Effect of Centrifuge Speed on Gel Extraction from Aloe Vera Leaves

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Abstract
This paper describes the extraction of gel from aloe vera leaves by use of method of centrifugation. The effect of different centrifuge speed, i.e., 2000, 5000 and 10,000 rpm at different temperature i.e., 5°C, 10°C and 32°C (ambient) and centrifuge holding duration i.e., 10, 20 and 30 min, on gel recovery and quality parameters like, viscosity of gel, refractive index of gel, was studied. An effort has been made to optimize the centrifuge speed for gel extraction from aloe vera leaves. It was concluded that the extraction of gel from aloe vera should be carried at 10000 rpm speed, 5°C temperature and 30 min duration, which yielded higher gel recovery and better quality of gel. Higher centrifuge speed leads more separation of gel molecules and fibers from aloe vera pulp to get clear gel.

Keywords: Aloe vera; Gel extraction; Centrifugation

Introduction
Aloe vera is a succulent that belongs to the liliaceae family. Aloe vera gel is the commercial name given to the fiber free mucilaginous exudate extracted from the hydroparenchyma of the succulent leaves of aloe vera (Aloe barbadensis Miller). Aloe vera gel (a clear, jelly-like material) is derived from tissue that comprises the inner portion of the leaves. Aloe vera gel is used as preservative coating for preservation of fruits [1] and food drinks for health supplements. Aloe gel is used for base in many cosmetic formulations and also used as medicine. Aloe vera contains biological active compounds which temperature sensitive and hence demands great care in processing. Aloe vera gel molecules are surrounded by the chain of sugar molecules surrounded [2]. Centrifugal action on Aloe gel break the chain of sugar molecules surrounded by gel molecules and leads more gel recovery and fibreless gel. Centrifugal separation of aloe vera gel is found its application in most prevalent gel extraction methods like hand filleting and whole leaf processing, from aloe vera (Figure 1).

Waller [3] had carried out study to investigate chemical constituents of aloe vera leaf gel and determined free amino acids, free monosaccharides and total saccharides released upon hydrolysis, sterols, and triterpenoids of the leaves of Aloe barbadensis Miller by extracting gel with water-acetone (1:1) and then acetone at room temperature. Shafi N [4] developed a commercially viable process for preparing a stable and pharmacological active crystalline substance from the fresh whole leaf. A review on processing of aloe vera leaf gel, has revealed aloe vera as a highly potential functional and valuable ingredient that exhibits relatively impressive biological functions of great interest in cosmetic, pharmaceutical and food industries [5]. It also revealed the present processing technologies viz., gel stabilization technique, biological activity of aloe leaf gel and the effect of heat treatment on various constituents of gel.

Aloe vera processing industry demands pure gel for its cosmetic, nutritional and medicinal use. Reynolds and Dweck [6] reported a review update for aloe vera leaf gel and pointed out that scarce information is available for aloe vera processing particularly extraction of gel, though it has acquired great commercial importance for medicinal use and cosmetics products. Therefore, it is worthwhile to explore methods of centrifugal separation of aloe vera gel that can lead to an increase of gel recovery and less fiber content in gel. In aloe vera leaf, the gel molecules are surrounded by a chain of sugar molecule. It is essential to break up this molecular chain and extract gel by application of centrifugal force. Under the centrifugal action, the speed of centrifuge play pivotal role in separation of gel from aloe vera pulp after removing the exudates. Yaron [7] have extracted gel from full sized mature leaves by centrifugation. After removal of the 'peel' the colorless hydroparenchyma was ground in a blender and centrifuged at 10,000 x g for 30 min at 4°C to remove the fibers. Considering the above fact, it is necessary to standardize the centrifuge speed for optimum quality of gel for its cosmetic, medicinal and nutritional use.

Materials and Methods

Materials
The effect of centrifuge speed on extraction of gel from aloe vera and subsequent recovery and quality of extracted gel was studied. The matured aloe vera (Aloe barbadensis Miller) leaves were obtained from Department of Botany, College of Agriculture, Junagadh Agricultural University, Junagadh [8]. The freshly harvested aloe vera leaves were stored at 4 to 5°C prior to experimentation.

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Methodology

The experiment was carried out by different unit operations like Aloe pulp grinding, Centrifugal separation and filtration of crude gel to obtain pure gel as shown in Figure 2.

**Centrifugal separation of gel:** The domestic blender (Boss make) was used to ground the fillets to obtain homogenized pulp. The 60 ml pulp on volume basis in three samples were centrifuged in cold type centrifuge (Remi Instruments,Mumbai, India) for separation of gel and fiber [7]. The centrifuge temperature was set 5°C, 10°C and 32°C (ambient) with different centrifuge speed. Centrifuge test tubes of 100 ml capacity were held for 10, 20 and 30 min duration with different combination of treatments and 40 % volume of test tubes was left to give sufficient space in for centrifugal action. The experiments were planned for 3 levels of centrifuge speed of (2000, 5000 and 10,000 rpm), at 5°C, 10°C and 32°C (ambient) centrifuge temperature for 10, 20 and 30 min centrifuge duration.

**Filtration unit:** The filtration unit was consisted of vacuum pump, Buchner flask and Buchner funnel. The gel, which was obtained after centrifuge operation, was mixed with Charcoal for gel purification. The gel then was filtered in the filtration unit with the help of Whatman No. 4 filter paper for further analysis.

After giving different treatment of centrifuge speed, samples were brought for purification to remove impurities. The charcoal was mixed with crude gel for purification. The vacuum filtration method was used to obtain pure gel from crude gel.

The pure gel was collected in the test tubes for further analysis.

**Fibre content:** Crude gel is defined as the gel obtained after the centrifuge operation of Aloe vera pulp, while pure gel is the gel obtained after purification of the crude gel. The fibre content is defined as the difference between the dry weight of the crude gel and that of the filtered gel (pure gel). It was measured by filtering the homogenate through a 2.0 μm muslin cloth followed by Whatman No. 4 filter paper under vacuum. Ten grams of the filtrate was placed in a dry glass petridish and dried at 105°C ± 2°C for 24 hours and its dry weight determined and the difference gives the fibre content [9].

**Evaluation of gel quality parameters:** Aloe gel quality was judged by its purity. Purity of gel was determined by the refractive index. The biological activity is the indication of more viscosity of gel. So higher viscosity of gel have more biological activity, which is important for its pharmaceutical and cosmetic use

**Viscosity**

The viscosity of a aloe vera gel is a very important property in the biochemical analysis as it is the indicator of active biological constituents. The Oswald viscometer was used for the measurement of viscosity of the aloe vera gel. After calibration the Oswald viscometer was filled to the lower calibration mark by applying suction with a rubber bulb and drawing the liquid analyte into the apparatus the time required for the volume of liquid between the two marks to drain from the bulb is measured. The tube at the lower end of the upper bulb has a fixed length and radius, which was used along with the pressure differential column between the upper and lower ends of the apparatus to measure the viscosity.

The kinematic viscosity (µ) of liquid and the time (t) required to pass 50cc of liquid were correlated by the expression

\[ \mu = 0.0026 t - 1.175 / t \]

Where,

\[ \mu = \text{Kinematic viscosity in Stokes} \]
\[ t = \text{time in seconds to collect 50 cc of gel.} \]

**The unit of kinematic viscosity was then converted in cm²s⁻¹**

**Refractive index:** Refractive index is the physical property of gel determines the purity of gel as compared to double distilled water and not total soluble solid content of gel. Gel with lowest refractive index, is the best treatment for extraction process. More refractive index indicates the impurities in the extracted gel.

The Abbey Refractometer was used for measurement of refractive index having range of refractive Indices between 1.3000 and 1.7000 with an accuracy of ± 0.0002. It was calibrated with known refractive indices i.e. doubled distilled water (1.3323) at 27°C ± 0.2°C. Two drops of aloe vera gel were placed on the Refractometer prism surface and closed carefully. The mirror was adjusted until the reading was sharp. The instrument was allowed to stand for a few minutes before the reading was taken so that the sample and instrument came to equilibrium. The reading was taken when the blue and yellow shade crossed the cross mark.

**Design of experiments**

The experiments were planned using four factor completely randomized design. The treatments consisted of 2 levels of acetone, 3 levels of centrifuge temperature, 3 levels of centrifuge speed and 3 levels of centrifuge duration. Independent variables were acetone i.e. without addition of acetone, and 10 % addition of acetone, centrifuge temperature i.e. 5, 10, and 32 (ambient)°C, centrifuge speed i.e. 2000, 5000 and 10,000 rpm and centrifuge duration i.e. 10, 20 and 30 min. Dependent variables were gel recovery, (%) viscosity of gel, (cm²s⁻¹) and refractive index of gel. Experiment was planned in Factorial Completely Randomized Design comprising of fifty four treatments with three replications. In all 54 experiments were conducted and results obtained were analyzed statistically. The analysis of variance along with the
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.S.</th>
<th>Cal. F</th>
<th>Tab. F 1%</th>
<th>S.E.M. ±</th>
<th>C.D. @ 5%</th>
<th>Test</th>
</tr>
</thead>
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<tr>
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<td>4372.784</td>
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<td>0.597</td>
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<td>0.521</td>
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<td>Sig.</td>
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<td>10.085</td>
<td>2.43</td>
<td>0.521</td>
<td>1.462</td>
<td>Sig.</td>
</tr>
<tr>
<td>Three levels of different centrifuge speed with two levels of acetone, three levels of temperature and three levels of holding time</td>
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<td>28.322</td>
<td>3.540</td>
<td>1.449</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

C.V, % = 2.68

d.f. = degree of freedom
S.S. = sum of squares
M.S.S. = mean of sum of squares
Cal. F = calculated F vale
Tab. F 1% = table F value
S.E.M. = standard error of mean
C.D. = critical difference
C.V. = coefficient of variation

Table 1: Analysis of variance table crude gel recovery.

level of significance was also determined. Subsequently, the principal components analysis was carried out to get the single optimum values of independent variables for the extraction gel from aloe vera leaves.

**Statistical Analysis**

The statistical analysis of experiment was carried out at Statistics Department, College of Agriculture, Junagadh Agricultural University, Junagadh, with Completely Randomized Design. The data of results were analyzed and interpreted by using Microsoft excel programme. The F-test was carried out to determine whether the effect was significant or not. Critical difference and Coefficient variation were considered for the interpretation of data.

**Results and Discussion**

**Effect of centrifuge speed on gel extraction process**

The centrifugation process was carried out to separate solid particles from pulp for getting crude gel. The aloe vera contains gel molecules surrounded by sugar molecules. Centrifugal force is required to break this chain of sugar molecules. It was seen from the results that, the gel recovery and viscosity increased with the increase of centrifuge speed, whereas refractive index decreased with the increase of centrifugal speed. The statistical analysis had shown that all the quality parameters were found to be significant at 5% Cd.

**Effect of centrifuge speed on gel recovery**

Results of analysis of variance for aloe vera gel recovery (%) from pulp are summarized in Table 1. It was clear from this analysis that variations among the various centrifuge speed and its interaction with acetone level, varying temperature for different holding time was found to be significant at 5% Cd. Therefore, the use of the complete randomized design in this experiment was essential to eliminate the variance among the various centrifuge speed with temperature and centrifuge durations as it was laboratory experiment. The results in the table also indicated that the effect of various centrifuge speed with temperature and centrifuge duration on gel recovery was statistically significant at 5% Cd. The gel recovery shows non-significant effect when all the independent variable was considered.

The results in Table 2 show that the mean values of aloe vera gel recovery at 10000 rpm centrifuge speed were significantly higher than 2000 rpm centrifuge speed as control. The average gel recovery of gel extraction at 10,000 rpm samples were about 30% higher than those of the controls (The maximum gel recovery i.e. 71.33 % from aloe vera leaf pulp, was found at 32°C temperature, 10000 rpm speed and 30 min duration with 10 % of acetone treatment and minimum recovery of 42.50 % was found at 5°C temperatures, 2000 rpm speed and 10 min duration for without acetone treatment). The higher gel recovery at higher speed i.e., 10,000 rpm may be attributed due the separation of solid particles from pulp and break down of the chain of sugar molecules. This was in accordance with findings by Yaron [7] who had extracted 300 ml gel from leaves weighing 800 g i.e., 37.5 % gel recovery from whole leaf and 75.0 % gel recovery from pulp considering 50 % aloe vera leaf pulp recovery at 10,000 x g centrifuge speed for 30 min at 4°C. This phenomenon suggested that the higher centrifuge speed may have an effect in breaking the intercellular forces in aloe vera leaf gel tissues, which may lead to release of more of gel molecules.

It was observed that increase in centrifuge speed with different temperature, and varying holding time increased the gel recovery (Table 2). When centrifuge speed was increased from 2000 rpm (42.50 – 56.50 %) to 5000 rpm (45.72 – 64.78 %), there was 7.0 to 15.0 % increase in gel recovery observed. Increase in centrifuge speed from 5000 rpm (45.72 – 64.78 %) to 10000 rpm (63.89 – 71.33 %) resulted about 12.0 to 40.0 % more gel recovery among various treatments. This may be attributed by the action of higher centrifugal force generated at high speed, which caused separation all the fibers and substance more efficiently and get more clear gel.
The results, it was evident that the higher centrifuge speed brought about increases in aloe vera gel recovery at 10000 rpm speed samples extracted for the same amount of time and temperature than 2000 rpm speed. In industrial practice, aloe vera gel should have more recovery at the same time clarity of gel i.e., purified gel. Therefore, it was desirable to extract the gel at higher centrifuge speed.

### Effect of centrifuge speed on Viscosity of gel

In Table 3, the analysis of variance for viscosity of gel extracted by different centrifuge speed with acetone treatment, varying temperature and different holding duration of sample was presented and their results were given in Table 5. The statistical analysis had shown that the effect of centrifuge speed and its interaction with temperature, acetone and duration, on viscosity of gel was found to be significant. But for different centrifuge speed and its interaction with centrifuge duration was found to be non-significant. Similar trend was found for interaction with centrifuge duration and acetone. There was no uniform trend in viscosity for varying centrifuge speed and duration (Table 4). This had shown that viscosity of aloe vera gel was statistically not affected by centrifuge duration but only depends on temperature and centrifuge speed.

As summarized in Table 5, the maximum viscosity was recorded 2.355 cm²s⁻¹ at 5°C temperature, 10000 rpm centrifuge speed and 10 min duration for 10 % addition of acetone treatment and minimum viscosity was found 0.521 cm²s⁻¹ at 32°C temperature, 2000 rpm centrifuge speed and 10 min duration for without addition of acetone treatment. This showed that about 220 % increases in viscosity of gel which mainly due to addition of 10 % acetone. It was observed that increase in centrifuge temperature decreased the viscosity of gel and addition of acetone resulted increase in viscosity of gel. Therefore, it was clear from this study that viscosity of gel may be increased by an acetone pretreatment. The probable cause of this effect may be attributed to physical changes that occurred during acetone pretreatment. The acetone pretreatment makes formation of micro layer over gel molecules and causes the gel more viscous.

It was seen observed that increase in centrifuge speed with different temperature, and varying holding time increased the viscosity (Table 4). When centrifuge speed was increased from 2000 rpm (0.521 – 2.098 cm²s⁻¹) to 10000 rpm (0.540 – 2.098 cm²s⁻¹), there was no difference found in viscosity of gel and similar trend was observed while Increasing centrifuge speed from 5000 rpm (0.540 – 2.098 cm²s⁻¹) to 10000 rpm (0.545 – 2.355 cm²s⁻¹) resulted marginal increase in viscosity of gel in various treatments.
The results of effect of centrifuge speed; with different proportion of acetone, varying temperature and duration on viscosity of gel (cm² s⁻¹).

### Table 4.

<table>
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<tr>
<th>Experimental conditions</th>
<th>Centrifuge duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without acetone</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Centrifuge speed 2000 rpm and 5°C temperature</td>
<td>0.635</td>
</tr>
<tr>
<td>Centrifuge speed 2000 rpm and 10°C temperature</td>
<td>0.584</td>
</tr>
<tr>
<td>Centrifuge speed 2000 rpm and 32°C temperature</td>
<td>0.521</td>
</tr>
<tr>
<td>Centrifuge speed 5000 rpm and 5°C temperature</td>
<td>0.650</td>
</tr>
<tr>
<td>Centrifuge speed 5000 rpm and 10°C temperature</td>
<td>0.592</td>
</tr>
<tr>
<td>Centrifuge speed 5000 rpm and 32°C temperature</td>
<td>0.543</td>
</tr>
<tr>
<td>Centrifuge speed 10000 rpm and 5°C temperature</td>
<td>0.635</td>
</tr>
<tr>
<td>Centrifuge speed 10000 rpm and 10°C temperature</td>
<td>0.595</td>
</tr>
<tr>
<td>Centrifuge speed 10000 rpm and 32°C temperature</td>
<td>0.545</td>
</tr>
</tbody>
</table>

**Effect of centrifuge speed on Refractive index of gel**

The statistical analysis of variance is given in Table 5 showed that the effect of centrifuge speed and its interaction with varying temperature, addition acetone and different holding duration on, refractive index of gel was found to be significant.

The results of effect of centrifuge speed; with different levels of acetone, temperature and duration on refractive index of gel presented in Table 6. The minimum refractive index was recorded 1.33427 at 10000 rpm centrifuge speed keeping at 5°C temperature, and holding it 30 min duration for 10 % addition of acetone treatment and maximum refractive index was found 1.33760 at 10°C centrifuge temperature and 2000 rpm centrifuge speed and 20 min duration for 10 % addition of acetone treatment. It was observed that increase in centrifuge speed, and duration decreased the refractive index of gel, and addition of acetone also results in increase in refractive index of gel. There was no uniform trend in refractive index for varying centrifuge temperatures (Table 6).

Increase in centrifuge speed with different temperature, and varying holding time resulted decrease in the refractive index (Table 6), which is a favourable aspect in gel extraction process. By visual observation it was perceived that when centrifuge speed was increased from 2000 rpm (1.33783 - 1.33607) to 5000 rpm (1.336530 - 1.33550), there was reduction found in refractive index of gel, while Increasing centrifuge speed from 5000 rpm (1.33653 - 0.33550) to 10000 rpm (1.33427 - 1.33587) lowered the refractive index of gel in various treatments. This may be due to more separation of fibers from pulp at higher centrifuge speed.

Obviously, further studies on the effect of acetone pretreatment with different centrifuge speed on the structural changes of aloe vera gel viscosity are essential to elucidate the exact mechanisms leading to the increase in viscosity.

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**Table 5.** Analysis of variance table for refractive index.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.S.</th>
<th>Cal. F</th>
<th>Tab. F 1%</th>
<th>S.E.M ± C.D. @ 5%</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three levels of Centrifuge speed</td>
<td>2</td>
<td>6.8x10⁻⁶</td>
<td>3.4x10⁻⁵</td>
<td>882.212</td>
<td>3.12</td>
<td>2.7x10⁻⁵</td>
<td>7.5x10⁻⁵</td>
</tr>
<tr>
<td>Three levels of Centrifuge speed with two levels of acetone</td>
<td>2</td>
<td>1.3x10⁻⁶</td>
<td>6.7x10⁻⁶</td>
<td>173.610</td>
<td>3.12</td>
<td>3.8x10⁻⁶</td>
<td>1.1x10⁻⁴</td>
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<td>4</td>
<td>8.7x10⁻⁷</td>
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<td>5.581</td>
<td>2.43</td>
<td>4.6x10⁻⁶</td>
<td>1.3x10⁻⁴</td>
</tr>
<tr>
<td>Three levels of Centrifuge speed with three levels of centrifuge holding time</td>
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<td>1.7x10⁻⁸</td>
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<td>4.2x10⁻⁸</td>
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<td>6.5x10⁻⁵</td>
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<td>9.1x10⁻⁷</td>
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<td>6.5x10⁻⁵</td>
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<td>4</td>
<td>5.5x10⁻⁷</td>
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<td>6.5x10⁻⁵</td>
<td>1.8x10⁻⁴</td>
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<tr>
<td>Three levels of different centrifuge speed with two levels of acetone, three levels of temperature and three levels of holding time</td>
<td>8</td>
<td>9.2x10⁻⁷</td>
<td>1.1x10⁻⁷</td>
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<td>2.01</td>
<td>1.1x10⁻⁸</td>
<td>3.2x10⁻⁴</td>
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<tr>
<td>Error</td>
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<td>4.2x10⁻⁶</td>
<td>4.0x10⁻⁶</td>
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<td></td>
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<tr>
<td>Total</td>
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</table>

C.V.,% = 0.015

d.f. = degree of freedom
S.S. = sum of squares
M.S.S. = mean of sum of squares
Cal. F = calculated F value
Tab. F 1% = table F value
S.E.M ± C.D. @ 5% = standard error of mean
C.D. = critical difference
C.V. = coefficient of variation
Conclusions

From the above results it was suggested that higher centrifuge speed found suitable for higher gel recovery and all quality parameters of gel extraction. Hence the gel extraction by centrifuge may be carried out at 10,000 rpm speed. This experiment shows that higher centrifuge speed break the chain of sugar molecules surrounded by gel molecules and leads more gel recovery and fibreless gel. It was recommended that the extraction of gel from aloe vera by the method of centrifuge should be carried out at the rate of 10,000 rpm centrifuge speed at 5°C centrifuge temperatures, for 30 min centrifuge duration so as to get higher gel recovery and good quality of gel.

References


Table 6: Effect of centrifuge speed with different proportion of acetone, varying temperature and duration on gel recovery (%) from pulp.