

DEPARTMENT OF CHEMICAL & MATERIALS ENGINEERING



CALIBRATING AN ACOUSTIC RIPENESS TESTER FOR KIWIFRUIT

Research Question:

How can firmness be correlated with the signals received from an acoustic fruit ripeness tester?

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in association with Plant & Food Research

Abstract

New Zealand's kiwifruit industry makes up the country's largest single horticultural export by volume and by value, with a revenue of \$930 billion in 2014 [1]. Plant and Food Research (PFR) is a Crown Research Institute based in Mt Albert, Auckland that supports the primary industry sector with research focused on kiwifruit. Fruit testing has applications that vary from research, industry, and export. Currently, fruit are tested to check firmness in a destructive manner (penetration tests) which means a sample cannot be used again. This leads to a large amount of waste and reduces the number of fruit samples in the batch. Non invasive ripeness testing methods have been considered for various different types of fruit such as melon. One in particular is the sonic ripeness testing method, which PFR have utilised to build a device that sends sound signals through kiwifruit to determine ripeness from the type of signal that is detected after passing through the fruit. There is a large amount of variation currently present in the test results, so synthetic models have been used to calibrate the device as they are more homogenous in structure. Hydrogels, due to their high water content and flexibility in the amount of cross links, were explored as an appropriate type of material with which to mimic kiwifruit properties. Carrageenan, agarose, and PDMS rubber (as a control) were prepared and tested using both a texture analyser and the acoustic device. The measured acoustic signal varies between materials depending upon the amount of voids, the surface area in contact with the piezoelectric plates, and changes in density throughout the material.

The findings from the study are that the firmness of a kiwifruit varies across its cross section, with elastic modulus of 5.8, 0.5, and 3 MPa at the core, inner pericarp, and outer pericarp respectively. In general, for carrageenan, the maximum amplitude of the peaks in the resulting acoustic signal may correlate to firmness, those for agarose show no visible trend, and those for PDMS suggests the opposite relationship, however the results are not statistically significant. There is a pattern visible where peaks are consistently around a certain frequency range for each type of material, which is likely to do with the structure of the material and its homogeneity. Contact area between the piezoelectric plates and the tested sample is a major factor in the amplitude of the signal, with more contact area resulting in a higher amplitude.

Although the results from this study are not statistically significant, they give a good indication of where the areas of improvement lie in this project of calibrating the acoustic ripeness tester. The complexity of kiwifruit structure presents a difficult challenge to overcome as there are multiple factors to consider, such as the seeds, the relative distribution of regions inside the fruit, the geometry of the overall shape and its origin, which may affect the ripening process of the fruit. The lack of existing research in this area also introduces another complexity. Further work is required for calibration of the acoustic ripeness tester, such as creating a database of data using kiwifruit samples and other materials of more complex structure than the homogenous samples used in this study.

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Introduction

The objectives of the project are to create synthetic testing models out of appropriate hydrogels, and subsequently use them to calibrate an ultrasonic ripeness tester in conjunction with Plant and Food Research in Mt Albert, Auckland. The project relevance comes with the current issues involved with fruit ripeness testing in the industry and for research purposes. To ensure consistent quality across all produce, certain characteristics must be quantified to determine ripeness. In the case of kiwifruit, according to Plant and Food Research, ripeness is measured by how hard the fruit is. When conducting research on fruits of different cultivars and locations, a lot of the time there are not enough samples of fruit to be able to test the fruit properties destructively. The industry standard test of whole fruit firmness is the maximum amount of compressive force required to push a probe into the kiwifruit, past the skin [2]. The properties can be hypothesised, but there is no way of knowing for sure unless it is cut, at which point the sample cannot be used for study again. A large scale application is in the fruit export industry. When the fruit is harvested from the vine, hardness is approximately 8 kg F. The fruit ripens in a storehouse and is ripe and able to be sold at 1 kg F hardness [3, 4]. This ripeness is usually measured destructively, as the fruit is packed into boxes, to be exported to market. This is an imprecise, invasive, time consuming process in industry. Finally, another application is quality control i.e. during transport. Power losses/fridge malfunction during long transport such as by ship will result in uncertainty of the quality of the fruit cargo. Having a non-invasive method of testing whether the fruit is still able to be sent to market will be extremely beneficial and efficient as not all the fruit cargo may have been subjected to the same conditions.

Some research has been conducted previously on non-invasive methods of determining fruit ripeness. Plant and Food Research has developed an ultrasonic ripeness testing device which passes sound waves through fruit, interpreting the resulting acoustic signal to determine its ripeness. Currently, they are having an issue with the device where two exactly identical fruit do not give similar acoustic signals.

A potential solution to this problem, which forms the basis of the overall project, is to create testing models using synthetic materials (more homogenous structures, specifically hydrogels and synthetic rubber), which will narrow down the variation and tolerance range in the signals from the device. The long term potential applications of the device are immense; especially in the fruit industry (fruit storage and packing could become significantly more automated). We discuss the basic concepts related to real and artificial fruit in order to understand and be able to make well-informed decisions on how to proceed with this problem. The main questions that will be answered include:

- How does standard industry testing of kiwifruit relate to its mechanical properties such as the elastic modulus?
- What kind of hydrogel structures will give the best approximation of kiwifruit flesh?
- How does the elastic modulus of the testing sample relate to the acoustic signal received from the ripeness testing device?

Literature Review/Background

Background in ultrasonic methods of fruit testing

Non-destructive methods of measuring fruit properties have great potential for use both in research and industry [5]. Ultrasound technology is an emerging field of research that can potentially be used for quality preservation and assurance of food safety [6]. Different frequencies, velocities, and attenuations can be used for different applications due to their influence on wave characteristics during travel through a medium [6]. High power and high intensity ultrasounds are defined as those between the range of 20 kHz and 500 kHz, with intensities higher than 1 W/cm^2 [6]. These ultrasounds affect the physical, mechanical, or chemical properties of foods and are therefore disruptive, with potential for applications in food processing, preservation, and food safety [6]. Low power and low intensity ultrasounds are those of frequencies higher than 100 kHz and intensities lower than 1 W/cm^2 , and are applicable for non-invasive analysis and measuring properties of foods during processing and storage [6]. This type of technology has been used previously for non-invasive evaluation of the composition of raw and fermented meat products, fish and poultry [6].

The drive towards non-destructive food quality testing is caused by the increasing need for better quality monitoring, which is usually undertaken via destructive testing, or human judgments, which are subjective [7]. The study notes that a survey of literature found that approximately 20% of non-destructive or non-invasive methods of food quality measurement used acoustic techniques [7]. Ultrasound waves are advantageous in that they are able to non-destructively extract the characteristic of the material that may have some correlation with quality assessment of fruits and vegetables [7]. Studies have found that the acoustic vibration characteristics of fruits and vegetables are directly related to their elastic modulus, mass and geometry [5]. The study notes that although the potential for application of ultrasound in the food industry was recognised since the 1970s, development of the technology did not progress as fast in the fresh fruit industry as it did in the food processing industry [7]. This was attributed to the lack of suitable equipment, as the sensor must be powerful enough to penetrate the fruit, but gentle enough so as to avoid damage to fruit tissue [7]. Advances in the area of instruments and sensors allowed research of this application to be stimulated and developed in more recent years [7].

Quality-related parameters in food

The use of ultrasounds in quality control processes has numerous different applications, most of which are primarily seen as a non-invasive alternative to destructive testing of food quality [6]. There are different methods of using ultrasounds to measure food properties; the acoustic device developed by Plant & Food Research (PFR) for kiwifruit testing uses the amplitude of a signal; another study explains the use of ultrasound velocities to measure the lipid, protein, moisture and

ash concentrations of cod fillets [6]. The study demonstrated that the ultrasound velocity of the fillets decreases in a linear fashion with increasing moisture content [6]. Calibration of ultrasound signals and food property is therefore an emerging area of research with immense potential for a wide range of industries [6]. Quality-related parameters correlated with ultrasound provide useful information in regards to understanding the natural processes of growth and maturation, in the course of harvesting, storage, and shelf life [7]. Firmness is usually the most important factor considered when evaluating the maturity of most fruits and vegetables, and it has also been found to correlate with ultrasound characteristics [7]. It is considered to be an accurate gauge of the change in tissue texture during preharvest, harvest and postharvest, as well as export and commercial sale [7]. The general method of measuring firmness is by compression or puncturing with various different types of probes (penetration tests) [7]. For fruit firmness testing, the standard test is using a destructive penetration device with a conical or curved puncture probe [7]. This is a time-consuming, destructive test, which is not always objective, and suggests the use of vibrational response analysis to measure the firmness (elastic properties) of fruits and vegetables as they have been found to be correlated [5, 8, 7]. The sonic vibration method uses the resonant frequency of fruit vibration modes to estimate elastic properties, and this has been often applied and verified to have a direct relationship to quality and ripeness [5]. A study carried out in 2006 verified the capacity of acoustic signal response to accurately measure firmness of mandarin fruit, by finding a correlation between the acoustic parameters (firmness index and elastic modulus) and fruit compression force [8]. A 2012 review finds that most cases attempting correlation between acoustic parameters and firmness carry out a similar method: firmness of the same sample was measured by both destructive penetration methods and ultrasound methods (with the non-destructive ultrasound test being carried out first), and then the obtained results were compared and correlated [7].

Piezoelectric effect in ultrasound generation

Ultrasound waves are generated using the piezoelectric effect: a ceramic crystal in a transducer produces a mechanical, acoustic wave from a short electrical pulse [7]. When an alternating voltage is applied across a particular crystal plate, mechanical vibration is produced, and this is then used for the generation and detection of sound waves [6]. The acoustic wave propagates through the material as a short ultrasonic pulse, and the signal is then received by another piezoelectric plate, converting it back into electrical energy [6, 7]. The study mentions two methods of receiving the signal: “pulse-echo” mode, where the same piezoelectric plate acts as a transmitter and a receiver alternately, and a “through-transmission” mode, where a second piezoelectric plate is used as the receiver [7]. The “through-transmission” mode is used in the device developed by PFR. Materials which produce piezoelectricity are used in ultrasonic transducers, such as some ceramics (barium titanate, lead zirconate) [6].

Analysis of ultrasound signal propagation

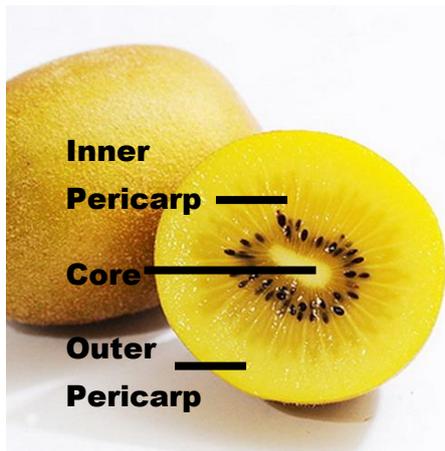
The ultrasonic signal passes through the tested material until the acoustic wave encounters a change in impedance, which correlates to changes in the material density or sound wave speed [7]. This can be related to changes in the nature of the material tissue, or the presence of a void or other reflecting body [7]. The change in impedance and the size of the reflecting body is associated with how much of the sound energy is reflected; and the remaining sound energy continues until it encounters the receiving plate, or the energy is dissipated [7]. Changes in the attenuation of the propagated signal can be caused by most physical or chemical changes in the material; with ultrasound energy usually propagating easily through solid and liquid industrial materials as well as most biological tissues [7]. The study notes that unlike these materials, analysing fruit and vegetable tissues with ultrasonic waves was found to be extremely difficult because they have very different acoustical properties [7]. Most fruit and vegetable tissue contain voids and pores scattered inhomogeneously, therefore they are highly attenuating materials and complicate the analysis and interpretation of received ultrasound signals [7].

Previous research done on this topic has allowed the identification of major acoustic factors used in quality measurement of fresh agricultural produce, and links between them and the physical characteristics of the produce [7]. As fruits and vegetable material exhibits both elastic and viscous characteristics, they can be considered viscoelastic and therefore complicate these relationships. In a number of studies, the effect of wave velocity as a parameter was found to be not sensitive enough to correlate with changes in fruit tissue [7]. The study cites research carried out in 1998 by Povey, which suggests that in some food systems, ultrasonic wave velocity was not a useful parameter in comparison to other major parameters such as attenuation [7]. Micro-scattering and absorption are the main causes of attenuation when a sound wave passes through a material [7]. Microscopic interfaces along the wave path scatter part of the wave, and other parts of the wave may be absorbed by the material as internal friction causes the transfer of energy from sound to heat [7].

Numerous studies have carried out attempts to use ultrasonic waves with fruit samples for the detection of tissue inhomogeneities like voids, seeds, stones, bruises, rots, foreign materials, or discontinuities, with difficulty [7]. Research done early in the 1980s with the purpose of using ultrasound technology to detect bruises in apples found difficulty with achieving sufficient penetration of the sound wave into the fruit, and other studies concluded that it was impossible to examine fruit interior successfully due to the signal's high attenuation [7]. A significant factor affecting attenuation, according to those studies, was frequency of the transmitted sound waves, with a 1983 study reporting very high attenuation of the signal with frequencies of 0.5 and 1 MHz propagated through the tissues of potato, cantaloupe, and apple. The same study suggested that a lower frequency and higher acoustic radiation power may yield better results in the non destructive testing of fruits and vegetables [7]. Using a lower frequency and a modified instrument, a study carried out in 1989 successfully measured the acoustic parameters of reflective loss, propagation velocities of acoustic waves through specimens of certain fruits and vegetables, and attenuation coefficients for potato, avocado, and carrot [7].

The physical properties of kiwifruit

There is a limited amount of literature on the mechanical properties of kiwifruit. The cultivar that the project will be focusing on is the Gold variety (*A. chinensis*), due to ease of accessibility and use (hairless, as opposed to the Green variety). The species/cultivar does not have any effect on the objectives of this project as long as it is kept constant throughout the duration of the project, as different varieties will have slightly different textural properties. Most research available today seems to be done on the Hayward variety of kiwi fruit (green), as it is the most widely available in the market.



Kiwifruit are comprised of four different tissue zones: the core, the inner pericarp, the outer pericarp, and the skin, in order from the centre of the fruit [2]. They differ in mineral, cell wall composition, and structure, and consequently have noticeable variance in mechanical properties [2]. The most dense region is the core, followed by the outer pericarp and then the softest region, the inner pericarp [2]. A study on the ripening of kiwifruit carried out by the Horticulture and Food Research Institute of New Zealand (which is now Plant and Food Research) showed the change in firmness during the ripening stage [2].

Figure 1: Schematic of kiwi fruit cross section

The mechanical properties of kiwifruit are important for texture analysis and product quality [9]. Handling, storing and processing procedures use firmness as a measure of the ripeness and maturity of fruit product, which then directly influences the quality control of fruit sent for the consumer market [5, 8, 9]. The precise manner of measurement can vary from study to study. The most common destructive testing method is a compressive puncture test using a texture analyser [9, 10]. The Horticulture and Food Research Institute of New Zealand defines whole fruit firmness as the maximum amount of force required to push a probe into the outer pericarp [2]. As the fruit ripens, its mechanical properties change dramatically; the flesh gets softer and there is a decrease in water content [4]. Water loss during ripening occurs due to the gradient in water content between the surrounding air and inside the fruit [4]. Temperature has an effect on the amount of water lost, with studies showing that fruit stored in ambient conditions lost three times as much water as those stored at a colder temperature, for the same time period [4]. The loss in firmness in the initial ripening stages is caused by a decrease in adhesion between cells, resulting in separation rather than cell rupture when the fruit is indented [2]. During the later ripening stages, there is further loss in adhesion and changes in the chemical composition of the cell wall, which leads to higher cell elasticity [2]. The study suggests that kiwifruit texture is determined by the chemical composition of the cell wall, and the relative numbers of large and small cells, as larger cells were found to be less likely to rupture [2].

General overview of hydrogels

Hydrogels are biomaterials that are made up of a hydrophilic network of cross-linked polymer chains, swollen with water [11, 12, 13, 14]. They are produced by the reaction of one or more monomers into chains of natural polymers such as collagen or alginate or synthetic polymers, such as polyvinyl alcohol (PVA) or polyacrylic acid (PAA) [12, 14]. Hydrogels are a relatively recent research focus, having received attention only in the last 50 years due to their potential use in biomedicine, e.g. tissue engineering [12, 14].

The high water content in synthetic hydrogels allows a large degree of flexibility, similar to natural tissue [11, 12,13,14,15]. The amount of water and crosslinking in the network are main factors contributing to the mechanical properties of the gel, and the potential of these factors to be manipulated makes hydrogels appropriate to be used in the modelling of fruit tissue and other biological materials [11, 12, 13, 14, 15]. The hydrophilic functional groups attached to the polymer backbone in hydrogels allow water to be absorbed into the gel structure, while the cross-links between network chains prevent dissolution [11, 12, 13, 14]. Hydrogels can be classified into different forms depending on the type of bonding and polymerisation [11, 12, 13]. The most common, broadest classification is by the type of cross-linking: physical or chemical gels [11, 12]. Chemically cross-linked networks have permanent covalent bonds, while physically cross-linked networks are held together by molecular entanglements and/or physical interactions such as ionic, hydrogen bonding, or hydrophobic interaction [11, 12]. These interactions in physically cross-linked gels are reversible, and fundamentally weaker than covalent bonding [11, 12].

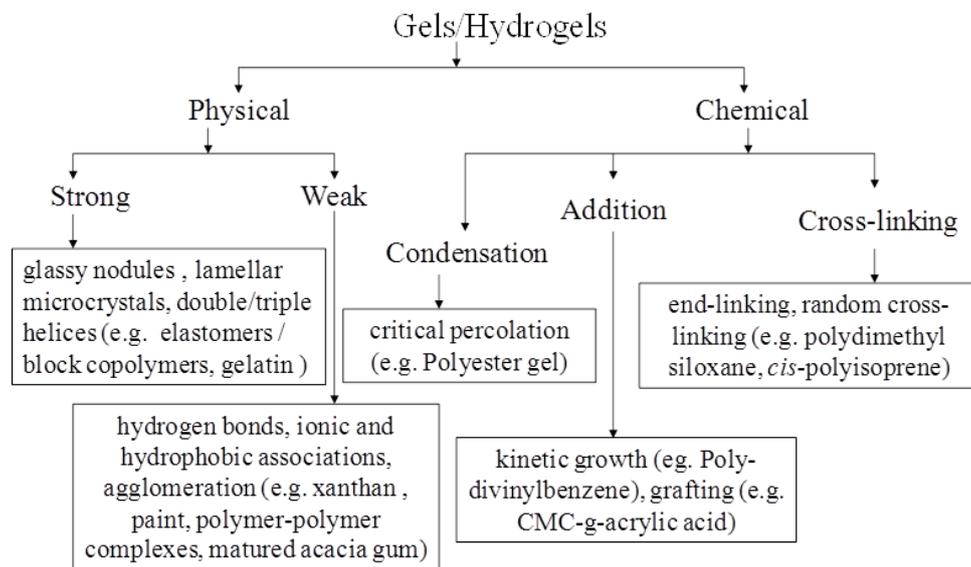


Figure 2: Classification of gels [11]

Stimuli-responsive hydrogels can respond to changes in the external environment such as temperature, pH, or electrolyte presence [11, 12]. These gels demonstrate significant reversible volume changes by swelling or deswelling in response to stimuli, which can be physical or chemical [11, 12]. This means gels can be designed to be controllable in terms of expansion and shrinkage in response to environmental stimuli [11, 12].

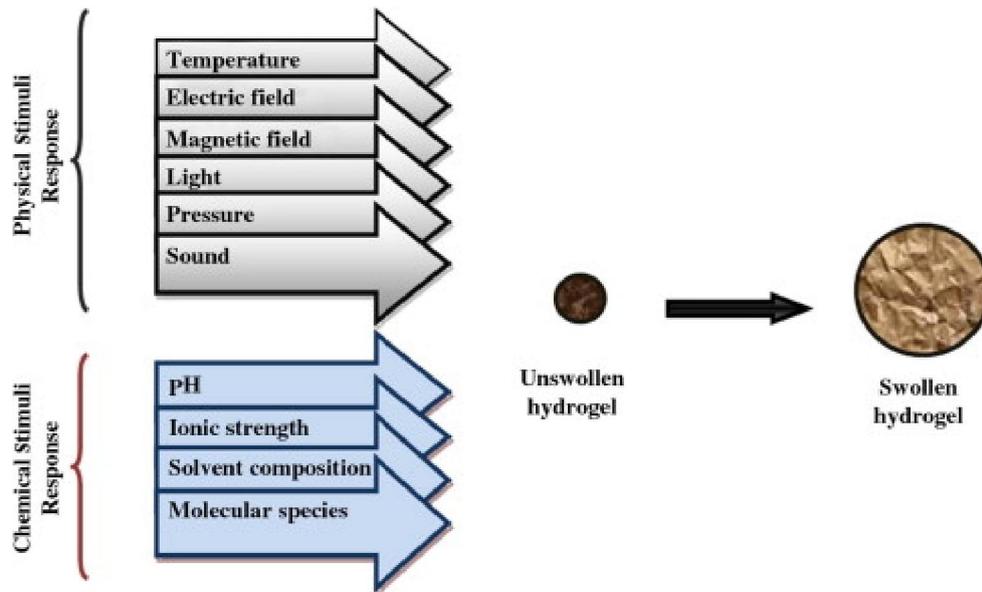


Figure 3: Hydrogel swelling response to external stimuli [12]

Most hydrogels have weak mechanical properties, which has severely limited their potential as a suitable material for a large range of applications [14, 16, 17, 18]. These properties are dependent upon the method of preparation of the gel, mainly the concentration of cross-linker, the initial degree of dilution of monomer, and the chemistry of the monomers [11, 12, 13, 14, 16, 17, 18, 19]. The mechanical property most relevant to this project is the elastic modulus, or Young's modulus. A hydrogel used to mimic natural kiwifruit tissue needs to be of similar elastic modulus in order to appropriately model the fruit for device calibration. The elastic modulus of hydrogels depends not only on the cross-link and charge densities of the polymer network, but also on the cross-linked polymer concentration after the preparation of the gel [13].

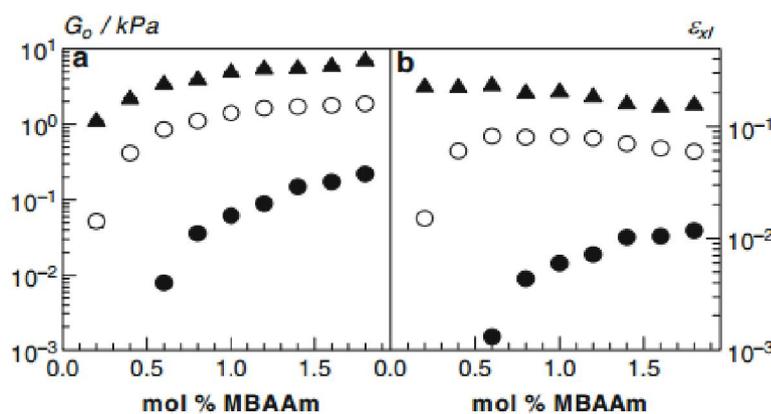


Fig. 1 The elastic modulus G_0 of PAAm hydrogels after preparation (a) and the cross linking efficiency ϵ_{xl} (b) shown as a function of MBAAm concentration. Initial monomer concentration $C_0 = 3$ (filled circle), 5 (open circle), and 7 w/v % (filled triangle). Reprinted from Orakdogan and Okay (2006a) with kind permission of Springer Science + Business Media

Figure 4: Elastic modulus of hydrogels as a function of cross-linker concentration [13]

From Figure 4, where polyacrylamide (PAAm) hydrogels that were prepared from AAm (initial monomer) and MBAAm (cross linker); it is clear that higher initial monomer % and mol % crosslinker lead to higher elastic modulus [13]. There are other studies that also confirm this trend [15, 17, 18]. Another notable characteristic of hydrogels is spatial inhomogeneity, as cross-link density distribution is only homogeneous in ideal gels [13]. A study found that spatial inhomogeneity increases with the cross-link density of the gel due to the increase in network defects and imperfections [13]. It was also found to decrease as the degree of ionisation increases, because there are more mobile counter ions and higher electrostatic repulsion forces [13].

A study done on the characterisation of the elastic modulus of hydrogels provides a useful set of different moduli measured from different types of hydrogels, however the lack of existing data for comparison is mentioned [15, 19]. The study states that the moduli reported for PEGDA and PDMS are consistently lower than those from other literature sources; however, this difference is to be expected due to the potential difference in measurement and preparation methods of hydrogels [15]. Another study done by Denisin and Pruitt also states that the reported elastic modulus for gels of the same composition varies drastically, likely due to different measurement methods [19]. This suggests that existing literature on the mechanical properties of different hydrogels should only be used as an indication when preparing hydrogels to match the elastic moduli of kiwifruit.

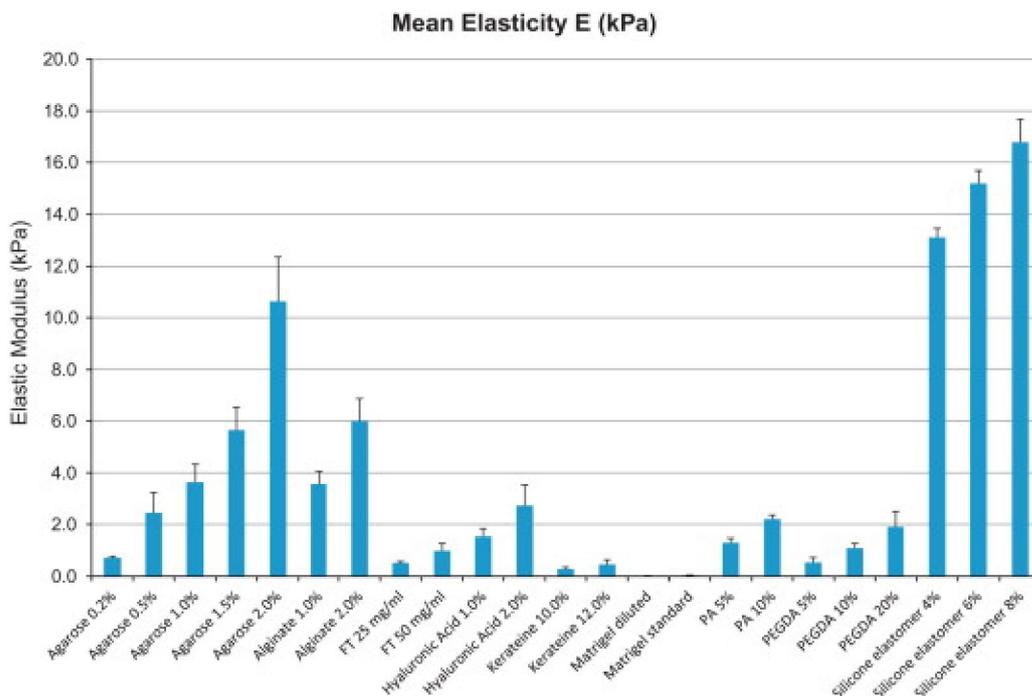


Figure 5: Bulk elastic modulus of different gels [15]

Tuning the stiffness of hydrogels

Numerous studies focus on the development of high strength hydrogels that can then be used to replicate natural tissue properties. One study reports a general method of using double network hydrogels to obtain gels of high mechanical strength, to be considered as a replacement for articular cartilage [18]. Their results showed that a double network hydrogel of PAMPS (poly(2-acrylamide-2-methylpropanesulfonic acid)) and PAAm (poly(acrylamide)) can sustain a stress of 17.2 MPa, more than twenty times that of the respective single network hydrogels [18]. The authors theorise that because the stress–strain curve overlaps with that of PAMPS gel at small strains, the first network contributes to higher elastic stress and the second network contributes to higher strain [18].

Another study done as a collaboration by Denisin and Pruitt looks at tuning the stiffness of polyacrylamide gels, for use in mechanobiology [19]. Adjusting the acrylamide monomer and cross-linker content has a direct effect on the elastic modulus of the resulting gel [19]. It was found that increasing crosslinker content results in increasing the elastic modulus of the gel, until a certain point where the modulus starts to decrease [19]. Another result of the study was that hydrogels are stiffer when polymerised at 4°C, as compared to room temperature, however it is mentioned that there is conflicting research on the relationship between polymerisation temperature and gel structure [19]. The study notes that the response of gel structure and elasticity due to temperature changes is not always in the same direction, due to the effect of linked factors (degradation, altered kinetics, molecular distribution) [19].

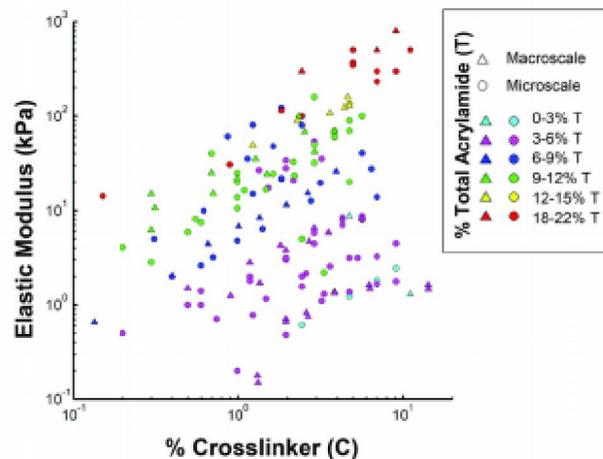


Figure 6: Elastic modulus as a function of cross-linker content for varying compositions of acrylamide [19]

A study suggests that a hybrid network of a crystalline polymer and a covalently cross-linked hydrophilic polymer produces a hydrogel with good mechanical properties and chemical stability [17]. The crystalline polymer is the source of crystallites which work as physical crosslinks, and the cross-linked polymer controls the swelling of the gel and the elasticity of the network [17]. The example provided in this study is a hydrophilic PAAm (polyacrylamide) network, cross-linked, and a PVA (polyvinyl alcohol) network which provides the crystallites [17]. The results show a crystallite-toughened hydrogel which has an elastic modulus measured at 5 MPa [17].

Experimental Procedure

Experiment Objectives

- To measure the elastic modulus of a ripe kiwifruit and synthetic (plastic) analogues
- Preparing samples out of gels
- Measure the firmness of the gels by using an acoustic ripeness testing device

A total of six different materials were initially prepared and tested: sodium alginate, hyaluronic acid, and gelatin in addition to the agarose, carrageenan and PDMS, however these three materials were not used for acoustic testing (see Appendices).

Section A: Preparing test samples

MATERIALS

- Distilled water
- k-carrageenan powder
- agarose powder
- Sylgard two-part silicone rubber
- calcium chloride
- 150mL beakers to use as moulds

CARRAGEENAN

Firstly, 1g of calcium chloride was dissolved in sufficient distilled water to form 50mL of solution in a 150mL beaker. 0.75g of k-carrageenan powder was added to the mixture to make it 1% carrageenan concentration, and the beaker was placed in the microwave until it reached approximately 70°C. The mixture was allowed to cool at room temperature for half an hour, and then placed in the refrigerator for a few hours until fully set. The set gel was slid out of the beaker and stored in the fridge until needed. This procedure was repeated six times so that six samples of k-carrageenan were prepared, of different carrageenan concentrations:

Table 1: Table showing the different k-carrageenan concentrations that were used as final test samples

Sample number	Carrageenan concentration (%)	Amount of k-carrageenan powder added (g)
1	1	0.5
2	1.5	0.75
3	2	1
4	2.5	1.25
5	3	1.5
6	3.5	1.75

AGAROSE

The first step in preparing an agarose gel was to measure 50mL of distilled water in a 150mL beaker. 0.5g of agarose powder was then added to the water, forming a 1% solution. The mixture was placed in the microwave for 30 seconds, or until the powder is entirely dissolved. The mixture was allowed to cool at room temperature for half an hour, and then refrigerated for an hour or until completely set. This procedure was repeated for six samples of agarose gel, of different agarose concentrations:

Table 2: Table showing the different agarose concentrations used for the agarose gel samples

Sample number	Agarose concentration (%)	Amount of agarose powder added (g)
1	1	0.5
2	1.5	0.75
3	2	1
4	2.5	1.25
5	3	1.5
6	3.5	1.75

AGAROSE - OTHER CONSIDERATIONS

- **Different core structure**

Kiwifruit consist of three regions of differing firmness. As the 6 samples that were prepared for each gel were of uniform concentration, this will not take all the complexity of a fruit into account. For this reason, another 2 samples were prepared as a method of observing what factors are of significant importance in the produced signal. A 50mL mixture of agarose was mixed, of 1.5% concentration. This was allowed to set in the refrigerator, and once it had set, a small cylinder was used to create a circular hole in the centre of the gel similar to the size of a kiwifruit core. Another gel mixture of 0.5% concentration was prepared and poured into the hole in the centre of the set 1.5% concentration gel. The beaker mould was placed back into the refrigerator so the 0.5% mixture could set. The process was repeated to create a second sample using a 0.5% base gel and a 1.5% gel for the core.

- **Different surface area**

Another possible consideration taken into account was the impact of surface area between the sample and the piezo test plates during the acoustic testing. 3 agarose gels of concentrations 1%, 2%, and 3% were prepared using the same method as for the original 6 samples, but of different shape and amount. The 150ml beaker was still used as a mould.



Figure 7: Agarose samples, top: 0.5% core in 1.5% base gel, bottom: opposite of the top composition configuration

Table 3: Agarose gel component measurements for preparation

Concentration	Agarose powder (g)	Distilled water (mL)
1%	1.5	150
2%	3	150
3%	4.5	150

PDMS - POLYDIMETHYLSILOXANE RUBBER

Sylgard two-part silicone rubber was used at various concentrations of catalyst to vary the modulus of the rubber. The monomer was measured by weight into a disposable plastic cup, after which the catalyst was added to the correct ratio to a sample of approximately 50g. The mixture was stirred with a disposable spoon, and then covered with aluminium foil. The rubber was left to set at room temperature for two days. This procedure was repeated to get six samples of PDMS with different monomer to catalyst ratios:

Table 4: Table showing the weights of monomer and catalyst used to obtain desired strength ratios for PDMS test samples

Sample number	Monomer to catalyst ratio	Monomer weight (g)	Catalyst weight (g)
1	50:1	49	1
2	40:1	49	1.3
3	30:1	48	1.6
4	20:1	48	2.4
5	10:1	45	4.5
6	5:1	40	8

Section B: Texture analyser testing

MATERIALS/EQUIPMENT

- Texture analyser and computer software
- Probe: conical 30mm base diameter, 60° angle
- Kiwifruit, Gold variety
- Gel samples of carrageenan, PDMS, and agarose

METHOD

- Kiwifruit

The kiwi fruit was sliced through the centre horizontally, and then 3 slices of around 1 cm thick were cut, to be used as testing samples. The texture analyser was set up and connected to the remote



Figure 8: Kiwifruit slice undergoing penetration test with the texture analyser

operation software on the computer. The test was set to a normal compression test, with a trigger load of 7g and speed of 0.5 mm/s.

The first slice was tested, with 3 different tests performed on the core region, 3 on the inner pericarp, and 3 on the outer pericarp region. Care must be taken to ensure placement is on the correct zone and that seeds and boundaries are avoided. This is repeated for the other two slices. The results are recorded in the form of load (g) - distance (mm) data. This data is averaged for each region across all three slices.

From this data and the probe surface area dimensions, the elastic modulus can be estimated for each of the three kiwi fruit regions.

- Prepared gel samples

In order to keep consistency, the same settings and probe on the texture analyser were used: a trigger load of 7g and a speed of 0.5 mm/s. 5 penetration tests were done on each sample surface, ensuring that they did not overlap and were evenly spaced across the entire surface, demonstrated in Figure 9. The readings were averaged before calculation of the elastic modulus.

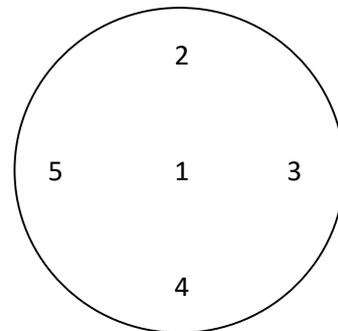


Figure 9: Placement of the 5 penetration tests for the texture analyser sample gel testing

ELASTIC MODULUS CALCULATION

The surface area of the probe can be found with the equation

$$SA_{cone} = \pi h^2 \frac{\tan\left(\frac{\theta}{2}\right)}{\cos\left(\frac{\theta}{2}\right)}$$

where the displacement of the cone, h, was set by the texture analyser at 5mm for all the tests. Using the force-distance data from the test, the data can be re-plotted with force and surface area of the cone. The elastic modulus was calculated by taking the average of the gradients of the linear regions of the curve.

Section C: Ultrasonic ripeness testing device

For the acoustic ripeness testing, the first step was to set up the computer signal software and switch on the power for the piezo trays. The test was set to cycle through the frequencies of 300 to 1200 Hz, as according to previous tests, with a step size of 10 Hz and step duration of 0.1s. The trays and plates are numbered as shown in Figure 11, therefore the start and stop positions must be identified in the setup. The testing samples were placed in the tray positions and the test was started. 10 runs were carried out before the test was stopped. The testing samples were then switched around into a different layout, and the 10 runs were carried out again. A minimum of 4 different layouts in the tray positions was used for all samples. A test for control was carried out by running the device with the same settings but with no samples, a blank run.



Figure 10: Acoustic ripeness tester set up; piezoelectric plates to the left, connected to a laptop with attached software



Figure 11: Numbering of the piezo plates for layout identification

Results and Discussion

Texture Analyser

The results from the texture analyser were analysed and compared using the factors of

- elastic modulus
- stress-strain curve
- homogeneity of results,
- relationship between elastic modulus and strength of the gel, or regions of fruit.

KIWIFRUIT, GOLD VARIETY

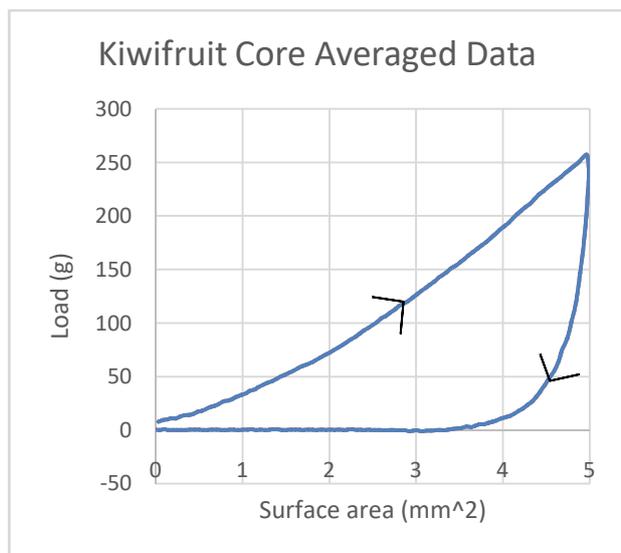


Figure 12: Force-surface area plot used to calculate elastic modulus, for kiwifruit, core region

The core of the kiwi fruit was found to be the stiffest, with an elastic modulus of 5.8 MPa, followed by the outer pericarp (3 MPa) and the inner pericarp found to be the softest region (0.5 MPa). The shape of the stress-strain curve remains very similar across all three regions; there is a significant amount of permanent deformation which is visible to the naked eye. It can also be seen that as the probe initially penetrates the fruit sample, there is some compression of tissue that takes place as the fruit deforms - this is suggested by the lower steepness of the curve in the beginning, in

accordance with theory and our hypothesis. The inner pericarp is the region which contains the juice sacks and the seeds, therefore it was

expected to be the softest. It is worth noting that the change in elastic modulus across the regions is not constant: the outer pericarp is roughly half as stiff as the core, but the inner pericarp is significantly less than half in stiffness than the outer pericarp. The maximum loading sustained follows the same trend as the elastic modulus when comparing across the three regions, suggesting that the viscoelastic response is very similar across all three regions of the fruit. The raw data was averaged in order to get a representative value for the bulk modulus, as there were slight, random differences in the data across different places in the fruit. This may be due to variation in the measurement technique, and inhomogeneity in the fruit itself.

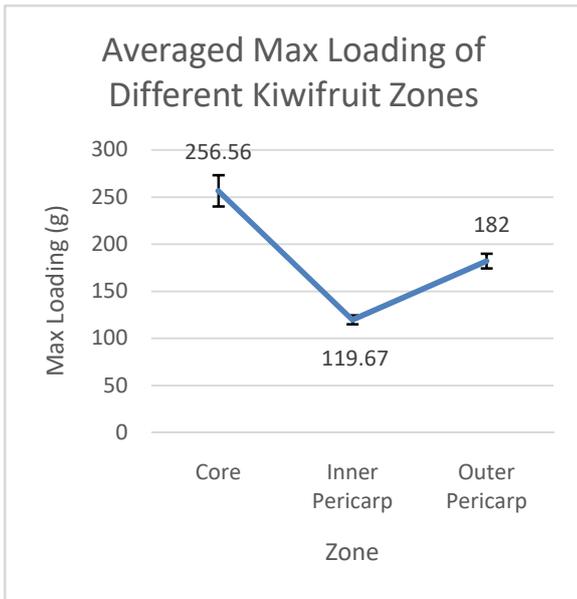


Figure 13: Kiwifruit regions, max loading sustained under penetration test

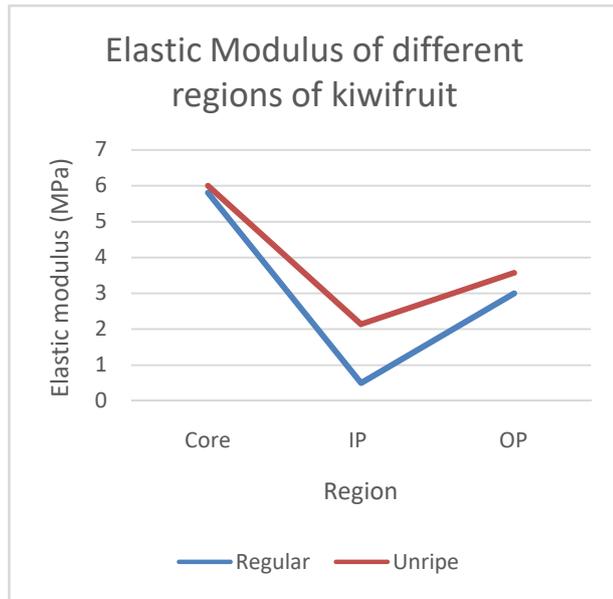


Figure 14: Elastic modulus of different kiwifruit regions

Another thing to note is the change in firmness as the fruit ripens. From Figure 14, the modulus results suggest that the inner pericarp region undergoes more significant softening during ripening when compared to the outer pericarp or the core. As these were only two samples however, a conclusion cannot be drawn from the data as it is likely that different fruit ripen in different ways.

The values calculated for elastic modulus suggest that the hydrogels required to appropriately model the fruit will need to be relatively high strength, such as silicone rubber or other gels which can be densely cross-linked.

CARRAGEENAN

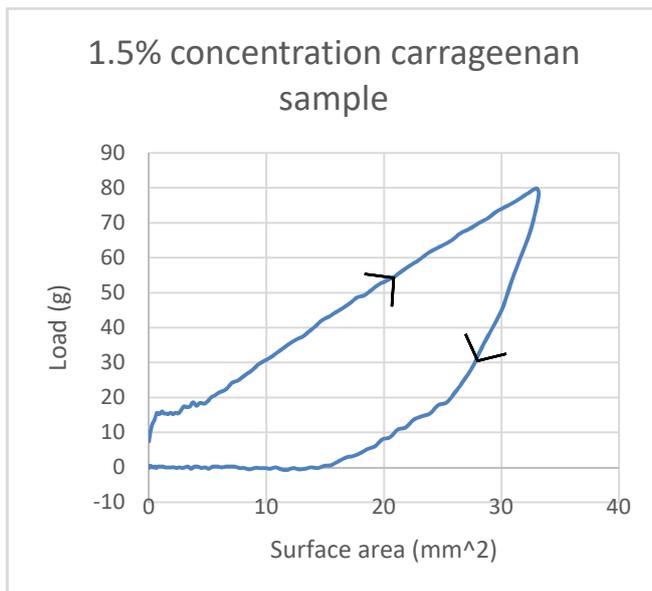


Figure 15: Elastic modulus curve for carrageenan

Carrageenan is usually used as a thickening agent in food, so a standard concentration for the gel varies as desired. 1.5% concentration produces a relatively firm, free standing gel. Comparing the viscoelastic response of the gel with that of kiwifruit, the carrageenan gel has a more consistent rate of deformation during loading: there is no visible "crimping" (the initial stretch of the material under the probe) as the slope of the loading curve is straight. In terms of firmness, the 6 trialled concentrations of carrageenan gel seemed to have similar strength to a kiwifruit, giving a range between 1.3 to 4.3 MPa. The relationship

between the firmness of the gel and its concentration was directly proportional as can be seen in Figure 16. There is some discrepancy between the expected hypotheses, such as the 2% gel having a higher elastic modulus than 2.5%. Carrageenan may have a set concentration value which is optimal for saturation and dissolving during the preparation process, going above this value may mean more inhomogeneity across the sample. Visually, it was easy to identify between gels as higher concentration produced gel of lower transparency. There was also an identifiable difference between the feel of the gel, higher concentration gels were firmer to the touch. The samples were visually homogenous, with no visible air bubbles or imperfections. As carrageenan gels are not purely elastic, the penetration left significant dents in the sample.

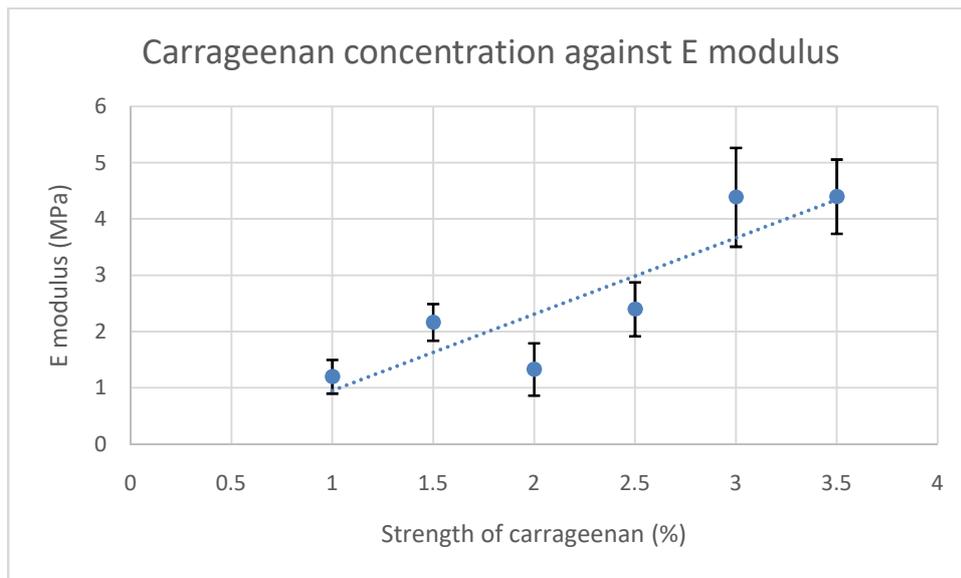


Figure 16: Relationship between concentration of carrageenan gel and e modulus

AGAROSE

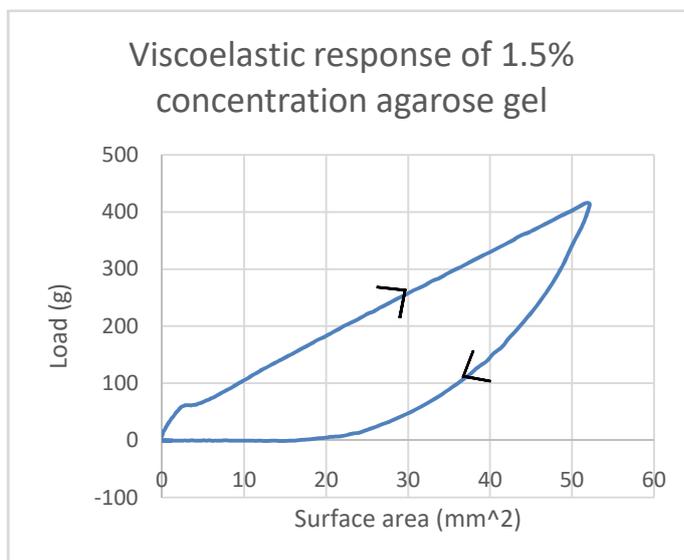


Figure 17: Elastic modulus graph for agarose

Figure 17 shows a stress strain curve very similar to carrageenan, but significantly stronger for the same concentration of agarose powder as carrageenan. Visually, it was also easy to differentiate between gels by their opacity and firmness to the touch. There were no visible imperfections and the relationship between strength and concentration was also directly proportional as expected. There were some inconsistencies, such as the 2% gel having a higher elastic modulus than that of 2.5% or 3%, but this may have been due to saturation

inconsistencies during preparation. Agarose is usually used for DNA analysis, at a concentration of 1%. There is also a large range of firmness values that can be achieved with agarose, as shown in Figure 18. The elastic modulus ranges from 0.5 to 12 MPa.

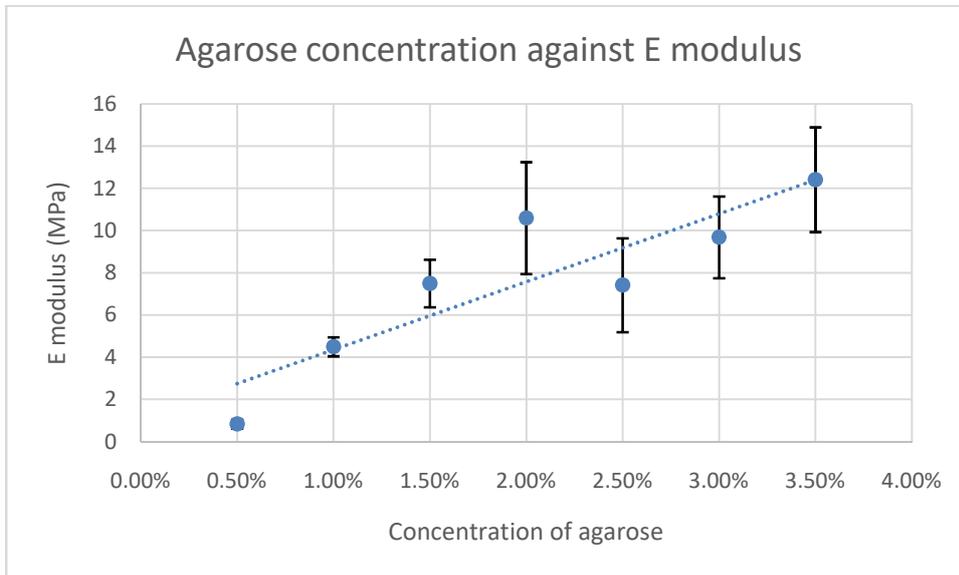


Figure 18: Relationship between agarose concentration and elastic modulus

PDMS

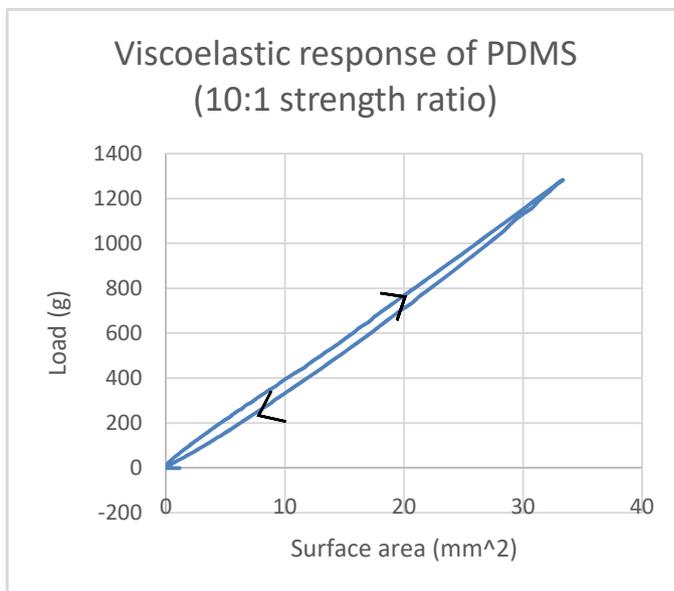


Figure 19: Elastic modulus graph for PDMS

The stress-strain curve of PDMS rubber was significantly different to that of kiwifruit, carrageenan and agarose. Figure 19 clearly shows a significantly elastic response, which was expected from the material. There was negligible permanent deformation and no visible dents in the samples after testing. 10:1 is the general preparation ratio used for PDMS rubber in other applications. It is also important to note the maximum load for this sample (1400g) which is significantly higher than can be expected from any viable concentration of agarose or carrageenan; but this also meets expectations as hydrogels are generally weaker than

synthetic rubber due to the high water content. This value corresponds to the high elastic modulus calculated in Figure 20. The relationship between the amount of cross linking agent added and the strength of the prepared rubber can be seen in Figure 20. There is a general correlation of higher catalyst amount and higher strength, but it is important to note that for the weaker rubber preparations, the jump in strength is not as high as that between 20:1 and 10:1 rubber.

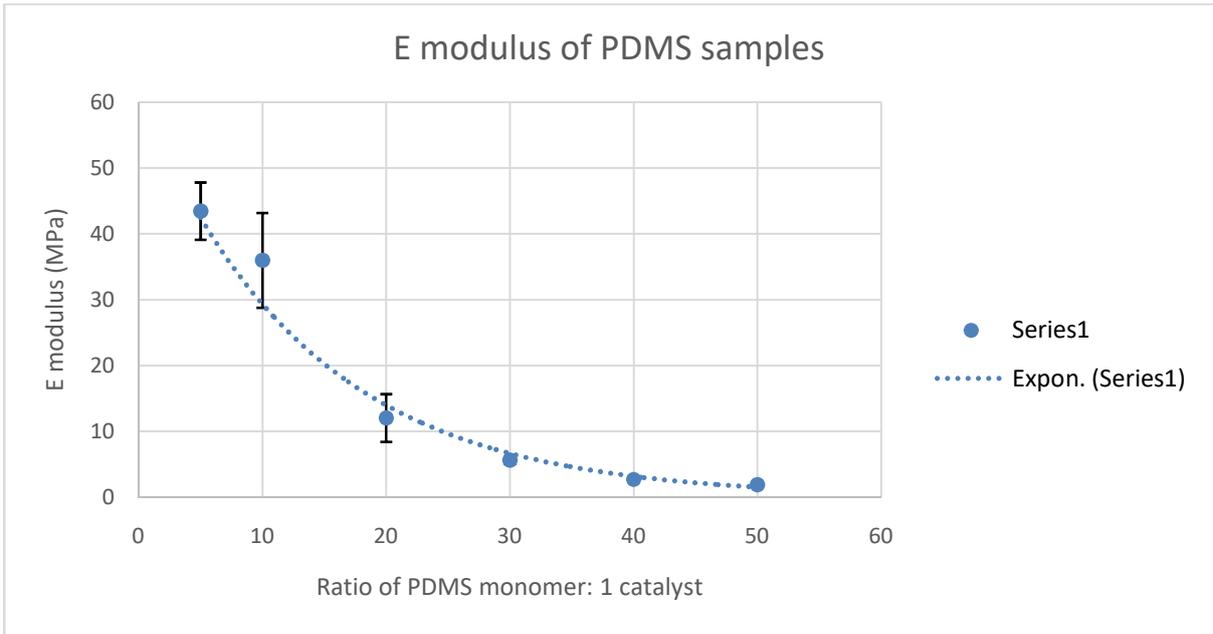


Figure 20: Relationship between strength of PDMS gel and elastic modulus

Piezo test results

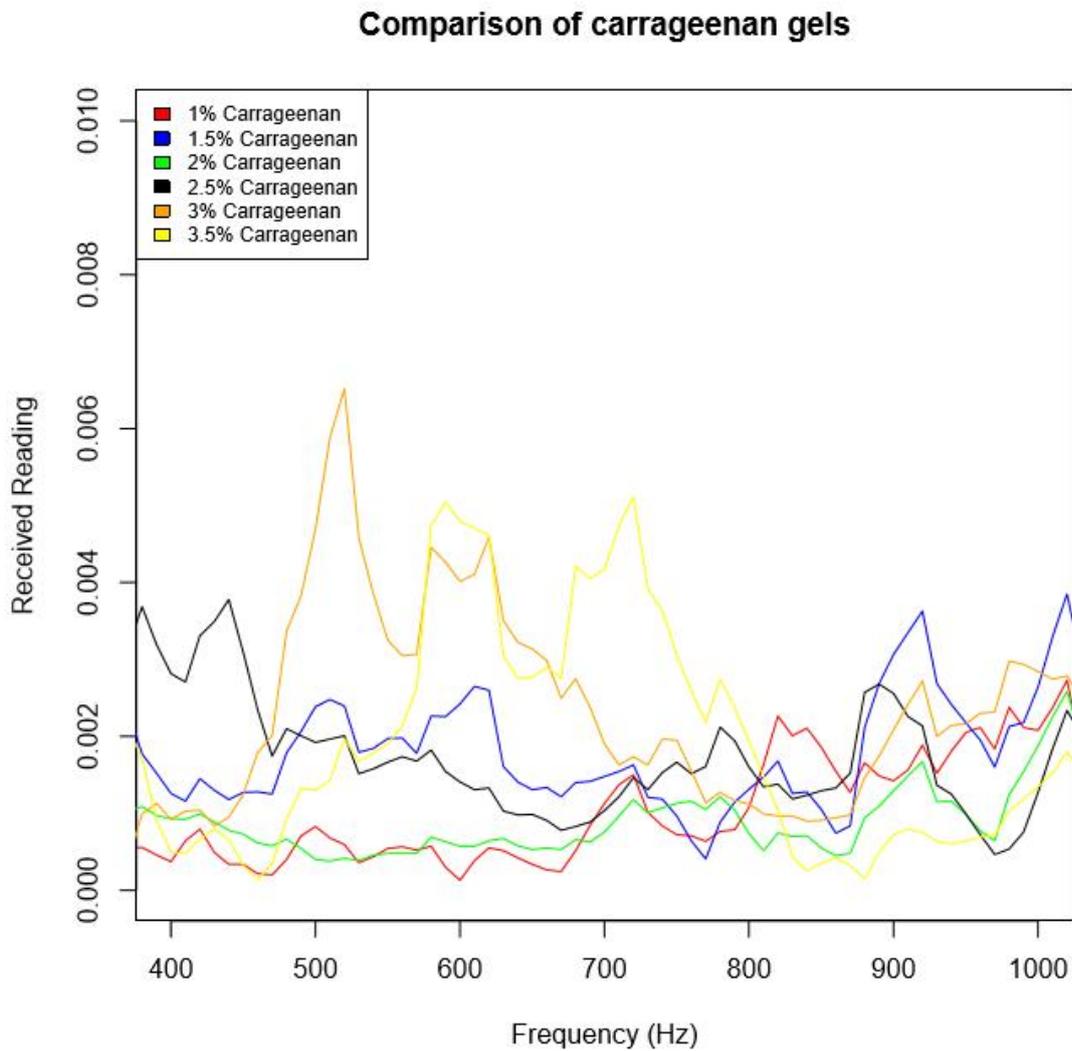


Figure 21: Acoustic signals received from the 6 carrageenan gels

The acoustic readings for all 6 carrageenan gel samples are shown in Figure 21. There is a significant amount of overlap between the signals for each gel, however the two strongest (3 and 3.5%) have clear peaks around the frequencies of 500-800Hz. Looking at the maximum peak height, the signals suggest a proportional relationship with the firmness of the gel (Figure 22). The general pattern across the tested frequency range is jagged, sharp peaks in an inconsistent format in consideration to other tested gel samples.

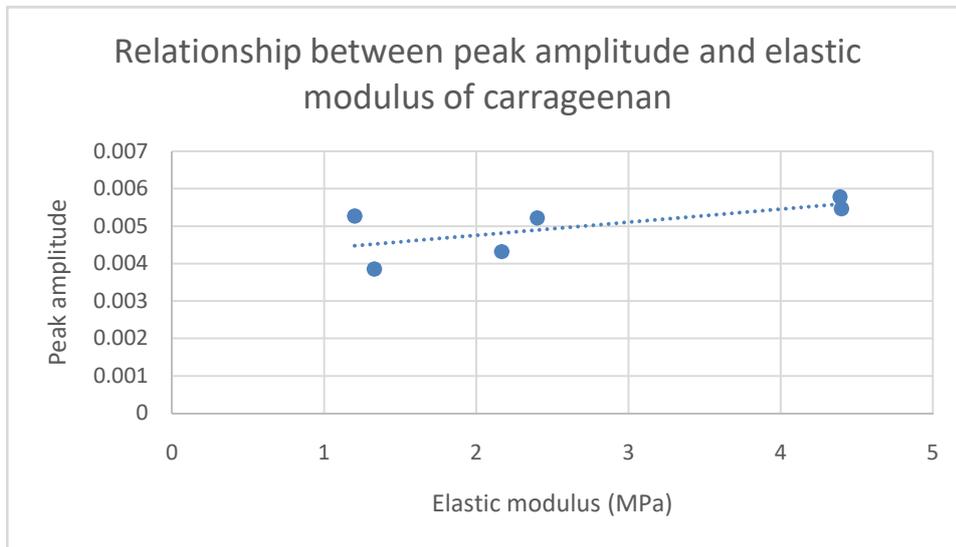


Figure 22: Carrageenan samples, peak amplitude against e modulus

Comparison of PDMS rubber samples

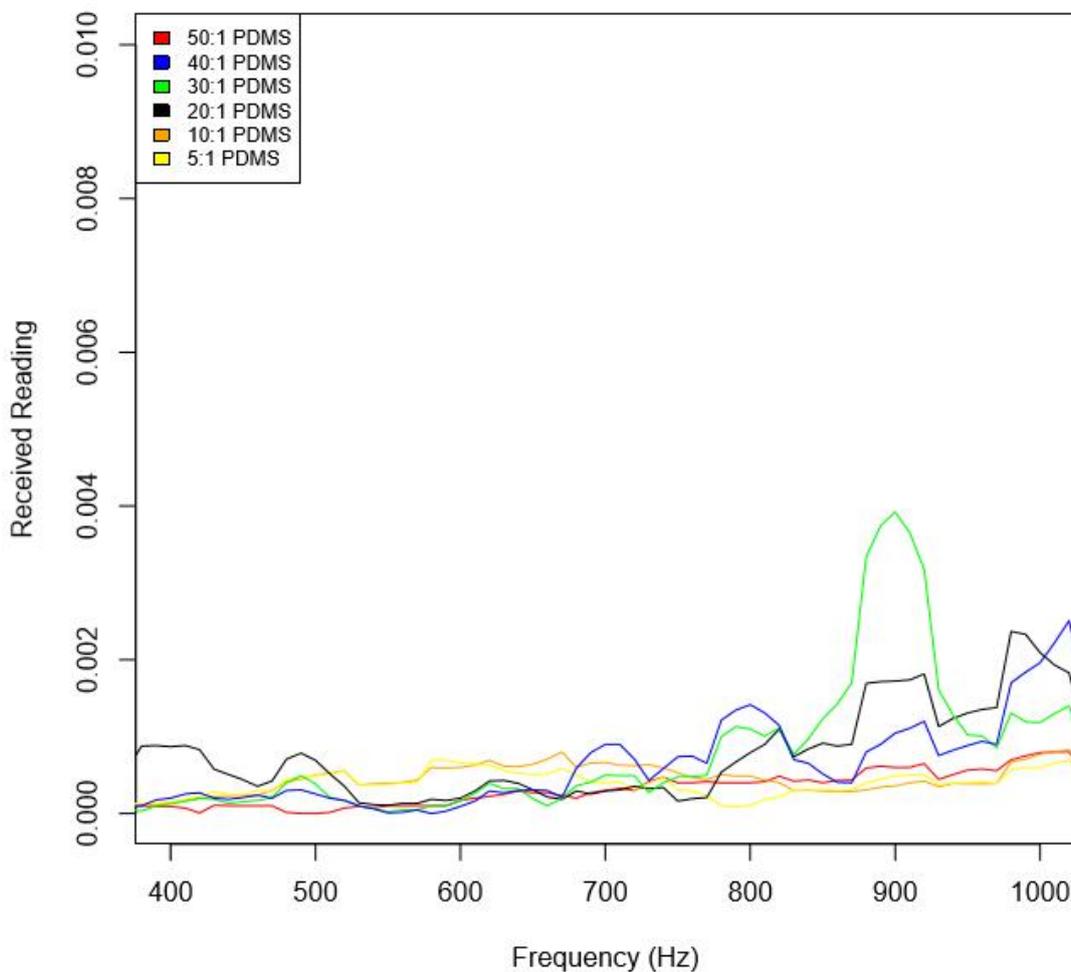


Figure 23: PDMS samples acoustic signals

From Figure 23, the acoustic signals received from the PDMS rubber samples are clearly not similar to those resulting from agarose or carrageenan gels. The amplitude of the general signal overall is lower, ranging around 0.001 as compared to 0.004 for the other tested materials. The peaks are less pronounced in PDMS samples, and the relationship between peak amplitude and rubber strength is not as clear, but it suggests a lower amplitude with higher elastic modulus (Figure 24). There seems to be a trend of higher amplitude with higher frequency for all 6 samples. The very low signals received at the lower end of the frequency range suggests that the rubber absorbs most of the energy at those frequencies, and at higher frequencies it shows a pattern more similar to those of the other tested materials.

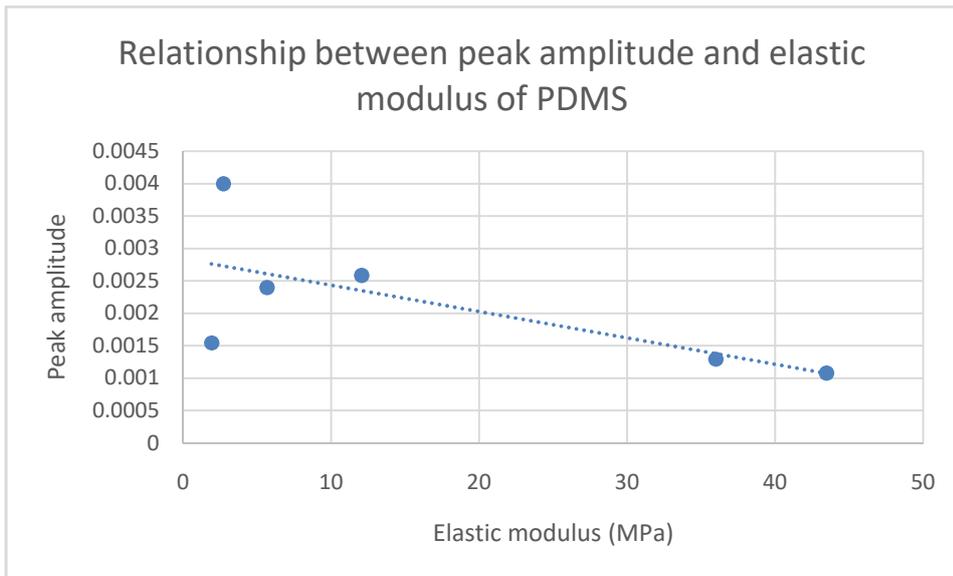


Figure 24: PDMS samples, relationship between peak amplitude and elastic modulus

Agarose gels gave signals of around the same amplitude as the carrageenan gels (Figure 25), however the pattern looks to be more consistent across agarose gels of different concentrations. The relationship between peak signal amplitude and firmness also looks to be relatively consistent, there is no particular trend visible (Figure 26). It is worth noting the placement of the peaks across the agarose samples seem to have some correlation with the frequencies. All gels have one or two significant peaks around the 500-600Hz frequency and also at 800-900 Hz, which suggests that the type of material plays a part in which frequency produces a peak. This may relate to how homogenous each material is across all six samples; as this same pattern is not noticeable in Figure 21, showing carrageenan gels.

Comparison of agarose gels

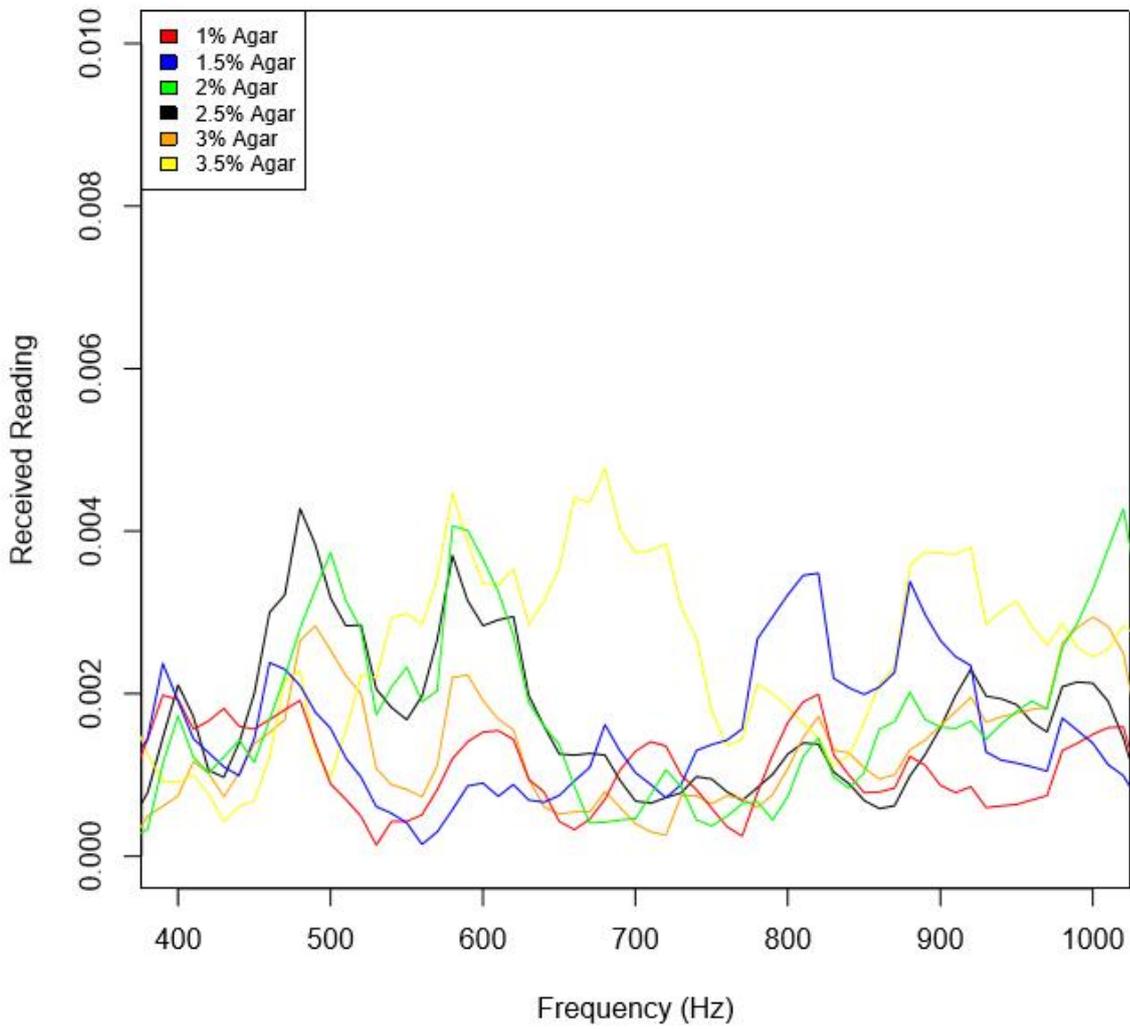


Figure 25: Acoustic signals from agarose samples

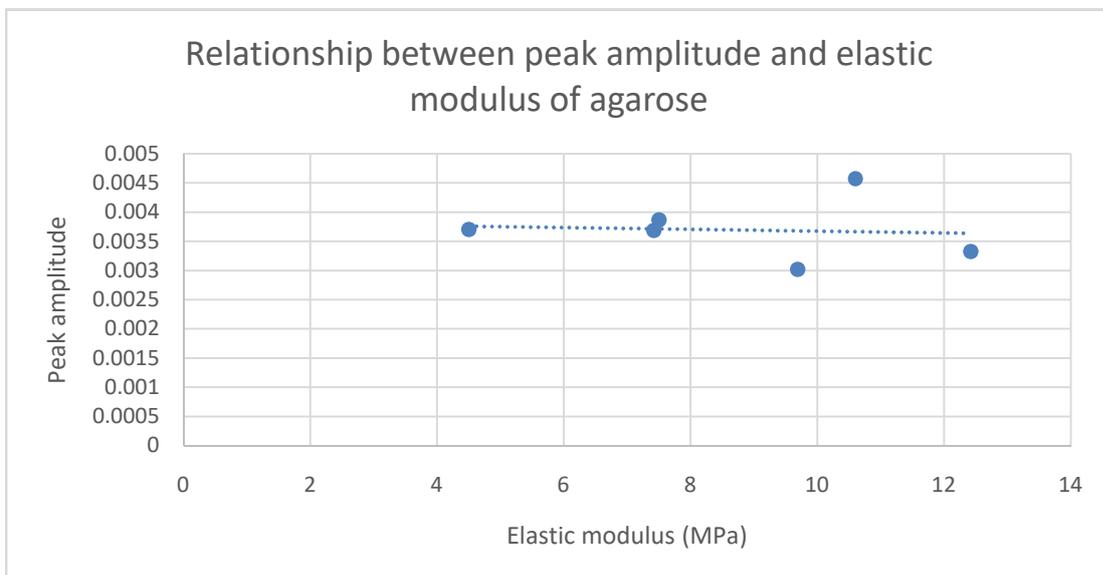


Figure 26: Relationship between peak amplitude and elastic modulus of agarose

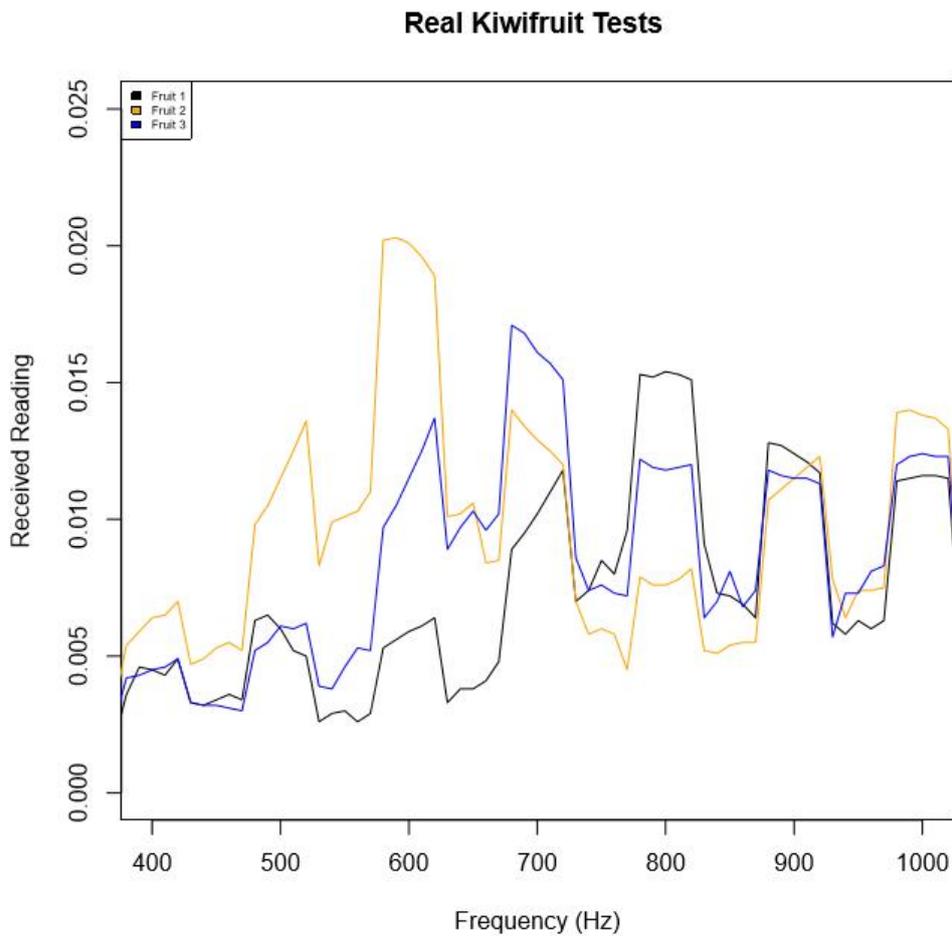


Figure 27: Acoustic signal data from real kiwifruit

From Figure 27, the acoustic signals from real kiwifruit are clearly significantly higher in general amplitude than the gels or the PDMS. There seems to be a pattern present with the placement of the peaks in the frequency range; this may be due to a number of reasons explored such as surface area, geometry of fruit, and width of regions inside the fruit cross section. The peaks are also different in fundamental shape, they appear flatter at the tip, wider, and more distinctly separated in particular at the higher end of the frequency range. The challenging issue for kiwifruit is that firmness for the fruit changes throughout the cross section, and the acoustic signal propagates across the entire cross section in this arrangement. This means the readings from the tester and the texture analyser are difficult to correlate.

Other Considerations

The agarose samples with a different internal core structure produce a different acoustic signal in the tester when compared to the original 6 agarose samples of the same concentration. The signal is generally higher in amplitude, although not as high as that of real kiwifruit. The peaks are also flatter and wider, in comparison to the uniform agarose or carrageenan samples, another similarity to real fruit.

Agar samples of different core structures

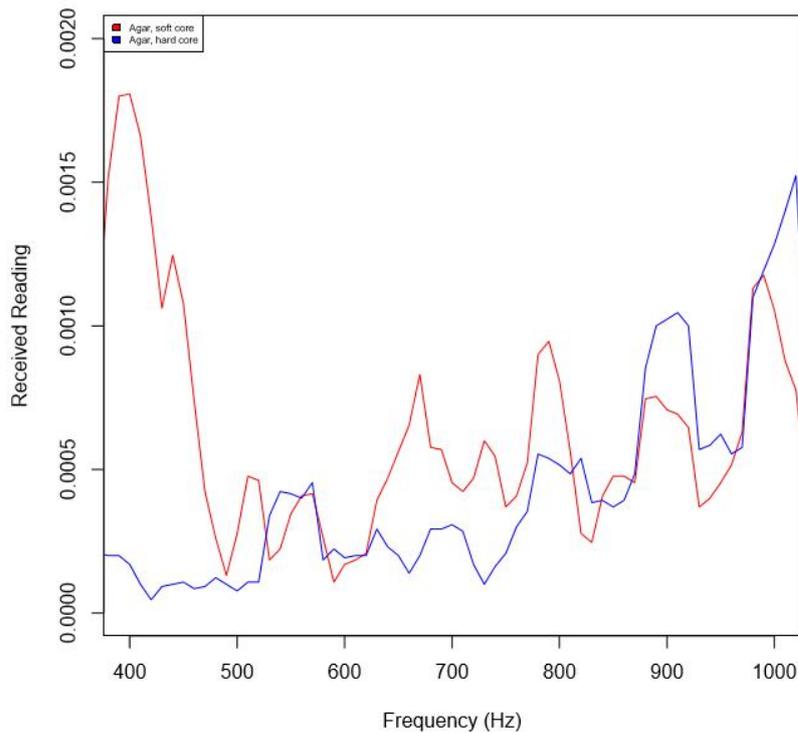


Figure 28: Agarose samples of different core structures

The acoustic signal is generated from the material's response to the propagation of acoustic waves through it. The presence of the peaks, from literature, most likely correspond to resonant frequencies of the material, voids such as air bubbles, and/or changes in density and other properties inside the material. The physical vibration of the material caused by the test may have some impact, as a softer testing sample will be more affected by this due to the set up of the test and placement of the sample across the piezo plates. Agarose and carrageenan are made up of agarose and carrageenan powder, respectively, dissolved in water. As cross links are formed, there is most likely a difference in the bond strengths of each link and densities of each separate structure in the network. This may account to the large number of seemingly random peaks produced in the signal. Although there were no visible air bubbles in the samples, due to the method of preparation, they must be considered as it is likely that they were present. PDMS rubber is highly amorphous due to its transparent and elastic nature, and therefore may also have a diverse range in individual bond strength across each sample.

Some major considerations that were taken into account were the factors of air bubbles, surface area, and device manufacturing inconsistencies. Air bubbles, not necessarily visible to the naked eye, were always to be expected due to the preparation method: agarose and carrageenan powder were mixed into water, and PDMS preparation involves stirring the monomer and catalyst together. Stirring and mixing manually introduces a considerable amount of air into a mixture, and unless it is removed by a degassing vacuum chamber or left to set extremely slowly, there will be air bubbles trapped inside the mixture especially due to its viscous nature. To determine the significance of this factor, a sample of agarose gel and PDMS were examined under an optical microscope, as can be seen in Figures 29 and 30. The agarose gel contains a large number of air bubbles scattered in a random formation across the sample; PDMS does not have nearly as many. This is likely to be

another contributing factor towards the differing acoustic signals; the sound energy is more likely to be absorbed by the denser PDMS structure, producing acoustic signals of lower amplitude and fewer peaks.



Figure 29: PDMS sample under optical microscope

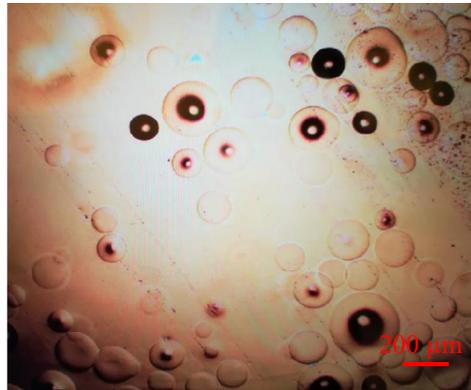


Figure 30: Agarose gel under optical microscope

Surface area was another factor that had the potential to have high impact on produced acoustic signal due to the set up of the ripeness tester. Due to the difficulty of creating the right mould for a kiwifruit shape, and the large amount of raw material required, the testing samples were created using the bottom of a small 150mL beaker. This is different to how a kiwifruit sits within the testing plates; as is demonstrated in Figure 32. The three agarose gel samples prepared to take this factor into account produced vastly different acoustic signals (Figure 33) in comparison to the signals produced by gels of the same concentration but different geometry in Figure 25. The signals are much higher in amplitude and the peaks are more similar to those produced by kiwifruit in that they are wider and less jagged. This suggests that geometry and surface area of contact between the piezo plate and the tested sample has a significant effect on the characteristics of the produced acoustic signal. This may be due to difference in physical vibration across the sample - there is a difference in the way the samples are balanced across the plates, which may affect the vibration that each sample undergoes.



Figure 31: Prepared samples sitting in tray positions, lower surface contact area



Figure 32: Higher contact surface area samples, more similar to kiwifruit

Agarose gels with higher surface area

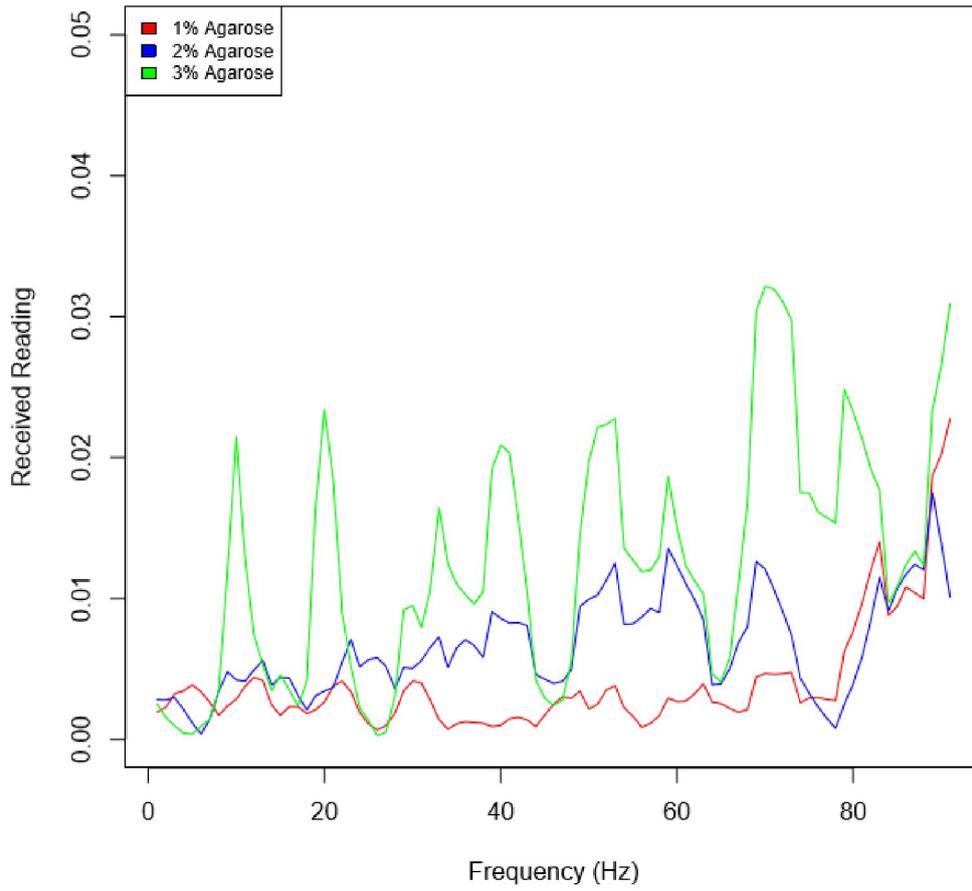


Figure 34: Agarose samples with higher contact surface area

Blank Piezo Tray Readings

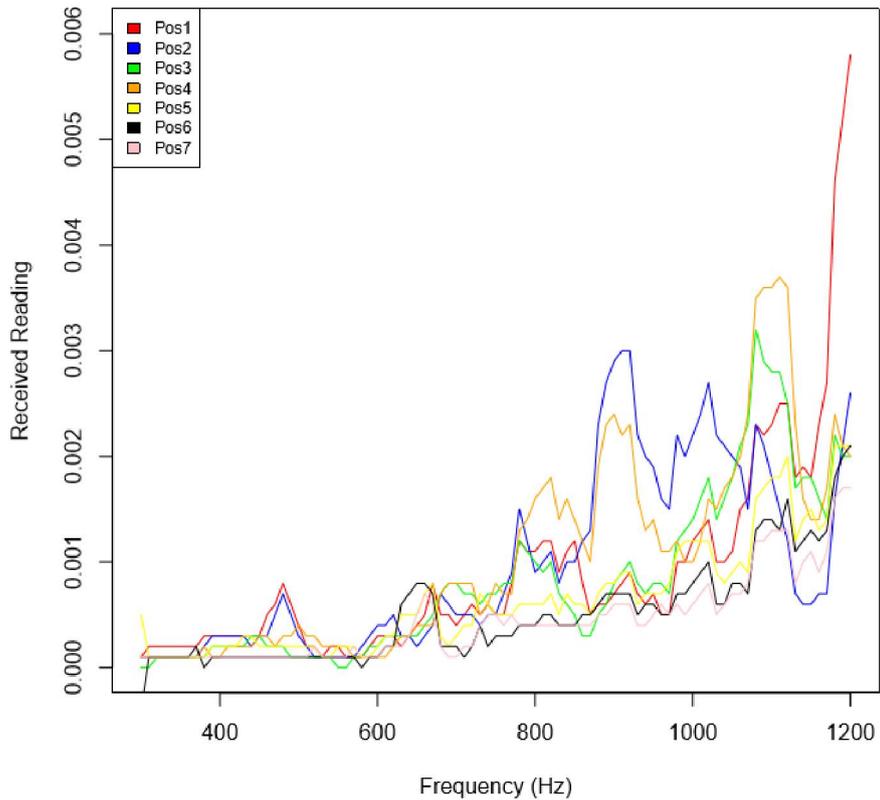


Figure 33: Blank piezo tray readings

As the ripeness tester is a prototype and manufactured manually, some difference between the different sets of plates was expected. The main source of this may be an inconsistency with how much silicone glue was used to attach the piezo plates to the main tray. From Figure 34, there is visible inconsistency between the positions, however it is on a small scale compared to the general amplitude of the readings of the tested samples. There is more variation at the higher end of the frequency range, from 800-1200 Hz, with one significant peak from the first position on the tray. This was mitigated by running multiple tests with the samples in different positions and averaging the results; which ensures that any trend from the result is not majorly affected by this inconsistency. It is important to investigate and note, for future usage and improvement of the tester.

Another source of error is the elastic modulus calculations for all the samples. As the modulus is calculated as the slope from a line of best fit, there is some random error present when judging the position of the line of best fit. Inhomogeneous samples are also another cause for error both for the calculation of the elastic modulus and for the acoustic ripeness tester readings. The variation in readings from the texture analyser is plotted through error bars (see Appendices). As the readings were averaged it can be assumed that this is negligible.

A point of consideration is that it is unlikely that two kiwifruit will be exactly identical. There will be differences in ripening, geometry, and distribution of internal regions, such as seeds and how large each region is respective to the others, as well as cell structure. This will lead to differences in acoustic signals which cannot be avoided. This study has allowed the identification of several experimental areas for future improvement and consideration. For proper calibration of the ripeness tester, surface area is a big consideration in that any future testing samples attempting to accurately mimic a kiwifruit structure. Another improved approach may be to build a database using kiwifruit categorised by cultivar, orchard, or similar so that over time, signals can be matched to differences noticed from regular penetrative testing which should be simultaneously carried out as usual. This reduces the amount of fruit samples or raw material required to carry out a thorough examination into acoustic signal analysis. Another area of exploration is using a different range of frequencies as examined in pre-existing literature.

Summary of Data

	Chemical structure	Concentration	Elastic moduli (MPa)	Maximum peak amplitude
Agarose	Agarose consists of supercoiled structures that are made up of agarose polymer chains wound into helical fibres. In solid form, it forms a 3D network of fibres with channels of ranging diameter, held together by hydrogen bonds [20, 21].	1%	0.84	0.0037
		1.5%	4.50	0.0039
		2%	10.60	0.0046
		2.5%	7.42	0.0037
		3%	9.69	0.0030
		3.5%	12.42	0.0033
Carrageenan	Carrageenan consists of large, flexible molecules wound into helical structures, similar to agarose [22].	1%	1.20	0.0053
		1.5%	2.17	0.0043
		2%	1.33	0.0038
		2.5%	2.40	0.0052
		3%	4.39	0.0058
		3.5%	4.40	0.0055
PDMS rubber	PDMS is a polymeric substance of flexible chains that are loosely entangled, which results in its highly viscoelastic behaviour. The dispersity depends upon the preparation method but PDMS generally has high homogeneity and a low polydispersity index [23].	50:1	1.92	0.0015
		40:1	2.72	0.0040
		30:1	5.67	0.0024
		20:1	12.06	0.0026
		10:1	36.00	0.0013
		5:1	43.48	0.0011

Table 5: Summary of all results

Conclusions

In conclusion, carrageenan, agarose, and PDMS rubber were the three materials used for signal analysis of the acoustic kiwifruit ripeness tester built by PFR Mt Albert. Although there were sources of error present and identified during the experiments, some findings were drawn:

- The firmness of a kiwifruit varies across its cross section, with the core twice as firm as the outer pericarp (elastic modulus of 5.8 MPa and 3 MPa respectively), and the inner pericarp (0.5 MPa) less than half the firmness of the outer pericarp.
- The viscoelastic response of a kiwifruit was best mimicked by hydrogels such as carrageenan and agarose, however PDMS rubber was also tested as a control
- In general, peak amplitude may correlate to firmness in carrageenan
- There is no visible trend between peak amplitude and agarose
- For PDMS rubber, peak amplitude may not be proportional to firmness; this is most likely due to the dense, highly amorphous structure of the rubber absorbing most of the propagating sound energy

The relationship between peak amplitude and elastic modulus of carrageenan, agarose, and PDMS is not statistically significant, however the results suggest the trends described above. Considerations taken into account were the amount of surface area in contact with the piezo plates, air bubbles, and the impact of inhomogeneous structure. Surface area in contact has a large impact on the acoustic signal, the higher the contact area, the larger the amplitude of the received acoustic signal. It is likely that air bubbles within the test sample are responsible for the random scattering of peaks seen in the acoustic signals received. The impact of an inhomogeneous structure was tested in the form of agarose gels prepared with a firmer core region, and vice versa. The resulting signals were more similar to those from kiwifruit in the width and placements of the peak.

There are numerous challenges present in this area of study, such as the complexity of kiwifruit structure, inconsistencies with manufacturing, and lack of pre-existing research in the overall topic but especially with more complex fruit structures such as in this case. The findings in this study require further study to be applicable for calibration of the acoustic ripeness tester, such as creating a database using kiwifruit samples and more complex testing structures rather than homogenous hydrogels. Using a different frequency range during acoustic testing is also potential area of further study.

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Appendices

Other trialled hydrogels

METHODS OF PREPARATION

Initially, some different kinds of materials were selected by considering their ease of accessibility, preparation, and suitability to the application. These included gelatin, PDMS, sodium alginate, agarose, k-carrageenan, and hyaluronic acid.

- Sodium alginate

The sodium alginate powder was stirred into distilled water until dissolved. This took around half an hour of stirring. Another solution of solution of calcium chloride and water was prepared, and combined into the alginate mixture. The alginate formed a set skin around it upon contact. The alginate was left in the chloride solution for a couple of hours until it has diffused sufficiently into the alginate blob, and then it was stored in the refrigerator.

- Gelatin

Various concentrations of gelatin were sprinkled over a small amount of cold water, mixed gently and set aside for five minutes to allow hydration. Warm water of the appropriate volume to make up the right concentration was then added to the mixture and stirred. The gelatin mixture was allowed to cool and set in the refrigerator for a couple of hours.

- Hyaluronic acid

Various concentrations of the HA powder were mixed into distilled water and stirred well. The mixture was left in the fridge for several hours to allow gel formation.

RAW RESULTS

- Sodium alginate

The difficulty using sodium alginate was the preparation method. When sodium alginate, dissolved in water, contacts the calcium chloride mixture, the external layer in direct contact with the chloride immediately gels, creating a skin around the rest of the alginate mixture. This characteristic is what makes sodium alginate a popular choice in cooking, for spherification. For the rest of the alginate to gel, it requires time and diffusion of the chloride from the outer skin. This means that it is difficult to control the cross-link density throughout the sample, as the centre will be softer than the outer edges. Also due to the formation of skin, it is difficult to create a mould shape using the alginate; as the mixture does not fit a mould. Analysis of the alginate using the texture analyser showed that the firmness of the sample was inconsistent across the surface. Although the range of measured elastic

modulus fit the desired range appropriate for kiwifruit, this inhomogeneity resulted in the decision being made to not use the gel for further testing:

Table 6: Measured elastic modulus results of sodium alginate gels of 1% and 2%

1% concentration		2% concentration	
Test on the same sample	Elastic modulus (MPa)	Test on the same sample	Elastic modulus (MPa)
1	3.4	1	4.4
2	3.7	2	4.2
3	1.5	3	6.3
4	1.9	4	6.7
5	2	5	10.6

- Gelatin

Gelatin was not used a tested material due to its stress-strain response under the texture analyser. As seen in Figure 35, the response is not similar to that of a kiwifruit, and is not a smooth curve; there is a lot of noise in the measurement.

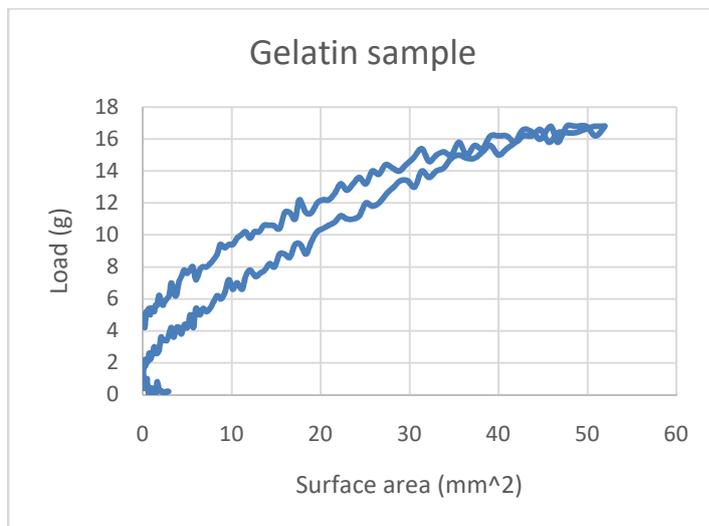


Figure 35: Stress-strain response of gelatin

- Hyaluronic acid

Hyaluronic acid was not used as a testing sample as the formed gel was very soft in structure, and the amount required to create a full size mould would have been unreasonably expensive in terms of sourcing raw material.

Safety and Laboratory Use: HAZOP

Table 7: HAZOP

Potential Hazards	Consequences	Preventative Action
Electrical equipment and wiring present	Injury/sprain from tripping over wires, electric shock (minor/major depending on situation)	Caution and care Ensure that cords are zip-corded neatly
Finger/hand getting under the hardness tester probe while in operation	Hand injury (minor/major depending on situation)	Make sure to keep hands away from the texture analyser when in operation.
Clothing or hair caught in the test	Injury, clothing damage	Keep clothing away from tester when in operation (loose sleeves, etc); tie hair back
Spillage of testing sample (i.e. if it is loaded in compression too quickly)	Causes a slip hazard, contaminates surrounding area unless cleaned thoroughly	Ensure that the probe is not set to an inappropriately high speed
Using a paring knife	Cuts	Store the knife out of the way when not in use, so that there is no chance of it being accidentally knocked off the table. Keep fingers out of the way when using the paring knife on the fruit.
Using an oven	Burns	Use appropriate safety gear when placing samples inside a hot oven and when taking them out (gloves)
Using calcium chloride	Hand injury, contamination	Use gloves and the fume cupboard.

