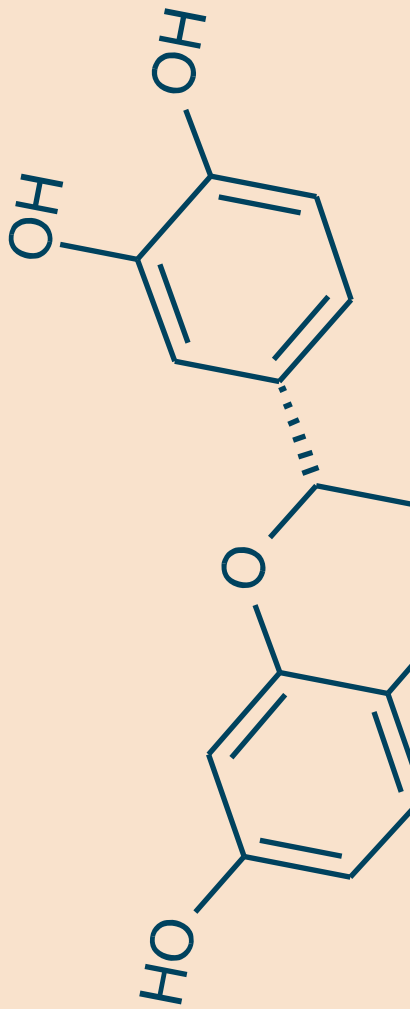


ENZOGENOL®



A natural extract of *Pinus radiata* pine bark containing a balanced blend of antioxidants including monomeric and oligomeric proanthocyanidins, organic acids, flavonoids, glycosides and esters.

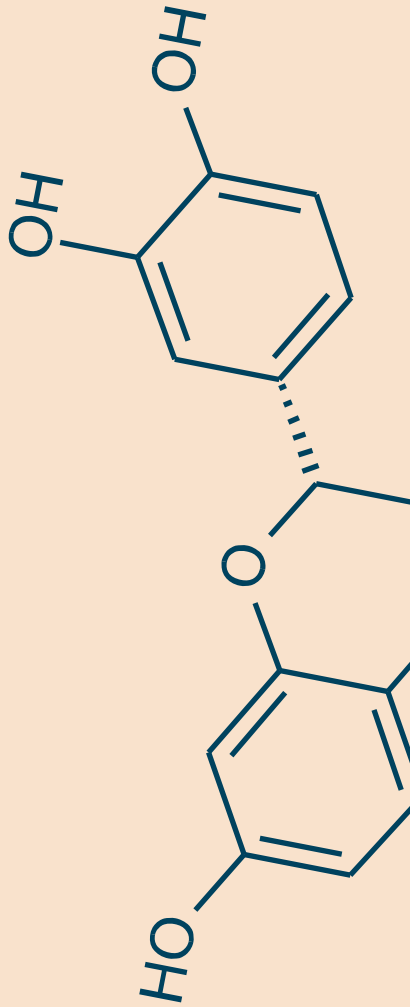


ENZOGENOL® is a registered trademark of ENZO Nutraceuticals Ltd

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ENZOGENOL®



ENZOGENOL® - an overview

The raw material source

The extraction process

Composition of ENZOGENOL® and other branded extracts

The effect of acidic and basic conditions on the molecular distribution of ENZOGENOL®

Antioxidant activity

- The peroxy radical scavenging antioxidant assay
- The superoxide inhibitory assay

Safety trials with ENZOGENOL®

- Safety
- Alpha and beta effects

Human clinical evaluation

Chromatographic studies

The naturally occurring dietary antioxidants

The higher proanthocyanidin

Manufacturing quality control summary

Product quality control summary

Specification data sheets

ENZOGENOL® -AN OVERVIEW

In the early 1990s a talented multi-disciplined team was assembled at the University of Canterbury to focus on developing more effective natural antioxidants. The team recognized the serious shortcomings of the up to then conventional methods which utilized toxic solvents to extract compounds.

Their early work convinced them that these conventional processes left behind certain important water soluble antioxidants, created solvent residues and were also frankly environmentally unsound.

The result of their quest was the creation of a totally new process using *only* pure water which for the first time could extract the full arsenal of naturally occurring antioxidants and concentrate them in a superior way using molecular selection. Patents have been granted in a number of countries for this breakthrough technology.

A new processing plant was constructed utilizing the pure water process and drawing on the valuable insights that had been gained in the laboratories. The hi-tech plant was located near an identified source of the bark from young fast growing *Pinus radiata* trees. This particular pine bark not only was a standardized source of organic material, but research had confirmed it was a unique and superior source of proanthocyanidins and flavonoids when compared to all other sources-including other pine barks.

The result of these efforts is ENZOGENOL® a new highly active antioxidant that contains improved levels of important proanthocyanidins and also incorporates other significant antioxidants that are lost in conventional solvent based processing.

Because the process uses only pure water there is no negative downstream impact on the environment - in fact the residual bark is an excellent clean organic mulch which is being used to grow vegetable crops.

In addition to capturing the monomeric and oligomeric proanthocyanidins the unique water only process is able to extract water soluble organic acids, flavonoid glycosides and esters that are proven antioxidants.

ENZOGENOL® is a balanced complex blend of water soluble proanthocyanidins and other proven naturally occurring dietary antioxidant compounds. Its general makeup is shown below:

PERCENT MASS *	
Monomeric and dimeric proanthocyanidins	8-10
Trimeric and tetrameric proanthocyanidins	10-12
Oligomeric and higher proanthocyanidins	64-66
Natural organic acids, flavonoids, glycosides, esters and sugars	14-16

* - determined using
HPLC (High Performance Liquid Chromatography),
GPC (Gel Permeation Chromatography),
TLC (Thin Layer Chromatography) and Total Phenol Analysis

THE EXTRACTION PROCESS

Following the success of the pilot plant a decision was made to build a world class production plant.

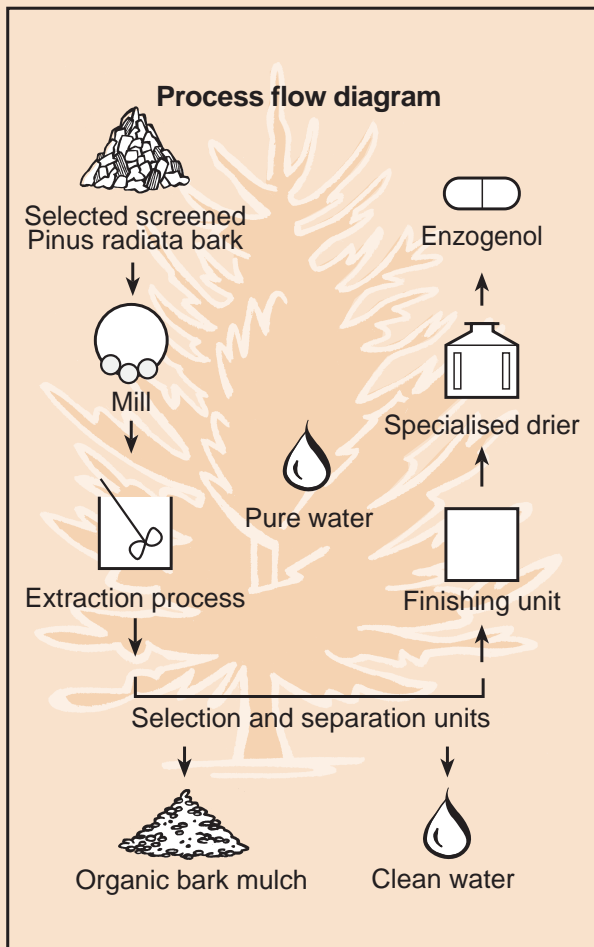
This plant was completed in 1996 and its operation has excelled in being able to fully utilise the pure water process and the molecular selection which makes ENZOGENOL[®] unique.

The simple goal is to consistently produce a biological extract with the very best specifications and antioxidant capability. The plant is run according to defined procedures with highly trained staff to ensure that each batch meets the highest standards. As well as excellent quality control which includes monitoring the make up of ENZOGENOL[®] using gel permeation chromatography, each batch is also assayed for antioxidant activity - ensuring that the manufacturer and the ultimate consumer of ENZOGENOL[®] receive full potency everytime.

THE RAW MATERIAL SOURCE

The bark is taken from age-selected fast-growing *Pinus radiata* pine trees which are plantation grown in sub-alpine conditions in the total absence of pesticides and synthetic fertilizers. New Zealand is located in the South Pacific thousands of kilometers away from heavy industrial pollution.

The trees are harvested for timber and at the debarking stage the clean bark is immediately transported to the nearby production facility thus ensuring freshness and eliminating any degradation of important compounds.



COMPOSITION OF ENZOGENOL[®] AND OTHER BRANDED EXTRACTS

All plant extracts are made up of many naturally occurring compounds. The range of compounds is related to the biological evolution of the source material, and these materials are not equal.

Over time this evolutionary process has also provided for the development of very complex compounds which provide sophisticated defence mechanisms for the plant. It is these compounds in particular that are important adjuncts in human health.

A well-balanced extract reflects the full spectrum of flavonoid compounds especially proanthocyanidins, across all ranges of molecular weight.

Pine bark extracts are important sources of the critical higher molecular weight proanthocyanidins. ENZOGENOL[®] is especially rich in these complex polymeric proanthocyanidins.

The bark from *Pinus radiata* undergoes a patented pure water and molecular selection extraction process which captures the whole range of naturally occurring compounds. Unlike many extraction processes that can break down or diminish chemical structures, the technique used to make ENZOGENOL[®] allows them to remain true to their natural state.

The following table shows the molecular weight distribution analysis of branded extracts using gel permeation chromatography

Compound type	Enzogenol [®]	Pine bark extract (PBE)	Grape seed extract 1 (GSE 1)	Grape seed extract 2 (GSE 2)	Green tea extract (GTE)
Carbohydrates, esters and organic acids	15%	33%	13%	13%	75%
Monomer, dimer and trimer proanthocyanidins	22%	27%	56%	44%	25%
Oligomeric proanthocyanidins	25%	22%	25%	30%	†
Polymeric proanthocyanidins	38%	18%	6%	13%	0%

† less than 1%

THE EFFECT OF ACIDIC AND BASIC CONDITIONS ON THE MOLECULAR DISTRIBUTION OF ENZOGENOL®

The aim of this study was to determine the fate of higher molecular weight proanthocyanidins (PAC's) under different biological conditions. No literature currently exists on how higher molecular weight PAC's react *in vivo*.

A reactor was set up to mimic stomach conditions. Acidic solutions of ENZOGENOL® were prepared using HCl (pH 2.5). Similarly a reactor was set up to mimic intestinal conditions. Basic solutions of ENZOGENOL® were prepared using NaHCO₃ (pH 8). Both reactors were maintained at 37°C. A control reactor was also set up using distilled water. The samples were analysed by using HPLC with a Size Exclusion Column (SEC) column and diode array detector.

Results

Higher molecular weight PAC's are converted to the smaller oligomeric PAC's over time in both acidic and basic conditions. This is demonstrated by the shift in retention time for ENZOGENOL® in acidic conditions from 4.2 - 6.5 minutes for the higher molecular weight PAC's to 8.6 - 9.5 minutes for oligomeric PAC's (table 1).

Base treated (pH 8) ENZOGENOL® also showed a shift towards lower molecular weight PAC's. However the rate of the depolymerisation reaction was slower in comparison to the acid treated ENZOGENOL®. ENZOGENOL® in distilled water was used as the control experiment.

Table 1: Retention Time (Molecular Size) of ENZOGENOL® in Acidic and Basic Conditions after 2 Hours Reaction Time

Condition	Retention time (min)	
	Higher Molecular Weight PAC's	Oligomeric PAC's
Control, Distilled Water	4.2 – 6.5	8.5
Acidic (pH 2.5)	nil	9.5
Basic (pH 8)	5.3	7.2 - 9.6

As food is digested in the stomach for approximately 2 hours, an additional experiment was carried out where the reactions in acidic and control conditions were continuously monitored over a period

Table 2 shows that the formation of oligomers from high molecular weight PAC's in stomach conditions (pH = 2.5) is a fast reaction. After the initial rapid changes in retention time, only slight changes in retention time were observed indicating that the further depolymerisation to smaller molecular weight compounds occurs at a slow rate.

Table 2: Retention Time (Molecular Size) of ENZOGENOL® in Acidic Conditions (pH 2.5) at Varying Time Intervals

Condition	Retention time (min)	
	Higher Molecular Weight PAC's	Oligomeric PAC's
Control, Distilled Water		
10 minutes	4.2 – 6.5	8.5
2.0 hours	4.2 – 6.5	8.5
Acidic (pH 2.5)		
10 minutes	nil	8.6
30 minutes	nil	8.6
1.0 hours	nil	8.7
1.5 hours	nil	9.5
2.0 hours	nil	9.5

Conclusions

The results clearly demonstrate the complete depolymerisation of higher molecular weight water-soluble PAC's to lower molecular weight oligomeric compounds in stomach conditions. The rapid formation of small flavonoid molecules can facilitate quick absorption by the digestive system.

ANTIOXIDANT ACTIVITY

An antioxidant is “any substance that when present in low concentrations compared to those of an oxidisable substrate, significantly delays or inhibits oxidation of that substrate” (Halliwell and Gutteridge, 1989).

Free radical research organisations throughout the world recognise that the only accurate way to measure the antioxidant ability of a substance is to measure the antioxidant protection of compounds from oxidation caused by free radicals.

The Porter method is sometimes used to analyse extracts and based on the measurement of coloured products formed during acid hydrolysis of the antioxidant. This method does not measure the damage of a substrate or the protection of the substrate from free radicals. It also does not detect the flavonoid monomers, and does not assess the biological activity of an extract.

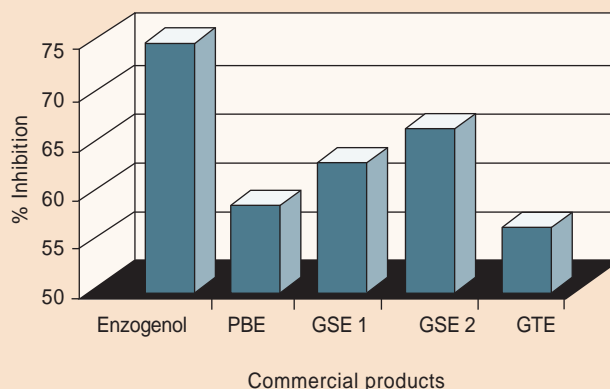
THE PEROXYL RADICAL SCAVENGING ANTIOXIDANT ASSAY

One of the most widely accepted scientific standard methods used to measure the antioxidant ability of different compounds is the Peroxyl Radical Scavenging Antioxidant assay. This assay is based on the procedure described by Pryor *et al* (1993) and measures the ability of a substance to inhibit the oxidation of a linoleic acid suspension by the free radical generating compound AAPH.

The AAPH radical generator reacts with oxygen to generate peroxy radicals which then initiate an oxidation chain reaction within the linoleic acid droplets. The rate of this oxidation reaction is monitored by measuring the formation of UV absorbing oxidised lipids. This assay measures the ability of these compounds to stop this lipid oxidation chain reaction. Such “chain-breaking” antioxidants inhibit the oxidation by reacting with and neutralising the reactive lipid intermediates.

In the following comparison assay, the stated compound was added to the reaction mixture to make the final concentration of 1µg/ml. The decrease in the rate of lipid oxidation that the test compounds caused is shown as a percentage of the control mixture where no inhibitor antioxidants were added. One hundred percent inhibition would indicate that the antioxidant was able to completely stop the chain reaction.

BRANDED EXTRACTS



Comparison of Antioxidant Activity using the Peroxyl Radical Scavenging Assay

REFERENCES

Halliwell, B and Gutteridge J.M.C (1989) Free Radicals in Biology and Medicine. 2nd Edition. Clarendon Press, Oxford

Pryor, W A, Cornicelli, J A, Devall, L J, Tait, B, Trivedi, B K, Witak, D T and Wu, M (1993) *J. Org. Chem.*, 58, (13), 3521-3531

THE SUPEROXIDE INHIBITORY ASSAY

Superoxide radicals are believed to be the most common free radicals formed within the body. This assay (known as the Nitro Blue Tetrazolium (NBT) assay) measures the ability of compounds to scavenge superoxide radicals using colorimetric enzymatic techniques.

In this assay water soluble NBT reacts to form a blue/purple tetrazolium salt when hypoxanthine is oxidised by xanthine oxidase. This oxidation reaction is inhibited by the antioxidants added resulting in less of the purple/blue salt being formed. The absorption of visible light of these tetrazolium salts is measured at 550 nanometres.

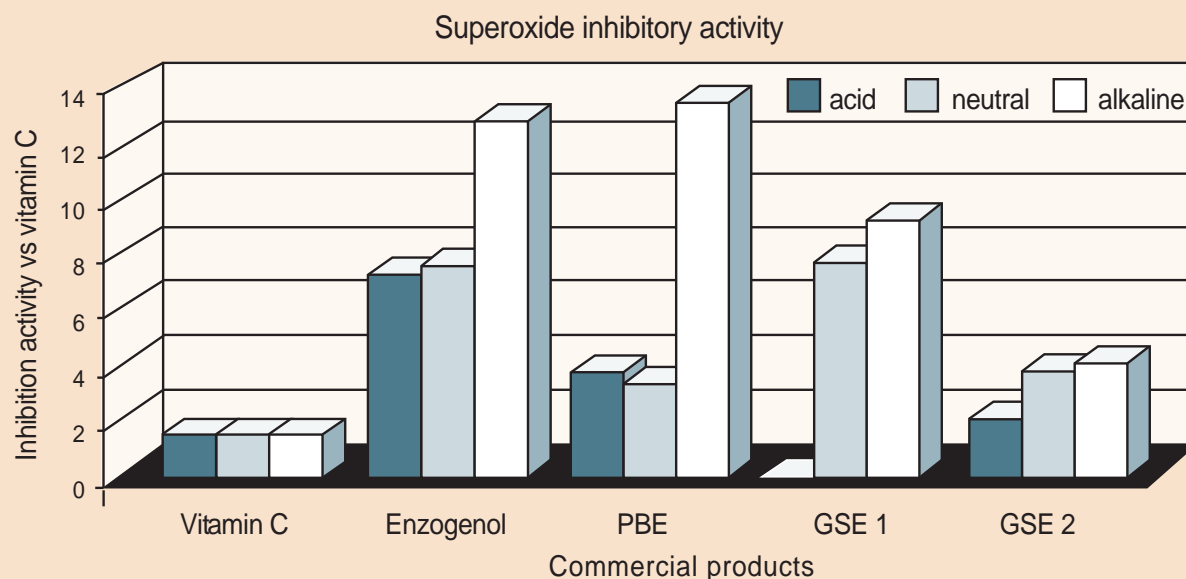
The scavenging activities of different branded antioxidants were conducted in acid and basic conditions that is representative of those encountered following ingestion. Neutral conditions were also examined.

The lower the concentration of antioxidant needed to reach the 50% enzyme inhibition (EC_{50}) level the more effective the substance is as an antioxidant.

The enzyme inhibition values can be standardised against Vitamin C as shown in the graph below. The greater the value the more effective the antioxidant activity.

A balanced extract requires a range of compounds to be active in a variety of sites and under conditions from acidic through to alkaline. ENZOGENOL[®] contains well over 2,000 compounds providing strong antioxidant activity throughout required conditions.

Comparison of Antioxidant Activity using the Superoxide Inhibitory Assay



REFERENCE

Sun, Y, Oberley, LW, Li, Y (1988) *Clin. Chem.* 34/3, 497-500

SAFETY TRIALS WITH ENZOGENOL®

A study was conducted over an 8 month period to discover the effect of ENZOGENOL® on mice. Their general condition, disease state and behaviour were closely observed daily. The primary aims of this study was to determine the effect of ENZOGENOL® on:

- Acute toxicity (if any)
- Chronic toxicity (if any)
- Incidence of tumours (if any) [This was a tumour prone variety of mice].
- Longevity

Mice were randomly allocated within sex to cages. The mice were divided into 3 groups, and were fed *ad libitum* on normal mouse food (Archer's Mills mouse pellets) with additional ENZOGENOL® as outlined below.

- Controls - no ENZOGENOL®
- NHD - Normal Human Dose equivalents of ENZOGENOL® adjusted for the difference in body mass
- 100NHD - 100 times Normal Human Dose. If ENZOGENOL® were toxic half the mice would die in about three days.

RESULTS

No acute or chronic toxicity was evident in this trial. Mice fed on ENZOGENOL® gained thicker, glossier fur; and their eyes became brighter, and their noses and tails pinker. They appeared to be more social with fewer negative interactions (biting etc neighbours). The controls were invariably third and last. Independent observers when asked to pick the healthiest looking mice all chose the 100 NHD group first followed by the NHD group.

Tumour and death rates were examined during this trial with the following results:

SURVIVAL RATES AND DEATHS

	Percent with 1 or more tumours	Percent dead due to tumours	Percent dead due to old age	Percent alive after 8 months
Control	50%	40%	20%	40%
NHD	40%	20%	10%	70%
100 NHD	13%	13%	13%	73%

From these results it can be seen that:

- Longevity in this trial nearly doubled with ENZOGENOL® (from 40% without ENZOGENOL® to 73% with ENZOGENOL®).
- The rate of tumour incidence decreased in a dose related manner with ENZOGENOL® (from 50 % without ENZOGENOL® to 13% with ENZOGENOL®).

Statistical Significance

The statistical significance of these results was established by two approaches:

1. A standard Fisherian technique testing trends in linear proportions, Snedecor and Cochran (1980) Statistical Methods, 7th Edition]
2. Modern resampling methods.

The linear trend analysis showed the results were highly significant ($P < 0.0001$). The details of the Fisherian statistical analyses are summarised in the following table.

FISHERIAN ANALYSIS

	TUMOUR INCIDENCE	DEATH RATE
Z-score	-1.982*	1.208
Gradient	-0.00338	-0.00211
Standard error	-0.00170	0.00175

The analysis in the table above shows that the death rate is not significantly related to dose and that the tumour rate is significantly related to dose rate.

Given that the sample sizes are small, a better means of analysis is to use the technique of resampling.

RESAMPLING RESULTS

	TUMOUR RATE	LONGEVITY
Mean p-value	0.001***	0.021*

Thus the decrease in tumour rate is highly significant, and the longevity increase is significant.

By How Much?

The answer to this question is difficult to apply to human populations, but if the mouse results are reliable and if they serve as a good model for humans, then a normal dose of ENZOGENOL[®] increases the number of people who survive to age X by 1.825 times. In other words old age survivorship at age X is nearly doubled. This effect is not strongly dose dependent as indicated by the non-significant test for linear trends.

However, the tumour incidence rate is dose dependent. For the 1-times dose (NHD) the rate is reduced to 80% of the normal value, and for the 100-times NHD it is reduced to 26% of the normal value.

CONCLUSIONS

- There is no evidence of acute toxicity
- There is no evidence of chronic toxicity
- The incidence of tumours is strongly reduced by ENZOGENOL[®] in a dose-dependent manner
- The longevity is increased in a (perhaps) weakly dose-dependent manner

ALPHA AND BETA EFFECTS

There are numerous ways in which ENZOGENOL[®] affected the mice. These can be split up into 2 groups:

ALPHA (α) EFFECTS

This is the scavenging or neutralising of free radicals. These effects do not change the way the mice (or humans) feel. There are no immediate health, psychological or emotional benefits.

The antioxidant is taken in the expectation it will prevent disease, cancer, heart attacks etc. There is no obvious change. In the mice the decreased tumour rate and the increased longevity are alpha effects.

BETA (β) EFFECTS

These are the changes in health, psychological or emotional state that you do notice, or others can notice in you. In this case the antioxidant is affecting metabolic processes with consequent changes in the physical, emotional or psychological state.

In the mice trials it was found that their coats had improved, they exhibited better social behaviour. Psychological measurement indicated increased curiosity and alertness. These are beta effects.

In people, when they comment on their increased alertness, improved skin condition, less flu and colds, faster recovery from soft tissue damage, improvement in joint movements or increased ability to cope with stress they are talking of beta effects.

HUMAN CLINICAL EVALUATION

Evaluation of the Clinical, Biochemical, Haematological and Haemodynamic Effects of Enzogenol® in Healthy Older People

A twelve week, open-label study was conducted on 24 healthy subjects. The mean age of participants was 64 years with a range of 56 to 75 years. There were 14 males and 10 females. A daily dosage of 480 mgs per day was administered. Parameters were measured at base line, six weeks and 12 weeks.

The parameters measured were:

Clinical: height, weight, body mass index, % body fat, blood pressure

Biochemical:

Renal: plasma creatinine, urine albumin-creatinine ratio

Liver: plasma albumin, bilirubin, alkaline phosphotase, AST, ALT, GGT

Lipid: cholesterol, LDL, HDL, triglycerides, apoB, chol:HDL

Glycemic: plasma glucose

Red blood cell:

rheology: high shear rate viscosity, osmotic fragility, plasma viscosity

Haematology:

Hb, Hct, MCV, MCH, WBC count + differential, platelet count

Plethysmography:

forearm blood flow, hyperaemic response

SUMMARY OF RESULTS

Measure	Change	Mean Values		
		Baseline	6 weeks	12 weeks
Weight (kg)	↓	75.8	74.9 ***	74.5 ***
Body mass index (kg/m ²)	↓	26.1	25.8 **	25.7 ***
Waist (cm)	↓	90.4	88.9 *	89.3
Body fat (%)	↓	30.9	30.3 *	29.3 ***
Systolic BP (mmHg)	↓	130	123 **	123 **
Diastolic BP (mmHg)	-	78	77	76
Mean arterial pressure (mmHg)	↓	95	92	92 *
Heart rate (beats/min)	-	65	65	66
Plasma viscosity (mPa.s)	↓	1.64	NA	1.58 ***
Basal blood flow (ml/min/dL)				
left forearm	↑	1.98	NA	2.32 *
right forearm	↑	2.14		2.47 (p=0.10)
Hyperaemic blood flow				
left forearm	↑	22.9	NA	27.3 **
right forearm (control)	↑	2.04		2.51*

P value of comparison with baseline * < 0.05 ** < 0.01 *** < 0.001

- Lipid profile - no significant change in any parameters
- Glycaemic control - no significant change
- Haematology - no significant change in any parameters
- Renal function - no significant change in any parameters
- Liver function - no significant change except in plasma albumin and plasma bilirubin, changes were small and not of clinical significance

CHROMATOGRAPHIC STUDIES

Chromatography is a very common method that allows scientists to separate closely related components of a complex mixture.

ENZOGENOL[®]'s molecular composition was investigated using the preparative separation methods of ultrafiltration and gel permeation chromatography (GPC) together with the analytical separation techniques of thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Some of the compound structures within ENZOGENOL[®] were investigated using nuclear magnetic resonance spectroscopy (NMR) also.

PREPARATIVE GPC

Both size exclusion and adsorption mode GPC were used to fractionate ENZOGENOL[®]. Size exclusion mode GPC resulted in the high molecular weight compounds being excluded from the gels' pores which allows them to move through the column rapidly. These will elute before the smaller compounds which are held up in the pores. Adsorption mode GPC (with a solvent gradient) resulted in a much improved separation of the lower molecular weight compounds (<1000 amu).

ANALYTICAL TLC *with selective visualisation reagents*

Preparative GPC fractions were then examined by silica TLC using iodine vapour and vanillin/HCl visualisation methods. The resulting thin layer chromatograms provided reproducible qualitative evidence for proanthocyanidin monomers, dimers, trimers, tetramers and higher oligomers as described in prior references.

ANALYTICAL HPLC *with diode array UV (190-400 nm) detection*

A reproducible reverse phase gradient separation method was used to characterise components by relative retention time and diode array UV spectrum. Known compounds were identified by both retention time and UV spectral comparison with commercial standards using this system.

ENZOGENOL[®] proanthocyanidins absorb strongly at 280 nm while other important antioxidant components are detected at 254 or 330 nm. The absorbances at each of these wavelengths are displayed in the following figures.

Fig 1a

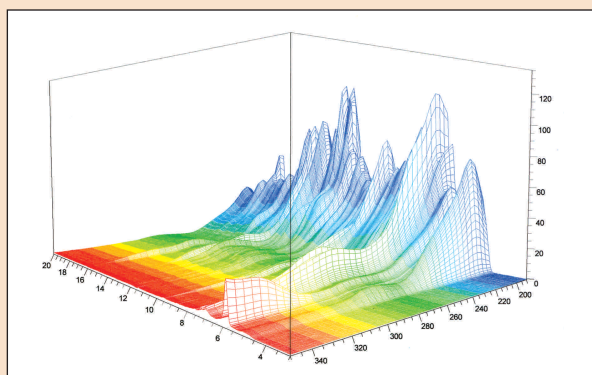
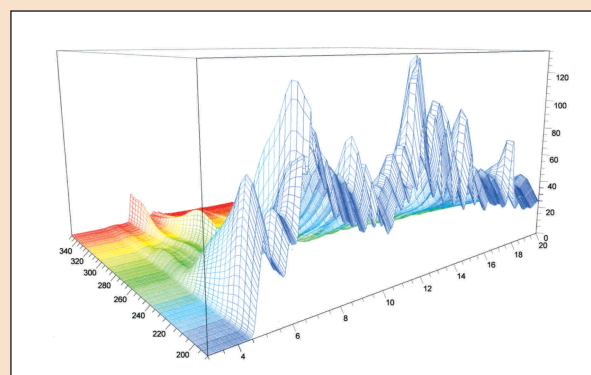
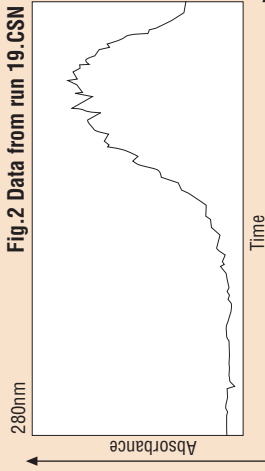


Fig 1b



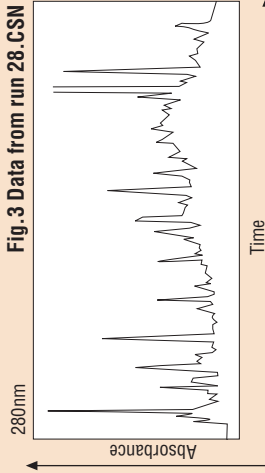
The Molecular Distribution of ENZOGENOL[®] from Monomeric through Oligomeric to Polymeric Proanthocyanidins

Fig.2 Data from run 19.CSN



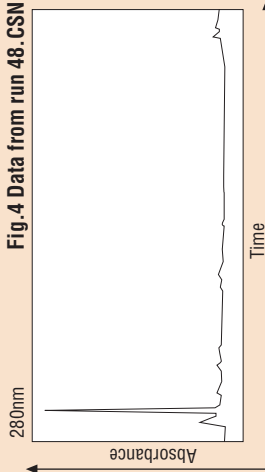
Higher oligomeric proanthocyanidin

Fig.3 Data from run 28.CSN



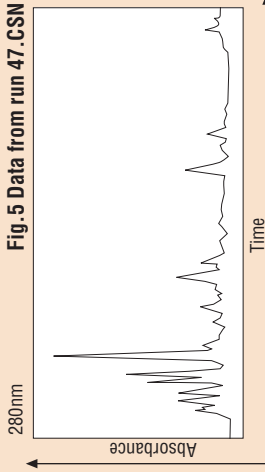
(+) - Catechin; procyanidin dimers B1, B3, B6; procyanidin trimer C2 & other proanthocyanidin trimers; quercetin;
(+) - Taxifolin; flavonoid glycosides¹; flavonoid esters¹; protocatechuic, gallic & other organic acids; astrigenin¹

Fig.4 Data from run 48.CSN



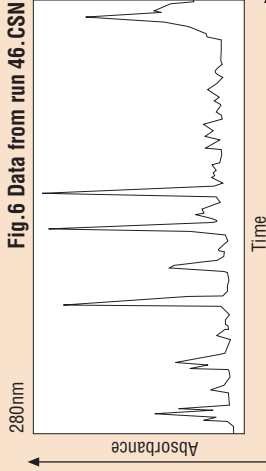
Flavonoid glycoside¹

Fig.5 Data from run 47.CSN



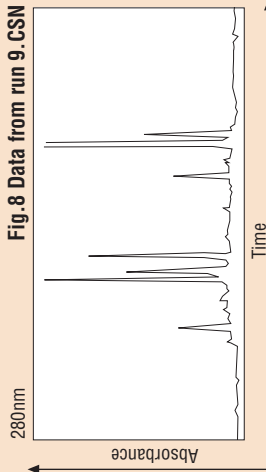
(+) - Gallocatechin¹; prodelphinidin dimer¹; flavonoid glycosides¹; flavonoid esters¹; protocatechuic acid

Fig.6 Data from run 46.CSN



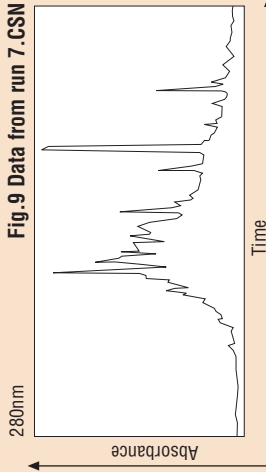
Flavonoid glycosides¹; flavonoid esters¹; organic acids¹

Fig.8 Data from run 9.CSN



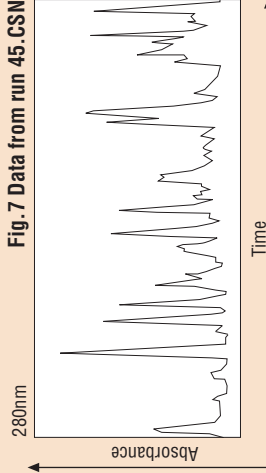
(+) - Catechin; procyanidin dimers B1, B3, B6;
(+) - Taxifolin, (-) - taxifolin; caffeic acid

Fig.9 Data from run 7.CSN



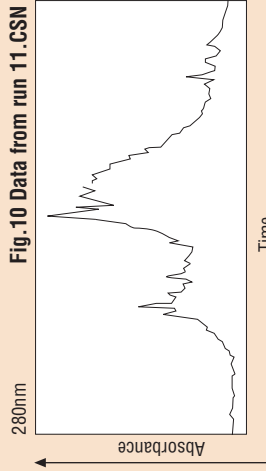
Procyanidin trimer C2, proanthocyanidin trimers; astrigenin¹

Fig.7 Data from run 45.CSN



Protocatechuic, vanillic, gallic, ferulic and other organic acids¹

Fig.10 Data from run 11.CSN

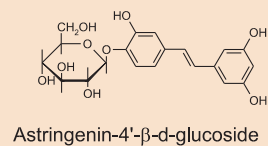
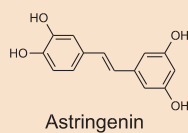
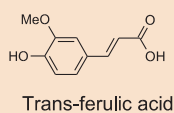
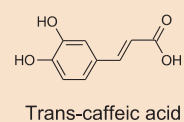
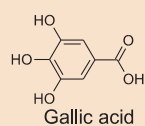
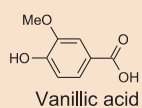
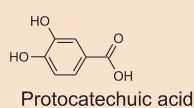
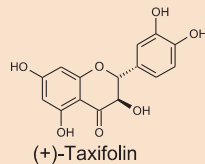
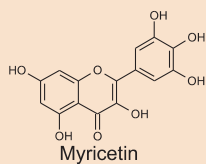
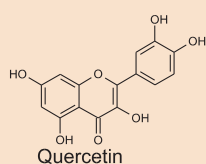
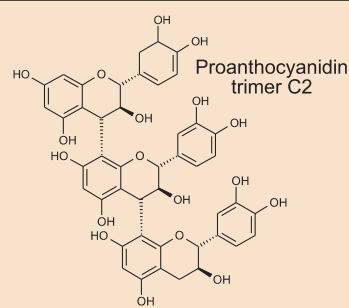
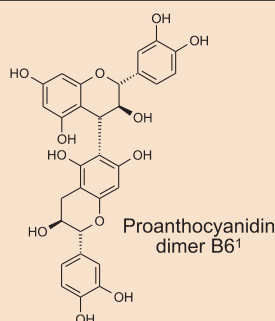
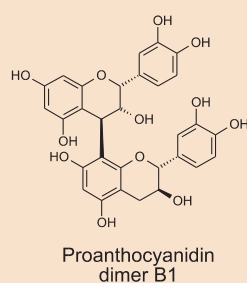
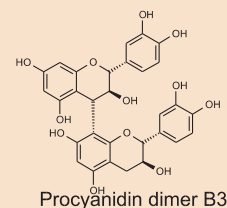
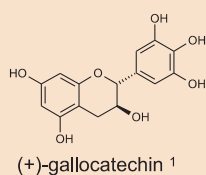
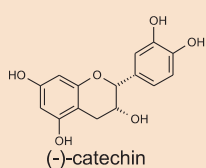
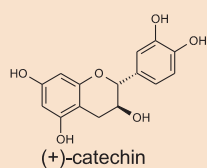


Proanthocyanidin tetramers; quercetin; pinosylvin¹, resveratrol¹

¹ to be confirmed

THE NATURALLY OCCURRING DIETARY ANTIOXIDANTS

ENZOGENOL® *Pinus radiata* pine bark proanthocyanidin complex contains a wide range of proven naturally occurring dietary antioxidants including the following monomeric-trimeric proanthocyanidin, flavonol, dihydroflavonol, flavonoid glycoside, flavonoid ester, organic acid and hydroxystilbene components.



¹ to be confirmed

THE HIGHER PROANTHOCYANIDIN

Flavonoids are a diverse group of polyphenolic compounds that exist naturally in plants.

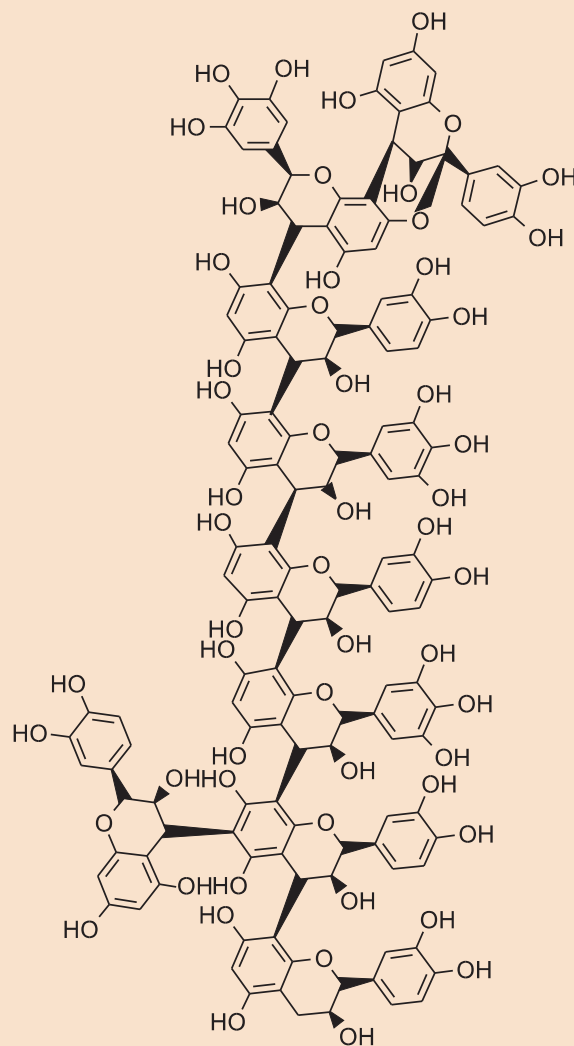
ENZOGENOL[®] contains a great deal of these important flavonoids and organic acids.

This diagram shows the hypothetical structure of one of the many possible discrete components that are contained in ENZOGENOL[®].

The structure shown is a higher molecular weight proanthocyanidin that is made up of 9 catechin units. Catechin is a monomeric flavonoid that when joined together can form new compounds. There are many ways the catechins are able to bond and also many ways they are orientated. For a 9-catechin structure which has 8 bonds joining the different catechins together there is an almost endless number of proanthocyanidin isomers possible.

While this structure is hypothetical it has been drawn with considerable knowledge of lower molecular weight proanthocyanidins reported to be present in *Pinus radiata* bark.

ENZOGENOL[®] contains a number of these proanthocyanidin isomers, which have similar properties. The research continues in identifying and quantifying these very complex compounds.



This higher molecular weight proanthocyanidin is made up from the monomer flavanol units (+)-catechin and (-)-epicatechin which are reported to be abundant constituents of polymeric proanthocyanidins.

MANUFACTURING QUALITY CONTROL SUMMARY

Raw Material Manufacturing Process

The only raw materials used in the production of ENZOGENOL[®] are pine bark from *Pinus radiata* and pure water. The process itself is solely physical extraction and purification. This includes filtration and membrane separation techniques.

The bark is thoroughly screened and washed before production. Only bark of a certain age, from a particular portion of the tree and free from contaminants is accepted. The bark is then ground before extensive washing (in pure water) takes place. The only water used in this process has been de-ionised, filtered and had all oxidants removed.

There are daily, weekly and monthly cleaning regimes in place. These are to ensure a constant high level of sanitation and cleanliness throughout the entire plant.

In-Process Controls

The process complies with Current Good Manufacturing Practice for Dietary Supplements. The process is fully documented and carried out according to the Production Manual. Log sheets are kept and signed off to ensure all the appropriate procedures are carried out and all the production parameters maintained. Standard thermometers, thermostats, pressure gauges, flow meters and sight glasses are used and their calibration maintained.

PRODUCT QUALITY CONTROL SUMMARY

Standardisation

The following four tests are carried out on each batch processed to ensure a high standard of consistency is maintained. Prior to processing the raw material is also screened and examined to ensure the bark is from the same part of tree and from trees of the equal age.

1. Gel Permeation Chromatography

This is our main tool for determining the exact chemical make up of the extract. ENZOGENOL[®] from each batch is passed through a column packed with Sephacryl S-100 HR Gel. A solvent gradient of increasing polarity is used to elute fractions of unique chemical groupings, which are then dried and weighed. From this it is able to determine the exact level of proanthocyanidin and other compounds and so gauge the product potency. The measurement of molecular weight distribution of the proanthocyanidins provide a valuable guide to ensure consistency of every batch.

2. Total Phenol

This is used on the whole extract to measure the level of the phenolic groups present relative to a (+)-catechin standard. A HACH Tannin-Lignin reagent is used (cat. 2560-42) and the absorbance measured at 700nm.

3. Total Tannin

This is a direct UV measurement of the sample, which utilises the aromatic conjugation present to approximate the level of tannin. The samples UV absorbance is measured at 278nm and compared to (+)-catechin.

Microbiology

3M Yeast and Mould count plates are used to test every batch and any reading greater than 20 cfu/g results in immediate rejection of the batch. Coliforms are not tested in this manner as ENZOGENOL[®] is very antibacterial and a living coliform has never been found in our extract. External laboratories are used periodically to carry out a full microbiological analysis.

Other Tests.

Periodically external laboratories are also used to carry out a heavy metal analysis and a herbicide/pesticide multiresidue-screening test.

SPECIFICATIONS

HEAVY METALS

The table below shows the amount of heavy metals in ENZOGENOL®

ELEMENT	LEVEL (PPM)
Arsenic	<1.4
Cadmium	<0.3
Copper	<3.5
Iron	<650
Lead	<0.5
Mercury	<0.02
Zinc	<35

MICROBIAL GROWTH

The table below indicates counts for ENZOGENOL®

TEST	COUNT
Aerobic Plate Count	< 15 cfu/g
Total Coliform	< 4 mpn/g
Faecal Coliform	< 4 mpn/g
Yeast and Mold	< 25 cfu/g

ABBREVIATIONS:

cfu colony forming unit
mpn most probable number

METHODS OF ANALYSIS

<i>Arsenic</i>	<i>Nitric/hydrochloric acid microwave digestion, determination by ICP-MS</i>
<i>Mercury</i>	<i>Nitric/hydrochloric acid microwave digestion. Cold Vapour AA Spectroscopy</i>
<i>Other Metals</i>	<i>Nitric/hydrochloric acid microwave digestion, determination by ICP-OES</i>

MATERIAL SAFETY DATA SUMMARY

Product Description	Powdered extract of <i>Pinus radiata</i> bark
Ingredients	Water soluble monomeric and oligomeric proanthocyanidins and organic acids, glycosides and esters.
Chemical Name (Actives)	Proanthocyanidins, flavonoids
Trade Name	ENZOGENOL®
Chemical Classification	Organic, nutritive
Usage	Dietary supplement
Hazard Classification	Slight
Fire, Explosion and Reactivity Data:	
Flash Point	Not applicable
Flammable Limits (Air)	Not applicable
Extinguishing Media	Water
Special Fire Fighting Procedures	Not applicable
Degree of Fire and Explosion Hazard:	Slight when exposed to high heat and flame
Stability	Stable
Hazardous Decomposition Products	None known
Routes of Exposure:	
Inhalation	May cause irritation of mucous membranes
Skin Contact	May cause irritation
Eye Contact	Direct contact causes irritation of the conjunctiva, cornea and iris
Ingestion	Oral LD ₅₀ (mice) > 5000 mg/kg
Effects of Overexposure	Possible cough, irritation or eyes or redness of skin may develop. No other known adverse effects
Emergency and First Aid Recommendations	
Eyes	Flush with clean water for at least 15 minutes. If irritation occurs and persists, obtain medical attention
Skin	Wash with soap and water. If irritation occurs and persists, obtain medical attention.
Inhalation	Remove victim to fresh air. If breathing is difficult or if any discomfort persists, obtain medical attention
Special Protection Information	General safety equipment including safety glasses, dust mask and gloves to reduce risk of irritation
Storage and Handling	To protect product quality, avoid excessive heat and store in airtight container in a dry place