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The Influence of Pervaporation on Ferulic Acid and Maltol in Dealcoholised Beer

Mateusz Jackowski [1,*], Magdalena Lech [1], Mateusz Wnukowski [2] and Anna Trusek [1,*]

[1] Department of Micro, Nano and Bioprocess Engineering, Faculty of Chemistry, Wroclaw University of Science and Technology, Norwida 4/6, 50-373 Wroclaw, Poland; magdalena.lech@pwr.edu.pl
[2] Department of Mechanics, Machines, Devices and Energy Processes, Faculty of Mechanical and Power Engineering, Wroclaw University of Science and Technology, Wybrzeze Wyspianskiego 27, 50-373 Wroclaw, Poland; mateusz.wnukowski@pwr.edu.pl
* Correspondence: mateusz.jackowski@pwr.edu.pl (M.J.); anna.trusek@pwr.edu.pl (A.T.)

Abstract: Non-alcoholic beer is becoming more and more popular every year. Due to the high demand for such drinks, numerous breweries decided to produce non-alcoholic beer. There are various methods to create a beer with a reduced alcohol content. Among them are biological methods influencing the biochemistry of the brewing process and physical methods focused on removing ethanol from ready beer. Thus far, the most popular methods are vacuum rectification and reverse osmosis. This work evaluated another method called pervaporation for non-alcoholic beer production. During the study, low-alcohol beer (0.58 vol.%) was achieved from standard beer (3.62 vol.%) using pervaporation. The colour of the product remained unchanged at level 7 EBC. The concentration of ferulic acid decreased from 11.5 to 9.1 mg/dm^3, and maltol was concentrated, reaching a concentration of 38 mg/dm^3 in the final retentate during a 5 h process.

Keywords: beer; pervaporation; dealcoholisation; non-alcoholic beer; maltol; ferulic acid

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1. Introduction

Beer is a popular drink across the world. In the European Union in 2020, beer production reached 341,037 hL [1]. Most of that is standard alcoholic beer, but the market for non-alcoholic beers is growing [2,3]. There are various methods for non-alcoholic beer production. Generally, those methods can be classified into two groups. First are biological approaches. They aim to reduce the amount of alcohol produced during fermentation. Such methods are arrested fermentation, using special yeasts and changing the mashing regime. The second approach is focused on the physical removal of ethanol from standard beer. For that purpose, there are two types of methods: thermal and membrane. Thermal methods like distillation or rectification aim to evaporate ethanol from standard beer. Membrane methods such as dialysis or reverse osmosis aim to separate the ethanol from the rest of the mixture using membranes [4,5]. Moreover, there is one more process that combines thermal and membrane methods. That process is called pervaporation.

Pervaporation is a process in which the feed (in the case of non-alcoholic beer production, it is a standard alcoholic beer) flows along a non-porous membrane. Membranes for pervaporation are usually integrated membranes consisting of support and a skin (separating) layer and made very often from polydimethylsiloxanes. Volatile compounds selectively adsorb and permeate through the membrane and, after desorption, are condensed on the other side of the membrane (Figure 1). The driving force of pervaporation is the chemical potential gradient across the membrane [6,7]. The process may be improved by using a vacuum, increasing the temperature or purging with inert gas on the permeate side. The separation process can be intensified by heating up the feed. It leads to the increasing diffusion coefficient. Hence, it creates higher permeate flux.

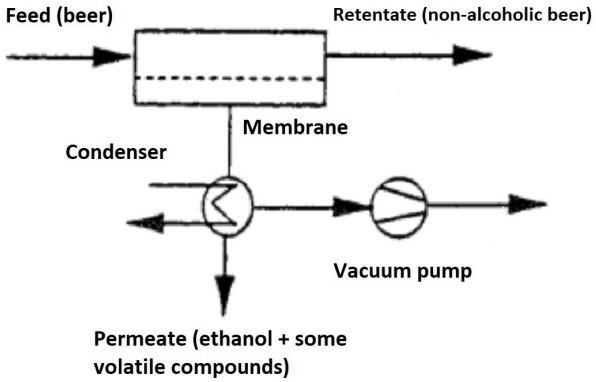

Figure 1. Scheme of pervaporation process.

The most challenging aspect of non-alcoholic beer production is to remove ethanol from the feed (alcoholic beer) without inflicting flavour compounds of dealcoholised beer. Although beer is a complex mixture of organic compounds, it is worth taking a closer look at two of them: ferulic acid and maltol.

Ferulic acid (Figure 2) is a compound delivered to beer from malt, especially when a special mashing regime is applied [8]. The abovementioned compound shows antioxidant properties [9,10] and inhibits growth of Listeria monocytogenes, which are the causative agent of listeriosis [11]. Moreover, ferulic acid may act as a neuroprotective agent, especially in the case of ischemic strokes [12].

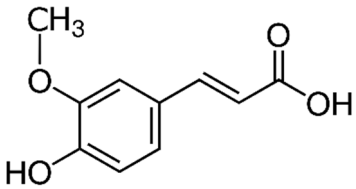

Figure 2. Structure of ferulic acid.

Maltol (Figure 3) is one of the health-promoting compounds in beer. It is derived from the malt and is responsible for the sweet and bread aromas [13–15]. This compound represents liver-protective [13] and neuroprotective properties [16]. Numerous studies show that maltol has anti-inflammatory properties [17,18].

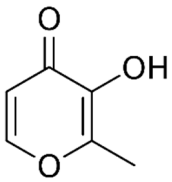

Figure 3. Structure of maltol.

Both listed compounds are good representatives of health-promoting agents of malt origin. Those compounds are partially responsible for beer's positive influence on human health. Moreover, after removing one of the toxins present in beer, which is ethanol, it is possible to create a health-beneficial drink [19].

In the literature, there are numerous publications describing the pervaporation of ethanol–water mixtures using various membranes. Chuntanalerg et al. [20] tested the performance of polybenzoxazine membranes and mixed matrix membranes for ethanol purification via pervaporation. The obtained separation factor of the pure PBZ membrane was more than 10,000 with the highest permeation flux of 1071 $gm^{-2}\,h^{-1}$ when using 25 wt% PBZ precursor with 15 wt% NaA zeolite loading. Claes et al. [21] examined PV as well. They used composite PTMSP-silica nanohybrid membranes for ethanol separation and received

ethanol–water separation factors up to 12 and fluxes up to 3.5 kg m^{-2} h^{-1}. Samanta and Ray [22] examined several ethanol-selective mixed matrix membranes prepared from the copolymer of butyl acrylate and styrene and an organophilic nano-size clay filler. The mixed matrix copolymer membrane containing 2% clay gave the best result, namely the permeate flux of 0.34 kg/m^2 h and an ethanol selectivity of 26.4 at 30 °C for 5 wt% ethanol in water.

However, there are few publications focusing on beer dealcoholisation using pervaporation. Although they are few in number, they deliver promising results. Di Matteo et al. [23] tested the pervaporation process supported by dialfiltration. They obtained low-alcohol beverages with 1.2 vol.% alcohol content. They proved also that this method has a negligible impact on the physical and chemical characteristics of beer before and after the treatment.

The pervaporation process is more popular in the food industry and can be applied in beer treatment successfully as well.

Thus far, the pervaporation process can be applied successfully in almost every type of food industry to recover naturally occurring ingredients responsible for taste, smell and nourishing properties. Additionally, they can be concentrated without the necessity of heating. The literature reports show that pervaporation can be an ideal process in which, after removing the alcohol, the product still retains the components naturally present in it. Dawiec-Liśniewska et al. performed pervaporation of fruit juice hydrolates. The study showed that most of the 38 analysed compounds were concentrated in the permeate [24]. Del Olmo et al. used pervaporation for the concentration of beer aroma. The experiment showed that some compounds like isoamyl acetate, isobutyl acetate and ethyl acetate were concentrated in the permeate [25]. Takacs et al. reported that Tokaji wine dealcoholised using pervaporation resulted in features close to the original character of alcoholic wine [26]. Moreover, Weschenfelder and his group presented that pervaporation is a promising alternative to concentrate aroma compounds from soluble coffee. They showed that almost all typical and valuable aromas from natural coffee were in the permeate in higher concentration than in the feed, especially 2,3-butanedione, which is responsible for the sweet, buttery, creamy and milky taste of coffee [27].

This work is focused on beer dealcoholisation using pervaporation. Although various methods of dealcoholisation were described so far, only a few are focused on the influence of the dealcoholisation procedure on health-promoting compounds present in beer. In this work, the pervaporation process was evaluated in order to assess its influence on the preservation of maltol and ferulic acid in dealcoholised beer.

2. Materials and Methods

2.1. Beer Production

The beer used for the experiment was prepared using the University Brewery. Samples for the tests were taken from a 50 L batch of lager beer. The beer production protocol was as follows. A total of 8 kg of pilsner malt (Viking malt, Strzegom, Poland) and 2 kg of unmalted barley were ground using a two-roller grinder (Roppi, Hungary). Then, starch materials were mixed with 40 L of tap water (MPWiK, Wrocław, Poland) and mashed. The mashing regime included temperature breaks at 60 °C for 30 min, then 70 °C for 20 min and finally 78 °C for 5 min in order to stop the enzyme activity. In the next step, the mash was filtered and sparged using 20 L of water at 80 °C. The obtained wort was boiled for 1 h. During that time, hops were added in two parts. The first 30 g of Hallertau hop (Browamator, Strzyżów, Poland) and 30 g of Oktawia hop (Browamator, Strzyżów, Poland) were added 1 h before the end of boiling. The second dose of 30 g of Hallertau hop and 30 g of Oktawia hop was added 15 min before the end of boiling. Before transferring the wort into the fermenter, it was centrifugated in order to remove hot trubs and cooled down to 12 °C. The wort extract was 10.5 Brix, and it was pitched with 25 g of bottom-fermenting yeasts (Mauribrew lager 497 Browamator, Strzyżów, Poland). Fermentation took 2 weeks

in 12 °C, and then the beer was maturated for 3 weeks in 5 °C. The final product had an ethanol content of 3.62 vol.%, an apparent extract of 2.7 Brix and a colour of 7 EBC.

2.2. Microfiltration

In order to remove turbidity, the beer was microfiltered using a polyethersulfone membrane (PolyMemTech, Warsaw, Poland, nominal pore size 0.14 µm, TMP = 2.2 bar). After microfiltration, beer parameters such as ethanol content, apparent extract and colour remained unchanged.

2.3. Pervaporation

Pervaporation was conducted at a temperature of 50 °C using a polydimethylosiloxane, flat, circular membrane (A = 177 cm^2, Sulzer PERVAP 4510, Sulzer, Winterthur, Switzerland). The membrane was selected due to its good performance in other applications [28]. The process was conducted for 5 h and under a pressure of 40 mbar on the permeate side. The retentate was circulated in a closed loop by a gear pump. The retentate flow was 30 L/h. The permeate flux is shown in the (Figure 4). During the experiment, samples of the permeate and the retentate were collected every hour in order to measure the ethanol level, colour, ferulic acid and maltol.

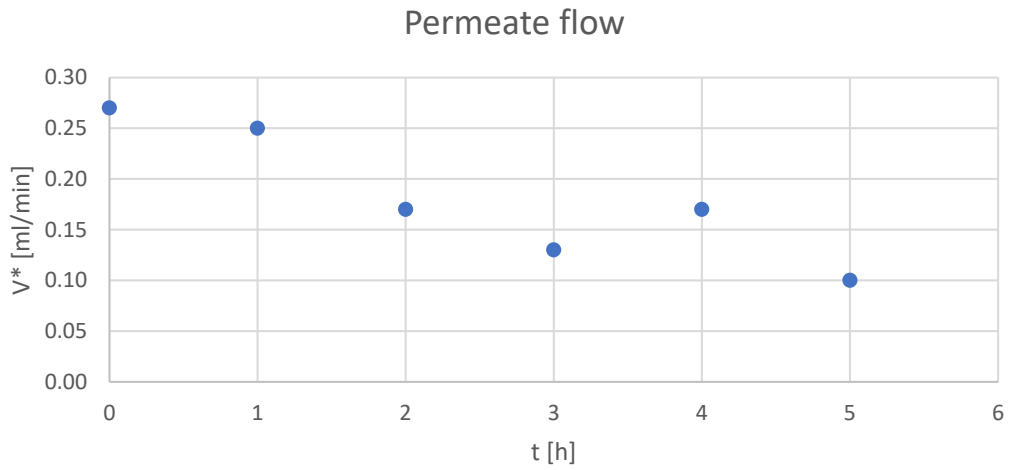

Figure 4. Permeate flow during pervaporation process.

2.4. Calculation of Separation Factor

The separation factor for ethanol in the pervaporation process was calculated using the formula below [29].

$$\beta = \frac{W_p / E_p}{W_f / E_f}$$

where

W_p—weight factor of water in permeate;
E_p—weight factor of ethanol in permeate;
W_f—weight factor of water in feed solution;
E_f—weight factor of ethanol in feed solution.

2.5. Calculation of Retention Factor

The retention factor for maltol and ferulic acid in the pervaporation process was calculated using the formula below [29].

$$R = 1 - \frac{C_p}{C_r}$$

where

C_p—concentration of maltol/ferulic acid in permeate;
C_r—concentration of maltol/ferulic acid in retentate.

2.6. Determination of Ethanol

The alcohol content was measured using a Shimadzu 2010 (Kyoto, Japan) gas chromatograph equipped with an FID detector. Samples were filtrated using 0.45 µm RC syringe filters in order to remove solid particles that may cause problems with the injecting port. Next, 0.5 µL of samples were injected into the injector using an AOC-20i autosampler (Kyoto, Japan). The injector temperature was set to 140 °C. The detector temperature was 200 °C. The split ratio was set at 30:1. Analysis was performed using a ZB-WAXplus column (L = 30 m, I.D. = 0.25 mm, df = 0.25 µm) with helium as a carrier gas. Flow through the column was set to 0.98 mL/min. The temperature program was set at 35 °C for 5 min. Then, it was raised to 85 °C (at 10 OC/min) and in the next step raised up to 200 °C (at 25 OC/min). The procedure ended with a hold at 200 °C for 1 min. The retention time of ethanol (Pol-Aura CAS: 64-17-5) was 2.78 s. Results were quantified using a calibration curve obtained from ethanol solutions in known concentrations.

2.7. Determination of Ferulic Acid

The ferulic acid was measured using a Waters LC Module I plus HPLC equipped with an Xterra RP18 (3 mm × 150 mm) column (Waters TM, Etten-Leur, The Netherlands). For the analysis, a mobile phase consisting of $H_2O/CH_3OH/H_3PO_4$ in proportions 540:450:10 with isocratic elution was used. The flow rate was set at 0.5 mL/min, and detection at 280 nm and 25 °C. The analysis was performed at ambient temperature. The samples were filtered through the 0.45 µm syringe filter (PES, Merck Milipore Burlington, MA, United States) before an injection. The volume of the injection was 10 µL. Under these conditions, the retention time of the ferulic acid (Sigma Aldrich, St. Louis, MI, USA, CAS: 537-98-4) was 2.33 min. The prior analysis calibration curve for ferulic acid (Sigma Aldrich, CAS: 537-98-4) was prepared in a range of concentrations from 0 to 25 mg/dm^3.

2.8. Determination of Maltol

The maltol content was analysed using an Agilent 7820 (Agilent, Santa Clara, CA, USA) gas chromatograph coupled with an Agilent 5977B MSD Electron ionisation mass spectrometer (Agilent, Santa Clara, CA, USA). Samples of 0.5 µL were introduced into the GC injector (200 °C; split = 2); helium was used as a carrier gas. The GC was equipped with a Stabilwax-DA column (30 m × 0.32 mm × 0.25 µm; Restek, Centre County, PA, USA), and the temperature profile was as follows: holding at 50 °C for 5 min, then increasing 10 °C/min to 200 °C and holding for 5 min. The retention time of the maltol was 17.29 min. Compound identification was done automatically by comparing mass spectra with the NIST-14 MS library (minimum match factor comparing to NIST library = 90%). The MS scanning range was m/z 10–450 with a frequency of 1.7 scans/s. The gain factor and EM Volts were 3.0 and 1708, respectively. The MS source and quadrupole temperatures were 230 °C and 150 °C, respectively. The presence of maltol was additionally confirmed by applying the maltol standard that was also used for its quantitative analysis.

2.9. Colour Measurement

The beer colour was measured using a Hitachi U-1900 spectrophotometer (Tokyo, Japan) at a wavelength of 430 nm. Measurements were performed using a 1 cm quartz cuvette. Colour in EBC units was calculated using the formula below [30].

$$\boldsymbol{EBC} = \boldsymbol{A}_{430} * \boldsymbol{f} * 25$$

where
A_{430}—absorbance using wavelength 430 nm;
f—dilution factor.

2.10. Organoleptic Tests

Organoleptic tests were performed with five respondents. Each responder assessed two 10 mL samples of degassed beer, one raw beer and one dealcoholised beer. In the questionnaire, respondents gave notes from 1 to 5 to the beer parameters: bitterness, sweetness, sourness, herbal aroma, sweet aroma, alcoholic aroma, yeast aroma and refreshes. Note 1 means that a given parameter is not perceived, and 5 means that the parameter is strongly perceived.

3. Results

3.1. Concentrations of Alcohol, Maltol and Ferulic Acid

The profiles of the measured ingredients are presented in Table 1. As shown, the ferulic acid is retained by the membrane totally. The situation looks quite different in the case of maltol, which is presented in the permeate however in a much lower concentration than in the retentate, so it acts like a typical pressure-driven membrane technic where the membrane is a transport barrier. Small change in the maltol concentration in the permeate was observed in the fourth hour. This may be linked with a change in the permeate flow through the membrane in the fourth hour.

Table 1. The concentrations of beer compounds in the retentate and permeate during the separation process.

	Time of the Process [h]	Ferulic Acid [mg/dm^3]	Ethanol [vol.%]	Maltol [mg/dm^3]
Permeate	1	0.0	5.79 ± 0.06	3.5 ± 0.2
	2	0.0	5.54 ± 0.06	3.9 ± 0.2
	3	0.0	4.54 ± 0.06	5.8 ± 0.3
	4	0.0	3.12 ± 0.06	8.8 ± 0.5
	5	0.0	2.40 ± 0.06	7.2 ± 0.4
Retentate	1	7.7 ± 0.2	3.46 ± 0.06	21.0 ± 0.3
	2	7.5 ± 0.3	2.27 ± 0.06	23.2 ± 0.3
	3	8.0 ± 0.2	2.04 ± 0.06	25.6 ± 0.2
	4	8.5 ± 0.3	0.58 ± 0.06	35.4 ± 0.4
	5	9.1 ± 0.3	0.57 ± 0.06	38.0 ± 0.4
Raw beer		11.5 ± 0.4	3.62 ± 0.06	22.0 ± 0.2

The dealcoholisation process resulted in a reduction in beer ethanol content from 3.62 vol.% to 0.58 vol.% after 4 h of the process. During the next hour, the ethanol was at the same level. The alcohol content is slightly too high for the standard of non-alcoholic beers in most European countries, but in some of them, like France or Spain, this level is acceptable for the abovementioned product [31].

3.2. Separation Process Parameters

The permeate flow changed throughout the process; for the first 1 h, the permeate flow was 0.25 mL/min. Between the first and second hour of the process, the flow decreased to 0.17 mL/min. In the next few hours, the decrease rate was slower, and the permeate flow reached 0.10 mL/min in the fifth hour. Nevertheless, a correlation between the permeate flow and the concentration of ethanol in the retentate is visible (Figure 4, Table 1). Lowering the concentration of ethanol lowers the flow rate of the permeate, which may be caused by the change in vapour pressure on the permeate side of the membrane.

This work aims to separate as much alcohol as possible from beer while retaining maltol and ferulic acid. Table 2 presents the calculated parameters.

Table 2. Parameters of separation process.

Time of the Process [h]	β_{Et} [-]	R_M [%]	R_{FA} [%]
1	0.59	83.3	100
2	0.62	83.2	100
3	0.76	77.3	100
4	1.13	75.1	100
5	1.47	81.1	100

The values of these parameters show that the process can be considered effective. The separation factor is, at this level, crossing the value of one after 40 min of the process (after removing water from the membrane). This indicates that the process should be carried out for much longer than an hour, which is rather common for a pervaporation system. Ferulic acid retention is 100%, which is the most desirable effect. The results obtained for maltol are slightly less satisfactory, but its retention remains at a high level. The average result for the entire process is 80%. Such difference may be caused by molecular difference between maltol and ferulic acid, where ferulic acid is a bigger compound in comparison with maltol (Figures 2 and 3). Moreover, there is a difference in hydrophobicity between ferulic acid and maltol where log P for ferulic acid is 1.51 and 0.09 for maltol [32,33].

3.3. Consumer Parameters

During the process, the colour of the dealcoholised beer remained unchanged and oscillated about 6–7 EBC, and the permeate was colourless (Table 3). The ferulic acid was concentrated in the retentate and was not observed in the permeate, but its level decreased from 11.5 mg/dm^3 to 9.1 mg/dm^3 in comparison to the raw beer. Such an observation may be caused by the thermal decarboxylation of ferulic acid into 4-vinyl guaiacol [8]. Maltol was concentrated in the retentate, reaching the level of 38 mg/dm^3. On the other hand, it was also identified in the permeate, reaching a maximal concentration of 8.8 mg/dm^3. Nevertheless, the concentration of maltol in the dealcoholised beer could lead to improvement in the health-promoting value of non-alcoholic beer. This process was carried out as a batch process. Hence, the separation coefficient according to the ethanol was the highest at the beginning of the separation due to the highest driving force. The results from Table 1 show that the process was efficient in the first 4 h.

Table 3. Colour of samples during pervaporation process.

	Time of the Process [h]	Colour [EBC]
Permeate	1	0
	2	0
	3	0
	4	0
	5	0
Retentate	1	7
	2	6
	3	6
	4	7
	5	6
Raw beer		7

Organoleptic tests indicate that dealcoholised beer has similar properties to standard beer in terms of bitterness, sourness, refreshens and herbal aroma. Yeast aroma and alcoholic aroma decreased during the process, whereas sweet aroma increased in dealcoholised beer (Figure 5).

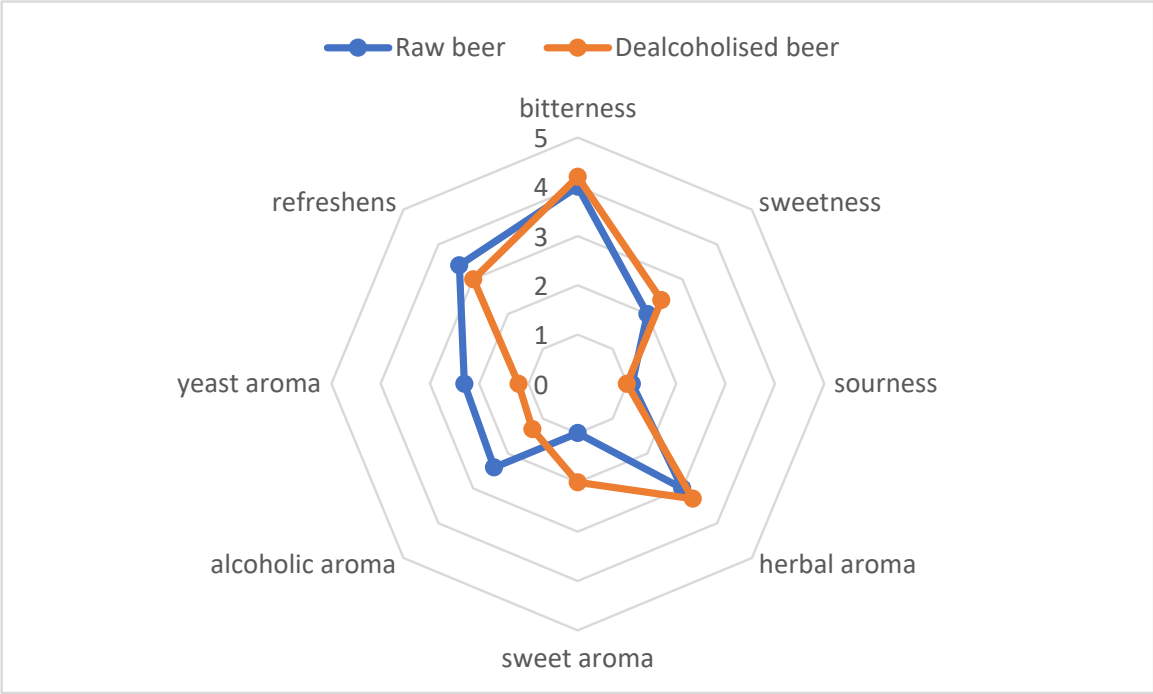

Figure 5. Comparison of organoleptic test results for raw beer and beer retentate after pervaporation process (dealcoholised beer).

4. Conclusions

Due to the scarce results in the literature, it was hard to compare all of the achieved results with previous works. Di Matteo et al. performed pervaporation for 7 h at 35 °C and a pressure of 25 mbar on the permeate side. The utilised membrane was Pervap 4060. During the experiment, the ethanol content was lowered from 4.71 vol.% to 1.11 vol.% Colour was nearly the same in the beer before and after the process. The ferulic acid decreased from 2.12 mg/dm^3 to 1.77 mg/dm^3 (Table 4) [23]. Although most of the ethanol was removed, such parameters are not sufficient to call this beer non-alcoholic. In both tests, reduction of the ferulic acid level was observed. Differences may occur due to the various parameter conditions, different feed and different membranes used in experiments. Unfortunately, the authors did not find any evidence of maltol concentration during the pervaporation.

Table 4. Comparison of pervaporation and other processes of ethanol removal.

	Parameter		Ethanol [vol.%]	Colour [EBC]	Ferulic Acid [mg/dm^3]	Maltol [mg/dm^3]	Reference
Dealcoholisation method	Pervaporation (presented work)	Raw beer	3.62	7	11.5	22.0	
		Dealcoholised beer	0.57	6	9.1	38.0	
	Osmotic distillation	Raw beer	4.8	7.6	n/d	n/d	[34]
		Dealcoholised beer	0.6	7.9	n/d	n/d	
	Membrane contactor	Raw beer	5.7	9.8	n/d	n/d	[35]
		Dealcoholised beer	1.0	10.7	n/d	n/d	
	Pervaporation	Raw beer	4.71	4.3	2.12	n/d	[23]
		Dealcoholised beer	1.11	3.9	1.77	n/d	
	Reverse osmosis	Raw beer	5.09	n/d	n/d	n/d	[36]
		Dealcoholised beer	0.40	n/d	n/d	n/d	
	Dialysis	Raw beer	3.8	n/d	n/d	n/d	[37]
		Dealcoholised beer	From 0.1 to 0.83	n/d	n/d	n/d	
	Vacuum distillation	Raw beer	4.11	10.1	n/d	n/d	[38]
		Dealcoholised beer	0.0004	10.1	n/d	n/d	

Note: n/d—no data.

There are numerous methods for ethanol removal from beer. Pervaporation was comparable in terms of ethanol removal to osmotic distillation. Techniques such as reverse osmosis, dialysis and vacuum distillation allowed for producing beer with an ethanol

concentration below 0.5 vol.% (Table 4), which is an acceptable ethanol concentration in non-alcoholic beers. Unfortunately, there is no data available on how the abovementioned methods influence maltol and ferulic acid concentrations in the finished product.

This experiment showed that pervaporation is a successful method for removing ethanol from beer and that the membrane Sulzer PERVAP 4510 seems to be feasible for further studies on beer dealcoholisation. This process does not affect beer colour but positively affects health-promoting-substance concentration in dealcoholised beer. Organoleptic tests showed that the final taste of the beer is similar to the raw materials used for pervaporation; the biggest decrease was observed in yeasty and alcoholic aromas. Nevertheless, due to the small sample size and small number of panellists, those results are not as reliable as those of full-scale consumer tests. The ferulic acid was partially removed from the dealcoholised beer, whereas the maltol was concentrated during the performed process. However, in comparison to the other dealcoholisation methods, e.g., rectification, the losses of the valuable ingredients are negligible. Pervaporation could be more appropriate due to the high selectivity of the ethanol than other volatile compounds, e.g., ferulic acid or maltol. As a result, much higher concentrations of these compounds were achieved in the obtained product, thus proving the coefficient factor estimated in this work. Nevertheless, pervaporation has its limitations, like the length of the time of the process and the low permeate flow. Such disadvantages may be overcome by using bigger pervaporation modules or multiple units working together. Another limitation of the abovementioned process is the relatively high price of pervaporation units, especially for those working on the scale required for industrial use in beermaking. Moreover, studies showed that pervaporation consumes more energy than other membrane processes like reverse osmosis [28]. On the other hand, pervaporation is a developing technique, and the appearance of new membranes in the future could make this process more feasible. Especially taking into account the concentration of health-promoting substances in dealcoholised beer, such a product could be considered a functional drink.

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