# Analysis of the properties of apple juice derived from Pulp Enzymation

# Testing the suitability of different gelling methods for the production of Apple Jam

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# Contents

**Abstract**

Apple juices are traditionally produced by grounding apples into a pulp and pressing the pulp through a filter to obtain a usually turbid juice. More recently, it was found that treating apple pulp with enzymes such as pectinases or cellulases (pulp enzymation) could have a clarification effect and produce a visually-appealing clear juice as well as increase the yield. The aim of this research is to investigate the differences, if any, between traditionally produced and pulp enzymation apple juice. Our tests affirm that pulp enzymation indeed produces a clear juice at roughly a 10% higher yield than traditional juice. Testing of the juice properties revealed subtle differences in the total sugar and reducing sugar content but were not significant. Notably, pulp enzymation produces a more acidic juice than traditional juice with a malic acid concentration of 0.40% and 0.26% respectively. Furthermore, the polysaccharide concentration of pulp enzymation apple juice was lower than that of traditional apple juice, at 1.5% and 3.6% respectively. As a further topic, 4 different methods of gelling in apple jam was also investigated, finding that the addition of citric acid and HM pectin produced the most desirable qualities in a jam compared to other methods.

# 1 Introduction

Traditional apple juices are produced by grounding apples into a pulp and pressing the pulp through a filter to obtain an often turbid juice containing a stable "cloud" assumed to consist of cell wall fragments or proteins surrounded by a coating of pectin[1]. This "cloud" is considered undesirable by most consumers who prefer a clear and shiny apple juice over a cloudy and turbid juice[2]. Hence, apple juice producers greatly appreciate techniques to remove the cloud particles in natural apple juice. One such technique is called "pulp enzymation", where pectin-degrading enzymes such as polygalacturonase (PG) are added to apple pulp before juicing. PG degrades the protective pectin coating of the cloud particles in the colloid, causing the exposure of dissimilarly-charged surfaces[2]. Aggregation or flocculation of the cloud particles then ensues due to electrostatic attraction, the large aggregates (flocs) then precicpitate out of the juice to form a desirable clear apple juice.

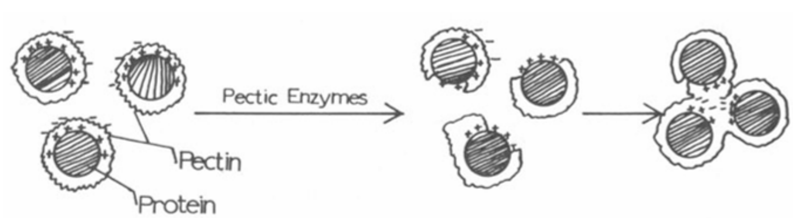


**Figure 1:** Proposed mechanism of flocculation of cloud particles in Apple juice[2]

With the desirable effects of pulp enzymation in mind, our research is aimed at characterizing the properties of pulp enzymation apple juice to detect, if any, the differences between the juices produced through pulp enzymation and traditional means. We have used Pectinex® Ultra SP-L, which contains pectinases from *Aspergillus Aculeatus* in our production of pulp enzymation juice[3]. Of the pectinases contained, the major component is polygalacturonase (PG).

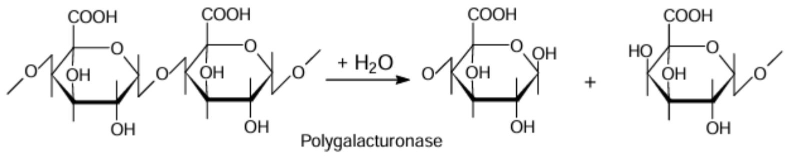


**Figure 2:** Activity of enzyme Polygalacturonase[1]

Pectin is a heteroglycan consisting of mainly $\alpha$-D-polygalacturonic acid chains that constitutes a major component of the plant cell wall[4]. Pectins also play a role in gel formation in various food products such as jams and syrups. Notably, different types of pectins can have different suitability in gel formation[4]. Pectins can be characterized according to the degree of methyl esterification of the carboxylic acid group on the galacturonic acid residues[1]. Pectins with more than 50% of the galacturonic acid residues in methyl ester form are called high-methoxy (HM) pectins, while pectins with under 50% are called low-methoxy (LM) pectins[1]. LM pectins tend to form a calcium pectate gel in the presence of $Ca^{2+}$ ions in an "egg box" dimer form[4]. The calcium pectate gel of LM pectin forms due to the electrostatic attraction between the negatively-charged deprotonated carboxylic acid residues in polygalacturonic acid and the positively-charged calcium ions[4]. On the other hand, HM pectins tend to form sugar-acid gels under low pH and high sugar concentrations (>65%)[1]. sugar-acid gels form due to the formation of junction zones in pectin chains by hydrogen bonding and hydrophobic interactions, these interactions are significant in HM pectin as there are lesser carboxylic acid groups with negative charge to promote repulsion between chains in HM pectin[1][4].

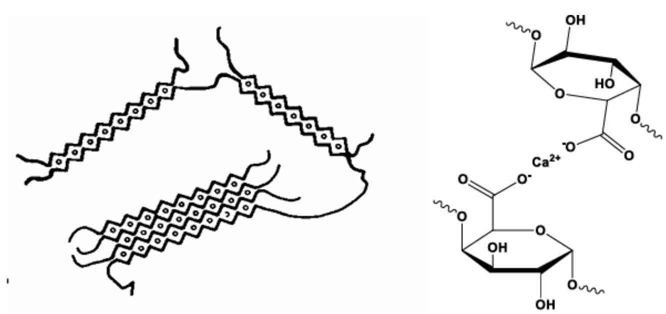


**Figure 3:** Mehcanism for formation of calcium pectate gels [1]

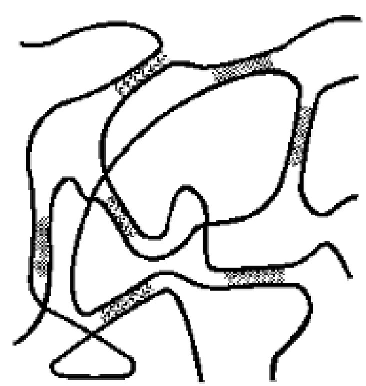

**Figure 4:** Formation of junction zones in a sugar-acid gel[1]

A section of this report documents our effort in determining what mode of pectin gelation is most suitable for the production of apple jam. Most typically, sugar-acid pectin gels are common in commercial apple jams, we expect this to be the safest approach.

# 2 Results and Discussion

We have tested 4 samples of apple juices that are produced according to the methods described in table 1. The type of apple used for the production of juices were generically of the Fuji Apple type. The naming convention used in Table 1 shall also be used throughout this report.

**Table 1:** Summary of juice samples

| Sample | Description |
|---|---|
| B1 | Traditional (blank) apple juice incubated at 40 °C for 1 hour |
| B2 | Traditional (blank) apple juice incubated at 40 °C for 2 hour |
| E1 | Pulp enzymation (enzyme) apple juice incubated at 40 °C for 1 hour |
| E2 | Pulp enzymation (enzyme) apple juice incubated at 40 °C for 2 hour |

## 2.1 Yield and Appearance of apple juices

The yields of the juices and comparison to the yields of other groups are summarized in Table 3. The clear juice obtained by pulp enzymation can be contrasted with the turbid traditional juice in Figure 5. The flocculation and sedimentation of cloud particles can be clearly observed in the E1 and E2 samples which have larger pellets than the B1 and B2 samples.

**Table 2:** Juice yields of different juice samples

| Sample | Yield (%) | Ref 1[a] (%) | Ref 2[a] (%) |
|---|---|---|---|
| B1 | 59.1 | 58.2 | 62.7 |
| B2 | 69.6 | 60.9 | 65.0 |
| E1 | 70.4 | 68.2 | 68.9 |
| E2 | 75.5 | 71.6 | 71.0 |

[a] Reference yields were taken from other groups of students

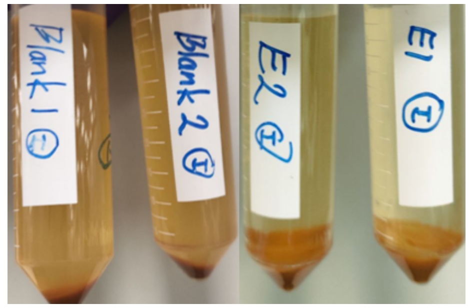


**Figure 5:** (left to right) B1, B2, E2, E1

The results show that increased incubation time results in a greater yield. This is possibly due to greater breakdown of cell walls by longer incubation. Additionally, the results show that pulp enzymation juices have a higher yield (70.2% - 75.5%) than traditional juices (59.1% - 69.6%). This is because the pectinases present in E1 and E2 have caused "liquefaction" of the apple pulp by degrading cell walls, more liquid is released from broken cells leading to greater yield[1].

## 2.2 Acidity of apple juices

The total titratable acids (TTA) of the different juices were measured and summarized in Table 3.

**Table 3:** pH and Total Titratable Acidity of apple juice samples

| Sample | Total Titratable Acidity[a] % (w/w)[a] | pH |
|---|---|---|
| B1 | 0.255 | 4.41 |
| B2 | 0.258 | 4.35 |
| E1 | 0.396 | 3.81 |
| E2 | 0.396 | 3.85 |

[a] malic acid

We observe higher acidity in pulp enzymation apple juices compared to traditional apple juices both in pH and total titratable acid. Furthermore, the results show that incubation time has little to no effect on acidity. The increased acidity of pulp enzymation juices is possibly due to the increased presence of polygalacturonic acid chains that have made it into the juice because of increased solubility as a result of cleavage by polygalacturonase (PG). Alternatively, the degradation of cell walls by pectinases could also cause a larger amount of inorganic acids to be released by broken cells.

The samples B1 and B2 align well with reported TTA values of 0.23% for Fuji Apple juice, while the samples E1 and E2 are more acidic than the reference values[5]. The results are also within the TTA (0.23% - 1.82 %) and pH ranges (3.37-4.24) for 175 different apple juices[6].

## 2.3 Sugar Profile of apple juices

The concentrations for total sugars, reducing sugars, polysaccharides and low molecular-weight (Mw) carbohydrates of the juice samples are given in Figure 6. The results were obtained by the Dubois-phenol sulphuric acid test, the Nelson Somogyi test, and ethanol precipitation of polysaccharides[7].

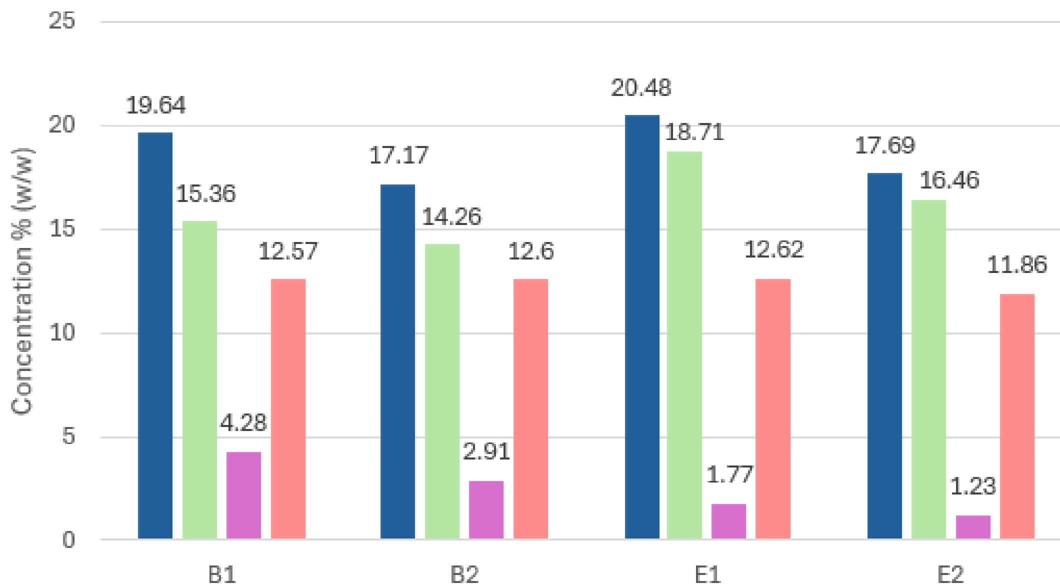


**Figure 6:** Total sugar concentration (blue), Low molecular-weight (Mw) carbohydrate concentration (green), polysaccharide concentration (purple) and reducing sugar concentration (red)

The results show that the total sugar concentration for pulp enzymation juices is slightly higher than the traditional juices (20.48% vs 19.64%, 17.69% vs 17.17%) , but the difference is not significant. As Pulp enzymation further degrades cell walls to release sugars from within apple cells, the slight increase in total sugars is understandable. Notably, the concentration of reducing sugars do not vary significantly accross the samples. This is contrary to expectations as the enzyme PG is expected to release more reducing ends as a result of the cleavage of glycosidic linkages in polygalacturonic acid[1]. It is difficult to correlate our obtained results for total sugar concentration to literature as a wide range of values are reported, ranging from 12.1% to 40.75% according to different sources [8][9]. However, our results for reducing sugar concentration do not differ significantly with the values reported by literature ranging from 11.51% to 11.24% [6][10].

The largest differences are observed in the polysaccharide and low Mw carbohydrate profile. The results show that the pulp enzymation juices have lower polysaccharide concentration than the traditional juices (4.28% vs 1.77%, 2.91% vs 1.23%). This can be explained by the activity of enzyme PG in the pulp enzymation process, where large polygalacturonic acid chains are broken into smaller chains[1]. Expectedly, the results also show a higher concentration of low Mw carbohydrates in the pulp enzymation juices compared to the traditional juices (18.71% vs 15.36%, 16.46% vs 14.26%). This further confirms the action of enzyme PG in breaking down large polysaccharides into low Mw carbohydrates such as oligosaccharides.

In general, the results show a trend of sugar concentration dilution as the incubation time increases from 1 hour to 2 hour. B1 has a total sugar concentration of 19.64% but B2 drops off to 17.17%, the same can be said for E1 to E2 with a drop from 20.48% to 17.69% in total sugar concentration. This trend appears in total sugar, polysaccharide and low Mw carbohydrate concentration, with the exception of reducing sugar concentration, which does not show much change. This trend can potentially be explained by the release of larger amounts of water as incubation time increases, giving a larger yield at the cost of a more dilute juice.

## 2.4 °Brix refractometry of apple juices

The amount of dissolved solids in the samples of apple juice was measured by °Brix refractometry and the results are summarised in Table 4.

**Table 4:** Amount of dissolved solids in juice as indicated by °Brix refractometry

| Sample | °Brix (%) |
|---|---|
| B1 | 13.4 |
| B2 | 13.0 |
| E1 | 14.7 |
| E2 | 14.3 |

Firstly, the results suggest that the pulp enzymation apple juices have a higher amount of dissolved solids than the traditional apple juice. This can be explained by the higher concentration of low molecular-weight carbohydrates (thus, higher solubility) of the pulp enzymation juice. A higher presence of inorganic acids such as malic acid as eluded by the larger acidity values may also contribute to the higher °Brix values.

Secondly, the results suggest that increased incubation time decreases the amount of dissolved solids in the final juice. This would suggest that longer incubation times increase the yield of juice at the cost of diluting it. Previous results also consistently show that 2 hour incubated juices have a lower sugar concentration than the 1 hour incubated juices, matching well with previously measured data.

Generally, the results align with the reported °Brix values of 13.9±1.0 % for Fuji Apple juice [5]. The results also fall within the reported range of °Brix values of 175 types of different apple juices, which is between 10.26 % to 21.62 % [6].

## 2.5 Gelation of pectin in apple jam

Apple jam was prepared using different additives that are summarized in Table 5:

**Table 5:** Summary of apple jam additives and expected gelling method

| Jam | Additives | Expected gel |
|---|---|---|
| 1 | Apples, Sugar | Sugar-acid gel |
| 2 | Apples, Sugar, HM pectin | Sugar-acid gel |
| 3 | Apples, Sugar, HM pectin, Citric acid | Sugar-acid gel |
| 4 | Apples, Water, Calcium Sulphate Dihydrate | Calcium pectate gel |

The properties of the resulting jams are discussed in Table 6. It can be observed in Figure 7 that jams 3 and 4 maintain their shape when sideways and are of higher viscosity than jams 1 and 2.

**Table 6:** Summary of resulting apple jams

| Jam | Viscosity | Description |
|---|---|---|
| 1 | least viscous | Undesirable because watery texture is unspreadable. |
| 2 | Syrup-like | Undesirable because of low spreadability. |
| 3 | Jam-like | Most desirable because of spreadable texture and appealing colour. |
| 4 | Jelly-like | Undesirable because of pale discolouration. |


**Figure 7:** Physical appearance of the jams laid sideways to demonstrate viscosity. (top, left to right) 1,2. (bottom, left to right) 3,4

Jam 4 posseses the most desirable qualities in an apple jam, having good texture and colour. This shows that addition of HM pectin and additional acid is required to produce a desirable viscosity in a sugar-acid gel in jam. The calcium pectate gel of jam 4 proves to be unsuitable as the method causes a discoloration to the jam, turning the color pale and unappetizing. This may be due to boiling the apples in water or some previously unknown discoloration effect of calcium sulphate on apples.

### 2.6 HM Pectin stability in different pH environments

We have tested the stability of a 0.5% HM pectin solution in acidic, basic and neutral solution by observing the ethanol precipitation of pectin after treating with acid or base[7]. However, we are unable to obtain any precipitation for any of the samples, meaning either that pectin degraded in all of the samples or that the experimental setup is flawed. Theoretically, HM pectins should be stable in acidic or neutral environments but degrade at basic conditions due to beta-elimination and saponification reactions[7]. An interesting observation made is that the treatement of HM pectin solution by base under heat causes the pectin solution to turn from alkaline to acidic, this can be explained by the saponification reaction cleaving the methoxy groups and releasing carboxylic acid groups that contribute to acidity.

## 3 Conclusions

Pulp enzymation is an effective method of producing clarified, non-cloudy apple juice which has subtle differences in composition compared to traditionally-produced apple juice. Notably, pulp enzymation produces apple juice at a higher yield (73% vs 64%), with a higher acid content (0.40% vs 0.26%), a higher concentration of low molecular weight carbohydrates (14.81% vs 17.59%), and a lower concentration of large polysaccharides (1.5% vs 3.6%). Furthermore, incubation of apples at 40 °C for 2 hours produced higher yields but more dilute juices compared to those incubated for 1 hour. These results demonstrate that pulp enzymation is a feasible method for apple juice manufacturers to not only clarify apple juices but also to increase juice yield. The apple juice produced by pulp enzymation has roughly the same total sugar content and is likely sweeter than traditional apple juice owing to the increased concentration of low molecular-weight carbohydrates, demonstrating that pulp enzymation does not affect the quality of the juice. However, manufacturers should still be aware of the increased acidity of the juices which may potentially be undesirable in commercial beverages.

We have also found that the addition of sugar, HM pectin and citric acid produces the most desirable qualities in an apple jam such as optimum texture and colour. On the other hand, calcium pectate apple jam has an undesirable discoloration with an overly rigid texture.

## References

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# A Appendix

**Figure 8:** Calculation for yields of apple juice

| Sample | Mass of Apples (g) | Mass of Juice (g) | yield (%) |
|---|---|---|---|
| B1 | 71.23 | 42.068 | 59.1 |
| B2 | 71.57 | 49.805 | 69.6 |
| E1 | 71.27 | 50.158 | 70.4 |
| E2 | 71.68 | 54.143 | 75.5 |

**Figure 9:** Liquefaction of apple pulp after incubation, (left to right) B1,E1,B2.E2

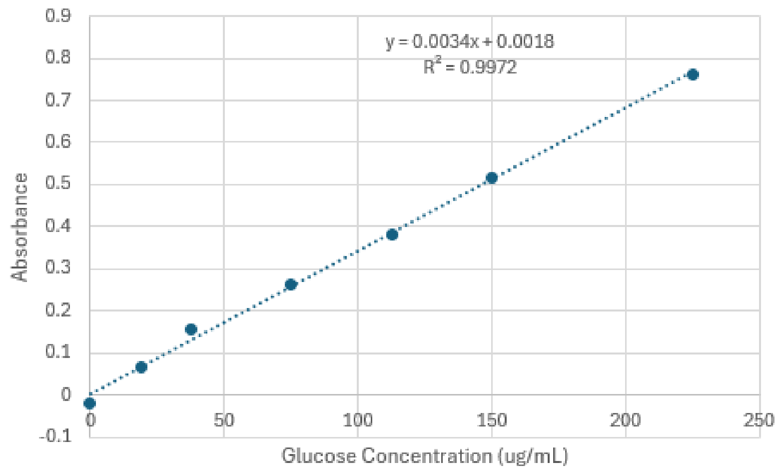


**Figure 10:** Calibration Curve for Total Sugar Assay

**Figure 11:** Calculation for the total sugar concentration and low Mw carbohydrate concentration

| Sample | Dilution | Absorbance | glucose concentration % (w/w) |
|---|---|---|---|
| B1 | 1500 | 0.447 | 19.64 |
| B2 | 1500 | 0.391 | 17.17 |
| E1 | 1500 | 0.466 | 20.48 |
| E2 | 1500 | 0.403 | 17.69 |
| PB1 | 1500 | 0.350 | 15.36 |
| PB2 | 1500 | 0.325 | 14.26 |
| PE1 | 1500 | 0.426 | 18.71 |
| PE2 | 1500 | 0.375 | 16.46 |

**Figure 12:** Calculation for the polysaccharide concentration

| Sample | Total Sugar Concentration % (w/w) | Low Mw carbohydrate concentration % (w/w) | Polysaccharide concentration % (w/w) |
|---|---|---|---|
| B1 | 19.64 | 15.360 | 4.28 |
| B2 | 17.17 | 14.260 | 2.91 |
| E1 | 20.48 | 18.710 | 1.77 |
| E2 | 17.69 | 16.460 | 1.23 |

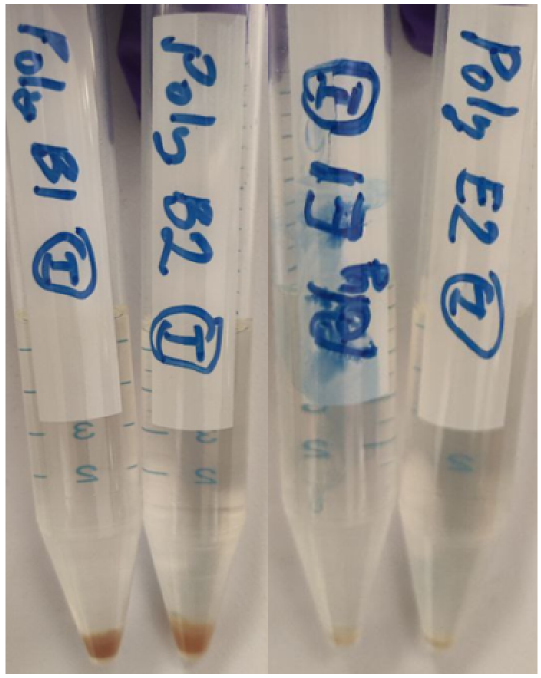


**Figure 13:** Ethanol Precipitation for polysaccharides, (left to right) B1,B2,E1,E2

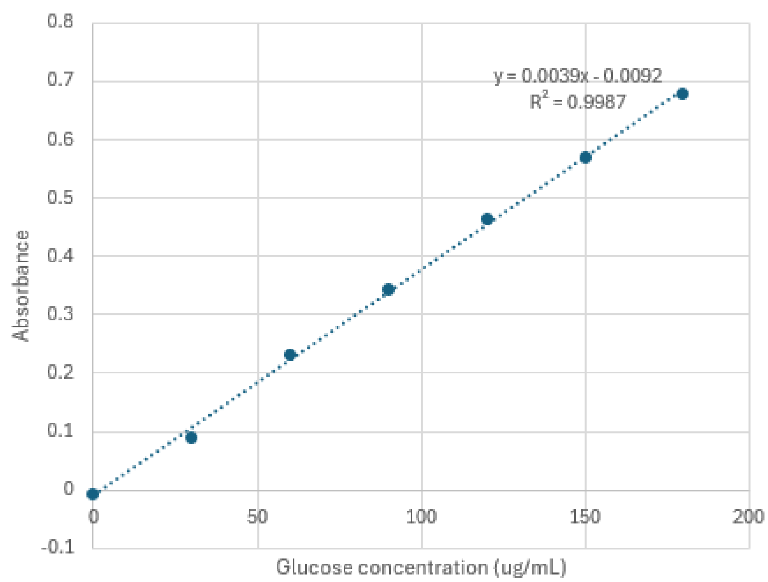


**Figure 14:** Calibration Curve for Reducing Sugar Assay

**Figure 15:** Calculation for Reducing sugar concentration

| Sample | Dilution | Absorbance | Glucose concentration % (w/w) |
|---|---|---|---|
| B1 | 1000 | 0.481 | 12.57 |
| B2 | 1000 | 0.482 | 12.59 |
| E1 | 1000 | 0.483 | 12.62 |
| E2 | 1000 | 0.464 | 12.13 |


**Figure 16:** Visual appearance of Jam