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Candidate session number	0			
Candidate name				
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Examination session (May or November)	MAY	Year	2015	

Diploma Programme subject in which this extended essay is registered: Biology  
(For an extended essay in the area of languages, state the language and whether it is group 1 or group 2.)

Title of the extended essay: The digestion rate of oatmeal after different pre-soaking treatments and predicting its Glycemic Index

### Candidate's declaration

*This declaration must be signed by the candidate; otherwise a grade may not be issued.*

The extended essay I am submitting is my own work (apart from guidance allowed by the International Baccalaureate).

I have acknowledged each use of the words, graphics or ideas of another person, whether written, oral or visual.

I am aware that the word limit for all extended essays is 4000 words and that examiners are not required to read beyond this limit.

This is the final version of my extended essay.

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## Supervisor's report and declaration

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Name of supervisor (CAPITAL letters) \_\_\_\_\_

Please comment, as appropriate, on the candidate's performance, the context in which the candidate undertook the research for the extended essay, any difficulties encountered and how these were overcome (see page 13 of the extended essay guide). The concluding interview (viva voce) may provide useful information. These comments can help the examiner award a level for criterion K (holistic judgment). Do not comment on any adverse personal circumstances that may have affected the candidate. If the amount of time spent with the candidate was zero, you must explain this, in particular how it was then possible to authenticate the essay as the candidate's own work. You may attach an additional sheet if there is insufficient space here.

In her Extended Essay, \_\_\_\_\_ showed a very thorough and accurate approach to the experimental investigation on the effect of pre-soaking times of oatmeal (prior to ingestion) on the digestive breakdown by alpha amylase and its correlation with the glycemic index.

The underlying ideas that helped her come up with a focused research question, the development of a method and the analytical and statistical tools that would allow for a reasoned argumentation were obtained through thorough research and the consultation of numerous scientific sources.

Although the method itself followed a relatively simplistic routine by using an iodine starch assay and a colorimeter to quantify the digestive breakdown of the starch contained in the grain, \_\_\_\_\_ had to spend a lot of time in preliminary trials in order to fine-tune the specifications in set up and controlled experimentation. During that time, \_\_\_\_\_ always considered the alterations to the method carefully, and by doing so developed a great sense for the scientific inquiry. Throughout the experimental phase, \_\_\_\_\_ engaged herself thoroughly and completely independently with the correct use and relevance of the apparatus.

\_\_\_\_\_ also displayed a great deal of independency and responsibility, barely requiring any outside help.

In addition to the applied techniques and procedure, the analytical component of \_\_\_\_\_ Extended Essay was elaborated on in detail and with the support of the well referenced research studies. The analysis of the data collected has been performed according to an appropriate choice of mathematical and statistical tools. The use of the Spearman's rank-test to test for a correlation between the obtained results and the predicted glycemic index the subsequent interpretation, were an extension to the already established results and allowed for a more meaningful and deeper interpretation of the data.

Throughout the interview, \_\_\_\_\_'s knowledge and understanding of the performed investigation became even more apparent. She clearly pointed out the significance and relevance of this investigation for real life situations and was able to deliver ample amount of scientific support for her results.

*This declaration must be signed by the supervisor; otherwise a grade may not be issued.*

I have read the final version of the extended essay that will be submitted to the examiner.

To the best of my knowledge, the extended essay is the authentic work of the candidate.

I spent 3 hours with the candidate discussing the progress of the extended essay.

Supervisor's signature \_\_\_\_\_

Date: 20.1.2015

**Assessment form (for examiner use only)**

Candidate session number	<b>0</b>
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**Achievement level**

Criteria	Examiner 1		Examiner 2		Examiner 3	
	maximum		maximum		maximum	
A research question		<input type="text" value="2"/>	2	<input type="text"/>	2	<input type="text"/>
B introduction		<input type="text" value="2"/>	2	<input type="text"/>	2	<input type="text"/>
C investigation		<input type="text" value="3"/>	4	<input type="text"/>	4	<input type="text"/>
D knowledge and understanding		<input type="text" value="2"/>	4	<input type="text"/>	4	<input type="text"/>
E reasoned argument		<input type="text" value="2"/>	4	<input type="text"/>	4	<input type="text"/>
F analysis and evaluation		<input type="text" value="3"/>	4	<input type="text"/>	4	<input type="text"/>
G use of subject language		<input type="text" value="3"/>	4	<input type="text"/>	4	<input type="text"/>
H conclusion		<input type="text" value="1"/>	2	<input type="text"/>	2	<input type="text"/>
I formal presentation		<input type="text" value="3"/>	4	<input type="text"/>	4	<input type="text"/>
J abstract		<input type="text" value="1"/>	2	<input type="text"/>	2	<input type="text"/>
K holistic judgment		<input type="text" value="3"/>	4	<input type="text"/>	4	<input type="text"/>
Total out of 36		<input type="text" value="25"/>		<input type="text"/>		<input type="text"/>

Name of examiner 1: \_\_\_\_\_ Examiner number: \_\_\_\_\_  
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Name of examiner 2: \_\_\_\_\_ Examiner number: \_\_\_\_\_  
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# The digestion rate of oatmeal after different pre-soaking treatments and its correlation to Glycemic Index

*Subject: Biology*

*Word Count: 3990*

## Abstract

The experiment investigates how the digestion rate of oatmeal by alpha amylase is changed by soaking the oats *Avena sativa* for different lengths of time, and thus how the Glycemic Index changed. The research question is therefore “How does the time allowed for pre-soaking treatment (2, 4, 6 and 8 hours prior to digestion) for *Avena sativa* affect the enzymatic breakdown of bacterial amylase, and how does this correlate with the Glycemic Index?” This could yield insight to the importance of pre-soaking oatmeal and its effect on our health, as longer pre-soaking treatment could allow for *Avena* to break up and release the starches used by the body for the production of energy.

unlike! by mass?  
by volume?

Equal amounts of *Avena* were mixed with equal parts water and set up on magnetic stirrers for 2, 4, 6 and 8 hours. The contents were then strained through a filter paper. The remaining liquid was collected into Eppendorf tubes. Equal parts of the liquid and of 1% alpha amylase were reacted inside cuvettes with iodine acting as an indicator. A colorimeter collected the absorbance for 5 minutes after the reaction was started. This data was then analysed and manipulated using statistical methods.

8 hours pre-soaking indicated the fastest absorption/second rate, followed by 6 hours. 2 hours and 4 hours pre-soaking showed very similar results, with the rate for 4 hours pre-soaking being slightly higher. A Spearman's Rank Correlation Test showed a clear correlation of  $R^2 = 1.0$  between pre-soaking time and absorption rate at 3s, indicating that the longest soaked *Avena* was digested most rapidly. This also indicated that the Glycemic Index was highest for this trial. However, it was concluded that due to the relatively small differences between absorption rates after different pre-soaking treatments, the differences in Glycemic Index are also relatively small.

?  
unclear.

**Word count: 298**

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*done + annotated*



## 1. Introduction

In the study conducted by Yiu, Wood and Weisz “Effects of Cooking on Starch and beta-Glucan of Rolled Oats”, the rate of starch digestion of rolled oats soaked, rapid cooked and gradually cooked were compared, with the rapid and gradual cooking showing the fastest rate, and soaked oats showing a slower rate (Yiu et al.). While this experiment showed that there is a difference between cooking and soaking oats, it did not go in depth into different soaking times of oatmeal. The following investigation will give insight into digestion after different soaking times, to further deepen the knowledge already available about oatmeal and its digestion rate.

### 1.1 Background information

#### 1.1.1 Oats

Oats, *Avena sativa*, are considered a healthy food for many reasons, including lowering cholesterol and reducing the risk of cardiovascular disease (“Oats”).

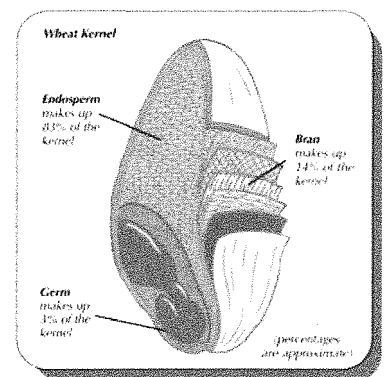
*Avena* are composed of three parts: the endosperm, which makes up 83% of the oat, the bran, which is 14% of the oat, and the germ, which is the smallest part of the oat, taking up 3% of its body mass (Figure 1).

The endosperm is what is most important for this investigation, as it contains the most carbohydrates out of the three components. Bran contains most of the soluble and insoluble fibres; the germ contains

most nutrients (Cooper). Oatmeal is one of the many popular dishes created out of oats, and it is often prepared by either soaking or cooking the oats. When the oats are cooked or soaked, the bran breaks down,

releasing a lot of the fibres and starches found inside the grain. This isn't the case when they are used fresh, without cooking or pre-soaking, yet this seems to be the method used more

often as people have less time in the morning. This is why pre-soaking (e.g. overnight in preparation for a breakfast) is a good solution. With its high content of starch, oatmeal is a good source of energy and often consumed by people who do sports. The starch is broken down to release glucose which in turn is converted to energy in respiration. Even though it is known that pre-soaking is helpful, some people pre-soak oatmeal for an hour before the meal, while others pre-soak overnight, which means it is soaked for 8 hours. It is unknown whether there is a difference between the amount of starch released after these treatments, which is how the research question “How does the time allowed for pre-soaking treatment (2, 4, 6 and



**Figure 1: Wheat kernel**  
(courtesy of Hemi Weingarten)

*8 hours prior to digestion) for Avena sativa affect the enzymatic breakdown of bacterial amylase, and how does this correlate with the Glycemic Index?"* was developed. RA.

### 1.1.2 Glycemic Index

This investigation will however also look at whether oatmeal can be considered a healthy meal under the nutritional framework of the Glycemic Index (GI). With increasing knowledge about nutrition, diets and healthy weight loss, the Glycemic Index has been a value which has often been used to monitor how healthy foods are. This index measures how different foods can raise the blood sugar after digestion. It does this through looking at the content of starch inside the foods (Montignac). In general, it can be said that a higher GI for a specific type of food contributes to a fast increase in blood glucose levels right after a meal, while a lower Glycemic Index allows blood sugars to rise more steadily after a meal. Fast rising blood sugar levels can contribute to insulin secretion in the body, possibly causing weight gain (Montignac). This is why the newest diets suggest, that only foods with low GI be consumed to keep blood glucose low. Because oatmeal is said to release glucose slowly, it should have a lower Glycemic Index. This also helps the organism feel full for longer, preventing urges to eat between meals.

### 1.1.3 Carbohydrates

Carbohydrates, or starches, are known to consist of two main forms of complex carbohydrates: amylopectin and amylose. Amylopectin is a large, highly-branched chain of carbohydrates with one reducing end group and numerous branches (Bemiller). It is a linear chain of 1 to 4 linked  $\alpha$ -D-glycopyranosyl with 1 to 6 branching found (Bemiller). In normal starches it takes up approximately 75% of the carbohydrate, while amylose takes up the other 25% (Bemiller). Amylose is a smaller compound, in a helical shape. It is also a linear chain of 1 to 4 linked  $\alpha$ -D-glycopyranosyl but lacks the branching characteristic for amylopectin (Bemiller). These two compounds are broken down by  $\alpha$ -amylase, an enzyme which catalyses the hydrolysis of starch into glucose. This enzyme attacks the 1 to 4 linkages found in the amylose and amylopectin, breaking them. It is secreted in the mouth and pancreas to help digest food we ingest. The starches are broken down to the monosaccharide glucose. ✓

### 1.1.4 The relationship between the amylopectin-amylose ratio and the Glycemic Index

As mentioned previously, the Glycemic Index (GI) measures how blood sugar is raised after consuming a certain food, with 100 being the highest and 0 being the lowest. With its high branching and many bonds, amylopectin can be broken down much easier and thus faster



than amylose, composed off a more linear structure. Hence, carbohydrate containing foods high in amylopectin are hydrolysed quickly, releasing glucose fast, meaning they have a high GI, while foods high in amylose release glucose slower, hence they have a lower GI. Hence, a fast digestion rate signifies that there is more amylopectin, while a slower digestion rate suggests more amylose. For a healthy diet a lower GI, so more amylose, is ideal, as glucose passes into the blood stream slower, releasing energy more steadily and keeping insulin levels low. The GI value of oatmeal is noted to be 55 (Atkinson et al), which is in the middle of the GI scale, suggesting that oatmeal is, in fact, digested relatively slowly, with a steady release of sugar. Naturally, a lower GI would mean a slower release, but the literature value for oatmeal can be considered as healthy.

1.1.5 Research Question: *How does the time allowed for pre-soaking treatment (2, 4, 6 and 8 hours prior to digestion) for Avena sativa affect the enzymatic breakdown of bacterial amylase, and how does this correlate with the Glycemic Index?* ✓

## 1.2 Hypotheses

**The digestion rate will be fastest for the oatmeal that soaks for 8h.** This is because this will break the bran up the most, releasing the most starch and as more starch is released, more can be digested by the amylase at one time. Theory suggests that **the longer the soaking time, the higher the Glycemic Index** because of the faster digestion rate at longer soaking times. The GI literature value for oatmeal was found to be 55 (Atkinson et al), but this does not specify how the oatmeal is prepared, therefore this experiment aims to investigate whether there really is a big difference between the individual soaking times and the digestion rates of their oatmeal, because if yes, it may be possible that soaking for longer amounts of time may be unhealthier. ✓

## 1.3 Apparatus

- 4x 600ml beakers
- 4x 400ml
- Tap water
- Scale ( $\pm 0.01$ g)
- 100ml measuring cylinder ( $\pm 0.5$ ml)
- 4x magnetic stirrers
- Filter paper

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- Funnel
- Styrofoam box
- Ice in a plastic bag
- 1.5ml pipettes ( $\pm 0.25$ ml)
- 40x Eppendorf tubes
- Computer with LoggerPro 3.8.5
- LabPro
- Vernier Colorimeter
- Cuvettes
- Iodine
- 1% amylase
- Thermometer

## 2. Methodology

### 2.1 Procedure

What was originally meant to be investigated by this experiment was the starch digestion rate by  $\alpha$ -amylase in different fruit and vegetables using Benedict's solution as an indicator of the reaction. This was meant to give insight into the GI and healthiness of different foods. Yet, this showed a lot of problems as all reactants and indicator had to be heated, meaning it was harder to measure the absorbance over time, as the mixture would cool down. Hence, instead of measuring sugar content using Benedicts, iodine was used to measure starch and how this decreases in the mixture. Iodine is an indicator which works well at room temperature, meaning that the experiment could be carried out with greater ease. It was also decided that rolled *Avena sativa* will be investigated, more precisely how it's soaking time affects starch digestion.

There were two main parts to the collection of data:

- Taking samples
- Taking readings

Four 600ml beakers were set up on magnetic stirrers. 40.00g of oats *Avena sativa* were measured out using a scale into each of the beakers. 300ml of tap water was poured into four 400ml beakers using 100ml measuring cylinders. The first beaker with water was then emptied into the first beaker with oats. A magnetic flea was then dropped into the beaker and

the magnetic stirrer was set onto the setting 4, while a timer was started. This was repeated with the other three beakers.

The filtering set up was prepared by folding a filter paper to fit a funnel, and putting both inside a dry 400ml beaker. After 2 hours, the stirrer was turned off, and all of the fluid was poured into the filter paper, including the oats inside the now formed oatmeal. The liquid drained out of the oatmeal was collected using a 1.5ml pipette into 10 Eppendorf tubes labelled with the correct pre-soaking treatment. These were filled to the 1.5ml line. The oatmeal with the filter paper was discarded and the funnel was rinsed and set up with another filter paper. This sample collection was repeated after 4, 6 and 8 hours.

Before the readings were taken, a bag of ice and a thermometer as well as amylase and Eppendorf tubes were inserted into a Styrofoam box. This ensured that both were at the same temperature, 23°C. The colorimeter was connected to the laptop using a LabPro. LoggerPro was set up on the computer to collect 1 sample every 3 seconds for 300 seconds. The colorimeter was then calibrated by inserting a cuvette filled with distilled water into it and pressing CAL.

1.5ml of the first liquid sample inside the Eppendorf tube was collected and transferred into a cuvette. 1.5ml of amylase was then added to the cuvette, and straight after this two drops of iodine were dropped inside. To mix the contents, the cuvette was flipped upside down once keeping a finger on the opening. The cuvette was then quickly inserted into the colorimeter and "Collect" was pressed on LoggerPro. This was repeated for all other trials of that treatment and for each other treatment.

## 2.2 Data processing

10 trials were done for each of the soaking times. The rate was calculated at each time interval except at 0s (this was excluded as it would give a rate of 0), using the following formula:

$$\text{rate} = \frac{\text{absorbance}}{\text{time}}$$

*what are the units of absorbance*

For example, in trial 1 for 2h soaking time, these were the values:

Time (s)	Absorbance
3	0.159

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Hence, the rate for this time interval would be:

$$rate = \frac{0.159}{3} = 0.0530 \text{ absorbance/second}$$

Then, the average of all of the rates for each trial and for each time interval was calculated using Excel 2013. The following formula was used:

=AVERAGE('TRIAL 1'!J5,'TRIAL 2'!J5,'TRIAL 3'!J5,'TRIAL 4'!J5,'TRIAL 5'!J5,'TRIAL 6'!J5,'TRIAL 7'!J5,'TRIAL 8'!J5,'TRIAL 9'!J5,'TRIAL 10 no 6h'!J5)

(This was the formula at 3s for 2h soaking time)

The standard deviation was also calculated on Excel 2013 using the following formula:

=STDEV('TRIAL 1'!J4,'TRIAL 2'!J4,'TRIAL 3'!J4,'TRIAL 4'!J4,'TRIAL 5'!J4,'TRIAL 6'!J4,'TRIAL 7'!J4,'TRIAL 8'!J4,'TRIAL 9'!J4,'TRIAL 10 no 6h'!J4)

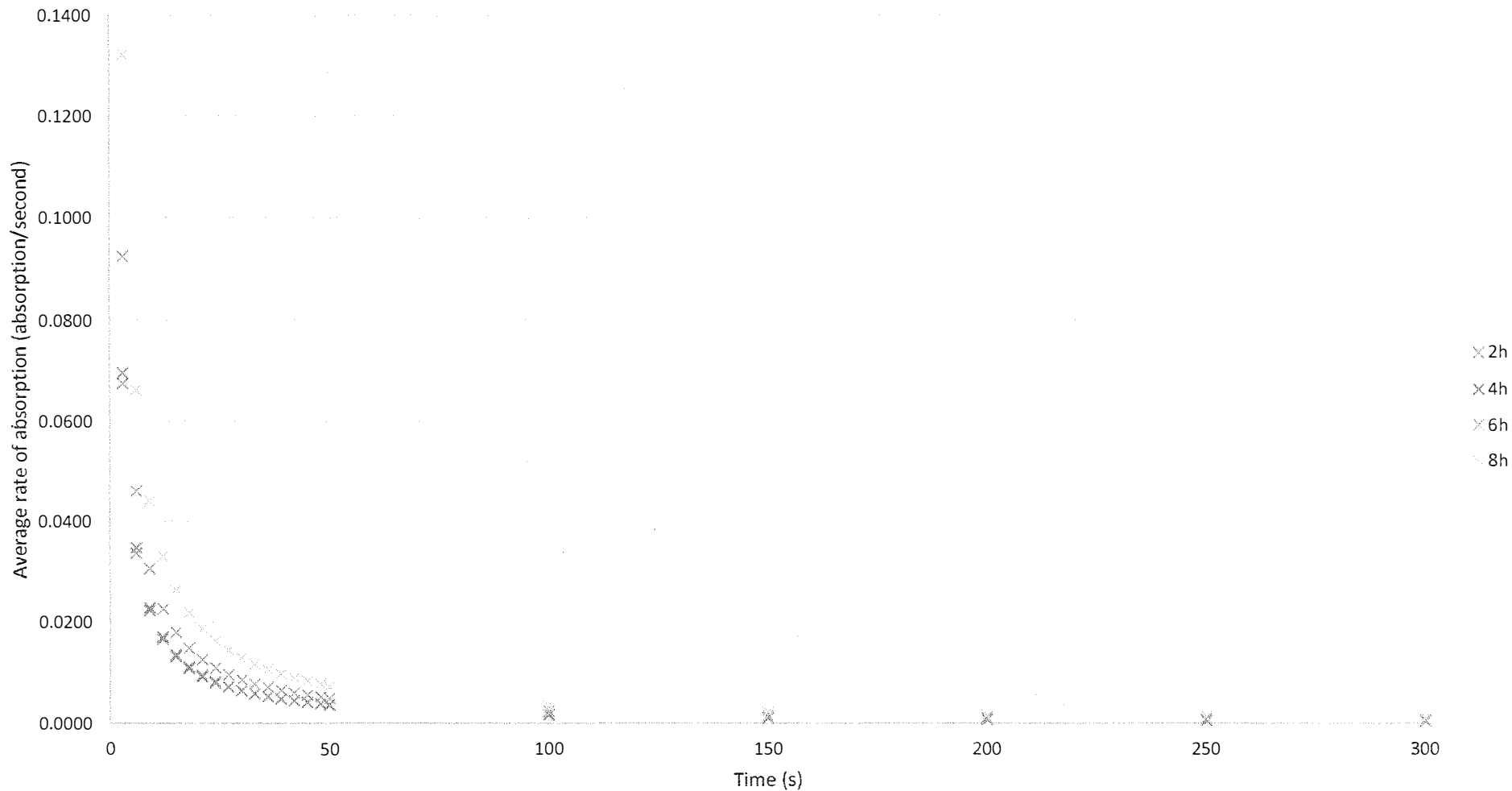
(Formula at 3s for 2h soaking time)

These calculations resulted in the table found in Appendix 1. For clearer observation of the trend in the data, absorption times were calculated for the most significant intervals (Table 1). These were plotted on Graph 1 as well as used for calculating a Spearman's Rank Correlation Coefficient (Table 2).

*Table 1 showing the average absorption/second at different time intervals for the 4 pre-soaking times*

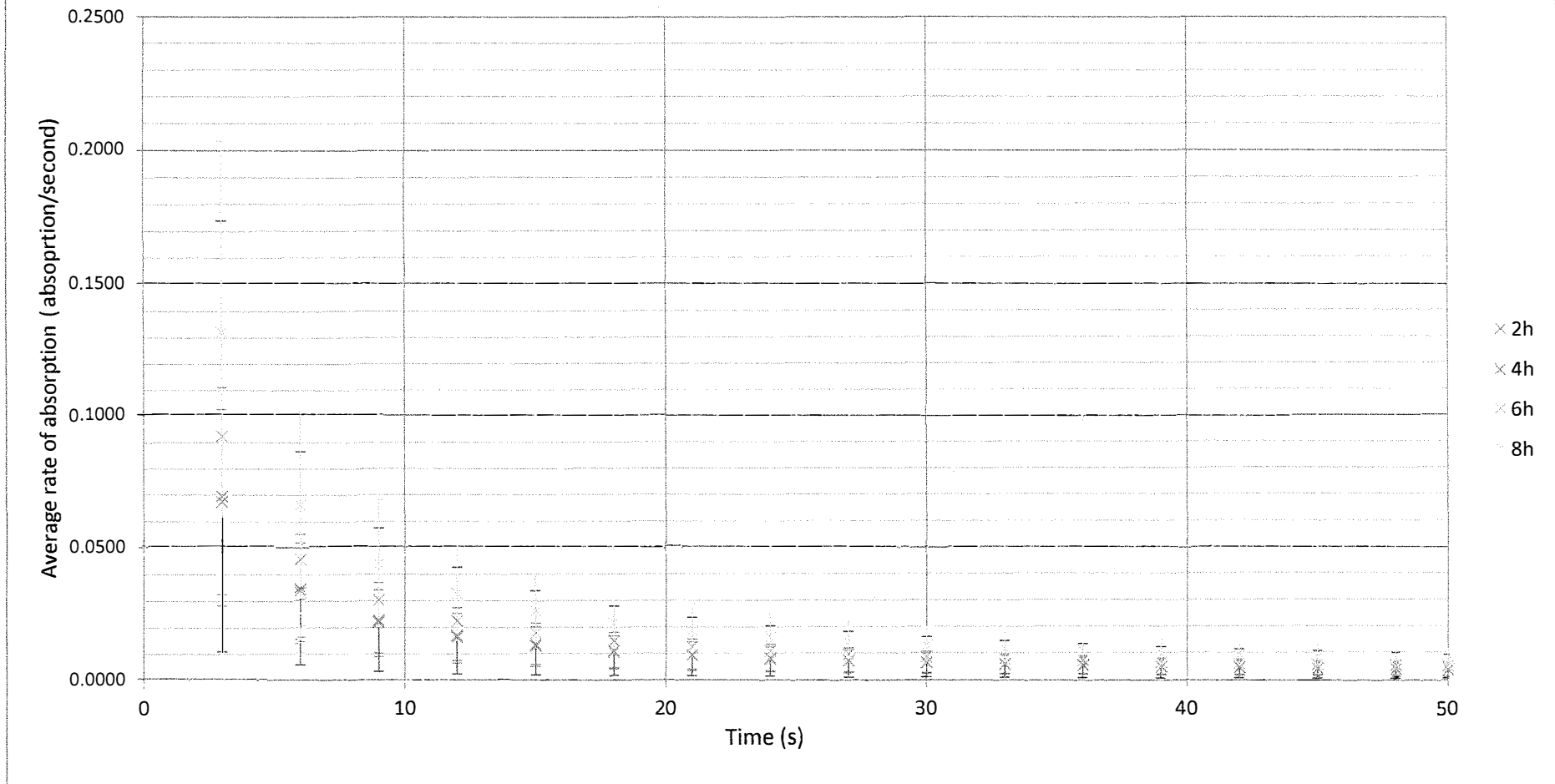
Equation used to calculate rate of absorption	$y = 0.2278x^{-1.046}$	$y = 0.2574x^{-1.084}$	$y = 0.3194x^{-1.063}$	$y = 0.4689x^{-1.06}$
Time interval (s)	2h	4h	6h	8h
3	0.0677	0.0697	0.0926	0.1323
50	0.00381	0.00371	0.00499	0.00742
100	0.00184	0.00175	0.00239	0.00356
150	0.00121	0.00113	0.00155	0.00231
200	0.000893	0.000825	0.00114	0.00171
250	0.000707	0.000648	0.000902	0.001347
300	0.000584	0.000531	0.000743	0.001110

Graph 1 to show the average rate of absorption per second in a reaction of amylase and oatmeal soaked for 2h, 4h, 6h and 8h



Graph 1 shows the average rate of absorption every 3 seconds for the first 50 seconds and after this the average rate every 50 seconds. This is to show the general trend in absorption for each soaking time.

Graph 2 to show average rate of absorption per second in the reaction of oatmeal with amylase on the interval 0s - 50s with error bars signifying the standard deviation



Graph 2 shows more clearly the trend in absorption rate in the 50s. After 50s, the absorption rate remains constant, hence why it is interesting to zoom into the interval shown on this graph.



2.2.1 Spearman's Rank Correlation Coefficient:

This test was carried out to investigate the nature of the relationship between rate of absorption and soaking time. It was done at 3 time increments: at 3s, 50s and 100s. After this, the other time increments listed in table 1 all resulted in the same coefficient as for 50s and 100s, hence only these three values were calculated. The following formula was used to calculate the rank (where  $n$  is the number of data and  $\sum d^2$  is the sum of differences between ranks squared):

$$r_s = 1 - \frac{6 \sum d^2}{n^3 - n}$$

Table 2 showing Spearman's Rank calculations for absorption rate at 3s, 50s and 100s.

Spearman's rank at 3s					
Soaking time	Rank	Average rate	Rank	Difference between ranks	Difference <sup>2</sup>
2	1	0.067667	1	0	0
4	2	0.069733	2	0	0
6	3	0.092556	3	0	0
8	4	0.132333	4	0	0
				Sum of differences <sup>2</sup>	0
				R <sup>2</sup> value	1
Spearman's rank at 50s					
Soaking time	Rank	Average rate	Rank	Difference between ranks	Difference <sup>2</sup>
2	1	0.003806	2	1	1
4	2	0.003706	1	-1	1
6	3	0.004993	3	0	0
8	4	0.007416	4	0	0
				Sum of differences <sup>2</sup>	2
				R <sup>2</sup> value	0.8
Spearman's rank at 100s					
Soaking time	Rank	Average rate	Rank	Difference between ranks	Difference <sup>2</sup>
2	1	0.001843	2	1	1
4	2	0.001748	1	-1	1
6	3	0.00239	3	0	0
8	4	0.003557	4	0	0
				Sum of differences <sup>2</sup>	2
				R <sup>2</sup> value	0.8

Can you justify 6 ds?

The  $R^2$  value is lower for 50s and 100s as the 4h time absorption rate is less than that for 2h, moving its rank from 2 to 1. As this is the case for all time intervals after 100s, all of these time intervals have the  $R^2$  value of 0.8 except for at 3s, where this is 1.0. This is why only 3s, 50s and 100s calculations are shown in Table 2.

### 3. Analysis

The digestion rate after all soaking treatments followed a similar trend: it was high at the beginning but quickly fell and then levelled off at around 50s at a rate of 0.04 (Appendix 1). There a negative exponential trend, with a steep fall in absorption in the first 20s (Graph 2) and then a slower, more gradual rate towards the end of the 50s interval. This suggests that the amylase is most active during this short time period at the start, with digestion happening at a rapid rate.

As predicted, pre-soaking for 8 hours showed the highest rate of absorption per second at the beginning, followed by the 6 hour treatment. 2 hours and 4 hours were surprisingly almost the same, the 4 hour oatmeal having only a slightly higher absorption rate than the 2 hour. Towards the end however, all 4 oatmeal show the same rate of absorption of around 0.0006 per second (Appendix 1). The standard deviations were very large at the start and then dramatically decreased as time progressed. This shows that at the start the data was wider spread, with less accuracy and very big discrepancies from the mean. Often, the standard deviation was as large as half of the mean. However, as time progressed, the data points grouped closer together, which resulted in smaller deviations. Table 3 shows why the standard deviation was often so large: The rate of digestion for the same soaking time was often very spread out.

*Table 3 showing the rate of absorption per second of the first five trials of oats soaked for 2h, at 3s*

Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0.0530 <i>0.05</i>	0.100 <i>0.10</i>	0.0777 <i>0.08</i>	0.0453 <i>0.05</i>	0.0563 <i>0.06</i>

Even though trials 1, 4 and 5 vary around 0.05, trials 2 and 3 are quite off. This is also reflected in the average: 0.0677. With such high fluctuations between the trials it would be hard to say whether or not there really is a difference between the individual soaking times, as there is a low level of precision. Furthermore, there is an overlap between error bars suggesting little precision between the averages at different points in time.

*With SD values of about 0.02 or 0.03 to 3<sup>rd</sup> + 4<sup>th</sup> dp. are misleading. Trials 1, 5 and 4 are effectively the same.*



While the data points for the singular treatments seem to show little precision and significant differences, there is a calculated correlation between absorption rate and soaking time. Table 2 shows the Spearman's Rank Coefficient calculated at 3s, 50s and 100s. 50s and 100s show an  $R^2$  value of 0.8, while at 3s the correlation is an excellent 1.0. This means that the general trend is a strong positive correlation, which proves the hypothesis "As soaking time increases, so does digestion rate". *supports!*

The 8h soaking time showed the highest rate of digestion at the start. This suggests that there more amylopectin than amylose present which is easier to digest, hence the highest GI can be found in this oatmeal. 6h has a slightly lower GI, whilst I suspect that 2h and 4h soaking time have similar Glycemic Indexes as they have similar digestion rates. It must be noted however that these differences are (most likely) minimal. The highest digestion rate at 3s is 0.132 absorption/second, while the lowest is 0.0677. This results in a range of only 0.0643 absorption/second and as the absorption scale goes from 0 to 1, the range shows just how small the differences are between the soaking times. The GI of oatmeal is a relatively low 55 (Atkinson et al) hence it can be suggested that even though 8h soaking time may have the highest GI of the four soaking times, this isn't so high so as to be considered unhealthy.

#### 4. Discussion:

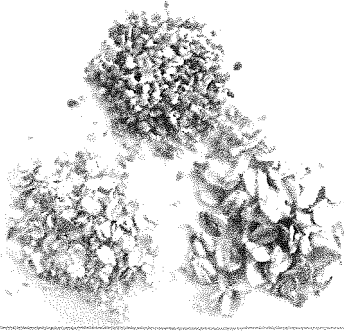
The results from my experiment show that, as predicted, a longer soaking time results in a shorter digestive rate, which in turn indicates a higher Glycemic Index. For a healthy diet however, it is suggested that the GI be kept quite low, so theoretically it isn't advisable for oatmeal to be soaked for as long as 8 hours. Yet realistically, as discussed above, the digestion rates for all soaking times reached around the same value after a quite short amount of time. This suggests that even though the GI could have varied between the different oatmeal, this difference was minimal, and in the end all starch is digested. Hence, the difference in GI must be minimal, and seeing as it was proven that the longest soaking time released the most starch, this can be advisable to all those who need a lot of energy during the day for sports and other activities. Yet the results, even though credible, may not be very reliable, and there are a few factors which suggest this. *So can you conclude that there is any difference at all?*

An important factor to be considered when evaluating the experiment carried out would be its validity in terms of comparing to digestion in humans. The first factor that subtracted from this validity was the use of bacterial amylase. The optimum temperature of alpha amylase lies between 50°C and 70°C (Rani), so to increase the activity of amylase enzymes, the

*line of argument is convincing.*

amylase would have had to been kept at this temperature before being reacted with the oats, instead of being kept at slightly below room temperature in a Styrofoam box. To make the experiment as close to real life conditions, human amylase would have had to be used, as this works at lower temperatures than bacterial amylase, around 37°C, the human body temperature. For more valid results, human amylase could be used and heated to around 37°C.

But not only was the temperature of the amylase important: what I noticed after a few trials was that the oats on the stirrer became heated up, especially after longer stirring hours such as 6 hours or 8 hours. This could have had an effect on how much starch was released by the oats, since not only were they soaked and stirred, but also heated slightly. This could have contributed to breaking down the hull of the oat and releasing more starch. To control the temperature of the mixture, plates which stir and heat at the same time could be used and a temperature such as 25°C, typical room temperature, could be set on them. This way, all the beakers would have been heated equally. Furthermore, a thermometer could be attached to a clamp stand and inserted into the beakers so that the actual temperature of the mixtures can be observed.



**Figure 2:** Clockwise from the top: Steel-cut, old-fashioned and quick-cooking rolled oats (courtesy of PJ Hamel)

The type of oats used in this experiment were old-fashioned oats (Figure 2). These are steamed and rolled out oat groats. As this is all the treatment that these oats undergo, none of the layers of the oats are lost during the process, meaning that the oat stays mostly intact. It must, however, be taken under account that steaming could remove a few of the nutrients found in the endosperm or bran. Using oat groats, which are oats from which only the hull is removed, and no other treatment is given to them, would have allowed for a fuller evaluation of the digestion of

the oat, as it is completely intact. However, it would have also been worth to compare the digestion rate of old-fashioned oats with steel-cut and rolled oats, so as to see which option is healthier, again through looking at the Glycemic Index of the individual oatmeal. ✓

Phytic acid was a variable which was not taken under account during the planning of this investigation, yet it could have played a very big role on controlling the digestion rate. Phytic acid is a form of storage of phosphorus found in seeds such as oats (Andrews). By having a very similar structure to the substrate, it can inhibit the work of amylase (Nagel). This is an example of competitive inhibition, where the inhibitor can attach itself to the active site of the

enzyme, prohibiting the actual substrate from being turned into the product. In this case, phytic acid could have attached itself to the active site of the alpha amylase, prohibiting it from hydrolysing starch into glucose. Phytic acid can be broken down by phytase. This enzyme can be found in the same seed as the phytic acid or in the gastrointestinal tracts of animals, but in different amounts (Nagel). Humans do not produce phytase, hence phytic acid could have posed a threat in my experiment. If not all enzymes were being used to break down the glucose released by the oatmeal, this could have led to an alteration in my results. Without the phytic acid, the digestion rate may be much higher, and may also show bigger differences between the soaking times. As explained by Nagel, not only would the oats have to be cooked at a slightly raised temperature, slightly because there is a limited amount of phytase present in oats, and overcooking would destroy this, but the oats would also have to be soaked in a slightly acidic solution to help activate phytase, so as to help it react with the phytic acid. This would be a significant improvement to my experiment.

#### 4.1 Further investigations

Behall and Howe's study on human subjects suggested, that there is indeed a clear correlation between the ratio of amylose to amylopectin and how insulin and glucose levels in the blood rise (Behall, Howe). Subjects fed with more amylose and less amylopectin had lower insulin and glucose levels in their blood in the hours following consumption, suggesting a low GI, while the exact opposite happened for those fed with more amylopectin and less amylose, where a high GI was suggested. What this study did not encompass was the digestion rate. Hence, it would be very interesting to carry out a study which changes the amylose to amylopectin ratio, investigating how this could affect the Glycemic Index as well as the digestion rate. Theoretically, amylose can be digested more slowly than amylopectin due to its straight structure, which would mean that a ratio with more amylose than amylopectin would decrease the Glycemic Index. It would also be interesting to see whether a longer soaking time releases more phytic acid, and whether or not this then inhibits the enzyme amylase, slowing the digestion rate down as less of the enzyme could react with the substrate.

#### 5. Conclusion

There was a very strong positive correlation between rate of digestion and hours of soaking at 3 seconds, however, the difference between the digestion rates of all four oatmeal decreased as time progressed, with the absorption rate being almost the same for each soaking time at the end of the 300 seconds for most trials. This suggests that while soaking time seems to

increase digestibility at the beginning of digestion, after 5 minutes the rate of digestion is the same for all oatmeal, meaning that all oatmeal is digested. This could suggest that there is no clear advantage in soaking oats for a longer time. The standard deviation calculated did, however, show discrepancies in the data, suggesting that data points were spread apart widely at the start of each trial, and became closer to each other as the trial progressed. Both the hypotheses were proven. The longest soaking time had the fastest digestion rate, from which it could be concluded that this oatmeal also had the highest GI. Yet, due to the small scale of the data points, as well as several uncontrolled factors, it was concluded that the differences in GI between 2, 4, 6 and 8 hours of soaking were not big enough to consider a high soaking time unhealthy or for it to show any additional benefits. While soaking oats for longer periods of time did not result in a big increase in digestibility, it may however help release the many nutrients found in the endosperm and bran, henceforth it is still recommended that oats be soaked for a significant amount of time before ingestion.

were supported, to an extent, depending on how you view the data.

here you are overstating the case.

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## Appendices

## Appendix 1

**Table to show the average rate of absorbance per second every 3 seconds for 300 seconds for oatmeal soaked at 2, 4, 6 and 8 hours being digested by alpha amylase, using iodine as an indicator.**

Time (s)	2h		4h		6h		8h	
	Average rate (absorbance/second)	Standard deviation	Average rate (absorbance/second)	Standard deviation	Average rate (absorbance/second)	Standard deviation	Average rate (absorbance/second)	Standard deviation
3	0.0677	0.0351	0.0697	0.0414	0.0926	0.0814	0.1323	0.0712
6	0.0336	0.0178	0.0347	0.0207	0.0461	0.0406	0.0663	0.0358
9	0.0224	0.0118	0.0229	0.0139	0.0305	0.0270	0.0442	0.0239
12	0.0167	0.0089	0.0171	0.0104	0.0226	0.0200	0.0330	0.0179
15	0.0133	0.0071	0.0136	0.0083	0.0180	0.0159	0.0264	0.0143
18	0.0110	0.0059	0.0112	0.0069	0.0149	0.0131	0.0219	0.0118
21	0.0094	0.0051	0.0096	0.0059	0.0126	0.0111	0.0187	0.0100
24	0.0082	0.0045	0.0083	0.0051	0.0110	0.0096	0.0163	0.0087
27	0.0073	0.0040	0.0073	0.0045	0.0097	0.0085	0.0145	0.0077
30	0.0065	0.0036	0.0066	0.0041	0.0087	0.0076	0.0130	0.0069
33	0.0059	0.0032	0.0059	0.0037	0.0078	0.0068	0.0117	0.0062
36	0.0054	0.0029	0.0054	0.0034	0.0072	0.0062	0.0107	0.0056
39	0.0050	0.0027	0.0049	0.0031	0.0066	0.0057	0.0099	0.0052
42	0.0046	0.0025	0.0046	0.0029	0.0061	0.0053	0.0091	0.0048
45	0.0043	0.0024	0.0043	0.0027	0.0056	0.0049	0.0085	0.0044
48	0.0040	0.0022	0.0040	0.0025	0.0053	0.0045	0.0079	0.0041
51	0.0038	0.0021	0.0037	0.0023	0.0049	0.0043	0.0074	0.0038
54	0.0036	0.0019	0.0035	0.0022	0.0046	0.0040	0.0070	0.0036
57	0.0034	0.0018	0.0033	0.0021	0.0044	0.0038	0.0066	0.0034
60	0.0032	0.0018	0.0031	0.0020	0.0042	0.0036	0.0062	0.0032
63	0.0030	0.0017	0.0029	0.0019	0.0039	0.0034	0.0059	0.0030
66	0.0029	0.0016	0.0028	0.0018	0.0038	0.0033	0.0056	0.0029
69	0.0028	0.0015	0.0027	0.0017	0.0036	0.0031	0.0054	0.0027
72	0.0026	0.0015	0.0026	0.0016	0.0034	0.0030	0.0051	0.0026
75	0.0025	0.0014	0.0024	0.0015	0.0033	0.0028	0.0049	0.0025
78	0.0024	0.0013	0.0023	0.0015	0.0031	0.0027	0.0047	0.0024
81	0.0023	0.0013	0.0022	0.0014	0.0030	0.0026	0.0045	0.0023
84	0.0022	0.0012	0.0022	0.0014	0.0029	0.0025	0.0043	0.0022
87	0.0022	0.0012	0.0021	0.0013	0.0028	0.0024	0.0042	0.0021
90	0.0021	0.0011	0.0020	0.0013	0.0027	0.0023	0.0040	0.0020
93	0.0020	0.0011	0.0019	0.0012	0.0026	0.0022	0.0039	0.0020
96	0.0019	0.0011	0.0019	0.0012	0.0025	0.0022	0.0038	0.0019
99	0.0019	0.0010	0.0018	0.0011	0.0024	0.0021	0.0036	0.0018
102	0.0018	0.0010	0.0017	0.0011	0.0024	0.0020	0.0035	0.0018

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105	0.0018	0.0010	0.0017	0.0010	0.0023	0.0020	0.0034	0.0017
108	0.0017	0.0009	0.0016	0.0010	0.0022	0.0019	0.0033	0.0017
111	0.0017	0.0009	0.0016	0.0010	0.0022	0.0018	0.0032	0.0016
114	0.0016	0.0009	0.0015	0.0010	0.0021	0.0018	0.0031	0.0016
117	0.0016	0.0009	0.0015	0.0009	0.0020	0.0017	0.0030	0.0015
120	0.0015	0.0008	0.0015	0.0009	0.0020	0.0017	0.0030	0.0015
123	0.0015	0.0008	0.0014	0.0009	0.0019	0.0017	0.0029	0.0014
126	0.0015	0.0008	0.0014	0.0009	0.0019	0.0016	0.0028	0.0014
129	0.0014	0.0008	0.0013	0.0008	0.0018	0.0016	0.0027	0.0014
132	0.0014	0.0008	0.0013	0.0008	0.0018	0.0015	0.0027	0.0013
135	0.0014	0.0007	0.0013	0.0008	0.0017	0.0015	0.0026	0.0013
138	0.0013	0.0007	0.0012	0.0008	0.0017	0.0015	0.0025	0.0013
141	0.0013	0.0007	0.0012	0.0007	0.0017	0.0014	0.0025	0.0012
144	0.0013	0.0007	0.0012	0.0007	0.0016	0.0014	0.0024	0.0012
147	0.0012	0.0007	0.0012	0.0007	0.0016	0.0014	0.0024	0.0012
150	0.0012	0.0007	0.0011	0.0007	0.0016	0.0013	0.0023	0.0012
153	0.0012	0.0007	0.0011	0.0007	0.0015	0.0013	0.0023	0.0011
156	0.0012	0.0006	0.0011	0.0006	0.0015	0.0013	0.0022	0.0011
159	0.0011	0.0006	0.0011	0.0006	0.0015	0.0012	0.0022	0.0011
162	0.0011	0.0006	0.0010	0.0006	0.0014	0.0012	0.0021	0.0011
165	0.0011	0.0006	0.0010	0.0006	0.0014	0.0012	0.0021	0.0010
168	0.0011	0.0006	0.0010	0.0006	0.0014	0.0012	0.0021	0.0010
171	0.0010	0.0006	0.0010	0.0006	0.0014	0.0012	0.0020	0.0010
174	0.0010	0.0006	0.0010	0.0006	0.0013	0.0011	0.0020	0.0010
177	0.0010	0.0006	0.0009	0.0006	0.0013	0.0011	0.0019	0.0010
180	0.0010	0.0006	0.0009	0.0005	0.0013	0.0011	0.0019	0.0010
183	0.0010	0.0005	0.0009	0.0005	0.0013	0.0011	0.0019	0.0009
186	0.0010	0.0005	0.0009	0.0005	0.0012	0.0010	0.0018	0.0009
189	0.0009	0.0005	0.0009	0.0005	0.0012	0.0010	0.0018	0.0009
192	0.0009	0.0005	0.0009	0.0005	0.0012	0.0010	0.0018	0.0009
195	0.0009	0.0005	0.0008	0.0005	0.0012	0.0010	0.0017	0.0009
198	0.0009	0.0005	0.0008	0.0005	0.0012	0.0010	0.0017	0.0009
201	0.0009	0.0005	0.0008	0.0005	0.0011	0.0010	0.0017	0.0008
204	0.0009	0.0005	0.0008	0.0005	0.0011	0.0009	0.0017	0.0008
207	0.0009	0.0005	0.0008	0.0005	0.0011	0.0009	0.0016	0.0008
210	0.0008	0.0005	0.0008	0.0005	0.0011	0.0009	0.0016	0.0008
213	0.0008	0.0005	0.0008	0.0004	0.0011	0.0009	0.0016	0.0008
216	0.0008	0.0005	0.0007	0.0004	0.0011	0.0009	0.0016	0.0008
219	0.0008	0.0004	0.0007	0.0004	0.0010	0.0009	0.0015	0.0008
222	0.0008	0.0004	0.0007	0.0004	0.0010	0.0008	0.0015	0.0008
225	0.0008	0.0004	0.0007	0.0004	0.0010	0.0008	0.0015	0.0007
228	0.0008	0.0004	0.0007	0.0004	0.0010	0.0008	0.0015	0.0007
231	0.0008	0.0004	0.0007	0.0004	0.0010	0.0008	0.0015	0.0007
234	0.0008	0.0004	0.0007	0.0004	0.0010	0.0008	0.0014	0.0007
237	0.0007	0.0004	0.0007	0.0004	0.0010	0.0008	0.0014	0.0007
240	0.0007	0.0004	0.0007	0.0004	0.0009	0.0008	0.0014	0.0007



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243	0.0007	0.0004	0.0007	0.0004	0.0009	0.0008	0.0014	0.0007
246	0.0007	0.0004	0.0006	0.0004	0.0009	0.0007	0.0014	0.0007
249	0.0007	0.0004	0.0006	0.0004	0.0009	0.0007	0.0013	0.0007
252	0.0007	0.0004	0.0006	0.0004	0.0009	0.0007	0.0013	0.0007
255	0.0007	0.0004	0.0006	0.0004	0.0009	0.0007	0.0013	0.0007
258	0.0007	0.0004	0.0006	0.0004	0.0009	0.0007	0.0013	0.0006
261	0.0007	0.0004	0.0006	0.0004	0.0008	0.0007	0.0013	0.0006
264	0.0007	0.0004	0.0006	0.0003	0.0008	0.0007	0.0013	0.0006
267	0.0006	0.0004	0.0006	0.0003	0.0008	0.0007	0.0012	0.0006
270	0.0006	0.0004	0.0006	0.0003	0.0008	0.0007	0.0012	0.0006
273	0.0006	0.0003	0.0006	0.0003	0.0008	0.0007	0.0012	0.0006
276	0.0006	0.0003	0.0006	0.0003	0.0008	0.0007	0.0012	0.0006
279	0.0006	0.0003	0.0006	0.0003	0.0008	0.0006	0.0012	0.0006
282	0.0006	0.0003	0.0006	0.0003	0.0008	0.0006	0.0012	0.0006
285	0.0006	0.0003	0.0006	0.0003	0.0008	0.0006	0.0011	0.0006
288	0.0006	0.0003	0.0005	0.0003	0.0008	0.0006	0.0011	0.0006
291	0.0006	0.0003	0.0005	0.0003	0.0008	0.0006	0.0011	0.0006
294	0.0006	0.0003	0.0005	0.0003	0.0007	0.0006	0.0011	0.0006
297	0.0006	0.0003	0.0005	0.0003	0.0007	0.0006	0.0011	0.0006
300	0.0006	0.0003	0.0005	0.0003	0.0007	0.0006	0.0011	0.0005

