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Original Article

Effect of chitosan on apple juice quality

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Abstract

The present study was undertaken to prepared chitosan by chemical method from shrimp shell and effect it's on the qualities of apple juice including chemical, microbiological and sensory qualities were examined , The results showed of addition of the chitosan as a clarifying agent was 0.4g/l at 25±2 Cº and 60min statistical analysis's showed that no significant on sensory character when added chitosan at 0.2-1g/l in apple juice, chitosan inhibited the growth of some spoilage of bacteria, mold and yeast , the analyses showed that increases in chitosan concentration extended the quality of the apple juice significantly (0.05),increasing pH range values (3.71-4.22) , reducing Brix value, enzymatic and non-enzymatic browning and controlling the spoilage during the storage time.

Introduction

Chitosan is a high molecular weight and a linear polymer that is composed of ß-1,4 linked glucosamine (GlcN) with various quantities of N-acetylated GlcN residues, Its is normally obtained by the alkaline deacetylation of chitin extracted from on a boundant source of shell fish exoskeletons or the cell walls of some microorganism and fungi (Shahidi *et al*.,1999).

Experimental evidence regarding the toxicity of chitosan has shown that it is not toxic , safe, and increase IgM production in human hybridoma cells (Hirano *et al*., 1990), chitosan already has a number uses in the food industry , Furda(1980) patented its use as a lipid-binding food additive , Knorr(1983) reported its emulsification property , dye absorption capacity , bioactivity and toughness makes it an attractive material for food industry, in the preservation of fruits, it has been used as a coating and antifungal agent , resulting in increase quality and storage ability (El-Ghaouth *et al*.1991) , Imery and Knorr(1988) also reported the use of chitosan for the reduction of titritabe acidity and color index of carrot and apple juice.

Processing of clarified fruit juices commonly involves the use of clarifying aids , including gelatins bentonite , silica sol, , tannins or combinations of these compounds (Soto-Peralta *et al*., 1989; Chatterjee *et al*.,2004) , chitosan with a partial positive charge has been shown to possess acid-binding properties (Imery and Knorr,1988) and to be effective in aiding the separation of colloidal and dispersed particles from food processing wastes (No and Meyers, 2000) While thermal pasteurization of juice significantly reduces the risk of food born disease , it can also reduce the nutrient content for this reason the scientific community and food industry focus their attention on non-thermal emerging technologies and alternative novel treatments to control undesirable microorganisms with less adverse effects on quality (Rosenthal and silva, 1997; Cardello *et al*., 2007)

There is little documentation on use of chitosan in juice , therefore present investigation was carried out to study the effect of utilizing chitosan on apple juice then study chemical and physical properties its , storage studies were also performed.

Material and Methods

Materials

Pink shrimp (*Parapenaeus longirostris*) waste was provided from local market of Basrah city including heads, shells and tails were removed from whole body for chitin and chitosan extraction, apple was purchased from a local market on the day of preparation.

Chitin and chitosan preparation

Chitin and chitosan were prepared from shrimp shell waste according to (limam *et al* ., 2011)

Preparation of apple juice

Apples were first washed with tap water after transported to the boratory , were detached from the nuts , then apples were cut and ground by a mixer (blender) the juice obtained by pressing the ground material was filtered through cheese cloth and then stored frozen at -18 ºc for different analysis.

Clarification of apple juice

Clarification of apple juice is normally performed by addition of clarification agent (chitosan concentration used from 0.2 to 1 g/l of juice , Similar experiments were conducted using of gelatin as clarifying aids for juice.all apple juice samples were flocculated at 25±2 Cº for(30,60,90) min. , the clear juice was obtained by measured optical density at 540 nm in a spectrophotometer (Sunny UV, 7804 , Japan) after centrifugation at 5000 rpm for 10 min. , unclarified juice was used as a control for comparison among clarification treatments , the clarified juice was stored until analysis at 4C(Oszminanski and wojdylo, 2007).

Quality parameters

Total soluble solids (TSS) were determined by using Abbe refractometer at 20ºc for apple juice with chitosan concentration used from 0.2 to 1 g/ L of juice in 0-10 day.

pH reading was determined using standard pH meter and glass electrode for apple juice with chitosan concentration used from 0.2 to 1 g/ L of juice in 0-10 day.

Potential browning was measured according to the methodology of Vina and Chaves , (2006), 10 ml of fresh apple juice was treated with ethanol (95%) for 60 min and then centrifuged at 4830 x g at 5ºc for 10 min , retaining the supernatant , after a further amount of ethanol was added to bring the final volume to 25 ml , absorbance at 320 nm of aliquots of these extracts was measured.

Microbiological examinations were done according to the standard method outlined by APHA(1984), these examination were included total aerobic count bacteria and yeast and mold count .

Sensory panel evaluation

The end products were presented to a taste panel of 10 judges selected randomly from colleagues at the Agriculture college/ Basrah university as well as expatriates , each panelist was presented with two samples , the panel asked to rate the product hedonically using a scale of 1 to 9 , where 1 was dislike extremely (Larmond , 1970 ; Fellers, 1985) the data so obtained was statistically analyzed by the analysis of variance method (Steel and Torrie , 1980) , the F value was calculated and the significance of probability at 5% level was determined .

Results and discussion

Chitin and chitosan production : from these results , it was observed that for chitin and chitosan production in alkaline (NaOH) concentration (3%) and acidic (HCl) concentration (1.25N)chitin was produced 9 g and chitosan was produced 8.7 g , the results indicate that when acid and base concentrations are increase in demineralization chitosan production slightly decrease due to extensive demineralization and deproteinization , the removal of protein , lipid , pigments and other inorganic acid bring a more white color end product (Fig.1), the end product which contains were chitin , must be whiter in color and the little brownish will be the lowest grade of end product which contain lowest chitin content due to incomplete demineralization and deproteinization , the products' (chitin and chitosan) were white and good quality and these concentrations are economic and safe to environment as it leaves less residual acid to soil.

Clarifying agent: The effect of chitosan on the clarification of apple juice vis gelatin is presented in Table.1 result showed were more than chitosan compared with gelatin in different time periods. The OD were (0.48 , 0.79) in conc. 0.2, 0.4g /L after 60,90 min. for chitosan and gelatin respectively from treatment ,and juices showed increased in luminosity with increasing chitosan concentration, these results could be explained by the clarification effect described in fruit juices associated to chitosan (Soto-Peralta *et al*., 1989 ; Chatterjee *et al*.,2004) , this result agreement with Yuanjun *et al*.(2006) were found chitosan 0.2 g/l , clarify time was 1h , the temperature of the juice was 35 ºC, gelatin commonly used as coagulant had been found to be much less effective in this respect than chitosan , processing of clarified fruit juices commonly involves the use of clarifying agents , including gelatin , bentonite , silica sol , tannins (simpson *et al*. 1994) , chitosan salts which carry a strong positive charge , have been shown to be effective as dehazing agents , chitosan is a good clarifying agent for grape fruit juices (Chen and Li , 1996) and highly effective fining agent for apple juice , which can afford zero turbidity products with 0.8 kg/ m^3 of chitosan (Soto-perlata *et al*., 1989) .Chaterjee *et al*.(2004) observed a reduction of 73, 76, 72 , and 61% of color using chitosan to clarity apple ,grape, lemon and orange juices ,respectively. Domingues *et al*.,(2012) were found that treatments employing chitosan ensures a turbidity reduction of almost 100% regarding to the raw juice or even to the juice centrifuged at 4000rpm , similar behavior is observed regarding to color removal.

Table.1 Effect of clarifying agents on the turbidity of apple juice

	Optical Density (540nm)										
Time (min.)	Control	Gelatin Conc.					Chitosan Conc.				
		0.2	0.4	0.6	0.8	1	0.2	0.4	0.6	0.8	1
30	1.42	1.36^{a}	1.20^{b}	1.09^{c}	0.97^{d}	0.97^{e}	1.22^{a}	0.88^{b}	0.92^{c}	0.91^{d}	0.89^{d}
60	1.42	122^{a}	1.05^{b}	0.97^{c}	0.92^{d}	0.90^{d}	1.03^{a}	0.51^{b}	0.61^{c}	0.61^{c}	0.59^{d}
90	1.42	1.15^{a}	0.98^{b}	0.92^{c}	0.79^{d}	0.80^{d}	0.86^{a}	0.48^{b}	0.48^{b}	0.49^{b}	0.50

Fig.1 picture of chitosan production from shrimp shell , (A) shrimp shell , (B)chitin , (C) chitosan

Total soluble solids(Brix) : The effect of chitosan on the TSS of apple juice presented in Fig.2 the resulted showed that chitosan reduced the Brix value of the apple juice , no significant changes over storage time were observed , this is in agreement with other authors (Rivas *et al*., 2006 ; Cartes *et al*. 2008), the Brix values in fresh apple juice were higher than those found in previous studies by the authors in juices enriched with chitin (9.8 to 11.4), these differences in the fresh juice without chitosan might be due to different factors , i.e genetic , growing environment , management practices and maturity time of the fruit (Quadir *et al*. 2006) the significant (0.05) quadratic reduction of Brix with the concentration of the positive charged polysaccharide to coagulate suspended solids, increasing the flocculation capacity of chitosan which could bind the sugar (Negatively charged)(Sapers, 1992).

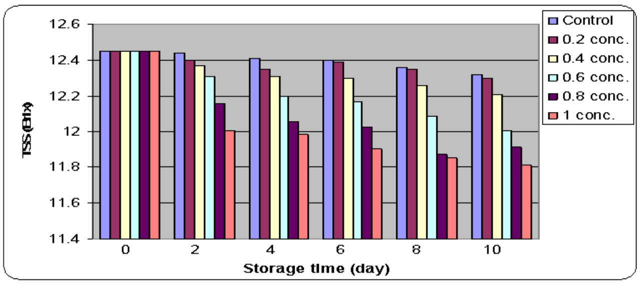

Fig.2 Effect of chitosan on TSS of apple juice in different time

pH : The effect of chitosan on the pH of apple juice presented in Fig.3 pH was significantly(0.05) affected by chitosan concentration and storage time , the pH increased significantly (0.05) with increasing chitosan concentration , this resulted in relatively high pH range values (3.71-4.22) can be observed in Fig.3 compared with previous studies (Kelebek *et al*. 2008), in fresh orange juice , who found pH values between 3.05 and 3.35 , while Martin-Diana *et al*. (2005) observed higher pH value in juices enriched with chitin , which ranged between 3.2 and 4.2 , this effect could be due to the capacity of chitosan to reduce fruit juice acidity (Imeri and Knorr , 1988) based on its acid-binding properties , when the ph is lower than 6.5 , chitosan carries appositive charge along its backbone (Einbu and Varum , 2003) , this might imply a decrease in the buffering property of the juice , but such effect was not observed in this study , as all the samples showed a similar ph increase over storage time and no interaction effect resulted from storage time and chitosan concentration. Also the ph increase the juices undergo over storage time has been associated to microbial spoilage (Cortes *et al*. 2008)

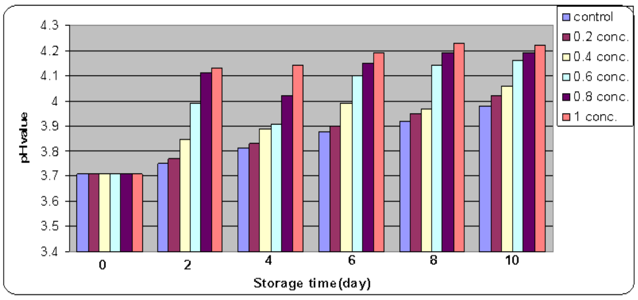

Fig.3 Effect of chitosan on pH of apple juice in different time

Browning potential: An interactive effect between chitosan concentration and storage time on the BP was also observed. In samples with lower or no chitosan concentration the BP increased rapidly over storage time, while it remained almost constant in samples with higher chitosan concentration (Fig. 5). Chitosan has been found to enhance the control of enzymatic browning in apple and pear juice (Sapers, 1992). This datum showed a significant relationship between chitosan concentration and inhibition of browning. The control of browning could be associated with the capacity to coagulate solids to which browning-related enzymes are bound. The antioxidant capacity of chitosan, similar to the capacity associated with phenolic compounds (Park *et al*., 2004), could also explain this browning reduction by inhibiting the oxidative process.

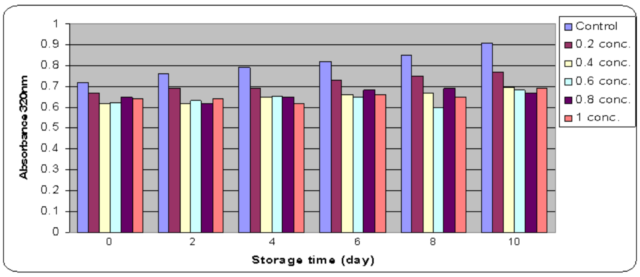

Fig.4 effect of chitosan on Browning potential of apple juice in different time

Microbiological tests: The log numbers of total count of bacteria and mold and yeast in juice during storage at 30 ºc and 4ºc for 30 day were also inhibited by presence of chitosan as shown in table (2) and (3)

Table.2 Change of microbial count (Log CFU/ml) of juice with added chitosan during storage at 30ºC for 30 day

Microorganisms	Storage (days)	Chitosan concentration(%)			
		0	0.01	0.1	1
Total bacteria	0	0.98^{a}	0.98^{a}	0.67^{b}	0.45^{c}
	5	2.48^{a}	2.45^{a}	2.45^{a}	2.31^{b}
	10	3.04^{a}	3^{a}	2.97^{b}	2.90^{c}
	15	3.30^{a}	3.21^{b}	3.09^{c}	2.97^{d}
	30	3.69^{a}	3.58^{b}	3.21^{c}	3.00^{d}
Yeast and mold	0	2.98^{a}	2.60^{b}	2.36^{c}	1.46^{d}
	5	4.48^{a}	4.42^{b}	4.01^{c}	3.20^{d}
	10	6.04^{a}	5.81^{b}	4.12^{c}	2.33^{d}
	15	6.30^{a}	6.11^{b}	5.27^{c}	4.51^{d}
	30	6.99^{a}	6.78^{b}	6.30^{c}	6.06^{d}

Table.3 Change of microbial count (Log CFU/ml) of juice with added chitosan during storage at 4ºC for 30 day**.**

Microorganisms	Storage (days)	Chitosan concentration (%)			
		0	0.01	0.1	1
Total bacteria	0	0.98^{a}	0.98^{a}	0.66^{b}	0.43^{c}
	5	2.45^{a}	2.41^{b}	2.40^{b}	2.26^{c}
	10	3.04^{a}	2.45^{b}	2.38^{c}	2.35^{c}
	15	3.30^{a}	3.00^{b}	2.95^{b}	2.91^{b}
	30	3.68^{a}	3.52^{b}	3.13^{c}	2.95^{d}
Yeast and mold	0	2.98^{a}	2.58^{b}	2.36^{c}	1.40^{d}
	5	4.48^{a}	4.40^{b}	3.93^{c}	3.11^{d}
	10	4.81^{a}	4.65^{b}	4.41^{c}	4.28^{d}
	15	6.04^{a}	5.80^{b}	4.10^{c}	2.31^{d}
	30	6.31^{a}	6.02^{b}	5.22^{c}	4.47^{d}

The mode of inhibition of chitosan on the growth of bacteria and fungi might be due to the interaction of chitosan with membranes or cell wall components , the mechanism underlying the inhibition of bacterial growth is thought to be that the cationic ally charged amino-group many combine with anionic components such as N-acetylmuramic acid , sialic acid and neuraminic acid on the cell surface , and may suppress bacterial growth by impairing the exchanges with the medium , chelating transition meal ions and inhibiting enzymes , due to the positive charge , resulting in increased permeability of the membranes and leakage of cell material from tissue , or due to water binding capacity and inhibition of various enzymes by chitosan (Jung *et al*. 1999 ; Limam *et al*. 2011). Chitosan also has bio absorption activity (Knorr , 1991) and can absorb nutrients of bacteria and many inhibit their growth .

Sensory Evaluation

Results of the sensory (hedonic) evaluation of using chitosan in apple juice are presented in table (4) . In all the treatments, the average score score ranged between 7-9 for colour ,taste and overall acceptability on a 9 – point hedonic scale , the data are the mean scores of 10 response . these data showed increased significantly with increasing chitosan concentration were observed for color , taste and overall acceptability of the control . This result agreement with chattejee *et al*.(2004) were found improvement of acceptability and appearance when treated with chitosan.

Table 4. Sensory evaluation scores of apple juice enriched with chitosan

Treatment		Characteristics evaluated		
		color	Taste	Overall acceptability
	Control	6.15^a	7.02^a	6.65^a
Chitosan Conc.	0.2	6.92^b	7.11^b	7.08^b
	0.4	7.19^b	7.13^b	7.18^b
	0.6	7.82^c	7.00^b	7.56^c
	0.8	8.01^d	7.20^c	7.70^d
	1	8.36^e	7.05^b	7.85^d

Conclusion

This paper shows the possible use of chitosan as a natural preservative ingredient to extend the shelf-life of fresh apple juice, variations in chitosan concentration in the range evaluated (0 to 2 g/l) were critical in some of the parameters studied i.e flavour , color , and total microbial load in the extension of the quality of the apple juice , especially in parameters such as browning and reduction of microbial counts .

Reference

America Public Health Association (1984) Standard Method of the Examination of Dairy Products.14th ed, A.P.H.A Inc Washington. D.C., U.S.A.

Cardello,A.V.; Schutz, H.G., and Lesher,L.L.(2007). Consumer perceptions of foods processed by innovative and emerging technologies. 8:73-83.

Chatterjee, S.; Chatterjee,S.; Chatterjee,B. and Guhas,A.(2004). Clarification of fruit juice with chitosan. Process Biochemistry. 39:2229-2232.

Chen, Y.and Li,C.(1996). Studies on the application of chitosan to clarification of grapefruit juice. Food science , Taiwan , 23:617-628.

Cortes, C.;Esteve,M.J. and Frigola, A.(2008). Colour of orange juice treated by high intensity pulsed lectric fields during refrigerated storage and comparison with pasteurized juice. Food control, 19:151-158.

Domingues, R.C.C.;Junior,S.B.F.;Silva,R.B.;Cardoso, V.L. and Reis, M.H.M.(2012). Clarification of passion fruit juice with chitosan: Effects of coagulation process variables and comparison with centrifugation and enzymatic treatments .Process Biochemistry, (47):467-471.

Einbu, A. and Varum, K.M.(2003).Structure –property relationship in chitosan in T. Priotr (Ed.), Chemical and functional properties of food sacchaides pp:223.

Elghaouth, A., Arul, J.; Ponnampalam,R. and Boulet, n.(1991). Chitosan coating effect on storability and quality of fresh strawberries, J. Food Sci., 56:1618-1620.

Fellers , P.J.(1985). Citrus. Sensory quality as related to root stock, cultivator, maturity and season. In "Evaluation of quality of fruit and vegetables." (Pattee, H.E.P. ed.) 83 Avi publishing co., Westport, C.T.

Hirano, S.;Itakura,C.; Seino,H.; Akiyama,Y; Nonaka, I.Kanbara,N. and Kawakami, T. (1990).Chitosan as an ingredient for domestic animal feeds.J.Agric.Food Chem. 38(5):1219-7.

Imeri, A. and Knorr,D.(1988). Effect of chitosan on yield and composition data of carrot and apple juice .J. Food Sci. 53(6):1707-9.

Jung,B.O.;Kim.C.H.;Choi,K.S.;Lee,Y.M.and Kim,J (1999) Preparation of amphiphilic chitosan and their antimicrobial activities . J. of Applied Polymer Science.72: 1713-1719.

Kelebek, H.; Canbas,A. and Selli, S.(2008).Determination of phenolic composition and antioxidant capacity of blood orange juice obtained from cvs. Moro and sanguimello (Citrus sinensis(L.)Osbeck) grown in Turkey. Food chemistry, 107: 1710-1716.

Knorr, D.(1983).Dye binding properties of chitin and chitosan . J. Food Sci.48:36-41.

Knorr, D.(1991).Recovery and utilization of chitin and chitosan in food processing waste management .Food Technol.,45:114-122.

Larmond, E. (1970). Method for sensory evalution of foods. Canada dep. Of Agric. Publ. No.1984.

Limam, Z.; Selmi,S.;Sadok,S. and Elabed ,A.(2011). Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties .African J. of Biotechnology, 10(4):640-647.

No, H.K.,Lee, K.S.and Meyers , S.P.(2000).Correlation between physicochemical characteristics and binding capacities of chitosan products . J.Food Sci. (65)7.

Oszmianski, J. and Wojdylo, A.(2007).Effects of various clarification treatments on phenolic compounds and color of apple juice .Eur. Food Res. Technol., 224:755-762.

Park, P.J.; Je, J.Y. and K im, S.K.(2004). Free radical scavenging activites of differently deacetylated chitosans using an ESR spectrometer. Carbohydrate polymers, 55:17-22.

Quadir, M.; Hickey,M.;Boulton,A. and Hoogers,R.(2006). Accumulation of total soluble solids in processing tomatoes. Acta H orticulturae, 724:97-102.

Rivas, A.;Rodrigo,D.; Martinez ,A.;Barbosa-Canovas, G.V. and Rodrigo,M.(2006). Effect of PEE and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. Food and science technology, 10: 1163-1170.

Rosenthal, A. and Silva, J.L.(1997). Foods under pressure. Food engineering .14: 37-39.

Sapers, G.(1992).Chitosan enhances control of enzymatic browning in apple and pear juice by filtration . J. Food Sci., 57(5): 1192-3.

Shahidi,F.;Arachchi, J.and Jeon ,Y.(1999).Food applications of chitin and chitosan .Trend food Sci. Technol.,10:37-51.

Simpson, B.K.; Gagane, N.and Simpson, M.V. (1994). Biotechnologyical applications, (Martin, A.M.,ed): 155-173. Chapman and Hall, London, UK.

Soto –Peralta,N.;Miller,H. and Knorr , D. (1989).Effects of chitosan treatments on the clarity and color of apple juice . j. Food sci., 54(2):495-6.

Steel, R.C.D.and Torrie,J.(1980). Principles and procedures of statistics .MC-Graw-Hill Book CO. New York.

Vina, S.Z. and Chaves ,A.S.(2006). Antioxidant responses in minimally processed celery during refrigerated storage. Food chemistry, 94:68-74.

Yuanjun, G.; Xueli,G.;Hefeng,Z.;Guanghui,W. and Yanlin, Z.(2006).Study on chitosan clarification technique of pear juice. Chinese Agricultural Scuence Bulletin, 22(11):350-355.

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