FUNGAL PRETREATMENT OF UNEXTRACTED AND PRESSURIZED HOT WATER EXTRACTED *EUCALYPTUS GRANDIS* WOOD CHIPS.

BY

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DECLARATION

I, the undersigned hereby declare that the work contained in this thesis is my own original work and has not in this entirety or part been submitted at any university for a degree.

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ABSTRACT

Unextracted (control) and PHWe *Eucalyptus grandis* wood chips were pulped at 15% active alkali (AA) and 1% antraquinone (AQ). Another batch of wood chips were then inoculated with fungal co-cultures of *Aspergillus flavipes* and *Pycnoporus sanguineus*. FCCi wood chips were incubated for four weeks; one PHWe inoculated experimental treatment was incubated for three weeks. The full pulping cycle (160 min) was used to digest the experimental treatments with the exception of one lot of PHWe wood chips that were pulped for 150 minutes. A further experimental treatment of PHWe wood chips was cooked at a reduced AA charge of 14% and 1% AQ. Analysis of variance (ANOVA) of the data from all the experimental treatments was conducted and the differences within the experimental treatments were determined using *Statistica* (v7, 1984–2006). The F-value (Fischer distribution) and the p-value as well as a non-parametric test known as the Mann-Whitney procedure was tested at the 95% confidence limit. For a further enhancement of the 95% confidence limit the screened yield data was tested by the Bootstrap method. Scanning electron micrographs clearly demonstrated the changed structure and appearance of the chip cross-sectional area after the different pretreatments.

Although the mean average results of all the screened pulp yields showed no significant statistical difference ($p > 0.05$), differences in screened yield of up to 2.5% were obtained. All the weighted means of the rejects showed a significant difference ($p < 0.05$). Other pulp properties like shive content, chemical consumption, Kappa number, handsheet brightness and strength tests showed mixed results i.e. rejected or accepted the hypothesis ($p >$ or $=$or < 0.05). The hypothesis that the combined PHWE and FCCI of wood chips would further increase the pulp yield had to be rejected. It is however anticipated that the combination of PHWE with successive co-culture fungal pretreatment would be very beneficial in obtaining higher pulp yields for fully bleached chemical pulp. Further research would be required to test this assumption. This investigation confirmed the expected beneficial effects of combined PHWE and FCCI pretreatments of wood chips on the strength properties. In addition the combined treatment also improved the initial bonding strength potential of the unbeaten fibres.

OPSOMMING

Onbehandelde en met onder druk, warm water uitgeloogde *Eucalyptus grandis* houtspaanders is respektiefwelik met 15% aktiewe alkali (AA) en 1% antrakinoon (AQ) verpulp. Hierdie is dan met swamkokulture van *Aspergillus flavipes* en *Pycnoporus sanguineus* inokuleer en respektiewelik vir drie en vier weke inkubeer. Onder druk uitgeloogde houtspaanders is ook vir 150 minute verpulp by 15% AA 1% AQ en by ‘n verminderde AA van 14%.

Pulpevaluasies is uitgevoer op alle eksperimentele behandelinge. Alle onder druk uitgeloogde en met swamkokultuur inokuleerde houtspaanders het ‘n laer pulpopbrengs, uitskot, skilferinhoud, Kappanommer en ‘n hoër RAA en helderheid opgelewer in vergelyking met die vars houtspaanders. Die vars en warm water uitgeloogde houtspaanders het soortgelyke pulpopbrengs opgelewer.

‘n Variansieanalise (ANOVA) van die data van alle eskperimentele behandelings is uitgevoer gebruikmakende van Statistica (V7, 1984 – 2006). Die F-waarde (Fischer-verspreiding) an die p-waarde so wel as ‘n parametriese toets (Mann-Whitney prosedure) is getoets by ‘n 95% betroubaarheidsgrens. Vir ‘n verdere verhoging van die 95% betroubaarheidsgrens van die pulpopbrengs, is die beskikbare data weer getoets met die Bootstrap-metode.

Alle gemiddelde pulpopbrengswaardes het geen beduidende statistiese verkil opgelewer nie ($p>0.05$), alhoewel verskille van tot 2.5% in pulpopbrengs verkry is. Alle gemiddelde uitskotwaardes het ‘n beduidende verskil getoon ($p<0.05$). Die ander pulpeienskappe soos skilferinhoud, verbruik aan chemikalieë, Kappagetal, handvel helderheid en sterktewaardes het gemengde resultate opgelewer maw verwerping of aanvaarding van die hipotese p> or =or < 0.05. Die hipotese dat die gekombineerde PHWE en FCCI van die houtspaanders die pulpopbrengs verder sou verhoog moes verwerp word. Daar word egter verwag dat die kombinasie van PHWE met opeenvolgende swamkokultuur behandeling baie voordelig sou wees op die pulpopbrengs van ‘n ten volle gebleikte chemiese pulp. Verdere navorsing is nodig om hierdie veronderstelling te toets. Die ondersoek het die verwagte woordelige effek van die gekombineerde PHWE en FCCI voorbehandelings van die houtspaanders op die papierstrkte-eienskappe bevestig. Bo en behalve dit, het die gekombineerde behandeling ook die aavanklikte bindsterkte potensiaal van die ongeklopte vessels verbeter.

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ABBREVIATIONS

0	No FCCI
1	14% Active alkali
2	15% Active alkali
20	Twenty minutes cooking
3	Three weeks incubation time
30	Thirty minutes cooking
4	Four weeks incubation time
AA	Active alkali
ANOVA	Analysis of variance
AQ	Antraquinone
CTC	Central Timber Cooperative
DED	Chlorine dioxide, Extraction, Chlorine dioxide (Bleaching stages)
DEDP	Chlorine dioxide, Extraction, Chlorine dioxide, Peroxide (Bleaching stages)
EA	Effective alkali
FCC	Fungal co-culture
FCCi	Fungal co-culture inoculated
FCCI	Fungal co-culture inoculation
FCCs	Fungal co-cultures
Inoc	Inoculation
PHW	Pressurised hot water
PHWe	Pressurised hot water extracted
PHWE	Pressurised hot water extraction
PLC	Programmable logic controller
RAA	Residual active alkali

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CHAPTER 1: LITERATURE REVIEW

1.1 IMPORTANCE OF WOOD IN PULP AND PAPER MANUFACTURE

Paper and paper products feature as an important necessity in our modern life. Paper provides the means for recording, storage and dissemination of information. It is the most widely used wrapping and packaging material, virtually all writing and printing is done on paper[1]. Pulping is the process by which wood is reduced to a fibrous mass. The existing commercial pulping processes are generally classified as mechanical, chemical or semichemical. The main chemical pulping processes today are the acid and alkaline pulping processes. Fibres are produced by loosening or dissolving lignin that binds the individual cellulosic fibres. Lignin is extracted by subjecting the raw wood material to suitable chemicals at extreme pH values, high temperature and pressure while retaining the cellulose. Chemical pulp processes produce papers of high strength, but these processes are hampered by relative low yield, high-energy demands and pollution constraints[2].

High yield pulps are rich in lignin which is hydrophobic in nature. As the amount of lignin increases there is deterioration in strength properties and the pulp requires more energy in mechanical treatments[3]. Most of high yield pulping units use softwoods. They are the reference species for thermomechanical pulps (TMP), chemi-thermomechanical pulps (CTMP); pressurized groundwood (PGW) and stone groundwood (SGW). New processes such as bleached chemi-thermomechanical pulp (BCTMP) and alkaline peroxide mechanical (AMP) have allowed a greater diversity in new raw materials as well as the use of hardwoods. It is also possible to produce high yield pulps using non-wood plants such wheat straw, flax, hemp and bamboo[4].

1.2 FOREST PRODUCTS BIOTECHNOLOGY

1.2.1 The Scope of Forest Products Biotechnology

In forestry and the forest products industry, any new developments related to biochemical or microbiological processes whether or not they include bioengineering, have been labelled as biotechnological processes [1]. Development of biotechnology for the pulp and paper industry started during the 1970's[5].

One of the goals of biotechnology applications for the pulp and paper industry is to identify, develop and make commercially available biological treatments to problems that commonly occur within the pulp and paper manufacturing process[6]. Biological applications have been reported in pulping, deinking [1], pitch removal [1, 7] and effluent management [6].

In biopulping, fungal inoculants have been employed to achieve a reduction in extractives content, improve pulp brightness levels and paper strength properties, as well as energy savings during mechanical pulping [6].

1.2.1.1 Effluent Treatment

Pulp mills are discharging effluents, which contain ample organic constituents, which could be reused as by-products if treated by microorganisms. Because of this and the nature of the effluent, pulp and paper mills have a significant effect on their surrounding environment. Mechanical and high-yield semichemical pulping mills are the least polluting with respect to toxic constituents, however such pulping processes also generate a large quantity of organic dissolved materials[8, 1]. Resin acids are responsible for much of the untreated softwood pulping effluent toxicity to aquatic organisms. Resin acids are found in chemical, mechanical and chemi-thermomechanical pulping (CTMP) effluents in concentrations ranging from two to several hundreds of milligrams per litre (mg/l). Dehydroabietic acid (DHA) and abietic acid (AA) are the most abundant resin acids representing respectively 19 to 23% and 14 to 30% of total resin acid. Batch studies indicated that resin acids inhibited anaerobes and probably were responsible for decreased efficiencies during the anaerobic treatment of pulp and paper effluents [9]. However, anaerobic treatment of resin acid-containing effluents is successful in reducing COD levels although to a lesser extent than in the aerobic treatment. The removal of resin acids by anaerobic lagoons or upflow anaerobic sludge bed (UASB) reactors ranged

from 44 to 63%. It has not been ascertained how much of the resin acid removal is achieved by means of biodegradation compared with absorption [9].

An advanced treatment process was developed during the last five years to improve the treated effluent quality in view of stronger environmental regulations and possibly for reuse of treated effluent in the pulp and paper industry [10,11,12,13]. Combination of ozone with fixed bed biofilm reactors is one of the most efficient tertiary effluent treatment processes to give maximum elimination of COD, colour and AOX with a minimum ozone dosage. Several laboratory and pilot tests with effluent from a full biological treatment works confirmed the expected targets [13]. A two-stage ozonation with intermediate biodegradation proved to be a valuable tool for obtaining high COD elimination efficiencies in the tertiary treatment of effluent with high persistent COD concentrations [13].

The use of enzymes is solely accepted in the pulp and paper industry to accelerate specific biological reactions. A recently patented multi-enzymatic microbial biostimulant overcame some environmental limits to enzymatic activity and increased the rate of biological activity[14]. These multi-enzymatic microbial biostimulants can drive biological treatment to lower more stable levels, reduce the rate of sludge build-up, and eliminate filamentous bulking and noxious odours from effluent treatment plants [14].
Savoie et al [11]; Robinson et al [12] demonstrated that the use of ultra filtration (UF) is technically feasible to process the biologically treated effluent for partial recycling. Furthermore, the quality of water produced from UF membrane treatment was suitable for recycling to the mill. It was hypothesized that this may require an increased use of biocides due to soluble residual nutrient compounds, which pass through the ultra filters, of which some would be recycled to the process.

Bleach plant effluents from the pulp and paper industry, generated during bleaching with chlorine-containing chemicals, are highly coloured and toxic due to the presence of chloro-organics, hence there is a need for treatment prior to discharge[15]. Fungal adsorption of colours in bleach plant effluent has recently attracted attention as a secondary treatment method. Christov et al [16], Magnus et al [17, 18], Tripathi et al [19] used a white-rot fungus and a mucoralean fungus and the results showed that a decolourisation of 53-73% could be attained using a hydraulic retention time of 23 hours.

Biochemistry and ecology of resin biodegradation contributed to a better understanding and to an improved performance of existing treatment systems and the development of new treatment systems for pulp and paper mill effluents. Using molecular genetic methods, a biochemical pathway for degradation of abietane resin acids has been partially elucidated by using *Pseudomonas abieatniphila* BKME-9. Genes encoding putative membrane-associated proteins, which are required for abietane metabolism, were identified. These proteins were assumed to function in cellular uptake of, or response to resin acids. The genetic evidence suggested that a mono-oxygenase is involved in the biochemical pathway [20].

The effects of pulp and paper mill effluents on the environment should be understood accurately because the quantity of water discharged from a mill is very large. The current effluent regulations are based on COD, BOD and AOX, but the measures do not reflect the actual environmental effect of effluent [21]. Bioassays are used to determine the effect of effluent discharge on the environment in several countries. The type of bioassay used in nearly every country is the sublethal toxicity test using fish, water fleas, green alga, sea urchins and luminescent bacteria as test organisms. Test conditions are slightly different from country to country.

Kachi et al [22] demonstrated in a laboratory experiment that most effluent samples showed sub-lethal effects upon aquatic life but no acute lethality to fish. Bioassays are not only used for effluent monitoring; but are also an effective procedure for toxicity identification evaluation (TIE) and toxicity reduction evaluation (TRE).

1.2.1.2 Tree Improvement

Wood is almost as important to humanity as food, and the natural forests from which most of the wood is harvested from are of enormous environmental value. However, these slow-growing forests are unable to meet the current demand resulting in the loss and degradation of forests [23]. Plantation forests have the potential to supply the bulk of humanity's wood needs on a long-term basis, and so reduce to acceptable limits to harvest pressures on natural forests. However, if they have to be successful, plantation forests must have a higher yield of timber than natural counterparts, on much shorter rotation times [23].

However, the long generation time of trees, presence of seasonal dormancy and prolonged periods required for evaluation of mature traits are strong limitations for classical breeding

and selection [24, 25]. Genetic engineering offers tree breeders the opportunity to add new genes into selected elite clones with little disturbance of the tree's genome [24].
Many research groups worldwide are currently focused on searching for new genes and developing reliable protocols for gene transfer in *Eucalyptus*. Traits such as herbicide and insect tolerance, rooting ability, lignin content and composition, cold tolerance, drought and salinity tolerance, wood morphology and chemistry are investigated, as they are considered amenable to gene transfer in *Eucalyptus* species [23-26]. The characteristics of most interest to the pulp and paper industry are wood fibre morphology and wood chemistry, which influence cost (growth, wood consumption) and paper quality (refinability, strength, porosity, bulk).

The application of genetic engineering is reported to produce better productivity and quality to help strengthen international competitiveness thereby creating more jobs in the process[27]. However, one of the most concerns for environmentalists is that in creating these genetically engineered species of trees, cross breeding may occur with their natural relatives and forever may change the characteristics of our natural forest lands[28].
In the latest research on transgenic trees conducted on field trials by Chiang [29] demonstrated that, transgenic trees may improve the efficiency of pulp production without detrimental environmental and ecological effects.

1.2.1.3 Single Cell Protein

An alarming rate in population growth has increased the demand for food production in third-world countries leading to a yawning gap in demand and supply. This has lead to an increase in the number of hungry and chronically malnourished people. This situation has created a demand for the production of innovative and alternative proteinaceous food sources. Single cell protein (SCP) production is a major step in this direction [30].

SCP is the protein extracted from cultivated microbial biomass. It can be used for protein supplementation of a staple diet by replacing costly conventional sources like soya meal and fishmeal to alleviate the burden of protein scarcity. Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final products cheaper [30]. This would offset the negative cost value of the residue

used as substrates to yield SCP. Further, it would make food production less dependent upon land and relieve the pressure on agriculture [30, 31].
Ziino et al [31] used continuous cultivation of *Geotrium candidum* grown on the orange peel extracts that produced a high protein, low-lipid content SCP which can be utilised as feed or protein extract source [31, 32]. The value of the food industry residues was raised using SCP.

1.2.1.4 Wood-Alcohol Fermentation

There is a worldwide interest in ethanol production from wood [33] and lignocellulosic residues as a substitute for fossil liquid fuel, since the combustion of ethanol produced from biomass makes no net contribution to the carbon dioxide in the atmosphere[34, 35]. The bioconversion of wood substrates to ethanol involves a number of sub-process steps including pre-treatment, fractionation, hydrolysis, fermentation and ethanol recovery [36]. The pre-treatment, fractionation and enzymatic hydrolysis are commonly recognised as a major component in the cost of producing ethanol from biomass [34, 35]. However one of the major drawbacks of these processes is the extensive degradation of wood components leading to low ethanol yields because of the wood sugar losses [37]. The resulting hydrolysis consists of complicated mixture of monosaccharides, lignin–derived products, extractives, organic acids and also degraded carbohydrates [35, 37].

It is known that the degradation products of wood sugars such as furfural, hydroxymethyl furfural; some organic acids such as formic acid and levulinic acid; and lignin derived products such as vanillin and catechol are highly potent inhibitors to most ethanol-producing microorganisms such as *Saccharomyces cerevisiae* [33, 34, 37,3839,40,41]. Moreover, the synergistic interactions of hydrolysates can further decrease ethanol production. Furthermore, it is difficult to predict the inhibiting effects of a hydrolysate because it is impossible to completely analyse its chemical structure. Thus, the effect of these inhibitors is not fully understood [37].

The use of conventional detoxifying techniques such as neutralisation, over-liming, and exposure to anion exchange resins and treatment with laccases has shown to enhance the fermentability of acid hydrolysates [37]. Indirect methods such as strain selection and adaptation

to inhibitory hydrolysates have also been effectively used to improve the fermentability of wood hydrolysates [37, 40].

Stenberg at al [42] obtained an improved ethanol yield from Douglas fir acid hydrolysates by progressive adaptation of a *Saccharomyces cerevisiae* strain and similar results were also reported for *Pichia stipitis*.

The fermentative production method such as simultaneous saccharification and fermentation (SSF) has proven to be a promising alternative over separate hydrolyses and fermentation (SHF) method [39, 43].

Zacchi et al [39, 43] stipulated some advantages of SSF over SHF. Firstly, the hydrolysis rate in SHF is strongly affected by end-product inhibition. In SSF, this inhibition is decreased because the fermenting organism consumes the glucose as soon as it is formed; hence the risk of contamination is lower. Secondly SSF is a one-stage process involving the enzymatic saccharification of cellulose and simultaneous fermentation of the fermentable sugar by yeast in one bioreactor hence it reduces capital costs.

One drawback of SSF, however, is the difference in optimal conditions regarding pH and temperature for hydrolysis and fermentation. Most of the organisms proposed for the fermentation of lignocellulosic hydrolysates such as *Saccharomyces cerevisiae, Zymomonas mobilis* and *Escherichia coli* limit the reaction temperature to below 40^0C, whereas the optimal temperature for hydrolysis is often claimed to be 50^0C [39, 43]. Below 40^0C the cellulases have a low activity, which in turn results in lower hydrolysis rate. The main drawback, however, is the difficulty in separating the yeast from the solid residue after the SSF process. This makes it difficult to recover and reuse the yeast in an industrial process [39].

Although SSF has been investigated extensively, there are still no guidelines for the optimal operating conditions for SSF of softwoods. Softwoods have been found to be more difficult to utilise than hardwoods because softwoods have lower lignin-extraction efficiencies, enzymatic rates and glucose yields than hardwoods [36, 43].

1.2.2 Pulp and Paper Biotechnology

1.2.2.1 Application of enzymes in the pulp and paper industry

1.2.2.1.1 Biological control of slime

Formation of slime deposits is a major problem facing paper making industries. The slime may be biological or no biological [44]. Biological deposits that are composed of varied microflora along with fibres, fillers and dirt are the most troublesome. Slime producing microbes secrete extracellular polysaccharides that gum up the process machinery [44]. These biological activities in paper making process waters are often the source of bad odours, corrosion problems, and slime deposits and consequently, reduced paper machine runnability and product quality. The specific nature of slime and its formation depends on the mill environment [44, 45].

The conventional slime control methods generally employ combinations of biocides. This leads to effluent toxicity, as well as high processing and treatment costs. Enzymes showed to be one of the alternative control measures of biological slime control. These enzymes attack the structures that the microorganisms use for attachment and improve biocide penetration into the slime layer. This results to a lesser amount of biocides required, that process economics is improved and effluent treatment is simplified [44,46]. Chemical bio-dispersants have been developed to control slime formation and deposition on the paper machine. Bio-dispersants exhibit a strong dispersing action on biological and organic deposits. The treatment by enzymes and bio-dispersants help reduce or eliminate odours and corrosion problems associated with microbiological deposits[47].

Malmqvist et al [48] demonstrated that installation of in-mill biological treatment in the white water system is also an alternative for facilitating closed paper mill water circuits. The introduction of a bioprocess in the white water circuit effectively lowered the amount of soluble matter and eliminated odours.

Verreanlt et al [49] developed a unique capacitance-based technology that is effective for preventing the plugging of paper machine shower water nozzles by microbial slime. A major

cost reduction was accomplished by replacing chemical biocide treatment with an installation of the Zeta Rod deposit control system. The results showed that within weeks the piping and nozzles of a wet felt shower system had been completely cleared of deposits, even as the chemical biocide addition rate was dramatically reduced. This technology causes rapid super-hydration of the existing biological slime deposits and prevents the attachment of free floating bacteria onto surfaces where they would colonise causing flow obstructions and corrosive deposits. This non-chemical and non-biological treatment strategy is being evaluated in all major mill-processing areas including: pulp and paper making, power input and output, recovery processes and effluent treatment.

1.2.2.1.2 Enzymatic pitch control

Pitch is the term used collectively for wood resins and resin acids, triglycerides, waxes, fatty alcohols, sterol esters, sterols, ketones and other oxidised compounds[50]. These lipophilic compounds are the most problematic in pulp and paper manufacturing, including deposition on the mill equipment, adverse effects on water adsorption by pulps, tearing of the paper due to sticky deposits on dryer rolls, discolouration and hydrophobic spots in the paper [50, 51, 52, 53, 54].

During wood pulping and refining of paper pulp, the lipophilic extractives in the parenchyma cells and softwood resin canals are released forming colloidal pitch. These colloidal particles can clog into larger droplets that deposit on the pulp or machinery forming pitch deposits or remain suspended in the process waters. Pitch deposition results in low quality pulp leading to technical shutdowns of the mill operation. Moreover the increasing need for recirculation process water in pulp mills is leading to an increase in pitch concentration, which results in higher deposition [51]. In addition, some wood extractives have a detrimental environmental impact when released into waste streams. Pitch problems originate with extractives in different types of wood but also depend on the pulping and bleaching processes.

The common solutions to minimise pitch deposition includes chemical methods, wood seasoning and the use of enzymes.

Microbial preparations currently on the market efficiently contribute to pitch removal in pine and other softwood mechanical pulping processes and in acidic sulfite chemical pulping and

toxicity reduction in the mill effluents. Enzyme preparations (added to the pulp or process waters) offer considerable advantages when compared to fungal inocula (applied to wood before pulping). This stems from the fact that enzyme treatments have shorter treatment times and greater specificity in the removal of wood components [51].

The use of enzymes in the pulp and paper industry has grown rapidly since the mid eighties. One of the best examples is the enzymatic control of pitch in softwood mechanical pulps using lipases. Lipases are a group of hydrolases that have been characterised from a variety of organisms. However, it is important to note that the addition of lipases to the pulp constitutes a prophylactic measure to prevent deposit formation but is not effective in the removal of previously formed pitch deposits.

Fleet et al [55] demonstrated that high concentrations of fatty acids affect lipase treatment of softwood thermomechanical pulps. The concentrations of total extractives and proportions of the different lipid classes in a pulp vary with these factors; tree species, the ratio of sapwood to heartwood, the wood seasoning and chemicals used in the process. Lipases differ from other enzymes in that their natural substrates i.e. tri-, di-, or monoglycerides with long chain fatty acids, have very low solubility in water. When lipases hydrolyse triglycerides, they liberate glycerol and free fatty acids. These products are surface-active. They tend to accumulate at interfaces in a triglyceride emulsion; they reduce the lipase's ability to access the substrate leading to decreased activity. Alternatively, when concentrations of fatty acids are high, they bind the active sites of the enzyme hence leading to decreased activity.

A recombinant lipase expressed in *Aspergillus oryzae,* called *Resinase,* has shown to hydrolyse approximately 95% of the triglycerides in a pine mechanical pulp. In addition, the *Resinase* treatment reduced the number of deposits, spots and holes in the paper, enabled a reduction in chemical dosage to control pitch deposition and permitted the use of higher amounts of fresh wood. Other industrial lipases such as *Lipidase 1000*, *Candiba* and *Aspergillus lipases* have been found to act on glycerides but do not degrade other extractives that form pitch deposits. Thus, enzymes acting on a broader range of substrates are being investigated.

Protein engineering techniques are being used to improve the performances of lipolytic enzymes in different industrial applications including pulp and paper manufacturing. Among the different factors to be improved by the above technique are substrate specificity, pH, temperature activity and stability. In a similar way, enzymes acting at high pH and temperature would be desirable for pitch bio-control in some chemical pulping processes [50-56].

1.2.2.1.3 Biodeinking

In a further attempt to improve the properties of waste paper, biotechnology has also been employed in the deinking of secondary fibre. Offices use more laser printers and copy machines every year; the volume of non-impact printed papers entering the recycled paper stream is increasing. Non-impact printed white office paper that include xerographic and laser printed paper are difficult to deink with conventional deinking methods. The reduced efficiency is due primarily to the strong adherence of the toner particles to the paper surface. Conventional deinking consists of pulping, selective floatation and dewatering processes. The dewatering process is also a substantial source of solid and liquid waste, and disposal of this waste presents problems to the environment[57].

Enzymatic deinking methods represent a new approach to convert these recycled papers into quality products. Various enzymes have been examined to improve the deinking performance of non-impact printed white office paper [57]. Biotechnology has shown that enzymes can be used to attack either ink or fibres. Lipases and esterases are used to degrade vegetable oil-based inks. Pectinases, hemicellulases, cellulases, and lignolytic enzymes are believed to alter fibre surfaces or bonds in the vicinity of ink particles thereby facilitating better ink removal by washing or floatation.

Research work on enzymes has largely focused on cellulases[58]. Hydrolysis of crystalline cellulose requires a three-part system comprised of endo-β-1,4-glucanases, exo-β-1,4-glucanases and β-1,4-glucosidases. Endoglucanases hydrolyse the amorphous cellulose and soluble derivatives by randomly splitting internal β-1,4- glycosidic linkages along the cellulose molecules. The hydrolysis products include glucose, cellobiose and other oligomers. Exoglucanase hydrolyse cellulose molecules from the non-reducing end and release glucose or cellobiose units. Glucosidases degrade cellobiose and other oligomers into glucose monomers.

According to a recent review, cellulase activity releases ink particles into suspension from fibres and reduces ink areas by one or combination of two mechanisms. In mechanism one, enzyme attack fosters disintegration of ink fibre complexes during pulping, thereby reducing the number and size of the residual ink particles. In mechanism two, enzymes attack at the sites where ink is bonded to fibres, thereby freeing ink particles from individual

fibres [58, 59, 60, 61].

Gubitz el al. [59] treated laser-printed paper with a purified endoglucanases from *Gloeophyllum sepiarium* (EGS) and *Gloeophyllum trabeum* (EGT), a xylanase from *Thermomyces lanuginosus* (X) and a mannase from *Sclerotium rolfsii* (M). Subsequent toner removal efficiency was assessed by image analysis. The enzyme effect was more pronounced in floatation deinking demonstrating 94% removal of toner using a combination of EGS and X. The use of pure EGT and EGS suggested that endoglucanases were responsible for most of the success in biodeinking.

Qin et al. [60] and Viestus et al [61] deinked old newspapers with endoglucanases and cellulobiohydrolases. The results showed that endoglucanases are essential enzymes in the old newsprint deinking. The synergism of endoglucanases and cellulobiohydrolases were beneficial to improve brightness and drainability of the deinked pulp. The potential benefits of enzyme-assisted processes includes higher brightness, better freeness and reduced water retention values, greater paper strengths, reduced chemical usage, lower bleaching costs, reduced liquid and solid waste disposal hence lower COD and BOD content in the effluent.

1.2.2.1.4 Biobleaching

1.2.2.1.4.1 Fungal bleaching

It is the treatment of pulp with fungal strains prior to a bleaching sequence. A considerable amount of research has been done on white rot fungi. White rot fungi degrade lignin and remove colour from effluents generated during bleaching. One major drawback of fungal biobleaching is the longer periods of treatment because of the slow reaction rate of the fungal inocculant with its substrate [1]. Modern environmentally sound trends in manufacturing of bleached pulp involve development of totally chlorine free (TCF) bleaching and zero liquid effluent (ZLE) processes. Lipophylic extractives are among the most problematic wood constituents in both TCF and ZLE pulps since they accumulate in water circuits resulting in manufacturing problems [62].

Gutierrez et al [62] used extractive degrading fungi such as *Bjerkandera adusta* to remove these compounds from *Eucalyptus* globulus using solid-state fermentation conditions. Results showed that 75% of problematic compounds were removed from the pulps and liquors.

Feijoo et al[63, 64] studied manganese (Mn) and manganese peroxidase (MnP) as essential components for Kraft pulp biobleaching with white rot fungi. However, the use of white rot fungi, in bleaching of EDTA extracted *Eucalyptus* oxygen delignified Kraft pulp, does not require manganese. Fungal organic acid metabolites added to the Mn-free culture were found to be stimulatory for brightness gains, delignification rates and MnP production.
Ahmed et al [65] used Kraft pulp obtained from white-rot fungi treated whole and bast Kenaf towards chlorine dioxide bleaching. In the case of bast Kenaf with 50% yield range and 12-13 Kappa number, only a minimum amount of chlorine dioxide was used to reach 78-80% brightness level. Pulp from white-rot fungi treated bast Kenaf could be bleached to 86% compared 78% ISO brightness for control bast pulp in DED bleaching stages and 88% compared to 80% ISO brightness for control when DEDP stages were applied.

Bajpai et al [66] bleached wheat straw soda pulps in which the feedstock was treated with different strains of *Ceriporiopsis subvermispora* and cooks were performed at reduced cooking alkali charges. Pretreated pulps showed better results than conventional control pulps (see Table 1).

Table 1: Bleaching data on wheat straw soda pulps in which the feedstock was treated with different strains of C. *subvermispora* and cooks were performed at reduced alkali charges [66].

Experimental treatments	**EA (%)**	**E-stage Kappa no.**	**E-stage brightness (% ISO)**	**H-stage brightness (% ISO)**	**Yellowness (% ISO)**	**Whiteness (% ISO)**
Control	12	4.4	38.5	81.3	5.63	67.5
Strain 1	10	3.8	39.7	82.7	5.11	70.1
Strain 2	10	3.2	41.0	83.7	4.31	72.9
Strain 2	9	4.2	39.2	82.3	5.21	71.1

1.2.2.1.4.2 Enzyme bleaching

At present the pulp and paper industry is under growing pressure from authorities, consumers and environmental groups, to reduce the effluent loads by using cleaner technologies of bleaching. Among various technological options available, enzyme prebleaching was considered as one viable alternative[67, 68, 69]. Research on enzyme prebleaching has been extensively conducted on a hemicellulose enzyme, xylanase. In the beginning, the production of the crude enzyme complex secreted from fungus in the bleaching process was carried out because purified enzymes were too expensive[70, 71].

These enzymes are target specific and speed up the bleaching reaction and by doing so, shorten the retention time, hence allowing for an increased pulp production[72, 73, 74]. The xylanase selectivily removes xylan from the surface and pores of the fibres. The morphological changes on the fibre surface such as cracks and peeling due to the enzyme treatment were observed using scanning electron microscopy (SEM)[75].

Bajpai et al [76] and Thibault et al [77] studied the role of different xylose degrading enzymes in pentosan removal and bleaching of high pentosan content pulp. Endo-xylanase was found to be the major enzyme in solubilizing pentosans[78]. Enzymes extracted from bacteria and engineered enzymes are also utilised.

White et al [79] demonstrated that engineered enzymes could operate at temperatures that are 5-10^0C higher than for natural enzymes. In the latest developments, new catalases have been discovered in two bacteria. This work has been patented [80]. Reid et al [81] also demonstrated that enzyme treatment improved the effectiveness of several cationic polymers, therefore increasing the retention of fines and filler particles and to lower the charges of cationic retention aids needed. Most researchers share similar points on the benefits of using enzyme pre-treatment. These benefits are the reduction in Kappa number[82, 83, 84] reduction of chemical demands, brightness gains [69, 70, 75, 77, 78, 85, 86, 87, 88, 89, 90], reduction in AOX levels and good strength properties [80, 88].

The use of enzyme cocktails is another available option for brightening the pulp. Surma-Slusarska et al [91] simultaneously used laccase and xylanase during hydrogen peroxide and ozone bleaching. The results showed that pre-treatment of pulp with xylanase increased the laccase access to lignin.

1.2.2.1.5 Biopulping

Biopulping is the fungal treatment of wood chips with lignin degrading fungi prior to any pulping process, that is, chemical, mechanical Organosolv [1]. The direct route of access into wood for all wood colonizing fungi is the ray cells because of their wideness hence providing ample space for hyphal growth. Furthermore, parenchyma cells of ray ducts function as storage cells, providing easily assimilated substances such as sugars and fat to the growing hyphae. Of utmost importance for the young growing hyphae is the high nitrogen content of the parenchyma cells. In the study by Dommisse [1], wood chips were supplemented with nutrients like urea and molasses. From ray cells, the hyphae move into the longitudinal elements such as tracheids. As soon as the tracheids of the wood are colonised, the nutritional situation of hyphae drops. Under nutritional starvation, lignin depolymerization is induced in most white-rot fungi. One major drawback of fungal pretreatments is the long incubation times needed for industrial scale application.

1.2.2.1.5.1 Biomechanical pulping

Mechanical pulps represent about 20% of the world total pulp production[92]. Mechanical pulping involves the use of mechanical force to separate the wood fibres and generate high yield pulps (up to 95%) rich in lignin but with relatively low strength properties compared to chemical pulps[93]. Mechanical pulping produces paper with high bulk, good opacity, and excellent printability. However, mechanical pulping is energy-intensive [93], produces paper with a higher pitch content and exhibits a higher colour reversion rate as compared to chemical pulps. Kraft pulp is often added to the mechanical pulp to impart strength but it is more expensive than mechanical pulp. These disadvantages limit the use of mechanical pulps in many grades of paper.

Biomechanical pulping is the fungal treatment of wood chips with lignin degrading fungi prior to the mechanical pulping process, that is, in the refining stages. Fungal treatments need long incubation times for industrial scale application. In contrast, enzymatic treatments only take few hours which means they are compatible with the mill processes and their effects on mechanical pulps depends largely on their penetration into the pulp. Enzymatic treatment induces an enzymatic refining, which facilitates fibrillation. Fibrillation enhancement is in agreement with the development of pulp properties. Tensile strength is improved while tear

index is slightly decreased due to fibre structure damage. By the application of enzymes, energy consumption is reduced in the process of mechanical refining [92-94, 95, 96, 97].

Ruel et al [92] have demonstrated that the action of lignolytic enzymes such as manganese peroxidase (MnP) and laccase on high yield pulp fibres was more efficient after the fibre structure was opened.

New developments had also demonstrated that a primary refined mechanical pulp treated with cellobiohydrolases resulted in energy savings between 10 to 40% in the secondary refining stage. Furthermore, there were no fibre length and paper strength modifications.

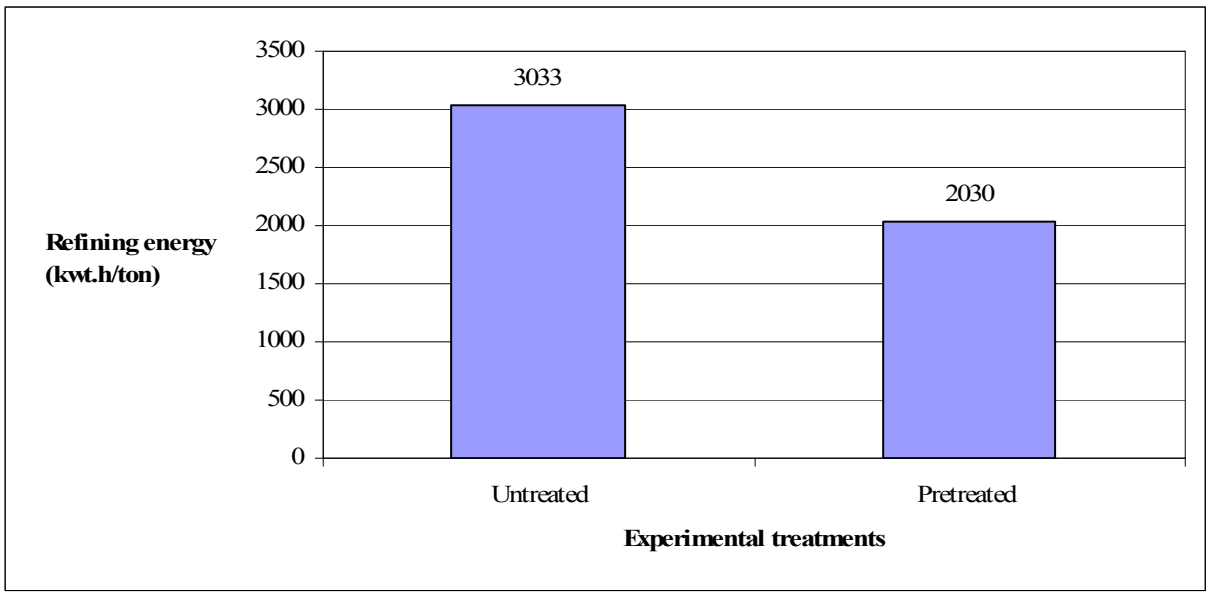

Figure 1: Refining energy reduction observed between untreated and pretreated *Eucalyptus grandis* wood chips[98].

1.2.2.1.5.2 Biochemical pulping

Biochemical pulping is a fungal pretreatment of wood chips prior to chemical pulping. The fungal pretreatment breaks down macromolecules i.e. hemicellulose and lignin, removes wood extractives and improves paper strength[99].

In the literature, it has been found that such treatment save chemicals, increases brightness and yield, decreases opacity, Kappa number and refining energy in various pulping processes[1].

Bajpai et al [66] pretreated wheat straw with *Ceriporiopsis subvermispora*, a lignin-degrading fungus, to study its effect on soda pulping. For soda pulping, COD load in the effluent was lowered as compared to the control experiments (see Table 2). Fungal pretreatment reduced the lignin and extractive content hence Kappa number of wheat straw by 16.5%, 44.3% and 22-27% respectively (see Tables 3 and 4).

Table 2: Comparing COD load (kg/ton of pulp) of effluents of biopulps in the chlorination (C), extraction (E) and hypochlorite (H) stages [66].

Experimental treatments	**EA (%)**	**C (kg/ton of pulp)**	**E (kg/ton of pulp)**	**H (kg/ton of pulp)**	**Total (kg/ton of pulp)**
Control	12	29.1	38.3	11.7	79.1
	10	32.1	48.3	7.5	88.5
Strain 1	12	25.4	37.1	8.3	70.8
	10	32.5	36.3	9.8	78.6
Strain 2	12	25.1	34.2	7.2	66.5
	10	26.9	38.7	8.1	73.7

Table 3: Comparing the effect of fungal treatment with strain 2 on cellulose, hemicellulose, lignin and extractive contents of wheat straw [66].

Chemical Compounds	**Control (%)**	**Pretreated**
Cellulose	44.6	50.2
Hemicellulose	27.8	28.6
Klason lignin	20.1	16.8
Total extractives	6.1	3.4

Table 4: Comparing yield, Kappa number, brightness and residual active alkali of wheat straw after soda pulping without and with fungal pretreatment [66].

Experimental treatments	Kappa number	Yield (%)	Brightness (% ISO)	Residual active alkali (RAA)
11% EA				
Control	30.9	46.4	31.1	1.3
Treated	24.1	46.6	38.2	1.4
12% EA				
Control	28.8	45.9	34.4	1.9
Treated	21.2	46.1	39.2	2.1

Table 5: Comparing the effect of cooking time at 12% effective alkali (EA) on unbleached soda pulp properties of wheat straw [66].

Experimental treatments	Cooking time, (min)	Kappa no.	Yield (%)	Brightness (% ISO)	Residual alkali (g/l)
Control	60	28.1	45.9	34.1	2.3
	45	30.1	46.5	33.9	2.5
	30	31.5	47.1	**33.1**	2.5
	15	-	-	-	-
Strain 2	60	21.9	46.1	38.2	2.5
	45	22.5	47.2	37.6	3.0
	30	24.1	47.8	37.1	3.2
	15	26.1	48.1	36.2	3.5

1.2.2.1.5.2.1 Bio-Kraft pulping

Bio-Kraft pulping has the potential to significantly reduce the chemicals, energy and water required to produce pulp. It involves treating wood chips with fungi to modify the lignin present and to make the cell wall more accessible to the Kraft liquor. The treated wood chips become easily delignified in the Kraft pulping process hence much milder chemical conditions would be required. Selective and effective fungi for Kraft pulping are still being researched [1].

Messner et al [100] conducted a study on fungal pretreatment of wood chips that were pulped using Kraft liquor. Literature results portrayed that there were losses in yield and brightness; tear index and Kappa number were improved (see Table 6).

Table 6: Physical properties of Kraft pulps that had been pretreated with fungal strains [100].

Property	**Fresh chips**	**Aged control**	***Ophiostoma piliferum***	***Phlebia tremellosa***
Pulp yield	55.7	54.8	54.9	54.7
Kappa number	15.4	15.2	14.5	13.2
ISO Brightness	32.4	33.0	32.9	33.7
Tear index	6.78	7.44	7.65	7.90
Tensile index	60.7	61.1	59.2	54.1

Klungness [98] reduced cooking time from 90 to 30 min. Better results were obtained at 30 min cooking time (see Table 7) below. This means that Kraft pulp mills could increase throughput and thus get more pulp production from the existing capital investment.

Table 7: Kraft biopulping of *Eucalyptus grandis* wood chips pretreated with *Ceriporiopsis subvermispora* at a reduced cooking and incubation time of two weeks [98].

	Cooking time 90 minutes	**Cooking time 30 minutes**
Pulp yield (%)	46	46
Brightness (%)	88.6	90.5
Burst index (kN/g)	4.6	4.8
Tear index (mNm^2/g)	7.8	8.0
Tensile index (Nm/g)	68.9	70.5
Breaking length (m)	7026	7193

1.2.2.1.5.2.2 Bio-Sulfite pulping

Historically, sulfite pulping has been dependent on calcium-based liquor of high acidity (pH 1-2). However, in the last half-century, more suitable bases of sodium, magnesium and ammonia have come into use. The use of these bases had extended the possible pH range to

less acidic conditions so that sulfite pulping could be now done at pH 3-5 and neutral sulfite semichemical (NSSC) could be done at pH 7-9 [101]. Akhtar et al [102] pretreated Loblolly Pine with two fungal strains and compared the yield and Kappa number to the control. The pretreated results showed better yield and reduced Kappa numbers (see Table 8).

Table 8: Comparing yield and Kappa number of Loblolly Pine after calcium-acid sulfite pulping without and with fungal pretreatment [102].

Treatment	Yield (%)	Kappa number
Control	47.6	26.8
Strain CZ-3	47.7	13.7
Strain SS-3	47.8	21.1

Biological alternatives that could aid the hemicellulose and lignin removal from dissolving grade pulp imply the use of microorganisms to pretreat wood chips prior to pulping for example biosulfite pulping.

Akhtar et al [102] used five screened strains of the white–rot fungus *Ceriporiopsis subvermispora* for their abilities to facilitate acid sulfite pulping and bleaching of *Eucalyptus grandis* wood chips for dissolving grade pulp. The results showed an increase in brightness (12%) and reduction in Kappa number (10-29%) which was attributed to increased lignin solubility.

On the other hand only one strain produced pulp yield comparable to that of the control [101], other strains gave lower yields depending on the duration of incubation. After the bleaching stage there was a yield gain (1%) obtained when other strains were used.

Furthermore, the study suggested that pre-treatment of wood chips with selected strains of white-rot fungi may be used as a means of improving the selectivity of both pulping and bleaching thereby increasing the final yield or brightness [102, 103]. It was also found that longer fungal treatment times led to greater Kappa reduction, a significant reduction in cooking time and lower shives content compared to the control results. This indicated that more complete pulping with fungal pre-treatment had occurred [104].

1.2.2.1.5.2.3 Bio-Organosolv pulping

Organosolv pulping is the treatment of wood chips with organic solvents in acid or alkaline solution under high pressure and temperature[104]. This process can avoid problems caused by sulfur emissions in Kraft pulping; hence, it is claimed as an environmentally friendly technique for obtaining cellulose pulps. Moreover, the use of wood residues [105] and low capital investment costs are the advantages that make small pulp mills feasible [104, 105].
The high delignification efficiency of several acid-catalysed organic solvent systems results in carbohydrate degradation. High acid concentration and pressure during the acid catalysed organosolv pulping is a necessary step to provide efficient delignification. Consequently, pulps with low papermaking quality were obtained [104].
Baeza et al [106] studied organosolv pulping of *Pinus radiata* and *Eucalyptus globulus* wood chips using formic acid/acetone at 70/30 vol / vol ratio. The formic: acetone system was found to be an excellent solvolytic pulping medium because good strength properties were obtained.

Uraki et al [105] used propylene glycol pulping of wood chips for various species of the Japanese Larch and Ceder families. Several desirable results were obtained such as low Kappa number, high bleachability, high α-cellulose content and high cellulose crystallinity. These properties suggested that these pulps could be used not only for paper but also as a source of highly crystalline cellulose. However, it has been postulated that if the lignin macromolecule is partially depolymerised in an initial step, mild cooking conditions are feasible hence carbohydrate degradation can be prevented. This pretreatment could be carried out by fungal degradation using selective white-rot fungi [104, 105].

Fungal pretreatment provides faster delignification rates hence biodegraded samples present a significantly increased xylan removal in the acid-organosolv pulping process [104, 107]. Consequently, the same residual lignin contents in the fungal pretreated samples were achieved at shorter reaction times [104, 108] hence, energy is saved [109] and pulps of increased strength properties [58] are obtained. Organosolv pulping has been claimed a pollution free technique [110].

Botello et al [111] studied the recovery of alcohol and by-products from ethanol and methanol water pulping liquor. The proposed recovery system consisted of three categories namely

black liquor flashing, lignin precipitation and precipitation distillation of the mother liquor. At the flash stage, 47 and 51% of the alcohols in the black liquor was recovered. The lignin recovery yield at the precipitation stage was 67% for ethanol black liquor and 73% for methanol black liquor. The precipitation distillation of mother liquor enabled a 98% of ethanol and 96% methanol recovery. The distillation residue contained significant amounts of sugars, furfural and acetic acid that could be recovered [111].

OBJECTIVES

Comprehensive research was carried out by Dommisse[1] on the use of fungal co-cultures as a pretreatment of wood chips and on the use of PHWE of wood chips as a pretreatment for wood pulp production. However at this stage the combination of both PHWE and pretreatment with fungal co-cultures was not investigated. As both the wood chip pretreatments individually produced improved pulping properties, it was decided to combine the two pretreatments and evaluate their performance on pulp yield and paper properties. The hypothesis that PHWE and fungal pretreatment together would improve liquor penetration and extractive removal and consequently pulp yield had to be tested.

In summary

I. To investigate the effect of a combined PHWE and FCCI of wood chips on pulp yield and paper properties.
II. To establish the effect of PHWE on the screened pulp yield, handsheet strength properties and residual active alkali during soda AQ pulping.
III. To evaluate the combined wood chip pretreatment with a more environmental friendly alkaline pulping method.

CHAPTER 2: MATERIALS AND METHODS

2.1 PULPING

2.1.1 Raw materials

The investigation was conducted on with *Eucalyptus grandis* wood chips and supplied by the Central Timber Cooperative (CTC) from Richards Bay. A six-component chip screen driven by an electric motor was used for chip screening. During operation, the tightly held screen components were shaken sideways for one minute. Oversized chips were mostly found in the unlabelled top screen and were rejected. The six to eight millimetre screen produced the correct wood chip size for pulping (8mm thickness) and was accepted. The undersized (less than 8mm thickness) wood chips were collected in the lower screen trays and rejected. The accepted wood chips were soaked in cold water for a week in order to regain moisture and facilitate better liquor penetration during cooking. After soaking, the accepted wood chips were put into 50 kg plastic bags and stored in cold storage at 4^0C until further use.

2.1.2 Digester

Laboratory alkaline anthraquinone pulping trials were conducted in a 15-dm^3 batch-type laboratory digester. The digester was charged with the equivalent of 1500g oven dry wood chips. The digester was electrically heated and oscillated through 45^0 vertical to the side to ensure proper chip-liquid contact and circulation of cooking liquor during the cooking cycle. In addition, the digester was equipped with pressure and temperature gauges, and the cooking cycle was controlled with a programmable logic controller (PLC) connected to a thermocouple that extended to the centre of the digester. Pressure was released with the aid of a blow-valve which was activated by a solenoid, controlled by the PLC and thus allowed blow-down at the specified times after the cooking schedule was completed.

2.1.2.1 Pressurized hot water extraction

Oven dry *Eucalyptus grandis* water soaked wood chips (1500g) and five litres of water were charged into a 15-dm^3 digester. Wood chips were cooked for an hour at a temperature between 100 and 170^{0}C and pressure between 500 and 800 KPa. The PHWE was conducted using the normal cooking programme for hardwoods as stated in section 2.1.2. At the end of one hour, the digester blow-down was activated from the cooking programme. At the end of the digester blow-down the cooking programme was stopped. After the digester had cooled down the lid was opened and dark coloured wood chips were obtained, rinsed in a 25l bucket and transferred into autoclavable plastic bags. The rinsed wood chips were kept in cold storage at 4^{0}C until further use.

2.2 MICROBIAL PROCEDURES

2.2.1 Fungal cultures

Dommisse[1] found that when *P. sanguineus* and *Aspergillus flavipes* were grown in co-culture a synergism existed between them. *A. flavipes* created a favourable environment for *P. sanguineus* by breaking down hemicelluloses and liberating essential monosaccharides to promote growth of *P. sanguineus*. Both fungi were found to be having an optimum growth temperature of 29^{0}C [1].

For this study, two fungal cultures were used i.e. *Aspergillus flavipes* PPRI 4965 (hemicellulytic) and *Pycnoporus sanguineus* PPRI 6762 (lignolytic). The *Aspergillus flavipes* strain was originally isolated from a *Quercus* spieces and the *Pycnoporus sanguineus* strain, was originally isolated from a beetle belonging to the family *Chrysomelidae* that in turn was obtained from *Rhus pyroides*. Both strains are held in the fungul culture collection of the Plant Protection Research Institute (PPRI) in Pretoria.

2.2.2 Agar preparation

Fifty gram of malt extract agar (MEA) was dissolved in boiling distilled water in a conical flask. The medium was sterilised by autoclaving at 121^0C for 20 min. Agar plates were subsequently prepared and later used for growing and storing the fungal cultures.

2.2.3 Broth for growing fungal pre-inoculum

A broth medium was prepared by adding 12.5g of 5% (w/v) molasses (Illovo Sugar) in 250ml water of a 250ml conical flask. Mouths of conical flasks were plugged with cotton wool stoppers and covered with aluminium foil caps. The cotton wool stoppers served as biological filters during the aeration and incubation of the culture. The contents of the conical flasks were then steam sterilised using an autoclave (20 min at 121^0C).

2.2.4 Preparation of fungal inoculum

For each fungal strain, a seven-day-old MEA plate culture was used to inoculate 20 ml of 5% (w/v) molasses in a 250ml conical flask. To obtain the fungal pre-inoculum the culture was incubated for four to seven days at 29^0C. The biomass i.e. mycelia was aseptically transferred from the conical flask using a sterile spatula to a sterile blender containing 20ml of the molasses broth. The content was homogenised by laboratory blender (Waring Commercial) for 1 min at 20 000 rpm to obtain a suspension. A 2ml portion of this homogenised pre-inoculum was then used to inoculate 250ml nutrient supplement contained in a 1000ml conical flask. This suspension was used as an inoculum for the wood chips.

2.2.5 Quantitative analysis of biomass

To determine the concentration of fungal biomass (g/l) in the above mentioned inoculum appropriate quantities of fungal suspension in nutrient supplement medium were filtered using pre-weighed filters (cellulose acetate, pore size 0.45µm). The filters containing the wet biomass were subsequently dried in a microwave oven (K.I.C. Mw5145T at setting 3.5 for 15 min) and weighed.

2.2.6 Nutrient supplement medium for fungal inoculum (for wood chip inoculation)

A nutrient rich liquid supplement containing 27.5g of 5% (w/v) molasses (Illovo sugar), 1.54g of 0.28% (w/v) urea and 550 ml water was prepared and dispersed into a 1000ml conical flasks. The flasks were subsequently sterilised for 20 min at 121^0C. Larger volumes were used in this instance to moisten the wood chips equally but the concentration was still the same.

2.2.7 Inoculation and incubation of wood chips

Three samples of unextracted wood chips (1500g oven dry) were loaded into autoclavable plastic bags and steam sterilised at 121^0C for 20 minutes. The samples were allowed to cool to room temperature and were inoculated with 20 ml inoculum of *A. flavipes* that was mixed with nutrient supplement (see section 2.2.6). The inoculation of the sterile wood chip samples in the autoclavable plastic bags was conducted under sterile conditions. The inoculated wood chips samples were each thoroughly mixed in their tightly closed autoclavable plastic bags and then incubated at 29^0C for a week in the incubation room. After a week 20 ml of *P. sanguineus* inoculum mixed with nutrient supplement (see section 2.2.6) was added into the previously inoculated wood chip samples. The contents of the thoroughly mixed wood samples in autoclavable plastic bags were transferred to a bioreactor (20 litre) for further 3 weeks incubation (i.e. total incubation period was 4 weeks) as shown in Figure 2. The same inoculation procedure was used for the PHWe wood chip samples (see section 2.1.2.1 for PHWE). Some PHWe wood chip samples were incubated for 2 weeks (i.e. total incubation period was 3 weeks).

The bioreactor inside contained a wire mesh sitting on a four legged stand (5.5cm height from the bioreactor base). An air inlet fitted on the top of the bioreactor right down underneath the stand, which supplies air to the fungal co-culture pretreated wood chips sitting on the wire mesh. An exhaust air outlet fitted on the top the bioreactor to a 250 ml conical flask containing a mixture of 30ml alcohol and 70ml water. The mixture served to prevent any contamination of the incubation room. An electrically plugged aquarium pump was used to pump air through a 0.45µm filter to an air humidifying (650ml/min) conical flask to the bioreactors.

Figure 2: Twenty-litre bioreactors with FCCI unextracted wood chip samples incubated at 29^0C for four weeks.

After four weeks of incubation of pretreated wood chip samples at 29^0C, a picture showing a clear growth of the fungal co-culture on wood chips inside of the bioreactor was taken (see Figure 3).

Figure 3: Top view of a twenty-litre bioreactor containing wood chips inoculated with fungal co-culture after incubation at 29^{0}C for four weeks.

2.3 PULPING CONDITIONS

Prior to pulping control wood chips, moisture content of the wood chips was determined using infrared drying.

Table 9: The pulping conditions used for Soda-AQ pulping of *E. grandis* wood chips [1].

Sodium Hydroxide Charge	15% AA
Anthraquinone Charge	1%
Liquor to wood Ratio	4.5:1
Initial cooking temperature	50^{0}C
Maximum cooking temperature	170^{0}C
Heat up time to 155^{0}C	90 min
Degas at 155^{0}C	10 min
Cooking time to 170^{0}C	25 - 30 min
Blow-down to 100^{0}C	30 min
Total pulping cycle	160 min

Moisture content of the PHWe and inoculated wood chips was determined from the unextracted control wood chips using an infrared drying. Same pulping conditions of unextracted and PHWe controls; unextracted inoculated and PHWe inoculated were used. One experimental treatment from PHWe wood chips was cooked at 14% AA and the other at 20 min at the temperature between 100^0C and 170^0C.

2.3.1 Pulping procedure

For each pulping trial that is; unextracted, PHWe, unextracted fungal inoculated and PHWe fungal inoculated wood chips; a fixed amount of 1500g of oven dry wood chips was transferred to a stainless steel mash basket and placed inside the digester. A mixture of antraquinone (1%) (Buckman Laboratories, Hammersdale), 15% or 14 % AA and water were prepared and added to the wood chips based on the oven dry mass of the wood chips.

At the end of cooking cycle, the digester pressure lid was opened and the stainless steel mesh basket that contained the cooked wood chips was removed. The black liquor was then removed from the digester and approximately 500 ml sample was collected in a glass bottle for chemical analysis. Cooked wood chips were washed through a 10-mesh stainless steel screen to separate fibre from rejects, and accept pulp was collected as solid matter retained on a 150-mesh stainless steel screen. All the pulping trails were done in triplicate to account for minor differences during various cooking cycles to take into account small differences in control conditions.

A summarised description was prepared for all the experimental treatments, which were unextracted, extracted, unextracted inoculated and extracted inoculated wood chips (see Table 10). Furthermore, a detailed schematic layout of the project was also compiled to give a better understanding of the work at hand (see Figure 4).

Table 10: Summary of various experimental treatments before wood chip digestion.

Oven dry (o.d.) Chip mass (g)	Experimental treatments (Wood chips)	Incubation times (weeks)	Description
1500	Unextracted	None	Wood chips were soaked in cold water for one week and pulped.
1500	Extracted	None	Wood chips were soaked in cold water for one week, PHWe and pulped.
1500	Unextracted inoculated	Four	Wood chips were soaked in cold water for one week, moisture content was measured. The correct amount of oven dry wood chips was steam sterilised at 121^0C for 20 minutes using autoclavable plastic bags. The wood chips were allowed to cool down to room temperature, then inoculated with *Aspergillus flavipes* for the first week, *Pycnoporus sanguineus* the following week and incubated at 29^0C.
1500	Extracted inoculated	Four	Wood chips were soaked in cold water for one week, moisture content was measured. The correct amount of oven dry wood chips was PHWe, thereafter rinsed because the inoculum grew very slowly on unwashed wood chips and steam sterilised at 121^0C for 20 minutes using autoclavable plastic bags. The wood chips were allowed to cool down to room temperature, then inoculated with *Aspergillus flavipes* for the first week, *Pycnoporus sanguineus* the following week and incubated at 29^0C until the fourth week.

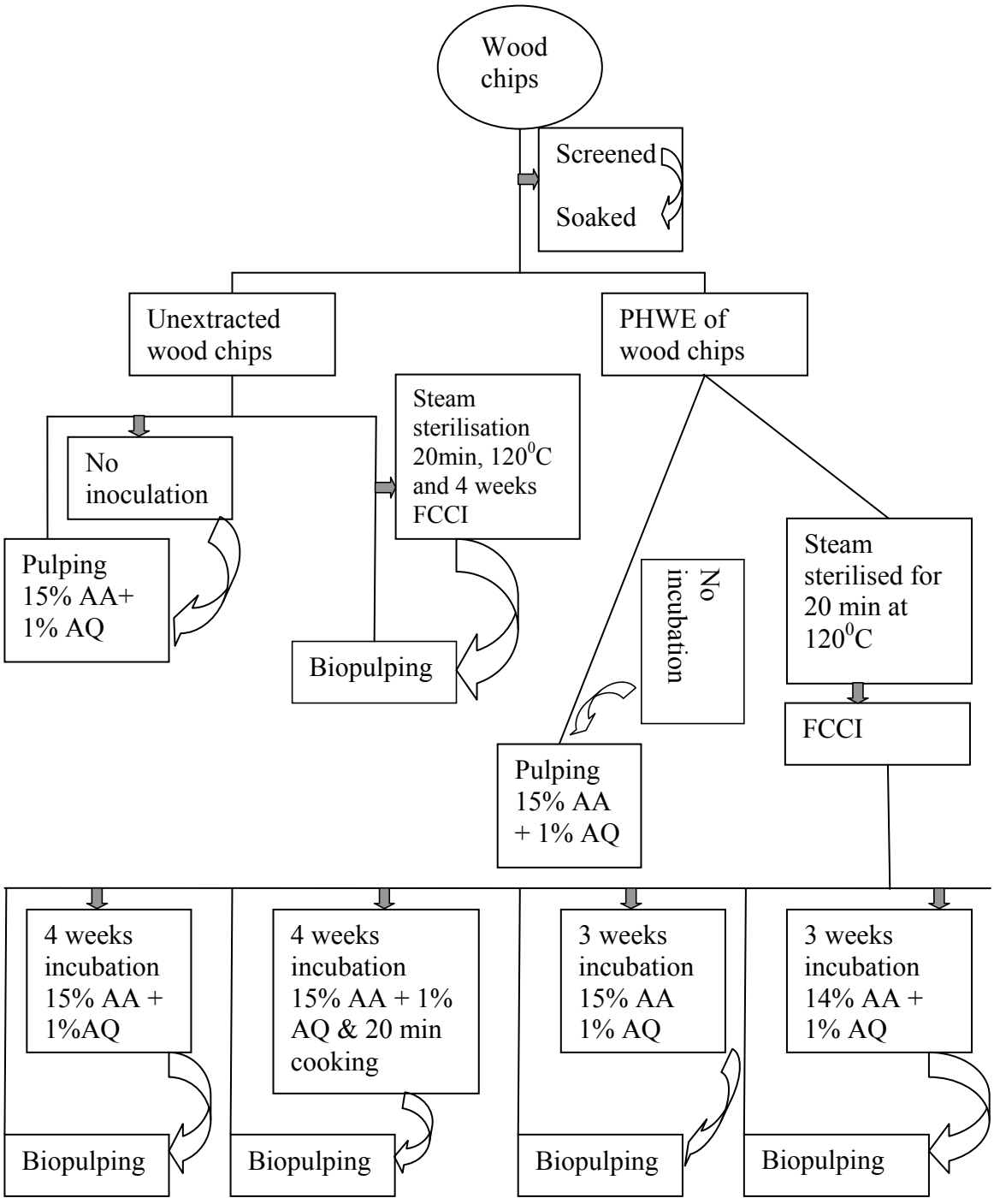

Figure 4: Schematic representation of project summary.

2.4 PULP EVALUATION

2.4.1 Rejects

Screened rejects were quantitatively collected as the material retained on the 10-mesh screen after washing of the cooked wood chips, transferred to an aluminium foil dish and placed in an infrared drying oven until completely dry. The samples were removed from the drying oven after this period, and placed in a desiccator for 24 hours before the mass was determined gravimetrically on an electronic balance. The rejects content was calculated as a percentage of the original dry mass of wood chips charged to the digester.

2.4.2 Shive content

Pulp retained on the 150-mesh screen was quantitatively collected before it was screened for shive content in a Packer slotted screen. The screen consisted of a 10 litre stainless steel cylindrical container, a stainless steel base plate with 0.15 mm slots through which the accept pulp was screened, and a pulsating rubber diaphragm that prevented clogging of the slots in the base plate. The pulp was screened and shives were collected as the material retained on the 150-mesh stainless steel base plate. The accept pulp on the other hand passed through the slots and was collected at the accept-outlet of the cylindrical container.

To determine the shive content for a given pulping trial, the shive sample from the total mass was quantitatively transferred to an aluminium foil dish and placed in an infrared drying oven until oven-dry. The shive content was calculated as a percentage of the original dry mass of wood chips charged to the digester.

2.4.3 Pulp yield

Screened pulp yield was calculated by quantitatively collecting the pulp retained on the 150-mesh stainless steel screen after the rejects had been removed and then transferred to a cotton linen bag. Wet pulp was then spin-dried to an approximate moisture content of 70%. The consistency of the pulp was determined as specified by TAPPI Standard Test Methods number T240 os-75 [112]. The pulp yield was calculated as a percentage screened yield.

2.4.4 Chemical consumption

The efficacy of the respective cooking trials was evaluated as the alkali consumed during the pulping cycle measured as residual active alkali (RAA). The evaluation was conducted according to TAPPI Standard Method number T625 om-85 [112].

2.4.5 Evaluation of pulp properties

2.4.5.1 Extent of delignification

The extent of delignification of the pulp was determined as the residual lignin content, recorded as the Kappa number according to TAPPI Standard Method number T236-cm–85[112]. The results were presented as mean values.

2.4.5.2 Pulp response to beating

Laboratory processing of pulp or beating was done to determine the papermaking quality of the pulp. This was conducted by subjecting the pulp to a controlled mechanical treatment in a standardised laboratory beater. Beating was conducted using a basalt lava Voith overhead beater. 800g o.d. pulp samples in water were beaten at 4% consistency. The response to beating was evaluated over time by collecting aliquots of 1200 ml pulp samples at regular intervals. These pulp samples were used to prepare handsheets.

2.4.5.3 Freeness of pulp

Freeness of the beaten pulp samples was determined according to TAPPI Standard Test Method number T227 os-58 [112], using a Schopper-Riegler freeness tester. Wetness of pulp was recorded as ^{0}SR.

2.5 EVALUATION OF PAPER PROPERTIES

2.5.1 Handsheet formation

Handsheets were prepared from the 1200 ml pulp samples collected for each interval of beating. Pulp suspensions were prepared at a consistency of approximately 0.2%. Handsheets were then formed with the aid of rectangular sheet former according to TAPPI Standard Method T205 os-71 [112]. Ten handsheets were formed for each aliquot of pulp collected at one and five minute intervals of beating. The respective wet handsheets were then dried on a photographic plate dryer between two sheets of blotting paper.

2.5.2 Paper strength properties

All handsheets were conditioned for 48 hours at 65% relative humidity and 20^0C before being tested. For the evaluation of various strength properties of the handsheets, appropriate samples were cut with the aid of a cutting die. For comparison, all strength properties were evaluated at a wetness of 38 ^{0}SR and handsheet evaluation was according to TAPPI Standard Test Methods [112].

2.5.2.1 Tensile strength

Tests for tensile strength of cut paper samples were determined according to TAPPI Standard Test Method number T404 om-87 [112].

2.5.2.2 Tear strength

Tests for tear strength of cut paper samples were determined according to TAPPI Standard Test Method number T403 om-91[112].

2.5.2.3 Burst strength

The burst strength of the cut paper samples was determined according to TAPPI Standard Test Method number T414 om-88 [112].

2.5.2.4 Handsheet brightness

The brightness of the cut paper samples was determined according to TAPPI Standard Test Method number T452 os-77 with a reflectance photometer (Zeiss Elrepho 65843, Germany). The instrument was calibrated with standard magnesium oxide (MgO) according to TAPPI Standard Test Method number T1207 os-72 [112] and measurements were taken at a directional reflectance of 457nm.

2.5.2.5 Scanning Electron Microscopy (SEM)

A microscopic study on the wood chips using a Leo® 143VP Scanning Electron Microscope was carried out to observe the effect of the various chip treatments. A microtome was used to prepare a special wood chip samples of eight millimetre thickness. These prepared wood chips were then placed inside a closed wire matchbox size chip basket to prevent loosing the chips during extraction and inoculation (see Figure 5). It was observed that the wood chips were not totally colonised by the fungi when placed in the match size chip basket, instead the fungi colonised the entire basket. The prepared wood chips were tied with a wire for identification purposes and tied on the basket walls to keep track on the special wood chips. The prepared eight millimetre thick wood chips were viewed and photographed cross-sectionally (see Figures 22-25). The same wood chips underwent the four experimental treatments i.e. non-extraction; one hour PHWE; non-extraction and FCCI; and one hour PHWE and FCCI.

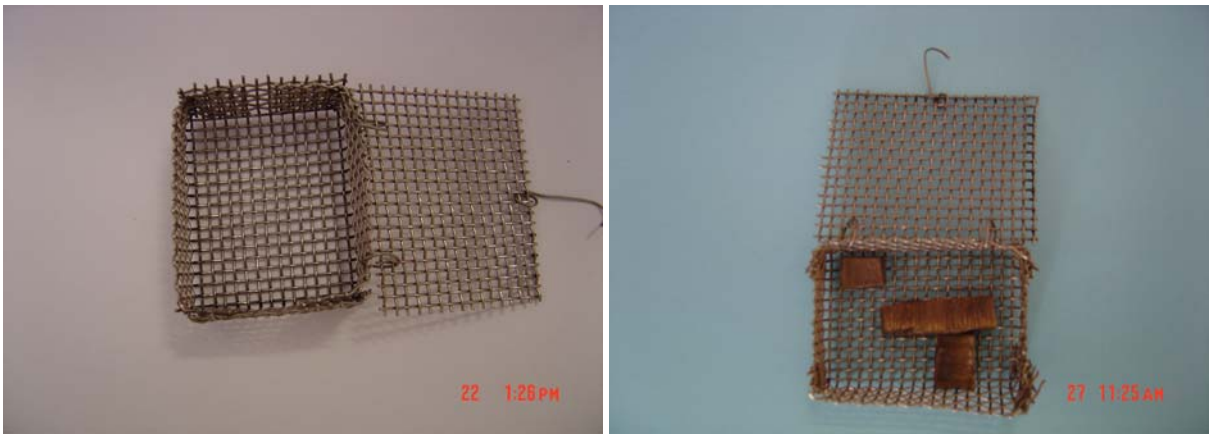

Figure 5: Match box size wood chip basket that was used to carry wood chips during the various treatments.

2.6 STATISTICAL ANALYSIS OF DATA

Analysis of variance (ANOVA) of data from all the experimental treatments and the differences within the experimental treatments was conducted using *Statistica* (v7, 1984–2006). F-value (Fischer distribution) with p-value as well as a non-parametric test called the Mann-Whitney test was tested at the 95% confidence limit. An advanced statistical procedure called Bootstrap was used to verify some results. Bootstrap method is a very computationally intensive data-based simulation method for assigning measures of accuracy to the statistical estimates. All the handsheet strength values were extrapolated at a freeness of 38 ^{0}SR and statistically analysed.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 SCREENED PULP YIELD

The screened yield results of all the experimental treatments were grouped together and statistically analysed amongst each other. The statistical analysis was conducted for seven sequences:

1. Unextracted and PHWe *Eucalyptus grandis* wood chips.
2. Unextracted FCCi and PHWe FCCi *Eucalyptus grandis* wood chips**.**
3. PHWe FCCi *Eucalyptus grandis* wood chips cooked at different charges.
4. PHWe FCCi *Eucalyptus grandis* wood chips cooked on different durations.
5. PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times.
6. Uninoculated and unextracted FCCi *Eucalyptus grandis* wood chips incubated at different times.
7. PHWE and PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times.

The results of the statistical analysis have been summarized in Table 11 and 19. Rejects, shives, RAA, brightness and strength properties were also statistically analysed in the same way as screened yield. The weighted means of sequences **1- 7** of the screened pulp yield (expressed in percentages) and the statistical analysis of all the treatments are shown in Figures 6 and 7-13 respectively. Sequence **1** (Figure 7) showed no significant difference in the mean average yields (p=0.89). This is however a positive result for the industry, because removal of extractives prior to pulping would minimise the usage of pulping chemicals.

Sequences **2** and **4-7** (Figures 8,10,11,12 and 13) showed lower percentage yields attributed to removal of extractives, some possible loss of lignin, and the fungal degradation of polysaccharide components. Messner et al [100] also reported pulp yield losses for fungal pretreated wood chips.

Only sequence **3** (Figure 9) indicated an improved pulp yield which however was not significant. Although statistically there was not significant increase in pulp yield, the results however indicated that this treatment improved the pulp yield which in turn would be a substantial benefit to the pulping industry. This higher yield could most certainly be attributed to the wood chip structure being more accessible to the pulping liquor, as illustrated in Figure 97. Bajpai et al [66] reported an increased pulp yield for fungal treated Kenaf.

Table 11: Summary of statistical analysis of all the experimental pulping treatments.

Sequences	Description	Yield (%)		Rejects (%)	Shives (%)	RAA (g/l)	Kappa number	% ISO Brightness
		Standard analysis	Bootstrap method					
1	Unextracted and PHWe	No significant difference p = 0.83	No significant difference p = 0.883	Significant difference p = 0.05	Significant difference p = 0.05	significant difference p = 0.05	Significant difference p = 0.05	Significant difference p = 0.02
2	Unextracted FCCi and PHWe FCCi	No significant difference p = 0.28	No significant difference p = 0.225	Significant difference p = 0.05	Significant difference p = 0.05	No significant difference p = 0.38	Significant difference p = 0.05	Significant difference p = 0.0311
3	PHWe FCCi *Eucalyptus grandis* wood chips cooked at different charges	No significant difference p = 0.66	No significant difference p = 0.673	Significant difference p = 0.05	Significant difference p = 0.05	No significant difference p = 0.51	Significant difference p = 0.05	Significant difference p = 0.0004
4	PHWe FCCi *Eucalyptus grandis* wood chips cooked on different durations	No significant difference p = 0.38	No significant difference p = 0.377	Significant difference p = 0.05	No significant difference p = 0.08	significant difference p = 0.05	Significant difference p = 0.05	Significant difference p = 0.0076
5	PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times	No significant difference p = 0.28	No significant difference p = 0.253	Significant difference p = 0.05	No significant difference p = 0.51	No significant difference p = 0.83	No significant difference p = 0.13	Significant difference p = 0.00059
6	Uninoculated and unextracted FCCi	No significant difference p = 0.51	No significant difference p = 0.438	Significant difference p = 0.05	No significant difference p = 0.38	Significant difference p = 0.05	No significant difference p = 0.08	No significant difference p = 1.0
7	PHWE and PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times	No significant difference p = 0.08	No significant difference p = 0.062	Significant difference p = 0.05	No significant difference p =0.13	Significant difference p = 0.05	No significant difference p = 0.66	Significant difference p = 0.02

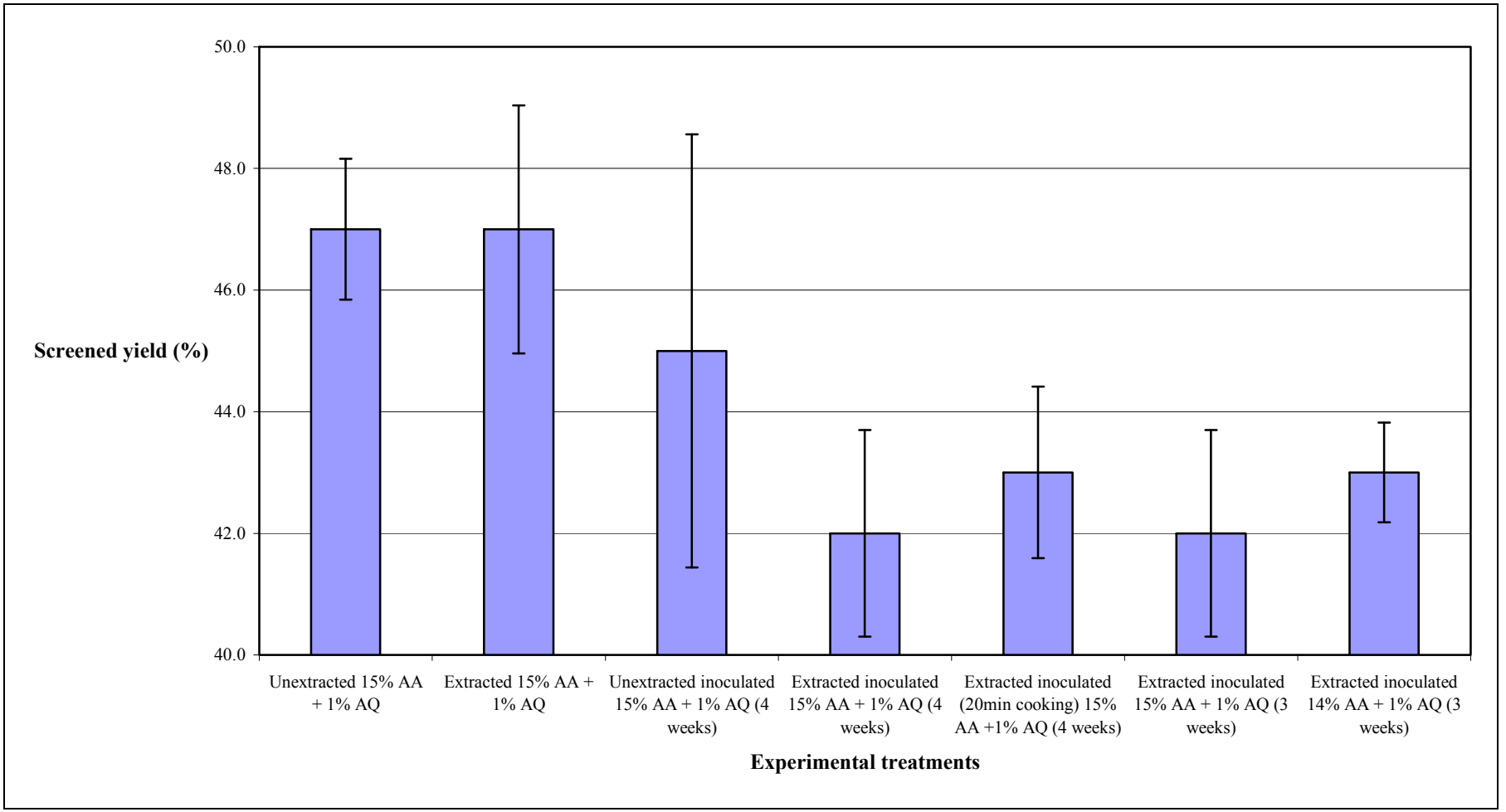

Figure 6: Screened pulp yield (%) of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

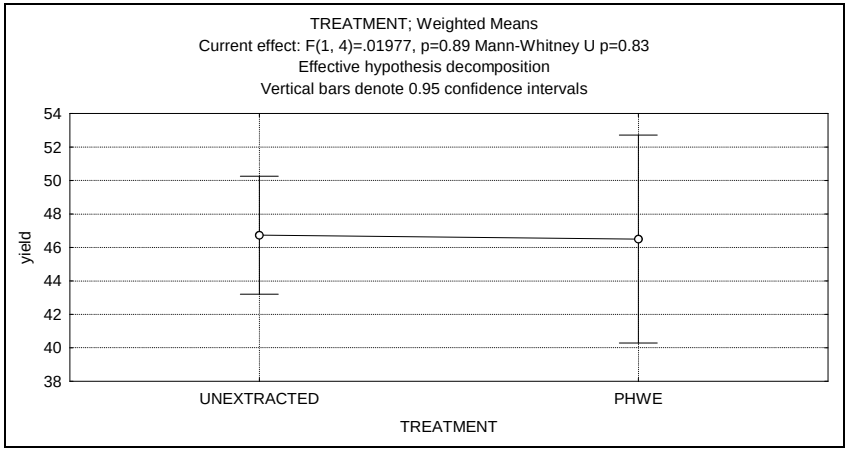

Figure 7: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

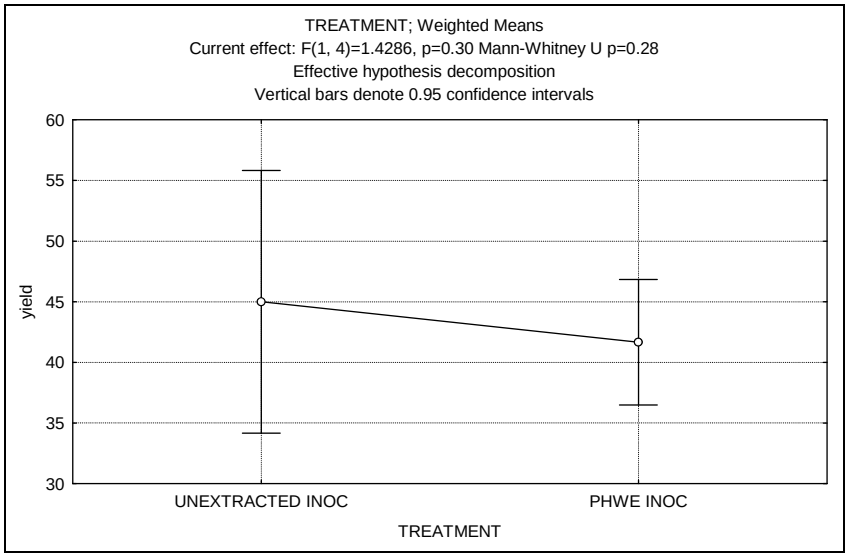

Figure 8: Representation of weighted means of unextracted FCCi and PHWe FCCi *Eucalyptus grandis* wood chips.

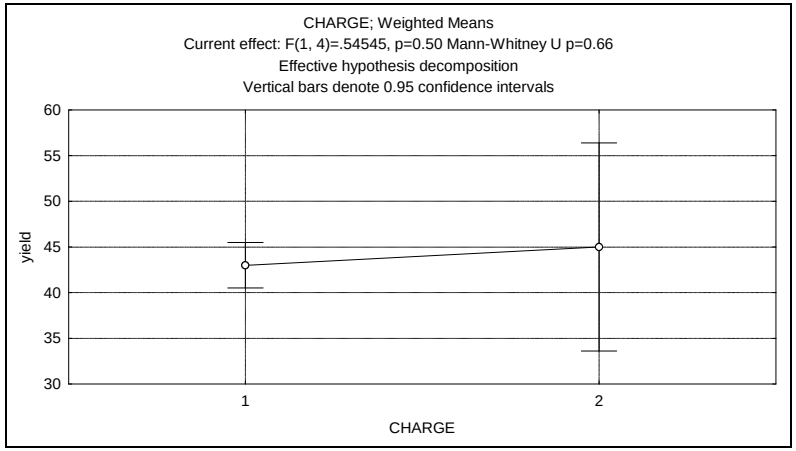

Figure 9: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (1 = 14% AA, 2 =15% AA).

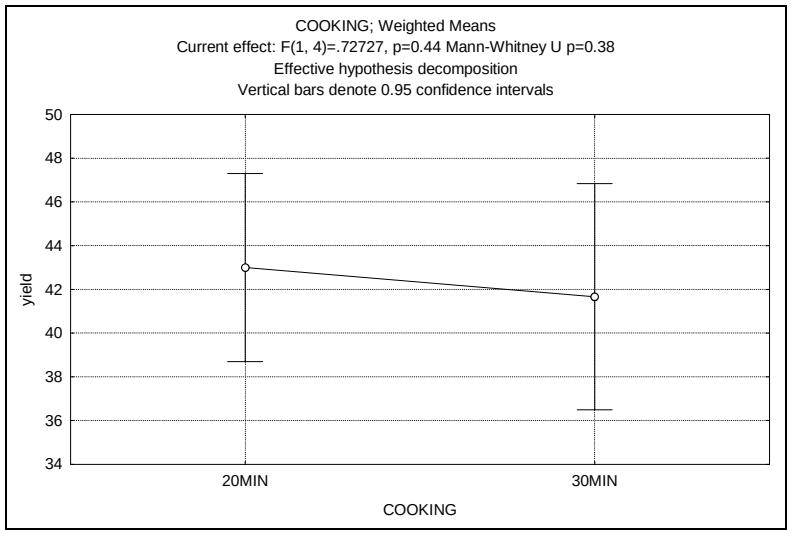

Figure 10: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

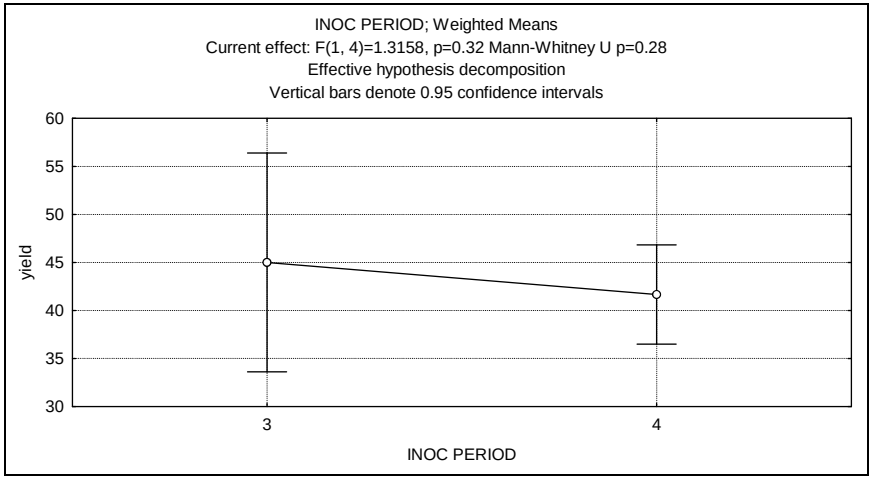

Figure 11: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (3 and 4 = weeks of incubation period).

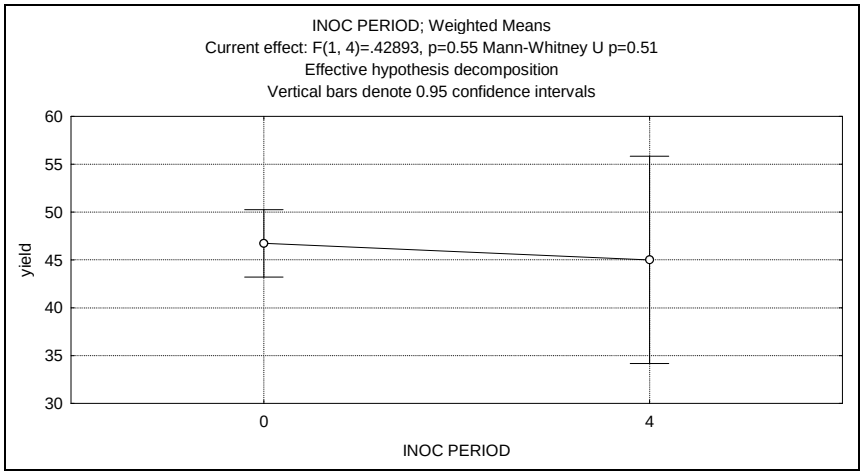

Figure 12: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0 = uninoculated; 4 = weeks of incubation period).

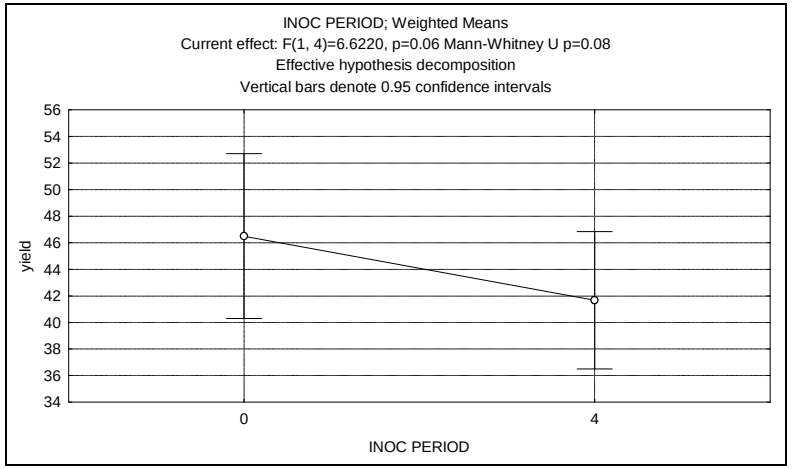

Figure 13: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

Due to the insignificant differences in the screened yields, further statistical analysis was conducted by an advanced testing procedure called Bootstrap method. Bootstrapping is a very computationally intensive data-based simulation method for assigning measures of accuracy to the statistical estimates. Also with the Bootstrap simulation no significant differences were recorded.

As the Bootstrap method did not improve the statistical significance of the results, it was decided to perform a sample size calculation (sample t-Test). It was found that a standardized effect (Es) of 0.3 would need approximately 230 pulping experiments to be conducted to obtain significant differences, as shown in Figure 21.

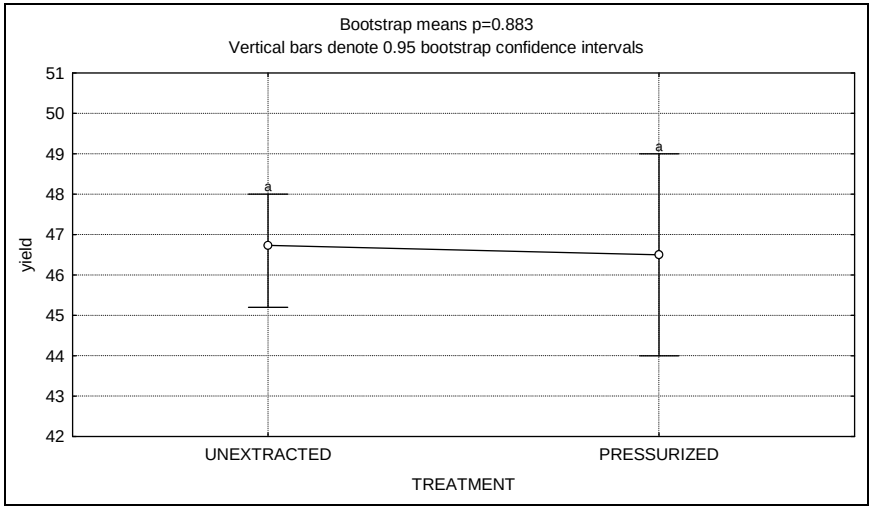

Figure 14: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

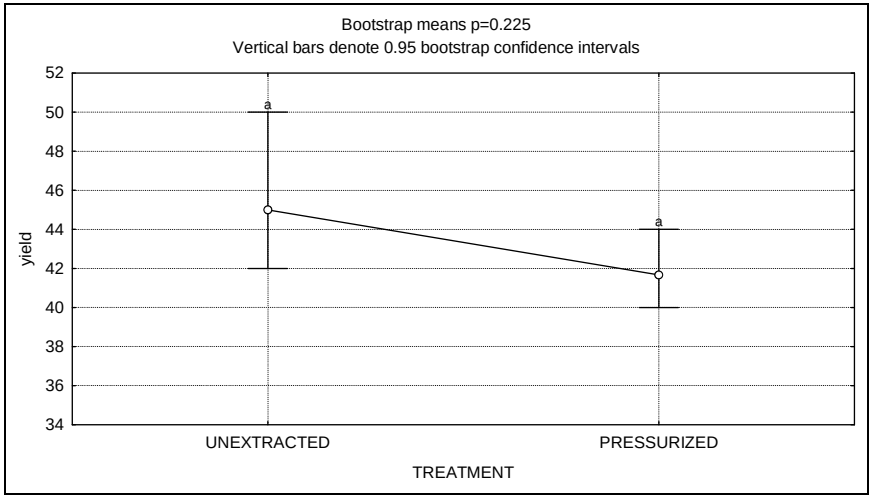

Figure 15: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

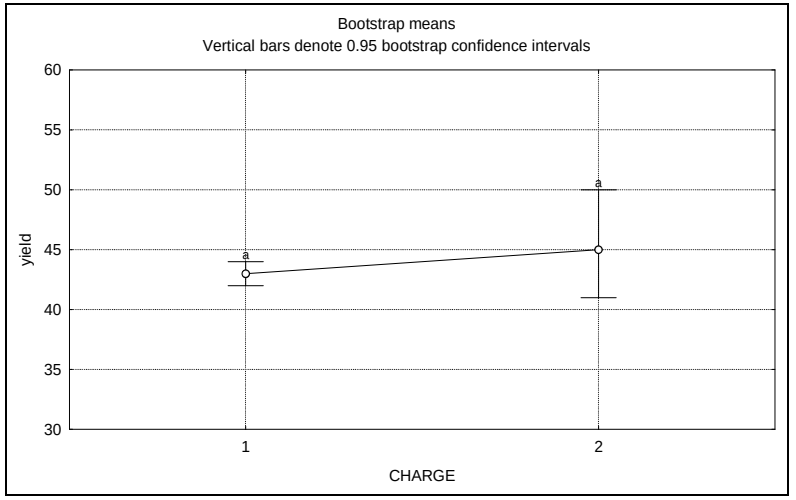

Figure 16: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (1 = 14% AA; 2 =15% AA).

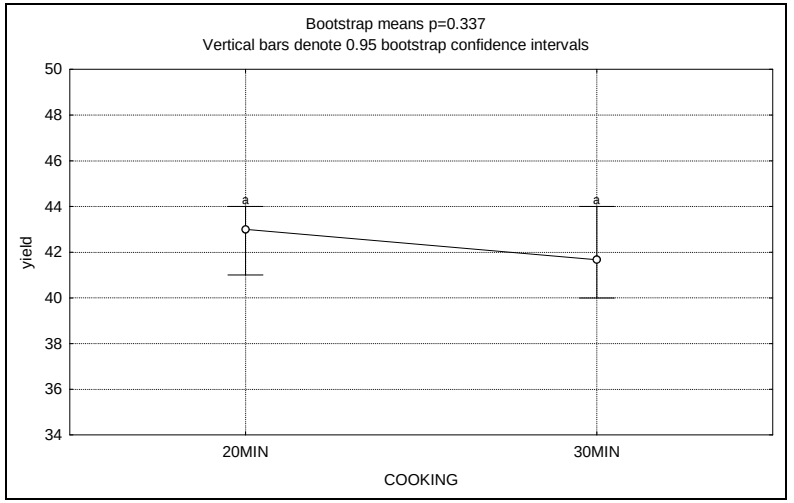

Figure 17: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

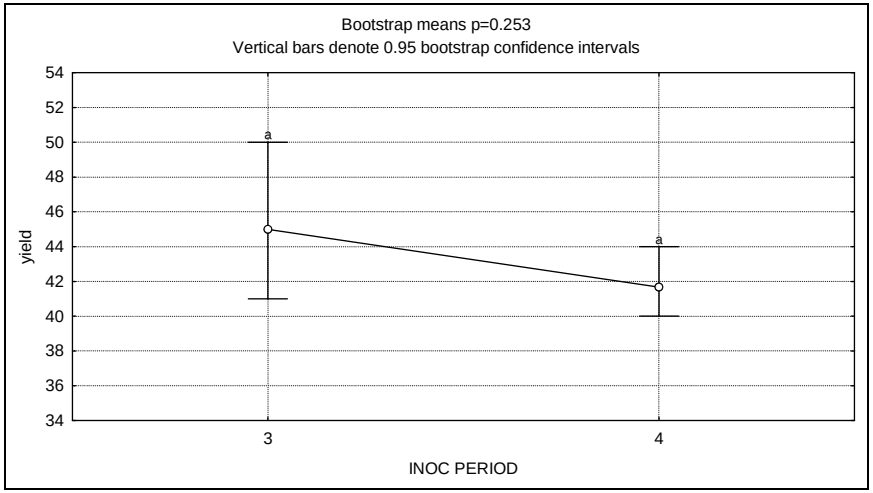

Figure 18: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (3 and 4 = weeks of incubation period respectively).

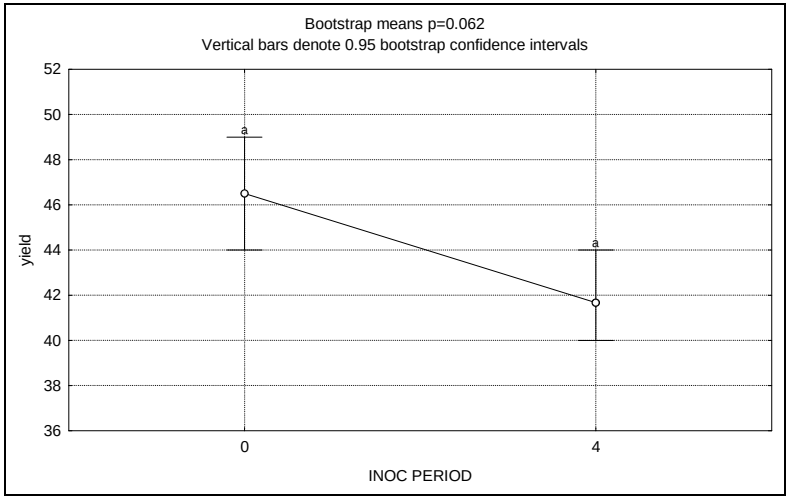

Figure 20: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

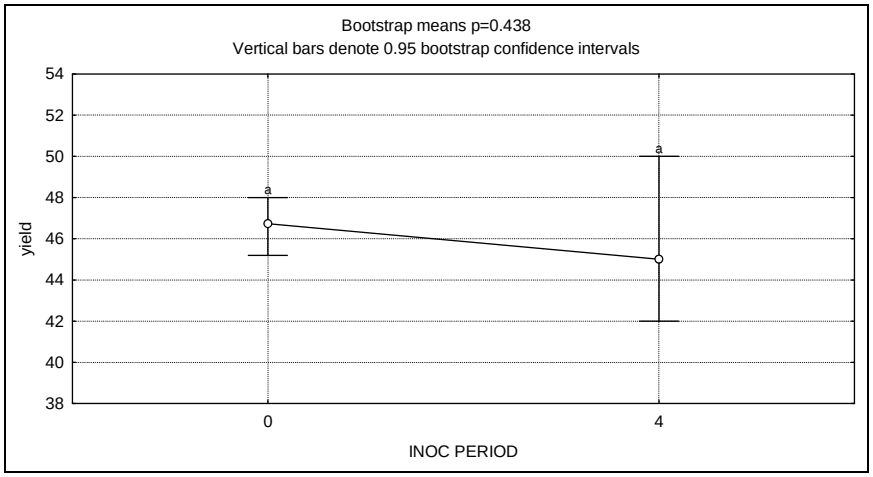

Figure 19: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0 = uninoculated; 4 = weeks of incubation period).

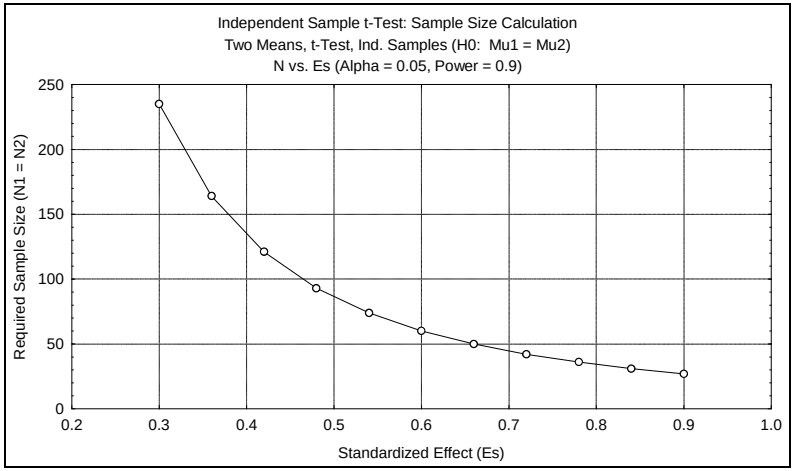

Figure 21: An independent sample t-Test (Sample Size Calculation) conducted after the bootstrap method.

3.2 PULP REJECTS

The results of the rejects (expressed in percentages) and the statistical analysis of all the treatments are shown in Figures 22 and 23-29 respectively. All the weighted means of sequences **1-7** (see section **3.1**) showed significant difference ($p < 0.05$) see Figures 23-29. In Figure 26, the p-values are different but in the normal distribution curve as shown in Figure 30 the residual values are normally distributed, thus the Mann-Whitney p-value can be accepted as significant. The use of PHWE and also FCCI prior to pulping improved the penetration of cooking chemicals into the wood chips thereby reducing screened rejects. Unextracted wood chips were characterised by intact cell walls as shown in Figure 95.

PHWE and fungal pretreatment were responsible for improved cooking liquor penetration and extractive removal. The fluffy appearance of the cross-sectional area and cell wall separation and rupture as shown in Figures 96 and 97 clearly demonstrate this.

Furthermore, percentage rejects were also lowered by FCCI, combination of FCCI and PHWE, combination of FCCI and PHWE at different cooking times, incubation time and charge. Unextracted FCCI, combination of PHWE and FCCI wood chips showed more collapsed cell wall structure as shown in Figures 97 and 98 indicating the break down of the complex lignin structure into monomers and oligomers.

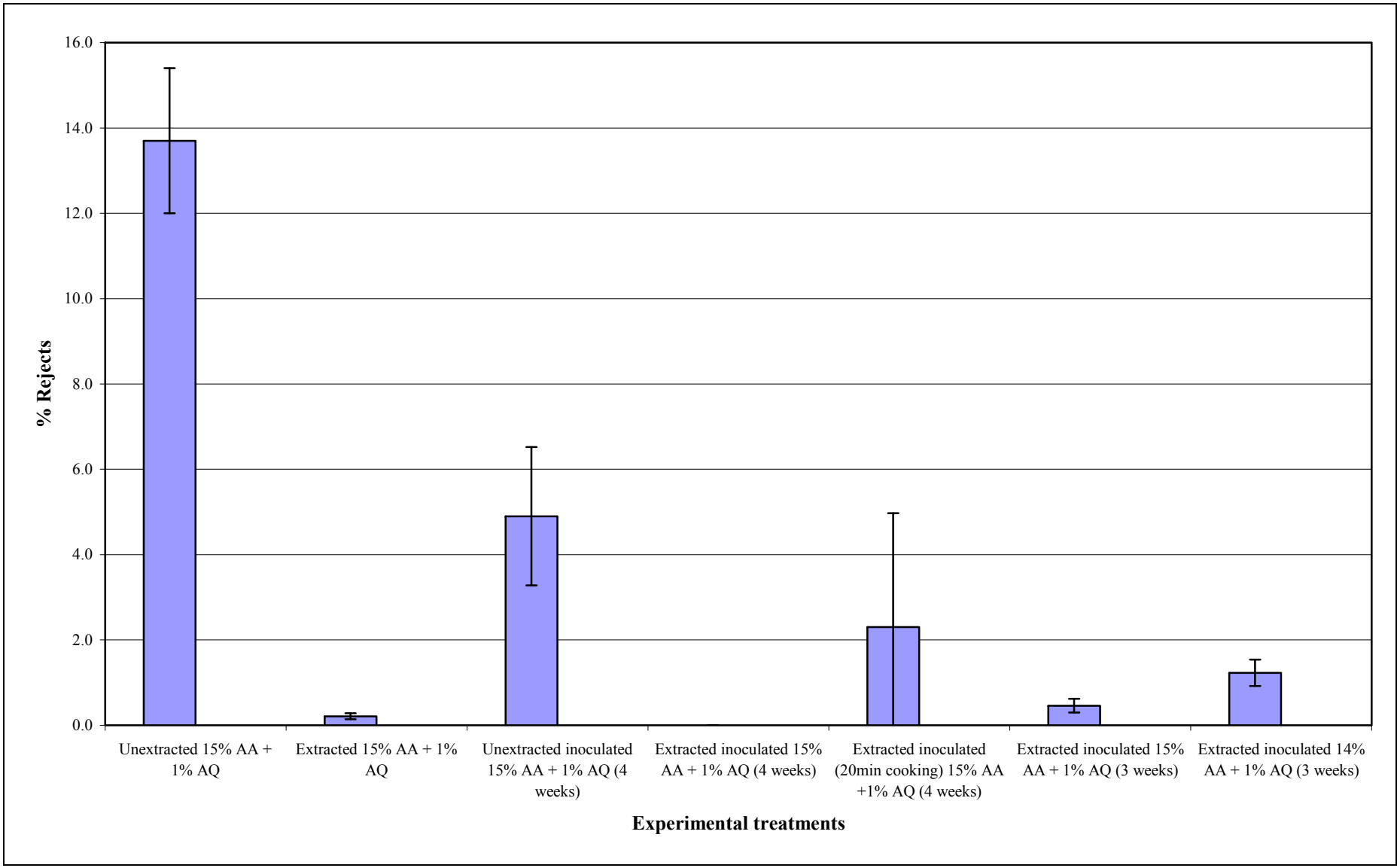

Figure 22: Rejects of Soda-AQ pulping of unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

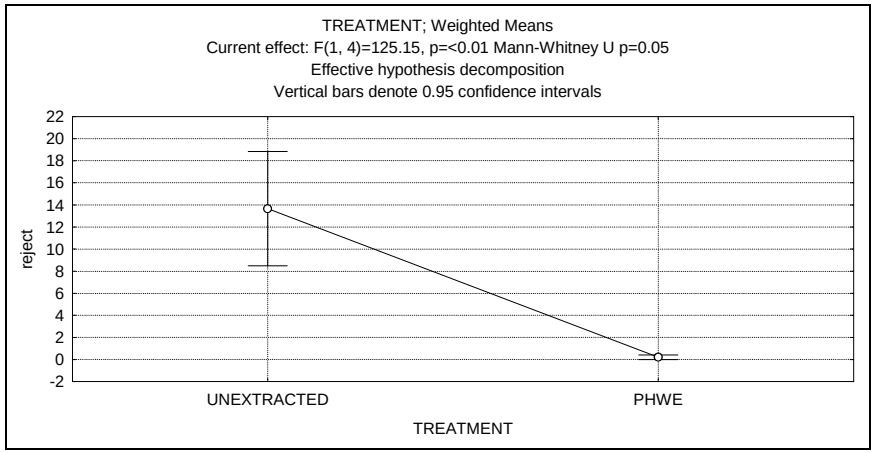

Figure 23: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

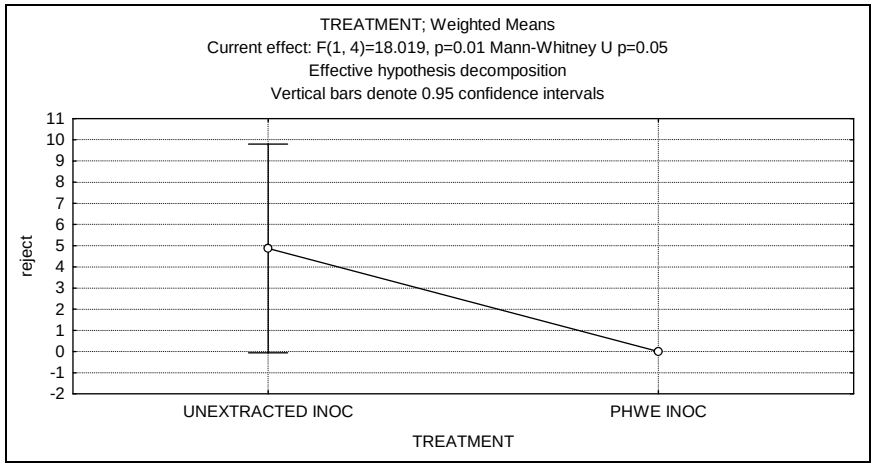

Figure 24: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

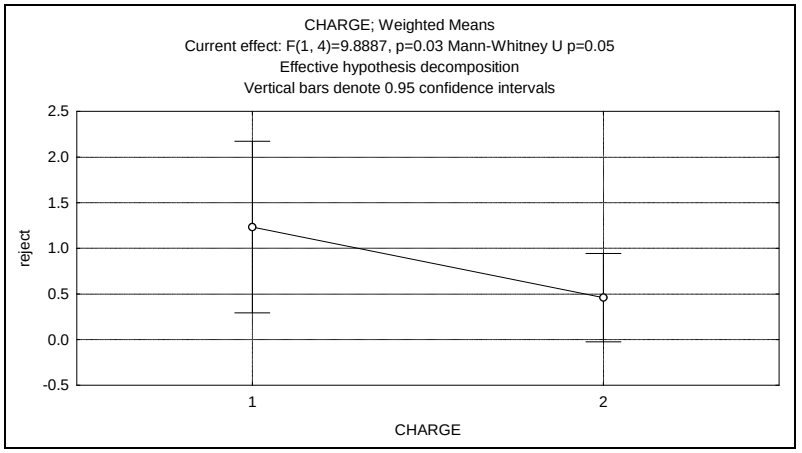

Figure 25: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood (1 = 14% AA; 2 = 15% AA).

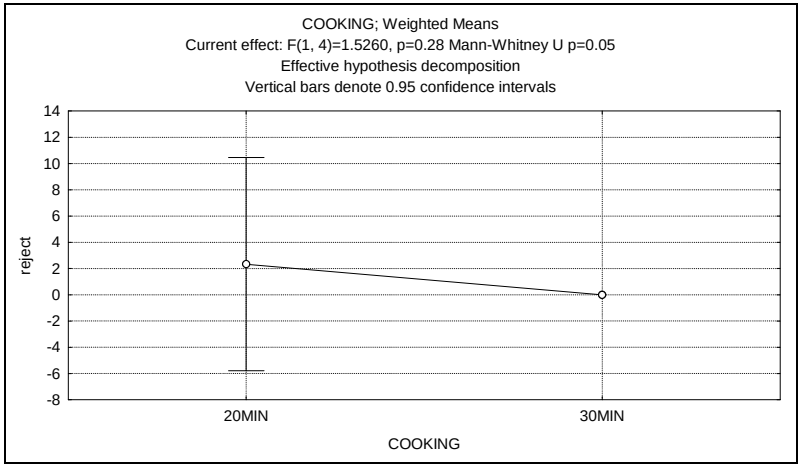

Figure 26: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood

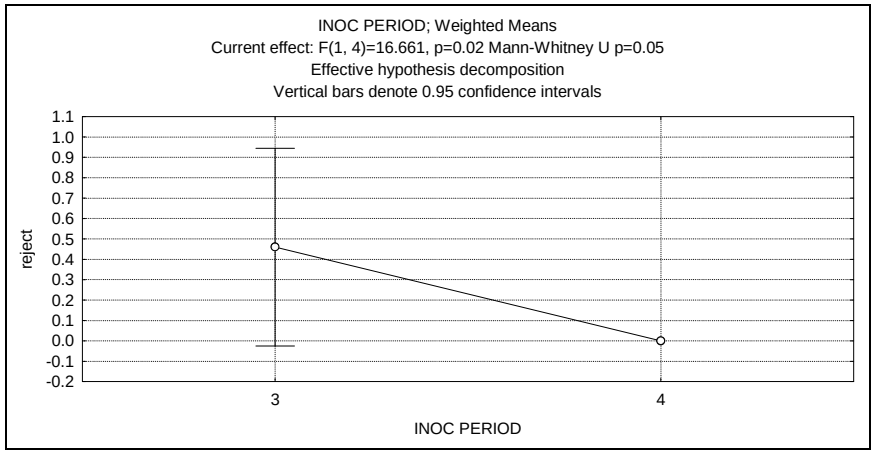

Figure 27: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips 3 and 4 = weeks of incubation period respectively).

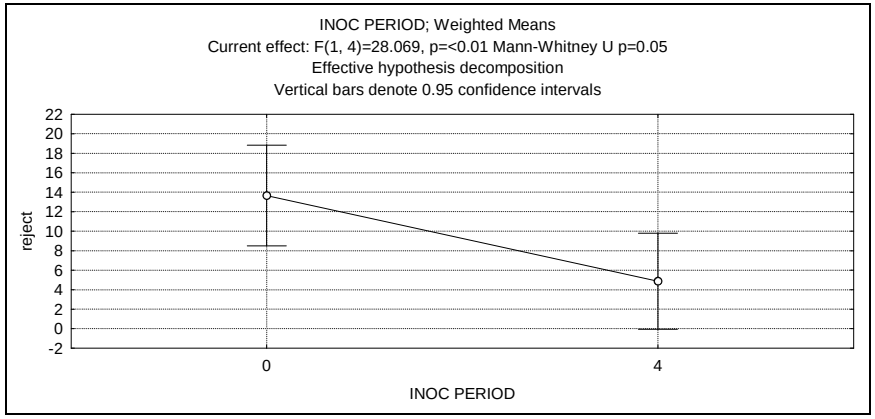

Figure 28: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

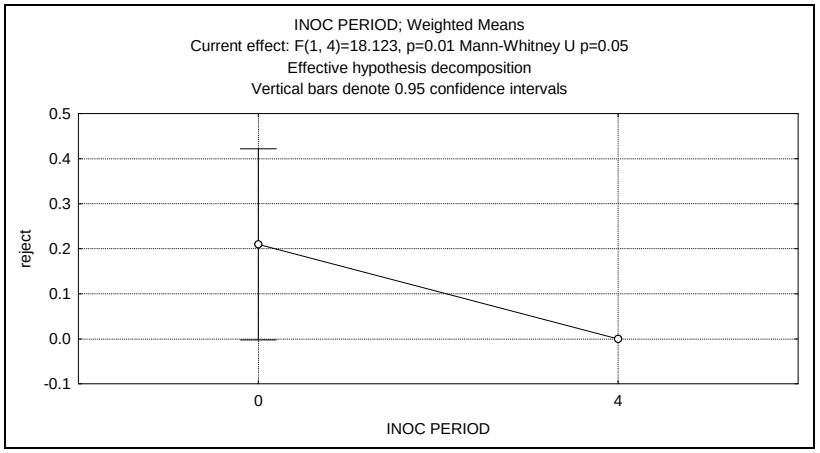

Figure 29: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

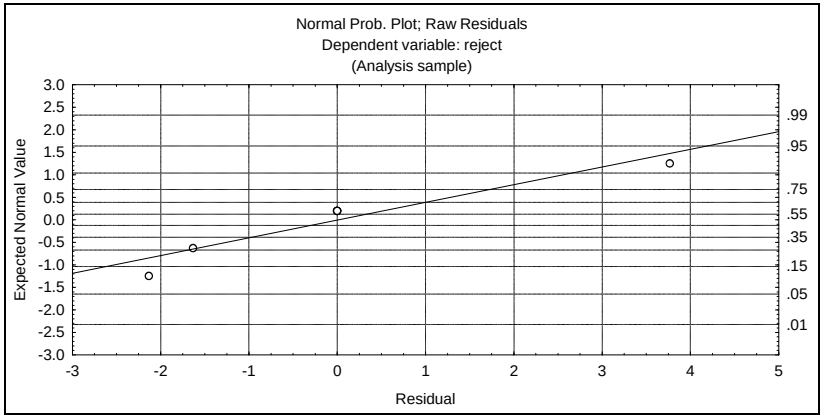

Figure 30: Normal probability plot of FCCI of PHWe *Eucalyptus grandis* wood chips.

3.3 SHIVE CONTENT

The results of the shives (expressed in percentages) and the statistical analysis of all the treatments are shown in Figures 31 and 32-38 respectively. The weighted means of sequences **1, 2** and **3** (see section **3.1**) showed significant difference ($p < 0.05$) as seen in Figures 32-34. Apart from the unextracted wood chips, the unextracted fungal co-culture inoculated wood chips showed higher shive content compared to the other treatments. These results suggest that the action of the fungal co-cultures was more on the outside of the chip than in the chip core and might have needed more incubation time to enhance delignification.

Due to the cell wall collapse in the PHWe wood chips as seen in Figure 96, more soluble extractives had been removed prior to pulping hence loosening and opening the wood structure thus resulting in a lower shive content. Thus the active alkali was able to penetrate deeper into the wood chip structure, hence reducing shives significantly. The weighted means of sequences **4, 5** and **7** (see section **3.1**) showed no significant difference ($p>0.05$) as seen in Figures 35, 36 and 38, even though there was a slight decrease in the shive content. On the other hand, the higher shive content might be attributed to the fact that the liquor penetration into the wood chips was insufficient. The weighted means of sequence **6** (see section **3.1**) showed an increase in the shive content but the increase did not appear to be any significant ($p>0.05$) as shown in Figure 37.

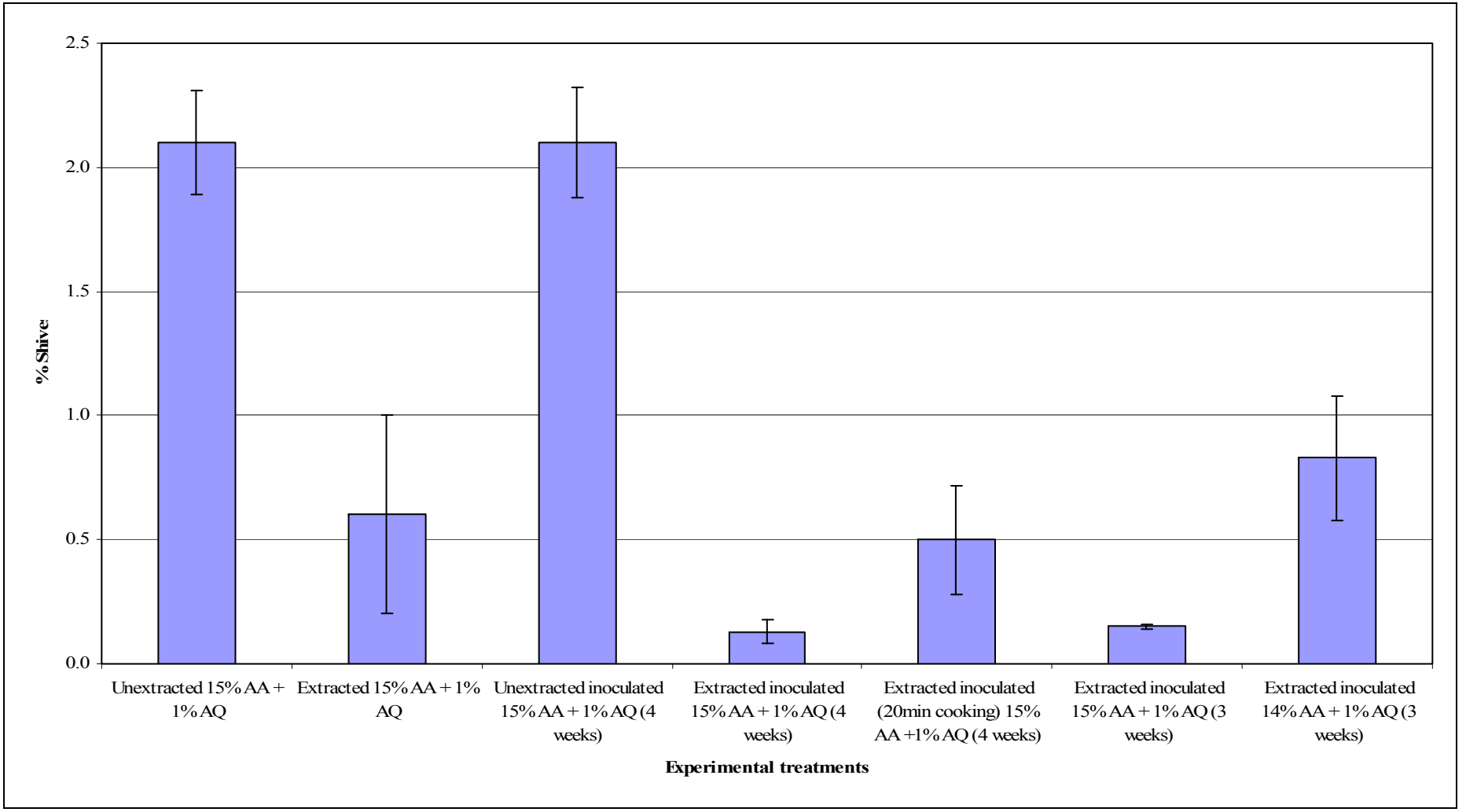

Figure 31: Shive contents of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

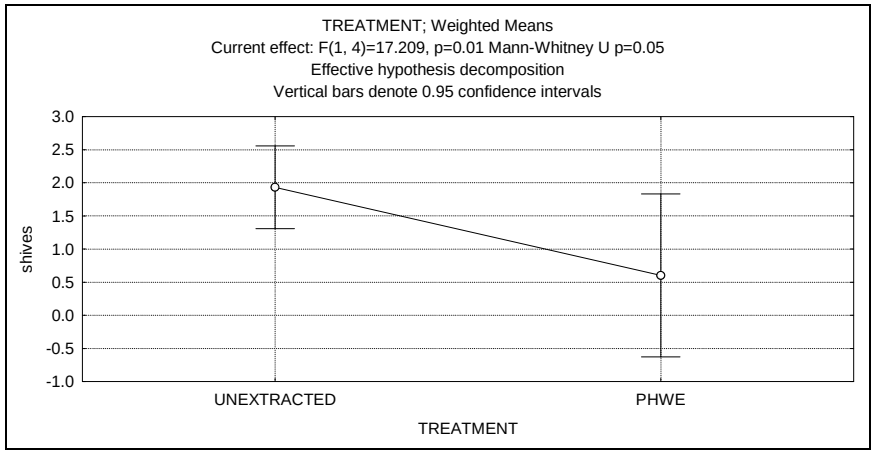

Figure 32: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

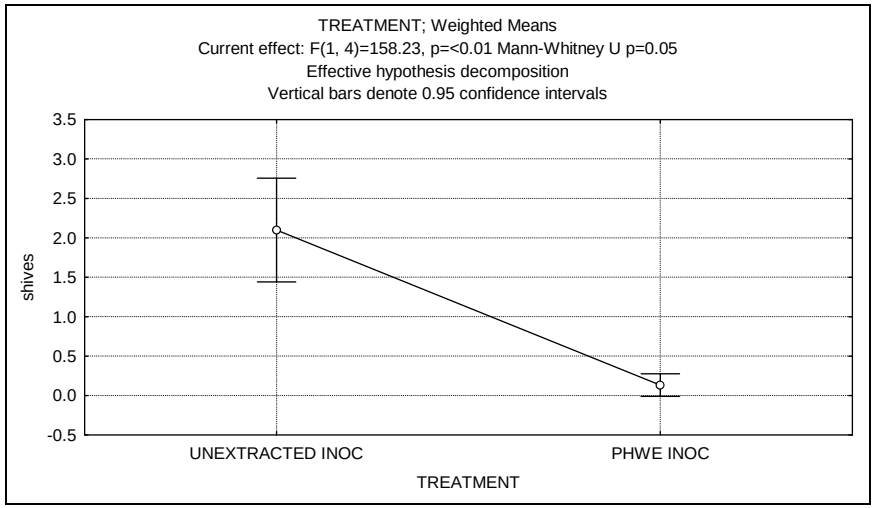

Figure 33: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

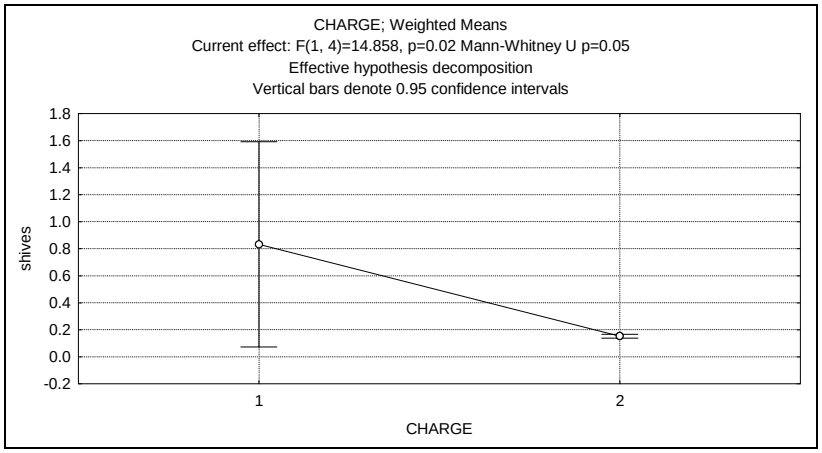

Figure 34: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood (1 = 14% AA; 2=15% AA).

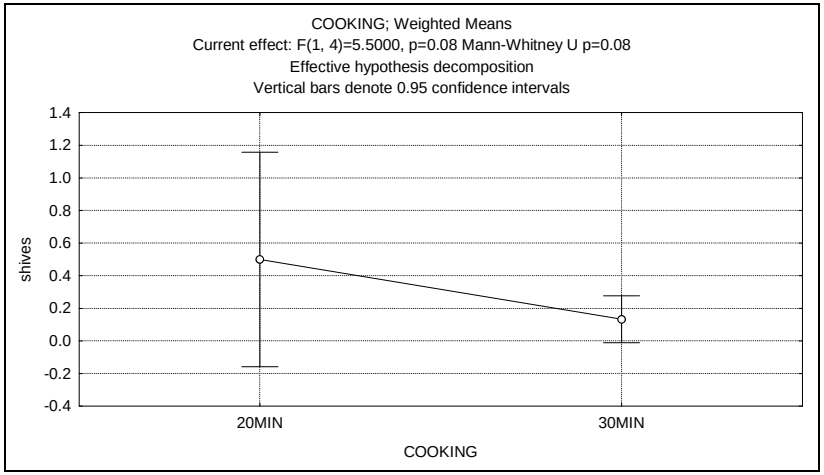

Figure 35: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

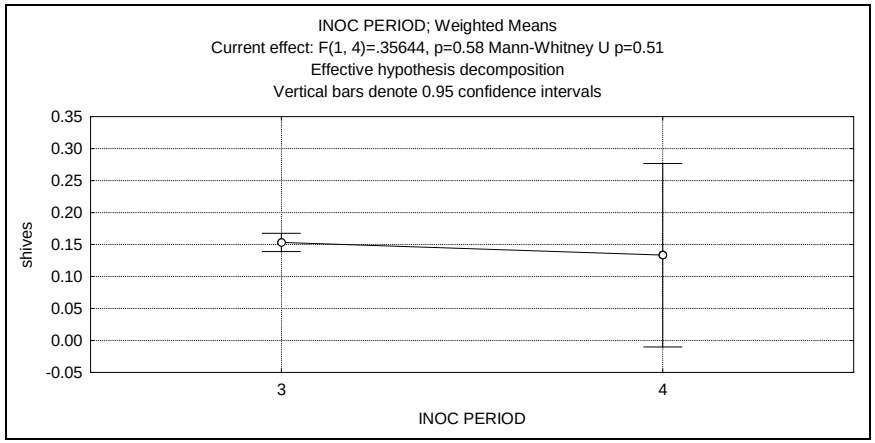

Figure 36: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (3 and 4 = weeks of incubation period).

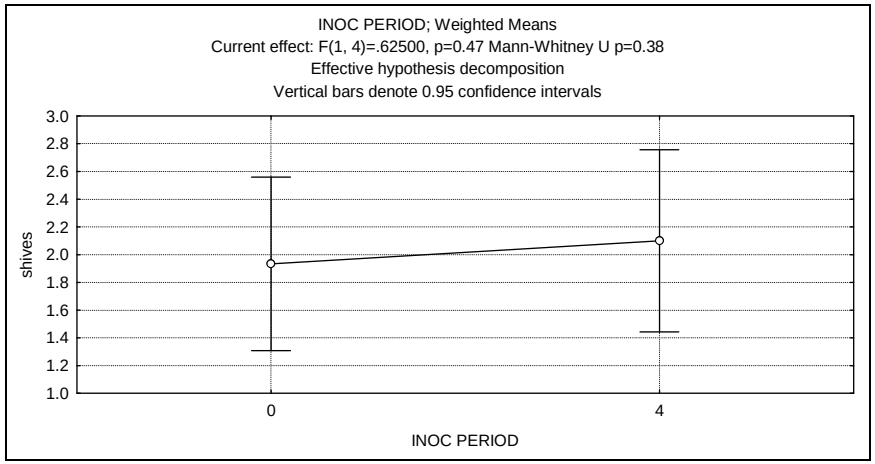

Figure 37: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

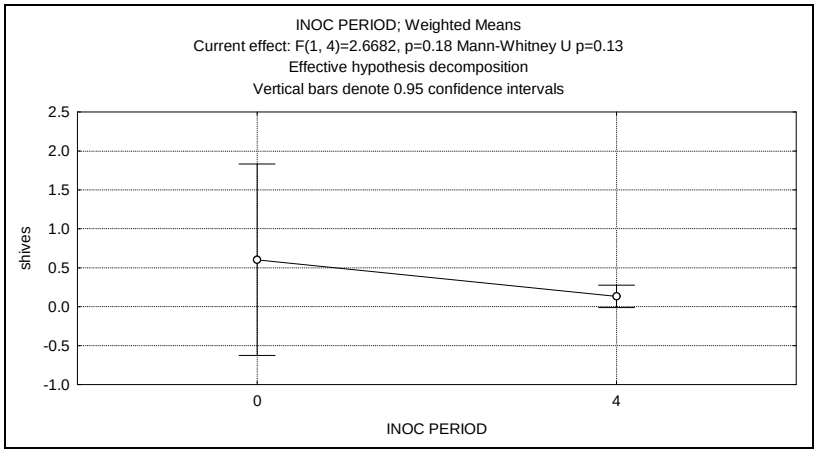

Figure 38: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

3.4 CHEMICAL CONSUMPTION

The results of the RAA (expressed in g/l) and the statistical analysis of all the treatments are shown in Figures 39 and 40-46 respectively. From Figure 39 it is evident that all the FCCi wood chips consumed less AA as compared to the unextracted and PHWe wood chips. The weighted means of sequences **4, 6** and **7** (see section **3.1**) showed significant difference (p = or < 0.05) as shown in Figures 43, 45 and 46. Bajpai et al [66] obtained similar results.

The weighted means of sequences **1, 2, 3** and **5** (see section **3.1**) showed no significant difference (p>0.05) as shown in Figures 40-42 and 44. In Figure 40, the p-values are different but in the normal distribution curve as shown in Figure 47 the residual values are normally distributed, thus the Mann-Whitney p-value can be accepted as significant. Reduced cooking time (20 minutes) of PHWe FCCi wood chip consumed more of active alkali as compared to other FCCi wood chips, but still less that unextracted and PHWe wood chips. By reducing the incubation time of the FCC pretreatment to three weeks for PHWe wood chips and cooked at 14% AA, RAA increased as compared to the cooking time of 20 minutes. This indicated that a reduction in cooking time on FCC pretreated wood chips could not save chemicals as compared to the full pulping cycle as shown in Figure 39. On the contrary, Bajpai et al [66] demonstrated that reduction in cooking time of wheat straw produced good results as shown in Table 4. This might be attributed to the pulping method used because wood chips need more chemicals or improved fungal strains to break down the lignin.

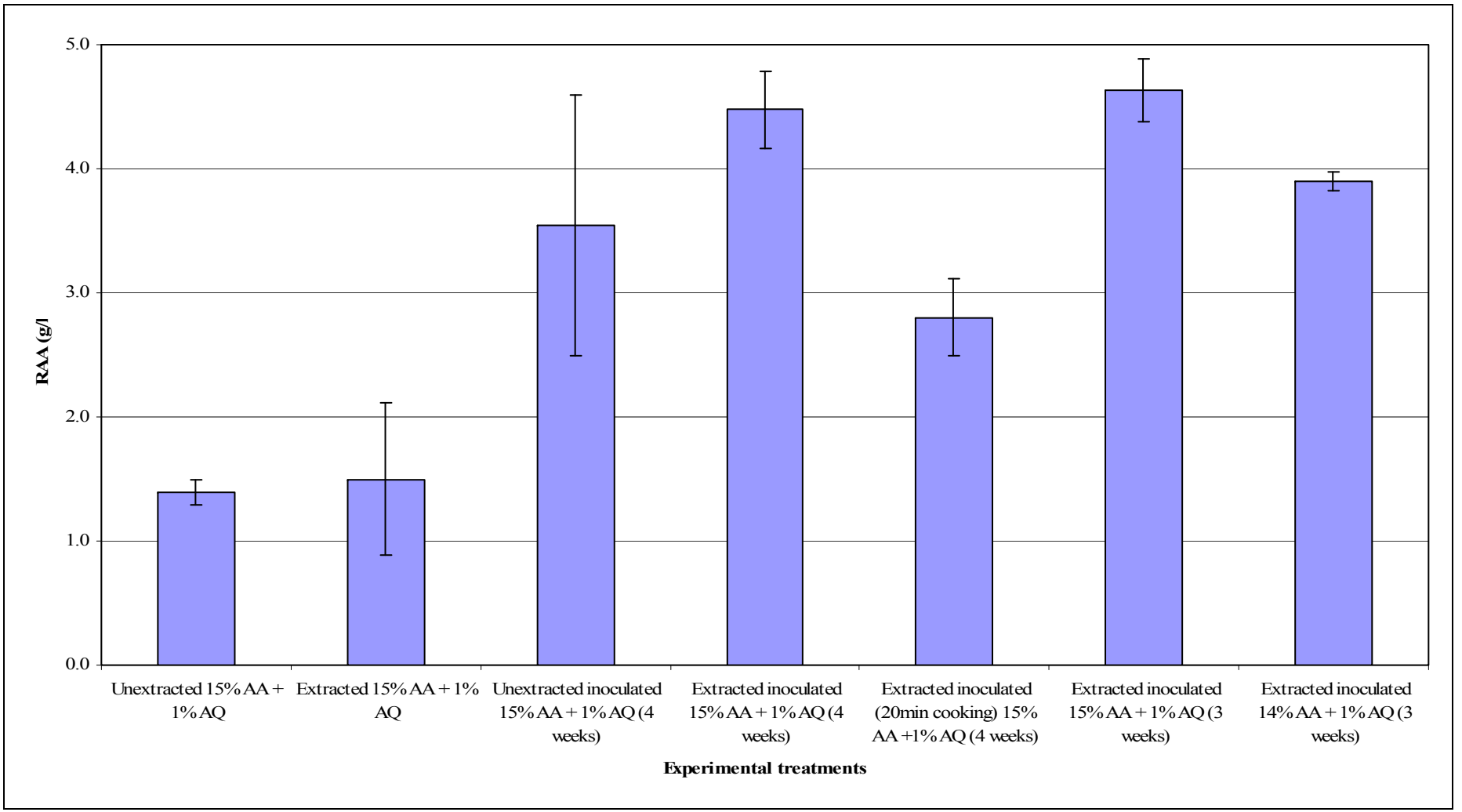

Figure 39: Residual Active Alkali (RAA) of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

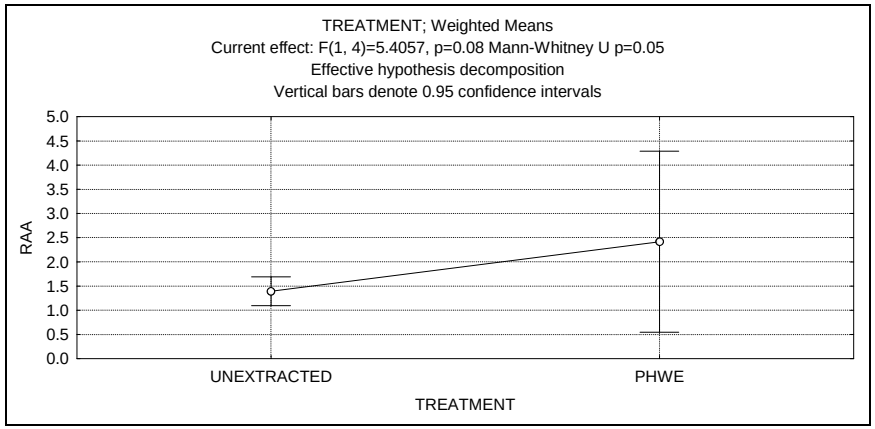

Figure 40: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

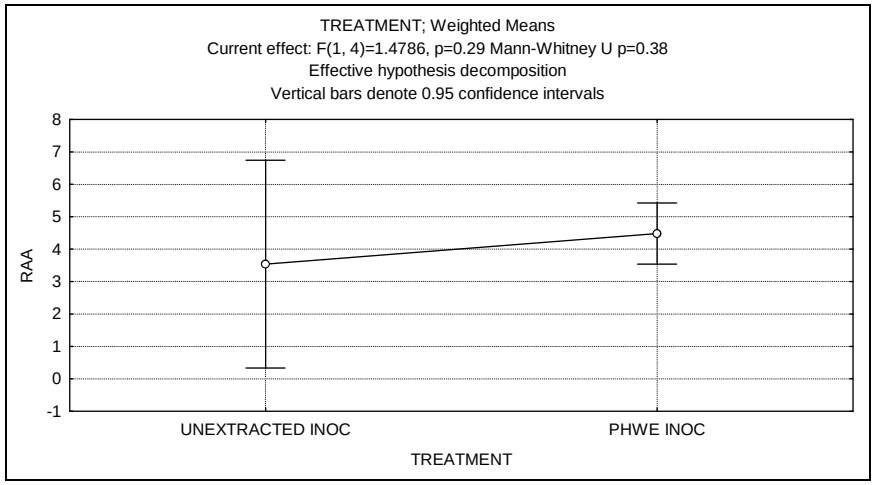

Figure 41: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

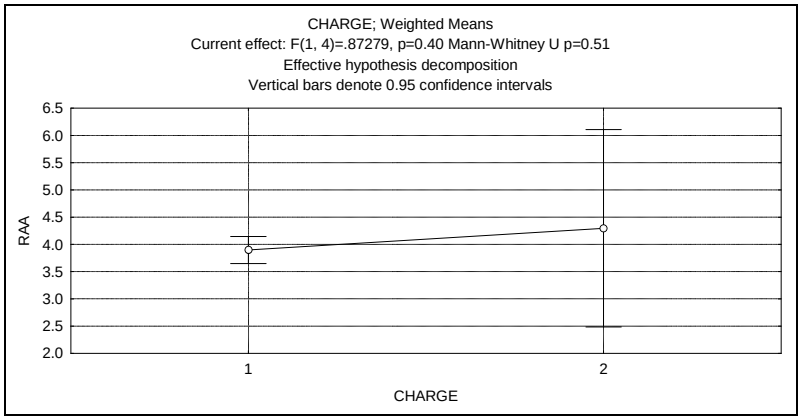

Figure 42: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood (1 = 14% AA; 2=15% AA).

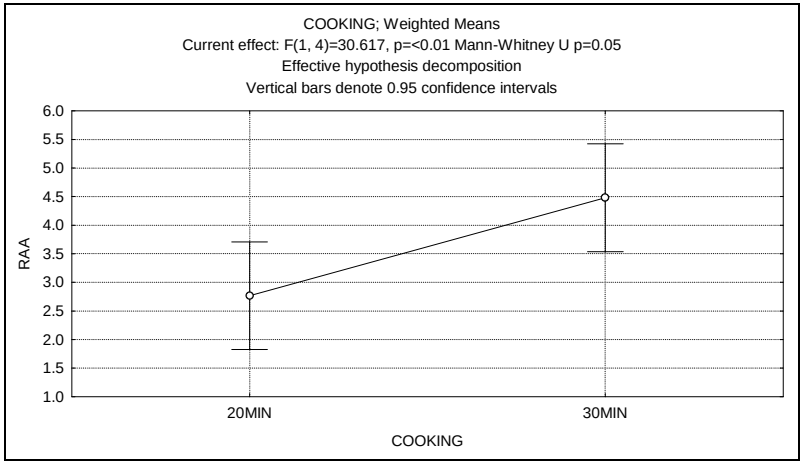

Figure 43: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

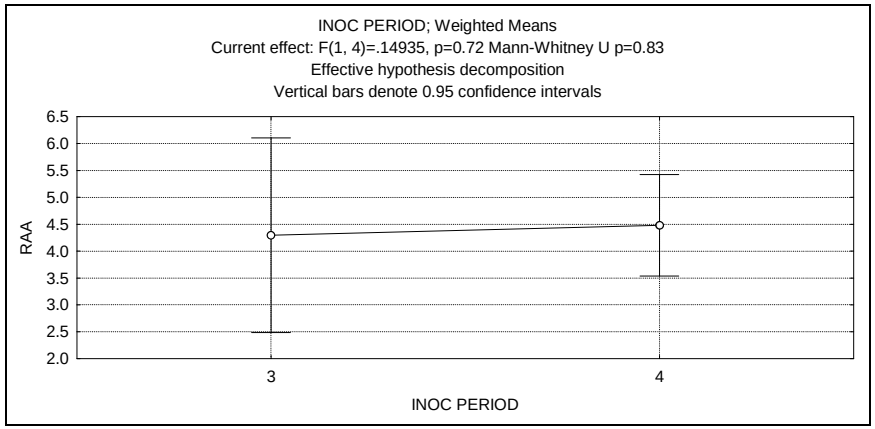

Figure 44: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips 3 and 4 = weeks of incubation period).

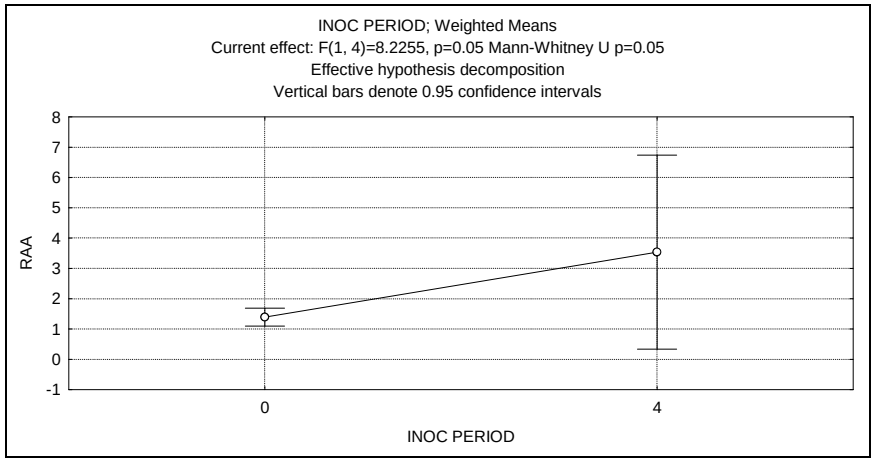

Figure 45: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips 0= uninoculated; 4 = weeks of incubation period).

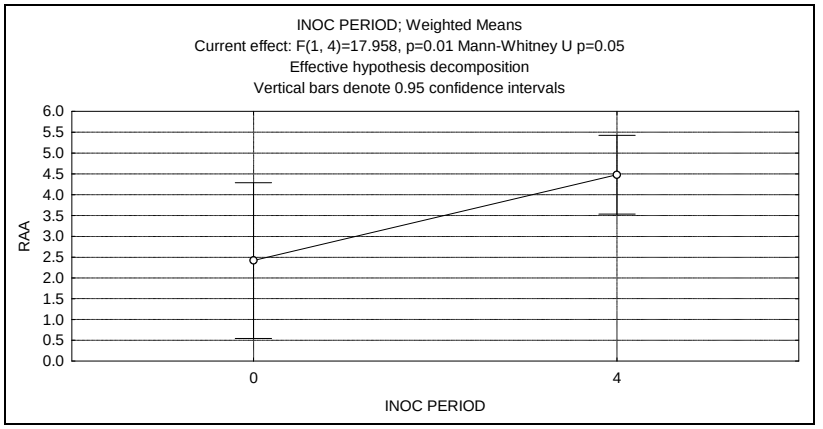

Figure 46: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips.

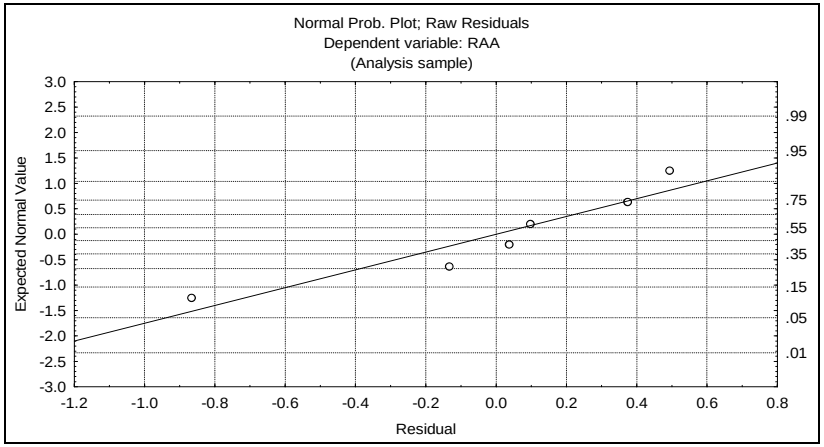

Figure 47: Normal probability plot of unextracted FCCI and PHWe FCCI *Eucalyptus* grandis wood chips.

3.5 KAPPA NUMBER

The results of the Kappa numbers and the statistical analysis of all the treatments are shown in Figures 48 and 49-55 respectively. The weighted means of sequences **1**, **2**, **3** and **4** (see section **3.1**) showed significant difference ($p = or < 0.05$) as seen in Figures 49-52. PHWE, FCCI period, alkali charge difference and cooking period showed to lower the Kappa number. PHWE removed the extractives; FCCI broke down the lignin structure making chemical degradation easier on the more open structure of the lignin. This phenomenon would also be of significant benefit for high brightness bleaching.

The unextracted and PHWe FCCi wood chips pulped at 14 and 15% AA respectively resulted in a highest Kappa number. All PHWe FCCi wood chips cooked at 15% AA gave lower Kappa numbers as seen in Figure 48. Unextracted FCCi and PHWe FCCi wood chips at 20 minutes cooking period produced a noticeable higher Kappa number than the PHWe and PHWe FCCi (incubated for three and four weeks cooked at 15% AA) wood chips as see in Figure 48. The weighted means of sequences **5-7** (see section **3.1**) showed no significant difference ($p = or > 0.05$) as shown in Figures 53-55 hence varying the incubation time from zero to four weeks and three to four weeks incubation proved to be statistically non significant. This could be attributed to the fact that lignin structure might was not thoroughly penetrated by the FCC to break down the wood chip structure so that the chemicals could gain excess into the wood chip structure.

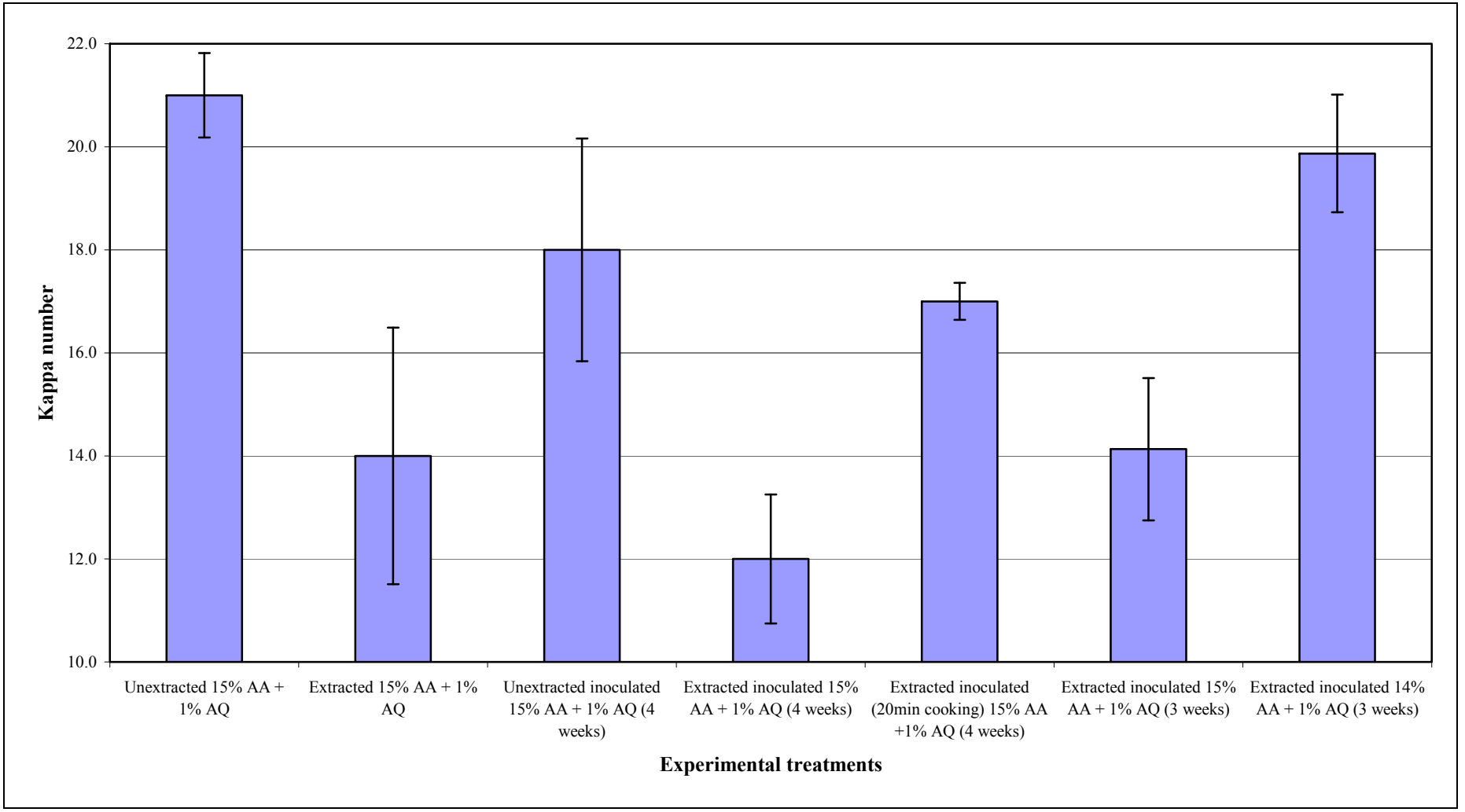

Figure 48: Kappa number of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

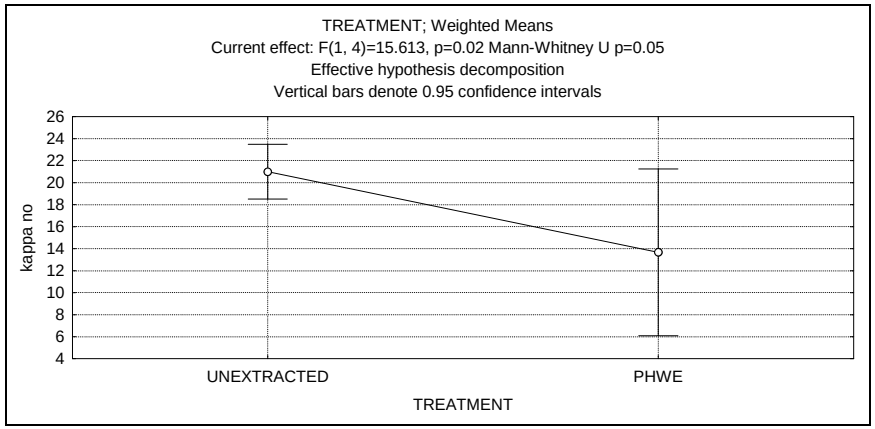

Figure 49: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

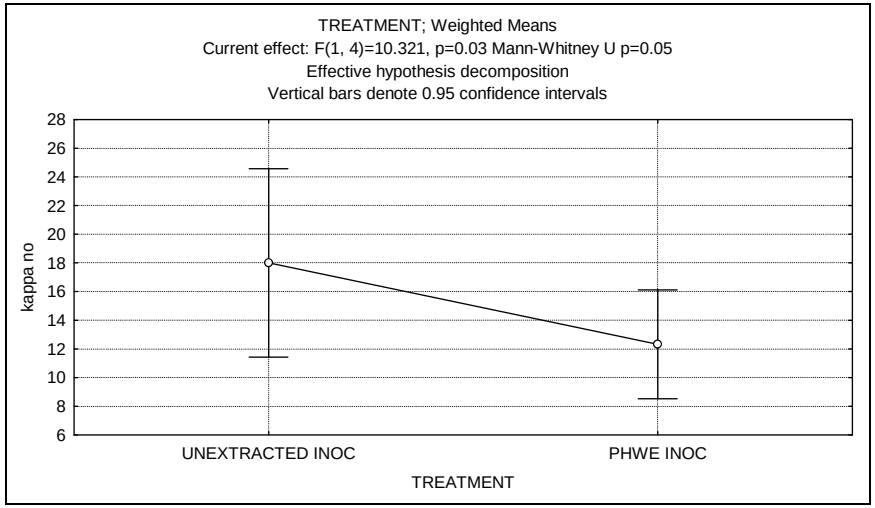

Figure 50: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

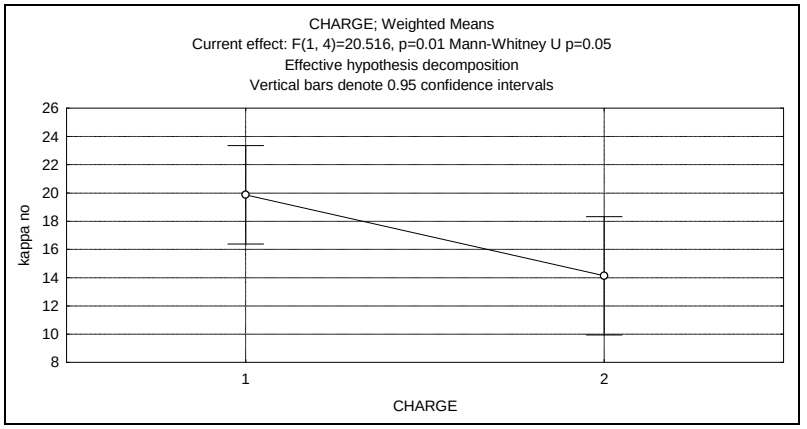

Figure 51: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood (1 = 14% AA; 2=15% AA).

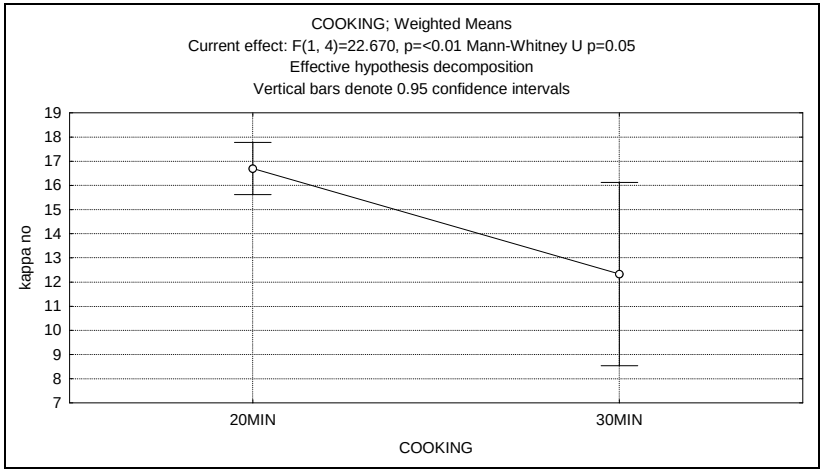

Figure 52: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

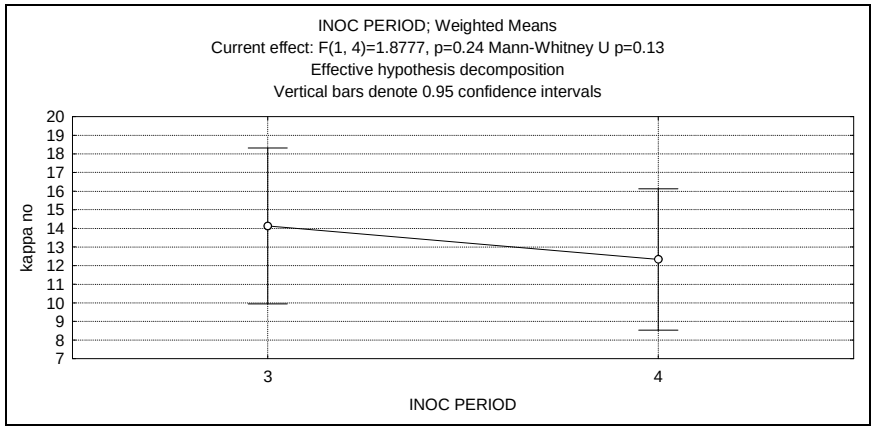

Figure 53: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (3 and 4 = weeks of incubation period).

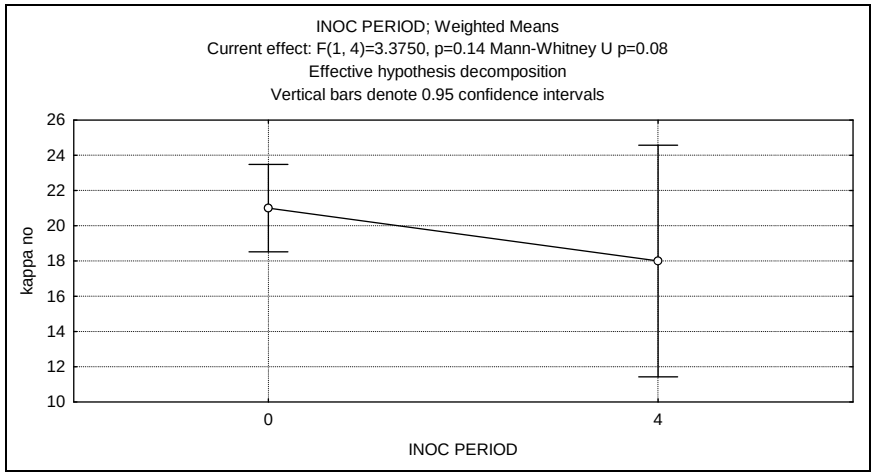

Figure 54: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of inoculation period).

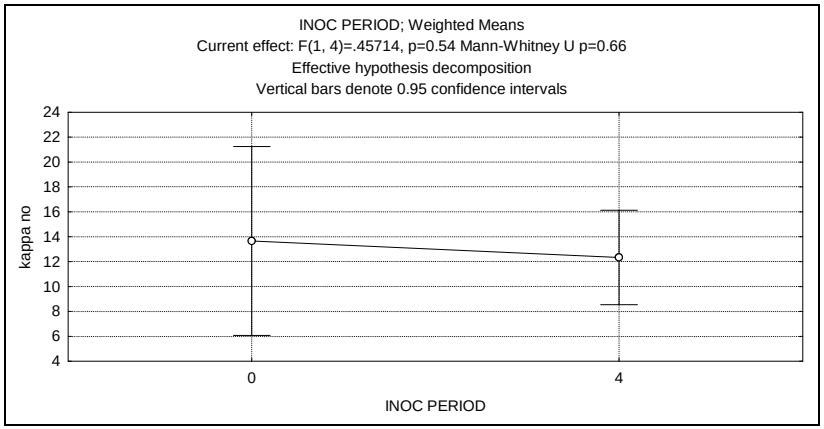

Figure 55: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips.

3.6 HANDSHEET BRIGHTNESS

The results of the handsheet brightness and the statistical analysis of all the treatments are shown in Figures 56 and 57-63 respectively The weighted means of sequences **1**-**5** and **7** (see section **3.1**) showed a significant difference ($p < 0.05$) as seen in Figures 57-61 and 63. PHWE, FCCI period, charge difference and cooking period showed to improve the handsheet brightness. The results would be attributed to the fact that PHWE removed the extractives; FCCI broke down the lignin structure making chemical degradation easier on the open structure of the lignin and release more extractives. This phenomenon would also be of significant benefit for high brightness bleaching also reduces the use of bleaching chemicals. The weighted means of sequence **6** (see section **3.1**) showed no significant difference ($p > 0.05$). Varying the incubation time (0-4 weeks) proved to be non-effective for the unextracted, FCC uninoculated and unextracted, FCCi wood chips. This could be attributed to the fact that lignin structure was not thoroughly penetrated by the FCC to open up the wood chip structure so that the chemicals could gain excess to the interior wood chip structure. It might also be attributed to the fact that the FCC perhaps were colonised more so on the wood chip surface than in the wood chip interior.

Wood chips that were PHWe, unextracted FCCi and PHWe FCCi, produced handsheets with a higher percent ISO brightness than the unextracted wood chips. One week of cold water soaking of the wood chips produced a colour change hence indicated that some extractives were removed and short chain sugars were dissolved. Furthermore, one-hour hot water extraction period also dissolved sugars and removed other extractives, which were not affected by cold water soaking as shown in Figure 56.

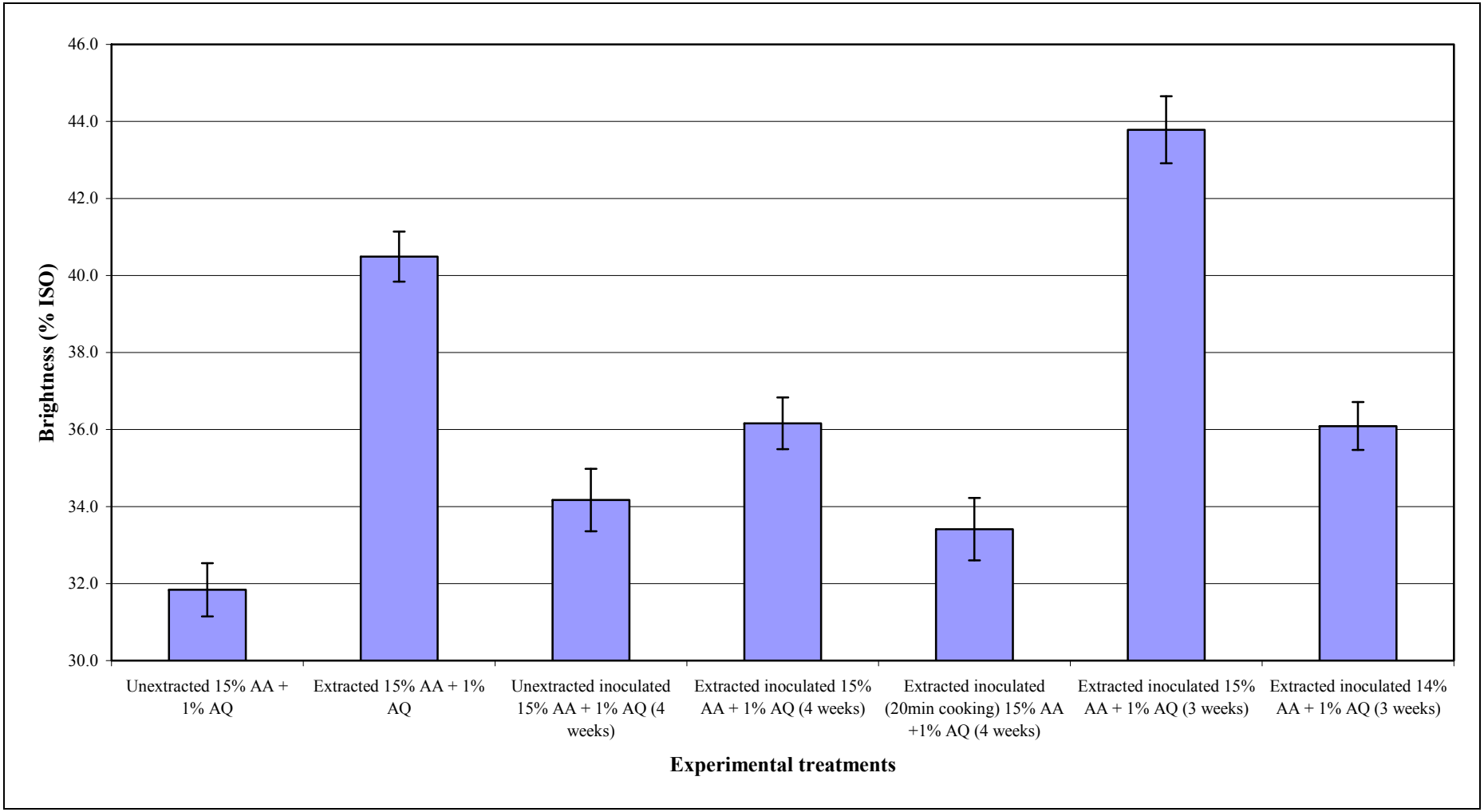

Figure 56: Brightness of handsheets from Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chips.

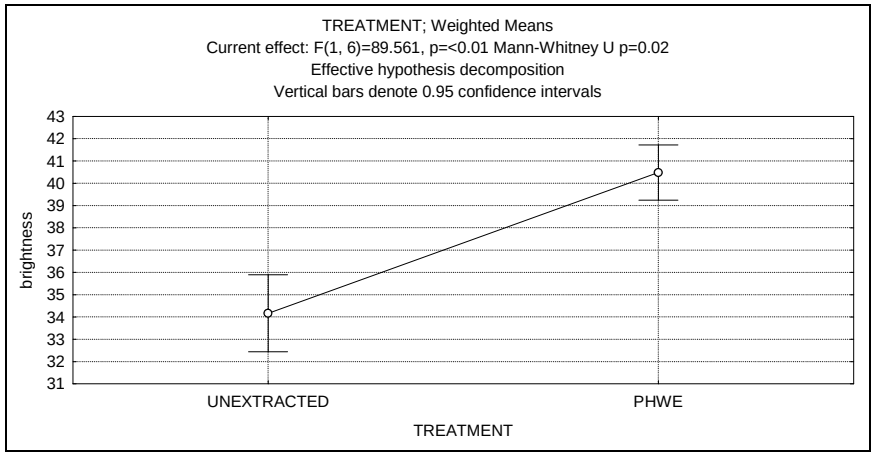

Figure 57: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips.

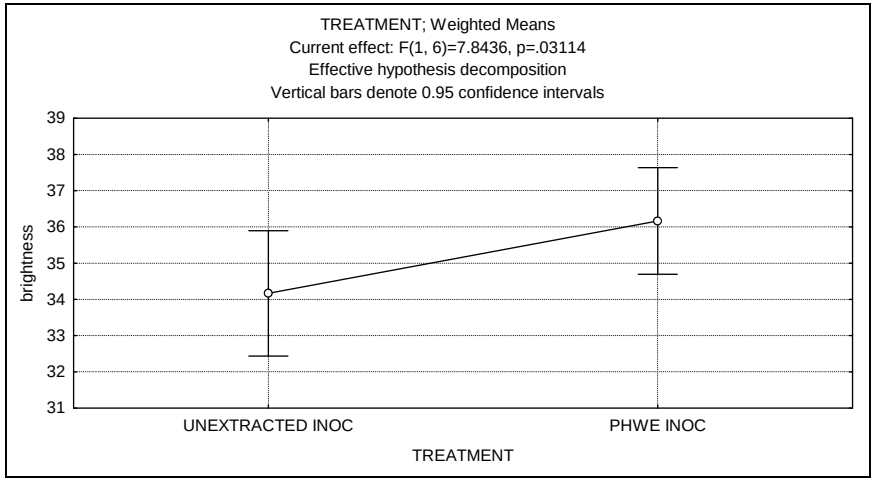

Figure 58: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips.

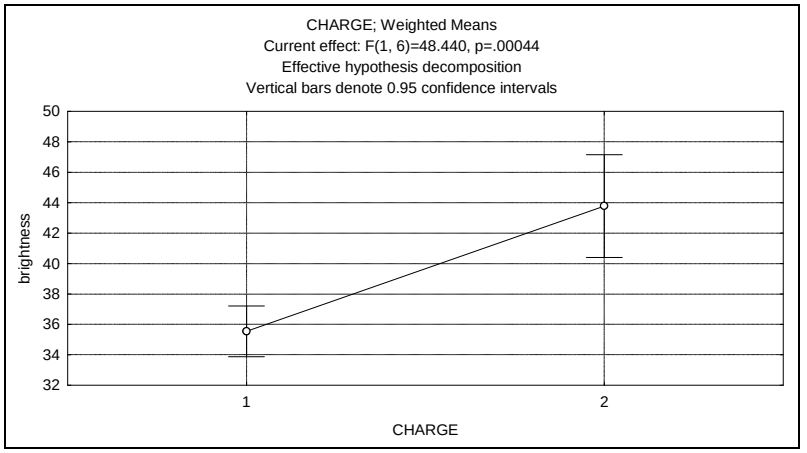

Figure 59: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood (1 = 14% AA; 2=15% AA).

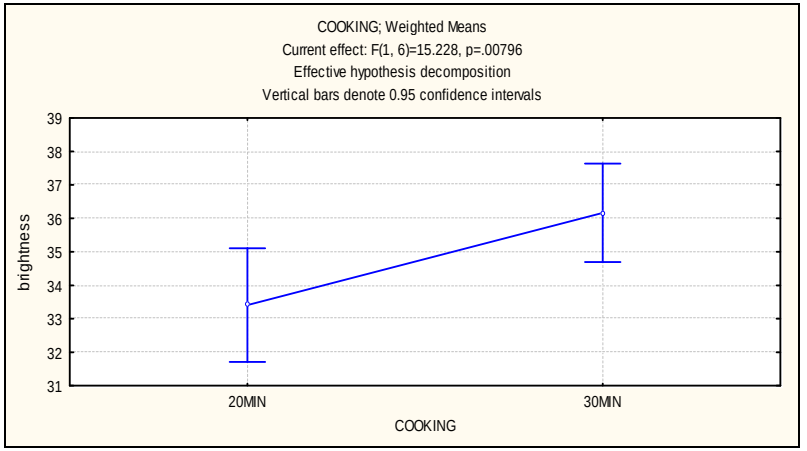

Figure 60: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips.

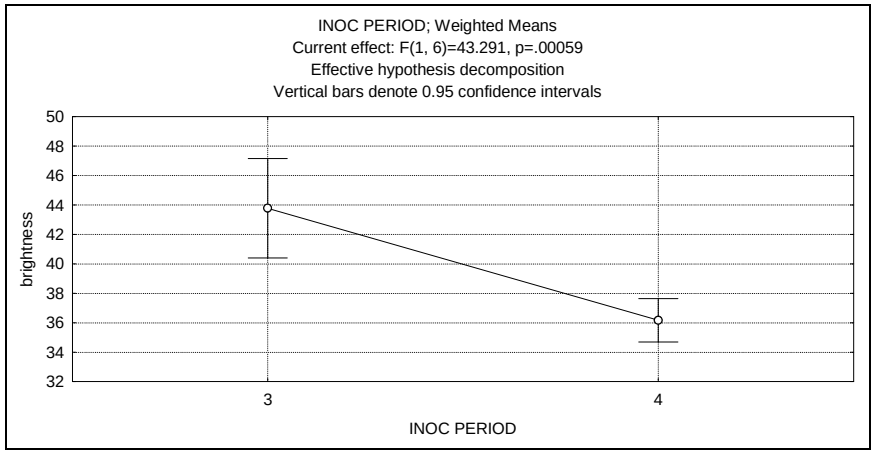

Figure 61: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips (3 and 4 = weeks of inoculation).

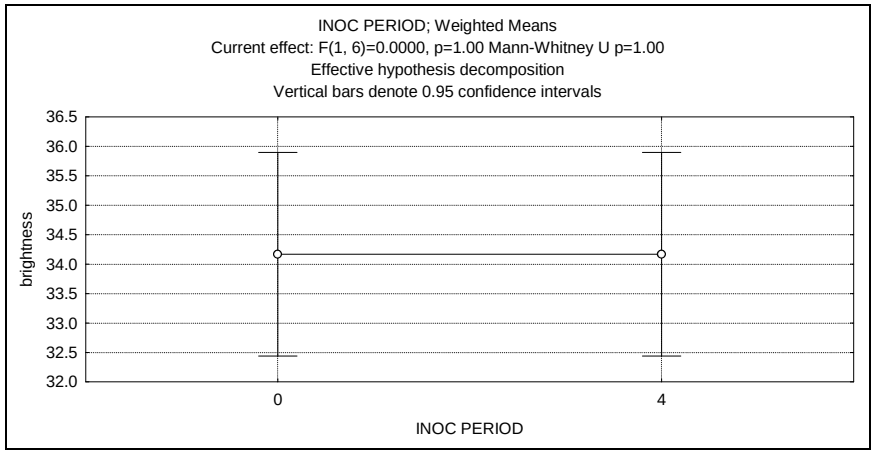

Figure 62: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of incubation period).

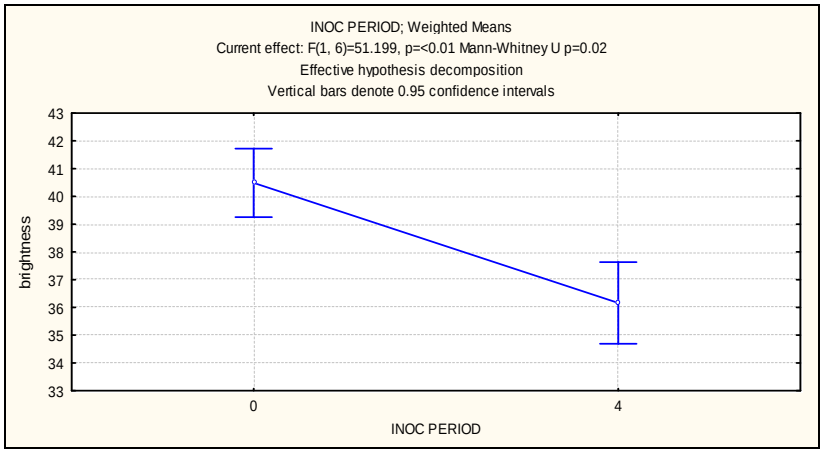

Figure 63: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips (0= uninoculated; 4 = weeks of inoculation).

3.7 PULP STRENGTH EVALUATIONS

Although the strength results shown in Table 12 are higher to those shown in Table 13, this could be attributed to the higher wetness values recorded for the unextracted wood pulp after beating. It seems that the PHWE might have altered the fibre surface properties giving it a slightly more hydrophobic character, thus negatively influencing fibre hydration during beating and as a result of the slower beating response fewer interfibre bonding surfaces were created.

Comparing these handsheet strength values for sequence **1** (Figure 72) at the 38 ^{0}SR freeness level it appeared that the weighted means of the burst index were not significant ($p>0.05$). The tear index on the other hand was significantly higher for the PHWe material as seen in Figure 79. Also the breaking length showed a significant difference with $p=0.00024$, but here the unextracted material resulted in the higher strength as seen in Figure 86. FCCI of unextracted wood chips improved all handsheet strength properties significantly. When comparing the strength test results shown in Tables 12 and 13 with the values shown in Tables 14 and 15, it is apparent that the fungal pretreatment of the wood chips produced pulps resulting in improved handsheet properties.

The FCCi chip material which had been PHWe before inoculation produced pulp with the highest strength development potential as revealed by the handsheet strength test results shown in Table 16 and 17 and Figures 68-70. The effect of PHWE and FCCI can also be seen from the improved initial bonding strength potential of the unbeaten fibres, as shown in Tables 14-16 and Table 18. The lower initial handsheet strength results shown in Table 17 can be attributed to the reduced incubation time of this specific fungal inoculated chip batch, although its strength development potential after beating was the best from all the strength test results. The handsheet strength development potential of the fungal inoculated material with a reduced incubation time and pulped with a reduced AA level, showed a noticeable decline.

Figure 64 summarizes the pulp freeness of all the experimental treatments. The extracted inoculated wood chips incubated for only 3 weeks and pulped at 14% AA and 1% AQ produced higher freeness values compared to all the other experimental treatments. The higher freeness values indicated that the pulp perhaps was over beaten, which reflected in the lower handsheet strength development as shown in Table 18. PHWe wood chips pulped at

15% AA and 1% AQ developed the lowest freeness values compared to all the other experiments. It seems that the PHWE might have altered the fibre surface characteristics rendering it slightly more hydrophobic. This would have had a negative influence on fibre hydration.

The burst index, tear index and breaking length values are presented in Figures 65-71. Both the unextracted FCCI and PHWe FCCI (3 weeks incubation and 14% AA) wood chips reached their full beating potential.

All the pulp material obtained from the extracted fungal inoculated wood chips with the longest incubation time had not reached its fullest strength potential after 3 minutes of beating time. The extracted chip material incubated for a shorter period did not show the same handsheet strength development (see Figure 65-71, green line). This clearly demonstrated that the duration of the incubation period plays an important role in the activation of cell wall fibrillation by the inoculated lignolytic fungal cultures.

The accelerated rate of beating of pulps obtained from fungal treated wood chips can be attributed to enhanced fibre swelling, fibrillation and flexibility. Cell wall punctures and cavities created by the fungal removal of lignin resulted in better water accessibility causing fibres to swell, thus showing enhanced beating rates. Dommisse [1] also observed that fungal pretreated wood pulp resulted in better water retention values, which suggested that the biodegraded wood pulp fibres were able to hydrate more readily during beating.

Table 12: Handsheet strength of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips (without FCCI) at 15% NaOH and 1% AQ.

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	19	26	35	49
Burst index (KPa.m^2/g)	0.70±0.26	1.67±0.35	2.51±0.52	3.75±0.43
Tear index (mN.m^2/g)	2.30±0.75	3.65±1.36	4.69±1.81	6.01±2.39
Breaking length (Km)	1.68±0.54	3.29±0.83	5.45±0.68	8.17±0.63

Table 13: Handsheet strength of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips (without FCCI) at 15% NaOH and 1% AQ.

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	17	24	32	37
Burst index (KPa.m^2/g)	0.43±0.08	0.78±0.16	2.31±0.23	2.63±0.38
Tear index (mN.m^2/g)	1.60±0.59	2.76±0.94	3.93±1.59	5.01±1.98
Breaking length (Km)	1.68±0.21	2.41±0.44	5.19±0.70	5.73±0.48

Table 14: Handsheet strength of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	21	31	36	48
Burst index (KPa.m^2/g)	0.71±0.12	2.51±0.78	4.50±0.42	4.80±0.30
Tear index (mN.m^2/g)	2.67±1.15	4.77±2.11	5.58±1.98	5.68±2.09
Breaking length (Km)	2.02±0.29	4.93±0.95	7.15±1.45	8.03±1.02

Table 15: Handsheet strength of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	22	26	36	46
Burst index (KPa.m^2/g)	0.86±0.24	2.12±0.34	**3.1**9±0.53	5.10±0.52
Tear index (mN.m^2/g)	2.31±1.13	4.04±1.93	4.90±2.15	6.14±2.65
Breaking length (Km)	2.43±0.30	4.72±0.62	5.89±0.95	7.95±0.83

Table 16: Handsheet strength of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced cooking time (20 min).

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	22	29	37	49
Burst index (KPa.m^2/g)	1.62±0.20	2.61±0.28	3.07±0.19	5.53±0.99
Tear index (mN.m^2/g)	3.51±1.53	4.89±1.96	5.21±2.06	6.51±2.64
Breaking length (Km)	2.58±0.22	5.25±0.45	5.25±0.41	9.38±1.15

Table 17: Handsheet strength of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced incubation time (3 weeks).

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	22	30	40	51
Burst index (KPa.m^2/g)	0.68±0.13	2.35±0.68	5.04±0.32	6.02±0.42
Tear index (mN.m^2/g)	2.02±0.85	4.02±1.78	6.06±2.22	7.69±3.65
Breaking length (Km)	1.47±0.30	4.34±1.37	8.16±0.56	10.22±0.49

Table 18: Handsheet strength of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 14% NaOH and 1% AQ at reduced incubation time (3 weeks).

Strength	**Beating Time (min)**			
	0	1	2	3
Wetness (^{0}SR)	22	30	40	55
Burst index (KPa.m^2/g)	0.70±0.08	1.19±0.22	3.78±0.33	3.76±0.73
Tear index (mN.m^2/g)	**3.1**9±1.81	3.56±1.60	5.54±2.03	5.70±2.20
Breaking length (Km)	2.00±0.27	3.08±0.37	7.61±0.75	7.59±0.61

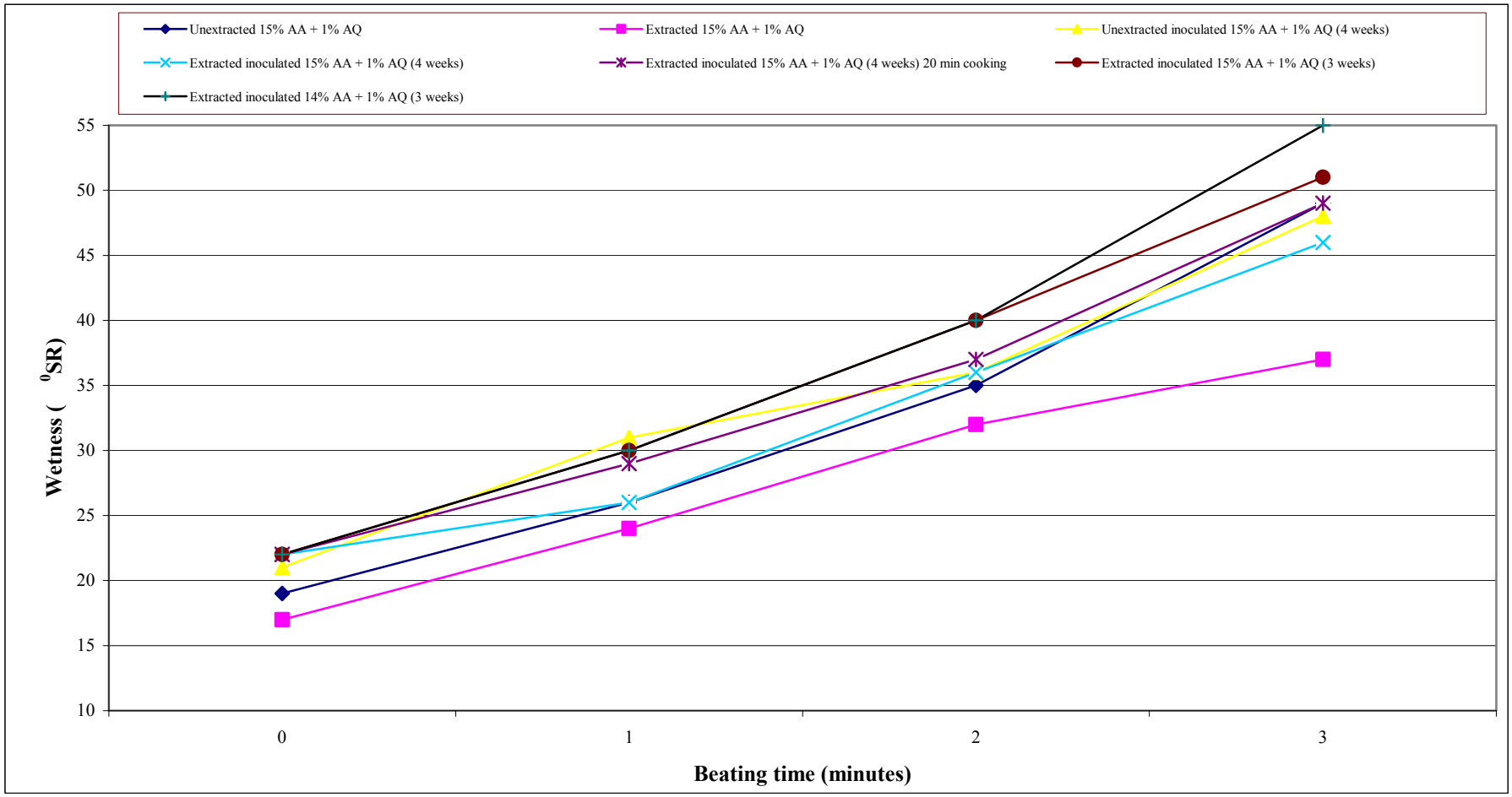

Figure 64: Relationship between wetness and beating time of unextracted, extracted, unextracted inoculated and extracted inoculated wood chip pulp.

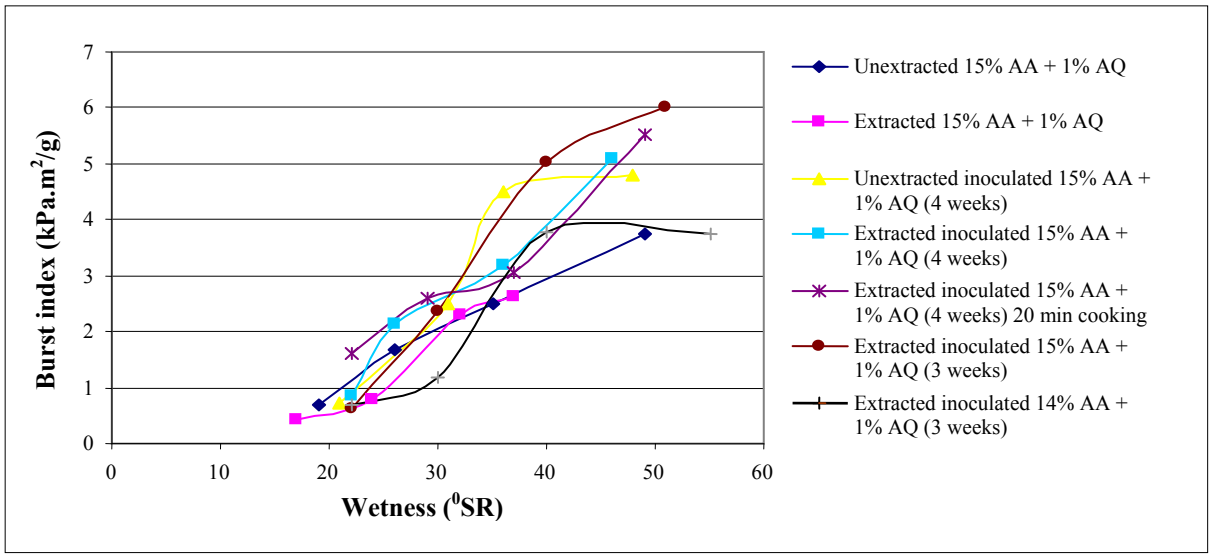

Figure 65: Burst index of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood pulp.

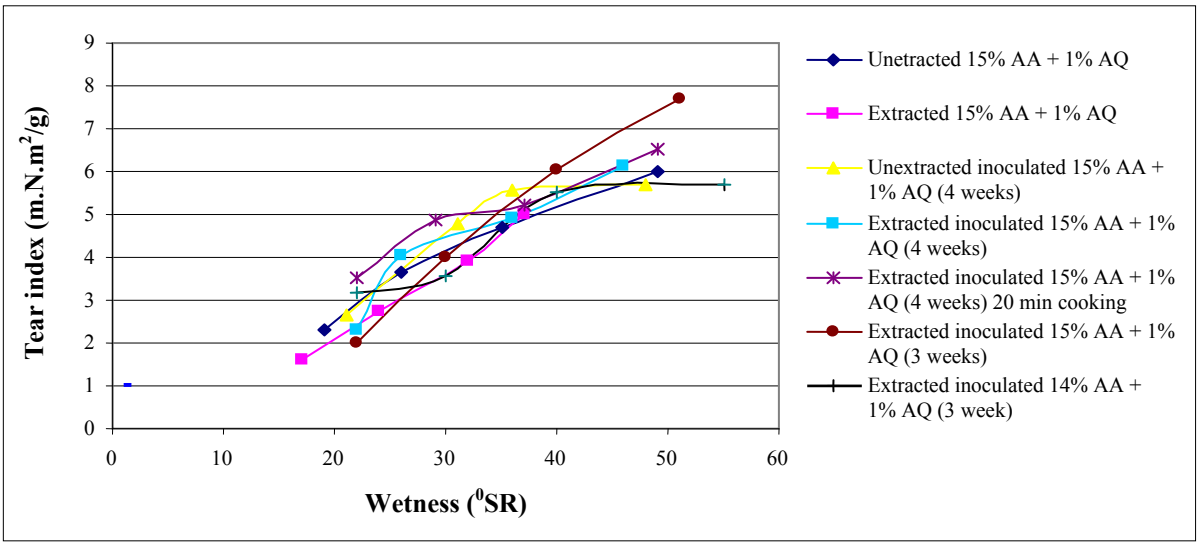

Figure 66: Tear index of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chip pulp.

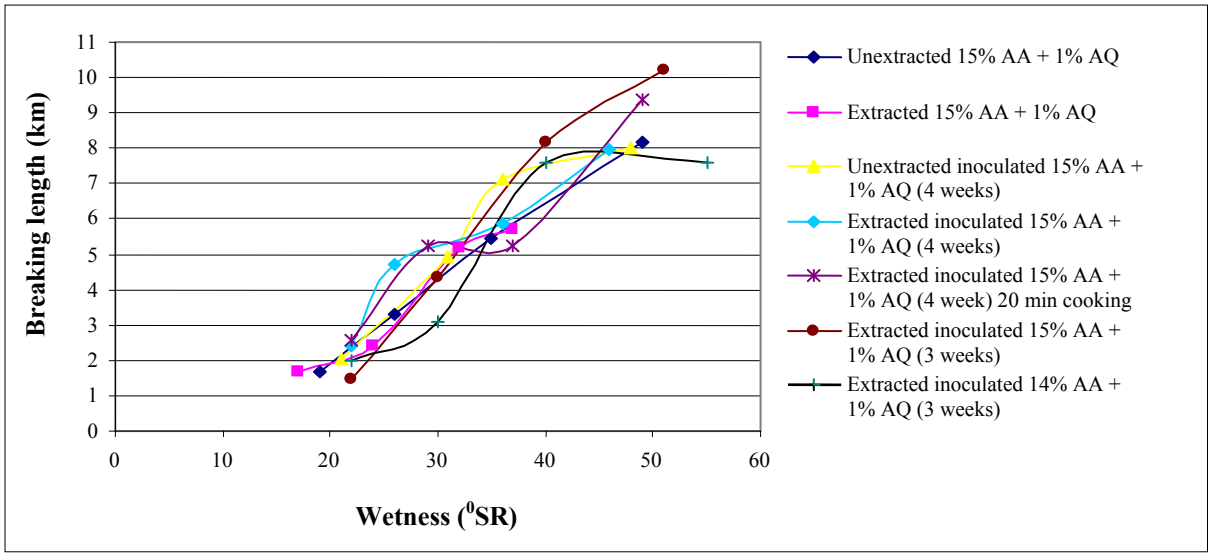

Figure 67: Breaking length of Soda-AQ pulped unextracted, extracted, unextracted inoculated and extracted inoculated *Eucalyptus grandis* wood chip pulp.

The handsheet strength test results are also presented in a bar graph version in Figures 68-70. All the handsheet strength values were extrapolated at a freeness of 38 ^{0}SR and were plotted as shown in Figure 71. The results shown in Figure 71 demonstrate that unextracted inoculated and PHWe FCCI treatment with three weeks incubation time gave superior handsheet strength properties. There seemed however be little difference whether the wood chips were PHWe and FCCI or not.

The statistical analysis of all the burst indexes is shown in Figures 72-78. The weighted means of sequences **2** and **5** (see section **3.1**) showed a negative significant difference ($p < 0.05$) as seen in Figures 73 and 76. The drop of burst index means that FCCI and duration of incubation (3-4 weeks) would not be an option for strength development. The weighted means of sequences **3**, **6** and **7** (see section **3.1**) showed a significant increasing difference ($p < 0.05$) as seen in Figures 74, 77 and 78. The increase of burst index can be attributed to the enhanced fibrillation of the cell wall surface, caused by FCCI as a chip pretreatment thus providing better inter-fibre bonding. Similar results were confirmed by Eriksson [5] and Dommisse [1]. The weighted means of sequences **1** and **4** (see section **3.1**) showed no significant difference ($p > 0.05$) as seen in Figures 72 and 75.

The statistical analysis of all the tear indexes is shown in Figures 79-85. The weighted means of sequences **1** and **6** (see section **3.1**) showed a significant increase in the tear index ($p <$

0.05) as seen in Figures 79 and 84. When comparing the unextracted wood chips with PHWe and FCCi material the tear index improved significantly, probably caused by the better fibrillation of the fibres leading to stronger handsheets. The weighted means of sequences **2** and **7** (see section **3.1**) also showed a significant difference in tear index ($p < 0.05$) as seen in Figures 80 and 85. The significant difference is negative meaning that the tear index dropped significantly. The results show that paper tear strength could only be improved when using PHWe and unextracted FCCi wood chips for pulp production. The weighted means of sequences **3-5** (see section **3.1**) showed no significant difference ($p > 0.05$) as shown in Figures 81-83. This means that increasing the charge from 14 to 15%; increasing cooking time 20-30 and increasing the incubation time from 3-4 weeks did not significantly improve the tear index.

The statistical analysis of all the breaking length results is shown in Figures 86-92. The weighted means of sequences **3, 4, 6** and **7** (see section **3.1**) showed a significant increasing difference ($p < 0.05$) as seen Figures 87, 88, 90 and 91. Increase of alkali charge from 14 and 15%, cooking time from 20 to 30 minutes and incubation time (0-4 weeks) all responded to a significant improvement in the breaking length. The weighted means of sequences **1, 2,** and **5** (see section **3.1**) showed a negative significant difference ($p < 0.05$) as shown in Figures 86, 87 and 90. The reduction in breaking length of handsheets made from the PHWE and PHWe FCCi wood pulp may be attributed to the removal of hemicelluloses as a result of the PHWE and FCC pretreatments.

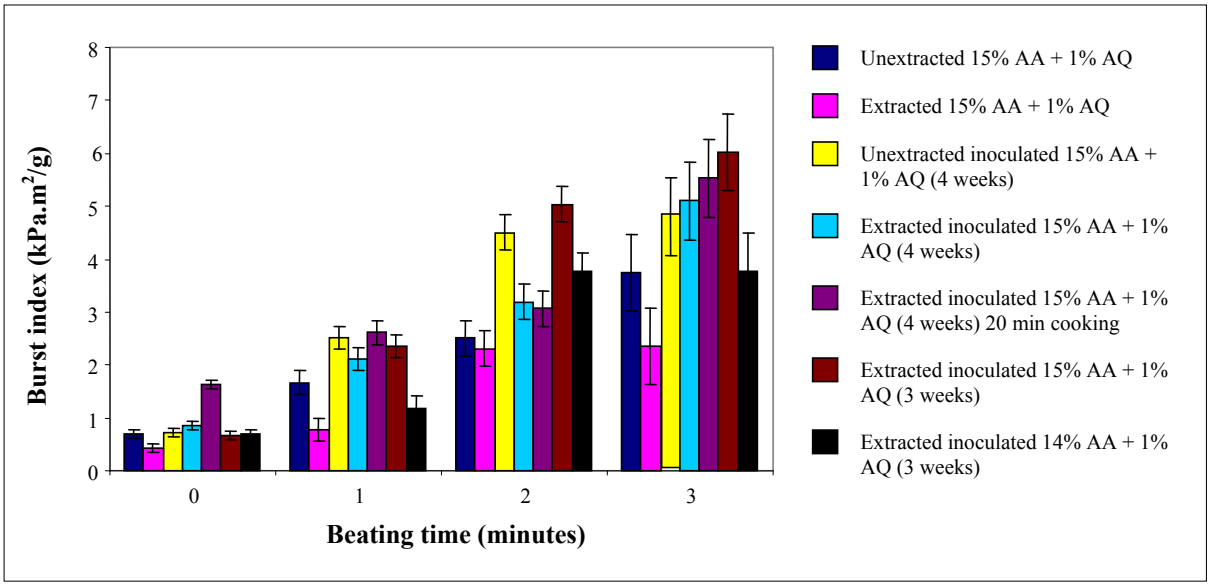

Figure 68: Relationship between burst index and beating time of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips.

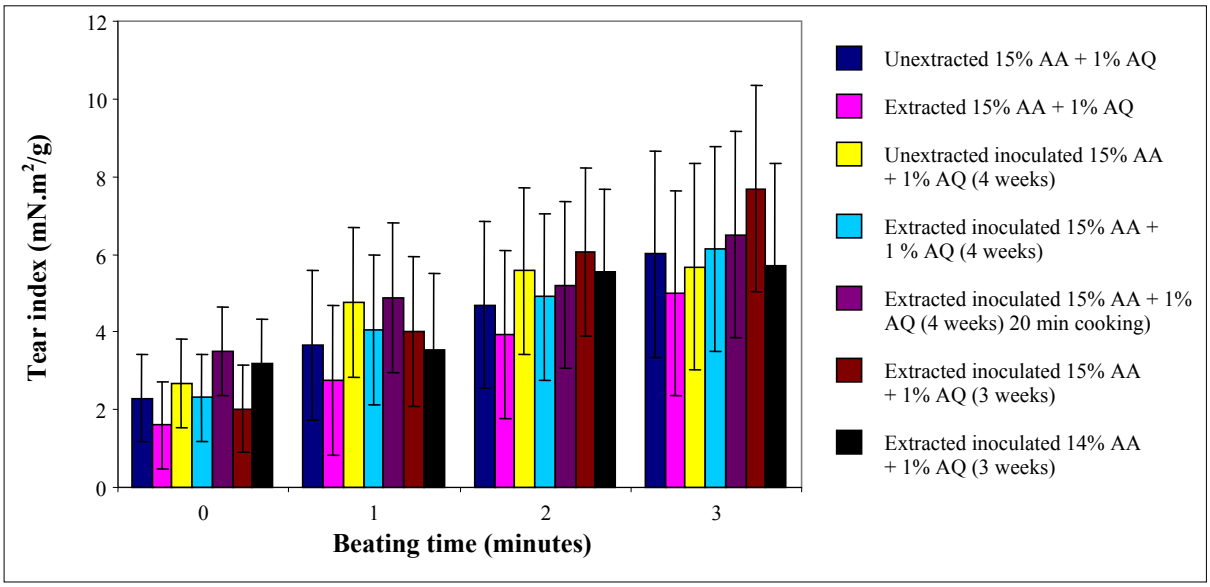

Figure 69: Relationship between tear index and beating time of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips.

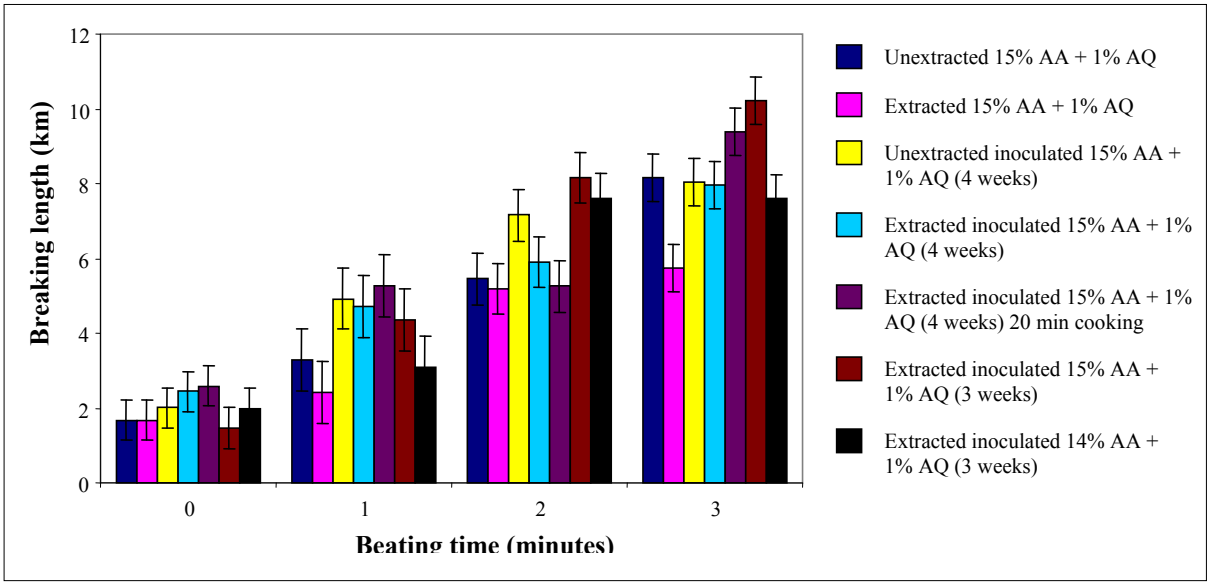

Figure 70: Relationship between breaking length and beating time of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips.

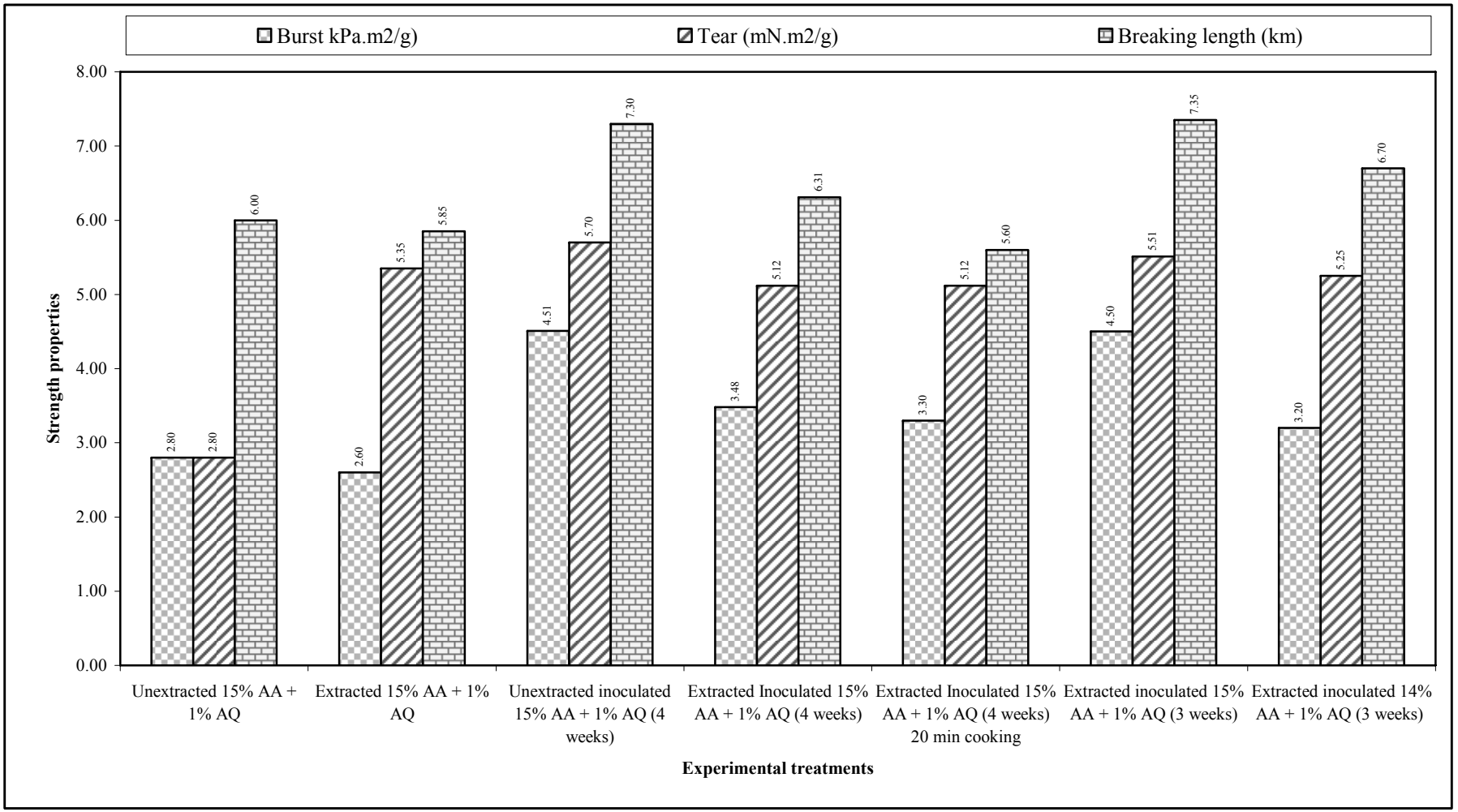

Figure 71: Strength properties of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips at 38 ^{0}SR.

Table 19: Summary of all the statistical analysis of the paper strength properties.

Sequences	Description	Burst index ($KPa.m^2/g$)	Tear index ($mN.m^2/g$)	Breaking length (Km)
1	Unextracted and PHWe	No significant difference p = 0.196	Significant difference p = 0.000	significant difference p = 0.0002
2	Unextracted FCCi and PHWe FCCi	Significant difference p = 0.00001	Significant difference p = 0.002	Significant difference p = 0.0002
3	PHWe FCCi *Eucalyptus grandis* wood chips cooked at different charges	Significant difference p = 0. 017	No significant difference p = 0.39	No significant difference p = 0.00002
4	PHWe FCCi *Eucalyptus grandis* wood chips cooked on different durations	No significant difference p = 0.20	No significant difference p = 1.00	Significant difference p = 0.01
5	PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times	Significant difference p = 0.0003	No significant difference p = 0.06	No significant difference p = 0.0003
6	Uninoculated and unextracted FCCi	Significant difference p = 0.01	Significant difference p = 0.01	Significant difference p = 0.01
7	PHWE and PHWe FCCi *Eucalyptus grandis* wood chips incubated at different times	Significant difference p = 0.01	Significant difference p = 0.02	Significant difference p = 0.01

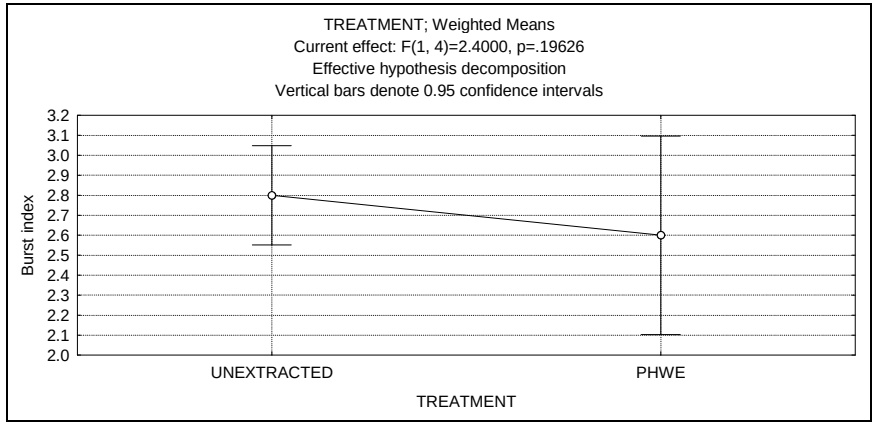

Figure 72: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

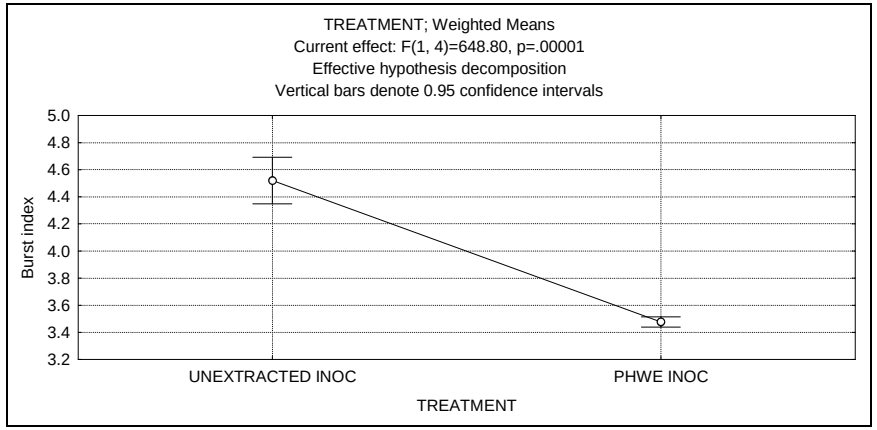

Figure 73: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips at 38 ^{0}SR.

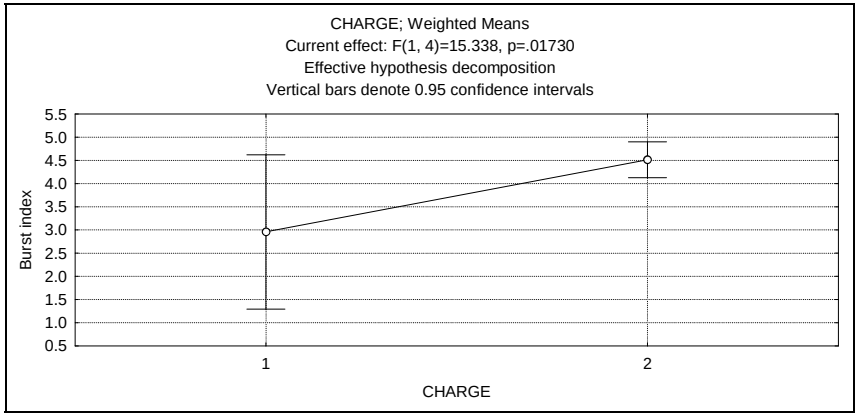

Figure 74: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood at 38 ^{0}SR.

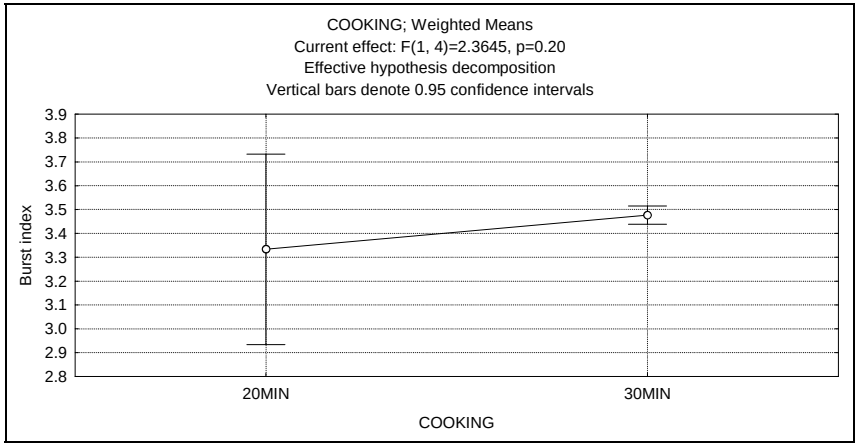

Figure 75: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

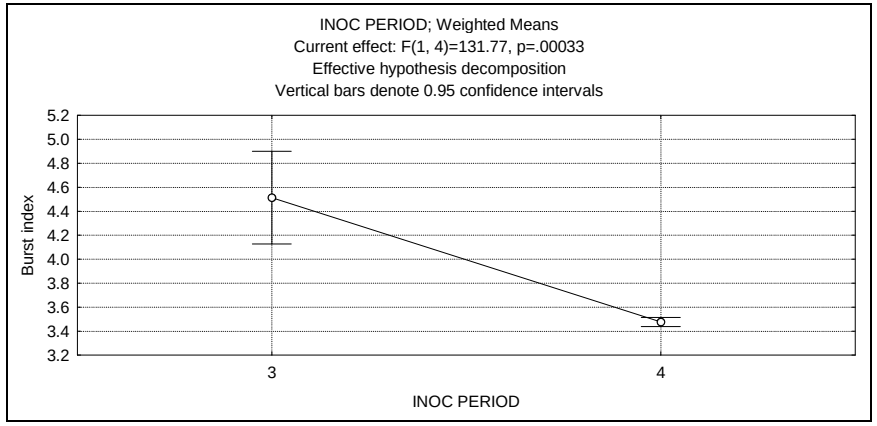

Figure 76: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

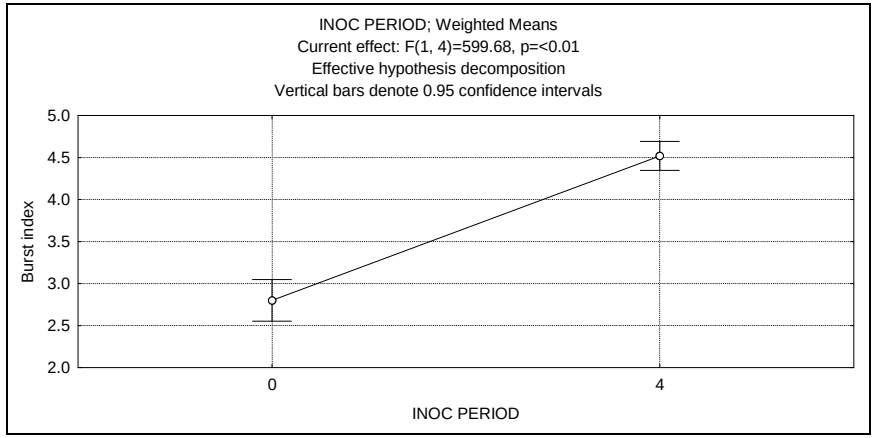

Figure 77: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips at 38 ^{0}SR.

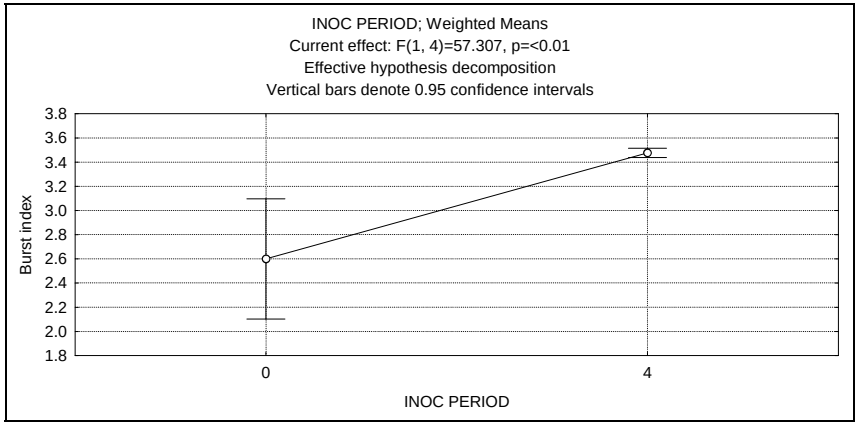

Figure 78: Representation of weighted means of PHWE and FCCI PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

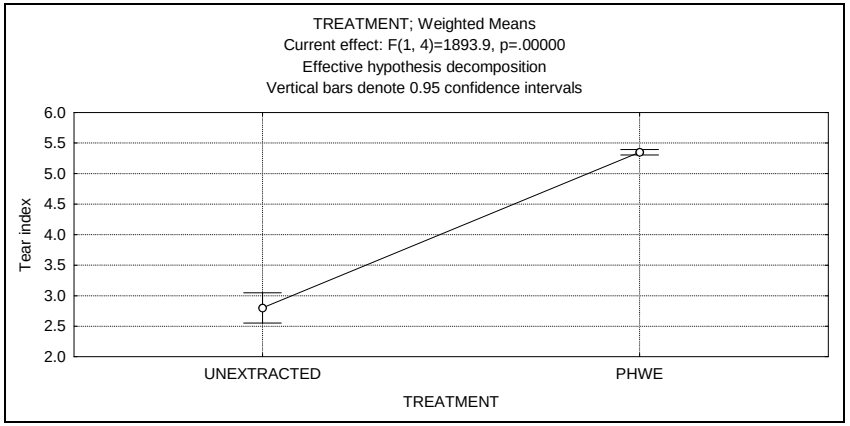

Figure 79: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

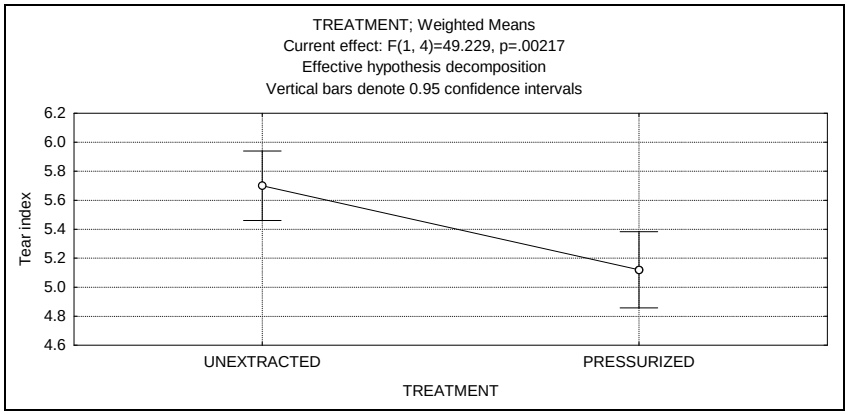

Figure 80: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips at 38 ^{0}SR.

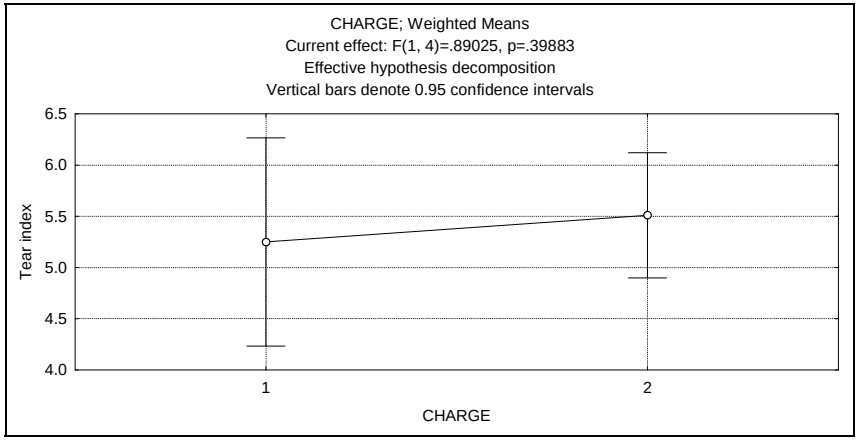

Figure 81: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

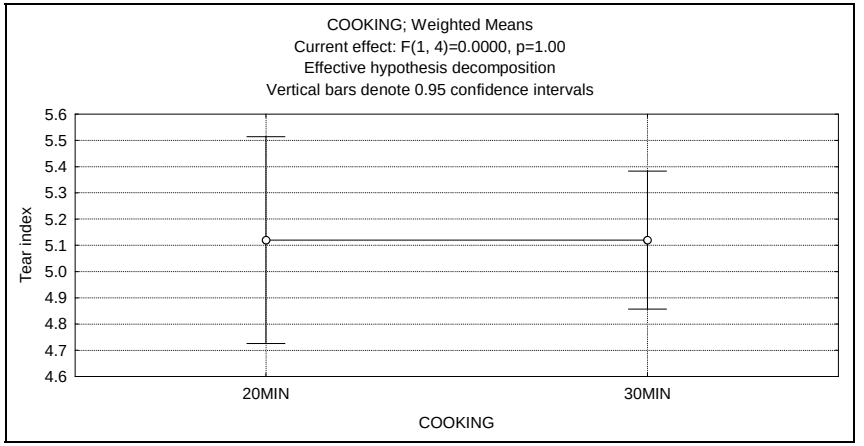

Figure 82: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

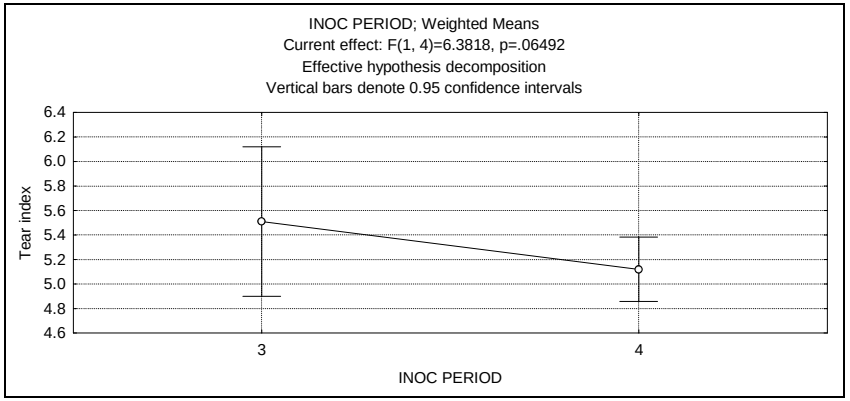

Figure 83: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

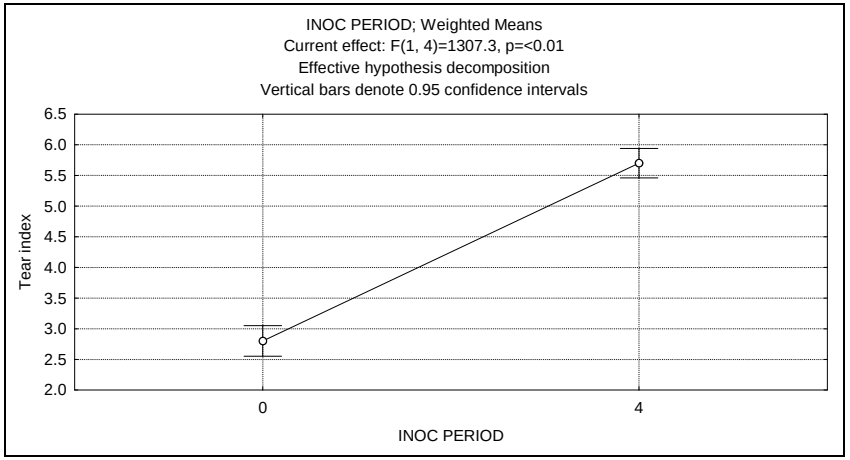

Figure 84: Representation of weighted means of uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips at 38 ^{0}SR.

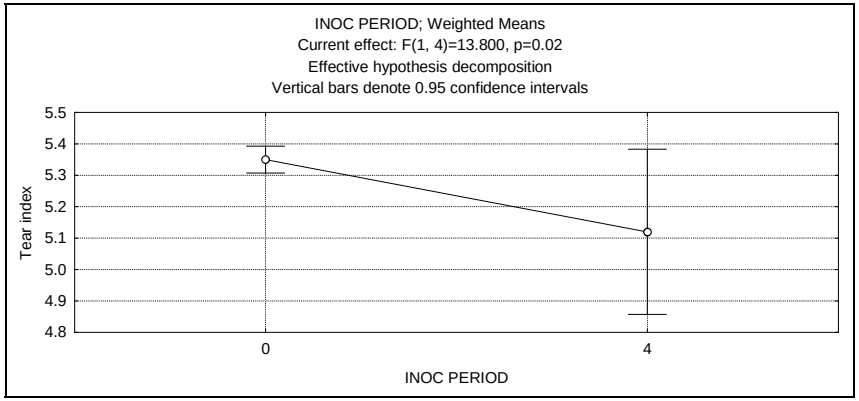

Figure 85: PHWE and FCCI PHWe *Eucalyptus grandis* wood chips versus tear index at 38 ^{0}SR.

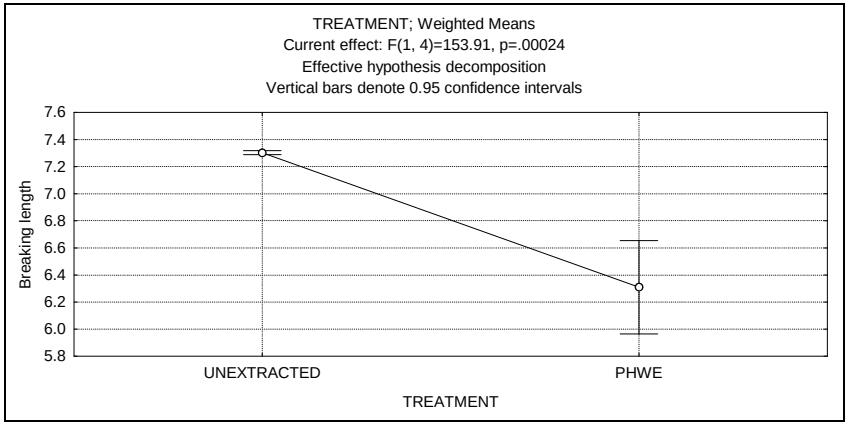

Figure 86: Representation of weighted means of unextracted and PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

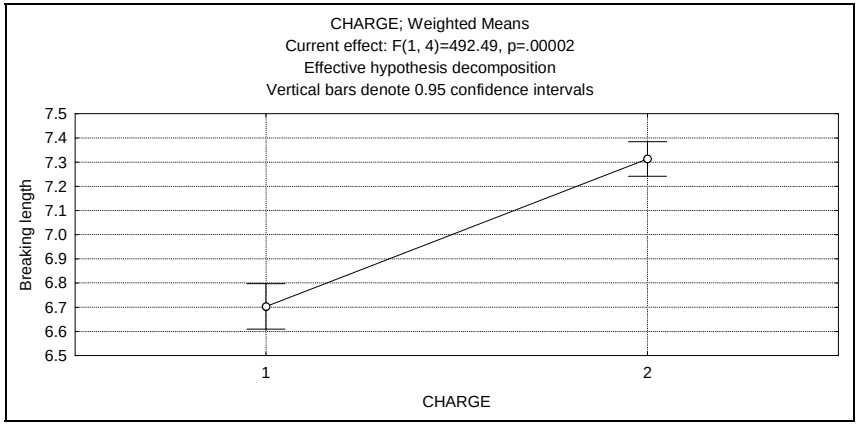

Figure 88: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

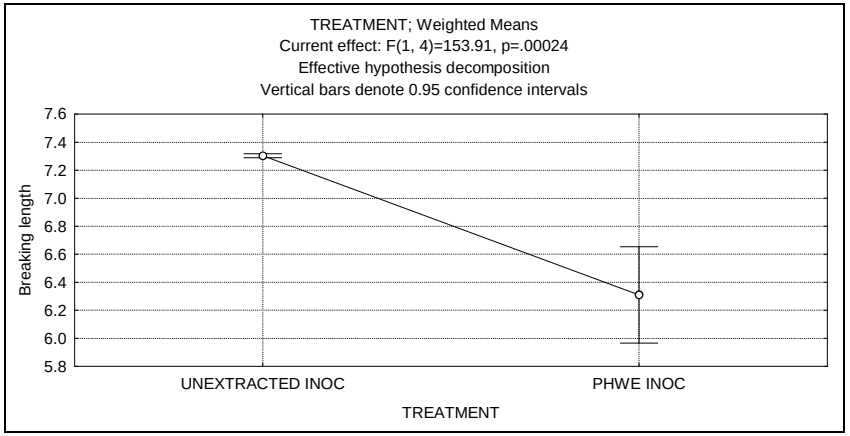

Figure 87: Representation of weighted means of unextracted FCCI and PHWe FCCI *Eucalyptus grandis* wood chips at 38 ^{0}SR.

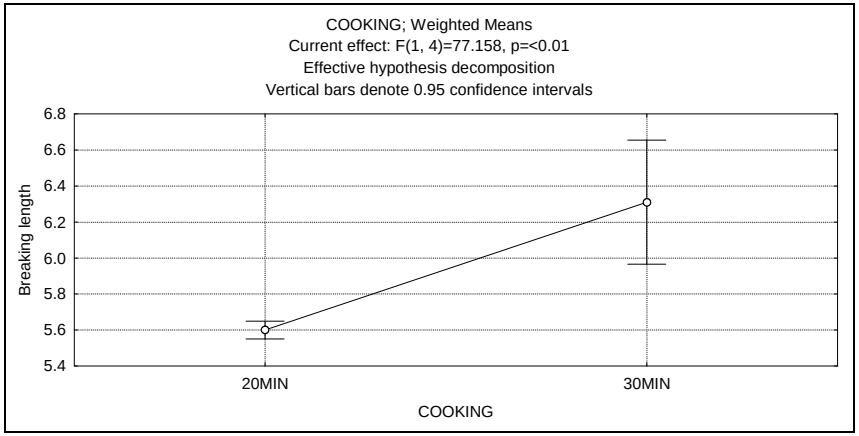

Figure 89: Representation of weighted means of FCCI of PHWe *Eucalyptus grandis* wood chips at 38 ^{0}SR.

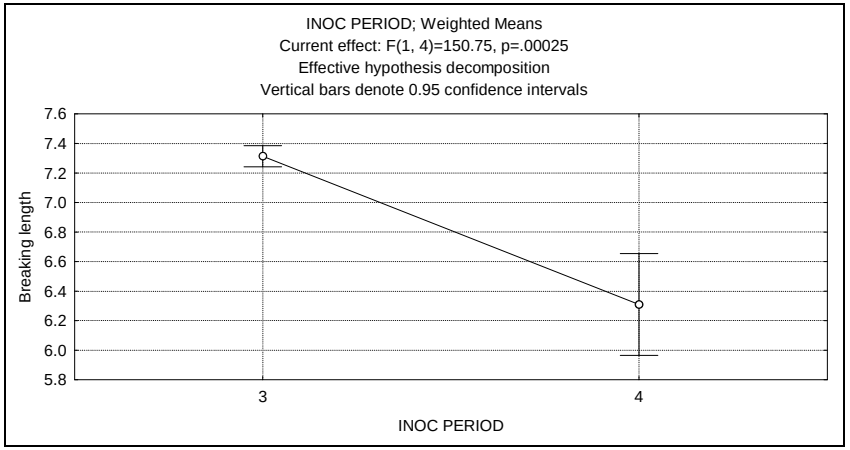

Figure 90: FCCI of PHWe *Eucalyptus grandis* wood chips incubated at different times versus tear index at 38 ^{0}SR.

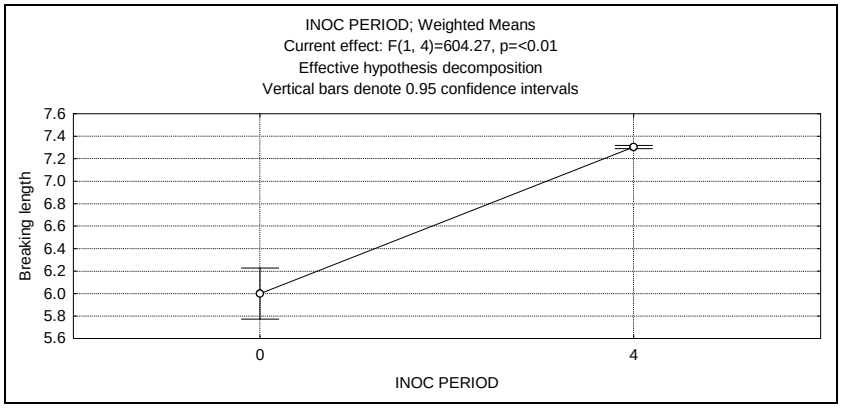

Figure 91: Uninoculated and FCCI unextracted *Eucalyptus grandis* wood chips versus tear index at 38 ^{0}SR.

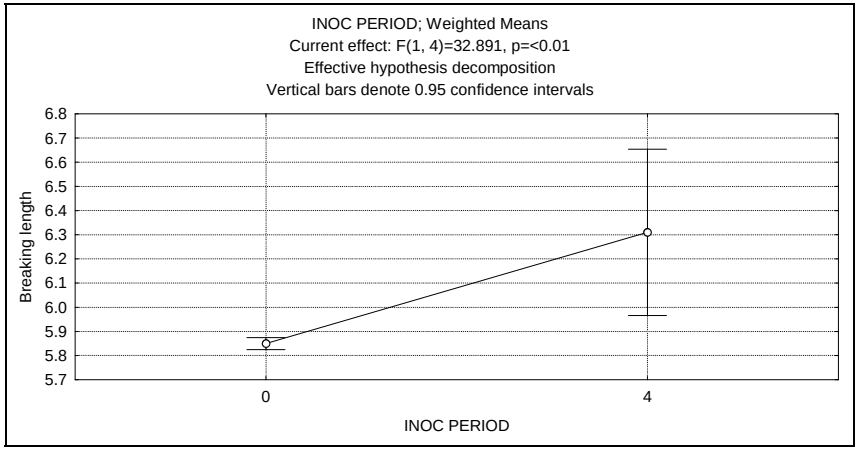

Figure 92: PHWE and FCCI PHWe *Eucalyptus grandis* wood chips versus tear index at 38 ^{0}SR.

3.8 MACRO- AND MICROSCOPIC APPEARANCE OF WOOD CHIPS

Figures 93 A & B illustrate unextracted and PHWe wood chips. PHWE produced a darker colour of the wood chips indicating that oxidation reactions occurred during the extraction period. After PHWE the wood chip surface felt semi-soft and the resulting effluent had a distinct colour illustrating removal of extractives.

Figure 94 illustrates the magnified cross-sectional surface of fresh *Eucalyptus grandis* wood chips.

After PHWE cell wall separation, deformation and swelling is apparent as shown in Figure 95. The cross-sectional appearance also seems to be more fluffy and of uniform character. The removal of extractives during this pressurized pretreatment period provided better accessibility to the water penetrating the cell wall structure and thus causing a hydration effect. This again provided an easier pathway for an improved penetration of the active alkali during pulping.

Figure 96 clearly shows advanced cell wall separation and rupture mostly apparent in the middle lamella area, caused by lignolytic fungal activity. This deformed wood chip structure beneficiated the improved pulping characteristics such as lower Kappa number and high RAA.

Figure 97 demonstrates the combined effect of pressurized wood chip extraction followed by FCCI. Advanced cell wall collapse and disorder is apparent.

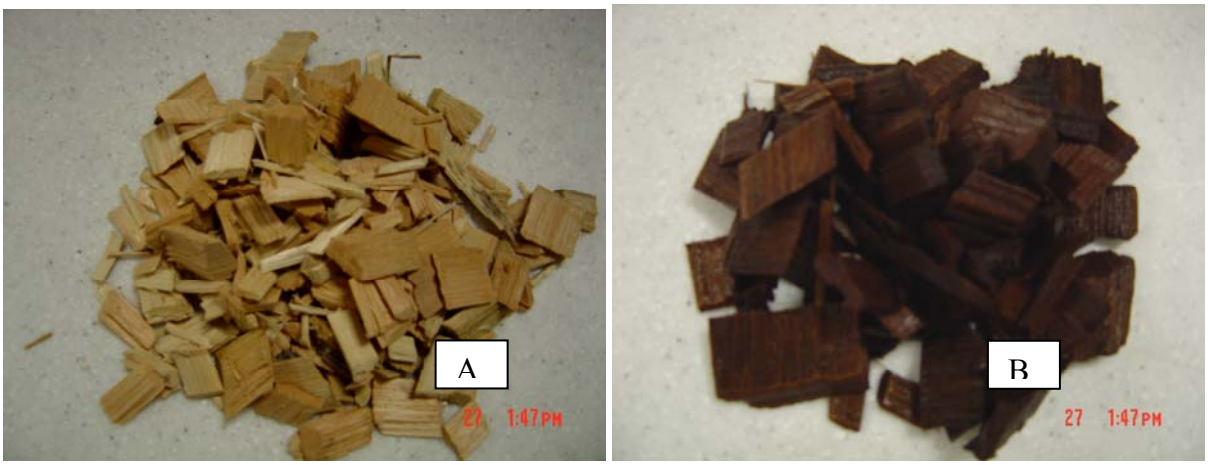

Figure 93: Unextracted (picture A) and one hour PHWe wood chips of *Eucalyptus grandis* (picture B).

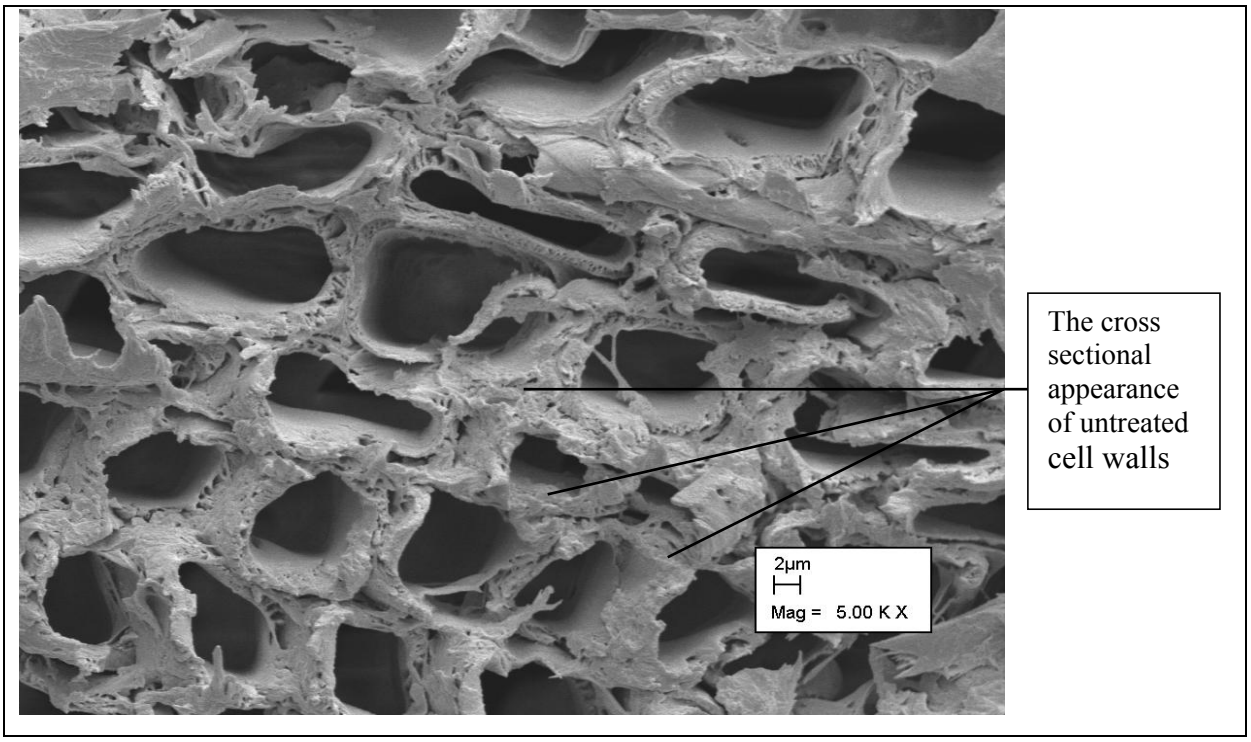

Figure 94: Cross section of unextracted *Eucalyptus grandis* wood chip showing cell wall intactness.

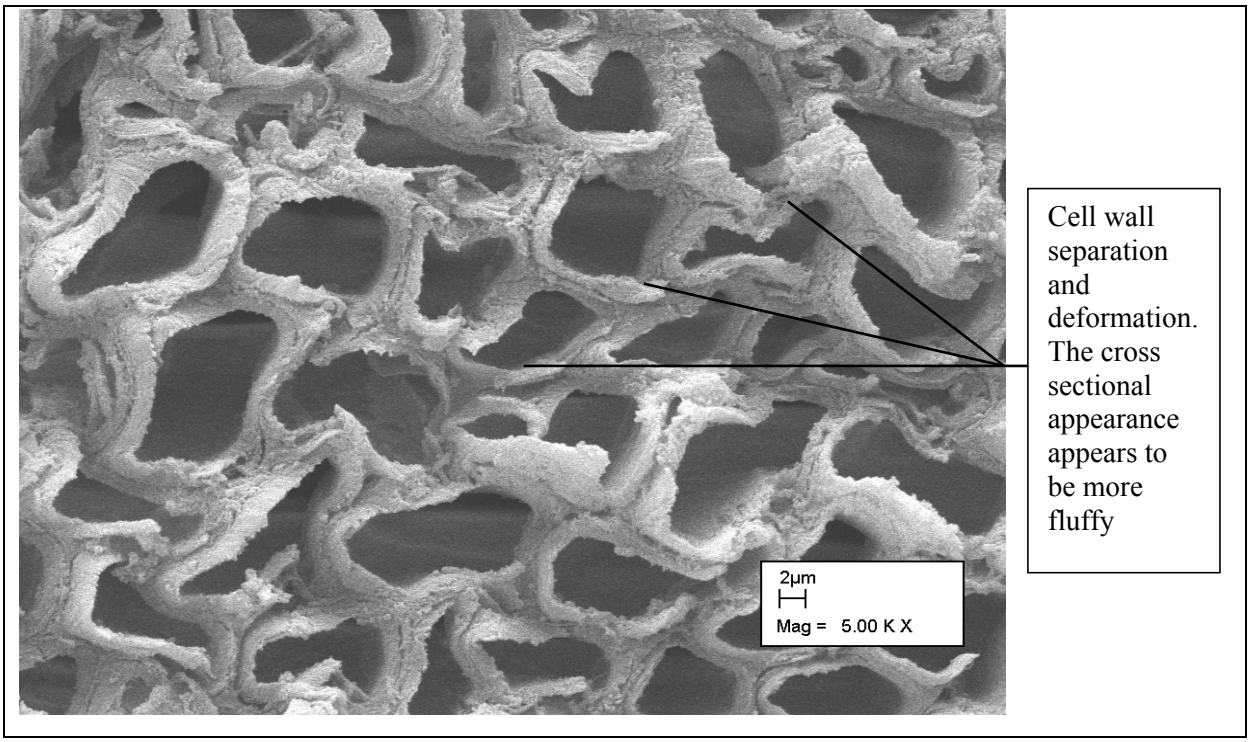

Figure 95: Cross section of extracted *Eucalyptus grandis* wood chip showing cell wall deformation.

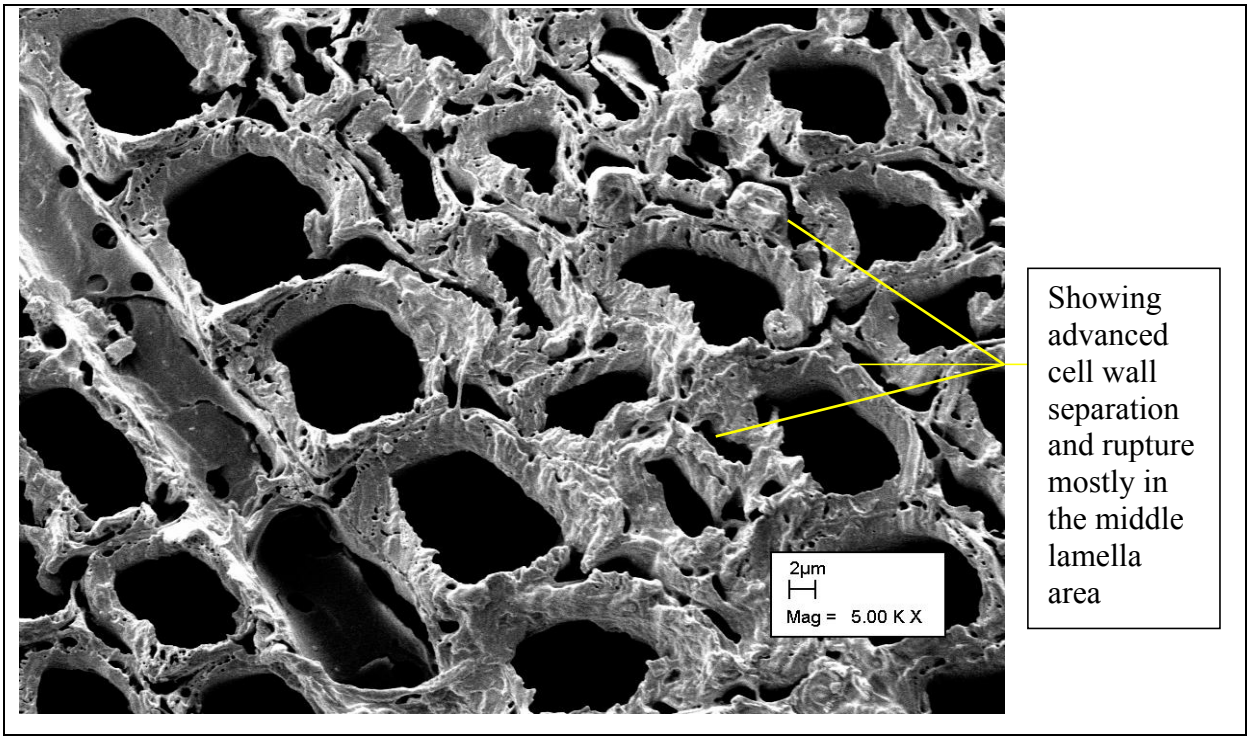

Figure 96: Cross section of unextracted FCCI *Eucalyptus grandis* wood chip showing cell wall break down and intercellular ruptures.

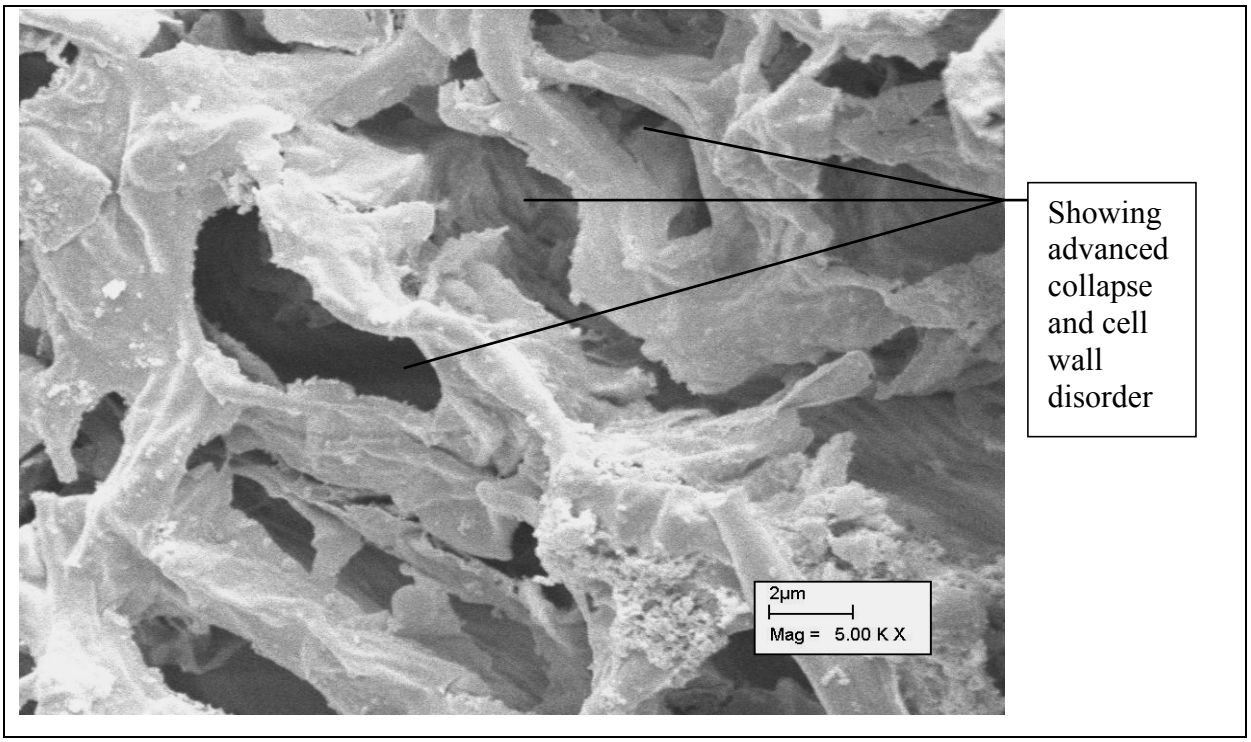

Figure 97: Cross section of extracted FCCI *Eucalyptus grandis* wood chip showing cell wall collapse and disorder.

CHAPTER 4: CONCLUSION

Screened Yield

- The weighted means of all the screened pulp yields showed no significant difference ($p > 0.05$). The fact that the removal of extractives prior to pulping did not reduce the pulp yield is a however a positive result for the pulping industry, because removal of extractives prior to pulping would minimize the usage of pulping chemicals, will result in lower pulp rejects and shives and the pulp residual lignin content. The fungal inoculation prior to pulping reduced the screened pulp yield which could be attributed to an additional loss in lignin (lower Kappa number), more removal of extractives and a possible degradation of polysaccharides as a result of fungal degradation. The hypothesis that the combined PHWE and FCCI of wood chips would further increase the pulp yield could not be achieved. It is anticipated that the combination of PHWE with successive co-culture fungal pretreatment would be very beneficial in obtaining higher pulp yields for fully bleached chemical pulp. Further research would be required to test this assumption.

Rejects

- The weighted means all of sequences showed a significant statistical difference ($p < 0.05$). The use of PHWE and also FCCI prior to pulping improved the penetration of cooking chemicals into the wood chips thereby reducing screened rejects. PHWE and fungal pretreatment were responsible for better extractive removal.

Shives

- Apart from the unextracted wood chips, the unextracted FCCi wood chips showed a higher shive content compared to other treatments. These results suggest that the action of fungal co-cultures was more on the outside of the wood chips than in the wood chip interior itself and might have needed more incubation time to enhance delignification.

Chemical consumption

- It was evident that all the FCCi wood chips consumed less active alkali as compared to the unextracted and PHWe wood chips.

Kappa number

- PHWE, FCCI period, alkali charge difference and cooking period showed a lower Kappa number. PHWE removed the extractives, FCCI broke down the lignin structure making chemical degradation easier on the more open structure of the chip surface. This phenomenon would also be of significant benefit for high brightness bleaching.

Handsheet brightness

- Most of the treatments showed an improved unbleached handsheet brightness, which underlined the beneficial effect of both PHWE and FCCI.

Handsheet strength development

- It is evident that PHWE altered the fibre surface properties giving it a slightly more hydrophobic character, thus negatively influencing fibre hydration during beating and resulting in lesser inter fibre bonding surfaces. This investigation confirmed the beneficial effects of fungal pretreatment of wood chips with co-cultures on paper strengths properties. The combined effect of PHWE and FCCI of wood chips before pulping resulted in the highest handsheet strength properties. This combined treatment also improved the initial bonding strength potential of unbeaten fibres and pulp reached their full beating potential within the allowed beating time.

  The handsheet strength development potential of the fungal inoculated material with a reduced incubation time and pulped with a reduced AA level, showed a noticeable decline.

  The PHWe chip material incubated for a shorter period did not show the same handsheet strength development. This clearly demonstrated that the duration of the incubation period plays an important role in the activation of cell wall fibrillation by the inoculated lignolytic fungal cultures. The increased rate of freeness development of pulps obtained from fungal treated wood chips can be attributed to enhanced fibre

swelling, fibrillation and flexibility. These phenomena demonstrated that strength properties can be developed better at a reduced refining energy.

Macro-and microscopic appearance of wood chips

- PHWE produced a darker coloured wood chip indicating that oxidation reactions occurred during the extraction period. Also the wood chip surface felt semi-soft and had a fluffy appearance. The scanning electron micrographs clearly illustrated the changed appearance of the chip cross-sectional area after the various chip pretreatments.

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APPENDIX A: TABULATED RESEARCH DATA

Table 1: Soda-AQ pulping of unextracted *Eucalyptus grandis* wood chips without FCCI at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	48	13	1.7	1.43	20
2	47	12	2.2	1.26	22
3	45.2	16	1.9	1.49	21
Average	**46.7**	**13.7**	**2.1**	**1.39**	**21**

Table 2: Soda-AQ pulping of extracted *Eucalyptus grandis* wood chips without FCCI at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	46.5	0.22	0.6	1.55	13
2	44	0.29	1.1	2.91	17
3	49	0.12	0.11	2.79	11
Average	**46.50**	**0.21**	**0.60**	**1.39**	**13.67**

Table 3: Soda-AQ pulping of unextracted, inoculated *Eucalyptus grandis* wood chips at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	50	7.1	2.2	2.05	19
2	43	3.3	1.8	4.34	15
3	42	4.2	2.3	4.22	20
Average	**45.00**	**4.90**	**2.10**	**3.54**	**18.00**

Table 4: Soda-AQ pulping of extracted, inoculated *Eucalyptus grandis* wood chips at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	44	0.0	0.2	4.91	12
2	40	0.0	0.1	4.34	11
3	41	0.0	0.1	4.19	14
Average	**42.00**	**0.00**	**0.13**	**4.48**	**12.33**

Table 5: Soda-AQ pulping of extracted, inoculated *Eucalyptus grandis* wood chips cooked for 20 minutes at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	44	0.2	0.2	3.2	16.2
2	44	0.7	0.6	2.6	16.9
3	41	6.1	0.7	2.5	17
Average	**43.00**	**2.30**	**0.50**	**2.80**	**16.70**

Table 6: Soda-AQ pulping of extracted, inoculated (3 weeks) *Eucalyptus grandis* wood chips cooked for 20 minutes at 15 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	40	0.26	0.16	4.98	12.20
2	44	0.47	0.15	4.38	14.9
3	41	0.65	0.15	4.53	15.3
Average	**42.00**	**0.46**	**0.15**	**4.63**	**14.13**

Table 7: Soda-AQ pulping of extracted, inoculated (3weeks) *Eucalyptus grandis* wood chips at 14 % NaOH and 1% AQ.

Sample	Screened Yield (%)	Rejects (%)	Shives (%)	RAA (g/L)	Kappa number
1	42	1.5	0.9	3.8	21
2	44	0.8	0.5	3.9	18.3
3	43	1.4	1.1	4.0	20.3
Average	**43.00**	**0.46**	**0.15**	**3.90**	**19.87**

Table 8: Handsheet brightness results (% ISO) for all *Eucalyptus grandis* Soda-AQ pulping trials.

Sample	Unextracted 15% AA + 1% AQ	Extracted 15% AA + 1% AQ	Unextracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks) 20 min cooking	Extracted inoculated 15% AA + 1% AQ (3 weeks)	Extracted inoculated 14% AA + 1% AQ (3 weeks)
1	35.27	41.47	35.27	37.24	33.49	46.03	35.16
2	34.05	40.41	34.05	36.22	33.36	45.01	34.97
3	32.72	40.50	32.72	36.22	34.70	42.68	37.11
4	34.63	39.57	34.63	34.98	32.09	41.39	34.95
Average	**31.84**	**40.49**	**34.17**	**36.16**	**33.41**	**43.78**	**35.55**

Table 9: Burst index ($KPa.m^2/g$) of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips at 38 ^{0}SR.

Sample	Unextracted 15% AA + 1% AQ	Extracted 15% AA + 1% AQ	Unextracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks) 20 min cooking	Extracted inoculated 15% AA + 1% AQ (3 weeks)	Extracted inoculated 14% AA + 1% AQ (3 weeks)
1	2.7	2.40	4.44	3.48	3.4	4.66	2.91
2	2.9	2.60	4.56	3.46	3.45	4.53	3.29
3	2.8	2.80	4.52	3.49	**3.1**5	4.35	3.40
Average	**2.8**	**2.6**	**4.51**	**3.48**	**3.3**	**4.5**	**3.2**

Table 10: Tear index ($mN.m^2/g$) of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips at 38 ^{0}SR**.**

Sample	Unextracted 15% AA + 1% AQ	Extracted 15% AA + 1% AQ	Unextracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks) 20 min cooking	Extracted inoculated 15% AA + 1% AQ (3 weeks)	Extracted inoculated 14% AA + 1% AQ (3 weeks)
1	2.9	5.33	5.81	5.00	5.30	5.45	4.90
2	2.7	5.36	5.66	5.20	5.00	5.30	5.15
3	2.8	5.36	5.63	5.16	5.06	5.78	5.70
Average	**2.80**	**5.35**	**5.70**	**5.12**	**5.12**	**5.51**	**5.25**

Table 11: Breaking length (Km) of unextracted, extracted, unextracted inoculated and extracted inoculated wood chips at 38 ^{0}SR.

Sample	Unextracted 15% AA + 1% AQ	Extracted 15% AA + 1% AQ	Unextracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks)	Extracted inoculated 15% AA + 1% AQ (4 weeks) 20 min cooking	Extracted inoculated 15% AA + 1% AQ (3 weeks)	Extracted inoculated 14% AA + 1% AQ (3 weeks)
1	6.02	5.84	7.30	6.47	5.58	7.33	6.73
2	5.90	5.85	7.31	6.23	5.62	7.28	6.66
3	6.08	5.86	7.30	6.23	5.60	7.33	6.72
Average	**6.00**	**5.85**	**7.30**	**6.31**	**5.60**	**7.35**	**6.70**

Table 12: Handsheet strength (burst index) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	65.00	0.78	105.00	1.24	190.00	2.24	260.00	3.11
	65.00	0.78	205.00	2.42	175.00	2.07	320.00	3.83
	95.00	1.13	110.00	1.30	160.00	1.89	295.00	3.53
	80.00	0.95	120.00	1.42	200.00	2.36	350.00	4.19
	55.00	0.66	135.00	1.59	275.00	3.25	355.00	4.25
	75.00	0.90	180.00	2.13	245.00	2.89	320.00	3.83
	20.00	0.24	135.00	1.59	300.00	3.54	280.00	3.35
	35.00	0.42	135.00	1.59	215.00	2.54	335.00	4.01
	60.00	0.72	135.00	1.59	195.00	2.30	260.00	3.11
	35.00	0.42	155.00	1.83	170.00	2.01	360.00	4.31
Average		**0.70**		**1.67**		**2.51**		**3.75**
Std Dev		**0.26**		**0.35**		**0.52**		**0.43**

Table 13: Handsheet strength (tear index) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (mN)	**mN**	**mN.m^2/g**	**1min (mN)**	**mN**	**mN.m^2/g**	**2min (mN)**	**mN**	**mN.m^2/g**	**3min (mN)**	**mN**	**mN.m^2/g**
	18.00	282.44	3.37	30.00	470.74	5.56	39.00	611.96	7.23	50.00	784.56	9.39
	9.00	141.22	1.69	14.00	219.68	2.60	17.00	266.75	3.15	23.00	360.90	4.32
	10.00	156.91	1.87	15.00	235.37	2.78	20.00	313.82	3.71	23.00	360.90	4.32
Average			**2.31**			**3.65**			**4.69**			**6.01**
Std Dev			**0.75**			**1.36**			**1.81**			**2.39**

Table 14: Handsheet strength (breaking length) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	2.40	2.92	3.73	4.50	4.93	5.94	7.20	8.79
	1.60	1.95	2.80	3.37	3.93	4.74	6.40	7.81
	0.93	1.14	3.47	4.18	4.80	5.78	6.40	7.81
	1.07	1.30	4.27	5.14	5.07	6.10	6.67	8.14
	1.07	1.30	4.27	5.14	3.87	4.66	5.60	6.84
	1.33	1.62	2.93	3.53	3.93	4.74	6.67	8.14
	1.20	1.46	3.20	3.86	5.47	6.59	6.53	7.98
	1.20	1.46	2.53	3.05	3.87	4.66	6.67	8.14
	1.93	2.35	3.33	4.02	4.40	5.30	7.47	9.12
	1.07	1.30	2.00	2.41	4.93	5.94	7.33	8.95
Average		**1.68**		**3.92**		**5.45**		**8.17**
Std Dev		**0.54**		**0.83**		**0.68**		**0.63**

Table 15: Handsheet strength (burst index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	40.00	0.51	60.00	0.77	200.00	2.38	185.00	2.24
	25.00	0.32	70.00	0.90	180.00	2.14	200.00	2.43
	30.00	0.38	55.00	0.71	200.00	2.38	195.00	2.36
	35.00	0.45	45.00	0.58	175.00	2.09	240.00	2.91
	25.00	0.32	90.00	1.16	175.00	2.09	165.00	2.00
	35.00	0.45	50.00	0.64	180.00	2.14	220.00	2.67
	45.00	0.57	60.00	0.77	175.00	2.09	225.00	2.73
	35.00	0.45	50.00	0.64	235.00	2.80	240.00	2.91
	30.00	0.38	70.00	0.90	205.00	2.44	215.00	2.61
	35.00	0.45	55.00	0.71	210.00	2.50	280.00	3.40
Average		**0.43**		**0.78**		**2.31**		**2.63**
Std Dev		**0.08**		**0.16**		**0.23**		**0.38**

Table 16: Handsheet strength (tear index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (mN)	**mN**	**mN.m^2/ g**	**1min (mN)**	**mN**	**mN.m^2/ g**	**2min (mN)**	**mN**	**mN.m^2/ g**	**3min (mN)**	**mN**	**mN.m^2/ g**
	12.00	188.29	2.40	20.00	313.82	4.05	33.00	517.81	6.17	41.00	643.34	7.80
	7.00	109.84	1.40	9.00	141.22	1.82	15.00	235.37	2.80	20.00	313.82	3.81
	5.00	78.46	1.00	12.00	188.29	2.43	15.00	235.37	2.80	18.00	282.44	3.43
Average			**1.60**			**2.76**			**3.93**			**5.01**
Std Dev			**0.59**			**0.94**			**1.59**			**1.98**

Table 17: Handsheet strength (breaking length) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips without FCCI at 15% NaOH and 1% AQ.

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.07	1.39	2.00	2.63	3.73	4.54	4.67	5.77
	1.20	1.56	2.13	2.81	5.20	6.32	4.93	6.10
	0.93	1.21	1.93	2.54	3.73	4.54	4.40	5.44
	1.47	1.91	2.07	2.72	4.27	5.19	3.87	4.78
	1.33	1.74	1.53	2.02	3.93	4.78	4.33	5.36
	1.47	1.91	1.47	1.93	3.73	4.54	4.67	5.77
	1.33	1.74	1.40	1.84	4.93	6.00	4.40	5.44
	1.33	1.74	2.00	2.63	3.93	4.78	5.20	6.43
	1.33	1.74	2.40	3.16	4.00	4.86	4.67	5.77
	1.40	1.82	1.40	1.84	5.20	6.32	5.20	6.43
Average		**1.67**		**2.41**		**5.19**		**5.73**
Std Dev		**0.21**		**0.44**		**0.70**		**0.48**

Table 18: Handsheet strength (burst index) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	60.00	0.75	250.00	3.17	300.00	3.81	390.00	4.87
	60.00	0.75	275.00	3.48	370.00	4.70	400.00	4.99
	40.00	0.50	250.00	3.17	400.00	5.08	380.00	4.74
	70.00	0.87	230.00	2.91	380.00	4.83	360.00	4.49
	45.00	0.56	225.00	2.85	330.00	4.19	400.00	4.99
	60.00	0.75	100.00	1.27	370.00	4.70	410.00	5.11
	60.00	0.75	135.00	1.71	340.00	4.32	340.00	4.24
	55.00	0.68	170.00	2.15	300.00	3.81	360.00	4.49
	70.00	0.87	105.00	1.33	370.00	4.70	420.00	5.24
	50.00	0.62	240.00	3.04	380.00	4.83	390.00	4.87
Average		**0.71**		**2.51**		**4.50**		**4.80**
Std Dev		**0.12**		**0.78**		**0.42**		**0.30**

Table 19: Handsheet strength (tear index) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (mN)	**mN**	**mN.m^2/g**	**1min (mN)**	**mN**	**mN.m^2/g**	**2min (mN)**	**mN**	**mN.m^2/g**	**3min (mN)**	**mN**	**mN.m^2/g**
	22.00	345.21	4.29	39.00	611.96	7.75	42.00	659.03	8.37	44.00	690.41	8.61
	10.00	156.91	1.95	17.00	266.75	3.38	22.00	345.21	4.38	23.00	360.90	4.50
	9.00	141.22	1.75	16.00	251.06	3.18	20.00	313.82	3.99	20.00	313.82	3.91
Average			**2.66**			**4.77**			**5.58**			**5.68**
Std Dev			**1.15**			**2.11**			**1.98**			**2.09**

Table 20: Handsheet strength (breaking length) of Soda-AQ pulp of unextracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.20	1.52	4.53	5.86	3.93	5.10	6.53	8.31
	1.20	1.52	4.27	5.51	5.47	7.08	6.93	8.82
	1.73	2.20	4.13	5.34	3.93	5.10	6.40	8.14
	1.87	2.37	5.07	6.54	5.87	7.60	5.60	7.13
	1.60	2.03	4.27	5.51	5.87	7.60	5.73	7.30
	1.60	2.03	3.93	5.08	5.60	7.25	7.13	9.08
	1.60	2.03	2.93	3.79	6.00	7.77	7.60	9.67
	1.80	2.28	3.33	4.31	5.07	6.56	4.93	6.28
	1.87	2.37	2.93	3.79	8.00	10.36	5.47	6.96
	1.47	1.86	2.80	3.62	5.47	7.08	6.73	8.57
Average		**2.02**		**4.93**		**7.15**		**8.02**
Std Dev		**0.29**		**0.95**		**1.41**		**1.02**

Table 21: Handsheet strength (burst index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	70.00	0.86	192.00	2.28	290.00	3.73	400.00	5.46
	78.00	0.96	226.00	2.68	210.00	2.70	330.00	4.50
	40.00	0.49	157.00	1.86	280.00	3.60	310.00	4.23
	60.00	0.74	224.00	2.66	250.00	3.21	350.00	4.77
	55.00	0.67	150.00	1.78	230.00	2.96	440.00	6.00
	60.00	0.74	180.00	2.14	285.00	3.66	330.00	4.50
	75.00	0.92	195.00	2.32	220.00	2.83	330.00	4.50
	73.00	0.89	171.00	2.03	230.00	2.96	340.00	4.64
	120.00	1.47	135.00	1.60	315.00	4.05	390.00	5.32
	70.00	0.86	159.00	1.89	175.00	2.25	370.00	5.05
Average		**0.86**		**2.12**		**3.19**		**4.90**
Std Dev		**0.24**		**0.34**		**0.53**		**0.52**

Table 22: Handsheet strength (tear index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (mN)	**mN**	**mN.m^2/ g**	**1min (mN)**	**mN**	**mN.m^2/ g**	**2min (mN)**	**mN**	**mN.m^2/ g**	**3min (mN)**	**mN**	**mN.m^2/ g**
	20.00	313.82	3.85	36.00	564.88	6.71	39.00	611.96	7.87	46.00	721.80	9.85
	6.00	94.15	1.15	12.00	188.29	2.24	14.00	219.68	2.82	18.00	282.44	3.85
	10.00	156.91	1.92	17.00	266.75	3.17	20.00	313.82	4.03	22.00	345.21	4.71
Average			**2.31**			**4.04**			**4.91**			**6.14**
Std Dev			**1.13**			**1.93**			**2.15**			**2.65**

Table 23: Handsheet strength (breaking length) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ.

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.60	2.00	3.20	3.88	3.60	4.72	5.93	8.26
	2.00	2.50	3.87	4.68	3.47	4.54	5.60	7.79
	1.73	2.17	5.00	6.06	5.33	6.99	4.60	6.40
	1.93	2.42	3.60	4.36	4.53	5.94	5.20	7.24
	2.13	2.67	4.40	5.33	4.93	6.47	6.93	9.65
	2.00	2.50	3.60	4.36	5.80	7.60	5.93	8.26
	2.47	3.08	4.40	5.33	4.93	6.47	6.00	8.35
	1.93	2.42	3.60	4.36	4.33	5.68	5.67	7.89
	1.67	2.08	3.73	4.52	4.13	5.42	5.20	7.24
	2.00	2.50	3.60	4.36	3.87	5.07	6.07	8.44
Average		**2.43**		**4.72**		**5.89**		**7.95**
Std Dev		**0.30**		**0.62**		**0.95**		**0.83**

Table 24: Handsheet strength (burst index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced cooking time (20min).

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	145.00	1.87	190.00	2.37	245.00	3.10	335.00	4.00
	105.00	1.36	255.00	3.18	228.00	2.88	420.00	5.02
	130.00	1.68	175.00	2.18	225.00	2.84	380.00	4.54
	135.00	1.75	200.00	2.49	258.00	3.26	320.00	3.83
	129.00	1.67	240.00	2.99	222.00	2.81	495.00	5.92
	98.00	1.27	211.00	2.63	258.00	3.26	520.00	6.22
	140.00	1.81	199.00	2.48	235.00	2.97	480.00	5.74
	105.00	1.36	192.00	2.39	261.00	3.30	540.00	6.45
	125.00	1.62	206.00	2.57	263.00	3.32	560.00	6.69
	138.00	1.78	220.00	2.74	240.00	3.03	520.00	6.22
Average		**1.62**		**2.60**		**3.08**		**5.46**
Std Dev		**0.20**		**0.28**		**0.19**		**0.99**

Table 25: Handsheet strength (tear index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced cooking time (20min).

	0min (mN)	**mN**	**mN.m^2/g**	**1min (mN)**	**mN**	**mN.m^2/g**	**2min (mN)**	**mN**	**mN.m^2/g**	**3min (mN)**	**mN**	**mN.m^2/g**
	28.00	439.35	5.68	39.00	611.96	7.63	41.00	643.34	8.13	54.00	847.32	10.25
	12.00	188.29	2.43	16.00	251.06	3.13	18.00	282.44	3.57	25.00	392.28	4.75
	12.00	188.29	2.43	20.00	313.82	3.91	20.00	313.82	3.97	24.00	376.59	4.56
Average			**3.52**			**4.89**			**5.22**			**6.52**
Std Dev			**1.53**			**1.96**			**2.06**			**2.64**

Table 26: Handsheet strength (breaking length) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced cooking time (20min).

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.93	2.55	4.53	5.77	3.87	4.98	8.80	10.86
	2.33	3.08	3.47	4.41	3.73	4.81	6.13	7.57
	2.13	2.81	4.00	5.09	3.73	4.81	8.67	10.69
	1.87	2.46	4.13	5.26	4.53	5.84	7.93	9.79
	1.87	2.46	4.53	5.77	4.40	5.67	7.53	9.30
	1.87	2.46	4.53	5.77	4.00	5.16	7.73	9.54
	1.93	2.55	3.87	4.92	4.00	5.16	7.60	9.38
	1.67	2.20	3.80	4.83	4.53	5.84	6.53	8.06
	2.00	2.64	3.73	4.75	4.53	5.84	6.27	7.73
	2.00	2.64	4.13	5.26	3.93	5.07	8.53	10.53
Average		**2.58**		**5.18**		**5.32**		**9.35**
Std Dev		**0.22**		**0.45**		**0.41**		**1.15**

Table 27: Handsheet strength (burst index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	70.00	0.84	140.00	1.76	440.00	5.48	550.00	6.53
	73.00	0.88	255.00	3.21	430.00	5.36	450.00	5.34
	45.00	0.54	190.00	2.39	390.00	4.86	488.00	5.79
	55.00	0.66	167.00	2.10	405.00	5.05	500.00	5.93
	60.00	0.72	134.00	1.69	410.00	5.11	550.00	6.53
	45.00	0.54	112.00	1.41	420.00	5.23	540.00	6.41
	50.00	0.60	142.00	1.79	410.00	5.11	490.00	5.81
	70.00	0.84	280.00	3.53	360.00	4.49	450.00	5.34
	40.00	0.48	215.00	2.71	420.00	5.23	533.00	6.32
	52.00	0.63	232.00	2.92	360.00	4.49	520.00	6.17
Average		**0.68**		**2.35**		**5.04**		**6.02**
Std Dev		**0.13**		**0.68**		**0.32**		**0.42**

Table 28: Handsheet strength (tear index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (mN)	**mN**	**mN.m^2/ g**	**1min (mN)**	**mN**	**mN.m^2/ g**	**2min (mN)**	**mN**	**mN.m^2/ g**	**3min (mN)**	**mN**	**mN.m^2/ g**
	17.00	266.75	3.22	33.00	517.81	6.52	47.00	737.49	9.19	69.00	1082.69	12.84
	7.00	109.84	1.32	13.00	203.99	2.57	22.00	345.21	4.30	29.00	455.04	5.40
	8.00	125.53	1.51	15.00	235.37	2.97	24.00	376.59	4.69	26.00	407.97	4.84
Average			**2.02**			**4.02**			**6.06**			**7.69**
Std Dev			**0.85**			**1.78**			**2.22**			**3.65**

Table 29: Handsheet strength (breaking length) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 15% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.47	1.80	4.53	5.83	5.40	6.86	8.33	10.08
	1.60	1.97	2.13	2.74	6.80	8.64	8.47	10.25
	1.20	1.48	2.00	2.57	6.73	8.56	8.67	10.49
	1.20	1.48	4.47	5.74	6.53	8.30	8.53	10.33
	1.20	1.48	2.33	3.00	6.87	8.73	8.47	10.25
	1.27	1.56	4.80	6.17	6.00	7.63	9.47	11.46
	1.33	1.64	4.60	5.91	6.07	7.71	8.33	10.08
	0.80	0.98	3.27	4.20	6.47	8.22	8.00	9.68
	0.80	0.98	2.93	3.77	6.53	8.30	8.33	10.08
	1.07	1.31	2.67	3.43	6.80	8.64	7.87	9.52
Average		**1.47**		**4.34**		**8.16**		**10.22**
Std Dev		**0.30**		**1.37**		**0.56**		**0.49**

Table 30: Handsheet strength (burst index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 14% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (KPa)	**KPa.m^2/g**	**1min (KPa)**	**KPa.m^2/g**	**2min (KPa)**	**KPa.m^2/g**	**3min (KPa)**	**KPa.m^2/g**
	70.00	0.85	121.00	1.44	350.00	4.08	240.00	2.69
	64.00	0.78	105.00	1.25	335.00	3.90	370.00	4.15
	63.00	0.77	101.00	1.21	338.00	3.94	200.00	2.25
	58.00	0.71	134.00	1.60	320.00	3.73	330.00	3.70
	50.00	0.61	65.00	0.78	338.00	3.94	340.00	3.82
	52.00	0.63	105.00	1.25	360.00	4.19	390.00	4.38
	50.00	0.61	90.00	1.07	285.00	3.32	397.00	4.46
	58.00	0.71	87.00	1.04	330.00	3.84	380.00	4.27
	60.00	0.73	106.00	1.27	260.00	3.03	310.00	3.48
	50.00	0.61	82.00	0.98	330.00	3.84	397.00	4.46
Average		**0.70**		**1.19**		**3.78**		**3.77**
Std Dev		**0.08**		**0.22**		**0.33**		**0.73**

Table 31: Handsheet strength (tear index) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 14% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (mN)	**mN**	**mN.m^2/g**	**1min (mN)**	**mN**	**mN.m^2/g**	**2min (mN)**	**mN**	**mN.m^2/g**	**3min (mN)**	**mN**	**mN.m^2/g**
	30.00	470.74	5.75	31.00	486.43	5.81	46.00	721.80	8.41	50.00	784.56	8.81
	10.00	156.91	1.92	12.00	188.29	2.25	23.00	360.90	4.20	24.00	376.59	4.23
	10.00	156.91	1.92	14.00	219.68	2.62	22.00	345.21	4.02	23.00	360.90	4.05
Average			**3.19**			**3.56**			**5.54**			**5.70**
Std Dev			**1.81**			**1.60**			**2.03**			**2.20**

Table 32: Handsheet strength (breaking length) of Soda-AQ pulp of extracted *Eucalyptus grandis* wood chips with FCCI at 14% NaOH and 1% AQ at reduced incubation time (3 weeks).

	0min (KN/m)	**Km**	**1min (KN/m)**	**Km**	**2min (KN/m)**	**Km**	**3min (KN/m)**	**Km**
	1.60	1.99	2.73	3.33	7.07	8.40	7.27	8.32
	1.87	2.32	2.53	3.08	6.53	7.76	5.93	6.79
	1.80	2.24	2.20	2.68	7.73	9.19	6.53	7.48
	1.67	2.07	2.73	3.33	6.13	7.29	6.67	7.63
	1.93	2.41	2.80	3.41	6.00	7.13	6.93	7.94
	1.73	2.16	2.53	3.08	5.67	6.73	7.60	8.70
	1.27	1.58	2.00	2.43	6.33	7.53	6.93	7.94
	1.47	1.83	2.60	3.17	5.67	6.73	6.13	7.02
	1.27	1.58	2.13	2.60	6.93	8.24	6.00	6.87
	1.53	1.91	3.00	3.65	6.00	7.13	6.27	7.18
Average		**2.01**		**3.08**		**7.61**		**7.59**
Std Dev		**0.27**		**0.37**		**0.75**		**0.61**