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Physicochemical Studies on Using Chitosan to meliorate the harmful of Nicotine, Tar and Heavy Metals as well as Microbial contents in Tobacco Industry

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Abstract

Chitosan nanoparticles used potential as antibacterial activity or anticancer activity. Chitosan used in a lot of fields such as tissue engineering, nerve and bone tissue, water engineering, biomedical, pharmaceutical materials, food chemistry and biotechnology. When looking to the risks of cigarette and tobacco smoking and the extent of its effect on the heart and lungs, and the high risk of developing cancer. In this study we can use chitosan to manufacture cigarette filter to reduce the risks of nicotine, tar, carbon monoxide and heavy metals from smoking. Make a chemical treatments on crayfish shells *Procambarus clarkii* to obtain chitosan powder and analysed using infrared spectrum and nuclear magnetic resonance spectrum. Use chitosan to manufacture filter rods and cigarettes then testing by chemical and physical experiments through using smoking machine, gas chromatography and ICP-OES. Conducting some experiments on molasses by adding different concentrations of chitosan to reduce the percentage of nicotine, increase its storage period and eliminate rotting problems in it while storage through chemical and microbiological tests. It was found that the weight of the filter rods containing chitosan was reduced compared to those that in treatment in which content of acetic acid concentrations of them. Also positive for nicotine, tar and heavy metals, and we found that in treatment in which chitosan was used with a concentration of 10 %, the proportion of nicotine in the molasses decreased by the amount of text compared to the rest of the prepared treatments and it was 0.24 %. Also the microbial content decreased in the same treatment which was 7.8 x 10^4 cfu/g, and thus the molasses rot will slow down. Chitosan is not a synthetic or manufactured substance, so can be used as a basic preservative instead of chemical and expensive preservatives, due to the ease of its preparation and according to international specifications.

Key words: *P.clarkii*, Chitosan, nicotine, tar, filter efficiency, heavy metals, microbial content, ICP-OES, NMR Spectrum. IR Spectrum.

1- Introduction

Procambarus clarkii was first introduced in 1984 at the estuary of Rasheed and Damietta, but no action was taken. It had a destructive impact upon the local biota and might lead to considerable stress on the freshwater ecosystems and therefore attacked agricultural banks and canals, they had affected many water courses in Giza and Qalyoubiya as well as attacking peasants. It caused big problems of fish wealth and fish production in Egypt by attacking fish farms than fish deaths [1] also it attacked fish inside nets, on the other hand, trammel nets used by fishermen in the Nile River were frequently torn by wrongly entrapped crayfish and cause loses for fishermen.

Chitosan nanoparticles was used potential as antibacterial activity or anticancer activity, they investigated the effect of chitosan nanoparticles protect crayfish *Procambarus clarkii* against WSSvirus. Chitosan (from crab shell) nanoparticles were prepared by ultrafine milling [2]. It was effects on immune stimulatory. Hence they can get chitosan and it used in a lot of fields such as tissue engineering [3], cartilage, nerve [4] and bone tissue [5], water engineering, biomedical, pharmaceutical materials,

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food chemistry [6] and biotechnology [7,8]. Used chitosan for the removal of mercury from wast water, and removal of Cu^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+} within the temperature range 25–60°Cat near neutral pH [9, 10].

All the people know the dangers of cigarette and tobacco smoking and the extent of its effect on the heart and lungs, and the high risk of developing cancer [11, 12]. And every year a lot of people die from diseases caused smoking but we cannot be prevent of smoke. This study aims to reduce the risk smoking by using the chitosan for reduce the risk each for nicotine, tar and carbon monoxide output from smoking, increase the removal efficiency for nicotine, tar and heavy metal. Reduce of nicotine percentage in molasses and eliminate rotting problems in molasses while storing.

1. Materials and Methods

2.1 Prepared and isolated chitosan:

First stage: **(**Demineralization) [13, 14] Treatment of 100 kg of crushed shell by 200 liters of water / 20 liters HCl 3-4 % then washed with water and drying by air.

Second stage: **(**Deproteinization) [15, 16] Treatment of 100 kg from previous product by 200 liter solution NaOH 4 % to convert the protein into an easily dissolved image then washed with water and drying by air.

The output at this stage is known as chitin.

Third stage: **(**Deacetylation) [17, 18,19] Treatment of 100 kg chitin by 200 liter solution NaOH 50% to convert the chitin into chitosan. Chitin and Chitosan chemical structure as shown in Fig. 1, and Schematic flow diagram for production of chitin and chitosan as shown in Fig. 2.

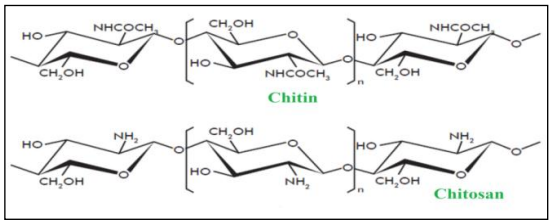

Fig. 1. Chitin and Chitosan chemical structure.

Every 100 kilos from *Procambarus clarkii* gives 35-37% chitosan which analysed by IR spectroscopy and NMR spectroscopy, the weight-average molecular weight of chitin is 1.03 $\times 10^6$ to 2.5 $\times 10^6$ Da, but the N-deacetylation reaction decreases it from 1 $\times 10^5$ to 5 $\times 10^5$ Da [20].

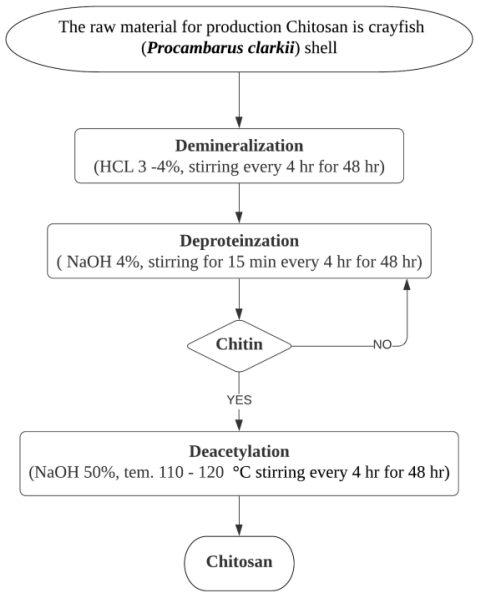

Fig. 2. Schematic flow diagram for production of chitin and chitosan.

2.2 Preparation of filter rods and cigarettes by using different concentrations from chitosan:

The filter tow (cellulose acetate 2.5Y30000) is bloomed as much as possible so that it develops its maximum filling capacity. The bloomed filter tow is compacted into the shape of the future cigarette filter and surrounded by paper, structure of cigarette is shown in Fig. 3. The filter is spread with individual filaments mostly now fed in the spray booth, where the rotating brushes create a fine spray of treatments which prepared in triacetate, this material gives the hardness to the filter rods, manufacturing filter rod 120 mm:

Negative Control: normal filter without any additives.

Positive Control: filter with concentration from acetic acid, will used in treatments.

Treatments make concentrates from chitosan 0.2, 1, 2 and 5 % in acetic acid 0.6M.

The blend used is Cleopatra 80mm is local brand make from the various sorts of tobacco.

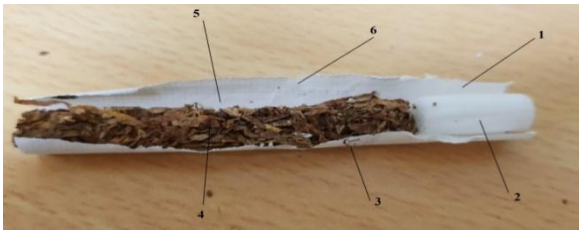

Fig. 3. Structure of cigarette. 1- Filter paper (aquafuge), 2- Filter plug, 3 - Ink, 4- Tobacco blend, 5-Cigarette warpaper, 6- Adhesive.

2.3 Preparation of sallome molasses blend by using different concentrations from chitosan:

As shown in Table 1, molasses consists of tobacco 100 Kg, Molasses 240 Kg, Glycerin 5 Kg, Sodium benzoate 100 g and Potassium sorbate 100 g.

Table1. Preparation of sallome molasses blend and treatments of chitosan

Additives	Additives quantity
Tobacco	100 Kg
Molasses	240 Kg
Glycerin	5 Kg
Sodium benzoate	100 g
Potassium sorbate	100 g

Positive Control: normal molasses blend is used.

Negative Control: molasses without any additives (sodium benzoate or potassium sorbate).

Treatments: make concentrates from chitosan 0.1, 0.2, 0.3, 5, 10 %.

Samples are incubated at 32 ° C and stirred continuously for 5 days.

Note: -The molasses was heated to 90 °C before adding to tobacco then glycerin was added at 65 °C but the best after 2 or 3 days from daily stirring.

Determination of nominal diameter [21, 22], ventilation [23] and draw resistance [24] for filter rods and cigarettes by using QTM device (Cerulean, UK). Then Smoking the cigarettes on an automated linear 20 part SM 450 smoking machine (Cerulean, UK) [25] to determine of total particulate matter [26] and determination of carbon monoxide in the vapour phase by NDIR method [27]. After smoking run the filter disc remove from trap. The folded disc placed in an appropriately shaped dry flask (maximum 150 ml for 44mm disc or 250 ml for 92mm) for make extraction of the nicotine and water in the particulate matter [28, 29].

Determination of arsenic, cadmium, chromium, nickel, lead, copper, iron, manganese and zinc ions in pieces of filter by using Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES, with Agilent Vapor Generation Accessory VGA 77[30].

Determination of sugar percentage [31], water content [32], ash, sand [33], nicotine [34] and total viable count [35, 36] were done by dilution plate count in sallome molasses.

2. Results and Discussion

We show in fig. 4 and table 2 infrared spectroscopy for a major group present in chitosan extracted from *Procambarus clarkia*.

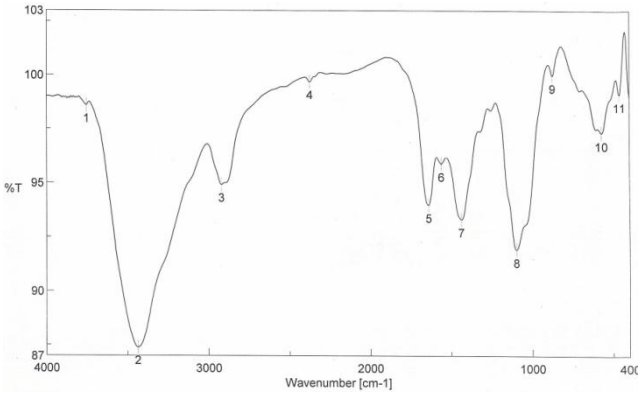

Fig.4. IR spectrum of the chitosan extracted from *Procambarus clarkia*.

Table 2. Wave length of the main bands IR spectrum of the chitosan extracted from *Procambarus clarkia*

Peaks	wave length (cm^{-1})
N–H and O–H stretching	3436.53
C-H stretching	2921.63
Amide I band	1640.16
Amide Π band	1561.09
CH2 bending	1437.67
C-O stretching	1099.23

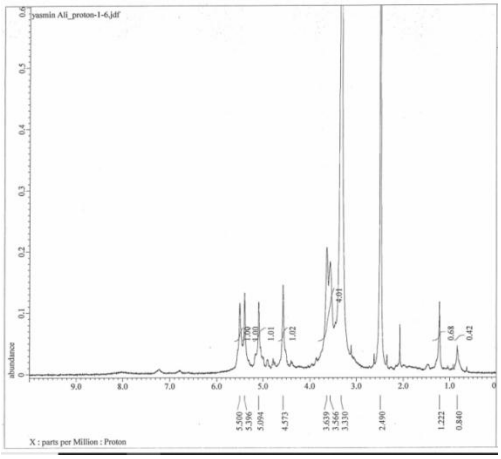

Fig. 5. H^1NMR spectrum of the chitosan extracted from *Procambarus clarkia*.

In fig.5 we can observe the H^1NMR spectrum for chitosan extracted.

When using a concentration of 0.2% from chitosan in treatment No. 1 of cellulose acetate rods, the results were obtained in Table 3.

Table 3. Results for filter rods, Treatments and control

	Negative control	Positive control	Treatments			
			1 (0.2%)	2 (1%)	3 (2%)	4 (5%)
Weight g/100rod	74.0±0.010	73.0±0.027	72.0±0.017	-	-	-
PD mm.WG	405.9±13.0	382.9±21.3	387.6±19.8	-	-	-
Triacetin %	7.0±1	7.0±1	6.2±1	-	-	-

Mean± standard deviation

From the table 3, we notice that when comparing treatment 1 with both the negative control and the positive control, we find that the weight of the filter rods decreased in treatment 1 and the positive control over the negative control, and the weights were respectively 72, 73 and 74 g / 100 rod, and all of them were less than the standard 74-79 g / 100 rod, the percentage of triacetin in all of them within the standard limits 6-8 % and the draw resistance of the treatment rods and the positive control within the limits, but the negative control is higher than the limits. In Treatments 2, 3 and 4 the resultes was negative because when raising the concentration of chitosan treatments, we encountered the problem of triacetin ruler clogging.

When looking at the results of physical tests for cigarettes treated with a concentration of 0.2 % of chitosan, the results were obtained in Table 4

Table 4. Physical results for cigarettes, Treatments and control

		Negative control	Positive control	Treatments			
				1 (0.2%)	2 (1%)	3 (2%)	4 (5%)
Weight	g/100Cig	1.03±0.035	0.95±0.031	0.99±0.037	-	-	-
PD	mm.WG	109.3±8.4	104.5±8.1	117.8±7.7	-	-	-
Ventilation	%	26.57±3.03	22.63±4.43	23.47±2.34	-	-	-

Mean± standard deviation

Looking at the results of the physical analysis of cigarettes, we find that the increase in the weight of the cigarettes in both of Treatment 1 and the negative control is outside the standard limits. But in positive control within the permissible limits, draw resistance or pressure drop (measured by millimeters water gauge) of cigarettes in both of passive control and positive control is within standard limits and Treatment 1 is slightly high 117.8 mm.WG. And in view of the ventilation we find it high in everyone.

When looking at the results of smoke analysis for cigarettes treated with a concentration of 0.2% of chitosan, the results were obtained in Table 5.

When looking at the results of the next table 5, we find that there are no noticeable differences in the results of the smoking of treatment 1and the negative

control, so the percentage of efficiency of filter for nicotine in Treatment 1 was 41.40% and negative control was 41.53%, but in positive control (content

of acetic acid) increased and become 45.90%, but not in the clear picture.

A change in the percentage of filter efficiency for tar, where it was in the negative control 54.14%, compared to the positive control, which became 55.54% and Treatment (1) 52.03%, not with the essential effect.

Table 5. Smoke results for cigarettes, Treatments and control

				Treatments			
		Negative control	Positive control	1 (0.2%)	2 (1%)	3 (2%)	4 (5%)
Nicotine	mg/Cig	1.11±0.031	1.02±0.041	1.11±0.040	-	-	-
Tar	mg/Cig	13.69±0.659	13.27±0.369	14.32±0.178	-	-	-
CO	mg/Cig	12.04±0.522	11.23±0.480	12.04±0.207	-	-	-
Efficiency of Nicotine	%	41.53±1.645	45.90±2.176	41.40±2.133	-	-	-
Efficiency of Tar	%	54.14±2.208	55.54±1.236	52.03±0.596	-	-	-

Mean± standard deviation

The insecticide nicotine hydrochloride (NCT) was formulated as nanoparticles composed of chitosan (CS) and sodium tripolyphosphate (TPP) to undermine its adverse impacts on human health and reinforce its physicochemical stability [37].

When looking at the amounts of heavy metals in Cleopatra bend and Treatment 1with a concentration of 0.2% of chitosan, the results were obtained in Table 6.

Table 6. The amounts of heavy metals (As, Cd, Cr, Pb, and Ni) in Cleopatra bend and treatment 1 (conc. 0.2% from chitosan) mg/kg

Element	Negative control	Positive control	Treatments			
			1 (0.2%)	2 (1%)	3 (2%)	4 (5%)
As	< 0.05	0.6	0.6	-	-	-
Cd	< 0.05	< 0.05	< 0.05	-	-	-
Cr	< 0.05	< 0.05	< 0.05	-	-	-
Pb	< 0.05	3.9	1.8	-	-	-
Ni	< 0.05	< 0.05	< 0.05	-	-	-

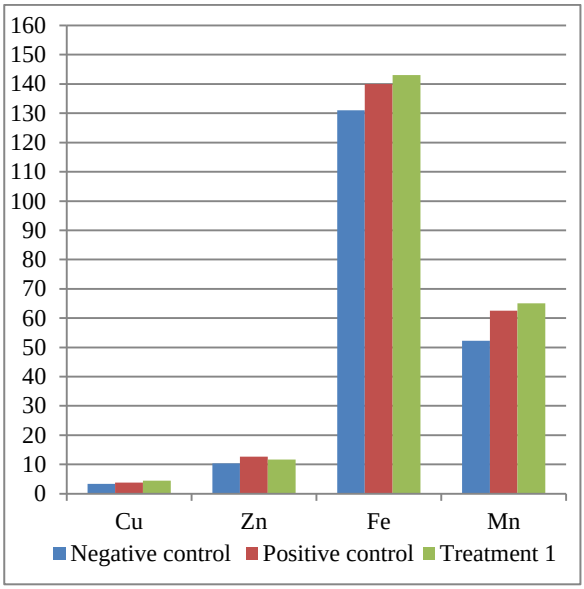

Fig. 6. The amounts of heavy metals (Cu, Zn, Fe and Mn) in Cleopatra bend and treatment 1 (conc. 0.2% from chitosan) mg/kg.

Looking at the heavy metal ratios, found effect for chitosan on filter and mainstream smoke, the results were useful. Where the highest concentration of arsenic in the filter was 0.107 µg/cigarette at a concentration of 10 mg chitosan, and in each of lead, nickel, chromium and cadmium it was 0.138, 0.195, 0.220 and 0.041 µg/cigarette respectively at a concentration of 30 mg of chitosan[38],

From table 6 we find that Cadmium, Chromium, and Nickel have no noticeable effect, but we find that the Arsenic ratio increased from <0.05 mg / kg for the negative control to 0.6 mg / kg in the filter chitosan treatment and the positive control, the Copper percentage increased from 3.3 mg / kg in negative control to 3.8 mg / kg in positive control, but increased in the filter treated with chitosan and becomes 4.5 mg / kg, and the percentage of Lead increased in the filter treated compared to the negative control and becomes 1.8 mg / kg and in the positive become 3.9 mg / kg, and when we looking at fig. 6 find that the ratios of Zinc, Iron and Manganese increased in the filter treated with chitosan, and their ratios were respectively 11.7, 143, and 65 mg / kg, which indicates the ability of chitosan to bind these minerals and reduce their harmful effect on the smoker.

The chitosan had antibacterial activity against Gram positive and Gram negative bacteria [39]. Chitosan is widely used as an antimicrobial agent either alone or blended with other natural polymers owing to its high biodegradability, and nontoxicity [40]. As shown in fig. 7 when comparing the results of the molasses positive control contain (Potassium sorbate + sodium benzoate as a preservative) and negative control that do not contain any preservatives with treatments of chitosan, as we found that the results of chitosan treatments with different concentrations 0.1, 0.2, 0.3, 5 and 10 % on the manufacture of molasses after experimenting with them had a positive effect on the viability of the molasses for a longer period, as it was found from the results that the number of total viable count in the treatments 3, 4, 5, 6 and 7 is better than the positive control containing the preservative used it was (2.0×10^6 cfu/g), and we also find that treatment No. 7 conc. 10% is less in the number of microbial cells (7.8×10^4 cfu/g) than the negative control was (1.2×10^5 cfu/g).

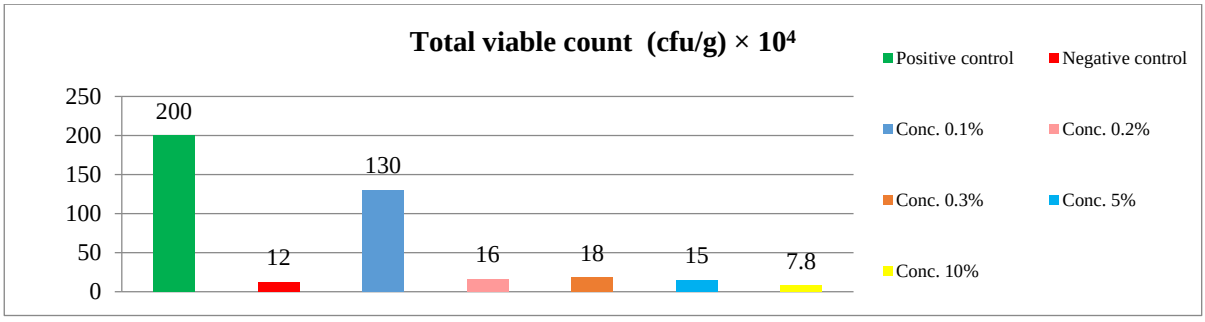

Fig. 7. Total viable count in sallome molasses.

Also, in table 7 we find a noticeable difference in the percentage of nicotine in the positive control from (0.41 %), than negative control (0.46 %) and the result of treatment 7, where it decreased it by 50 % which became (0.24 %).

Accordingly, we see that the use of chitosan with conc. 10 % was the best ever, and it can be applied and use this substance as a preservative and reduce the percentage of nicotine as a substitute for the one used in order to preserve the product and the smoker.

3. Conclusions

Chitosan is not a synthetic or manufactured substance, so can be used as a basic preservative instead of chemical and expensive preservatives, due to the ease of its preparation and according to international specifications.

Accordingly, use a high concentration from chitosan more than 0.2 % in filter rods will be more effect.

We see that the use of chitosan in molasses by conc. 10 % was the best ever. It can be applied and use this substance as a preservative and reduce the percentage of nicotine as a substitute for the one used in order to preserve the product. The smoker so must be change the preservative material percentage.

4. Conflicts of interest

There are no conflicts to declare.

5. Acknowledgments

The authors are gratefully acknowledged for the support of this work from Eastern Company S.A.E,6thOctober, Giza, Egypt.

Table 7. Results of sugar, water content, ash, sand and nicotine percentage in sallome molasses

Tests	Positive Control	Negative Control	Treatments				
(%)	1	2	3 (0.1%)	4 (0.2%)	5 (0.3%)	6 (5%)	7 (10%)
Water content	18.02±1.110	17.84±0.247	18.05±0.374	17.95±0.185	16.88±0.036	19.73±0.214	19.98±0.253
Total Sugar	29.17±0.025	25.95±0.055	31.03±0.045	34.38±0.175	31.56±0.095	28.34±0.460	30.88±0.135
Reduced Sugar	13.24±0.260	15.04±0.160	15.31±0.315	14.72±0.035	15.27±0.060	14.93±0.065	15.32±0.015
Sand	0.50±0.065	0.48±0.053	0.60±0.006	0.42±0.010	0.48±0.010	1.40±0.020	0.73±0.015
Ash	10.68±0.140	10.12±0.115	10.17±0.025	10.57±0.115	10.59±0.045	10.60±0.200	8.98±0.075
Nicotine	0.41±0.006	0.46±0.006	0.38±0.006	0.44±0.015	0.41±0	0.32±0.033	0.24±0.011

Mean± standard deviation

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7. Arabic Abstract

دراسات كيميائية وفيزيائية للشيتوزان المستخدم في صناعة الدخان

تهدف هذه الدراسة إلى اجراء معاملات كيميائية على قشور الاستاكوزا النيلية الموجودة بنهر النيل للحصول على مسحوق الشيتوزان ،ثم استخدام هذا المسحوق في تصنيع فلاتر السجاير وذلك تقليل مخاطر كل من النيكوتين ،القطران وأول أكسيد الكربون الناتج عن التدخين ، وزيادة كفاءة حجز النيكوتين والقطران والمعادن الثقيلة من خلال اسخدام ماكينة التدخين الالى ، جهاز التحليل الكروماتوجرافي الغازى و جهاز اشعاع البلازما الحثية ، وكذلك اجراء بعض التجارب على المعسلات باضافة تركيزات مختلفة من الشيتوزان لتقليل نسبة النيكوتين وزيادة وقت تخزينه والقضاء على مشاكل التعفن به اثناء التخزين من خلال الاختبارات الكيميائية والميكروبيولوجية. تم تحليل مسحوق الشيتوزان المتحصل عليه باستخدام طيف الأشعة تحت الحمراء وطيف الرنين المغناطيسى النووى. وبعد ذلك تم تحضيرتركيزات من الشيتوزان في حامض الخليك ٠,٦ مولر عند إضافة تركيز ٠,٢٪ في مرحلة تصنيع الفلتر وقد تبين أن وزن اصابع الفلتر المحتوية على مادة الشيتوزان قد انخفض مقارنة بالمستخدم والمحتوى علي حامض الخليك فقط ، كما أنها موجبة للنيكوتين والقطران والمعادن الثقيلة ، ووجدنا أن في المعاملة التى تم استخدام الشيتوزان بتركيز ١٠ ٪ ، انخفضت نسبة النيكوتين في المعسلات بمقدار النص مقارنة بباقي المعاملات المحضرة وبلغت ٠,٢٤ ٪ ، كما انخفض المحتوى الميكروبي في المعسل بنفس المعاملة وكان ٧,٨× ١٠^{٤} خلية ميكروبية / جم وبالتالي سيتباطأ تعفن المعسل وزيادة فترة تخزينه.