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Leather bioprocess intensification: Ultrasound assisted novel enzymatic hair-loosening system for leather processing

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The use of enzymes for hair loosening in leather processing provides a cleaner option. However, there is need to improve the efficiency of these unit operations involving bioprocess by way of process intensification techniques, such as, the use of ultrasound. In this paper, the use of ultrasound has been studied in native enzyme papain (A) based hair-loosening system and compared with commercially available enzyme Microdep-C (B), combination of enzyme system (A+B) and conventional Na_2S system. Various process parameters, such as, enzyme concentration, time and ultrasound power on the hair-loosening efficacy, were studied. Enzyme concentration (5-6%) and ultrasound (100 W) were found to give better hair loosening under given process conditions. The results indicated that papain based unhairing system was better and the use of ultrasound provided better hair-loosening efficacy. The reason for the ultrasound driven enhancement could be due to better diffusion of enzymes through skin matrix to reach the hair-bulb cite for interaction and dispersion of unhairing formulation employed. Thus, the present process provides novel, eco-friendly, enzymatic unhairing system in leather processing based on native enzyme or a combination of enzyme system.

Keywords: Animal skin, bio-processing, enzymatic process, diffusion, hair-loosening, leather processing, sonochemistry, ultrasound

Introduction

In recent years, enzyme aided bioprocessing of leather provides potential alternative to chemical based processing. In fact, there is a need for developing viable, cleaner bioprocessing techniques for unhairing because of the growing environmental concern in leather processing industry. Sodium sulphide, which is conventionally used as sharpening (unhairing) agent in liming process, poses environmental problems[1]. Consequently, unhairing process has been given wide attention for development of sulphide-free processes in leather making. Enzymes useful in degrading proteins (other than collagen) or proteoglycans, present in hair root cementing substances, are applied for unhairing process. Several eco-friendly unhairing systems have been studied earlier for their potential benefits and reviews on the subject matter have been available in the literature[1-3]. Enzyme based applications are useful but also involve diffusion and distribution problems in the skin/hide matrix. Since ultrasound has been found to improve the diffusion of substances through the skin/leather matrix[4], there is a need to improve enzymatic systems more effective by way of augmentation technique with the use of ultrasound. Ultrasound has been reported to give enhancement in the activities of -amylase and invertase towards hydrolysis of starch and sucrose, respectively[4]. The positive effect of ultrasound on enzyme-based bioprocesses has also been studied[5-7] and several reviews on the use of ultrasound in leather processing are available[8-10]. In the present paper, the use of ultrasound in enzymatic unhairing systems has been studied in order to harvest the benefits of ultrasound for intensification of bio-processing of leather, presently named as sono-bio-leather technology. Enzyme papain known for meat tenderization as well as commercially available unhairing enzyme Microdep-C has been studied for hair loosening efficacy under ultrasonic field.

Materials and Methods

Experimental Set-up

Ultrasonic (US) experiments were performed using ultrasonic probe (VCX 400, Sonics and Materials, USA, 20 kHz and 0-400W) in a glass vessel with provisions to set required output power time and

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temperature. Control experiments were performed in absence of ultrasound keeping the unhairing formulation similar to ultrasonic experiments. US and control experiments were carried out under stationary condition with temperature maintained at around 32°C.

Purified papain (CASR NO. 9001-73-4) was purchased from S D Fine Chemical Pvt. Ltd., India. Commercial unhairing enzyme Microdep-C was obtained from Textan Chemicals Pvt. Ltd., India. Commercial grade sodium sulphide flakes (60% purity) and lime powder (70% purity) were used for the experiment. Distilled water was used in all the unhairing experiments.

Process Details (% based on soaked wt)

Fresh goatskins from a slaughter house based on Chennai were collected. Skins were cut opened, trimmed and thoroughly washed in water. Common salt (30%) was applied on the flesh side of the skin and preserved under refrigerated condition in order to avoid any hair slip. Skins were ensured for the absence of any hair slip before taking in to various experiments. Skins were cut in to a 6×6 cm^2 size parallel to backbone for comparison of the processes with and without ultrasound following the IUP/1 prescribed method of sampling and analysis[11]. The individual skin sample was marked for different experimental trails and weighed. Further, the skins were soaked in (300%, w/w on salted skin wt) plain water for 3 h with three changes of water[1]. The skins were taken out and water from the skins was allowed to drain out. Then individual weight of the skin was noted as soaked wt for further processing. For all the experiments, the individual skin was taken in a clean glass beaker, into which 300% (w/w) water and 5% lime were added. For unhairing studies, individual skin was treated with papain (2-6%), Microdep-C (2-6%) and sodium sulphide (Na_2S; 3%) individually or in a combination of these enzyme systems. Further, each individual enzyme treatment or a combination of these enzyme treatments were subjected to various US power (60, 80 & 100 W) and then unhairing process was compared with the control process. In case of US experiments, ultrasonic treatment was given for 6 h then kept overnight without ultrasound. For all the experiments, ease of unhairing was checked after 6 h of treatment and after overnight (15 h).

Unhairing (hair-loosening) Index Scale (UI)

The ease of hair loosening from skin was manually checked during the course of unhairing process and it was indexed (UI) on the basis of 1-5 scale and then transformed into % unhairing efficacy as shown in Table 1.

Results

Unhairing efficacy (%) of enzymes papain (2-6%) and Microdep-C (2-6%) was studied and compared with traditional system of Na_2S with and without US treatment (60, 80 & 100 W). The results indicate that 5-6% of both papain and Microdep-C provided good loosening of hair (Table 2). Therefore, 5% of papain and Microdep-C was taken as their optimum concentration for the hair loosening process (only optimal results are shown). However, when these results were compared with conventional Na_2S (3%), latter was found superior. Further, it has been observed that 100 W US treatments improved the unhairing efficacy of both the enzymes and Na_2S (Table 2).

Table 1—Unhairing index (UI) scale based on ease of hair-loosening

UI scale (% efficacy)	Hair-loosening ease
1 (1-20)	No loosening
2 (21-40)	Moderate loosening
3 (41-60)	Good loosening
4 (61-80)	Very good loosening
5 (81-100)	Excellent loosening

Table 2—Effect of ultrasound (60, 80 and 100 W) with enzyme papain, Microdep-C and Na_2S under optimum concentrations on hair loosening as compared to control (without ultrasound) after 6 and 15 h of treatment

Enzyme (%)	Optimum conc. (%)	Unhairing efficacy (%)							
		After 6 h				After 15 h			
		Ultrasound power			Control	Ultrasound power			Control
		60W	80W	100W		60W	80W	100W	
Papain	5	60	60	80	60	60	80	100	61-80
Microdep-C	6	60	60	80	60	60	80	100	81-100
Na_2S	3	81-100	81-100	81-100	60	100	100	100	80

Table 3—Effect of combination of enzyme systems and influence of ultrasound (60, 80 and 100 W) on hair loosening as compared to control (without ultrasound) after 6 and 15 h of treatment

Combination process Papain (P), Microdep-C (M), Na_2S (S) (-%)	Unhairing efficacy (%)							
	After 6 h				After15 h			
	Ultrasound power			Control	Ultrasound power			Control
	60W	80W	100W		60W	80W	100W	
P-2+M-2	40	40	60	40	60	60	80	60
P-2+S-2	100	100	100	80	100	100	100	80
M-2+S-2	40	60	100	80	60	80	100	80
P-1+M-1+S-1	80	80	100	81-100	100	100	100	81-100
P-3+M-3	20	40	60	20	40	40	80	60
P-3+M-3+S-0.5	60	60	100	60	80	80	100	80

Effect of combination of systems with papain (P), Microdep-C (M) and Na_2S (S) on unhairing was studied with and without ultrasound and the results are presented in Table 3. The results indicate that combination system with P (1%)+M (1%)+S (1%) gave the 100% unhairing efficacy with both ultrasound and control processes. Similar efficacy of unhairing was also observed with 100 W US in combinations with P (2%)+S (2%), M (2%)+S (2%) and P (3%)+M (3%)+S (0.5%).

Discussion

Normally, hair-loosening in animal skin is practiced as one of the unit operations in leather processing and it has been taken as a model to study the enzyme based processes under ultrasonic field. In the present study, enzymes papain and Microdep-C were studied in unhairing process of goatskin. The results indicate that 5-6% use of these enzymes provided good hair loosening after 6 h of enzyme treatment and further ease of hair loosening could be achieved in 15 h time. The use of ultrasound (100 W) on enzyme based unhairing systems gave significant improvement to papain as well as various combination systems after both 6 and 15 h under the given process conditions (Table 2). Thus, use of ultrasound provided improvement in the efficiency of the native enzyme based unhairing processes. In case of combination of enzyme systems, papain (1%)+Microdep-C (1%)+Na_2S (1%) was found most effective for both ultrasound and without ultrsound processes. A similar efficacy of unhairing was also observed with 100 W ultrasound in combinations with papain (2%)+Microdep-C (2%), Microdep-C (2%)+Na_2S (2%) and papain (3%)+Microdep-C (3%)+Na_2S (0.5%). The general properties of resultant leather produced from these enzyme-based systems with ultrasound were also found to be good. Therefore, ultrasound application afforded either minimization of percentage enzyme or process time in order to effect good hair loosening. The reasons for the enhancement with ultrasound could be due to, i) improved diffusional mass transfer of enzymes through the skin matrix under the ultrasonic field, so that enzymes could reach the hair root cementing substances target site and perform the hair loosening action in a better manner; ii) better dispersion of enzyme based unhairing formulation due to sonication helping diffusion process; and iii) possible enhancement in enzyme activity due to ultrasound under the given process conditions, which needs to be further studied. Our recent publication[12] indicates significant decrease in the aggregate size of commercial unharing enzyme due to the use of ultrasound. Therefore, the use of ultrasound assisted native enzyme based unhairing system could be a potential viable option for cleaner leather processing for effective development of sulphide free unhairing system. The present study gives clue for the enhancement in enzyme-based hair loosening system using ultrasound and effectiveness in diffusion process of generic biomolecules through substrates.

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