

## **PRODUCTION OF NATURAL CLOUDING AGENT FROM LIME PEEL**

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### **ABSTRACT**

This study was planned to produce natural clouding agent extracts and concentrates from lime peel by enzymatic treatments.

Results indicate that clouding agent extracts and concentrates treated with enzymes were higher in total solids, total soluble solids, total sugars, total acidity, total soluble pectin and free amino nitrogen compared to the untreated ones. Viscosity of the untreated extracts and concentrates were higher compared to the enzymatic treated ones.

Cloud values of drinks prepared from the aforementioned extracts and /or concentrates produced by enzymatic treatments were lower compared to the untreated samples. On the other hand, drinks prepared by adding 15% and 2% of these extracts and concentrates recorded lower cloud values than the other concentrations.

Cloud stability of drinks produced by clouding agent extracts and concentrates and stored for 30 days at room temperature indicated that, after five days a slight decrease in cloud stability occurred then became stable. Moreover, clouding agents extracts and concentrates produced by enzymatic treatments (Pectinex Ultra SPI or Citrozym Cloudy 100 L enzyme) were more stable than that of the untreated samples.

Organoleptic evaluation of lime drinks prepared by adding 15% and 2% of clouding agent extracts and concentrates produced by Citrozym Cloudy 100 L enzyme were the best concerning the overall acceptability than the other treatments.

Accordingly, it can be recommended that natural clouding agent extracts and concentrates produced from lime peel could successfully be used to produce drinks by adding 15% and 2% of clouding agent extracts and concentrates produced by enzymatic treatments.

### **INTRODUCTION**

Since the use of brominated oils and artificial cloudifiers in citrus beverages had been prohibited in several countries, the demand for suitable, natural cloudifiers processed from citrus raw materials increased. The enzyme technology had proved to be superior than the purely mechanical process. Selected enzyme preparations made it possible to extract more cloud from the peel and reduce the viscosity prior to concentration (Janser, 1995). All freshly extracted fruit juices, particularly those from citrus, are naturally turbid due to the suspension of cellular organelles and cell wall fragments (Baker, 1980). Therefore consumers expect fruit- based or fruit-flavored drinks to be cloudy.

Natural or citrus blends were used (0.06 to 0.26%) as clouding agents in bottled and canned drinks (Braddock and Kesterson, (1974). These clouding agents had improved flavour and shelf-life, but the intensity of cloud will be limited. Douglas, 1972 Patented a process to prepare a cloud fortified

citrus fruit juice by the addition of finely divided albedo to juice. Brominated vegetable oils had been successfully employed as clouding agents since the 1940s (Tateo, 1977).

A beverage clouding agent for use in place of brominated oils or can sugar fatty acids ester was obtained from the extraction of orange pulp by Shionogi Co. Ltd as a Patent (Japanese Patent, 1973). (Felton and Kapp, 1976) described a clouding agent based on combinations of essential oils, vegetable oil and natural and synthetic flavour oils with edible natural oil soluble gum resins. Cloud of orange emulsion was 70% of the original; but ultrasonic treatment of concentrate increased this to about 80%. A method for making a clouding agent from the waste water from pectin pomace production was described by (Tateo, 1977; Braddock and Crandall, 1978). Potential use of waste by- products from juice extraction was discussed by (Crandall *et al.*, 1983). Citrus fruit by- products and wastes contain large amounts of colouring material and are potentially useful as clouding agents in soft drinks. Waste of Valencia orange and grapefruit peel emulsions extracted from citrus oil recovery units were evaluated by (Bryan *et al.*, 1973) as potentially valuable clouding agents for beverage concentrates.

(Villadsen, 1968) and (Larsen, 1969) described the preparation of such clouding agents from the albedo of citrus by the action of pectolytic enzymes. (Braddock and Kesterson, 1979) studied some properties of clouding agents manufactured by enzymatic treatment of orange and grapefruit peel. There was a significant difference in yields as orange peel gave approximately three times. (Herrera *et al.*, 1979) used a commercial pectinase to hydrolyse pectin in the leach water of orange. (Abd El- Fadeel, 1990) produced a natural clouding agent from the peel of Baladi orange by two methods i.e. using enzyme treatment or heating under pressure. (El-Shamei and El-Zoghbi, 1994) produced two beverage clouding agents from peels of Valencia orange and of Baladi lemon. Three enzyme preparations were used under their estimated optimum conditions to hydrolyse pectin of the peel . (Abd El-Latif, 1995) studied appropriate technical method to produce a natural clouding agent from orange peel. Three methods namely enzyme treatment, heating and drying, were tried. Finally, (Janser, 1995) studied Citrozym CEO enzyme to improve citrus oil recovery. This study aimed to produce natural clouding agent extracts and concentrates from lime peel by enzymatic treatments. It was also extended to study physical and chemical properties of clouding agent extracts and concentrates, producing natural drinks from these materials, studying some physical properties of drinks produced therefrom, and also to evaluate the organoleptic qualities of these products.

## **MATERIALS AND METHODS**

Lime peel was used to prepare the clouding agent. Nine commercial enzyme preparations, Citrozym Cloudy 100L, Pectinex Ultra SPI, Citrozym CEO, Cellubrix L, Pectinex AFP-L (Novo Nordisk Ferment Ltd Dittingen, Switzerland), Ultrazyme 100G (Schweizerische Ferment AG, Basel, Schweiz), Rohament P, Rohament K (Röhm GmbH, Darmstadt, Germany) and Maxazyme GOL (Gist Brocades, Delft, Holland) were used.

**1-Processing methods:**

- Preparation of natural clouding agent extracts and concentrates from lime peel are shown in Fig. (1).
- Extraction of lime oils using Citrozym CEO enzyme:  
 Flavedo layer was taken from lime peel during producing natural clouding agent extracts and concentrates to obtain essential oil. This essential oil was used as a natural flavouring and coloring agent to produce beverages using natural clouding agent instead of the artificial ones. Citrozyme ECO enzyme was used to improve the extraction of essential oil from lime peel. The yield of essential oil was increased to the range of 5-15% (Fig.2).
- Preparation of lime drinks from lime peel clouding agent extracts and concentrates is shown in Table (1 and 2).

**2-Analytical methods:**

Moisture content, total solids, total soluble solids, total titratable acidity, pH value and free amino nitrogen were determined according to A.O.A.C. (1995). Ascorbic acid content was determined according to the method described by the Association of Vitamin Chemists (1947).

Total sugars content, reducing and non-reducing sugars were assayed according to Bernfeld (1995) and Miller (1959) methods. The viscosity of clouding agent extracts and concentrates were measured using Brookfield viscometer Model DV-III Rheometer. Anhydrogalacturonic acid content was determined according to Ahmed and Labavitch (1977). Color index was measured according to Ranganna (1977) using spectrophotometer, (Beckman) DU 7400 at O.D. 420nm. Browning index was determined by the method of Meydav et al., (1977). Cloud values were measured using Beckman DU7400 spectrophotometer at 660 nm as mentioned by Senn *et al.*, (1955).

Cloud stability assay:

Twenty ml from each drink were kept into two calibrated covered test tubes. The two tubes were allowed to stand undisturbed at 25°C for 30 days. The height of the cloudy phase during sedimentation was recorded daily in the first week and every two days thereafter. The degree of stability was measured from the following equations:

$$\text{Degree of stability} = \frac{\text{Hight of cloudy phase}}{\text{Total hight}} \times 100$$

Organoleptic evaluation: The organoleptic evaluation of drinks produced from lime peel clouding agent extracts and concentrates were carried out as described by Notter *et al.*, (1959). Color, flavor, apperance and overall acceptability were evaluated using ten trained persons from Food Technology Research Institute. The quality attributes were scored on a scale from 1 to 10.

Statistical analysis: The organoleptic data were statistically analyzed using the ANOVA procedure of the SPSS statistical package for the IBM computer (SPSS, 1987).

**Table1**

**Table2**

**Fig. (1): Preparation of natural clouding agent extracts and concentrates from lime peel.**

**Fig. (2): Extraction of lime oil using Citrozym CEO enzyme.**

## RESULTS AND DISCUSSION

### 1-Physical and chemical properties of natural clouding agent extracts produced from lime peel:

Results in Table (3) represent physical and chemical properties of clouding agent extracts produced from untreated lime peel and that produced by enzymatic treatments (Pectinex Ultra SPL and Citrozym Cloudy 100L enzymes). It could be noticed that by adding such enzymes to lime peel extracts, the values of total solids, total soluble solids, total sugars and reducing sugars were increased compared to the untreated samples. This increase could be due to the action of the previous enzymes on the cell wall of lime peel. These results are in agreement with that obtained by (Voragen *et al.*, 1983; Sreenath *et al.*, 1987; and Abd El-Latif, 1995).

**Table (3): Physical and chemical properties of natural clouding agent extracts produced from lime peel.**

Properties	Untreated sample (control)	Enzymatic treatments	
		Pectinex Ultra SPL	Citrozym Cloudy 100L.
Moisture %	96.31	95.92	95.88
Total solids %	3.69	4.08	4.12
Total soluble solids %	2.60	3.30	3.80
Total titratable acidity % (as citric acid)	0.634	0.773	0.811
pH value	3.42	3.38	3.28
Free amino nitrogen (mg/100ml)	40.19	44.35	41.58
Total sugars %	1.00	1.15	1.25
Reducing sugars %	0.42	0.69	0.72
Non-reducing sugars %	0.58	0.46	0.53
Total soluble pectin (AGA %)*	0.975	1.384	1.436
Ascorbic acid (mg/100ml)	0.064	0.064	0.064
Color index (O.D at 420nm)	0.129	0.104	0.105
Browning index (O.D at 420nm)	0.170	0.217	0.195
Viscosity (centipoise)	161.10	11.95	9.00

\*Calculated as anhydrogalacturonic acid %

The same table showed an increase in total acidity of the treated samples. The opinion of (Rombouts and Pilnik, 1980) could explain this increase. They indicated that pectin esterase of the added enzyme degraded pectins causing deesterification of methoxyl esters.

The pH values of clouding agent extracts produced by using Pectinex Ultra SPL and Citrozym Cloudy 100L enzymes were decreased compared to the untreated extracts as a result of increasing in total acidity. High values of total soluble solids, total acidity and reducing sugars of lime peel extract were recorded by using Citrozyme Cloudy 100L enzyme treatment.

Table (3) show that clouding agent extracts prepared by enzymatic treatments recorded slight increase in free amino nitrogen contents than that of untreated extracts. This increase may be due to hydrolysis of the protein components of peel cell wall as a result of proteolytic enzymes because commercial pectinases contain high activities of pectic enzymes such as PE, PG and PL in addition to the side activities of hemicellulose, cellulases,



proteases, phenolases and other minor activities (Rombouts and Pilnik, 1978).

The same table shows that clouding agent extracts produced by enzymatic treatments had high amount of total sugars compared to the untreated extracts. The same findings were given by (Abd El-Latif, 1995).

Table (3) also indicates that total soluble pectin (AGA) of lime peel clouding agent extracts produced by enzymatic treatments were higher than that of untreated samples. These increases could be due to conversion of insoluble pectin to the soluble form by the pectic enzymes used.

The values of pectic fractions of clouding agent extracts produced by enzymatic treatments were higher than those of the untreated samples (El-Shamei & El-Zoghbi, 1994 and Abd-El-Latif, 1995).

The same table show no differences between ascorbic acid contents in the extracts treated with enzymes and the untreated samples.

Furthermore, table (3) demonstrates that color index of clouding agent extracts produced by enzymatic treatments were lower compared to the untreated samples. Browning index of lime peel clouding agent extracts was higher by using enzymatic treatments than that of the untreated extract. This increase may be attributed to the Maillard reaction and Strecker degradation caused by heating.

While data in table (3) indicate that addition of Pectinex Ultra SPL and Citrozym Cloudy 100L enzymes resulted in a decrease in the viscosity of treated sample. This may be due to the action of the enzymes added in degrading of the high molecular weight of pectic substances compared to lower molecules. This explanation is in agreement with that of (Sreenath *et al.*, 1987).

## **2-Physical and chemical properties of natural clouding agent concentrates produced from lime peel:**

The physical and chemical properties of natural clouding agent concentrates produced from lime peel are given in Table (4). The clouding agent extracts were prepared by treating the peel with Pectinex Ultra SPL and Citrozym Cloudy 100L enzymes to compared to those concentrated under vacuum by rotary evaporator at 40°C.

**Table (4): Physical and chemical properties of natural clouding agent concentrates produced from peel.**

Properties	Untreated sample (control)	Enzymatic treatments	
		Pectinex Ultra SPL	Citrozym Cloudy 100L.
Moisture %	87.11	79.64	79.60
Total solids %	12.89	20.36	20.40
Total soluble solids %	11.30	18.90	18.90
Total titratable acidity % (as citric acid)	2.566	3.295	4.182
PH value	3.32	3.25	3.18
Free amino nitrogen (mg/100gm)	519.75	533.61	526.68
Total sugars %	3.83	5.25	5.06
Reducing sugars %	1.54	2.67	2.61
Non-reducing sugars %	2.29	2.58	2.45
Total soluble pectin (AGA %) *	3.976	8.529	8.235
Ascorbic acid (mg/100gm)	1.77	1.77	1.77
Color index (O.D at 420nm)	0.102	0.096	0.090
Browning index (O.D at 420nm)	0.071	0.105	0.133
Viscosity (CP)	7132	4019	1434

\*Calculated as anhydrogalacturonic acid %

It could be noticed that the clouding agent concentrates produced by enzymatic treatments recorded higher values of total solids, total soluble solids, total and reducing sugars than that of the untreated concentrates. This increase may be due to the enzymes actions on the cell wall of the peels resulting in degrading it into its components showing an increase in the values assayed (Voragen *et al.*, 1983 and Sreenath *et al.*, 1984). The evaporation ratio of the extracts treated with enzymes were higher compared to the untreated samples. These results are in agreement with those of (Abd El-Fadeel, 1990) who found that orange clouding agent concentrates prepared by enzymatic treatments had higher values of total solids, total soluble solids, total acidity and reducing sugars compared to those prepared by heating under pressure.

The previous Table show that total acidity of the clouding agent concentrates treated with enzymatic treatments were higher than that of the untreated ones. This may be due to the enzymatic treatments which produced clouding agents extracts with high values of total acidity.

The pH values of the clouding agent concentrates produced by enzymatic treatments were lower than that of the untreated concentrates as a result of increasing the total acidity. Data in the same tables indicate that the free amino nitrogen of the clouding agent concentrates produced by enzymatic treatments were higher than that of the untreated samples. This increase may be due to hydrolysis of the protein in the clouding extracts as a result of proteolytic enzymes found in commercial pectic enzymes used .

Table (4) show an increase in total soluble pectin of the clouding agent concentrates produced by enzymatic treatments compared to the untreated samples. This increase may be due to the conversion of insoluble pectin into the soluble form by pectic enzymes used and the total soluble solids of the concentrates treated with enzymes were higher than the untreated concentrates. These results are in accordance with those found by (Abd El-Fadeel, 1990).

Furthermore, no difference was recorded in ascorbic acid content of clouding agent concentrates produced by enzymatic treatments (Pectinex Ultra SPL and Citrozym Cloudy 100L enzymes ) as well as in the untreated concentrates.

Otherwise table (4) shows that browning index of the lime concentrates produced by Citrozym Cloudy 100L enzyme was higher than other concentrates of lime.

The increase in browning index may be due to Maillard reaction.

Table (4) show that viscosity of untreated lime peel clouding agent concentrates were higher than that of concentrates produced by enzymatic treatments. This could be due to the action of these enzymes causing degradation of high molecular pectic substances into lower molecules. These results are in agreement with those of (Sreenath *et al.*, 1987 and El- Shamei & El- Zoghbi, 1994).

### **3-Cloud values of lime drinks prepared from lime peel clouding agent extracts and concentrates:**

Figures (3 and 4) show that adding 15% of untreated lime peel clouding agent extracts, treated with pectinex Ultra SPL and Citrozym Cloudy 100L enzymes to lime drinks recorded lower cloud values (56.20, 47.08, and 46.20), respectively.. Otherwise adding 2% of these concentrates produced by the previous treatments to lime drinks recorded lower cloud values (52.03, 47.67 and 48.57), respectively.

### **4-Cloud stability of lime drinks produced by clouding agent extracts and concentrates:**

#### **4-1-Cloud stability of lime drinks produced by lime peel clouding agent extracts:**

The cloud stability of the prepared lime drinks as influenced by storing at room temperature for 30 days, was investigated and the results obtained are shown in table (5). It could be noticed that cloud produced by enzymatic treatments then added to the drinks were more stable than that of untreated samples. After 5 days there was a little change in cloud stability then cloud stability did not change until 30 days.

#### **4.2- Cloud stability of lime drinks produced by lime peel clouding agent concentrates :**

Data in Table (6) show the cloud stability of lime drinks produced by adding lime peel clouding agent concentrates then stored at room temperature for 30 days. The results indicate that lime peel clouding agent concentrates produced by Pectinex Ultra SPL enzyme was more stable in cloud stability compared to the same produced by Citrozym Cloudy 100L enzyme. Thus clouding agent concentrates produced by enzymatic treatments were more stable than that of the untreated concentrate. Fig3-4

Fig3-4

Table5

Table6

#### **5- Organoleptic evaluation of drinks prepared from lime peel clouding agent extracts and concentrates:**

Table (7) shows that lime drinks prepared by lime peel clouding agent extract treated with enzymes gained the highest scores for all sensory attributes than the untreated and the extracts treated with Citrozym Cloudy .100 L enzyme which recorded slight increase than that of Pectinex Ultra SPL enzyme.

Otherwise, lime drinks prepared by adding 15% of clouding agent extracts obtained the highest scores for all the judged parameters followed by lime drinks containing 10% and 5%. These results were statistically analyzed and showed that lime drinks prepared by adding extracts treated with Pectinex Ultra SPL and Citrozym Cloudy 100 L enzymes obtained the highest score with highly significant difference than the untreated in color, appearance and overall acceptability. Besides, no significant differences could be noticed between the two enzymes used. While, no significant differences were observed in flavour between clouding agent extracts treated with enzymes and untreated. On the other hand, lime drinks prepared by adding of 15% clouding agent extract obtained the highest score with highly significant differences being found between 15% and 10% of clouding agent extract in overall acceptability. Also, an interaction occurred between enzymatic treatments and concentrations in appearance of lime peel clouding agent extract with highly significant difference.

Organoleptic evaluation of lime drinks prepared from lime peel clouding agent concentrate . Table (8) indicates that drinks prepared by lime peel clouding agent concentrate produced by Citrozym Cloudy 100 L enzyme obtained the highest scores for all the judged parameters followed by the treatment with Pectinex Ultra SPL enzyme, while, the untreated clouding agent concentrate recorded the lower scores. On the other hand, lime drinks prepared by adding 2% concentrates obtained the highest scores for all sensory attributes followed by lime drinks containing 1.5% and 1%.

Statistical analysis of lime drinks prepared by adding lime peel clouding agent concentrate produced by enzymes recorded the highest score with highly significant difference than the untreated in color, appearance and overall acceptability. While, no significant difference in flavour between concentrate produced by enzymes and the untreated. Otherwise, lime drinks prepared by adding 2% clouding agent concentrate obtained the highest score with highly significant difference, for all sensory attributes with the exception of flavour which had no significant difference between 2%, 1.5% and 1% of clouding agent concentrate. Also, an interaction could be seen between enzymatic treatments and concentrations of lime peel clouding agent concentrates in appearance with highly significant difference. However, interaction existed between enzymatic treatments and concentrations in the overall acceptability with significant difference.

It could be noticed that lime drinks prepared by adding clouding agent extracts and concentrates treated with Citrozym Cloudy 100 L enzyme then adding 15% of clouding agent extracts and 2% of clouding agent concentrates were the best in the overall acceptability than the other treatments.





Table8

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إنتاج مواد معكرة طبيعية من قشور الليمون البلدي  
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استهدفت هذه الدراسة إنتاج مستخلصات و مركبات عوامل تعكير طبيعية من قشور الليمون البلدي باستخدام المعاملات الإنزيمية . و قد أوضحت النتائج أن مستخلصات و مركبات المواد المعكرة المنتجة بواسطة المعاملات الإنزيمية كانت اعلي في نسب المواد الصلبة الكلية و المواد الصلبة الذائبة الكلية و السكريات الكلية و الحموضة الكلية، و البيكتين الذائب الكلي و النيتروجين الأميني الحر و ذلك بالمقارنة بالعينات غير المعاملة. كما وجد أن لزوجة مستخلصات و مركبات المواد المعكرة الغير معاملة إنزيمياً كانت اعلي من تلك المنتجة بواسطة المعاملات الإنزيمية.

ووجد أن قيم عكارة للمشروبات المصنعة من مستخلصات و مركبات المواد المعكرة و التي تم معاملةها بواسطة الإنزيمات كانت أقل من قيم عينات الكنترول. بينما سجلت المشروبات المصنعة باستخدام 15% من مستخلصات المواد المعكرة، 2% من المركبات قيم عكارة اقل من التركيزات الأخرى.

و تبين حدوث نقص طفيف في ثبات عكارة المشروبات المحضرة باستخدام مستخلصات و مركبات المواد المعكرة و المخزنة لمدة 30 يوم عند درجة حرارة الغرفة بعد خمسة أيام من التخزين و بعد ذلك لم يحدث تغير ملحوظ في ثبات عكارة هذه المشروبات.

كما تبين أن مستخلصات و مركبات المواد المعكرة لقشور الليمون البلدي و المنتجة بواسطة المعاملات الإنزيمية (سواء إنزيم Pectinex Ultra SPL أو إنزيم Citrozym Cloudy 100L ) كانت أكثر ثباتاً عن العينات غير المعاملة.

و قد أظهرت نتائج التقييم الحسي أن مشروبات الليمون البلدي و المصنعة باستخدام مستخلصات و مركبات المواد المعكرة و بنسبة 15%، 2% علي الترتيب و المعاملة بإنزيم Citrozym Cloudy 100L كانت هي الأفضل من حيث القابلية العامة عن المعاملات الأخرى بناء علي إمكانية التوصية باستخدام مستخلصات و مركبات المواد المعكرة المنتجة من قشور ثمار الليمون البلدي و المعاملة إنزيمياً في إنتاج المشروبات مع إضافتها بنسبة 15%، 2% لكل من المستخلصات و المركبات علي الترتيب.

