AP450
New processing opportunities for pome fruit - Extraction of apple polyphenols from apple thinnings

Steve Martin
Tasmanian Department of Primary Industry and Fisheries

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NEW PROCESSING OPPORTUNITY FOR FRUIT

THE EXTRACTION OF APPLEPOLYPHENOLS FROM APPLE THINNINGS

SUMMARY

In 1994, the apple industry was approached by Sunward International from Shizuoka-Ken, Japan, to investigate the possibility of extracting a natural antioxidant or polyphenol from immature apples or thinnings. In December 1994, with support of funds from the Horticultural Research and Development Corporation, a trial processing of 10 tonnes of fruit was undertaken by Cascade Beverage Company in partnership with Sunward and the Tasmanian Apple and Pear Growers Association.

The trial processing, whilst satisfactory, failed to prove whether the project was commercially viable and it was resolved to undertake a second trial in December 1995, when a much larger commercial scale trial was undertaken and nearly 80 tonnes of fruit processed.

A number of associated experiments were undertaken to evaluate the relationship between apple size and polyphenol content, and apple variety and polyphenol content. The results of these experiments, which were undertaken over two years, are recorded.

BACKGROUND

Consumers of fresh products are becoming extremely concerned about the health issues in relation to the food they eat, and in particular food additives. As a result, there is a demand for food additives that are of a natural origin. It has been shown that aquis solutions containing polyphenols when added to meat and fish, increased storage life without loss of freshness or colour change.

The polyphenols of green tea have been shown to reduce the incidence of cancer and have antibacterial activity (Oguni and Hara, 1990).

Apples are known to contain large quantities of polyphenolic substances (Bardon and Bramlage, 1993) and it is also reported that apple peels contain antioxidant activity. It is the oxidation of one of these polyphenolic compounds that leads to the major problem of superficial scald, which develops during long term storage of apples. As different cultivars of apples have different scald susceptibilities, this implies that
different cultivars of apples might have different yields of polyphenolic materials with different antioxidant activity.

Macheix (1970) showed that the polyphenolic content of Calville Blanch apples was maximum (on a fresh-weight basis) at 40 days after the fruit had set and that these levels fell dramatically during the following 15 days which corresponded with an acceleration in fruit growth. This data suggests that apples of different size will yield different quantities of polyphenolic compounds on a wet weight basis. However, it must be realised that on a dried basis, the optimum yield of polyphenolic material may be at a different growth stage.

The project is a completely new opportunity for the apple industry and the trials were necessary to establish whether yield and product quality specifications could be achieved before further investment was made.

Apple thinnings are currently just dropped on the ground, therefore any returns that can cover approximately 50% of thinning costs would be of major benefit to almost all growers, since apple thinning costs are one the major single costs to the industry.

Experimental objectives

• to establish a commercially feasible collection process for apple thinnings to a pre-specified size;

• to understand the basic storage parameters for apple thinnings to maximise polyphenol yield; and

• to produce a satisfactory yield of polyphenols to acknowledged specifications.

METHODOLOGY

Collection Process

In 1994, in conjunction with participating growers, a course economic analysis was made of the various methods of collection of the thinnings. Two techniques were initially tried - the first was the collection into picking bags, the second was to drop the thinnings to the ground onto plastic sheets and then the thinnings were deposited in bins and boxes. It was reported that in Aomori in Japan, the growers preferred collecting thinnings by dropping them to the ground onto plastic sheets.

In orchards in the Huon, this was found to be unsatisfactory as thinning ladders slipped on plastic sheets on slightly sloping ground. Growers adopted the technique of picking into picking bags. Initial observations
recognised that this increased thinning time by at least 20%. In 1995, a more detailed analysis was undertaken and a table of results are presented in Table 1. This involved additional record keeping by key growers and observation of picking rates, handling and storage costs.

Storage Parameters

It was necessary to establish a methodology for the measurement of polyphenols and to clarify the most appropriate storage conditions, including whether cold storage affects the level of polyphenols in thinnings fruit. The 1994 preliminary trials indicated that thinning apples could be cold stored without loss of polyphenol level. The need to confirm the impact of storage was seen as an important factor in understanding the future logistics of collecting large tonnages of thinning fruit and further trials were undertaken in 1995.

PRODUCTION OF A COMMERCIALLY ACCEPTABLE SAMPLE OF POLYPHENOL PRODUCT

Whilst the other issues are of importance, the initial experiment conducted in December 1994 centred around using the existing commercial facilities at Cascade Beverage Company with relatively minor modifications to extract a commercially acceptable product. The plant modifications included improved loading and handling equipment, including the ability to wash and scrub much smaller fruit sizes, of between 10 mm to 25 mm diameter, and modifications to pumping equipment to enable much denser fruit pulp to be circulated.

Whilst the product is processed in a not dis-similar manner to apple concentrate, a number of steps are considerably different, especially in the later stages of the primary processing. Secondary processing currently cannot be done in Tasmania and samples of the primary processed liquor were air freighted to Japan for immediate secondary processing. The balance of the juice was concentrated, frozen and air freighted to Japan in 1994. In 1995, all juice was frozen and then shipped by sea.

RESULTS

Collection Process

In 1994, growers evaluated that the best method of collection was in a picking bag, rather than dropping the thinnings onto plastic sheets and collecting the thinnings into bins. Evaluation of the costs of the collection process are outlined in the attached table (Table 1).
Storage Parameters

Several bags of Fuji and Delicious were taken from the cool store at Sheilds Packing Shed on 16 December 1994 and placed in a 5 degree refrigerator at New Town Research Laboratories (NTRL). Cultivars were kept separate in plastic bags. A number of preliminary experiments were undertaken to evaluate the apple polyphenol standard and the SO₂ concentration versus absorbency to assist in establishing the concentrations of SO₂ required to prevent oxidation in filtered apple juice.

A number of tests were undertaken to establish the possibility of loss of polyphenols from the filtrate and to establish whether the cloudiness of unfiltered samples may, in part, explain the higher absorbancies in unfiltered samples. The result of these experiments were that it was concluded that the juice samples need to be filtered before conducting polyphenol determinations.

From the results in 1994/5, there were a number of technical issues that needed to be discussed with the technical experts handling the process in Japan. There were subsequently two visits to Japan to discuss technical issues and representatives of Cascade and TAPGA visited the Japanese polyphenol factory at Hirosaki in Aomori Prefecture to gain a more intimate understanding of the technical issues.

The affect of fruit size on polyphenol content

Initially apples were juiced and the unfiltered juice diluted to 200 ml with deionised water. No SO₂ was added, however the juice was kept cool and measurements made quickly to avoid oxidation.

Summary for unfiltered juice of Delicious apples at 1:200 ml dilution with deionised water:

<table>
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<th>Size category (mm diameter)</th>
<th>Absorbance (1:200) at 280 nm</th>
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<td>1.71</td>
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Summary for unfiltered juice of Fuji apples at 1:200 ml dilution with deionised water:

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Analysis of polyphenol content of apples with storage

Apple thinning from Shield coolstore were processed during December 1994 by Cascade, and after storage of approximately 2 months at 5 degrees by NTRL.

<table>
<thead>
<tr>
<th></th>
<th>Juice type</th>
<th>SO2 conc.</th>
<th>TSS</th>
<th>Absorbance (1:250 ml) at 280 nm</th>
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<td>Cascade</td>
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<td>6.7</td>
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<tr>
<td>NTRL</td>
<td>Delicious (ii)</td>
<td>100 ppm</td>
<td>8.0 *</td>
<td>1.31 *</td>
</tr>
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</table>

(i) juice from primary pressing of mixed cultivar (Fuji and Delicious) apple thinning.
(ii) juice from Delicious apple thinning after storage of approximately 2 months in sealed plastic bags at 5 degrees.

* NTRL TSS and absorbance values are means of 4 samples.

Absorbance of juice from apple thinning collected from throughout Tasmania and processed by Cascade was considerably lower than juice from comparably sized apples originating from Shields coolstores and those processed at NTRL. It is possible that this difference in polyphenol level was due to sampling procedure.

NTRL samples were removed from the top of bulk bins whereas Cascade samples were more random. This may imply that fruit in the centre or bottom of bulk bins had lost polyphenol activity by the time it reached Cascade, perhaps due to the high temperatures after picking that these fruit would have experienced compared with those on the top of the bins.

Alternatively, the combination of apple thinning of Delicious, Fuji (which were relatively low in polyphenol content) and possibly other cultivars in Cascade pressings, or the wider geographic origin of the fruit processed by Cascade, may have contributed to the lower absorbance measured by Cascade. Further experimentation is therefore required to clarify the effect of storage on polyphenol content of apple thinning.

Further work undertaken in 1995 does show that polyphenol levels are influenced by variety.

Storage

The preliminary trial in 1994 established that if fruit was kept in cold storage at about 1 degrees Celsius, the level of polyphenol from the preliminary experiments remained relatively constant. However, an important practical point was established that the bins needed to be half filled and cooled quickly, otherwise heating occurred, which, it was believed, would lower polyphenol levels.

In 1995, bins were water dumped to reduce field heat as rapidly as possible before the bins were topped up and put in coolstore.
Once collected in the coolstore, transport from farm to factory posed no difficulties and in 1995 fruit was despatched in approximately 10 or 20 tonne lots to fit with Cascades pressing capacity.

PRODUCTION OF A COMMERCIALY ACCEPTABLE POLYPHENOL PRODUCT

Plant Modifications

In 1994, once Cascades processing equipment was modified, the processing proceeded satisfactorily. There was some concern about the ability of the small apples to float satisfactorily. This proved unfounded and handling throughout the factory was satisfactory. Modifications were made to ensure the pipelines could handle the additional solids from the small apples during the processing. As a result of the 1994 preliminary trial, a number of additional modifications were made to the processing facility which resulted in a dramatic improvement in yield and polyphenol content in the 1995/96 pressings.

Establishing a satisfactory standard technique for polyphenol determination

In regards to the testing of the polyphenol levels, standard polyphenol concentrations were brought from Japan and tested in colorimetric tests equipment at Cascade. The results matched exactly. However, in 1994, there was some concern at the level of suspended material in the concentrate at the end of the first processing stage affected the polyphenol concentration measurements. This was an issue that was subsequently a matter of extensive discussion in 1995 and a suitable standard technique was adopted involving centrifuging of the concentrate as previously discussed.

Shipment to Japan

Once processed and concentrated, 8 drums were air freighted to Japan for secondary processing. In future, to avoid uneconomic transport costs, there is a need to consider producing polyphenol concentrate in 72 drum lots, which is equivalent to a frozen container size, which could be sent at -18°C to Japan.

Another initial issue that was required to be addressed after shipment was negotiated regarding the tariff on the product. The import tariff was 26% for apple juice to Japan, however zero tariff was levied on the shipment sent in February 1996.
Results on 1995/96 Trial

The results of the trial held in 1995/96 are outlined in the attached tripartite paper (See Appendix II) which addressed the key processing issues identified from the trial of 1994/95.

As outlined in the conclusions, there was substantial improvement and progress in the processing technology.

In regards to issues relating to fruit variety versus size versus polyphenol content, a number of subsidiary experiments were undertaken which are presented in the graphs Appendix III, IV and V.

The outcome from these experiments clearly demonstrate that polyphenol concentration varies depending on the variety with Crofton variety consistently producing almost half the level of polyphenol as Red Fuji or the Delicious spur types. Consistently, Red Fuji and the Delicious spur types produced the highest level of polyphenol, whatever the harvesting date.

The results also show that by 5 January when fruit diameter had reached approximately 4cm, polyphenol concentration was approximately 25% of the maximum polyphenol level produced in fruit harvested on 28 November.

The significant observation is that the new and primary current major variety for the apple industry, Fuji, spur type Delicious and High Early apples provide high levels of polyphenol and since these varieties require heavy thinning to produce high quality export fruit, there is clearly a benefit in collecting the fruit for the commercial production of polyphenols from apples. Collecting thinnings of Croftons for polyphenol production may not be cost effective, especially during the last two weeks of December when polyphenol levels start to decline rapidly.

In 1996/97 harvesting period, collection periods for specific varieties may need to be specified to maximise the polyphenol yield from thinnings collected.

CONCLUSION

Initial research using apple thinnings from the 1994/95 crop, indicated that Tasmanian fruit thinnings provided an excellent source of desirable apple polyphenols. The processing technique, however, needed further refinement because yield and level of polyphenol was not to the expectations of the Japanese partner.
It was agreed, following extensive negotiations during 1995, that a further trial of 50 - 80 tonnes would be undertaken to establish whether the losses were the result of lack of sufficient technical understanding and the need for further modifications of equipment. The 1995/96 season produced excellent results, with yield and polyphenols exceeding expectations.

With the project now passing the critical experimental stage, the three partners have the confidence to process commercial quantities of fruit. The next stage is to turn the technical possibility into a new commercial product and registration of the product as a food additive in Australia has commenced.

Apart from the market opportunities in Japan, there is also market development prospects in Australia, with interest already shown by the Queensland Prawn Industry and the South Australian Tuna Industry in using the product for preserving fish products for export.

The important issue for growers is to maintain an attractive price for the additional inconvenience and extra time in picking the small fruit. In 1995, a bonus was paid to growers as an incentive to provide raw material for processing. In 1995/96, the price paid per tonne of thinning was at a more commercial level of $450 per tonne. In 1996/97 it is hoped to process a further 150 tonnes with similar returns to growers. However, monitoring polyphenol levels during the critical picking period will assist in maximising yield and justify economically attractive prices for growers. Negotiations later in July 1996 will establish the basis for a long term commercial relationship.

ACKNOWLEDGMENT

It is acknowledged that without the support of the Horticultural Research and Development Corporation, it was unlikely that this project would have proceeded and this potential new opportunity would not have been undertaken in Australia. The Corporation's support is therefore gratefully acknowledged by the Tasmanian Apple and Pear Growers Association.

REFERENCES


of Primary Industry and Fisheries and Cascade Beverage Company for the Tasmanian Apple and Pear Growers Association.

## THINNING.XLS

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Grid Results are $ per Tonne
APPENDIX II

【Report on Second Trial Production of Tasmanian Concentrated Apple Juice】

31. January 1996

Kanda
1st Development Dept.
The Nikka Whiskey Distilling co., ltd. Technical Laboratory

(Participating Organizations)

(Purpose)
Last year, in cooperation with all concerned above, we trial-produced concentrated apple juice from 24 tons of thinned out apples of 24 ton, as a counter-seasonable material of apple phenon. These raw apples were very small in diameter. The density of polyphenol of the apples was high, and it was judged that they had quality. Juice from thinned out apples has little change of constituent due to concentration process and added hydrochloric acid and we judged that it is possible to supply these apples as material of concentrated apple juice from the quality point of view.

This time, we placed great importance on the problems of yield, points to be improved in the production process, and problems of quality control listed below, which were enumerated by the experimental production of last year.

1. Yield
Please refer to the data arranged by Mr. Rod Haigh (another report) about the quantity of the juice and concentrated juice of each lot and its analysis value. The Nikka Whisky distilling Co., LTD also analyzed samples from Cascade, and the level of the values are about the same as the report. Yield (polyphenol / apple) per apple 0.753% of very high density, and yield from the pressed juice is 96.8% of very high compared with 84.0% yield of last year. It was drastically improved despite of deducted decreasing rate of phlorizin (6.0%).

It seems that improvement of the problems in the production process which are pointed out last year made a contribution. Because we had drumming per lot done by Cascade in the concentrating process (refer to the section on preservation of juice for the reason), yield only in the concentrating process is not so good. But it seems that it certainly increased if the juice is concentrated and drummed continuously.
Mr. Aoyama has already reported about the points to be improved in production line. Refer to the report. In addition to this, an autotitrator, an electric balance, and a specific gravity balance are introduced as quality control related devices, therefore we judged that the line is more improved than we pointed out last year.

2. Analysis Method

Clarification process is done to make juice clear when analyzing. Nikka Laboratory does it with the high speed centrifugal method, and Hiroshiki factory does it with a filter disk. We had no filter disk at the first trial, so the value tended to be higher. Mr. Rod Haigh pointed out that we should consider difference of absorption rate between the filter disk used at Nikka and Cascade, and we compared filtration methods and two kind of filter disk.

As a result, we judged that the way is better that the original juice of 5 ml is first filtered, then the filtered juice is disposed, the same original juice filtered again with the same filter, and the filtered juice is served to diluted. If filtered this way, we found no difference between two kind of filter disk.

3. Preservation of Juice

In this production, it is planed that the subsequent concentrated juice after the second press will be drummed all together tanked in one. However by this method, the first pressed concentrated juice will be left for 3 days at 33°C. Therefore we left the first pressed concentrated juice overnight at 45°C, and checked discoloration.

As a result, we found out that discoloration into brown of the juice left overnight at 45°C is progressed compared with the same cold storaged concentrated juice. Following the result, we judged that it is preferred to drum the juice after concentration as soon as possible and to keep it in a freezer. So we decided that the drumming will be done at each concentration this time. It seems that it decreased loss in process to preserve, blend, continuously concentrate and drum juice as a pre-concentrated juice, because the pre-concentrated juice excels in preservativity or let produced quantity of juice per day to be equal to concentrated quantity with controlling speed of concentration.

4. Phloridzin

Now phloridzin is mostly removed from produced applephenon with enzyme to decomposite it. Though we treated it with the enzyme at Cascade last year, we decided that the enzyme treatment process is done in Japan this time, and we only did pectinase treatment to clarify the juice.

(1) As juice is more clarified, action efficiency of the enzyme is better. It leads to lower cost.
(2) The production process of applephenon from concentrated juice is: the concentrated juice is once diluted, filtered, and column-refined. Therefore it is more efficient that the enzyme treatment to the juice is done when it is diluted.
However phloridzin is not a material of applephenol, but a kind of polyphenol. So Nikka offered that we deduct the removed quantity of phloridzin by enzyme from the total quantity of polyphenol in the concentrated juice. In conclusion, we regard the result of last year as important as Mr. Aoyama's report, and reached an agreement with Cascade that the deducted ratio will be 6% if total phloridzin content will exceed 6% (concentration ratio) of total polyphenol, and we will have special conference if the content is under 6%.

We had already confirmed the method to measure phloridzin concentration (high speed liquid chromatography: HPLC), but CUB (parent company of Cascade, Asahi Breweries LTD is a shareholder) in Melbourne pointed out that there may be any trouble in purity of phloridzin, because a shoulder of the standard phloridzin (made by Tokyo Chemical Industry Ltd., we brought it with ourselves; it is not a standard phloridzin used usually due to problem of date of delivery) is found out at its peak. We checked the two kinds of standard phloridzin with two kinds of separation method (HPLC) the other day, but they have enough purity (already reported). So there should be another reason. We will appreciate, if CUB obtain and check the standard phloridzin.

The table below shows concentration of phloridzin and content ratio of it to total polyphenol of each lot.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Phloridzin mg/L (NIKKA)</th>
<th>Phloridzin mg/L (CUB)</th>
<th>Phloridzin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 conc.</td>
<td>9814</td>
<td>9623</td>
<td>8.4</td>
</tr>
<tr>
<td>P2 conc.</td>
<td>6497</td>
<td>5870</td>
<td>8.2</td>
</tr>
<tr>
<td>P3 conc.</td>
<td>7518</td>
<td>-</td>
<td>7.0</td>
</tr>
<tr>
<td>P4 conc.</td>
<td>5470</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>P5+6 conc.</td>
<td>7616</td>
<td>-</td>
<td>6.3</td>
</tr>
</tbody>
</table>

The table says that the concentration of phloridzin to the concentration of polyphenol exceeds mostly 6.0%, so we guess the condition above has no problem, but there is a lot below 6.0% as P4 conc. However if they are concentrated all together in one, these values are averaged. This problem is difficult to understand, and reference is not possible now.
1. Continuous concentration and drumming aiming at real production. We hope that Cascade will examine to make it possible to control concentration speed matching production speed of juice and to secure juice preservation tank in order to continuously proceed with the process from filtered juice referred in. (3. Preservation of Juice) to concentration and drumming.

2. How to understand unevenness of quality (total polyphenol and phloridzin concentration). For example, brix and total polyphenol of the concentration juice sample P1 conc. and P4 conc. which were sent this time are mostly same, but phloridzin concentration is differed 1.8 times (table. above). And when P1 conc. was sent to Japan, it was clear and the difference of measured value of its polyphenol concentration between Cascade and Nikka was under 1% and on the same level. But P4 conc. had muddiness and sediment, and the difference of measured value between two companies was over 10% and high as shown in the attached sheet. The same tendency applies to the pressed juice of same lot. We will examine about it further.

Mr. Rod Haigh is doing research on Tasmanian apple from wide point of view of varietype-size. So we asked him to send data if the data will be arranged.

(Conclusion)

As above, there are more substantial improvements and progresses in technology than the first trial. The trial was substantial and had been making steady progress. Among them, we repeated many discussions about analysis technology, and we judged that we had reached to high repeatability method. So the value became more reliable. Especially, the high level opinions and deepening understanding of all concerns are beyond description. Each has his own position, but we felt they were very wonderful.
### 95 Tasmanian Immature Apple Juice Concentrate

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Mass (L)</th>
<th>Polyphenol CASCADE (mg/L)</th>
<th>Polyphenol NIKKA (mg/L)</th>
<th>Ratio of nikka/cascade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Press 1 a</td>
<td>6.074</td>
<td>8.565</td>
<td>8.528</td>
<td>98.4%</td>
</tr>
<tr>
<td>Press 1 b</td>
<td>6.074</td>
<td>9.015</td>
<td>9.008</td>
<td>99.9%</td>
</tr>
<tr>
<td>Press 2 T3</td>
<td>6.683</td>
<td>6.682</td>
<td>6.458</td>
<td>96.6%</td>
</tr>
<tr>
<td>Press 2 T4</td>
<td>6.756</td>
<td>5.140</td>
<td>5.198</td>
<td>101.1%</td>
</tr>
<tr>
<td>Press 3 T67</td>
<td>7.055</td>
<td>7.180</td>
<td>7.082</td>
<td>98.6%</td>
</tr>
<tr>
<td>Press 3 T68</td>
<td>7.055</td>
<td>7.105</td>
<td>7.022</td>
<td>98.8%</td>
</tr>
<tr>
<td>Press 4 T61</td>
<td>6.859</td>
<td>6.756</td>
<td>6.644</td>
<td>98.3%</td>
</tr>
<tr>
<td>Press 4 T62</td>
<td>6.933</td>
<td>9.245</td>
<td>7.314</td>
<td>79.1%</td>
</tr>
<tr>
<td>Press 5 T63</td>
<td>7.046</td>
<td>6.390</td>
<td>5.982</td>
<td>90.9%</td>
</tr>
<tr>
<td>Press 5 T64</td>
<td>5.683</td>
<td>9.185</td>
<td>8.606</td>
<td>93.7%</td>
</tr>
<tr>
<td>Press 6 T62</td>
<td>7.191</td>
<td>7.355</td>
<td>6.988</td>
<td>95.0%</td>
</tr>
<tr>
<td>Press 6 T68</td>
<td>5.756</td>
<td>8.600</td>
<td>8.072</td>
<td>93.9%</td>
</tr>
<tr>
<td>P 1 conc.</td>
<td>891</td>
<td>115,543</td>
<td>118,377</td>
<td>100.7%</td>
</tr>
<tr>
<td>P 2 conc.</td>
<td>1.113</td>
<td>74,852</td>
<td>89,961</td>
<td>93.5%</td>
</tr>
<tr>
<td>P 3 conc.</td>
<td>1,274</td>
<td>106,888</td>
<td>96,454</td>
<td>90.3%</td>
</tr>
<tr>
<td>P 4 conc.</td>
<td>724</td>
<td>108,252</td>
<td>95,682</td>
<td>88.4%</td>
</tr>
<tr>
<td>P 5+6 conc.</td>
<td>1,334</td>
<td>119,627</td>
<td>109,650</td>
<td>91.7%</td>
</tr>
<tr>
<td>D 7 (94)</td>
<td>182</td>
<td>56,350</td>
<td>49,620</td>
<td>88.1%</td>
</tr>
<tr>
<td>D 8 (94)</td>
<td>182</td>
<td>48,700</td>
<td>45,180</td>
<td>92.8%</td>
</tr>
<tr>
<td>D 11 (94)</td>
<td>67</td>
<td>43,600</td>
<td>41,080</td>
<td>94.2%</td>
</tr>
</tbody>
</table>
APPENDIX VI

TASMANIAN APPLE AND PEAR GROWERS
ASSOCIATION INCORPORATED

RECONCILIATION OF POLYPHENOL PROJECT:

Amount contributed by Participants -
Sunward $13,589.00
Cascade $13,589.00
TAPGA $13,411.00

$40,589.00

Amount required by HRDC -
Sunward $13,411.00
Cascade $13,411.00
TAPGA $13,411.00 = $40,233.00

Reimb. to Sunward & Cascade due $177.00 ea = $354.00

AMOUNT PAID TO HRDC 2 March '95 AP450 = $40,232.00

FINANCIAL PROVISIONS Ex HRDC -

Approved expenditure items:
Salaries $0.00
Travel $13,600.00
Operating $55,600.00
Capital $6,000.00 Total $75,200.00

Sources of Funds
Industry funds $37,600.00
HRDC funds $37,600.00 Total $75,200.00

PAYMENTS TO PARTICIPANTS
SUNWARD 22/3/95 INV Y0002 Y293,358 = $A 4,583.00
23/3/95 INV Y0001 $A 25,600.00 Total $A30,183.

$75,200.00
less 30,183.00 = $45,017.00

equates to Cascade/TAPGA share $22,508.50 ea.