Problems faced in processing of passion fruits: microbial contamination and oxidation of the juice.

By

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Thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in Food Science and Technology of the Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka , Buttala, Sri Lanka.

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DECLARATION

The work described in this thesis was carried out by me at CPC Agrifoods Ltd. (manufactures of KIST and Knorr products, packers of MARMITE and aseptic processing of fruit juices).Weniwelgodella, Kondagammulla Road , Demanhandiya ,Katana and faculty of Applied Sciences under supervision of Dr.K.K.D.S.Ranaweera and Mrs. Udhyani Jayasuriya. A report on this has not been submitted to any other University for another degree.

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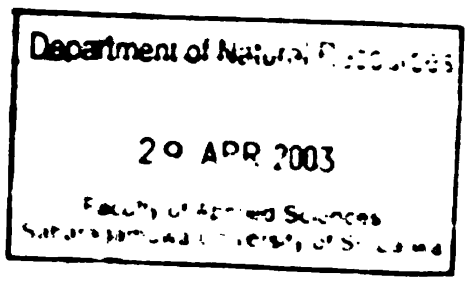

AFFECTIONATELY DEDICATED TO

MY PARENTS AND TEACHERS

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ABSTRACT

This study was conducted to investigate the effectiveness of current passion fruit juice processing procedure and to carry out quality improvements at CPC Agrifoods Ltd. Major objectives were to reduce microbial contamination and oxidation during the process of fruits for on receiving to finished aseptic passion fruit juice.

Fruit sample were collected from received fruits, after primary washing, after sorting and after secondary washing for fruit surface microbial evaluation by using swab testing method. Fruit juice samples were collected from juice collector bin, chiller tank and aseptic sample bag. Microbial evaluation was done by using after suitable dilution and checked oxidation effect by determination of colour ,pH , acidity changes, and taste/ aroma sensory evaluation.

Effectiveness of machinery surface cleaning was determined by swab microbial evaluation. Atmospheric microbial count was evaluated.

At primary washer chlorine level was monitored at different levels in relation to microbial content and at secondary washer monitored microbial content with time.

Statistical evaluation of results was done by using paired one sample T-test of MINITAB computer package , obtained P values and expressed conclusions. Based on results effective steps and improvements were encountered scientifically for better and hygienic out put.

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CHAPTER 01

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Passion fruit (*Passiflora edulis*) is a promising fruit grown in tropical and sub tropical countries including Sri Lanka. Passion fruit has a big potential for value addition activities. Therefore, processing intended for value added products has become one of the most important component in fruit cannery industries. On the other hand, production of value added passion fruit based products makes this seasonal fruit available to the consumer throughout the year. However, problems like contamination of raw materials, semi-final or final products during the manufacturing process can causes deterioration of the product thereby lowering its quality.

As far as deterioration of processed passion fruit juce is concerned, there can be two major reasons for this contamination;

1. Microbial activity.
2. Oxidation

Prevalence of microorganism is determined by several factors like availability of nutrients in the juice and its pH value. Juices of yellow fassion fruit and purple are reported to have lower pH values higher acidic contents being 2.8-3.3 (Boyle et al. 1955) and pH 2.6-3.2 (Pruthi, 1963) respectively. The acidic levels are accordingly higher ranging from 3.0 to 5.0% (Boyle et al. 1955) in yellow fruits juice and from 2.4-4.0 % in purple fruit juice (2.4-4.8% ; Pruthi 1963). Water content of the juice is approximately 84%. Therefore prevalence of microorganisms in the juice can be characterized as follows;

1. Acetic acid bacteria -causes anaerobic and aerobic fermentation
2. Lactic acid bacteria
3. Yeast
4. Molds

Microbial activity on juices may result in the following -:

- Spoilage of fruit juice
- Reduction of shelf life
- Accumulation of Micotoxin that may be health hazardous to consumers
- Violation and legislation due to Micotoxin
- Deterioration of sensory characters (specific colour, flavour, taste and texture)
- Increase of wastage
- Reduce demand for the products and thereby the reduction of the profit from the industry.

Oxidation can occur if juice or damaged fruit is exposed and mixed with atmospheric air. Oxidation can lead to the following consequences -:

- Browning of the product
- Reduction of nutrition value
- Reduction of shelf life
- Decline of sensory quality and there by reduction of the demand and profit.

1.2. Objectives

Therefore, the main objective of the present study was to control the microbial growth by reducing the microbial population through monitoring the control parameters in order to reduce the microbial load entering to the Aseptic process during the heat treatment on fruit juices.

Specific objectives are following here:

- Study on the effect of chlorine content of rinsing water in primary fruit washer on the microbial load and study the chlorine reduction rate.
- Study the relationship between atmospheric microbial population and microbial count on fruit extracts.
- Study the effect of initial microbial load of juices before the aseptic process in relation to microbial population after incubation of aseptic juices under accelerated shelf life conditions.

- Study the effectiveness of cleaning and sanitizing of fruit processing section.
- Study the changes in extracted juices in chiller storage tank with relation to retention time and temperature.

This research project was carried out at CPC Agrifoods Ltd, Katana.

CHAPTER 02

LITERATURE REVIEW

2.1 Origin and distribution

The edible commercial species of Passion Fruit had originated on the edges of South American rainforests in the Amazon region of the Brazil and possibly in Paraguay and Northern Argentina. The purple Passion Fruit (*Passiflora edulis*) is adapted to the cooler subtropics or at high altitudes in the tropics, while the golden Passion Fruit (*P. edulis f. flavicarpa*) is more suited to tropical lowland conditions.

Passion Fruit became popular in most of the tropical and subtropical world, reaching South Africa, Hawaii, California and Florida by the end of the 19th century and Kenya, Sri Lanka and Fiji by the middle of the 20th century. More than 80 percent of world's Passion Fruit production is by Brazil, Venezuela, South Africa, Australia, Sri Lanka, Papua New Guinea, Fiji, Hawaii, Taiwan and Kenya. The production in Hawaii and Papua New Guinea has declined in the recent past while new countries such as New Zealand, West Samoa, Malaysia, Congo, Angola, Peru, Colombia and West Indies have begun production (Henegedara *et al*, 2002).

2.2 Botany of Passion Fruit

Passion Fruit is a member of *Passifloraceae* family of which, there are roughly 400 members. Few of them have edible fruits. Genus *Passiflora* has 60 species of edible Passion Fruits and a few members are listed here (Bose and Mitra 1990).

Passiflora edulis

P quadrangularis (giant granadilla)

P. lingularis (sweet granadilla)

P laurifolia (Yellow granadilla or bell apple)

P malifomis (Sweet calbash)

P. mollissima (Banana Passion Fruit)

P antiquensis

P. incarnata (vine apricot or wild Passion Fruit)

P caerulea

P alata

P.coccinea

P.mixta

P.popenovii

P.sedmanni

P.serratodigitata

2.3 Cultivation of Passion Fruit

2.3.1 Climate and soil

Climate

Passion Fruits are adapted to tropical and sub tropical areas with high rainfall. The purple Passion Fruit crops best at higher altitudes above 2000 m in the tropics, while the golden Passion Fruit and hybrids between the two forms are superior in the lowlands.

Passion Fruits are sensitive to frost and are killed or severely injured by prolonged temperatures below freezing, but can withstand light frosts. They may survive short periods below -2^0C. Young vines and actively growing shoots are more susceptible than mature hardened plants. Species with high chilling tolerance are : *P.edulis f. flavicarpa, P.incarnata, P.edulis,* and *P. caerulea* (Patterson et al.,1976-1978). In cooler sites, it is desirable to select the north and east facing slopes (Southern Hemisphere) to increase temperatures in the orchard. At the present state of knowledge, it is suggested that commercial Passion Fruit sites should be free of frosts.

Passion Fruits are limited in their adaptation to extremes of temperatures. Temperatures below 15-18^{0}C restrict vegetative growth and flowering, while temperatures above 30-32^{0}C promote growth at the expense of flowers and fruits. Low temperatures can also reduce pollination, there being no pollen germination below 20^{0}C. Heaviest yield is obtained between 20^0 and 30^{0}C, a compromise between flowering and excessive vegetative growth.

Passion Fruit may survive drought conditions, but will not grow and crop. Consequently, unless irrigation is available, high well-distributed rainfall in excess of 1200 mm per annum is usually considered essential for commercial Passion Fruit growing.

Because Passion Fruit evolved on the margins of tropical rainforests, they are very sensitive to wind damage. Cold southerly and southeastern winds in Australia restrict vegetative growth and increase the severity of PWV - Passion Fruit

restrict vegetative growth and increase the severity of PWV - Passion Fruit Woodiness Virus. Strong cyclonic winds cause branch breaking, flower drop and fruit rub and sometimes vine death. Hot-dry northern and north-western winds at flowering can dehydrate flowers, while fruits do not stay on vines if subjected to continued strong winds. It is vital for Passion Fruit to be planted in a protected site with permanent windbreaks around to cover if winds are strong.

It has been suggested that Passion Fruit is a long-day plant, requiring day lengths in excess of 10.5 hours to flower and fruit (Weston and Bowers, 1965; Vallani *et al.*, 1976). However, this is in conflict to the flowering of vines in warm winters in southern Queensland. It is more likely that the observed depression of flowering under short day is a response to reduced sunlight (Bose and Mitra .1990).

Soil

Passion Fruit vines are grown in many soil types but light to heavy sandy loams, of medium texture are most suitable, and pH should be from 6.5 to 7.5 with moderate salinity. If the soil is too acidic, lime must be added. Good drainage is essential to minimize the incidence of collar rot. They will grow on infertile soils, although the yield is reduced without fertilizers (Bose and Mitra et al 1990).

2.3.2 Propagation

Seeds, cuttings or grafting, may propagate Passion Fruit.

Seed

Passion Fruit vines are usually grown from seeds. With the yellow form, seedling variation provides cross-pollination and helps overcome the problem of self-sterility. Some say that the fruits should be stored for a week or two to allow them to shrivel and become perfectly ripe before seeds are extracted. If planted soon after removal from the fruit, seeds will germinate in 2 to 3 weeks. Cleaned and stored seeds have a lower and slower rate of germination. Sprouting may be hastened by allowing the pulp to ferment for a few days before separating the seeds, or by chipping the seeds or rubbing them with fine sandpaper. Seeds are planted 1/2 inches (1.25 cm) deep in beds, and seedlings may be transplanted when 10 inches (25 cm) high. If taller–up to 3 ft (0.9 inches)–the tops should be cut back and the plants heavily watered.

Vegetative propagation

Some growers prefer layers or cuttings of matured wood with 3 to 4 nodes. Cuttings should be well rooted and ready for setting out in 90 days. Rooting may be quickened by hormone treatment. Grafting is an important means of perpetuating hybrids and reducing nematode damage and diseases by utilizing the resistant yellow Passion Fruit rootstock. If seeds are available in the early spring, seedlings for rootstocks can be raised 4 inches (10 cm) apart in rows 24 inches (60 cm) apart and the grafted plants will be ready to set out in late summer.

If seeds cannot be obtained until late summer, the seedlings are raised and grafted in pots and set out in the spring. Scions from healthy young vines are preferred to those from mature plants. The diameter of the selected scion should match that of the rootstock. Either a cleft graft, whip graft, or side-wedge graft may be made.

If approach-grafting is to be done, a row of potted scions must be placed close alongside the row of rootstocks so that the union can be made at about 3/4 of the height of the plant (Morton *et al* 1987).

2.3.3 Passion Fruit cultivation in Sri Lanka

As in Kenya and Fiji, Passion Fruit cultivation in Sri Lanka commenced in the mid- 20th century. Until 1970 Passion Fruit was grown as a mixed home garden crop in the wet zone areas. The commercial cultivation started in 1973 as a result of market links and produced 3,700 Mt. of juice (Abeysinghe,1973). Passion Fruit cultivation was promoted in the 1970-77 regime through land settlement projects and market promotion programs. Thus the five-year Development Plan (1970-75) targeted to cultivate 5,000 acres or to produce 37,000 Mt. of fruits by 1976. It was planned to achieve production targets by maintaining a higher standard of orchard management, fertilization, weed control and pest control. It was also expected to expand the acreage the high yielding yellow-fruited variety and to increase average yield from 5 tons to 7-10 tons per acre. However the total extent decreased after 1977 due to the gradual withdrawal of state intervention and limited market opportunities.

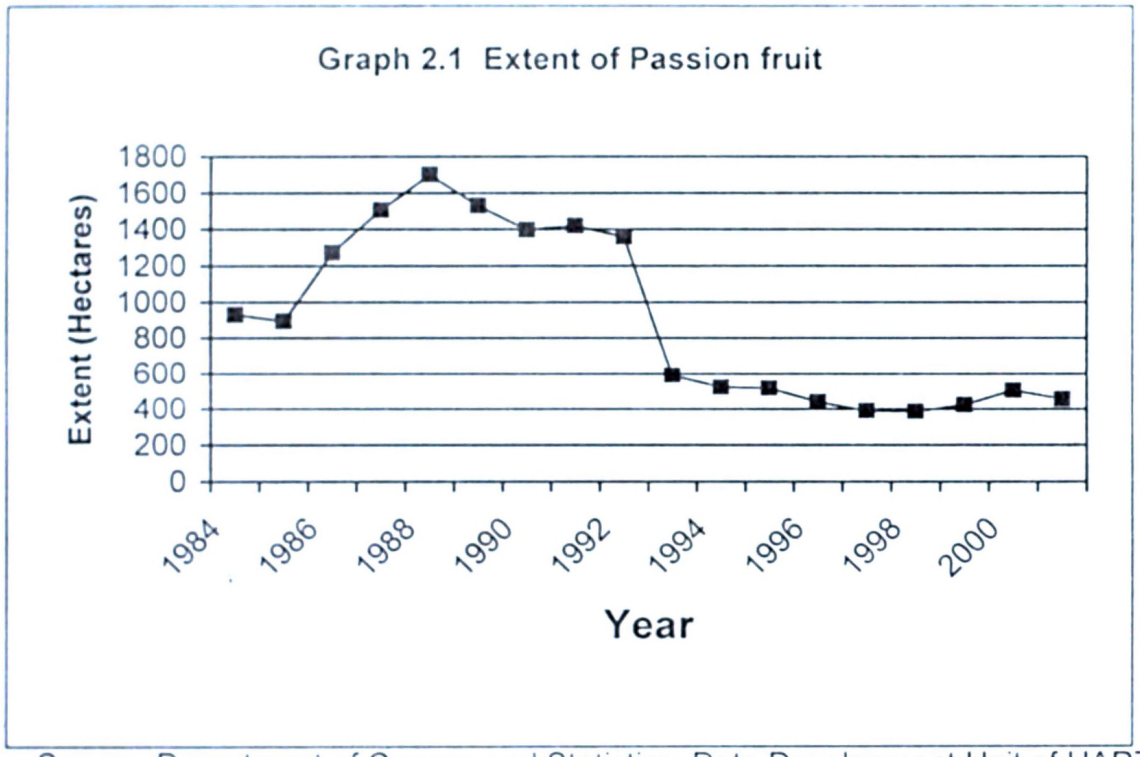
2.3.4 The extent and production

The overall extent and production over the past 16 years (1984-2001), indicate in table 2.1. Though the total extent of Passion Fruit cultivation has increased from 930 hectares in 1984 to 1701 hectares in 1988 it has gradually decreased to 1,359 hectares in 1992 and dropped to 591 hectares in 1993. Since then it has dropped further and it was 425 hectares in 1999. Then it has increased and it was 507 hectares in 2000. Then extent has dropped and it was 457 hectares in 2001. This was mainly due to low returns from Passion Fruit cultivation and low market price. Compared with tea and rubber the price of Passion Fruit dropped sharply and consequently many growers switched from Passion Fruit to tea.

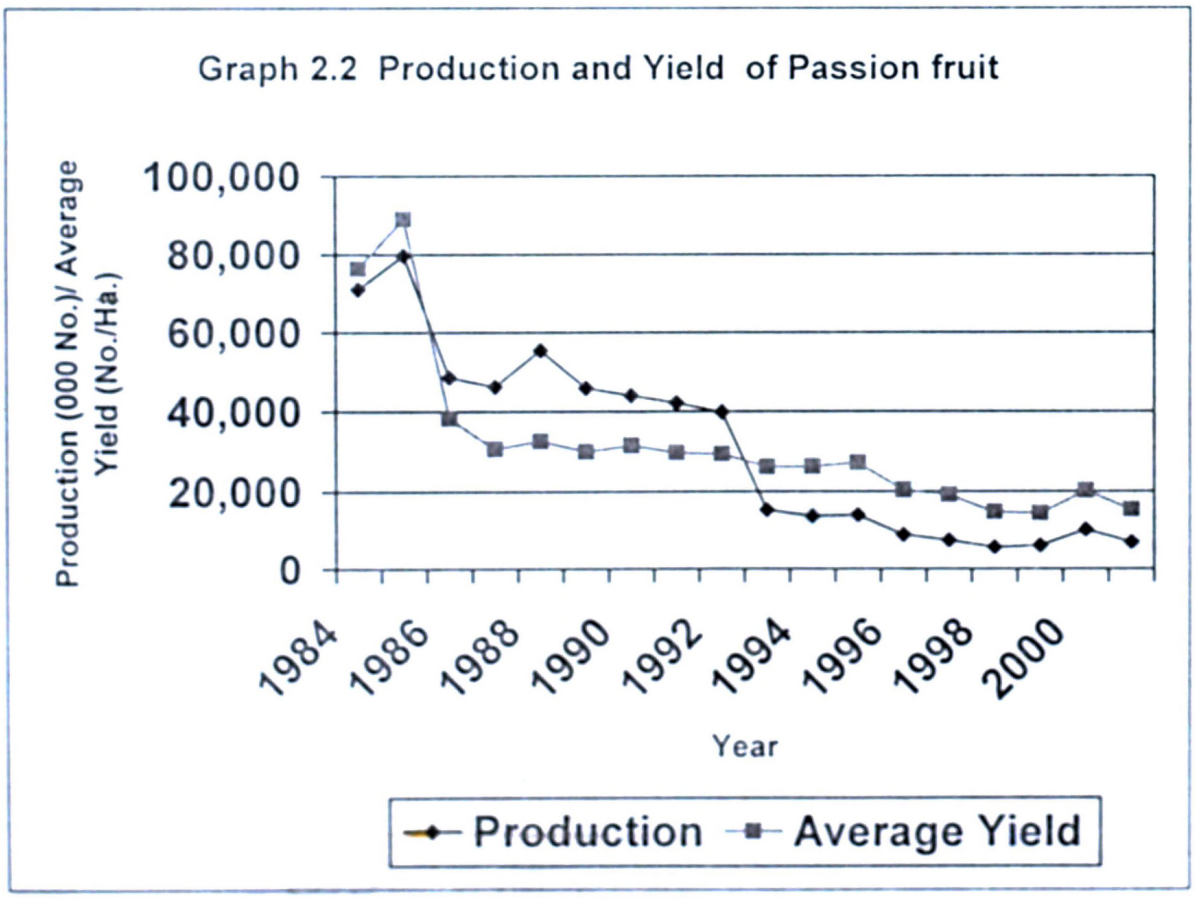
Table 2.1 Extent, Production and Average Yield of Passion Fruit in Sri Lanka

Year	Extent (Hectares)	Production (000 No.)	Average yield(no/H)
1984	930	71,100	76,452
1985	895	79,748	89,104
1986.	1,272	48,626	38,228
1987	1,507	46,177	30,642
1988	1,701	55,506	32,631
1989	1,531	45,901	29,981
1990	1,397	43,963	31,470
1991	1,420	42,143	29,678
1992	1,359	39,957	29,402
1993	591	15,423	26,096
1994	525	13,769	26,227
1995	519	14,090	27,148
1996	441	9,018	20,449
1997	392	7,534	19,219
1998	388	5,771	14,874
1999	425	6,202	14,593
2000	507	10,260	20,237
2001	457	7,072	15,475

Source ; Department of Census and Statistics, Data Development Unit of HARTI



Source :Department of Census and Statistics, Data Development Unit of HARTI



2.3.5 Types of variety cultivated in Sri Lanka

Three varieties of Passion Fruit are cultivated in Sri Lanka currently.

(a) The yellow fruited variety (*Passiflora edulis forma flacicarpa*) (figure 2.4)

(b) The purple fruited variety (*Passiflora edulis*) (figure 2.3)

(c) A mixed variety improved by crossing yellow and purple varieties

The yellow fruited variety and crosses in which the fruit is predominantly yellow are better suited for cultivation in the lower and medium elevation of Sri Lanka, while the purple fruited variety is more suited for cultivation in more cooler climates at higher elevation (above 3,000 feet). The mixed variety called the Rahangala variety is also cultivated mainly in the upcountry areas at higher elevations.

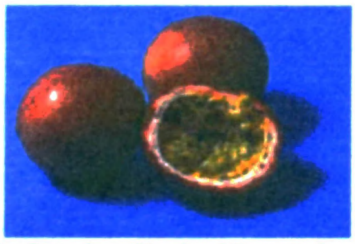


Figure 2.3 Purple Passion Fruit (*Passiplora edulis*)

Figure 2.4 Yellow Passion Fruit (*Passiflora f. flavicarpa edulis*)

2.3.6 **Extent of Passion Fruit by major growing areas**

Passion Fruit cultivation was concentrated in few districts in the wet zone. Thus, as indicated in Table 2, 68% of total extent of Passion Fruit was grown in two districts, Kalutara (48%) and Rathnapura (20%). The balance (32%) was grown in the Colombo, Gampaha, Kurunegala , Matara and Hambontota districts (figure 2.5).

Table 2.2 Extent of Passion Fruit by Major Growing areas (Hectares)

Period	Area				
	Kalutara	Rathnapura	Colombo	Others*	Sri Lanka
1985	485	41	22	347	895
1990	871	49	47	430	1,397
1991	930	52	51	387	1,420
1992	877	73	48	361	1,359
1993	275	106	28	182	591
1994	260	105	22	138	525
1995	262	104	34	119	519
1996	208	81	39	113	441
1997	184	75	25	108	392
1998	187	79	25	97	.388
1999	199	100	21	105	425
Avg. (95-99)	208	88	29	108	433
%	48.04	20.28	6.65	25.03	100.00

Source ;fruits and vegetables ,HARTI Agricultural commodity Review

2.4 Production seasons

The Passion Fruit production of the country enters the commercial channels in the second half of the year starting June and ending around November. Within that six-month period more than eighty percent of the Island's total production of Passion Fruit is harvested and marketed. Although there are some districts that reported production in January to May period, the aggregated output is not much and for all practical purposes this period can be considered as lean months. July is the peak harvest month for Sri Lanka and also for the district of Matara; December for the Colombo district and October-November period for Kalutara district. The differences in the district production patterns may be attributed to variations in agro climatic conditions (Kuhonta and Wijekoon ,1973).

2.5 Yield

Yield depends on several factors including cultivar, seasonal weather and vine management. When Passion Fruit Woodiness Virus (PWV) is not severe, commercial hybrids in subtropical Queensland yield up to 20 to 25 tonnes per hectare. In contrast, yield up to 30 to 50 tonnes per hectare has been recorded for the golden Passion Fruit, *P mollissima* and *P quadrangularis* in Hawaii, Colombia and Northern Queensland. The purple Passion Fruit is less productive with maximum yields of about 5 to 10 tonnes per hectare where Passion Fruit woodiness virus is not severe. The yields of Passion Fruit species in different countries are presented in Table 2.3.

Table 2.3 Yields of *P.edulis* f.*flavicarpa* and hybrids in various countries

country	Species	Density (Vines/ha)	Yield(t/ha)	References
Australia	*P.edulis* × *P.edulis* f. *Flavicarpa*	850	10.0-25.0	Menzel *et al.*(1988)
Brazil	*P.edulis* f. *flavicarpa*	667-1665	2.4-21.6	Manica *et al.*(1978)
Cameroon	*P.edulis* f. *flavicarpa*	2500	6.0-21.8	Haury (1979)
Fiji	*P.edulis* f. *flavicarpa*	550	13.6-37.4	Partridge (1972)
Hawaii	*P.edulis* *P.edulis* f. *flavicarpa*	1200 1200	5.0-10.0 25.0-50.0	Akamine and Girolami (1959) Abeysinghe (1973)
India	*P edulis*	1100	4.6-7.2	Singh *et al.*(1980)
Kenya	*P.edulis*	1200-1500	3.0-66.0	Lippmann *et al.*(1978)
Newzyland	*P.edulis*	1320	10.0-12.0	Sale and Alexander (1986)
South Africa	*P edulis*	1600	18.9-24.4	Bester *et al.*(1978)
Sri Lanka	*P.edulis* *P edulis* f. *flavicarpa*	1000 1000	4.0-6.0 6.0-30.0	Abeysinghe (1973) Abeysinghe *et al* (1973)

Source : fruits ; Tropical and Subtropical (Bose and Mitra 1990)

Average yield for purple-gold hybrids in Australia is about 10 to 15 tonnes per hectare, with more favourable growing conditions in Queensland compared with northern New South Wales accounting for higher productivity. Vines may produce commercial crops for 6 to 8 years. However, the average life of vines is usually no

more than 3 to 4 years, especially in warm environments where disease and pests are difficult to control in dense canopies.

2.6 Marketing

The domestic Passion Fruit market is mainly controlled by few companies involved in the fruit processing and canning industries.

companies are ;

- CPC AGRIFOODS LTD.
- LANKA CANNERIES LTD
- KELANI VALLEY CANNERIES LTD.
- SCAN PRODUCTS MANF. (PVT) LTD

Marketing arrangements are done through:

(a) Regional Collectors

Regional collectors posted in all the growing areas collect Passion Fruit from small growers and local agents and send it to processing companies.

(b) Out growers Schemes

A few companies involved in fruit juice and canning industries implement these schemes. The necessary inputs and advice are given to out growers registered with companies, which purchase total product at the market price. These out growers' schemes are operated in Gampaha, Rathnapura and Kalutara districts.

2.7 Export

More than 90% of local production of Passion Fruits are used for making various Passion Fruit products such as juice, pulp, jam, jelly, syrup and cordials etc. Many of these final products of pulp, cordial or syrup are exported. According to custom reports, Passion Fruit products have been exported to 35 countries from 1994 to 1998 (Appendix 1).

According to some reports the overseas markets for Passion Fruits in 1995 to 1999 have changed. Appendix 1 shows that Japan, Singapore, Switzerland and U.A.E. were the main buyers before 1997 according for 72% of total exports. Since 1998, however, Germany, Netherlands and Philippine became the major buyers and accounted for 67% of total exports.

2.8 Consumption

According to Consumer Finance and Socio Economic Survey (1996/97) of the Central Bank of Sri Lanka, overall average per capita consumption of Passion Fruit per annum was 0.72 and it varied according to urban (0.36), rural (0.72) and estate (0.24) sectors respectively (table 2.3). The per capita average consumption is around 1 or above among income categories above Rs. 6,000/= per annum while it vary 0.24-0.48 among income categories less than Rs. 6,000/= per annum. According to Table 2.3, consumption is very low in the estate sector compared to the rural and urban sectors. However, it shows that the average consumption in the rural sector has decreased from 1.20 in 1986/87 to 0.72 in 1996/97 (Henegedera *et al*, 2002).

Table 2.3 Per Capita Consumption of Passion Fruit per Annum by Income Group and Sectors (1996/97)

Income Group	Urban (No)	Rural (No.)	Estate (No.)	All Sec. (No.)
0-300	-	-	-	-
301-600	-	-	-	-
601-1200	-	-	-	-
1201-1800	-	0.24		0.24
1801-2400	-	0.12	-	-
2401-3000	-	0.36	-	0.36
3001-4500	-	0.48	-	0.48
4501-6000	-	0.24	-	0.24
6001-7500	-	1.20	0.48	1.08
7501-9000	-	0.96	-	0.72
9001-12000	0.36	0.96	2.16-	0.84
12001-15000	0.24	1.08	-	0.84
15001-30000	0.60	1.80	-	1.44
Over 30000	1.20	-	-	0.60
Overall Avg.	0.36	0.72	0.24	0.72

Source: Consumer Finance and Socio Economic Survey
Central Bank of Sri Lanka

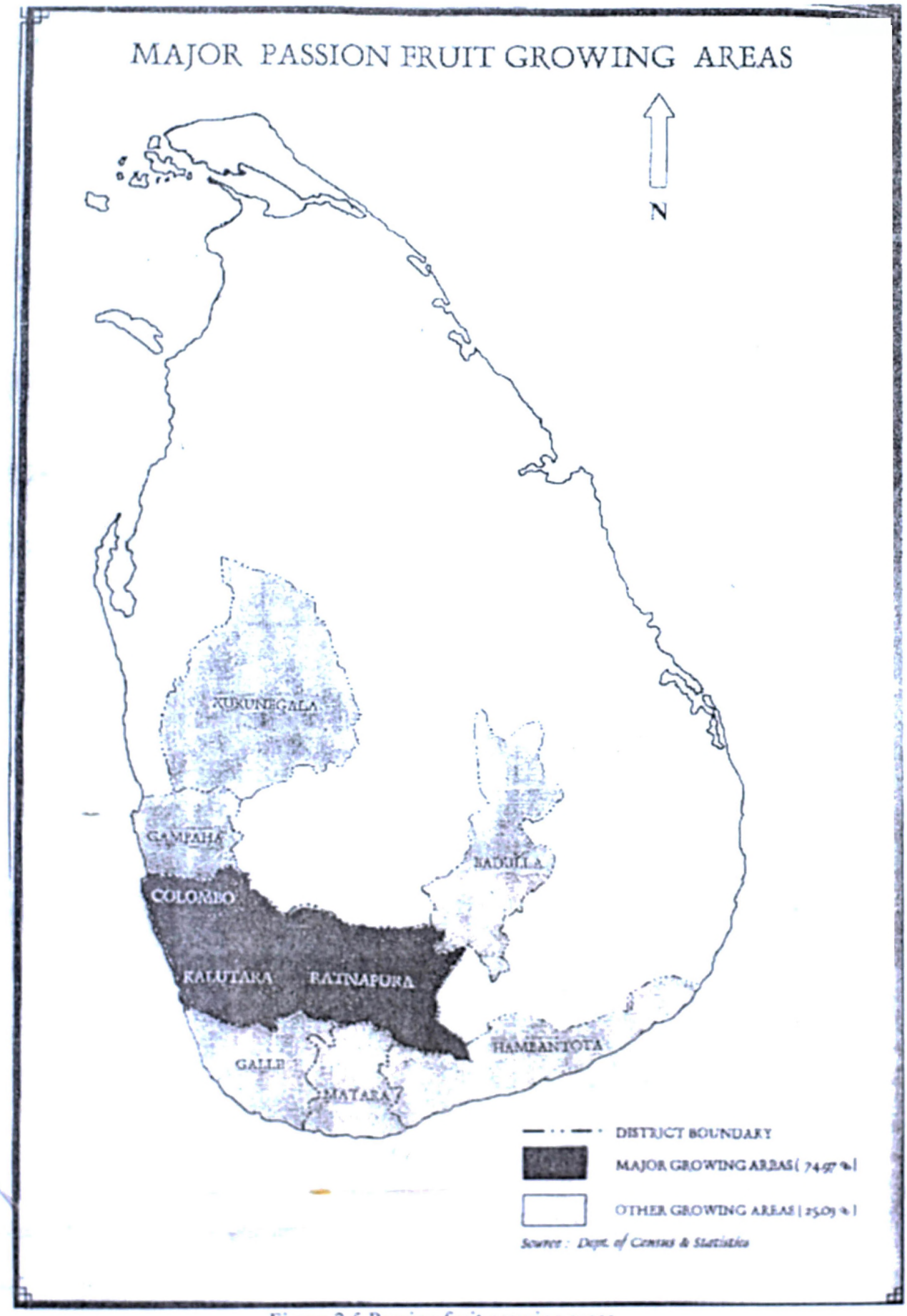


Figure 2.5 Passion fruit growing areas

2.9 Composition of Passion Fruit and juice

Passion Fruit juice, a fragrant, acidic, yellow/orange-pulp is extracted from round or egg-shaped fruits (T.C.Harvey,1993). The juice has a strong intensive aromatic flavour with a soluble solids content of about 15%. The acidity is high with a brix / acid ratio averaging 5 and a pH value of 2.6-3.2. Carotene, Vitamins A and C are present in quite high quantities (Hooper.J). The major nutrients found in Passion Fruit vary according to growing conditions and geographic location.

Pruthi (1963) reviewed the composition of the fruits of the passiflora species earlier in a comprehensive treatise. A later review by Chan (1980) provided more recent information on the nutrient composition of seven different species of Passion Fruit and also provided information on the flavor chemistry of both the yellow and purple Passion Fruits. Another recent review by Casmir *et al* (1981) provided a comprehensive review of Passion Fruit processing technology and its effect on the chemistry of Passion Fruit flavors. Some of the earliest reports on the composition of the various Passion Fruits grown through the world were Munsell *et al.*(1950 A, B, C) who reported on the composition of Central American Passion Fruits; Jewell (1933) who reported on Australian Passion Fruit; Seale and Sherman (1960) and Wenkam and Miller (1965) who reported on the composition of Hawaiian grown purple yellow Passion Fruits; and Pruthi and Lal (1959) who reported on the composition of purple Passion Fruit grown in India. Researches are reported which lists the nutrient composition of both purple and yellow Passion Fruits which given in Table 2.5.

Figure 2.6Yellow Passion Fruit (*Passiflora F. flavicarpa edulis*) pulp

Table 2.5 Nutritive composition of the purple and yellow varieties of Passion Fruit juice

Nutrient	Units	Amount in 100g of juice	
		Purple	Yellow
Proximate;			
Water	g	85.62	84.21
Food energy	kcal	51	60
Protein	g	0.39	0.67
Total lipid	g	0.05	0.18
Carbohydrate	g	13.60	14.45
Fiber	g	0.04	0.17
Ash	g	0.34	0.49
Minerals;			
Calcium	mg	4	4
Iron	mg	0.24	0.36
Magnesium	mg		17
Phosphorus	mg	13	25
Potassium	mg		278
Sodium	mg		6
Vitamins;			
Ascorbic acid	mg	29.8	18.2
Riboflavin	mg	0.131	1.101
Niacin	mg	1.460	2.240
Vitamin B_{12}	mcg	0	0
Vitamin A	IU	717	2,410

Source ; Percival *et al* (2000)

2.9.1. Sugar

The total carbohydrates is the second largest constituent of Passion Fruit juice. Major type of carbohydrate is sugar. It can be divided in to three major sugars as Glucose, Fructose and Sucrose.

Table 2.6 Values are reported in mg/g of juice

	Fructose	Glucose	Sucrose
Yellow Passion Fruit	14.5	19.8	9.1
Purple Passion Fruit	16.2	20.1	8.1

Source: passionfruit juice composition and Potential Health Benefits (Percival *el al.* 2000).

The sugars in the purple variety is an average of 17.3% (Pruthi 1963) and the sugars in the yellow variety average of 15% (Boyle et al. 1955). Because of the purple Passion Fruits higher average sugar content, it's sugar acid ratio (5.1) is higher than that of the yellow variety (3.8). fructose is at least 1.5 times sweeter than sucrose; therefore purple Passion Fruit has higher sweetness rating followed by the yellow variety.

Trace amounts of seven carbon sugars such as mannoheptulose (D-manno-heptulose) and sedoheptulose (D- altro-heptulose) were reported to be present in Passion Fruit by Ogata *et al* (1972).

2.9.2. Starch

Passion Fruit contains appreciable amounts of starch. Pruthi (1963) reported the starch content of purple Passion Fruit juice as 1.0 to 3.7%. Cillie and Joubert (1950) isolated and characterized the starch from the purple variety. Kwok *et al* (1974) isolated and characterized the starch granules from both purple and yellow Passion Fruits that were grown in Hawaii. The starch content was found to be higher in the purple variety (0.74%) than in the yellow variety (0.06%). Alpha amylase was effective in reducing the viscosity of Passion Fruit juice in which the starches gelatinized.

The phenomena of Passion Fruit starch gelation at temperatures greater than 55°C have been reported by Casmir (1974), Mollenhauer (1954), and Fonseca (1976). Because of its low gelation temperature (55-58.5°C), Passion Fruit starch causes severe problems during the heat processing of Passion Fruit juice. Seale and Sherman (1960) noted that the high starch content of passion fruit juice caused gelatinous deposits to accumulate on the heating surfaces of the heat exchangers. This resulted in localized scorching with resultant drop in heat exchanger efficiency and deterioration in juice flavour (Harvey T Chan, Jr,1993).

2.9.3 Organic Acid

Other than its unique and distinctive flavor, the high acid content of Passion Fruit is its most distinctive characteristic and is important in the formulation and processing of products containing this fruit. The ranges in pH and total acid content (expressed as citric acid, w/w) have been reported for the yellow (pH 2.8-3.3, 3.0-5.0% acid; Boyle *et al*,1955) and purple Passion Fruit (pH 2.6-3.2,2.4-4.8% acid; Pruthi 1963) juice. Using gas and thin-layer chromatographic methods, Chan et al. (1972) isolated and identified the nonvolatile organic acids in purple and yellow Passion Fruit juices. For yellow Passion Fruit, citric acid was the predominant acid, followed by malic. The other acids present in yellow Passion Fruit, but in much lesser amounts, were lactic, malonic and succinic acid (table 2.7). although purple Passion Fruit was found to contain the same acids as yellow Passion Fruit, the relative abundance of each of the acids differed markedly. Citric acid was the most abundant acid (Harvey T Chan ,Jr,1993).

Table 2.7 Quantitative determination of organic acids in yellow and purple Passion Fruit

Acid	Yellow (meq/100g)	Purple (meq/100g)
Citric acid	55.00	13.10
Malic	10.55	3.86
Lactic	0.58	7.49
Malonic	0.13	4.95
Succinic	trace	2.42
Ascorbic	0.06	0.05
Volatile acids	0.11	0.12
Total	**66.43**	**31.99**
Total titrable acids	**65.83**	**32.01**

Source : Chan *et al.*(1972)

2.9.4 Amino Acid

Pruthi (1963) reviewed the composition and technology of Passion Fruit juice. He gives the total nitrogen content as ranging from 96-192 mg 100ml; of which about half is amino nitrogen. The chief free amino acids are leucine, proline and threonine

with smaller amounts of valine, tyrosine, aspartic acid, glycine, arginine and lysine (Hulme, 1971).

2.9.5 Phytochemicals- Carotenoids

Phytochemicals are a class of compounds that are found exclusively in plants that are non-nutritive but have far-reaching health benefits, usually acting as potent antioxidants (Percival,S.S *et al* ,2000). The three main carotenoids in purple Passion Fruit are beta-carotene, gamma-carotene and phytofluence. In addition, the presence of beta –apo-12'-carotenal, beta- apo-8'-carotenal, cryptoxanthin, auroxanthin and mutatoxanthin, were reported by Leuenberger sand Thommen (1972).

2.9.6 Enzymes

The presence of a catalase enzyme in yellow Passion Fruit was reported by Ross and Chang (1958). Aung and Ross (1965) attained 100% inactivation of Passion Fruit catalase by heating the juice at 79°C for 75 sec. Pectinmethylesterase was reported to be present in purple Passion Fruit juice by Pruthi and Srivas (1963). The enzyme was inactivated by heating at 80°C for 60 sec. Hashinaga *et al.*(1978) detected the presence of two proteases in purple Passion Fruit juice. Using casein as the substrate, pH optimal for the acid protease was 2.3 and for the SH-protease the pH optimal was 5.7.

2.9.7 Vitamins and Minerals

Vitamin A and C are present in quite high quantities (Table 2.5). One glass of Passion Fruit juice provides about 50% of the dietary reference intake for adult men and 60% of Vitamin C. Labeling requirements would allow Passion Fruit juice to be labeled as an excellent source of vitamin C (refer table2.8).

Table 2.8 Content of Vitamin C in different Exotic Fruits

Fruit	Vitamin C (mg/100g) Fresh fruit
Passion Fruit	67.78
Grapefruit	64.78
Kiwi	67.23
Mango	25.32
Papaya	88.20
Pineapple	30.60
Lemon	51.30
Orange	49.80

Source; Percival *et al* .2000

Passion Fruit juice also provides minerals as Ca, Mg, K, Zn, Cu and Se (table 2.5).

2.9.8 Plant Sterols

Plant sterols are found in all plant foods and have been reported to have an ability to lower blood cholesterol. Increasing the consumption of plant foods that are high in sterols may have a positive impact on health, although the benefits may also be due to other factors in plants, such as the amount of soluble fiber.

Passion Fruit contains the highest amount of plant sterol compared to the other fruit and the second highest sterol content out of all the fruits and vegetables. Compared to broccoli, Brussels sprouts, cauliflower and black olives, Passion Fruit is sweet alternative to those other sterol-dense vegetables.

2.9.9 Alkaloids and Cyanogenic compound

Both alkaloid and Cyanogenic type compounds have been reported in Passion Fruit juice (Casmir *et al*.1981). Lutomski *et al.* (1975) detected the presence of seven alkaloids with four being identified as harman, harmin, harmol and harmalin. Pharmacological tests showed that the juices had slight sedative effects. Gondwe (1976) showed Passion Fruits both the purple and yellow as Cyanogenic. However, it was concluded that the small amounts present in ripe fruit were insignificant to be of any toxicological significance.

2.9.10 Volatile Compound

Flavour and aroma are volatile compounds in Passion Fruit juice. Casmir and Whitfield (1978) have conceived a method to assess the flavour value of each flavor component which they term "Flavor Impact Value". Out of the 300 volatile flavorants in Passion Fruit, only 22 of the peaks were identified as having Passion Fruit flavour. The flavour impact values for the15 compounds responsible for Passion Fruit flavour is shown in Table 2.9.

Table 2.9 Flavour impact values of Passion Fruit volatile compounds

Compound	Flavour impact value	Conc. In juice (ppm)	Contribution to flavor profile (%)
• 6-(But-2-enylidene)-1,5,5-trimethycyclohex-1-ene	79	1.1	30
• (Z)- Hex-3-enyl butanoate	41	0.8	11
• Hexyl butanoate	6.8	4.1	9
• Ethyl (Z)-oct-4-enoate	62	0.4	8
• Beta-ionone	410	0.05	7
• Edulan I	23	0.8	6
• Ethyl (Z)-octa-4,7-dienoate	239	0.06	5
• Linalool	30	0.05	5
• Ethyl hexanoate	1.3	7.6	3
• Heptan-2-ol	1.7	5.3	3
• (Z)-Hex-3-enol	26	0.3	3
• S compounds (unidentified)	76	0.1	3
• Hexanol/nonan-2-one	1.8	4.0	3
• Rose oxide	45	0.2	2
• Methyl butanoate	0.7	8.3	2

Source: Casmir *et al.*(1981).

2.10 Health benefits of Passion Fruit juice

The new dietary recommendations for healthy peoples encourages the intake of fruits and vegetables due to their anticancer and other health promoting properties. Passion Fruit would be an attractive addition to the American diet, providing unique flavor, plenty of antioxidants and an increase in the variety of the diet.

Passion Fruit juice is an excellent source of antioxidants such as vitamin C and beta-carotene. Vitamin C and beta-carotene have the distinct ability to neutralize damaging free radicals from the watery and fatty parts of the body. They protect our cell from DNA damage and help prevent the formation of pre-cancerous cells. Vitamin C protects LDL- cholesterol from oxidative damage and may protect against cardiovascular disease. Beta carotene not only has its own health benefits, but it is also converted to vitamin A, which in turn assists with normal cell growth and differentiation, vision, reproduction and skin health.

Passion Fruit juice is also a good source of potassium. Due to the high incidence of hypertension in the world, many people are required to take multiple medications for high blood pressure, some which help eliminate water from the body. Another common heart condition typically experienced by older persons is Congestive Heart Failure (CHF). Associated with CHF is the retention of fluids in the lower legs, in the lungs and around the heart muscle. This condition, known as edema, often requires people with CHF to take medications known as diuretics to help remove water. Many types of diuretics are potassium –depleting drugs. Passion Fruit contains a greater potassium content than oranges. The addition of an exotic, flavorful fruit juice high in potassium may help many people find ways to increase their dietary intake of this nutrient while taking these type of drugs.

Further research is needed on terpenoids and their potential anti cancer properties. Research using animal cancer models, have shown that some monoterpenes have anticarcinogenic properties therefore allowing potential as anticancer. A benefit of dietary sources of monoterpenes appears to be their low toxicity, which puts them in a unique position as a new novel class of anticancer drugs.

In 1995, the monoterpene limonene was tested in phase 01 clinical trials in the United Kingdom (Gould M.N. 1997). The naturally occurring limonene derivative, perillyl alcohol, has been found to be more potent than limonene itself and may also prove to be a potent anticarcinogenic compound. Perillyl alcohol will be the focus of US trials and if proven to be effective will open the door for further research into the possible therapeutic properties of other terpenoids. Yellow Passion Fruit juice may possess similar therapeutic potential and should be thoroughly researched.

2.10.1. Composition and health benefits.

Changes that occur post-harvest, during handling and processing may affect the health benefits of the fruit and juice. Common changes include vitamin, mineral and physiochemical losses. Some of the greatest losses occur due to the removal of the rind, lack of pulp and post –harvest-ripening techniques. The rind and pulp contain many health attributes. They are a significant source of pectin and phytochemicals such as phytosterols. This waste product may be able to utilize in a functional food designed to provide cardiovascular health benefits related to its antioxidant capacity. The amounts of these substances may be decreased during the juicing process. In some cases, the Phytochemicals found in the juice may be concentrated during the juicing process.

Significant amounts of protein, fat and ash are removed during the juicing process. The juice contains 81.6% less protein, 95% less fat and 38.7% less ash than the fresh fruit. This analysis however, is limited because the analyses are derived so differently and the techniques used to derive the values are so different.

The major change in composition is the difference in vitamin C content in naturally versus artificially ripened fruit. The average loss of ascorbic acid associated with artificial ripening of different was 32-42% (Vinci *et at*,1995). This is a considerable amount of nutrient loss, which indicates that naturally ripened Passion Fruit may yield higher nutrient content and possess more impressive health benefits.

2.10.2 Possible precautions for allergies

Passion Fruit, like other foods, may not be appropriate for all people. Although Passion Fruit is in many ways like other citrus fruits eaten in the United States, people with multiple food allergies or food sensitivities should introduce new foods cautiously. Passion Fruit has also been included in studies concerning "latex fruit syndrome". Some people with confirmed allergies to latex also experience allergies to some fruits. Further research is needed to discover the exact immune response associated with this phenomenon.

As reported, Passion Fruit does not contain significant amounts of salicylic acid, which is a known allergen for many people. Other phytochemicals found in yellow Passion Fruit, except for cyanogenic glycosides found primarily in the immature fruit, have not been reported to cause deleterious health effects (Percival ,Talcott and Kellenberger, 2000).

2.11 Passion Fruit products

Passion Fruit, an exotic tropical product, has slowly but steadily gained acceptance in the international market. Compared to other tropical fruits, it is still at an early stage of development. The potential uses of Passion Fruit in the food industry is being explored and broadened.

i. Passion Fruit juice for either carbonated and uncarbonated beverages (nectars, fruit juice beverages, soft drink)
ii. Mixed drinks- carbonated and uncarbonated with other fruit juices such as orange, grapefruit, pineapple and guava.
iii. Syrups and squashes as foundation for party drinks and sweet dishes.
iv. As an ingredient for mix fruit jam and jelly.
v. Flavoring for ice cream, soft ice and sherbet.
vi. Creation of exotic food mixes out of traditional milk products, e.g. milk Passion Fruit shakes, Passion Fruit yogurt, and fruit salad with Passion Fruit juice.
vii. As filling for confectionery, cakes, chocolates, etc.

2.12 Byproduct of Passion Fruit

The raw Passion Fruit edible matter proportion (flesh and seeds ; no skin) of purchased 0.42 (Kirk and Sowyer, 1991). In the extraction of juice from Passion Fruit, about 2/3 of bulk is refuse, of which 90% is rind and about 10% is seeds (Pruthi 1963; Otagaki and Matsumotom,1958). Because of its serious disposal problem, several studies on its possible utilization have been conduct. Martin and Reuter (1949) isolated pectic substances from purple Passion Fruit skin. Sherman *et al.*(1953) isolated and characterized the pectin from yellow Passion Fruit and found the pectin to have good jelling properties with a methoxyl content of 8.9-9.2%.

The composition of purple and yellow Passion Fruit rinds is shown in Table 2.10. Both the yellow (Otagaki and Matsumoto,1958) and purple (Pruthi,1960) Passion Fruit rinds were found to be high in carbohydrates, low in ether extractable material and moderate in crude protein. The seeds yielded clear bland oil of good quality.

Passion Fruit rinds were found to be satisfactory as a supplementing foodstuff for dairy cows. The rinds dehydrated readily without lime pretreatment and the dried material was acceptable to dairy cattle at 22% of the ratio (Otagaki and

Matsumoto,1958). As a result of this study, Passion Fruit rinds are now commercially utilized as feed for dairy animals in the Hawaiian Islands.

Further chemical analysis of Passion Fruit rinds (Susheela *et al*, 1960) showed the presence of starch, sucrose, fructose, glucose, citric acid, malic acid, and tannic acid. Feeding studies with Passion Fruit seed oil showed it to be similar to groundnut (peanut) oil with respect to its growth promoting value and digestibility coefficient when fed at the 5% level in poor southern Indian and synthetic diets (Pruthi,1963).

Table 2.10 Composition of purple and yellow Passion Fruit rind

Composition	Fresh purple Rind (%)[1]	Dried yellow Rind (%)[2]
Moisture	81.92	16.80
Crude protein	2.56	4.58
Ether extract	0.12	0.33
Ash	1.47	6.76
Crude fiber	5.01	25.66
Nitrogen free extract	7.14	45.87
Pentosans	-	15.70
Lignin	-	6.50
Pectin	1.78	20.00

[1] *source:* Pruthi (1963),

[2] *source :* Otagaki and Matsumoto (1958).

2.13 Passion fruit Juice processing and preservation at CPC

2.13.1 Harvesting

Generally farmers harvest mature ripened passion fruit including immature, spoiled or diseased fruits from vines. They collect fruits into polypropylene bags or plastics crates. Also might even collect fallen fruits from the ground.

2.13.2 Transportation and storage

Wholesale suppliers pack passion fruit in polypropelene bags or plastic crates. They are transported using lorries. Unloaded fruits are temporarily stored at production line 1 storages, under room temperature conditions (about 31 ^{0}C).

2.13.3 Washing and inspection

Fruit wash at primary washer and secondary washer.

Primary washer

Primary washer is built like a wash tank. Material – fiberglass. Method of Fruit washing is by soaking in water with water sprays. Water in the bath is Chlorinated. Recommended chlorine content is 300 ppm with contact time of 10 minutes.

Inspection

The fruit is fed to an inspection belt , where manual sorting is done to remove culls , immature fruits , spoiled fruits and extraneous materials. (after primary washer)

Secondary washer

This has a zigzag travel path to increase washing time when fruit is transferred with flowing fresh water. Major objective is to remove remaining chlorine from fruits.

2.13.4 Juice extraction from fruits

In countries where Labour is relatively cheap, passion fruit is extracted manually from the harvested fruits by spooning or reaming. Automatic means of use of unopened fruit with suction needles (Hubbard, 1973) too has been recorded.

Several kinds of mechanical extractors are available.

1. Centrifugal Extractor.
2. Converging cone Extractor.
3. Passypress Extractor.

Passypress Extractor

Passypress Extractor is the mechanism available at CPC Agrifoods Ltd. The Italian food machinery manufactures S.A. Bertuzzi have developed an extractor, particularly for the yellow passion fruit, which they call the passypress extractor and which resembles some citrus juice extractors. The passion fruit are compressed between two rollers, one rubber- covered and the other having stainless steel teeth. A diaphragm moving up and down ensures that the rollers and the skins are fractured. The toothed roller then presses the broken fruit against a screen so that the pulp flows through. Extractors are available with feed capacities of 1-4 tons/hr (Casmir, 1981).

2.13.5 Preservation of passion fruit

2.13.5.1 Methods of fruit juice preservation

Preservation can be defined as a process by which foods are treated to retard decay or spoilage. There are many reasons for preserving passion fruit juice. To have a supply of these foods throughout the year , rather than seasonally at harvested time. In case of a passion crop failure caused by natural disaster such as drought ,wind, hail, flood, fire, freezing or insect and disease infestation ,or by human disasters ,such as war .The preservation of previously processed excess food becomes paramount.

With preservation , one can obtain a more varied diet because a crop can then be used throughout the year and crops native to only a small area can be transported and used anywhere in the world . One of the reasons for developing countries to have food shortages is that they do not have facilities for preservation and transportation of foods. Thus certain areas have a temporary surplus of fruits while other areas have a shortage.

Fresh fruit deteriorate rapidly if held at ambient temperatures. Preservation allows the holding of foods so that they can be used as ingredient for mixed foods. Many of our convenience foods are combinations of various foods. Some systems used to preserve food also destroy many of the organisms and toxic factors that are hazards in food products.

Three methods on applied for preservation of passion fruit in this company.

- Pasteurization & Aseptic filling – (without using preservatives)
- Pasteurization & Chemical preservation
- Freezing preservation

When the juice is extracted, it is transferred to an Aseptic system. Receiving tank of Aseptic system is equipped with a dearator which removes overhead air that can cause oxidation. Receiving juice is also pre heated (50 ^{0}C) which helps dearation & 2nd cycle of heating.

Pasteurization

Then de-aerated, pre heated passion juice is subjected to heating (pasteurization). This is done by on line transferring of juice via a tubular type heat exchanger in aseptic system. Pasteurization is done at 95 ^{0}C for 60 seconds. (Pasteurization parameters are determined by pH and texture of juice, Passion fruit juice is of low pH and the texture is thin, there for require reduced temperature and short time). Heated product is pre-cooled and cooled to below 40^{0}C before filling into aseptic Bags. Passion fruit is heat sensitive and turns brown due to non-enzymatic browning with time. Therefor the aseptic juice can stored under low temperatures between 5 – 15^{0}C only for 18 months.

Chemical preservation and chilling preservation

This method used once an aseptic bag has being opened and a balance require storage. Done by addition of Sodium Metabisulphite solution and mixing to contain 300 ppm of sulfur dioxide. Storage is in chiller for maximum of 3 months. Low temperature reduces chemical reactions and also will control if any microbial contamination has occurred when the contents were exposed.

2.14 Packaging

Aseptic packaging is widely used for a range of fruits products. For example tomato paste, fruit desserts, pudding and fruit juices. Passion fruit juice processing also uses aseptic packaging. It is a bulk aseptic system.

2.14.1 Bulk aseptic systems

Bulk aseptic systems are of growing importance in the worldwide distribution of product such as tomato paste, fruit purees and fruit juice concentration. They are normally done using aseptic bag systems in the drum. Bag in box system is also practiced by some.

2.14.2 Aseptic bag

200 liters preformed laminated aseptic bags used for filling aseptic juice. The bag used is an Elpo style barrier bag, which packs 180 Kg – 200 kg of pasteurized juice. Capacity of sample bag is 5 Kg. Both bags are drawn in to a sterilized chamber in aseptic system prior to opening of cap. Then the bag is filled automatically and capped before transferring out of sterilized chamber. The bag is received from supplier sterilized using Gamma irradiation process to a level of 15.0 Kgy.

Material- Metalized laminate –outer layer
Linear polyethylene blend - inner layer
Linear polyethylene blend - inner layer
Metalized laminate –outer layer

Drum is HDPE plastic drum with insert type lid and screw on ring for processed fruit juice storage. Capacity is 220 liters.

2.15 Problems faced in processing of passion fruits

2.15.1 Pest and diseases

Pest

Several insect pests attack passion fruit vine causing economic losses because of fruit blemishes and/or loss of plant vigour and productivity (Sale and Alexander, 1986). The major pests are fruit fly (not in all countries), citrus mealybug ,California red scale and passion vine mite. Pests that are sometimes important are fruit spotting bug, green vegetable bug, soft brown scale, passion fruit hopper, aphids, thrips, rutherglen bug and broad mite.

Diseases

Several diseases limit the production of passion fruit (Sale and Alexander, 1986). The major diseases of passion fruit are septoria Spot, brown spot, Phytophthora blight, Alternata spot, woodiness virus and base rot.

Phytopthora rot of roots and Fusarium wilt (*Fusarium oxysporum)* and nematodes are not

problems because of the use of resistant *Passiflora edulis* f. *flavicarpa* root stocks.
Septoria spot (*Septoria passiflorae*), Brown spot (*Alternaria passiflorae*) and Alternata spot (*Altrnāria alternata*) infect leaves, stems and fruit of passion fruit.

2.15.2 Pre harvest and post harvest practices

The quality of fresh fruits or the products processed from fresh fruits is governed by a number of pre harvest and post harvest factors (Kader and Barrett, 1996). The important preharvest factors that influence fruit quality are the following;

- The genetics-selection of the right cultivars and rootstocks;
- Climate-temperature,
- Light and wind factors and cultural practices-soil type,
- Soil nutrient and water supply,
- Pruning,
- Thinning,
- Pest controls.

Post harvest factors that influence fruit quality are:

- Environmental-temperature,
- Relative humidity
- Atmospheric composition of storage,
- Handling methods

Post harvesting handling systems involving the channels through which the harvested fruit reach the processor or consumer and time period between harvesting and consumption-delays between harvesting and cooling or processing may cause losses in fruit quality (Kader and Barrett, 1996).

2.15.3 Biochemistry of fruits and its implications on processing

2.15.3.1 Browning reaction during processing

(a) Polyphenol oxidases

Polyphenol oxidase is widely distributed in fruits. It has been given two entries in the international Union of Biochemistry classification, namely as EC 1.14.18.1,monophenol mono –oxygenase, and EC 1.10.3.1, catechol oxidase. Common trivial names for mono-phenol mono oxygenase are tyrosinase,phenolase,and cresolase. Common trivial names for catechol oxidase are diphenol oxidase,o-diphenolase, phenolase, polyphenol oxidase and tyrosinase (Enzyme Nomenclature,1984). Polyphenol oxidases can catalyze many reactions involving phenolic compounds found naturally in many fruits. When the mono-phenol p-cresol is the substrate, it is oxidized to 4-methyl catechol, a diphenol. The oxygen for the hydroxylation comes from the atmosphere. Thus the enzyme acts as a monooxygenase. When the diphenol cresol is the substrate, it is dehydrogenated to o-benzoquinone. Quinones are highly reactive compounds and undergo further oxidative polymerization to form brown-colored pigments (Whitaker,1996).

Total phenolic compounds in ripe fruits 1.4 mg/100g fruit weight (Pruthi *et al*, 1961)

(b) Non enzymatic browning in fruit products

Fruit juices usually undergo a number of non-enzymatic reactions depending on their composition, concentration and storage conditions (Eskin *et al*, 1971). Maillard browning reactions occur between reducing sugars and α-amino groups of amino acids, peptides and proteins. The reaction between amino acids α-dicarbonyls, known as the streacker degradation in the maillard reaction, also leads to brown pigment formation. Lipid oxidation can give rise to reducing substances that can react with amino acids to form brown pigments and off-flavors.

Ascorbic acid destruction in fruit juices can take place under aerobic or anaerobic conditions under normal processing temperatures (Rojas and Gerschenson, 1997). They found that under anaerobic conditions the destruction of ascorbic acid and thus the browning reactions increase with increase in pH from 3.5 to 5.0. Addition of tin or lysine increased ascorbic acid loss and browning under aerobic conditions the degradation of ascorbic acid was retarded in the presence of glucose.

Caramelization of sugars can take place at relatively high temperatures under acid or alkaline conditions in the absence of amino acids giving rise to non enzymatic browning is associated with unpleasant, burned and bitter flavors.

(c) Browning reactions due to chlorophyll degradation reactions

Chlorophyll present in immature passion fruit rind. Chlorophylls are porphyrins containing the basic tetrapyrrol ring, of which one is reduced. The four rings are coordinated with Mg^{**}. The Mg^{**} is easily reduced in acid solutions found in the fruit by H^{*}. Giving rise to dull olive green and further degradation leads to brown pigment formation (Gross, 1991).

The passion fruit is now taking on an increasing importance as a source of juice. The fruit is small and has a hard rind, so that juice extraction presents problems; centrifugation of the cut fruit is one method adopted. Further problems are the presence of starch and the heat sensitivity of the aroma components (Pruthi, 1963; Charley, 1968). Unless the amount of starch can be reduced by centrifugal or other means following juice extraction, the formation of gels makes subsequent preservation by heat treatment difficult. Preservation of the juice by freezing overcomes both these problems.

(d) Changes occurring during juice extraction

The process of milling fruit disorganizes the cellular structure and brings enzymes normally associated with structural components or other wise segregated into contact with soluble substrates and with oxygen from the air or from the intercellular spaces (Smock and Newbert, 1950).

Other changes that may occur are the oxidation of ascorbic acid, where suitable enzyme systems are present and other oxidative changes that can affect quality and flavor.

CHAPTER 03

MATERIAL AND METHODOLOGY

3.1 Material, equipment and machinery required for culture, examination and measurement of microorganisms.

3.1.1. Equipment for culture

The basic equipments needed for culturing microorganisms are as follows:

A. **Containers:** These are specific vessels in which sterile media are kept ready for use and in which cultures are grown. Those which are used most frequently are :

i. Petri dishes
ii. Culture tube
iii. Screw cap glass bottles: these are of 20-30 ml capacity such as McCartney bottles.
iv. Conical flasks
v. A Inoculating needle
vi. Pipettes
vii. Autoclave
viii. Incubator
ix. Inoculation room

This could be closed and sterilized small room for use inoculation at QA lab. Tables, bench sterilized by using 99% alcohol solution.

3.1.2 Culture media

OXOIDE is a reputed supplier of culture media.

3.1.2.1 Nutrient Agar

Code number: CM3

This media is used for determine total plate count.

Formula

Chemicals	g/liter
'Lab Lemco' powder*	1.0
Yeast extract	2.0
Peptone	5.0
Sodium Chloride	5.0
Agar	15.0

pH 7.4 ± 0.2

- Lab Lemco is a beef extract

Preparation

Suspend 28 g in one liter of distilled water. Bring to the boil to dissolve completely sterilized by Autoclaving at 121°C for 15 minutes.

3.1.2.2 Potato Dextrose Agar

Code number: CM 139

a medium recommended for the detection and enumeration of yeast and moulds in butter and other dairy and food products.

Formula

Chemicals	g/liter
Potato extract	4.0
Glucose	20.0
Agar	15.0

pH 5.6 ±0.2

Preparation

Suspend 39 g in one liter of distilled water. Bring to dissolve completely sterilized by Autoclaving at 121°C for 15 minutes. Mix well before pouring.

3.1.2.3 Brilliant green Bile (2%) Broth

Code number :CM 31

This medium is used to detect or confirm the presence of members of the *coli–aerogenes* group; the brilliant green content suppresses anaerobic lactose fermenters, such as *Clostridium perfingens* and the medium is recommended for the 44^{0}C confirmatory test for *Escherichia coli.*

Formula

Chemicals	g/liter
Peptone	10.0
Lactose	10.0
Ox bile (purified)	20.0
Brilliant green	0.0133

pH 7.4 ± 0.2

Preparation

Add 40 g to one liter of distilled water, mix well, distribute into containers fitted with Durham's tubes and sterilize by autoclaving at 121°C for 15 minutes.

An alternative procedure is to heat the dissolved broth at 100°C for 30 minutes; a recommended procedure when preparing double strength broth.

3.1.3 Sterilization of culture medium

The process of complete elimination or killing of all microbes is called sterilization. A sterile object, in the microbiological sense, is free of all living microorganisms.

3.1.3 .1 Methods of sterilization

Several sterilization processes can be adopted in the laboratory, depending upon the material to be sterilized. Three types of sterilization processes are usually adapted such as the physical, chemical and gaseous ones.

1. Physical Methods of Sterilization
   a. *Moist heating*
   b. *Filtration*
   c. *Radiation*
2. Sterilization through chemicals
3. Gas Sterilization

In practical used method is moist heating.

Moist heating

Moist heat is usually provided by steam under pressure in an autoclave (P-SELECTA), and is a reliable method of sterilization for most materials. Since moist heat is more efficient in penetrating materials it is used for sterilizing laboratory media. Usually test tubes or flasks containing media are autoclaved at 15-20 lbs. pressure per sq. inches for 30 minutes, at a temperature of 121^{0}C. Steam is supplied either from a central source or is generated within the autoclave and allowed to come down to zero pressure before opening the lid.

3.1.4 Sample preparation

Sample collection for microbiological analysis

Fruits – sample collection from production line 1.

Point of receiving – transported trays

–Primary washer

–Sorting belt

–Secondary washer

Fruits sample collected in to clean individual poly bags . Sealed each bag with rubber and transferred to microbiology lab.

Juice collection– extracted raw juice collected from collecting 250 ml into sterilized bottle at

beginning and end of production run.

After aseptic filling – by using aseptic sample bag (5 kg).

Air microbial sampling

Using prepared nutrient agar plates and potato dextrose agar plates. Opened plates are kept in 10 minutes and closed from sample collected areas .

Physical chemical parameters

Checked chemical and physical parameters are:

Brix values

Acidity

pH

Temperature

Chlorine level

Readings of Brix, acidity and pH were checked using same samples drawn for microbiological analysis after required volume for microbial tests were drawn out. Temperature was taken by on line sampling .

Since microorganisms are on the surfaces of equipment and as well as fruits , the sampling and analysis of surfaces are important. The system to used swab cotton method. Some of the surface sampling systems that have been used are listed in table 3.1

Table 3.1 Surface sampling methods

Swab	Contact systems
Cotton	Agar-Syringe
Alginate	Agar-sausage
Glass sampler	Agar plate (RODAC)
Cylinder template	Tape
Scrape	Membrane filter pad
Excise tissue	Agar spray
Wash rinse	Drip or exuded juice
Vacuum probe	Abrasive discs

Source: Banwart, G.J , 1998 Basic Food Microbiology.

Several diluents have been used except after aseptic samples. Although AOAC (1985) recommended the used of Butterfields buffered phosphate. 0.1 % peptone water is also accepted. Dilutions 10^{-1}, 10^{-2}, 10^{-3} are used for MPN methods.

3.1.5 Inoculation

Inoculation is the method by which microorganisms are transferred from any source to the sterilized medium for their cultivation in laboratory.

3.1.5.2 Method of inoculation

Following steps are involved in inoculating the Brilliant Green Bile Broth (BGBB) medium contained in a culture tube procedure.

1. To take freshly sterilized BGBB medium in which inoculation is to be done.
2. Filled in to sterilized tube 9ml of BGBB by using sterilized pipette.
3. To hold the culture e tube in between thumbs and forefinger of the left hand in such a way that cap should face towards the body.
4. To remove the cap over a flame.
5. To keep the mouth of tube and pipette of end near the flame.
6. To take out 1ml of culture by using pipette and transfer it on medium in the culture tube.

7. To keep again the mouths of test tube near the flame and then, replace the cap.

Following steps are involved in inoculating Nutrient Agar and Potato Dextrose Agar contained in a petri dish procedure.

1. To keep the end of pipette near the flame.
2. Take 1 ml of sample from diluted sample bottle.
3. To keep again the end of pipette near the flame.
4. Pouring of sample into slightly opened (keeping one end is closed) a petri dish and closed.
5. Removed the cotton plug over a flame.
6. Poured into slightly opened petri dish and closed.
7. Replace the cotton plug over a flame.
8. Mixed contents by gently swirling it clockwise and counter clockwise. Avoid spillage on petri dish lid.

3.1.6 Incubation

After inoculation, the inoculated petri dishes and culture tubes were kept at the desired temperatures for allowing the growth of the inoculated microorganism. This is referred to as incubation. The optimal values of temperature and other physical parameters of growth differ with different microorganisms. Most of the organisms however are incubated in aerobic conditions at 25-35^{0}C temperature.

Nutrient agar cultures and Brilliant Green Bile Broth culture tubes were kept in incubator at 37 $\pm$1^{0}C for 48 $\pm$ 2 hours.

Potato dextrose agar cultures were kept in incubator at 25-30^{0}C for 3 to 5 days.

3.1.7 Estimating the number of microorganisms

Several procedures were be used to estimate the microbial population (table 3.2)

Table 3.2 Systems to estimate the microbial load of food

Direct microscopic count (DMC)	Electrical
Breed clump count	Conductance
Electronic particle count	Impedance
Pour plate (APC,SPC)	Capacitance
Spread plate	voltage drop
Spiral plate	Spectrophotometric (optical density)
Drop plate	Adenosine triphosphate (ATP)
Plate loop	Reductase tests
Roll tube	Easicult-TTC
Oval tube	Respiration rates
Burri strip/slant	*limulus* amoebocyte lysate
Little plate	Chemical indicator
Tube dilution	pH
Most probable numbers (MPN)	Agar droplets
Membrane filter	Millipore sampler
Hydrophobic grid (HGMF)	Bactoscan
Direct epifluorescent filter technique (DEFT)	Microcalorimetry
Microtiter-Spot plate	Flow cytometry
Dry rehydratable film	
Petrifilm	

Source: Banwart, G.J, 1998 Basic Food Microbiology

Used methods

Direct microscopic colony count- Total plate count , yeast and mold count using different morphological colony characteristics.

Most probable number- By using several tubes at each dilution and recording the positive (showing growth) tubes and negative (no growth) tubes. At least three diluents are needed. Used dilutions are 10^{-1}, 10^{-2} and 10^{-3}. Their relationship of positive and negative tubes has been determined mathematically and MPN tables have been derived (Appendix 3).

3.2 Physical and Chemical tests

3.2.1 Measuring of chlorine content in primary washer

Chlorine is used as a disinfectant to control microorganism (Richardson *et al.*1998). Chlorine solution use at PD 1(Production 1 section) primary washer for fruits wash and disinfection. In there chlorine content should be 300ppm . That is the standard content. It measured by comparing the color using the Lovibond comparator (Lovibond 2000).

3.2.1.1 Equipment

250 ml volumetric flask

Pipette 10 ml

Lovibond comparator (Lovibond 2000)

Reagents

Othotoludine

3.2.1.2 Procedure

Sample collected from PD 1 primary washer tank. 2.5 ml of sample in to a 250 ml volumetric flask. Added distilled water up to 250 ml mark and volumetric flask and was agitated well. Then Filled 10ml into the cell of comparotor and added drop of Orthotoludine solution and mixed well. Then compared the colour against the comparotor. Observed the reading of chlorine content ppm.

Reading	**chlorine ppm in sample**
1ppm	100ppm

3.2.2 Measuring of pH in passion fruit juice

Passion fruit juice is high acidic .H^+ concentration is high.

$pH = -\log [H^+]$

Therefore pH is low. Measure pH by using electrical pH meter (ORION model 420A)

3.2.2.1 Equipment

Electrical pH meter

Beaker

Wash bottle

Reagents

Buffer solution pH 4 and pH 7

3.2.2.2 Procedure

General.

Pressed power button

(Electrode solution pH indicated 6.74 and "READY" displayed.

Pressed "MODE" button till arrow points to pH.

Pressed "2 ND" button and then "CAL" button, then "P – 1" displayed.

(This indicated, it is ready for the 1st buffer calibration)

Calibration

Rinsed electrode with distilled water and dried with tissue.

Inserted electrode to 1st buffer solution, "READY" displayed.

(The reading displayed is the 1st buffer solution reading) 1st buffer should be near the electrode isopotential point (pH 7).

pressed " YES" button, then "P – 2" displayed. (This indicates that it is ready for the 2nd buffer

Calibration

Rinsed electrode with distilled water and dried with tissue.

Inserted electrode to 2nd buffer solution, "READY" will be displayed, (The reading displayed is the 2nd buffer solution reading).

Pressed "YES" button. "SLP" displayed. (Sloop should be between 92– 102). Then "MEASURE" displayed which indicates that the calibration was finished and the sample pH checked.

Sample checking

Rinsed electrode with distilled water and dried with tissue.

Inserted electrode to sample solution, "READY" displayed.

(Received reading after stabilisation)

3.2.3 Measuring of acidity in passion fruit juice

Passion fruit juice is high acidic, because it contain high citric acid content. Measure acidity juice consists of weighing and titration using by Sodium Hydroxide solution and phenolphthalein solution.

3.2.3.1 Equipment

Electronic Balance-(minimum 0.1g readability)

Beaker

Titration flask

Spoon/ spatula

Burette

Reagents

0.1M Sodium Hydroxide (0.1 M NaOH)

Phenolphthalein solution

Distilled water

3.2.3.2 Procedure

Mixed passion fruit sample well and weigh out about 2.0g sample by using a spatula or a spoon. Added distilled water and dissolved well. Then added few drops of phenolphthalein indicator solution and mixed well. Then titrated against 0.1 M NaOH solution. Endpoint is colorless to pink.

Calculation

Express acidity as % (m/m) citric acid

1ml 0.1M NaOH = 0.00citric acid

if Ac is titre, then

* Acidity = Ac × $\frac{0.007}{2.0}$ × 100% (m/m) Citric Acid

* Round off 1st decimal point

3.2.4 Measuring of Brix of passion juice

Total Soluble Solid (TSS) in liquid solution can be measure as a Brix value. When TSS is high , Brix is high. As well as TSS is low Brix is low.

Brix determination by using Refractometer .

Used type kyowa – range 0-90%

ATAGO –renge 0-95%

3.2.4.1 Procedure

Cleaned prism of Refractometer with a soaked tissue.

Wiped dry using tissue.

Smeared sample as a thin layer on prism.

Looked through eyepiece and focused for a clear reading using focus knob.

Meter reads Brix directly. Displayed line of light/dark gave value of Brix.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Microbial population results and discussion

4.1.1 Sample to be used in microbial data on processing steps

- Receiving fruits
- After primary washed fruits
- After sorted fruits
- After secondary washed fruits
- Raw juice in collector bin
- Aseptic filed juice in sample bag

Table 4.1 Microbiological testing results in receiving passion fruits

Organisms	TPC/g	Yeast/g	Molds/g	Coliforms /g
Average colonies	7156500	8177457.5	600014.33	2400

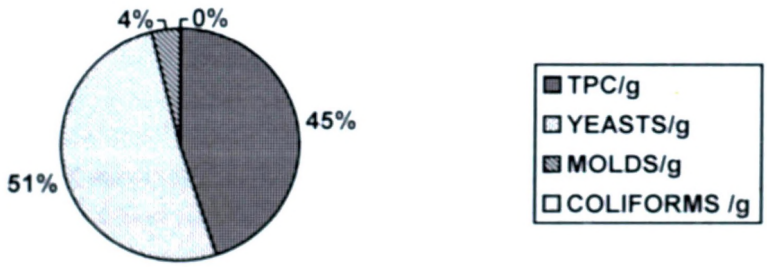


Figure 4.1 microbes of received fruit

Coliforms observed 2400 colonies per gram, however it less than 1% from total microbes.

Table 4.2 Microbilogical testing results in after primary washed fruits

Organisms	TPC/g	Yeast/g	Molds/g	Coliforms/g
At start average col.	40581.25	38587.5	3627.37	Didn't check
At end Average col.	23201.67	744066.25	65005.62	Didn't check
Total Average col.	31891.46	391326.87	34316.50	Didn't check

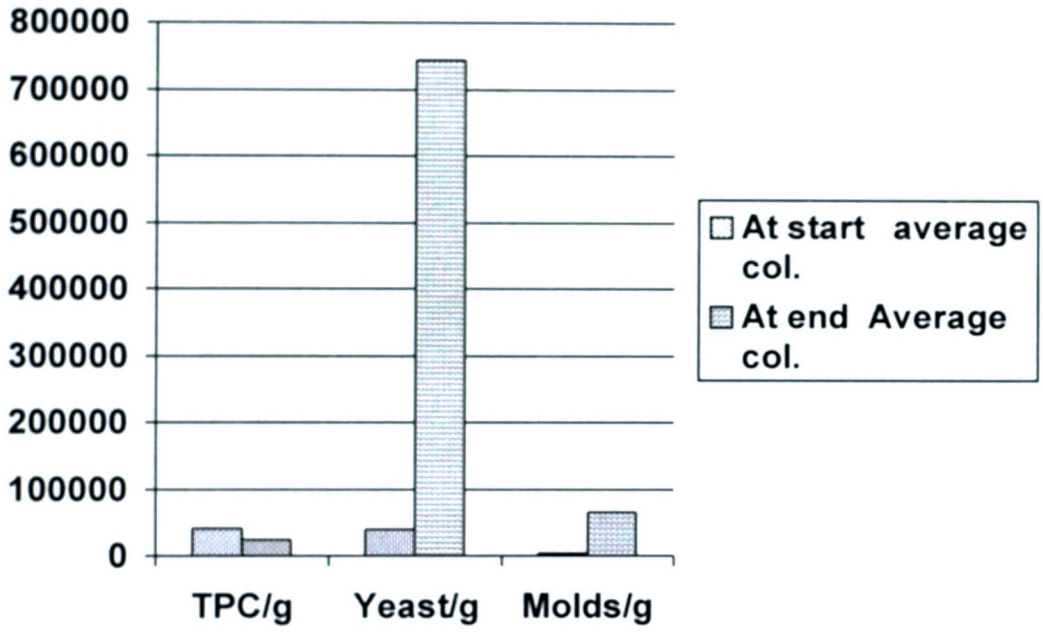


Figure 4.2 Combination of at start with at end of production

According to comparing we can determine as follows

-With Received fruit

TPC is not reduced.
Yeast is reduced.
Molds is reduced.

- At start with at end of the production

TPC is not reduced.
Yeast is not reduced.

Table 4.3 Microbiological testing results in after sorted passion fruits

Organisms	TPC/g	Yeast/g	Molds/g	Coliforms/g
At start average col.	68912.5	87737.5	1875.06	Didn't check
At end Average col.	53791.25	33592.5	3875.37	Didn't check
Total Average col.	61351.87	60665	2875.21	Didn't check

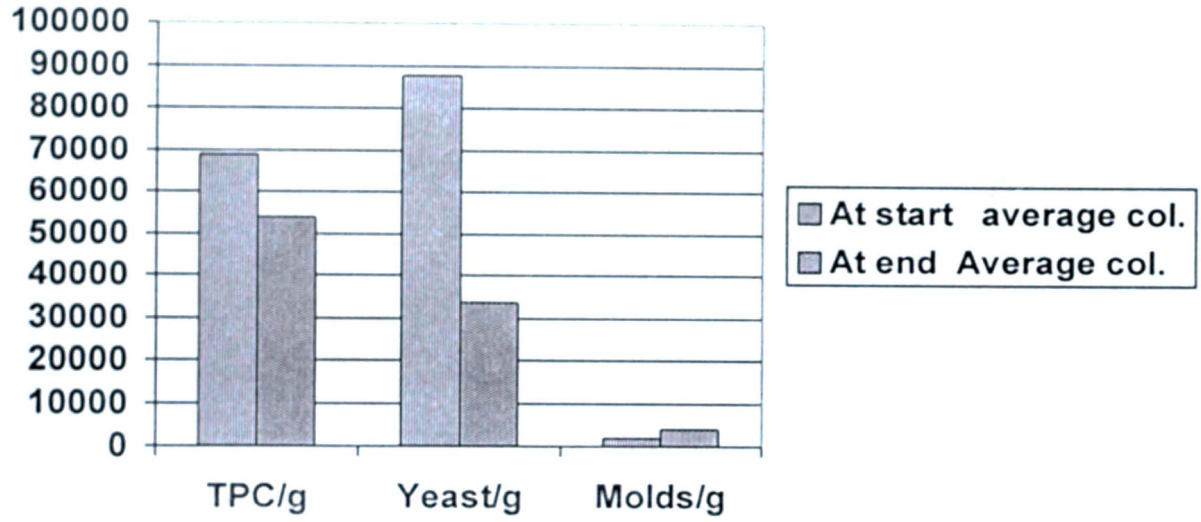


Figure 4.3 Combination of at start with at end of after sorted fruits

With received fruits-TPC not reduced significantly. Yeast reduced.
At start with at end –TPC and Yeast are not reduced significantly.

Table 4.4 Microbiological testing results in after secondary washed passion fruits

Organisms	TPC/g	Yeast/g	Molds/g	Coliforms/g
At start average col.	33500	49436.25	1881.06	Didn't check
At end Average col.	62488.75	147087.5	6375	Didn't check
Total Average col.	47994.37	98261.87	4128.03	Didn't check

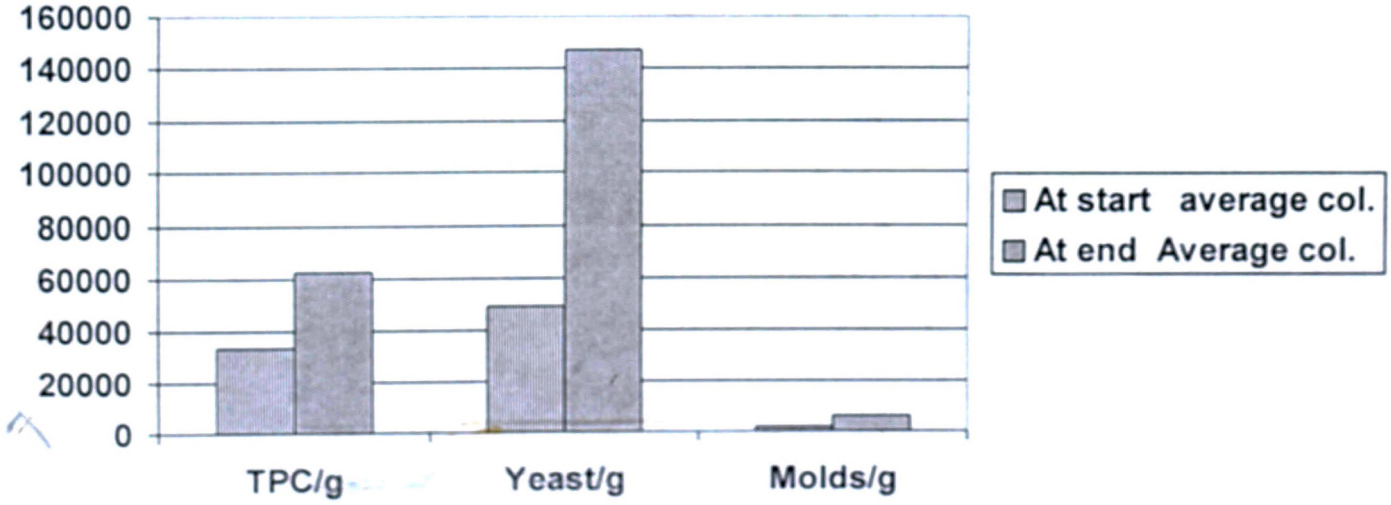


Figure 4.4 Combination of at start and at end of secondary washed fruits

With received fruit – TPC not reduced but Yeast and mold reduced significantly.

With primary washer –not reduced TPC ,Yeast and Mold

At start with at end –increased all microbes.

Table 4.5 Microbiological testing results of raw juice in collector bin

Organisms	TPC/g	Yeast/g	Molds/g	Coliforms/g
At start average col.	723333.35	1072500.1	382083.32	1207.5
At end Average col.	1022250	692500	73000	Didn't check
Total Average col.	872791.67	882500.05	227541.66	1207.5

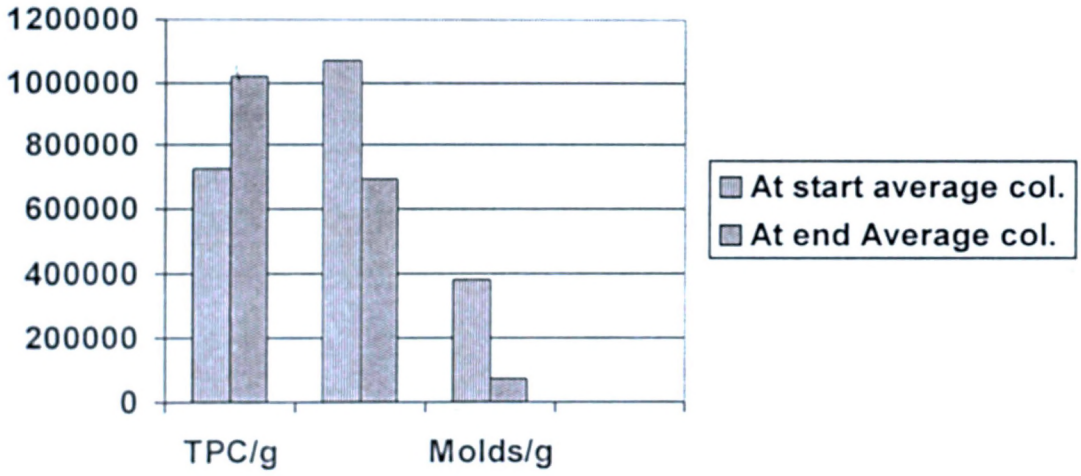


Figure 4.5 Combination of at start with at end

With received fruit –not reduced significantly all of microbes.

Table 4.6 Microbiological testing results of aseptic filled passion juice in sample bag

Organisms	TPC/g	Yeast/g	Molds/g	Coliform/g
Average col.	0	0	0	0

When after aseptic process all microbes eliminated. Incubated sample also present zero counts.

4.1.2 Microbial population variation through the aseptic juice processing

Table 4.7 Microbial level through the processing

	TPC/g	Yeast/g	Molds/g
Received fruit	7156500	8177457.5	600014.33
After Primary washer	31891.46	391326.87	34316.50
After sorted washer	61351.87	60665	2875.21
After secondary washer	47994.37	98261.87	4128.03
Raw passion juice	872791.67	882500.05	227541.66
Aseptic product	0	0	0

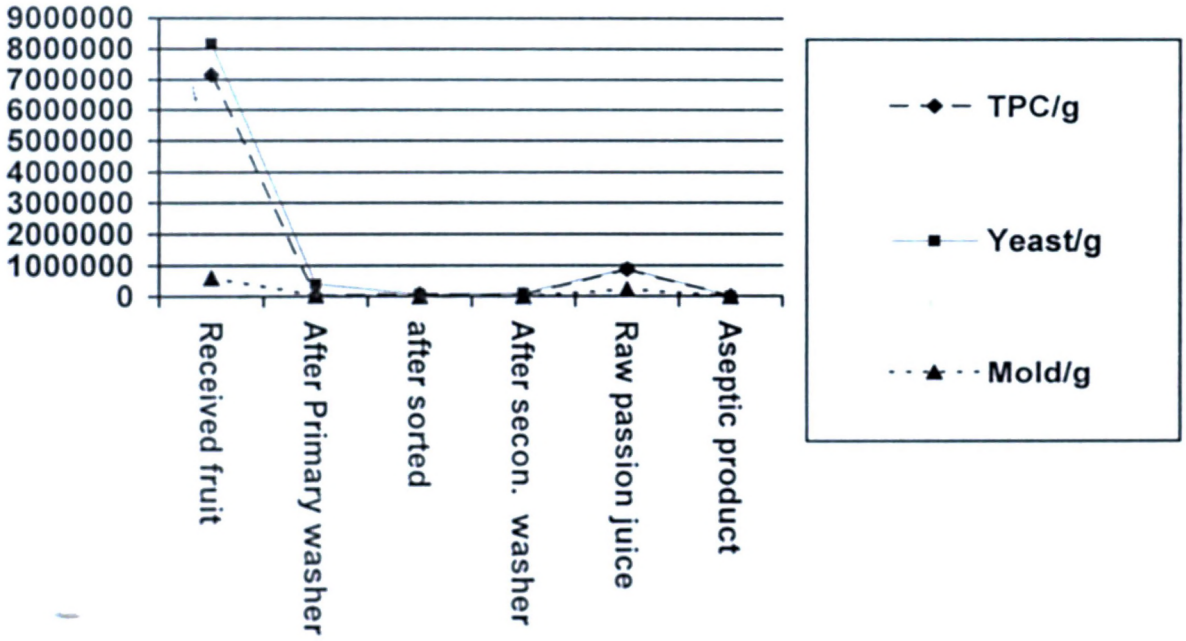


Figure 4.6 Combination of microbes through aseptic processing

In raw juice microbial levels higher than after primary washed fruit. However all microbes reduced to no colony forming units just after aseptic processing. But during shelf life of 18 months a small count may be present within set standards due to growth of dormant colonies.

4.1.3 Microorganisms on surface of machinery equipment and packing

Swab test method used for this practical

Sample point

- Sorting belt
- Passypress
- Juice outlet
- Destoner
- Drum inside

Table 4.8 Microbial counts of sorting belt

Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	290	120	40
After cleaning	20	20	0
Total average	155	70	20

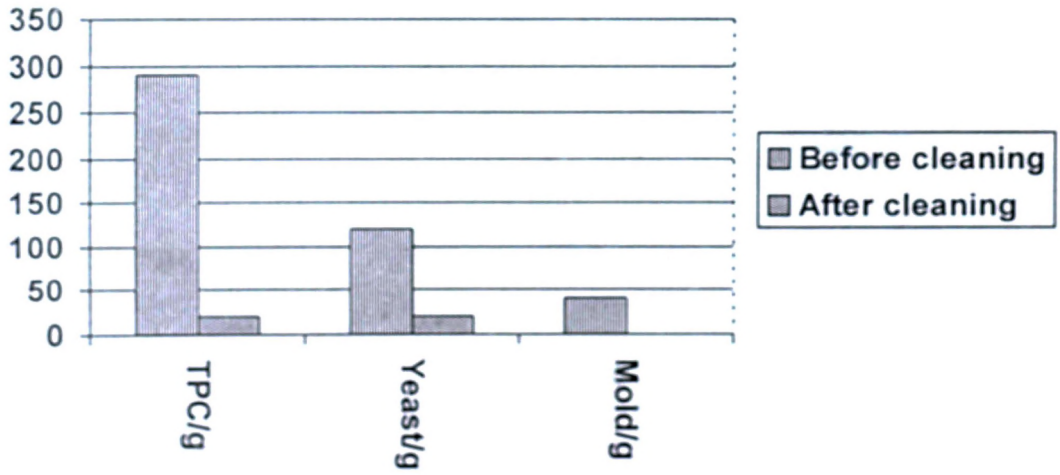


Figure 4.7 Before cleaning and after cleaning

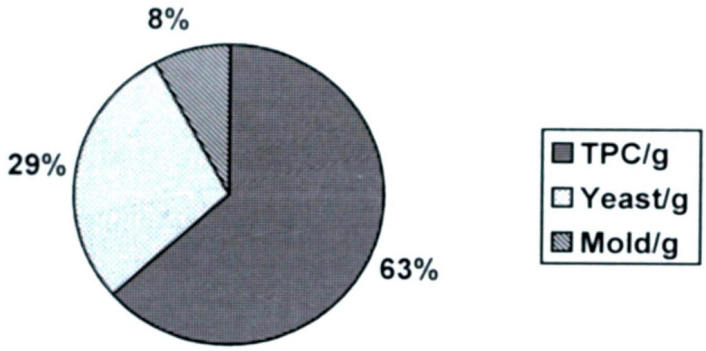


Figure 4.8 Distribution of microbes in sorting belt

As per results of before cleaning with after cleaning, general reduction of microbes was evident but cleaning were not 100% effective.

Table 4.9 Microbial counts of Passypress

Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	60	10	10
After cleaning	50	20	0
Total average	55	15	5

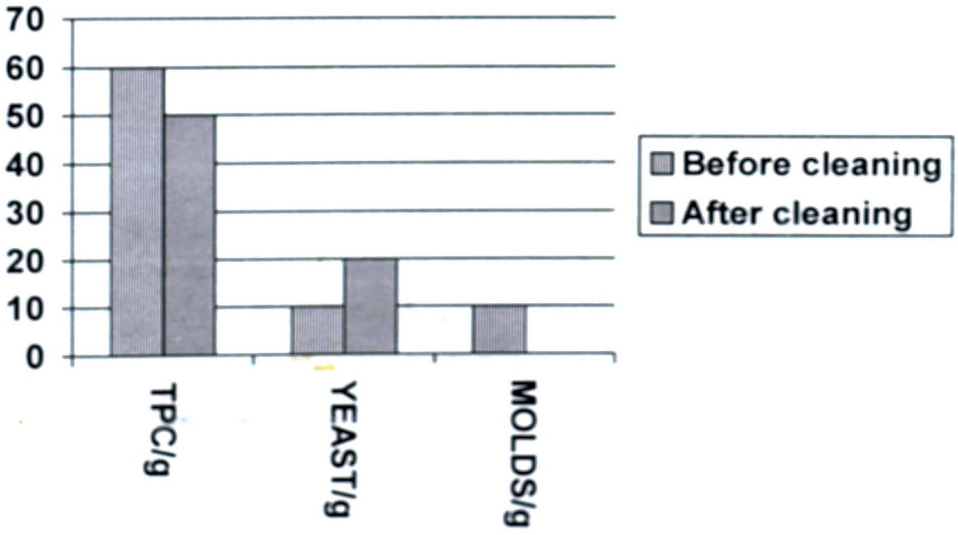


Figure 4.9 Combination of before and after cleaning of Passypress

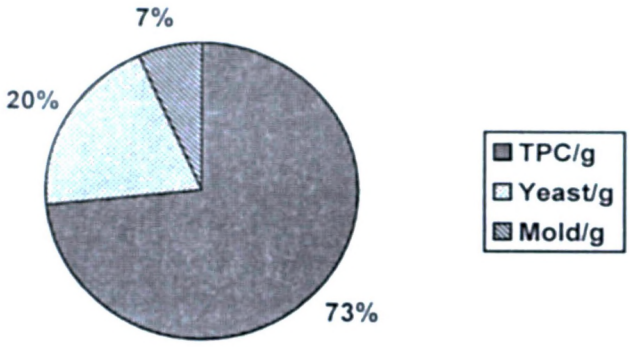


Figure 4.10 Average microbial counts in Passypress

Passypress cleaning was not effective. (corrective actions taken)

Table 4.10 Microbial counts of juice outlet

Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	70	30	90
After cleaning	10	1190	20
Total average	40	610	55

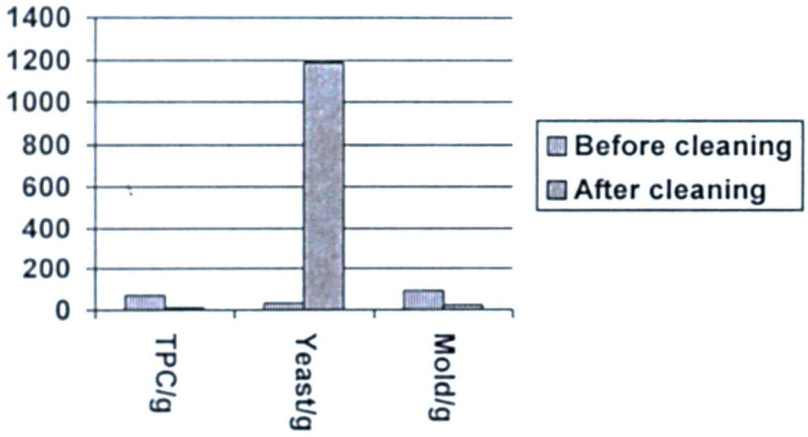


Figure 4. 11 Before with after cleaning of juice outlet

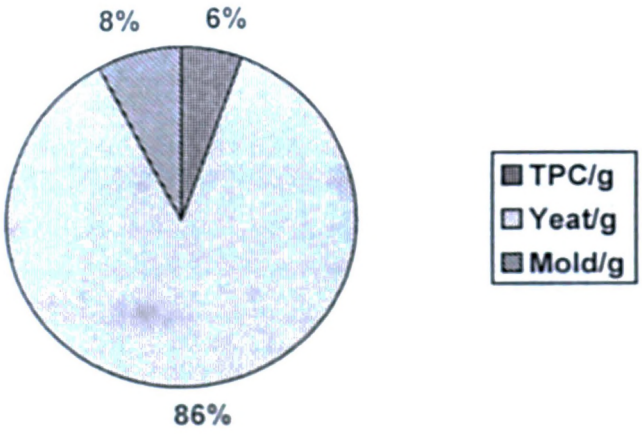


Figure 4.12 Microbes on juice outlet

In there cleaning was not effective. (Corrective actions taken)

Table 4.11 Microbial counts of Destoner

Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	650	50	30
After cleaning	60	0	20
Total average	355	25	25

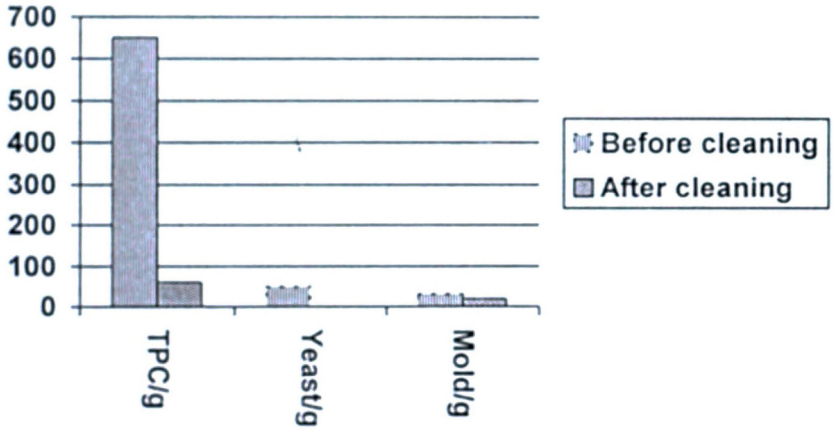


Figure 4.13 Combination of before with after cleaning Destoner

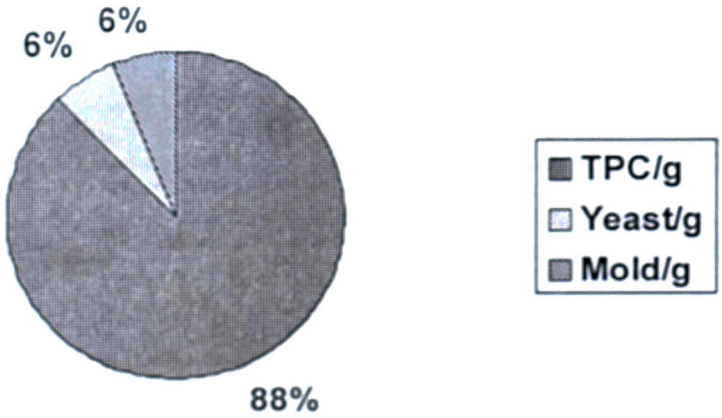


Figure 4.14 microbes on Destoner

Cleaning of Destoner was not 100% effective, but acceptable. (corrective actions taken)

Table 4.12 Microbial counts of Collector bin

Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	450	50	60
After cleaning	120	40	20
Total average	285	45	40

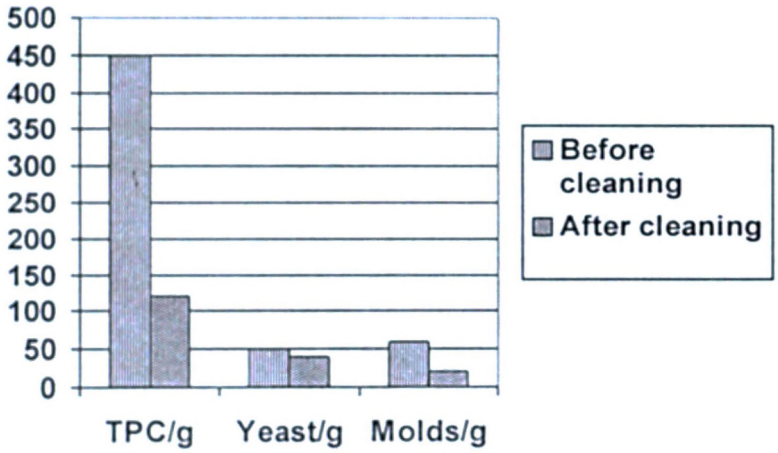


Figure 4.15 Before and after cleaning microbes on collector bin

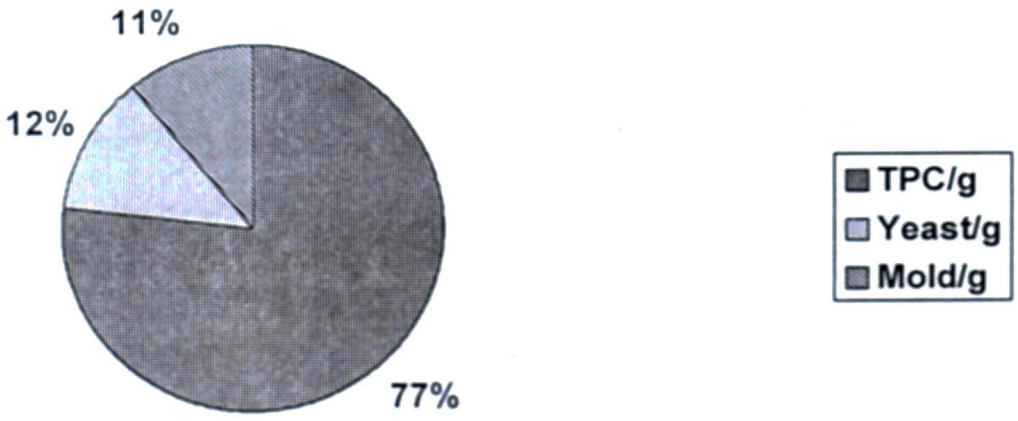


figure 4.16 microbes on collector bin

Table 4.13 Microbial counts of Drum inside
Area 81 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
After cleaning	tntc	tntc	57
Average			

tntc –too numerous to count
Drum inside cleaning very ineffective. (A strict corrective action is implemented.)

4.1.4 Microorganisms of atmosphere

Microorganisms of fruit processing area determined by using opened culture media poured plates on position.
Sample were collected from

- Fruit keeping area
- Primary washer area
- Sorting area
- Secondary washer area
- Juice collector area
- Aseptic filling area

Table 4.14 Microorganisms in fruit keeping area
Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	20.33	5.67	17.33
After cleaning	21.33	10.67	10.00
Total average	20.83	8.17	13.67

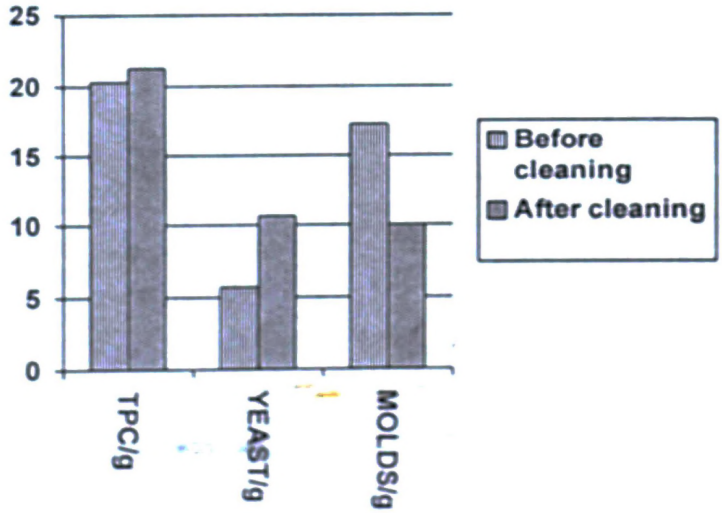


Figure 4.17 Before with after cleaning of fruit keeping area

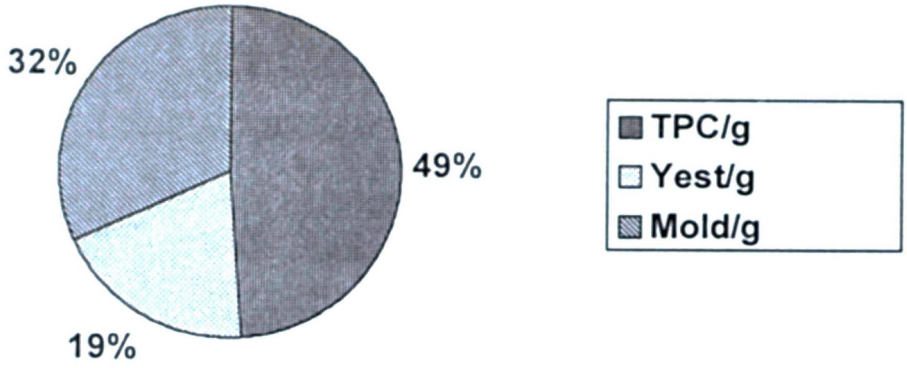


Figure 4.18 Microbes of fruit keeping area

In this area cleaning was not effective.

Table 4.15 Microorganisms in primary washer area

Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	10	4	34.5
After cleaning	18	7	6
Total average	14	5.5	20.25

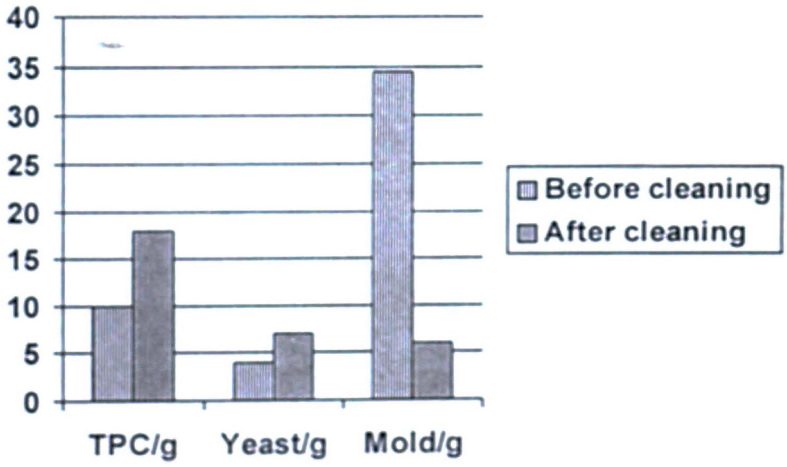


Figure 4.19 Combination of before with after cleaning in primary washer area

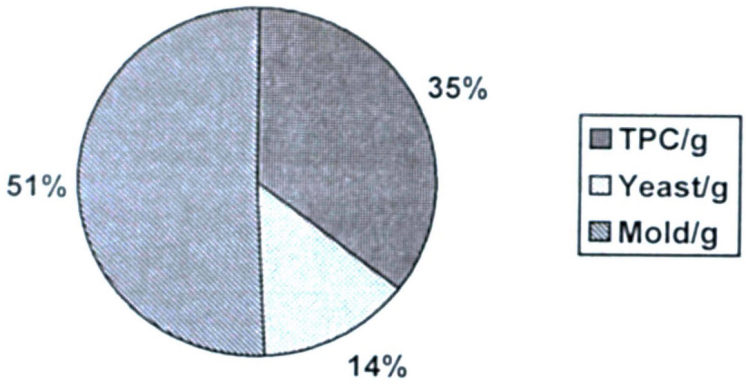


Figure 4.20 microbes in Primary washer area

High number of molds observed in primary washer area however reduced to a considerable level after cleaning. After cleaning TPC and Yeast have been increased.

Table 4.16 Microorganisms in sorting area

Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Mold/g
Before cleaning	31.67	21.67	50.67
After cleaning	19.67	5.67	5.33
Total average	25.67	13.67	28

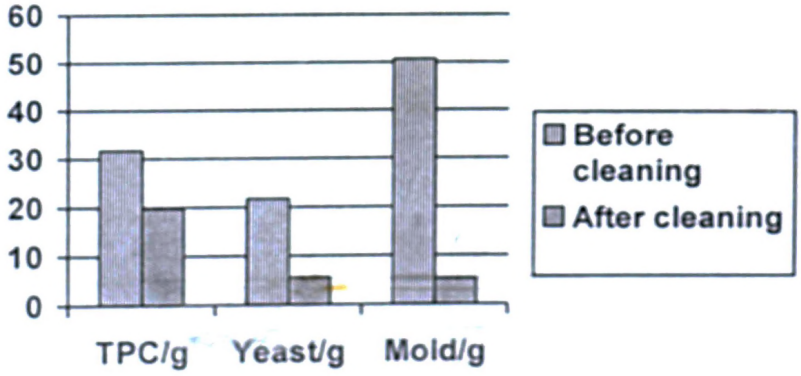


figure 4.21 Cleaning of sorting area

In sorting area major microbes are Mold and TPC.

Table 4.17 Microorganisms in secondary washer area

Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	286	25	51
After cleaning	26	3.5	4.5
Total average	156	14.25	27.75

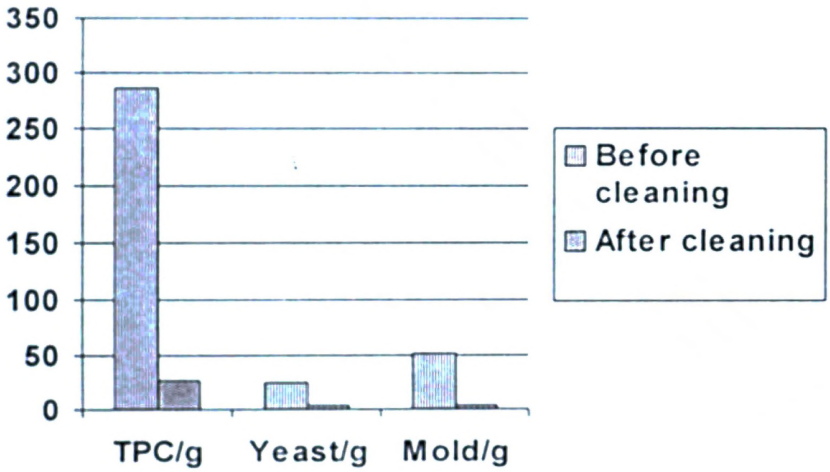


Figure 4.22 before with after cleaning of secondary washer area

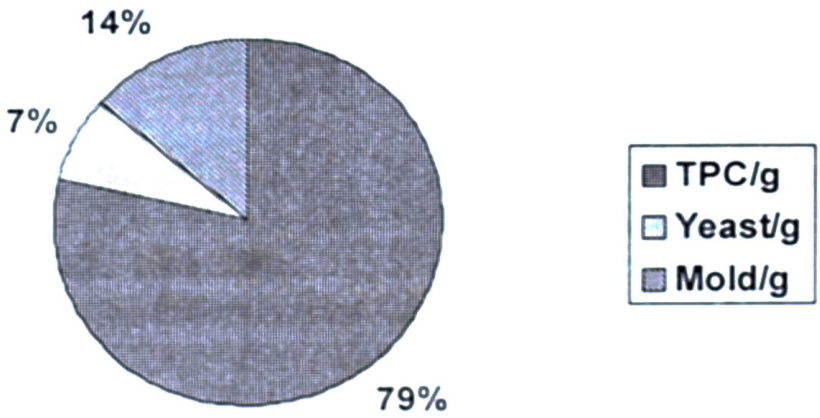


Figure 4.23 microbes in secondary washer area

In secondary washer area TPC counts is very highly than others.

Table 4.18 Microorganisms in juice collector area

Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	243.5	365	7.33
After cleaning	32.33	36.67	13.33
Total average	137.92	200.83	10.33

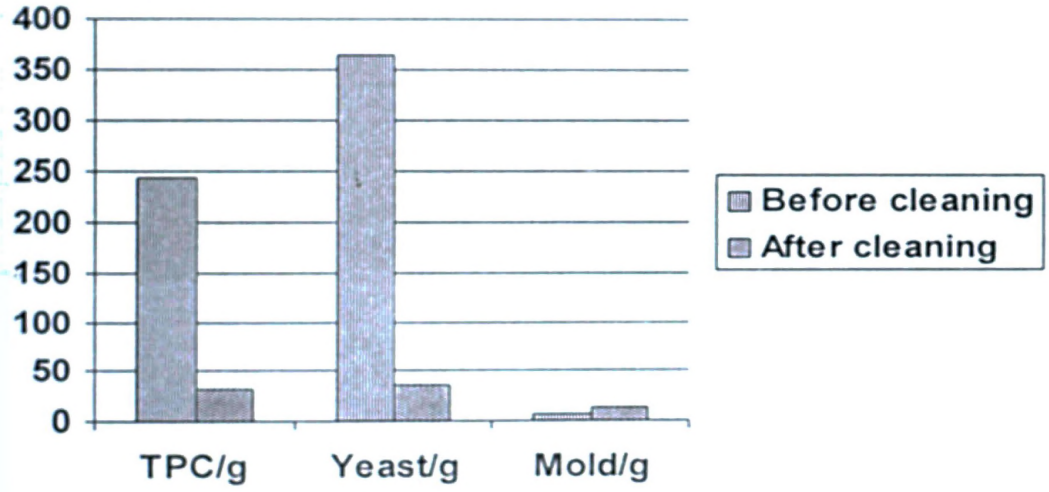


Figure 4.24 microbes in juice collector area

In juice collector area major microbes in atmosphere are Yeast and TPC.

Table 4.19 Microorganisms in aseptic plant area

Area 64.20 cm^3 Exposed time 15 minutes

Microorganisms	TPC/g	Yeast/g	Molds/g
Before cleaning	21	9	5
After cleaning	8	0	0
Total average	14.5	4.5	2.5

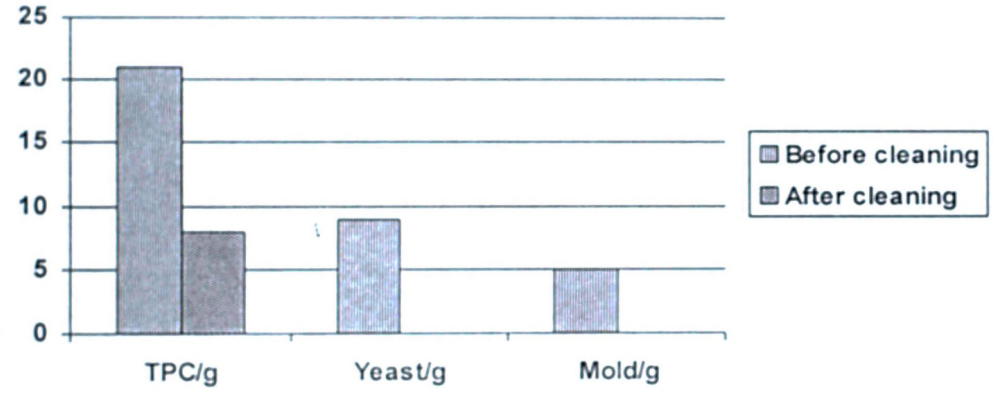


Figure 4.25 combination of before with after cleaning Aseptic plant area

Cleaning of aseptic plant good,

4.1.5 Microbial level according to chlorine content in primary washer

I was determined chlorine level in primary washer at processing by same time interval and taken fruit sample cultured.

Table 4.20 Microbial level according to chlorine content

Microorganisms	TPC/g	Yeast/g	Molds/g
At 100 Average col.	274833.33	265700	5433.33
At 150 Average col.	3500	25250	5700

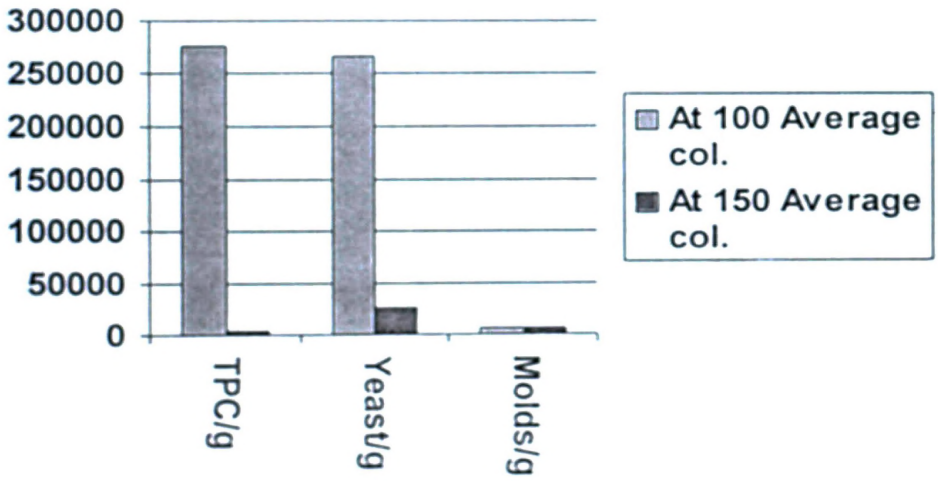


Figure 4.26 chlorine level with microbes

Chlorine level at 150 ppm reduced microbes more than at 100 ppm.

4.1.5 microbial level in secondary washer with time

Table 4.21 microbial level with time in secondary washer

Microorganisms	TPC/g	Yeast/g	Molds/g
At start	90500	120500	2000
Average col.			
After 10 minutes	108000	192000	4000
Average col.			
After 20 minutes	126500	84500	3500
Average col.			

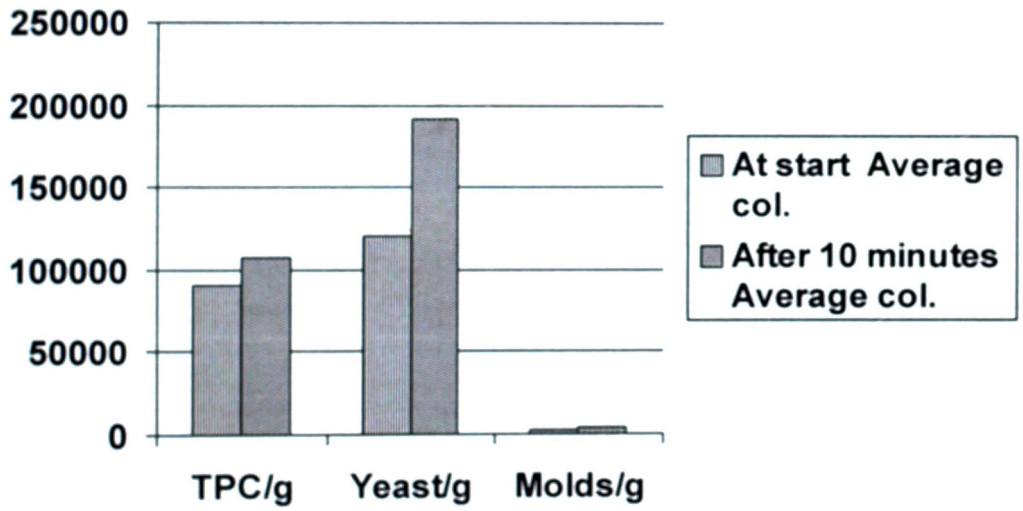


Figure 4.27 microbial level in secondary washer with time

Microbial level increased with time in secondary washer.

4.2 Oxidation of passion juice

Sample collected from chiller tank and checked various parameters with time

Table 4.22 changes in chiller tank

Parameters	Temperature	pH	Acidity
At start	29.5	2.97	5.65
After 20 minutes	29.8	3.06	3.63
After 40 minutes	32	2.97	4.27

One of the sample bag was incubated (99 days) from 2002/10/15 to 2003/01/22 in incubator at 37°C.

Table 4.23

Parameters	Brix	pH	Acidity
2002/10/15	11	2.98	2.9
2003/01/22	11.5	2.7	3.52

Incubated sample is slightly bitter with a peely taste.
Colour was slightly changed. (discoloured)

Microbiological analysis results indicated no colony forming units after incubation.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATION
5.1 Conclusion

A high number of colony forming Microbial population was observed throughout the juice processing. Contributory factors include felled fruits collect from dirty soil. Coliforms contaminate from soil and *E.coli* in case of mixing of fecal matter to soil. Suppliers transport fruit packed in polypropeline bags, thereby increase temperature in the bag, which provides favourable temperature for increase of microbial population. Also the package of polypropeline bags and plastic crates used are dirty, which contributes to microbial contamination.

At primary washer, microbial level increased at end of the production in comparison to the start of production. Reason for that increase is due to accumulation with time because water is recycled. Chlorine content reduces rapidly due to presence of organic matter and Chlorine release to atmosphere. Recommended chlorine content (300 ppm) and expose time (10 minutes) were not strictly followed. Observed average chlorine content was 125 ppm and exposed time varied 5 - 20 minutes. All fruits were not properly rinsed even thou over head jet system is used.

After sorting, microbial count reduced as highly contaminated fruits were removed physically.

At secondary washer, water is recycled, which results in increase of contamination with time. Mainly due to exterior of sorted fruits too carry some amount of microbes. Also exposure to atmosphere too contributes to contamination.

Raw juice, contained a high amount of microbes. Juice sacks in fruits are exposed when the fruits are crushed in passypress and any microbes in peel contaminate the juice sacks. Juice is very nutritive and is highly favourable for increase of microbial population. During the juice extraction process temperature of juice increases to about 35-40 ^{0}C. This is the optimum temperature for growth of microbes. Also contamination occur through improperly cleaned machinery and equipment (Passypress, destonor, juice outlet, conveyor belt, juice collecting bin and connecting pipes).

Pasteurization of passion juice is done at 95°C for 60 seconds (If pasteurization was improper, it can lead to spoilage But Aseptic System at CPC Agrifoods which has an automatic machine sterilization cycle that ensures that the machine is fully sterilized

prior to production run of juice pasteurization, yields well treated out put, provided temperature and time settings are done accurately. This function is checked by microbiological analysis of samples and in case on non conformances of having any count, the product will be re-worked). Machinery cleaning if improper or if heat resistant microbes were present or dormant spores were present. This can lead to spoilage of aseptically processed juice, if the packaging used was sterile & sealed properly. After about 6 months some microbes (especially molds) may appear on surface of juice in 180 Kg aseptic bag in drums. If drum cleaning was improper contamination once the bag is opened.

Received fruit temporary storage is in same location as juice processing area, near primary washer and sorting area. This opens chances of possible cross contamination from received fruits and dirty trays.

Oxidation of juice occurs at all steps once juice sacks are opened in extraction section. In chiller tank the rate reduces due to low temperature and enzymatic browning stops after pasteurization. Yet non-enzymatic browning can takes place during shelf life. Tubes of aseptic system may contain encrustation of caramelized juice, lining the walls of tubes, which may become loose and find its way to pasteurized juice.

Maillard browning reactions occur between reducing sugars and α-amino groups of amino acids, peptides and proteins. The reaction between amino acids α-dicarbonyls, known as the streacker degradation in the maillard reaction, also leads to brown pigment formation.

5.2 Recommendation

Post harvest practices

Should harvest only healthy fruits when the peel is yellow purple indicating that fruit is ripe. Most suitable method is to collect fallen ripened fruits, clean, dry and pack in crates. Fruit collected should be prevented from coming into contact with soil.

Suitable harvesting time is about 9 (00) a.m to 4 (00) p.m in dry days. If fruit is exposed to wet conditions it becomes susceptible for growth of fungis.

Control

Bacteria and viruses

A correctly constructed HACCP plan for production of fruit should take account of the possibility that fruit may carry pathogenic bacteria and viruses. Fruits and fruit products are frequently consumed raw without having been exposed to a process that can reliably eliminate pathogens. When this is the case; it is essential that measures are taken to prevent contamination of fruit with pathogens of fecal origin at all points in the growing, processing, storage, distribution and preparation chain. (Brackelt, 1992).

Packing and transportation

Should use clean crates for packing and transportation of passion fruit. Unloaded fruits should be stored separately in well ventilated cool and dry room to prevent cross contamination and deterioration.

When processing it is recommend to initially inspect and remove spoiled and/or damaged fruits. This can reduce chlorine consumption rate and reduce contamination of chlorinated water, which will result in using chlorinated water with better productiveness.

Primary washing

Washing of fruit, usually one of the first processing steps, will remove much of the original micro flora. As an improvement for primary wash bath, introduction of a slowly moving agitator will increase fruit washing process. A brush wash followed by a rinse with chlorinated water was found to reduce the microbial population on the surface of oranges by 95% (Murdock and Brokaw,1958). This method is possible to apply for passion fruit after suitable modifications. As a strict control maintenance of standard chlorine content (300 ppm) and expose time (10 minutes) has to be implemented. The chlorine determination equipment (Lovbond comparator) which is used presently is not convenient.

Secondary inspection is needed for further remove-spoiled fruits.

Secondary washer

Fresh water should be made available at least after one hour of re-circulation to minimize recontamination. Only a little amount of water is used in comparison to primary washer in this, therefor it is possible.

Passion juice should be pumped to chiller tank or aseptic plant as soon as after extraction because temperature of extracted juice is about 35-40°C, which is optimum for growth of microflora. Temperature in chiller tank should be controlled to maintain at aimed 15^{0}C to reduce microbial growth rate and to slow down other reactions.

Cleaning

Cleaning is a highly important factor in food processing. Machinery, equipment and atmosphere must be maintained hygienically. Also the juice bag holding drums too should be properly cleaned to primarily prevent bringing in to Aseptic section contaminated packaging material and secondarily to prevent contamination of Aseptic juice once the bag is opened.

Plant environment is susceptible to be contaminated by people if strict hygienic practices are not used. Good Manufacturing Practices are highly regarded with audits in plant to ensure proper application. Uniforms, caps, boots and hand washing with antiseptic detergent at entrance are practiced. As an effective method of personal foot cleaning before entering processing room a foot washer with antiseptic solution can be used. However recontamination could be minimized by use of foot dryer after foot washing. Detergents Chlorine solution of foot washer must be replaced two or three time of a day.

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APPENDIX I

Table

Exports of passion fruit juice by Country of Destination

Country	1995		1996		1997		1998		1999	
	Kg	Rs.	Kg	Rs.	Kg	Rs.	Kg	Rs.	Kg	Rs.
Afghanistan	177	21,334	-	-	-	-	-	-	-	-
Australia	1,323	120,327	3,191	298,975	2,456	260,132	2,301	239,962	2,392	245,682
Bangladesh	-	-	-	-	-	-	-	-	10	2,430
Belgium	-	-	13	432	-	-	-	-	-	-
Canada	5,216	436,811	6,607	515,734	4,565	418,000	3,052	293,377	1,138	111,801
China	-	-	-	-	-	-	900	84,926	-	-
Denmark	-	-	9	1,051	-	-	-	-	-	-
Egypt	-	-	-	-	-	-	9	1,800	-	-
Elselvador	46	5,211	-	-	-	-	-	-	-	-
Ethiopia	-	-	-	-	-	-	20	1,050	-	-
France	812	67,772	152	14,183	1,060	94,675	362	39,150	245	19,860
Germany	5,704	479,692	1,916	186,708	523	55,997	10,297	1,265,524	13,093	1,968,939
Greece	1,020	104,719	86	8,241	-	-	-	-	-	-
Hongkong	-	-	717	68,288	-	-	-	-	-	-
India	-	-	-	-	-	-	50	5,440	-	-
Italy	270	24,895	178	22,451	-	-	637	89,336	929	135,929
Japan	18,300	1,534,264	26,970	2,635,231	18,261	1,904,288	393	33,447	30,891	4,088,023
Kuwait	-	-	50	3,823	-	-	-	-	-	-
Lebanon	-	-	-	-	-	-	12	2,535	54	6,434
Malaysia	122	12,111	-	-	106	14,159	-	-	136	8481
Maldives	2,037	208,491	7,559	858,559	1,503	139,845	4,783	670,768	7,124	768,660
Netherland	542	55,382	-	-	430	38,530	16,283	2,226,734	13,115	1,945,689
Newzealand	68	6,927	53	4,835	45	5,005	45	7,032	345	33,323
Norway	171	16,533	192	18,676	45	5,821	75	9,353	205	27,008
Oman	2,490	255,176	1,007	108,372	1,697	114,764	1332	121,589	1572	224,442
Philipine	-	-	-	-	12,276	1,345,570	13,950	1,521,768	-	-
Poland	-	-	-	-	-	-	-	-	8	2,430
Qatar	-	-	268	22,232	37	4,021	84	7,939	-	-
Russia	*	76,407	-	-	-	-	-	-	-	-
Singapore	391	71,697	221	18,535	46	4,830	-	-	-	-
Sweden	888	127,434	-	-	-	-	-	-	-	-
Switzerland	*	2,281	198	14,934	309	28,943	242	22,241	-	-
U.A.E	1,406	140,631	864	69,153	9,060	280,731	1,444	135,165	1,356	192,320
U.K.	13,173	1,090,565	7,415	671,783	15,996	1,927,329	2,818	334,641	8,485	1,155,503
U.S.A.	293	30,820	936	91,086	945	129,014	802	165,472	768	66,024
Total	54,449	4,889,480	58,602	5,633,282	69,360	6,771,654	60,024	7,279,249	81,866	11,003,111

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Bangladesh	-	-	-	-	-	-	-	-	10	2,430
Belgium	-	-	13	432	-	-	-	-	-	-
Canada	5,216	436,811	6,607	515,734	4,565	418,000	3,052	293,377	1,138	111,801
China	-	-	-	-	-	-	900	84,926	-	-
Denmark	-	-	9	1,051	-	-	-	-	-	-
Egypt	-	-	-	-	-	-	9	1,800	-	-
Elselvador	46	5,211	-	-	-	-	-	-	-	-
Ethiopia	-	-	-	-	-	-	20	1,050	-	-
France	812	67,772	152	14,183	1,060	94,675	362	39,150	245	19,860
Germany	5,704	479,692	1,916	186,708	523	55,997	10,297	1,265,524	13,093	1,968,939
Greece	1,020	104,719	86	8,241	-	-	-	-	-	-
Hongkong	-	-	717	68,288	-	-	-	-	-	-
India	-	-	-	-	-	-	50	5,440	-	-
Italy	270	24,895	178	22,451	-	-	637	89,336	929	135,929
Japan	18,300	1,534,264	26,970	2,635,231	18,261	1,904,288	393	33,447	30,891	4,088,023
Kuwait	-	-	50	3,823	-	-	-	-	-	-
Lebanon	-	-	-	-	-	-	12	2,535	54	6,434
Malaysia	122	12,111	-	-	106	14,159	-	-	136	8481
Maldives	2,037	208,491	7,559	858,559	1,503	139,845	4,783	670,768	7,124	768,660
Netherland	542	55,382	-	-	430	38,530	16,283	2,226,734	13,115	1,945,689
Newzealand	68	6,927	53	4,835	45	5,005	45	7,032	345	33,323
Norway	171	16,533	192	18,676	45	5,821	75	9,353	205	27,008
Oman	2,490	255,176	1,007	108,372	1,697	114,764	1332	121,589	1572	224,442
Philipine	-	-	-	-	12,276	1,345,570	13,950	1,521,768	-	-
Poland	-	-	-	-	-	-	-	-	8	2,430
Qatar	-	-	268	22,232	37	4,021	84	7,939	-	-
Russia	*	76,407	-	-	-	-	-	-	-	-
Singapore	391	71,697	221	18,535	46	4,830	-	-	-	-
Sweden	888	127,434	-	-	-	-	-	-	-	-
Switzerland	*	2,281	198	14,934	309	28,943	242	22,241	-	-
U.A.E	1,406	140,631	864	69,153	9,060	280,731	1,444	135,165	1,356	192,320
U.K	13,173	1,090,565	7,415	671,783	15,996	1,927,329	2,818	334,641	8,485	1,155,503
U.S.A	293	30,820	936	91,086	945	129,014	802	165,472	768	66,024
Total	54,449	4,889,480	58,602	5,633,282	69,360	6,771,654	60,024	7,279,249	81,866	11,003,111

APPENDIX 2

The UK soft drinks market (% volume.1988)

Carbonates	50
Squash/cordials	33
Fruit juice /fruit drinks	14
Bottled water	2
Still drinks	1
	100%

Source :Canadiean (Griffith 1990)

Definition

Fruit juice

Fruit juice is a generic term with covers both pure juice and juice drinks.
According to the fruit juice and fruit nectar regulations 1977,pure juices are defined as 100% pure juice made either by dilution of juice concentrate back to its original strength or by squeezing juice from fresh fruit imediately pror to packinmg. They must not contain any added ingredients.

Juice drinks

Juice drinks are made from fruit juices which have been diluted , to which sugar , sweeteners, flavours, colours and preservatives may be added .they generally contain around 10% fruit juice.

Fruit nectars

Fruit nectars are part of the fruit drink sector and generally contain between 25% and 50% fruit juice. the term nector permits the addition of water and sugar to achieve the right consistency and flavour.

APPENDIX 3

Analysis of microorganisms population by using paired T test-1 sample of MINITAB. Computer package.

H_0 ::No difference between two situation

H_1 : Have difference between two situation

Since P > 0.05 we do not reject H_0 at 5% significance level

TPC

Received fruit Vs primary washer

Received fruit	afpri wash	c1
26000	850	25150
34000	1800	32200
18000	1600	16400
4900000	610	4899390
45000000	12000	44988000
1300000	12000	1288000
2000000	360000	1640000
2000000	430000	1570000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-6807393	15510433	5483766	-1.24	0.13

Received fruit Vs after sorted fruit

Received fruit	af sorting	C2-c1
26000	460	25540
34000	840	33160
18000	850	17150
4900000	480	4899520
45000000	130000	44870000
1300000	170000	1130000
2000000	7000	1993000
2000000	14000	1986000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-6869296	15441223	5459297	-1.26	0.12

APPENDIX 4

Received fruit Vs after secondary washed fruit

recieved fuit	sec wash	c2-c1
26000	50000	24000
34000	29000	-5000
18000	62000	44000
4900000	140000	-4760000
45000000	50000	-44950000
1300000	66000	-1234000
2000000	11000	-1989000
2000000	82000	-1918000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-6848500	15478578	5472504	-1.25	0.13

Received fruit vs raw passion juice

recieved fuit	raw juice	c2-c1
26000	980000	954000
34000	340000	306000
18000	170000	152000
4900000	70000	-4830000
45000000	500000	-44500000
1300000	9000	-1291000
2000000	80000	-1920000
2000000	60000	-1940000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-6633625	15407246	5447284	-1.22	0.13

At start Vs at end of processing in primary washed fruit

at start	at end	c2-c1
850	1600	750
1800	610	-1190
12000	360000	348000
12000	430000	418000
52000	29000	-23000

APPENDIX 5

T-Test of the Mean

Test of mu 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	5	148512	215685	96457	1.54	0.90

At start Vs at end of processing in after sorted fruit

at start	at end	c2-c1
410	850	440
840	480	-360
130000	7000	-123000
170000	14000	-156000
68000	160000	92000
57000	44000	-13000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	6	-33320	91035	37165	-0.90	0.21

At start Vs at end of processing in secondary washed fruit

at start	at end	c2-c1
50000	62000	12000
29000	140000	111000
50000	66000	16000
11000	82000	71000

T-Test of the Mean

Test of mu = 0 Vs mu > 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	4	52500	47388	23694	2.22	0.94

APENDIX 6

Primary washed fruit Vs secondary washed fruit

pri wash	sec wash	c2-c1
850	1600	750
1800	310	-1490
1600	50000	48400
610	29000	28390
12000	62000	50000
12000	140000	128000
360000	50000	-310000
430000	66000	-364000
97000	11000	-86000
52000	82000	30000

T-Test of the Mean
Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	10	-47595	162027	51237	-0.93	0.19

Yeast

Received fruit Vs primary washer

rec fruit	pri wash	c2-c1
490	850	360
11000	930	-10070
18000	1600	-16400
3700000	17000	-3683000
35000000	10000	-34990000
8500000	640000	-7860000
10000000	230000	-9770000
4100000	42000	-4058000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-7548389	11684785	4131195	-1.83	0.055

APPENDIX 7

Received fruit Vs after sorted fruit

rec fruit	af sort	e2-e1
490	3300	2810
11000	2600	-8400
18000	760	-17240
3700000	980	-3699020
35000000	200000	-34800000
8500000	210000	-8290000
10000000	82000	-9918000
4100000	580000	-3520000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
e2-e1	8	-7531231	11652399	4119745	-1.83	0.055

Received fruit Vs after secondary washed fruit

rec fruit	af sec	e2-e1
490	890	400
11000	2600	-8400
18000	2900	-15100
3700000	3800	-3696200
35000000	13000	-34987000
8500000	17000	-8483000
10000000	340000	-9660000
4100000	480000	-3620000

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
e2-e1	8	-7558663	11705106	4138380	-1.83	0.055

Received fruit Vs raw passion juice

rec fruit	raw juice	e2-e1
490	1400000	1399510
11000	1200000	1189000
18000	470000	452000
3700000	200000	-3500000
35000000	0	-35000000
8500000	190000	-8310000
10000000	380000	-9620000
4100000	1100000	-3000000

APPENDIX 8

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-7048686	12035396	4255155	-1.66	0.071

Primary washed fruit Vs secondary washed fruit

pri wash	sec wash	c2-c1
850	890	40
930	2600	1670
1600	2900	1300
17000	3800	-13200
10000	13000	3000
640000	17000	-623000
230000	340000	110000
42000	480000	438000

Test of mu = 0 vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-10274	290668	102767	-0.10	0.46

At start Vs at end of processing in primary washed fruit

at srart	at end	c2-c1
850	930	80
17000	640000	623000
10000	230000	220000
42000	2200000	2158000
98000	340000	242000

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	5	6486'6	87[illegible]4	[illegible]9	1.66	0.91

at start Vs at end of processing in after sorted fruit

at start	at end	c2-c1
3300	760	-2540
2600	980	-1620
200000	3000	-197000
210000	6000	-204000
82000	580000	498000
61000	71000	10000

APPENDIX 9

T-Test of the Mean

Test of mu = 0 Vs mu ≠ 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	6	17140	255646	104367	0.16	0.56

At start Vs at end of processing in after secondary washed fruit

at start	at end	c2-c1
890	2900	2010
2600	3800	1200
13000	340000	327000
17000	480000	463000
95000	97000	2000
86000	78000	-8000

T-Test of the Mean

Test of mu = 0 Vs mu ≠ 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	6	131202	208848	85262	1.54	0.91

APPENDIX 10

Molds

Received fruit Vs after primary washed fruits

rec. fruit	aft pri	c2-c1
0	9.5	10
160	15.0	-145
12	30.0	18
300000	2000.0	-298000
1000000	8000.0	-992000
2300000	0.0	-2300000
1000000	0.0	-1000000
100000	9500.0	-90500

T-Test of the Mean

Test of mu = 0 Vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-585077	813505	287618	-2.03	0.041

Received fruit Vs after secondary washed fruits

rec fruit	af sec	c2-c1
0	41.0	41
160	7.5	-153
12	0.0	-12
300000	0.0	-300000
1000000	0.0	-1000000
2300000	0.0	-2300000
1000000	0.0	-1000000
100000	1000.0	-99000

T-Test of the Mean

Test of mu 0 vs mu < 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-587390	813246	287526	-2.04	0.040

APPENDIX 11

Received fruit Vs raw passion juice

rec fruit	raw juice	c2-c1
0	180000	180000
160	390000	389830
12	20000	19988
300000	50000	-250000
1000000	2000000	1000000
2300000	17000	-2283000
1000000	75000	-925000
100000	110000	10000

T-Test of the Mean

Test of mu = 0 Vs mu > 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-232271	991643	350599	-0.66	0.26

Primary washed fruit Vs secondary washed fruits

af pri	af sec	c2-c1
9.5	41.0	31.5
15.0	7.5	-7.5
30.0	0.0	-30.0
2000.0	0.0	-2000.0
8000.0	0.0	-8000.0
0.0	0.0	0.0
0.0	0.0	0.0
9500.0	1000.0	-8500.0

T-Test of the Mean

Test of mu = 0 Vs mu > 0

Variable	N	Mean	StDev	SE Mean	T	P
c2-c1	8	-2313	3731	1319	-1.75	0.061

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