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Microbial and enzymatic changes in natural soursop puree during storage

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Abstract

Microbial and enzymatic comparisons were made on natural soursop puree stored at 15, 4 and −20°C without pasteurisation and with pasteurisation at 79°C for 69 s. Results showed that pasteurisation caused significant decrease in microbial count in soursop puree from 6.4×10^3 to 8.5×10^1 cfu per g. Generally, storage at −20°C exhibited greater stability of samples as compared to those kept at 4 and 15°C. In contrast to microbial reduction, pasteurisation caused significant increase in cloud from 0.318 to 0.529 and completely inactivated the pectinesterase enzyme. The puree packed into foils kept at 4°C exhibited decreased cloud loss as compared to others packed in cans and bottles kept at 15 and −20°C. No pectinesterase enzyme regeneration was observed for pasteurised puree in this study during 12 weeks of storage time.

1. Introduction

Due to their high value and wide appeal to consumers, fruits and vegetables have always been of interest to food scientists as raw materials for processed products. The most frequent reason for quality deterioration of food products is the result of microbial activity and this often results in food moulding, fermenting and change in acidity. In addition, native enzymes in fruits may cause desirable and undesirable effects before, during or after processing of fruit juices.

Maintaining cloud is important to eye appeal and retention of certain flavour compounds associated with cloud matrix. The instability of the cloud has been attributed to pectinesterase. This enzyme is capable of de-esterifying the pectin; the low methoxyl pectin formed reacts with calcium ions, causing cloud loss in various fruit juices (Versteeg, 1979). For these products, thermal treatments are generally employed to inactivate both microbes and enzymes, which sometimes cause off flavours, and juice concentrates must be stored at −20°C to keep degradative processes at a minimum. Many consumers are concerned with the wholesomeness of products which have undergone minimal heat treatment. Pasteurisation of such product is directed at the enzyme pectinesterase (PE). Thermal treatment directed solely at micro-organisms will not fully inactivate the heat stable isozyme(s) of PE. Unchecked, PE may produce cloud loss in juices (Sadler, Parish, & Wicker, 1992). Although several studies on fruit juice processing using pasteurisation have been conducted, most of these are on pectinesterase inactivation or microbial reduction in citrus juice. Soursop fruit contains pectinesterase enzyme and information regarding the effect of pasteurisation on microbial and PE activity in natural soursop puree is not available.

Soursop (*Annona muricata* L.) is one of the popular exotic fruits in Malaysia. The white cottony pulp is very juicy, varying in flavour from acid to sweet and highly regarded because of its distinctive aroma and flavour (Bueso, 1980). As such, it offers potential for the soft drink industry; in addition, the puree can be used for manufacturing various juice blends, nectar, jam, jelly, ice cream and preserves. The effects of processing temperatures and additives on the quality and shelf life of frozen soursop pulp were studied by Sanchez Nieva, Hernandez, and Iguina de George (1970). Our present objective is to examine microbial and enzymatic changes in soursop puree which has been pasteurised at optimum thermal conditions of 79°C for 69 s (Umme, Asbi, Junainah, Jamilah, & Salmah, 1997) stored in different packaging material and temperatures.

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2. Materials and methods

2.1. Preparation of soursop puree

Fully ripe soursop (*A. muricata* L.) fruits of commercial variety were collected directly from the trees of orchards Planter's Heaven, Nilai, Negeri Sembilan of Malaysia. The fruits were washed under running tap water, hand-peeled, cored, deseeded and the pulp macerated using a local made stone grinder (stone size 10 in). Water was added in the ratio of 1:2 (w/v, pulp:water) to facilitate the maceration process and it was repeated twice to achieve a smooth-textured puree. The pH and total soluble solids of prepared soursop puree were 3.7 and 6.0°Brix, respectively, with no added sugar.

2.2. Thermal pasteurisation treatment

The soursop puree was pasteurised at optimum conditions of 79°C for 69 s. The deaerated puree was heated in a tubular heat exchanger with a 5.8 m, 23 mm ID hold tube (APV Co. Ltd., Singapore) at 2.1 litre/min and the samples were then rapidly cooled to 9°C with chilled water. The cooled puree was packed into three different types of container and stored as described until use.

2.3. Storage test

Thermally pasteurised and unpasteurised soursop purees were packed in laminated aluminium foils (LAL), indigenous lacquered cans (LC) and plastic bottles (HDP). They were stored at 15, 4 and −20°C for a period of 12 weeks. The presence of *Escherichia coli*, survival of total mesophilic bacteria and yeast and mould, activity of residual enzymes and cloud stability in juice prepared from puree as fresh, after pasteurisation and during storage were measured.

2.4. Sampling

Samplings for analysis of soursop puree were done at 0, 1, 3, 6, 9 and 12 weeks. Four replicate samples from each of the three storage conditions and packages were randomly selected at each evaluation. Mixing the puree was accomplished by inverting the containers at least 10 times; they were then aseptically opened. Samples for microbial analysis were aseptically removed from the container prior to sampling for other analyses.

2.5. Microbiology

Samples were serially diluted with sterile 0.1% peptone solution and plated by the pourplate technique (AOAC, 1984) in duplicate. Yeast and mould counts were conducted with rose-bengal chloramphenicol agar (DRBC agar base; Oxoid, UK). Total mesophilic bacterial (TMB) counts and *E. coli* were conducted with orange serum agar (OSA; Becton Dickson, Cockeysville, MD 21030, USA) and eosin methylene blue agar levine (EMB; Gibco diagnostics, Madison, Wisconsin, USA), respectively. Plates were incubated for 3 days at 30°C for yeast and mould and 48 h at 35°C for mesophilic bacteria and *E. coli* prior to counting.

2.6. Enzyme extraction

Extraction of pectinesterase enzyme was done by the Arbaisah, Asbi, Junainah, and Jamilah (1996) method. The soursop puree was blended at medium speed with extraction solution (1.92 M NaCl; pH 8.4) using a Waring blender (model 7011 S) for 1 min. The ratio of the fruit puree to the extractant was 1:1 (w/v, puree:soln) and the pH was maintained by addition of NaOH. The slurry was centrifuged at 15,000×***g*** for 30 min using a refrigerated centrifuge (Beckman, model J2 - 21 M/E) and the supernatant was collected for enzyme assay. All procedures were carried out at 4°C.

2.7. Assay of pectinesterase (PE) activity

The pectinesterase activity of soursop puree as fresh, after pasteurisation and during storage was measured at pH 7.0, 30°C in 1% pectin, containing 0.15 M NaCl according to the procedure of Korner, Zimmermann, and Berk (1980) (Table 1). The initial reaction velocity was measured by automatic titration of the liberated carboxyl groups with 0.02 M NaOH for 10 min in a Titralab Autotitrator model VIT 90/ABU 93/SAM 90 (Radiometer, Copenhagen, Denmark). One unit represented the hydrolysis of 1microequivalent of ester per min. PE was assayed in six replicates.

2.8. Determination of cloud stability

Cloud stability measurement was carried out as per the method of Krop et al. (1974). The puree was diluted with water (1:2 w/v, puree:water) to make juice. After filtration with muslin cloth, 10 ml juice was centrifuged (Clements GS 15) for 10 min at 360×***g***. The extinction of the supernatant was then measured at 660 nm in a 1 cm cuvette (Shimadzu UV-1201 UV–VIS Spectrophotometer) and this value is considered a measure for the cloudiness.

2.9. Statistical analysis

All data were assessed by SAS PROC GLM and Duncan's multiple range test (SAS Institute, 1985). Data were analysed as a factorial with four replications, six storage times, three packaging, three storage temperatures and two treatments (with and without pasteurisation).

Table 1
Effect of pasteurisation on the pectinesterase enzyme activity (units[a]) of natural soursop puree during storage

Storage temperature (°C)	Type of packaging	Pasteurised puree storage time (week)						Unpasteurised puree storage time (week)					
		0	1	3	6	9	12	0	1	3	6	9	12
−20	Foil	0	0	0	0	0	0	12.9ax	11.9bx	11.1cx	10.7cy	9.97dy	9.95dy
−20	Can	0	0	0	0	0	0	12.9ax	11.8bx	11.4bcx	11.1cx	10.96cx	10.9cx
−20	Bottle	0	0	0	0	0	0	12.9ax	11.6bx	11.3bx	11.2bx	11.2bx	11.0bx
4	Foil	0	0	0	0	0	0	12.9ax	12.8ax	12.1bx	11.5cx	10.1dx	10.1dx
4	Can	0	0	0	0	0	0	12.9ax	12.2ay	12.1 ax	11.3abx	10.3bx	10.2bx
4	Bottle	0	0	0	0	0	0	12.9ax	12.0ay	11.9ax	10.4by	–[b]	–
15	Foil	0	0	0	0	0	0	12.9ax	–	–	–	–	–
15	Can	0	0	0	0	0	0	12.9ax	–	–	–	–	–
15	Bottle	0	0	0	0	0	0	12.9ax	–	–	–	–	–

[a] Definition of units in Materials and Methods.
[b] —Sample already unaccepted.
a–e = Means within a row with different letters are significantly different ($p < 0.05$).
x–y = Means within a column with different letters are significantly different ($p < 0.05$).

3. Results and discussion

3.1. Microbiological trends

At 15°C there was a 1 log cycle increase in microbial population of unpasteurised puree at the 1st week. The puree quality was unacceptable by the 3rd week as a result of fermentation and, as such, their evaluations were discontinued.

There was a gradual increase of 2 log cycles in bacterial population for unpasteurised puree during the 6th week of storage but this dropped after the ninth week at 4°C in all packages (Fig. 1a and b). However, the bacterial populations for puree in foils and cans were lower than those for puree in bottles. Yeast and mould counts in unpasteurised puree at 4°C, for all packaging types, remained unchanged during the first week of storage (Fig. 1b); but the total plate count fell rapidly at the same period. Then, in the 3rd week, total plate counts remained numerically similar to those of yeast and mould (Fig. 1a). This indicated that soursop puree medium was more favourable for yeast and mould at 4°C than for bacteria. Yeast growth was favoured by the presence of sugar and acid pH. Fruit juices are readily fermented by yeasts while their acid pH discourages most bacterial growth (Marion Bennion, 1980). Similar trends in reduction were noted by others (Gow-Chin Yen & Hsin-Tang Lin, 1996; Sadler, Parish, & Wicker, 1992).

Samples stored at −20°C were stable in terms of TMB and Y&M counts (Fig. 2a and b). This may be due to the sudden mortality on immediate freezing and the cells which were still viable immediately after freezing died gradually when stored in the frozen state (Splittstoesser, 1978; Jay, 1978).

Pasteurisation at 79°C for 69 s reduced microbes of the puree from 6.4×10^3 to 8.5×10^1 cfu per g. Unlike microbial counts for guava puree of pH 3.8 (adjusted) at 4°C (Gow-Chin Yen & Hsin-Tang Lin, 1996), both TMB and Y&M counts for pasteurised soursop puree in this study fell during 1st week of storage at 4°C (Fig. 1a and b). These results are in agreement with Sadler et al. (1992) who showed that OSA and APDA counts fell rapidly during first 7 days storage of orange juice with pH of 3.52 and 3.78 at 4°C. Our observation showed that the microbes in soursop puree slowly increased to 1 log cycle in foils and cans, 2 log cycles in bottles at the ninth week and fell thereafter during storage of 12 weeks (Fig. 1a and b). This suggested that Y&M which initially survived thermal treatment apparently adapted poorly to puree at 4°C. This can be attributed to the low pH (3.7) or presence of endogenous organic acids in ripe soursop, such as malic acid followed by citric, oxalic and acetic acid (Paull, Deputy, & Chen, 1983; Magda, 1991). Organic acids exert their antimicrobial effect through undissociated molecules (Rusul & Ang, 1994). The activity of the acids is pH-dependent, because pH determines the extent of their dissociation.

As with the growth trend in unpasteurised puree at 4°C, pasteurised puree packed in bottles increased by more than 3 log cycles, and in foils nearly by 2 log cycles at the 6th week and declined in growth thereafter at 15°C. Unlike in foils and bottles the bacterial population in cans suddenly dropped after the 1st week and numbers steadily decreased until the 12th week (Fig. 3a and b). One of the possible explanations is the formation of hydrogen ions (H^+) produced by corrosion inside the can by the acidic soursop puree or by microbial action on the puree. Owing to their ability to produce large amounts of acids, lactic acid bacteria often inhibit the development of other organisms in juices and are capable of causing their own autolysis (Hsu, 1975).

Similar to the unpasteurised puree, pasteurised puree kept at −20°C showed sudden decreases in TMB and Y&M in all packaging types and remain unchanged thereafter (Fig. 2a and b). No growth were observed for

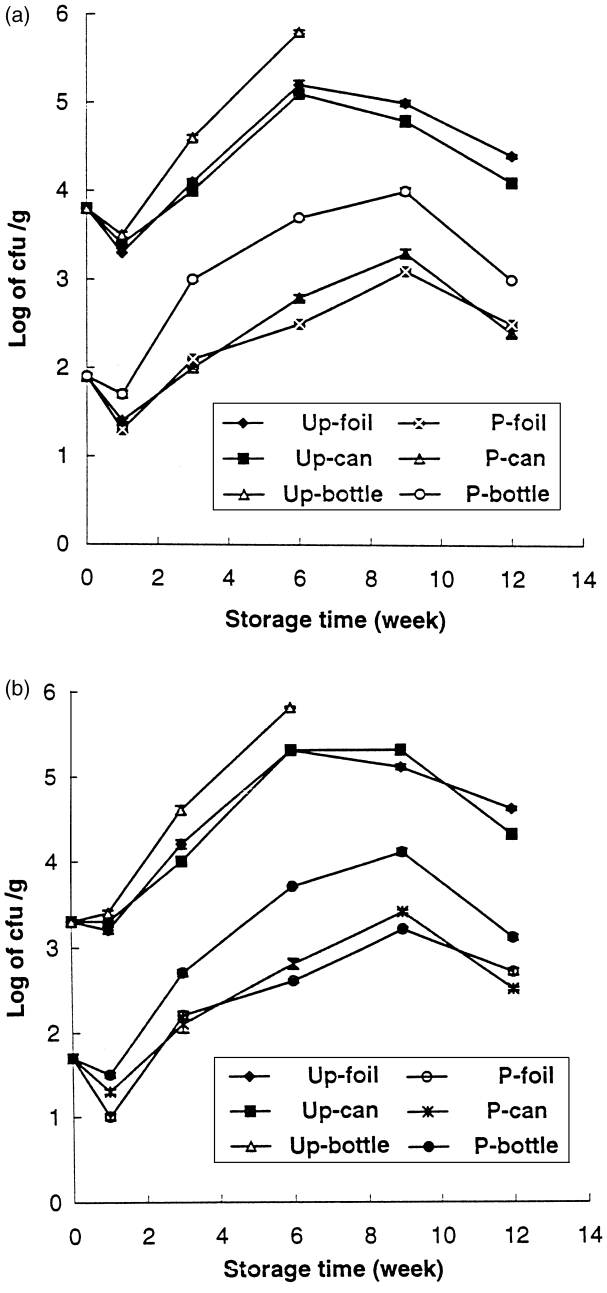

Fig. 1. (a) Effect of pasteurisation on the changes in populations of TPC in different packaging during storage at 4°C. (b) Effect of pasteurisation on the changes in populations of Y&M counts in different packaging during storage at 4°C.

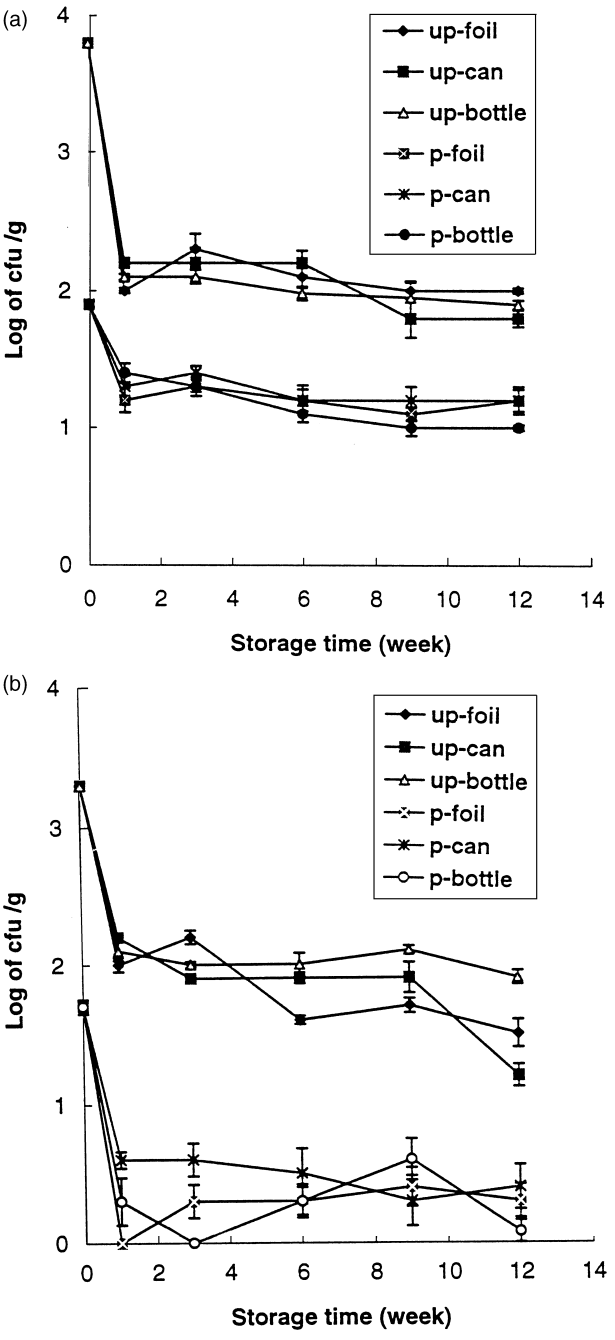

Fig. 2. (a) Effect of pasteurisation on the changes in populations of TPC in different packaging during storage at −20°C. (b) Effect of pasteurisation on the changes in populations of Y&M counts in different packaging during storage at −20°C.

E. coli in all types of pasteurised or unpasteurised puree stored at different temperatures.

Results in Table 2 indicated that, the treatment, type of packaging materials, storage temperatures and time of storage significantly ($p < 0.05$) affect the microbial growth. Apart from −20°C storage, 4°C seemed to be the best suitable temperature for puree storage to inhibit the growth of the mesophilic bacteria, mould and yeast. However, no significant difference was observed between foils and cans in inhibiting microbial growth, except for puree packed in bottles which showed significantly ($p < 0.05$) higher mould and yeast counts compared to the others.

3.2. Cloud stability

Cloudiness is an important quality attribute for soursop juice. Cloud loss was apparent within 2 days in unpasteurised puree at 4°C packed in cans and bottles

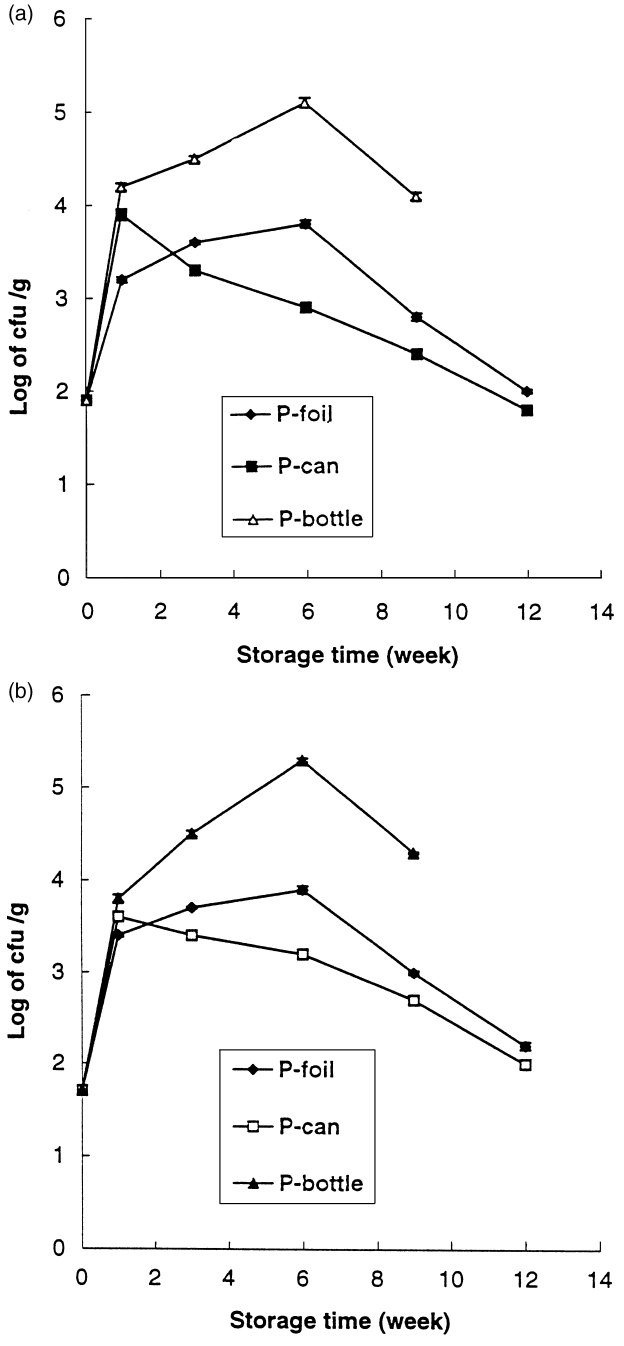

Fig. 3. (a) Effect of pasteurisation on the changes in populations of TPC in different packaging during storage at 15°C. (b) Effect of pasteurisation on the changes in populations of Y&M counts in different packaging during storage at 15°C.

and rapid loss continued until 12 weeks, whereas, in foils, slight reduction in cloud occurred after 1 week and showed greater loss by the 9th week (Fig. 4). These trends suggested rapid losses of cloud in unpasteurised puree during storage as a result of microbial fermentation and degradation of cloud pectin by PE activity in the puree. Significant but low correlations were present between TMB, Y&M and cloud loss ($r = -0.31$ and -0.28). On the other hand, significant and negative but high correlations ($r = -0.82$) between PE activity and cloud stability were noted. According to Nussinovith and Rosen (1989), moulds potentially produce a cloud loss in aseptically processed juices. Soursop puree and other tropical fruit juices contain varying amounts of PE (Fayyaz, Asbi, Ghazali, Cheman, & Jinap, 1994), which catalyse the hydrolysis of the methoxy groups to free carboxyl groups and are then bound by ions present in the juice. They alter the colloid stabilising power of the pectin and increase the rate of cloud loss (Versteeg, Rombouts, Spaansen, & Pilnik, 1980). Cloud had decreased to 0.089 in bottles at the 6th week and became unacceptable thereafter; clouds dropped to 0.1 and 0.095 in foils and cans, respectively, by the end of storage of puree.

Unpasteurised puree in all packages stored at −20°C exhibited sharp drop in cloud by the 1st week and continued a slow and steady loss up to the 12th week; however, puree packed in foils showed less cloud loss than in cans and bottles (Fig. 5). Our observation is consistent with the results of others who prepared orange juice from frozen oranges which contributed to the poor cloudiness (Owusu-Yaw, Marshall, Koburger, & Wei, 1988). As ice separates out during thawing, the concentration of the solution phase increases. There is therefore a greater potential for cross-linking between the polymer molecules. This results in reduction of solubility, aggregation and loss of consistency (Reid, 1994).

Heating at 79°C for 69 s caused a significant ($p < 0.05$) increase in cloud stability of the soursop puree from 0.318 to 0.529 (Fig. 4). Heat treatment caused some disintegration of the larger cloud particles and enhanced cloud stability (Mizrahi & Berk, 1970). Braverman (1949) assumed that the turbidity is increased as a result of extraction of pectic substances into the serum. However, such a solubilisation is due to β-eliminative depolymerization of pectin molecules (Greve, McArdle, Gohlke, & Labavitch, 1994) associated with the cloud matrix.

The cloud stability in pasteurised puree was almost stable at 4°C (Fig. 4), showing very little cloud loss during storage for 12 weeks. Statistical analysis of the puree samples stored at 4°C showed that the puree in foils and bottles (abs at 660 nm was&0.504 and 0.502) were not significantly ($p > 0.05$) different from each other but only slightly significantly ($p < 0.05$) different from puree in cans (abs at 660 nm was ~0.498). Puree stored in these conditions had a low initial bacterial load and inactive PE enzyme which could afford some protection against the loss of cloud.

Apparent cloud loss began within one week in cans and bottles, and after 1 week in foils at 15°C (Fig. 6). Purees packed in foils showed slow decrease in cloud by the end of 12 weeks storage and those packed in bottles followed the same and became unacceptable after the

Table 2
Main effects of processing, packaging, storage temperature and storage time on the quality of natural soursop puree[a]

	TPC (log cfu/g)	Y&M (log cfu/g)	Cloud (O.D at 660 nm)	PE activity (unit/g)
Processing:				
Pasteurised	2.30B	2.05 B	0.47A	0.00B
Unpasteurised	3.31A	3.16A	0.24B	11.6A
Packaging:				
Foil	2.16B	1.91B	0.49A	11.5B
Can	2.13C	1.93B	0.48B	11.6AB
Bottle	2.64A	2.33A	0.44C	11.7A
Storage temperature:				
−20°C	1.32C	0.56C	0.43C	11.4A
4°C	2.49B	2.46B	0.52A	11.6A
15°C	3.15A	3.20A	0.46B	–
Storage time:				
0 week	2.87B	2.54C	0.42A	12.9A
1st week	2.40D	2.13D	0.41B	12.1B
3rd week	2.80C	2.56C	0.38C	11.6C
6th week	3.12A	2.98A	0.35D	11.0D
9th week	2.77C	2.72B	0.34E	10.5E
12th week	2.26E	2.06E	0.32F	10.4E

[a] Means separated in columns by main effects of Duncan's multiple range test. Numbers followed by the same letter are not significantly different.

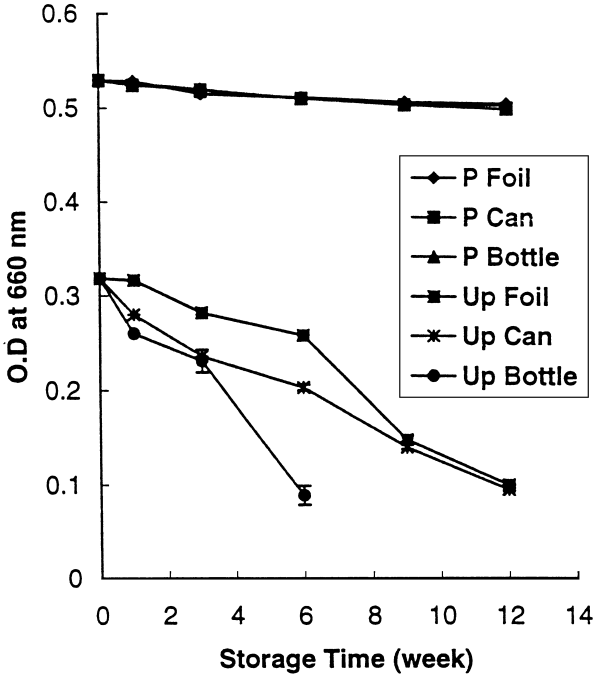

Fig. 4. Change of cloud at different packaging stored at 4°C.

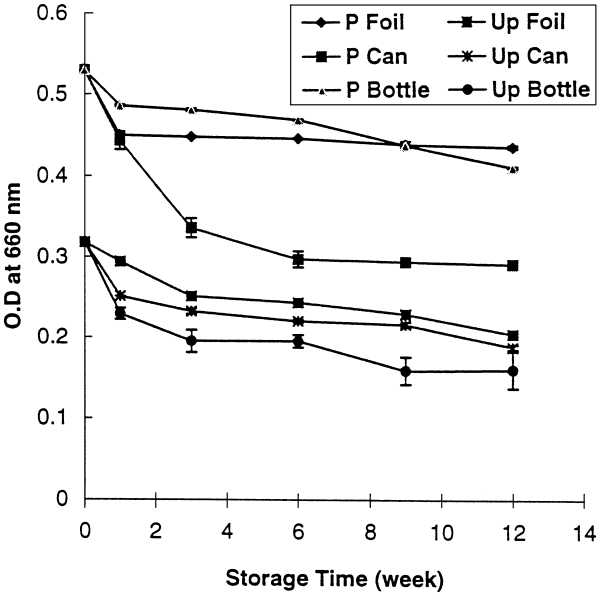

Fig. 5. Change of cloud at different packaging stored at −20°C.

ninth week. Higher cloud loss was noted in cans after the 6th week and lost up to 56.5% at the end of storage. This might be attributed to H^+ ion produced: (a) by chemical spoilage from the action of acidic puree on iron and tin from the can; (b) by microbial spoilage; or (c) by interactions between the sugar break down products (triose sugars, acetaldehyde, pyruvic aldehyde, acetal, crotonaldehyde, etc.) from the heated puree and the tin can, as with can corrosion. These reactions and breakdown are manifested by visible changes in the puree such as flocculation, cloud formation or gelation as well as non-typical texture and appearance (Woodroof, 1975).

As with unpasteurised puree, pasteurised puree kept at −20°C showed a sudden decrease in cloud stability within 1 week, but was stable thereafter in foils and bottles. Pasteurised puree packed in cans exhibited cloud loss up to 55% at the end of the 12 week storage period (Fig. 5).

Pasteurisation, storage temperature, storage time and packaging did have a significant ($p > 0.05$) effect on the cloud stability of soursop puree indicated by the main

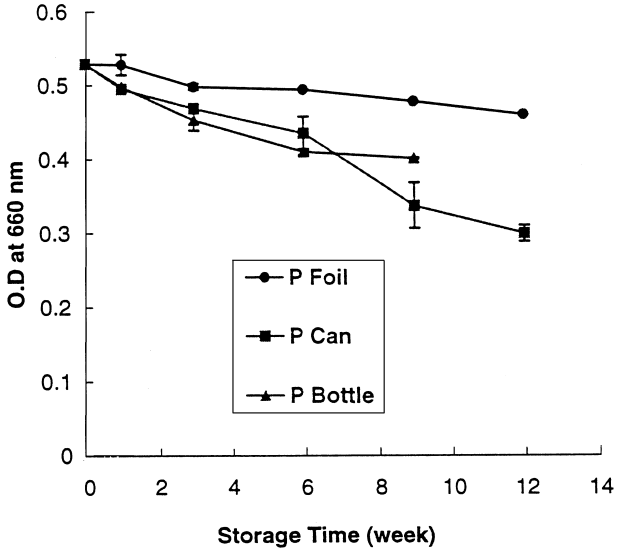

Fig. 6. Change of cloud at different packaging stored at 15°C.

effects during 12 weeks of storage (Table 2). The change in cloud during storage in can and bottle was more rapid at 15 than at 4°C. This indicated that cloud stability of soursop puree during storage was highly temperature-dependent. Overall, the cloud of juices prepared from puree packed in foil at 4°C was very stable ($p > 0.05$) after 12 week.

3.3. Pectinesterase inactivation

Table 1 summarises the changes in activities of PE in soursop puree pasteurised with optimum conditions stored at different temperatures in different packaging. The PE activity of soursop puree was completely ($p > 0.05$) inhibited by pasteurisation at 79°C for 69 s. Four per cent PE activities still existed in guava puree heated at 88–90°C (Gow-Chin Yen & Hsin-Tang Lin, 1996). In grape fruit juice, loss in PE activity predominantly came from the heat-labile isozyme of PE (Seymore, 1990). Regeneration of thermally inactivated PE has been reported in cucumbers (McFeeters, Fleming, & Thompson, 1985). Laratta et al. (1995) noted that pasteurised tomato puree often displayed pectinesterase activity around 10^{-2}–10^{-3} units PE per ml product. However, no PE regeneration was found for pasteurised natural soursop puree in our study.

According to Arbaisah (1996) the D values of pasteurisation at 70°C, 5 min, were necessary to inactivate 90% of both purified soursop PE I and PE II at pH 7.5; and the Z values of 8.5 and 8.6°C were found for PE I and PE II, respectively. As the heating time and temperature to inactivate the pectinesterase decreased with pH, the time–temperature reported here in this study were considered enough for commercial pasteurisation of natural soursop puree.

The PE activity of unpasteurised puree decreased during storage (significant main effect of time). However, the PE activity did not differ notably due to packaging during 12 weeks and no difference in activity showed between storage temperatures (Table 2). No significant storage temperature×packaging interactions were found for the PE activity of unpasteurised soursop puree.

Up to 30 and 18% loss in PE activity has been observed (Gow-Chin Yen & Hsin-Tang Lin, 1996) in unheated guava puree at pH 3.8, 4°C, during 60 days storage and orange juice at pH 3.52, 4°C, for 50 days (Sadler et al., 1992), respectively. A similar 19% loss in PE activity was observed for unpasteurised soursop puree in our study (Table 2) at the end of the 12 week storage period.

4. Conclusion

Pasteurisation of natural soursop puree at 79°C for 69 s inactivated the microbes, completely inactivated the pectinesterase enzyme and stabilised the cloud in natural soursop puree. Out of four storage temperatures used, storage at −20°C showed the lowest microbial count in pasteurised puree for both TPC and Y&M and followed by those at 4 and 15°C. In unpasteurised puree, microbial proliferation accelerated more rapidly after the third week. So, it can be concluded that the optimum conditions for pasteurisation at 79°C for 69 s for enzyme inactivation and nutrient retention were also sufficient to inactivate micro-organisms. Out of three packaging materials used, laminated aluminium foils at 4°C had the lowest rate of decrease in cloud stability and the lowest rate of increase in microbial counts; it was found to be most suitable for the stability of pasteurised soursop puree during storage. Inversely, pasteurised puree packed in lacquered cans exhibited greater loss in cloud and natural consistency. Some of the unknown destabilising effects were caused by metal-complexing reactions and change in pH by acidic soursop puree. Due attention should be paid to selecting proper can lacquers for the respective fruits.

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