

**PRODUCTION OF PLANT PROTEINASE FROM JACK FRUIT  
(ARTOCARPUS INTEGRIFOLIS) AND ITS INFLUENCE  
ON RHEOLOGICAL AND SENSORY CHARACTERISTICS  
OF LOW FAT YOGURT**

[8]

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**ABSTRACT**

Adding a proteolytic enzyme extraction from Jack fruit (*Artocarpus integrifolis*) in combination of fermentation process in yogurts manufacture was tried to improve yogurt flavour and rheological properties. Experimental yogurts milk contained control, 3.9 (T1), 7.8 (T2) and 11.7 (T3) units/ml milk from crude extracts of plant proteinase. The pH of the product treated with plant proteinase was lower than the control. However, The rate of acidity development during storage slightly increased with increasing the addition of plant proteinase level and progress of storage period of yogurt. The proteolytic activity of all yogurts gradually increased until the end of storage period (15 days). Yogurts made from milk treated with plant proteinase preparations were less firm compared with control at all storage periods, where T3 showed more less firm after 15 days of storage being 20.17 g/100g. Generally, increasing units of plant proteinase preparations decreased the firmness. On the other hand, yogurt made from milk pretreated with plant proteinase had higher syneresis, and apparent viscosity than the untreated product. The greatest viscosity was found in T2 and T3 of 433 and 479 mPa.s respectively, compared with control of 299 mPa.s at 15 days storage. The results indicated that there is an inverse relationship between the amount of units of plant proteinase preparations and susceptibility of yogurt to syneresis. The T2 gained the highest scores (85 points) followed by the control (81.5 points) after 15 days of storage, while yogurt of T3 showed a low scoring being 75. From the foregoing results, it is recommend to use Jack fruit (*Artocarpus integrifolis*) as a source of plant proteinases and utilize it to develop a high quality yogurt at a level of 7.8 units of plant proteinases / ml milk.

**Key words:** Proteinase, Jack fruit (*Artocarpus integrifolis*), Yogurt, Curd tension, Syneresis, Viscosity

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## INTRODUCTION

Proteolytic enzymes have been found in both dicotyledonous and monocotyledonous plants. Representative of the former are the fig (*Ficus carica*), papaya (*Carica papaya* L.), milkweed (*Asclepias speciosa*), and euphorbian (*Euphorbia lathuris*); of the latter, the pineapple (*Ananas comosus* L.), Cardosin B (*Cynara cardunculus*) (Goodenough and Owen, 1987; Maksimenko *et al* 1990; Zimacheve *et al* 1994; Silva and Malcata, 1999) and cereals. It appears that there are two major groups of these proteinases, one requiring free SH groups for activity, having their optimum pH at ~7.0 and strong milk-clotting activity; the other group lack active SH groups, have a more alkaline optimum pH, and an inferior milk-clotting power. Both groups of plant proteinases are unusually heat resistant, often maintaining their activity at temperature of 60 to 70°C. Proteinases produced by psychrotrophic bacteria can withstand pasteurization (72°C for 15 s), and treatment at ultrahigh temperatures (138°C for 2 s), with important implications on the quality of milk and heat-treated dairy products (Cousin, 1982). Ismail *et al* (1999) has found in Jack fruit (*Artocarpus integrifolis*) which planted recently several important enzymes for dairy field such as proteinase,  $\beta$ -galactosidase. Also, proteinase can be produced from the seeds of this fruit as a source of an enzyme suitable for use with some dairy products (Al-Tanboly, 2003). Certain of the plant proteinases have a number of food technological and medical usages. Plant proteinases are interesting because they are natural products which can be easily extracted by aqueous infusion, no legal barriers and low cost. A

part from flavour, quality of yoghurt and other fermented milks is largely dependent on its texture (Lankes *et al* 1998 and Savello & Dargan 1995, 1997). Texture is defined as a group of physical properties of food influenced by its structural elements and perceived by human senses. Ultrafiltration of milk for yoghurt production is one of several valuable techniques used to increase milk dry matter content, necessary to obtain compact coagulum and appropriate viscosity of yoghurt (Domagala and Kupiec, 2003). Of special interest, protein hydrolysates by proteinase are mainly used for medical purposes, as medical diets for human breast cancer cells and colon cancer (Parodi 1998). To achieve desirable flavor as well as texture properties in yoghurt, it was tried to produce it from milk enriched by adding suitable proteolytic enzyme besides the action of yogurt starter bacteria. The aim of this study was to determine the influence of a novel plant proteinase from Jack fruit (*Artocarpus integrifolis*) on fermentation activity, proteolysis and rheological properties of yogurt.

## MATERIAL AND METHODS

### Jak fruit (*Artocarpus integrifolis*)

Jak fruit was obtained from the Horticulture Institute, Agric. Res. Centre, Ministry of Agriculture, Cairo, Egypt.

### Extraction of enzyme

For maximum extraction of enzyme it was prepared according to the method described by (Al-Tanboly, 2003).

### Yogurt culture

Combined yogurt culture, *Streptococcus salivarius subsp. thermophilus* (EMCC 1225) and *Lactobacillus delbrueckii subsp. bulgaricus* (EMCC 1322) were obtained from the Egyptian Microbial Culture Collection of Cairo MIRCEN (EMCC), Faculty of Agriculture, Ain-Shams University, Egypt.

### Yogurt culture preparation

The culture was prepared by inoculated at 4% into sterile 11.5% reconstituted non-fat dry milk. It was subcultured at least twice for 8 hrs at 42°C for uniform coagulation, then stored overnight at 4 +1°C, at which time the yogurt to be prepared.

### Yogurt preparation

Low fat yogurt making steps were mainly carried out according to Tamime and Robinson (1985). Yogurt mix consisted of 11.5% reconstituted non-fat dry milk with 2% added culture and incubation temperature at 42°C for 3 hrs. Before addition of culture, levels of crude extracts of plant proteinase of 3.9 (T1), 7.8 (T2) and 11.7 (T3) units of plant proteinase /ml milk, or no enzyme (control) were added. The resulting yogurt was kept in the refrigerator at 4 +1°C when their pH was between 4.8-4.9 and was analyzed at 1, 3, 7 and 15 days. All treatments were carried out in three replicates.

### Chemical analysis

In Yogurt samples, pH was measured by pH - meter 646 with glass electrodes, Ingold, Knick, Germany. Titratable acidity (°SH) was done with Soxhlet

Henkel method as described by Scott (1981). The proteolytic activity (PA) was measured according to (Otani *et al* 1991) using tyrosine as standard as suggested by (Greenberg, 1957).

### Rheological properties analysis

#### The curd tension (firmness)

Curd tension of yogurt was estimated as described by Chandrasekhare *et al* (1957). The apparatus used consists of knives of constant weight (5g) H-shaped with needle in the middle ending with a hook, and a thread crossing a freely rotating wheel attached to the knife at one end, and a pan (5g) at other. The knife was placed in a 100 ml cup yoghurt mixture inoculated with the yoghurt starter and incubated at 42 +1°C until set. The curd tension was measured as weight in grams to remove the knife from the yoghurt.

#### Susceptibility to syneresis

Syneresis (whey separation) was determined by using the drainage whey (ml/100 ml) yogurt as described by Hassan *et al* (1999). The amount of free whey collected after 30 min at room temperature (23 +1°C) was taken as the index of susceptibility to syneresis.

#### Viscosity

Viscosity was measurement using Hoeppler viscometer at 20°C according to Żbikowska and Żbikowski, (1996).

#### Sensory evaluation

The yogurt were evaluated organoleptically by a team of experienced yogurt graders from staff members of N.R.C. The yogurt were calculated from

three characteristics gave a maximum total of 100 points. The appearance was awarded a maximum of 10 points, consistency was awarded a maximum of 30 points and flavor 60 points.

## RESULTS AND DISCUSSION

### Titratable acidity and pH of yogurt during storage

Figures 1 and 2 shows the changes in pH and titratable acidity of yogurt during storage. One day after manufacture, the mean pH of the yogurts ranged from 4.7 to 4.65 with no differences effect of treatments. Yogurt pH dropped to a range of 3.7-3.81 after 15 days of storage of yogurt with different proteinase treatments. When data from all sampling periods was combined for each treatment, the mean pH of the product treated with plant proteinase was lower than the control. The greater proteolysis that occurred in this product may have stimulated acid production during storage. However, The acidity range was 1.11-1.26% after 15 days of storage of the proteinase yogurt treatments. This result similar to that observed by Cousin (1982) and Farrag (2002).

### Proteolysis of yogurt during storage

Data for Proteolytic activity measured as liberated tyrosine/ml/min of the reaction mixture show continuation of proteolysis of different proteinase yogurt treatments during storage (Fig. 3). The proteolytic activity of all yogurt treatments slightly increased until the end of the storage period (15 days). Several proteolytic activity values of yogurt treatments were higher as compared to those of the control. Yogurt treatment

(T3) continued to exhibit a higher rate of proteolysis formation than other treatments (T1 and T2). This result is in agreement with at of El-Tanholy *et al* (2001) who reported that yoghurt fortified with increasing level of fortification to 15% protein hydrolysates from different sources (rice bran, soybean, sesame) showed greater positive effect on proteolytic activity than control.

### Rheological properties analysis of yogurt during storage

#### The curd tension (firmness)

The curd tension (firmness) of plant proteinase yogurt treatments is shown in Figure (4). Yogurts made from milk treated with plant proteinase preparations were less firm compared with control at all storage periods. While T3 showed 20.17 g it became more less firm after 15 days of storage. Generally, increasing units of plant proteinase preparations decreased the firmness. These results in agreement with those reported by Gassem and Frank (1991) and Domagala and Kupiec (2003).

#### Susceptibility to syneresis

Syneresis (whey separation) in yogurt is an important parameter, on which the quality of yogurt is evaluated. The results in Fig. 5 reveal that there is an inverse relationship between the amount of units of plant proteinase preparations and susceptibility of yogurt to syneresis. The greatest syneresis was found in T3 at 1 and 3 days of storage being of 34.90 and 33.50 ml/100ml respectively, compared with control being 25.10 and 24.80 ml/100ml in the same order. The lowest

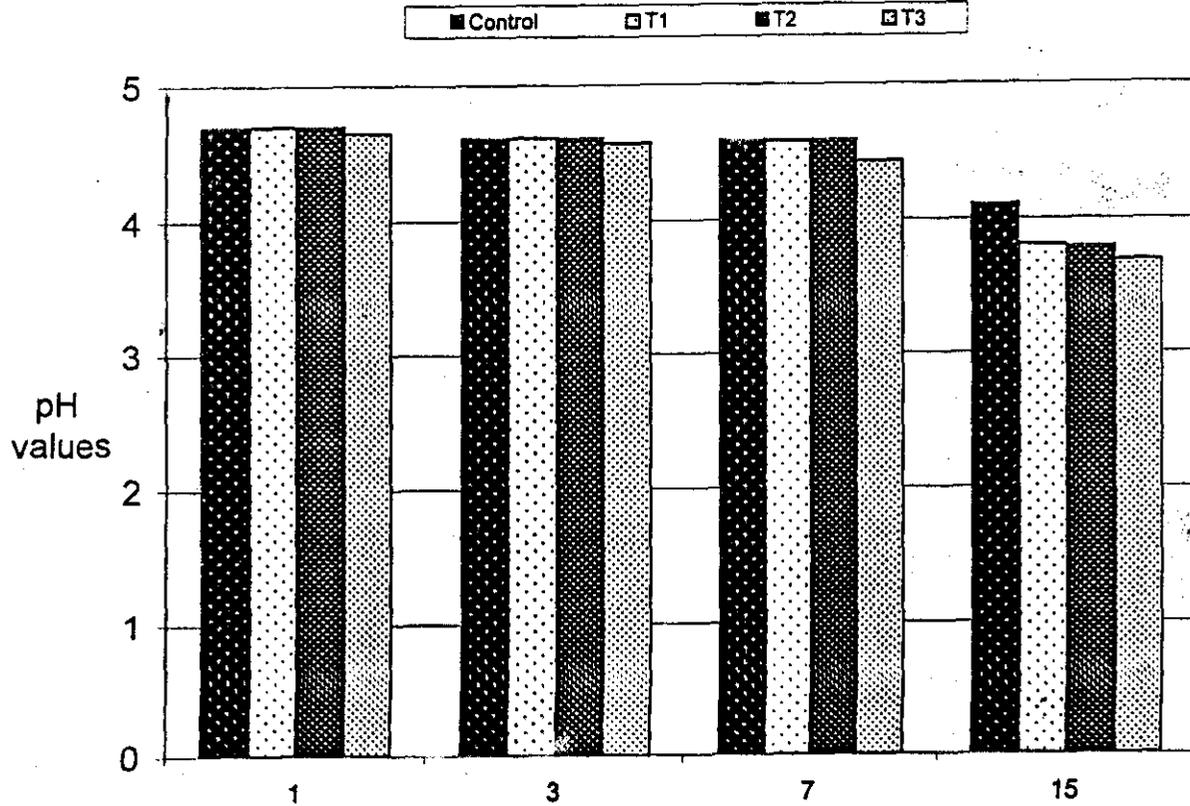


Fig. 1. Effect of different plant proteinase levels of yogurt on changes in pH during storage

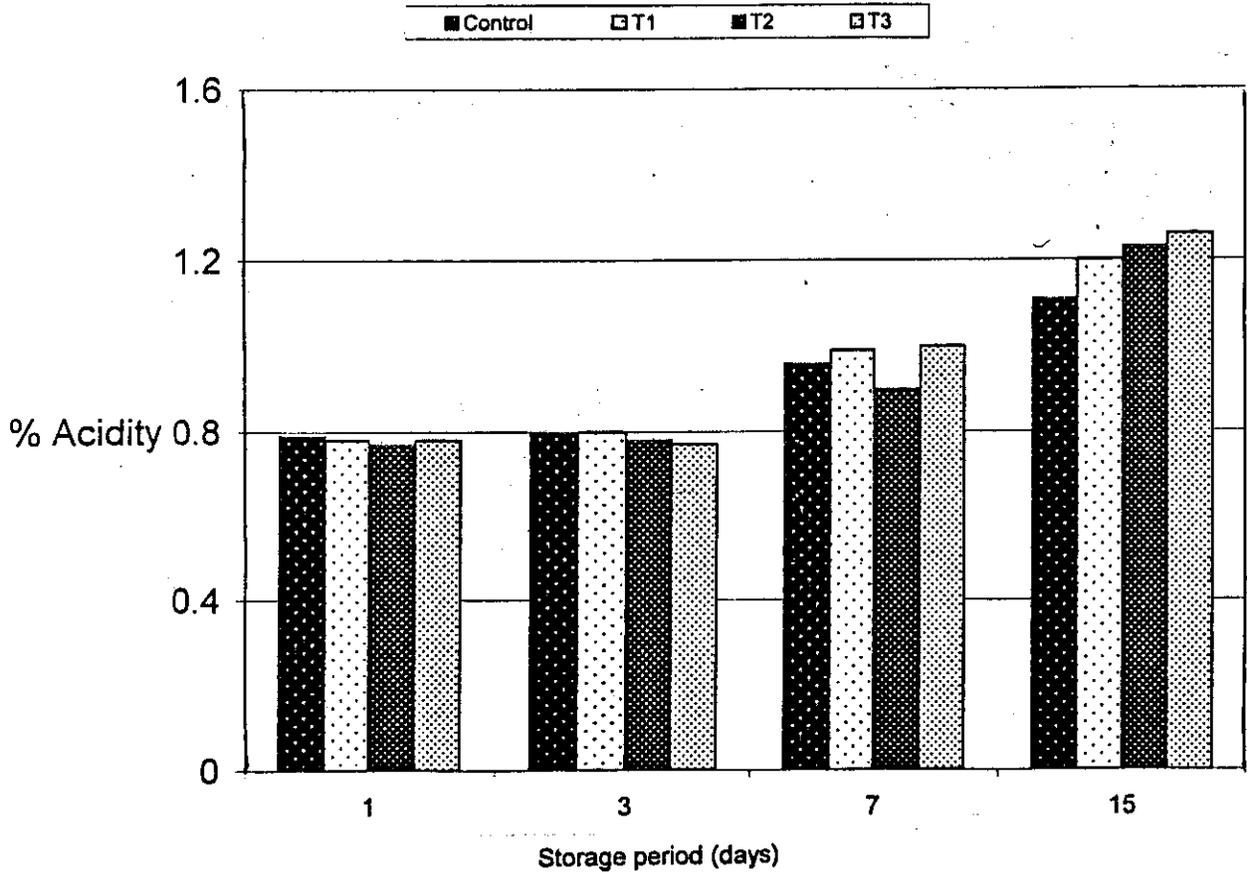


Fig. 2. Effect of different plant proteinase levels of yogurt on changes in acidity during storage

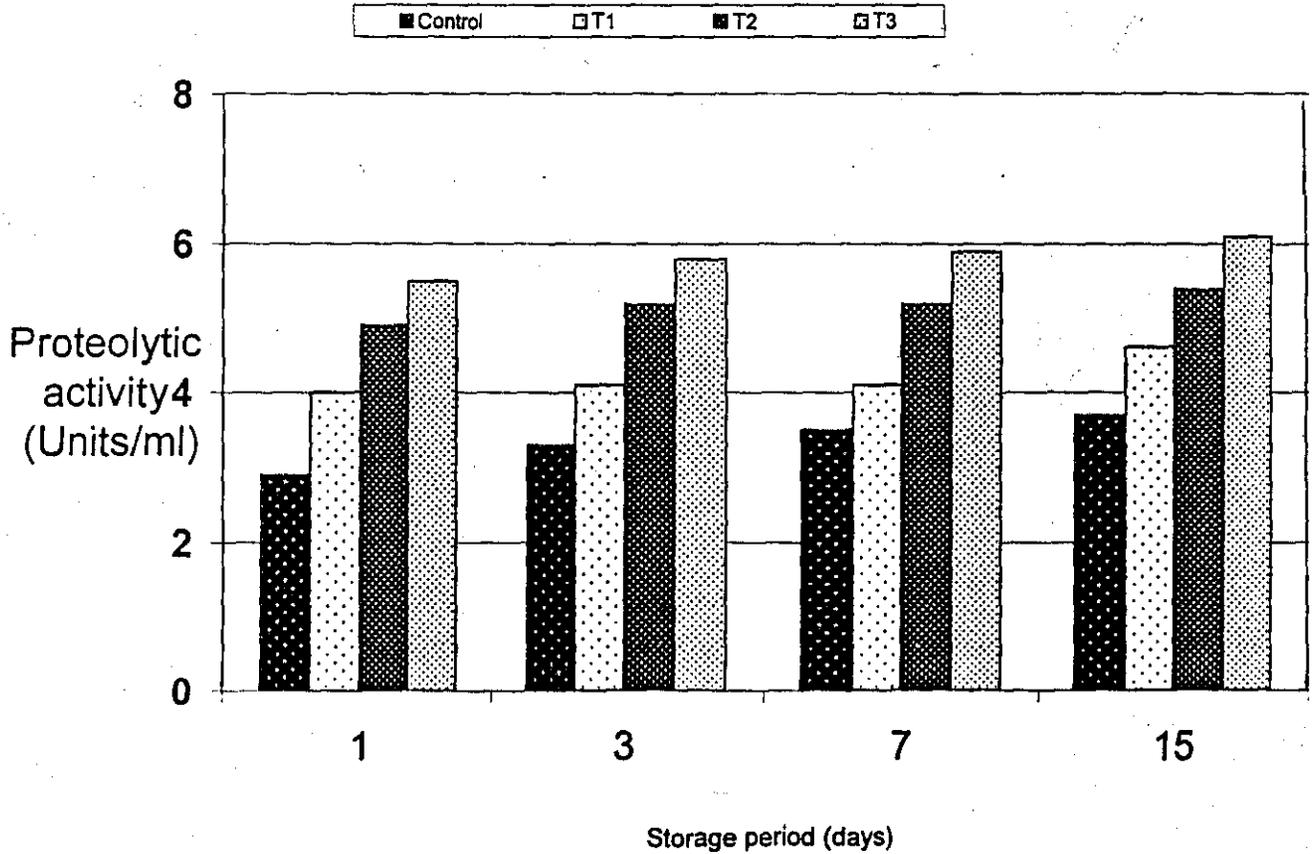


Fig. 3. Effect of different plant proteinase levels of yogurt on changes in proteolysis during storage

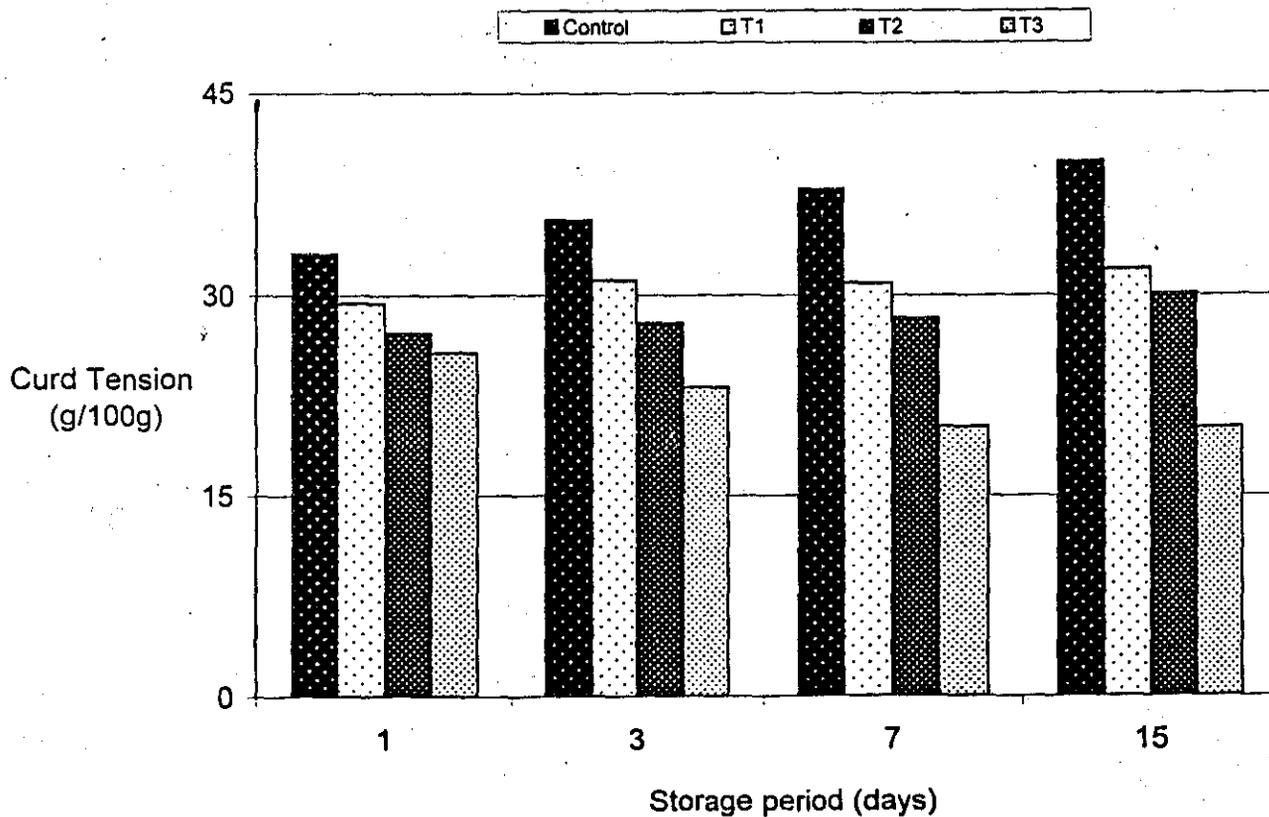


Fig. 4. Effect of different plant proteinase levels of yogurt on changes in curd tension (g/100 g) during storage

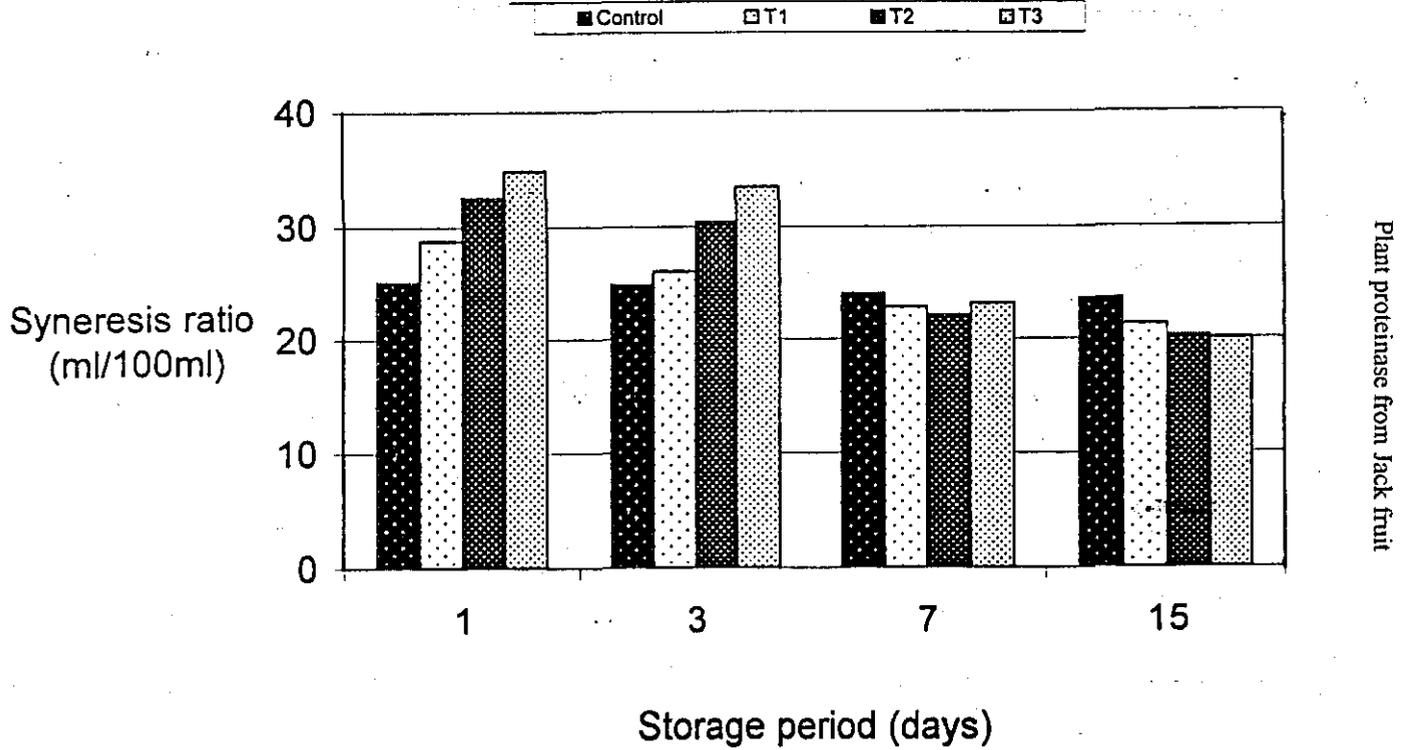


Fig. 5. Effect of different plant proteinase levels of yogurt on changes in syneresis ratio (ml/100) during storage

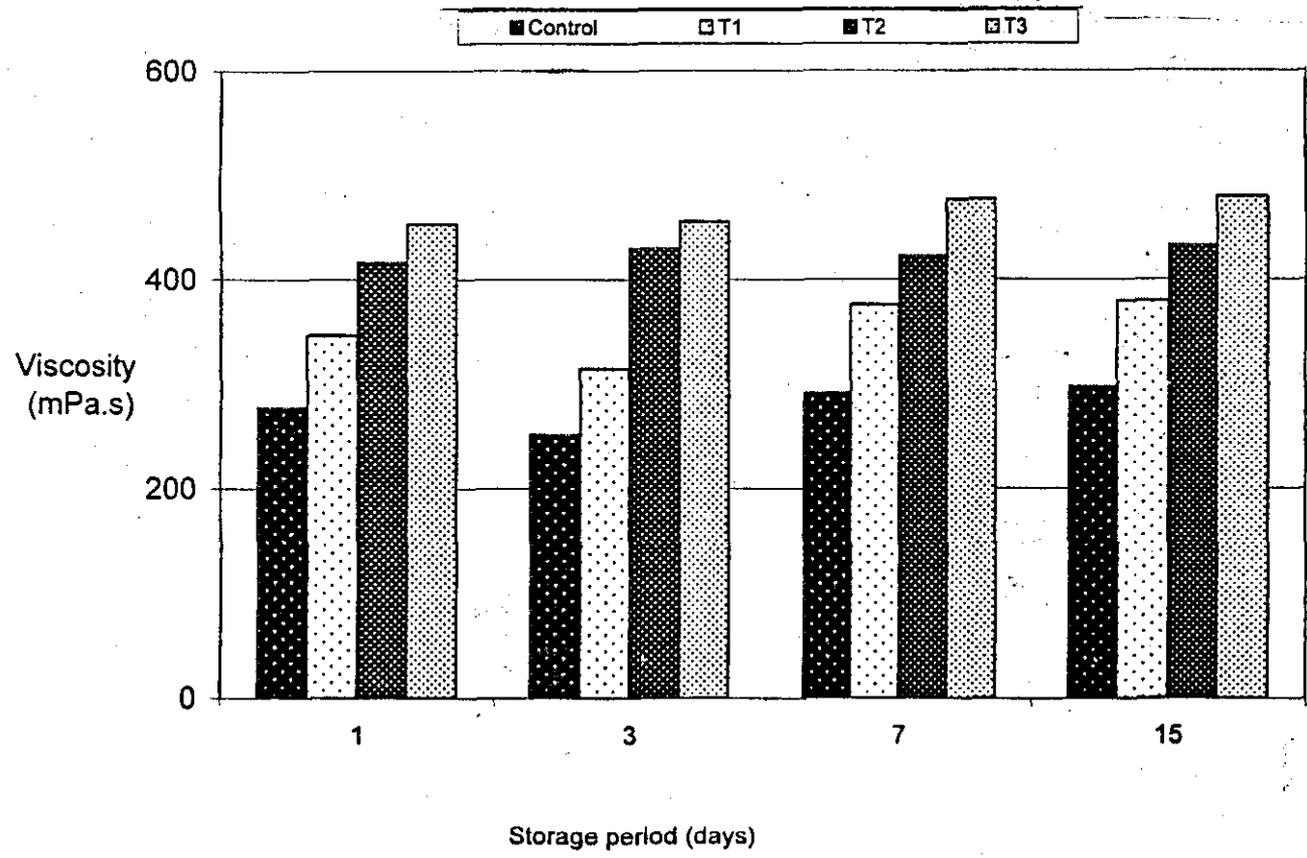


Fig. 6. Effect of different plant proteinase levels of yogurt on changes in viscosity (mPa.s) during storage

Table 1. Effect of different plant proteinase levels on scores of yogurt during storage

Storage Period (days)	Treatment	Appearance (10)	Flavor (60)	Consistency (30)	Total (100)
1	Control	10	57	28	95.0
	T1*	10	57	28	95.0
	T2**	10	58	28	96.0
	T3***	10	54	28	92.0
3	Control	9.0	55	27	91.0
	T1	9.0	52	27	88.0
	T2	9.5	57	27.5	94.0
	T3	9.0	50	26	85.0
7	Control	8.5	51	26	85.5
	T1	8.5	49	26	83.5
	T2	8.0	55	27.5	90.5
	T3	8.0	47	25	80.0
15	Control	8.5	48	25	81.5
	T1	8.0	46	24	78.0
	T2	8.5	51	25.5	85.0
	T3	8.0	45	22	75.0

Control: yogurt no enzyme; T1\*: yogurt added with plant proteinase extracts (3.9 units / ml); T2\*\* yogurt added with plant proteinase extracts (7.8 units / ml); T3\*\*\*: yogurt added with plant proteinase extracts (11.7 units / ml).

syneresis was found in T2 and T3 after 7 and 15 days storage of (20.00, 20.25) and (23.10, 20.0) ml/100 ml respectively. These findings agree with that report by Chawala and Balachandran, 1993.

### Viscosity

Figure 6 shows the effect of added plant proteinase on the viscosity (mPa.s) after 1, 3, 7 and 15 days of yogurt storage. Yogurts made from milk treated with plant proteinase preparations had greater viscosity than the control. The viscosity values were 279, 347, 417 and 453 mPa.s at the first day samples which increased after 15 day storage to 299, 380, 433 and 479 mPa.s for control, T1, T2 and T3 respectively. Moreover, the greatest viscosity was found in T2 and T3 of 433 and 479 mPa.s respectively, compared with control of 299 mPa.s at 15 days storage. The development of acidity may contribute to this increase (Farrag, 2002). These results are similar to those reported by Gassem and Frank, (1991) and Domagala and Kupiec, (2003).

### Sensory evaluation

Table (1) shows the organoleptic evaluation of plant proteinase yogurt treatments during 15 days storage periods. Yogurt treatments showed no marked difference in appearance. Consistency scores for all treatments decreased during storages up to 15 days. With regard to flavour, it was gradually decreased in all yogurt treatments. The T2 gained the highest score at the end of storage period (15 days) being 85 point followed by the control 81.5 point. On the other hand T3 showed the lowest score point being 75. These results had the same trend as El-Tanboly *et al* (2001).

### Conclusion

From the foregoing results, it could be recommended to use using Jack fruit (*Artocarpus integrifolius*) as a source of plant proteinases in order to improve yogurt quality at a level of 7.8 units of plant proteinases /ml milk.

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مجلة حويلات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، ٤٩م ، ع(١) ، ١٠٩ - ١٢٣ ، ٢٠٠٤  
**إنتاج إنزيم البروتينيز النباتي من نبات الجاك فروت وتأثير استخدامه على  
 الصفات الريولوجية والحسية لليوجهورت منخفض الدهن**

[٨]

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الخشرة ومعدل طرد الشرش واللزوجة هذا فضلا عن التحكم الحسي على اليوجهورت خلال فترة التخزين من ١ إلى ١٥ يوم ثم مقارنة النتائج بالعينة الخالية من الإنزيم (الكنترول).

وأوضحت النتائج أنه لا توجد فروق جوهرية فى قيم الـ pH بين اليوجهورت المعامل بإنزيم البروتينيز النباتي مقارنة بالكنترول خلال فترة تخزين ١-٧ يوم ولكن عند ١٥ يوما من التخزين انخفض الـ pH من ٨١ و ٣ الى ٧٠ و ٣ كما لوحظ أن قيمة الـ pH اليوجهورت المعامل كانت أقل من قيمة pH للكنترول ومن الناحية الأخرى بالنسبة للحموضة ودرجة نشاط تحلل البروتين كانت هناك زيادة تدريجية طفيفة بنهاية فترة التخزين لليوجهورت المعامل وأظهرت المعاملة الثالثة (٧ و ١١ وحدة أنزيمية / ١ مل من لبن اليوجهورت منخفض الدهن) أعلا نشاطا لتحلل البروتين عن المعاملات الأخرى.

وبالنسبة للصفات الريولوجية فقد أوضحت النتائج أن معدل طرد الشرش فى

فى هذا البحث تم استخدام إنزيم البروتينيز النباتي المستخلص من نبات "الجاك فروت" ودراسة تأثيره على بعض الصفات خاصة الريولوجية والحسية لليوجهورت منخفض الدهن بعد أن تم عزله ودراسة صفاته سابقا فى الجزء الاول من الدراسة وذلك لما له من أهمية تكنولوجية فى صناعة منتجات الألبان حيث تم تحضير عدة معاملات من اليوجهورت منخفض الدهن مع مستخلص الإنزيم.

- ١- كنترول للمقارنة .
- ٢- المعاملة الأولى ( ٣ و ٩ وحدة أنزيمية / ١ مل من لبن اليوجهورت منخفض الدهن).
- ٣- المعاملة الثانية ( ٧ و ٨ وحدة أنزيمية / ١ مل من لبن اليوجهورت منخفض الدهن).
- ٤- المعاملة الثالثة ( ٧ و ١١ وحدة أنزيمية / ١ مل من لبن اليوجهورت منخفض الدهن).

وأجريت بعض التحاليل الكيمائية لدراسة التركيب الكيمائي لليوجهورت منخفض الدهن وكذلك تتبع التحلل البروتيني ودراسة الصفات الريولوجية من قوة تماسك

وخلصت النتائج أيضا إلى زيادة لزوجة اليوجهورت المعامل بانزيم البروتينيز النباتي مقارنة بالكنترول وثباتها خلال فترة التخزين. هذا ولقد حصلت المعاملة الثانية على أعلى درجات التحكيم فى العينات الطازجة وخلال مراحل التخزين.

مما سبق يتضح أنه يمكن إنتاج يوجهورت منخفض الدهن ذو جودة عالية من الصفات الريولوجية والحسية بإضافة (٧ و٨) وحدة أنزيمية / ١ مل من لبن اليوجهورت منخفض الدهن) من أنزيم البروتينيز النباتي المستخلص من نبات "الجاك فروت".

يوجهورت المعاملة الثالثة كان أعلى قيمة (٣٤ و٣٥-٣٣ مل/١٠٠ مل يوجهورت) من الكنترول (٢٥ و٢٤-٢٤ مل/١٠٠ مل يوجهورت) خلال فترة تخزين ١-٣ يوم فى حين أن يوجهورت المعاملة الثانية والثالثة كانت الأقل قيمة (٢٠ و٢٥ و٢٠) و(٢٠ و٢٣-٢٠) مل/١٠٠ مل يوجهورت بعد فترة تخزين ٧-١٥ يوم. وأظهرت النتائج أيضا أن معاملات اليوجهورت الأولى والثانية كانتا أقل قيمة فى درجة الصلابة من يوجهورت الكنترول بعد فترة تخزين ١-٧ يوم فى حين أن يوجهورت المعاملة الثالثة كان الأقل قيمة بعد فترة ١٥ يوما من التخزين.

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