Effect of Enzymatic Modification on Galactomnnan Gums and their Rheological properties

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Abstract— Galactomannan gums represents valuable and sustainable alternative to traditional Synthetic polymers and increasingly applied in growing number of industrial fields In their native and chemically modified and bio modified form. The aim of the present work is to apply an enzymatic modifications on Fenugreek gum With galactose: mannose ratio 1:1 which represent an innovative approach that are alternative to classical chemical modifications etc. (depolymerization, ,cationization ,carboxymethylation Enzymatic modifications offer the advantage of exploiting specific reactions, creating New modified Galactomannan, while developing a mild more controllable and Ecofriendly biochemical process. In this study green modification of Galactomannan Were applied using two different Enzymes Catalase and laccase enzyme, also the storage time effect on enzymatic modified fenugreek gum was studied at the present work.

Index Terms— Galctomnnan, Gums, laccase, enzyme, Catalase e, Fenugreek, ecofriendly.

1 INTRODUCTION

Galactomannan gum have attracted considerable attention as well as industrial interest especially at an ecofriendly printing textile. Galactomannan is polysaccharides found especially in the endosperm of several leguminous seeds, are composed of mannan backbone linked seeds, are composed of mannan backbone linked together by B-(1-4) glycoside linkages and having galactose side chain residues linked by ∞-(1-6)([1])One of the sources of galactomannan is Fenugreek seeds which has 1:1galactose: mannose ratio.And it has wide applications as a thickener, rheology modifier and emulsion stabilizer in several industrial sectors, such as food, textile, personal care, oil operations, paper and coating. According to the intended applications, a variety of chemical application are performed on fenugreek gum. For example (carboxymethilation, carbamation, depolymerization and cationization) the purpose of this modification is to improve their solubility and their rheological properties and functional properties in order to optimize and extend its industrial users [(2)]. Enzymatic modification represents an innovative eco-friendly approach to classical chemical modification. At present work one main type of enzymatic reaction can be applied on Fenugreek gum using oxidoreductose enzymes (laccase, catalase) enzymes. Laccase enzyme, p-diphenol oxidase (systematic name – benzenediol: oxygen oxidoreductase (3). It belongs to oxidoreductase enzymes) which comprises an extended group of blue multi copper oxidases known to oxidiase a wide range of substrates, mainly phenols and substituted amines by catalyzing the four electron reduction of molecular oxygen to water of substrates, mainly phenols and substituted amines by catalyzing the four electron reduction of molecular oxygen to water coupled with the oxidation of small organic substrates [(4)] .

2.1 Materials and methods

2.1.1 Plant Seeds:

Dry clean Fenugreek was cultivated in Egypt, provided by Hraz Agricultural Seed & Medical Plants Company, Ahmed, Cairo, Egypt.

2.1.1 Plant Seeds:

Dry clean Fenugreek was cultivated in Egypt, provided by Hraz Agricultural Seed & Medical Plants Company, Cairo, Egypt.

2.1.2 Enzymes:

The following enzymes were selected and used throughout the present work

Laccase enzyme:(Lava Zyme Lite 1540) were supplied by Egypt dye star company, Cairo, Egypt .

Catalase enzyme: (Peroxfinac 40/p) were supplied by Glory Chemical company, Cairo, Egypt.

2.2 Methods:

2.2.1- Extraction of the gum

For the separation of the Galactomannan gum from the aforementioned seeds, the endosperm was thoroughly separated from the hull and the germ.The procedure adopted was carried out as follows:

- The clean dry seeds were crushed mechanically.
-The crushed seeds were sieved to remove the germ which possesses the lowest hardness from the other components.
-The remaining parts which comprise the endosperm and hull are soaked in water over night to allow the endosperm which is composed mainly from Galactomannan gum to swell
- The swelled gum was isolated from the hull via filtration using a very fine silk fabric.
- The gum was precipitated from viscous solution by adding ethyl -alcohol, filtered and finally air dried, grinded to affine powder. [(5)]

2.3. Enzyme prepration.

2.3.1 Laccase enzyme preparation:

Laccase enzyme (Lava zyme lite 1540) were applied at powder form directly with two different concentration (300gm,

500gm under optimum condition of the enzyme (PH =5-5.5, Temp: 65 °C, time: 20-30minutes).

After extraction of the gum from Fenugreek seeds the oxidation of galactomannan by laccase enzyme was conducted as follows:

250 gm from fenugreek gum paste at concentration of 2%, was added gradually to the laccase enzyme during mechanical stirring.

2.3.2 Catalase enzyme preparation:

catalase enzyme (Perooxfin)were applied at solution form directly with two different concentration (350gm , 600gm)under optimum condition of the enzyme (PH =5-5.5),temp: (40 °C -50°C) time: 10 minutes) .

After extraction of the gum from fenugreek) seeds the modification of Galactomannan by catalase enzyme was conducted as follows:

250 gm from fenugreek gum paste at concentration of 2%, was added gradually to the catalase enzyme during mechanical stirring.

2.4 Rheological measurement:

The rheomat viscometer consists principally of two co-axial cylinders. The inner one is axially connected to a motor and can rotate with 15 speeds. The other one is kept stagnat. Both cylinders are usually kept immersed in thermostated water – bath at 25.0 ± 0.1°C.

The modified Galactomannan pastes (Fenugreek, Guar,) were poured carefully in the vicinity between the two cylinders so as to avoid air inclusion and kept for 15 minutes to obtain the thermostate temperature. The rheological properties were measured under the following experimental conditions: μ (viscosity)= τ/D

-μ: Apparent viscosity in poise.

- τ: Shearing stress(dyne.cm) $^{-2}$
- D: Rate of shear [6,11].

3.Results and Discussion.

3.1.Study the effect of the Rheological properties of bio-Modified Fenugreek thickeners based on Galactomannnan

.2.3.1 Laccase enzyme preparation:

Since the Galactomannan gums on both the native form and the modified one are generally used in the form of viscous solutions, it is of great importance to investigate the viscosity and rheological properties of the pastes for the aforementioned Galactomannan Gums. For this reason all the investigated pastes were prepared in a concentration of 2%; their Rheological properties were measured using Rheomat-15. The data obtained are represented in Figures (1-2) as shown below:

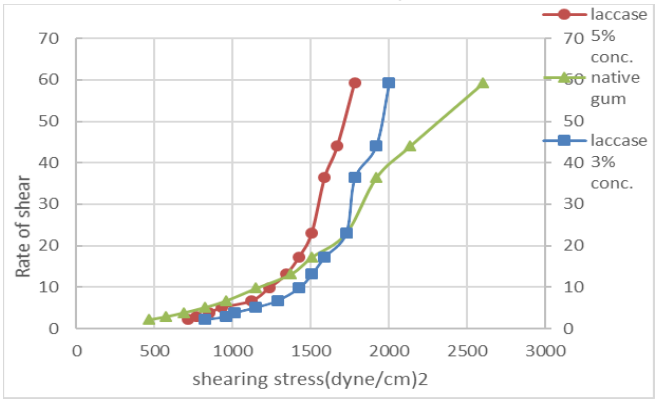

Figure (1) Rheological properties of Galactomannan Gums isolated from fresh Fenugreek seeds before and after being modified with Laccase enzyme with two different concentration

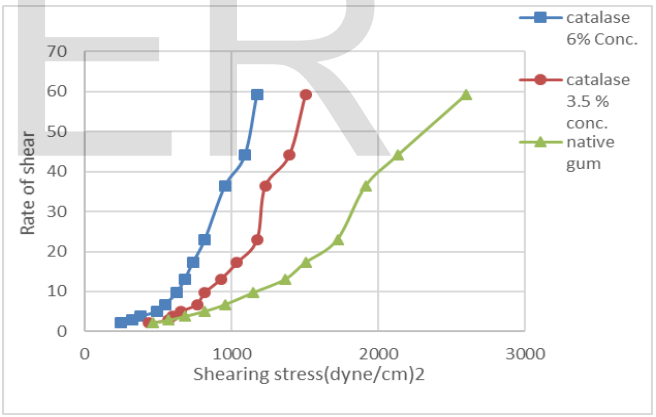

Figure (2) Rheological properties of Galactomannan Gums isolated From fresh Fenugreek seeds before and after being modified wit Catalase enzyme with two different concentration

As it is obvious from the reheogrames (1 and 2), that all the examined pastes i.e. modified Galactomannan gums obtained from fenugreek, as well as native gum are characterized by a non- Newtonian pseudo plastic behavior, this is due to the relation between shearing stress and the rate of shear is not a straight line and concaved towards the rate of shear axis as well as the up and down curves coincident. This means that, if the pastes are subjected to a large applied force a structural breakdown will occur, leads the viscosity to decreases. By removing the force, the pastes retain their original viscosity immediately and their rebuilding need no time. We can have a brief look on the two pervious figures we can obviously recognize that the bio modification of the Fenugreek gum irrespective of the kind of enzyme used i.e. Laccase or Catalase

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enzyme in most cases cause rheogrames to be closer towards The rate of shear axis after modification and hence means decrease in the apparent viscosity when compared to the native. This phenomena holds true irrespective of the kind or even the concentration of the enzyme used. This phenomena can be acceptable since the modification of the gum with any kind of enzyme cause increase in the solubility hence breakage of the chains particles cause the viscosity to be decreased.

Table (1) Effect of different concentrations of laccase enzyme on the apparent viscosity of fenugreek gum

Rate of shear (sec^{-1})	Apparent Viscosity in poise of fresh fenugreek gum on using		
	Laccase enzyme 3%	Laccase enzyme 5%	Native fenugreek gum
2.18	376.789	326.5505	213.5138
2.927	327.4001	261.9201	196.44
3.851	263.0641	220.4051	177.746
5.139	223.7712	181.1481	159.8365
6.779	189.8304	165.5967	141.363
9.771	145.7128	126.0976	117.6911
13.12	114.779	102.2576	104.3445
17.26	92.00695	82.48899	87.24797
23.03	74.8997	65.38862	74.8997
36.38	48.91974	43.65146	52.68279
59.22	33.7511	30.05235	43.92266

It is clear from table (1) which represents the effect of using two different concentration of laccase enzyme (3%, 5%) and comparing it with unmodified Fenugreek gum on the apparent viscosity of the pastes. It was found that the apparent viscosity highly increased as result of laccase modification. This phenomena was achieved irrespective of laccase enzyme used for example the apparent viscosity increased from 213.5138 poise at rate of shear 2.18 to 376.789 poise and 326.5505 poise for 3% concentration and 5% Laccase enzyme concentration.

Also it seems that the apparent viscosity increases 376.789 poise at rate of shear 2.18 while using laccase enzyme with 3% concentration rather than 5% concentration the apparent viscosity is 326.5505 poise at the same rate of shear.As a laccase enzyme belongs to oxidoreductase enzymes as it shown at scheme [5] which comprises an extended group of blue multi copper which oxidases a wide range of substrates, mainly phenols and substituted amines by catalyzing the four electron reduction of molecular oxygen to water coupled with the oxidation of small organic substrates[7] .Oxidation enzymatic modification causes to a various extent not only an increase in the viscosity of the Fenugreek gum but also modification of their rheological profiles suggesting formation of stable elastic gel. This phenomenon is more pronounced with Fenugreek gum as if the primary hydroxyl groups that undergo oxidation are exclusively or preferentially those of the Galctose side chains and because of the carbonyl groups which were generated by the enzymatic reaction which eventually form hemiacetalic bonds between generated carbonyl group and free OH groups causing internal crosslinking of fenugreek gum and their structuring to yield elastic gels [7].

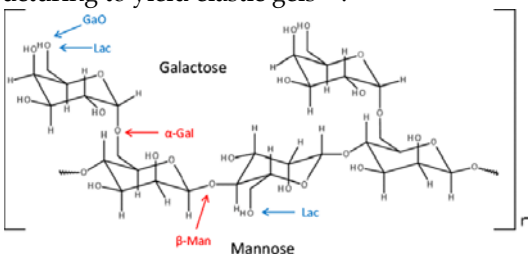

Figure (5) General structure of galactomannans.

Fenugreek is the polysaccharide where this transition is more dramatic that's because of the highest galactose content 1:1galactose: mannose ,that means it comprises high spaces and the ratio between the galactose with its small chains are almost equal to that of mannose with its long chains , and hence these high ratio of galactose and more voids and spaces found in fenugreek gum leads to facility and mobility to occur for the enzymes molecules and hence more chains cause the structure of the gum to be more solid and interlocked ended in high viscosity[9].

Table (2) Effect of different concentrations of Catalase enzyme on the apparent viscosity of fenugreek gum

Rate of shear (sec^{-1})	Apparent Viscosity in poise of fresh fenugreek gum on using		
	Catalase enzyme 3.5%	Catalase enzyme 6%	Native fenugreek gum
2.18	200.9541	113.0367	213.5138
2.927	196.44	112.2515	196.44
3.851	156.4165	99.53778	177.746
5.139	127.8692	95.90193	159.8365
6.779	113.0904	80.77888	141.363
9.771	84.06509	64.4499	117.6911
13.12	70.95427	52.17226	104.3445
17.26	60.28042	42.83082	87.24797
23.03	51.12201	35.66652	74.8997
36.38	33.86751	26.3414	52.68279
43.46032	31.66395	24.83447	48.42721
59.22	25.42891	19.88078	43.92266

Table (2) represents the apparent viscosity of modified Fenugreek gum using Catalase enzyme in two concentrations 3.5% and 6%concentration.

As it is clear from the data the effect of using two different concentration of catalase enzyme (3.5%, 6%) concentration for example at rate of shear 2.18 the apparent viscosity of the unmodified Fenugreek gum was found to be 213.5138 poise while when using catalase enzyme with 3.5% and 6% concentration the apparent viscosity was decreases to 200.9541 poise and113.0367 poise respectively at the same rate of shear.

The apparent viscosity of using Catalase enzyme with concentration 6% in the modification of fenugreek gum have been decreased compared with the native gum. The decrease in apparent viscosity may be due to the breakage of chains occurred. This phenomena was found depending on the concentration of Catalase enzyme i.e. 3.5% conc. The decrease in the viscosity was so slightly 200.9541 poise on compared to the native gum which was found to be 213.5138 poise.

While at high concentration 6% conc. The apparent viscosity decreases to high extent to reach 113.0367 poise at the same rate of shear.

Catalase enzyme is one of oxidoreductase enzymes, the basic mechanism of the working of this enzyme that catalyze the conversion of hydrogen peroxide to water and molecular oxygen [10].

$$2H2O \longrightarrow 2H2O + O2$$

3.2. Effect of storage time on the Reheologilocal properties of Galctomnnan Gums isolated from Fenugreek seeds:

The influence of storage and validity time on the rheological properties of the enzymatic modification of Galactomannan gums isolated from fenugreek seeds as well as native Fenugreek Gum are measured and illustrated in tables and figures below. All the investigated pastes were prepared in a concentration of 2%; and stored for (1day, 7days and 30 days) respectively, their Rheological properties were measured using Rheomat-15. The data obtained are represented in Figures (3and 4).

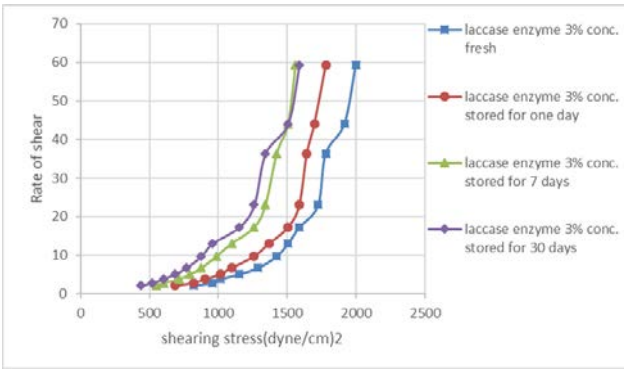

Figure (3) Rheological properties of Galactomannan Gums isolated from Fenugreek Seeds modified with Laccase enzyme at different storage time

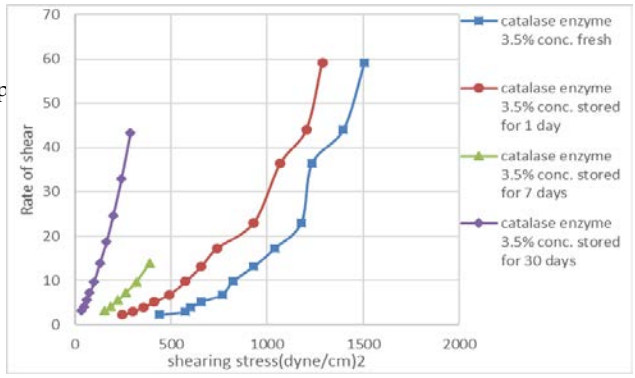

Figure (4) Rheological properties of Galactomannan Gums isolated from Fenugreek Seeds modified with Laccase enzyme at different storage time.

As it is clear from (Figures 3and 4), that the examined pastes i.e. modified Galactomannan gums obtained from fenugreek, as well as native gum at different storage time are characterized by a non- Newtonian pseudo plastic behavior, where the up and down flow curves are coincident where the modification of Fenugreek gum with even Laccase or Catalase enzyme doesn't affect the rheological properties.

In addition to that different storage time of the enzymatic modification does not affect the rheological properties of the fenugreek gum where it is still acquire pseudo plastic behavior.

While It is clear from figure (3and 4) that the storage time although doesn't affect the rheological properties but it affects the apparent viscosity of both modified and native fenugreek gums. The fresh native Fenugreek gum rheograme is far from the rate of shear axis although the location of the rheograms which were shifted nearer from the rate of shear axis as long as the storage time increased. and the re- rheograme of the examined pastes are following that order from the nearest to the farest from the rate of shear axis (stored samples for (30 days, 7 days, 1 day and fresh) respectively which indicate a decrease in the apparent viscosity comparing with the fresh fenugreek gum. This means that the storage time because ofmore solubility to the gum and the chains may be under go breakage to somehow, leads the viscosity to decrease.

Table (3) the apparent viscosity of the modified Fenugreek Gum with Laccase enzyme 3% at different storage time (fresh, 1day, 7 days and 30days

Apparent Viscosity in poise of modified fenugreek gum with laccase enzyme 3% conc. after			
Fresh	1day storage	7 Days storage	30 Days storage
376.789	313.990826	251.1927	200.9541
327.4001	280.62863	205.7943	177.7315
263.0641	234.624773	184.8559	156.4165
223.7712	197.131738	154.5087	133.1971
189.8304	161.557752	129.2462	113.0904
145.7128	128.899806	100.8781	89.66943
114.779	104.344512	83.47561	73.04116
92.00695	87.2479722	72.97103	66.62572
74.8997	68.9552757	58.25532	54.68867
48.91974	45.1566795	39.13579	36.87795
43.46032	38.493424	34.14739	34.14739
33.7511	30.0523472	26.3536	26.81594

Table (4) the apparent viscosity of the modified Fenugreek Gum with Laccase enzyme 6% at different storage time (fresh, 1day, 7 days and 30days

Apparent Viscosity in poise of modified fenugreek gum with laccase enzyme 5% conc. after			
Fresh	1day storage	7 Days storage	30 Days storage
326.5505	301.431193	263.7523	188.3945
261.9201	261.920055	224.5029	168.3772
220.4051	227.514931	191.9657	142.1968
181.1481	186.475968	159.8365	122.5414
165.5967	153.479864	133.2851	105.0125
126.0976	120.493296	103.6803	89.66943
102.2576	95.9969512	83.47561	75.12805
82.48899	79.3163384	68.21205	61.86674
65.38862	61.8219713	59.4442	51.12201
43.65146	41.3936229	39.13579	34.62012
37.87256	37.2517007	35.38912	31.04308
30.05235	29.1276596	27.27828	25.42891

Table (3and 4) represents a comparison between the different storage time on the viscosity of modified gums using laccase enzyme (3% and 5%) As we mentioned before that laccase enzyme concentrated 3% was giving slightly better viscosity compared with laccase enzyme 5% and it is clear from table (3 and 4) that there is a slightly decrease in the viscosity after storing the paste for (oneday,7 days and 30days) respectively but it is still have a good viscosity properties. For example the apparent viscosity was 188.3945 poise and 200.9541 poise after a month storage for 3% and 5% laccase enzyme concentration, This may be due to the carbonyl groups which generated by the enzymatic reaction which eventually form hemi-acetalic bonds between generated carbonyl group and free OH groups causing internal crosslinking of fenugreek gum. That cause an acceptable viscosity of the paste and make the storage doesn't affect the prepared gums properties [9]. another phenomena occurred which is the kind of modification with Laccase enzyme, that irrespective of the concentration of Laccase used all the examined samples were found to be valid up to three months and still without being rotted (while it was in the wet (liq-uid) form during all this period of time).This means that the rotting of the gum with laccase enzyme have been solved as it considered one of the major problem of the thickeners which have been extracted from a natural seeds which is rotting easily

Table (5) the apparent viscosity of the modified Fenugreek Gum with Catalase enzyme 3.5% at different storage time (fresh, 1day, 7 days and 30 days.

Apparent Viscosity in poise of modified fenugreek gum with Catalase enzyme 3.5% conc. after			
Fresh	1day storage	7 Days storage	30 Days . storage
200.9541	125.5963	49.74735	11.05497
196.44	121.6057	44.25593	11.32128
156.4165	106.6476	40.68207	10.95287
127.8692	95.90193	36.34774	10.55257
113.0904	84.81782	32.88804	10.22196
84.06509	70.05424	28.06377	9.251793
70.95427	56.34604	0	8.726603
60.28042	49.17613	0	8.028989
51.12201	40.42206	0	7.326354
33.86751	29.35184	0	6.64436
31.66395	27.31791	0	5.80669
25.42891	21.73016	0	0

Table (6) the apparent viscosity of the modified Fenugreek Gum with Catalase enzyme 6% at different storage time (fresh, 1day, 7 days and 30 days.

Apparent Viscosity in poise of modified fenugreek gum with Catalase enzyme 6% Conc.			
Fresh	1day storage	7 Days storage	30 Days storage
113.0367	75.3577982	6.909354	0
112.2515	65.4800137	6.175245	0
99.53778	56.8787328	5.476433	0
95.90193	53.278848	5.862539	31.96731
80.77888	44.4283818	5.333195	28.27261
64.4499	39.2303756	5.242683	22.95579
52.17226	33.3902439	4.822596	20.62225
42.83082	28.5538818	4.712667	17.83326
35.66652	40.42206	4.448144	12.79439
26.3414	29.35184	3.966782	12.41723
24.83447	27.31791	3.688956	10.17156
19.88078	21.73016	3.306923	8.784651

Table (5 and 6) represent the effect of storing for 1day, 7 days and 30 days on the apparent viscosity of Fenugreek gum at 3.5% and 6% Catalase enzyme concentration as it clear from the data that there is a It is clear that the viscosity have been decreased to some extent After 1 day storage for example the apparent viscosity was 125.5963 poise and 75.3577982 poise at 3.5% and 6% Catalase enzyme concentration.

After 7days storage the decreasing of the viscosity become clearer while using Catalase enzyme 6% concentration for example the apparent viscosity was 6.909354 poise while the apparent viscosity was 49.74735 poise at 3.5% Catalase enzyme concentration which shows better viscosity results. And after 30 days the apparent viscosity totally decreased for example the apparent viscosity was 11.05497 poise.

4 Conclusion

In present work the enzymatic modification of galactomannan gums which extracted from fenugreek gum oxidized by laccase, catalase enzymes which classified as oxdioreductose enzymes was compared to a native fenugreek gum and studying their rheological properties .

It seems from the rheograms that the modification of fenugreek doesn't affect the rheological properties on both native and modified form as they still characterized as a non- Newtonian pseudo plastic pastes.

Oxidoenzymatic modification using laccase enzyme increase the apparent viscosity of the Fenugreek gum and that's may be due to the carbonyl group as it mentioned before.

The apparent viscosity of using Catalase enzyme in the modification of Fenugreek gum have been decreased compared with the native gum. And also studying the effect of storage time as the samples stored for (fresh, 1day, 7 days and 30 days)It seems that the storage doesn't affect the rheological properties as that all examined pastes using (catalase, laccase) enzyme are still described as a non- Newtonian pseudo plastic pastes.

The storage time is a slightly decrease in the viscosity after storing the paste for (one day, 7 days and 30days) while using Laccase enzyme, But it is still have a good viscosity properties after a month storage, and it remains till 3 months without being rotted. The storage doesn't affect the prepared gums properties. This means that the rotting of the gum with laccase enzyme have been solved as it considered one of the major problem of the thickeners which have been extracted from a natural seeds which is rotting easily. As we see that fresh sample couldn't stand for one month storage. And the viscosity decreased to a great extent after 7 days storage. Although catalase enzyme it is clear that the storage time affect the apparent viscosity as it decreased as long as the storage time increased but it still stands without being rotted till one month storage which is better than native gum

5. ACKNOWLEDGMENT

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