

Valorization of Oilseed Cake through the Isolation of Cellulose by Autohydrolysis

Matteo Cipriani, Laura Principato, Giorgia Spigno, Andrea Bassani*

Università Cattolica del Sacro Cuore, Department for Sustainable Food Process (DiSTAS), Via Emilia Parmense 84, 29122 Piacenza, Italy.

andrea.bassani@unicatt.it

Oilseed cakes are by-products of the oil extraction process that are commonly used as animal feed due to their high protein content. However, they can be valorized also in other ways producing for instance protein isolates or bioenergy. In this work, the application of a lignocellulosic fractionation process on an oilseed cake to recover cellulose is tested. Indeed, oilseed cake can have a relevant content of cellulose that can be isolated. It is important to point out that the first hydrolysis step is carried out as an auto-hydrolysis novel process instead of a conventional mild acid hydrolysis to reduce the environmental impact of the process itself. The first aim of this work is to investigate, discuss and verify the applicability of such a fractionation process on oilseed cake. In addition, to further reduce the potential environmental impact of the process, the effects of variation of both NaOH concentration and temperature in the alkaline hydrolysis are investigated and discussed as the second aim. Therefore, experimental tests are carried out at three different NaOH concentrations (i.e., 1.5, 4.0, 16.0% v/v) and two different temperatures (i.e., 25 and 60°C) to make an initial assessment of the optimal conditions for cellulose recovery and reduced environmental impact.

1. Introduction

Europe is a significant producer of various oilseed crops, such as rapeseed, sunflower, soybeans, and more. These oilseeds are processed to extract oil, and the by-product remaining after oil extraction is known as oilseed cake or meal. Oilseed cakes are valuable sources of protein and are commonly used as animal feed due to their nutritional content. However, they can be valorized also in different ways because are rich in non-starch polysaccharides, antioxidants, fibers, vitamins and minerals. Indeed, applying the suitable technology, oilseed cake can be used for the production of protein isolate, bioenergy, biopolymer, pesticides, or bioelectricity (Sarkar et al., 2021). Another potential way to valorize this residue could be the recovery of the cellulose fraction due to its quite high content in oilseed cake (Fernández-Cegri et al., 2013). Cellulose recovered has very good properties such as renewability, biodegradability, lightweight and good mechanical characteristics (Kassab et al., 2019), and can be used as packaging filler or for paper production. Different processes are available in the literature for the isolation of cellulose from by-products and most of these involve acid and/or alkaline treatments. For instance, Sumesh et al. (2020) obtained fibers with a cellulose content of around 82.3% w/w from peanut oil cake by performing an alkaline treatment with 4% w/v of NaOH at 80°C for 1.5 hours followed by a bleaching step using 0.7% v/v NaClO₂ at 100°C and further treatment with 4% w/v NaOH at 20°C. Also Hu et al. (2017) proposed a mild treatment for cellulose recovery from argan press cake. Such treatment was done at 80°C with an aqueous solution of 12% NaOH and 8% sodium sulfate, followed by bleaching in different stages with various solutions containing, among others, NaOCl, H₂O₂ and NaOH. Specifically regarding the isolation of cellulose from oilseed cakes, Kassab et al. (2019) obtained microfibrils of cellulose by treating sunflower cake three times with NaOH 4% w/v at 80°C for 2 hours followed by a bleaching step with acid hydrolysis using sulfuric acid. All these treatments are effective in isolating cellulose but have a significant impact on the environment as they require several treatments with NaOH and, in some cases, even acid hydrolysis. For this reason, the reduction or removal of the use of alkaline or acid solvents is crucial for the environmental sustainability of the process. In this framework, autohydrolysis, which is a high temperature (160-200°C) and mid-pressure (12-14 bars) treatment, can be a potential solution. Indeed autohydrolysis is attractive because doesn't need any acid

or alkaline chemicals but just water (Carvalho et al., 2016). At optimal conditions, autohydrolysis can remove almost all of the hemicellulose from the by-product treated and partially degrade the lignin, while retaining most of the cellulose. For this reason, it is often necessary to combine this treatment with others (e.g., alkali treatment) to isolate the cellulose while removing the lignin. For example, Fiorentini et al. (2022) recovered cellulose from wheat straw through three consecutive treatments: autohydrolysis, alkaline hydrolysis with NaOH and bleaching. The aim of this work was to investigate the potential isolation of cellulose from oilseed cakes by exploiting the process of autohydrolysis. For this purpose, it was decided to follow the process proposed by Fiorentini et al. (2022). In addition, while the latter focused on optimizing the operating conditions of the autohydrolysis step, in this work it was decided to investigate in-depth the alkaline hydrolysis step by evaluating its effectiveness as a function of different NaOH concentrations and treatment temperatures to evaluate a potential reduction in NaOH use, thus improving the environmental impact of the process itself.

2. Material and Methods

The samples of oilseed cake were subjected to a lignocellulosic fractionation process reported in Figure 1 to isolate cellulose. The process is similar to that used for the extraction of cellulose from wheat straw (Fiorentini et al., 2022). The tests were carried out starting from samples of oilseed cake, supplied by the Cereal Docks company, grounded to a size of 2 mm with a laboratory hammer mill. Briefly, the autohydrolysis was carried out in a high-pressure laboratory reactor (Highpreactor BR-1000, Berghof, Germany) into which the grounded samples and water were loaded with a ratio of 1:20 w/v. Autohydrolysis was carried out at 195°C for 15 minutes, selecting the optimal condition for cellulose recovery highlighted by Fiorentini et al. (2022).

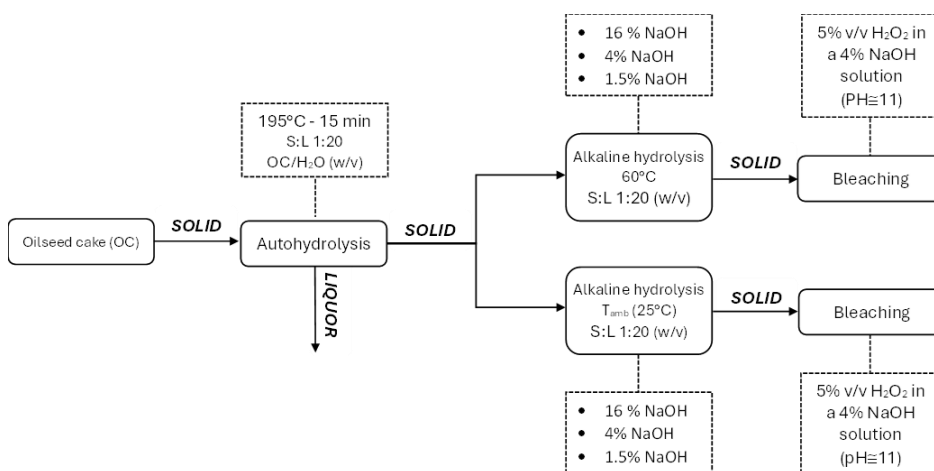


Figure 1 – Fractionation process for cellulose isolation from oilseed cake - Experimental plan

The solid resulting from auto-hydrolysis was filtrated using Whatman paper filters (N°589/2) and the solid residue was then dried at 60°C for 24 hours. To quantify lignin, cellulose, and hemicellulose content in the solids obtained from the auto-hydrolysis the analysis of structural carbohydrates was carried out. Then, the recovered solid was treated with different NaOH solutions with a solid-to-liquid (S/L) ratio of 1:20 w/v, to investigate the effect on cellulose isolation as a function of the different NaOH concentrations used. Bassani et al. (2020) tested a sodium hydroxide concentration of 16% w/v at ambient temperature (about 25°C), while Camarero et al. (2004) carried out an alkaline hydrolysis treatment at 60°C using a NaOH concentration of 1.5%. Therefore, it was decided to compare a hydrolysis treatment at 25°C and 60°C for 24 hours at three different concentrations of NaOH (i.e. 1.5%, 4%, 16% w/v). The alkaline hydrolysis treatments were conducted in an orbital shaker. All the solids obtained were then recovered and washed with distilled water until neutral pH was reached and finally dried at 60°C for 24 hours. Then the solids recovered were again characterized in terms of cellulose, hemicellulose and lignin. Finally, a bleaching phase was carried out with a 5% v/v H₂O₂ solution in a 4% NaOH solution (pH approximately equal to 11). The solution was added to the solid residue in a 1:20 w/v ratio and the mixture was placed in the orbital shaker at 45°C, 120 rpm, for 8 hours. The solid was recovered through a filtration phase, on Whatman paper filters (N°589/2), washed with 50% acetic acid (v/v) in a ratio of 1:20 w/v, and finally brought to neutral pH by washing with distilled water. The solid was then dried at 60°C for 24 hours and stored for subsequent fiber composition analysis.

2.1 Fat and Protein Content

The fat and protein contents were determined following the procedure provided by Švarc-Gajić J. et. al. (2020). Briefly, the fat content was evaluated via the Soxhlet method, using petroleum ether as solvent, while the protein content was estimated using the Kjeldahl method.

2.2 Water and ethanol extractives

The determination of extractives in the by-product was performed by applying the method also used by Miliotti et. al. (2019). Briefly, it consists of a two-step extraction using hot water for the first (100°C) and ethanol (95% v/v) for the second, performed both in a Soxhlet extractor.

2.3 Structural Carbohydrates

The analysis was performed by applying the method reported by Bassani et. al., 2020, starting from the extractives-free sample (EFS). Briefly, EFS were subject to two acid hydrolysis (72% H₂SO₄ at 30°C for 1 hour and 4% (v/v) H₂SO₄ at 121°C for 1 hour). Then the lignin content was calculated by weight difference with the initial sample while hemicellulose and cellulose were estimated from glucose and xylose content in the liquor obtained by applying a factor of 0.88 and 0.9 to xylose and glucose content respectively.

3. Results and Discussion

3.1 Raw material characterization

The characterization of oilseed cake is provided in Table 1. It can be observed that moisture, protein and ash contents are quite similar to the values reported by Fernández-Cegrí et al. (2013) which are about 7.0%, 25.3% and 6.8% respectively. On the other hand, regarding the fiber composition, only cellulose seems to have a significantly different value (about 25.3% in the work of Fernández-Cegrí et al. (2013)). This seems to be related to the analytical technique (i.e. Van Soest NDF fiber analysis) to quantify the fiber amount instead of using structural carbohydrates like in this study. It is interesting to point out that if the cellulose content presented by Fernández-Cegrí et al. (2013) was considered, the total composition of the oilseed cake would be about 100%. However, it was decided for consistency and better comparison with subsequent analyses to retain the result obtained through structural carbohydrate analysis.

Table 1: Oilseed cake characterization

	Composition		Composition
Moisture content (%)	7.34 ± 0.08	Ashes (% on dw)	5.79 ± 0.24
Fat (% on dw)	0.20 ± 0.07	Acid insoluble lignin (% on dw)	13.05 ± 0.70
Water Extractives (% on dw)	16.40 ± 1.23	Acid soluble lignin (% on dw)	1.45 ± 0.01
Ethanol Extractives (% on dw)	3.33 ± 0.20	Hemicellulose (% on dw)	9.05 ± 0.30
Proteins (% on dw)	25.27 ± 0.72	Cellulose (% on dw)	13.68 ± 0.27

3.2 Fractionation Process

The fractionation process reported in Figure 1 was analyzed starting from the autohydrolysis step. As mentioned in “Material and Methods” section, regarding the autohydrolysis treatment, the optimal operating conditions derived from Fiorentini et. al. (2022), were selected. As can be seen from Table 2, the main treatment objectives, which are hemicellulose removal and high cellulose recovery yield, were achieved, reflecting what was reported by Fiorentini et. al. (2022). It is important to point out that a cellulose recovery slightly higher than 100% is due to the high variability of the analysis. Therefore, it can be reasonably assumed that cellulose recovery is around 100%. On the other hand, the much higher recovery than 100% of lignin is due to the formation of lignin-like structures. These can be generated during acid and thermal pretreatments, such as autohydrolysis, due to polymerization or condensation reactions of compounds derived from furfural and hydroxymethylfurfural. The latter are products of degradation of xylose, glucose and sugary compounds already present in the extractives (Shinde et al., 2018). For this reason, it is possible to estimate that the actual recovery of lignin is equal to or slightly lower than 100%. This consideration is also supported by subsequent analysis following the alkaline hydrolysis and bleaching steps where a reduction in lignin recovery below 100% has been observed as the effect of the formation of lignin-like structures is diminished. The solid from the autohydrolysis pretreatment, suitably recovered, filtered and dried, was then subjected to the subsequent alkaline hydrolysis and bleaching treatments to go on to isolate the cellulose, consequently reducing the lignin content and eliminating the hemicellulose fraction. Table 3, Table 4, and Table 5 show the results for the alkaline hydrolysis treatments at different concentrations of NaOH, room temperature (i.e. about 25 °C) and the subsequent bleaching step.

Hemicellulose underwent further degradation is reduced as NaOH content increases and is almost eliminated in the final bleaching step. Regarding the lignin content, this is effectively reduced to a maximum of about 21% w/w after the alkali step with 1.5% w/v of NaOH.

Table 2: Composition of the solid residue and recovery percentage after autohydrolysis

	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	34.37 ± 0.56	123.26 ± 2.01
Acid soluble lignin (% on dw)	1.37 ± 0.31	43.97 ± 9.98
Cellulose (% on dw)	33.27 ± 3.32	113.74 ± 11.37
Hemicellulose (% on dw)	2.50 ± 0.32	12.94 ± 1.68

The lignin was then further degraded during bleaching treatment to a maximum of about 72%, resulting in a minimum lignin content in the final solid of about 14% w/w. For alkaline hydrolysis with 4% and 16% w/v of NaOH, no substantial differences related to lignin reduction are shown.

Table 3: Composition of the solid residues and recovery percentage after alkaline hydrolysis (16% w/v NaOH, ambient temperature) and bleaching.

	Alkaline Hydrolysis (16% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	28.13 ± 1.75	72.53 ± 4.51	15.10 ± 1.17	28.34 ± 2.20
Acid soluble lignin (% on dw)	0.49 ± 0.01	11.45 ± 0.25	0.37 ± 0.04	6.19 ± 0.64
Cellulose (% on dw)	40.89 ± 1.93	100.49 ± 4.73	55.23 ± 0.30	98.82 ± 0.54
Hemicellulose (% on dw)	0.39 ± 0.08	1.45 ± 0.29	0.43 ± 0.04	1.17 ± 0.11

Table 4: Composition of the solid residues and recovery percentage after alkaline hydrolysis (4% w/v NaOH, ambient temperature) and bleaching.

	Alkaline Hydrolysis (4% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	29.50 ± 2.46	81.85 ± 6.83	19.23 ± 1.71	41.33 ± 3.68
Acid soluble lignin (% on dw)	0.61 ± 0.06	15.12 ± 1.38	0.77 ± 0.16	14.89 ± 3.02
Cellulose (% on dw)	43.64 ± 2.99	115.42 ± 7.91	53.25 ± 0.80	109.08 ± 1.64
Hemicellulose (% on dw)	1.21 ± 0.15	4.86 ± 0.60	0.63 ± 0.63	1.96 ± 0.22

Table 5: Composition of the solid residues and recovery percentage after alkaline hydrolysis (1.5% w/v NaOH, ambient temperature) and bleaching.

	Alkaline Hydrolysis (1.5% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	21.19 ± 0.79	58.42 ± 2.16	14.14 ± 0.07	28.25 ± 0.13
Acid soluble lignin (% on dw)	0.47 ± 0.02	11.56 ± 0.57	0.77 ± 0.09	13.76 ± 1.63
Cellulose (% on dw)	39.96 ± 2.72	105.02 ± 7.16	61.23 ± 3.59	116.64 ± 6.84
Hemicellulose (% on dw)	1.98 ± 0.12	7.87 ± 0.49	0.72 ± 0.09	2.07 ± 0.26

These considerations are in agreement with what has been reported by Wunna et al. (2017) that investigated, although on a different raw material, the effect of alkaline pretreatment highlighting the maximum degradation of lignin at NaOH concentrations of 2.0% w/v. Regarding cellulose, its content increases up to a maximum of about 61% w/w during the alkaline step using 1.5% w/v of NaOH. Cellulose recovery remains about 100% for all cases at different NaOH concentrations although it is interesting to note that cellulose recovery begins to drop slightly as the NaOH concentration increases. This may be attributed to a more severe hydrolysis, which in turn initiates the degradation of cellulose. It was then decided to carry out the same treatments, varying only the temperature (i.e. 60°), to investigate the effect of the temperature variation on cellulose isolation. The results are shown in Table 6, Table 7, and Table 8 and reflect what was found for the alkaline hydrolysis treatment at

ambient temperature. Indeed, the hemicellulose is almost completely removed, while the lignin is degraded to a maximum of about 85% by alkaline hydrolysis treatment with 1.5% NaOH, reaching, after bleaching, a lignin content in the residual solid of about 8% w/w. Regarding cellulose, its total recovery can again be found, with a fraction of about 55% w/w for all the various cases in the final solid recovered. Again, a slight reduction in cellulose recovery can be found through treatment with higher NaOH content.

Table 6: Composition of the solid residues and recovery percentage after alkaline hydrolysis (16% w/v NaOH, 60°C) and bleaching.

	Alkaline Hydrolysis (16% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	30.98 ± 1.76	79.88 ± 4.54	13.25 ± 1.33	24.86 ± 2.49
Acid soluble lignin (% on dw)	0.56 ± 0.06	12.88 ± 1.30	0.83 ± 0.12	13.98 ± 1.95
Cellulose (% on dw)	37.24 ± 2.45	91.54 ± 6.01	58.51 ± 0.90	104.67 ± 1.61
Hemicellulose (% on dw)	0.79 ± 0.34	2.95 ± 1.27	0.60 ± 0.02	1.63 ± 0.05

Table 7: Composition of the solid residues and recovery percentage after alkaline hydrolysis (4% w/v NaOH, 60°C) and bleaching.

	Alkaline Hydrolysis (4% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	29.09 ± 1.26	80.69 ± 3.48	16.62 ± 2.74	35.70 ± 5.88
Acid soluble lignin (% on dw)	0.68 ± 0.01	16.90 ± 0.15	0.81 ± 0.03	15.68 ± 0.51
Cellulose (% on dw)	43.26 ± 7.17	114.42 ± 18.97	55.36 ± 2.09	113.41 ± 4.28
Hemicellulose (% on dw)	0.98 ± 0.16	3.91 ± 0.66	1.39 ± 0.32	4.30 ± 0.98

Table 8: Composition of the solid residues and recovery percentage after alkaline hydrolysis (1.5% w/v NaOH, 60°C) and bleaching.

	Alkaline Hydrolysis (1.5% w/v NaOH)		Bleaching	
	Composition (% on dw)	Recovery (%)	Composition (% on dw)	Recovery (%)
Acid insoluble lignin (% on dw)	22.05 ± 2.07	60.78 ± 5.69	7.83 ± 2.16	15.65 ± 4.32
Acid soluble lignin (% on dw)	0.50 ± 0.01	12.48 ± 0.17	0.62 ± 0.01	11.14 ± 0.13
Cellulose (% on dw)	41.71 ± 0.04	109.59 ± 0.12	55.18 ± 1.25	105.11 ± 2.38
Hemicellulose (% on dw)	2.08 ± 0.14	8.28 ± 0.56	2.14 ± 0.30	6.16 ± 0.86

Comparing the cases at different temperatures, it can be seen that there is a positive effect of temperature in terms of reducing lignin content. For instance, considering the optimal scenario targeted in this study (i.e., alkaline hydrolysis with 1.5% w/v NaOH), the lignin content drops from about 14% to 8% w/w and consequently, the lignin removal increased from about 72% to 85%. However, it is useful to point out that treatment at 60°C has higher energy consumption so it will be necessary to investigate, as a future step of this work, the cost-effectiveness of treatments at higher temperatures. Another interesting aspect to be investigated will be the effect of the treatment time. It may be feasible to reduce this duration by operating at higher temperatures to attain comparable results, thus offsetting the increased costs associated with elevated operating temperatures. Moreover, it is interesting to point out that although a lignin removal between about 60% and 85% could be considered high, further investigation can be done to further reduce the lignin content and thus purify the final cellulose obtained by increasing the severity of the alkali treatment (e.g., with higher temperatures and/or longer treatment times). However, this could lead to increased degradation not only of lignin but also of cellulose. Therefore, an optimization of the process will be necessary through also the use of phenomenological mathematical models and/or models based on the response surface methodology (Dhara et al., 2023).

4. Conclusions

In this work a new process for cellulose isolation by enhancing oilseed cakes as a raw material was investigated. This process involves three treatment steps, namely alkaline hydrolysis with NaOH and bleaching preceded by an autohydrolysis step. The latter allows almost all of the hemicellulose and some of the lignin to be degraded,

replacing the traditional and more impactful acid hydrolysis treatment. Moreover, the effects of NaOH concentration and temperature on the alkaline hydrolysis treatment were investigated. It was observed that the best conditions for cellulose isolation were obtained through alkaline hydrolysis with 1.5% w/v of NaOH at 60°C where a lignin degradation of 85%, while cellulose was recovered almost totally. From the point of view of environmental impact, the use of a low concentration of NaOH is a positive aspect as opposed to using higher temperatures (i.e., 60 °C). Therefore, in the future it will be necessary to investigate these aspects further to find the optimal operating conditions for alkali treatment, also introducing treatment time as an additional factor. Finally, to make this isolation process more economically and environmentally sustainable, it would be interesting to analyze the content of the liquor produced by the autohydrolysis step (Figure 1), which could potentially be rich in phenolic and antioxidant compounds.

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