other components of pea beans (after fractionation), was additionally added to the supernatant after separation of the protein fraction, resulting in a stable DM of 10%. Thus, we managed to obtain SLW, which can be easily incorporated into the existing scheme for the production of ethanol from dry grind, taking into account the necessary technological features. In fact, use SLW to replace process water for mixing, and regulate the amount of dry matter in the medium by adding additional corn milling. This approach may allow SLW to be considered a raw material for industry due to its ease of integration into existing production.

After analyzing the content of the main components of the standardised SLW (conditional starch content, protein content), it was found that their values are within approximate limits to the indicators of the starting raw material (corn). This, in turn, makes it possible to calculate the amounts of enzymes and excipients, as well as control the leading indicators of fermentation efficiency, in particular ethanol yield.

To confirm the feasibility of preparing a nutrient medium using standardised SLW and corn and further ethanol fermentation, a series of experiments was conducted with varying DM levels in the wort.

Fig. 2 shows the dynamics of ethanol accumulation in the medium during fermentation. Fermentation was stopped after the same test time for all samples — 72 hours after adding yeast to the wort at a temperature of 32± 0.1 °C. The choice of fermentation time is due to practical considerations: the volume of industrial fermenters for starch-containing raw materials in Ukraine is usually calculated for this time.

The results of fermentation tests showed that, for all samples of combined wort with a concentration of 25–29% w/v DM, the process can be practically completed within the specified time. This is also evidenced by the identical ethanol yield from conditioned starch in all three samples, 0.6±0.004 l/kg (see Table 1). The practical completion of the fermentation process for the wort with the highest DM content in 72 hours indicates the possibility of increasing DM in the wort and

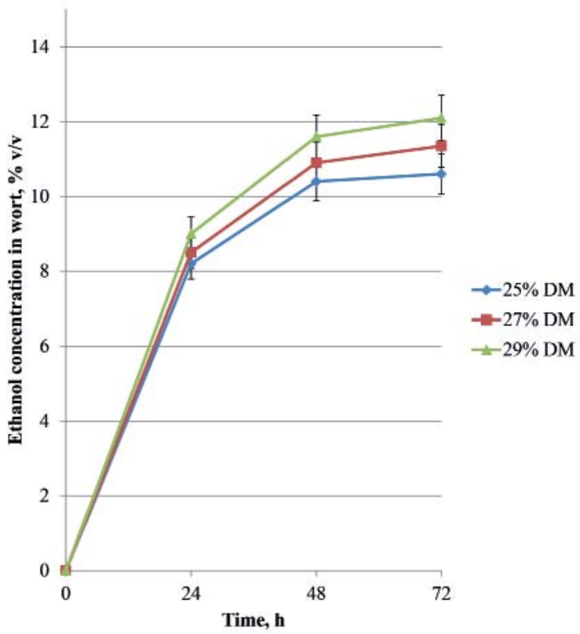

Fig. 2. **Dynamics of ethanol accumulation for samples using SLW and milled corn and different content of DM**
* — $P < 0.05$.

using VHG (Very High Gravity) technology to reduce the volume of liquid involved in the technological process. In industrial conditions, this enables reducing energy requirements by up to 44% steam consumption at the distillation/rectification and dehydration stages of bioethanol processing, and the processing of the distillation by-product [12].

Analysis of the obtained data, given in Table 1, showed that the final ethanol content in the fermented wort is determined only by the concentration of DM, regardless of the ratio of pea and corn starch, since the main technological parameters and the amount of additional components remained unchanged. In this case, the required amount of enzymes for each sample was added according to the manufacturer's recommendations, increasing it in proportion to the DM concentration. It should also be noted that the enzyme complex did not contain cellulase, which is used to further increase the yield of ethanol by hydrolysis of the cellulose and hemicellulose structures present in corn grain and pea. Thus, the sugars used by yeast to produce ethanol during fermentation were obtained only from the starch in the complex medium.

According to the literature, the average ethanol yield is 0.665 l/kg of corn starch [11]. Based on this, the possible ethanol content in the fermented medium only from corn starch contained in corn is 9.22% v/v., 8.38% v/v, 7.54% v/v for 29%, 27% and 25% w/v DM in the wort, respectively. The ethanol concentration in fermented wort in all tests was higher (Table 1). This means that the yeast also used the fermentable sugars of pea starch to produce ethanol.

The average ethanol yield from the conditional starch contained in the combined medium was 0.6±0.004 l/kg for all samples – less than the standard ethanol yield from corn starch. That is, not all the starch contained in the samples was sufficiently hydrolyzed. Since the laboratory tests used standart conditions for preparing wort from corn, this result may indicate that during mashing and liquefaction, starch in the SLW was insufficiently hydrolyzed.

Table 1. Results of test experiments with samples of combined wort

Indicators	Sample 1	Sample 2	Sample 3
DM content, % w/v	29±0.2	27±0.2	25±0.2
*Pea to corn starch ratio, g/g	62.9/138.6	62.9/126.0	62.9/113.4
*Corn grind (g)	220	200	180
*Amylex® 6T (1:100), ml	5	4.7	4.4
*Diazyme® TGA (1:100), ml	9.2	8.6	8
*Alphalase® AFP (1:100), ml	1.35	1.26	1.17
*Optimash® SA (1:100), ml	0.83	0.78	0.73
*Optimash® Ptytase (1:100), ml	1.35	1.26	1.17
Ethanol in fermented wort, % v/v**	12.1±0.05	11.35±0.05	10.6±0.05
Yield, l/kg starch	0.6±0.003	0.6±0.006	0.6±0.004

* Data for 1 liter of medium. ** — $P < 0.05$.

The ethanol yield in samples with different ratios of pea and corn starch was practically the same or within the limits of the determination error. This is probably explained by the fact that the ratio of pea to corn starch in all samples was close.

Thus, to increase the product yield, the specifics of pea starch should be taken into account, for which it is necessary to further optimize the conditions and combinations of thermostable enzymes for liquefaction processing of the nutrient medium. This is especially need for the use of SLW for the VHG fermentation technology with high DM content [13].

Conclusions

The results of laboratory tests confirm the possibility of using combined wort with starch liquid wastes after wet fractionation of pea and milled corn to produce bioethanol with appropriate selection of enzymes and technological modes of wort preparation. For samples of combined wort with a concentration of 25–29% w/v DM, the average ethanol yield from conditional starch is 0.6±0.004 l/kg, lower than from corn starch (0.665 l/kg). Further research will aim to develop technological solutions for VHG fermentation of combined wort, taking into account the characteristics and structure of pea starch.

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Author contributions

Vovk Ye.A. — conceptualization, study design, data collection, and drafting the manuscript.

Tsygankov S.P. — developed the concept and design of the study, conducted methodological validation

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ВИКОРИСТАННЯ ВІДХОДІВ ФРАКЦІОНУВАННЯ ГОРОХУ ПРИ ВИРОБНИЦТВІ БІОЕТАНОЛУ

Є.А. Вовк, С.П. Циганков

Державна установа «Інститут харчової біотехнології та геноміки Національної академії наук України», Київ

E-mail: vovkeu@gmail.com

Одержання протеїну та очищених протеїнових ізолятів із бобових культур за технологією технології вологого фракціонування призводить до утворення рідинних крохмалевмісних відходів, утилізація яких є значною проблемою. Такі відходи можна розглядати як сировину для біопалива, зокрема біоетанолу. Таким чином можна буде досягти подвійного ефекту — утилізації крохмалевмісних рідинних відходів (КРВ) та часткової заміни харчової сировини для енергетичних потреб.

Мета. Дослідити можливість приготування комплексного поживного середовища з використанням стандартизованого КРВ після фракціонування гороху з додаванням подрібненої кукурудзи для подальшої ферментації з метою отримання біоетанолу.

Методи. Стандартизований КРВ одержували методом вологого фракціонування за лабораторних умов, вміст крохмалю визначали за ГСТУ 46.045-2003, вміст проеїну за методом К'єльдаля відповідно до методики AOAC 979.09. Біоетанол одержували в лабораторному ферментері з використанням процесу одночасного оцукрювання та ферментаціїю. Основні показники у ферментованих зразках визначали за допомогою методів відповідно до СОУ 15.9-37-242:2005.

Результати. Для усіх зразків комбінованого сусла з концентрацією в діапазоні 25–29% сухих речовин (СР) процес практично може бути завершено за 72 години. Вміст етанолу у ферментованому суслі становив 10.6±0.05—12.1±0.05% об. для зразків сусла із зазначеною концентрацією СР.

Висновки. Під час бродіння комбінованого сусла гороховий крохмаль, який також присутній у суслі, збільшує концентрацію етанолу в ферментованому суслі. Кінцевий вихід етанолу з крохмалю об'єднаного сусла низький (0.6±0.004 л/кг), нижчий, ніж тільки з кукурудзяного крохмалю (0.665 л/кг). Для розробки технології бродіння дуже високої щільності необхідно підібрати відповідні типи ферментів і параметри гідроферментативного приготування комбінованого сусла.

Ключові слова: горох, рідкі відходи перероблення крохмалю, фракціонування, ферментація, затор, ферментація середовища надвисокої густини (VHG), біоетанол.

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