*Chemistry of Natural Compounds, Vol. 46, No. 6, 2011*

# INFLUENCE OF CELLULOLYTIC ENZYMES ON OIL YIELD FROM LOW-OIL-CONTENT PLANT SEEDS

**Sh. Ya. Mirzaakhmedov,[1*] D. T. Ruzmetova,[1] N. Sh. Berdiev,[1] Kh. B. Ashurov,[2] and Sh. I. Salikhov[1]**

UDC 577.15+663.15+577.112

*Enzymatic hydrolysis of tomato and pomegranate seeds was studied using an enzyme preparation from the fungus* Aspergillus terreus. *The optimum hydrolysis conditions for tomato and pomegranate seeds were pH 4.0, 40°C, and incubation time 2 d. Preliminary enzymatic treatment of the studied tomato and pomegranate seeds increased the oil yield from them by cold pressing by 21.3 and 34.8%, respectively.*

**Keywords:** enzyme, enzymatic hydrolysis, *Aspergillus terreus*, tomato and pomegranate seeds, cold pressing, oil.

Edible oils are produced in the oil and fat industry by hot pressing of seeds and extraction methods [1]. The high temperature or organic solvent usually change the natural composition of the oil and its biologically valuable properties. The properties of the oil are preserved upon cold pressing of seeds but the yield decreases. If it is considered that plant seed husks consist of cellulose, hemicellulose, and pectin-like substances [2], then the seed walls need to be destroyed beforehand using hydrolytic enzymes in order to increase the cost effectiveness of cold pressing.

The goal of the present investigation was to study the role of cellulolytic enzymes of *Aspergillus terreus* on the yield of valuable oils from tomato and pomegranate seeds. The studied seeds were hydrolyzed using a preparation of enzymes from the fungus *A. terreus* that was isolated by us earlier [3]. The enzyme treatment was carried out under nine reaction conditions, i.e., at pH values 3–5 and at temperatures in the range 20–40°C. The incubation time was 72 h. The amount of free glucose in the reaction mixtures was measured and the degree of hydrolysis was determined every 24 h. Table 1 presents the results.

Table 1 shows that tomato seed husks more or less underwent enzymatic hydrolysis under all reaction conditions. Experiments performed at pH 4, 40°C, and pH 5, 40°C, were especially notable among them. Under these conditions the enzyme preparation produced 0.34 and 0.31 μM free glucose after one day of incubation. The yield of hydrolysis products increased sharply to 1.0 and 0.75 μM as the incubation time increased to two days. Increasing the incubation time further to three days did not increase the degree of hydrolysis.

The action of the enzyme preparation on the degree of hydrolysis of pomegranate seed husks was studied in parallel. Like in the preceding instance, the experiment performed at pH 4 and 40°C was especially notable. Under these conditions the reaction medium contained 0.24 μM glucose as the hydrolysis product after incubation for one day with enzyme preparation. The product yield increased to 0.8 μM after the second day. The amount of glucose remained unchanged after the third day of incubation. An examination of the results in both instances for experiments performed at pH 4 and 40°C showed that the product yield after two days of incubation increased noticeably compared with the amount of product obtained from the experiment performed at pH 5 and 40°C.

An examination of the results for enzymatic hydrolysis of tomato and pomegranate seed husks under identical conditions (pH 4, 40°C) showed that the enzyme preparation hydrolyzed more effectively polysaccharides of tomato seeds. This may be due [4] to the lower content in them of difficultly soluble cellulose fractions (20.04%) compared with pomegranate seeds (31.36%). Thus, it was found that the optimum conditions for enzyme hydrolysis of tomato and pomegranate seed husks are incubation time 2 d, pH 4, and 40°C.

1) Institute of Bioorganic Chemistry, 100125, Tashkent, Republic of Uzbekistan, fax: (99871) 262 70 63, e-mail: mirzaakhmedov@mail.ru; 2) Institute of Electronics, 100125, Tashkent, Ul. F. Khodzhaeva, 33, Republic of Uzbekistan. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 737–739, November–December, 2010. Original article submitted August 17, 2010.

0009-3130/11/4606-0869  

TABLE 1. Enzymatic Hydrolysis of Tomato and Pomegranate Seed Husks

| pH | T, °C | Glucose from seeds, μM | | | | | |
|---|---|---|---|---|---|---|---|
| | | tomato | | | pomegranate | | |
| | | 1st day | 2nd day | 3rd day | 1st day | 2nd day | 3rd day |
| 3 | 20 | 0.06 | 0.06 | 0.06 | 0.05 | 0.06 | 0.06 |
| | 30 | 0.09 | 0.09 | 0.09 | 0.08 | 0.08 | 0.08 |
| | 40 | 0.18 | 0.27 | 0.27 | 0.09 | 0.09 | 0.09 |
| 4 | 20 | 0.06 | 0.06 | 0.06 | 0.09 | 0.09 | 0.09 |
| | 30 | 0.25 | 0.56 | 0.56 | 0.14 | 0.16 | 0.16 |
| | 40 | 0.34 | 1.0 | 1.0 | 0.24 | 0.8 | 0.8 |
| 5 | 20 | 0.06 | 0.06 | 0.06 | 0.08 | 0.09 | 0.09 |
| | 30 | 0.28 | 0.6 | 0.6 | 0.14 | 0.15 | 0.15 |
| | 40 | 0.31 | 0.75 | 0.75 | 0.18 | 0.25 | 0.25 |

TABLE 2. Oil Yield from Tomato and Pomegranate Seeds, %

| Seeds | Before enzyme treatment | After enzyme treatment | Before enzyme treatment | After enzyme treatment |
|---|---|---|---|---|
| | oil yield from hydrocarbon extraction | | oil yield from cold pressing | |
| Tomato | 20.50 | 23.12 | 14.20 | 17.22 |
| Pomegranate | 15.60 | 16.01 | 2.10 | 2.83 |

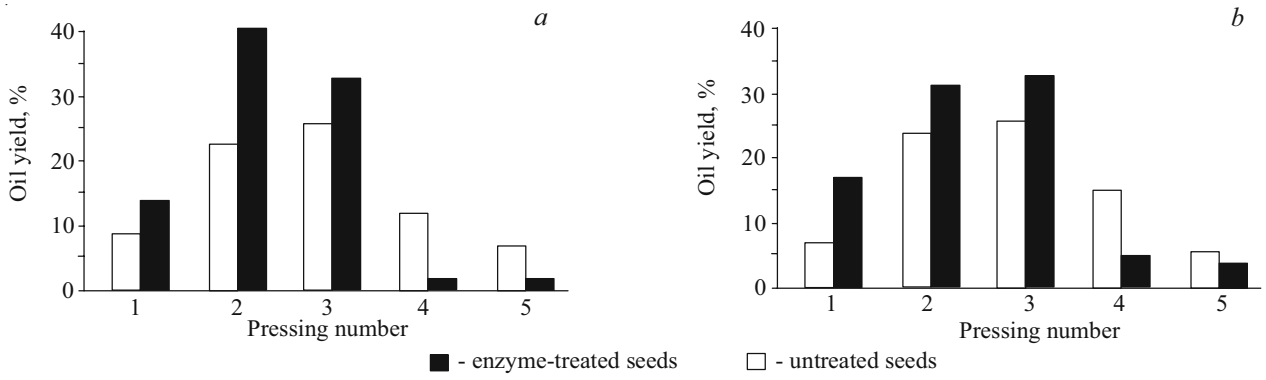


Fig. 1. Oil yield from seeds of tomato (*a*) and pomegranate (*b*).

Tomato and pomegranate seeds were subjected to enzyme treatment under the conditions found by us. The studied seeds were separated from enzyme liquid after the hydrolysis time expired, washed thoroughly with water, and dried. The mass losses of the tomato and pomegranate seeds were 3.6 and 5.7%, respectively, compared with control seeds.

Cold pressing of seeds was performed five times on a screw press-granulator. Figure 1 shows the results from pressing seeds and compares the oil yield from enzyme-treated and untreated tomato and pomegranate seeds after each cold-pressing step. Stepwise pressing of control seeds increased the oil yield three times and then gradually reduced it whereas three pressings were sufficient to obtain the majority of the oil from seeds treated beforehand with enzymes in order.

In both instances the maximum oil yield was observed for three pressings of pomegranate seeds. The oil yield in subsequent pressing steps (4th and 5th) from enzyme-treated seeds decreased compared with the control. This was apparently related to a reduction in the sorption capacity of the cellulose.

Table 2 presents oil yields from tomato and pomegranate seeds obtained by extraction and cold pressing.

The difference between the oil yields from hydrocarbon extraction and cold pressing is at the background value judging from the oil content remaining in the pulp. It was determined that the background oil values after enzyme treatment decreased to 5.9% and 13.18 for tomato and pomegranate seeds, respectively, compared with values of 6.3% and 13.5 in pulp from control seeds.

Thus, enzyme treatment of tomato and pomegranate seeds caused noticeable structural changes, as a result of which their background oil content relative to a control decreased by 0.4 and 0.32%, respectively. This increased the oil yield from enzyme-treated tomato and pomegranate seeds by 21.3% and 34.8 compared with the control.

## EXPERIMENTAL

**Reagents.** Na-CMC (Sigma, USA); acetic acid (Merck, Germany); NaOH (Merck, Germany); $Na_2SO_4$, Na/K-tartrate, $Na_2CO_3$, $NaHCO_3$, $Cu_2SO_4$, ammonium molybdate, sodium arsenate, and conc. $H_2SO_4$ (Russia); and extraction hydrocarbons were used. Enzyme preparation was isolated from culture liquid of *A. terreus*.

**Enzyme Treatment of Seeds.** Enzyme preparation (10 mg, 10 activity units) was dissolved in $CH_3COONa$ buffer (50 mL, 10 mM, pH 3). Seeds (20 g) from tomato *Lycopersion esculentum* Mill. or pomegranate *Punica granatum* L. were treated with enzyme solution and incubated for 3 d at 20°C. The experiment was also repeated at pH 4 and 20°C; pH 5 and 20°C; pH 3 and 30°C; pH 4 and 30°C; pH 5 and 30°C; pH 3 and 40°C; pH 4 and 40°C; and pH 5 and 40°C. Large-scale hydrolysis was performed using enzyme preparation (1 g) dissolved in $CH_3COONa$ buffer (5 L, 10 mM, pH 4) adding tomato or pomegranate seeds (2 kg) and incubating for 2 d at 40°C.

**Determination of Glucose from Endoglucanase Activity [3].** The studied solution (1 mL) was treated with Somogyi reagent (2 mL), refluxed on a water bath for 15 min at 100°C, cooled, treated with Nelson reagent (2 mL) and distilled water (4 mL), stirred, and left undisturbed. Then the intensity of the blue color was measured spectrophotometrically at 520 nm. The control was glucose. The enzyme activity was calculated from a calibration curve constructed for glucose.

**Instruments and Equipment.** Enzymatic destruction of seeds was carried out in a 5-L fermenter. Cold pressing of seeds used a screw press-granulator built at the Institute of Electronics, AS RU. The throughput of the screw press was 100 kg/h of processed material [5].

## REFERENCES


1. V. M. Kopeykovski, *Food Industry*, Moscow, 1982.
2. N. C. Carpita and D. M. Gibeaut, *Plant J.*, **3**, 1 (1993).
3. Sh. Ya. Mirzaakhmedov, Zh. F. Ziyavitdinov, Z. R. Akhmedova, A. B. Saliev, D. T. Ruzmetova, Kh. B. Ashurov, D. Fessas, and S. Iametti, *Khim. Prir. Soedin.*, 489 (2007).
4. S. Y. Mirzaakhmedov, J. F. Ziyavitdinov, D. N. Dalimov, A. B. Soliev, G. N. Dalimova, K. B. Ashurov, S. T. Azizov, M. K. Bhat, S. I. Salikhov, and S. T. Nazarbekova, *Polysaccharides and Polysaccharidases in Food and Agriculture*, 121 (2007).
5. Kh. B. Ashurov, S. T. Azizov, Zh. T. Abdurakhmonov, and A. V. Zinov′ev, Uzb. Pat. IAP 20030229, 2002.