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Original research

Enzymatic Dehulling of Sesame (*Sesamum indicum L.*) Seeds Using Cellulase and Xylanase

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A B S T R A C T

Enzymatic dehulling of sesame (*Sesamum indicum L.*) seeds was investigated in this research work. Since cellulose and hemicellulose are two main ingredients of sesame hull, cellulase and xylanase were used for sesame seeds dehulling. A full factorial experimental design was employed to investigate the effects of total enzyme activity in solution, the ratio of cellulase to xylanase activity, and mass percentage of seeds in solution on the dehulling process. Analysis of variance showed that all the three factors affected the process significantly. Efficiencies as high as 95% dehulling in 15 min was obtained with total enzyme activity of 10 U/ (g seeds), the cellulase to xylanase activity ratio of 1, and the sesame seed mass percentage in solution of 10% (w/w). In a separate experiment the rate of dehulling was determined as a function of time and seeds mass percentage in solution, and a rate equation was suggested for the rate of dehulling. Investigations, moreover, showed that the spent enzyme solution from one batch of dehulling could be reused up to four times without significant loss of enzyme activity.

Keywords: Sesame seeds; dehulling; Cellulase; Xylanase

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1. Introduction

Sesame (*Sesamum indicum L.*) is cultivated worldwide for its seeds. Sesame seeds are rich in unsaturated fatty acids (around 50%), and proteins (around 20%). The seeds also contain considerable amounts of carbohydrates, minerals, and insoluble fibers (Badifu and Akpagher,1996; Egbekun and Ehieze, 1997; Gandhi and Srivastava, 2007; Nzikou *et al.,* 2009). Sesame seeds are covered with a hull which comprises cellulose, hemicelluloses, pectin, and oxalic acid (Toker, 2000). The percentage of cellulose, hemicellulose, and pectin in the hull are around 31%,7%, and 0.06%, respectively (Elleuch *et al.,* 2007). Oxalic acid constitutes around 2.2% of the sesame seeds, which exist mostly in the hull of the seeds. Presence of oxalic acid in sesame hull has a negative impact on its taste and nutritional values (Manikantan et al., 2015). Therefore, sesame seeds are normally dehulled before being used.

A wet method is traditionally used to dehull sesame seeds. The seeds are soaked in water for a few hours, and stored for 12 hours after removing from water. The loosened hulls are then removed mechanically in a special designed apparatus (Gungor, 2004).

The traditional method for sesame dehulling is time consuming. Water is also used in large amounts, which is a main drawback in arid areas. Soaking sesame seeds in dilute alkali solutions has been proposed to accelerate the process of dehulling (Yehia *et al.*, 2002). Using alkali solutions accelerate corrosion of equipment, and deteriorate the quality of dehulled seeds. Considerable amounts of water are also needed to wash the dehulled seeds thoroughly. Other chemicals were also investigated for sesame dehulling. Carbonell-Barrachina *et al.* (2009) used NaClO, H_2O_2, $Na_2S_2O_5$, HCl, and H_2SO_4 to assist sesame dehulling, and investigated the effect of these chemical on the microstructure of the sesame seeds. Among the chemical tested, NaClO and H_2O_2 were more effective in dehulling with minimum damage to the microstructure of the sesame seeds, resulting in light colored seeds.

Application of enzymes may accelerate sesame seeds dehulling without imposing problems caused by chemical solutions. Enzymes can attack sesames hulls specifically without damaging the interior body

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of the seeds. Despite promising advantages that can be expected from the application of enzymes in sesame seeds dehulling, limited published data are found in the literature on this subject. Gungor (2004) used Peelzyme-I (a proprietary mixture of enzymes) to dehull sesame seeds in a specially designed apparatus. More than 95% of the seeds were dehulled in 15 min. The work, however, was mainly focused on the design of the apparatus, and the effect of Peelzyme-I on dehulling was not investigated in details. Ozcan and Tanriverdi (2023) used a commercially available cellulolytic enzyme mixture (Viscozyme L) for the dehulling of the sesame seeds, and compared the results with traditional non-enzymatic method. The results showed the superiority of the enzymatic method. Song *et al.* (2013) used a mixture of cellulase, xylanase, and pectinase for dehulling of sesame seeds. Optimization of the influencing factors on sesame seeds dehulling resulted in the yield of 98% in 30 min at the temperature of 50°C. The latter two works, however, lacked information on the rate of dehulling as a function of sesame seeds mass percentage, and possible reuse of spent enzyme solutions.

The potential application of enzymes to valorize seed hulls, and to facilitate oil extraction from oilseeds have been investigated by researchers. These are closely related to the subject of this work since in both cases depolymerization of polysaccharides in seed hulls and cell's structure would results in the release of valuable ingredients from seed hulls and/or enhance oil extraction from the seeds. Zhou *et. al.* (2023) applied Viscozyme, Alcalase, and feruloyl esterase successfully to release avenanthramides, phenolic acids, free sugars, and organic acids from oat hulls. Enzymatic pretreatment of oilseeds has been reviewed by Vovk et. al. (2023).

The objective of this research work is to compare the effect of cellulase, xylanase, and a mixture of both on sesame seeds dehulling. The quantification of the effect of the individual enzymes, and a mixture of the enzymes with known activities is a subject not investigated by other researchers. The rate of dehulling is also investigated as a function of the mass percentage of sesame seeds in solution, for the first time in this work. Investigation on the effect of temperature and pH, and possible reuse of spent enzyme solutions are other objectives of this research work.

2. Material and Methods

2.1. Materials

Cellulase and xylanase were purchased from Sigma Aldrich Company. The materials were in the form of powders with the specific activity of 800 U_c/g and 2500 U_x/g for cellulase and xylanase, respectively. Each unit of cellulase activity (U_c) is defined as the amount of the enzyme that releases 1µmol/min of glucose from cellulose at 37°C and pH=5. Each unit of xylanae activity (U_x) is defined as the amount of the enzyme that releases of 1µmol/min of xylose from hemicellulose at 40°C and pH=5. Citric acid and sodium citrate were purchased form Merck. The materials were used to prepare buffer solutions. Sesame seeds were obtained from a local market.

2.2. Methods

2.2.1. Dehulling procedure

Sesame seeds and enzymes (based on the amounts described below) were added to 100 mL of citrate solution (pH=5) in a beaker. The resulting mixture was agitated using a disk type agitator (7cm diameter) with the rotation speed of 330 rpm. The solution temperature was kept constant at desired temperatures (mentioned below for each specific experiment) using an automatic heater. After termination of the process, the sesame seeds were separated from the solution using Whatman paper. The seeds were dried to a constant weight, and the dehulled seeds were separated manually. The seeds were weighed, and the dehulling efficiency was calculated as:

$$\text{Efficiency} = \frac{\text{weight of dehulled seeds}}{\text{initial weight of seeds}} \times 100 \qquad (1)$$

2.2.2. Effective parameters on dehulling efficiency

A full factorial experimental design was employed to determine the effect of mass percentage of seeds in solution, total enzyme activity, and the ratio of cellulase to xylanase activities on the efficiency of sesame seeds dehulling. Three levels were considered for each factor. The design required 27 experiments. The experiments were done in duplicate. Three blank experiments (in enzyme free solutions) were also done for each level of the amount of seed in solution. All these experiments were done at 25°C, pH=5, and the contact time of 15 min. Table 1 shows the experimental design and the results. To find out the effect of temperature and pH on dehulling efficiency, in a separate experiment, run 19 in Table 1 was repeated under different temperatures and pHs.

2.2.3. Determination of dehulling rate as a function of time and mass percentage of sesame seeds

Rate of sesame seeds dehulling was determined as a function of time and the amount of sesame seeds in solutions. For this purpose, buffer solutions (pH=5) containing 20% (w/w) sesame seeds and enzyme activity of 10 (U_c+U_x)/g seeds, with cellulase/xylanse activity ratio of 1 were prepared in five beakers. Dehulling operation was performed as described in 2.2.1. The process was terminated in beakers in 5 min intervals. The amount of unhulled sesame seeds was determined as a function of time, and a rate equation was suggested for enzymatic sesame seeds dehulling. Using the rate equation, the dehulling process was modelled in a batch system, and an equation was obtained for the prediction of unhulled sesame seeds amount as a function of time (Sec.3.2). An experiment was conducted to verify the accuracy of the suggested model. Buffer solutions (pH=5) containing 10% (w/w) sesame seeds and enzyme activity of 10 (U_c+U_x)/g seeds, with cellulase/xylanse activity ratio of 1 were prepared in five beakers. Dehulling operation was performed as described in 2.2.1. The process was terminated in beakers in specified time intervals, and the amount of unhulled sesame seeds was determined as a function of time. The concentration of the seeds was compared with the concentration predicted by the suggested model.

2.2.4. Reuse of spent enzyme solution

Sesame seeds were dehulled under the condition of 10%(w/w) seeds amount, 10 (U_c+U_x)/g seeds, cellulase/xylanase activity ratio of 1U_c/U_x, process time of 15 min, and solution temperature of 25°C. After the process, the solution was filtered, replenished with distilled water to initial value, and reused for sesame seeds dehulling. The operation was repeated several times, and dehulling efficiency was calculated after each batch.

2.2.5. Data analysis

Analysis of variance (ANOVA) using the software MINITAB 18 was performed to determine the significance of the factors mentioned in Sec. 2.2.2. The regression analysis calculations to determine the constants in the rate equation (Sec. 3.2) was performed by MATLAB 2020a.

Table 1. Factorial design for dehulling efficiency as a function of sesame seeds mass percentage, total enzyme activity, and celluase to xylanase activity ratio. pH=5, T=25°C, Contact time=15 min.

Run	Sesame percentage. (%w/w)	Total enzyme activity (U_c+U_x)/g seeds	Ratio of cellulase/xylanase U_c/U_x	Dehulling Efficiency
1	10 (balnk)	0	-	28.5±1.25
2	10	1	∞	37±2
3	10	1	1	65±3
4	10	1	0	35.6±0.6
5	10	5	∞	57.6±8.3
6	10	5	1	88.7±3.7
7	10	5	0	52.5±2.5
8	10	10	∞	78±10
9	10	10	1	95±3
10	10	10	0	68.3±0.7
11	20 (blank)	0	-	14.4±0.9
12	20	1	∞	19±2.3
13	20	1	1	33.2±2.7
14	20	1	0	18.6±0.4
15	20	5	∞	29.4±4.6
16	20	5	1	45±6
17	20	5	0	27.1±3.7
18	20	10	∞	39.4±1.6
19	20	10	1	55.2±1.8
20	20	10	0	34.4±1.3
21	30 (blank)	0	-	9.2±0.7
22	30	1	∞	11.3±0.5
23	30	1	1	20.7±2.3
24	30	1	0	11.2±0.9
25	30	5	∞	18.5±1.3
26	30	5	1	28.9±1.3
27	30	5	0	16.6±1.7
28	30	10	∞	25±2.1
29	30	10	1	36.1±0.7
30	30	10	0	22±1.5

3. Results and Discussion

3.1. Dehulling efficiency as a function effective parameter

Dehulling efficiency of sesame seeds was determined as a function of the amount of sesame seeds in solution, total enzyme activity, and cellulase/xylanase activity ratio using a full factorial experimental design. The results have been presented in Table 1. Dehulling efficiency was evidently affected by the presence of enzymes. For the sesame concentration of 10%(w/w), the dehulling efficiency of 28.5% was obtained for the blank sample, while the dehulling efficiency ranged from 35.6% to 95% in enzyme(s) containing solutions. For the sesame concentration of 20%(w/w), the dehulling efficiency for the blank sample was 14.4%, and for the enzyme(s) containing solutions this figure ranged from 18.6% to 55.2%. Similarly, for the sesame concentration of 30%(w/w) the dehulling efficiency of 9.2% was obtained for the blank sample, and it ranged from 11.2% to 36.1% in enzyme(s) containing solution. From Table 1 it is also inferred that with the increase in total enzyme(s) activity per unit mass of sesame seeds in solution, the dehulling efficiency increases, and a mixture of cellulase/xylanase performs better than each of the enzymes alone. The largest dehulling efficiency for this set of experiments (95%) was obtained at the sesame seeds amount of 10%(w/w), total enzyme activity of 10 (U_c+U_x)/g seeds, and cellulase/xylanse activity ratio of 1. Analysis of variance for the data in Table 1 indicated that the effect of all the factors on dehulling efficiency were statistically significant (P_{value}<0.05).

Cellulose together with hemicellulose and (to a lower percentage) pectin form a rigid polysaccharide composite layer around sesame seeds. Cellulose is a biopolymer consists of glucose monomers linked by $\beta 1 \rightarrow 4$ glucoside bounds. This biopolymer exists in crystalline form and is not affected easily by chemical agents (Chen, 2014). Cellulases, which are a group of hydrolytic enzymes, are able to depolymerize cellulose. Cellulases are characterized by their activity which is a measure of their ability to release glucose from cellulose per unit time (Jasani *et al.* 2016). Hemicellulose is a heteropolymer comprised of 5 and 6 carbon sugars, with xylose in larger amounts compared to other sugars. Xylanases are hydrolytic enzymes that can release xylose from hemicellulose which results in its disintegration (Polizeli, *et al*., 2005). Application of enzymes is a common practice in fruit, food, and textile processing (Godfery and West,1996). This work verifies, in general, the works published by Song *et al.* (2013), Ozcan and Tanriverdi (2023), and Gungor (2004) as the only specific works we could find on the enzymatic dehulling of sesame seeds. Song *et al.* (2013) reported weak effect of pectinase on sesame dehulling. This result is expected as pectin comprises only around 0.06% of the sesame hull (Elleuch *et al.,* 2007). Comparing the effect of cellulase and xylanse, the authors reported that xylanse performs slightly better than cellulase when applied alone. Our findings in Table 1, however, contradict their results in this case. The results in Table 1 shows that, when applied alone, cellulase performs slightly better than xylanase. The findings of Song *et al.* (2013) in this case can be challenged by lack of error bars. The results in Table 1, at the other hand, consistently proves better performance of cellulase. The fact that cellulose percentage in sesame hull is larger than hemicellulose may be the reason for higher effectiveness of cellulase. Synergistic effects of cellulase and xylanase on sesame dehulling has been reported by Song *et al.* (2013). The same result is verified in this work. Overall, Song *et al.* (2013) reported more than 98% dehulling efficiency for a solution containing 25% seasame seeds at 50 °C in 15 min. Similarly, we obtained near complete dehulling for a solution containing 20% sesame seeds at 45 °C in 15 min (Fig.1). Although from the works of Ozcan and Tanriverdi (2023), and Gungor (2004) it can be inferred the positive effect of enzymes on sesame dehulling, comparison of their work with others is not possible as the details of the enzyme types and compositions have not been provided by those authors.

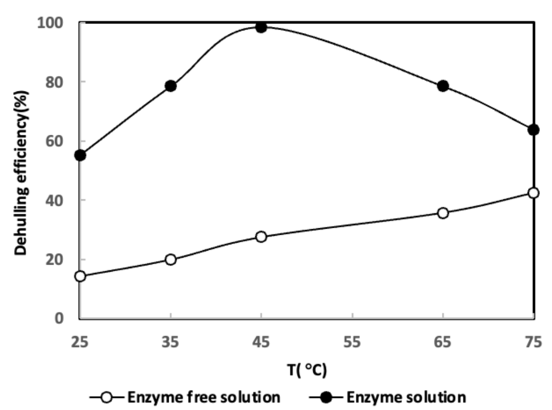

Fig. 1. The effect of temperature on dehulling efficiency. Sesame seed conc.:20%(w/w), Total enzyme activity:10(U_c+U_x)/g seeds, Ratio of cellulase/xylanase:1, pH:5, Process time:15 min.

The effect of temperature on the dehulling efficiency of sesame seeds in enzyme solutions has been presented in Fig.1. The efficiency increased with the increase in temperature up to 45°C. Beyond this temperature a gradual decrease in efficiency was observed. The decrease in efficiency can be attributed to deactivation of enzymes at higher temperatures. Dehulling efficiency in enzyme free solution increased steadily with temperature.

Although maximum efficiency occurred at pH=5, there was no drastic variations in dehulling efficiency in the pH range of 4-9. For the sesame seeds amount of 20%(w/w), total enzyme activity of 10 (U_c+U_x)/g seeds, cellulase/xylanse activity ratio of 1, and the contact time of 15 min, the dehulling efficiency was 48.2% at pH=4. The efficiency increased to the optimum value of 55.2% at pH=5, and decreased to 50% at pH=9. Adjustment of pH, therefore, may be eliminated in practice without considerable loss in dehulling efficiency (Fig. 2).

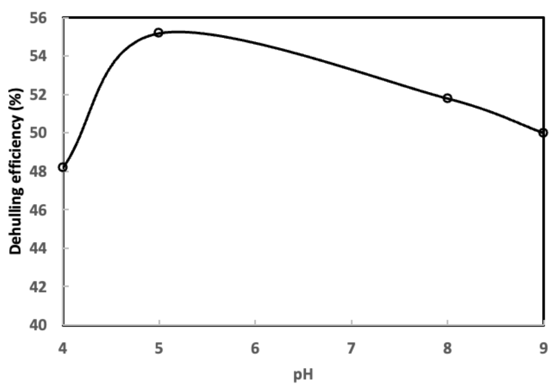

Fig. 2. The effect of pH on dehulling efficiency. Sesame seed conc.:20%(w/w), Total enzyme activity:10 (U_c+U_x)/g seeds, Ratio of cellulase/xylanase:1, T=25°C, Process time:15 min.

3.2. Dehulling rate as a function of sesame seeds concentration

Data on the amount of unhulled sesame seeds as a function of time have been shown in Fig. 3. The figure indicates that the slope of the curve increases progressively as time passes, and declines later with the decrease in unhulled seeds amount. Following rate equation, therefore, is proposed for the rate of sesame seeds dehulling:

$$v = \frac{k_1 t C_s}{k_2 + C_s} \quad (2)$$

in which:

v: rate of sesame seeds dehulling (g seeds/ (L of buffer solution. min))

C_s: Unhulled sesame seeds amount (g seeds/L of buffer solution)

t: time (min)

k_1: constant (g seeds/ (L of buffer solution. min^2))

k_2: constant (g seeds/ (L buffer solution))

To determine the constants in the rate equation, the process of seeds dehulling in a batch system is modelled as:

$$\frac{dC_s}{dt} = -\frac{k_1 t C_s}{k_2 + C_s} \quad (3)$$

Equation 3 is solved analytically:

$$\frac{t^2}{2} = \frac{k_2}{k_1} ln \frac{C_{s0}}{C_s} + \frac{1}{k_1}(C_{s0} - C_s) \quad (4)$$

in which:

C_{s0}: the initial amount of sesame seeds in solution (g seeds/L of buffer solution).

Equation 4 was fitted to the experimental data to estimate the constants. Using linear regression techniques, k_1 and k_2 were obtained as 1.695 and 78.14, respectively. The regression procedure has been summarized in Table 2. To verify the validity of equation 4 for the prediction of unhulled seeds amount as a function of time, the equation was used to predict the dehulling process in a solution with the initial seeds concentration of 100 g seeds/ (L buffer solution). The process was also carried out experimentally, and the concentration of unhulled seeds was measured at specific time intervals. Fig. 3 presents the results. The figure demonstrates that the model can predict the amount of unhulled seeds as a function of time with reasonable accuracy.

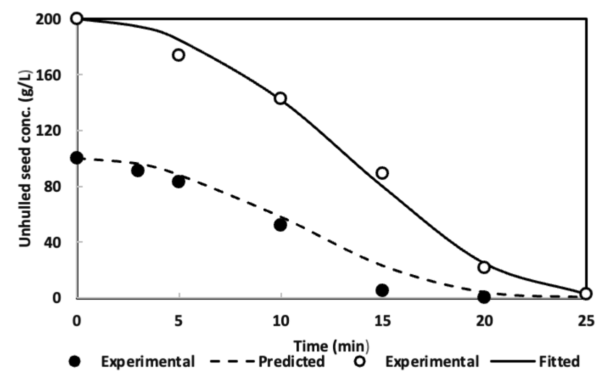

Fig. 3. Unhulled sesame seeds amount as a function time. Total enzyme conc.:10 (U_c+U_x)/g seed, Cellulase/xylanase ratio:1, Temperature:25°C, pH:5.

3.3. Reuse of spent enzyme solution

Reusing spent enzyme solutions for sesame seeds dehulling makes the process more efficient in cases of enzyme and water consumption. The efficiency of an enzyme solution for sesame seeds dehulling when the solution was used in consecutive batches is shown in Fig. 4. The dehulling efficiency decreased from 95% to 85% after four batches, and it decreased to 55% after seven batches. The decline in efficiency can be attributed to the loss of enzymes with the moisture content of the dehulled sesame seeds, and denaturation of the enzyme under the sheer stress caused by the agitator. The results, however, indicates that the solution can be reused up to four consecutive batches without losing much efficiency.

Table 2. Factorial design for dehulling efficiency as a function of sesame seeds mass percentage, total enzyme activity, and cellulase to xylanase activity ratio. pH=5, T=25°C, Contact time=15 min.

t(min)	C_s(g/L)	$Y = \frac{t^2}{2}$	$X_1 = ln\frac{C_{s0}}{C_s}$	$X_2 = (C_{s0} - C_s)$
0	200	0	0	0
5	173.8	12.5	0.14	26.2
10	142.8	50	0.34	57.2
15	89.6	112.5	0.8	110.4
20	21.6	200	2.22	178.4
25	3	312.5	4.2	197

Regression results: $\frac{t^2}{2} = 46.103 ln\frac{C_{s0}}{C_s} + 0.59(C_{s0} - C_s)$

$R^2 = 0.996$; k_1=1.695, k_2=78.14

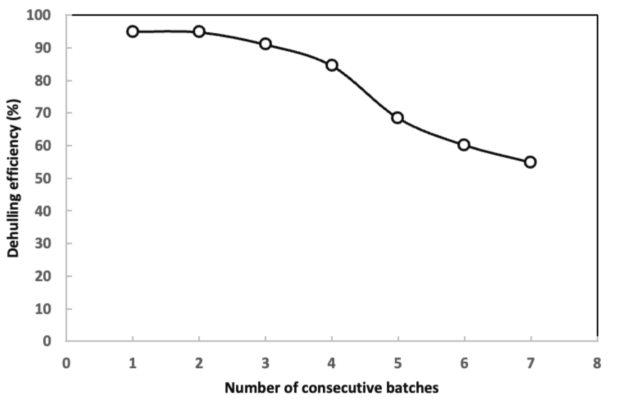

Fig. 4. Decline in dehulling efficiency of an enzyme solution after reusing in consecutive batches. Sesame seeds conc. 10%(w/w). Total enzyme conc.:10(U_c+U_x)/g seed, Cellulase/xylanase ratio:1, Temperature:25°C, pH:5, Time: 15 min.

4. Conclusion

Using enzymes for sesame seeds dehulling is practically feasible. Celulase and xylanase are two effective enzymes for sesame seeds dehulling. A mixture of cellulase and xylanase is more effective than each of the enzymes alone. The rate of enzymatic dehulling can be modelled as a function of time and the amount of unhulled seeds in solution, and an analytic equation can be obtained to predict dehulling efficiency as a function of time in a batch system.

Further research is needed to determine the effect of enzymatic dehulling on the quality of the seeds. The amount of adsorbed enzymes on the hulled seeds, and the ways to deactivate the adsorbed enzymes needs to be determined. Enzymes are active biological agents, and residual enzymes on the hulled seeds should be deactivated before the end use of the seeds. In this research work for the runs with the mixture of the enzymes, the ratio of the cellulose to xylanase activities was 1. This might not be the optimum ratio, and more research is needed to find the optimum ratio.

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Conflict of interest

The authors declare that there is no conflict of interest.

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