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RESEARCH ARTICLE

CHARACTERIZATION AND FERMENTATIVE UTILIZATION OF TANNERY FLESHINGS USING *LACTOBACILLUS PLANTARUM*

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ABSTRACT

Solid wastes create a major problem for leather industry, among which animal fleshing constitutes about 56-60% of the pre-tanned solid wastes. The current work aims at the fermentative utilization of animal fleshing, for its reuse in other industries. Lactic acid fermentation is an *in situ* acid generating technology for recovery of biomolecules from solid wastes. Wet leather fleshings were delimed with 0.1% H₂O₂ and 0.2N HCl and their proximate composition was analyzed. Fleshings were fermented using *Lactobacillus plantarum*. Fermentation of Steam cooked (80°C for 15 minutes) fleshings, mixed with 19.5% (v/w) *L.plantarum*, 20% (w/w) sugar and 2% (w/w) common salt at 30±2°C yielded lowest pH (2.9) and highest lactic acid content (4.05g/L) on 24th day of fermentation. The fermented mixture was lyophilized to obtain a biomass which could be used in livestock/aquaculture feeds. The current work thus highlights the nutritive value of fleshings and their recycling in an eco-friendly manner.

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INTRODUCTION

Leather industry which plays a crucial role in the country's economy, contributes to environmental pollution by dumping the solid wastes into the land and the water bodies. Even though disposal methods such as landfill disposal, thermal incineration and anaerobic digestion are available, they again contribute to pollution and cause additional economic burden to the tanners. Solid wastes generated in leather industries contribute mainly skin trimmings, keratin wastes, fleshing wastes, chrome shaving wastes and buffing wastes that constitute protein as the main component (Kanagaraj *et al.*, 2006). In this present study, Animal fleshing - a proteinaceous solid waste obtained from untanned hides/skins has been focused, as this contributes about 56-60% of the wastes generated from pre-tanning operations in tanneries. Instead of disposing them, it would be better to recycle them and reuse them as a raw material for another industry.

A growing body of research has documented the beneficiary effects of few microbes leading to the evolution of probiotics. The concept of probiotics was evolved around 20th century. Probiotic by definition is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller *et al.*, 1989). An expert with the Joint Food and Agriculture Organization of the United Nations / World Health Organization (FAO/WHO), stated that probiotics are live microorganisms which when consumed in adequate amounts, confer a health benefit for the host

(FAO/WHO, 2001). Among the probiotic bacteria used, the lactic acid bacteria stand out for their easy multiplication, production of antimicrobial compounds (bacteriocins, hydrogen peroxide, organic and lactic acids), and the stimulation of the non-specific immune response of the host (Gatesoupe, 2008). They have been used as starter cultures for various fermented products such as yoghurt, cheeses and sausages, helping in lowering of the food pH and so preventing the growth of spoilage microbes. Lactic acid fermentation is a simple but well developed acid generating technology which was used to recycle carcasses since 1984. This fermentation produces end products which are pathogen-free as well as nutrient rich. Lactic acid - a natural, low-pH, effective preservative helps in the preservation of the wastes through ensilation and also helps in the recovery of biomolecules from various kinds of solid wastes (Jini *et al.*, 2011). The present study aims at i) analysis of physical parameters of the limed as well as delimed fleshings; ii) proximate composition analysis of the fleshings; iii) fermentation of the fleshings using a probiotic bacterium *Lactobacillus plantarum* and to lyophilize the fermented flesh mixture to obtain a nutrient-enriched biomass as a final product.

MATERIALS AND METHODS

Sample Collection

Pre-tanned Animal fleshings (Limed Fleshings [LFs]) were collected in sterile polythene covers from E.K.M Leather

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Processing Company in Erode District of Tamil Nadu, India. The samples were transferred immediately to the laboratory for further analysis.

Physical analysis of limed fleshings

The Physical analysis of LFs was followed by [A.P.H.A, 2005](#) method. Physical parameters like pH, temperature, colour, odour, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), and Total Solids (TS) were analysed for the LFs immediately after it has been transferred to the laboratory.

Deliming of Limed fleshings

Deliming of LFs was accomplished by [Bhaskar et al., 2009](#) method. Minced fleshings were dispersed in potable water at 1 : 10 (w/v) containing H₂O₂ (0.1% v/v of wash water). Material was allowed to stand for 30 minutes with occasional stirring before draining liquid to collect solids. Collected solids were again subjected to H₂O₂ treatment and water drained after 30 minutes. Obtained solids were then treated with 0.2N HCl prepared in demineralized (DM) water (1 : 10 w/v) containing H₂O₂ (0.1% v/v of wash water). Material was allowed to stand for 30 minutes before draining solution and repeated treatment with water containing HCl and H₂O₂. Solids obtained after this treatment were termed as Delimed Tannery Fleshings (DFs).

Physical analysis of Delimed fleshings

The Physical analysis of DFs was followed by [A.P.H.A, 2005](#) method. Physical parameters like pH, temperature, colour, odour, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), and Total Solids (TS) were analysed for DFs immediately after it has been delimed.

Characterisation of Delimed fleshings

The delimed fleshings were analysed for the total nitrogen, crude protein, total fat, carbohydrate, moisture, ash, and crude fiber content.

Total nitrogen and protein content of delimed fleshings

The total protein content of DFs was determined by [Kjeldahl](#) method [A.O.A.C, 2005](#). The sample was first digested in H₂SO₄, using Na₂SO₄ and CuSO₄ as catalyst, at 450°C for 2 and half hours converting all N to NH₃ and the ammonia was then distilled and titrated against 0.1N HCl. The appearance of violet colour was found to be the end point.

The % of Nitrogen present in the DFs was calculated using the following formula –

$$\% N = \frac{\text{Burette reading} \times \text{Normality of HCl} \times 1.4007}{\text{Weight of the sample (g)}}$$
$$\% P = \% N \times 6.25$$

Total fat content of delimed fleshings

The Total Fat content of DFs was determined using [A.O.A.C, 2005](#) method. About 1g of the DF sample was placed in the

Soxhlet extraction apparatus and 150 ml of petroleum ether was added and the sample was extracted for 6 hours at 60-80°C. It was then allowed to cool and the ether was evaporated. The weight of the extraction flask along with the dried extract minus the weight of the empty extraction flask gives the weight of the total fat being extracted.

The % of total fat in DFs was calculated using the following formula –

$$\text{Total Fat \%} = \frac{\text{Weight of the fat (g)}}{\text{Weight of the sample (g)}} \times 100$$

Carbohydrate content of delimed fleshings

The carbohydrate content of DFs was estimated based on [Hedge et al., 1962](#) method. 1g of the sample was taken and it was homogenized and centrifuged. To the clear supernatant (1ml), 4 ml of anthrone reagent was added and the test tubes were kept in a boiling water bath for 8 minutes. The test tubes were taken out and cooled rapidly and finally the colour developed was measured at 630 nm in a spectrophotometer.

The % of Carbohydrates in DFs was calculated using the following formula –

$$\% \text{ Carbohydrates} = \frac{\text{Concentration of Carbohydrates in the sample (mg)}}{\text{Volume of the test sample (ml)}} \times 100$$

Moisture content of delimed fleshings

The moisture content of DFs was determined by [A.P.H.A, 2005](#) method. About 1g of the wet DF was taken on previously weighed concave glass and was kept in a hot air oven at 105°C for about 5 hours. After drying, it was cooled in a dessicator and weighed.

The % of moisture content was calculated using the following formula –

$$\% \text{ Moisture} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100$$

Ash content of delimed fleshings

The ash content of DFs was determined by [A.P.H.A, 2005](#) method. About 1g of the oven dried DF was taken in a silica crucible. The crucible containing the sample was ignited on the hot plate till the sample gets charred. Then the crucible along with the sample was kept in a muffle furnace and heated at 550-600°C for 4 h. Finally, it was allowed to cool and the ash formed was weighed.

The % of ash content was calculated using the following formula –

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Crude fiber content of delimed fleshings

The crude fiber content of DFs was determined by A.O.A.C, 2005 method. About 1g of the sample was taken and 100 ml of 1.25% sulfuric acid was added into it and was boiled for 30 minutes, filtered and washed several times with hot water till it is free from acid. Then 100ml of 1.25% Sodium hydroxide solution was added into the acid free sample and was boiled, filtered and washed several times with hot water till it is free from alkali. It was then dried in the hot air oven at 105°C. The dry weight of the sample was noted. Finally, the dried sample was transferred into a crucible and ignited in muffle furnace at 550-600°C for 2 hours. It was then cooled in a dessicator and the weight of the ashed sample was noted.

The % of Crude Fiber in DFs was calculated using the following formula –

$$\% \text{ Crude Fiber} = \frac{\text{Weight aft drying} - \text{Weight after ashing}}{\text{Weight of the sample}} \times 100$$

Fermentation of delimed fleshings

Lyophilized culture of *Lactobacillus plantarum* (NCDC No-025) was obtained from National Dairy Research Institute, Karnal. DFs were ground into smaller pieces to facilitate fermentation (Johnston *et al.*, 1998). Distilled water was added into the chopped fleshings in the ratio of 1 : 1 and the fleshings were steam cooked at 80°C for 15 minutes (Amit *et al.*, 2009). Overnight culture of *Lactobacillus plantarum* in MRS brot (19.5% v/w) and sugar (20% w/w) were added. Common salt (2% w/w) was added and the fermentation mixture was held at 30 ± 2°C for 30 days with occasional mixing daily. pH, and the lactic acid content was monitored once in every 6 days.

The amount of lactic acid present in the fermented mixture was determined by transferring 20ml of the fermented mixture into a conical flask. About 1ml of phenolphthalein indicator was added into the flask. This was titrated against 1N NaOH (standardized) for the appearance of pink colour.

The amount of lactic acid produced in gm/litre of the fermented mixture -

$$= \frac{\text{Strength of NaOH} \times \text{Titre value} \times \text{Gram Equivalent weight of lactic acid}}{\text{Volume of the Sample used for each Titration}}$$

Lyophilization

The fermented flesh mixture was subjected to Lyophilization. It was performed using VIRTIS Lyophilizer to obtain biomass.

RESULTS

Instead of disposing the animal wastes from tanneries, which are made up of proteins and lipids, an alternative method of converting them into an usable form is being executed in the present study.

Physical analysis of limed fleshings

Data of the physical analysis of the sample (LFs) is tabulated as follows –

Table 1Physical Parameters of Limed Fleshings

Serial No.	Parameter	Obtained values	Indian Standards
1.	Colour	Greyish brown	--
2.	Odour	Pungent	--
3.	Ph	12	5.5-9.0
4.	Temperature	30°C	45°C
5.	Total Suspended Solids(TSS)	954 mg/L	600mg/L
6.	Total Dissolved Solids(TDS)	20,000 mg/L	2100mg/L
7.	Total Solids	20,975 mg/L	2700mg/L

All the parameters were above the permissible limits laid down by the Central Pollution Control Board (CPCB).

Deliming of limed fleshings

Table 2 Deliming of Limed Fleshings

S.No	No. of Washes	pH	H ₂ S Smell
1	1*	11.6	Strong
2	2*	10.9	Moderate
3	3*	8.35	Mild
4	4*	6.87	No Smell

*Wash with 0.2 N HCl and 0.1% (v/v of wash water) of H₂O₂

Physical analysis of delimed fleshings

Data of the physical analysis of the sample (DFs) is tabulated as follows –

Table 3 Physical parameters of Delimed Fleshings

Serial No.	Parameter	Obtained values	Indian Standards
1.	Colour	Pinkish brown	--
2.	Odour	Normal	--
3.	pH	6.8	5.5-9.0
4.	Temperature	30°C	45°C
5.	Total Suspended Solids(TSS)	215 mg/L	600mg/L
6.	Total Dissolved Solids(TDS)	2000 mg/L	2100mg/L
7.	Total Solids	2215 mg/L	2700mg/L

After deliming, all the physical parameters were within the permissible limits laid down by the Central Pollution Control Board (CPCB). Thus indicating complete removal of lime from the fleshings.

Characterisation of delimed fleshings

Data of characterisation of Delimed Fleshings is as follows –

Table 4 Characterisation of Delimed Fleshings

Serial No.	Characterised parameters	Obtained Value (%)
1.	Total Nitrogen Content	2.5
2.	Crude Protein	15.6
3.	Total Fat	26.1
4.	Carbohydrates	4.3
5.	Moisture	80
6.	Ash	18
7.	Crude Fiber	1.1

Fermentation of delimed fleshings

Cooked delimed fleshings became more fragile and suitable for feasible fermentation. Thermal Hydrolysis of collagen took

place leading to structural changes where protein molecule was hydrolyzed into smaller peptides. **Fermentation of delimed fleshings with *Lactobacillus plantarum* produced lowest pH (2.9) and highest lactic acid content (4.05g/L) on 24th day of fermentation.** A small decrease in the lactic acid content was found at the 30th day of the fermentation, hence, the fermentation was stopped, and the fermented mixture was lyophilized.



1st Day of fermentation



16th Day of fermentation



24th Day of fermentation

Figure 1 Changes occurring during fermentation of Delimed Fleshings

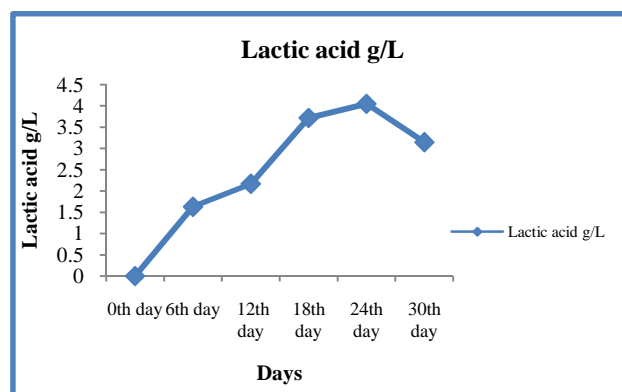


Figure 2 Lactic acid content of the fermented flesh mixture:

Lyophilization

The fermented flesh mixture was lyophilized. From 1 kg of the mixture, around 650g of semi-solid, nutrient enriched biomass of fleshings was obtained, which may be used as a raw material (ingredient) for feed industries.

DISCUSSION

As the pH and the total solids of limed fleshings were higher than the Indian Standards (Table 1), Deliming was performed using 0.2N HCl and 0.1% H₂O₂. Hydrochloric acid is a strong acid that deactivates pathogenic micro organisms and also reduces the pH of LFs by removing the lime content. Hydrogen peroxide is a very powerful bactericide and viricide that possess no undesirable residues, improves feed efficiency and is environmentally beneficial (Rach *et al.*, 1997).

Proteins are large, complex molecules made up of amino acids which are essential for growth and repair of tissues and to provide energy. The nitrogen content (2.5%) obtained was multiplied with 6.25 (Protein factor) to know the crude protein content of the sample (Table 4). **Crude Protein % and 15.6**, which was higher than the values obtained by Bhaskar *et al.*, 2007 (6.9% only). Similarly, the fat content obtained by Bhaskar *et al.*, 2007 was also lower (7.5%) than the value obtained in the present study (26.1%).

Fermentation using Lactic acid bacteria has a better impact than other conventional methods like acid ensilaging, as it was eco friendly, cost effective and also provided beneficial effects like antibacterial activity and antioxidative properties. LAB was best known as starter culture due to their versatile metabolic characteristics such as acidification activity, proteolytic activity and synthesis of metabolites like bacteriocin (Jini *et al.*, 2011). Fall in pH during fermentation of fleshings was due to decrease in sugar content and increase in lactic acid content.

Lyophilization, also called as Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. This technique maintained the stability of each and every component of the sample in its native form, thus eliminating the need for refrigeration of the final product.

CONCLUSION

Delimiting made the fleshings vulnerable for fermentation. Fermentation of the animal fleshings with a probiotic bacterium *Lactobacillus plantarum* is an eco-friendly method due to their GRAS status. However toxicological studies has to be performed before using this biomass as a feed ingredient for animals. If in the case of fats, they could be transesterified and used in biodiesel production or used as such, in glue manufacturing industries. Biogas production in an eco-friendly manner using fleshings as a substrate for anaerobic digestion could also be encouraged. Thus, fermentative utilization of tannery fleshings will be a promising green technology to reuse and recycle them for other industries and this will decrease the problems faced by the tanners and their economic burdens for disposal.

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