



## International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2026; 11(2): 23-31

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Received: 02-12-2025

Accepted: 22-01-2026

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## Utilization of microbial lipases for bone degreasing in gelatin preparation

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**DOI:** <https://www.doi.org/10.22271/veterinary.2026.v11.i2a.3015>

### Abstract

One of the main basic materials used to prepare gelatin is the cattle bone. There are numerous additional biological contaminants, such as leftover protein and fat, in the crushed bones. In order to determine the quality and characteristics of gelatin that would satisfy the intended purpose, collagen purity is crucial. Purified collagen is typically obtained by removing unwanted contaminants through chemical and physical pre-treatment. Regular chemical pre-treatment has the drawbacks of causing environmental contamination and bone deterioration. The employment of enzymes lessens the adverse effects on the environment by lowering the amount of chemicals, energy, wash water and waste that is subsequently produced. These days, a wide range of industries use lipases extensively as degreasing agents. There were three stages of the study viz., Temperature optimisation for incubation; concentration of lipase; microbial lipase incubation period, leading to the development of a standard for the lipase enzyme-based degreasing of crushed bone. The effectiveness of fat reduction percentage before and after enzyme therapy was essentially the basis for selecting the ideal conditions throughout the investigation. The results indicated that incubation temperature of 47.5 °C, lipase concentration of 1% of bone weight and incubation duration of two hours had significantly beneficial effect on lipase activities in degreasing of crushed bone. Using the standardised procedure, the fat content of bones was found to be reduced by 22.42% when subjected to the action of microbial lipase. Thus, the enzyme lipase can be used as one of the best options for degreasing of the crushed bones.

**Keywords:** Microbial lipases, incubation temperature, incubation period, concentration, degreasing, bones

### 1. Introduction

The demand for gelatin is increasing worldwide especially in food and pharma industry. Gelatin is a product obtained by the partial hydrolysis of collagen, derived from the white connective tissue, bones and skin of animals. Type A gelatine is derived from an acid-treated precursor and Type B gelatine is derived by alkali-treatment (U.S. pharmacopoeia, 2011). The gel forming quality of gelatin is one of its important physical properties. The gelling ability is directly proportional to amount of gelatin present in solution, which varies according to different applications. The behaviour of gelatin gels is influenced by concentration of gelatin, pH and temperature (Schreiber, 1976)<sup>[63]</sup>. The most important property that makes gelatin very favourable to be used in the food industry is its low melting temperature, molten at human body temperature and has higher gel strength, when compared to other common gelling agents (Boran *et al.*, 2010)<sup>[2]</sup>. The uniqueness of the gelatin is its ability to form thermos-reversible gels with a melting temperature close to body temperature and its solubility in water (Zhou *et al.*, 2006)<sup>[80]</sup>. Johnston (1990)<sup>[25, 26]</sup> reported that the properties of gelatin such as gel strength, viscosity, setting behaviour and melting point depend on their molecular weight and amino acid composition. The major food applications of gelatin are in confectionery (for providing texture, chewiness and foam stabilization) and jelly desserts (to provide creaminess, fat reduction and mouth feel), in meat products (to provide water-binding) and in dairy products (To provide texturization and stabilization) (Nishimoto *et al.*, 2005; Karim and Bhat, 2009)<sup>[53, 29]</sup>.

Manning and Schryver (1921)<sup>[42]</sup> suggested that the gelatin is extracted by hydrolysis of ossein and by decalcification of bovine bone. Eastoe (1955)<sup>[3]</sup> and Stainsby *et al.* (1977)<sup>[66]</sup> suggested that, gelatin is a fundamental pure protein that can be obtained by controlled hydrolysis of a fibrous insoluble protein collagen which is widely found in bone, skin and connective tissue of mammalian origin. Most of the commercial gelatin is sourced from cattle bone, hides, and pig skins and more recently from various species of fish. The cattle bone is used as one of the principal raw materials for gelatin preparation. The crushed bones mainly contain the extraneous biological impurities like residual protein and fat. The purity of collagen is very important to establish the quality and other properties of gelatin to meet the intended end use. The extraneous substances are removed by chemical and physical pre-treatment to give purified collagen. The pretreatment methods can cause both physical and chemical changes in the collagen molecules. (Haug and Draget, 2009)<sup>[13]</sup>. Pre-treating of the collagenous raw material is generally followed in the commercial line of gelatin preparation to improve rate of extraction and quality of gelatin. The pre-treatment of gelatin involves short time soak in the dilute acid solution or prolonged soak in a saturated lime solution. Although there are several physical and chemical methods available for removal of these biological impurities, certain limitations like longer pre-treatment time affect various physico-chemical properties of the gelatin. Chemical processes used have inherent drawbacks from environmental point of view. The use of acid or alkali during pre-treatment process also needs high capital investment, specially designed equipment and control systems. The disposal of unwanted by-products formed during pre-treatment with chemicals may involve high cost. High energy consumption and chemicals as well as harmful by-products have a negative impact on the environment. Therefore, it has become vitally important to develop new techniques to remove the extraneous impurities using some biological systems. By using enzymes, some or all the drawbacks of using chemical can be eliminated. Enzyme reactions may often be carried out under mild conditions. They are highly specific and involve high reaction rates. As only small amounts of enzymes are needed in order to carry out reactions even on an industrial scale, enzymatic pre-treatment can be one of the best suitable alternative methods to remove the biological impurities attached with bones. The advantages of enzymatic treatment may also include prevention of physical or chemical damage to bones which might happen during routine chemical cleaning process. Enzymes treatment also reduces the negative impact on the environment by reducing the consumption of chemicals, wash water, energy and the subsequent generation of waste. Lipases are now extensively used to remove the fat between collagen fibres during leather processing in many parts of the world and they require no cofactors to catalyze hydrolytic reactions and remain active in the presence of organic solvents (Sangeetha *et al.*, 2011)<sup>[61]</sup>.

## 2. Materials and Methods

### 2.1 Source of materials

Crushed, washed and dried bones obtained from Nitta Gelatin India Pvt. Limited, Koratty, Kerala were used as the raw material during the study of standardization of procedure. Enzyme Lipase (Lipozyme TL 100 L) supplied by Novozymes, India was used as source of microbial lipase for the experiment.

## 2.2 Experimental design

The present study was conducted as three different experiments to optimize three different parameters viz., 1. Incubation temperature for microbial lipase 2. Concentration of the microbial lipase 3. Optimisation of the Incubation duration.

### 2.2.1 Optimising the Incubation Temperature (IT)

Since no literature was available regarding the incubation temperature for degreasing of bone, preliminary studies were conducted at different temperatures (45, 50, 55 and 60 °C) and the lipase activities were found only at temperature ranging from 45 to 50 °C. The incubation temperature (IT) was evaluated by using three different temperatures of incubation in a thermostatically controlled water bath *i.e.*, 45 °C, 47.5 °C and 50 °C keeping the lipases concentration as 1% of the bone weight and time of incubation as one hour. The samples were divided into four groups as follows

- C: control (crushed bone without incubation)
- T<sub>1</sub>: crushed bone subjected to IT of 45 °C
- T<sub>2</sub>: crushed bone subjected to IT of 47.5 °C
- T<sub>3</sub>: crushed bone subjected to IT of 50 °C.

The control and the samples were analysed for fat percentage, Hunter color and weight loss. The most effective IT among T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was selected based on their highest fat reduction percentage in the samples.

### 2.2.2 Optimisation of the concentration of the Microbial Lipase (ML)

Studies were conducted to evaluate the concentration of ML at three different levels *i.e.*, 0.75% (T<sub>4</sub>), 1% (T<sub>5</sub>) and 1.25% (T<sub>6</sub>) of the bone weight. The best selected IT of experiment-1 was kept as incubation temperature for this experiment and incubation time was fixed as one hour. The samples were divided into four groups as follows

- C<sub>2</sub>: Control 2 (T<sub>2</sub>, the best selected treatment of experiment 1)
- T<sub>4</sub>: Crushed bone treated with ML at 0.75% of bone weight and IT of 47.5 °C
- T<sub>5</sub>: Crushed bone treated with ML at of 1% of bone weight and IT of 47.5 °C
- T<sub>6</sub>: Crushed bone treated with ML at of 1.25% of bone weight and IT of 47.5 °C

The control and the samples were analysed for fat percentage, hunter color and weight loss. The best ML concentration among T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> was selected based on their highest fat reduction percentage in the samples. The experiment was repeated six times. Treatment T<sub>5</sub> was selected as best concentration and used for experiment 3

### 2.2.3 Optimisation of the Incubation Period (IP)

Studies were conducted to evaluate the best incubation period by using three different incubation period viz., one hour, two hour and three hours at the best selected IT of experiment:1 and best selected concentration of ML of experiment:2. The sample was divided into three groups as:

- C<sub>3</sub>: Control 3 = T<sub>5</sub>
- T<sub>7</sub>: Crushed bone subjected to 1 hr. IP at IT of 47.5 °C and ML of 1%
- T<sub>8</sub>: Crushed bone subjected to 2 hr. IP at IT of 47.5 °C and ML of 1%
- T<sub>9</sub>: Crushed bone subjected to 3 hr. IP at IT of 47.5 °C and ML of 1%

The control and the samples were analysed for fat percentage, hunter color and weight loss. The best IP among T<sub>7</sub> and T<sub>8</sub> was selected based on their highest fat reduction percentage in the samples. The experiment was repeated six times. Treatment T<sub>8</sub> was selected as the standardised procedure for degreasing of bone using lipase.

## 2.3 Fat percentage

The fat percentage of the bones before and after treatment with lipase was determined by the standard procedure of AOAC (1990) using socsplus apparatus.

### 2.3.1 Fat reduction percentage (FRP)

FRP= (Fat% of sample before treatment) - (Fat% of sample after treatment)

## 2.4 Weight loss percentage

The weights of the crushed bones before and after treating with microbial lipase were recorded.

$$\text{Weight yield (\%)} = \frac{\text{Weight of the crushed bone after enzyme treatment}}{\text{Weight of the crushed bone before enzyme treatment}} \times 100$$

Weight loss (%) = 100 - Weight yield (%).

## 2.5 Hunter lab Colour (L\*, a\*, b\*)

Colour of the enzyme pre-treated crushed bone was determined using Hunter Lab Mini Scan XE Plus Spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination. The instrument was set to measure Hunter L\*, a\* and b\* using illuminant 45/0 and 10° standard observer with an aperture size 2.54cm. It was calibrated using black and white calibration tiles before starting of the measurement and colorimeter score recorded with 'L' of black equals zero and 'L' of white equals 100, 'a' of lower numbers equals more green (less red), higher numbers equals more red (less green) and 'b' of lower numbers equals more blue (less yellow), higher numbers equals yellow (less blue). The colour co-ordinates L\* (lightness), a\* (redness), b\* (yellowness) of the samples were measured thrice and mean values were taken.

## 2.6 Statistical analysis

Each experiment was repeated six times and the data obtained from fat reduction, weight loss and Hunter lab value were statistically analysed as per Snedecor and Cochran (1994) by repeated measure ANOVA, one-way ANOVA and Kruskal-Wallis test using SPSS software version 24.

## 3. Results

The study was conducted to optimize the conditions required for the action of microbial lipase for the degreasing of crushed bones in gelatin production line. The present study was conducted in three phases, viz., 1. Optimization of incubation temperature 2. Optimization of concentration of microbial lipase 3. Optimization of incubation period of microbial lipase. In all the phases of the study the fat percentage was analysed for least fat retention after the enzyme action. The Hunter colour was determined to compare the colour difference before and after the enzyme action. The weight loss of samples was also determined before and after the enzyme action to evaluate the effect of the enzyme on the weight loss of the samples.

### 3.1. Optimization of the incubation temperature (IT)

Based on the experimental design the incubation temperature, viz., 45 °C, 47.5 °C and 50 °C were taken as three different

actions of lipase. This study was undertaken for selecting an optimum incubation temperature for microbial lipase activity. The best incubation temperature was assessed, primarily on the reduction of fat percentage on the sample.

#### 3.1.1. Fat

The fat percentage of the control and the samples subjected to different incubation temperature are presented in the table 1. The fat percentage of C<sub>1</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 2.21 ± 0.25, 2.28 ± 0.33, 1.52 ± 0.17, 2.17 ± 0.17, respectively. It can be noted that among the treatment samples T<sub>2</sub> showed significantly ( $p < 0.01$ ) lower fat per centage than control C<sub>1</sub> and T<sub>1</sub> and showed no significant difference with T<sub>3</sub>. The control T<sub>1</sub> had significantly ( $p < 0.01$ ) higher and T<sub>2</sub> had significantly ( $p < 0.01$ ) lower fat retention per centage than that of others in the group.

**Table 1:** Effect of incubation temperature on lipase activity

| Treatment      | Fat percentage            |
|----------------|---------------------------|
| C <sub>1</sub> | 2.21 ± 0.25 <sup>ab</sup> |
| T <sub>1</sub> | 2.28 ± 0.33 <sup>a</sup>  |
| T <sub>2</sub> | 1.52 ± 0.17 <sup>b</sup>  |
| T <sub>3</sub> | 2.17 ± 0.17 <sup>ab</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.01$ )

C<sub>1</sub>- Control 1(Crushed bones without incubation temperature)

T<sub>1</sub> - Treatment 1(Crushed bones with IT of 45 °C at 1 hr. IP and ML of 1%)

T<sub>2</sub> - Treatment 2(Crushed bones with IT of 47.5 °C at 1 hr. IP and ML of 1%)

T<sub>3</sub> - Treatment 3(Crushed bones with IT of 50 °C at 1 hr. IP and ML of 1%)

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration

#### 3.1.2. Weight loss

The weight of the samples was measured before and after enzymatic treatment to determine the percentage of weight loss of the samples due to enzyme action and are depicted in tables 2.

The weight loss per centage of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 2.16 ± 1.01, 7.1 ± 2.42, 1.40 ± 0.35 respectively. The weight loss was found to be significantly ( $p < 0.05$ ) higher for treatment T<sub>2</sub> than T<sub>1</sub> and T<sub>3</sub>.

**Table 2:** Effect incubation temperature of microbial lipase on weight loss

| Treatment      | Weight loss%             |
|----------------|--------------------------|
| T <sub>1</sub> | 2.16 ± 1.01 <sup>b</sup> |
| T <sub>2</sub> | 7.1 ± 2.42 <sup>a</sup>  |
| T <sub>3</sub> | 1.40 ± 0.35 <sup>b</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

T<sub>1</sub> - Treatment 1(Crushed bones with IT of 45 °C at 1 hr. IP and ML of 1%)

T<sub>2</sub> - Treatment 2(Crushed bones with IT of 47.5 °C at 1 hr. IP and ML of 1%)

T<sub>3</sub> - Treatment 3(Crushed bones with IT of 47.5 °C at 1 hr. IP and ML of 1%)

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration

#### 3.1.3. Colour

The Hunter colour L\* a\* b\* colour values of the control and the three treatments of crushed bones when treated at different incubation temperatures i.e., 45°C, 47.5°C and 50°C are given in tables 3.

**Table 3:** Effect of incubation temperature of microbial lipase on colour of crushed bones

| Parameters      | C <sub>1</sub>             | T <sub>1</sub>            | T <sub>2</sub>             | T <sub>3</sub>            |
|-----------------|----------------------------|---------------------------|----------------------------|---------------------------|
| L* (lightness)  | 75.47 ± 0.52 <sup>ab</sup> | 73.88 ± 0.42 <sup>b</sup> | 79.07 ± 0.76 <sup>a</sup>  | 69.08 ± 1.11 <sup>c</sup> |
| a* (redness)    | 2.75 ± 0.09 <sup>a</sup>   | -0.13 ± 0.21 <sup>c</sup> | 2.89 ± 0.10 <sup>a</sup>   | 1.69 ± 0.13 <sup>b</sup>  |
| b* (yellowness) | 19.41 ± 0.16 <sup>ab</sup> | 11.92 ± 0.20 <sup>b</sup> | 19.47 ± 0.21 <sup>ab</sup> | 16.46 ± 0.14 <sup>b</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.01$ )

C<sub>1</sub>- Control 1(Crushed bones without incubation temperature)

T<sub>1</sub> - Treatment 1(Crushed bones with IT of 45 °C at 1 hr. IP and ML of 1%)

T<sub>2</sub> - Treatment 2(Crushed bones with IT of 47.5 °C at 1 hr. IP and ML of 1%)

T<sub>3</sub> - Treatment 3(Crushed bones with IT of 47.5 °C at 1 hr. IP and ML of 1%)

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration

The lightness (L\*) value of C<sub>1</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> ranged from 69.08 - 79.07. The redness (a\*) value varied from -0.13 - 2.89. The yellowness (b\*) values ranged from 11.92 - 19.47. Lightness value was significantly ( $p < 0.01$ ) higher recorded in T<sub>2</sub> and T<sub>3</sub> was significantly ( $p < 0.01$ ) lower among all. The redness value was significantly ( $p < 0.01$ ) higher for T<sub>2</sub> and no significant difference was noted among T<sub>1</sub> and C<sub>1</sub> for redness. The yellowness value was significantly higher for T<sub>2</sub> and lowest for T<sub>1</sub>. On the basis this observation it was found that the incubation temperature had influence on the lightness value, the redness and yellowness value.

### 3.2. Optimization of enzyme concentration

Three different levels of microbial lipase, viz., 0.75%, 1% and 1.25% of the bone weight were taken and added in the wash water as per the experimental design. The study was undertaken for selecting the best level of concentration of lipase. The best level was assessed, primarily on the reduction of fat percentage on the sample.

#### 3.2.1. Fat

The fat percentage of the control and the samples subjected to different concentration of lipases are presented in the Table 4. The protein percentage of C<sub>2</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> were 1.50 ± 0.27, 2.44 ± 0.34, 1.39 ± 0.18, and 2.44 ± 0.34, respectively. Among the treatments sample treated with 1% (T<sub>5</sub>) showed significantly ( $p < 0.01$ ) lower fat per centage than T<sub>4</sub> and T<sub>6</sub>. T<sub>6</sub> had significantly ( $p < 0.01$ ) higher and T<sub>5</sub> had significantly ( $p < 0.01$ ) lower fat retention per centage than that of others in the group. There was no significant difference in values of fat percentage. The addition of lipase above 1% did not show significant benefit in cleaning process. T<sub>5</sub> was selected as the best concentration.

**Table 4:** Effect of enzyme concentration of lipase

| Treatment      | Fat percentage           |
|----------------|--------------------------|
| C <sub>2</sub> | 1.50 ± 0.27 <sup>b</sup> |
| T <sub>4</sub> | 2.45 ± 0.34 <sup>a</sup> |
| T <sub>5</sub> | 1.39 ± 0.18 <sup>b</sup> |
| T <sub>6</sub> | 2.44 ± 0.34 <sup>a</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

C<sub>2</sub> - Control 2 = T<sub>2</sub>

T<sub>4</sub> - Treatment 1Crushed bone with ML of 0.75%, at 1 hr. IP and IT of 47.5 °C

T<sub>5</sub> - Treatment 2Crushed bone with ML of 1%, IT of 47.5 °C and 1 hr. IP at 2hrs.

T<sub>6</sub> - Treatment 3Crushed bone with ML of 1.25% at IT of 47.5 °C and 1 hr. IP at 3hrs.

IT - Incubation temperature, IP - Incubation period, ML - lipase concentration

#### 3.2.2. Weight loss

The weight of the samples was measured before and after enzymatic treatment to determine the percentage of weight

loss of the samples due to enzyme action and are shown in Table 5.

**Table 5:** Effect of concentration of microbial lipase on weight of crushed bones

| Treatment 2    | Weight loss%            |
|----------------|-------------------------|
| T <sub>4</sub> | 2.2 ± 0.28 <sup>b</sup> |
| T <sub>5</sub> | 6.9 ± 0.38 <sup>a</sup> |
| T <sub>6</sub> | 2.3 ± 0.53 <sup>b</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

T<sub>4</sub> - Treatment 1Crushed bone with ML of 0.75%, at 1 hr. IP and IT of 47.5 °C at 1hr.

T<sub>5</sub> - Treatment 2Crushed bone with ML of 1%, IT of 47.5 °C and 1 hr. IP at 2hrs.

T<sub>6</sub> - Treatment 3Crushed bone with ML of 1.25% at IT of 47.5 °C and 1 hr. IP at 3hrs.

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration.

The weight loss per centage of T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> ranged between 2.2- 6.9 respectively. The weight loss of the samples treated with 1% of lipase (T<sub>5</sub>) was significantly ( $p < 0.05$ ) higher among treatments.

#### 3.2.3. Colour

The colour characteristics of the control and the three treatments of crushed bones when treated at different concentrations of enzyme i.e., 0.75%, 1% and 1.25% are given in table 6.

**Table 6:** Effect of concentration of lipase on colour of crushed bones

| Parameters      | C <sub>2</sub>            | T <sub>4</sub>             | T <sub>5</sub>             | T <sub>6</sub>            |
|-----------------|---------------------------|----------------------------|----------------------------|---------------------------|
| L* (lightness)  | 76.48 ± 1.06 <sup>a</sup> | 75.55 ± 1.55 <sup>ab</sup> | 70.98 ± 0.67 <sup>b</sup>  | 62.27 ± 0.51 <sup>c</sup> |
| a* (redness)    | 1.37 ± 0.58 <sup>c</sup>  | 3.41 ± 0.15 <sup>b</sup>   | 3.83 ± 0.15 <sup>ab</sup>  | 6.63 ± 0.10 <sup>a</sup>  |
| b* (yellowness) | 15.69 ± 1.43 <sup>c</sup> | 23.06 ± 0.17 <sup>ab</sup> | 23.15 ± 0.32 <sup>ab</sup> | 27.08 ± 0.37 <sup>a</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.01$ )

C<sub>2</sub> - Control 2 = T<sub>2</sub>

T<sub>4</sub> - Treatment 1Crushed bone with ML of 0.75%, at 1 hr. IP and IT of 47.5 °C

T<sub>5</sub> - Treatment 2Crushed bone with ML of 1%, IT of 47.5 °C and 1 hr. IP at 2hrs.

T<sub>6</sub> - Treatment 3Crushed bone with ML of 1.25% at IT of 47.5 °C and 1 hr. IP at 3hrs.

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration

The lightness (L\*) value of C<sub>2</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> varied from 62.27 - 76.68. The redness (a\*) values of C<sub>1</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> ranged from 1.37 -6.63. The yellowness (b\*) value of C<sub>2</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> were 15.69 ± 1.43, 23.06 ± 0.17, 23.15 ± 0.32, 27.08 ± 0.37 respectively. The lightness (L\*) value was significantly ( $p <$

0.01) higher recorded with C<sub>2</sub> and T<sub>6</sub> recorded significantly ( $p < 0.01$ ) lower among all. The increase in concentration of microbial lipases significantly ( $p < 0.01$ ) lowered the lightness value at incubation temperature of 47.5 °C. Whereas the redness and yellowness value significantly ( $p < 0.01$ ) with increase in concentration of lipase.

### 3.3. Optimization of incubation period for enzyme lipase

Based on the experimental design three different incubation period of application of lipase, viz., 1hr, 2hrs and 3hrs were taken as three treatments T<sub>7</sub>, T<sub>8</sub>, T<sub>9</sub> and the best incubation temperature and concentration of experiment 1 and 2 respectively are used for this experiment. The best incubation period was assessed, primarily on the reduction of fat percentage on the sample.

#### 3.3.1. Fat

**Table 7:** Effect of incubation duration on lipase activity

| Treatment      | Fat percentage           |
|----------------|--------------------------|
| C <sub>3</sub> | 1.62 ± 0.19 <sup>b</sup> |
| T <sub>7</sub> | 3.11 ± 0.46 <sup>a</sup> |
| T <sub>8</sub> | 2.22 ± 0.25 <sup>b</sup> |
| T <sub>9</sub> | ± 0.56 <sup>ab</sup>     |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

C<sub>3</sub> - Control 3 = T<sub>5</sub>

T<sub>7</sub> - Treatment 1(Crushed bones subjected to 1% of enzyme for 1 hr at 47.5 °C)

T<sub>8</sub> - Treatment 2(Crushed bone subjected to 1% of enzyme for 2 hrs at 47.5 °C)

T<sub>9</sub> - Treatment 3(Crushed bone subjected to 1% of enzyme for 3 hrs at 47.5 °C)

IT - Incubation temperature, IP - Incubation period, ML - Lipase concentration

The fat percentage for different treatment varied from 2.22 ± 0.25 - 3.11 ± 0.46. The fat percentage was significantly ( $p < 0.05$ ) lower and higher in T<sub>7</sub> and T<sub>8</sub> respectively. No significant difference was noted among T<sub>8</sub> and T<sub>9</sub>. The result of this experiment indicates that incubation temperature at 47.5 °C, lipase concentration at rate of 1 percent of the bone weight and incubation of 2h was selected as standardized procedure for cleaning of crushed bones for the gelatin preparation.

#### 3.3.2. Weight loss

**Table 8:** Effect of incubation duration of microbial lipase on the weight of crushed bones

| Treatment 3    | Weight loss%             |
|----------------|--------------------------|
| T <sub>7</sub> | 2.63 ± 0.28 <sup>b</sup> |
| T <sub>8</sub> | 6.43 ± 0.95 <sup>a</sup> |
| T <sub>9</sub> | 3.80 ± 0.83 <sup>b</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.01$ )

T<sub>7</sub> - Treatment 1(Crushed bones subjected to 1% of enzyme for 1 hr at 47.5 °C)

T<sub>8</sub> - Treatment 2(Crushed bone subjected to 1% of enzyme for 2 hrs at 47.5 °C)

T<sub>9</sub> - Treatment 3(Crushed bone subjected to 1% of enzyme for 3 hrs at 47.5 °C)

The weight loss percentage of T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> were 6.43 ± 0.95<sup>a</sup>, 2.63 ± 0.28<sup>b</sup> and 3.80 ± 0.83<sup>b</sup> respectively. The weight loss percentage of the sample incubated for two hours showed significantly ( $p < 0.01$ ) higher weight loss among treatments.

#### 3.3.3. Colour

The colour characteristics of the control and the three treatments of crushed bones when treated at different incubation duration of enzyme i.e., 1hr., 2hrs. and 3hrs are given in table 9.

The lightness values of C<sub>3</sub>, T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> were 70.98 ± 0.83<sup>b</sup>, 80.81 ± 0.83<sup>a</sup>, 74.87 ± 0.65<sup>ab</sup> and 74.87 ± 0.65<sup>ab</sup> respectively. The redness values of C<sub>3</sub>, T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> were 3.83 ± 0.15<sup>b</sup>, 3.72 ± 0.11<sup>c</sup>, 4.20 ± 0.07<sup>b</sup> and 4.55 ± 0.48<sup>a</sup>. The values of yellowness of C<sub>3</sub>, T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> were 23.15 ± 0.32<sup>b</sup>, 20.44 ± 0.44<sup>c</sup>, 22.85 ± 0.41<sup>b</sup> and 23.44 ± 0.32<sup>a</sup> respectively. Lightness (L\*) was significantly ( $P < 0.01$ ) higher recorded with T<sub>7</sub> recorded significantly ( $p < 0.01$ ) lower T<sub>8</sub> and C<sub>3</sub> among all. The redness was significantly higher for T<sub>9</sub> and T<sub>8</sub> and lower for T<sub>7</sub>. No significant difference was obtained among T<sub>9</sub> and C<sub>3</sub>. The yellowness value of treatment of T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> were significantly higher in T<sub>9</sub> and C<sub>3</sub> and values lowest in T<sub>7</sub> and T<sub>8</sub>.

**Table 9:** Effect of incubation period of lipase on colour of crushed bones

| Parameters      | C <sub>3</sub>            | T <sub>7</sub>            | T <sub>8</sub>             | T <sub>9</sub>             |
|-----------------|---------------------------|---------------------------|----------------------------|----------------------------|
| L* (lightness)  | 70.98 ± 0.67 <sup>c</sup> | 80.81 ± 0.83 <sup>a</sup> | 74.87 ± 0.65 <sup>ab</sup> | 74.87 ± 0.65 <sup>ab</sup> |
| a* (redness)    | 3.83 ± 0.15 <sup>ab</sup> | 3.72 ± 0.11 <sup>c</sup>  | 4.20 ± 0.07 <sup>b</sup>   | 4.55 ± 0.48 <sup>a</sup>   |
| b* (yellowness) | 23.15 ± 0.32 <sup>b</sup> | 20.44 ± 0.44 <sup>c</sup> | 22.85 ± 0.41 <sup>b</sup>  | 23.44 ± 0.34 <sup>a</sup>  |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

C<sub>3</sub> - Control 3 = T<sub>5</sub>

T<sub>7</sub> - Treatment 1(Crushed bones subjected to 1% of enzyme for 1 h at 47.5 °C)

T<sub>8</sub> - Treatment 2(Crushed bone subjected to 1% of enzyme for 2 h at 47.5 °C)

T<sub>9</sub> - Treatment 3(Crushed bone subjected to 1% of enzyme for 3 h at 47.5 °C)

### 3.4. Determining the efficiency of microbial lipase on quality of raw crushed bones

The efficiency of the standardized process was validated on the raw crushed bones by treating these bones with lipase enzyme at optimized conditions standardised procedure for pre-treatment (SPPT) i.e., incubation temperature at 47.5 °C, 1% lipase concentration and incubation period for 2h and then determining its efficiency by comparing the fat percentage of the samples before and after the enzyme treatment are compared in the Table 10.

**Table 10:** Efficiency of lipase on fat degreasing using standardized conditions

| Treatment | Fat                      |
|-----------|--------------------------|
| Control   | 2.23 ± 0.18 <sup>a</sup> |
| SPPT      | 1.73 ± 0.31 <sup>b</sup> |

Mean ± SE with same superscript in a column does not differ significantly ( $p < 0.05$ )

Control - without any treatment; SPPT - standardized procedure for pre-treatment

The standardized procedure showed significant ( $p < 0.05$ ) higher value for fat reduction percentage in comparison to that of the control samples. Statistically significant difference was observed in mean fat percentage in raw crushed bones before and after the enzymatic wash ( $p < 0.05$ ). The fat percentage were the least in raw bones after the treatment with lipase compared to the raw crushed bones before the treatment inferring that the enzyme was efficient in the washing of crushed bone under standardized conditions.



**Fig 1:** Raw crushed bones which has not been pre-treated



**Fig 2:** Dried crushed bones before enzyme action



**Fig 3:** Dried crushed bones after enzyme treatment

## 4. Discussion

### 4.1. Optimization of incubation temperature (IT)

The demand for industrial enzyme, particularly for microbial lipase is increasing in recent past due to its wide range of application. The activity of microbial lipase depends on the incubation temperature. The lipase enzyme isolated from thermostable gram positive bacteria had optimum activity at 30 °C - 60 °C. The maximum stability of lipase enzyme obtained from *Lactobacillus* sp was in the range of 30-50 °C at the pH range of 8-9 (Balakrishnan, 2011) [1]. Cai *et al.* (2009) [82] reported that lipases obtained from *Pseudomonas* showed higher lipase activity in the incubation temperature range of 40 - 60°C. In aqueous media the enzyme lipozyme obtained from *Thermomyces lanuginosus* is stable being active over the pH range of 7-11 and it maintains activity reasonably well at 55-60° C although the recommended temperature for application between 30-40 (Fernandes *et al.* 2004) [4]. However, in the present study significantly ( $p < 0.05$ ) higher lipase activity was seen at incubation

temperature of 47.5 °C. Higher and lower temperatures than 47.5 °C showed lower enzyme activity.

### 4.2. Optimization of concentration of microbial lipase (ML)

Lipase produced from bacteria, yeasts and fungi shows diversified physical, chemical and biological properties. we expected increasing the concentration level of enzyme increase the degreasing effect, however in the present study, 1% microbial lipase concentration was found to show significantly ( $p < 0.05$ ) higher degreasing effect on crushed bones when compared to 0.75% and 1.25% concentration level, it might be due to the effect of the incubation temperature we applied.

### 4.3. Optimization of incubation duration (ID)

The longer an enzyme is incubated with its substrate, the greater the amount of product that will be formed. However, optimizing incubation duration for the enzyme to act on the substrate is important as different enzymes need different incubation duration and conditions. In the present study the optimal incubation duration of lipase was found to be two hours and with increase in the incubation duration to three hours showed no significant effect in degreasing, so incubation duration of two hours is selected for the standardization procedure.

### 4.4. effect of enzyme incubation parameters on colour of crushed bones

Products like bones which are rich in fats have a possibility of undergoing certain colour changes depending upon the processing conditions. The samples prepared in this study have undergone enzyme action under different conditions of incubation temperature, concentration of microbial lipase and incubation duration and are expected to show variable tones of browning. The change in colour was measured objectively in terms of lightness, redness and yellowness values. According to the present study the action of microbial lipase had a significant effect on the colour of the crushed bones. The three parameters i.e. incubation temperature, concentration of enzyme and incubation duration had different effects on the colour of the crushed bones.

The data revealed that increase in temperature increased lightness of bones. Maximum whiteness was observed at T<sub>2</sub>. But the lightness decreased with further temperature increase. So T<sub>2</sub> can be concluded as temperature at which microbial lipase have maximum activity. Redness of the bones had a notable decrease at increased temperature. Initial temperature increase caused increased yellowness which in turn can be termed as increased greyness, whereas decreased yellowness was observed with increase in temperature. The incubation duration also had a significant ( $p < 0.01$ ) effect on the lightness, redness and the yellowness of the sample. The highest value of lightness was observed in 2hrs incubation duration with 47.5 °C and 1 per cent as incubation temperature and concentration respectively, whereas it had the lowest redness among the other samples indicating that 47.5 °C incubation temperature, 1% concentration and 2h duration were the optimal condition for the action of microbial lipase in cleaning of crushed bones.

### 4.5. Effect of enzyme incubation parameters on weight loss of crushed bones

The data revealed that there was significant ( $p < 0.05$ ) reduction in weight of the samples after the treatment with

lipase under standardized conditions (Table 4.3.2) which indicate that the enzyme is effective in removing unwanted residual fat from the crushed bones when we subject the enzyme under an optimum condition.

#### 4.6. Determining the efficacy of microbial lipase on raw crushed bones

Statistically the efficacy of lipase in degreasing process was validated by analyzing fat percentage before and after treating the bones with lipase enzyme under the standardized condition. Sirisha, *et al.* (2010) [64] reported that maximum activity of lipase was observed at 36 °C temperature and at pH 7 during their study using oil contaminated soil. However, in the present study, it was observed that lipase had a significant ( $p < 0.05$ ) effect in degreasing (there was 22.42 percent reduction in fat) under optimized conditions of 47.5 °C, 1% enzyme concentration for two hrs of incubation. From the above study it can be suggested that enzyme lipase under standardized conditions can be used as an alternative to chemical pretreatment of crushed bone.

#### 5. Conclusion

The present investigation was undertaken to standardize the optimum conditions for lipase activities in the process of bone degreasing for gelatin preparation. The investigation was conducted in three phase to develop a standardized procedure for degreasing of crushed bone using lipase enzyme. In the first phase optimization of incubation temperature of the lipase activity was standardised with the enzyme, microbial lipase shows maximum activity at 47.5 °C. In the second phase optimization of the lipase concentration was done and the maximum fat reduction was noted at concentration of one percent-treatment  $T_5$ . In the last phase of study, the maximum fat reduction due to the activity of the lipase enzyme was noted at the incubation duration of two hours ( $T_8$ ). Thus, the incubation temperature of 47.5 °C, lipase concentration of one percentage of bone weight and incubation duration of two hours had more beneficial effect on lipase activities in degreasing of crushed bone used for gelatine preparation has been standardised. Then the efficiency of lipase was validated using the standardised procedure by analysing the fat percentage before and after the enzyme treatment on the bone collected from the slaughter houses. The fat content of bones was found to be reduced by 22.42% when subjected to the action of microbial lipase. From the above investigation it is inferred that enzymes lipase can be used as the best possible option for degreasing of the crushed bones.

#### 6. Acknowledgement

The authors gratefully acknowledge the facilities and support provided by the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

#### References

- Balakrishnan P. Production of lipase enzyme from *Lactobacillus* spp. and its application in the degradation of meat. *World Appl Sci J.* 2011;12(10):1798-1802.
- Boran G, Mulvaney SJ, Regenstein JM. Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. *J Food Sci.* 2010;75:565-571.
- Eastoe JE. The amino acid composition of mammalian collagen and gelatin. *Biochem J.* 1955;61:589-900.
- Fernandes MLM, Krieger N, Baron AM, Zamora PP, Ramos LP, Mitchell DA. *J Mol Catal B Enzym.* 2004;30:43-49.
- Gimenez B, Aleman A, Montero P, Gomez-Guillen MC. Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. *Food Chem.* 2009;114:976-983.
- Gimenez B, Turnay J, Lizarbe MA, Montero P, Gomez-Guillen MC. Use of lactic acid for extraction of fish skin gelatin. *Food Hydrocol.* 2005;19:941-950.
- Glicksman M. Gelatin. In: *Gum Technology in the Food Industry. Food Science and Technology Monographs.* New York: Academic Press; 1969.
- Gelatin Manufacturers Institute of America. Gelatin handbook. New York: GMIA; 2012.
- Gomez EJ, Bravo L, Gomez-Guillen MC, Aleman A, Montero P. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chem.* 2009;112:18-25.
- Gomez-Guillen MC, Gimenez B, Montero P. Extraction of gelatine from fish skins by high pressure treatment. *Food Hydrocolloids.* 2005;19:923-928.
- Grossman S, Gan R, Bergman M, Holon. Process for the production of gelatine from fish skins. US patent. 1992;19:1-8.
- Hasan F, Shah AA, Hameed A. Methods for detection and characterization of lipase: A comprehensive review. *Biotechnol Adv.* 2009;27:782-798.
- Haug I, Draet KI. Gelatin. In: Phillips GO, Williams PA, editors. *Handbook of Hydrocolloids.* 2nd ed. Cambridge: Woodhead Publishing; 2009.
- Hayatudin H. Move efforts to produce halal medicinal products. *J Halal.* 2005.
- Heidemann E, Roth W. Synthesis and investigation of collagen model peptides. *Adv Polym Sci.* 1982;142:203.
- Herpandi HN, Adzitey F. Fish bone and scale as a potential source of halal gelatin. *J Fish Aquat Sci.* 2011;6(4):379-389.
- Hinterwaldner R. Raw materials. In: Ward AG, Courts A, editors. *Science and Technology of Gelatin.* London: Academic Press; 1977. p. 132-145.
- Holzer D. Gelatin production. US patent. 1996;5:484-888.
- Hou WS, Bromme D, Zhao Y. Characterization of novel cathepsin K mutations in the pro and mature polypeptide regions causing pycnodysostosis. *J Clin Invest.* 1999;103:731-738.
- Irvine SA, Agarwal A, Lee BH, Chua HY, Low BC, Machluf M, *et al.* Printing cell-laden gelatin constructs by free-form fabrication and enzymatic protein crosslinking. *Biomed Microdevices.* 2015;17:1-8.
- Jacobson RE. Photographic gelatin II. In: Cox RJ, editor. London: Academic Press; 1967. p. 233-252.
- Jaeger KE, Dijkstra BW, Reetz MT. Bacterial biocatalysts: Molecular biology, three-dimensional structures and biotechnological applications of lipases. *Annu Rev Microbiol.* 1999;53:315-351.
- Jeevithan E, Bao B, Bu Y, Zhou Y, Zhao Q, Wu W. Type II collagen and gelatin from silvertip shark (*Carcharhinus albimarginatus*) cartilage: Isolation, purification, physicochemical and antioxidant properties. *Mar Drugs.* 2014;12:3852-3873.
- Johns P, Courts A. Physical tests for gelatin and gelatin

- products. In: Ward AG, Courts A, editors. *The Science and Technology of Gelatins*. New York: Academic Press; 1977. p. 507-554.
25. Johnston BFA. Gelatin. In: Harris P, editor. *Food Gels*. London: Elsevier Applied Science Publishers; 1990. p. 233-289.
  26. Johnston-Banks FA. Gelatin. In: Harris P, editor. *Food Gels*. New York: Elsevier; 1990. p. 255-274.
  27. Jones RT. Gelatin: Manufacture and physico-chemical properties. In: Podczek F, Jones BE, editors. *Pharmaceutical Capsules*. 2<sup>nd</sup> ed. London: Pharmaceutical Press; 2004. p. 23-60.
  28. Jones RT. Gelatin: Physical and chemical properties. In: Ridgway K, editor. *Hard Capsules: Development and Technology*. London: Pharmaceutical Press; 1987.
  29. Karim AA, Bhat R. Fish gelatine: Properties, challenges, and prospects as an alternative to mammalian gelatines. *Food Hydrocolloids*. 2009;23:563-576.
  30. Kim SK, Jeon YJ, Lee BJ, Lee CK. Purification and characterization of gelatin from the bone of cod (*Gadus macrocephalus*). *Korean J Life Sci*. 1996;6:14-26.
  31. Kubota A. The structure of the end region of collagen macromolecules. *Annu Rep Nippon Hikaku Kenkyujo*. 1963;9:1-11.
  32. Laboureur P, Villalon M. Enzymes in food lactoglobulin. *Adv Food Nutr Res*. 1972;44-81.
  33. Ledward DA. Gelation of gelatin. In: Mitchell JR, Ledward DA, editors. *Functional Properties of Food Macromolecules*. London: Elsevier; 1986. p. 171-174.
  34. Lesuisse E, Colson SK. Purification and preliminary characterization of the extracellular lipase of *Bacillus subtilis* 168, an extremely basic pH-tolerant enzyme. *Eur J Biochem*. 1993;216:155-160.
  35. Lim YP, Mohammad A. Physicochemical properties of mammalian gelatin in relation to membrane process requirement. *Food Bioprocess Technol*. 2011;4(2):304-311.
  36. Lin YK, Lin TY, Su HP. Extraction and characterisation of telopeptide-poor collagen from porcine lung. *Food Chem*. 2011;124(4):1583-1588.
  37. Lithauer D, Ginster A, Skein E. *Pseudomonas luteola* lipase: A new member of the 320-residue *Pseudomonas* lipase family. *Enzyme Microb Technol*. 2002;30:209-215.
  38. Lobo L. Effect of gelatin on rupture and coalescence during emulsification. *J Colloid Interface Sci*. 2002;254:165-174.
  39. Machius M, Wiegand G, Huber R. Crystal structure of calcium-depleted *Bacillus licheniformis*  $\alpha$ -amylase at 2.2 Å resolution. *J Mol Biol*. 1995;246:545-559.
  40. Malcata FX, Hill CG, Amundson CH. Use of a lipase immobilized in a membrane reactor to hydrolyze the glycerides of butter oil. *Biotechnol Bioeng*. 1991;38:853-868.
  41. Matsushita O, Yoshihara K, Katayama S, Minami J, Okabe A. Purification and characterization of a *Clostridium perfringens* 120-kilodalton collagenase and nucleotide sequence of the corresponding gene. *J Bacteriol*. 1994;176:149-156.
  42. Manning AB, Schryver SB. Studies on gelatine. Part I. The dynamics of gelatine formation from ossein. *Biochem J*. 1921;15:523-529.
  43. Manwaring WH. Gelatine and physical endurance. *Calif West Med*. 1940;53(3):110-110.
  44. Merkel JR, Dreisbach JH, Ziegler HB. Collagen lytic activity of some marine bacteria. *Appl Microbiol*. 1974;29:145-151.
  45. Montero P, Borderias J. Emulsifying capacity of collagenous material from the muscle and skin of hake (*Merluccius merluccius* L.) and trout (*Salmo irideus* Gibb): Effect of pH and NaCl concentration. *Food Chem*. 1991;41:251-267.
  46. Montero P, Álvarez C, Marti MA, Borderias AJ. Plaice skin collagen extraction and functional properties. *J Food Sci*. 1995;60:1-3.
  47. Morales TI, Woessner JF. PZ-peptidase from chick embryos: Purification, properties, and action on collagen peptides. *J Biol Chem*. 1976;252:4855-4860.
  48. Morimoto N, Kakudo N, Matsui M, Ogura T, Hara T, Suzuki K, et al. Exploratory clinical trial of combination wound therapy with gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: Study protocol. *BMJ Open*. 2015;5:1-6.
  49. Muyonga JH, Cole CGB, Duodu KG. Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatine. *Food Hydrocolloids*. 2004;18:581-592.
  50. Nagai T, Suzuki N. Isolation of collagen from fish waste material—skin, bone and fins. *Food Chem*. 2000;68(3):277-281.
  51. Nagai T, Wora WW, Suzuki N, Nakamura T, Ito T, Fujiki K, et al. Isolation and characterization of collagen from rhizostomous jellyfish (*Rhopilema asamushi*). *Food Chem*. 2000;70(2):205-208.
  52. Nishihara T, Miyata T. The effects of proteases on the soluble and insoluble collagens and the structure of insoluble collagen fiber. *Collagen Symposium*. 1962;3:62-93.
  53. Nishimoto M, Sakamoto R, Mizuta S, Yoshinaka R. Identification and characterization of molecular species of collagen in ordinary muscle and skin of the Japanese flounder (*Paralichthys olivaceus*). *Food Chem*. 2005;90:151-156.
  54. Norland RW. Fish gelatin. In: Voight MN, Botta JK, editors. *Advances in Fisheries Technology and Biotechnology for Increased Profitability*. Lancaster (PA): Technomic Publishing Co.; 1990. p. 325-325.
  55. Papon P, Leblon J, Meijer PHE. Gelation and transitions in biopolymers. In: *Physics of Phase Transitions*. Berlin: Springer; 2007. p. 189-213.
  56. Patil KJ, Chopda MZ, Mahajan RT. Lipase biodiversity. *Indian J Sci Technol*. 2011;4(8):971-982.
  57. Poppe J. Gelatin. In: *Thickening and Gelling Agents for Food*. 2<sup>nd</sup> ed. London: Blackie Academic and Professional; 1997. p. 144-168.
  58. Postlethwaite AE, Seyer JM, Kang AH. Chemotactic attraction of human fibroblasts to type I, II and III collagens and collagen-derived peptides. *Proc Natl Acad Sci USA*. 1978;75:871-875.
  59. Reich G, Walther S, Stather F. The influence of the age of cattle and pigskin on the yield and quality of gelatins obtained after acid conditioning. *Investigation of Collagen and Gelatin IV*. Freiberg (LSA): Deutsche Lederinstitut; 1962. p. 24-30.
  60. Robinson JJ. Comparative biochemical analysis of sea urchin peristome and rat tail tendon collagen. *Comp Biochem Physiol B Biochem Mol Biol*. 1997;117(2):307-313.

61. Sangeetha R, Arulpandi I, Geetha A. Bacterial lipases as potential industrial biocatalysts: An overview. *Res J Microbiol.* 2011;6:1-24.
62. Schallmey M, Singh A, Ward OP. Developments in the use of *Bacillus* species for industrial production. *Can J Microbiol.* 2004;50:1-17.
63. Schrieber R. Edible gelatin: Types, properties, use and applications in the food industry. *Gordian.* 1976;356-364.
64. Sirisha E, Rajasekar N, Naras ML. Isolation and optimization of lipase producing bacteria from oil contaminated soils. *Adv Biol Res.* 2010;4(5):249-252.
65. Snedecor GW, Cochran WG. Statistical methods. 8<sup>th</sup> ed. Ames (IA): Iowa State University Press; 1989.
66. Stainsby G. The physical chemistry of gelatin in solution. In: Ward AG, Courts A, editors. *The Science and Technology of Gelatin.* London: Academic Press; 1977. p. 109-135.
67. Stainsby G. Gelatin gels. In: Pearson AM, Dutson TR, Bailey AJ, editors. *Advances in Meat Research.* 4<sup>th</sup> ed. New York: Van Nostrand Reinhold; 1987. p. 209-222.
68. Stickley FL. Photographic gelatin. In: *Proceedings of the Fifth RPS Symposium*; 1987. p. 107-110.
69. Thakur S. Lipases: Sources, properties and applications. *Int J Sci Eng Res.* 2012;3(7):1-29.
70. Torres CF, Moeljadi M, Hill CG. Lipase-catalyzed ethanolysis of fish oils: Multi-response kinetics. *Biotechnol Bioeng.* 2003;83(3):274-281.
71. Tybursky A, Wasiak P, Cegiela A. Application of composite protective coatings on the surface of sausages with different water content. *Acta Sci Pol Technol Aliment.* 2010;9(2):151-159.
72. United States Pharmacopeia 34-National Formulary 29 and Food Chemicals Codex 7. Rockville (MD): United States Pharmacopeial Convention; 2011.
73. Veis A. *Macromolecular chemistry of gelatin.* New York: Academic Press; 1964.
74. Wang Y, Regensteim JM. Effect of EDTA, HCl, and citric acid on Ca salt removal from Asian (silver) carp scales prior to gelatin extraction. *J Food Sci.* 2009;74(6):426-431.
75. Wiseman A. Introduction to principles. In: *Handbook of Enzyme Biotechnology.* 3<sup>rd</sup> ed. London: Ellis Horwood; 1995. p. 3-8.
76. Yang H, Shu Z. The extraction of collagen protein from pigskin. *J Chem Pharm Res.* 2014;6:683-687.
77. Zhong HY. Protein sequencing by mass analysis of polypeptide ladders after controlled protein hydrolysis. *Nat Biotechnol.* 2004;22:10-15.
78. Zhang LQ, Zhang YD, Xu L, Li XL, Yang XC, Xu GL, et al. Lipase-catalyzed synthesis of RGD diamide in aqueous water-miscible organic solvents. *Enzyme Microb Technol.* 2001;29:129-135.
79. Zhong S, Teo WE, Zhu X, Beuerman RW, Ramakrishna S, Yung LY. Formation of collagen-glycosaminoglycan blended nanofibrous scaffolds and their biological properties. *Biomacromolecules.* 2005;6:2998-3004.
80. Zhou P, Mulvaney SJ, Regensteim JM. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. *J Food Sci.* 2006;71:313-321.
81. Balakrishnan P. Production of lipase enzyme from *Lactobacillus* spp. and its application in the degradation of meat. *World Appl Sci J.* 2011;12(10):1798-1802.
82. Cai YJ, Wang L, Liao XR, Ding YR, Sun J. Purification and partial characterization of two new cold-adapted lipases from mesophilic *Geotrichum* sp. SYBC WU-3. *Process Biochem.* 2009;44:786-790.
83. Fernandes MLM, Krieger N, Baron AM, Zamora PP, Ramos LP, Mitchell DA. *J Mol Catal B Enzym.* 2004;30:43-49.
84. Lithauer D, Ginster A, Skein E. *Pseudomonas luteola* lipase: A new member of the 320-residue *Pseudomonas* lipase family. *Enzyme Microb Technol.* 2002;30:209-215.
85. Snedecor GW, Cochran WG. Statistical methods. 8<sup>th</sup> ed. Ames (IA): Iowa State University Press; 1994. p. 313-313.
86. United States Pharmacopeia 34-National Formulary 29 and Food Chemicals Codex 7. Rockville (MD): United States Pharmacopeial Convention; 2011.

**How to Cite This Article**

Basha AMHS, Veenamol EM, Sathu T, Uma R, Jayavardhanan KK. Utilization of microbial lipases for bone degreasing in Gelatin preparation. *International Journal of Veterinary Sciences and Animal Husbandry.* 2026;11(2):23-31

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