Lipoxygenase-1 Leaching from Soybean Flour Employing Stirred Tank Contactor

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ABSTRACT: *The present work examines leaching of lipoxygenase-1 from soybean flour employing stirred tank vessel. The effect of operating parameters, impellers speed, operational period, temperature, pH and scale-up were considered. The acetic acid pH: 5.2 increased the leaching of lipoxygenase- 1. The sensitivity of agitator speed and geometrical-scale-up on the enzyme leaching has been conducted. The effect of agitator speed on the geometrically- scaled- up reactor has shown that similar amount of enzyme is leached at lower speed. The result is in para with the conventional system. The importance of operational period from 5 to 50 min on the enzyme leaching was evaluated and higher enzyme leaching obtained within 10 min. of operation. The Sigmaplot-6 for statistical verification and developing a correlation for the enzyme leaching was used.*

KEY WORDS: : *Enzyme leaching, Lipooxygenase-1, Operational period, Soybean flour, Stirred tank contactor.*

INTRODUCTION

The lipoxygenase can be produced from plants, animals and microbial sources; however, production of lipoxygenase-1 from microbial at present is economically less attractive [1]. A soybean contains protein, lipid, cellulose and hemicellulose, sugars, crude fiber, B vitamins, calcium, zinc and is rich in iron [2]. It is reported that the soybean flour remaining after oil leaching contains hydroperoxide lyase, hydroperoxide peroxygenase

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and hydroperoxide isomerase enzymes, proteins, phospholipids and vitamins [3-5]. Aqueous leaching of soybean oil is the most promising in the succession of n-hexane oil leaching, with several technical, socio-economic and environmental benefits [6,7]. The dispersion of solid in liquid in baffled stirred vessel is one of the key issues in the process industries such as oxidation, hydrogenation, ammonolysis, fermentation, wastewater treatment, and typical leaching. Understanding of suspension mechanism of flour slurry by propeller impeller is required for a reliable process prediction conducted in stirred tank reactors. Though the solid suspension has been studied in the past for the solid-liquid system, relatively less information is available on the leaching of lipoxygenase-1 using propeller impellers in stirred vessel. The mass transfer is often rate determining for overall process, which is strongly influenced by the flow generated by impellers in the vessel. In the present study, defatted soybean flour was used for leaching of lipoxygenase-1 employing baffled stirred tank reactors. The soybean lipoxygenases can significantly improve some food quality by off-flavoring the products [8]. The importance of hydroperoxides of soybean lipoxygenase reaction with fatty acids such as linoleic acid or linolenic acids in the production of fine chemicals are available [9]. Lipoxygenase are also useful in bleaching of wheat flour, improving dough rheology, inhibition studies of enzyme, and to catalyze the specific addition of molecular oxygen to polyunsaturated fatty acids yielding 1,3-cis, trans-diene-5-hydroperoxides [10,11]. In particular, *Lius* [12] has reported that the lipoxygenase appears to be regiostereoselective and has a preference to oxygenate certain polyunsaturated fatty acids that produce its conjugated unsaturated hydroperoxides. The lipoxygenase metabolites of arachidonic acid and linoleic acid have been employed in the treatment of diseases such as asthma, allergies and cancers. Further, recent discovery reported by Wecksler et al. has enhanced the possibility to be used for human drug therapy [13]. In view of lipoxygenase-1 beneficial properties, there is considerable interest in developing extracting technique potential for scale-up, which yields reliable quantity of enzyme. Our attention was drawn to the lack of significant experimental data with respect to the effect of propeller type of mixer assembled in a stirred vessel on the leaching of lipoxygenase-1 from soybean flour. Therefore, the effect of operating parameters including stirrers speed, operational period, temperature, pH and the geometrical scale-up on the leaching of lipoxygenase-1 has been examined.

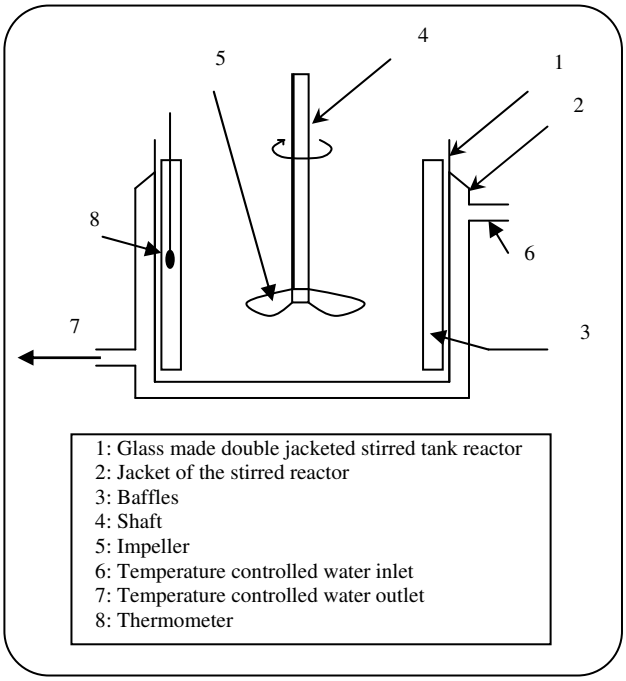

Fig. 1: Schematic diagram of the experimental set- up.

EXPERIMENTAL SECTION

Materials: soybean seed, chemicals and reagents Soybean seed of William's variety, ethanol, and nitrogen gas cylinder was purchased from local market. All other chemicals were of analytical grade and purchased from Merck and Sigma Companies.

Equipment

The schematic diagram of the experimental set-up has been numbered and presented in Fig. 1. A baffled stirred vessel made of glass of 150 mL total volume was used. The propeller impeller assembly was mounted on a shaft and positioned at standard location of 1/3 D_T and driven by Stuart Scientific SS3 A.C motor. A similar reactor of 625 mL total volume was used to study the effect of scale-up.

Preparation of soybean flour and leaching of lipoxygenase-1

The soybean seed was sieved, washed, dried and milled at about 40 ± 2°C. Analytical grade N-hexane

was employed for leaching of soybean oil from the flour. Immersion type of solvent leaching was employed to defat the soybean flour [2]. The defatted flour was air-dried over night to become solvent free. N-hexane is used because of its leaching power and low boiling point properties. The flour was filled in airtight ambered color bottles, stored in an appropriate place (4 ± 1°C) and used within a month. Further, five g of defatted flour was introduced into the stirred tank reactor containing 50 mL of medium and agitation terminated at appropriate time. The suspension was centrifuged for 15 min at 16300g, and the precipitate discarded. The pH of the supernatant was adjusted to about 9 using 1 M NaOH. The mixture was heat treated for 5 min at 70 ◦C, the precipitated proteins were discarded and the supernatant used as partially purified lipoxygenase-1.

Analysis

Substrate preparation

To prepare 25 mL of linoelic acid of 10 mM, 90 mg of Tween 20 and 70 mg of linoelic acid was, respectively, added into oxygen free deionized water. In addition, the medium was stirred and nitrogen gas bubbled to 0.55 mL of 0.5N NaOH. Oxygen free deionized water was added till its total volume became 25mL. The LOX-1 measurement method reported by Axelerd et al. and modified by Sigma Company has been used to analysis its hydrolytic action on linoleic acid [5]. A control solution without the supernatant was run in tandem. Further, 0.1 mL each of supernatant, and substrate, and 2.8 mL of buffer were mixed in quartz cuvette by a plastic stirrer for few seconds prior to measurement. The enzyme activity has been expressed in terms of 0.001 changes in A_{234} per min at pH 9 and 25°C, where 0.1 mL of enzyme mixture after heat treatment and recovery was added in a 3.00 mL of reaction volume employing UV-Shimadzu 2101PC continuous spectrophotometer. One unit of enzyme is the amount of enzyme, which catalyzes the formation of 1 μmole of hydroperoxides of linoleic acid per min at specified conditions. Protein determination was carried out using the Bradford method [14].

RESULTS AND DISCUSSION

Leaching of edible oil by N-hexane compare to alcohols has the advantages such as high power of leaching, low boiling point and ease of evaporation [15]. Alcohols have disadvantage of higher latent heat of vaporization compared to N-hexane. Further, there is a wealth of available information on the use of N- hexane at industrial scale, therefore we used it for soybean flour oil leaching despites its slight toxic effect [16].

Effect of temperature

The effect of temperature on lipoxygenase-1 leaching revealed that as the temperature was increased from 4 to 10 ºC, with 10 ºC intervals up to 40 ºC, the leached enzyme in terms of activity increased and has been presented in Fig. 2. Further, as the medium temperature was increased from 40 to 50 ºC, no significant effect on the enzyme activity was detected.

Effect of agitator speed

The developed flow in the mixing vessel is associated with shape factors and physico-cheimacl properties of the reactor content. The turbulence of the medium, velocity of the circulated fluid and flow rate affect the effectiveness of dispersion and mixing operations in a reactor. Turbulence and circulation increase with impeller speed, however, type and size of stirrer influence the flow rate in the form of developed flow and power consumption. The sensitivity of stirrer speed in a wide range of operations from 1.66 to 10 rps on the enzyme leaching was investigated and has been reported in Fig. 3. As illustrated, with increase in impeller speed from 1.66 to 5 s^{-1} the enzyme activity increases, which indicate that the rate of mass transfer also increases. Moreover, increasing the stirrer speed to 10 rps has not shown any significant effect on the enzyme leaching. The mass transfer in the form of the solid phase (soybean flour) to the liquid phase enhances due to increase in stirrer speed [17-20]. However, *Kikuchi et al.* have shown that in a solid-liquid system the mass transfer coefficient in turbulent flow stirred tank increases by the B^{th} power of the contactor speed [21]:

$$Sh = 2 + A\left(\varepsilon^{1/3} dp^{3/4} / \nu\right)^{B} Sc^{1/3} \quad (1)$$

Where, A, and B are constants. In addition, dp is particle diameter, (m), and Sh is the Sherwood number, (-), and Sc the Schmidt number, (-). ν efers to velocity, (m / s), and ε refers to dissipation energy (w), which is usually proportional to the third power of the stirrer speed in the turbulent flow stirred tank. Therefore, the mass transfer

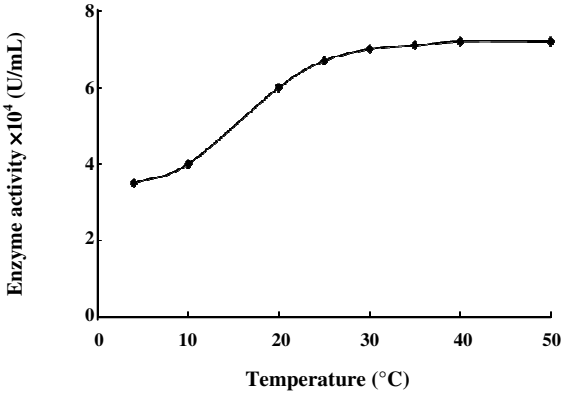

Fig. 2: Effect of temperature on extraction of lipoxygenase-1.

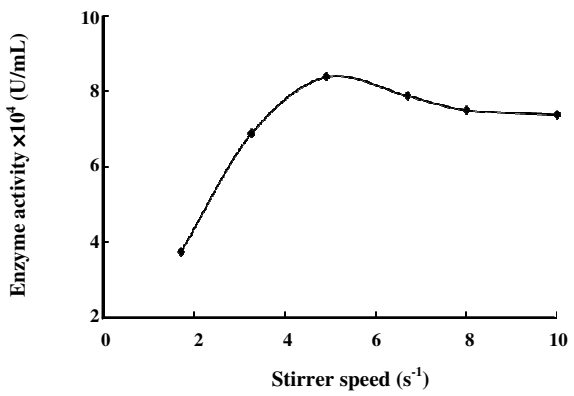

Fig. 3: Effect of stirrer speed on extraction of lipxygenase-1.

coefficient k_L, (m / s) increases by the B^{th} power of the agitator speed. High mass transfer rate has been observed at about 5 rps, which indicates that full velocity profile has been developed in the vessel and by increasing the agitator speed does not affect the external mass transfer resistance [22]. However, increasing the stirrer speed to 10 rps did not significantly affect the enzyme leaching. In addition, the mass transfer of solid–liquid is influenced by several factors such as flow pattern [23, 24], shear of impeller and complex behavior of dissipation energy [25, 26]. Further, slight enzyme losses have been detected at high agitation speed for example 10 rps, which may be due to deactivation of enzyme at the gas-liquid interface. The present result is consistent with those reported by other workers [27, 28], considering variation due to raw materials and solvent used for different enzymes leaching.

Effect of operational period

The effect of operational period from 5 to 50 minutes on the enzyme leaching is depicted in Fig. 4. It is depicted that as the operational period is increased from 5 to 10 min, the enzyme activity considerably increases. The study was performed at about 5 revolutions per second. During the initial 5min of operation, the amount of enzyme leached was low and the results were inconsistent and irreproducible. It was further concluded that the highest enzyme activities can be obtained at about 10 minutes of operational time. It appears that 5 min of operation is insufficient for the medium to reach equilibrium. The soya flour subjected to leaching has reached equilibrium within 10 minutes of operation like solid-liquid system.

Effect of pH

The effect of 5 g of defatted flour in approximately 50 mL of medium made of different species viz., acetic acid, sodium acetate, sodium phosphate, sodium borate, and sodium propionate on the LOX-1 leaching was studied. The result of the present work suggests that certain acidic medium at about pH 5.2 has considerable effect on the enzyme leaching, which may be due to sound solubility of LOX-1 in such aqueous medium. Further, sodium borate and basic buffer of sodium phosphate have indicated inverse effects on the enzyme leaching. The leaching of the enzyme in terms of activity using sodium propionate and sodium acetate was much lower than using acetic acid medium.

Correlation developed

The influence of operational period, temperature and agitator speed on the leaching of the enzyme is presented by:

$$U = 0.0001\, t^{0.167}\, T^{0.29}\, r^{0.95} \quad (2)$$

Where r refers to agitator speed (s^{-1}), t is operational period (s), T is the operating temperature (K), and U is the enzyme activity (U/ mL). The correlation coefficient (R^2) of the equation equals to 0.97. The correlation (2) agreement is effective (S.D. 6.9), and captures the effect of parameters, since the trends in the data are consistent and there is little scatter.

Effect of geometrical scale-up

For industrial applications it is often useful to study the scale up factor for the characterization of operational condition. The effect of agitator speed in scaled-up vessel

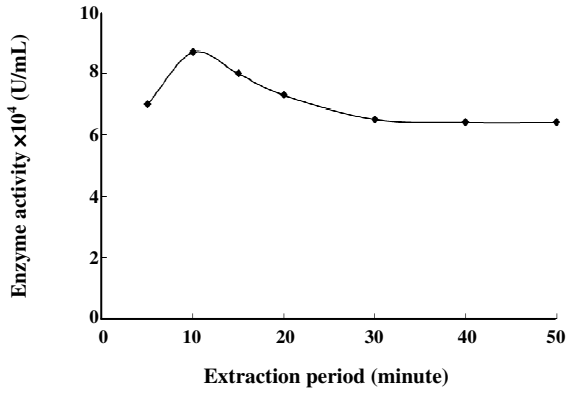

Fig. 4: Effect of operational period on extraction of lipoxygenase-1.

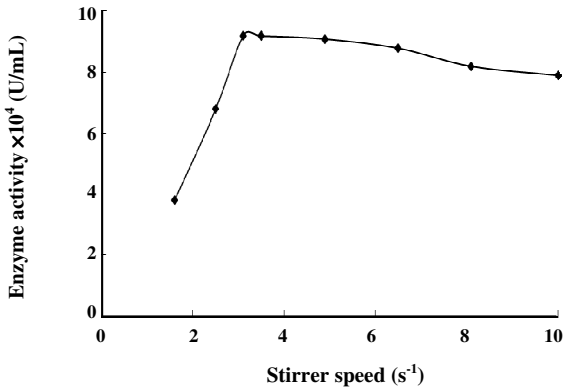

Fig. 5: Effect of stirrer speed on extraction of lipoxygenase-1 in scaled-up stirred vessel.

of 500 mL effective volume containing 50 g of defatted flour from 1.66 to 10 rps on lipoxygenase-1 leaching has been presented in Fig. 5. As shown, the amount of enzyme leached in the scaled up vessel at 3 rps equals that of the smaller vessel at 5 rps. This is because when the scale-up factor is the same, equal amounts of the enzyme is leached, which may be due to full axial development through the whole vessel at lower speed of 3 rps for a different type of enzyme and medium conditions [21]. Further, increasing the agitator speed from 3 to 10 rps has shown no significant effect on the enzyme leaching.

CONCLUSIONS

The process demonstration results showed that medium pH 5.2 and temperature 40 ºC have potential effects on the enzyme leaching. During 10 min of operation and at 5 rps higher enzyme was extracted. As the geometrical scale-up was performed, similar amount of lipoxygenase-1 was extracted at lower speed of 3 rps. The correlation coefficient is higher than 0.97 and the trends in the data are consistent and there is little scatter. Since a well-developed velocity profile at 3 rps in the larger reactor of similar identification is likely the reason. Overall, this work demonstrates the potential for application of propeller mixing assembly in a baffled stirred vessel providing a simple and effective combination with added advantages of ease of geometrical scale-up, for extraction of lipoxygenase-1from naturally available compound such as soybean flour. We presume this combination could be used at industrial scale since it covers operational simplicity, low cost, high yield of products and applicability to large scale leaching.

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