

PRODUCTION OF CHITOSAN FROM SHRIMP SHELLS BY MICROWAVE TECHNIQUE AND ITS USE IN MINCED BEEF PRESERVATION

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Abstract: This work was undertaken to figure out the optimum conditions for transformation of chitin to chitosan via microwave and to determine chitosan physical properties, antioxidant and antimicrobial activities to evaluate the possibility of using such by-products in preparing some functional foods. The obtained results revealed that deacetylation degree (DA) 93 % attained by chitin remediation with 50% aqueous sodium hydroxide sol at 1:25 solid:liquid ratio for 10 min into a microwave using a power 800W. Purified chitosan was characterized for molecular weight (4200 KDa), solubility (94%), water holding capacity (505%), oil holding capacity (321%) as well as intrinsic viscosity (13.2 dl/g). Samples of meat containing chitosan (0.02%) have the minimal experiment into the microbiological count, e.g. total count, yeasts and moulds. The anti-microbial effectiveness of chitosan was frequently higher than that of sorbistat-K or their blend by adding 0.01% chitosan and the same percent from sorbistat-K. Sensual attributes of samples contained chitosan, i.e. odour, texture and colour, were greater than other samples (sorbistat-K and control treatments). Though, chitosan nominated like a potent, eco-friendly and naturalistic substitution to reserve meat and protect its comprehensive quality.

Key words: chitosan; microwave; antioxidant activity; natural preservative

Introduction

Artificial anti-oxidative substances like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were utilized to hold up oxidation of lipids in meat (1). Nonetheless, contemporary attention over their utilization has generated a necessity and induced research for alternate antioxidants, predominately from naturalistic origin. The usage of naturalistic preservatives with meat products increases the storage shelf life is a hopeful technology as nu-

merous vegetative materials posse antimicrobial and antioxidant characteristics (2). Functional substances supplement into meat products might upgrade the health and nutritional attributes and extension their storage shelf-life (3).

The meat industry is rottenly suffering from many critical challenges related to well-being and hygienic of the final product (4). Microbial pathogens transference to the products can be carried out during processing steps or from outer parts of the carcass (5). The removing of contamination or disinfection of the carcass

body is hard to be realized by the use of a co-administered antimicrobial agent. In addition, commonly used antimicrobial and preservative substances possess artificial and alchemical properties, offering a lot of harmful effects and prospect hazards to consumer health (5).

Shrimp considered as one of the world's leading fishery products, especially in Egypt (6). The product is mainly exported under freezing conditions, passing through the shells and heads removing process (7). The economic value of raw shrimp shell substances and heads is little and they are considered as biological wastes or sold to factories of animal feeds (8). In the processing of shrimp industry, shrimp produce a large amount of biological waste, accounting for 45-55% of the raw weight of shrimp (9). Also, these biological wastes are able to be utilized in producing high-added value substances like chitosan and chitin. Chitosan, consisted of D-glucosamine units linked with β -linkage, is a cationic amino-polysaccharide which produced by partially deacetylation of chitin (10). If the acetyl- glucosamine groups proportions are higher (above 50%), it is named chitin, however if the proportion is lower than 50%, the ingredient is called chitosan (11, 12). The conventional methods for chitin extraction from crustaceans are chemical processes which involve the use of strong acid for demineralization and strong base for deproteinization. However, these methods reduce the quality and increase the costs and environmental problems (13, 14). Chitosan can be characterized in terms of its quality, intrinsic properties (purity, molecular weight, viscosity, and degree of deacetylation) and physical forms (15).

In modern decades, microwave radiation has received considerable attention for its ability to speed up the reaction duration as compared to else procedures which transfer heat by conventional heating procedures, and can also perform heat transfer more uniformly in food compositions (16, 17). Therefore, the aim of the present study was to figure out the optimum conditions to get chitosan from chitin via microwave and to evaluate its physical properties, antioxidant and antimicrobial activities to determine the

possibility of using this chitosan as preservatives in minced beef.

Materials and methods

Materials and preparation of samples

All chemical used in this study of HPLC grade (99.9% purity) were obtained from Sigma company of chemicals and drugs, St. Louis, MO, USA. Wastes of shrimp (shells and heads) have been acquired from local restaurant in Kafr El-sheikh governorate, Egypt. The wastes were dried in an electric oven then were ground up to pass through 20 mesh screen sieve, the yielded powder samples were mixed, and stored in polyethylene bags, then kept at room temperature until use.

Chemical composition

Moisture, crude protein, ether extract, ash, crude fiber contents of investigated samples (shrimp waste, crude chitin and chitosan) were performed using the methods given in the (18).

Isolation of chitin

Various chitin extraction stages were carried out according to Synowiecki and Al-Khateeb (19). Demineralization process was operated by mixing 4 g shrimp waste with 40 ml hydrochloric acid solution 2% (v/v) for 12 hrs at 30 °C, and the mixture was centrifuged for 15 min at 4000 rpm, then the precipitate twice treated by distilled water. Deproteinization of the precipitate was operated by adding sodium hydroxide solution 4% (w/v) to precipitate at liquid to solid ratio (10:1 v/w) for 12 hrs at 30 °C, and separation of different insoluble alkaline fractions by centrifugation for 15 min at 4000 rpm, then was twice treated with distilled water and was dried overnight at 40 °C. The product obtained after drying was marked as pure chitin.

Chitosan production via microwave route

The various stages for chitosan production have been done as briefed via Sahu, et al. [13]. Chitosan was prepared from isolated chitin using various concentrations of sodium hydroxide aqueous solution (NaOH 30%, NaOH 40% and NaOH 50 %) at several sample to solvent ratios

(1:10, 1:15, 1:20 and 1:25) at different microwave power (800W, 900W and 1000W) for various irradiation durations (5, 10, 15 and 20 minutes). chitosan yield recovered from each step was determined.

Chemical, physical and functional attributes for chitosan

Extracted and commercial chitosan deacetylation grade was measured using the method of Qin et al., (20). Molecular weight and solubility of extracted and commercial chitosan were determined according to the method of (21). Water binding capacity and oil binding capacity of extracted chitosan sample were measured using the method of (22).

Chitosan usage to preserve minced beef

Fresh clean meat specimens were treated with the disinfectant sodium hypochlorite (100 g meat piece was soaked into 10 ppm aqueous solution of sodium hypochlorite for 60 min followed by twice washes in distilled water). Pieces of meat were finely minced, blended and split to four groups. The first group (G1) untreated samples, G2 treated with 0.02% chitosan, G3 treated with 0.02% sorbistat-K, and G4 treated with a mixture of 0.01% chitosan and 0.01% sorbistat-K. All samples were stored at 5°C for seven days.

Microbiological analyses

Total count of aerobic microorganisms and fungi and yeasts were carried out at zero time and the end of storage period in triplicate as previously described (23).

Sensory assessment

After storage period, twenty semi-trained panelists from food technology department, kafrelshiekh University evaluated texture, color, and odour according to the method described by Pohlman, et al. (4).

Statistical analysis

All data (except phenolic content) were done in triplicate independent analyses and expressed as mean \pm standard deviation. Data of antibacterial activity were analyzed using one-

way ANOVA according to (24) procedure. The significance level was set at $p \leq 0.01$.

Results and discussion

Chemical composition of shrimp waste

Shrimp wastes were chemically analyzed for their contents of moisture, crude protein, ether extract, ash, crude fiber, total carbohydrates and the results were tabulated in Table (1). The data in Table (1) indicate that shrimp waste is considered a good source of protein, crude fiber and total carbohydrates which are important from the nutritional point of view. The results showed that the moisture, crude protein, ether extract, ash, crude fiber and total carbohydrate contents were 74.38, 11.55, 6.47, 32.21, 7.81 and 41.96%, respectively. These results were in harmony with those of (25, 26). Also, the data in the same table revealed that chitin content of shrimp wastes was 36.43% (on dry weight bases). According to the pervious results, shrimp wastes considered as a good source for chitin which can be modified to chitosan.

Production of chitosan by microwave

Effect of sodium hydroxide concentration (%) on chitosan yield

Table (2) shows the effect of using different concentrations (%) of sodium hydroxide on the chitosan yield. The modification process by microwave heating was carried out using different concentrations of sodium hydroxide (30, 40 and 50%) at 800W microwave power for 5 min and solid : liquid ratio (1:10). It could be observed that, there was an increment in chitosan yield as a function for increasing sodium hydroxide concentration. The highest chitosan yield (53.2%) was obtained with NaOH concentration (50%). These results were in harmony with those of (17).

Effect of solid : liquid ratio on chitosan yield

In this experiment, chitin modification process was carried out at 800W microwave power for 5 min where, sodium hydroxide (50%) was used as a solvent. The effect of solid: solvent ratio on chitosan yield was studied and the results were presented in Table (2). The results

revealed that chitosan yield was significantly increased with increasing solid: liquid ratio from 1:10 to 1:20. Meanwhile, chitosan yield was decreased when solid: liquid ratio reached up 1:20. The decrement in chitosan yield after reaching the optimum condition of solid: liquid ratio may be due to the degradation effect of alkali solution on chitosan (27). From such results, it could be noticed that, the highest chitosan yield was obtained at 1:20 of sample: solvent ratio. Where at this ratio, the percent of chitosan yield was 67.4%. These results are almost in agreement with those reported by (17).

Effect of microwave power on chitosan yield

In this experiment, chitin modification process was carried out using sodium hydroxide (50%) as a solvent at 1:25 solid: liquid ratio for 5 min. The effect of microwave power on chitosan yield was studied and the results were presented in Table (2). The results revealed that, chitosan decreased with increasing microwave power from 800 to 1000. From such results, it could be noticed that, the highest chitosan yield was obtained at microwave power (800 W). At this microwave power; the percent of chitosan yield was 67.4%. These results are in agreement with those found by (17). The obtained results may be due to thermal degradation of chitosan as a function to microwave power (28).

Effect of using different modification periods on chitosan yield

In this experiment, the modification process of chitin was carried out using 800 W as microwave power, sodium hydroxide (50%) as a solvent at 1:25 solid: liquid ratio for different periods (5, 10 and 15 min) and the results are illustrated in Table (2). The results indicated that, chitosan yield (%) increased gradually with extending the modification time from 5 to 10 min. This means that, the optimum period for chitin modification to chitosan was 10 min. These results are in agreement with those found by (17). The previous results may be explained by (29) who reported that, prolonging the modification time after reaching the optimum time leads to

degradation of chitosan and lost into the reaction solvent.

Gross chemical composition of chitosan

Table (3) show that, the total carbohydrates content of chitosan prepared from shrimp shell wastes was 89.11%. It had low percentages of crude protein 0.98%, ether extract 1.30%, ash 0.31% and 2.21% crude fiber, respectively. Similar results were found by (30, 31). A high quality grade of chitosan should have less than 1% of ash content (32).

Physicochemical and functional properties of chitosan

The produced chitosan had a degree of deacetylation and molecular weight higher than commercial chitosan (Table 4). The deacetylation degree and molecular weight are important parameters for chitosan, as they affect its functional properties (33). The degree of deacetylation of chitosan is important for its application in the industry. Thus, certain researchers (34) suggested that the term chitosan should be used when the degree of deacetylation is above 70%. Solubility, water holding capacity, oil holding capacity and intrinsic viscosity values of the produced chitosan were 94, 5.05, 3.21 and 10.13, respectively. Moreover, produced chitosan has water holding capacity and oil holding capacity compared with commercial chitosan. Our results were in the same line with those obtained by (31, 32).

Using chitosan in minced beef preservation

Microbiological criteria

The influence of adding chitosan and sorbistat-K into minced meat, on the amount of microorganisms, is presented in Figure 1. It was evidenced that the addition of 0.02% chitosan had the strongest effect for lowering the microbial count toward all examined microbial species. The combination of chitosan and sorbistat-K, at percentage of 0.01% from each, was the second strongest supplement, whereas the application of sorbistat-K was the weakest treatment to reduce microbial count. The total

Table 1: Proximate chemical composition of shrimp waste (on dry weight basis)

	Shrimp wastes
Moisture	74.38
Crude protein (N x 6.25)	11.55
Ether extract	6.47
Ash	32.21
Crude fiber	7.81
Total carbohydrates	41.96
Chitin	36.43

Total carbohydrates were calculated by difference

Table 2: Effect of process variables on chitosan producing by microwave from shrimp waste

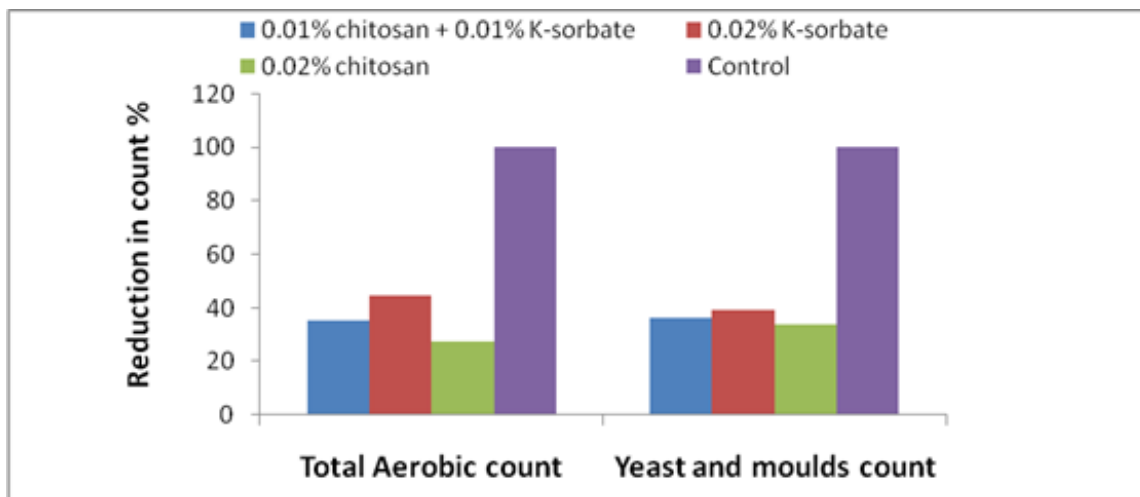
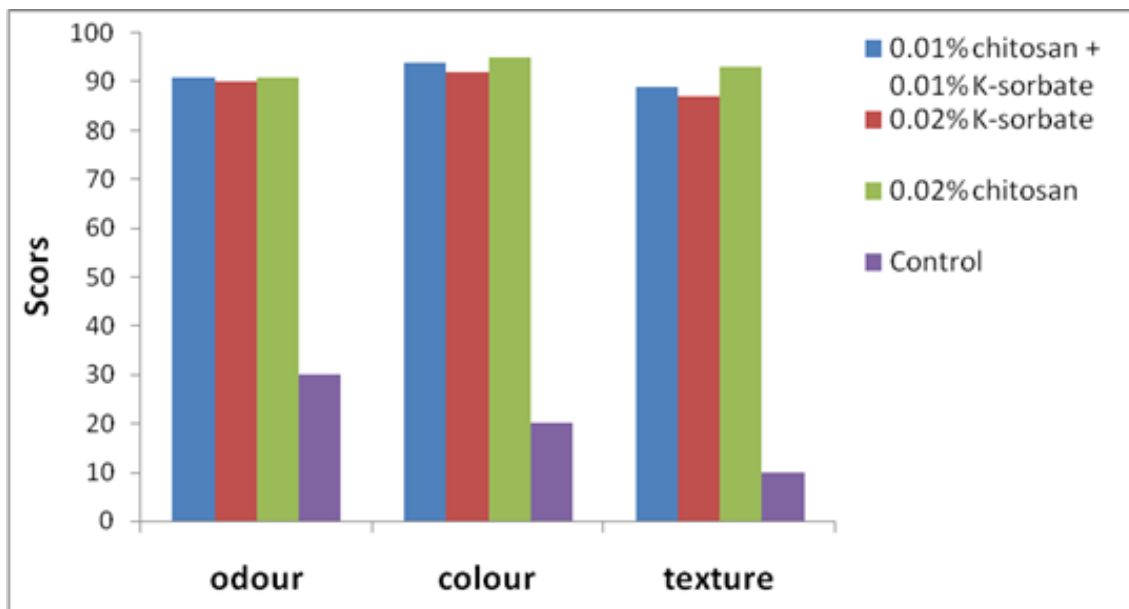
Type of experiment	Sodium hydroxide Concentration (%)	Ratio (sample/solvent)	Time (min)	Microwave power (W)	Chitosan yield (%)
Sodium hydroxide concentration					
	30	1:10	5	800	52.40
	40	1:10	5	800	52.80
	50	1:10	5	800	53.20
Sample/solvent ratio					
	50	1:10	5	800	53.20
	50	1:15	5	800	60.40
	50	1:20	5	800	67.40
	50	1:25	5	800	63.10
Microwave power					
	50	1:20	5	800	67.40
	50	1:20	5	900	60.40
	50	1:20	5	1000	54.80
Time					
	50	1:20	5	800	67.40
	50	1:20	10	800	69.83
	50	1:20	15	800	61.09

Table 3: Gross chemical composition Isolated chitosan

Components %	Samples chitosan
Moisture	8.30
Crude protein (N x 6.25)	0.98
Ether extract	1.3
Ash	0.31
Crude fiber	2.21
Total carbohydrates	89.11

Table 4: Physicochemical properties of prepared chitosan

Components %	Samples	Produced chitosan	Commercial chitosan
Degree of deacetylation		93	85
Molecular weight (k Daltons)		4200	300
Solubility		94	99
Water holding capacity		5.05	3.12
Oil holding capacity		3.21	1.56
Intrinsic viscosity		10.13	15.92

**Figure 1:** Effect of treating minced beef with chitosan and potassium sorbate on microbial count**Figure 2:** Effect of treating minced beef with chitosan and potassium sorbate on sensory characteristics

Sensory evaluation

There was a significant improvement in the values of sensual attributes of minced beef as a result for usage of chitosan as a preservative. After ending the storage duration, the sensory attributes (e.g. colour, odour and texture) values of untreated beef samples were highly poor, compared with other samples contained chitosan or sorbistat-K (Fig. 2). Chitosan get a higher efficiency than sorbistat-K in maintaining meat texture and odour quality, whilst, meat samples integrated with sorbistat-K had the highest values in colour. The average for texture, odour and colour were 93.1, 91.0 and 94.9% for 0.02% chitosan preserved meat; 87.1, 89.9 and 92.0% for 0.02% sorbistat-K and 9.9, 30.0 and 20.1 % for control, respectively. Lipid auto-oxidation and myoglobin-oxidation are the essential reason for meat discoloration and oxidative rancidity or another un acceptable odour or flavour components (36). With minimizing the fat auto-oxidation level, there is a prospective that the deterioration in red meat colour will be minimized as they are related reactions (37).

Conflict of interest

The authors declare that they have no conflict of interest.

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