CHEMICALLY ACIDIFIED WET STORAGE OF CASSAVA STARCH IN INDIA

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ABSTRACT

The Natural Resources Institute (NRI) is co-operating with Indian counterpart organisations to assist with the development of the cassava starch and *sago* industry in Tamil Nadu (India). Improved storage of wet starch is one area that has received particular attention. Adverse quality changes (reductions in paste viscosity and clarity) during conventional wet storage lead to a 20% loss of income for the industry. These quality losses are attributable to the growth of amylolytic lactic acid bacteria (ALAB) during storage. Laboratory experiments and field trials have shown that low concentrations of acetic acid can be used to prevent the growth of ALAB during storage. Cassava starch treated with 2% (v/v) acetic acid prior to storage, had a viscosity value 32% higher than the minimum value specified in the Indian standard for textile starch after wet storage. Starch stored conventionally under the same conditions had a viscosity value 14% below the minimum value required for textile starch.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop which serves as a staple food for large numbers of people in the tropical and subtropical regions of the world. In India cassava roots are either consumed directly or processed into starch or cassava *sago*. Cassava *sago* is a popular food item in many parts of India, that is made industrially by roasting and drying small pellets of wet cassava starch extracted from peeled roots. S*ago* production is concentrated in the southern state of Tamil Nadu where much of the Indian cassava crop is grown.

The highest grades of cassava starch and *sago* are processed directly from wet starch without storage. However, in most factories the capacity to crush roots is greater than the capacity to further process the extracted starch. Extracted starch that cannot be processed immediately (approximately 40% of production) is transferred to open granite lined tanks for storage under wet conditions. This occurs mainly during November and December when the volumes of roots delivered to the factories are at their peak and cloudy weather restricts sun-drying. Starch is usually stored until the cassava harvest is complete, which is typically 2-5 months after the start of storage.

Long-term storage of cassava starch under wet conditions results in adverse quality changes which reduce the grade and sale price of the resultant dry starch or *sago*. In 207 starch factories surveyed during 1994 in Tamil Nadu approximately 25% of starch production was low quality (no paste/glucose grade) wet stored starch (Graffham 1995). It was estimated that wet starch storage cost these factories 15.21 million (152 lakh) Indian Rupees in lost income during the 1993-94 processing season. Assuming that the 850 sago and starch factories in Salem District have the same size distribution/production ratios, the loss to the industry as a whole in 1993-94 was approximately 60 million (600 lakh) Indian Rupees. Wet storage has less effect on sago producers who rely on colour rather than viscosity for grading their products. However most producers agree that wet

storage leads to a one to two grade reduction in the quality of sago produced from wet stored starch. This represents a 20% loss in income (Graffham 1995).

The objectives of the present study were to investigate the microbiology and physico-chemistry of wet starch storage in India with a view to identifying the reasons for adverse quality changes occurring during the process, and to develop a means for preventing reductions in quality during wet storage.

MATERIALS AND METHODS

Preparation of cassava starch

Cassava starch was extracted from peeled roots using a rotary crusher, separated from extraneous material by passing through a series of nylon mesh screens (50, 80, 150, 200, 280 and 350 mesh), and allowed to sediment in an open tank overnight. The supernatant and suspended materials were removed, and the starch resuspended in water. Starch milk was fed into a settling tank and allowed to settle for 8-12 hours. Further purification was achieved by gentle agitation of the suspension during settling (to prevent fine contaminants settling with the starch granules).

Wet storage of cassava starch (unacidified control)

A granite lined storage tank (length: 1.5m, width 1.5m and depth 1.1m) containing 100 litres of water was used for wet starch storage. The tank was filled with settled starch (approximately 45% moisture). The starch was covered with a layer of water (5cm deep) throughout the six week storage period.

Acidified storage of cassava starch

A granite lined storage tank of the same dimensions as the control tank was loaded with a mixture of wet cassava starch and food/textile grade acetic acid (96%) diluted in water. The volume of acid was calculated to provide a final acid concentration of 2% v/v after addition of the starch to the tank. Within one day, the starch settled within the tank to leave a clear layer of acidified water on top of the starch. This covering layer with 2% v/v acetic acid was maintained throughout the six week storage period.

Sampling

During the storage period, samples of starch were collected at weekly intervals for analysis. The layer of surface water was removed from the tank before sampling to prevent interference with sample collection. Samples (1.5kg) were collected by inserting a soil drill (1.2m long by 30mm in diameter) into the starch at randomly selected points. The statistical approach for sampling was based on that of Springer & McClure (1988).

Preparation of samples for microbiological analysis

Cores of starch were trimmed aseptically using a sterile scalpel to remove material which had come into contact with the soil auger, transferred to sterile plastic bags, packed into a refrigerated container and taken to the laboratory for analysis. The time between sampling and analysis at the laboratory was less than one hour.

Preparation of samples for physico-chemical analysis

Samples of starch (1000g) were divided into two lots of 500g each, placed in plastic trays lined with aluminium foil and either sun dried for 1-2 days or oven dried at 40-50°C for 2 days. Dried samples were ground manually (to avoid damage to starch granule structure) using a roll bottle technique and passed through a 180μm sieve.

Effect of washing on pasting characteristics of chemically acidified starch

To assess the possible effect of acid residues on the functional properties of cassava starch selected samples of chemically acidified starch (oven and sun dried) were washed in an excess of water and oven dried in a laboratory oven (40-50°C for 2 days).

Microbiological Investigations

For the determination of viable counts of bacteria, samples of starch (10g) were homogenised and serially diluted in maximum recovery diluent (Oxoid). Suitable dilutions were plated in triplicate onto a range of selective media. *Enterobacteriaceae* were enumerated on Violet Red Bile Glucose Agar (Oxoid) incubated aerobically for 1-2 days. Lactic acid bacteria were enumerated on de Man, Rogosa and Sharpe Medium (Oxoid) incubated under microaerophilic conditions for 3-4 days. Yeasts and moulds were enumerated on Dichloran Rose-Bengal Chloramphenicol Agar (Oxoid) incubated aerobically for 3-5 days.

Leuconostoc sp. were enumerated after aerobic incubation for 1-2 days on a high sucrose medium. The high sucrose medium contained ($g\ l^{-1}$) in distilled water: tryptone, 10; yeast extract, 5; sucrose, 100; sodium azide (1%), 5ml; agar, 15 (pH 6.4-6.6).

Homo and heterofermentative lactic acid bacteria were enumerated on modified HHD medium (McDonald *et al.* 1987) incubated under microaerophilic conditions for 3-4 days. The modified HHD medium contained ($g\ l^{-1}$) in distilled water: fructose, 1.8; peptone, 10; yeast extract, 2.5; casamino acids, 3; KH_2PO_4, 2; tween 80, 1; meat extract, 2.5; $NaC_2H_3O_2$, 2; $MgSO_4$, 0.1; $MnSO_4$, 0.05; bromocresol green (0.1g bromocresol green dissolved in 30ml of 0.01M NaOH), 20ml; agar, 20 (pH 7.0).

Amylolytic lactic acid bacteria were enumerated on ALB Agar. After incubation under microaerophilic conditions for 7 days, plates of ALB were stained with Lugol's iodine solution. Colonies exhibiting zones of clearing were recorded as amylolytic. ALB Agar had the same formulation as the modified HHD medium, but with the following

exceptions: (g l^{-1}) fructose, 0.5; Lintners soluble starch, 1.3; and bromocresol green was not added.

All microbiological media were incubated at 26°C.

pH value, pasting characteristics, swelling volume, swelling power, solubility and reducing value

pH values were measured in triplicate by inserting a pH electrode into a suspension of the starch sample (10g starch/20ml distilled water). Pasting characteristics of 5% starch suspensions were measured using both Brabender Visco-Amylograph and Redwood viscometer. Swelling volume, swelling power and solubility were measured for replicate samples using the method of Leach *et al.* (1959). Reducing values were measured in triplicate using the ferricyanide method of Schoch (1964).

RESULTS AND DISCUSSION

CONVENTIONAL WET STARCH STORAGE

Microbiology of conventional wet starch storage

Freshly processed cassava starch contained a mixed microflora of lactic acid bacteria, *Enterobacteriaceae*, yeasts (low numbers) and unidentified aerobic bacteria. *Leuconostoc* species were not detected in freshly processed starch, but were present at relatively high numbers in starch after one week of wet storage (Figure 1).

After two weeks of storage, homofermentative lactic acid bacteria dominated the microflora of the starch (Figure 1). *Enterobacteriaceae* and *Leuconostoc* spp. were excluded from the starch microflora after three weeks and the population of yeasts was reduced to low levels after four weeks (Figure 1).

During the fermentation, the percentage of lactic acid bacteria capable of degrading starch increased from 9% in fresh starch to 58% in starch stored under wet

conditions for three weeks. However, after four weeks of storage, amylolytic lactic acid bacteria represented less than 7% of the total lactic acid bacterial population (Figure 2). During the first two weeks of storage, colonies grown on ALB medium produced large zones of starch hydrolysis indicating organisms with highly active amylase systems. After two weeks of storage, the level of amylolytic activity of the individual organisms isolated declined reaching a minimum in the fourth week, even though the numbers of lactic acid bacteria remained high throughout the four week period. During the same period there was a decrease in pH value from 4.1 to 3.4 (Figure 1).

These observations indicate that there is a link between pH value and amylolytic activity. Amylolytic lactic acid bacteria appeared to show optimal activity in the range pH 3.6 to 4.1, but very little activity was detected when the pH value was less than 3.5 (based on number of organisms, and size and clarity of amylolytic zones on ALB Agar).

However, the effect of pH is difficult to determine as it seems likely that substrate availability could also affect changes in the population of amylolytic lactic acid bacteria. Fresh starch granules contain amorphous regions which are readily accessible to microbial enzymes (French 1984). During storage, the amorphous regions would be expected to be gradually degraded reducing the amount of substrate available for the microorganisms.

The progressive increase in the percentage of lactic acid bacteria showing amylolytic activity over a three week period (Figure 2) would appear to indicate that a process of adaptation was taking place, which favoured microorganisms having the ability to utilise starch (main carbon/energy source in the tank) under the conditions in the tank (low pH and anaerobic).

Physico-chemical changes during conventional storage

Samples of freshly processed starch gave double pasting peaks and two values for peak viscosity (Table 1) indicating the presence of two distinctive groups of associative forces

in the starch granules. Two stage pasting is a characteristic of starch extracted from roots of cassava variety M4 (Moorthy 1994), the variety that was being processed by the factory at the time of the study. Cassava starch stored for one week under wet conditions gave a single pasting peak indicating that one set of associative forces was disrupted during the first week of storage. Acids and amylolytic enzymes attack initially the more accessible amorphous regions of starch granules (French 1984) which indicates that the associative forces responsible for a second pasting peak are likely to be in the amorphous regions of the starch granules.

Wet storage of cassava starch led to progressive reductions in paste viscosity values. After only one week, hot paste stability was reduced greatly (Table 1). Reductions in paste stability in the presence of enzymes or mineral acids can be attributed to depolymerisation which weakens the structure of the swollen granules causing them to break up under the shear stresses present in the Brabender Visco-Amylograph (Radley 1968; French 1984). In addition low pH (pH <4.0) has been shown to reduce paste stability by weakening intergranular forces (Radley 1968; Cereda & Wosiacki 1985). Wet storage of cassava starch reduces the pH value from 4.1 to 3.4 within four weeks (Figure 1), and in addition amylolytic activity by amylolytic lactic acid bacteria would be expected to weaken the structure of the starch granules.

When cassava starch was stored under wet conditions, there was an initial rise in swelling volume in the first week followed by a progressive fall with time (Table 2). During the experimental period, swelling power decreased with time and there were corresponding increases in solubility and reducing value (Table 2). These results support those obtained with the Brabender Visco-Amylograph indicating that a combination of low pH and amylolytic enzymes were degrading the starch granules during storage.

The initial rise in swelling volume is probably related to initial enzymic attack on the amorphous regions of the granule (Franco *et al.* 1988). Weaker granule structures expand more readily but are more susceptible to shear in the Brabender Visco-

Amylograph, hence the reduction in viscosity and paste stability after one week of storage. Continued enzymic attack on the amorphous regions of starch granules leads to low paste stability and reductions in swelling volume and swelling power (French 1984; Swinkels 1985).

The increases in solubility and reducing value during wet storage are clear indicators that enzymic attack is also causing changes at a molecular level within the starch granule. In freshly processed starch, the amylose and amylopectin polymers theoretically only contain one reducing end group per molecule. However when amylolytic enzymes start to degrade these polymers, the number of reducing end groups will naturally increase thus giving a direct indicator of enzymic attack at a molecular level. Obviously as the starch polymers are broken down there will be a corresponding increase in the levels of soluble sugars hence the increase in starch solubility seen during wet storage.

CHEMICALLY ACIDIFIED STORAGE OF WET CASSAVA STARCH

Effect of chemically acidified (acetic acid) wet storage on cassava starch quality

Acidification of cassava starch with acetic acid (2% v/v) prior to wet storage reduced the population of ALAB by 3-4 log cycles after one week of wet storage. The population of lactic acid bacteria and level of amylolytic activity (Fig. 2) remained at a low level throughout the storage period. This observation was in marked contrast to the high levels of ALAB in the conventionally stored starch which only reduced after several weeks when the pH value of the starch went below pH 3.4.

Acidified storage with acetic acid (2% v/v) preserved starch quality better than the conventional storage technique. The paste viscosity (measured in Redwood seconds and Brabender units) of the acidified stored starch was slightly lower than that of the fresh starch (Table 3), however, this can be attributed to the presence of acetic acid. When the acetic acid was washed out before drying, the paste viscosity of stored starch was shown to be superior to that of the fresh starch (Tables 3 & 4). The apparent

improvement in quality over that of fresh starch is most probably due to the high efficiency of the washing procedure used for the experimental trials.

Conventional wet storage for six weeks reduced the paste viscosity of cassava starch to 14% lower than the minimum value specified in the Indian Standard for textile grade cassava starch (IS 1605-1977). Chemically acidified wet storage, followed by washing to remove the acid, produced a product with a paste viscosity 32% higher than the minimum value specified in IS 1605-1977 (Table 4).

CONCLUSION

In this communication, it has been proposed that the growth of amylolytic lactic acid bacteria is responsible for the reduction in quality of cassava starch during wet storage. The use of acetic acid at a 2% level to acidify the starch prior to wet storage has been demonstrated to offer the potential to prevent the growth of these microorganisms and so prevent damage to the starch. Preliminary economic evaluations (not presented here) indicate that the process will be economcally feasible. Further work is planned to confirm the economic benefits of the process, assess the environmental implications and optimise the quantity of acid used.

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DEDICATION

The first author would like to dedicate this paper to the memory of his friend the late Thiru L V Palaniswamy of Sri Velmurugan Traders, Salem (India). Thiru Palaniswamy was dedicated to the future prosperity of the Indian cassava starch and *sago* industry and gave unstinting support and friendship to all who sought to improve the industry through research and development.

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Table 1. Pasting characteristics of 5% suspensions of conventionally stored wet cassava starch measured using a Brabender Visco-Amylograph.

	Pasting characteristic *			
Storage time (weeks)	**Peak viscosity**	**Viscosity at 95°C**	**Viscosity after 20 minutes at 95°C**	**Viscosity at 50°C**
STARCH SUN-DRIED FOR 8 HOURS:				
0	380/410 [a]	360	280	260
1	390	290	80	60
2	300	240	140	80
3	270	200	90	60
4	250	150	70	40
STARCH OVEN-DRIED FOR 48 HOURS (40-50°C):				
0	450/510 [a]	440	400	270
1	410	380	230	140
2	400	350	170	100
3	380	340	140	100
4	270	170	70	60

* Pasting characteristics: all values for viscosity in Brabender units (Bu)

a Denotes double pasting peak

Table 2. Changes in swelling volume, swelling power, solubility and reducing value of cassava starch during conventional wet storage.*

Storage time (weeks)	Swelling volume ml/g starch	Swelling power	Solubility %	Reducing value
STARCH SUN-DRIED FOR 8 HOURS:				
0	44.7	30.5	16.1	0.87
1	51.3	23.9	22.6	1.93
2	43.4	16.4	26.4	2.20
3	37.8	13.5	37.5	2.27
4	28.8	7.3	47.1	3.33
STARCH OVEN-DRIED FOR 48 HOURS (40-50°C):				
0	50.3	30.9	16.8	0.66
1	55.0	31.3	17.1	1.66
2	52.8	27.5	20.2	1.93
3	52.5	26.5	29.7	2.20
4	31.6	7.8	42.3	3.33

* The values are mean values of four replicate determinations

Table 3. Effect of conventional and chemically acidified wet storage for 6 weeks on the paste viscosity of cassava starch.

Samples (all unwashed)	Paste viscosity in Redwood seconds *	Peak viscosity in Brabender units	Viscosity at 50°C in Brabender units
Fresh starch (week 0)	51	280	260
Conventionally stored starch (week 6)	38	180	40
Chemically acidified starch (2% acetic acid) (week 6)	48	280	160

* - The minimum requirement for IS 1606-1977 is 44 Redwood seconds (measured with a Redwood No 1 Viscometer at 75°C).

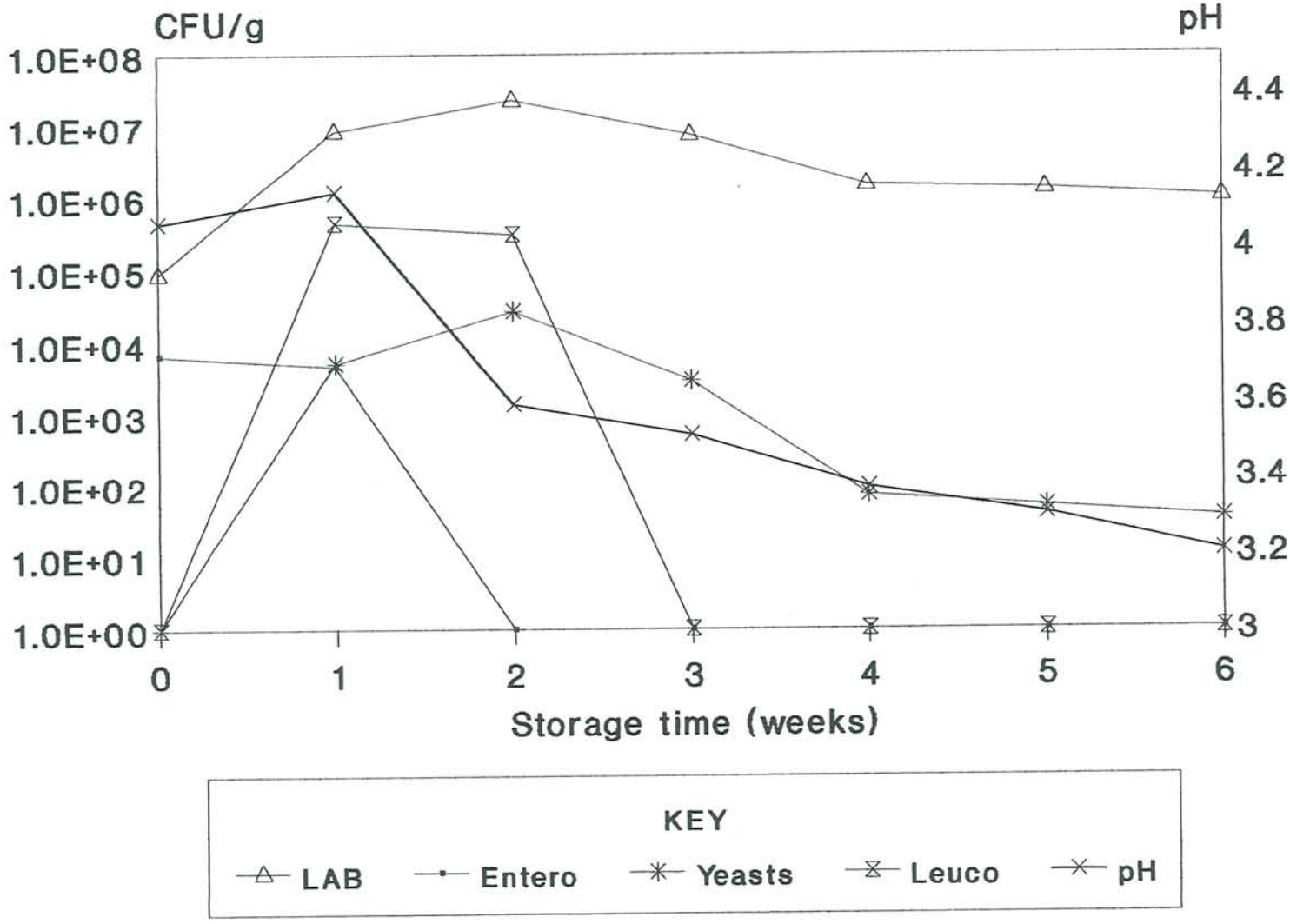
Table 4. Effect of washing on the paste viscosity of conventionally and chemically acidified (2% acetic acid) cassava starch wet stored for 6 weeks.

Sample	Paste viscosity in Redwood seconds	Peak viscosity in Brabender units	Viscosity at 50°C in Brabender units
Conventionally stored starch washed prior to drying	38	180	140
Chemically acidified starch washed prior to drying	58	380	360
Chemically acidified starch washed after drying	48	300	200

Legends to Figures

Figure 1. Changes in the microflora and pH value of cassava starch stored under wet conditions for 6 weeks (△lactic acid bacteria, ■ *Enterobacteriaceae*, ⧖*Leuconostoc* spp.,✱ yeasts and ✕ pH value). All values shown are mean results for triplicate determinations.

Figure 2. Changes in the percentage of lactic acid bacteria (LAB) showing amylolytic activity during conventional and chemically acidified storage of wet cassava starch. All values shown are mean results for triplicate determinations.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1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
4.4
4.2
4
3.8
3.6
3.4
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Storage time (weeks)
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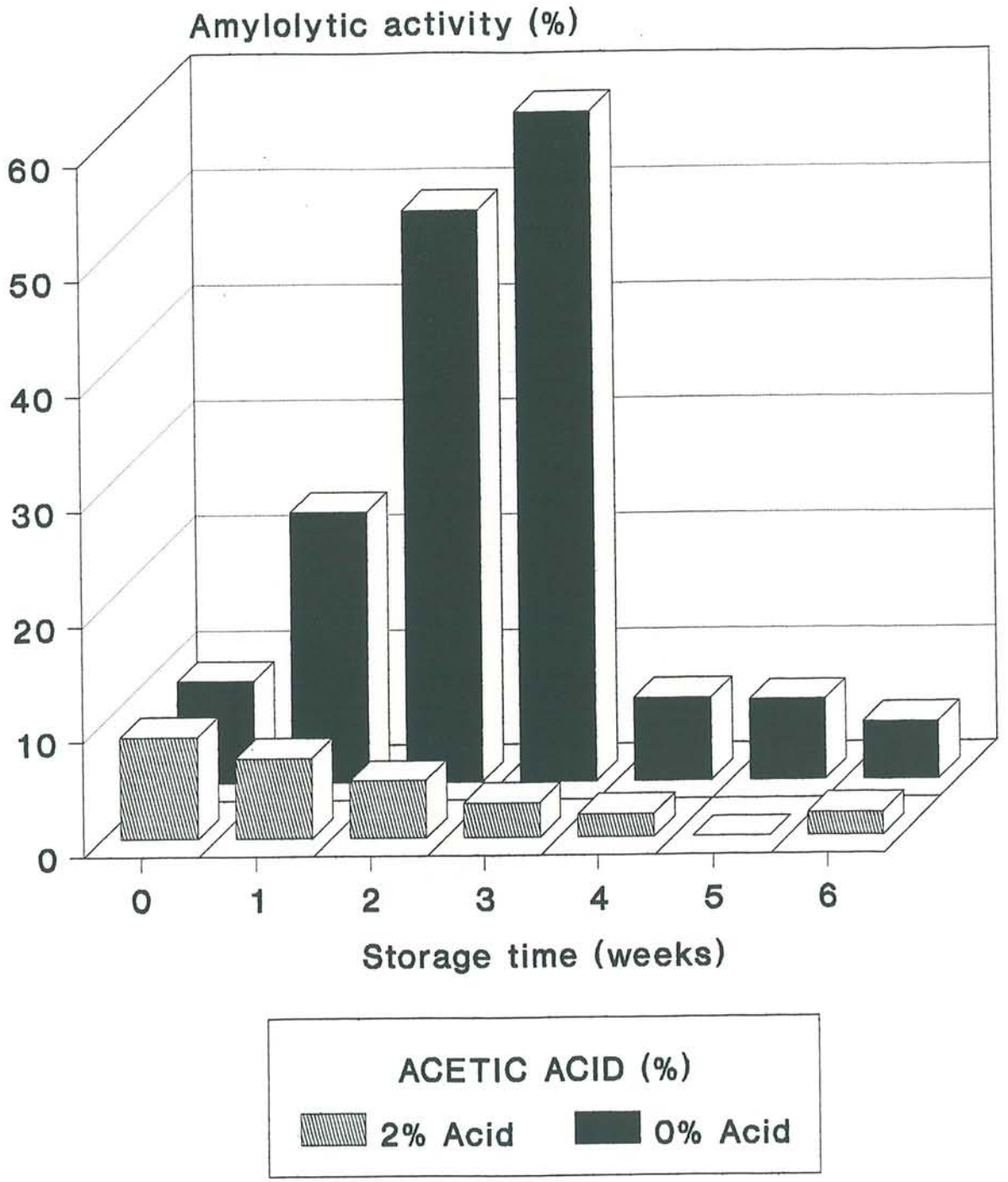

% colonies showing amylolytic activity