International Journal of Sciences & Applied Research

www.ijsar.in

Physicochemical and functional properties of chitosan extracted from crab (*Scylla serrata)* by different chemical processing sequence

B. V. Gaikwad, J. M. Koli*, S. T. Sharangdher, S. B. Patange

College of Fisheries, Shirgaon, Ratnagiri-415629, India.

Correspondence Address: * Dr. J. M. Koli, Assistant Professor, Fish Processing Technology and Microbiology, College of Fisheries Shirgaon, Ratnagiri-415629, India.

Abstract

Chitosan (CS) was prepared from crab shells using the same chemical process as described for the other crustacean species, with minor adjustments in the treatment conditions. The influence of modifications of the CS production process on the physiochemical and functional properties of the CS obtained was examined. For instance, changing the sequence of DC for the production of crab chitosan affected its properties. DCMPA resulted in an increase in yield (53%) and lower DD (23.63%). Though out the study, DMCPA treatment showed no changes in physicochemical and functional properties were noticed. DMPCA resulted in an increase in solubility (87.21%) and emulsion capacity (10.27%). Compared to shrimp CS (selected as an example of CS from a different crustacean source) .The physicochemical and functional properties of the prepared crab CSs were enhanced, compared to control and commercial samples, by varying the processing step sequence.

Keywords: Crab (*Scylla serrata*) Shells; Chitosan, Physicochemical and Functional properties

Introduction

Chitosan is a derivative of chitin obtained by treating the chitin in a concentrated sodium hydroxide bath at elevated temperature. The process cleaves the N-acetyl group from the C2 carbon and replaces it with a hydrogen atom. The result is an amine group (NH2), creating a natural cationic polymer, meaning it carries a positive ionic charge when dissolved in acidic solutions. Chitosan is chemically similar to cellulose, a plant fiber, and displays most of the features similar to plant fibers. Chitosan is non-toxic, non-hazardous and biodegradable, making it ideal in many uses involving humans, including food additives, cosmetics, beauty and personal care products, and medical materials.

Chitin has been known to exist since the early 1800's, but only in the last couple of decades has the extraction of chitin from crustacean shells been industrialized as a means to convert the seafood waste into something useful. Chitosan in particular has seen an increase in usage as environmental concerns with synthetic plastics increase. Also, with concerns over the usage of petroleum-based products, such as synthetic plastics, efforts are being made to use materials derived from renewable natural resources, such as chitin and chitosan.

The global market for chitosan was reported by Global Industry Analysts, Inc. in "Chitin & Chitosan: A Global Strategic Business Report" (October 2012) is forecasted to reach more than 40,645 metric tons by 2018
Chitosan (CS) and its derivatives are examples of value-added materials. They are produced from chitin, which is a natural carbohydrate polymer found in the skeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes.
Insects, such as butterflies and ladybugs, also have chitin in their wings and the cell walls of yeast, mushrooms and other fungi also contain this substance (Shahidi and Abuzaytoun, 2005; Tharanathan and Kittur, 2003). Industrial-scale CS production involves four steps: demineralization (DM), deproteinization (DP), decoloration (DC) and deacetylation (DA) (Shahidi and and Abuzaytoun, 2005; Tharanathan and Kittur, 2003). Despite the widespread occurrence of chitin in nature, presently crab and shrimp shells remain the primary commercial sources.
Chitosan is usually prepared from chitin and chitin has been found in wide range of natural sources (crustaceans, fungi, insects, annelids, molluscs, coelenterate etc.) (Tharanathan and Kittur, 2003). However chitosan is only manufactured from crustaceans (crab, krill, and crayfish) primarily because a large amount of the crustaceans exoskeleton is available as product of food processing. Crab shell is made up of three basic components. These are chitin, protein and a calcium salt of which chitin is most important for scientific studies. Chitin is a fairly completely acetylated polysaccharide in nature, being only second after cellulose (Adole and Omogbai, 2012).

Materials and methods

Preparation of chitosan

Chitin extraction from crab shells was carried out as described previously for other crustacean shells by an alkali-acid treatment with minor modifications of the treatment conditions (Fernandez-kim, 2004).
Five crab CSs labeled (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA,) were prepared by changing ofthe order of the four sequential preparation processes. For example, DPMCA denotes sequential steps of deproteinization + demineralization + decolorization + deacetylation. DPMCA was taken as the traditional processing method (control sample).
Depending upon the production order, samples (referred to as crab shells, demineralized or decolorized samples) were deproteinized by treating with 3.5% (w/w) NaOH solution for 2 hrs at 65^0C with constant stirring at a solid to solvent ratio of 1:10 (w/v) (No *et al*., 1989). Demineralized at room temperature with 1N HCL for 30 min at ambient temperature with a solid to solvent ratio of 1:15 (w/v) (No et al., 1989). for 15 min and decolorized with acetone for 10 min and dried for 2 hrs under hood, followed by bleaching with 0.32 % (v/v) solution of sodium hypochloride (containing 5.25% available chlorine. After each step, the solid was filtered off, washed with distilled water to neutral pH. Chitin deacetylation was carried out at 15 psi/121 °C using 50% sodium hydroxide solution for 15 min. After this step, samples were filtered off, washed with distilled water to neutral pH and dried in an oven at 60 °C for 24 hrs. Commercial shrimp Chitosan was used as a to compare with the crab CSs produced in this study.

Determination of physicochemical and functional properties: yield, moisture, ash and nitrogen content

Chitosan (CS) yield was determined by comparing weight measurements of the raw material and of the CS obtained after treatment. Moisture content of the samples was determined according to the standard method (AOAC, 1999) with minor modification. Moisture of samples was determined by drying the samples at 60°C for 24 hrs or until the weights were constant. It was then calculated by percentage of weight loss comparing to the initial weight of the samples. Ash and nitrogen contents of CSs were measured according to a previously described procedure (AOAC, 1999).

Determination of viscosity, Degree of deacetylation

Chitosan solutions at the concentration of 6.67% (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60 ^{0}C for the determination of viscosity. The viscosity (cP) of 10 ml of the solution was determination using Brookfield digital viscometer (Model DV –E Brookfield Engineering, USA) equipped with a No.1 spindle at 40 ^{0}C ±1 ^{0}C (Cho *et al*., 2006).

However, the DD The degree of deacetylation of chitin and chitosan were measured according to Pradhan and Bedakar, (2002) For actual determination, 1 g dried chitosan sample was taken and refluxed with 20 ml of 12 N sulfuric acid for twenty minutes. The clear solution obtained was cooled and 50 ml distilled water was added to it. The resulting acetic acid was distilled out and titrated directly with 0.1 N sodium hydroxide until end point was pink using phenolphthalein as an indicator. Taking into consideration the molecular weight of chitin $(C_8H_{13}NO_5)n$ for 100% acetylation and that of chitosan $(C_6H_{11}NO_4)n$ for 100% deacetylation did the calculations for degree of deacetylation

Emulsifying capacity

The method of Yasumatu *et al*, (1972) was used to determine emulsifying capacity. Emulsions were prepared with 1g of each sample, 50 ml of cold distilled water (4 ^{0}C) and 50 ml of sunflower oil. The gelatine samples were dispersed with a homogenizer/blender. Each blended samples was equally into 50 ml of centrifuge tubes. One centrifuge tubes was directly centrifuge at 4000 × g for 10 min while the other was centrifuged under the same conditions after heating in a water bath at 80 ^{0}C for 30 min and cooling to room temperature (25 ^{0}C). The hight of emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying capacity, using following formulae:

Statistical analysis

The data were analysed using appropriate statistical method (Snedecor and Cochran, 1967; Zar, 2005). Using ANOVA technique significant difference between the treatments was known. The significant difference between the mean of treatments was further subjected Tukey Test.

Results and discussion

The present work represents the first attempt to investigate various physicochemical and functional properties of crab chitosan. The variation in physicochemical and functional properties of crab CS with changes in the five sequential processes of preparation was investigated. The results are shown in Figures 1-4 and Tables 1-4

Figure 1 presents the percentage yields of chitosan and CS from crab obtained in this study. The different CS, labeled DCMPA, DMCPA, DMPCA, DMPAC and DPMCA were prepared by changing the order of the five sequential preparation processes. For example, DPMCA denotes sequential steps

of deproteinization + demineralization + decolorization + deacetylation. DPMCA represents the traditional processing method and was selected as the control sample.

The yields depended on the CS extraction method, as DCMPA gave the highest CS yield (53%) and DMPAC gave the lowest (41%)but was not different from the control DPMCA (42%). Brzeski, (1982) reported 14% yield of chitosan from krill and 18.6% from prawn waste (Alimuniar and Zainuddin, 1992).

The results of this work demonstrated that there was significant difference in the % moisture (2.37 and 5.4 %) between the five CSs prepared from crab chitosan (Table 2). on a dry basis compared with 2.42% for shrimp chitosan. Since CS is ahygroscopic polymer (Khan *et al*., 2002), it is possible that the commercial samples were affected by moisture absorption during storage (Fernandes-kim, 2004). The moisture adsorption may be important by affecting water holding capacity of CSs, when it comes to its processing and applications (Chandumpai *et al*., 2004).

Ash content of crab chitosans compared with that of the commercial shrimp chitosan. The crab chitosans contained less than 3% ash with a range of 1.82 % to 3.02 % **(Table 2).** Commercial shrimp chitosan products contained less than 3 % ash. Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of calcium carbonate. Elimination of the demineralization resulted in products having 31 - 36% ash (Bough *et al*., 1978). The ash content in chitosan is an important parameter.A high qualitygrade of chitosan should have less than 1% of ash content (No *et al*., 1995).

No and Meyers (1995) have shown that the nitrogen content of CSs from various sources ranged from 7.06 to 7.97%. In this study, the nitrogen content of the CS products was in the 0.9% - 1.91% range (Table 2).

Effects of sequential process modifications on CS Viscosity, solubility and DD

The viscosity, degree of deacetylation (DD) and solubility resulting from the various CS preparation methods are shown in Table 3. Among the five samples and the commercial CS used in this study, DMPCA showed the highest viscosity (33.6cP) while the sample (DMPAC) had the lowest viscosity.

This was in contrast to the findings of No and Meyers, (1995) who demonstrated that the viscosity of CSs varied considerably, from 60 to 5110 cP, depending on the species and preparation methods used. In our study, significant differences were found between the viscosity of DCMPA samples (33.6cP) and commercial CS (126cP), which was higher than average of crab CS.

The DD is an important parameter affecting solubility, chemical reactivity, and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 95% (Martino *et al*., 2005). This study (Table 3) revealed that, DD was >70% for all CSs except for DMPCA, which had a DD of 67%. The results from the present work showed that the Artemia cyst CS had a lower degree of acetylation than crustacean CSs (Fernandez-kim 2004).

Brine and Austin, (1981) noted that lower solubility values suggest incomplete removal of protein. Since the chemical basis of this method is based on the reaction with the amino group, the presence of protein contaminants remaining in the sample during the analysis process could adversely interfere with the results.

In the present study, crab chitosan samples and the commercial chitosan, demonstrated an excellent solubility ranging from 81.78 to 88.78 % with significant difference, while

the DCMPA showed lower solubility (81.78%).

Effects of sequential process modifications on CS Bulk density, Emulsion capacity

According to Cho *et al*., (1998) and Brine and Austin, (1981) the bulk density of crawfish and commercial chitin and chitosan varies, and this can be attributed to species or sources of chitosan and the methods of preparation.

In the present study, bulk density of crab chitosan samples were in the range of 0.75 – 1.08 g/ml. commercial shrimp chitosan observed bulk density 0.35g/ml and highest bulk density of crab chitosan DMPAC (1.08g/ml) (Table 4).

Cho and No, (1999) noted that lower bulk density may indicate that the chitosan is more porous and may have been subjected to a lower alkali concentration treatment for deproteinization.

In the present study, crab chitosan samples demonstrated an emulsion capacity ranging from 3.35 to 10.27 % (Table 4) and commercial shrimp chitosan the emulsion capacity from 9.65%.highest emulsion capacity of crab chitosan DMPCA (10.27%).stabilizer, suspending agent or film former.

Del Blanco *et al.,* (1999) stated that the degree of deacetylation is a determining factor in the emulsifying properties of chitosan, and chitosan with intermediate DD is a less effective emulsifier while chitosan with higher DD tends to produce poor emulsification. The optimum %DD of chitosan for sunflower oil emulsification is 81 and 89. In our study, the DD of samples ranged from 23.63% to 52.17% yet they still had an effect on emulsion.

Table 1: Crab chitosan production yield (dry weight basis).

Sample	Yield (%)
DCMPA	53
DMCPA	49
DMPCA	52
DMPAC	41
DPMCA(CONTROL)	42

Table 2: Percentage ash, moisture and nitrogen of crab and commercial shrimp chitosan samples.

Chitosan samples [a]	Ash %	Moisture%	N %
DCMPA	2.67 ± 0.49	4.6±0.21	1.23 ±0.02
DMCPA	2.45 ±0.45	5.42±0.33	1.24 ± 0.02
DMPCA	1.87 ± 0.26	5.37±0.21	1.3 ± 0.03
DMPAC	2.55 ±0.17	2.37±0.33	0.9 ± 0.03
DPMCA(CONTROL)	3.02 ± 0.22	3.27±0.17	1.91 ± 0.02
SHRIM CHITOSAN	2.8 ±0.29	2.42±2.28	1.98 ±0.02

Mean ± standard deviation of triplicate determinations

Table 3: Viscosity (cP), Solubility (%) and Degree of deacetylation (%) of crab and commercial shrimp chitosan samples.

Chitosan samples	Viscosity (cP)	Solubility (%)	Degree of deacetylation (%)
DCMPA	33.2 ±3.09	81.78±0.50	23.6375±0.12
DMCPA	32.4±2.87	87.13±0.42	40.1775±0.05
DMPCA	33.6±3.30	87.21±1.25	23.645±0.25
DMPAC	32.2±2.5	86.56±2.16	40.7325±0.54
DPMCA(CONTROL)	33±3.316	86.43±1.40	52.175±0.17
SHRIM CHITOSAN	126±11.08	88.55±0.92	68.175±0.17

Mean ± standard deviation of triplicate determinations

Table 4: Emulsion capacity (%), Bulk density (g/ml) of crab and commercial shrimp chitosan samples.

Chitosan samples	Emulsion capacity (%)	Bulk density (g/ml)
DCMPA	9.37±0.30	0.89±0.006
DMCPA	9.42±0.33	0.96±0.008
DMPCA	10.27±0.35	0.92±0.01
DMPAC	3.35±0.36	1.08±0.02
DPMCA(CONTROL)	5.45±0.36	0.75±0.08
SHRIM CHITOSAN	9.65±0.20	0.35±0.08

Mean ± standard deviation of triplicate determinations

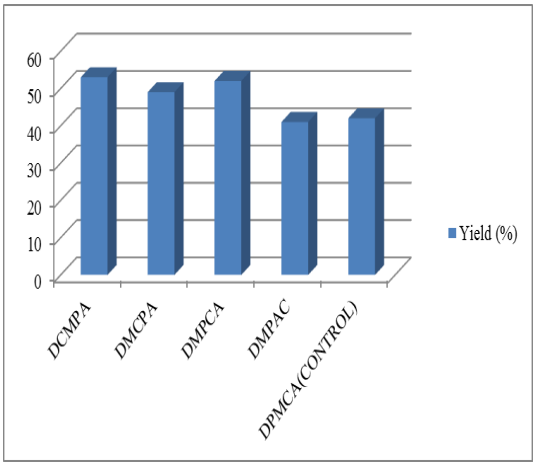

Fig. 1: Crab chitosan production yield (dry weight basis).

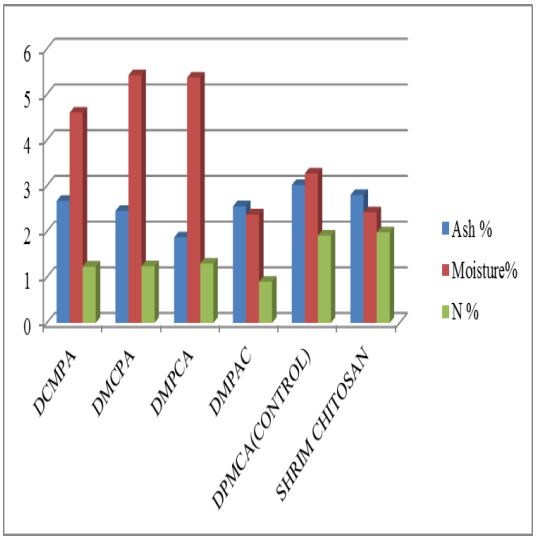

Fig. 2: Percentage (%) Ash, moisture and nitrogen of crab and commercial shrimp chitosan samples.

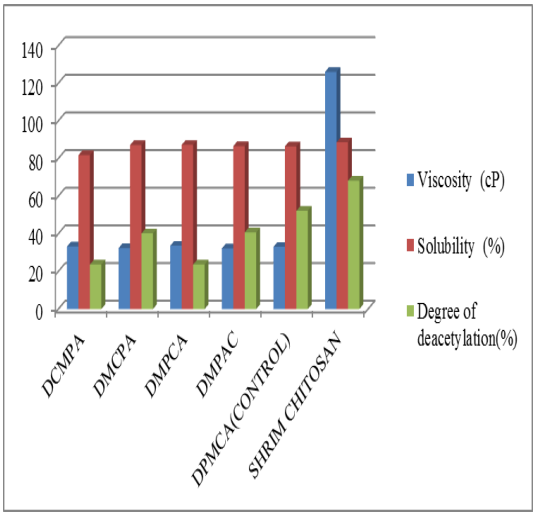

Fig. 3. Viscosity (cP), Solubility (%) and Degree of deacetylation (%) of crab and commercial shrimp chitosan samples

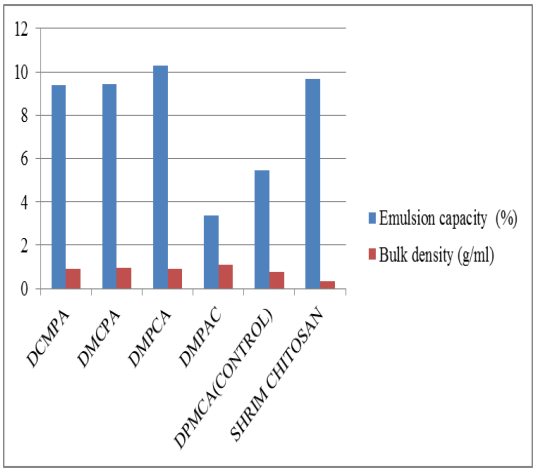

Fig. 4. Emulsion capacity (%), Bulk density (g/ml) of crab and commercial shrimp chitosan samples

Conclusions

Throughout the literature on chitosan, the main emphasis is on its quality and physicochemical properties which vary widely with crustacean species and preparation methods. Upon this emphasis, this research study was attempted to monitoring the modification of processing protocols of the chitosan production using crab shell waste, and to determine whether such modifications had any effect on the various physicochemical and functional properties of chitosan. From our results, we found that specific physicochemical and functional properties of chitosan have affected by process protocol alteration/modification.

Overall, the results indicated that process modification in crab chitosan production yielded some differences on each characteristic over the control and commercial products. However, it will be very ambiguous to conclude that only one modified process is the optimum for the production of chitosan because the interests of applications may vary from one study to another and even from one industry to another, and as seen in our study. In view of the foregoing, it is our recommendation that for the purpose of achieving uniformity and proper product quality control for particular usage of chitosan, the relationship between the process protocols/conditions and the resulting specific characteristics of chitosan products must be monitored constantly and properly.

In this review, an attempt has been made to increase the understanding of the importance and characteristics of the chitosan by describing various aspects, including the chemical properties, biological properties, processing and applications. In view of this, the present study will attract the attention of entrepreneurs, industrialists, academicians and environmentalists. Fish processing industries produce enormous crustacean shell waste were discarded, is a good waste management practice leading to additional economic benefit by producing chitin and chitosan, for upliftment of socioeconomic status of coastal people. However, chitin and chitosan is a biodegradable product therefore, it helps to maintain the environmental sustainability.

References

Adole, T., Omogbai, B. A., 2012. Antibacterial effect of crab shell extract on klebsiell pneumoniae and propers mirabilis. *J. of pharmacy and biol sci.*, 01 - 06

Alimuniar, A., Zainuddin, R., 1992. An economical technique for producing chitosan. In Advances *in Chitin and Chitosan* (Brine *et al*., Ed) .627. *Elsevier Applied Science, Essex, UK.*

AOAC. 1999. *Official methods of analysis of the Association of Official Analytical Chemistry*, 15th ed. The Association of Official Analytical Chemistry Inc: Washington, DC,USA

Bough, W.A., Salter, W.L., Wu, A.C.M., Perkins, B.E., 1978. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. 1. Chemical composition, viscosity, and molecular weight distribution of chitosan products. *Biotec. Bioeng*., 20, 19-31.

Brine, C.J., Austin, P.R. , 1981. Chitin variability with species and method of preparation. *Comp.Biochem. Physiol*. 69(B), 283-286.

Brzeski, M.M. 1982. Concept of chitin/chitosan isolation from Antarctic Krill (Euphausia superba) shells ona technique scale. In *Proceedings of the Second International Conference on Chitin and Chitosan;* (S.Hirano and S. Tokura Ed.), 15. The Japan Society of Chitin and Chitosan, Sapporo, Japan.

Chandumpai, A., Singhpibulporn, N.,Faroongsarng, D., Sornprasit, P., 2004. Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, *Loligolessoniana* and *Loligo* formosana. CarbohydPolym.**,** 58, 467-474.

Cho, S.M., Jahncke , M.L., Chin, K.B. , Eun, J.B., 2006. The effect of processing condition on the properties of gelatin from skate (Raja *Kenojei*) skins. *Food hydrocolloid.* 20, 810-816.

Cho, Y.I., No, H.K., Meyers, S.P., 1998. Physicochemical Characteristics and Functional Properties of various Commercial Chitin and Chitosan Products. J. of Agr.l and Food Chemi. 46(9), 3839-3843.

Cho,Y.I., No, H.K., 1999. Effective Deproteinization Under Autoclaving Conditions for Preparation of Chitin. J. of Korean Society for Chitin and Chitosan. 4(3), 152- 155.

Del Blanco L.F., Rodriguez M.S., Schulz P.C., Agullo, E., 1999. Influence of the deacetylation degree on chitosan emulsification properties. Colloid Poly Sci.. 277, 1087-1092.

Fernandez-Kim, S.O., 2004. Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols. Dissertation, Louisiana State University: Baton Rouge, La,USA,**.**

Khan, T., Peh, K., Chang, H.S., 2002. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. J. Pharm. Pharm. Sci.**,** 5, 205-212.

Martino, A.D., Sittinger, M., Risbud, M.V., 2005. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. Biomaterials**,** 26, 5983-5990.

No, H. K., Hur, E.Y., 1998. Control of foam formation by antifoam during demineralization of crustacean shell in preparation of chitin. J. of Agri. and Food Chem., 46(9), 3844 -3846.

No, H.K., Meyers, S.P., 1995. Preparation and characterization of chitin and chitosan-a review. J. Aquat. Food Prod. Tech.**,** 4, 27-52.

Pradhan, V.R., Bedekar, A.N. , 2002. Degree of deacetylation of chitosan:

Acetic acid distillation using sulfuric acid, Eco. Env. Cons. 8(4), 345-348.

Shahidi, F.; Abuzaytoun, R., 2005. Chitin, chitosan, and co-products: chemistry, production, application,and health effects. Adv. Food Nutr. Res.**,** 49, 93-135.

Snedecor,G,W., Cocharn,W.G., 1967. In statistical method, 585, Oxford and IBH publishingco.,USA.

Tharanathan, R.N., Kittur, F.S., 2003. Chitin – the undisputed biomolecule of great potential, Crit. Rev. Food Sci.Nutr., 43(1). 67-87.

Yasumatu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., Ishii, K.,1972. whipping and emulsifying properties of soybean product. Agri.Biol.chem., 36, 719 – 726

Zar,H.J., 2005. Biostastical analysis 4th edn., 663 Dorling Kindersley India Pvt Ltd., Delhi.