The study of the extraction of pectin from dried lemon peels

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This dissertation is submitted in fulfillment of the requirements for the degree of Masters of Chemical Engineering, Faculty of Engineering, University of KwaZulu Natal

As the candidate's Supervisor I agree/do not agree to the submission of this thesis

7 April 2009
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Date of the submission of the thesis

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L. Lerotholi

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ABSTRACT

Pectin is a polysaccharide found in plant cell walls. It is a linear molecular chain of D-galacturonic acid units linked by α 1→4 glycosidic bonds. Pectin is widely used in the food industry. The main sources of pectin are citrus fruits and apple. In South Africa, pectin is still imported whereas it can be produced from waste peels that result from citrus processing. A consortium was formed by CSIRBio/Chemtek, Chemin, Kat River Co-op and University of KwaZulu Natal (UKZN) to investigate pectin production. UKZN was to develop the drying (for off-season purposes) and extraction technology required for pectin production. The project was categorized into two phases; the drying and the extraction phases.

Tray drying tests were followed by batch tests in a fluidized bed dryer with the inlet ait at 150 ° C. Five process variables were chosen for the study of the extraction phase; temperature, time, pH, peel size and peel to water ratio. Three variables were chosen as the response variables; Yield (% Yield), Degree of Esterification (% DE) and Galacturonic Acid content (% GA).

A duplicated 2^5 factorial design was conducted on dried peels, followed by a duplicated 2^4 design (peel size excluded as a process variable) on fresh wet peels. A 2^4 design was also performed on wet peels stored at atmospheric conditions for two days. Lastly, a central composite design was conducted on dried peels in order to find the optimum condition of extraction.

Lemon peels were successfully dried at 150 ° C for 24.8 minutes to 10 % moisture content. Fresh wet peel extractions resulted in a lower % Yield, a similar % GA and a greater % DE than that of dried peels. Storing wet peel for two days degraded the pectin severely, showing the need for drying the peel in order to preserve it. The optimum conditions for pectin extractions were 86 ° C, a pH of 1.97, an extraction time of 1hr15min, peel size of 2-1 mm and peel to water ratio of 1:37.5. At this condition, the % Yield was found to be 22.22, % GA was 85.19 and % DE was 74.89.

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NOMENCLATURE

Applicable Section	Symbol	Representation
Drying	A	Area of drying surface (m^2)
	X	Moisture ratio
	X_e	Equilibrium moisture ratio
	X_{cr}	Critical moisture ratio
	M_D	bone dry mass of material (kg dry solid)
	M_i	Initial mass of material (kg)
	M_{H_2O}	mass of moisture within the material (kg H_2O)
	% moisture	percentage moisture content of the material
	CDTA	Cyclohexanediamine tetraacetic acid
Extraction	DE	Degree of esterification
	EDTA	Ehtylenediamine tetraacetic acid
	GA	Galacturonic acid content
	IPA	Isopropanol
	m/m	Ratio of mass of substance per mass of another (g/g)
	MAE	Microwave assisted extraction
	CI	Confidence interval
Statistical Analysis	DOF	Degrees of Freedom
	H_0	Null hypothesis
	MS	Mean square
	SS	Sum of squares
	x	Investigated variable
	y	Response variable
	$\bar{y}$	Average response variable
	ε	Random error
	τ	Error due to the difference in the mean
	μ	Mean
	R^2	Correlation coefficient
	R^2_{adj}	Adjusted correlation coefficient
	R^2_{model}	Model correlation coefficient

CHAPTER 1- INTRODUCTION

1. Introduction

All plant cell walls have a similar basic construction although they vary enormously in composition and physical properties. The cell wall varies from plant to plant, species to species and even within the same species. It also differs depending on the maturity of the plant (Harris, 2005). Pectin is obtained from primary walls of dicotyledons and is a water-soluble polysaccharide often referred to as a hydrocolloid.

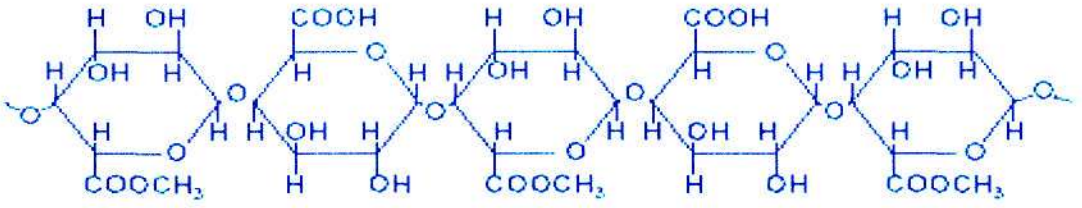

Fig 1: *Pectin molecule – homogalacturonan block (www.cpkelco.com/pectin)*

1.1 Structure

Pectin is made up of homogalacturonan, rhamnogalacturonan I and to a less extent a complex polysaccharide of rhamnogalacturonan II. The homogalacturonan block makes up most of the pectin. It is a linear molecular chain of D-galacturonic acid units linked by α 1→4 glycosidic bonds (Refer to Fig 1 above). The galacturonic acid units are methyl esterified and, in some pectin, are also acetyl esterified at some of the carboxyl ends (Walter, 1991). The rhamnogalacturonan I block is made up of a linear chain of galacturonic acid inter-dispersed with rhamnose residues. The rhamnose residue has side chains made up of arabinans, galactans and type I arabinogalactans (Willard et al., 2001). The side chain sugars are bonded to the rhamnose through 1 → 4 positions, although 1→2 and 1→3 attachments have also been reported. This gives the pectin a 'hairy' structure at this block. The homogalacturonan blocks are often referred to as the non-hairy or 'smooth' regions and the rhamnogalacturonan blocks the hairy regions.

There were several contradictions about the structure of pectin. Some believed that the rhamnose units in the rhamnogalacturonan chain are evenly dispersed while others believed that they are uneven (Rees & Wright 1971 and de Vries et al., 1982). The latter belief seems to have been accepted within the pectin industry and studies utilizing enzymatic degradation and ß-elimination have proved it true (Thibault, 1983 and de Vries, 1986).

1.2 Classification of pectins

Pectins are classified according to their degree of esterification (DE). The degree of esterification is the ratio of methyl esterified galacturonic acid units to the total galacturonic acid units in the pectin (Stephen and Charms, 2006). The DE has important effect on the functional properties of the pectin especially on their solubility, thickening and gelling properties. Commercial pectins typically have a DE ranging from 55-78% (Stephen and Charms, 2006). Pectins of a DE greater than 50% are known as high molecular (HM) pectins and those with a DE less than 50% are known as low molecular (LM) pectins. LM pectins can also be further processed to produce amidated (AM) pectins by de-esterification of the LM pectin in an ammonia medium.

1.2.1 HM pectins

HM pectins can be categorized into rapid set, medium set and slow set pectins. Rapid set pectins have a DE between 71-77%, medium set 66-69% and slow set between 58-65%. Depending on the DE, HM pectins require different pH conditions to gel;

- pH 3.1-3.4 for rapid set pectins
- pH 2.6-2.9 for slow set pectins

HM pectins also require the presence of a high concentration of solids (> 55%) before they can gel, with sucrose used mainly for commercial pectin (Thibault & Ralet., 2003). This limits the use of HM pectin to sweetened products.

1.2.2 LM pectin

LM pectins do not require solids to gel, but rather gel in the presence of calcium ions. They do not require a low pH, but gel at a pH range of 2-6. Even though high concentrations of solids are not needed, the high calcium content results in a bitter after-taste (Thibault & Ralet, 2003). LM pectins of a DE of about 40% are termed slow set and those of a DE around 30% are termed rapid set.

1.2.3 AM pectins

These types of pectins do not require a high solid content to gel, but like LM pectins require the presence of calcium. Nevertheless, the calcium content needed for AM pectins is less than that required for LM pectins. Amidation produces firmer gels than conventional LM pectins (Flutto, 2003) that have the ability to re-heal after shearing. Unfortunately, pectin gelation is also delayed in the process.

1.3 Uses

HM pectin is used mostly on food jams and jellies, bakery fillings and diary products as a gelling agent. It is also used as a stabiliser in acidic and fermented milk products such as sour milk, yoghurts and conserves. According to Willats et al. (2006), the homogalacturonan block binds electrostatically to the surface of the casein particle. This prevents them from agglomerating, which would cause them to sediment. HM pectins are also used in fruit juices to increase the body and improve the mouth feel of the juice (Flutto, 2003).

LM pectins together with AM pectins are also used in the manufacturing of jams and jellies, milk products and in fruit juice preparation, but for low sugar content products. LM pectin has a low water binding ability and is used in sugar-free jams for diabetics. A carrageenan can also be added to milk products to attain the same outcome as the pectins, and is more economical, however, pectin ensures against casein precipitation at lower pH values, thus increasing the shelf life of the product. LM pectin gels are thermoreversible and as a result can be used for glazing purposes in the bakery industry. In yoghurt making, the pectin can be combined with gums to reduce fruit colour migration into the yoghurt phase.

Pectins also increase the total dietary fibre intake which is recommended to be 20-35 g/day for adults (Harris and Smith, 2006). Dietary fibre is said to reduce the risk of type II diabetes, cardiovascular diseases, constipation and colorectal cancer (Champ et al, 2003). Moreover, pectin is utilized in the pharmaceutical industry. It is used to stabilize emulsions in liquid medication. It is also known to have an anti-diarrheal effect and is thus used in making anti-diarrheal tablets. It has also been reported to have wound healing and bactericidal effects.

1.4 Sources of pectin

The main sources of pectins are citrus and apple fruits. They not only have high pectin content, but are also by-products of the juice production industry (May, 2000). Citrus peel has been reported to have a pectin content of 25-35% (m/m on a dry basis) and apple pomace of 10- 15% (m/m on a dry basis). Sugar beet pulp, a by-product of the sucrose production industry, and sunflower heads, a by-product of the seed oil industry, are also under investigation for use industrially. Sugar beet has been reported to have a pectin content of 10-20% and sunflower 15-25% m/m on a dry basis (William et al., 2005).

1.5 Background

South Africa is the third largest citrus exporter in the world, after USA and Spain (CSIR, unpublished results). This means that a large quantity of citrus by-products is discarded as waste or used as low value cattle feed, while it could be used in one of the most lucrative and growing industry of pectin production. South Africa was reported to import pectin of about 250 tonnes valued at 77.61 R/kg fob and export 6.667 tonnes valued at 57.44 R/kg fob (ytd Oct) in 2002 (CSIR, unpublished results).

A consortium was formed by CSIRBio/Chemtek together with Chemin, Kat River Co-op and the University of KwaZulu Natal (UKZN). The members of this consortium have taken it upon their shoulders to improve the pectin industry in South Africa by taking advantage of its large citrus industry and developing the technology needed for the production of HM pectin. HM pectin production can be handled, at a later stage, by small and medium enterprises (SMME's) within the country, who in turn can sell their produce to large scale LM pectin producers.

The first phase of this project was to develop the technology required, optimize and commercialize a HM pectin production process. Commercialization will involve the construction and operation of a pilot scale (15tpa) prototype production facility. In the second phase, production will then be ramped to 150-300 tpa pectin production and Kat River Citrus Co-op will provide 10 000 tpa of citrus material to meet this demand. The envisaged production at a later stage when SA ventures into the international market is 1000 – 1 500 tpa. Granor Passi, the largest citrus producer in SA will be able to supply increased demand for citrus peels as it produces about 60 000 tpa of wet peel which is equivalent to about 1500 tpa pectin produce (~2.5% (m/m) of pectin on a wet basis). This means more income for citrus producers and generation of new jobs from plant operation.

Due to the fact that fresh fruit is not available throughout the year and because the peels degrade with time, it is necessary to dry the wet peel, to maintain pectin availability during the off-season. The role of UKZN was to develop the drying technology needed for pectin extraction. Usually, pectin is dried from 85% moisture content to 10% moisture content.

1.6 Project Objectives

The aims of this project were to suitably dry lemon peels and characterize and optimize the extraction process for pectin production. In an attempt to suitably dry the lemon peels, the most appropriate dryer and drying conditions were examined with the aid of previous research by the CSIR team. Pectin was then extracted from the dried peel and the resultant yield (% Yield), percentage galacturonic acid content (% GA) and degree of esterification (% DE), were then determined. Pectin from dried peels and fresh wet peels was then compared to prove the suitability of the drying method and conditions established. In order to ascertain the need for drying, the degradation of pectin extracted from wet peel stored at atmospheric conditions was assessed.

1.7 Outline of presentation

In the chapter (Chapter 2) that follows, the literature and theory relevant for the study are presented. This chapter was categorized into three sections; drying (2.1), extraction (2.2) and experimental design and analysis (2.3). Chapter 3, details the equipment, the experimental designs and procedures used in conducting all the experiments undertaken. Chapter 4 then

reports the results found and concurrently discusses them. Results from drying tests (4.1) are discussed first and then extraction results (4.2) follow. The extraction results are further grouped into dry peel first order modeling results (4.2.1), comparison of fresh wet peel and dried peel results (4.2.2), effect of storage on wet peels (4.2.2) and lastly, optimization of dried peel extraction with respect to the three response variables, % Yield, % GA and % DE (4.2.3). Chapter 5 presents the conclusions of the study and recommendations for future work and Chapter 6 details all references used for the study. Finally, Chapter 7 outlines all the calculations undertaken and the results obtained for this study in the presented appendices.

CHAPTER 2-LITERATURE SURVEY AND THEORY

2.1 Drying of the peel

The raw material peel for the pectin industry is mainly from the juice manufacturing industry. Consequently, the pectin quality is dependent on the quality of the peel processed from this industry. The quality of pectin is affected by the ripeness of the fruit (Turakhohaev et al., 1999 and Arancibia & Motsenbocke, 2004). If the juice manufacturer uses ripe fruit, then the resultant pectin will be of poor gelling properties.

The peel waste is recovered in its wet state and in this state is perishable as it is prone to attacks by moulds. The moulds produce pectic enzymes such as pectin methylesterase, which causes de-esterification, polygalacturonas, pectin-lyase and pectate-lyase, which are responsible for pectin degradation (May, 1990). The wet peel also contains natural methylesterase, which is still active even if moulds do not attack. With these enzymes active, the pectin is soon undesirable for use in most of its applications. In its wet state, according to May (1990), it is inadvisable to store the peel more than a few hours, unless it is specially treated. Even after it is treated it is not advisable to keep for more than a few days at most (May, 1990). If the peel is stored at low temperatures (less than 4 °C), Attri & Maini (1995) state that no significant loss in the quality is detected for up to 6 days. Transportation of the peel in its wet state therefore poses a problem to the pectin producer. If the pectin plant is located too far from the juice manufacturer, the pectin peel may arrive degraded and de-esterified.

Wet peel is inexpensive, but the resultant variability of the content of pectin, which relies on a number of factors that cannot be controlled, can be detrimental to the pectin producer (May, 1990). It is therefore advisable to dry the peel to ensure consistent good quality pectin. If a drought or adverse climatic conditions affect the content of the peel in a certain region, the pectin manufacturer can get dried peel from any other region. If a crop from the supplier is not of the required pectin content, then another supplier can be used even from an outlying juice producing plant (May, 1990). Since fruits are not available throughout the year, it will still be necessary for the pectin plant to be adapted for the processing of dry peel to ensure availability during the off-season. Thus drying of the peel is imperative in the pectin industry.

Drying of the peel can have an adverse affect on the quality of the pectin as pectin is a fairly heat-labile material (May, 1990 and Tuchnina et al., 1994). The temperature has to be high enough to denature all enzymes and destroy all moulds without destroying the pectin. If the process is monitored closely, good quality pectin can still be extracted. Fishmann et al. (2000) states that short heating improves the plant matrix structure thereby assisting in pectin release from the matrix. As a result the drying process can assist in liberating more pectin from the peel, but the time for drying has to be kept short as de-esterification and degradation of the pectin can occur. Hence a fluidized bed dryer was selected for this project.

2.1.2 Drying

Drying is defined as the removal of moisture from a material through vaporization (Perry, 1999 and Keey, 1992). There are many ways of removing water from materials; the key factor that defines drying is that the moisture should be removed by vaporization. The heat to effect vaporization is usually externally added, by convection, conduction or radiation. Internal methods, although rare, can also be employed such as heating via dielectric means (Keey 1992). The liquid removed is in most cases water, but removal of other solvents is also considered.

In food dehydration, drying is used as a preservation technique. Many micro-organisms cannot reproduce in the absence of water, thus increasing the shelf life of food. Moreover, enzymes within the material, which degrade the food, are inhibited in the absence of water. Geankopolis (1993), states that biological matter cannot function if the water content is reduced to about 10%.

2.1.2.1 Choice of Source of heat: Conduction, Convection, Radiation or Dielectric heating

Conduction:

Conductive heating is used mostly when the material to be dried is not temperature sensitive and is very thin or wet (as with slurries) (Keey, 1972). The heat is passed to the material by a heated surface with which it is in contact. This method of heating is thermally economical as all the heat from this heated surface is passed to the material (Perry, 1999).

Radiation:

Radiation can be used to supply energy to the material to increase the rate of evaporation. It is usually used for highly specialized purposes, such as in pharmaceuticals preparation (Van't Land, 1991). Various sources can be used such as solar and microwave radiators. Most materials absorb well in 4 – 8 μm wavelengths; as a result infra-red radiant heating is used (Keey, 1972)

Dielectric:

In dielectric drying, energy is given off within a moist material when place in a rapidly oscillating electric field, and this dries the material (Keey, 1972). Dielectric heating is generated internally throughout the entire material therefore aiding in its uniform drying, a quality difficult to attain with other heating methods. Because of the high frequency equipment needed in this method of heating, it is not industrially viable and is thus only used in special cases (Perry 1999, Keey 1972).

Convection:

According to Keey (1972), convective heating is preferred to all other types as the drying conditions can readily be controlled by the temperature and humidity of the heating medium that evaporates and conveys away the moisture. The temperature of the material being heated can never exceed that of the incoming drying medium, there is therefore, some assurance against overheating. Various type of material can be dried through this method, but the most

preferred are of the free-flowing granular type. The downfall of convective heating, though, is that it is sometimes thermally inefficient due to high sensible heat losses in the outlet heating medium (Cook & DuMont, 1991).

2.1.2.2 Direct or Indirect Drying

There are many types of dryers because most are adapted to the material dried. They therefore are best classified according to the heating method they use; indirect or direct dryers.

In indirect dryers, the material to be dried is contacted with the hot surface in order to heat the material by conduction and or radiation (Keey, 1972). The heat is added from outside the system, thus making the system non-adiabatic. Because these dryers mainly use conduction for heating, they are not suitable for thermal-labile material. In order to be adapted for this type of material a vacuum is usually employed (Cook & DuMont, 1991). Because of the contact heating, the particles in contact with the heating surface are the primary recipients of the heat and in turn heat the others. This can be a slow process as heat conduction is low in most solids (Cook & DuMont, 1991). This effect can be lessened by spreading the fluid paste or slurry thinly on the surface. In indirect drying, the temperature of the material attains the temperature of the heating surface, thus the material can be damaged if the heat temperature is high.

In direct dryers, the material is contacted directly with the heating medium. The heating medium is a hot gas which normally is air. If perfect insulation is assumed, the dryer operates under adiabatic conditions as all the heat is provided internally. Because the external particle surface is exposed to the hot heating gas, the material is heated more uniformly than with indirect dryers. The solid surface temperature is usually at its adiabatic saturation temperature when there is still moisture in the solid material. This ensures against heat degradation of material because the temperature is low enough. Because this type of dryer depends on convective rather than conductive heat transfer, the drying time of the material is shortened and thus the residence time within the dryer is reduced.

Most natural (organic) products are damaged at elevated temperatures as the cell wall matrix and other cell components are degraded. Lemon peels are heat-sensitive as pectin degrades if

exposed to elevated temperatures for a long time. It thus seems appropriate that direct drying rather than indirect be employed to dehydrate the peel.

2.1.2.3 Batch-wise or continuous drying

Drying can be either batch-wise or continuous. In batch-wise drying, the feed material is inserted into the dryer for a set period of time and the product removed thereafter. In contrast, in continuous drying the feed material is continuously added into the dryer and the product continuously removed. Batch drying ensures the same residence time for the product while a continuous dryer normally has a distribution in the residence time, which results in a variability in the product quality.

The choice between the two can be narrowed down by the production rate. Van't Land (1991) states that a continuous, rather than batch-wise dryer, can be chosen if the capacity of the dryer exceeds 100kg/hr (approximately 876 tpa). He also shows that the choice is not as clear cut as it also depends on the equipment preceding and following the dryer in a plant.

This project was aimed at developing pectin technology for an initial scale of 15 tpa for pilot scale operation and ramping that up to 150-300 tpa production. The project was later envisaged to produce 1000-1500 tpa when ventures into international market are undertaken.

In the first two cases batch-wise operations can be undertaken, but for the industrial scale operation (1000-1500 tpa) a continuous dryer should be employed. The extraction process at industrial scale, which follows the drying phase, is also continuous and thus does not affect the continuity of the drying phase. A continuous process is also often favored as it requires less handling than a batch-wise operation (Keey, 1992). The drying phase can be made batch-wise however in the case that the drying is only done to avail the peels for off-season purposes. Since citrus fruits are not available for four months of the year, production rate required for the four months at industrial scale will be 333-500 tpa only. Since this stated produce can be run over the 8 months when the peels are in season batch-wise operation is preferable.

2.1.2.4 Reasons for drying materials

Drying is usually carried out to achieve a particular moisture content, in most cases not all the moisture is removed from the material. There are various reasons for drying materials, two of which are outlined below (Cook & DuMont, 1991):

- Preservation of the material: Bacteria thrive in moist places, thus if the moisture is removed the shelf-life is increased

- Reducing transportation and packaging costs: The decreased volume of dried material in turn decreases the amount of packaging material and reduces the volume of equipment used in transportation of the material

2.1.3 Choice of Dryer

It was decided that pilot plant tests would be used for this project, operated in a batch-wise mode and that direct drying be used (Refer to Section 2.1.2.2 and 2.1.2.3). Batch-wise because the production rate required at pilot plant scale was only 15 tpa and direct drying was used because the lemon peels are heat-labile. There are several other characteristics that have to be looked into in choosing the best dryer for a particular material.

Various authors have discussed the typical selection criterions for a dryer. Sazhin (1984) considers the scale of production, the form and properties of the material and the drying time as the basis of classification. Strumillo and Kundra (1986), consider the structure of the particle, thermal resistance, the grading of the material (either poly-dispersed or mono-dispersed) and whether the materials moisture can be classed as superficial or bound. Van't Land (1991) outlines a simpler method; a decision tree for both batch-wise and continuous drying. Refer to Fig 2.1 and Fig 2.2:

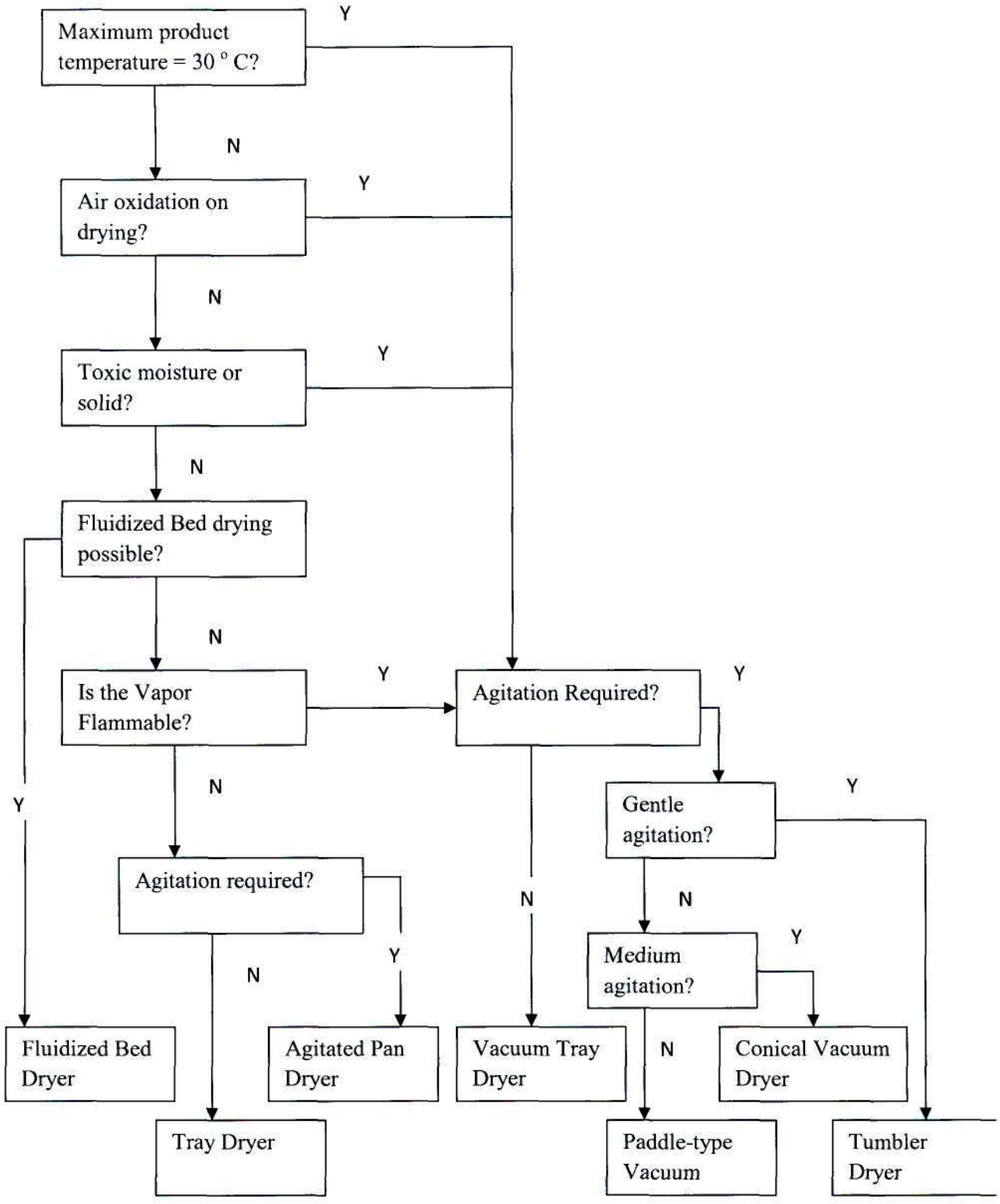

Fig 2.1: *Decision Tree for a batch-wise operated dryer (After Van't Land, 1992)*

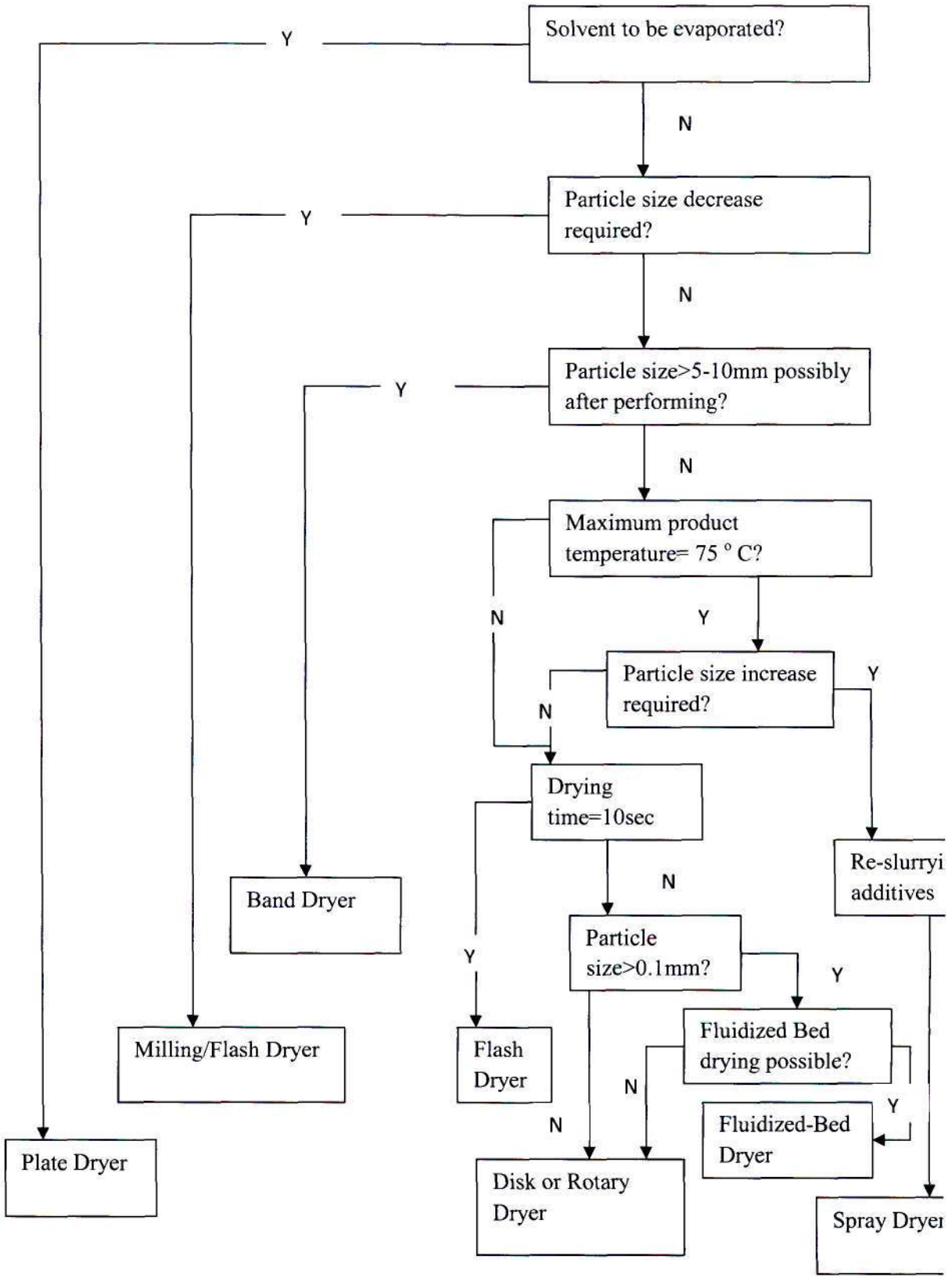

Fig 2.2: *Decision Tree for a continuous operated dryer (After Van't Land, 1992)*

The first decision tree (Fig 2.1) considers a batch-wise dryer, and from this tree it can be seen that if the maximum product temperature of less than 30 °C is not a required, then a vacuum is not required. If the product is oxidized during the drying period, then a vacuum dryer or an inert gas should be used. The use of a vacuum dryer can also be essential if the product or the vapor removed is toxic or flammable; the vapor can easily be drawn off and dust formation is avoided (Keey, 1992 and Van't Land, 1991). A fluidized bed dryer is always preferable because scale up of such a dryer is easy. Thus, if oxidation does not occur and neither the product nor the vapor released is toxic or flammable, it is normally the first choice dryer (Keey, 1992). If it is not possible to use a fluidized bed, then other dryers can be opted for.

The decision tree for a continuous dryer (Fig 2.2) considers mostly the product size, drying time and temperature. If the solvent within the material dried is evaporated and then recovered, a direct/conductive (tray dryer) dryer is the best option as its use reduces the size of the condenser needed (Van't Land, 1991). If there is a need to reduce the particle size of the product, then a flash dryer that incorporates a mill can be used. If the particle size of the feed material is less than 5-10 mm, but greater than 0.1mm, the drying time is greater than 10 seconds and the maximum product temperature is less than 75 ° C, then a fluidized bed dryer is the best option for a dryer. A flash dryer can be used if the required drying time is less than 10 seconds. If the particles size of the material dried is less than 0.1 mm, a convective or conductive dryer with a rotating shell or with agitation, such as a disk or rotary dryer, can be used. A spray dryer can be employed if an increase in particle size is required and re-slurrying with the aim of adding the required additives to the product is essential (Van't Land, 1991).

In this project, the product as well as the vapor emitted from the dryer was not toxic and no oxidation occurred during the drying. Therefore a batch-wise fluidized bed dryer was used to dry the lemon peels before the extraction of pectin. Because the project is a stepping stone to the design of an industrial dryer, from the decision tree by Van't Land (Fig 2.2), a continuous fluidized bed dryer will still be appropriate on an industrial scale to dry the lemon peels.

2.1.4 Fluidized Bed Drying

In this type of drying, hot air is passed through a bed of material supported on a grid. This support grid is simultaneously used as a gas distributor. As the gas (hot air) velocity is increased, there is a loosening of the bed material and frictional drag on the particles leads to an

increase in the pressure drop across the bed (Keey, 1992). As the velocity is further increased, incipient fluidization velocity is reached. At this velocity, the frictional drag is equal to the effective weight of the bed (Vanecek et al., 1966). Above the incipient fluidization velocity, the bed begins to expand until it reaches a state similar to that of a boiling liquid (Geankopolis, 1993).

The gas velocity, exit material moisture content, residence time of the material and throughput of the material determine the size of the dryer. The dryer usually incorporates several other units such as a cyclone, or any other filter medium, in order to trap particles entrained in the exit gas stream after drying.

The dryer can either be operated in a batch-wise mode or in a continuous mode. For small scale productions, a batch-wise dryer can be used, especially if the preceding operations are also batch-wise. Usually a continuous dryer is used for large scale production especially dryers operated on an industrial scale (Van't Land, 1991).

The material flow in a fluidized bed dryer can either be of a form approximating perfect mixing (as in a tank type dryer) or that approximating plug flow (as in a through type dryer) (Strumillo and Kudra, 1986). Perfect mixing flow can be approximated when the material to be dried is difficult to fluidize and the material is thus introduced into an active bed and is mixed with already dry and hot material. Intensive drying is used in this form of drying. Plug flow can be approximated when the material can be easily fluidized. Plug flow ascertains product particles of highly uniform moisture content unlike in perfect mixing where a wide range is attained. Multi-stage dryers can be used where both types of flows can be attained. If a material that is difficult to fluidize is considered, a tank type dryer can be used followed by a through type dryer to ascertain the uniformity of the exit product.

The main advantages of a fluidized bed dryer are outlined by Strumillo and Kudra (1986) and Keey (1992). They state that that a fluidized bed dryer is easy to service and maintain, even at an industrial scale. There are no moving parts and thus it is safe and easy to maintain. It offers good heat and mass transfer conditions and good mixing properties that ascertain uniformity of the material dried. There is also a possibility of using other heating sources such as radiators.

A fluidized bed drying is also characterized be relatively short drying times and large feed capacities (Keey, 1992). It is therefore one of the best methods of drying available. There are other drying methods that ensure extremely short exposure times of the material to heat, but all these are expensive on an industrial scale.

2.1.4.1 Drying Kinetics

The drying kinetics of a material show the change of the average moisture content and average temperature of the material dried with time (Strumillo and Kudra, 1986). They are essential in calculating and computing the amount of moisture evaporated, the drying time required for a particular material and energy consumption. They can be influenced by both the internal conditions of the material and the external conditions of drying. Internal conditions refer to the physico-chemical properties of the material in consideration. External conditions refer to heat and mass transfer between the material and its surroundings such as:

- Heating medium temperature
- Humidity
- Relative velocity of the heating medium
- Total pressure at which the dryer is operated

According to Strumillo and Kudra (1986), a drying process is best explained in diagrams of the following:

- Drying curve – moisture content of the material versus the drying time
- Drying rate curve- the drying rate versus the material moisture content
- Temperature curve- the material temperature versus the material moisture content

This data for constructing these diagrams are usually found from laboratory experiments by measuring the moisture content and drying temperature in time.

2.1.4.2 Laboratory experiments

In order to determine rate of drying of a material, the material to be dried is usually spread out on a tray such that only the top surface is exposed to the surrounding drying air stream (Geankopolis, 1993). The moisture drop of the material with time is then recorded. Great care must be taken to ensure that the temperature of the inlet air, its humidity and velocity remain constant throughout the experiment. The drying curve, which portrays the moisture content of the material in time, can then be graphed.

The moisture ratio of a sample at time (t), is given as

$$X = \frac{M_i - M_D}{M_D} = \frac{M_{H_2O}}{M_i - M_{H_2O}} \frac{\frac{M_{H_2O}}{M_i} * 100}{\frac{M_i - M_{H_2O}}{M_i} * 100} = \frac{\%\text{moisture}}{100 - \%\text{moisture}} \quad (\text{kgH}_2\text{O/kg dry solid})$$

.....(2.1)

Where M_i = initial mass of material (kg)

M_D = mass of bone material (kg dry solid)

M_{H_2O} = mass of the moisture within the material (kg H_2O)

% moisture = the percentage moisture content of the material

The drying rate is defined as follows:

$$\text{Drying rate} = -\frac{M_D}{A}\frac{dX}{dt} \quad (\text{kg H}_2\text{O/m}^2\text{hr}) \quad \text{..........} (2.2)$$

Where A = Area of the drying surface (m^2)

$\frac{dX}{dt}$ = the change in moisture content with time (kg H_2O/ (kg dry solid * hr))

From the drying curve, which is a plot of the moisture ratio versus the time, the gradients at particular time intervals can be computed. The gradients can then be plotted against the moisture ratio to yield the drying rate curve. From this curve, the critical moisture content can be computed, the constant rate and falling rate can be distinguished and the equilibrium moisture content determined. Typical drying and drying rate curves are given in Fig 2.3 and 2.4:

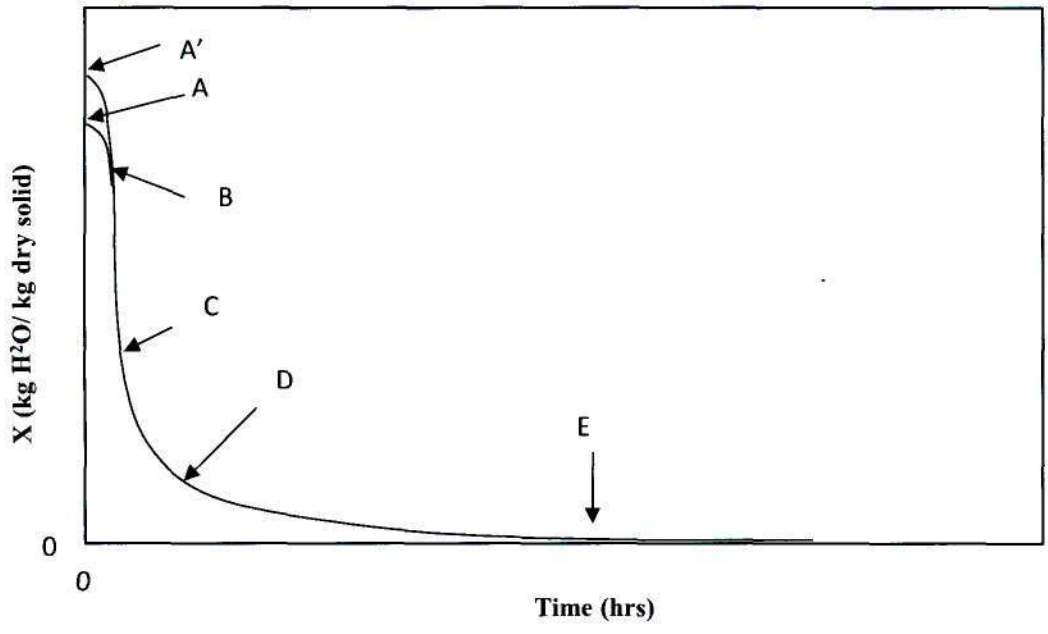

Fig 2.3: *Drying Curve*

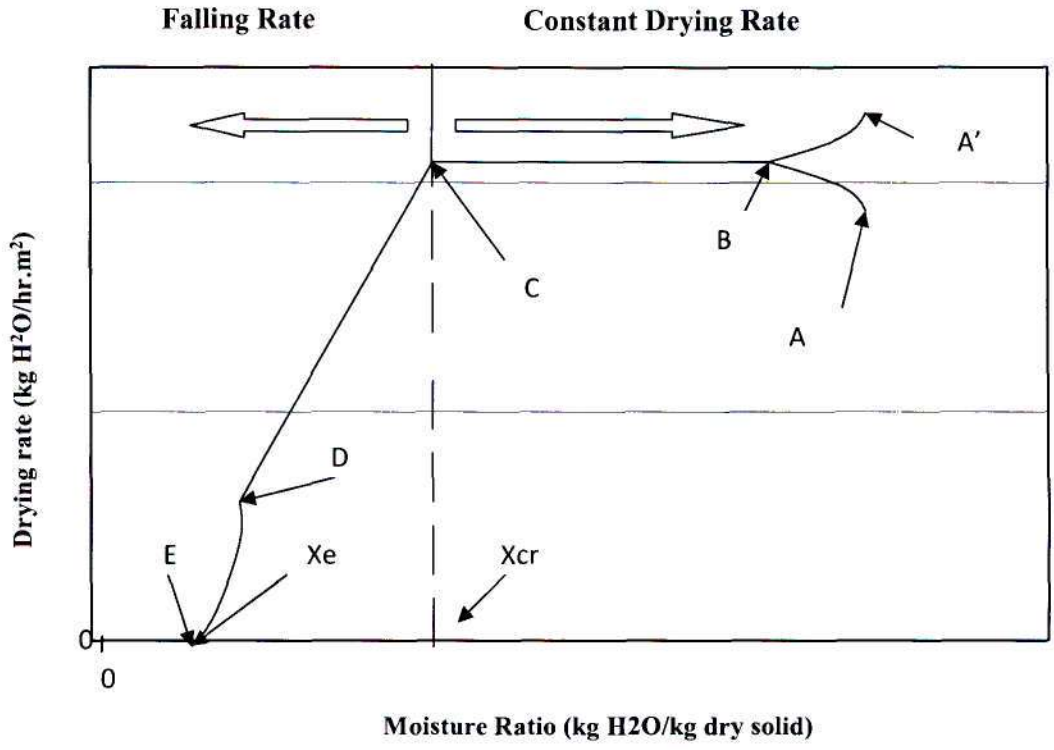

Fig 2.4: *Drying Rate Curve*

When the material enters the dryer it normally comes in at a lower temperature than the heating medium and this initially reduces evaporation from the solid surface. The temperature then increases to the equilibrium temperature and this is shown by the A to B curve on the graphs above. Alternatively, the material can enter the dryer at a higher temperature and thus initial evaporation rate is higher than the equilibrium rate, as depicted by the curve A' to B.

From point B to C, a constant drying rate period is attained. In this period unbound water is removed from the surface of the solid at the particles adiabatic saturation temperature. This period continues on as long as the rate the water moves to the surface of the particle is the same as the rate of evaporation from its surface.

Once there is imbalance in the two rates, point C on the curves, the drying rate decreases and the period that follows is known as the falling rate period. At point C, the corresponding moisture ratio is known as the critical moisture ratio; at this point the particle is no longer wetted.

The drying rate continues to fall from point C to D, the moisture within the particle is vaporized and the vapor makes its way to the surface of the solid. The discontinuity at point D occurs as the surface becomes completely dry and internal vaporization dominates.

In general, the two prominent regions are those from B to C and then to D. The other regions might take too short a time and the change may be undetectable. Relatively small amounts of moisture may be removed in the falling rate period, but in contrast the drying time may be long. In food dehydration, however, the constant rate period is usually short and the dominating period is the falling rate period.

2.2 Extraction

Pectin is industrially extracted with hot acidic water in a variety of extraction units such as stirred tanks or pots or counter-current extractors (Joye & Luzio, 2000). This is regarded as the conventional method of the extraction process. The quality of the pectin obtained is largely dependent on the extraction conditions and thus these have to be optimized.

Different experimental methods such as microwave extraction, pressure extraction, soxhlet extraction, flash extraction and auto-clave extraction have been investigated in other studies. The conventional method (hot acid extraction) is still preferred industrially as it liberates pectin of good quality while at the same time being economically viable (May, 1990). The use of different extraction media or solvents for pectin extraction has also been studied, but hot acidic water is still favoured above them all. A detailed discussion of the above concepts is outlined below.

2.2.1 Conventional Extraction Method

Traditionally, pectin has always been extracted at higher temperatures, 70 – 90 °C, using acidic water as the extraction medium (Stephen & Churms, 2006). There are five basic steps to the extraction of pectin:

- Exposure of the peel to hot acid
- Filtration to remove the peel from solution
- Precipitation of the pectin from solution
- Recovery of the extracted pectin
- Drying of the pectin to produce powder.

The details of each step are different for different pectin producers. The first step has to be optimized in terms of the extraction conditions to achieve good quality pectin. The removal of the peels from solution can be a tedious process, as the solution tends to become viscous while the peel becomes mushy with time. This can be overcome by increasing the amount of extraction solvent which will result in a less viscous solution. There is a compromise however, between efficient drying and operating costs (May, 1990). Industrially, rotary vacuum filtration is often used to handle the mushy peel-solvent solution (Joye & Luzio, 2000).

If liquid pectin is required, the filtrate is then clarified and stored. If however powdered pectin is required, the filtrate is exposed to an alcohol medium to precipitate out the pectin. The filtrate though, has to be concentrated prior to precipitation to ensure a high pectin concentration in the filtrate and in order to use less alcohol for the precipitation. The temperature has to be kept fairly low, and thus vacuum evaporation is employed. A 1:1 ratio (m/m) of filtrate and precipitating agent is needed to precipitate out all the pectin from solution. Iso-propanol (IPA), ethanol or methanol can be used, but IPA is preferred (May, 1990). The alcohol used can be recovered by distillation and this is often the determining factor of the profitability of the plant.

The pectin can also be precipitated out using an aluminium salt; aluminium chloride is usually used (Joye & Luzio, 2000). The advantage of using the salt is that there is no need to concentrate the filtrate and certain impurities are more easily removed. The pectin is removed by flotation or by screens as a greenish-yellow aluminium pectate precipitate (May, 1990). The

yellowish pectin extract is then exposed to an acid-alcohol mixture to remove the aluminium and is then neutralized. This precipitation method however, results in a lower pectin recovery. It is also not possible to precipitate out highly esterified pectin (HM pectin) with this method. Exposure to the acid-alcohol solution de-esterifies the pectin and if not optimized, can degrade the pectin. As a result, most pectin producers prefer alcohol precipitation over aluminium precipitation (May, 1990).

The precipitated pectin can then be isolated either centrifugally or by filtration. The isolated pectin is then dried under vacuum and then ground. To date, the same concept is still used predominantly for pectin extraction.

2.2.2 Other Extraction Methods

Y. Liu et al. (2006) investigated the effect of different extraction methods on the pectin yield and pectin properties. In the study, soxhlet extraction, microwave extraction and hand-pressure extraction were compared. Pressure extraction was seen to extract the peel oil together with pectin, thus introducing an impurity to the pectin extract. Microwave Assisted Extraction (MAE) was seen to work efficiently with high initial extraction rates of the pectin. With the elapse of time though, the pectinaceous material was degraded. Soxhlet extraction proved to be effective, but because it can only be run at water boiling temperatures, the pectin was also degraded. A combination of microwave and soxhlet extraction methods was seen to work better than the two methods apart.

Fishman et al. (2003) investigated the use of steam injection under pressure in the extraction of the pectin from orange albedo. They found a high pectin extraction rate. They also found that as the time of extraction increased, the molecular weight and intrinsic viscosity of the pectin decreased, resulting in a pectin of poor gelling properties. Thus if this method is used, the time of extraction needs to be stringently monitored.

Autoclave extraction was studied by Martinez-Sanchez et al. (2005). Pectin was extracted using a weak acid at high pressures in an autoclave. A pectin yield of 26.5% in 30 minutes was realized. This method reduces the corrosive and contaminant effect that conventional extraction

results in. The drawback though, is the increased equipment cost and maintenance required for high pressure vessels.

The advantage of using extraction methods with short extraction times (rapid extraction methods ~ 3-15 min) such as MAE and steam injection under pressure, over conventional extraction methods (30min to 5hrs extraction times), is that fewer hydrogen bonds within the pectin are broken, resulting in a pectin of high molar mass (MW) and viscosity. The short heating improves the plant matrix structure, thereby assisting in pectin release from the matrix (Fishman et al., 2000, 2001). Although these methods result in an increased extraction rate and pectin quality, their use in industry is still at the moment non-economical.

2.2.3 Extraction conditions

In any extraction process, the extraction conditions have to be monitored closely in order to attain the desired product. Some of these conditions, that are important in pectin extraction, are discussed hereafter.

2.2.3.1 Extraction medium / solvent type

There are different ways of extracting the pectin from the cell wall, and the pectin extracted is often named according to the way it has been extracted. Water-soluble pectins are extracted with cold/hot water or dilute salt solution. Chelator-soluble pectins are extracted with calcium chelating agent solutions such as EDTA, CDTA and hexametaphosphate. Protopectins are extracted with alkali solutions or hot dilute acids (Van Buren, 1991).

Bucher (1984) investigated the use of different solvents in the extraction of pectin. Among them were CDTA and EDTA. The results showed more galacturonan yield with the chelating agents and oxalatic acid than by milder hydrogen-bond breaking agents (Bucher, 1984).

Alkaline extraction is carried out by exposure of the pectin source to KOH or NaOH solutions. The result is a carboxylate salt, which is then acidified to release the pectinecious carboxylic acid, which in turn is precipitated out of solution. Chelating agents are disadvantageous in their

difficulty to remove residual chelatos. Alkaline extraction has the effect of decreasing the DE and the length of the main chain of GA by ß-elimination (Rambouts & Thibault, 1986), thus producing pectin of low MW and poor gelling qualities.

These disadvantages make the use of an acid medium the most attractive method to use for pectin extraction. According to Thibault and Rambouts (1986), the greatest yield of pectin is obtained by using a hot acid as the extraction medium. May (1990) also adds that this is the most suitable way of pectin production industrially. Moreover, although many solvents have been investigated for pectin extraction, water is still the most preferred solvent in the food industry because it is cheap, readily available, non-toxic and non-flammable (Liu et al., 2006)

Levigne et al. (2001) studied the effect of the type of acid used to acidify hot water on the resultant pectin characteristics. Two acids were compared; HCl and HNO_3. The results showed the type of acid used to be insignificant on the resultant pectin's chemical and physicochemical properties. On the other hand, Turakhozhaev et al. (1999) found that the type of acid used had an effect on the yield of the pectin extracted. They investigated the use of HCl, HNO_3 and H_3PO_4. HNO_3 had the least yield followed by HCl, while H_3PO_4 gave the greatest yield. A combination of HCl and H_3PO_4 however proved to be more effective than when the two were used individually.

2.2.3.2 Temperature

Masbehi et al. (2004) investigated the effect of temperature change on the pectin yield and content. Two temperatures were investigated; 80 °C and 90 °C. They reported that the higher the temperature, the higher the pectin yield obtained. Prolonged heating however hydrolysed and degraded the pectin, resulting in pectin of low molecular weight and DE. Pagan et al. (2001) investigated the effect of two temperatures, 60 °C and 80 °C, on the yield and pectin quality. The DE and viscosity were shown to decrease with increase in temperature.

2.2.3.3 Extraction time

Masbehi et al. (2004) investigated the effect of the extraction time (3 and 4 hrs) on sugar beet pectin yield and quality. They showed that the yield attained was not significantly greater after 4

hours than 3 hours, but the pectin degradation was more pronounced. They therefore chose the optimum time of extraction as 3 hrs. Previous work by Fishman and co-workers (1999 and 2006) investigated the use of microwave-assisted extraction (MAE) from lime pectin extraction. This work also showed the same trend; that time had a negative effect on the pectin quality. Pagan et al. (2001), varied the time of peach pectin extraction from 10 to 80 minutes at a 10 °C interval. The highest yield and quality of the pectin was attained after 60 minutes extraction time.

2.2.3.4 pH

At a lower pH value, the yield of the pectin was seen to increase in most extraction studies. The reason was explained by BeMiller (1986) as a result of the repression of hydrated carboxylate ions at higher hydrogen ion concentration experienced at the lower pH. A lose of charge then results as the carboxylate group is converted into a slightly hydrated carboxylic acid. The polysaccharide then becomes less repulsive, encouraging gel formation of the pectin, which then precipitates out of solution.

This increase in the yield however, is normally accompanied by the degradation of the pectin to low quality pectin. An example can be seen from Fishman et al. (2006), who showed that at a constant extraction time, the molecular weight and intrinsic viscosity decreased as the pH of the acidified water was decreased from 3 to 1. Pagan et al. (2001) also varied the extraction pH from 1.2 to 2.53 and noted the yield and the pectin quality. There was a decrease in the yield with increase in pH, but a general increase in DE with increase in pH.

2.2.3.5 Peel size

The effect of the peel size on the extraction response parameters has not been extensively investigated. Most investigations worked on a fixed peel size. Robert et al. (2006) investigated the effect of milling on the pectin yield and composition. In this study it was found that milled chicory roots yielded much more pectin of a higher quality than unmilled roots. Mira and Blasco (1996) in their experiments noted that the intra-particle diffusion resistance was significant in larger particle sizes (5-10mm) compared to the smaller sized peel. Thus in this current study, peels within the size range of 2-4mm were compared to those of less than 1mm size to establish if indeed the diffusion resistance was insignificant for peels of less than 5 mm.

2.2.3.6 Peel to water mass ratio

Liu et al. (2006) investigated the effect of peel to solvent mass ratio on the pectin yield. Only the peel albedo was used in their experiments. Three ratios were chosen for the investigation; 1:12.5, 1:25 and 1:50. All the other extraction parameters (extraction time, temperature, pH and procedure employed) were held constant. The results showed the highest yield to be obtained at 1:12.5 and a decreased trend in the yield from 1:12.5 to 1:50. A similar investigation using MAE on orange peel albedo was conducted by Fishman et al. (2000). In their work, 1:5 and 1:25 mass to volume (m/v) albedo to solvent (acidified water) ratios were investigated. Contrary to the previous study's results, Fishman's results showed an increase in the pectin extracted with increase in the ratio.

2.2.4 Analysis of pectin

There are several methods of analysis outlined by different authors used in pectin investigations. In this project two analytical qualities of the pectin are studied; the Galacturonic Acid content (GA) and the Degree of Esterification (DE).

2.2.4.1 Galacturonic Acid content

The amount of pectin in the extract can be quantified by the amount of galacturonic acid units present in the extract. IFJU Method 26 (1964/1996) states that the two principle methods used to quantify the GA content are precipitation methods and photometric methods. A detailed outline of the precipitation method is given by the Food Chemical Codex (1972), Ranganna (1986) and Walter (1991). The method entails titration of prepared pectin solutions with NaOH and back titration with HCl.

Garna et al. (2004), describe a method using m-hydroxybiphenyl, where the GA content is determined photometrically from a standard curve. Thibault (1979) and Ahmed & Labvitch (1977) also outline an automated m-hydroxybiphenyl method. The GA content can also be determined by high performance anion exchange (HPAEC). Garleb et al. (1991) modified it by using HPAEC with a pulsed amperometric detector (HPAEC- PAD) and this gave better results of the GA content. Yapo et al. (2005) used the HPAEC-PAD method and modified it after complete hydrolysis of the pectin as described by Garna et al. (2004).

2.2.4.2 Degree of Esterification

The DE can be found by similar titrimetric method used in determining the GA content (McCredy, 1970). High pressure liquid chromatography (HPLC) can also be used after alkaline de-esterification of the pectin using 0.2M NaOH for 2hrs at 4 °C to quantify the amount of methanol in the pectin. This method is also completely detailed by Voran et al. (1986). The DE is then found as a ratio of the methanol to the total galacturonic acid content.

2.2.5 Method Chosen for analysis

This project is an extension of the work already done by CSIR. CSIR used the titration method to quantify the galacturonic acid content (%GA) and degree of esterification (%DE) of the pectins in their experiments. In order to have comparative results, the same method was adopted for pectin analysis in this study. This method is further detailed under the Experimental Procedure section (Chapter 3). It entails several steps. First, the extracted pectin is acid washed with hydrochloric acid for a set time. The acid washed extract is then dried in an oven at 105 °C for 2½ hours. Approximately 0.5g of the acid washed pectin is weighed and mixed with an acid-alcohol mixture and phenolphthalein. This is then titrated against 0.1 N NaOH until a faint pink colour is observed, then the titer is noted. 20 ml of 0.5 N NaOH is then added to the solution followed by the same amount of 0.5 N HCl. The indicator is added yet again, and the solution is titrated against 0.1N NaOH until the faint pink colour. The second NaOH titer is also noted. The noted volumes of NaOH can then be used to compute the pectin's chemical properties (% GA and % DE).

2.3 Experimental Design and Analysis in pectin production

Experimental Design and Analysis of Data are concepts that are difficult to separate from each other, if the two are divorced from each other, it may be difficult for the researcher to reach sensible and legitimate conclusions from the resulting data (Chatfield, 1978).

2.3.1 Experimental Design and Analysis

In experimental design, it is often imperative to use statistics as a tool for collecting, analyzing and interpreting data (Montgomery, 2005). It is important to identify the objective of the experiment, the dependent and independent variables, otherwise known as the 'factor' and the 'response' respectively, and the best design method that will result in substantial and consistent data analysis and interpretation that are in line with the objective.

Factors can be quantitative or qualitative. A quantitative factor is a variable that can be measured and whose values can be arranged in the order of magnitude. A qualitative factor on the other hand is a variable with values that cannot be arranged in the order of magnitude (Chatfield, 1978). An experimenter may be interested in investigating the effect of a combination of different values of different factors. The different values of a factor are referred to as 'levels' and the particular combination of different factor levels a 'treatment'. An 'effect' of a factor is the change of the response that results from the change in the factor level (Montgomery, 2001). 'Interactive effects' and 'main effects' can be computed. Main effects result from a change in the level of one particular factor while interactive effects result from the variation of levels of two or more factors at a time.

It is vital that the results obtained are as precise as possible and that systematic errors are avoided in an experiment. To combat this problem, techniques such as randomization, replication, blocking and analysis of covariance are used (Caulcutt, 1991 and Montgomery, 2001). It is not always possible to perform all of them due to experimental constraints, thus it is important to examine the design and chose which are possible to incorporate. Randomization and replication are the most common (Chatfield, 1978) and thus are discussed next.

2.3.1.1 Randomization

As a good experimentation practice, randomization is almost always employed. Montgomery (2001) states that randomization is a technique employed by experimenters to ensure against systematic errors in the investigation.

Ideally an experiment should be free of nuisance factors which may result in trends in the response variable that are not solely resultants of the investigated factors (Lipson & Sheth, 1973 and Chatfield, 1978). Nuisance factors are factors that are uncontrolled and lead to variations in the experiment that cannot be accounted for.

Nuisance factors can be functions of time, place and experimental units, thus this variables have to be controlled in order to cut out the resulting response noise. If tests are performed on different experimental units, then the units should be chosen at random, if the experiments are performed successively in time then the different runs should be chosen at random. In some cases it is imperative that the experimental place should be the same for all the runs, so that differences in e.g. climatic conditions do not result in errors in the response (Chatfield. 1978)

2.3.1.2 Replication

This refers to the numbers of observations made on a particular treatment. Montgomery (2001) states that a replica is an independent repeat of each factor combination. Replication makes it possible to estimate the size of error in the experiment and thus the calculation of the experimental precision. The entire experimental treatments, including the replica, should be randomized in order to determine a non-biased error.

2.3.2 Factorial design

There are different designs that can be used to investigate different objectives in an experiment. "A factorial design, sometimes referred to as a 'complete factorial design', is conducted if the researcher is interested in investigating how the response variable is affected by the changes in the different factors and the combination of the different factor levels that result in the maximum or minimum of the response variable" (Chatfield, 1978). In general, a factorial design can be used in the investigation of optimum conditions of a particular process.

In a complete factorial design, if factor **A** has **a** levels and factor **B** has **b** levels, then the number of runs necessary for the investigation is **a** × **b**. If for precision the experiment is conducted with **n** replicas, that is, **n** observations at each combination of factor levels, then the number of runs will be **a** ×**b** × **n**.

2.3.3 Factorial design versus One-at-a-time design

One of the most common designs is a 'one-at-a-time' experiment. In this design the researcher keeps the other factors constant while varying just one of the factors at a time and monitors the change in the response variable (Caulcutt, 1991). The factorial design is different in that all the possible combinations of the factor levels are investigated. The factorial design is therefore more powerful in that it not only investigates the effects of the individual factors (main effects) but also the effect of all the possible interactions of the different factor levels (interactive effects). In the case where interactions are not of vital importance, then a one-at-a-time design would be economical as fewer experiments are conducted, but if interaction of the factors may be of importance, then a factorial design would be the most preferred as it requires fewer experiments (Montgomery, 2001). If interactions are of importance, the optimum conditions of the experiment may be overlooked by the one-at-a-time design.

Montgomery (2001) states three advantages of a factorial experiment over a one-at-a-time experiment. The three advantages of a factorial experiment are listed next.

- More efficient: fewer number of experiments are required to investigate both main effects and interactive effects
- Interactions can be determined easily
- It allows the effect of a single factor to be evaluated at different levels of other factors, leading to valid conclusions over a range of experimental conditions

2.3.4 2^k Factorial Design

If two levels, a high (+) and a low (-), of a particular factor, with a total of **k** factors are investigated, then the number of experimental runs would be 2^k. This type of experiment is therefore referred to as a 2^k factorial design. It is usually conducted if a lot of variables are to be investigated and a time constraint is imposed on the duration of the experiment (Lipson & Sheth, 1973 and Chatfield 1978). It thus limits the number of experiments that have to be conducted if all the factor levels were to be investigated.

The two levels of each factor have to be carefully selected, since only a linear estimate of the effects can be investigated with the variation of two points. If the two are close to optimum conditions then this region can be sufficient explained by linear estimates as the response stays fairly constant within this region (Montgomery, 2001). Previous knowledge of the process is thus imperative in such a design.

2.3.5 Analysis of Variance (ANOVA) in experimental design

This is a statistical technique that is used to determine and analyse the extent of the variability in an experiment (Montgomery, 2001). For simplicity, an experiment with only one factor under investigation will be considered first and the resultant conclusions will be extrapolated to include cases where many factors are investigated.

In a single factor experiment, where **a** levels of the factor **A** are under investigation (**a** treatments are conducted) and the experiment is replicated **n** times, the total sample variability (or total sum of squares of deviations) can be expressed as:

$$SS_{TOTAL} = \sum_{i=1}^{a}\sum_{j=1}^{n}(y_{ij} - \bar{y}_{grand})^2 \quad \ldots\ldots (2.3)$$

Where $\bar{y}_{grand}$ is the grand average, which is the average of the sum of the total responses at all observations.

It can be shown mathematically that Equation 2.3 of the total sum of squares (SS_{TOTAL}) can be expressed as:

$$SS_{TOTAL} = \sum_{i=1}^{a}\sum_{j=1}^{n}(y_{ij} - \bar{y}_{grand})^2 = n\sum_{i}^{a}(\bar{y}_i - \bar{y}_{grand})^2 + \sum_{i=1}^{a}\sum_{j=1}^{n}(y_{ij} - \bar{y}_i)^2$$

....(2.4)

Equation 2.4, shows the total sum of squares to be a partition of the sum of squares of the differences 'between' the average observation at each level of factor **A** and the grand average, and that of the differences 'within' observations at each level of factor **A** and the average observation at each level (Montgomery, 2005). Simply put, the total sum of squares can be expressed as the deviation '**between**' samples sum of squares plus the deviation **'within'** samples sum of squares (Caulcutt, 1991). The deviation 'within', can only be attributed to random error. Thus the total sum of squares can be expressed as:

$$SS_{TOTAL} = SS_{Treatments} + SS_{ERROR} \quad \text{.....(2.5)}$$

In the single factor ANOVA illustrated above, **a** treatments are conducted. In a case where two factors, **A** and **B**, are investigated at different levels, where the total number of levels per factor is **a** and **b** respectively, then **a*b** treatments are conducted. When three factors, **A**, **B** and **C**, are studied at different levels, where the total number of levels for each factor is **a**, **b** and **c** respectively, then **a*b*c** treatments are conducted. The same is true for any number of factors. In the case where many factors are investigated Equation 2.5 can be written as:

$$SS_{TOTAL} = SS_{Model} + SS_{ERROR} \quad \text{.....(2.6)}$$

SS_{Model} in Equation 2.6 represents all the sum of squares resulting from all the different 'effects'. In the case where two factors, A and B, are investigated then:

$$SS_{Model} = SS_A + SS_B + SS_{AB} \quad \text{.....(2.7)}$$

The variability that identifies the ANOVA is obtained by two estimates, as shown previously; that of the sample variance 'within' treatments (SS_{ERROR}) and 'between' treatments ($SS_{Treatments}$). It can thus be concluded that if the treatment means are the same, the two estimates will be the same. If the two variances are different, it is assumed and can be proved mathematically, that the difference is due to the differences in treatment means (Montgomery, 2001).

The variance of the experiment, s^2, is more appropriately expressed as the mean square instead of the sample square. For an experiment studying the effect of factor **A** on the response, where factor **A** is investigated at **a** levels and replicated **n** times, the mean squares of the variability within and between treatments is given as:

$$MS_{ERROR} = \frac{SS_{ERROR}}{N - a} \quad \text{and} \quad MS_{Treatments} = \frac{SS_{Treatments}}{a - 1} \text{........} (2.8)$$

Where N is the total number of observations.

In the case where the treatment means are equal, the mean square error (MS_{ERROR}) is assumed to give an estimate of the variance (s^2) of the experiment and is similar to the mean square of the treatments ($MS_{Treatments}$).Thus a hypothesis that there is no difference in the treatment means can be tested by a comparison of the MS_{ERROR} and $MS_{Treatments}$. This is one form of statistical testing of experimental data. If more factors are investigated, then $MS_{Treatments}$ becomes MS_{Model} which is described by the following Equation:

$$MS_{Model} = \frac{SS_{Model}}{DOF_{Model}} \text{..} (2.9)$$

DOF_{Model} in Equation 2.9 is the number of degrees of freedom of all treatment combinations of all the factors. In the case where two factors, **A** and **B**, are investigated, at **a** and **b** levels respectively, the DOF will be:

$DOF_{Model} = (a * b) - 1$ .....(2.10)

2.3.6 ANOVA for a 2^k Factorial Design

In an ANOVA, the variation of a factor has an effect on the response. The effect of a certain variable or variable combination, say **A** and the combination of **A** and **B** is termed Effect A and Effect AB, respectively. The different 'effects' can be measured in an experiment and their computation is shown later on.

In an experiment with two factors A and B, let the 'effect' of **A** at a high level, be denoted by **a**, and the 'effect' of **B** at a high level, by **b**. If in a 'treatment' either 'effect' **A** or **B** is at its low level, then this is shown by the exclusion of **a** or **b** in this 'treatment' representation. If both 'effects' are at their low level, the treatment is denoted by **(1)**. A 2^2 treatment combination table of the above example is as follows:

Table 2.1: *A 2^2 Treatment combination*

	A_{low}	A_{high}
B_{low}	(1)	a
B_{high}	b	ab

In each 'treatment', the 'effect' of a factor can be evaluated as follows:

$$\text{Effect A} = \frac{1}{2^k * n} * [(a-1)(b+1)] = \frac{1}{2^k * n} * [\text{Contrast A}] \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.11)$$

$$\text{Effect B} = \frac{1}{2^k * n} * [(a+1)(b-1)] = \frac{1}{2^k * n} * [\text{Contrast B}] \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.12)$$

The 'effect' of the combination of the factors **A** and **B** (**AB**) can be evaluated from:

$$\text{Effect AB} = \frac{1}{2^k * n} * [(a-1)(b-1)] = \frac{1}{2^k * n} * [\text{Contrast AB}] \ldots\ldots\ldots\ldots\ldots\ldots (2.13)$$

The different 'contrasts' of the above 'effects' can be shown to be

$$\text{Contrast A} = (a-1)(b+1) = ab + a - b - 1 \rightarrow ab + a - b - (1) \ldots\ldots\ldots\ldots (2.14)$$

$$\text{Contrast B} = (a+1)(b-1) = ab - a + b - 1 \rightarrow ab - a + b - (1) \ldots\ldots\ldots\ldots (2.15)$$

$$\text{Contrast AB} = (a-1)(b-1) = ab - a - b + 1 \rightarrow ab - a - b + (1) \ldots\ldots\ldots\ldots (2.16)$$

From the above Equations 2.3.14 to 2.3.16, it can be seen that if a variable (or factor) is included in the 'contrast name', as in contrast **A**, then in order to calculate this contrast, **-1** is added to the factor in question, as in (a **-1**). The opposite is true for a factor not included in the 'contrast name' (say **B** in the case where contrast **A** is calculated); then **+1** is added to this factor, as in (b + **1**). Put together, contrast A becomes (a – 1)* (b+1). The ±1 that results from the multiplication of the brackets (Refer to Equations 2.3.14 to 2.3.16), is replaced by ±(1), where (1) represents the 'treatment' where both factor **A** and **B** are at their low levels.

This can be expanded to any 2^k factorial design. In the case of a 2^3 factorial design, with factors **A**, **B**, **C**, then the following would be true:

$$\text{Contrast A} = (a-1)(b+1)(c+1) \rightarrow abc + ab + ac - bc + a - b - c - (1) \ldots\ldots\ldots (2.17)$$

$$\text{Contrast AB} = (a-1)(b-1)(c+1) \rightarrow abc + ab - ac - bc - a - b + c + (1) \ldots\ldots\ldots (2.18)$$

The contrasts of **B**, **C**, **AC**, **BC** and **ABC** can be found by following the same procedure in order to compute the corresponding factor 'effects'.

In a 2^k factorial design, because it exibits orthogonal properties, the sum of squares of each 'effect' are easily calculated from the following Equation:

$$SS(I) = \frac{(\text{Contrast }(I))^2}{2^k * n} \quad \dots\dots (2.19)$$

Where - SS (I) is the sum of squares of an arbitrary 'effect' of factor I

- Contrast (I) is the contrast of factor I
- **n** is the number of replicas
- **k** is the number of factors investigated

For a 2^k factorial design, there is a way of determining the 'treatment' combinations required in the calculation of the different 'effects', this is known as the Yates Algorithm.

2.3.6.1 Yates' Algorithm

Frank Yates, as cited by Montgomery (2005), proposed a method for performing and an analysis of variance (ANOVA) on a factorial design. In this technique, the order of the treatment combinations proposed by Yates is always maintained (Chatfield, 1978). The combination which begins is always (1) and the rest follow. Table 2.3.2 shows examples of 2^k factorial designs and the corresponding Yates Order/ Algorithm of the *treatment* combinations:

Table 2.2: *The order of combinations in Yates' Algorithm for 2^2 to 2^5 factorial experiments*

Factorial design	**Factors**	**Yates Order/Algorithm of treatment combinations**
2^2	A, B	(1), a, b, ab
2^3	A, B, C	(1), a, b, ab, c, ac, bc, abc
2^4	A, B, C, D	(1), a, b, ab, c, ac, bc, abc, d, ad, bd, abd, cd, acd, bcd, abcd
2^5	A, B, C, D, E	(1), a, b, ab, c, ac, bc, abc, d, ad, bd, abd, cd, acd, bcd, abcd, e, ae, be, abe, ce, ace, bce, abce, de, ade, bde, abde, cde, acde, bcde, abcde

The signs of each treatment combination in the Yates Algorithm used in the calculation of factor 'effects' for a 2^k factorial design can be shown to exhibit orthogonal characteristics. A table of the signs or coefficients for each treatment combination can be easily found from constructing an orthogonal table using the Yates order for a specific 2^k factorial design. In the case of a 2^2 factorial design the orthogonal table would be as follows:

Table 2.3: *Treatment combination coefficients used in the calculation of contrasts for a 2^2 factorial design*

	Factor Effects			
Treatment Combination	**I**	**A**	**B**	**AB**
(1)	+	-	-	+
A	+	+	-	-
B	+	-	+	-
Ab	+	+	+	+

Column **I** is found by multiplying all the other signs in the other columns of the Table 2.3 and is an identity column; which proves the orthogonal character of the treatment combinations.

2.3.6.2 Statistical Analysis

The response variable at each 'treatment' combination can be estimated from the following equation:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad \text{.....(2.20)}$$

Where - the subscript 'i' represents the different treatment levels and 'j' the number of replicas

- μ is the overall mean of all treatments given previously as $\bar{y}_{grand}$
- τ is a parameter unique to the *i*th treatment
- ε is the experimental random error (estimated by SS_{ERROR})

Equation 2.20 is called the Effects Model. It shows that the deviation of the response from the mean is due to the difference in the treatment combination and the random error particular to the treatment. If however the experiment is properly randomized, it can be assumed that the error term is normally and independently distributed with a zero mean and a variance σ^2. If this is the case, then the response variable y_{ij} is normally distributed with mean $\mu + \tau_i$ and variance σ^2 (Montgomery, 2005). The total sum of squares (SS_{TOTAL}) can also be shown to be normally distributed. Thus the SS_{TOTAL} divided by the total variance (σ^2) can be shown to be chi-square distributed with N - 1 Degrees of freedom (DOF_{TOTAL}), where N is the total number of experimental runs.

The null hypothesis (H_o), that the treatment means are the same, or rather that τ_i of all treatments is zero can thus be looked into; this involves a comparison of MS_{ERROR} and $MS_{Treatments}$. The null hypothesis and the two mean squares are given by the following equations for a total of **a** treatment combinations:

$$H_o = \mu_1 = \mu_2 = ... = \mu_a \quad \text{or} \quad H_o = \tau_1 = \tau_2 = ... = \tau_a = 0 \qquad \text{.....(2.21)}$$

$$MS_{ERROR} = \frac{SS_{ERROR}}{DOF_{ERROR}} \qquad \text{.....(2.22)}$$

$$MS_{Treatments} = \frac{SS_{Treatments}}{DOF_{Treatments}} \qquad \text{.....(2.23)}$$

Because SS_{TOTAL} can be shown to be distributed as chi-square, the F-test can be used to check the null hypothesis (H_o) that the treatment means are equal. This is given by:

$$F_0 = \frac{MS_{Treatments}}{MS_{ERROR}} \qquad \text{.....(2.24)}$$

If the null hypothesis is incorrect, the $MS_{Treatments}$ is greater than σ^2. Thus H_0 should be rejected if:

$$F_0 > F_{\alpha, DOF_{Treatments}, DOF_{ERROR}} \qquad \text{.....(2.25)}$$

In a 2^k factorial design, the statistical analysis used in ANOVA can best be summed up in a tabular format. For a 2^2 factorial design, of two factors A and B, the table would look as follows:

Table 2.4: *An example of a 2^2 factorial design ANOVA table*

Source of the variation (Effects)	**Sum of Squares (SS)**	**Degrees Of Freedom (DOF)**	**Mean Square (MS)**	**F_0**
A	SS(A)	a-1 = 1	$\frac{SS(A)}{DOF_A}$	$\frac{MS(A)}{MS_{ERROR}}$
B	SS(B)	b-1 = 1	$\frac{SS(B)}{DOF_B}$	$\frac{MS(B)}{MS_{ERROR}}$
AB	SS(AB)	(a-1)(b-1) = 1	$\frac{SS(AB)}{DOF_{AB}}$	$\frac{MS(AB)}{MS_{ERROR}}$
Model	SS_{Model}	$DOF_A * DOF_B * DOF_{AB}$	$\frac{SS_{Model}}{DOF_{Model}}$	$\frac{MS_{Model}}{MS_{ERROR}}$
Error	SS_{ERROR}	$2^k(n-1)$	$\frac{SS_{ERROR}}{DOF_{ERROR}}$	
Total	SS_{TOTAL}	$n\,2^k - 1$	$\frac{SS_{TOTAL}}{DOF_{TOTAL}}$	

The F value ($F_{\alpha,DOF_{Treatments},DOF_{ERROR}}$) can be found from statistical F-distribution tables at a confidence interval of 1-a. If the null hypothesis is rejected at the specified confidence interval for a specific 'effect', then this 'effect' is significant in determining the value of the response variable.

The above information of the 'significant effects' is vital in experimental modeling. Only the 'significant effects' are used in the final experimental empirical model. The 'effects' which satisfy the null hypothesis do not play an important role in determining the resultant response variable. This information can also be tested graphically by plotting a **normal probability plot** of the 'effects'. Those 'effects' that deviate from normality are said to be 'significant'. The normal probability plot is constructed by first arranging the calculated 'effects' in an increasing order. Then the normal probability of a particular 'effect' is calculated by:

$$\text{Normal Probability} = P_j * 100 \quad \ldots \ldots \ldots (2.26)$$

Where $P_j = \frac{j - 0.5}{N}$

j is the number of the 'effect' after arranging all the 'effects' in increasing order

N is the total number of all the calculated 'effects'

The normal probability is then plotted against the calculated 'effect' estimates. The 'effect' (main effect or interactive effect) that is seen to be an outlier when a straight line is fitted to the plot is considered to deviate from normality and thus is significant in changing the response variable.

2.3.7 Empirical Modeling

In a factorial design, the experimental results can be expressed in terms of an empirical model. There are three main models that are used in experimental analysis:

- Means Model
- Effects Model
- Regression Model

In a 2^k factorial design, Montgomery (2001) states that the experimental results are best described in terms of the regression model, although the means model and effects model can also still be used. The regression model can either be linear or quadratic in most cases. Higher order models can be computed, but these usually become too complex to provide practical result interpretation. A linear regression model is shown in the following empirical equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=2}^{k} \beta_{ij} x_i x_j \quad \ldots \ldots \ldots (2.27)$$

Also expressed as:

$$y = \beta_0 + \beta_i x_i + \beta_j x_j + \cdots + \beta_k x_k \quad \ldots\ldots\ldots (2.28)$$

Where - y is the measured variable or response variable

- x_i , $x_j \ldots x_k$ stands for the 'coded' experimental factors (Refer to Equation 2.30)
- ß's are called regression coefficients or regressors

In the ANOVA procedure, the regression coefficients can be determined by the following Equation 2.29:

$$\beta_i = \frac{\text{Effect (I)}}{2} \quad \ldots\ldots\ldots (2.29)$$

Where - effect (I)'represents any calculated main effect or interactive effect (Refer to Equation 2.11-2.13)

$ß_o$, unlike all the other regressors, is just the average of all observed response values, previously referred to as $\bar{y}_{grand}$ in section 2.3.5.

The 'coded' variables included in the model above are calculated from 'natural' variables.

Let **x'** represent the 'natural' variable in the units measured, then the corresponding 'coded' variable (**x**) is expressed as:

$$x = \frac{x' - \left(\frac{x'_{low} + x'_{high}}{2}\right)}{\left(\frac{x'_{high} - x'_{low}}{2}\right)} \quad \ldots\ldots\ldots (2.30)$$

Where - x'_{low} is the value of factor x at its low level and x'_{high} is the value of factor x at its high level

Equation 2.30 transforms the 'natural' variable into a 'coded' variable. With the coded variables, the low level of a factor becomes -1 and the high level of a factor becomes +1.

For a 2^2 experiment (with two variables A and B also represented as factor x_1 and x_2), Equation 2.28 becomes:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad \ldots\ldots (2.31)$$

For quadratic modeling, Equation 2.27 becomes:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 \quad \ldots\ldots (2.32)$$

For a 2^2 experiment, Equation 2.32 then becomes:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \quad \ldots\ldots (2.33)$$

2.3.7.1 Linear or Quadratic Modeling

A linear representation of the experimental data is often preferred to any other higher order representation, as the former enables ease of data interpretation. If a quadratic model can best describe the data, however, it can be used. It is therefore often essential to determine if the experimental data can be sufficiently defined by a linear or quadratic model.

This is done by running several experiments, usually between 3-5, at the central point of all variable levels; that is, half way between the high level and the low level. Because the high level of a factor in the 'coded' variable transform is +1 and that of the low level is -1, then the center point is coded 0. This is called centre point analysis.

The average response of just the center points ($\bar{y}_c$) and the average response of just the factorial runs ($\bar{y}_F$), referred to in section 2.3.5 as $\bar{y}_{grand}$, are then computed. The pure quadratic sum of squares ($SS_{Pure\ quadratic}$) is then calculated as follows:

$$SS_{Pure\ quadratic} = \frac{n_F n_c (\bar{y}_F - \bar{y}_c)^2}{n_F + n_c} \quad \ldots\ldots (2.3.30)$$

Where – n_F is the number of factorial runs and n_c the number of centre point runs

The Degrees of freedom for the pure quadratic term is one. Therefore, the mean square of the pure quadratic term can be calculated and the resultant F_0 computed. A comparison of this F_0 with that found from statistical F-distribution tables will then determine the significance of the quadratic term. If insignificant, then the linear model is assumed correct. If significant, then another set of experiments are conducted to compute the quadratic model regression coefficients. The central composite design (CCD) is one of the most useful designs that are commonly used to determine these coefficients.

2.3.7.2 Central Composite Design (CCD)

In order to develop the factorial design first order model into a second order model, a central composite design can be developed. This design measures the centre points of the design and the star points. Centre points are experiments conducted at the 'coded' variable level of zero for all investigated variables. Star points are conducted at zero level (0 coded variable) for all factors save for one, which is measured at its high and then its low level (applicable for FCCD).

There are two common types of CCD's, the spherical CCD (SCCD) and the face-centered CCD (FCCD). With the SCCD, the plotted area of the investigated domain (in 'coded' variables) on a 3-D surface, can be represented by a sphere, while with the FCCD the investigated area can be represented by a cube. This means that with FCCD, the coordinates of the star points can never be less than -1 (low level of the investigated factor) or greater than +1 (high level of the investigated factors) while with SCCD they are always less or greater -1 and +1 respectively. The FCCD is usually common in investigations as it requires fewer measurements at the center point to give accurate results (Montgomery, 2005).

2.3.7.3 Statistical Analysis in Regression Modeling

With a regression model, the null hypothesis is tested by:

$$H_0 = \beta_1 = \beta_2 = \ldots = \beta_k \quad (2.31)$$

The test for the null hypothesis still continues as described in Equations 2.24 and 2.25. The model correlation coefficient (R^2) can then be used to determine if the model best represents the experimental data, or as Montgomery (2001) puts it, to "explain the total variability of the experiment explained by the model". The model correlation coefficient is defined by the following Equation:

$$R^2 = \frac{SS_{Model}}{SS_{TOTAL}} \quad (2.32)$$

The adjusted model correlation coefficient (R_{adj}^2) is however more preferred; this is because the normal correlation coefficient (R^2) has a tendency of increasing with the number of factors investigated even if the 'effects' of the factors are shown to be insignificant. The adjusted correlation coefficient is described by the following Equation:

$$R_{adj}^2 = \frac{\left(\frac{SS_{Model}}{DOF_{Model}}\right)}{\left(\frac{SS_{TOTAL}}{DOF_{TOTAL}}\right)} \qquad (2.33)$$

2.3.7.4 Model Adequacy Checking

The ANOVA procedure is only adequate if the assumptions made in order to test the null hypothesis that the treatment means are equal are valid for the experiments conducted. Firstly, it is assumed that the experimental errors are normally and independently distributed, with zero mean and a constant variance s^2. Secondly, it is assumed that the effects model adequately explains the variance of the response (Montgomery, 2001). One way to check if these assumptions are not violated is to examine the residuals.

Residuals are differences between the observed response and the corresponding predicted response. To ensure model adequacy, graphical analysis of the residuals is undertaken. A normal probability plot of the residuals is one of the most common and useful graphical procedures used. If the residuals are normally distributed, then the plot resembles a straight line. Another useful plot is that of the residuals versus the fitted or predicted values. This plot should be 'structure-less'.

2.3.7.5 Matrix notation in statistical analysis

Apart from using the ANOVA, the method of least squares can be used in determining the model regression coefficients (ß's) and thus the predicted value of the response. In the discussion that follows, in order to distinguish the observed response value from the predicted value, y is used to represent the observed value and $\hat{y}$ the predicted value.

The regression model can be written in terms of the **observed** response as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon} \qquad (2.34)$$

$$\mathbf{y} = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_k \end{bmatrix}, \mathbf{X} = \begin{bmatrix} 1 & x_{11} & \dots & x_{1k} \\ 1 & x_{21} & \dots & x_{2k} \\ \vdots & \vdots & \vdots & \vdots \\ 1 & x_{n1} & \dots & x_{nk} \end{bmatrix}, \boldsymbol{\beta} = \begin{bmatrix} \beta_0 \\ \beta_1 \\ \vdots \\ \beta_k \end{bmatrix} \text{ and } \boldsymbol{\varepsilon} = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix}$$

Where - y_i is the response variable at the *i*th observation

- x_{ij} the level of factor x_j at the *i*th observation

- $ß_j$ the regression coefficient corresponding to factor x_j

- e_i the random error at the *i*th observation

Least squares estimators are then found. These are values that minimize the square of the error. If L is defined as the square of the error as follows:

$$L = \sum_{i=1}^{n} \varepsilon_i^2 = \boldsymbol{\varepsilon}'\boldsymbol{\varepsilon} = (\mathbf{y} - \mathbf{X}\boldsymbol{\beta})'(\mathbf{y} - \mathbf{X}\boldsymbol{\beta}) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2.35)$$

L can then be shown to be:

$$L = \mathbf{y'y} - \boldsymbol{\beta}'\mathbf{X}'\mathbf{y} - \mathbf{y}'\mathbf{X}\boldsymbol{\beta} + \boldsymbol{\beta}'\mathbf{X}'\mathbf{X}\boldsymbol{\beta} = \mathbf{y'y} - 2\boldsymbol{\beta}'\mathbf{X}'\mathbf{y} + \boldsymbol{\beta}'\mathbf{X}'\mathbf{X}\boldsymbol{\beta} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2.36)$$

The least squares must satisfy the following Equation in order to minimize the squared error:

$$\left|\frac{dL}{d\boldsymbol{\beta}}\right|_{\hat{\boldsymbol{\beta}}} = -2\mathbf{X}'\mathbf{y} + 2\mathbf{X}'\mathbf{X}\hat{\boldsymbol{\beta}} = \mathbf{0} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2.37)$$

Where $\hat{\boldsymbol{\beta}}$ is the least squares estimate of the regression coefficients (ß)

Equation 2.37 then simplifies to:

$$\mathbf{X'X\hat{\beta} = X'y} \quad \ldots\ldots (2.38)$$

The estimated least squares regression coefficients ($\hat{\boldsymbol{\beta}}$) can then be calculated as follows:

$$\mathbf{\hat{\beta} = (X'X)^{-1}X'y} \quad \ldots\ldots (2.39)$$

The **fitted** regression model used to predict the response variable thus becomes:

$$\mathbf{\hat{y} = X\hat{\beta}} \quad \ldots\ldots (2.40)$$

The residual error (**e**), which is the difference between the observed and predicted values of the response variable, can thus be calculated from:

$$\mathbf{e = y - \hat{y} = y - X\hat{\beta}} \quad \ldots\ldots (2.41)$$

From the calculated residuals (Equation 2.41), the error sum of squares can then be computed:

$$SS_{ERROR} = \sum_{i=1}^{n} e_i^2 = \mathbf{e'e} = \mathbf{(y - X\hat{\beta})'(y - X\hat{\beta})} = \mathbf{y'y - \hat{\beta}'X'y} \quad \ldots\ldots (2.42)$$

The variance can then be measure as:

$$\sigma^2 = \frac{SS_{ERROR}}{n - k + 1} \quad \ldots\ldots (2.43)$$

Where 'n' is the total number of observations and 'k' is the is the number of factors investigated

The solution given in Equation 2.39 for the least squares estimates of the regression coefficients ($\hat{\boldsymbol{\beta}}$), is used by MATLAB in fitting the regression model (Equation 2.40) to

experimental data. Individual regression coefficients in the model can then be tested for significance, because some coefficients are not important in predicting the response variable.

A procedure called stepwise regression is used to test the effect of all variables and variable interactions on the variability of the response model. In this procedure, the regression coefficients are tested for significance and MATLAB includes into the model the next most significant regression coefficient. The first coefficient added is usually $ß_o$, then the others follow successively depending on their level of significance. Coefficients not significant at 95% confidence interval (CI) are left out of the model. MATLAB has a built-in function called stepwisefit which performs this task.

2.3.7.6 Response Surface Methodology

After the adequacy of the model has been established, the response is usually optimized. A response surface can be generated from a factorial design with more than one investigated factor. If the response of a 2^2 factorial experiment is given by

$$y = f(x_1, x_2) + \varepsilon \quad \ldots\ldots (2.44)$$

Then the surface generated is given by

$$S(y) = f(x_1, x_2) \quad \ldots\ldots (2.45)$$

This surface is called the Response Surface. Techniques such as method of steepest ascent or descent can be used. In the case where multiple responses are optimized, one of the most common methodologies is to overlay the contour plots of the individual plots in order to determine the best operating experimental conditions that will satisfy all the response requirements. Montgomery (2001), states that it becomes difficult to overlay contour plots with more than three design variables as a contour plot is two-dimensional. This means that k-2 of the design factors must be held constant to construct the contour. Trial and error techniques then have to be used to ascertain which of the responses to hold constant and the levels to select to obtain the best view of the surface.

CHAPTER 3-EXPERIMENTAL EQUIPMENT, MATERIAL AND PROCEDURE

3.1 Equipment and Material

The material and the equipment used in running the experiments conducted during this project are reported in the discussion that follows. The detail of the equipment used is in section 3.1.1 and section 3.1.2 presents the material used.

3.1.1 Equipment

De-juicer/mincer:

Fresh lemons were de-juiced to produce the required peels using the Kenwood 1000 de-juicer. This unit was also used to mince the peel to the required size by inserting a mincer sieve plate of the required size (mincer plates with 3 mm, 6 mm and 9 mm hole diameters were used).

Grinders:

These were used for grinding dried peels to the required sizes. The Kenwood FP101T was used to produce the larger particle sizes; 2-4 mm and 1-2mm. The Russell Hobbs Satin coffee grinder (Model no 10934) was used to attain the small sized peels (less than 1 mm)

Moisture analyzer:

An IR-200 Moisture Analyzer was used to measure the required moisture content of the either fresh or dried peels. The analyzer has a heater hood that is ventilated. Approximately 0.5g sample was placed in a pan in the heater hood at a particular time and the heater hood was then closed. The sample was then heated until bone dry using infra red radiation from four parallel quartz infrared heaters located in the heater hood. The moisture analyzer uses the principle of "loss on drying" to measure the moisture content (IR-200 Moisture Analyzer Operation Manual).

The moisture analyzer was calibrated for lemon peel analysis; the temperature of the infra-red heater was set at 105 °C and the moisture loss was reported until it fell below 0.05% per minute.

pH meter:

A Jenway 3310 manual temperature adjustable pH meter was used to read off the pH of the required solutions. The accuracy of the pH meter is reported to be ±0.02. The pH meter was calibrated on a weekly basis using buffers (±0.02) purchased from BDH laboratories.

Automatic burette:

A Walu Continuous E 8580 automatic burette, of 25ml capacity, was used to titrate 0.1 N of sodium hydroxide solution to prepared pectin solutions in order to obtain the Degree of Esterification (DE) and Galacturonic Acid (GA) content of the pectin. The burette is reported to have an accuracy of ±0.01 ml.

Fluidized bed dryer:

A pilot scale fluidized bed dryer was used to dry minced wet lemon peels (Refer to Fig 3.1 for the diagram). The dryer consists of a feed chute or feeder (1) where the feed enters and drops into the fluidized bed section of the dryer (2) in which fluidization takes place. The air that fluidizes the feed is blown into the dryer by a fan (6). The fan's frequency controller (5) is then used to set the inlet air velocity which controls the extent of fluidization within the bed. The air velocity is measured by a pitot tube (4) fitted to the inlet pipe of the gas combustor (3). In the combustor the air is heated up to a set temperature and this air is then used to dry the material within the bed. As the air exits the dryer, some of the dried material particles may be carried out with the air, a cyclone (7) is therefore used to trap the entrained particles. The product is removed from the dryer after a run via an exit chute (8).

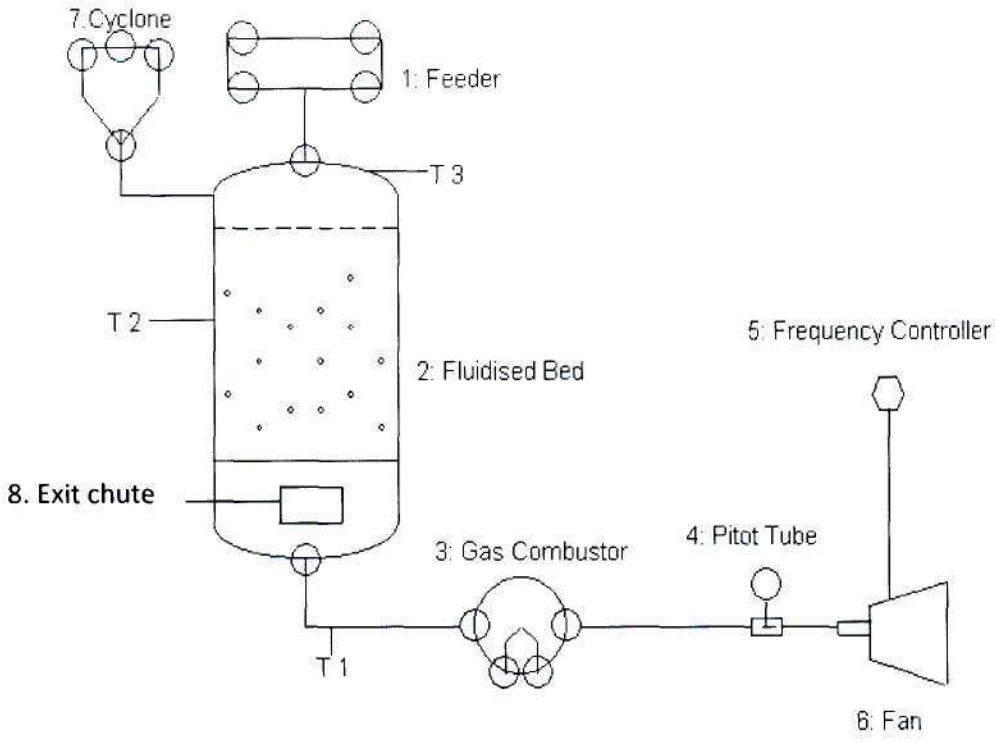

Fig 3.1: *Pilot Plant Fluidized Bed Dryer*

Water bath:

Two thermostat GD100 water baths maintained at 90 ° C, 80 ° C and 70 ° C were used to maintain the peel-water solutions at the required temperatures when extracting the pectin. The water bath was covered with a thick layer of polystyrene caps to prevent loss of heat to the environment.

Heating plates and stirrers:

FMN (model number STR-MH) heating plate and magnetic stirrer bar plates were used for all the heating and stirring required in the conducted experiments.

Filter pump:

A Speedivac ES150 vacuum pump was used to filter off all the required solutions in the experiment.

Filter wadding:

A filter wadding (PY013) was used as the filter medium in filtering off the mushy water-peel solution during the extraction procedure.

Vacuum dryer:

A Haraeus (model number 7404437) thermostat vacuum oven maintained at 50 °C and approximately 13.33 kPa pressure was used to dry the pectinaceous material after extraction. The vacuum was produced by a Gast (model number DOA-V505-BN) vacuum pump connected to the oven.

Convectional tray dryer:

A Labcon (model number FSOE-D8) convectional tray dryer maintained at 105 °C was used to dry the pectin after acid washing to prepare it for analysis. This dryer was also used to dry fresh peel samples at 100 °C and 150 °C for computing lemon peel drying kinetics.

3.1.2 Materials

Lemon (*Citrus limon*) Peels:

Lemon peels were supplied by Kat River Co-op for dried peel extraction experiments. Fresh lemons were also bought from market for preliminary drying experiments as well as wet peel extraction experiments.

Solutions:

All solutions used were of standard analytical grades obtained from chemical companies.

3.2 Experimental Procedure

The two important experimental phases of this project are outlined in the sections to follow. Section 3.2.1 details out the drying phase experiments and section 3.2.2 details out the extraction phase experiments.

3.2.1 Drying

The drying phase procedures include tray drying experiments and fluidized bed drying experiments. The material used is presented first, and then the experiment details are outlined.

3.2.1.1 Material used for drying experiments

Lemons were purchased from market and minced to three grade sizes using the mincer described in section 3.1.1.

Table 3.1: *The different investigated grades of wet peel for tray drying experiments*

Peel Grade	**Size**
Small	~ 3 mm
Medium	~ 6 mm
Large	~ 9 mm

3.2.1.2 Tray Experiments

Peel of a specific size was weighed into petri dishes of known areas. The peel was arranged evenly on the petri dishes. These were then placed into a tray dryer with an automatic temperature controller. At different time intervals a dish was taken out of the dryer and the moisture content of the peel was measured using a moisture analyser. This was done until the peel was found to be bone dry. The experiments were conducted at two temperature settings of the dryer; 100 ° C and 150 ° C. All experiments were duplicated and the average value was used for all calculations conducted.

3.2.1.3 Pilot Scale Fluidized Bed Drying

Refer to the design of the dryer given under the Equipment Section 3.1.1, Fig 3.1.

22 litres of peels were used for a given a run. From preliminary test runs, it was found that the small and medium sized peel agglomerated when dried, thus only the large sized peel was used for further testing. The frequency controller was turned allowing the air in at a known velocity. The LPG gas was ignited in the combustion chamber and the temperature was allowed to reach 150 ° C. The peel was then added and the air velocity was controlled to attain good fluidization within the bed. Samples were then taken at different time intervals and the moisture content of these was measured using the moisture analyser.

3.2.2 Pectin extraction and analysis

This study focuses on optimising the conditions of pectin extraction from dried peels. Extraction of pectin from wet peels was also investigated to compare the results obtained with those found from dried peel extraction. The sections that follow (3.2.2.1 to 3.2.2.2) present the preparation of the material used in the experiments, the extraction procedure, acid washing procedure and pectin analysis procedure. The experimental design for dried peel, fresh wet peels and stored wet peel extractions are detailed out in chapter 4.

3.2.2.1 Material used for extraction experiments

For dried peel extractions, lemon peels were supplied by Kat-River Citrus Co-op and dried in the fluidized bed drier (Fig 3.1) at 150 ° C to ~10% moisture content. The dried peels were then ground (Kenwood FP101T), sieved and stored in sealed plastic bags at atmospheric conditions. The peels were stored in different bags according to size; 2-4 mm size, 1-2 mm size and less than 1mm sized peels.

For fresh wet peel extractions, lemons purchased from market were de-juiced, de-pulped and the peels were minced to 3mm sized particles (Kenwood 1000 de-juicer). The moisture content of the peel was measured and an average moisture content of 83 % was found. The peel was sealed in plastic bags and then stored in a refrigerator at – 7 ° C. Before the peel was used for any run it had to be thawed and weighed.

For stored wet peel extractions, lemon from market was prepared the same way as in fresh wet peel extractions. However, instead of the peel being stored at -7 ° C, it was stored at atmospheric conditions for two days.

3.2.2.2 Extraction procedure

20.0 g (on a dry basis), m_i, of the peel of the size investigated (less than 1mm, 1-2 mm and 2-4mm) was weighed into four beakers. Distilled water at the temperature of interest (70 ° C, 80 ° C and 90 ° C) was adjusted to the pH studied (1.5, 1.75 and 2). The acidified water was then added to the beaker to make up the respective studied mass ratios (1:25, 1:37.5 and 1:50) and beaker was then put into a water bath for the required duration (0.5, 1.15 and 2 hours). The pH was monitored closely at five minute intervals for the first thirty minutes, then at thirty minute intervals for the remaining time, and adjusted to the pH investigated as need be.

The beakers were then removed from the water bath after the required duration of the experiment, and the contents were vacuum filtered using a filter wadding (PY013) as the medium of filtration. The filtrate was then cooled, weighed and its pH was adjusted to 4.5. Pure Iso-propanol (IPA) was then added to the filtrate at a mass ratio of 1:1.5 (mass of filtrate to mass of IPA) to precipitate the pectin out of solution. The precipitated pectin was then filtered, washed three times and dried at 60 °C overnight. The following day it was weighed and the mass was recorded as m_e.

3.2.2.3 Acid Washing

The following day, the pectin was weighed, ground using a mortar and piston and put into a beaker. 100 ml of 60 % IPA was then added followed by 5 ml of 32% HCl. The contents of the beaker were thereof stirred for 10 minutes and filtered. The pectin was washed thoroughly with 60% IPA until no chloride ions were present in the filtrate. This was ensured by an addition of 3 drops of $AgNO_3$ solution which would result in a white precipitate in the presence of chloride ions. The washed pectin was then dried at 105 ° C for 2½ hours as stipulated by the Food Chemical Codex (FCC, 1996).

3.2.2.4 Pectin Analyses

The dried pectin (at 105 ° C) was ground using a mortar and piston, then approximately 0.5g was weighed (mass m_s) into a beaker. 2 ml of 60% IPA were added to wet the sample, followed by 100 ml of de-carboxylated distilled water and was stirred until complete dissolution of the pectin. 5 drops of phenolphthalein were added to the beaker. The temperature of the beaker contents was noted and the pH meter was adjusted for the temperature. The pectin solution was then titrated with 0.1N NaOH until a light pink colour was observed (pH of ~8.5). The volume of the titer was noted as V_1 (ml).

20ml of 0.5N NaOH was added to the beaker and the contents were thereof stirred for 10 minutes. The solution turned to a dark pink colour on addition of the NaOH. 20 ml of 0.5N HCl was then added and the contents were stirred until the solution was colourless. 3 drops of phenolphthalein were added and the solution was titrated with 0.1N NaOH until a faint pink colour. The volume of the NaOH was noted as V_2 (ml). The percentage yield, %GA, and %DE were then calculated from the following equations as stipulated by the Food Chemical Codex (1981):

$$\% \text{ DE} = 100 * \frac{V_2}{V_t} \quad \ldots\ldots (3.1)$$

$$\% \text{ GA} = V_t * \frac{19.41}{m_s} * 100 \quad \ldots\ldots (3.2)$$

$$\% \text{ Yield} = \frac{m_e}{m_i'} * 100 \quad \ldots\ldots (3.3)$$

Where: V_t (ml) = $V_1 + V_2$

m_s is the mass of pectin analysed after acid washing (mg)

m_e is the mass of the extract dried at 60 ° C (g)

m'_i is the initial mass of dried peel, m_i, on a dry basis (g)

CHAPTER 4 – EXPERIMENTAL DESIGN

4.1 Dried Peel Extraction

Dried peel extraction experiments were designed in two ways. First, a 2^5 factorial design was carried out in order to characterise the relationship of five investigated variables on three response variables, the percentage yield (% Yield), percentage galacturonic acid content (% GA) and percentage degree of esterification (% DE). The five investigated variables were:

- Temperature (A)
- Time of extraction (B)
- pH (C)
- Size of peels (D)
- Dry peel to water mass ratio (E)

Secondly, a face centered central composite design (FCCD) was undertaken in order to obtain the optimum region of operation of the extraction process. The optimum region encompasses the highest possible percentage yield, at the highest % DE and highest % GA.

4.1.1 2^5 Factorial Design

A 2^5 factorial design method was used to analyse the effect of the process variables on the response variables. The process variables were investigated at two levels as follows; 70 °C and 90 ° C for temperature, ½ and 2 hours for the extraction time, 1:25 and 1: 50 dry peel to water mass ratio, 1.5 and 2.5 for pH and peel sizes of less than 1 mm and 2-4 mm. Coded variables were used after standardization of the levels. The smallest value of the levels was coded -1 and largest was coded +1.

Only four samples could be analysed daily. Two replicas were initially designed for, but due to a high reproducibility error a third replica was performed. Only the second and third replicas are reported in the results section as less variability was noted between the replicas (within 15% repeatability error).

An Analysis of Variance (ANOVA) was performed on the variables to test for their individual and interactive effects on the response variables. A first order multiple linear regression equation was then fitted to model the experimental results (Refer to Equation 2.27).

4.1.2 Central Composite Design

The 2^5 factorial design was further developed into a central composite design, by measuring the center points and star points. The face centered design (FCCD) was chosen because the interest area lied within the investigated process variable levels (factor levels). The center points were thus evaluated at the coded value of 0 (corresponds to natural variables of 80 °C temperature, extraction time of 1.15 hours, pH of 2, peels size of 1-2mm and peel to water mass ratio of 1:37.5) for all investigated factors. The star points of a certain process variable were measured at -1 and +1 level of the variable while all other variables were kept at 0 level. An example of a measured star point for the temperature variable would be at -1 level of temperature, 0 level of extraction time, 0 level of pH, 0 level of peel size and 0 level of dry peel to water mass ratio.

From this design, a second order model was then evaluated in order to fit the results better than the first order model (Refer to Equation 2.32). An examination of the resulting contour plots of the developed second order model then revealed the optimum operating conditions for the extraction process.

4.2 Fresh Wet Peel Extraction

Pectin can be extracted from fresh wet peel or dried peel. In order to compare the effect of drying on the pectin quantity and quality, fresh wet peel extractions were conducted using the same process conditions as in dried peel extractions.

4.2.1 2^4 Factorial Design

Four experimental variables, similar to those studied for dried peel extraction, at similar process variable levels, were investigated. Only four of these process variables were investigated because the size of the peel could not be minced to a size smaller than 3 mm for fresh peels. The four variables were:

- Temperature (A) at 70 ° C and 90 ° C
- Extraction time (B) at ½ hours and 2 hours
- pH (C) at 1.5 and 2.5
- Peel (on a dry basis) to water mass ratio (E) at 1:25 and 1:50

A 2^4 factorial design method was used to analyse the effect of the variables on the percentage yield (% Yield – w/w on a **dry basis**), percentage galacturonic acid content (% GA) and percentage degree of esterification (% DE). The individual variable levels investigated were similar to those of considered for corresponding variables in the 2^5 factorial design of dried peel. All runs were duplicated.

A similar ANOVA was performed on the wet peel data to test for their individual and interactive effects of the response variables. A first order multiple linear regression equation was then fitted to model the experimental results (Refer to Equation 2.27). The results were then compared to those of dried peel extractions.

4.3 Stored Wet Peel Extraction

The effect on the response variables of two days storage of wet peel at atmospheric conditions was also investigated in order to validate the need for drying the peel.

A 2^4 factorial design similar to that conducted for fresh wet peel extractions was undertaken for stored wet peels. Similar factors and factor levels were investigated. The difference in the results obtained (between stored wet peel and fresh wet peel) was then noted and graphed.

CHAPTER 5 – RESULTS AND DISCUSSION

The experiments were divided into two phases, the drying phase and the extraction phase. The drying phase is discussed first.

5.1 Drying of the Peel

In the drying phase, tray experiments were conducted first, followed by pilot plant fluidized bed drying experiments and the results are presented and discussed in succession.

5.1.1 Tray Drying

Drying experiments were first of all conducted in a tray dryer in order to get drying characteristics of the lemon peels. At the outset, it was important to prove that most of the drying occurs in the falling rate period rather than in the constant rate period. In the falling rate period unlike the constant rate period, mass and heat transfer coefficients play no significant role in the drying of the material and material properties affect the drying rate significantly. If this was proven, then the drying characteristics of the lemon peels computed from tray drying experiments could be used as a conservative representation of those that would result in the pilot plant fluidized bed dryer. Conservative because they would not give the exact characteristics, but could serve as preliminary approximate indications of the drying rate.

From previous experiments conducted by the CSIR team, the range of temperature appropriate for the drying of the peel was given as 100 $^{\circ}$ C – 150 $^{\circ}$ C. In this temperature range, the degradation of the peel was minimized. The minimum temperature (100 $^{\circ}$ C) and the maximum temperature (150 $^{\circ}$ C) of the considered range were thus chosen for investigation in a tray dryer to compare their effect on the drying characteristics of the lemon peel. Concurrently, the effect of the size of the peels on the drying characteristics was also investigated. The drying rate curves at the different temperatures and for the different sized peels were constructed (Appendix A, Fig A.1 and A.2), and from them, the critical moisture content (CMC) as well as the residence time required to achieve 10% moisture content of the peel were computed. At 10 % moisture content, the peel is considered dry enough to inactivate bacteria and enzyme activity within the peel. The findings are discussed hereafter.

5.1.1.1 Critical Moisture Content (CMC)

From the experiments conducted, an increase in temperature was seen to increase the critical moisture content (Refer to Fig 5.1a). When temperature is increased, external mass and heat transfer rates are increased, which in turn increases the rate at which the saturated moisture is evaporated from the peel. As the mass and heat transfer rates are increased, they result in a decrease in the constant drying period, as a result, the critical moisture ratio or content increases. These results were in agreement with Perry (2003), who states that the critical moisture content increases with increased drying rate.

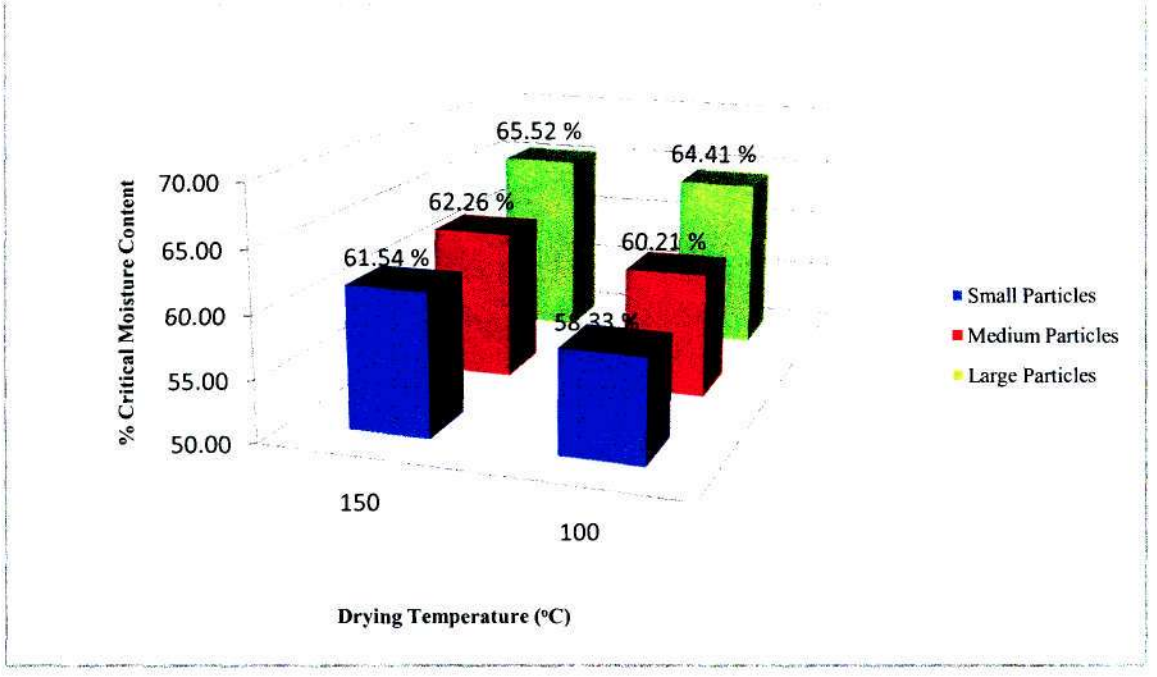

Fig 5.1a: *Percentage Critical Moisture Content of the different peel sizes dried at different temperatures.*

From Fig 5.1a above, the critical moisture content of the peel was seen to increase with increase in the size of the peel particles dried. This is because the intra-particle mass transfer becomes significant with bigger particles, which is the basis of the falling rate period. When the falling rate is increased, the critical moisture ratio or content is in turn increased.

5.1.1.2 Residence time required to achieve 10 % moisture content in tray drying

Fig 5.2a shows the residence time required to achieve 10 % moisture content of the peel at the drying temperatures of 100° C and 150° C. It is clear from the bar graph that the residence time was shorter at the higher temperature than the lower temperature for all peel sizes. This is because at higher temperatures the temperature gradient between the peel particles and the drying medium is greater than that at lower temperatures, thus providing a greater driving force for heat transfer and in turn mass transfer (Geankopolis, 1993).

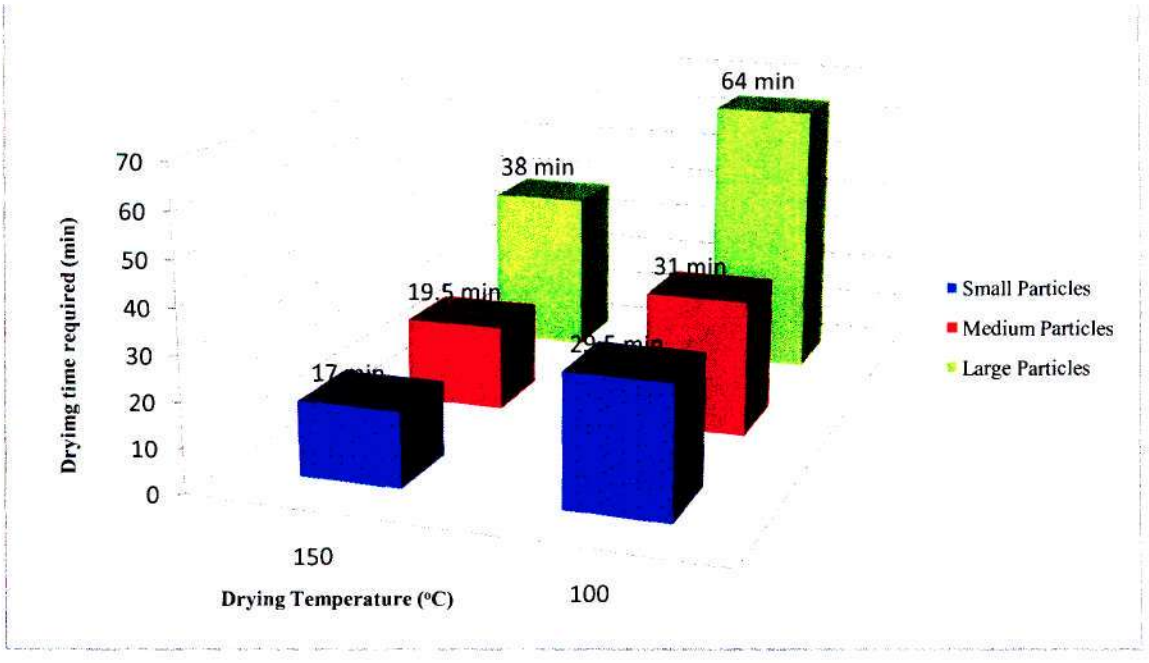

Fig 5.2a: *The residence time required in the dryer to achieve 10 % moisture content at different drying temperatures and for different peel sizes.*

Another observation from Fig 5.2a is that the difference in the residence time between the small sized (3mm) and medium sized (6mm) particles was not as pronounced as that between the medium sized (6mm) and the large sized (9mm) particles, although the difference in sizes is a constant 3mm between the two. An increase in the size of the peel means an increase in the amount of moisture within the particle. Because of the increased unsaturated moisture within the particle, the falling rate period increases. An increase in the particle size also means an increase in the distance the internal moisture travels to the surface of the particle. Intra-particle diffusion resistance thus becomes more pronounced for large sized particles.

There are two stages in the falling rate period; the unsaturated surface drying stage and the internal moisture movement drying stage. If internal moisture movement becomes more significant than the unsaturated surface drying in the falling rate period, the overall time for drying the material increases, as the former takes the longest time and is a function of the material thickness. It can therefore be concluded that for the small and medium peel sizes, internal moisture movement from the material was less significant than for the large sized peel. This resulted in the noted increase in the residence time for large sized peel. Mira and Blasco (1996) in their experiments also noted the intra-particle diffusion resistance to be significant in larger particle sizes (5-10mm) compared to the smaller sized peel, which was in line with this study's findings.

5.1.2 Fluidized Bed Drying Tests

A continuously operated fluidized bed dryer was chosen as the best dryer to use for lemon peel drying in industry (Refer to Section 2.1.3, pg 12). For small scale drying of the peel, a batch operated fluidized bed dryer was chosen.

A shorter residence time means a decrease in the cost of the dryer as the size of the required fluidized bed dryer decreases. From tray drying tests, small sized peel followed by medium sized peel were seen to have a shorter residence time than the large sized peel (Fig 5.2a). If the small or medium sized peel was dried rather than the large sized peel, then the cost of the required dryer would be reduced. It was found however in practise that the small and medium sized peels tend to agglomerate and thus proved difficult to fluidize. Therefore, only the large sized peel was effectively fluidized and therefore dried. Consequently, the large peel particle size was chosen for further investigation. Since at 150 $^{\circ}$ C, the residence time to achieve 10 % moisture content of the peel was found to be shorter, the inlet air temperature, T1 (Refer to Fig 3.1, pg 53), of the fluidized bed dryer was maintained at approximately 150 $^{\circ}$ C.

5.1.2.1 Residence Time required to achieve 10 % moisture content in fluidized drying

The time taken to achieve 10 % moisture content of the peel was found to be 24.8 min and at this time, the temperature within the bed was found to be 80 ° C (Refer to Fig 5.3a). The time was found to be shorter than that found in tray drying experiments (38 min at 150 ° C) for large sized peels. The difference was expected as more intensive drying occurs in a fluidized bed dryer due to greater heat and mass transfer rates within the dryer. The tray drying results though, gave a close enough residence time to give a conservative estimate of that expected in the fluidized bed dryer. This is because from tray drying experiments the critical moisture content of the large sized peel particles at dried at 150 ° C was found to be 65.52%, indicating that most of the drying occurs in the falling rate period. Thus, external conditions were less significant in the determination of the drying rate of the peel compared to internal material properties.

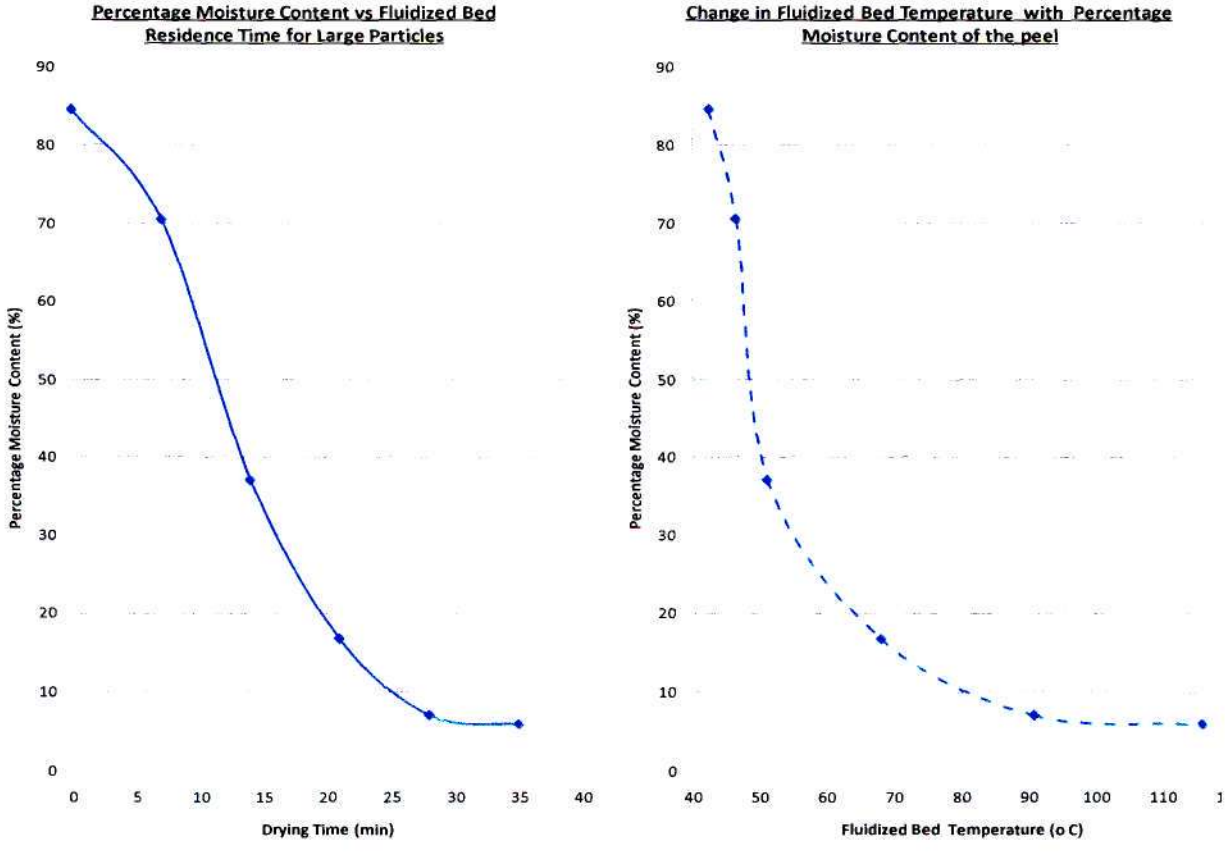

Fig 5.3a: *Change in the percentage moisture content of the peel with drying time and fluidised bed temperature.*

5.2 Peel Extraction

The main focus of this section of the study was to optimize the conditions of pectin extraction from peels dried in the fluidized bed pilot plant dryer. Only large sized wet peels (~ 9 mm) were dried (Refer to section 5.1.2) and were milled to get the different sizes investigated in the extraction phase experiments. Firstly, dried peel extraction was characterized in terms of the investigated extraction parameters. Extraction of pectin from wet peels was also undertaken to compare the results obtained with those found from dried peels. First order empirical models in conjunction with an analysis of variance (ANOVA) for the extraction process were developed for both the dried and wet peel. The first order empirical model for dried peels was then further developed into a second order model in order to find the optimum region of operation for the extraction process. A central composite design (CCD) was used to develop the second order model as described by Montgomery (2005).

The following discussion looked into the effect of the investigated process variables or factors on the response (monitored) variables for dried peels (5.2.1). The results found from wet peel extractions were then compared to those attained from dried peels in order to determine the superior starting material for the extraction process (5.2.2). The effect of two days storage on the response variables for wet peel extraction was also investigated in order to substantiate the need for drying the peel (5.2.3). Then lastly, the optimum conditions of operation for dried peel extraction were chosen (5.2.4).

5.2.1 Extraction from Dried Peel (Factorial Design)

The effects of five process variables, namely temperature (A), time (B), pH (C), peel size (D) and dried peel to acidified water ratio (E), on three response variables were investigated. The three monitored response variables were the percentage yield (% Yield) of the extract, percentage galacturonic acid content (%GA) and the percentage degree of esterification (% DE) of the pectin extract. The response variables were calculated according to Equations 3.1 to 3.3. The following discussion looks at the resultant response variables achieved experimentally and the effect of the process variables on these responses. Section 5.2.1.1 looks at the % Yield response, section 5.2.1.2 looks at the % GA response and section 5.2.1.3 at the % DE response

5.2.1.1 Extraction Yield (% Yield) for dried peel extraction

The range of the attained yield, the extraction conditions that resulted in the lowest and highest yield, the developed empirical model and significant variables are presented and discussed in the sections that follow (5.2.1.1(a) to 5.2.1.1(d)). Then lastly, the investigated process variables found to be significant in this work are compared to those found in literature (5.2.2.1(e)).

5.2.1.1(a) The achieved range of the yield (% Yield)

The average percentage yield (of the two replicas investigated) found in this study ranged from 2.87 % to 30.33 % on a dry basis. This range was found to be within the range obtained by Levigne et al. (2001) for sugar beet pectin (2.3% - 35.4 %). The range reported by Levigne et al. (2001) was chosen for comparison with this work because it was found to be the closest range reported in the literature reviewed to that found in this work. Yapo et al. (2005) found a range from 4.1 % to 16.2 % while Robert et al. (2006) reported a range from 3.1 % to 24.2 % and Mesbahi et al. (2004) found a range from 5.7 % to 22.4 %. These few works cited, show pectin yield ranges which are much lower than that found in this study and by Levigne et al. (2001). Levigne et al. (2001) worked investigated pH at a low level of pH 1 compared to that of 1.5 which was investigated in this study, which is why their highest percentage yield of 35.4% was greater than that found in this study (30.58%); a decrease in the pH causes cell degradation and thus results in an increase in the extract liberated from the peel.

5.2.1.1(b) Lowest and Highest percentage yield (% Yield) achieved

The smallest average percentage yield, 2.87%, was found at a temperature of 70 °C, extraction time of 30 minutes, pH of 2.5, peel size of 2-4 mm and a dried peel to water mass ratio of 1:25. The greatest average percentage yield in this study was found to be 30.33 %. This yield was found at a temperature of 90 ° C, an extraction time of 2 hours, pH of 1.5, peel size of less than 1 mm and a dried peel to water mass ratio of 1: 25.

The greatest yield was found under most harsh conditions of temperature, time and pH (90 °C, 2 hrs, 1.5 respectively) while the lowest yield was found when these variables were at their mildest (70 ° C, 30 min, 2.5 respectively). This is because at the harshest conditions (high temperature, longer extraction time and low pH), the cell wall degrades and this makes it easy for the pectin to be extracted, while at the mildest conditions (low temperature, shorter

extraction time and high pH), the cell wall still remains intact, making it difficult for the pectin to be extracted. Robert et al. (2006) and Yapo et al. (2005) also found the same outcome from their investigations. The small sized peel had a greater surface area exposed to the extraction medium; this therefore increased the rate at which mass transfer occurred. This explained why the greatest yield found was that of pectin extracted from small sized peel (less than 1 mm) and the least yield found for large sized peels (2-4 mm). The least and greatest yield were both found at the lowest peel to water mass ratio. This implied that an increase in this variable did not significantly affect the extraction yield, but an analysis of variance was still needed to confirm this assumption.

5.2.1.1(c) Empirical model and correlation coefficient for the % Yield response for dried peel extraction

A first order model was developed to represent the variation of the % Yield response in an empirical formula which explained the effect of the change in the investigated factors on the percentage yield (Equation 5.1). A model adequacy check was then undertaken before the empirical model was accepted in order to verify that the assumption of a normal distribution was adhered to in all the investigations as this is assumed in the analysis of variance (ANOVA) methodology. A probability plot of the residuals of the experimental and predicted findings verified that the assumption was observed (Refer to Appendix B, Fig B.1). The developed model is expressed in a tabular format in Table 5.1.

Empirical Model expression:

$$\%\ \mathbf{Yield} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.1)$$

Table 5.1: *Empirical model developed for the %Yield response in coded variables for dried peel extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effects	Regressor Coefficient (ß)	Value of Regressor Coefficient (ß)
Main Effects			$ß_0$	14.91
	A	x_1	$ß_1$	4.26
	B	x_2	$ß_2$	2.16
	C	x_3	$ß_3$	-3.94
	D	x_4	$ß_4$	-3.25
	*E	x_5	$ß_5$	0.19
Interactive Effects	AB	x_{12}	$ß_{12}$	0.99
	AC	x_{13}	$ß_{13}$	-0.84
	AD	x_{14}	$ß_{14}$	-0.38
	AE	x_{15}	$ß_{15}$	-0.32
	BD	x_{24}	$ß_{24}$	0.83
	CD	x_{35}	$ß_{34}$	0.54
	DE	x_{45}	$ß_{45}$	-0.23
	ABC	x_{123}	$ß_{123}$	-0.62
	ACD	x_{134}	$ß_{134}$	-0.31
	ACE	x_{135}	$ß_{135}$	0.25
	BCD	x_{234}	$ß_{234}$	-0.60
	*BDE	x_{245}	$ß_{245}$	-0.18
	ABCD	x_{1234}	$ß_{1234}$	-0.30
	ABCE	x_{1235}	$ß_{1235}$	0.91
	ABDE	x_{1245}	$ß_{1245}$	-0.40
	**BCDE	x_{2345}	$ß_{2345}$	0.15
	ABCDE	x_{12345}	$ß_{12345}$	-0.43

The unmarked effects are significant at 99 % and lower CI while those marked with (*) are significant at 97.5 % and lower CI and those marked as (**) are significant at 95 % and lower CI.

One of the most essential values that show whether the predicted model best explains the experimental results is the correlation coefficient. A correlation coefficient close to 1 shows the developed model to best predict the experimental findings. An analysis of variance (ANOVA) was undertaken to examine the effect of the investigated variables on the percentage yield response. From this analysis, the adjusted correlation coefficient (R^2_{adj}) was calculated mathematically (Equation 2.33). The correlation coefficient can also be found graphically by plotting the predicted findings (found using the empirical model shown in Table 5.1) versus the experimental findings. This is shown in Fig 5.1. These two correlation coefficients can differ if in the predicted model (used in plotting Fig 5.1) effects significant only below 95 % CI are also included.

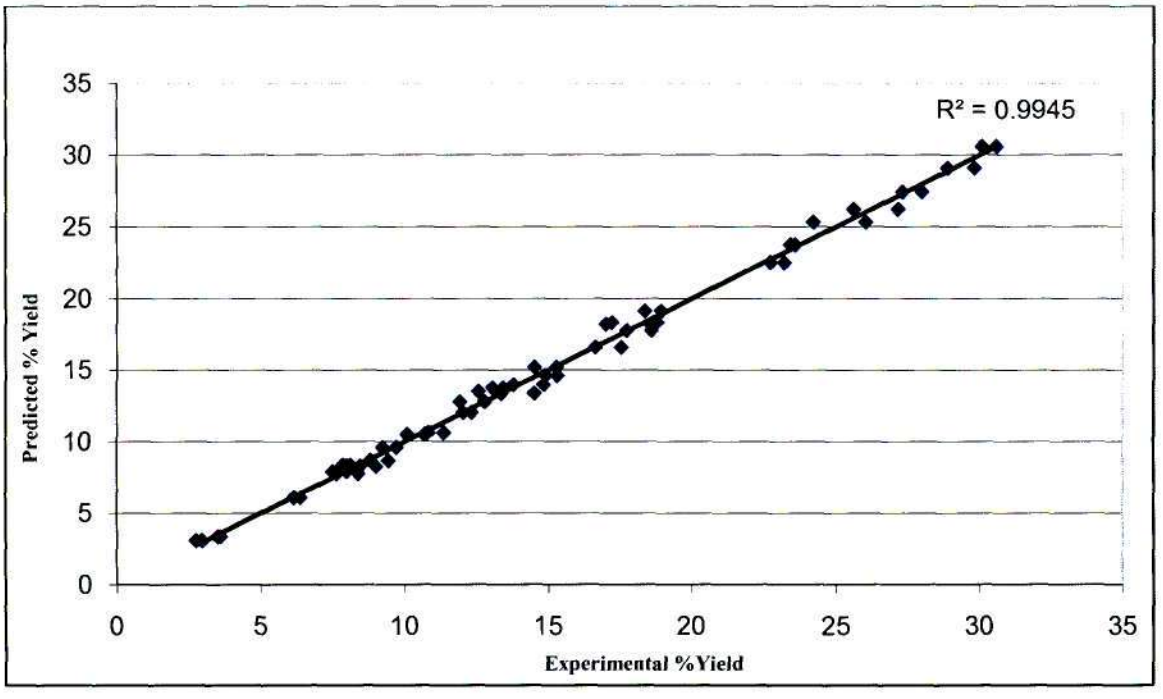

Fig 5.1: *The correlation plot of the experimental and predicted results for the %Yield response for dried peel extraction (only significant effects were used in calculating the predicted values)*

The adjusted correlation coefficient was found to be 0.994 and was exactly the same as the graphical correlation coefficient shown in Fig 5.1. The value found for the correlation coefficient showed that the chosen process variables or factors explained 99.4 % of the variation of the percentage yield response. This proved that the five process variables investigated in this study were the most influential parameters on the resultant yield attained in the extraction process, and thus controlling these variables would ascertain the required pectin yield.

5.2.1.1(d) Significant variables for the % Yield response for dried peel extraction

Examination of the Empirical Model:

The empirical model shown in Table 5.1 only includes significant terms, at 95% and higher confidence intervals, found from an examination of the ANOVA. Usually this confidence interval (95 % CI) is used in most analyses. The first order model developed is given in coded variables (Refer to Equation 2.30). Montgomery (2005), states that in coded variable notation, the magnitude of the regression coefficients is directly comparable, as they are dimensionless.

From the model developed, almost all effects and effect interactions were identified as significant in predicting the percentage yield. The model also showed the main effects of temperature (A), time (B), pH (C) and peel size (D) as the *most* significant in predicting the percentage yield as their corresponding regression coefficients (ß) were the largest and they were shown to be significant from a CI of 99 %. The main effect of dried peel to water mass ratio (E) was only significant at 97.5 % and lower CI and not 99 % CI, and the resultant regression coefficient (ß) of this main effect was much less than those of the previously mentioned main effects.

The other second order and higher order interaction effects were found to be significant from a CI of 99%, save for BDE and BCDE which were only found significant from a CI of 97.5 and 95 % respectively. An examination of their corresponding regression coefficients however, showed them to be far less than those of the main effects (A, B, C and D) save for the dried peel to water mass ratio main effect (E). It was therefore deduced, that the main effects of temperature, time, pH and peel size were the *most* important effects to control to attain a good yield of the extract. This was further proved by a probability plot of the effects and contribution chart of the effects.

Examination of Normal Probability Plot of effects:

A normal probability plot of the effects (Refer to pg 41 and 42 for an explanation of the plot) is given in Fig 5.2. From the probability plot, significant effects are detected by their deviation from normality; the most outlying effects are thus considered to be the most significant.

The probability plot showed many significant effects (higher order effects), but the furthest from normality and thus the *most* significant were identified as the main effects of temperature (A), time (B), pH (C) and peel size (D). These findings agreed with those suggested from examining the empirical model as stated previously.

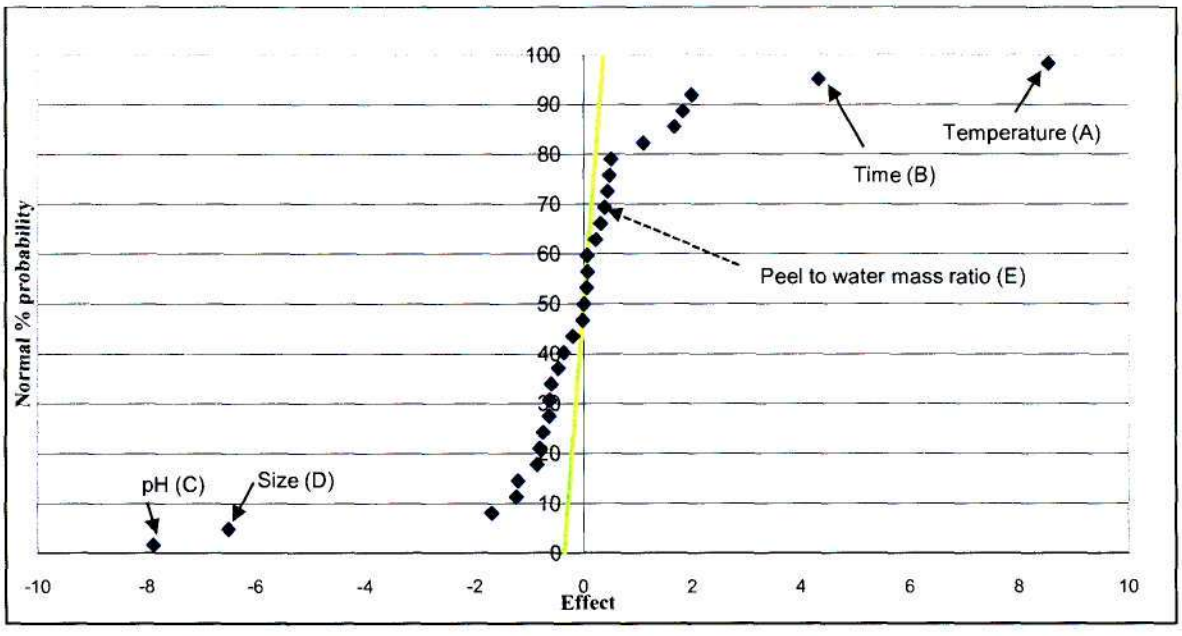

Fig 5.2: *Normal probability plot of the effects for percentage yield (% Yield) response for dried peel extraction*

Examination of the Percentage Contribution of the effects:

Fig 5.3 shows the main effects identified previously (A, B, C and D) and their percentage contribution on the variation of the percentage yield response. The contribution of each effect was calculated according to Equations B.8a and B8b (Appendix B).

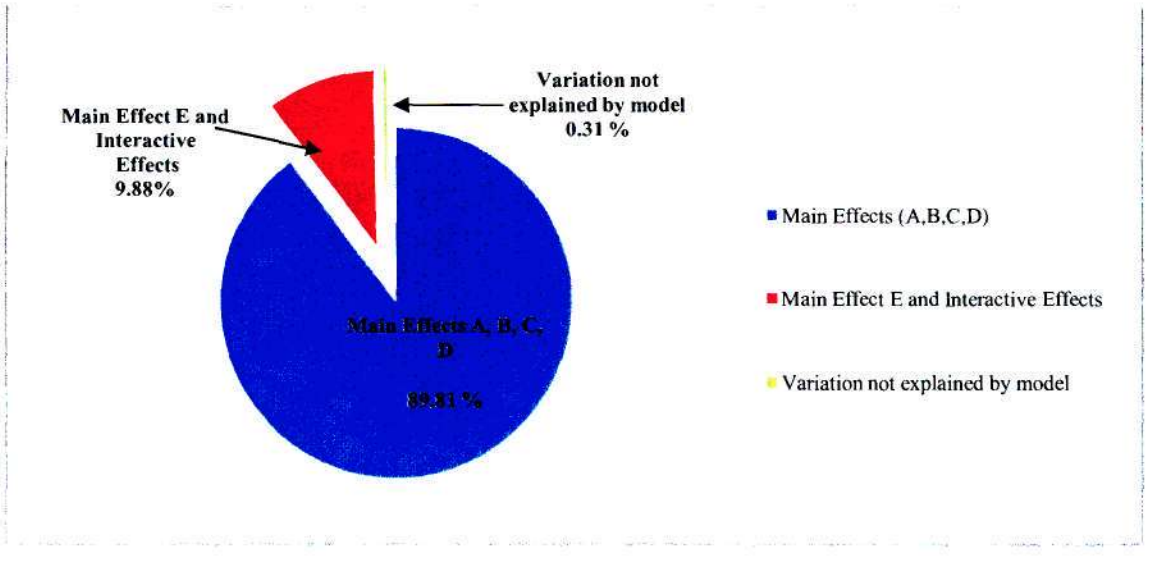

Fig 5.3: *Percentage contribution of effects on the % Yield response variable for dried peel extraction*

A calculation of the percentage contribution of the four *most* significant main effects (A, B, C and D) on the variation of the yield, showed that just these four variables explained 89.81% of the variation of this response. The interactive effects that were shown to be significant by ANOVA and are included in the model only explained 9.88% of the variation of the yield. This thus showed that control of the main effects of temperature, time, pH and peel size was sufficient to attain the required yield, the interactive effects of the process variables were found to be less significant and thus could only affect the process minimally.

From the chart, only 0.31 % of the variation in the yield was not explained by the model. This further showed that the investigated variables were sufficient enough to determine the percentage yield of the extract.

Characterization of the most significant effects using the developed model:

The regression coefficients of the main effects of temperature (A) and time (B) obtained from the model were found to be positive (4.46 and 2.16 respectively). This showed that an increase in the temperature from 70 ° C to 90 ° C increased the yield and an increase in time from 0.5 hrs to 2 hrs also increased the yield. The coefficient of temperature was found to be greater than that of time, indicating that a unit increase in temperature increased the yield more than a unit increase in time. The regression coefficient of temperature was found to be approximately double that of time, thus showing that a unit increase in the temperature when compared to that in time resulted in a double increase of the yield.

The regression coefficients of pH (C) and peel size (D) were found to be negative (-3.94 and -3.25 respectively). This indicated that and increase in pH from 1.5 to 2.5 decreased the percentage yield of the extract and an increase in peel size from a size of less than 1 mm to 2-4 mm size also decreased the percentage yield.

5.2.1.1(e) Comparison of literature and summarised experimental findings on the effects of the significant variables on the % Yield response

Effect of Temperature on the % Yield response:

In this present study, the main effect of temperature (A) was seen to be significant (from 99% CI) in explaining the variation in the % Yield response. All the other interactive terms of temperature when compared to the main effect were found to be less significant in determining the yield. Therefore, generally, an increase in temperature was seen to increase the resultant yield of the extract. Similarly, Robert et al. (2006), Yapo et al. (2005) Mesbahi et al. (2004) and Levigne et al. (2001), all noted an increase in the yield with increase in temperature. The contradiction came when the significance of the temperature variable was assessed according to the ANOVA method.

Contrary to this works findings, Yapo et al. (2005) and Levigne et al. (2001) found the main effect of temperature to be insignificant at a CI of 95 %. Robert et al. (2006), however, working at a CI of 90 % found the temperature to be significant. Yapo et al. (2005) and Levigne et al. (2001) studied pectin extraction from sugar beet, while Robert et al. (2006) studied pectin extraction from chicory roots. Because different plants have different matrix structures of the cell walls, the effect of a process parameter on different plant material can have different effects on their processing. It was thus assumed that the type of material from which the pectin is extracted may influences the significance of the temperature effect on the extraction yield, hence the difference in the significance of the main effect of temperature in this work and in that in the literature reviewed. At higher temperatures, there is an upset in the structure of the cell wall plant matrix making it easy for the extract to move into the extraction medium, hence the reason why the yield increased with increase in the temperature.

Effect of the extraction time on the % Yield response:

In this work, the main effect of the extraction time (B) was found to be highly significant (from a CI of 99%). Interaction terms of the time variable were seen to be less significant that the main effect of time, therefore it could be concluded that an increase in the extraction time increased the resultant extract yield (within the range studied - 30 min to 2 hours). Similar to the results found, Robert et al. (2006) and Yapo et al. (2005) reported an increase in the yield with

increase in extraction time from 1 to 4 hours, and in their findings, the main effect of time was reported to be significant. Levigne et al. (2001), on the other hand, found the main effect of time to be insignificant. The reason for the discrepancy with Levigne et al. (2001) was thought to have been because they investigated a time domain of 30 to 90 minutes, and this time domain was thought to have been too short to observe a change in the yield, thus a change in the yield with the variation of the time factor was seen to be insignificant in their study. An increase in the time of extraction allows sufficient time for the plant matter to be exposed to the extraction medium, hence the observed increase in the yield with the extraction time within the investigated time domain.

Effect of pH on the % Yield response:

The main effect of the pH was also found to be significant from a CI of 99% and its increase was found to decrease the resultant yield of the extract. The interactive effects of this variable with other process variables were seen to be less significant in determining the % Yield response than the main effect. As thus, an increase in pH was found to reduce the yield significantly. Similar results were found by Levigne et al. (2001), who reported the pH to be the most significant main effect that affects the yield. Contrarily, Robert et al. (2006), found the pH to be insignificant in their investigation. Robert et al. (2006) investigated a lot of process parameter effects on the response variable at once; therefore, it was assumed that some of the effects that were significant were masked in the process of investigation. A decrease in the pH degrades the plant cell wall matrix making it easy for the pectin to be liberated from the plant, hence the observed decrease of the yield with the increase in the pH of the extraction medium.

Effect of peel size on the % Yield response:

The findings of this study showed the main effect of peel size (D) to significantly affect the yield (from a CI of 99%), and an increase in the peel size was seen to decrease the extraction yield. Because the interactive effects of the peel size with other process variables were seen to be less significant than the main effect of peel size, it could thus be deduced that generally the smaller sized peels (less than 1 mm) yield a greater extract than the large sized peels (2-4mm). This is because the surface area exposed to the extraction medium is greater for small sized peel which increases the rate of mass transfer. Robert et al. (2006) investigated the effect of milling on the extraction yield. Similar to the results found in this study, they also found that milling the start material significantly increased the yield of the extracted pectin.

Effect of dried peel to water mass ratio on the % Yield response:

The effect of dried peel to water mass ratio (E) was found insignificant at 99% CI, but significant from a CI of 97.5 %. Therefore, this effect was less significant than the main effects of temperature, time, pH and peel size. Although the main effect of peel to water mass ratio (E) was considered less significant, an examination of the regression coefficient (ß = 0.19) of this effect showed it to increase the yield with its increase. Slightly similar results were found by Robert et al. (2006). In their study they found this effect to be insignificant in determining the percentage yield, although it was generally seen to result in its increase.

5.2.1.2 Galacturonic Acid (%GA) for dried peel extraction

The amount of galacturonic acid content present in the extract shows the purity of the extract; the greater the percentage galacturonic acid (% GA), the greater the content on the pectin in the extract.

5.2.1.2(a) The achieved range of the Galacturonic Acid (% GA) content

The average galacturonic acid content (of the two replicas investigated) attained in this investigation ranged from 78.60 to 88.64 %. Industrial pectins have been reported to have at least a percentage galacturonic acid content of 65% (Food Chemical Codex, 1981). The pectin obtained in this study was thus found to be within the range acceptable in industry.

From the literature reviewed for this study, where the investigators use the same experimental design and statistical analyses similar to that used in this study, the highest % GA quality range was that found by Yapo et al. (2005); it ranged from 35.2% to 76.3 %. Other works reported a similar or lower % GA; to mention but a few, Levigne et al. (2001) found a range of 29.5 - 52.8 %, Kalapathy and Proctor (2000) found a range o f 68 – 72 % and Micard and Thibault (1999) that of 63.5 – 71.9 %. All these researchers extracted pectin from different sources other than lemon peels (sugar beet, chicory roots and soy hull). Pectins extracted from lemon peel have superior qualities than those from other sources (Harris and Smith, 2006), which is why the range found in this study exceeded that of these other investigations. Joye and Luzio (2000) however, worked with lemon peels, although their methodology was different from that used in this study, they found a % GA range of 70 – 80 %, which was comparable to that found in this study.

5.2.1.2(b) Lowest and Highest percentage galacturonic acid (% GA) achieved

The lowest average percentage galacturonic acid content (78.60 %) obtained in this study, was found at an extraction temperature of 70 ° C, time of extraction of 30 minutes, extraction pH of 1.5, a peel size of less than 1 mm sized peel and a dried peel to water mass ratio of 1:25. The greatest average percentage galacturonic acid (% GA) content was found to be 88.64 %. This was attained at a temperature of 70 ° C, an extraction time of 2 hours, pH of 2.5, peel size of less than 1mm and dried peel to water mass ratio of 1: 50.

Because interactions of the process variables were found to be significant in explaining the variability of the % GA response (shown in section 5.2.1.2(c) and 5.2.1.2(d)), it was difficult to predict what process conditions would significantly decrease or increase % GA from examining process conditions that resulted in the lowest and highest % GA attained. An analysis of variance was required to explain the effect of the process variables on the % GA response.

5.2.1.2(c) Empirical model and correlation coefficient for the % GA response for dried peel extraction

A linear first order empirical model was developed from the experimental findings to predict the variation of the % GA response. In order to ascertain that the model developed was adequate, a normal probability plot of the residuals was constructed. This plot showed no anomalies from normality and thus the model was considered adequate (Appendix B, Fig B.2). The model is given in Table 5.2 and its expression is given by Equation 5.2:

$$\%\,\mathbf{GA} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.2)$$

Table 5.2: *Empirical model developed for the % GA response in coded variables for dried peel extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effect	Regression coefficient	
Main Effects	-	-	$ß_0$	84.52
	**A	x_1	$ß_1$	0.69
	B	x_2	$ß_2$	1.47
	***C	x_3	$ß_3$	0.59
Interactive Effects	AC	x_{13}	$ß_{13}$	-0.94
	AD	x_{14}	$ß_{14}$	-0.84
	CE	x_{35}	$ß_{35}$	0.68
	ABD	x_{124}	$ß_{124}$	0.68
	ACE	x_{135}	$ß_{135}$	0.72
	ABCE	x_{1235}	$ß_{1235}$	-0.87
	ACDE	x_{1345}	$ß_{1345}$	-0.89

The unmarked effects are significant at 99 % and lower CI while those marked with (*) are significant at 97.5 % and lower CI, those marked as (**) significant at 95 % and lower CI and those with (***) are significant at 90% and lower CI.

In order to evaluate whether the predicted model in Table 5.2 best explains the experimental results, the correlation coefficient was calculated. The adjusted correlation coefficient (R^2_{adj}) only takes into account significant effects, and with the correlation coefficient adjusted, its value became 0.488. This meant that the investigated process variables only explained 48.8 % of the variation in the % GA found in this study. This value was found to be low and therefore indicated that the investigated process variables did not best explain the variation in the % GA response. It could therefore be concluded that there were other significant process variables that needed to be controlled in order to ascertain a desired % GA in the extraction process. The graphical correlation coefficient was also found by plotting the predicted % GA versus the experimental % GA. This is shown in Fig 5.4.

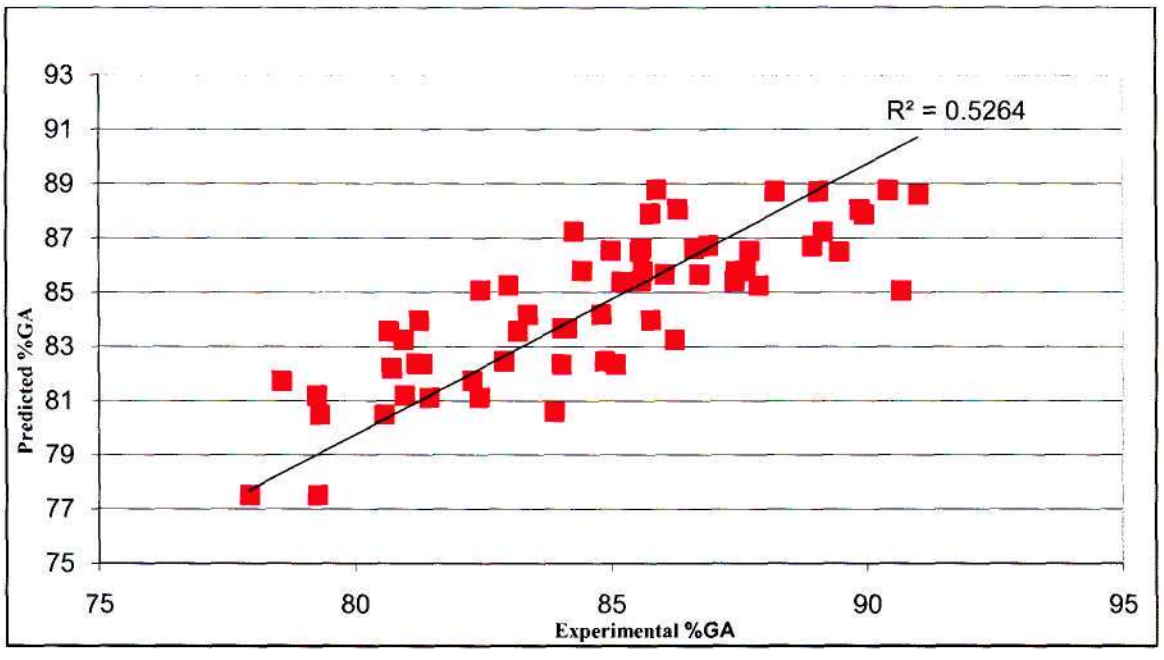

Fig 5.4: *The correlation plot of the experimental and predicted results for the % GA response for dried peel extraction (only significant effects were used in calculating the predicted values)*

From the Fig 5.4, the graphical correlation coefficient was found to be 0.526. This value was found to be different from that of the calculated adjusted correlation coefficient (R^2_{adj} = 0.488) because in the developed model (used to compute the predicted % GA values for finding the graphical correlation coefficient), effects significant at a confidence interval (CI) of 90 % (insignificant above 90 % CI) were included. It is usual to only include effects significant at a CI of 95 % only, but because the adjusted model correlation coefficient (R^2_{adj}) was low, the CI was lowered in order to better represent the experimental values. Robert et al. (2006) also reported his findings at a CI of 90 %. The graphical correlation coefficient indicates that the investigated variables only explained 52.6 % of the variation of the % GA response even when effects significant at 90% CI were included in the model. This value is still low and therefore still indicates that the investigated variables did not best explain the variation in the % GA response.

5.2.1.2(d) Significant variables on the % GA response for dried peel extraction

Examination of Empirical Model:

Significant effects on the resultant percentage galacturonic acid (% GA) content of the extract were evaluated by using the statistical F-test. From the model developed (Table 5.2), only the

main effect of time (B) was the most important main effect as it was found to be significant from a confidence interval (CI) of 99 %, temperature (A) only became significant from a CI of 95% while pH (C) only became significant from a CI of 90 %. The main effects of peel size (D) and dried peel to water mass ratio (E) were found to be insignificant at 90 % CI, however, interactive terms of these variables were found to be significant from a CI of 99 %.

From the examination of the regression coefficients (ß), the second order interactions as well as the third and forth order interactive effects all had comparable regression coefficients, therefore none could be assumed to be more significant than the others. This was further shown by the fact that they were all significant from a CI of 99 %. It is usually assumed that higher order interactions are less significant than lower orders interactions (Montgomery, 2005), but for the % GA response it was not so.

Examination of Normal Probability Plot of effects:

The normal probability plot of the effects (Refer to pg 41-42 on the normal probability plot) was also constructed to validate the significance of the effects. The plot is shown in Fig 5.5.

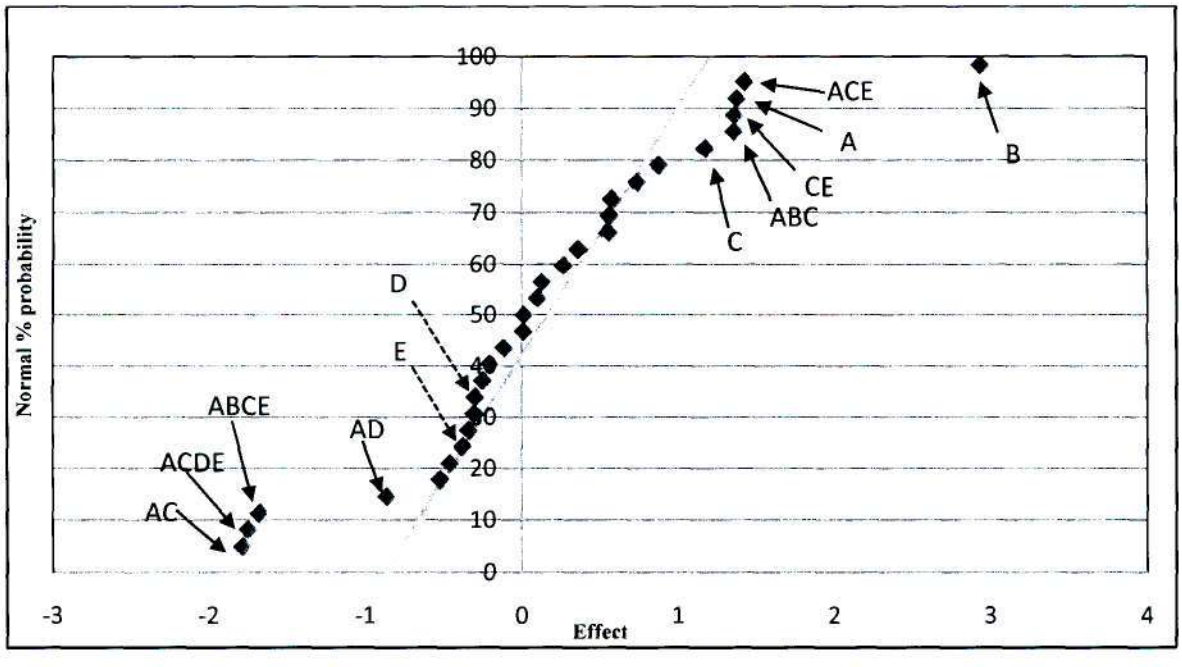

Fig 5.5: *Normal probability plot of the effects for % GA response for dried peel extraction (where A is the temperature variable, B the extraction time, C the pH, D the peel size and E the peel to water mass ratio)*

The effects found significant from the F-test were also identified by the normal probability plot (Fig 5.5) as significant. The significant effects are identified as those that lie away from normality. From the graph (Fig 5.5), it was evident that the main effect of time (B) was the most significant of effects as it deviated most from normality (furthest outlier in Fig 5.5). The main effects of temperature (A) and pH (C) were also noted to be significant, but not as significant as that of time the main effect of peel size (D) and peel to water mass ratio (E) were seen to be insignificant as they did not deviate from normality. The labelled interactive terms (AC, AD, CE, ABD, ACE, ABCE, and ACDE) were also seen to be significant. Because interactive terms were significant for this response variable, proper monitoring of the extraction variables is in order as an increase in two or more variables that could individually increase the percentage galacturonic acid (% GA) could result in the % GA decreasing due to the effect of their interaction.

Examination of the Percentage Contribution of the effects:

The percentage contribution of the effects was investigated to analyse which of the effects had a greater impact on the variation in the percentage galacturonic acid (% GA) content of the extracted pectin (Refer to Equations B.8a – B.8b (Appendix B) on the percentage contribution). Fig 5.6 shows the percentage contribution of the effects on the variation of the % GA response.

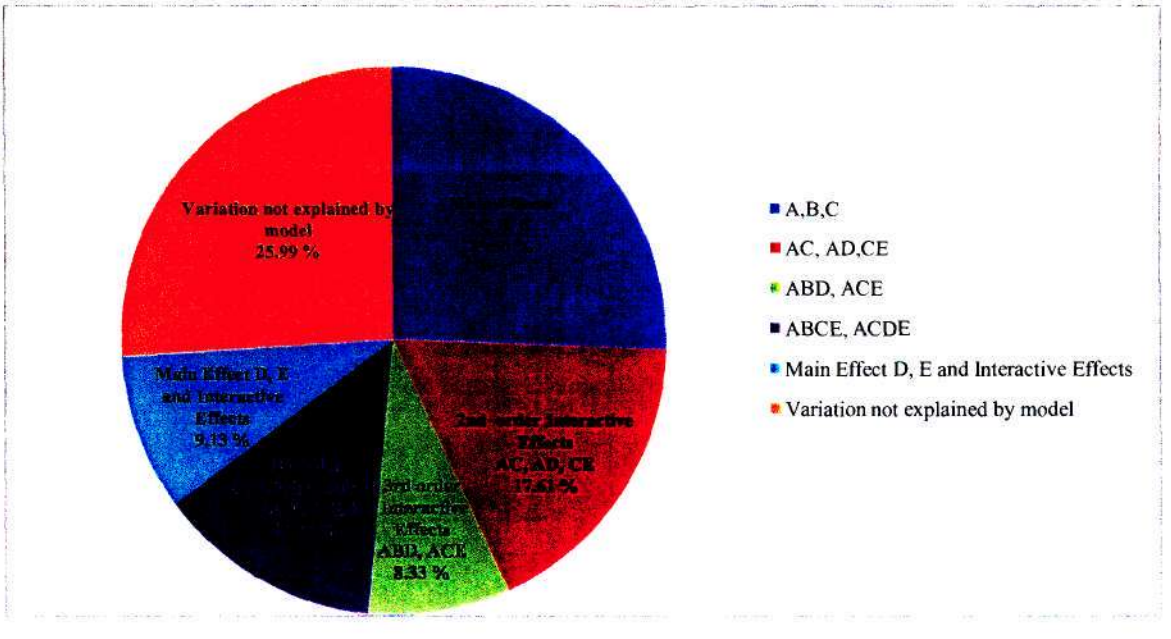

Fig 5.6: *Percentage contribution of effects on the % GA response for dried peel extraction*

From the pie chart (Fig 5.6), the main effect of temperature (A), time (B), and pH (C) were seen to contribute the greatest (25.56 % contribution) to the variation of the % GA response. These were followed by second order interactions of temperature-pH (AC), temperature-peel size (AD) and pH-dried peel to water mass ratio (CE), at a contribution of 17.61 %. Two forth order interactions of temperature-time-pH-peel to water mass ratio (ABCE) and temperature-pH-peel size-peel to water mass ratio (ACDE) came third in rank at a contribution of 13.38 %. In the fourth place followed the third order effects of temperature-time-peel size (ABD) and temperature-pH-peel to water mass ratio (ACE) at a contribution of 8.33%. The rest of the interactive effects together with the main effects of peel size (D) and dried peel to water mass ratio (E) contributed 9.13% to the variation of the % GA; within this 9.13% contribution, these main effects (D and E), only accounted for 0.64% of the contribution.

From the graph, it was also evident that the process variables chosen for investigation did not properly model the percentage galacturonic (% GA) response as a contribution of 25.99 % of unaccounted variability was found. This was in agreement with the conclusion previously made from the examination of the correlation coefficient. Levigne et al. (2001) investigated the impact of temperature, time and pH on the GA content and also found the GA content to be moderately impacted by these variables in their study.

Characterization of the most significant effects using the developed model:

From the F-Test and normal probability plot of effects, the main effects of temperature (A), extraction time (B) and pH (C) were shown to be significant. The regression coefficients of these effects were found to be positive and their values were 0.69, 1.46 and 0.59 respectively. As mentioned previously, the regression coefficients are in coded variables and therefore a direct comparison of the effects is possible (Montgomery, 2005). The effect of time was found to more than double that of temperature and pH over the level of variable domains investigated, showing it to be more significant in determining the % GA variation than the other two. The positive regression coefficients of the mentioned main effects (A, B and C) showed that an increase in these main effects individually, resulted in an increase in the percentage galacturonic acid (% GA) content. This means that an increase in time from 30 minutes to 2 hours or an increase in temperature from 70 $^{\circ}$ C to 90 $^{\circ}$ C or an increase in pH from 1.5 to 2.5 increased the % GA.

The impact of interactive terms in the variation of % GA response was found to be very significant; significant second order and higher order interactions together accounted for a total contribution of 39.32 % to % GA variation, as compared to that of 25.56 % found for significant main effects (Refer to Fig 5.6). Although individually an increase in temperature and pH increased the % GA content, but because the regression coefficient of the interactive effect term of temperature-pH was found to be negative (-0.94), increasing them both lowered the % GA content. The main effects of peel size and peel to water mass ratio were found to be insignificant on the % GA content, but an increase in the temperature and the peel size simultaneously (AD) was found to be significant and resulted in the lowering of the resultant % GA, as the regression coefficient of this interaction was found to be negative (-0.84). The opposite was true for an increase in the pH in unison with an increase in the peel to water mass ratio (CE); rather than lowering the % GA, it increased it. This was anticipated as the regression coefficient of this interaction was found to a positive value of 0.68.

Increasing the temperature, time and peel size altogether (ABD), had the effect of increasing the % GA (positive ß of 0.68); the interaction of temperature and time (AB) and time and peel size (BD) were insignificant, and that of temperature and peel size (AD) was found to lower the pH, but the interaction of the three, ABD, increased the % GA. The interaction of temperature, pH and peel to water mass ratio (ACE) had a regression coefficient that was positive (0.72). This meant that an increase in the three variables simultaneously increased the % GA content of the extract.

The regression coefficients of the forth order interactions of temperature, time, pH and peel to water mass ratio (ABCE) and of temperature, pH, peel size and peel to water mass ratio (ACDE) were found to be both negative. This means a concurrent increase in the variables that make up the interactive terms lowered the % GA content of the extract. In the case of ACDE, the third order interaction of ACE was found to increase the %GA, but increasing the peel size (D) at the same time, results in a decrease in the resultant % GA.

From these findings, it was deduced that to control the resultant % GA of the extract, interactive terms as well as main effects have to be monitored carefully as increasing two or more process variables together may result in an increase or decrease of the % GA content of the extracted pectin.

5.2.1.2(e) Comparison of literature and summarised experimental findings on the effects of the significant variables on % GA content

Effect of Temperature on the % GA response:

In this study the effect of increasing the temperature (A) variable independently from 70 °C to 90 °C, was found to increase the % GA response marginally (significant only from 95% CI). When the temperature variable interacted with the other variables of study (AC, AD, ABD, ACE, ABCE, ACDE), the effect of the interaction on the % GA variation was seen to be more significant (significant only from 99% and lower CI). These interactions either increased the % GA (ABD, ACE) or decreased it (AC, AD, ABCE, ACDE) as evident when inspecting their regression coefficients (Refer to section 5.2.1.2(d), pg 83-84).

Slightly similar results were found by Yapo et al. (2005) and Levigne et al (2001). Yapo et al. (2005) found that the % GA was moderately influenced by temperature (A) and temperature-time (AB) interaction and Levigne et al. (2001) found temperature (A) and the interaction of temperature and pH (AC) to moderately influence the resultant % GA. Like in this study, both researches found a moderate influence of the temperature main effect on the % GA, but Yapo et al. (2005) found a moderate influence of the AB interaction while in this study this interaction was found insignificant. Levigne et al. (2001) only noticed a moderate influence of the AC interaction while in study this interaction was found highly influential on the % GA response. In this study, two more variables that Yapo et al. (2005) and Levigne et al. (2001) did not investigate were considered (peel size (D) and peel to water mass ratio (E)), hence the significance of other interactions of the temperature variable that they did not report.

Effect of Time on the % GA response:

Increasing the time variable (B) separately within the investigated range of our study was found to significantly increase the % GA of the extract more than all other variables. The interaction of the time variable with other variables did not achieve a large change in the % GA, although increasing the extraction time with temperature and peel size concurrently (ABD) appreciably increased the % GA while a simultaneous increase with temperature, pH and peel to water mass ratio (ABCE) considerably decreased the % GA. Because the main effect of time was seen to be far more significant than the interactions, it could generally be deduced that an increase in time

increased the % GA content of the pectin extract. Contrary results were found by Robert et al. (2006), Yapo et al. (2005) and Levigne et al. (2001). Robert et al. (2006) found the time variable to be insignificant in determining the outcome % GA content of the extract. Yapo et al. (2005) found a moderated influence of time (B) and temperature-time interaction (AB) on the resultant % GA in their study. Levigne et al. (2001) found the main effect of time to be insignificant in their study. The reason for the discrepancy was thought to have been because the other researchers extracted pectin from a different source (chicory roots or sugar beet) to that of study (lemon peels). Therefore for lemon peel extraction, the extraction time was found to significantly influence the % GA content of the pectin extract.

Effect of pH on the % GA response:

The main effect of the pH (C) variable was only found to be significant from a CI of 90%. Increasing this variable therefore only had a minimal effect on the resultant % GA. Interaction terms of pH and other variables (temperature-time (AC), pH-peel to water mass ratio (CE), temperature-pH-peel to water mass ratio (ACE), temperature-time-pH-peel to water mass ratio (ABCE) and temperature-pH-peel size-peel to water mass ratio (ACDE)) were seen to be more significant (from a CI of 99%) and either increased (CE, ACE) or decreased (AC, ABCE, ACDE) the % GA content when the interaction variables were increased. Slightly similar results were found by Levigne et al. (2001) who reported a moderate influence of pH(C) and pH-temperature (AC) effect on the resultant % GA content of the extract. A moderate influence of the main effect of pH was also realised in this study, but contrary to Levigne et al. (2001), the AC interaction was found significant in determining the % GA content of the pectin. Contrary results were reported by Yapo et al. (2005) who found the pH (C) to be the most significant variable that influenced the % GA content, while Robert et al. (2006) found it to be insignificant. Because of the difference in the pectineceous source materials, these results were different from each other and different from our work.

Effect of peel size on the % GA response:

The influence of the main effect of peel size (D) on the % GA content was found to be insignificant in this study (even at a CI of 90 %). The interactive effects of this variable however, with other variables of study, were found to significantly impact the % GA content (from a CI of 99%). An increase of these interactive terms increased (ABD) or decreased (AD, ACDE) the % GA. Similar results were found by Robert et al. (2006) who investigated the

effect of milling on the % GA content. In their study they found the effect of milling to be insignificant in determining the % GA content of the extract as in this study.

Effect of dried peel to water mass ratio on the % GA response:

The main effect of dried peel to water mass ratio (E) was found to be insignificant in determining the % GA content of the extract even at a confidence interval (CI) of 90 %. As with the peel size variable, interactive terms of this variable (E) with the other process variables were found to be significant at from a CI of 99 %. Simultaneously increasing the peel to water mass ratio with the other variables either increased (CE, ACE) or decreased (ABCE, ACDE) the % GA content. Similar results to that found in this study were found by Robert et al. (2006) who investigated ratios of 1:29 and 1:51 on a dry basis. In their investigation they found the dried material to water ratio to be insignificant in determining the resultant % GA. In their study, they did not investigate interactive effects, thus the interactive terms that may have still been significant, as in this study, were not reported.

5.2.1.3 Percentage degree of esterification (%DE) for dried peel extraction

The percentage degree of esterification (% DE) of pectin indicates whether the pectin extracted is high molecular (HM pectin- DE>50%) or low molecular (LM pectin- DE<50 %). This is important in determining the type gel that results as pectin is mostly used as a gelling agent and HM pectin is usually industrially used to form gels. According to Thibault and Ralet (2003), HM pectin is further divided into slow set (DE of 58-65%), medium rapid set (DE of 66-69%). rapid set (DE of 71-74%) and ultra rapid set (DE of 74 – 77 %); the more rapid the pectin sets, the better the gelling properties of the pectin.

5.2.1.3(a) The range of the achieved degree of esterification (% DE)

The average (two replicas were investigated) percentage degree of esterification (% DE) found in our range of experimental conditions ranged from 59.29 % to 73.79 %. This showed that HM pectin was extracted from the peels as required by the pectin research consortium. The % DE range found also showed that the resulting pectin ranged from a slow set to rapid set type.

Yapo et al. (2005) found a range of 14.4 – 65.6 % in their study, Kalapathy and Proctor (2000) found a range 56 – 60 % and Joye and Luzio (2000) found a range of 64 – 77 %. Yapo et al. (2005) worked with beet pectin, Kalapathy and Proctor (2000) with soy hull pectin while Joye and Luzio (2000) worked with lemon peels. Harris and Smith (2006) in their findings reported that pectins extracted from lemon peels were of superior quality to all others; this explained why the range found in this study was greater than that reported in the reviewed literature and much more comparable to that of Joye and Luzio (2000).

5.2.1.3(b) Lowest and Highest degree of esterification (% DE) achieved

The least percentage degree of esterification (% DE) obtained in this study (59.29 %) was found at an extraction temperature (A) , extraction time (B) , pH (C), peel size (D) and peel to water mass ratio (E) of 90 ° C, 2 hours, 1.5, less than 1 mm and 1: 25 respectively. The greatest % DE was found when the temperature, extraction time, pH, peel size and peel to water mass ratio were at 90 ° C, 30 min, 2.5, less than 1 mm and 1:50 respectively.

From the reviewed literature Yapo et al. (2006) reported the least % DE at a temperature, time and pH of 90 ° C, 4 hours and 1.5 respectively and found the greatest % DE at a temperature of 80 ° C, an extraction time of 1 hour and pH of 2.5. Levigne et al. (2001) in their study found the least % DE at an extraction temperature, time and pH of 95 ° C, 90 minutes and 1 respectively. They found the greatest % DE at a temperature of 75 ° C, an extraction time of 30 minutes and a pH of 3. In both this reviewed studies, the least % DE was found at harshest conditions of the studied levels of temperature, time and pH and the greatest % DE was found at the mildest of these conditions. Similarly, in this study, the least % DE was found at the harshest conditions of temperature, time and pH. The greatest % DE was found at the mildest conditions of the studied levels of time and pH, but not temperature.

5.2.1.3(c) Empirical model and correlation coefficient for the % DE response for dried peel extraction

An empirical model was developed to explain the variation of the percentage degree of esterification (% DE) of the pectin extract in terms of the process variables investigated. A model adequacy check was performed on the model in order to prove that the experimental investigations followed a normal distribution, which is the basis of the ANOVA method used to

calculate the regression coefficients of this model. This was done by plotting a probability plot of the residuals. Any outliers from normality signify an inadequacy of the model. The model was proven adequate by this plot (Appendix B, Fig B.3). The developed model expression is given in Equation 5.3 and the model is shown in Table 5.3:

Model expression:

$$\%\ \mathbf{DE} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.3)$$

Table 5.3: *Empirical model developed for the % DE response in coded variables for dried peel extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effect	Regression coefficient	
	-	-	$ß_0$	68.49
	A	x_1	$ß_1$	-1.81
	B	x_2	$ß_2$	-0.56
	C	x_3	$ß_3$	2.30
Main Effects	***D	x_4	$ß_4$	0.39
	AB	x_{12}	$ß_{12}$	-0.68
	AC	x_{13}	$ß_{13}$	0.70
	BC	x_{23}	$ß_{23}$	0.65
	DE	x_{45}	$ß_{45}$	-0.70
	***ABE	x_{125}	$ß_{125}$	-0.41
	*ADE	x_{145}	$ß_{145}$	-0.52
	***CDE	x_{345}	$ß_{345}$	0.40
	ABCE	x_{1235}	$ß_{1235}$	-0.61
Interactive	*BCDE	x_{2345}	$ß_{2345}$	-0.53
Effects	**ABCDE	x_{12345}	$ß_{12345}$	-0.48

The unmarked effects are significant at 99 % and lower CI while those marked with (*) are significant at 97.5 % and lower CI, those marked as (**) are significant at 95 % and lower CI and those with (***) are significant at 90% and lower CI.

The analysis of variance (ANOVA) was performed on the experimental results and from it the adjusted correlation coefficient (R^2_{adj}) was calculated (Refer to Equation 2.33). This correlation coefficient only takes into account effects significant at a CI no less than 95 %. The R^2_{adj} was found to be 0.817. This meant that the model developed explained 81.7 % of the variability in the % DE response. This correlation coefficient was found to be less than that found for the % Yield response (0.994), but much greater than that found for the % GA response (0.488). It could therefore be concluded that the investigated process variables explained the variation in

the % DE response well. The graphical correlation coefficient was also found graphically by plotting the predicted results (calculated by using the empirical equation shown in Table 5.3) versus the experimental results; the graph is shown in Fig 5.7.

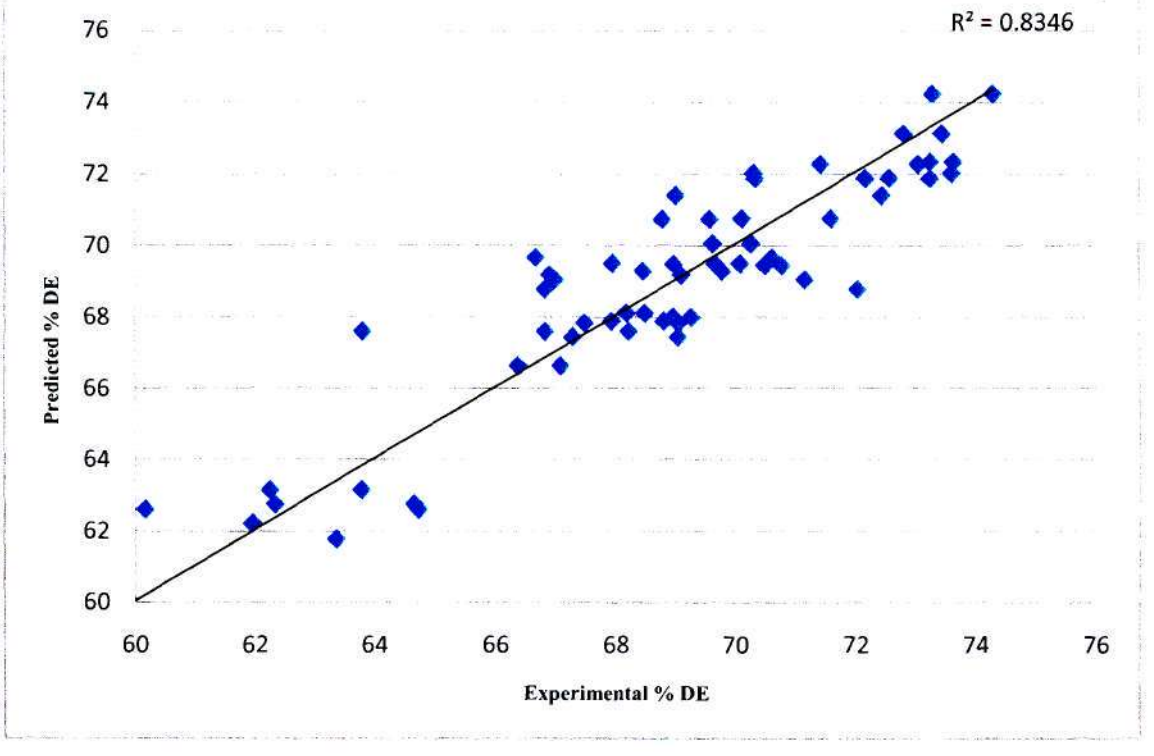

Fig 5.7: *The correlation plot of the experimental and predicted results for the % DE response for dried peel extraction (only significant effects were used in calculating the predicted values)*

The correlation coefficient found graphically (0.834) was greater than the adjusted correlation coefficient (0.817). This was because in the model, effect terms that were significant only from a confidence interval (CI) of 90 % were included in order to predict the results better.

5.2.1.3(d) Significant variables on the %DE for dried peel extraction

Examination of Empirical Model:

From the empirical model in Table 5.3, the main effects of temperature (A) and pH (C) seemed to be the most significant in determining the % DE of the resultant pectin. This was shown by their high regression coefficients compared to all the other significant terms. The main effect of time (B) was found to be significant at a confidence interval of 99 %, but the regression

coefficient of this effect (-0.56) was found to be lower than that of temperature (-1.81) and pH (2.30). The main effect of peel size (D) was only found significant from a CI of 90 %, while the main effect of peel to water mass ratio (E) was found insignificant at this CI.

Second order interactive effects of temperature-time (AB), temperature-pH (AC), time-pH (BC) and peel size-peel to water mass ratio (DE) were found to be significant at a CI of 99 %. The higher order interactions were found to be significant at lower CI (lower than 99 % CI) save for the forth order interaction of temperature, time, pH and peel to water mass ratio (ABCE) which was found significant at 99% CI.

Examination of Normal Probability Plot of effects:

A plot of the normal distribution of effects (Refer to pg 41 and 42 on the normal probability plot) was used to verify the significance of the effects. The plot is given in Fig 5.8:

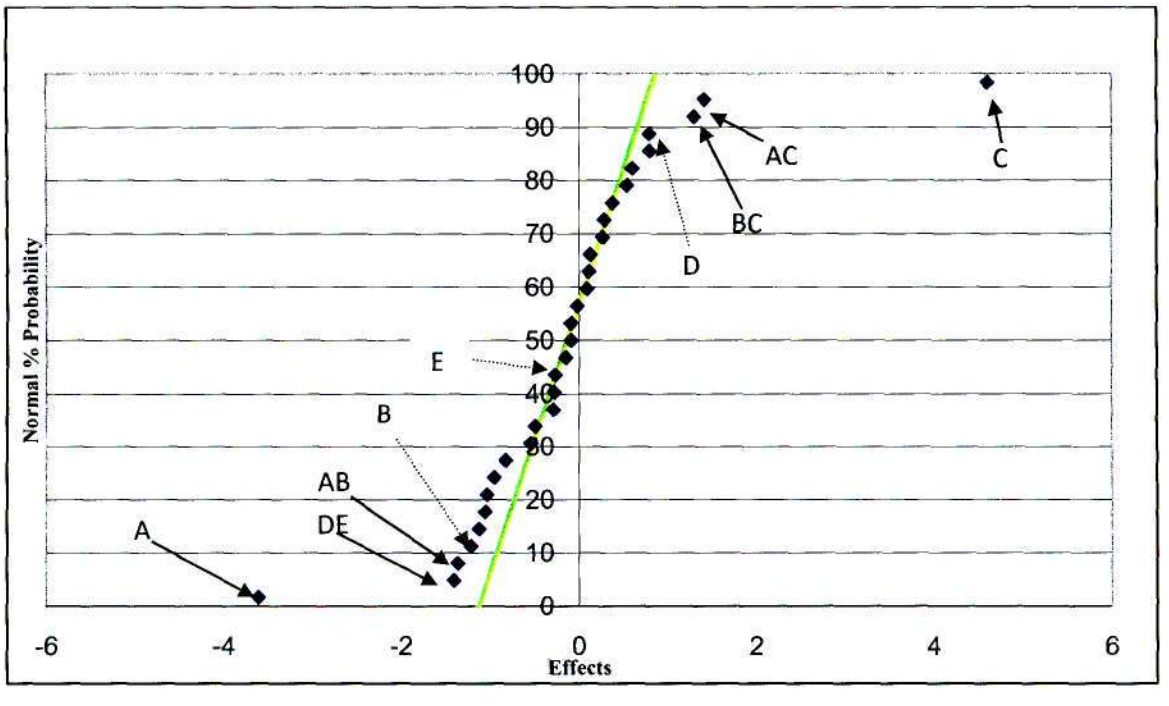

Fig 5.8: *Normal probability plot of the effects for % DE response for dried peel extraction (where A is the temperature variable, B the extraction time, C the pH, D the peel size and E the peel to water mass ratio)*

Usually, higher order interactive effects are not included in the model (Montgomery, 2005), as such only main effects and second order effects were shown on the graph above. It was evident though that the other higher order interactions (not labelled on the graph to reduce its cluster- ABE, ADE, CDE, ABCE,BCDE, ABCDE) were also significant as a deviation from normality of these effects was unmistakable from the graph. The main effect of pH (C) and temperature (A) were identified to lie the farthest from normality and as thus were the *most* significant. The main effects of time (B) and peel size (D) were shown to be less significant than temperature and pH, with peel size (D) close to insignificance. The dried peel to water mass ratio (E) main effect was not identified as significant at all and thus was not identified in the graph. Second order interactions of temperature and pH (AC), time and pH (BC), temperature and time (AB) and peel size and dried peel to water mass ratio (DE) were found to be significant. These results agreed with those deduced from the developed empirical model. The percentage contribution of the effects was calculated and is reported next; this identified if the main effects and second order effects identified were sufficient in predicting the % DE.

Examination of the Percentage Contribution of the effects:

The percentage contribution of the effects identifies the importance of the effects in predicting the response variable. The contributions of the effects were calculated and are shown in the pie chart in Fig 5.9.

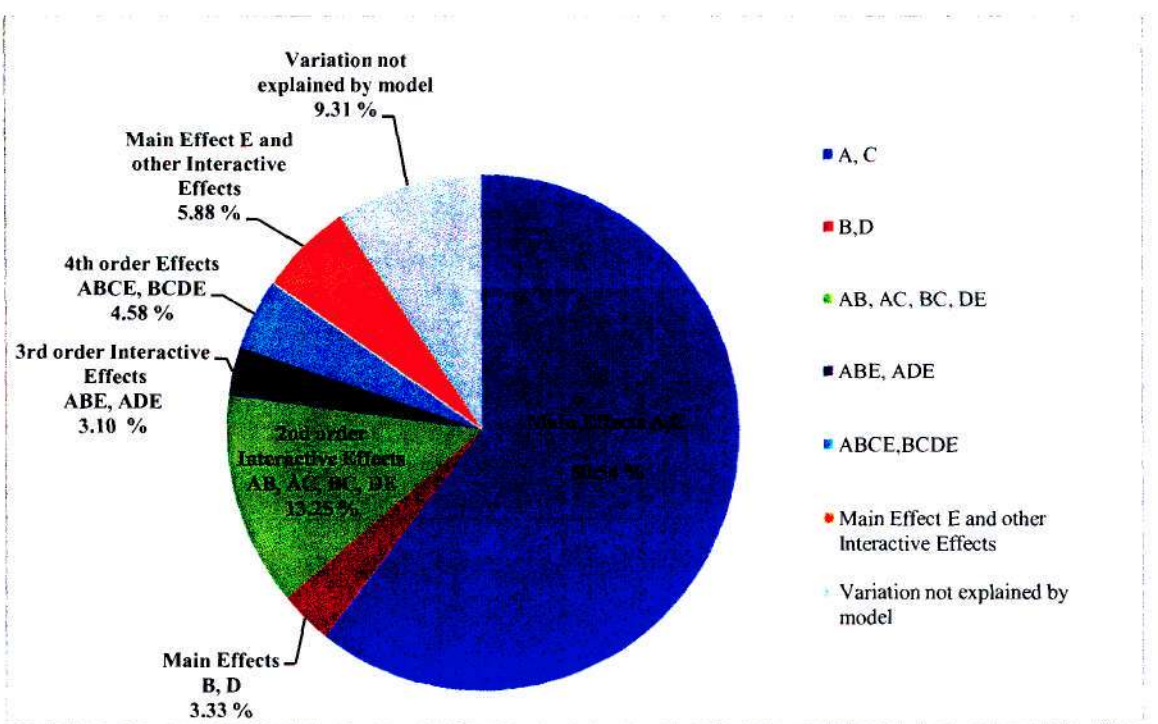

Fig 5.9: *Percentage contribution of effects on the % DE response for dried peel extraction*

Equations B.8a and B.8b (Appendix B) show how the percentage contribution for each effect was calculated. From the pie chart in Fig 5.9, the main effects of temperature (A) and pH (C) were found to have a contribution of 60.54 %. This meant that these effects explained 60.54 % of the variation of % DE of the pectin in this study. Second order interactions of temperature-time (AB), temperature-pH (AC), time-pH (BC) and peel size-peel to water mass ratio (DE), contributed 13.25 % towards the resultant variation of the %DE. A combination of this contributions showed that the main effects, A and C, together with the second order interactions, AB, AC, BC and DE, explained a total of 73.79 % of the variation of % DE. These effects were identified as the most important to monitor in order to determine the resultant %DE of the extract. For much better control though, the other significant effects should still be monitored.

Characterization of the most significant effects using the developed model:

From the model developed (Table 5.3), the main effect of pH (C) was found to have the largest positive regression coefficient (2.30). This showed that an increase in the pH increased the percentage degree of esterification (%DE) drastically. This was also proven by the fact that the lowest % DE of the extract was found at the low level of pH (1.5), while the greatest % DE was attained at the high level of pH (2.5) (Refer to section 5.2.1.3(b)) . The main effect of peel size (D) had a positive regression coefficient (0.39), which meant that an increase in this variable resulted in an increase in the % DE. The regression coefficient of pH (C) was found to be about 6 times greater than that of peel size (D) and therefore showed that a change in the pH had much more impact on the % DE than the peel size. The main effect of temperature (A) and time (B) had negative regression coefficients of -1.8 and – 0.56 respectively. This showed that an increase in either effect decreased the % DE. The regression coefficient of temperature was found to be approximately three times greater than that of time. Thus an increase in temperature had a much greater impact on the % DE than an increase in the extraction time.

An increase of temperature (A) and time (B) variables individually as shown above had the impact of decreasing the %DE. If increased concurrently they were found to decrease the % DE further as an interaction term of the two, AB, was found to be significant and to have a regression coefficient of -0.68. An increase in peel size and peel to water mass ratio simultaneously (CE) also had the effect of lowering the %DE (ß = -0.70), even though the change in the main effect of the peel to water mass ratio (E) was insignificant in determining the

% DE and an increase in the main effect of the peel size (D) almost insignificantly increased the %DE on its own. Increasing the temperature and pH (AC) concurrently had the effect of increasing the % DE and an increase in time and pH (BC) also had the impact of increasing the % DE. This was concluded from the fact that their regression coefficients were found to be positive at 0.70 and 0.65 respectively.

The interaction of temperature, time and peel to water mass ratio (ABE) and temperature peel size and peel to water mass ratio (ADE) both had negative regression coefficient of -0.41 and -0.52 respectively. Increasing the temperature (A), time (B) and peel to water mass ratio (E) simultaneously (ABE) and the temperature (A), peel size (D) and peel to water mass ratio (E) concurrently (ADE) had the effect of lowering the % DE, even though the main effect of peel to water mass ratio (E) was found to be insignificant in determining the % DE. The interaction of pH, peel size and peel to water mass ratio (CDE) had a positive regression coefficient (ß = 0.40), which meant that increasing CDE had the effect of increasing the % DE. All these significant interactive effects involved the main effect of peel to water mass ratio (E) that on its own was found insignificant.

Forth order interactions of temperature, time, pH and peel to water mass ratio (ABCE) and of time, pH, peel size and peel to water mass ratio (BCDE) were found to have negative regression coefficients (ß of -0.61 and -0.53 respectively). This meant that increasing all the variables involved in the interactive terms concurrently had the impact of decreasing the % DE. A fifth order interaction of all the variables investigated in this study (ABCDE) was also found to be significant in determining the %DE. The regression coefficient of this interaction was found to be -0.48, which meant that an increase of all the investigated process variables had the impact of decreasing the % DE of the pectin extracted.

5.2.1.3(e) Comparison of literature and summarised experimental findings on the effects of the significant variables on the degree of esterification (% DE)

Effect of Temperature on the % DE response:

In this study the temperature was found to have a significant effect on the resultant % DE of the pectin in the extract. Its main effect, A, and its interaction with time (AB) were found to lower

the % DE while its interaction with pH (AC) was found to increase the % DE. Other higher order terms of significant temperature interactions with the other investigated variables had the effect of lowering (ABE, ADE, ABCE and ABCDE) the % DE. Generally, the temperature seemed to appreciably decrease the % DE of the extract. Similar results were found by Yapo et al. (2005) and Mesbahi et al. (2004), who found the pectin extracted at higher temperatures to have a low % DE. This is because an increase in temperature degrades and de-esterifies the galacturonic acid chain in the pectin.

Effect of Time on the % DE response:

An increase in the main effect of the extraction time (B) was seen to decrease the % DE. The interaction of time and temperature (AB) was also found to decrease the % DE, while the interaction of time and pH (BC) was observed to increase the % DE of the pectin in the extract. All other higher order time interactions (ABE, ABCE, BCDE, and ABCDE) lowered the % DE of the pectin. In general, an increase in time seemed to decrease the % DE except when the pH was increased simultaneously. Yapo et al. (2005), Mesbahi et al. (2004) and Levigne et al. (2001) all found the same results; an increase in the time decreased the % DE. The contradiction was in the significance of the extraction time variable in determining the variability of % DE response.

Similar to this work, Levigne et al. (2001) found the time variable be significant in explaining the variability of the % DE response. Yapo et al. (2005) and Mesbahi et al. (2004) found contrary results. This was because in their study they investigated an extraction time of 1 to 4 hours, unlike in this study, where the extraction time was investigated from 30 minutes to 2 hours. Levigne et al. (2001) also investigated the time variable from 30 to 90 minutes, which explains why their results were in agreement with our work. Levigne et al. (2001) also found the main effect of time (B) to be moderately significant in determining the % DE of the extract, while the time-pH (BC) interactive term was highly significant. Similarly, in this study, the main effect of time (B) was found to be significant as well as the time pH interactive term (BC), with the interactive term found more significant than the main effect.

Effect of pH on the % DE response:

The pH (C) was found to be the most significant term in determining the resultant % DE of the pectin. An increase in the pH was found to result in an increase in the % DE. Interactive terms of pH and temperature (AC) and pH and time (AB) were also found to be significant and resulted in an increase in the % DE. Higher order interactions were observed to increase (CDE) or decrease the (ABCE, BCDE, ABCDE) % DE. All higher order interactions were less significant than the main effect and the second order interactions. All in all, an increase in the pH was seen to generally increase the % DE of the extract.

A decrease in the pH degrades the pectin; HM pectin becomes LM pectin; which is why the % DE was seen to increase with an increase in pH in this study. Similar results were found by Yapo et al. (2005) and Joye and Luzio (2000) who found the pH to be the most significant effect in their study. Levigne et al. (2001) found slightly different results: they found the main effect of pH (B) to have a moderate influence on the resultant % DE, while its interaction with time (BC) was found to be the most significant term in their study. The results found in this study also compared well with those found by Levigne et al. (2001), in that, the pH-time (BC) interaction was found to be significant.

Effect of peel size on the % DE response:

In this study, the effect of the peel size (D) was found to be less significant in determining the % DE (significant from a CI of 90 %.) than its interaction with other variables (significant at higher CI). An increase in the peel size could therefore increase (D, CDE) or decrease (DE, ADE, BCDE, ABCDE) the % DE depending on the interaction of the variables investigated. In most studies the effect of peel size on the degree of esterification of the pectin was not investigated. Robert et al. (2006) reported the effect of milling on the yield and galacturonic acid content, but not on the degree of esterification.

Effect of dried peel to water mass ratio on the % DE response:

The main effect of dried peel to water mass ratio (E) was found insignificant in determining the % DE in this study. However, its interactive effect with peel size (DE) as well as higher order interactions had a significant impact on the resultant % DE. Similarly, Robert et al. (2006) did

not find the main effect of peel to water mass ratio (E) to be significant in explaining the variability of the % DE response in their study.

5.2.2 Extraction from Fresh Wet Peels

The main purpose in this study for performing extraction experiments on wet peels was for comparison of the obtained results with those found from dried peel extraction. In the discussion that follows, a comparison of these results to those of dried peels is reported. The three response variables, the percentage yield (w/w on a **dry basis**), the percentage galacturonic acid content and the degree of esterification of the extract, were still monitored similarly to dried peel extractions.

Only four variables were investigated in this study; the temperature (A), the extraction time (B), the pH (C) and the peel to water mass ratio (E) on a **dry a basis**. The peel size variable (D), investigated for dried peels, was left out as the wet peel could not be minced to less than 1 mm size as in dried peel preparation. The minced wet peels investigated were approximately 3 mm and hence the results could be compared to the that found for large (2-4 mm) dried peel.

5.2.2.1 Extraction Yield (% Yield) for fresh wet peel extraction

The extraction yield response variable was measured the mass of the dried extract divided by the mass of the initial peel on a dry basis. Refer to Equation 3.3.

5.2.2.1(a) The achieved range of the yield (% Yield)

The range of the percentage yield achieved for fresh wet peels was 3.45 – 22.47 %. The range found for dried peels as reported earlier was 2.87 – 30.33 %. The range found for the wet peels was smaller than that achieved for dried peels. This may have been because of the added advantage of dried peels that they could be ground to a smaller size, thus increasing the mass transfer rate of the pectin into solution.

5.2.2.1(b) Lowest and Highest percentage yield (% Yield) achieved

The lowest yield found for wet peels was 3.45 % while that of dried peels was 2.97 % and the greatest percentage yield for wet peel was found to be 22.47 % while that of dried peels was 30.33 %. These conditions are shown in Table 5.4:

Table 5.4: *Comparison of extraction conditions required to achieve the lowest and highest percentage yield response for dried and wet peels*

Process Variables	Lowest % Yield		Highest % Yield	
	Dried Peels	Wet Peels	Dried Peels	Wet Peels
Temperature (° C)	70	70	90	90
Time (hrs)	0.5	0.5	2	2
pH	2.5	2.5	1.5	1.5
Peel to water mass ratio (w/w)	1:25	1:25	1:25	1:50

The lowest percentage yield for both wet and dried peel was found at the same conditions of extraction, while the greatest percentage yield was found at the same conditions of temperature, time and pH, but at different peel to water mass ratios.

5.2.2.1(c) Empirical model and correlation coefficient for the % Yield response for fresh wet peel extraction

A first order empirical model was developed to explain the variability of the response in terms of the investigated process variables. The expression of the developed model is given in Equation 5.4 and the model is shown in Table 5.5. A model adequacy check of this model was also conducted in order to ascertain that the normal distribution of data assumed for the ANOVA methodology utilised in this study was adhered to. The adequacy test (a normal probability plot of the residual) is shown in Fig C.1 (Appendix C), and from the plot no anomalies from normality were seen.

Model Expression:

$$\%\,\mathbf{Yield} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.4)$$

Table 5.5: *Empirical model developed for the %Yield response in coded variable notation for fresh wet peels extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effect	Regressor Coefficient (ß)	Value of Regression coefficient (ß)
Main Effects	-	-	$ß_0$	11.39
	A	x_1	$ß_1$	3.18
	B	x_2	$ß_2$	1.91
	C	x_3	$ß_3$	-3.14
	*E	x_5	$ß_5$	0.28
Interactive Effects	AB	x_{12}	$ß_{12}$	0.48
	AC	x_{13}	$ß_{13}$	-0.39
	AE	x_{15}	$ß_{15}$	0.35
	BC	x_{23}	$ß_{23}$	-0.44
	*CE	x_{35}	$ß_{35}$	-0.99
	ABC	x_{123}	$ß_{123}$	-0.44
	ABE	x_{125}	$ß_{125}$	0.45
	**BCE	x_{235}	$ß_{235}$	-0.26
	*ABCE	x_{1235}	$ß_{1235}$	0.30

The unmarked effects are significant at 99 % and lower CI while those marked with (*) are significant at 97.5 % and lower CI and those marked as (**) are significant at 95 % and lower CI.

The adjusted correlation coefficient (R^2_{adj}), which was found when only the significant effects were taken into consideration, was calculated to be 0.986 (Refer to Equation 2.33). This meant that the model explained 98.6 % of the variability in the % Yield response. Graphically, the correlation coefficient was found by plotting the predicted results versus the experimental results (Fig 5.10):

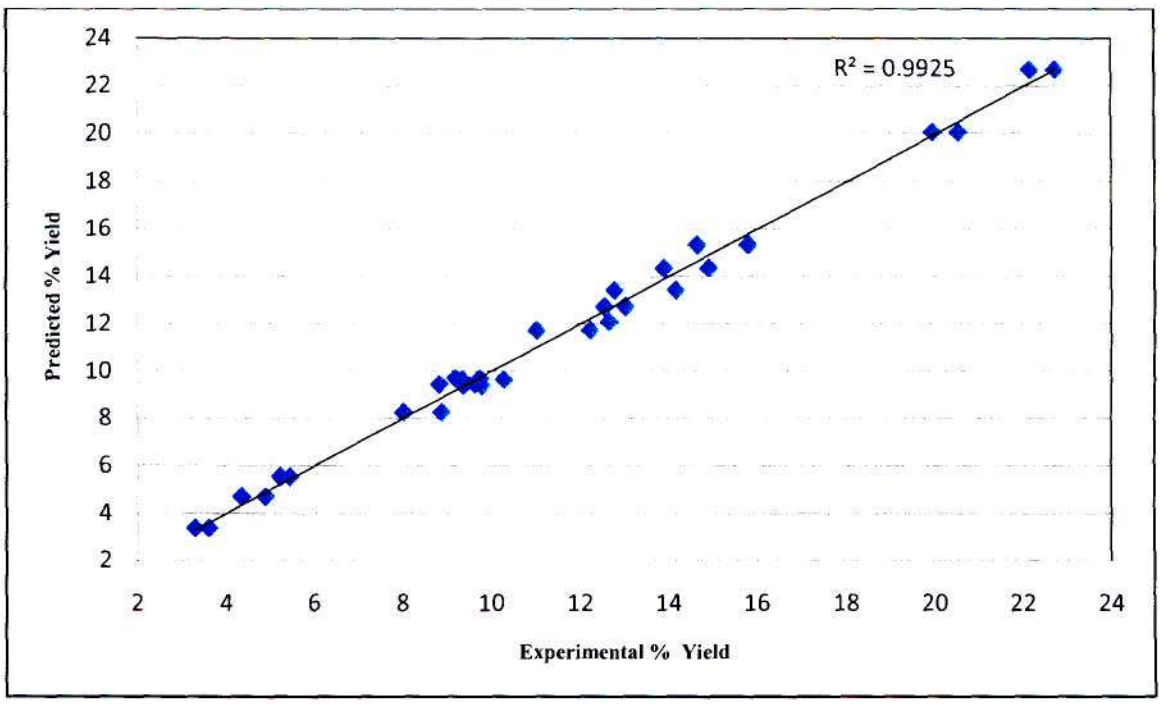

Fig 5.10: *The correlation plot of the experimental and predicted results for the % Yield response for fresh wet peels extraction (only significant effects were used in calculating the predicted values)*

From the graph, the correlation coefficient was found to be 0.992. This graphical correlation coefficient is similar to that found for dried peels, which was 0.994. A correlation coefficient close to 1 shows that the model developed explains the experimental data well. Both these correlations are close to 1 and therefore both show that the developed models explained the variability of the response (% Yield) adequately.

5.2.2.1(d) Significant factors or variables for fresh wet peel extraction

Examination of Empirical Model:

From the empirical model and statistical F-test, the *most* significant effects were found to be the main effect of temperature (A), time (B) and pH (C). The main effect of peel to water mass ratio (E) was only found significant at 97.5 % confidence interval. The other interactive effects were also found significant, but compared to the mentioned *most* significant main effects, they were less significant.

Similar results were found for dried peels as the main effects of temperature (A), time (B), pH (C) and peel size (D -not investigated with wet peels) were found to be *more* significant than other significant interactive effects. The peel to water mass ratio (E) main effect was also only found significant at 97.5 % CI.

Examination of Normal Probability Plot of effects:

The normal probability plot of effects (Refer to pg 41-42 on the normal probability plot) for fresh wet peels also established the same conclusions found from examining the developed model. This plot is shown in Fig 5.11:

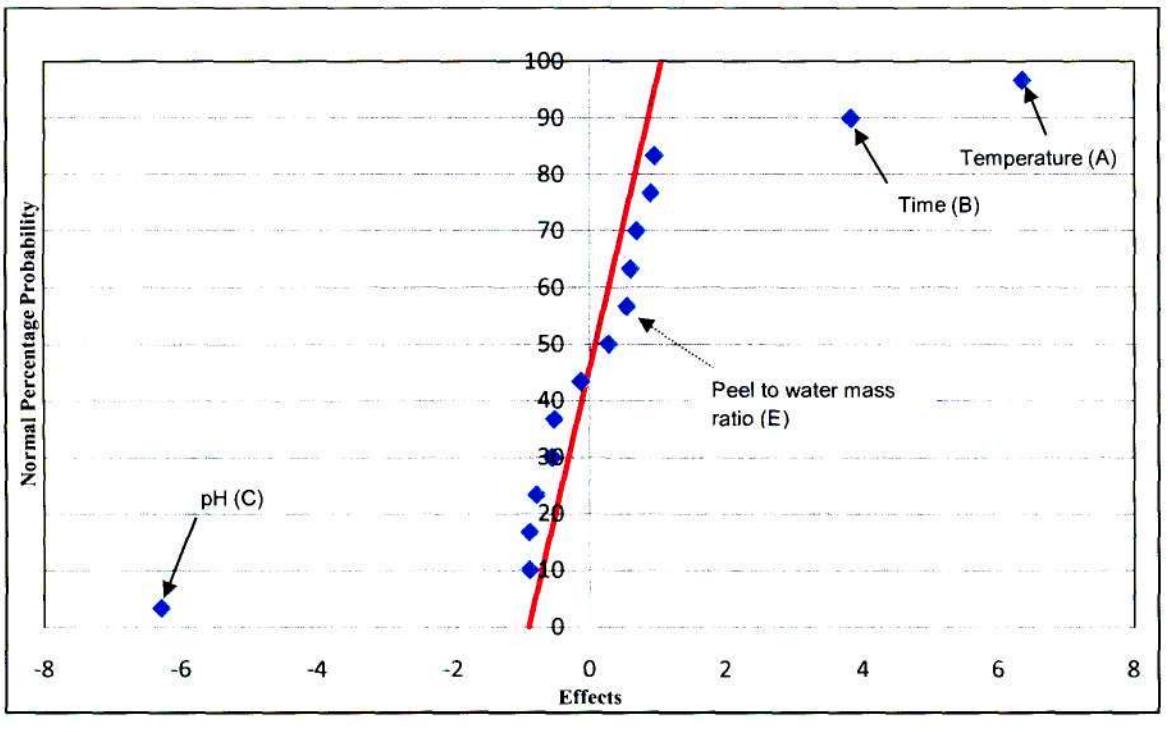

Fig 5.11: *Normal probability plot of the effects for the % Yield response for fresh wet peels extraction*

This plot also shows that the most significant effects were the main effect of temperature (A), extraction time (B) and pH (C) in predicting the percentage yield, as this effects lie the farthest from normality. All other significant interactive effects and main effect of peel to water mass ratio (E) were found to be less significant when compared to the most significant main effects. Similar results were found for dried peel extraction.

Examination of the Percentage Contribution of the effects:

The percentage contributions of the effects on the resultant percentage yield of the extract are presented in Fig 5.12. The contribution of each effect was calculated according to Equations C.7a and C7b (Appendix C).

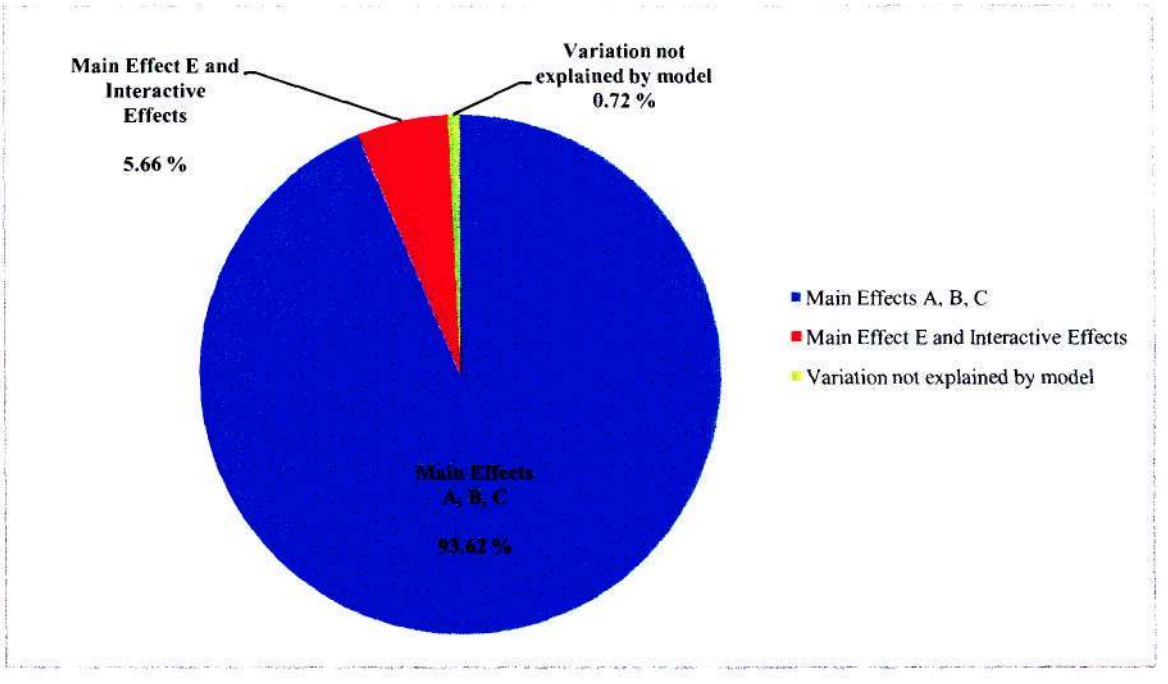

Fig 5.12: *Percentage contribution of effects on the % Yield response for fresh wet peels extraction*

The main effects of temperature (A), time (B) and pH (C) contributed 93.62 % to the model and the main effects of peel to water mass ratio (E) and interactive effects only contributed 5.66% to the variation of the percentage yield. Variations not explained by the model only contributed 0.72 %.

The main effects of temperature (A), time (B) and pH (C) for wet peels contributed 93.62 % to the variation of the percentage yield while this same effects for dried peels (together with the peel size (D) variable) contributed 89.81 % to the variation of the yield. These main effects, predicted the yield better in the case of wet peels than dried peel extraction.

Characterization of the most significant effects using the developed model:

The regression coefficients (ß) of temperature (A) and time (B) were found to be positive. This meant that increase in temperature from 70 ° C to 90 ° C, resulted in an increase in the yield, and an increase in the extraction time from 30 minutes to 2 hours, also resulted in an increase in the percentage yield. The main effect of temperature (ß = 3.18) was observed to increase the percentage yield more than that of time (ß = 1.91) as the coefficient of temperature (A) was greater than that of time (B). On the other hand, the regression coefficient of the main effect of pH (C) was found to be negative (ß = -3.14). This meant that an increase in the pH from 1.5 to 2.5 resulted in a decrease in the percentage yield.

5.2.2.1(e) A summarised comparison of wet and dried peel findings on the effects of the significant variables on the percentage yield

Effect of Temperature on the % Yield response for fresh wet peels extraction:

For both the peel types an increase in temperature (A) was observed to increase the resultant percentage yield. At elevated temperatures the plant matrix is improved resulting in the increased liberation of the pectin from the plant. This was also confirmed by Fishman et al. (2000).

Effect of Time on the % Yield response for fresh wet peels extraction:

An increase in the extraction time (B) was observed to result in an increase in the percentage yield for wet peels (within the time domain investigated in this study). The same was observed for dried peels. In both cases, the effect of time on the yield was found to be highly significant (from 99 % CI). As the time of exposure of the peel to the extraction medium increased from 30 min to 2 hours, the plant cell wall was broken down causing an increased liberation of the pectin with time. Yapo et al. (2005) also confirmed these findings in their study; they observed an increase in the yield with time in their study.

Effect of pH on the % Yield response for fresh wet peels extraction:

An increase in pH (C), from 1.5 to 2.5, was observed to decrease the resultant percentage yield for wet peels. Similar results were found for dried peels. A decreased pH facilitates in the

hydrolysis of the pectin in the peel into solution thereby increasing the extraction yield, which is why at an increased pH the pectin yield was seen to decrease. Pagan et al. (2000) found similar results; they reported a decrease in the yield resulting from an increase in pH from 1.4 to 2.

Effect of dried peel to water mass ratio on the % Yield response for fresh wet peels extraction:

For both wet and dried peels, the effect of peel to water mass ratio (E), although significant, was found to be less significant than that of the other main effects. An increase in the peel to water mass ratio main effect was observed in both cases to result in an increase in the % Yield response. The reason may be because an increase in the peel to water mass ratio causes an increase in the concentration gradient of the pectin, thereby increasing its diffusion into the extraction medium.

5.2.2.2 Galacturonic Acid (%GA) for fresh wet peel extraction

As mentioned previously, the % GA shows the purity of the pectin in the extract. Therefore, for good quality industrial pectin, the % GA should be greater than 65% (Food Chemical codex, 1981).

5.2.2.2(a) The achieved range of the percentage galacturonic acid (% GA)

The range of the percentage galacturonic acid (% GA) content achieved for wet peels was found to be 77.89 – 88.40 %. This was comparable to the range found for dried peels, which was 78.60 – 88.64 %. This range showed that good quality pectin was extracted from both wet and dried peels.

5.2.2.2(b) Lowest and Highest percentage galacturonic acid (% GA) achieved

The extraction conditions at which the lowest and highest % GA content (Identified from the range given in 5.2.2.2(a)) of the pectin extract for both fresh wet peels and dried peels were found are shown in Table 5.6:

Table 5.6: *Comparison of extraction conditions required to achieve the lowest and highest percentage galacturonic acid content for dried and wet peels*

Process Variables	Lowest % GA		Highest % GA	
	Dried Peels	Wet Peels	Dried Peels	Wet Peels
Temperature (° C)	70	90	70	70
Time (hrs)	0.5	0.5	2	2
pH	1.5	1.5	2.5	2.5
Peel to water mass ratio (w/w)	1:25	1:50	1:50	1:25

The extraction time and pH required to achieve the least % GA for both types of peels were found to be the same. The temperature and peel to water mass ratio however were found to differ; the low level of temperature (70 ° C) and of the peel to water mass ratio (1:25) were required for dried peel, while the high level of temperature (90 ° C) and of peel to water mass ratio (1:50) was required for wet peel.

The same conditions of extraction for the temperature, extraction time and pH were required to attain the greatest % GA, only the peel to water mass ratio differed; the high level of 1:50 was required for dried peel while the low level of 1:25 was required for wet peel.

5.2.2.2(c) Empirical model and correlation coefficient for the % GA response for fresh wet peel extraction:

A first order empirical model was developed to explain the variability of the % GA response in terms of the investigated process variables. The adequacy of the model was checked by the normal probability plot of the residuals (Appendix C.2.3). The model was found adequate as no residuals were seen to deviate much from normality (Refer to Fig C.2, Appendix C). The developed empirical model is given in Table 5.7 and the expression in Equation 5.5:

Model Expression:

$$\%\,\mathbf{GA} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.5)$$

Table 5.7: *Empirical model developed for the % GA response in coded variable notation for fresh wet peels extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effect	Regression coefficient (ß)	Value of Regression coefficient (ß)
Main Effect	-	-	$ß_o$	82.44
	A	x_1	$ß_1$	-1.96
Interactive Effect	**BCE	x_{235}	$ß_{235}$	-1.35

The unmarked effects are significant at 99 % and lower CI while those marked as (**) significant at 95 % and lower CI.

The adjusted correlation coefficient for the model, which only takes into account significant effects, was calculated according to Equation 2.33 and found to be 0.296. Graphically, the correlation coefficient was found by plotting the experimental versus the predicted results. The graph is shown in Fig 5.13:

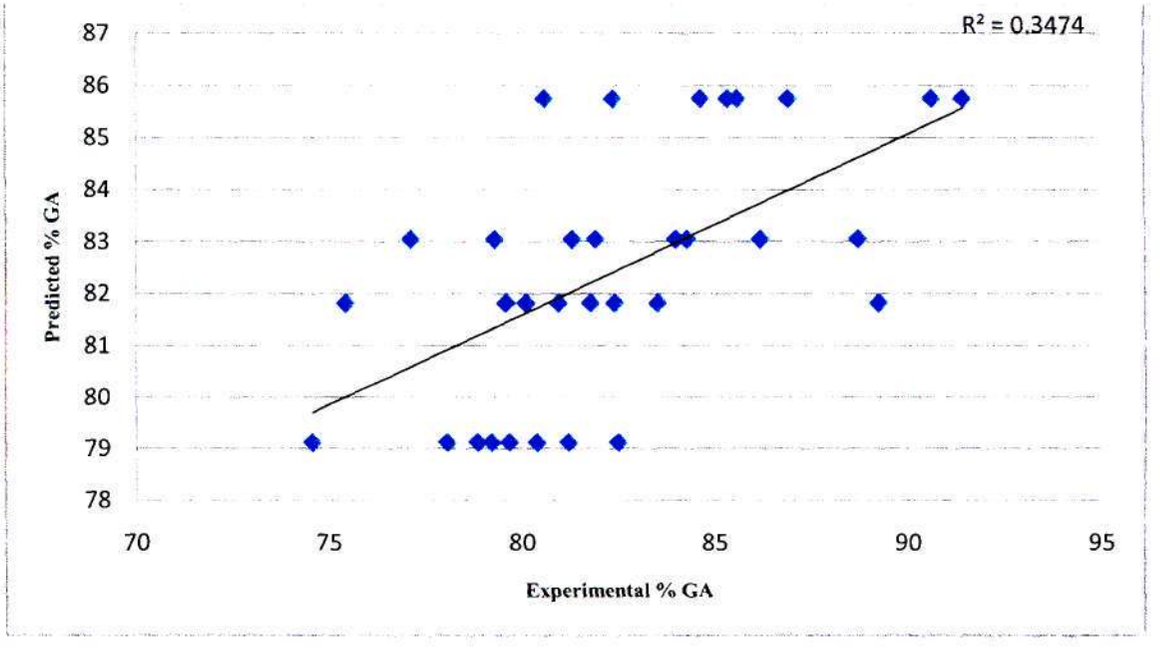

Fig 5.13: *The correlation plot of the experimental and predicted results for the % GA response for fresh wet peels extraction*

From this plot, the correlation coefficient was found to be 0.347. The developed model was thus shown to explain 34.7 % of the variability of the percentage galacturonic (%GA) acid content of the extracts found. This correlation coefficient was far less than 1, which showed that the

process conditions chosen either did not significantly cause the resulting variation in the % GA or the model developed was not sufficient in predicting the % GA values. The graphical correlation coefficient found for wet peels was much less than that found for dried peels (52.6 %), but both showed that the models developed did not best explain the variability of the % GA.

5.2.2.2(d) Significant factors or variables for fresh wet peel extraction

Examination of Empirical Model for fresh wet peels extraction:

From the model the only highly significant effect (from 99 % confidence interval (CI)) was found to be the main effect of temperature (A). The interactive effect of time-pH-peel to water mass ratio (BCE) was found to be significant only at 95 % CI. The other main effects (time (B), pH (C) and peel to water mass ratio (E)) and interactive effects were found to be insignificant (even at 90 % CI).

For dried peels, the main effect of time (B) was found to be most significant effect (at 99 % CI. The main effect of temperature (A) and then pH (C) followed, but these main effects were only significant at 95 % CI and 90 % CI respectively. The interactive effect of BCE was found significant for wet peel extractions but was insignificant at 90% CI for dried peel extraction, while second order interactions and some higher order interactions that were found insignificant for wet peels, were significant at 99% CI for dried peel extraction.

From these observations, it can be deduced that drying of the peel changes the extraction conditions that would have been significant in determining the % GA of pectin in wet peel extraction. Drying the material changes the structure of the peel and thus the diffusion mechanism of pectin from the peel changes with drying. Since the extraction is based on diffusion, the extraction conditions that may result in the optimum conditions of extraction for wet peel and dried peel consequently may differ.

Examination of Normal Probability Plot of effects for fresh wet peels extraction:

The normal probability plot of effects was also used to identify significant effects. The plot was constructed (Refer to pg 41-42) and the results are shown in Fig 5.14:

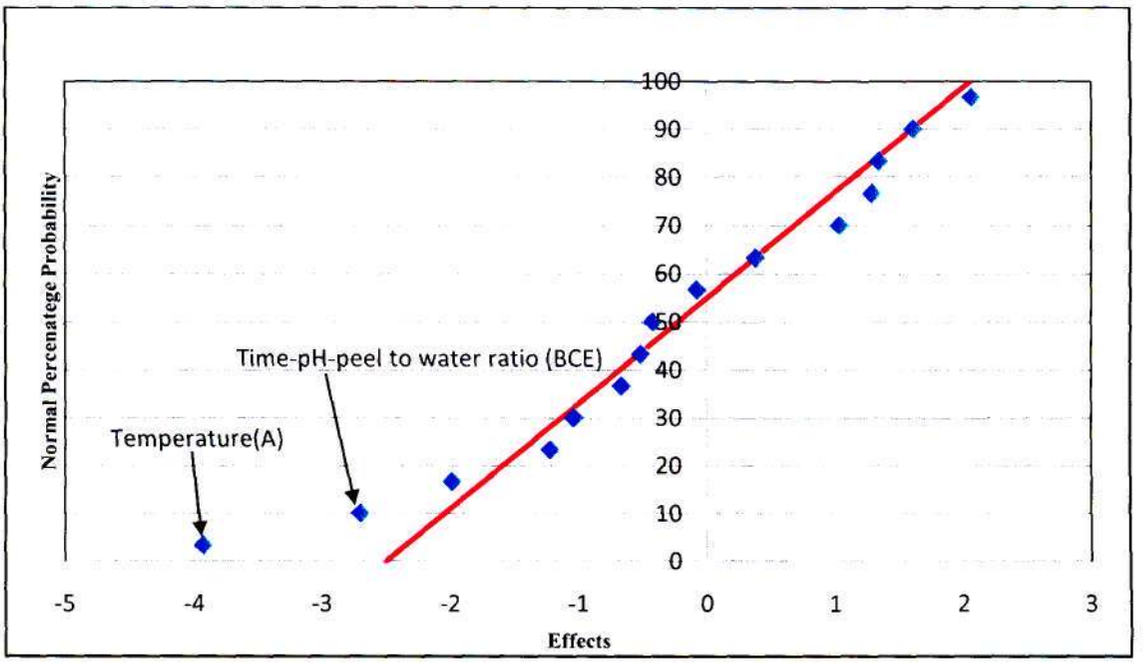

Fig 5.14: *Normal probability plot of the effects for the %GA response for wet peel extraction*

- From an examination of the graph, the main effect of temperature (A) and to a less extent, the interactive effect of time-pH-peel to water mass ratio (BCE), were the only effects identified as significant. These confirmed the results found from the examination of the empirical model

Examination of the Percentage Contribution of the effects for fresh wet peels extraction:

The percentage contribution of the main effects and interactive effects on the percentage galacturonic acid content response are presented in Fig 5.15. The contribution of each effect was calculated according to Equations C.7a and C7b (Appendix C).

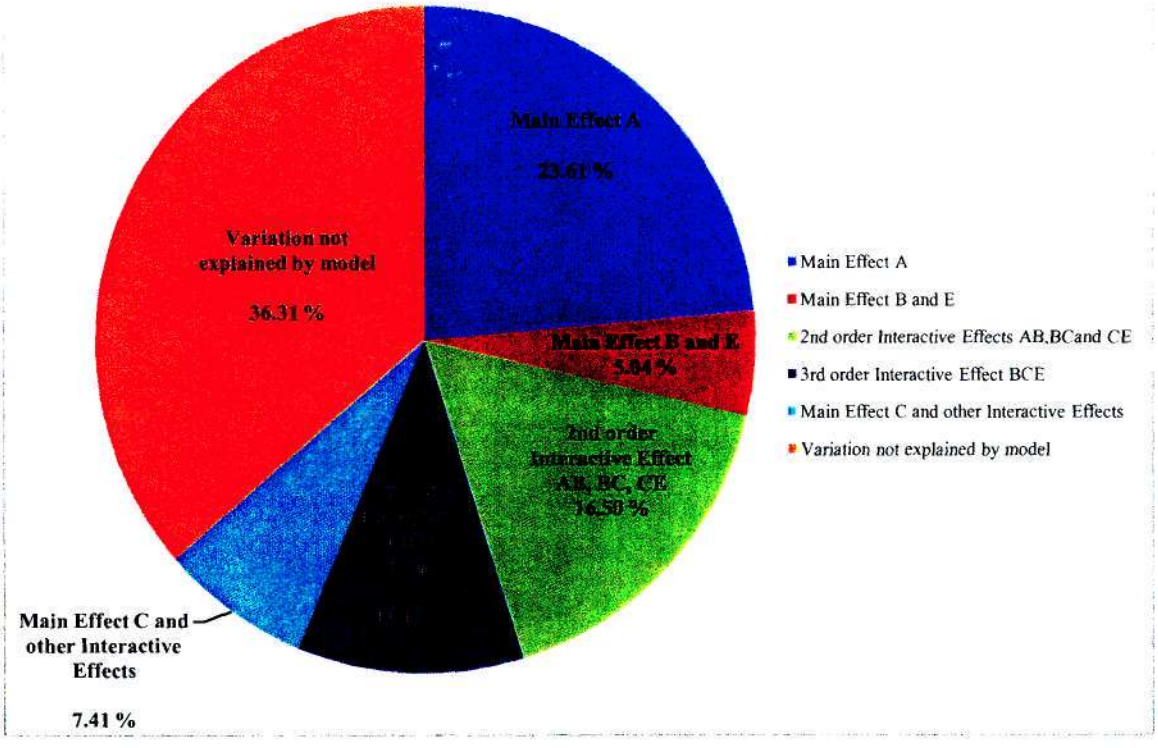

Fig 5.15: *Percentage contribution of effects on the % GA response for fresh wet peel extraction*

From the chart in Fig 5.15, the main effect of temperature (A) and the interactive effect of time-pH-peel to water mass ratio (BCE) together contributed 34.74 % to explaining the variation of the percentage galacturonic acid (% GA). The main effects of time (B) and peel to water mass ratio (E) contributed 5.04 %, but from the examination of the F-test, these effects were not even significant at 75 % confidence interval (CI). Second order interactive effects of AB, AC and BE were found to contribute 16.50 % to the variation of the % GA. Although these interactive effects showed a high contribution to the % GA content, they were found significant only at 75 % CI. This was considered too low a CI to include them in the empirical model, normal practise is to include effects significant at the lowest value of 95 % CI, although 90% CI can also be used. Other interactive effects and the main effect of pH (C) contributed 7.41 % to the variation of the % GA response and they were insignificant even at 75 % CI. A contribution of 36.31 % to the variation of % GA could not be explained by the developed model.

These results found from dried peels showed the main effects of temperature (A), time (B) and pH (C) to account for 25 99 %, while for wet peels only the main effect of temperature (A) accounted for 23.61 % of the % GA variation. Second order interactions, that affected the % GA

the most, only accounted for 6.52 % variation and were found insignificant at 90 % CI for wet peel extractions, while for dried peel they accounted for 17.61 % variation and they were significant at 99% CI. The variation not explained by the model for wet peels (36.31 %) was found to be greater than that found in dried peel extraction (25.56 %). Showing that the model developed for dried peels better describes the variation of the % GA than that of wet peels. Both contributions though, showed a great variation that could not be explained by either the investigated process variables or the model. The added investigation of the peel size (D) may have reduced the noise imposed by the peel size (D) on the response variable (% GA) and hence dried peel had a lower unaccounted variability than wet peels.

Characterization of the most significant effects using the developed model for fresh wet peels extraction:

Both regression coefficients (ß) of the main effect of temperature (A) and the interactive effect of time, pH and peel to water mass ratio (BCE) were found to be negative. This meant that an increase in the extraction temperature from 70 $^{\circ}$ C to 90 $^{\circ}$ C decreased the percentage galacturonic (% GA) content of the extract and increasing the time, pH and peel to water mass ratio concurrently, also had the effect of reducing the % GA of the extract.

5.2.2.2(e) A summarised comparison of wet and dried peel findings on the effects of the significant variables on the %GA response

Effect of temperature on the % GA response for fresh wet peels extraction:

The main effect of temperature was found to be more significant in determining the percentage galacturonic acid (% GA) of the extract for wet peel extraction (from 99% CI) than for dried peel extraction (only significant from 95 % CI). The interactive effects of temperature were found significant for dried peel, but not for wet peel in determining the % GA. In general, increasing the temperature was found to decrease the % GA for wet peel extractions, while increasing the temperature increased the % GA for dried peel extractions. This was attributed to the fact that drying the peel changed the plant matrix structure of the peel cell wall. Thus for dried peels, instead of the temperature degrading the peel and therefore lowering the % GA with

its increase, it helped hydrolyse it into solution. For wet peels, the increase in temperature degraded the galacturonic chain and thus caused the observed decrease of the % GA response. Yapo et al. (2005) reported the temperature to moderately influence the % GA in their investigations. Similar results were attained for dried peel extractions, but for wet peels, the temperature was seen to be the most, rather than moderate, significant variable that determined the % GA of the extract.

Effect of Time on the % GA response for fresh wet peels extraction:

The effect of the extraction time on the percentage galacturonic acid (%GA) was found to be insignificant for wet peel, but for dried peel it was found to be the most significant of all variables explored. The drying process appears to have changed the cell wall structure of the peel, as thus, the time of exposure to the extraction medium affected the dried peel and the wet peel differently; for dried peels, elongated time of exposure hydrolysed the peel significantly liberating the pectin into solution, thus causing the observed increase in the % GA with time, while for wet peels the effect was not as pronounced. Levigne et al. (2001), found the time insignificant in determining the resultant % GA, which was in agreement with results from wet peel extraction. Robert et al. (2006) found the extraction time to be significant, which was in agreement with dried peel extractions. This may have been because both these researches worked on different start materials (sugar beet and chicory roots respectively), which is why their results differed.

Effect of pH on the % GA response for fresh wet peels extraction:

The main effect of pH was found to be less significant (only significant from 90 % CI) than its interactive terms (found significant at 99 % CI) for dried peels. For wet peel extractions, this effect (pH) was found insignificant in determining the % GA of the extract; it only became significant when it interacted with time and peel to water mass ratio (BCE) (significant from 95 % CI). Because the plant cell wall structure of the peel was changed by the drying process, the pH affected the peel differently, with different interactions of the pH variable resulting in different outcomes of the % GA content of the pectin. Levigne et al. (2001) found the pH main effect to be a key factor in explaining the % GA variation, but Robert et al. (2006) found contrary results. The source material for the pectin in both these works was different (sugar beet and chicory roots), hence the difference in the impact of the pH on the % GA content of the

extract. The wet peel results were thus in agreement with Roberts et al.'s (2006) findings and dried peel extractions leaned towards Levigne et al.'s (2001) findings.

Effect of dried peel to water mass ratio on the % GA response for fresh wet peels extraction:

The main effect of peel to water mass ratio in both wet and dried peel was found to be insignificant (even at 90 % CI). The interactive effects of the peel to water mass ratio were found significant in both peel type extractions, although the interactive effect found significant for wet peel extraction (BCE) was found insignificant for dried peel.

5.2.2.3 Degree of Esterification (%DE) for fresh wet peel extraction

As mentioned previously, the degree of esterification (% DE) identifies the type of pectin extracted, with HM pectin found when the % DE >50 % and LM pectin when the % DE<50 %. In this study HM pectin was desired.

5.2.2.3(a) The achieved range of the percentage degree of esterification (% DE)

The achieved range of the percentage degree of esterification (% DE) found for wet peels was 68.44 – 78.91 %. The range found for dried peels was 59.29 – 73.79 %. As explained in section 5.2.1.3, this range (for dried peels) showed that slow set to rapid set HM pectin was extracted. In the case of wet peels, the range shows that medium rapid set to ultra rapid set HM pectin was extracted (Refer to Section 5.2.1.3 for further explanation on the pectin types). This means that pectin extracted from wet peels had slightly superior gel qualities to that extracted from dried peels.

5.2.2.3(b) Lowest and Highest percentage degree of esterification (% DE) achieved

The lowest and greatest percentage % DE found for wet peels and dried peels were found at slightly different extraction conditions. These are shown in Table 5.8:

Table 5.8: *Comparison of extraction conditions required to achieve the lowest and highest percentage degree of esterification response for dried and wet peels*

Process Variables	Lowest % DE		Highest %DE	
	Dried Peels	Wet peels	Dried Peels	Wet peels
Temperature (° C)	90	90	70	70
Time (hrs)	2	0.5	2	2
pH	1.5	1.5	2.5	2.5
Peel to water mass ratio (w/w)	1:25	1:25	1:50	1:25

In the case of the lowest degree of esterification (% DE), all conditions of extraction were the same for both peel types, save for the extraction time; the lowest % DE was attained at an extraction time of 30 minutes for wet peels, but 2 hours for dried peels. In order to attain the greatest degree of esterification, the only different parameter of extraction was found to be the peel to water mass ratio for the two peel types; the least peel to water mass ratio of 1:25 resulted in the greatest % DE for wet peels, while a ratio of 1:50 resulted in the greatest % DE for dried peels. All other parameters were the same.

4.2.2.3(c) Empirical model and correlation coefficient for the % DE response for fresh wet peel extraction

A first order empirical model was developed to explain the variability of the percentage degree of esterification (% DE) in terms of the investigated process variables. The model was then checked for its adequacy (a normal distribution of the experimental data was assumed in constructing the model); this was done be plotting a normal probability plot of the residuals. The plot is presented in Appendix C, Fig C.3, and from the plot, the model was found adequate as no deviations from normality were noted. The developed empirical model is given in Table 5.9 and its expression is presented in Equation 5.6:

Model Expression:

$$\%\,\mathbf{DE} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_{12} + \beta_3 x_3 + \beta_{13} x_{13} + \cdots + \beta_{12345} x_{12345} \ldots\ldots\ldots\ldots (5.6)$$

Table 5.9: *Empirical model developed for the % DE response in coded variable notation for fresh wet peels extraction (only accounts for significant effects)*

Effects	Factor Effect	Model Representation of Effect	Regression coefficient (ß)	Value of Regression coefficient (ß)
			$ß_o$	73.97
	A	x_1	$ß_1$	-1.31
	B	x_2	$ß_2$	1.22
Main Effect	C	x_3	$ß_3$	2.31
Interactive Effect	***AC	x_{13}	$ß_{13}$	0.72

The unmarked effects are significant at 99 % and lower CI while that marked with (***) are significant at 90% and lower CI.

The adjusted correlation coefficient, which only takes into account significant effects, was calculated according to Equation 2.33 and found to be 0.613. This meant that the process conditions investigated accounted for 61.3 % of the variability in the % DE response. A correlation coefficient close to 1 shows an excellent representation of the experimental results by the developed model. In this case, the adjusted correlation coefficient showed that the developed model only moderately explained the % DE variation. The correlation was also found graphically by plotting the experimental results versus the predicted model results (only significant effects at 90 % CI were included in the model). The graph is given in Fig 5.16:

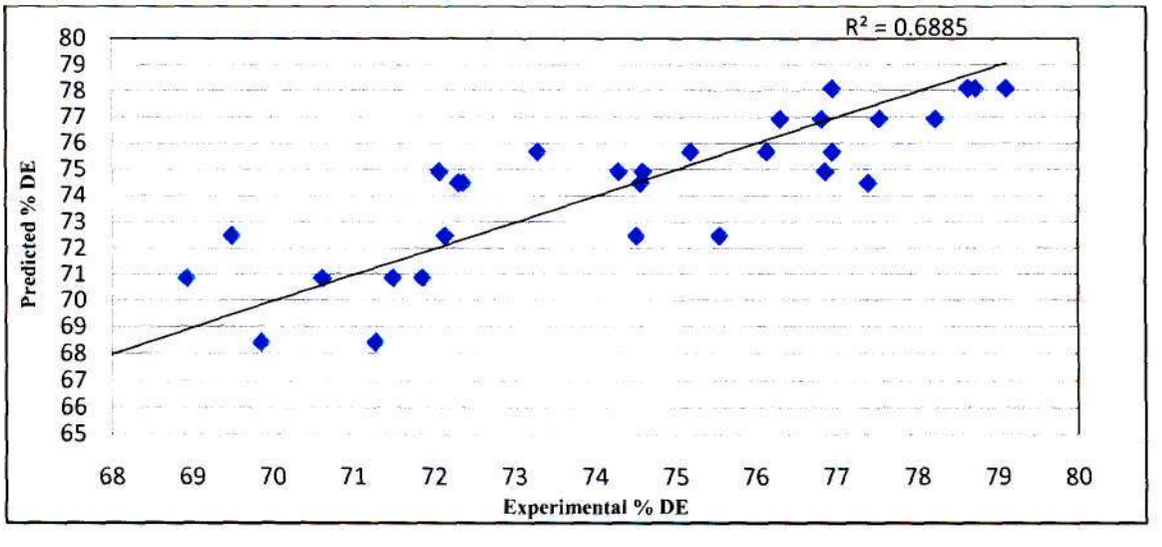

Fig 5.16: *The correlation plot of the experimental and predicted results for the % DE response for fresh wet peel extraction (only significant effects were used in calculating the predicted values)*

The plot in Fig 5.16 shows a correlation of 0.688 which meant that 68.8 % of the variability in the degree of esterification was explained by the model developed. This correlation was found to be greater than the adjusted correlation coefficient because significant effects at 90% CI were included in the model (Table 5.9), which was used to determine the predicted results used in the plot, in calculating the adjusted correlation coefficient, such effects are excluded. The graphical correlation coefficient for dried peels was found to be 0.843. This showed that the model developed for dried peels explained the variability of the degree of esterification better than that of wet peels.

5.2.2.3(d) Significant factors or variables for fresh wet peels extraction

Examination of Empirical Model for fresh wet peels extraction:

From the examination of the model, only main effects of temperature (A), time (B) and pH (C) were significant at 99 % confidence interval (CI). The main effect of peel to water mass ratio (E) was found insignificant at 90 % CI. The only second order interaction term that was found significant at 90 % CI was that of temperature and pH (AC), all other second order interaction and higher order interactions were insignificant at this CI.

All these effects found significant in wet peel extractions (A, B, C and AC) were also found significant in dried peel extractions, although other interactive effects were also found significant for dried peels. The main effect of peel to water mass ratio (E) was also found insignificant for dried peel extractions at 90 % CI.

Examination of Normal Probability Plot of effects for fresh wet peels extraction:

The normal probability of effects (Refer to pg 41-42 on the normal probability plot) was also plotted to affirm the results found from the empirical model and statistical F-test. The plot is given in Fig 5.17:

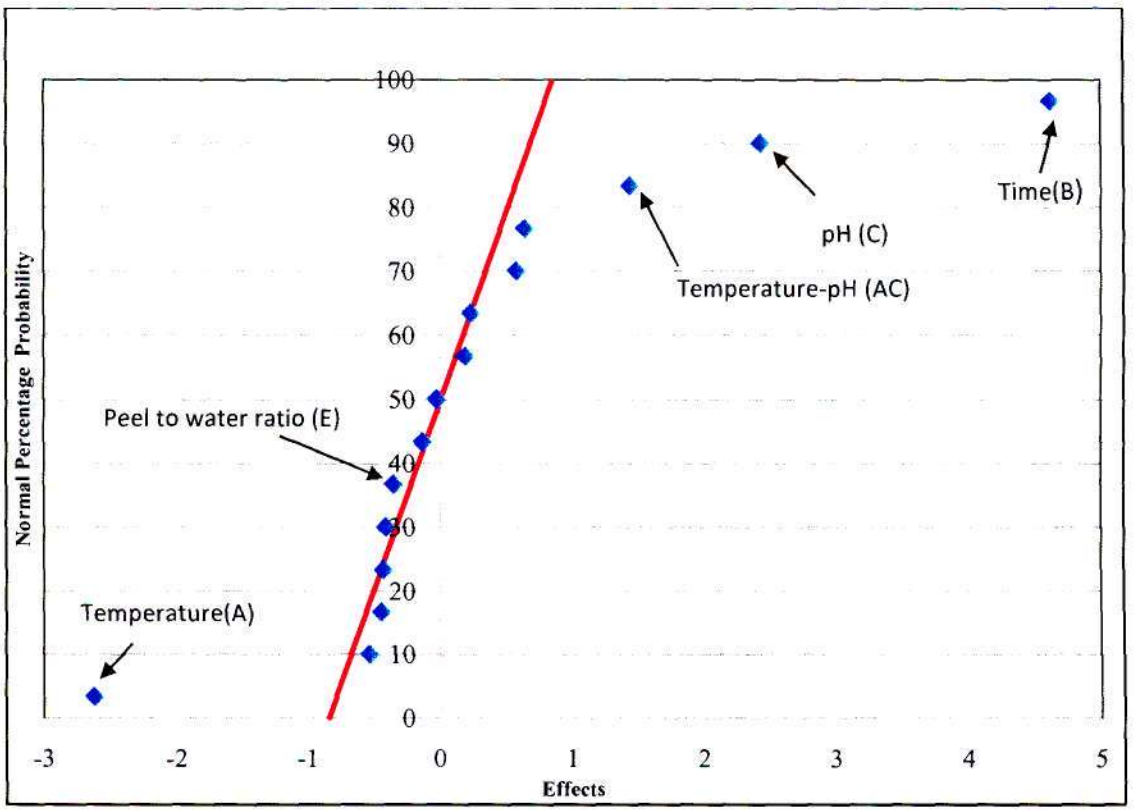

Fig 5.17: *Normal probability plot of the effects for the % DE response for fresh wet peels extraction*

From the graph the main effect of temperature (A), of time (B), of pH (C) and the interactive term of temperature and pH (AC) were observed to deviate from normality. This showed that they were significant in determining the variability of the percentage degree of esterification (% DE). The main effect of peel to water mass ratio (E) as well as other interactive effects (not labelled on the graph in order to reduce cluster) were found insignificant as they did not deviate from normality.

Examination of the Percentage Contribution of the effects for fresh wet peels extraction:

Fig 5.18 presents the percentage contribution of the effects (main effects and interactive effects) to the percentage degree of esterification response. The contribution of each effect was calculated according to Equations C.7a and C7b (Appendix C).

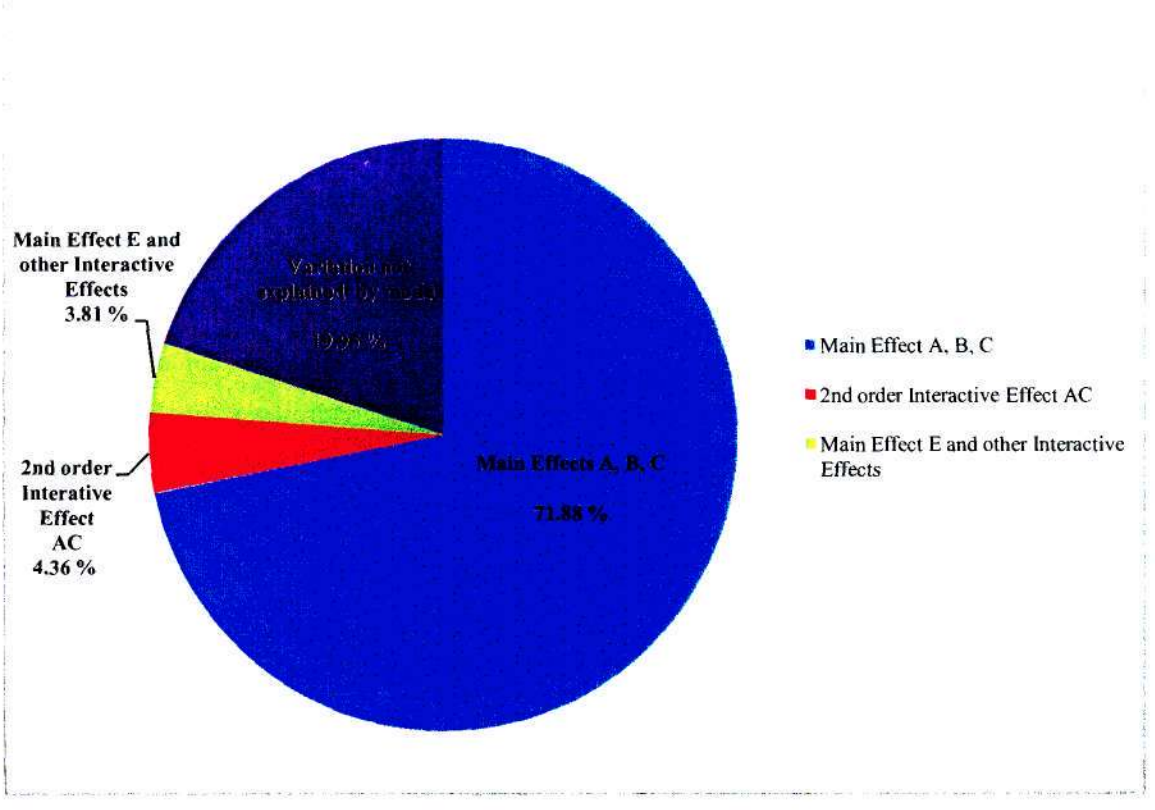

Fig 5.18: *Percentage contribution of effects on the % DE response for fresh wet peel extraction*

From Fig 5.18, the main effects of temperature (A), time (B) and pH (C) were found to contribute 71.88 % to the variability of the percentage degree of esterification (% DE). These main effects together with the temperature- pH interactive term (AC), contributed 76.24 % to the variability of the % DE response. The main effect of peel to water mass ratio (E) as well as other interactive terms only contributed for 3.81% to the variation of the % DE.

For dried peels, the main effects of temperature (A), time (B), pH (C) and peel size (D- not investigated with wet peel) contributed 63.87 % to the variation of the % DE. This was less than the 71.88 % found for wet peel extraction. The variation that could not be explained by the model was 9.31 % for dried peels while for wet peels it was 19.95 %. This could be because interactive terms found significant for dried peel extractions, improved the model accuracy, and the added investigation of the peel size (D) reduced the noise imposed by this parameter on the response variable (% DE).

Characterization of the most significant effects using the developed model for fresh wet peels extraction:

From the empirical model, the regression coefficients of time (B) and pH (C) were seen to be positive, with the coefficient of pH (2.31) much greater than that of time (1.22). These meant that an increase in pH from 1.5 to 2.5 had the effect of increasing the percentage degree of esterification (% DE), and increasing the time from 30 minutes to 2 hours also had the effect of increasing the % DE. The regression coefficient of the main effect of temperature was found to be negative (-1.31). This showed that increasing the temperature from 70 °C to 90 °C had the effect of decreasing the % DE of the pectin. The regression coefficient of the interactive term of temperature and pH (AC) was positive showing that an increase in the temperature and pH simultaneously had the effect of increasing the % DE of the pectin.

5.2.2.3(e) A summarised comparison of wet and dried peel findings on the effects of the significant variables on the percentage degree of esterification (% DE)

Effect of Temperature on the % DE response:

The main effect of temperature (A) was found significant for both wet and dried peel extractions. An increase in the main effect of temperature (A) had the effect of decreasing the % DE for both dried peel and wet peels. This showed that the temperature de-esterified the pectin chain for both peel types which caused the noted decrease in the % DE. An increase in temperature thus proved to be detrimental for both dried and wet peel.

Effect of Time on the % DE response:

In both wet and dried peel extractions the time of extraction was found significant in determining the resultant % DE of the pectin. An increase in the time of extraction increased the % DE for wet peels while it decreased the % DE for dried peels. The increased time of exposure to the extraction medium for wet peels helped in the liberation of the pectin from the peel, hence the observed increase in the % DE, while for dried peel the same increase increased the rate of de-esterification of the pectin molecule, resulting in the noted decrease in the % DE of the extract. The drying process seems to have changed the structure of the cell wall matrix of the peel causing the extraction time to have different effects on the two peel types.

Effect of pH on the % DE response:

The pH of the extraction in both wet and dried peel extractions was found to be a key factor in determining the resultant % DE. An increase in the pH was seen to increase the % DE for both peel types. The interaction of pH and time was also found significant in determining the % DE of the pectin for both peel types; an increase in the two increased the resultant % DE. Although low pH of the extraction medium assists in the liberation of pectin from the peel, it also has the adverse effect of causing the de-esterification of the pectin chain, which results in a low % DE. As a result an increase in the pH was seen to result in an increase in the % DE

Effect of dried peel to water mass ratio on the % DE response:

The peel to water mass ratio was found insignificant for wet peels. For dried peels the main effect of the peel to water mass ratio was also found insignificant, but unlike in wet peel extraction, the interaction of this variable with other process variables was seen to be significant in determining the % DE of the pectin.

5.2.3 Effect of storage on pectin extracted from Wet Peel

In order to substantiate the need for drying the pectin, fresh peel was minced and was kept for two days at room temperature. The juice manufacturing industry supplies the raw material (wet peel) needed for pectin production; as a result, the peel will have to be transported to the pectin plant from the juice manufacturing plant. Consequently, the pectin content may be degraded and de-esterified on arrival at the pectin producing plant. For this reason, peel extraction experiments, similar to those conducted on fresh wet peels, were conducted to assess the extent of degradation and de-esterification of the pectin given two days storage at atmospheric conditions.

Each investigated variable was represented by a letter; temperature is represented by letter **a**, time by **b**, pH by **c**, peel to water mass ratio by **e**. The different 'treatments' shown on the graphs that follow, represent the different experiments undertaken at different variable levels; when the variable was at its high level the letter representation of the variable was included in

the treatment. Thus treatment **ab,** meant the extraction experiment was performed at the high level of temperature and extraction time, but a low level of the other variables (**c** and **e**) investigated.

5.2.3.1 Effect on the Extraction Yield (% Yield)

Fig 5.19 shows the effect of storing the peels for two days at atmospheric conditions on the extraction yield. Similar experimental treatments as those undertaken for fresh wet peels were conducted for stored peel extractions and these were compared.

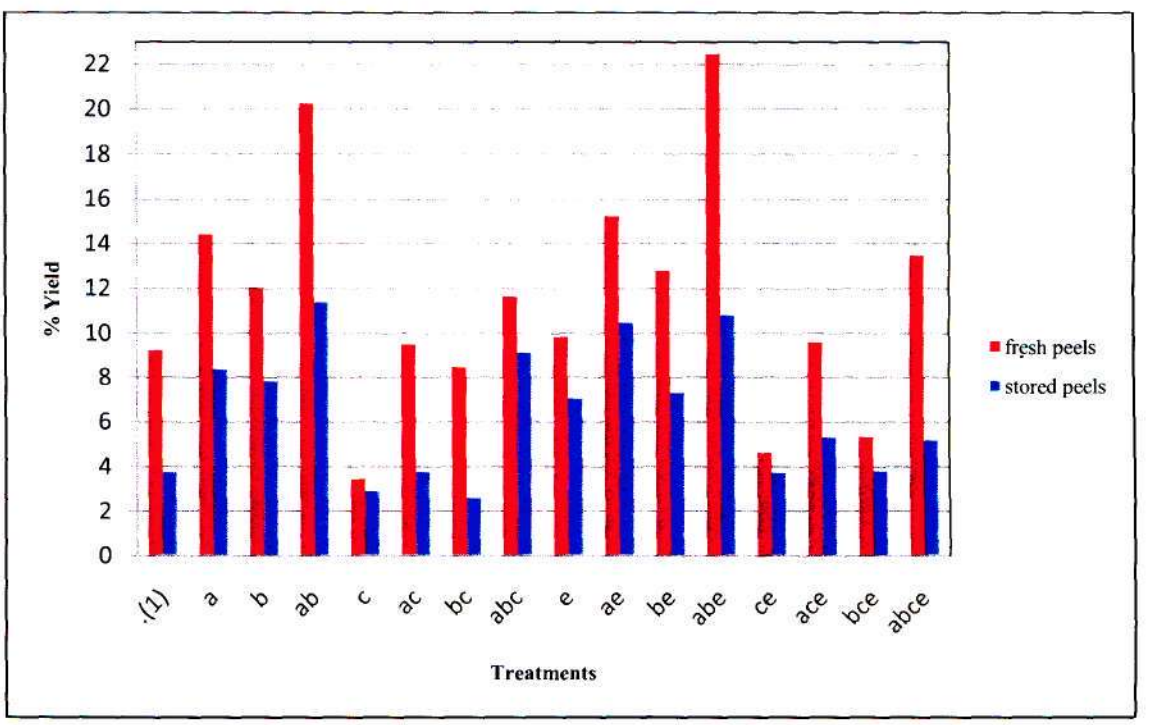

Fig 5.19: *The difference in the percentage yield (% Yield) of the extract for fresh wet peels and stored wet peels*

From the graph, it was evident that the yield of the stored peels was less than that of fresh peels for all treatment combinations. May (1990), stated that it is inadvisable to store the peel more than a few hours, unless it is specially treated.

The range of the percentage yield found for fresh wet peels was 3.45 % to 22.47 %, while that of stored wet peels was 2.62 % to 11.40 %; the greatest yield found for fresh peels (22.47 %)

was almost twice the yield found for stored peels (11.40 %). This showed that storage at atmospheric conditions had a drastic effect on the yield.

5.2.3.2 Effect on the Galacturonic Acid (% GA) content

Similarly, the percentage galacturonic acid (% GA) content of the extract was greatly affected by the storage time (Fig 5.20).

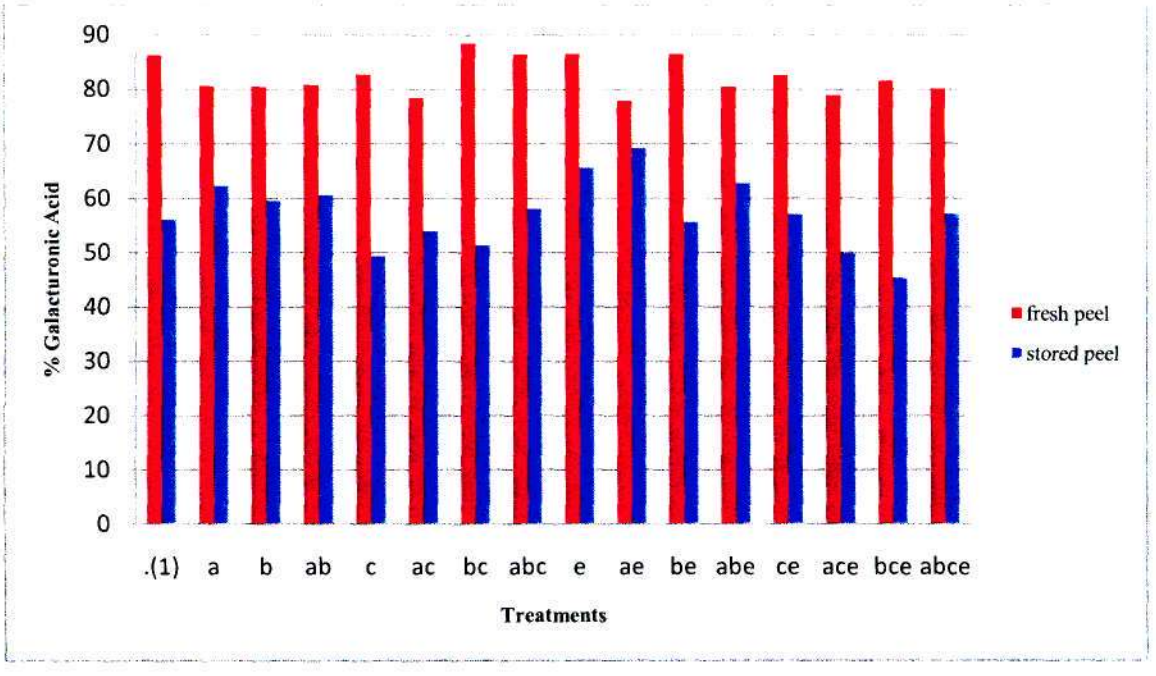

Fig 5.20: *The difference in the percentage galacturonic acid (% GA) content of the extract for fresh wet peels and stored wet peels*

The % GA content of the extract shows the amount of pectin in the extract; the greater the % GA the greater the pectin content in the extract. As thus, it was evident from Fig 5.20 that the extract from stored peels was of less pectin purity. Pectin of low purity is said to have low gelling properties.

According to USP XXII (Food Chemical Codex, 1981), the lowest limit of % GA for good quality pectin is no less than 74 % (% GA >74 %). The % GA range for stored wet peel was found to range from 49.44- 69.39 % while that of fresh wet peel was 77.89 – 88.40 %. As thus,

the pectin extracted from fresh peels was found to be within the acceptable USP XII limit, while the stored peel was below the required % GA imit. The reason for this degradation in pectin quality in stored peels was because of the activity of enzymes during storage. May (1990) gives three types of enzymes responsible for pectin degradation, these are polygalacturonas, pectin-lyase and pectate-lyase. It was shown in this study that within two days of storage, these enzymes had degraded the pectin content of the peel considerably.

5.2.3.3 Effect on the Degree of Esterification (% DE)

Fig 5.21 shows the effect of storage on the percentage degree of esterification (% DE) of fresh and stored wet peel found at different experimental treatments.

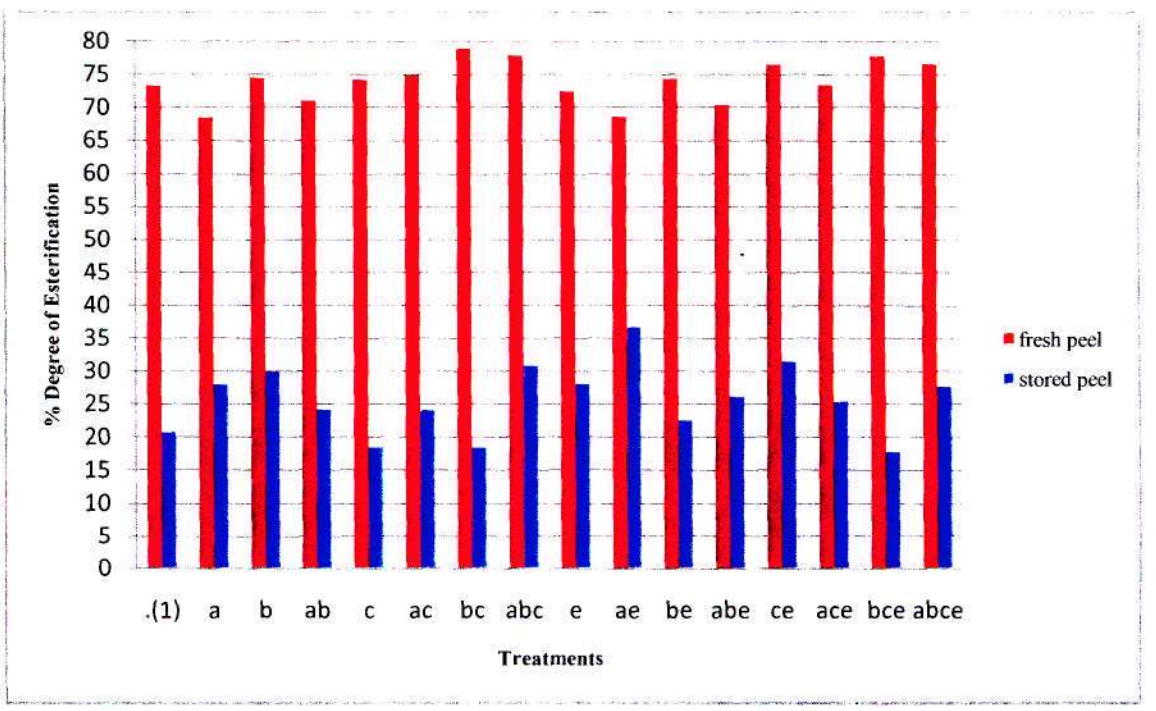

Fig 5.21: *The difference in the percentage degree of esterification (%DE) of the extract for fresh wet peels and stored wet peels*

The degree of esterification (% DE) was severely affected by storage. The range of the % DE found from stored peel investigations was 17.80 – 36.74 %, while that for fresh peel was 68.64 – 78.91 %. The least % DE found for fresh peels was more than three times greater than that of stored peel and the greatest % DE for fresh peels was almost twice that of stored peel. The %

DE of the extract from stored peel showed LM pectin (% DE < 50 %) rather than the required HM pectin (% DE > 50 %) to have been extracted.

Wet peel is said to contain natural methylesterase, which during storage of the peel de-esterifies the pectin within the peel; this can render the pectin undesirable for most applications (May, 1990). Our results were confirmed by Pagan et al. (2000), who also showed that stored peel results in pectin of lower % DE than fresh peel.

5.2.4 Optimization of Dried Peel extractions

In order to optimise the extraction process, contour plots of second order models of the three investigated responses were overlaid. The discussion that follows shows the development of these models. The models are then compared to first order linear models. The resultant contour plots are then shown and the optimum operating conditions are identified.

5.2.4.1 Development of 2nd order model using Central Composite Design (CCD)

The functional relationship of the investigated process variables and the monitored (response) variables was developed into a 2nd order model by using a central composite design (CCD). A face centred design (FCCD) was used because the area of interest lay between the chosen low and high levels of the five investigated process variables. MATLAB was used to calculate the regression coefficients. Stepwisefit function was then used to identify the coefficients that were significant at 95 % confidence interval and these coefficients were used in the model. Only main effects, main effect quadratic terms and second order interactions were used in the model; higher order interactions were not included as is common in such second order models (Montgomery, 2005). The developed empirical models represented in coded variables were found to be:

$$\%\text{Yield} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_{12} + \beta_{13} x_{13} + \beta_{24} x_{24} + \beta_{34} x_{34}$$

$$= 14.65 + 4.20 x_1 + 2.19 x_2 - 3.94 x_3 - 3.23 x_4 + 0.99 x_{12} - 0.84 x_{13}$$

$$+ 0.83 x_{24} + 0.54 x_{34} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5.7)$$

$$\%\text{GA} = \beta_0 + \beta_2 x_2 + \beta_{13} x_{13} + \beta_{14} x_{14} + \beta_{11} x_1^2$$

$$= 86.64 + 1.39 x_2 - 0.94 x_{13} - 0.84 x_{14} - 2.24 x_1^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5.8)$$

$$\%\text{DE} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_{12} + \beta_{13} x_{13} + \beta_{23} x_{23} + \beta_{45} x_{45} + \beta_{22} x_2^2$$
$$+ \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{55} x_5^2$$

$$= 78.46 - 1.75 x_1 - 0.58 x_2 + 2.33 x_3 + 0.46 x_4 - 0.68 x_{12} + 0.70 x_{13} + 0.65 x_{23}$$
$$- 0.70 x_{45} - 2.50 x_2^2 - 2.37 x_3^2 - 2.56 x_4^2 - 2.49 x_5^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5.9)$$

5.2.4.2 Comparison of first and second order model Correlation Coefficients

The correlation coefficients (R^2_{adj}) of the above models were calculated to be 0.909 for the percentage yield (% Yield) response, 0.244 for the galacturonic acid content (% GA) response and 0.874 for degree of esterification (% DE) response.

The coefficient of the percentage yield response was found to be slightly less than that of the first order model developed previously (0.994). This was because in the first order model third, fourth and fifth order interaction effects were included in the model while in the CCD model, only second order interactions were included. The model (% Yield) was still seen to be linear as no quadratic terms were found significant in its development. The adjusted correlation coefficient of this CCD model still showed that the model sufficiently represented the experimental results.

The second order model correlation coefficient (R^2_{adj} = 0.224) for the % GA response, was found to be half that of the first order model (0.488). The decrease was still attributed to the

inclusion of the higher order interactions in the first order model. This second order model, as with the first order model, showed that the chosen variables and or variable levels in this study did not sufficiently explain the variation of the % GA response. Similar results were found by Levigne et al. (2001).

The correlation coefficient for % DE response (R^2_{adj} = 0.874) was found to be greater for the second order model compared to the first order model (0.817). This showed that quadratic effects were very significant in explaining the variation of this response.

5.2.4.3 Multiple Response Analysis

Three response variables were investigated in this study; therefore the optimum region for the extraction simultaneously considers all the responses. In order to optimise this process, the contours of the developed 2nd order models were constructed by varying two of the investigated parameters between -1 and 1, while holding the rest at 0 level (coded variable levels were used). The contours of the different response variables were then superimposed in order to identify the optimum region. The most favourable region incorporated the highest % Yield and %GA, while a % DE at or above 74% (Ultra rapid set pectin) was most preferred. The graphs that follow show the overlaid contours and also identify the optimum conditions: the x-value and y-value of the two varied extraction variables (in coded variables) and the level of the optimum percentage yield are identified on the graphs. The blue contours represent the % Yield response, the maroon contours represent the % GA response and the red dotted contours represent the % DE response in all contour plots.

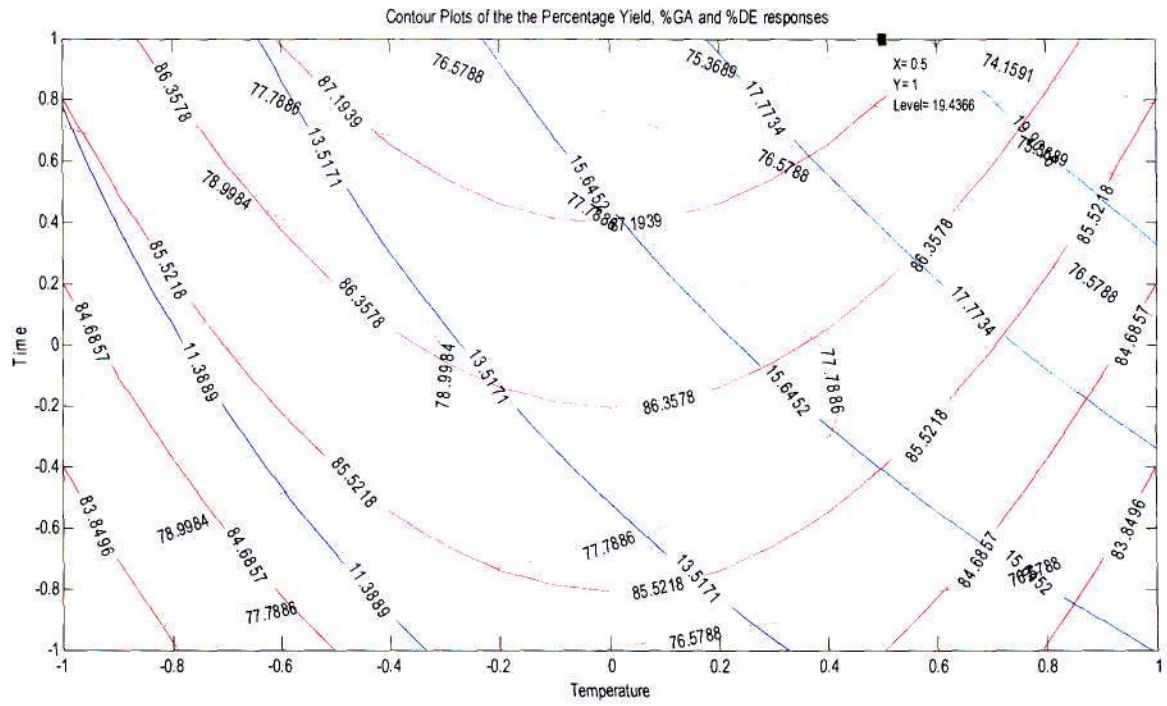

Fig 5.22: *Contour plots for the monitored responses when varying temperature and time variables and holding the other variables constant*

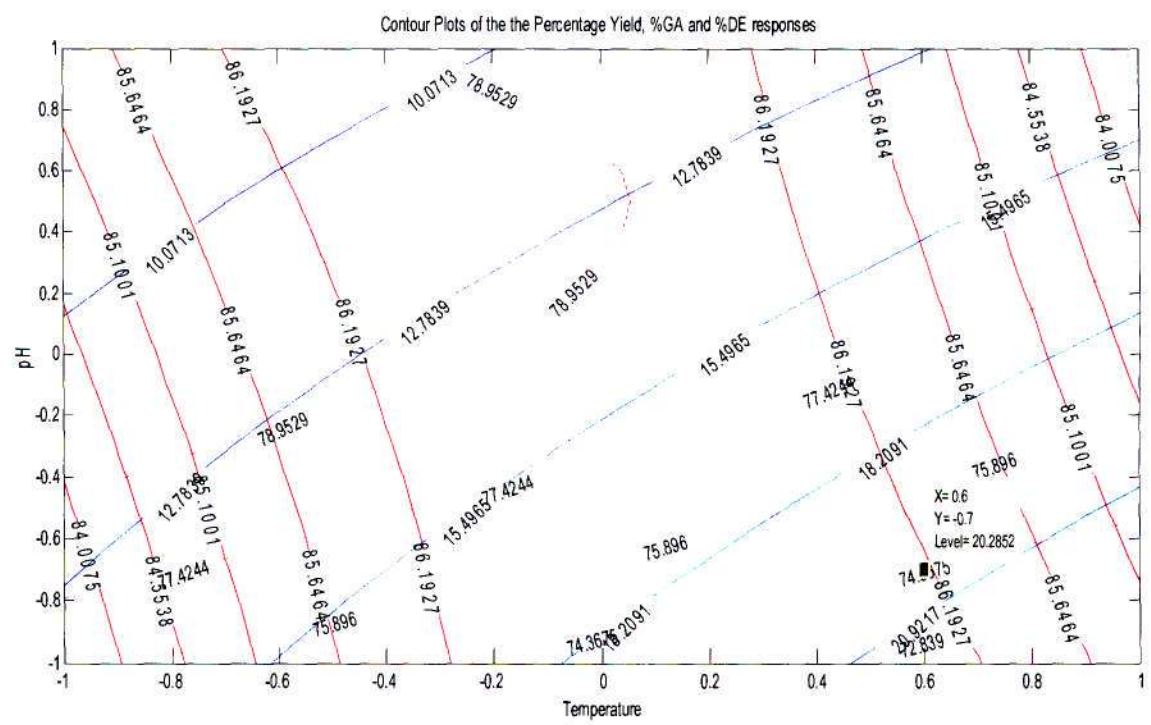

Fig 5.23: *Contour plots for the monitored responses when varying temperature and pH variables and holding the other variables constant*

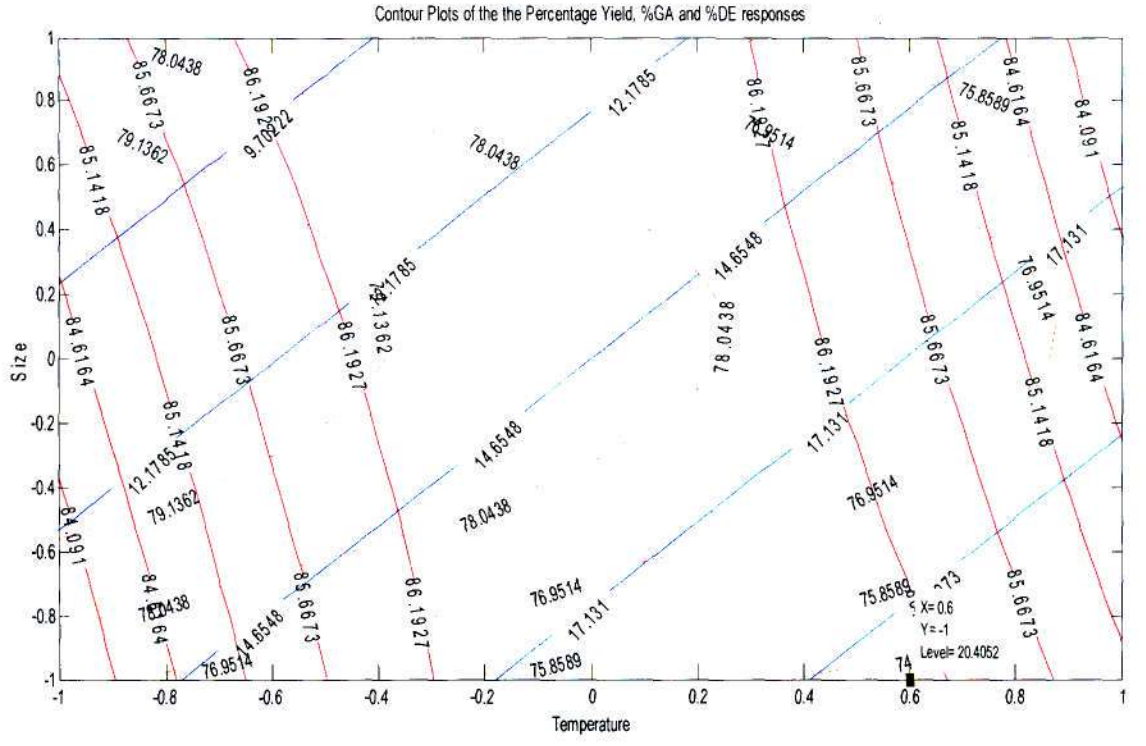

Fig 5.24: *Contour plots for the monitored responses when varying temperature and peel size variables and holding the other variables constant*

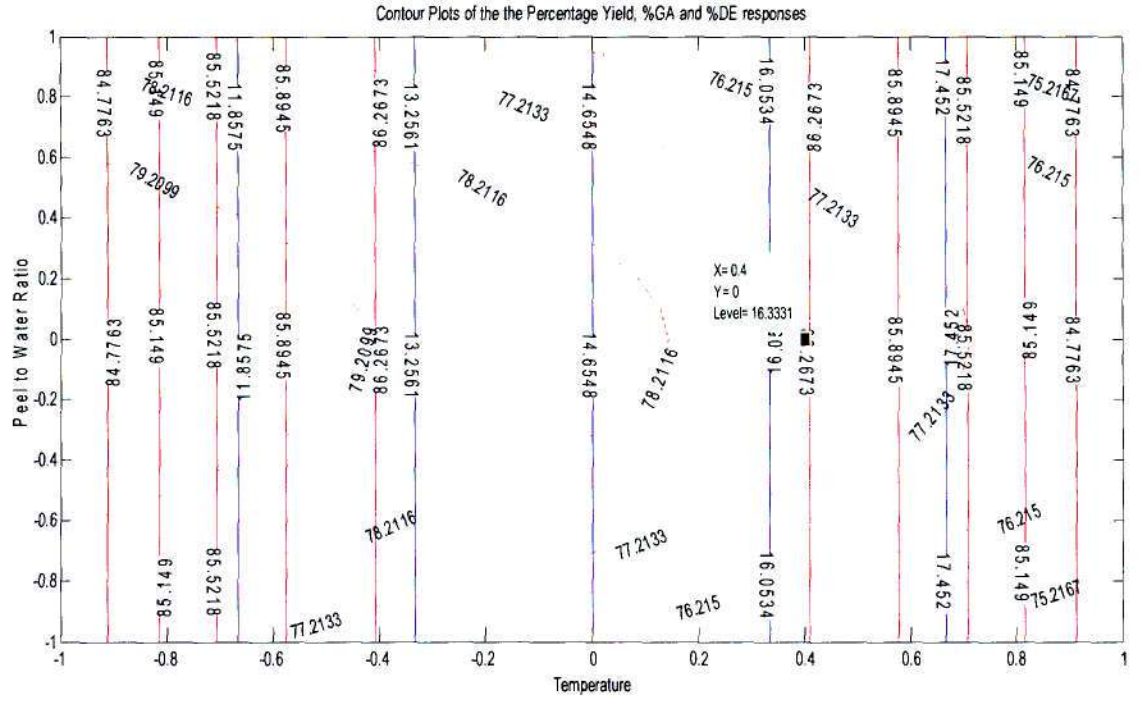

Fig 5.25: *Contour plots for the monitored responses when varying temperature and peel to water mass ratio variables and holding the other variables constant*

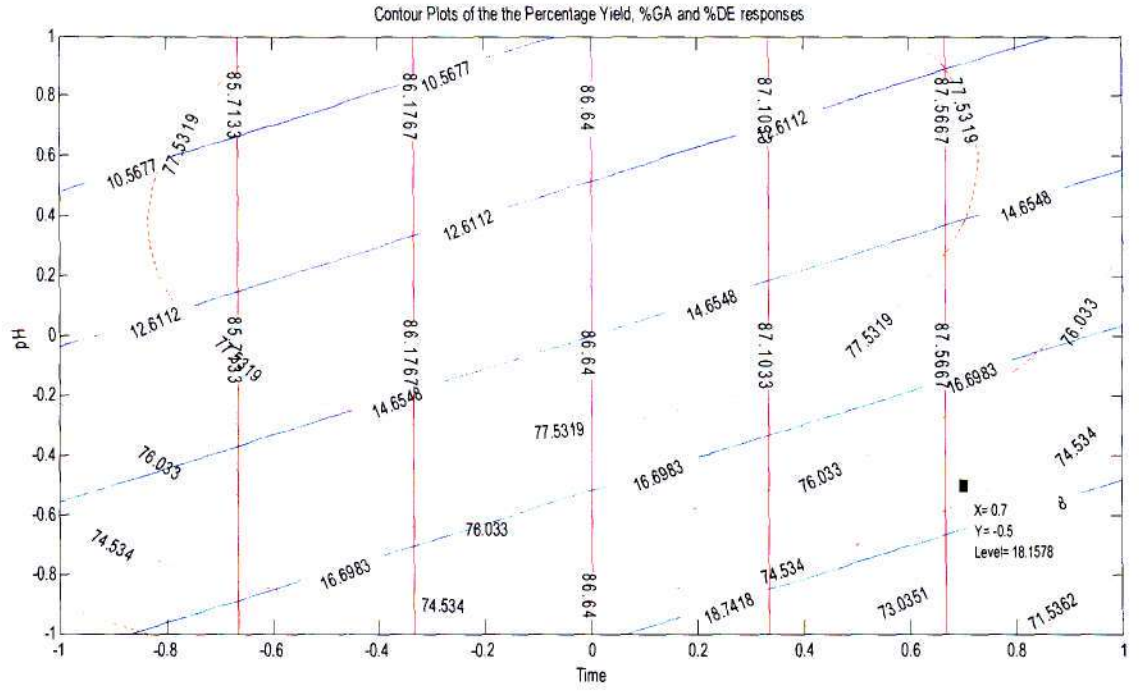

Fig 5.26: *Contour plots for the monitored responses when varying time and pH variables and holding the other variables constant*

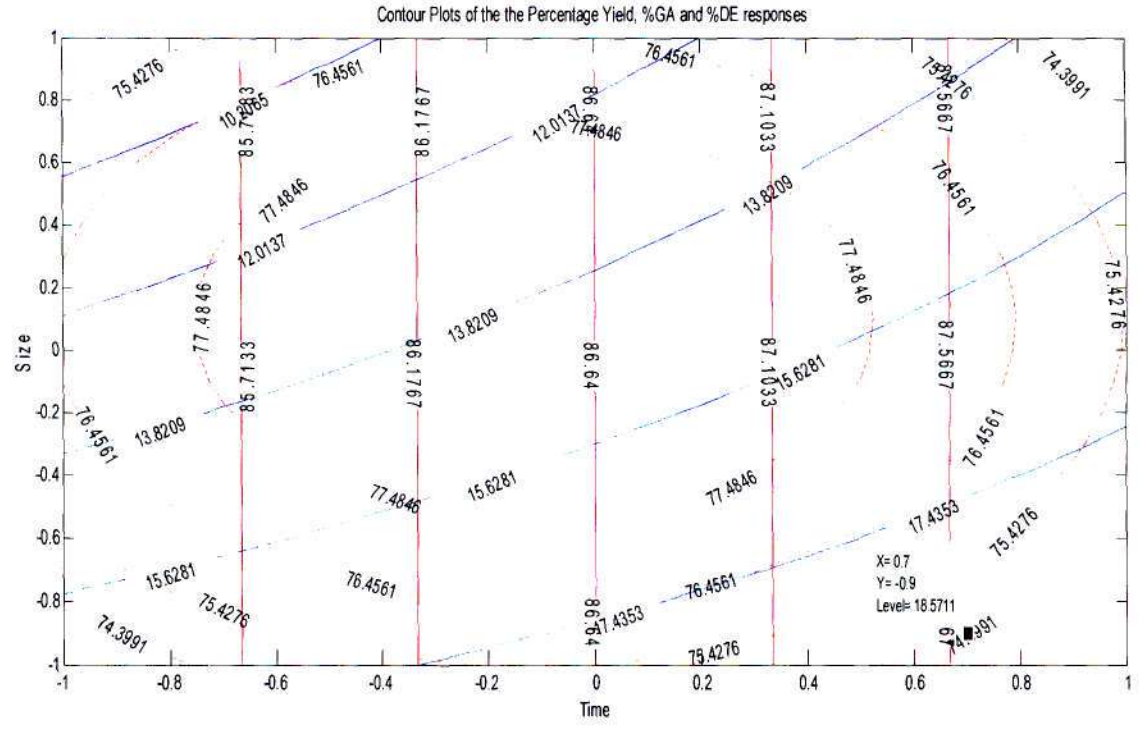

Fig 5.27: *Contour plots for the monitored responses when varying time and peel size variables and holding the other variables constant*

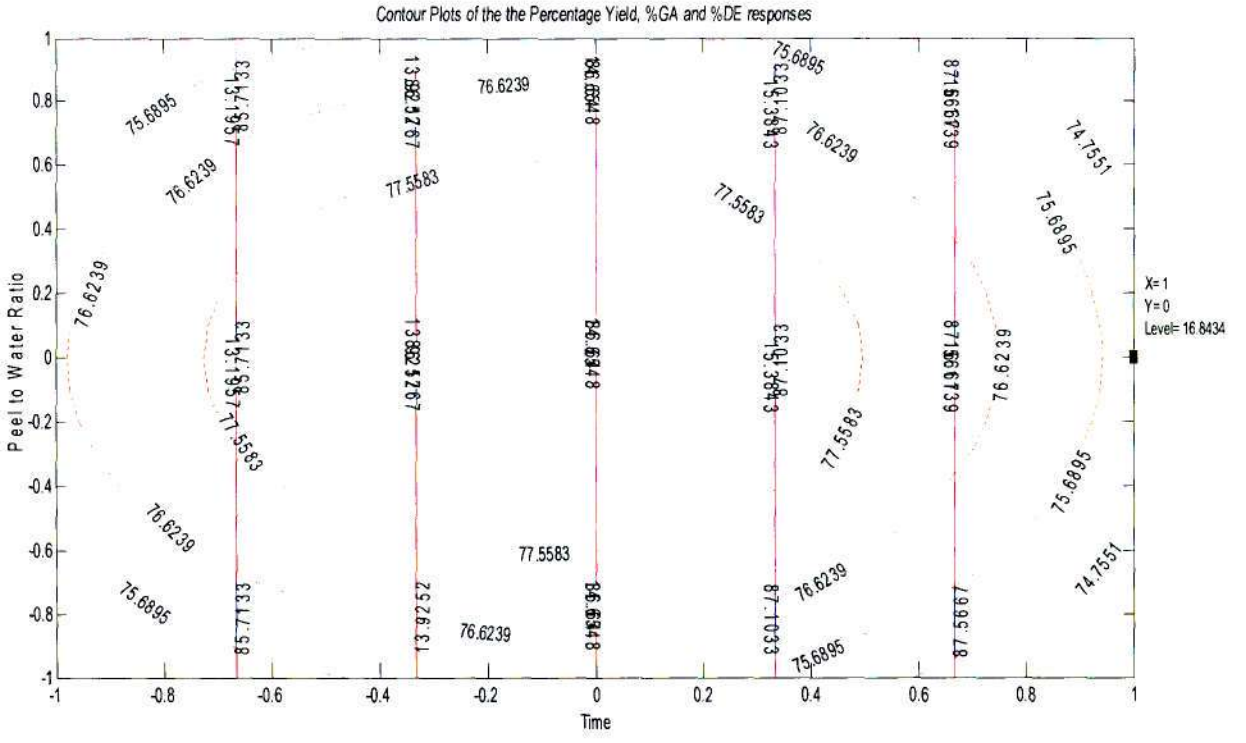

Fig 5.28: *Contour plots for the monitored responses when varying time and peel to water mass ratio variables and holding the other variables constant*

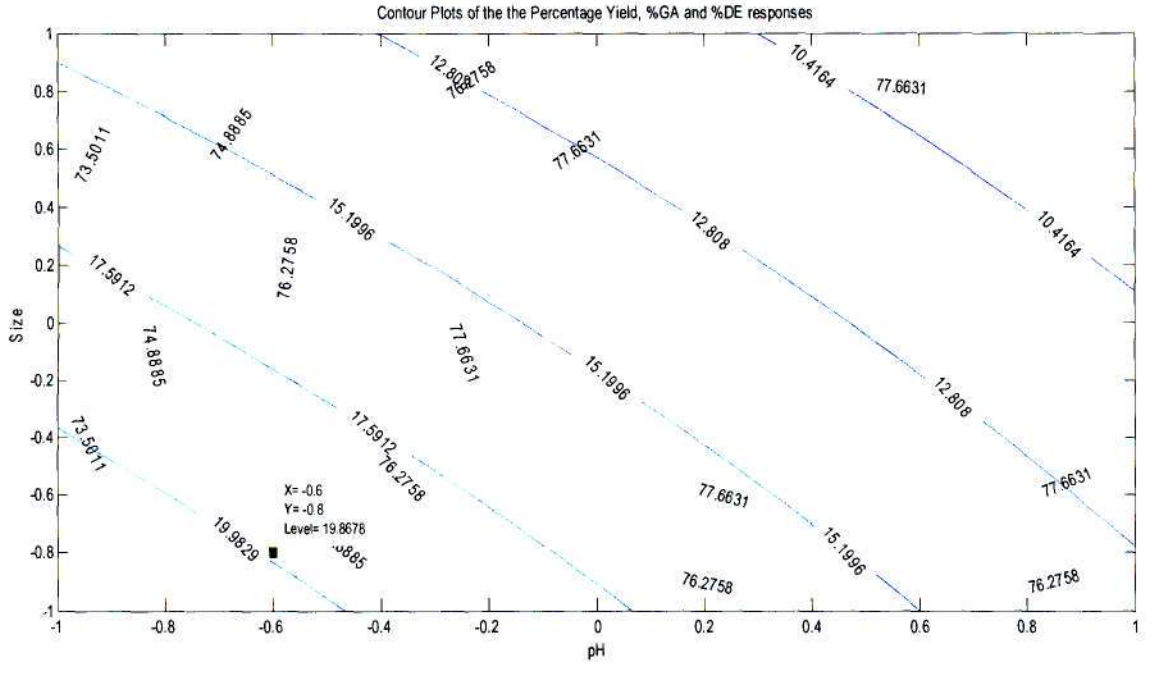

Fig 5.29: *Contour plots for the monitored responses when varying pH and peel size variables and holding the other variables constant*

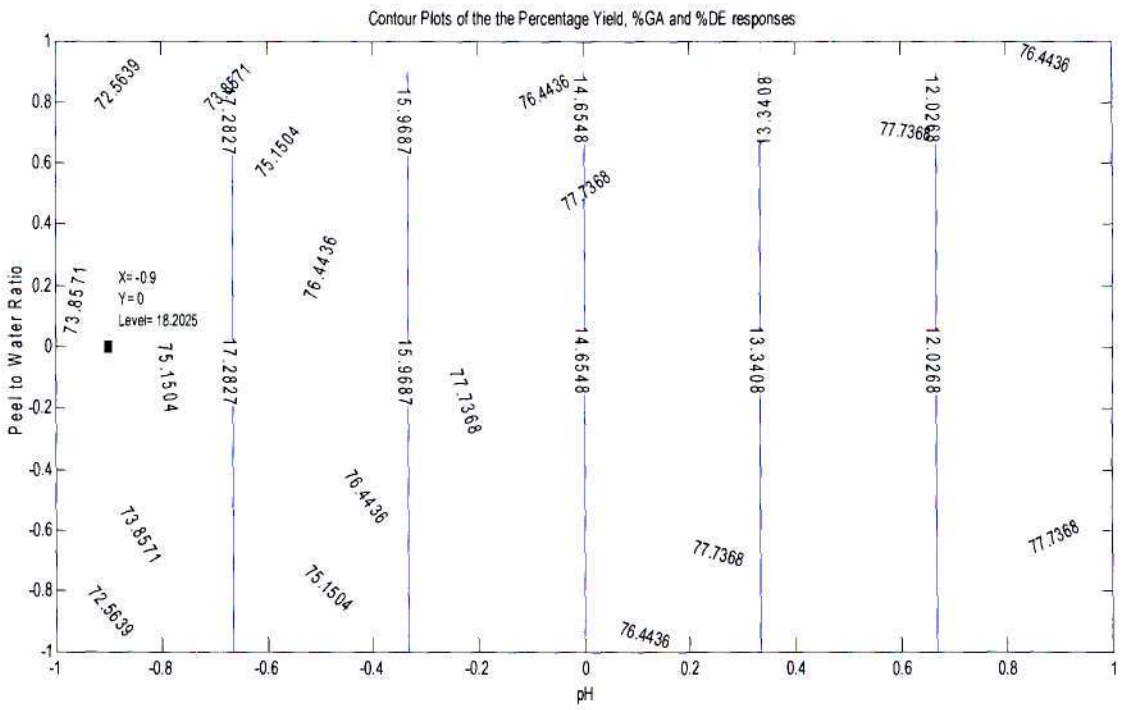

Fig 5.30: *Contour plots for the monitored responses when varying pH and peel to water mass ratio variables and holding the other variables constant*

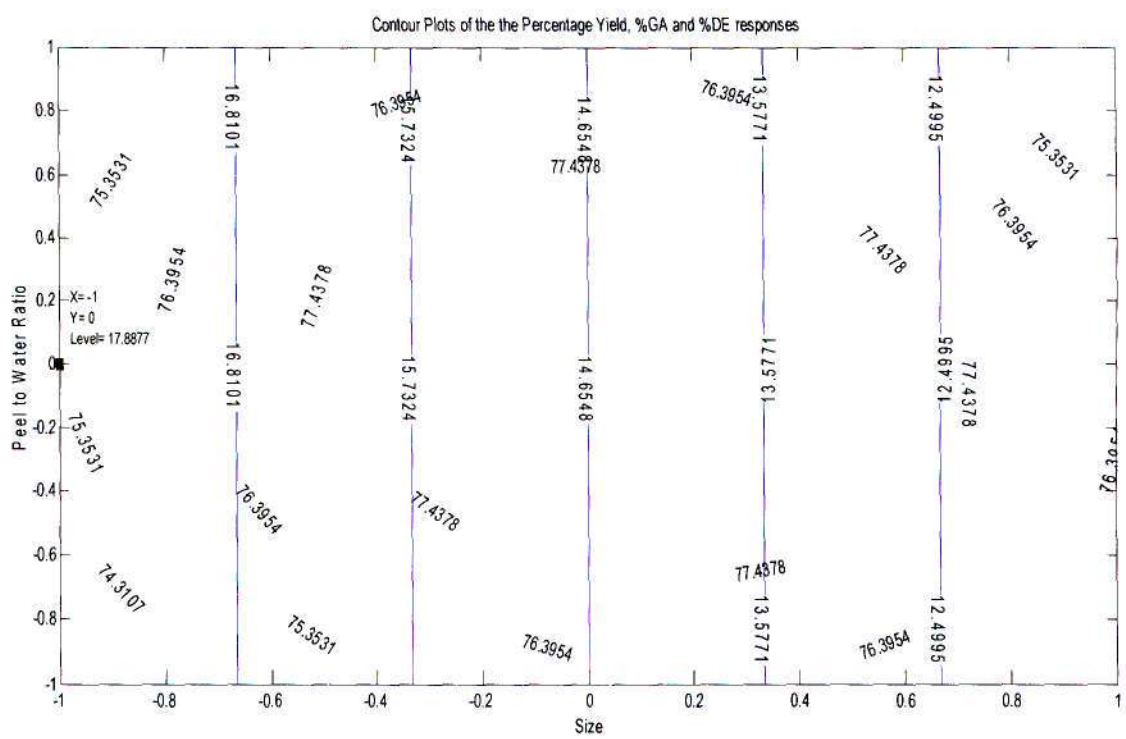

Fig 5.31: *Contour plots for the monitored responses when varying peel size and peel to water mass ratio variables and holding the other variables constant*

4.2.4.4 Optimum Region

The best operating condition was identified from the plot of contours in Fig 5.24. From this plot, the percentage yield was found to be 20.41%, the % GA was 86.34% and % DE was 74.37%. The process variables investigated at this condition were such that the temperature was at 0.6 level (86 ° C), the size of the peels was at -1 level (less than 1 mm size), while the extraction time, pH of extraction and peel to water mass ratio were at 0 level (1hr15min, 2 and 1:37.5 respectively). The problem that may arise at this condition is that the required peel size may be too small (less than 1mm size) for use in some extraction equipment, making it difficult to separate the peels from the solution after the extraction. In such cases the next best optimum condition which was identified from Fig 5.23 may be used. For this optimum condition, the percentage yield was found to be 20.29%, % GA was 86.23% and % DE was 74.33%. At this condition, the temperature was at 0.6 level (86 ° C), the pH of extraction was at -0.7 level (1.97), while the extraction time, size of the peels and peel to water mass ratio were at 0 level (1hr15min, 2-1 mm and 1:37.5 respectively).

This later optimum condition was opted for, because the extraction equipment already used by CSIR, required peels greater than 1mm. The resultant responses at this condition were verified experimentally. An average percentage yield of 22.2%, % GA of 85.19% and % DE of 74.89% were found.

CHAPTER 6 – CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Lemon peels were successfully dried in a fluidized bed dryer with incoming air set a 150 ° C, and 24.8 minutes was required to achieve 10 % moisture content of the peel. Of the three fresh peel sizes that were investigated, the largest peel size (9 mm) was found appropriate for use as it did not agglomerate when fluidized. Tray drying experiments were used to identify the critical moisture content of the peels and predict a conservative representation of pilot plant fluidized bed dryer experiments.

After drying, the peels were ground further and the effect of five process variables (temperature, time, pH, peel size and peel to water mass ratio) on three response variables (percentage yield, galacturonic acid content (% GA) and degree of esterification (% DE)) in the investigation of pectin extraction from dried peels, was effectively carried out. The variables explained the variation of the percentage yield and % DE successfully, but the variability of the % GA response was not sufficiently explained by the variable and or levels. The first order models developed for the percentage yield and % GA responses proved to explain the variation of these responses better than the developed second order models, while in the case of the % DE response the opposite was true. This was attributed mainly to the fact that higher order interactions were included in first order modelling while only second order interactions were considered for second order modelling. At the optimum condition of operation (appropriate for the CSIR equipment), the percentage yield was found to be 22.22%, % GA was 85.19% and % DE was 74.89%. At this condition, the process variables were set at 86 ° C, a pH of 1.97, an extraction time of 1hr15min, peel size of 1-2 mm and peel to water mass ratio of 1:37.5.

Fresh wet peels were found to yield less pectin than dried peels (3.45-22.47% for wet peel and 2.87-30.33% for dried peel). The % GA of the two investigated peel types was found to be similar (77.89-88.40% for wet peel and 78.60-88.64% for dried peel), while the % DE for wet peels was found to be greater than that of dried peels (68.44-78.91% for wet peel and 59.29-73.79% for dried peel). This showed that drying the peel liberates more pectin, but the quality with respect to the degree of esterification is slightly lowered. By optimizing the extraction process however (including star points and center point experiments), optimum conditions

realised a % DE of 74.89% for dried peel extractions. In order to yield the same quantity of pectin, less starting material was needed for dried peel than wet peel. Therefore the extraction equipment required for fresh wet peel processing would be bigger and thus more costly than that required for dried peel processing.

Storing fresh peel at atmospheric conditions for two days at atmospheric conditions had a detrimental effect on the pectin yield, % GA and % DE. The yield was lowered from a range 3.45 % to 22.47 % to 2.62 % to 11.40 %, the % GA was lowered from 77.89 - 88.40% to 49.44 - 69.39%, while the % DE was lowered from 68.44 - 78.91% to 17.80 - 36.74%. This proved the need for drying the peel in order to ascertain good quality pectin throughout the year. The drying therefore need not only be to ascertain pectin production during the off-season, but to preserve the pectin during the production season.

6.2 Recommendations

- The gelling properties as well physicochemical properties of the extracted pectin should be looked into to ensure good quality pectin.
- The impact of the acid washing step on the resultant chemical composition of the pectin in the extraction process should also be investigated.
- The scale up of the pilot plant fluidized bed dryer to an industrial scale size for the production of pectin needed per annum should be undertaken. In the case of a continuous drying the construction of a dryer must ensure uniform residence time for all particles necessary to achieve 10% moisture content.
- Design of all the drying and extraction equipment should be undertaken in order to establish preliminary costs for pectin production.
- An environmental impact assessment for pectin production should be carried out in order to ensure that off-gases and ultimate disposal of the peel meets green engineering standards.

CHAPTER 7 – REFERENCES

1. Ahmed, A. R., Labavitch, J. M. A. 1977, "A simplified method for accurate determination of cell wall polyuronides content", *Journal of Food Biochemistry*, vol. 1, no. 1, pp 361–365.

2. Arancibia, R. A., Motsenbocke, C. E. 2004, " Pectin ultra-degradation decreases the force required to detach ripe fruit from the calyx in tabasco pepper", *Journal of the American Society of Horticultural Science*, vol 129, no. 5, pp 642-648.

3. Aravantinos-Zafiris, G., Oreopoulou, V., Tzia, C. and Thomopoulos, C. D. 1994, "Fibre Fraction from Orange Peel Residues after Pectin Extraction", *Lebensmittel-Wissenschaft und –Technologie*, vol. 27, no. 5, pp 468-471.

4. Attri, B. L., Maini, S. B. 1996, "Pectin from galgal (Citrus pseudolimon Tan.) Peel", *Bioresource Technology,* vol.55, no.1, pp 89-91.

5. BeMiller, J. N. 1986, "Pectins: Structure and Properties" in *Chemistry and Functions of Pectins* American Chemical Society, Washington, DC, pp 2-12.

6. Berardini, N., Knodler, M., Schieber, A. and Carle, R. 2005, "Utilization of mango peels as a source of pectin and polyphenolics", *Innovative Food Science and Emerging Technologies*, vol.6, no.4, pp 442-452.

7. Bitter, T., Muir, H. M. A 1962, "modified uronic acid carbazole reaction", *Analytical Biochemistry*, vol.4, no.4, pp 330-334.

8. Bucher, A. C. 1984, *A Comparison of solvent systems for extraction of pectic substances from fruits and vegetables*, Cornell University, pp 3-15.

9. Caulcutt, R. 1991, *Statistics in Research and Development,* Chapman and Hall, Florida.

10. Champ, M., Langkilde, A.-M., Brouns, F., Kettlitz, B. and Collet, Y. L. B. 2003, "Advances in dietary fibre characterization", *Nutritional Research Reviews*, vol.16, pp 71-82.

11. Chang, K. C., Dhurandhur, N., You, X. and Miyamoto, A.1994, "Sunflower head residue pectin extraction as affected by physical conditions", *Journal of Food Science*, vol.59, no.6, pp 1207-1210.

12. Chatfield, C. 1978, *Statistics for Technology: A course in Applied Statistics*, John Wiley and Sons Inc., New York.

13. Cook, E. M., DuMont, H. D. 1991, *Process Drying Practice*, McGraw-Hill, United States of America.

14. Cuthbert, D. 1976, *Applications of Statistics in Industrial Experimentation*, John Wiley and Sons, Inc., Canada.

15. Davies, R. J. 1982, *Pectin extraction from New Zealand fruit wastes*, Dept of Scientific and Industrial Research Industrial Process Division, New Zealand.

16. de Vries, J. A., Rambouts, F. M., Voragen, A. G. J. and Pilnik, W. 1982, "Enzymatic degradation of apple pectins", Carbohydrate Polymers, vol. 2, pp 25.

17. de Vries, J. A., Voragen, A. G. J., Rambouts, F. M. and Pilnik, W. 1986, "Structural studies of apple pectins with pectolytic enzymes" in *Chemistry and functions of pectins* American Chemical Society, Washington, DC, pp 38.

18. Dea, I. C. M., Madden, J. K. 1986, "Acetylated pecttic polysaccharides of sugar beet", *Food Hydrocolloids*, vol.1, no.1, pp 71-88.

19. Diaz-Rojas, E. I., Pacheco-Aguilar, R., Lizardi, J., Arguelles-Monal, W., Valdez, M. A., Rinaudo, M. and Goycoolea, F. M. 2004, "Linseed pectin: gelling properties and performance as an encapsulation matrix for shark liver oil", *Food Hydrocolloids*, vol.18, no.2, pp 293-304.

20. Donaghy, J. A., McKay, A. M. 1994, "Pectin extraction from citrus peel by polygalacturonase produced on whey", *Bioresource Technology*, vol.47, no.1, pp 25-28.

21. Donner, L. W. 1985, "Analytical methods for determining pectin composition" in *Chemistry and Function of Pectin* American Chemical Society, Washington, DC, pp 13-22.

22. Evageliou, V., Ptitchkina, N. M. and Morris, E. R. 2005, "Solution viscosity and structural modification of pumpkin biopectin", *Food Hydrocolloids*, vol.11, no.19 6, pp 1032-1036.

23. Faravash, R., Ashtiani, F. 2008, "The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace", *Food Hydrocolloids*, vol. 22, no.1, pp 196-202.

24. Fishman, M. L., Chau, H., Hoagland, P. and Ayyad, K. 1999, "Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure", *Carbohydrate Research*, vol. 323, no.1, pp 126-138.

25. Fishman, M. L., Chau, H. K., Hoagland, P. D. and Hotchkiss, A. T. 2006, "Microwave-assisted extraction of lime pectin", *Food Hydrocolloids*, vol.20, no.8, pp 1170-1177.

26. Fishman, M. L., Walker, P. N., Chau, H. K. and Hotchkiss, A. T. 2003, "Flash extraction of pectin from orange albedo by steam injection", *Biomacromolecules*, vol.4, no.4, pp 880-889.

27. Fishman, M. L., Friedman, R. B. and Huang, S. J. 1994, *Polymers from agricultural coproducts*, American Chemical Society, Washington, DC.

28. Food Chemical Codex, 1981.

29. Garleb, K. A., Bourquin, L. D. and Fahey, G. C. 1991, "Galacturonate in pectic substances from fruits and vegetable: comparison of anion exchange HPLC with pulsed amperometric detection to standard calorimetric procedure", *Journal of Food Science*, vol.56, no.1, pp 423-426.

30. Garna, H., Mabon, N., Wathelet, B. and Paquot, M. 2004, "New Method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains", *Journal of Agricultural and Food Chemistry*, vol.52, no.1, pp 4652-4659.

31. Geankopolis, C. J. 1993, *Transport Processes and Unit Operations*, Prentice-Hall Inc, New Jersey.

32. Gerritz, H. W. 1935, "Extraction of Pectin from Apple Thinnings", *Industrial & Engineering Chemistry*, vol. 27, no.12, pp 1458-1459.

33.Guillon, F., Thibault, J. -F. 1989, "Methylation analysis and mild acid hydrolysis of the "hairy" fragments of sugar-beet pectins", *Carbohydrate Research*, vol.190, no.1, pp 85-96, 97-108.

34.Iglesias, M. T., Lozano, J. E. 2004, "Extraction and characterization of sunflower pectin", *Journal of Food Engineering*, vol.62, no.3, pp 215-223.

35.Ishii, T., Matsunaga, T. 2001, "Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan", *Phytochemistry*, vol.57, no.6, pp 969-974.

36.Jiang, C. -M., Lai, Y. -J., Lee, B. -H., Chang, W. -H., Wu, M. -C. and Chang, H. -M. 2002, "Changes in physico-chemical properties of pectin from jelly fig (Ficus awkeotsang Makino) seeds during extraction and gelling", *Food Research International*, vol.35, no.1, pp 31-35.

37.Johnstin, R., Denton, M. C. 1923, "The Relation of Alcohol Precipitate to Jellying Power of Citrus Pectin Extracts", *Industrial & Engineering Chemistry*, vol.15, no.8, pp 778-780.

38.Joslyn, M. A., de Luca, G. 1957, "The formation properties of aluminium pectinates", *Journal of Colloid Science*, vol.12, no.1, pp108-130.

39.Joye, D. D., Luzio, G. A. 2000, "Process for selective extraction of pectins from plant material by differential pH", *Carbohydrate Polymers*, vol. 43, no.4, pp 337-342.

40.Kalapathy, U., Proctor, A. 2000, "Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin", *Food Chemistry*, vol.73, no.4, pp 393-396.

41.Keey, R. B. 1992, *Drying of Loose and Particulate Materials*, Hemisphere Publishing Corporation, United States of America.

42.Keey, R. B. 1972, *Drying Principles and Practice,* Pergamon, Oxford, United Kingdom.

43.Koubala, B. B., Mbome, L. I., Kansci, G., Tchouanguep M. F., Crepeau, M. J., Thibault, J. -F. and Ralet, M. C. 2007, "Physicochemical properties of pectins from ambarella peels (Spondias cytherea) obtained using different extraction conditions", *Food Chemistry*, vol.106, no.3, pp 1202-1207.

44. Leroux, J., Langendorff, V., Schick, G., Vaishnav, V. and Mazoyer, J. 2003, "Emulsion stabilizing properties of pectin", *Food Hydrocolloids*, vol. 17, no.4, pp 455-462.

45. Levigne, S., Ralet, M. –C. and Thibault, J. –F. 2002, "Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design", *Carbohydrate Polymers*, vol.49, no.2, pp 145-153.

46. Lipson, C., Sheth, N. J. 1973, *Statistical Design and Analysis of Engineering Experiments*, McGrawHill, New York.

47. Liu, Y., Shi, J. and Langrish, T. A. G. 2006, "Water-based extraction of pectin from flavedo and albedo of orange peels", *Chemical Engineering Journal*, vol.120, no.3, pp 203-209.

48. Lopez da Silva, J.A., Rao, M. A. 2006, "Pectins: Structure, Functionality and Uses" in *Food polysaccharides and their application* Taylor and Francis, NW, pp 354.

49. Manabe, M., Naohara, J., Sato, T. and Okada, J. 1988, "Nippon Shokuhin Kogyo Gakkaishi", *Chem. Abstr.*, vol.109, no.35, pp 497-501.

50. Martínez-sánchez, C. E., Herman-Lara, E., Varela-Santos, E., Dominguez-Jeronimo, L. M., Cisneros-Avendaño, M., Contreras-Esquivel, J. C., Vivar-Vera, M. A. and Nogueira-Terrones, H. 2005, "Pectin extraction from passion fruit peel by autoclaving", *Food Chemistry: Effects of food processing, formulation and component interactions* IFT Annual Meeting, New Orleans, Louisiana.

51. May, C. D. 1990, "Industrial pectins-sources, production and applications", *Carbohydrate Polymers*, vol. 12, no.1, pp 79.

52. McCredy, R. M. 1970, *Methods of food Analysis*, Academic Press, New York.

53. Michel, F., Thibault, J. -.F, Mercier, C., Heitz, F. and Pouillaude, F. 1985, "Extraction and characterization of pectins from sugar beet pulp", *Journal of Food Science*, vol.50, pp 1499-1502.

54. Mira, B., Blasco, M. 1996, "Supercritical CO^2 extraction of essential oils from orange peel", *Journal of Supercritical Fluids*, vol.9, no.2, pp 238 – 243.

55. Montgomery, D.C., Runger, G. C., Hubele, N. F. 2001, *Engineering Statistics*, John Wiley and Sons, Inc., New York.

56. Montgomery, D.C. 2005, *Design and Analysis of Experiments*, John Wiley and Sons, Inc, United States of America.

57. Montgomery, R. 2004, "Development of biobased products", *Bioresource Technology*, vol. 91, no.1, pp 1-29.

58. Nagayama, T. 1997, "Decrease in Organic Solvent Extractable Ethion by Grapefruit Pectin during Processing", *Journal of Agricultural and Food Chemistry*, vol.45, no.12, pp 4856-4860.

59. Pagan, J., Ibarz, A. 1999, "Extraction and rheological properties of pectin from fresh peach pomace", *Journal of Food Engineering*, vol.39, no.2, pp 193-201.

60. Pagán, J., Ibarz, A., Llorca, M., Pagán, A. and Barbosa-Cánovas, G. V. 2001, "Extraction and characterization of pectin from stored peach pomace", *Food Research International*, vol.34, no.7, pp 605-612.

61. Ramganna, S. 1986, *Handbook of analysis and quality control for fruit and vegetable products*, McGraw Hill, New Delhi, India.

62. Rees, D. A., Wight, A. W. 1971, "Polysaccharide conformation. Part VII. Model building computation for a a-1,4 galacturonan and the kinking function of L-rhamnose residues in pectic substances", *Journal of Chemical Society*, pp 1366.

63. Renard, M. G. C., Thibault, J. -F. 1993, "Structure and properties of apple and sugar-beet pectins extracted by chelating agents", *Carbohydrate Research*, vol.244, no.1, pp 99-114.

64. Robert, C., Devillers, T., Wathelet, B., VanHerck, J. -C. and Paquot, M. 2006, "Use of a Plackett-Burman Experimental Design to Examine the Impact of Extraction Parameters on Yields and Compositions of Pectins Extracted from Chicory Roots (Chicorium intybus L.)", *Journal of Agricultural and Food Chemistry*, vol.54, no.19, pp 7167-7174.

65. Rombouts, F. M., Thibault, J. -F. 1986, "Feruloylated pectic substances from sugar-beet pulp", *Carbohydrate Research*, vol.154, no.1, pp 177-187.

66. Ros, J. M., Schols, H. A. and Voragen, A. G. J. 1998, "Lemon albedo cell walls contain distinct populations of pectic hairy regions", *Carbohydrate Polymers*, vol.37, no.2, pp 159-166.

67. Ros, J. M., Schols, H. A. and Voragen, A. G. J. 1996, "Extraction, characterisation, and enzymatic degradation of lemon peel pectins", *Carbohydrate Research*, vol.282, no.2, pp 271-284.

68. Sabir, M. A., Sosulski, F. W. and Campbell, S. J. 1976, "Polymetaphosphate and oxalate extraction of sunflower pectins", *Journal of Agricultural and Food Chemistry*, vol.24, no.2, pp 348-350.

69. Sahari, M. A., Akbarian, M. A. and Hamedi, M. 2003, "Effect of variety and acid washing method on extraction yield and quality of sunflower head", *Food Chemistry*, vol.83, no.1, pp 43-47.

70. Sakamoto, T., Sakai, T. 1995, "Analysis of structure of sugar-beet pectin by enzymatic methods", *Phytochemistry*, vol. 39, no.4, pp 821-823.

71. Sazhin, B. S. 1984, *Principles of Drying Technology*, Khimiya, Moskva.

72. Schieber, A., Hilt, P., Streker, P., Endresz, H. -U., Rentschler, C. and Carle, R. 2003, "A new process for the combined recovery of pectin and phenolic compounds from apple pomace", *Innovative Food Science and Emerging Technologies*, vol.4, no.1, pp 99-107.

73. Schols, H. A., Vierhuis, E., Bakx, E. J. and Voragen, A. G. J. 1995, "Different populations of pectic hairy regions occur in apple cell walls", *Carbohydrate Research*, vol. 275, no.2, pp 343-360.

74. Shi, X. Q., Chang, K. C., Schwarz, J. G., Wiesenborn, D. P. and Shih, M. C. 1996, "Optimizing pectin extraction from sunflower heads by alkaline washing", *Bioresource Technology*, vol.58, no.3, pp 291-297.

75. Singthong, J., Ningsanond, S., Cui, S. W. and Douglas Goff, H. 2005, "Extraction and physicochemical characterization of Krueo MaNoy pectin", *Food Hydrocolloids*, vol.19, no.5, pp 793-801.

76. Strumillo, C., Kudra, T. 1986, *Drying: Principles, Applications and Design*, Gordon and Breach Science Publishers, Switzerland.

77. Thibault, J. F. 1983, "Enzymatic degradation and ß-elimination of the pectic substance in cherry fruits", *Phytochemistry*, vol.22, no.7, pp 1567.

78. Thibault, J. -F. 1979, "Automatisation du dosage des substances pectiques par la méthode au métahydroxydiphényle", *Lebensmittel Wissenschaft und Technologie*, vol.12, no.1, pp 247–251.

79. Thibault, J. –F., Renard, M. G. C., Axelos, M. A. V., Roger, P. and Crépeau, M. -J. 1993, "Studies of the length of homogalacturonic regions in pectins by acid hydrolysis", *Carbohydrate Research*, vol.238, pp 271-286.

80. Thomas, M., Guillemin, F., Guillon, F. and Thibault, J. -F. 2003, "Pectins in the fruits of Japanese quince (Chaenomeles japonica)", *Carbohydrate Polymers*, vol.53, no.4, pp 361-372.

81. Thomas, M., Thibault, J. -F. 2002, "Cell-wall polysaccharides in the fruits of Japanese quince (Chaenomeles japonica): extraction and preliminary characterization", *Carbohydrate Polymers*, vol.53, no.4, pp 361-372.

82. Tuchnina, I. V., Khaustov, I. P. and Osinskii, V. P. 1994, "Development of equipment for the drying of pectin", *Chemical and Petroleum Engineering*, vol.30, no.9-10, pp 413-416.

83. Turakhozhaev, M. T., Khodzahaeva, M. A., Ivanova, I. A., Sagdullaev, B. T. and Kim, K. N. 1999, "Pectins from the peel of Citrus unshiu", *Chemistry of Natural Compounds*, vol.35, no.5, pp 502-504.

84. Turquois, T., Rinaudo, M., Taravel, F. R. and Heyraud, A. 2000, "Extraction of highly gelling pectins from sugar beet pulp" in *Katsuyoshi Nishinari Hydrocolloids* Elsevier Science, Amsterdam, pp 229-234.

85. Van Buren, J. P. 1991, "Function of Pectin in Plant Tissue Structure and Firmness" in *The Chemistry and Technology of Pectin* San Diedo Academic Press, San Diego.

86. Vanecek, V., Markvart, M. and Drbohlav, R. 1966, *Fluidized Bed Drying*, Leonard Hill Books and SNTL-Publishers of Technical Literature, Prague.

87. Van't Land, C. M. 1991, *Industrial Drying Equipment: Selection and Application*, Marcel Dekker, New York.

88. Wang, S., Chen, F., Wu, J., Wang, Z., Liao, X. and Hu, X. 2007, "Optimization of pectin extraction assisted by microwave from apple pomace using response surface methodology", *Journal of Food Engineering*, vol.78, no.2, pp 693-700.

89. Wiesenborn, D. P., Wang, J., Chang, K. C. and Schwarz, J. G. 1999, "Comparison of continuous and batch processes for pectin extraction from sunflower heads", *Industrial Crops and Products*, vol.9, no.3, pp 171-181.

90. Yang, H., Lai, S., An, H. and Li, Y. 2006, "Atomic force microscopy study of the ultrastructural changes of chelate-soluble pectin in peaches under controlled atmosphere storage", *Postharvest Biology and Technology*, vol.39, no.1, pp 75-83.

91. Yapo, B. M., Robert, C., Etienne, I., Wathelet, B. and Paquot, M. 2007, "Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts", *Food Chemistry*, vol.100, no.4, pp 1356-1364.

92. Yapo, B. M., Wathelet, B. and Paquot, M. 2007, "Comparison of alcohol precipitation and membrane filtration effects on sugar beet pulp pectin chemical features and membrane filtration effects on sugar beet pulp pectin chemical features and surface properties", *Food Hydrocolloids*, vol.21, no.2, pp 245-255.

93. Zhongdong, L., Guohua, W., Yunchang, G. and Kennedy, J. F. 2006, "Image study of pectin extraction from orange skin assisted by microwave", Carbohydrate Polymers, vol.64, no.4, pp 548-552.

94. http://www.cpkelco.com/pectin/industrial/molecular_structure.htlm, Aug 2007.

APPENDICES

Appendix A shows the calculations undertaken for the drying of the lemon peels, Appendix B presents calculations for dried peel extraction, Appendix C presents calculations for fresh wet peel extraction, Appendix D presents calculations undertaken for stored wet peel calculations and Appendix E presents optimization calculations for dried peel extraction.

Appendix A - Drying of wet peels

Lemon peels were minced to different sizes and dried at different temperatures in order to develop the drying characteristics of the peel. These provided conservative estimates of drying times for fluidized bed drying tests.

A.1 Calculations of the moisture ratio at different time intervals for tray drying experiments

Table A.1 shows the sizes of the minced peel and how they were classified.

Table A.1: *Particle size classification*

Particles	Size (mm)
Small	~3
Medium	~6
Large	~9

For small particles dried at 150 ° C at initial moisture content of 89.12 %, the corresponding moisture ratio was calculated as follows:

$$X = \frac{\text{moisture content}}{100 - \text{moisture content}} = \frac{89.12\%}{100\% - 89.12\%} = 8.19 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (A.1)$$

Similar calculations were done for all particle sizes at different time intervals and are given in Table A.2 and A.3

Table A.2: *Moisture content of peels at different time intervals at 150° C*

Time (min)	Moisture Content (%)			Moisture Ratio		
	Small Particles	Medium Particles	Large Particles	Small Particles	Medium Particles	Large Particles
0	89.12	80.22	80.91	8.19	4.06	4.24
10	44.77	59.24	74	0.81	1.45	2.85
20	4.05	9.21	57.94	0.04	0.10	1.38
40	3.25	2.05	7.08	0.03	0.02	0.08
60	4.49	2.74	2.07	0.05	0.03	0.02
90	3.52	2.05	1.99	0.04	0.02	0.02

It was due to experimental errors that the value of the moisture content of the peels was observed to increase from 40 to 60 minutes time interval in some instances in Table A.2.

Table A.3: *Moisture content of peels at different time intervals at 100° C*

Time (min)	Moisture Content (%)			Moisture Ratio		
	Small Particles	Medium Particles	Large Particles	Small Particles	Medium Particles	Large Particles
0	85.03	79.29	80	5.68	3.83	4.00
10	71.55	68.18	78.22	2.51	2.14	3.59
15	52.92	-	72.54	1.12	-	2.64
20	38.96	40.19	66.71	0.64	0.67	2.00
30	10.41	12.26	56.77	0.12	0.14	1.31
40	5.77	5.94	40.27	0.06	0.06	0.67
55	-	-	16.12	-	-	0.19
60	4.83	4.41	12.96	0.05	0.05	0.15
70	4.63	4.22	7.68	0.05	0.04	0.08
90	2.98	3.23	6.69	0.03	0.03	0.07

A.2 Calculation of the drying rate

The moisture ratio was then plotted against the corresponding drying time for all particles dried at 150 ° C and 100 ° C and the results are shown in Fig A.1 and Fig A.2. The gradients of this plots were then used to create the drying curves as follows:

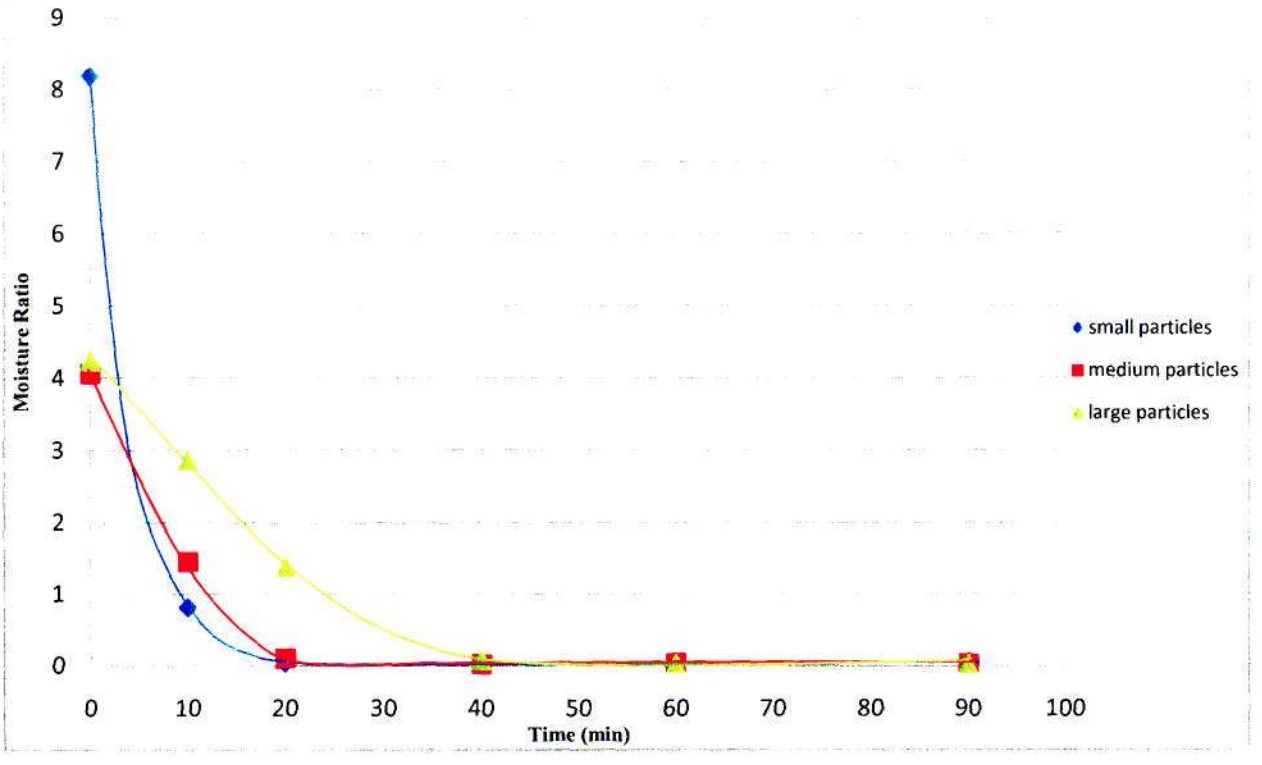

Fig A.1: *moisture ratio plotted against the corresponding drying time for particles at 150 ° C*

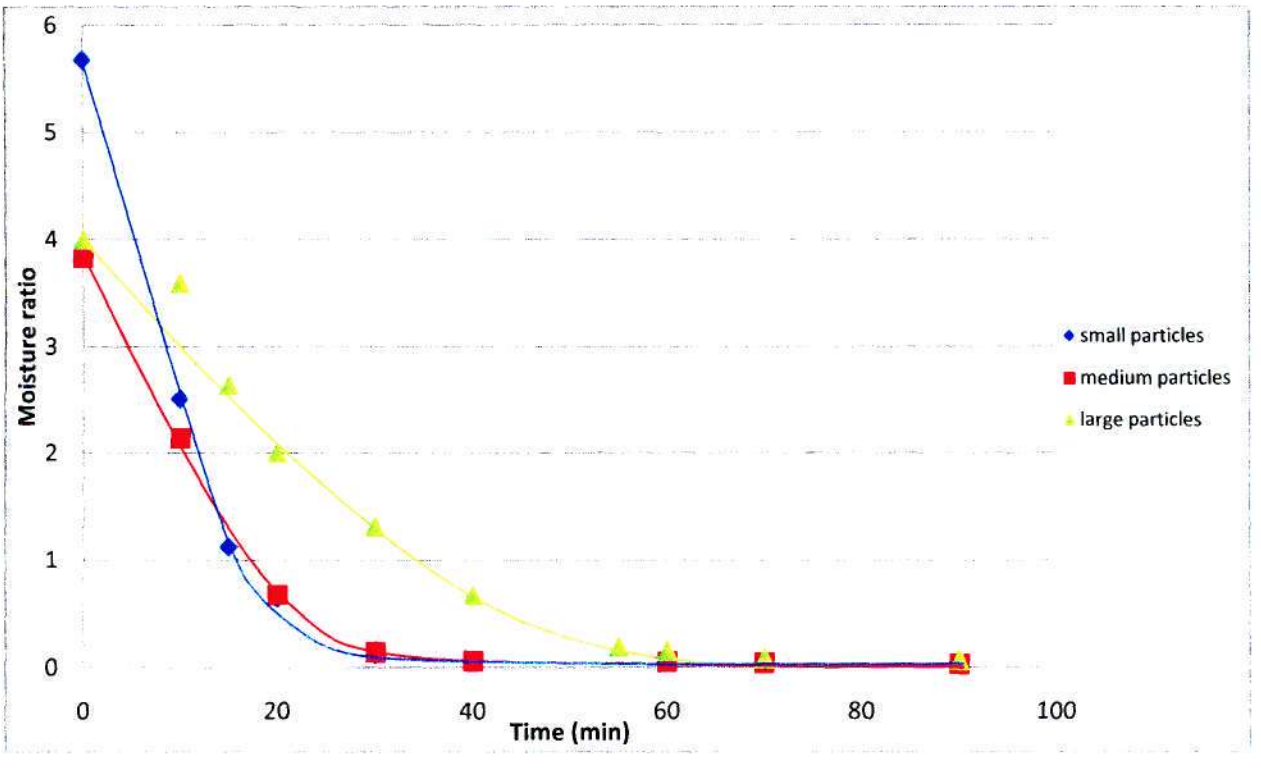

Fig A.2: *moisture ratio plotted against the corresponding drying time for particles at 100 ° C*

For small particles at 150 °C (Refer to Fig A.1), the gradient between points X = 8.19 and X = 1.6 was calculated as follows:

$$\frac{dX}{dt} = \frac{8.19 - 1.6}{8.2} = 0.804 kg_{H_2O}/kg_{dry\ sample}.min \ldots\ldots\ldots (A.2)$$

The bone dry mass of the particles was found to be 0.003264 kg

The area of the petri dish was calculated to be 0.00567 m^2

The drying rate was then calculated as follows:

$$\text{Drying Rate} = \frac{M_D}{A} * \frac{dX}{dt} \ldots\ldots\ldots (A.3)$$

$$\text{Drying Rate} = \frac{0.003264\ kg_{dry\ sample}}{0.00567\ m^2} * 0.804 \frac{kg_{H_2O}}{kg_{dry\ sample}.min}$$

$$= 0.46 kg_{H_2O}/min.m^2$$

The corresponding drying rates were calculated similarly and are shown in Fig A.3 and Fig A.4

A.3 Calculation of the critical moisture content

Critical moisture ratios were identified from drying rate curves and these were converted into corresponding critical moisture content values. These curves are given in Fig A.3 to A.4 and Table A.4 shows the critical moisture ratios with their corresponding moisture content.

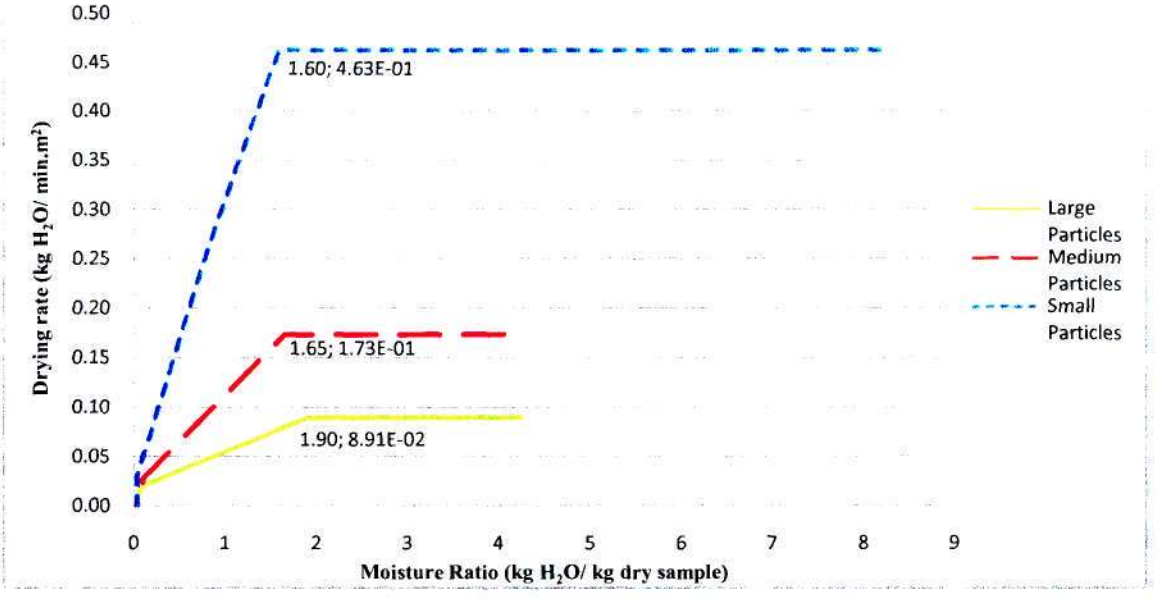

Fig A.3: *Drying rate curves for the three particle sizes dried at 150 ° C*

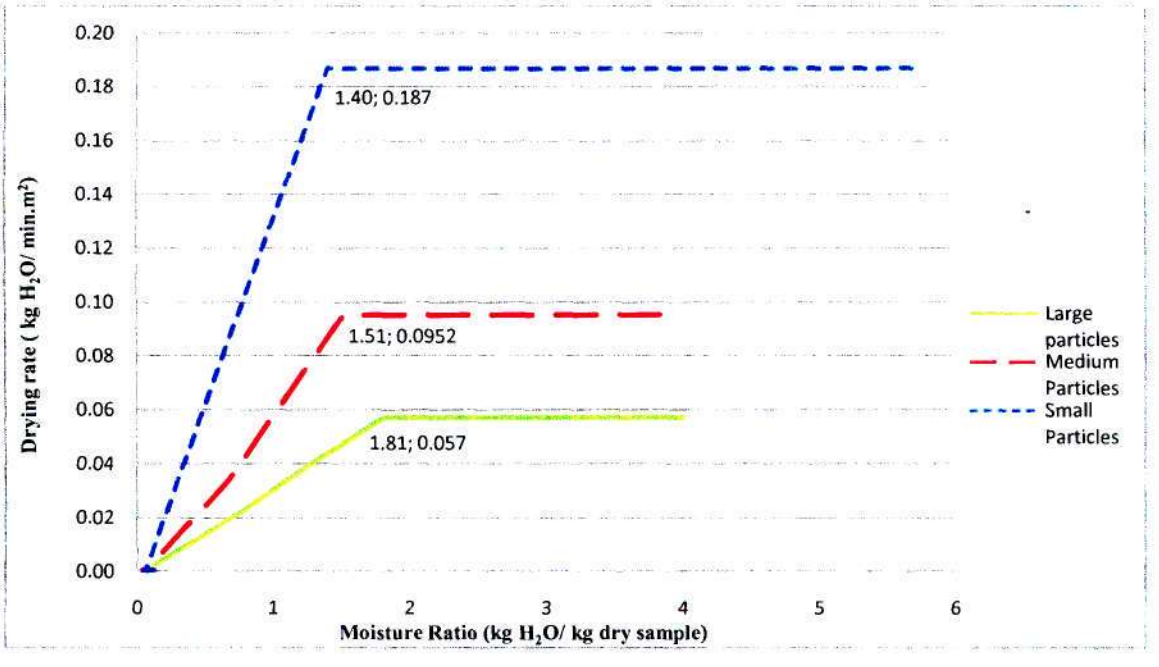

Fig A.4: *Drying rate curves for the three particle sizes dried at 100 ° C*

For small particles dried at 150 ° C, the critical moisture ratio was read off the drying rate vs moisture ratio graph for small particles dried at 150 ° C and the corresponding moisture content was calculated:

$$\text{moisture content} = \frac{100X}{1+X} = \frac{100 * 1.6}{1 + 1.6} = 64.54\ \% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(A.5)}$$

Similar critical moisture content values were calculated for all particle sizes dried at different temperatures and are presented in Table A.4:

Table A.4: *Critical moisture ratio and content for different particle sizes dried at 150 ° C and 100 ° C*

Particle Size	Critical Moisture Ratio (kg/kg)		Critical Moisture Content (%)	
	Drying Temperature (° C)		Drying Temperature (° C)	
	150	100	150	100
Small	1.60	1.40	61.54	58.33
Medium	1.65	1.51	62.26	60.21
Large	1.90	1.81	65.52	64.41

A.4 Time required to achieve 10 % moisture content

The time taken to achieve 10 % moisture content for the different peel sizes at different drying temperatures was identified from graphical analysis of moisture content versus drying time plots. These plots are given in Fig A.5 to A.7. The resulting time required to achieve this moisture content for the different sized peel at different temperatures is shown in Table A.5.

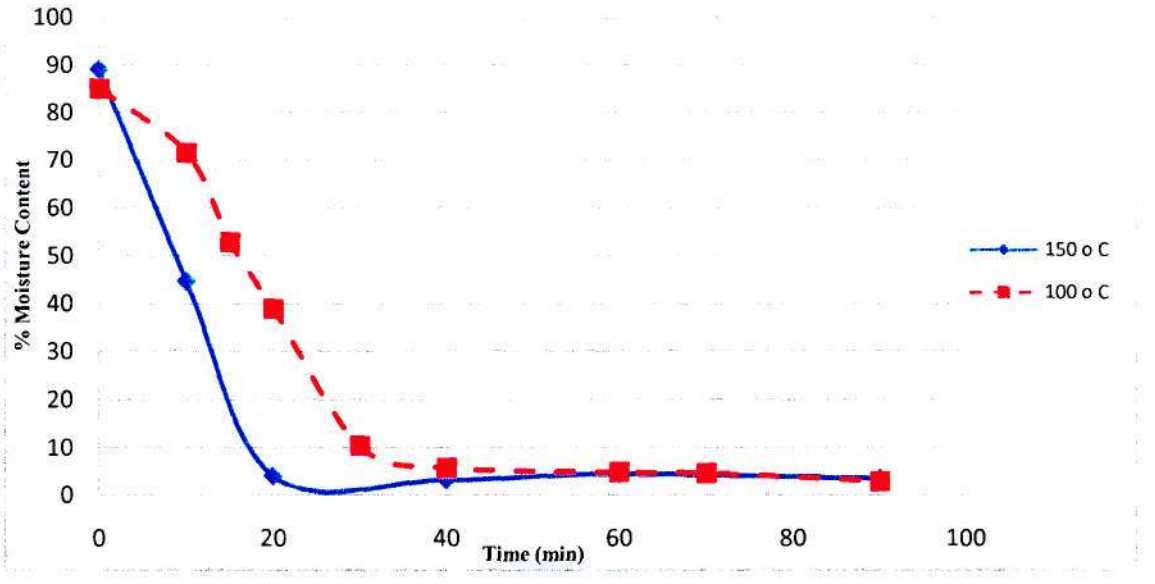

Fig A.5: *Change in moisture content with time for small sized particles*

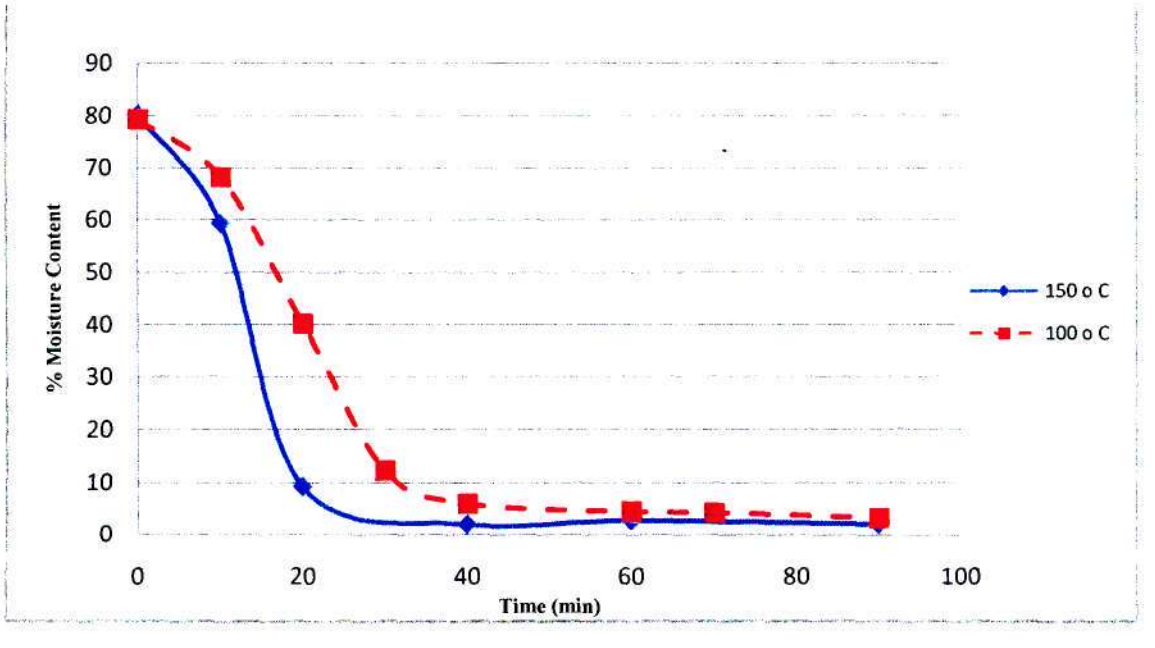

Fig A.6: *Change in moisture content with time for large medium particles*

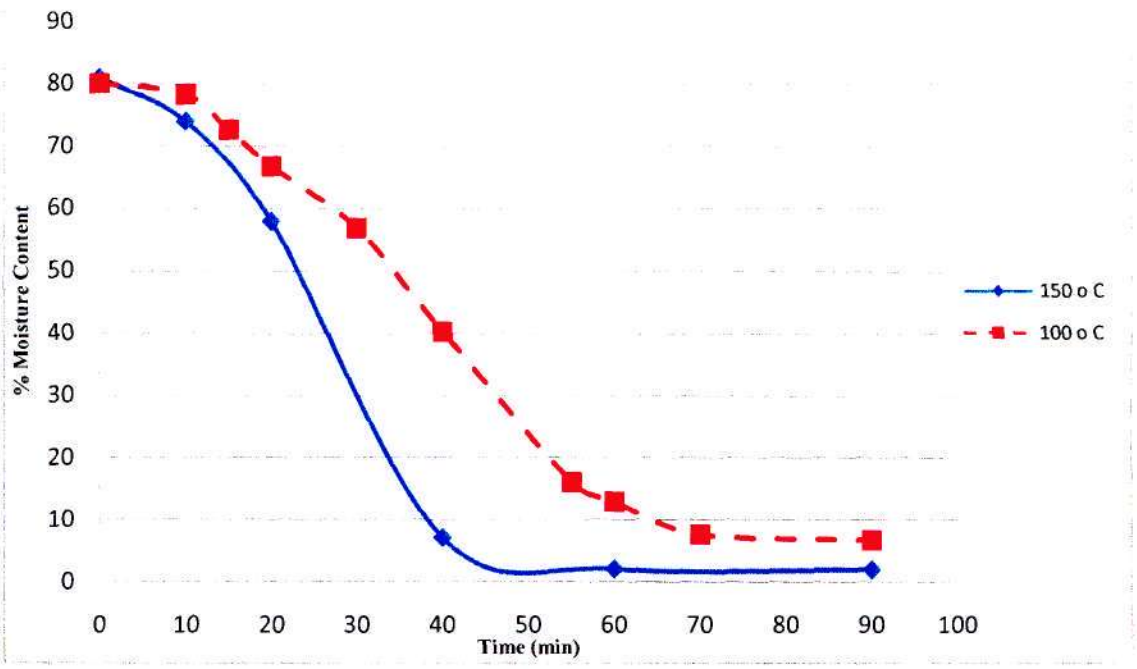

Fig A.7: *Change in moisture content with time for large sized particles*

Table A.5: *Time require to achieve 10 % moisture content for different peel sizes dried at 150 ° C and 100 ° C in a tray dryer*

Particle Size	Time (min) taken to achieve 10 % Moisture Content	
	Drying Temperature (° C)	
	150 ° C	**100 ° C**
Small	17	29.5
Medium	19.5	31
Large	38	64

A.5 Fan frequency control calibration calculations for the fluidized bed dryer

In order to set the air velocity within the dryer to a set value, the fan frequency use to blow air into the fluidized bed dryer had to be controlled. The frequency of the fan was calibrated to the velocity of the air within the pipe leading (measured using a pitot tube) to the inlet of the dryer. This air velocity within the pipe was then used to calculate the fluidisation velocity within the bed. (Refer to Fig 3.1 on the Fluidized Bed Dryer)

Calculations at 5 Hz frequency

$$P_{abs} = P_{atm} + P_{gauge} \quad \text{(A. 6)}$$

Where the gauge pressure is given by the pressure drop across the pitot tube

$$P_{gauge} = \Delta P_{pitot} = 5\text{kPa and } P_{atm} = 100.2\ \text{kPa}$$

The absolute pressure (Equation A.6) thus becomes

$$P_{abs} = 100.2 + 5 = 105.2\ \text{kPa}$$

The density of the air was calculated as follows:

$$\rho_{air}(T) = \rho_{ref} * \frac{T_{ref}}{T} * \frac{P_{abs}}{P_{ref}} \quad \text{(A. 7)}$$

$$\rho_{air}(298.35) = 1.2 * \frac{293}{298.35} * \frac{105.2}{101.3} = \frac{1.224\text{kg}}{\text{m}^3}$$

From the Equation of a pitot tube the velocity of the air in the pipe was calculated as:

$$v_{air} = \sqrt{\frac{2(P_i - P_s)}{\rho_{air}}} \quad \text{(A. 6)}$$

$$v_{air} = \sqrt{\frac{2(32\text{kPa})}{1.224\text{kg/m}^3}} = 7.23\text{m/s}$$

Air velocities for frequencies from 5 – 40 Hz were calculated similarly and are given in Table A.6:

Table A.6: *Pilot plant frequency control calibration*

Frequency (Hz)	ΔP (kPa)	T2 (K)	T_{air} (K)	P_{abs} (kPa)	ρ_{air} (kg/m^3)	v_{air} (m/s)
5	5	318.35	298.35	105.2	1.224	7.23
10	3	318.45	298.45	103.2	1.2	8.37
15	4	318.35	298.35	104.2	1.212	9.48
20	5	318.45	298.45	105.2	1.223	11.79
25	7	318.55	298.55	107.2	1.246	12.67
30	7.5	319.15	299.15	107.7	1.25	15.18
35	9.5	320.35	300.35	109.7	1.268	16.59
40	11	321.15	301.15	111.2	1.282	18.06

The corresponding calibration graph was the plotted and used to set the air velocity to the required value (Fig A.8).

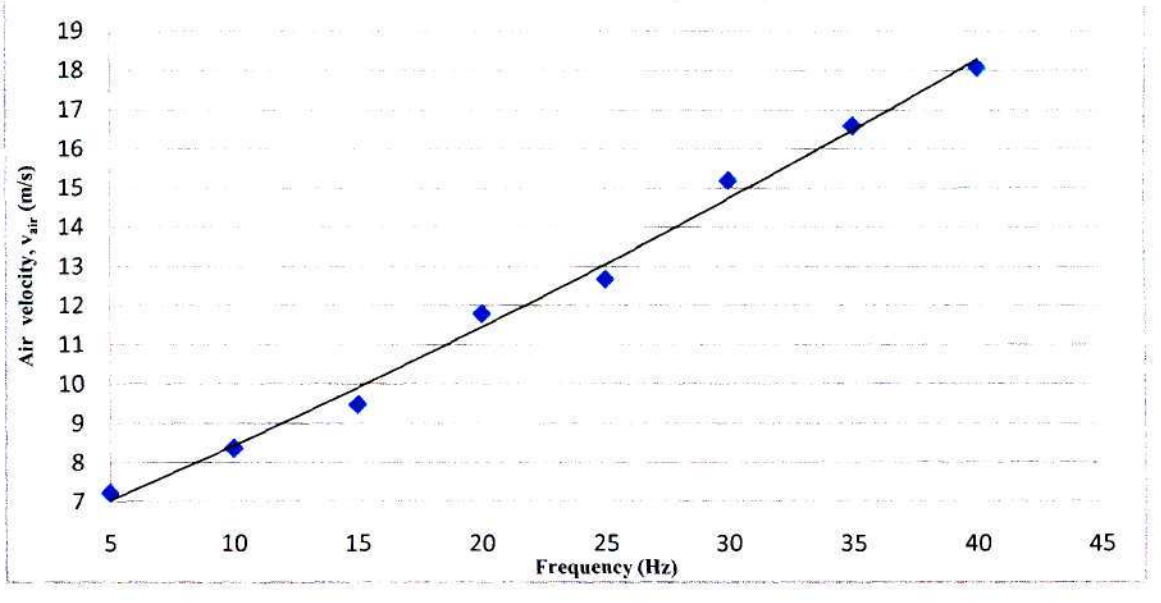

Fig A.8: *Frequency calibration graph*

It was found from practise that a frequency of 35 Hz achieved good fluidization and this was used in pilot plant tests.

The fluidization velocity was calculated using the following Equation A.7:

$$\dot{V}_{pipe} = \dot{V}_{bed} \quad \text{(A. 7)}$$

Which shows that the volumetric air flow rate in the pipe and in the bed are the same. From Equation A.7, the fluidization velocity was calculated from:

$$v_{air} * A_{pipe} = v_f * A_{bed} \quad \text{(A. 8)}$$

Where A_{pipe} ($= \pi r^2 = \pi(0.12)^2 = 0.045\ m^2$) is the cross sectional area of the pipe, A_{bed} ($= 0.345 * 0.345\ m^2 = 0.119 m^2$) is the cross sectional area of the bed and vf is the fluidization velocity of the air. Therefore:

$$v_f = \frac{v_{air} * A_{pipe}}{A_{bed}} \quad \text{(A. 9)}$$

For 35 Hz, v_{air} from the Fig A.8 was found to be 16.3m/s. Therefore:

$$v_f = \frac{16.3 * 0.045}{0.119} = 6.195\ m/s$$

A.6 Fluidized bed tests

The data presented in Table A.7 was collected during wet peel drying in the fluidized bed dryer and used to plot and identify the time required for the peel to reach 10 % moisture content (large sized peel at 150 ° C was dried) and to show the temperature dynamics within the bed (T2) during the drying (Refer to Fig A.9 and A.10). Refer to Fig 3.1 on the Fluidized Bed to see the placement of temperature sensors T1, T2 and T3.

Table A.7: *Data collected for fluidized bed drying tests*

Time (min)	T1 (° C)	T2 (° C)	T3 (° C)	Pitot Tube ΔP (kPa)	Moisture Content (%)
0	147	42.6	44.9	0.2035	84.6
7	155	46.6	46.6	0.197	70.52
14	157	51.2	50.8	0.1975	37.08
21	159	68.1	72.2	0.2	16.7
28	159	90.8	97.7	0.21	7.08
35	155	116	116.1	0.205	5.9

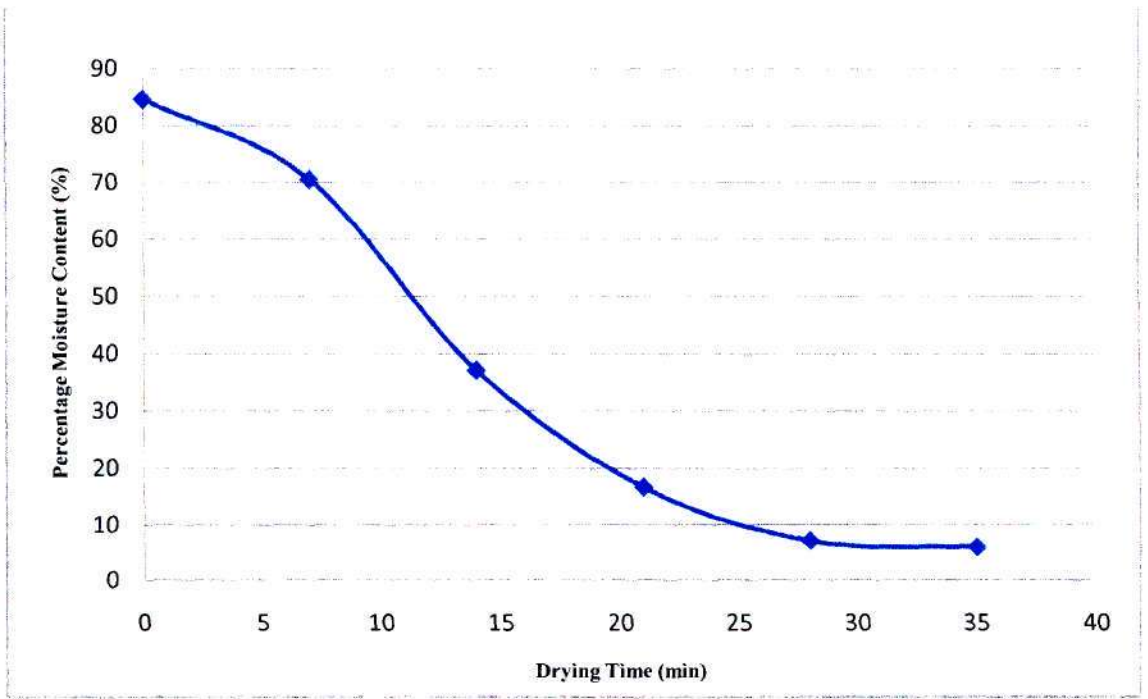

Fig A.9: *Change in moisture content of the peel with time*

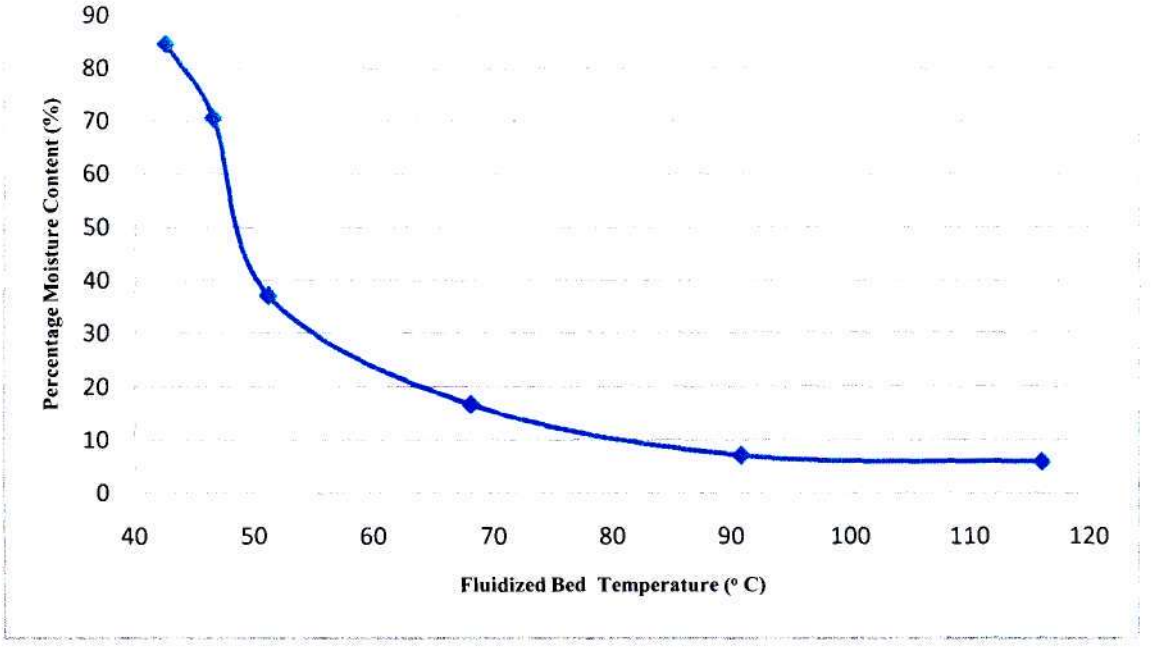

Fig A.10: *Change in the moisture content with temperature change*

Appendix B - Extraction Experiments for dried peel (factorial design)

Appendix B.1 shows the calculations undertaken for the analysis of variance carried out on dried peel experimental results. Appendix B.2 then shows the calculations undertaken when modeling the results.

B.1.1 Analysis of Variance calculations

Shown in Table B.1 are the experimental data collected for dried peel 2^5 factorial design (the two replica values are shown for each treatment combination).

Table B.1 *Experimental data for dried peel 2^5 factorial design*

Factor 1 A-Temp (° C)	Factor 2 B-Time (hrs)	Factor 3 C-pH	Factor 4 D- Size (mm)	Factor 5 E-p:w ratio	Response 1 -% Yield	Response 2 -% GA	Response 3 - % DE	Treatment Combinatior
70	0.5	1.5	<1	1:25	17.21	77.93	68.97	**.(1)**
70	0.5	1.5	<1	1:25	18.79	79.26	69.27	**.(1)**
70	2	1.5	<1	1:25	14.88	80.65	69.05	**b**
70	2	1.5	<1	1:25	15.31	83.17	67.28	**b**
70	0.5	2.5	<1	1:25	6.15	83.37	73.61	**c**
70	0.5	2.5	<1	1:25	6.37	84.81	70.32	**c**
70	2	2.5	<1	1:25	12.02	88.94	69.01	**bc**
70	2	2.5	<1	1:25	12.32	86.91	72.44	**bc**
70	0.5	1.5	4 - 2	1:25	7.99	84.03	69.63	**d**
70	0.5	1.5	4 - 2	1:25	7.86	85.09	70.27	**d**
70	2	1.5	4 - 2	1:25	10.8	86.05	69.78	**bd**
70	2	1.5	4 - 2	1:25	11.35	86.73	68.46	**bd**
70	0.5	2.5	4 - 2	1:25	2.76	87.42	73.05	**cd**
70	0.5	2.5	4 - 2	1:25	2.97	85.59	71.43	**cd**
70	2	2.5	4 - 2	1:25	7.97	82.99	70.34	**bcd**
70	2	2.5	4 - 2	1:25	7.48	87.89	73.25	**bcd**
70	0.5	1.5	<1	1:50	18.61	81.44	70.62	**e**
70	0.5	1.5	<1	1:50	17.74	82.42	66.67	**e**
70	2	1.5	<1	1:50	18.37	84.04	68.49	**be**
70	2	1.5	<1	1:50	18.94	84.13	68.18	**be**
70	0.5	2.5	<1	1:50	10.06	77.1	70.5	**ce**
70	0.5	2.5	<1	1:50	10.68	83.89	70.78	**ce**
70	2	2.5	<1	1:50	9.23	86.64	73.45	**bce**
70	2	2.5	<1	1:50	9.7	85.6	72.81	**bce**
70	0.5	1.5	4 - 2	1:50	8.44	81.3	69.1	**de**
70	0.5	1.5	4 - 2	1:50	8.98	81.19	66.9	**de**
70	2	1.5	4 - 2	1:50	11.92	80.71	69.05	**bde**
70	2	1.5	4 - 2	1:50	12.77	78.67	67.48	**bde**
70	0.5	2.5	4 - 2	1:50	3.6	85.21	71.6	**cde**
70	0.5	2.5	4 - 2	1:50	3.51	82.13	70.12	**cde**
70	2	2.5	4 - 2	1:50	7.62	88.21	74.29	**bcde**
70	2	2.5	4 - 2	1:50	8.37	89.06	73.29	**bcde**
90	0.5	1.5	<1	1:25	26.05	90.41	64.66	**a**
90	0.5	1.5	<1	1:25	24.23	85.9	62.33	**a**
90	2	1.5	<1	1:25	30.58	85.2	58.82	**ab**
90	2	1.5	<1	1:25	30.08	91	59.75	**ab**
90	0.5	2.5	<1	1:25	16.64	82.28	71.16	**ac**
90	0.5	2.5	<1	1:25	17.55	78.56	66.99	**ac**
90	2	2.5	<1	1:25	17.01	90.66	67.93	**abc**
90	2	2.5	<1	1:25	18.57	82.44	68.81	**abc**
90	0.5	1.5	4 - 2	1:25	14.84	85.78	66.82	**ad**
90	0.5	1.5	4 - 2	1:25	13.78	81.24	72.04	**ad**
90	2	1.5	4 - 2	1:25	27.31	85.57	64.73	**abd**
90	2	1.5	4 - 2	1:25	27.99	89.46	60.17	**abd**
90	0.5	2.5	4 - 2	1:25	8.79	80.57	69.68	**acd**
90	0.5	2.5	4 - 2	1:25	9.42	79.31	68.98	**acd**
90	2	2.5	4 - 2	1:25	12.57	85	73.64	**abcd**
90	2	2.5	4 - 2	1:25	13.32	87.72	73.25	**abcd**
90	0.5	1.5	<1	1:50	27.16	84.88	66.82	**ae**
90	0.5	1.5	<1	1:50	25.61	82.91	68.22	**ae**
90	2	1.5	<1	1:50	28.89	85.62	63.8	**abe**
90	2	1.5	<1	1:50	29.83	87.44	61.95	**abe**
90	0.5	2.5	<1	1:50	13.43	89.86	69.58	**ace**
90	0.5	2.5	<1	1:50	13.07	86.31	68.79	**ace**
90	2	2.5	<1	1:50	22.74	85.77	70.09	**abce**
90	2	2.5	<1	1:50	23.22	89.94	67.95	**abce**
90	0.5	1.5	4 - 2	1:50	14.51	80.96	62.25	**ade**
90	0.5	1.5	4 - 2	1:50	15.27	79.25	63.79	**ade**
90	2	1.5	4 - 2	1:50	23.44	84.27	59.36	**abde**
90	2	1.5	4 - 2	1:50	23.59	89.14	63.36	**abde**
90	0.5	2.5	4 - 2	1:50	7.83	86.25	72.57	**acde**
90	0.5	2.5	4 - 2	1:50	8.11	80.94	72.17	**acde**
90	2	2.5	4 - 2	1:50	14.51	87.63	66.37	**abcde**
90	2	2.5	4 - 2	1:50	13.36	84.43	67.08	**abcde**

B.1.2 Summing of the treatment replicas

In order to calculate the contrasts of each 'effect' the two response replicas had to be summed up, the calculations are shown in Equation B.1 and also given in Table B.2.

In the case of treatment **c** (pH at a high level and all other variables at their low level) under the % Yield response, the resultant sum was found as follows (Refer to Table B.1 for the corresponding treatment response values).

Sum(c) = Replicate 1 + Replicate 2 = 6.15 + 6.37 = 12.52... ... ...(B.1)

Table B.2: *Table of sums of the treatment combination replicas for dried peel extraction*

Treatment Combination	%Yield	%GA	%DE
c	12.52	168.18	143.93
b	30.19	163.82	136.33
(1)	36	157.19	138.24
bc	24.34	175.85	141.45
a	50.28	176.31	126.99
abc	35.58	173.1	136.74
ac	34.19	160.84	138.15
ab	60.66	176.2	118.57
d	15.85	169.12	139.9
bcd	15.45	170.88	143.59
cd	5.73	173.01	144.48
bd	22.15	172.78	138.24
acd	18.21	159.88	138.66
abd	55.3	175.03	124.9
ad	28.62	167.02	138.86
abcd	25.89	172.72	146.89
ce	7.11	167.34	141.72
be	24.69	159.38	136.53
e	17.42	162.49	136
bce	15.99	177.27	147.58
ace	26.5	176.17	138.37
abce	58.72	173.06	125.75
ae	52.77	167.79	135.04
abe	45.96	175.71	138.04
de	36.35	163.86	137.29
bcde	18.93	172.24	146.26
cde	20.74	160.99	141.28
bde	37.31	168.17	136.67
ade	29.78	160.21	126.04
abcde	27.87	172.06	133.45
acde	15.94	167.19	144.74
abde	47.03	173.41	122.72

B.1.3 Calculating 'effect' contrasts

The Yates algorithm table was (Table B.3) used to predict the sign of each treatment combination used in calculating the contrast of a particular 'effect' for the 2^5 factorial design.

Table B.3: *Yates algorithm for a 2^5 factorial design*

	Factorial Effect																													
Treatments	I	A	B	AB	C	AC	BC	ABC	D	AD	BD	ABD	CD	ACD	BCD	ABCD	E	AE	BE	ABE	CE	ACE	BCE	ABCE	DE	ADE	BDE	ABDE	CDE	ACDE	BCDE
(1)	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+
a	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+
b	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	+	-	+	-	-	+	-
ab	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
c	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	+	-	-	+	+	-	-
ac	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	-	-	+	+	-
bc	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	-	+	-	+	-	+
abc	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
d	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-
ad	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-
bd	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+
abd	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+
cd	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
acd	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+
bcd	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
abcd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
e	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-
ae	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+	+	+	-	
be	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+
abe	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+
ce	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+
ace	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+
bce	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-
abce	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
de	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+
ade	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+
bde	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-
abde	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-
cde	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
acde	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-
bcde	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
abcde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

From Table B.3 (Yates Algorithm), the sign of the sum of each treatment response value used in the calculation of a particular 'effect' contrast was determined and the 'effect' contrast was calculated as follows:

For Effect A:

Contrast A = - sum((1)) + sum(a) –sum(b) +sum(ab) –sum(c) +sum(ac) –sum(bc) +sum(abc)

-sum(d) +sum(ad) –sum(bd) +sum(abd) –sum(cd) + sum(acd) – sum(bcd)

+sum(abcd) -sum(e) +sum(ae) –sum(be) +sum(abe) –sum(ce) +sum(ace)

-sum(bce) + sum(abce) – sum(de) + sum(ade) –sum(bde) + sum(abde)

-sum(cde) + sum(acde) -sum(bcde) + sum(abcde)... ... ... (B.2)

= - 36 + 50.28 - 30.19 + 60.66 - 12.52 + 34.19 - 24.34 + 35.58- 15.85 +28.62

- 22.15 + 55.30 - 5.73 + 18.21 - 15.45

+ 25.89 - 36.35 + 52.77 - 37.31 + 58.72 - 20.74 + 26.5

- 18.93 + 45.96 - 17.42 + 29.78 - 24.69 + 47.03

- 7.11 + 15.94 - 15.99 + 27.87

= 272.52

The contrasts for other 'effects' were then calculated similarly with the aid of Table B.3 and are shown in Table B.4:

Table B.4: *Table of 'effects' and their corresponding contrasts for dried peel extraction*

	CONTRASTS		
Effect	%Yield	%GA	%DE
A	272.53	44.13	-115.58
B	138.05	94.09	-35.98
AB	63.39	17.67	-43.6
C	-252.17	37.59	147.26
AC	-53.87	-60.31	45.08
BC	0.09	18.37	41.32
ABC	-39.69	-12.09	19.06
D	-208.01	-9.69	25.2
AD	-24.03	-53.63	12.02
BD	53.37	0.45	2.98
ABD	-0.65	43.47	-4.76
CD	34.87	4.23	8.58
ACD	-20.11	3.21	3.96
BCD	-38.67	-10.87	-0.68
ABCD	-19.01	-9.41	-2.82
E	12.15	-14.59	-8.44
AE	-20.47	23.59	-2.78
BE	1.73	-3.57	9.02
ABE	15.19	-16.67	-26.36
CE	2.11	43.61	3.54
ACE	15.81	45.75	-15.68
BCE	13.97	-6.53	-17.48
ABCE	58.39	-55.99	-38.94
DE	-14.89	-27.59	-45.04
ADE	-6.39	11.47	-33.18
BDE	-11.83	8.59	-8.9
ABDE	-25.81	0.53	-9.28
CDE	7.15	28.05	25.42
ACDE	2.21	-57.09	17.28
BCDE	9.77	17.87	-33.72
ABCDE	-27.41	-7.99	-30.5

B.1.4 Calculation of the sum of squares (SS) of each 'effect'

The sum of squares (SS) for each Effect was then determined as follows:

$$SS = \frac{(\text{contrast})^2}{n * p} \quad \dots\dots\dots \quad (B.3)$$

Thus the sum of squares of Effect A (SS (A)) was determined to be:

$$SS(A) = \frac{(272.52)^2}{2 * 2^5} = 1160.51$$

The sum of squares for all other 'effects' were calculated as in above and are shown in Table B.5.

B.1.5 Calculation of the model sum of squares

The rest of the 'effect' sums of squares were calculated. These were then summed to obtain the model sum of squares for each response as follows:

$$SS_{model} = \sum SS_{Effect} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (B.4)$$

For the % Yield response, the model sum of squares was found to be:

SS_{model} = SS(A) +SS(B) + SS(AB) + SS(C) +SS(AC) + SS(BC) + SS(ABC)

+SS(D) +SS(AD) +SS(BD) +SS(ABD) +SS(CD) + SS(ACD) + SS(BCD)

+SS(ABCD) +SS(E) +SS(AE) +SS(BE) +SS(ABE) +SS(CE) +SS(ACE)

-SS(BCE) + SS(ABCE) + SS(DE) + SS(ADE) +SS(BDE) + SS(ABDE)

+SS(CDE) + SS(ACDE) +SS(BCDE) + SS(ABCDE)

SS_{model} = 1160.51 + 297.78 +62.79 + 993.59 + 45.34 + $1.27*10^{-4}$ + 24.61

+ 676.07 + 9.02 + 44.51 + $6.60*10^{-3}$ +19.00 + 6.32 + 23.37

+ 5.65 + 2.31 + 6.55 + $4.68*10^{-2}$ + 3.61 + 0.07 + 3.91

+ 3.05 + 53.27 + 3.46 + 0.64 + 2.19 + 10.41

+ 0.80 + 0.08 + 1.49 + 11.74

= 3472.15

The model sums of squares for the other responses were then calculated using the same equations and are shown in Table B.5

B.1.6 Calculation of the total sum of squares

Unlike the calculation of the sum of squares for each Effect and for the model, individual replicates of the treatment response results rather than their sum are used in the calculation of this sum of squares.

For a 2^5 factorial design, the total sum of squares is calculated from the following Equation B.5:

$$SS_{TOTAL} = \sum_{f=1}^{2}\sum_{g=1}^{2}\sum_{h=1}^{2}\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{n} y_{fghijk}^2 - \frac{(y_{grand})^2}{2^5 * n} \quad \text{(B. 5)}$$

Where the five subscripts (f, g, h, i, j) represent the different variables investigated at two levels each. The subscript k represents the number of **n** replicates.

In the experiments conducted the total sum of squares was found as follows:

Equation B.5 above was broken down and calculated as:

$$SS_{TOTAL} = \mathbf{X} - \mathbf{Y} \quad \text{(B. 6)}$$

Where $\mathbf{X} = \sum_{f=1}^{2}\sum_{g=1}^{2}\sum_{h=1}^{2}\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{n} y_{fghijk}^{2}$ and $\mathbf{Y} = \frac{(y_{grand})^2}{2^5 * n}$

y_{grand}, known as the grand total, should be distinguished from $\overline{y}_{grand}$, which in the grand mean.

$$\mathbf{X} = (6.15^2 + 6.37^2) + (14.88^2 + 15.31^2) + (17.21^2 + 18.79^2) + (12.02^2 + 12.32^2)$$
$$+ (26.05^2 + 24.23^2) + (17.01^2 + 18.57^2) + (16.64^2 + 17.55^2) + (30.58^2 + 30.08^2)$$
$$+ (7.99^2 + 7.86^2) + (7.97^2 + 7.48^2) + (2.76^2 + 2.97^2) + (10.80^2 + 11.35^2) + (8.79^2 + 9.42^2)$$
$$+ (27.31^2 + 27.99^2) + (14.84^2 + 13.78^2) + (12.57^2 + 13.32^2) + (3.60^2 + 3.51^2)$$
$$+ (11.92^2 + 12.77^2) + (8.44^2 + 8.98^2) + (7.62^2 + 8.37^2) + (13.43^2 + 13.07^2)$$
$$+ (28.89^2 + 29.83^2) + (27.16^2 + 25.61^2) + (22.74^2 + 23.22^2) + (18.61^2 + 17.74^2)$$
$$+ (9.23^2 + 9.70^2) + (10.06^2 + 10.68^2) + (18.37^2 + 18.94^2) + (14.51^2 + 15.27^2)$$
$$+ (14.51^2 + 13.36^2) + (7.83^2 + 8.11^2) + (23.44^2 + 23.59^2)$$
$$= 17705.70$$

$$y_{grand} = 6.15 + 6.37 + 14.88 + 15.31 + 17.21 + 18.79 + 12.02 + 12.32 + 26.05 + 24.23 + 17.01$$
$$+ 18.57 + 16.64 + 17.55 + 30.58 + 30.08 + 7.99 + 7.86 + 7.97 + 7.48 + 2.76 + 2.97$$
$$+ 10.80 + 11.35 + 8.79 + 9.42 + 27.31 + 27.99 + 14.84 + 13.78 + 12.57 + 13.32 + 3.60$$
$$+ 3.51 + 11.92 + 12.77 + 8.44 + 8.98 + 7.62 + 8.37 + 13.43 + 13.07 + 28.89 + 29.83$$
$$+ 27.16 + 25.61 + 22.74 + 23.22 + 18.61 + 17.74 + 9.23 + 9.70 + 10.06 + 10.68$$
$$+ 18.37 + 18.94 + 14.51 + 15.27 + 14.51 + 13.36 + 7.83 + 8.11 + 23.44 + 23.59$$
$$= 954.01$$

Therefore

$$\mathbf{Y} = \frac{954.01^2}{2^5 * 2} = 14222.60$$

It follows, therefore, that the total sum of squares for the response % Yield was found as:

SS_{TOTAL} = 17705.70 - 14222.60 = 3483.01

The total sums of squares for the rest of the responses were also calculated similarly and are presented in Table B.5

B.1.7 Calculation of the error sum of squares

The error sum of squares (SS_{ERROR}) was estimated as follows:

$$SS_{ERROR} = SS_{TOTAL} - SS_{model} \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots . (B.7)$$

For the response % Yield, the error sum of squares was found to be:

SS_{ERROR} = 3483.01 – 3472.15 = 10.86

The same Equation was used to calculate the error sum of squares for the other responses and these are given in Table B.5

B.1.8 Percentage contribution of each 'effect' to the variability in the response

$$\% \text{ Contribution (I)} = \frac{\text{SS (I)}}{\text{SS}_{\text{TOTAL}}} \quad \text{(B. 8a)}$$

Where ***I*** can be any main or interactive 'effect'

The percentage contribution of variables not explained by the model on a particular response variable was calculated as follows:

$$\text{Variation not explained by model} = 100 - \sum \% \text{ Contribution (I)} \quad \text{(B. 8b)}$$

The percentage contribution to the variability in the % Yield response for Effect A was calculated as follows:

$$\% \text{ Contribution (A)} = \frac{\text{SS (A)}}{\text{SS}_{\text{TOTAL}}} = \frac{1160.51}{3483.01} = 33.32\%$$

The other contributions for other 'effects' were calculated likewise (Refer to Table B.5)

The variation not explained by the model for the % Yield response was calculated from Equation B.8b as:

$$\sum \% \text{ Contribution (I)}$$

$$= 33.32 + 8.55 + 1.80 + 28.53 + 1.30 + 0.00 + 0.71 + 19.41 + 0.26 + 1.28 + 0.00 + 0.55 + 0.18 + 0.67 + 0.16 + 0.07 + 0.19 + 0.00 + 0.10 + 0.00 + 0.11 + 0.09 + 1.53 + 0.10 + 0.02 + 0.06 + 0.30 + 0.02 + 0.00 + 0.04 + 0.34$$

$$= 99.69\%$$

$$\text{Variation not explained by model} = 100 - 99.69 = 0.31\%$$

B.1.9 F-Testing for the significance of each 'effect'

The mean square of each 'effect' was computed by dividing the 'effect's' sum of squares with its corresponding degree of freedom (DOF). The same was done to compute the mean error sum of squares.

For Effect A

$$MS(A) = \frac{SS(A)}{DOF(A)} = \frac{1160.51}{1} = 1160.51 \quad \text{(B.9)}$$

Where each 'effect' has a DOF equal to 1

The DOF of the error was calculated as follows:

$$DOF_{model} = 2^k - 1 = 2^5 - 1 = 31 \quad \text{(B.10)}$$

$$DOF_{TOTAL} = (2^k * n) - 1 = (2^5 * 2) - 1 = 63 \quad \text{(B.11)}$$

$$DOF_{ERROR} = DOF_{TOTAL} - DOF_{model} = 63 - 31 = 32 \quad \text{(B.12)}$$

Where **k** is the number variables investigated and **n** the number of replicas

It therefore followed that

$$MS_{ERROR} = \frac{SS_{ERROR}}{DOF_{ERROR}} = \frac{10.86}{32} = 0.34 \quad \text{(B.13)}$$

The F value was computed as follows:

$$F_0 = \frac{MS(A)}{MS_{ERROR}} = \frac{1160.51}{0.34} = 3420.86 \quad \text{(B.14)}$$

$F_{1-\alpha,v1,v2}$ at 95 % confidence interval (CI)= 4.152

Where *v1* = DOF(numerator) =DOF(A) and *v2* = DOF (denominator) =DOF_{ERROR}

If $F_o > F_{1-\alpha,v1,v2}$ then the 'effect' is significant as thus Effect A was found to be significant at 95 % CI.

The significance of all other 'effects' was computed similarly and are shown in Table B.5

The sum of squares of each 'effect', its corresponding percentage contribution, degrees of freedom, mean square and F value (F_o) were calculated as shown in Appendix B.1.4 to B.1.9 and the results are shown below:

Table B.5: *Analysis of Variance Table for the 2^5 factorial design for dried peel extraction*

	SS			%contribution			DOF			MS			Fo		
Effect	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%DE
A	1160.51	30.43	208.73	33.32	4.07	23.08	1	1	1	1160.51	30.43	208.73	3420.86	5.02	79.28
B	297.78	138.33	20.23	8.55	18.52	2.24	1	1	1	297.78	138.33	20.23	877.77	22.81	7.68
AB	62.79	4.88	29.70	1.80	0.65	3.28	1	1	1	62.79	4.88	29.70	185.07	0.80	11.28
C	993.59	22.08	338.84	28.53	2.96	37.46	1	1	1	993.59	22.08	338.84	2928.82	3.64	128.70
AC	45.34	56.83	31.75	1.30	7.61	3.51	1	1	1	45.34	56.83	31.75	133.66	9.37	12.06
BC	0.00	5.27	26.68	0.00	0.71	2.95	1	1	1	0.00	5.27	26.68	0.00	0.87	10.13
ABC	24.61	2.28	5.68	0.71	0.31	0.63	1	1	1	24.61	2.28	5.68	72.56	0.38	2.16
D	676.07	1.47	9.92	19.41	0.20	1.10	1	1	1	676.07	1.47	9.92	1992.85	0.24	3.77
AD	9.02	44.94	2.26	0.26	6.02	0.25	1	1	1	9.02	44.94	2.26	26.60	7.41	0.86
BD	44.51	0.00	0.14	1.28	0.00	0.02	1	1	1	44.51	0.00	0.14	131.19	0.00	0.05
ABD	0.01	29.53	0.35	0.00	3.95	0.04	1	1	1	0.01	29.53	0.35	0.02	4.87	0.13
CD	19.00	0.28	1.15	0.55	0.04	0.13	1	1	1	19.00	0.28	1.15	56.00	0.05	0.44
ACD	6.32	0.16	0.25	0.18	0.02	0.03	1	1	1	6.32	0.16	0.25	18.63	0.03	0.09
BCD	23.37	1.85	0.01	0.67	0.25	0.00	1	1	1	23.37	1.85	0.01	68.87	0.30	0.00
ABCD	5.65	1.38	0.12	0.16	0.19	0.01	1	1	1	5.65	1.38	0.12	16.64	0.23	0.05
E	2.31	3.33	1.11	0.07	0.45	0.12	1	1	1	2.31	3.33	1.11	6.80	0.55	0.42
AE	6.55	8.70	0.12	0.19	1.16	0.01	1	1	1	6.55	8.70	0.12	19.30	1.43	0.05
BE	0.05	0.20	1.27	0.00	0.03	0.14	1	1	1	0.05	0.20	1.27	0.14	0.03	0.48
ABE	3.61	4.34	10.86	0.10	0.58	1.20	1	1	1	3.61	4.34	10.86	10.63	0.72	4.12
CE	0.07	29.72	0.20	0.00	3.98	0.02	1	1	1	0.07	29.72	0.20	0.21	4.90	0.07
ACE	3.91	32.70	3.84	0.11	4.38	0.42	1	1	1	3.91	32.70	3.84	11.51	5.39	1.46
BCE	3.05	0.67	4.77	0.09	0.09	0.53	1	1	1	3.05	0.67	4.77	8.99	0.11	1.81
ABCE	53.27	48.98	23.69	1.53	6.56	2.62	1	1	1	53.27	48.98	23.69	157.03	8.08	9.00
DE	3.46	11.89	31.70	0.10	1.59	3.50	1	1	1	3.46	11.89	31.70	10.21	1.96	12.04
ADE	0.64	2.06	17.20	0.02	0.28	1.90	1	1	1	0.64	2.06	17.20	1.88	0.34	6.53
BDE	2.19	1.15	1.24	0.06	0.15	0.14	1	1	1	2.19	1.15	1.24	6.45	0.19	0.47
ABDE	10.41	0.00	1.35	0.30	0.00	0.15	1	1	1	10.41	0.00	1.35	30.68	0.00	0.51
CDE	0.80	12.29	10.10	0.02	1.65	1.12	1	1	1	0.80	12.29	10.10	2.35	2.03	3.83
ACDE	0.08	50.93	4.67	0.00	6.82	0.52	1	1	1	0.08	50.93	4.67	0.22	8.40	1.77
BCDE	1.49	4.99	17.77	0.04	0.67	1.96	1	1	1	1.49	4.99	17.77	4.40	0.82	6.75
ABCDE	11.74	1.00	14.54	0.34	0.13	1.61	1	1	1	11.74	1.00	14.54	34.60	0.16	5.52
Model	3472.15	552.65	820.21	99.69	74.01	90.69	31	31	31	112.00	17.83	26.46			
Error witho	10.86	194.09	84.25	0.31	25.99	9.31	32	32	32	0.34	6.07	2.63			
TOTAL	3483.01	746.74	904.46	100.00	100.00	100.00	63	63	63	55.29	11.85	14.36			

B.1.10 Model Correlation Coefficient and Adjusted Model Correlation Coefficient

The model correlation coefficient was calculated as follows

$$R^2 = \frac{SS_{model}}{SS_{TOTAL}} \quad \text{(B.15)}$$

The correlation coefficient (R^2) for the response % Yield was calculated as follows:

$$R^2 = \frac{3472.15}{3483.01} = 0.997$$

The model correlation coefficients for the other responses were also calculated likewise.

The adjusted model correlation (R_{adj}^2) coefficients were also calculated as follows:

$$R_{adj}{}^2 = 1 - \frac{\left(\frac{SS_{ERROR}}{DOF_{ERROR}}\right)}{\left(\frac{SS_{TOTAL}}{DOF_{TOTAL}}\right)} \quad \text{(B.16)}$$

The adjusted correlation coefficient for the % Yield response was thus evaluated as follows:

$$R_{adj}{}^2 = 1 - \frac{\left(\frac{10.86}{32}\right)}{\left(\frac{3483.01}{63}\right)} = 0.994$$

The other adjusted correlation coefficients for the other responses were also computed similarly and presented in Table B.6

Table B.6: *Table of correlation coefficients for the three responses for dried peel extraction*

Response	R^2_{Model}	R^2_{adj}
%Yield	0.997	0.994
%GA	0.740	0.488
%DE	0.907	0.817

B.2 Modeling

The calculations undertaken when modeling the response results for dried peel extractions are shown in the following section

B.2.1 Calculating 'effect' estimates

The 'effect' estimates were calculated as follows:

$$\text{Effect estimate} = \frac{1}{2} * \frac{(\text{contrast})^2}{n * p} \quad \text{(B. 17)}$$

Where **n** is the number of replicas and **p** the total number of experiments conducted

For the extraction experiments conducted **p** = 2^5 and **n** = 2

Thus Effect A was calculated as follows:

$$\text{Effect A} = \frac{1}{2} * \frac{272.52}{2 * 2^5} = 8.52$$

All the other 'effect' estimates were calculated similarly and are shown in Table B.7

Table B.7: *Table of Effect Estimates for the three responses for dried peel extraction*

Effect	Effect Estimation		
	%Yield	%GA	%DE
A	8.52	1.38	-3.61
B	4.31	2.94	-1.12
AB	1.98	0.55	-1.36
C	-7.88	1.17	4.60
AC	-1.68	-1.88	1.41
BC	0.00	0.57	1.29
ABC	-1.24	-0.38	0.60
D	-6.50	-0.30	0.79
AD	-0.75	-1.68	0.38
BD	1.67	0.01	0.09
ABD	-0.02	1.36	-0.15
CD	1.09	0.13	0.27
ACD	-0.63	0.10	0.12
BCD	-1.21	-0.34	-0.02
ABCD	-0.59	-0.29	-0.09
E	0.38	-0.46	-0.26
AE	-0.64	0.74	-0.09
BE	0.05	-0.11	0.28
ABE	0.47	-0.52	-0.82
CE	0.07	1.36	0.11
ACE	0.49	1.43	-0.49
BCE	0.44	-0.20	-0.55
ABCE	1.82	-1.75	-1.22
DE	-0.47	-0.86	-1.41
ADE	-0.20	0.36	-1.04
BDE	-0.37	0.27	-0.28
ABDE	-0.81	0.02	-0.29
CDE	0.22	0.88	0.79
ACDE	0.07	-1.78	0.54
BCDE	0.31	0.56	-1.05
ABCDE	-0.86	-0.25	-0.95

From all the estimated 'effects', only the significant were used in modelling the results. Normal probability plots were constructed and the plots in conjunction with the F-test identified significant 'effects'. Table B.8 shows the 'effect' names and corresponding 'effect' estimates arranged in decreasing magnitude. The corresponding graphs that identified significant terms were shown under the Discussion section (Chapter 5), Fig 5.2, Fig 5.5 and Fig 5.8.

Table B.8: *Rearranged effect estimates used in normal probability plots for the three responses for dried peel extraction*

Cumulative frequency	Normal probability					
	% Yield		% GA		%DE	
	Effect	%Yield	Effect	%GA	Effect	%DE
98.39	A	8.52	B	2.94	C	4.60
95.16	B	4.31	ACE	1.43	AC	1.41
91.94	AB	1.98	A	1.38	BC	1.29
88.71	ABCE	1.82	CE	1.36	D	0.79
85.48	BD	1.67	ABD	1.36	CDE	0.79
82.26	CD	1.09	C	1.17	ABC	0.60
79.03	ACE	0.49	CDE	0.88	ACDE	0.54
75.81	ABE	0.47	AE	0.74	AD	0.38
72.58	BCE	0.44	BC	0.57	BE	0.28
69.35	E	0.38	BCDE	0.56	CD	0.27
66.13	BCDE	0.31	AB	0.55	ACD	0.12
62.90	CDE	0.22	ADE	0.36	CE	0.11
59.68	CE	0.07	BDE	0.27	BD	0.09
56.45	ACDE	0.07	CD	0.13	BCD	-0.02
53.23	BE	0.05	ACD	0.10	ABCD	-0.09
50.00	BC	0.00	ABDE	0.02	AE	-0.09
46.77	ABD	-0.02	BD	0.01	ABD	-0.15
43.55	ADE	-0.20	BE	-0.11	E	-0.26
40.32	BDE	-0.37	BCE	-0.20	BDE	-0.28
37.10	DE	-0.47	ABCDE	-0.25	ABDE	-0.29
33.87	ABCD	-0.59	ABCD	-0.29	ACE	-0.49
30.65	ACD	-0.63	D	-0.30	BCE	-0.55
27.42	AE	-0.64	BCD	-0.34	ABE	-0.82
24.19	AD	-0.75	ABC	-0.38	ABCDE	-0.95
20.97	ABDE	-0.81	E	-0.46	ADE	-1.04
17.74	ABCDE	-0.86	ABE	-0.52	BCDE	-1.05
14.52	BCD	-1.21	DE	-0.86	B	-1.12
11.29	ABC	-1.24	AD	-1.68	ABCE	-1.22
8.06	AC	-1.68	ABCE	-1.75	AB	-1.36
4.84	D	-6.50	ACDE	-1.78	DE	-1.41
1.61	C	-7.88	AC	-1.88	A	-3.61

B.2.2 Regression model

The regression model coefficients (regression coefficients) of significant 'effects' were computed.

The model was given by the following expression

$$\%\text{Yield} = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_i x_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B.18)$$

Where β_o was evaluated as the grand mean ($\bar{y}_{grand}$)

$\beta_1 \ldots \beta_i$ were regression coefficients corresponding to individual 'effects'

For the % Yield response:

$$\beta_o = \bar{y}_{grand} = \frac{y_{grand}}{N} = \frac{954.01}{64} = 14.91 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B.19)$$

Where y_{grand} was calculated previously (in Appendix B.1.6)

For Effect A (which was found significant), the corresponding regression coefficient (in coded variables) was found to be:

$$\beta_1 = \frac{\text{Effect (A)}}{2} = \frac{8.52}{2} = 4.26$$

The other regression coefficients were calculated similarly to give the following model:

$$\begin{aligned}\%\text{Yield} = {} & 14.91 + 4.26x_1 + 2.16x_2 - 3.94x_3 - 3.25x_4 + 0.19x_5 + 0.99x_{12} - 0.84x_{13} \\ & - 0.38x_{14} - 0.32x_{15} + 0.83x_{24} + 0.54x_{34} - 0.23x_{45} - 0.62x_{123} - 0.31x_{134} \\ & + 0.25x_{135} - 0.60x_{234} - 0.18x_{245} - 0.30x_{1234} + 0.91x_{1235} - 0.40x_{1245} \\ & + 0.15x_{2345} - 0.43x_{12345} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (B.20)\end{aligned}$$

Under the Discussion section (Chapter 5), Table 5.1, Table 5.2 and Table 5.3 show all the empirical models for the three responses; only significant regression coefficients are shown.

B.2.3 Calculation of Residuals

The experimental and calculated response variables were then computed in order to check the model adequacy.

The residual of each treatment combination was calculated as follows:
For treatment **c** (*the first replica*) the predicted % Yield was calculated using Equation B.20 as follows:

$$\begin{aligned}\text{predicted \% Yield } (\mathbf{c}) &= 14.91 + 4.26(-1) + 2.16(-1) - 3.94(+1) - 3.25(-1) + 0.19(-1) \\ &+ 0.99(-1 * -1) - 0.84(-1 * +1) - 0.38(-1 * -1) - 0.32(-1 * -1) \\ &+ 0.83(-1 * -1) + 0.54(+1 * -1) - 0.23(-1 * -1) - 0.62(-1 * -1 * +1) \\ &- 0.31(-1 * +1 * -1) + 0.25(-1 * +1 * -1) - 0.60(-1 * +1 * -1) \\ &- 0.18(-1 * -1 * -1) - 0.30(-1 * -1 * +1 * -1) \\ &+ 0.91(-1 * -1 * +1 * -1) - 0.40(-1 * -1 * -1 * -1) \\ &+ 0.15(-1 * +1 * -1 * -1) - 0.43(-1 * -1 * +1 * -1 * -1)\end{aligned}$$

$$= 6.10$$

Therefore the residual of treatment c for the first replica was calculated as:

Residual (**c**) = experimental % Yield (**c**) – predicted % Yield (**c**) … … … … … … … ... (B.21)

$$= 6.15 - 6.10 = 0.05$$

All other residuals were computed (Table B.9) for each treatment in a similar manner and a probability plot of the residuals was then plotted to check for model adequacy

Table B.9: *Residual calculations for the different treatments used for model adequacy checks for dried peel extraction*

	% Yield				% GA				% DE			
cum freq	Treament	%Yield exp	%Yield pred	Residual	Treatment	%GA exp	%GApred	Residual	Treatment	%DE exp	%DE pred	Residu
99.22	abcde	14.51	13.38	1.13	abc	90.66	85.07	5.59	ad	72.04	68.78	3.26
97.66	ac	17.55	16.61	0.94	ce	83.89	80.57	3.32	abd	64.73	62.60	2.13
96.09	ae	27.16	26.23	0.93	acde	86.25	83.25	3.00	ac	71.16	69.05	2.11
94.53	ad	14.84	13.97	0.87	abd	89.46	86.51	2.95	a	64.66	62.75	1.91
92.97	e	18.61	17.79	0.82	d	85.09	82.33	2.76	b	69.05	67.44	1.61
91.41	abe	29.83	29.09	0.74	bcd	87.89	85.26	2.63	abde	63.36	61.76	1.60
89.84	acd	9.42	8.69	0.73	ae	84.88	82.45	2.43	c	73.61	72.02	1.59
88.28	a	26.05	25.32	0.73	ab	91.00	88.61	2.39	bcd	73.25	71.87	1.38
86.72	bd	11.35	10.63	0.72	bc	88.94	86.72	2.22	ce	70.78	69.45	1.33
85.16	abce	23.22	22.50	0.72	abce	89.94	87.90	2.04	abcd	73.64	72.33	1.31
83.59	b	15.31	14.62	0.69	cd	87.42	85.42	2.00	(1)	69.27	68.00	1.27
82.03	de	8.98	8.30	0.68	abde	89.14	87.25	1.89	bde	69.05	67.84	1.21
80.47	bcde	8.37	7.77	0.60	abcde	87.63	85.80	1.83	ce	70.50	69.45	1.05
78.91	abd	27.99	27.43	0.56	ad	85.78	83.96	1.82	bc	72.44	71.41	1.03
77.34	(1)	18.79	18.36	0.43	ace	89.86	88.06	1.80	(1)	68.97	68.00	0.97
75.78	abc	18.57	18.25	0.32	(1)	79.26	77.51	1.75	e	70.62	69.66	0.96
74.22	bc	12.32	12.04	0.28	d	84.03	82.33	1.70	abcd	73.25	72.33	0.92
72.66	c	6.37	6.10	0.27	abe	87.44	85.78	1.66	abc	68.81	67.89	0.92
71.09	b	14.88	14.62	0.26	a	90.41	88.77	1.64	cde	71.60	70.76	0.84
69.53	cde	3.60	3.36	0.24	e	82.42	81.11	1.31	cd	73.05	72.28	0.77
67.97	abce	22.74	22.50	0.24	abcd	87.72	86.54	1.18	acde	72.57	71.88	0.69
66.41	bd	10.80	10.63	0.17	bd	86.73	85.66	1.07	ade	63.79	63.14	0.65
64.84	ce	10.68	10.51	0.17	c	84.81	84.17	0.64	ae	68.22	67.60	0.62
63.28	cde	3.51	3.36	0.15	ac	82.28	81.74	0.54	abce	70.09	69.51	0.58
61.72	de	8.44	8.30	0.14	be	84.13	83.66	0.47	bd	69.78	69.30	0.48
60.16	acd	8.79	8.69	0.10	ae	82.91	82.45	0.46	abcde	67.08	66.64	0.44
58.59	bce	9.70	9.61	0.09	(1)	77.93	77.51	0.42	be	68.49	68.12	0.37
57.03	ade	15.27	15.20	0.07	bd	86.05	85.66	0.39	bce	73.45	73.12	0.33
55.47	c	6.15	6.10	0.05	be	84.04	83.66	0.38	acde	72.17	71.88	0.29
53.91	bcd	7.97	7.93	0.04	bcde	89.06	88.72	0.34	d	70.27	70.06	0.21
52.34	ac	16.64	16.61	0.03	e	81.44	81.11	0.33	acd	69.68	69.48	0.20
50.78	ab	30.58	30.58	0.00	bc	86.91	86.72	0.19	bcde	74.29	74.23	0.06
49.22	bde	12.77	12.78	-0.01	cd	85.59	85.42	0.17	be	68.18	68.12	0.06
47.66	abcde	13.36	13.38	-0.02	acd	80.57	80.49	0.08	abc	67.93	67.89	0.04
46.09	bc	12.02	12.04	-0.02	bce	86.64	86.62	0.02	de	69.10	69.18	-0.08
44.53	e	17.74	17.79	-0.05	abe	85.62	85.78	-0.16	b	67.28	67.44	-0.16
42.97	cd	2.97	3.08	-0.11	cde	85.21	85.39	-0.18	abe	61.95	62.21	-0.26
41.41	abd	27.31	27.43	-0.12	ade	80.96	81.20	-0.24	abcde	66.37	66.64	-0.27
39.84	bcde	7.62	7.77	-0.15	b	83.17	83.56	-0.39	bce	72.81	73.12	-0.31
38.28	abde	23.59	23.76	-0.17	bcde	88.21	88.72	-0.51	bde	67.48	67.84	-0.36
36.72	ad	13.78	13.97	-0.19	c	83.37	84.17	-0.80	a	62.33	62.75	-0.42
35.16	abcd	13.32	13.52	-0.20	abd	85.57	86.51	-0.94	d	69.63	70.06	-0.43
33.59	abe	28.89	29.09	-0.20	bce	85.60	86.62	-1.02	acd	68.98	69.48	-0.50
32.03	be	18.94	19.15	-0.21	de	81.30	82.36	-1.06	cde	70.12	70.76	-0.64
30.47	acde	8.11	8.36	-0.25	de	81.19	82.36	-1.17	ae	66.82	67.60	-0.78
28.91	abde	23.44	23.76	-0.32	acd	79.31	80.49	-1.18	ab	59.75	60.58	-0.83
27.34	cd	2.76	3.08	-0.32	abcde	84.43	85.80	-1.37	bd	68.46	69.30	-0.84
25.78	ace	13.43	13.76	-0.33	bde	80.71	82.20	-1.49	cd	71.43	72.28	-0.85
24.22	d	7.99	8.35	-0.36	abcd	85.00	86.54	-1.54	ade	62.25	63.14	-0.89
22.66	bce	9.23	9.61	-0.38	ace	86.31	88.06	-1.75	bcde	73.29	74.23	-0.94
21.09	ce	10.06	10.51	-0.45	ade	79.25	81.20	-1.95	ace	69.58	70.74	-1.16
19.53	bcd	7.48	7.93	-0.45	abce	85.77	87.90	-2.13	bcd	70.34	71.87	-1.53
17.97	d	7.86	8.35	-0.49	bcd	82.99	85.26	-2.27	abce	67.95	69.51	-1.56
16.41	ab	30.08	30.58	-0.50	acde	80.94	83.25	-2.31	c	70.32	72.02	-1.70
14.84	acde	7.83	8.36	-0.53	abc	82.44	85.07	-2.63	ab	58.82	60.58	-1.76
13.28	ae	25.61	26.23	-0.62	ad	81.24	83.96	-2.72	ace	68.79	70.74	-1.95
11.72	ace	13.07	13.76	-0.69	a	85.90	88.77	-2.87	ad	66.82	68.78	-1.96
10.16	ade	14.51	15.20	-0.69	b	80.65	83.56	-2.91	ac	66.99	69.05	-2.06
8.59	be	18.37	19.15	-0.78	abde	84.27	87.25	-2.98	de	66.90	69.18	-2.28
7.03	bde	11.92	12.78	-0.86	ac	78.56	81.74	-3.18	abd	60.17	62.60	-2.43
5.47	abcd	12.57	13.52	-0.95	cde	82.13	85.39	-3.26	abde	59.36	61.76	-2.40
3.91	a	24.23	25.32	-1.09	ab	85.20	88.61	-3.41	bc	69.01	71.41	-2.40
2.34	(1)	17.21	18.36	-1.15	ce	77.10	80.57	-3.47	e	66.67	69.66	-2.99
0.78	abc	17.01	18.25	-1.24	bde	78.67	82.20	-3.53	abe	63.80	67.60	-3.80

The residuals were then plotted and the plots showed that the data was normally distributed as no significant anomalies were evident. Figs B.1 to B.3 show the plots.

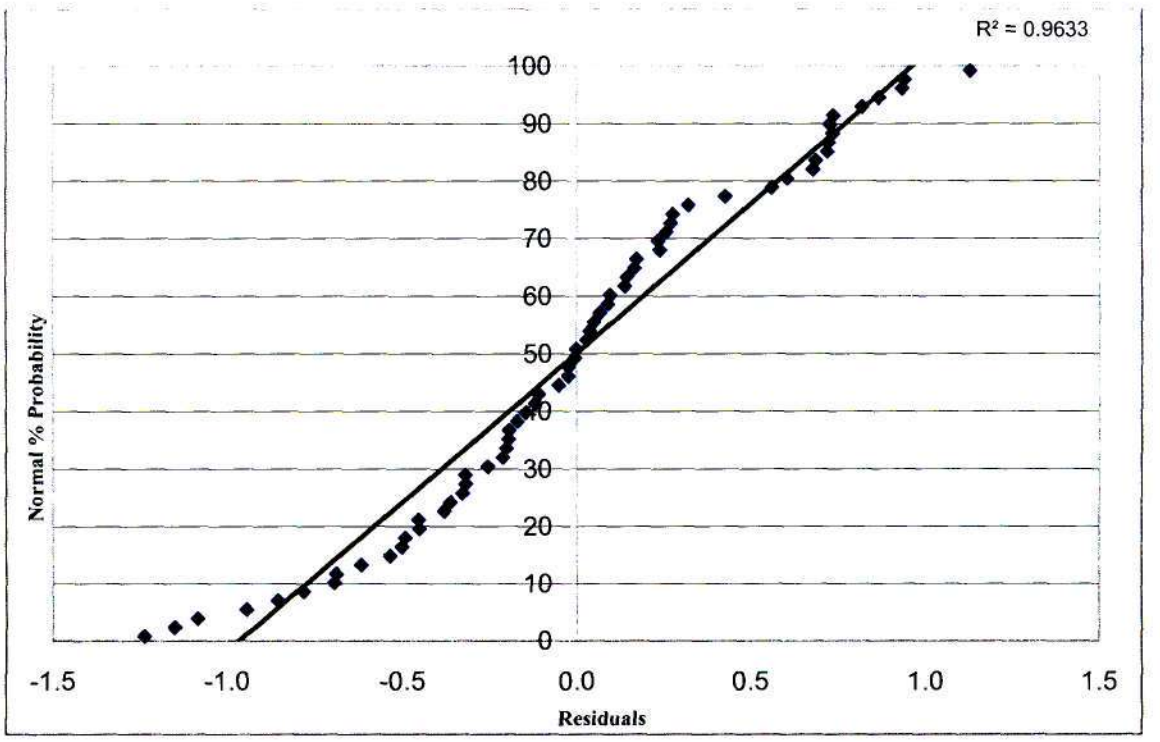

Fig B.1: *Normal probability plot of residuals for the % Yield response for dried peel extraction*

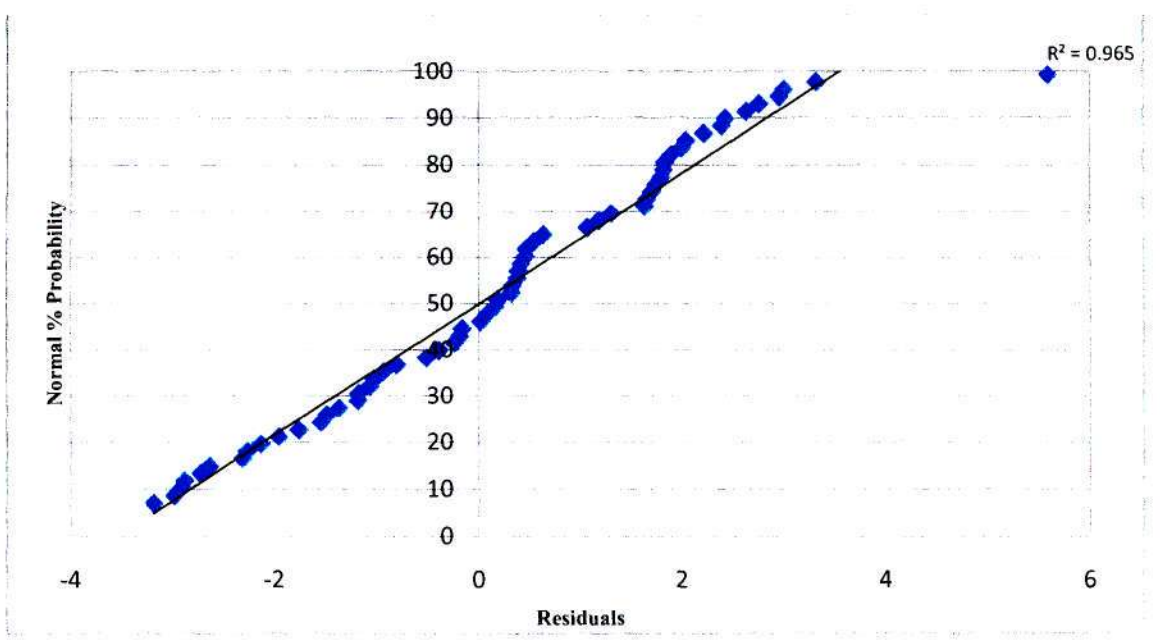

Fig B.2: *Normal probability plot of residuals for the % GA response for dried peel extraction*

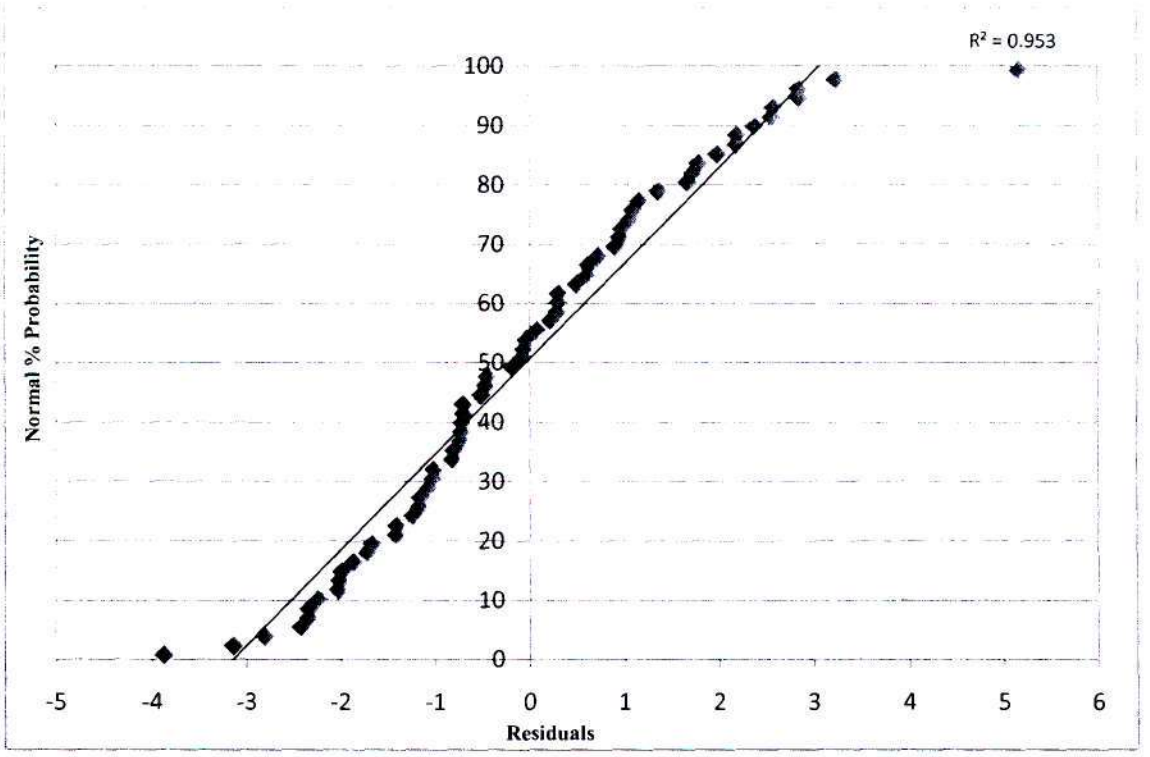

Fig B.3: *Normal probability plot of residuals for the % DE response for dried peel extraction*

Appendix C – Extraction Experiments for fresh wet peel (factorial design)

In order to compare dried peel pectin quantity (% Yield) and quality (% GA and % DE) with fresh wet peel extractions, a 2^4 factorial design on fresh wet peel extractions was conducted. Appendix C.1 shows the calculations undertaken for the analysis of variance carried out on fresh wet peel experimental results. Appendix C.2 then shows the calculations undertaken when modeling the results.

C.1.1 Analysis of Variance calculations

Shown in Table C.1 are the experimental data collected for dried peel 2^4 factorial design (the two replica values are shown)

Table C.1: *Experimental Data for fresh wet peel 2^4 factorial design*

Treatment Combination	NATURAL VARIABLES				RESPONSE VARIABLES		
	Factor 1 – A:Temp (°C)	Factor 2- B:time (hrs)	Factor 3- C:pH	Factor 4- E: p:w	%Yield	%GA	%DE
.(1)	70	0.5	1.5	1:25	8.84	86.91	74.52
.(1)	70	0.5	1.5	1:25	9.63	85.60	72.14
c	70	0.5	2.5	1:25	**3.30**	86.20	75.20
c	70	0.5	2.5	1:25	**3.61**	79.32	73.29
a	90	0.5	1.5	1:25	13.90	81.77	71.28
a	90	0.5	1.5	1:25	14.92	79.59	65.63
ac	90	0.5	2.5	1:25	9.20	78.87	77.40
ac	90	0.5	2.5	1:25	9.76	78.08	72.30
e	70	0.5	1.5	1:50	9.37	84.29	75.56
e	70	0.5	1.5	1:50	10.30	88.72	69.49
ce	70	0.5	2.5	1:50	4.35	84.65	76.15
ce	70	0.5	2.5	1:50	4.90	80.60	76.96
ae	90	0.5	1.5	1:50	15.81	81.20	69.86
ae	90	0.5	1.5	1:50	14.66	74.58	67.43
ace	90	0.5	2.5	1:50	9.38	82.40	74.57
ace	90	0.5	2.5	1:50	9.79	75.45	72.36
b	70	2	1.5	1:25	11.34	84.00	76.87
b	70	2	1.5	1:25	12.67	77.15	72.07
bc	70	2	2.5	1:25	8.03	91.44	79.10
bc	70	2	2.5	1:25	8.90	85.35	78.72
ab	90	2	1.5	1:25	20.56	82.49	71.49
ab	90	2	1.5	1:25	19.97	79.23	70.62
abc	90	2	2.5	1:25	12.24	83.52	77.54
abc	90	2	2.5	1:25	11.04	89.24	78.23
be	70	2	1.5	1:50	12.56	82.36	74.60
be	70	2	1.5	1:50	13.03	90.63	74.30
bce	70	2	2.5	1:50	5.22	81.91	76.96
bce	70	2	2.5	1:50	5.44	81.31	78.63
abe	90	2	1.5	1:50	22.75	80.94	68.93
abe	90	2	1.5	1:50	22.18	80.11	71.86
abce	90	2	2.5	1:50	14.18	80.39	76.32
abce	90	2	2.5	1:50	12.79	79.68	76.83

C.1.2 Summing of the treatment replicas

In order to calculate the contrasts of each 'effect', the two response replicas had to be summed up, the calculations are shown in Equation C.1.

In the case of treatment **c** (pH at a high level and all other variables at their low level) under the % Yield response, the resultant sum was found as follows (Refer to Table C.1 for the corresponding treatment response values)

Sum(c) = Replicate 1 + Replicate 2 = 3.30 + 3.61 = 6.91... ... ...(C.1)

All replicates were summed and the results are shown in Table C.2.

Table C.2: *Table of sums of the treatment combination replicas for fresh wet peel extraction*

Treatment Combination	SUM		
	%Yield	%GA	%DE
(1)	18.47	172.51	146.66
a	28.82	161.37	136.90
b	24.01	161.15	148.94
ab	40.53	161.71	142.11
c	**6.91**	165.51	148.49
ac	18.96	156.95	149.70
bc	16.93	176.79	157.82
abc	23.28	172.76	155.77
e	19.67	173.01	145.05
ae	30.47	155.78	137.28
be	25.59	173.00	148.89
abe	44.93	161.05	140.79
ce	9.25	165.25	153.10
ace	19.18	157.85	146.93
bce	10.67	163.21	155.58
abce	26.96	160.07	153.15

C.1.3 Calculating 'effect' contrasts

The Yates algorithm table (Table C.3) was used to predict the sign of each treatment combination used in calculating the contrast of a particular 'effect' for the 2^4 factorial design.

Table C.3: *Yates Algorithm for a 2^4 factorial design*

Treatment	Factorial Effect															
	I	A	B	AB	C	AC	BC	ABC	E	AE	BE	ABE	CE	ACE	BCE	ABCE
(1)	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+
a	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-
b	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	-
ab	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+
c	+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-
ac	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+
bc	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+
abc	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
e	+	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-
ae	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+
be	+	-	+	-	-	+	-	+	+	-	+	-	-	+	-	+
abe	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
ce	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+
ace	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
bce	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
abce	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

From Table C.3, the sign of the sum of each treatment response value used in the calculation of a particular 'effect' contrast was determined and the 'effect' contrast was calculated as follows:

For Effect A:

Contrast A = - sum((1)) + sum(a) –sum(b) +sum(ab) –sum(c) +sum(ac) –sum(bc) +sum(abc)

-sum(e) +sum(ae) –sum(be) +sum(abe) –sum(ce) + sum(ace) – sum(bce)

+sum(abce) ... ...(C.2)

= - 18.47 + 28.82 – 24.01+ 40.53 – 6.91 + 18.96 – 16.93 + 23.28

- 19.67 + 30.47 – 25.59+ 44.93 – 9.25 + 19.18 – 10.67

+ 26.96

= 101.64

The contrasts for other 'effects' were then calculated similarly with the aid of Table C.3 and are shown in Table C.4:

Table C.4: *Table of 'effects' and their corresponding contrasts for fresh wet peel extraction*

	CONTRASTS		
Effect	%Yield	%GA	%DE
A	**101.64**	-62.88	-41.89
B	61.18	21.52	38.93
AB	15.37	25.79	3.06
C	-100.36	-1.18	73.91
AC	-12.37	16.61	23.01
BC	-14.08	33.04	9.25
ABC	-14.05	-8.20	-2.13
E	8.81	-19.52	-5.60
AE	11.09	-16.54	-7.07
BE	-2.00	-10.62	-6.84
ABE	14.44	-6.71	3.74
CE	-8.86	-31.74	-0.41
ACE	4.54	20.66	-8.49
BCE	-8.27	-43.17	-6.55
ABCE	9.70	6.15	10.27

C.1.4 Calculation of the sum of squares (SS) of each 'effect'

The sum of squares (SS) for each 'effect' was then determined as follows:

$$SS = \frac{(\text{contrast})^2}{n * p} \quad \text{(C.3)}$$

Thus the sum of squares of Effect A (SS (A)) was determined to be:

$$SS(A) = \frac{(101.64)^2}{2 * 2^4} = 322.82$$

The sum of squares for all other Effects were calculated as in above and are shown in Table C.5.

C.1.5 Calculation of the model sum of squares

The rest of the 'effect' sums of squares were calculated. These were then summed to obtain the model sum of squares for each response as follows:

$$SS_{model} = \sum SS_{Effect} \quad \ldots\ldots (C.4)$$

For the % Yield response, the model sum of squares was found to be:

$$SS_{model} = SS(A) + SS(B) + SS(AB) + SS(C) + SS(AC) + SS(BC) + SS(ABC)$$
$$+ SS(E) + SS(AE) + SS(BE) + SS(ABE) + SS(CE) + SS(ACE) + SS(BCE)$$
$$+ SS(ABCE)$$

$$SS_{model} = 322.82 + 116.96 + 7.38 + 314.73 + 4.78 + 6.20 + 6.17$$
$$+ 2.42 + 3.85 + 0.12 + 6.51 + 2.45 + 0.64 + 2.13 + 2.94$$
$$= 800.31$$

The model sums of squares for the other responses (% GA and % DE) were then calculated using the same Equation C.4 and are shown in Table C.5.

C.1.6 Calculation of the total sum of squares

Unlike the calculation of the sum of squares for each 'effect' and for the model, individual replicates of the treatment response results rather than their sum, are used in the calculation of this sum of squares.

For a 2^4 factorial design, the total sum of squares is calculated from the following Equation C.5:

$$SS_{TOTAL} = \sum_{f=1}^{2}\sum_{g=1}^{2}\sum_{h=1}^{2}\sum_{i=1}^{2}\sum_{k=1}^{n} y_{fghik}^2 - \frac{(y_{grand})^2}{2^4 * n} \quad \text{(C.5)}$$

Where the five subscripts (f, g, h, i) represent the different variables investigated at two levels each. The subscript k represents the number of **n** replicates.

In the experiments conducted the total sum of squares was found as follows:

Equation C.5 above was broken down and calculated as:

$$SS_{TOTAL} = \mathbf{X} - \mathbf{Y}$$

Where $\boldsymbol{X} = \sum_{f=1}^{2}\sum_{g=1}^{2}\sum_{h=1}^{2}\sum_{i=1}^{2}\sum_{k=1}^{n} y_{fghijk}^2$ and $\boldsymbol{Y} = \frac{(y_{grand})^2}{2^4 * n}$

$$\mathbf{X} = (8.84^2 + 9.63^2) + (3.30^2 + 3.61^2) + (13.90^2 + 14.92^2) + (9.20^2 + 9.76^2) + (9.37^2 + 10.30^2)$$
$$+ (4.35^2 + 4.90^2) + (15.81^2 + 14.66^2) + (9.38^2 + 9.79^2) + (11.34^2 + 12.67^2)$$
$$+ (8.03^2 + 8.90^2) + (20.56^2 + 19.97^2) + (12.24^2 + 11.04^2) + (12.56^2 + 13.03^2)$$
$$+ (5.22^2 + 5.44^2) + (22.75^2 + 22.18^2) + (14.18^2 + 12.79^2)$$
$$= 4960.61$$

$$y_{grand} = 8.84 + 9.63 + 3.30 + 3.61 + 13.90 + 14.92 + 9.20 + 9.76 + 9.37 + 10.30 + 4.35$$
$$+ 4.90 + 15.81 + 14.66 + 9.38 + 9.79 + 11.34 + 12.67 + 8.03 + 8.90 + 20.56 + 19.97$$
$$+ 12.24 + 11.04 + 12.56 + 13.03 + 5.22 + 5.44 + 22.75 + 22.18 + 14.18 + 12.79$$
$$= 364.62$$

Therefore

$$\boldsymbol{Y} = \frac{364.62^2}{2^4 * 2} = 4154.70$$

It follows, therefore, that the total sum of squares for the response % Yield was found as:

SS_{TOTAL} = 4960.61- 4154.70 = 805.91

The total sums of squares for the rest of the responses were also calculated similarly and are presented in Table C.5.

C.1.7 Calculation of the error sum of squares

The error sum of squares (SS_{ERROR}) for each response variable was estimated as follows:

$$SS_{ERROR} = SS_{TOTAL} - SS_{model} \quad \text{(C.6)}$$

For the response % Yield, the error sum of squares was found to be:

SS_{ERROR} = 805.91 – 800.31 = 5.79

The same Equation C.6 was used to calculate the error sum of squares for the other responses and these are given in Table C.5.

C.1.8 Percentage contribution of each 'effect' to the variability in the response

In order to assess the contribution of each effect on the variability of the investigated response, the percentage contribution of effect was evaluated as followed:

$$\% \text{ Contribution (I)} = \frac{\text{SS (I)}}{\text{SS}_{\text{TOTAL}}} \quad \text{(C. 7a)}$$

Where ***I*** can be any main or interactive 'effect'.

The percentage contribution of variables not explained by the model on a particular response variable was calculated as follows:

$$\text{Variation not explained by model} = 100 - \sum \% \text{ Contribution (I)} \quad \text{(C. 7b)}$$

The percentage contribution of Effect A to the variability in the % Yield response was calculated as follows:

$$\% \text{ Contribution (A)} = \frac{\text{SS (A)}}{\text{SS}_{\text{TOTAL}}} = \frac{322.82}{805.91} = 40.06\ \%$$

The other contributions for other 'effects' were calculated likewise (Refer to Table C.5)

The variation not explained by the model for the % Yield response was calculated from Equation C.7b as:

$$\sum \% \text{ Contribution (I)}$$

$$= 40.06 + 14.51 + 0.92 + 39.05 + 0.59 + 0.77 + 0.77 + 0.30 + 0.48 + 0.02 + 0.81 + 0.30 + 0.08 + 0.26 + 0.36$$

$$= 99.28\%$$

$$\text{Variation not explained by model} = 100 - 99.28 = 0.72\%$$

C.1.9 F-Testing for the significance of each 'effect'

As in Appendix B.1.9, the mean square of each 'effect' was computed by dividing the 'effect's' sum of squares with its corresponding degree of freedom (DOF). The same was done to compute the mean error sum of squares.

For Effect A

$$MS(A) = \frac{SS(A)}{DOF(A)} = \frac{322.82}{1} = 322.82 \quad \text{(C.8)}$$

Where each 'effect' has a DOF equal to 1
The DOF of the error was calculated as follows:

$$DOF_{model} = 2^k - 1 = 2^4 - 1 = 15 \quad \text{(C.9)}$$

$$DOF_{TOTAL} = (2^k * n) - 1 = (2^4 * 2) - 1 = 31 \quad \text{(C.10)}$$

$$DOF_{ERROR} = DOF_{TOTAL} - DOF_{model} = 31 - 15 = 16 \quad \text{(C.11)}$$

Where **k** is the number variables investigated and **n** the number of replicas

It therefore followed that

$$MS_{ERROR} = \frac{SS_{ERROR}}{DOF_{ERROR}} = \frac{5.79}{16} = 0.36 \quad \text{(C.12)}$$

The F value was computed as follows:

$$F_0 = \frac{MS(A)}{MS_{ERROR}} = \frac{322.82}{0.36} = 892.61 \quad \text{(C.13)}$$

$F_{1-\alpha,v1,v2}$ at 95 % confidence interval (CI)= 4.49

Where $v1$ = DOF(numerator) =DOF(A) and $v2$ = DOF (denominator) =DOF_{ERROR}

If $F_o > F_{1-\alpha,v1,v2}$ then the 'effect' is significant as thus Effect A was found to be significant at 95 % CI.

The significance of all other 'effects' was computed similarly and are shown in Table C.5.

The sum of squares of each Effect, the corresponding percentage contribution, degrees of freedom, mean square and F value (F_o) were calculated as shown in Appendix C1.4 to C1.9 and the results in Table C.5:

Table C.5: *Analysis of Variance Table for the 2^4 factorial design for fresh wet peel extraction*

Effect	SS			% contribution			DOF			MS			Fo		
	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%DE	%Yield	%GA	%
A	322.82	123.55	54.85	40.06	23.61	14.45	1	1	1	322.82	123.55	54.85	892.61	10.40	
B	116.96	14.48	47.35	14.51	2.77	12.47	1	1	1	116.96	14.48	47.35	323.41	1.22	
AB	7.38	20.78	0.29	0.92	3.97	0.08	1	1	1	7.38	20.78	0.29	20.41	1.75	
C	314.73	0.04	170.69	39.05	0.01	44.96	1	1	1	314.73	0.04	170.69	870.26	0.00	
AC	4.78	8.63	16.55	0.59	1.65	4.36	1	1	1	4.78	8.63	16.55	13.22	0.73	
BC	6.20	34.12	2.67	0.77	6.52	0.70	1	1	1	6.20	34.12	2.67	17.13	2.87	
ABC	6.17	2.10	0.14	0.77	0.40	0.04	1	1	1	6.17	2.10	0.14	17.06	0.18	
E	2.42	11.91	0.98	0.30	2.28	0.26	1	1	1	2.42	11.91	0.98	6.70	1.00	
AE	3.85	8.55	1.56	0.48	1.63	0.41	1	1	1	3.85	8.55	1.56	10.63	0.72	
BE	0.12	3.53	1.46	0.02	0.67	0.39	1	1	1	0.12	3.53	1.46	0.35	0.30	
ABE	6.51	1.41	0.44	0.81	0.27	0.11	1	1	1	6.51	1.41	0.44	18.01	0.12	
CE	2.45	31.47	0.01	0.30	6.01	0.00	1	1	1	2.45	31.47	0.01	6.78	2.65	
ACE	0.64	13.33	2.25	0.08	2.55	0.59	1	1	1	0.64	13.33	2.25	1.78	1.12	
BCE	2.13	58.25	1.34	0.26	11.13	0.35	1	1	1	2.13	58.25	1.34	5.90	4.90	
ABCE	2.94	1.18	3.29	0.36	0.23	0.87	1	1	1	2.94	1.18	3.29	8.13	0.10	
MODEL	800.13	333.33	303.88	99.28	63.69	80.05	15	15	15	53.34	22.22	20.26			
ERROR	5.79	190.05	75.75	0.72	36.31	19.95	16	16	16	0.36	11.88	4.73			
TOTAL	805.91	523.37	379.64	100	100	100	31	31	31	26.00	16.88	12.25			

C.1.10 Model Correlation Coefficient and Adjusted Model Correlation Coefficient

The model correlation coefficient was calculated as follows

$$R^2 = \frac{SS_{model}}{SS_{TOTAL}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (C.14)$$

The correlation coefficient (R^2) for the response % Yield was calculated as follows:

$$R^2 = \frac{800.31}{805.91} = 0.993$$

The model correlation coefficients for the other responses were also calculated likewise.

The adjusted model correlation (R_{adj}^2) coefficients were also calculated as follows:

$$R_{adj}^2 = 1 - \frac{\left(\frac{SS_{ERROR}}{DOF_{ERROR}}\right)}{\left(\frac{SS_{TOTAL}}{DOF_{TOTAL}}\right)} \quad \text{(C. 15)}$$

The adjusted correlation coefficient for the % Yield response was thus evaluated as follows:

$$R_{adj}^2 = 1 - \frac{\left(\frac{5.79}{16}\right)}{\left(\frac{805.91}{31}\right)} = 0.986$$

The other adjusted correlation coefficients for the other responses were also computed similarly and presented in Table C.6

Table C.6: *Table of correlation coefficients for the three responses foe fresh wet peel extraction*

Response	R^2	R^2_{adj}
% Yield	0.993	0.986
% GA	0.637	0.296
% DE	0.800	0.613

C.2 Modeling

The calculations undertaken when modeling the response results for fresh wet peel extractions are shown in the following section

C.2.1 Calculating 'effect' estimates

The 'effect' estimates were calculated as follows:

$$\text{Effect estimate} = \frac{1}{2} * \frac{(\text{contrast})^2}{n * p} \quad \ldots\ldots\ldots\ldots (C.16)$$

Where **n** is the number of replicas and **p** the total number of experiments conducted

For the extraction experiments conducted **p** = 2^4 and **n** = 2

Thus Effect A was calculated as follows:

$$\text{Effect A} = \frac{1}{2} * \frac{101.64^2}{2 * 2^4} = 6.35$$

All the other 'effect' estimates were calculated similarly and are shown in Table C.7

Table C.7: *Table of 'effect' estimates for the three responses for fresh wet peel extraction*

	Effect Estimate		
Effect	%Yield	%GA	%DE
A	6.35	-3.93	-2.62
B	3.82	1.35	2.43
AB	0.96	1.61	0.19
C	-6.27	-0.07	4.62
AC	-0.77	1.04	1.44
BC	-0.88	2.07	0.58
ABC	-0.88	-0.51	-0.13
E	0.55	-1.22	-0.35
AE	0.69	-1.03	-0.44
BE	-0.12	-0.66	-0.43
ABE	0.90	-0.42	0.23
CE	-0.55	-1.98	-0.03
ACE	0.28	1.29	-0.53
BCE	-0.52	-2.70	-0.41
ABCE	0.61	0.38	0.64

From all the 'effects' calculated, only the significant were used in modelling the results. Normal probability plots were constructed and the plots in conjunction with the F-test identified these significant effects. Table C.8 shows 'effect' names and corresponding 'effect' estimates arranged in decreasing magnitude. The corresponding graphs that identified significant terms were shown in the Discussion section (Chapter 5), Fig 5.11, Fig 5.14 and 5.17.

Table C.8: *Rearranged effect estimates used in normal probability plots for the three responses for fresh wet peel extraction*

Cumulative frequency	Normal Probability					
	% Yield		% GA		% DE	
	Effect	%Yield	Effect	%GA	Effect	%DE
96.67	A	6.35	BC	2.07	C	4.62
90.00	B	3.82	AB	1.61	B	2.43
83.33	AB	0.96	B	1.35	AC	1.44
76.67	ABE	0.90	ACE	1.29	ABCE	0.64
70.00	AE	0.69	AC	1.04	BC	0.58
63.33	ABCE	0.61	ABCE	0.38	ABE	0.23
56.67	E	0.55	C	-0.07	AB	0.19
50.00	ACE	0.28	ABE	-0.42	CE	-0.03
43.33	BE	-0.12	ABC	-0.51	ABC	-0.13
36.67	BCE	-0.52	BE	-0.66	E	-0.35
30.00	CE	-0.55	AE	-1.03	BCE	-0.41
23.33	AC	-0.77	E	-1.22	BE	-0.43
16.67	BC	-0.88	CE	-1.98	AE	-0.44
10.00	ABC	-0.88	BCE	-2.70	ACE	-0.53
3.33	C	-6.27	A	-3.93	A	-2.62

C.2.2 Regression model

The regression model's coefficients of significant 'effects' were computed.

The model was given by the following expression:

$$\%\text{Yield} = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_i x_i \quad \ldots\ldots (C.17)$$

Where β_o was evaluated as the grand mean ($\bar{y}_{mean}$)

$\beta_1 \ldots \beta_i$ are regression coefficients corresponding to individual 'effects'

For the % Yield response:

$$\beta_o = \bar{y}_{grand} = \frac{y_{grand}}{N} = \frac{364.62}{32} = 11.39 \quad \text{(C. 18)}$$

Where y_{grand} was calculated previously (Appendix C.1.6)

For Effect A which was found significant, the corresponding regression coefficient (in coded variables) was found to be:

$$\beta_1 = \frac{\text{Effect (A)}}{2} = \frac{6.35}{2} = 3.18 \quad \text{(C. 19)}$$

The other regression coefficients were calculated similarly to give the following model:

$$\begin{aligned} \%\text{Yield} = 11.39 &+ 3.18x_1 + 1.91x_2 - 3.14x_3 + 0.28x_5 + 0.48x_{12} - 0.39x_{13} + 0.35x_{15} \\ &- 0.44x_{23} - 0.28x_{35} - 0.44x_{123} + 0.45x_{125} - 0.26x_{235} \\ &+ 0.30x_{1235} \quad \text{(C. 20)} \end{aligned}$$

The Discussion section (Chapter 5), Table 5.5, Table 5.7 and Table 5.9 show all the empirical models for the three responses with significant regression coefficients.

C.2.3 Calculation of Residuals

The experimental and calculated response variables were then computed in order to check the model adequacy.

The residual of each treatment combination was calculated as follows:

For treatment **c** (*the first replica*) the predicted yield was calculated using Equation C.20:

$$\text{predicted \% Yield } (\mathbf{c}) = 11.39 + 3.18(-1) + 1.91(-1) - 3.14(+1) + 0.28(-1) + 0.48(-1 * -1) - 0.39(-1 * +1) + 0.35(-1 * -1) - 0.44(-1 * +1) - 0.28(+1 * -1) - 0.44(-1 * -1 * +1) + 0.45(+1 * -1 * -1) - 0.26(-1 * +1 * -1) + 0.30(-1 * -1 * +1 * -1)$$

$$= 3.37$$

Residual (**c**) = experimental % Yield (**c**) – predicted % Yield (**c**)… … … … … … .(C.21)

= 3.30– 3.37 = - 0.07

All other residuals were computed for each treatment in a similar manner (shown in Table C.9). And a probability plot of the residuals was then plotted to check for model adequacy

Table C.9: *Residual calculations for the different treatments used for model adequacy checks for fresh wet peel extraction*

	% Yield				% GA				% DE			
cum freq	Treatment	%Yield exp	%Yield pred	Residual	Treatment	%GA exp	%GA pred	Residual	Treatment	%DE exp	%DE pred	Residual
98.44	abce	14.18	13.40	0.77	abc	89.24	81.82	**7.42**	e	75.56	72.48	3.08
95.31	e	10.30	9.63	0.67	bc	91.44	85.75	**5.69**	ac	77.40	74.48	2.92
92.19	bc	8.90	8.26	0.64	e	88.72	83.05	**5.67**	a	71.28	68.42	2.86
89.06	a	14.92	14.33	0.59	be	90.63	85.75	**4.88**	.(1)	74.52	72.48	2.04
85.94	b	12.67	12.08	0.59	ab	82.49	79.12	**3.37**	b	76.87	74.91	1.96
82.81	abc	12.24	11.72	0.52	c	86.20	83.05	**3.14**	ae	69.86	68.42	1.44
79.69	ae	15.81	15.31	0.50	ae	81.20	79.12	**2.08**	abc	78.23	76.91	1.32
76.56	ab	20.56	20.06	0.50	abc	83.52	81.82	**1.70**	ce	76.96	75.66	1.30
73.44	ace	9.79	9.38	0.41	abce	80.39	79.12	**1.27**	abe	71.86	70.85	1.01
70.31	be	13.03	12.72	0.32	e	84.29	83.05	**1.24**	bc	79.10	78.09	1.01
67.19	c	3.61	3.37	0.24	.(1)	86.91	85.75	**1.16**	ab	71.49	70.85	0.64
64.06	ce	4.90	4.70	0.19	b	84.00	83.05	**0.95**	abc	77.54	76.91	0.63
60.94	.(1)	9.63	9.44	0.19	ace	82.40	81.82	**0.58**	bc	78.72	78.09	0.63
57.81	abe	22.75	22.67	0.08	abce	79.68	79.12	**0.56**	bce	78.63	78.09	0.54
54.69	ac	9.76	9.69	0.07	ab	79.23	79.12	**0.10**	ce	76.15	75.66	0.49
51.56	ace	9.38	9.38	0.00	a	81.77	81.82	**-0.05**	ace	74.57	74.48	0.09
48.44	c	3.30	3.37	-0.08	.(1)	85.60	85.75	**-0.15**	abce	76.83	76.91	-0.08
45.31	ab	19.97	20.06	-0.09	ac	78.87	79.12	**-0.26**	ab	70.62	70.85	-0.23
42.19	bce	5.44	5.54	-0.09	bc	85.35	85.75	**-0.40**	be	74.60	74.91	-0.31
39.06	be	12.56	12.72	-0.16	abe	80.94	81.82	**-0.88**	.(1)	72.14	72.48	-0.34
35.94	bc	8.03	8.26	-0.23	ac	78.08	79.12	**-1.04**	c	75.20	75.66	-0.46
32.81	e	9.37	9.63	-0.26	ce	84.65	85.75	**-1.10**	abce	76.32	76.91	-0.59
29.69	bce	5.22	5.54	-0.31	bce	81.91	83.05	**-1.14**	be	74.30	74.91	-0.61
26.56	ce	4.35	4.70	-0.35	abe	80.11	81.82	**-1.71**	ae	67.43	68.42	-0.99
23.44	a	13.90	14.33	-0.43	bce	81.31	83.05	**-1.75**	bce	76.96	78.09	-1.13
20.31	ac	9.20	9.69	-0.48	a	79.59	81.82	**-2.23**	abe	68.93	70.85	-1.92
17.19	abe	22.18	22.67	-0.49	be	82.36	85.75	**-3.39**	ace	72.36	74.48	-2.11
14.06	.(1)	8.84	9.44	-0.60	c	79.32	83.05	**-3.74**	ac	72.30	74.48	-2.17
10.94	abce	12.79	13.40	-0.62	ae	74.58	79.12	**-4.54**	c	73.29	75.66	-2.37
7.81	ae	14.66	15.31	-0.66	ce	80.60	85.75	**-5.16**	a	65.63	68.42	-2.79
4.69	abc	11.04	11.72	-0.68	b	77.15	83.05	**-5.91**	b	72.07	74.91	-2.84
1.56	b	11.34	12.08	-0.74	ace	75.45	81.82	-6.37	e	69.49	72.48	-2.98

The residuals were then plotted and the plots showed that the data was normally distributed as no significant anomalies were evident. Figs C.1 to C.3 show the plots.

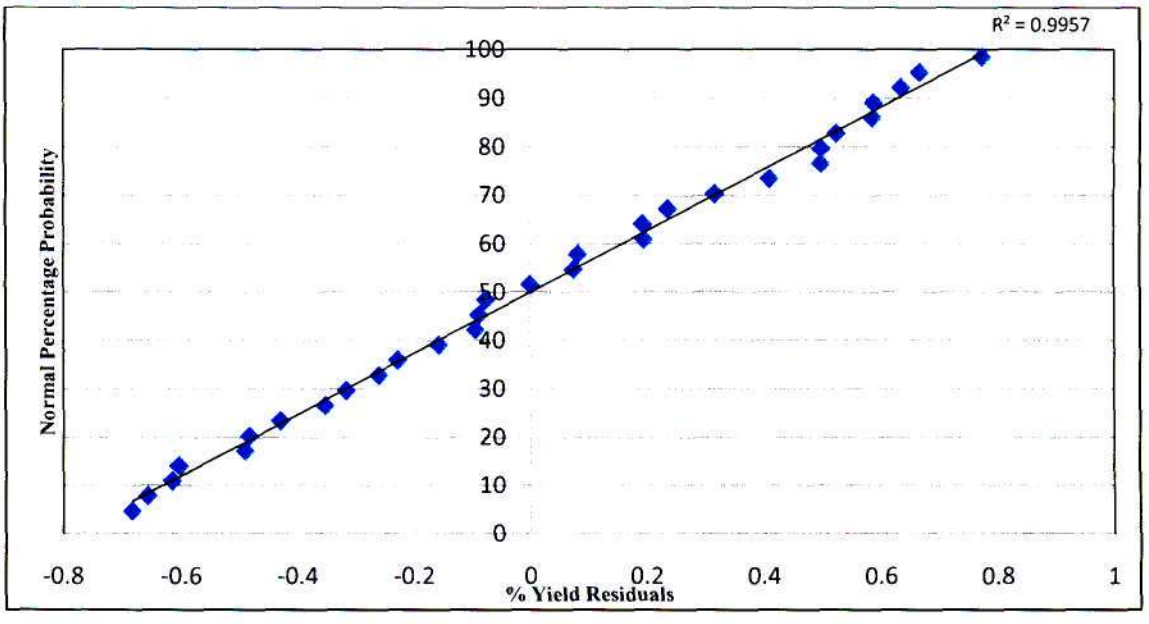

Fig C.1: *Normal probability plot of residuals for the % Yield response for fresh wet peel extraction*

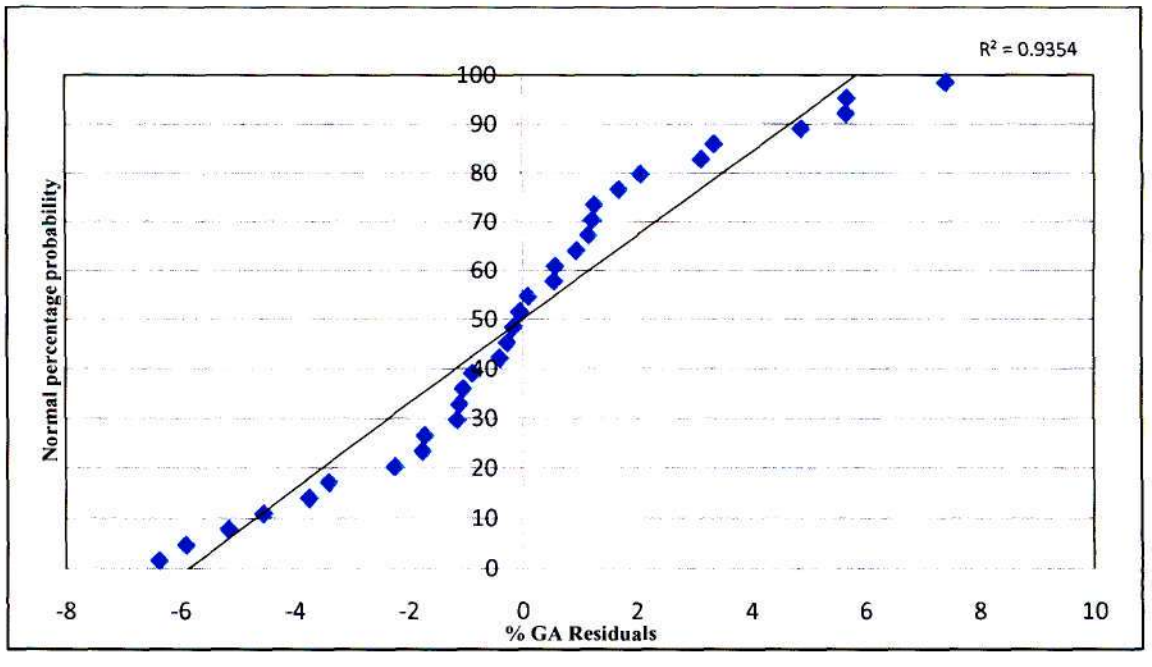

Fig C.2: *Normal probability plot of residuals for the % GA response for fresh wet peel extraction*

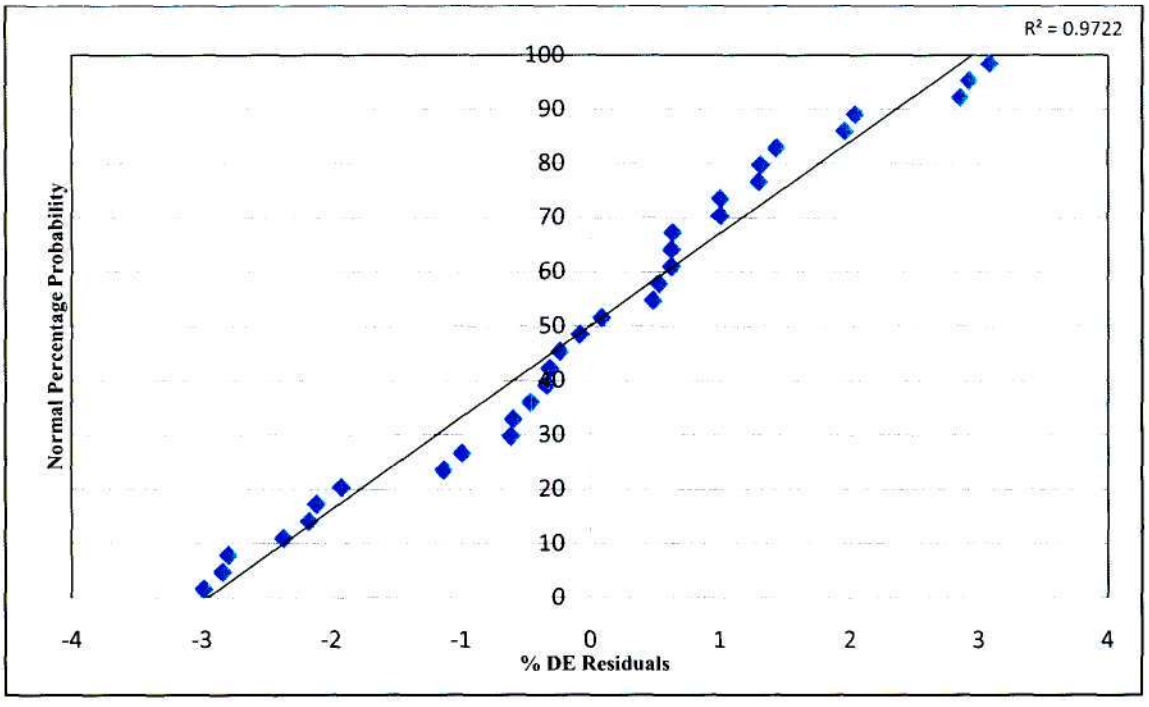

Fig C.3: *Normal probability plot of residuals for the % DE response for fresh wet peel extraction*

Appendix D - Extraction experiments for stored wet peel

Extraction experiments on lemon peels stored at atmospheric conditions for 2 days were carried out and the results were compared to fresh wet peel extractions.

Table D.1: *Experimental data for stored wet peel 2^4 factorial design (single replicate)*

Treatment Combination	Factors				Responses		
	Factor 1 – A:Temp (°C)	Factor 2- B:time (hrs)	Factor 3 – C:pH	Factor 4 - E: p:w	%Yield	%GA	%DE
.(1)	70	0.5	1.5	1:25	3.77	56.22	20.80
b	70	2	1.5	1:25	7.86	59.73	29.92
c	70	0.5	2.5	1:25	2.92	49.44	18.55
bc	70	2	2.5	1:25	2.62	51.47	18.46
e	70	0.5	1.5	1:50	7.07	65.79	28.15
be	70	2	1.5	1:50	7.33	55.72	22.55
ce	70	0.5	2.5	1:50	3.73	57.15	31.55
bce	70	2	2.5	1:50	3.82	45.33	17.80
a	90	0.5	1.5	1:25	8.40	62.46	28.03
ab	90	2	1.5	1:25	11.40	60.76	24.26
ac	90	0.5	2.5	1:25	3.78	54.10	24.14
abc	90	2	2.5	1:25	9.13	58.20	30.87
ae	90	0.5	1.5	1:50	10.50	69.39	36.74
abe	90	2	1.5	1:50	10.82	62.88	26.18
ace	90	0.5	2.5	1:50	5.32	49.81	25.47
abce	90	2	2.5	1:50	5.19	57.22	27.76

The response values found for stored wet peel were then compared to the average value of those calculated for fresh wet peel

The average was computed as follows:

$$y_{average} = \frac{\text{replicate 1} + \text{replicate 2}}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (D.1)$$

For treatment **c** (for fresh wet peels) the average value was calculated to be (Refer to Table C.1):

$$y_{average} = \frac{3.30 + 3.61}{2} = 3.45$$

All the other response averages were computed similarly and are presented in Table D.2

Table D.2: *Experimental data for fresh wet peel average response values for the 2^4 factorial design*

	Factors				Responses		
Treatment Combination	Factor 1- A:Temp (° C)	Factor 2- B:time (hrs)	Factor 3- C:pH	Factor 4- E:s:s	%Yield	%GA	%DE
.(1)	70	0.5	1.5	1:25	9.24	86.26	73.33
b	70	2	1.5	1:25	12.01	80.57	74.47
c	70	0.5	2.5	1:25	3.45	82.76	74.24
bc	70	2	2.5	1:25	8.46	88.40	78.91
e	70	0.5	1.5	1:50	9.84	86.51	72.53
be	70	2	1.5	1:50	12.80	86.50	74.45
ce	70	0.5	2.5	1:50	4.62	82.62	76.55
bce	70	2	2.5	1:50	5.33	81.61	77.79
a	90	0.5	1.5	1:25	14.41	80.68	68.45
ab	90	2	1.5	1:25	20.26	80.86	71.06
ac	90	0.5	2.5	1:25	9.48	78.47	74.85
abc	90	2	2.5	1:25	11.64	86.38	77.88
ae	90	0.5	1.5	1:50	15.24	77.89	68.64
abe	90	2	1.5	1:50	22.47	80.53	70.39
ace	90	0.5	2.5	1:50	9.59	78.93	73.47
abce	90	2	2.5	1:50	13.48	80.04	76.57

The comparison was done graphically and the results were shown and discussed under the Discussion section (Chapter 5), Figs 5.19, 5.20 and 5.21.

Appendix E – Dried peel optimization

E.1 Experimental Design

A central composite design (CCD) was used in order to optimize the extraction process with respect to the three experimental response variables. Star points and centre points were added to the factorial design in order to make up a complete CCD. Table E.1 gives the coded variables and their corresponding natural variables. Table E.2 gives the added experimental data of star and centre points.

Table E.1: *Coded variables and their corresponding natural variable values for the CCD design*

	Natural Variable				
Coded Variable	Temp (°C)	Time (hrs)	pH	Size (mm)	p:w
-1	70	0.5	1.5	less than 1	1:25
0	80	1:15	2	2-1	1:37.5
1	90	2	2.5	4-2	1:50

Table E.2: *Experimental data of the centre points and star points used in the CCD*

Points	Coded Variables					Responses		
	Temp (°C)	Time (hrs)	pH	Size (mm)	p:w	%Yield	%GA	%DE
Centre points	0	0	0	0	0	19.59	90.96	77.99
	0	0	0	0	0	15.70	89.58	78.01
	0	0	0	0	0	19.31	89.21	77.09
	0	0	0	0	0	17.90	91.60	77.64
Star points	-1	0	0	0	0	9.80	83.23	79.07
	-1	0	0	0	0	10.59	83.58	78.15
	1	0	0	0	0	16.44	82.85	78.04
	1	0	0	0	0	16.74	80.51	75.86
	0	-1	0	0	0	10.74	87.39	77.12
	0	-1	0	0	0	11.20	86.61	77.51
	0	1	0	0	0	16.89	87.75	74.59
	0	1	0	0	0	15.83	86.68	76.56
	0	0	-1	0	0	17.89	83.11	74.27
	0	0	-1	0	0	16.28	88.35	73.43
	0	0	1	0	0	9.57	91.07	79.12
	0	0	1	0	0	8.72	91.65	79.48
	0	0	0	-1	0	16.52	81.44	75.66
	0	0	0	-1	0	15.00	89.03	73.98
	0	0	0	1	0	10.18	83.22	77.13
	0	0	0	1	0	9.51	78.91	78.71
	0	0	0	0	-1	11.31	86.45	76.68
	0	0	0	0	-1	11.12	84.08	74.21
	0	0	0	0	1	14.61	84.83	77.52
	0	0	0	0	1	14.11	80.88	77.40

E.2 MATLAB Code

MATLAB was used in optimizing the extraction process and the code is given hereafter. The graphs resulting from this optimization were shown under the Discussion section (Chapter 5), Figs 5.22 to 5.31. The resulting Equations from the MATLAB calculations were also given under the same section, Equations 5.7, 5.8 and 5.9.

MATLAB code:

```
% OPTIMIZATION OF THE EXTRACTION OF PECTIN FROM LEMON PEELS:
% involves the development of second order empirical equations for the
% three response variable investigated (% Yield, % GA and % DE) and
% overlaying of the resultant contour plots of the three response
% variables to identify the optimum operating conditions

%GENERATING MODELS OF THE RESPONSE VARIABLES FROM PROCESS VARIABLES

close all, clear all, clc     %Clears the work space

pectinccd;          %imports data from pectinccd m-file:matrix A where
                    %matrix A is the Face-Centred Central Composite
                    %FCC) Design matrix of the experimental data where
                    %columns 1 to 5 are the process variable columns
                    %(temperature (x1), time (x2),pH (x3), peel size(x4)
                    %and peel to water mass ratio (x5) respectively) and
                    %6 to 8 are the response variable columns (Yield,
                    %%GA and %DE respectively)

Yield = A(:,6);
GA = A(:, 7);
DE = A(:,8);

D=x2fx(A(:,1:5),'quadratic'); % Generates a matrix with main effect
                              % variables in the first columns,
                              % followed by columns of interaction
                              % terms then lastly by the quadratic
                              % terms

 %Stepwisefit function was used to input only the regressor
 %coefficients significant at 95% confidence interval (CI).
 %regression coefficients with 90% CI and less were eliminated
 %from the model

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield)

intercept_ones = ones(88,1);       %generating a vector of ones

grand_mean = stats.intercept;      %creating a variable of the grand
                                   % mean of the response which is
                                   % returned as the 'intercept' by
                                   % the stepwisefit function
```

```
yint = grand_mean.*intercept_ones; %creates a column vector of the
                                    %grand mean
b_use = inmodel'.*b;               %only the significant terms are
                                   %used in the model:
                                   %'inmodel' identifies these
                                   %coefficients

%calculates the adjusted correlation coefficient (R^2adj) of the model
%for the percentage yield response


R2adj_yield = 1- ((stats.SSresid/stats.dfe)/...
              (stats.SStotal/(stats. df0 + stats.dfe)))

Yield_model = yint + D*b_use;      %The response is generated from
                                   %only the significant correlation
                                   %coefficients

%The same procedure is used to compute the percentage galacturonic
%acid (GA) content model and the corresponding adjusted correlation
%coefficient (R^2adj)

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);
intercept_ones = ones(88,1);
grand_mean = stats.intercept;
yint = grand_mean.*intercept_ones;
b_use = inmodel'.*b;
R2adj_GA = 1- ((stats.SSresid/stats.dfe)/...
              (stats.SStotal/(stats. df0 + stats.dfe)))
GA_model = yint + D*b_use;

%The same procedure is used to compute the percentage degree of
%esterification (DE) content model and the corresponding adjusted
%correlation coefficient (R^2adj)

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);
intercept_ones = ones(88,1);
grand_mean = stats.intercept;
yint = grand_mean.*intercept_ones;
b_use = inmodel'.*b;
R2adj_DE = 1- ((stats.SSresid/stats.dfe)/...
              (stats.SStotal/(stats. df0 + stats.dfe)))
DE_model = yint + D*b_use;

%GENERATING CONTOURS FOR OPTIMIZATION

%Temperature (x1) and Time (x2) main effects, interactions and
%quadratic effects contours looked into for the three responses
%( Yield, GA and DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;                %Only significant correlation
                                    %coefficients used (95% CI)
```

```
[x1,x2] = meshgrid(-1:.1:1,-1:.1:1); %generating a mesh grid of the
                                     %temperature (x1) and time (x2)
                                     %variables


% generating the response by varying the temperature (x1) and time(x2)
% while holding all other variables at 0 level (coded variable level)

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(3).*x2 +...
    b_use(7).*x1.*x2+ b_use(17).*x1.^2 + b_use(18).*x2.^2 ;


%generating a contour plot of the response in the temperature (x1) and
%time (x2) variable domain

figure(1)

[C,h]=contour(x1,x2,y,5);     % 5 contours generated for legibility


% contour and axes labelling
set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Time')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x2] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(3).*x2 ..
    + b_use(7).*x1.*x2 + b_use(17).*x1.^2 + b_use(18).*x2.^2 ;


[C,h]=contour(x1,x2,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Time')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);
```

```
grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x2] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(3).*x2...
    + b_use(7).*x1.*x2 + b_use(17).*x1.^2 + b_use(18).*x2.^2 ;


[C,h]=contour(x1,x2,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Time')

hold on


%Temperature (x1) and pH (x3) main effects, interactions and quadratic
%effects contours looked into for the three responses (Yield, GA and
%DE)

%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(4).*x3...
    + b_use(8).*x1.*x3 + b_use(17).*x1.^2 + b_use(19).*x3.^2 ;

figure(2)

[C,h]=contour(x1,x3,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('pH')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(4).*x3
    + b_use(8).*x1.*x3 + b_use(17).*x1.^2 + b_use(19).*x3.^2 ;
```

```
[C,h]=contour(x1,x3,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('pH')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(4).*x3...
    + b_use(8).*x1.*x3 + b_use(17).*x1.^2 + b_use(19).*x3.^2 ;

[C,h]=contour(x1,x3,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('pH')

hold on



%Temperature (x1) and Size(x4) main effects, interactions and
%quadratic effects contours looked into for the three responses
%(Yield, GA and DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep,history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(5).*x4...
    + b_use(9).*x1.*x4 + b_use(17).*x1.^2 + b_use(20).*x4.^2 ;

figure(3)

[C,h]=contour(x1,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Size')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')
```

```
hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(5).*x4 ...
    + b_use(9).*x1.*x4 + b_use(17).*x1.^2 + b_use(20).*x4.^2 ;

[C,h]=contour(x1,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Size')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(5).*x4...
    + b_use(9).*x1.*x4 + b_use(17).*x1.^2 + b_use(20).*x4.^2 ;

[C,h]=contour(x1,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Size')

hold on


%Temperature (x1) and Peel to water mass ratio (x5) main effects,
%interactions and quadratic effects contours looked into for
%the three responses ( Yield, GA and DE)



%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);
```

```
grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(6).*x5...
    + b_use(10).*x1.*x5 + b_use(17).*x1.^2 + b_use(21).*x5.^2 ;

figure(4)

[C,h]=contour(x1,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Peel to water mass ratio')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(6).*x5...
    + b_use(10).*x1.*x5 + b_use(17).*x1.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x1,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Peel to water mass ratio')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x1,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(2).*x1 + b_use(6).*x5...
    + b_use(10).*x1.*x5 + b_use(17).*x1.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x1,x5,y,5);
```

```
set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Temperature')
ylabel('Peel to water mass ratio')

hold on


%Time (x2) and pH (x3) main effects, interactions and quadratic
%effects contours looked into for the three responses (Yield, GA and
%DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(4).*x3...
    + b_use(11).*x2.*x3 + b_use(18).*x2.^2 + b_use(19).*x3.^2 ;

figure(5)

[C,h]=contour(x2,x3,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('pH')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(4).*x3...
    + b_use(11).*x2.*x3 + b_use(18).*x2.^2 + b_use(19).*x3.^2 ;

[C,h]=contour(x2,x3,y,5);
set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('pH')

hold on
```

```
%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x3] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(4).*x3...
    + b_use(11).*x2.*x3 + b_use(18).*x2.^2 + b_use(19).*x3.^2 ;


[C,h]=contour(x2,x3,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('pH')

hold on


%Time (x2) and Size (x4) main effects, interactions and quadratic
%effects contours looked into for the three responses (Yield, GA and
%DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(5).*x4...
    + b_use(12).*x2.*x4 + b_use(18).*x2.^2 + b_use(20).*x4.^2 ;

figure(6)

[C,h]=contour(x2,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Size')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;
```

```
[x2,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(5).*x4...
    + b_use(12).*x2.*x4 + b_use(18).*x2.^2 + b_use(20).*x4.^2 ;



[C,h]=contour(x2,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Size')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(5).*x4...
    + b_use(12).*x2.*x4 + b_use(18).*x2.^2 + b_use(20).*x4.^2 ;


[C,h]=contour(x2,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Size')

hold on


%Time (x2) and Peel to water mass ratio (x5) main effects,
interactions
%and quadratic effects contours looked into for the three responses
%( Yield, GA and DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(6).*x5 ...
    + b_use(13).*x2.*x5 + b_use(18).*x2.^2 + b_use(21).*x5.^2 ;
```

```
figure(7)

[C,h]=contour(x2,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Peel to water mass ratio')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(6).*x5...
    + b_use(13).*x2.*x5 + b_use(18).*x2.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x2,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Peel to water mass ratio')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x2,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(3).*x2 + b_use(6).*x5...
    + b_use(13).*x2.*x5 + b_use(18).*x2.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x2,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Time')
ylabel('Peel to water mass ratio')

hold on
```

```
%pH (x3) and Size (x4) main effects, interactions and quadratic
%effects contours looked into for the three responses (Yield, GA and
%DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x3,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(5).*x4...
    + b_use(14).*x3.*x4 + b_use(19).*x3.^2 + b_use(20).*x4.^2 ;

figure(8)

[C,h]=contour(x3,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Size')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x3,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(5).*x4...
    + b_use(14).*x3.*x4 + b_use(19).*x3.^2 + b_use(20).*x4.^2;



[C,h]=contour(x3,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Size')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);
```

```
grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x3,x4] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(5).*x4...
    + b_use(14).*x3.*x4 + b_use(19).*x3.^2 + b_use(20).*x4.^2 ;


[C,h]=contour(x3,x4,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Size')

hold on


%pH (x3) and Peel to water mass ratio (x5) main effects, interactions
%and quadratic effects contours looked into for the three responses
%( Yield, GA and DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use= inmodel'.*b;

[x3,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(6).*x5...
    + b_use(15).*x3.*x5 + b_use(19).*x3.^2 + b_use(21).*x5.^2 ;

figure(9)

[C,h]=contour(x3,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Peel to water mass ratio')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')

hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x3,x5] = meshgrid(-1:.1:1,-1:.1:1);
```

```
y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(6).*x5...
    + b_use(15).*x3.*x5 + b_use(19).*x3.^2 + b_use(21).*x5.^2;



[C,h]=contour(x3,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Peel to water mass ratio')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x3,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(4).*x3 + b_use(6).*x5...
    + b_use(15).*x3.*x5 + b_use(19).*x3.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x3,x5,y,5);
set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('pH')
ylabel('Peel to water mass ratio')

hold on


%Size(x4) and Peel to water mass ratio (x5) main effects, interactions
%and quadratic effects contours looked into for the three responses
%( Yield, GA and DE)


%Considering the YIELD Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,Yield);

grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x4,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(5).*x4 + b_use(6).*x5...
    + b_use(16).*x4.*x5 + b_use(20).*x4.^2 + b_use(21).*x5.^2 ;

figure(10)

[C,h]=contour(x4,x5,y,5);
```

```
set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Size')
ylabel('Peel to water mass ratio')
title('Contour Plots of the Percentage Yield, %GA and %DE responses')
hold on


%Considering the GALACTURONIC ACID CONTENT (GA) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,GA)


grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x4,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(5).*x4 + b_use(6).*x5...
    + b_use(16).*x4.*x5 + b_use(20).*x4.^2 + b_use(21).*x5.^2 ;


[C,h]=contour(x4,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Size')
ylabel('Peel to water mass ratio')

hold on


%Considering the DEGREE OF ESTERIFICATION (DE) Response

[b,se,pval,inmodel,stats,nextstep, history]=stepwisefit(D,DE);


grand_mean = stats.intercept;
b_use = inmodel'.*b;

[x4,x5] = meshgrid(-1:.1:1,-1:.1:1);

y = grand_mean + b_use(1)+ b_use(5).*x4 + b_use(6).*x5 ...
    + b_use(16).*x4.*x5 + b_use(20).*x4.^2 + b_use(21).*x5.^2;


[C,h]=contour(x4,x5,y,5);

set(h,'ShowText','on','TextStep',get(h,'LevelStep')*2)
xlabel('Size')
ylabel('Peel to water mass ratio')

hold on
```