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Production and Characterization of Chitosan from Fish Scales

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Abstract: *The chitosan has attracted considerable interest in various fields due to its unique biological activities, such as biocompatibility, biodegradability, nontoxicity, antimicrobial activity, antitumor activity and immune-enhancing effect. These properties make chitosan a promising candidate for medicine, food, cosmetic, water treatment, biomedical engineering industries and many agricultural uses. Despite enormous applications of chitosan, there is a limited research on it. The current work is focus on extraction, production and assessment of physical, chemical and functional properties of chitosan from fish scales. Fish scales were collected from Ilesha, Osun state and sundry for 24hrs before grinding with blender. 10g of powdered form of fish scales was used for chitin extraction and chitosan production. Chitosan was produced by prepared by changing of the order of the four sequential preparation processes (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA,). DPMCA denotes sequential steps of deproteinization + demineralization + decolorization + deacetylation. DPMCA was taken as the traditional processing method (control sample). Physicochemical and functional properties such as Nitrogen content, ash content, moisture content, viscosity, solubility, degree of deacetylation, emulsion capacity, bulk density, water binding capacity and fat binding capacity.The results showed that 3.65 and 0.85g of chitin and chitosan were produced with 36.5 and 23.29% by DMCPA methods. Physicochemical and functional properties recorded were: Nitrogen content (7.23%), ash content (0.17%), moisture content (0.77%), viscosity (37cP), solubility (74.35%), degree of deacetylation (65.33%), emulsion capacity (94.61%), bulk density (1.04g/ml), water binding capacity (682%) and fat binding capacity (363.33%). Demineralization, deproteinization and deacetylation and decolorization (DMPAC) produced the best chitosan in terms of quantities and properties with respect to physicochemical and functional.*

Keywords: Chitosan, chitin, fish scales, Production, Decolourization, demenirelaization, deproteinization and deacetylation

I. INTRODUCTION

Fish industrial processing plants and fish markets produce crustaceans (shrimp, crabs, prawns, lobster, and krill) as byproducts. Every year, the shellfish processing industry produces huge waste that could be an environmental hazard. About 75% of the total weight of discarded as by-products (Kuddus and Ahmad, 2013). In Saudi Arabia, the total production of shrimp is 40 000 tons in 2010 with 95 percent exported. Exported shrimp is processed, packed and frozen and sold both heads-on and head-less (FAO, 2015). Utilizing these wastes could develop added-value products that possess physico-chemical and biological properties which can be applied in many fields.

Chitin is a natural polysaccharide and the second most abundant organic compound in nature after cellulose; it is widely distributed in marine invertebrates, insects, fungi, and yeast (Knor, 1982). Chitosan is the deacetylations process of chitin a polysaccharide (b-(1-4)-N-acetyl-D-glucosamine). Chitin is the constituent of the crustacean such as shrimps, and crabs, cartilage of the squid, and outer cover of insects, it also occur as ordered crystalline microfibrils forming structural components (Rinaudo, 2006). Chitosan is normally insoluble in aqueous solutions above pH 7.0; In its crystalline form however, in dilute acids (lower than pH 6.0) such as acetic acid the protonated free amino groups on glucosamine make the molecule soluble (Martino *et al*., 2005).

Chitosan, produced by alkaline deacetylation of chitin, is considered one of the most abundant polysaccharides on the earth especially in coastal regions and well-known for renewable, non-toxic, biocompatible and degradable (Bhatnagar and Sillanpa, 2009). As the only natural alkaline and cationic polysaccharide, chitosan has great potentials in wastewater treatment, because its amine and hydroxyl groups act as active sites for heavy metal and anionic organic pollutants (Crini, 2008).

The chitosan has attracted considerable interest in various fields due to its unique biological activities, such as biocompatibility (Hsu, 2011), biodegradability (Kim *et al*., 2011), nontoxicity (Shi *et al*., 2016), antimicrobial activity (Rabea *et al*., 2009), antitumor activity (Toshkova *et al*., 2010) and immune-enhancing effect (Li, 2013). These properties make chitosan a promising candidate for medicine (Tan, 2013), food (Qiu, 2014), cosmetic (Ray *et al*., 2011), water treatment (Bhatnagar and Sillanpa, 2009), biomedical engineering industries (Upadhyay *et al*., 2013) and many agricultural uses (Xing *et al*., 2015).

Despite the above stated applications, the quantity and quality of chitosan produced is still major problems as the quantity produced is not enough for industrial application while the quality of chitosan produced is not satisfactory. In a bid to improve the quantity and quality of chitosan, this study examined the production of chitosan from fish scales using different production process through alteration of earlier traditional methods used for its production and subsequent characterization of produced chitosan to ascertain its quality.

II. MATERIALS AND METHODS

A. Materials

Fish scales were obtained from in Ilesha, Osun State. The area was within the latitude and longitude of 7° 37' 40.40" N and 4° 44' 29.80" E respectively. The samples collected were put in ice for storage during transportation to the laboratory. Fish scales were completely separated from the waste in the laboratory, cleaned, washed in pure water and dried at 60°C in an oven and finally blended with blending machine.

Other materials include Sodium hydroxide (NaOH) and Hydrochloric acid (HCl, 36.5g/mol). All chemical were of analytical grades. Five fish scales Chitosan labeled (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA,) were prepared by changing of the 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).

B. Extraction of chitosan

i. Deproteinization

With a ratio of ground fish scales to the solution of 1:20 (w/v) and a constant stirring for 2 h at 90 °C to remove the protein, the grounded Fish scales was treated with 2.0 % of potassium hydroxide (KOH) solution. The filtrates were washed with tap water for 30 mins until pH neutral (pH, 7) after the samples were filtered under vacuum. This was followed by drying of deproteinized shells in the oven for 24 hrs at 60 °C (Shahidi and Synowiecki, 1991.

ii. Demineralization

Demineralization of the deproteinized fish scales was carried out through addition of 2.5 % (w/v) of hydrochloric acid (HCl) in a ratio of ground shell to the solution of 1:20 (w/v) at room temperature (20 °C) for 6 h to remove the mineral content. The samples were washed for 30 mins with tap water until pH neutral (pH, 7) after filtration under vacuum. The demineralized shells were dried in the oven for 24 h at 60 °C (Shahidi and Synowiecki, 1991)

iii. Decolouration and dewatering

Decolourizing was carried out by treating the samples with acetone for 10 mins and dried at ambient temperature for 2 h and followed by removal of the resulting residues. Fish scales chitin was obtained by washing the decolourized shells in the running tap water, rinsed, filtered and dried for 24h at 60 °C (Shahidi and Synowiecki, 1991).

iv. Deacetylation of chitin

This was done according to the method described by Yen *et al*. (2009). The chitin was treated with 40 % (w/w) aqueous sodium hydroxide (NaOH) with a ratio of chitin to the solution of 1:15 (w/v) at 105 °C for 2 h. Then, the filter pump was used to filter the chitin and the chitosan was obtained by washing the filtered chitin with the deionized water until pH neutral (pH, 7). The chitosan obtained was then dried at 60 °C for 24 h in the oven.

C. Characterization of chitosan

i. The yield

This was determined by comparing weight measurements of the Chitosan obtained after treatment and that of the raw material.

A yield was calculated as follows:

Yield of chitin (%) = $\frac{(\text{Extracted chitin (g)}}{\text{Grinded Fish scales(g))}}$ x100 (1)

Yield of chitosan (%) = $\frac{(\text{Extracted chitosan(g)}}{\text{Extracted chitin(g))}}$ x100

ii. Moisture, Ash and Nitrogen Contents

This was determined according to the method described by standard method [AOAC, 1990] with minor modification. The samples were dried for 24h at 60°C until the weights are constant. It was then calculated by percentage of weight loss comparing to the initial weight of the samples. Ash and nitrogen contents of chitosan were measured according to a previously described procedure.

iii. Determination of degree of deacetylation (DD)

This was determined by method of direct titration described by Kjartansson (2008) with some modification. Chitosan samples (0.1 g) were dissolved in 25 ml of 0.06 M HCl at room temperature for an hour. The solution was diluted to 50 ml before being titrated with a 0.1 N NaOH under constant stirring to pH 3.75. The volume of NaOH at pH 3.75 was acquired and recorded. Titration was continued to pH 8 and the total volume of NaOH (0.1 M) was recorded. The degree of deacetylation was then calculated using the following equation:

DD= $\frac{(161.16*(V2-V1)N}{W1}$

where, 161.16 is the mass of chitosan monomer, V1 and V2 are the volumes of NaOH solution used, *N* is the strength of the NaOH solution (0.1 M) and W1 is the mass of sample after correction for moisture. The degrees of deacetylation (DD) of the samples were be determined in triplicate.

iv. Water binding capacity

This was measured as described by method of Ocloo *et al*. (2011). To a centrifuge tube, 0.5 g of chitosan and 10 ml of distilled water ware added. To dissolve the chitosan, the mixture was vortexes for 1 min before left for 30 mins at an ambient temperature and the tube was shaken for 5 s every 10 minutes before being centrifuged for 25mins at 3,200 rpm. After supernatant decantation, the tube was weighed again. The water binding capacity was then calculated as the following equation:

WBC (%) = $\frac{\text{Water bound(g)}}{\text{Sample weight(g))}}$ x 100

v. Fat binding capacity

This was measured using a modified method of Wang and Kinsella (1976). This was initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding 10 ml of oil (five types of oil: soybean oil (Pure Wesson® Congra Foods, Irvine, CA. USA), canola (Pure Wesson®), corn (Pure Wesson®), sunflower (Pure Wesson®), and olive (San Marc' Can-America Inc. Tampa, FL. USA)) and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every 10 min and centrifuged (Model # Z383K, HERMLE-National Labnet Company, Woodbridge, NJ. USA) at 3,500 rpm (6,000 x *g*) for 25 min. After the supernatant was decanted, the tube was weighed again. FBC was calculated as follows:

FBC (%) = $\frac{\text{Fat bound(g)}}{\text{Sample weight(g))}}$ x 100

All experiments were triplicated.

vi. Solubility

This was determined according to the method described by Fernandez-kim (2004). Chitosan powder (0.1 g in triplicate) was then placed into a centrifuge tube (known weight) then dissolved with 10 ml of 1 % acetic acid for 30 mins using an incubator shaker operating at 240 rpm and 25 °C. The solution was then immersed in a boiling water bath for 10 mins, cooled to room temperature (25 °C) and centrifuged at 10,000 rpm for 10 mins. The supernatant was decanted. The un-dissolved particles was washed in distilled water (25 ml) and then centrifuged at 10,000 rpm. The supernatant was removed and un dissolved pellets were dried at 60 °C for 24 h. Finally, the particles were weighed and the percentage of the chitosan solubility was determined. The solubility of chitosan was calculated using the following equation:

Solubility (%)=(Initial weight of tube + chitosan) (Final weight of tube +chitosan)×100/(Initial weight of tube + chitosan)–(Initial weight of tube)

vii. Viscosity

The viscosity of extracted chitosan was determined according to the method by Ocloo *et al*. (2011). The extracted chitosan was diluted in 1 % of acetic acid at 1 % concentration on a dry basis. The viscosity of the extracted chitosan was determined using a Brookfield viscometer (Spindle no. 2 at 50 rpm at 25 °C) with values reported in centipoise units (cP).

viii. Emulsifying capacity

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

D. Statistical analysis

Data were determined using appropriate statistical analysis in triplicate.

III. RESULT AND DISCUSSION

A. Production of Chitin and Chitosan from Fish scales using different methods

Figure 1a shows the quantity of Chitin produced from fish scales using various method of production. The different Chitins, labeled DPMCA, DMCPA, DMPCA and DCMPA, were produced by altering the known traditional method of production process. For example, DPMCA denotes sequential steps of deproteinization + demineralization + decolorization + deacetylation. DPMCA stands for the traditional production method and was considered as the control sample.

The highest quantity of chitin (3.65g from 10 g of grinded fish scales) was obtained with DMCPA production process while chitin production through DMPAC gave the lowest (2.53g from 10 g of powdered Snail fish scales) chitin (Figure 1a). The highest chitin yield (36.5 %) was recorded with DMCPA as extraction method while the least chitin yield (28.7 %) was obtained with DPMCA as production method (Figure 1b).

The highest quantity of chitosan (3.55g from 7.24g of chitin) was produced in this work by DMCPA as production process while DPMCA as method gave the least quantity of chitosan (0.85g from 3.65 g of chitin) Figure 2a. The maximum and least yields of chitosan (23.29% and 19.51%) were recorded through DMCPA and DMPAC as production process (Figure 2b).

This might be connected to fact that when demineralization and deproteinization come behind decoloration and demineralization steps, there will be increased in Chitin and chitosan production. Similar result was reported by Tajik *et al*. (2008) that recorded 29.3%, 34.5%, 30.1%, 31.6% of chitin and 19.2%, 23.1%, 22.8%, 22.9% of chitosan from Brine Shrimp (*Artemia urmiana*) Cyst Shells through different processing processed (DPMCA, DMCPA, DMPCA and DCMPA).

B. Characterization of Chitosan produced by different Production Processes

i. Ash content

Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of calcium carbonate. The ash content from chitosan in this study is very low ranging from 0.17 to 0.76% figure 3. This might be link to effectiveness of demineralization step in removal of mineral content of fish scales. This is in line with work of Tajik *et al*. [2008] that reported low ash that ranged between 0.19 and 0.51% with various production processes

ii. Moisture

The present report shows a minimal variation and a significant difference in the % of moisture content, varied from 0.77 to 1.32% through different production process including traditional methods Figure 4. The low moisture content observes in this study is of importance because adsorptions of moisture affect water holding capacity of chitosan in relation to its processing and several

applications (Chandumpai *et al*., 2008). In related view, Gaikwad *et al*. (2015) reported significant difference in the % moisture ranging from 2.37 to 5.4 % among the five Chitosan prepared from crab chitosan.

iii. Nitrogen content

The nitrogen content reported for chitosan in this work ranged from 7.23 to 7.86% (Figure 5). This is related to work of Tajik *et al*. (2008) and No and Meyers (1995) that both reported nitrogen content between range of 7.06 to 7.97% and 7.32-7.51% respectively.

iv. Solubility

An excellent solubility was recorded for chitosan in this work of ranging from 68.45 to 75.25 % with significance variation; the minimum solubility obtained with production process through DMPCA (68.45%) while the highest was recorded by DMCPA (75.25%) production protocols (Figure 6). The higher solubility obtained in this work reveals complete protein removal (1981). This work is similar to the report of Gaikwad *et al*. (2015) that demonstrated an excellent solubility varied from 81.78 to 88.78 %.

v. Degree of Deacetylation

The solubility, chemical reactivity, and biodegradability were affected by degree of deacetylation. This study showed that degree of deacetylation was >70% for all Chitosan except for DMPAC, which had a DD of 65.33% (Figure 7). This work shows that the chitosan obtained from fish scales had degree of deacetylation ranged from 65.33 to 90% (Figure 7). The amount of positively charged groups available for flocculating a negatively charged material will be reduced by lower degree of deacetylation.

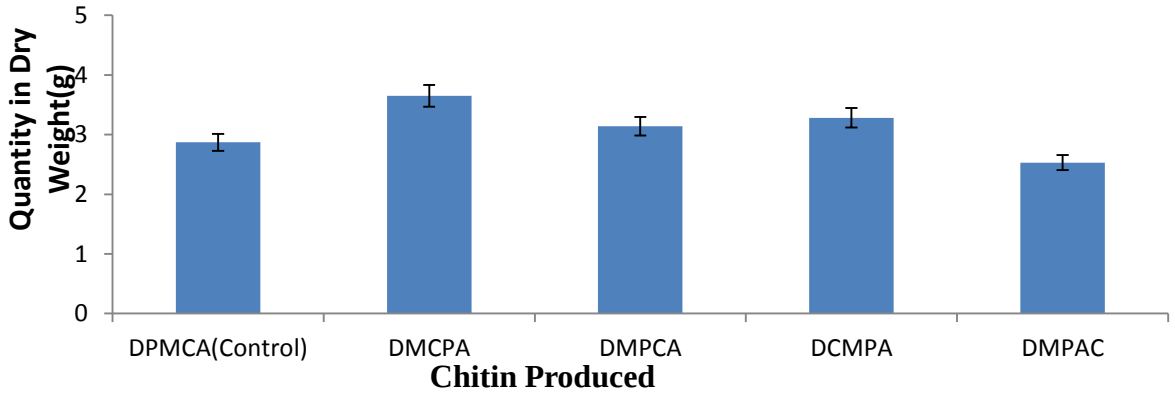

Figure 1a: Quantity of Chitin produced from fish scales using different production process

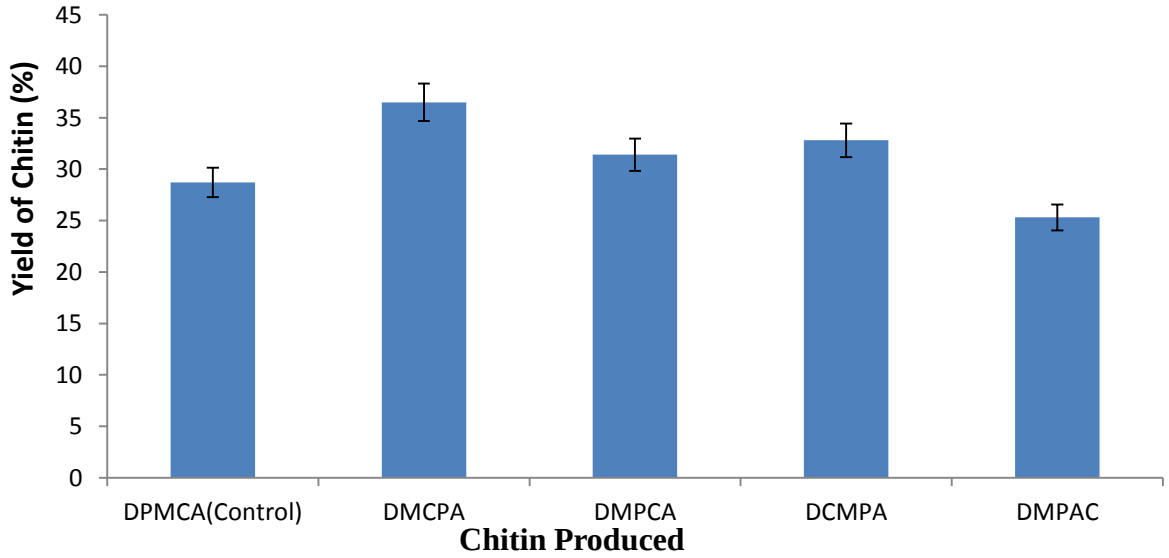

Figure 1b: Yields of Chitin produced from fish scales using different production process

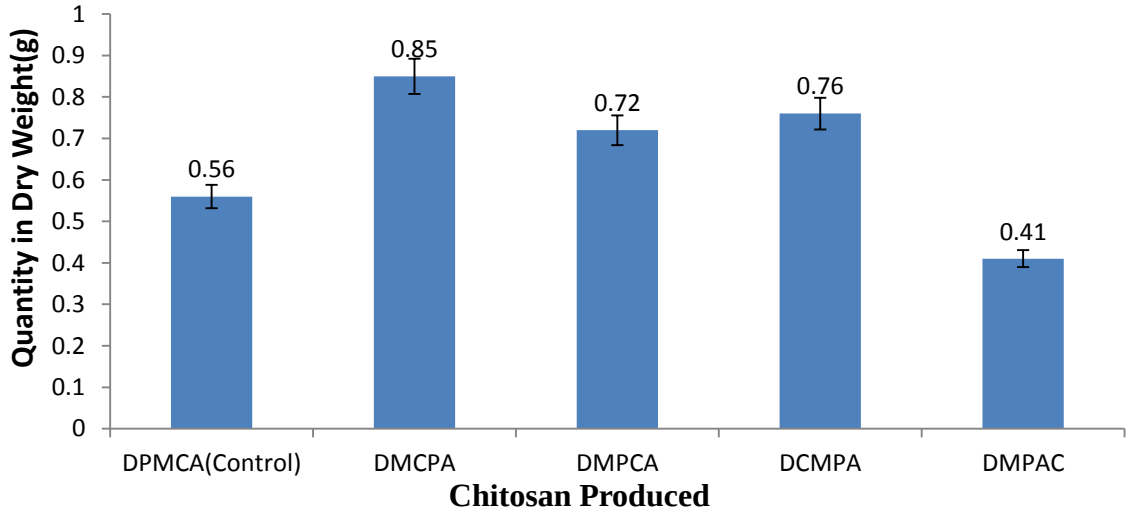

Figure 2a: Quantity of Chitosan produced from fish scales using different production process

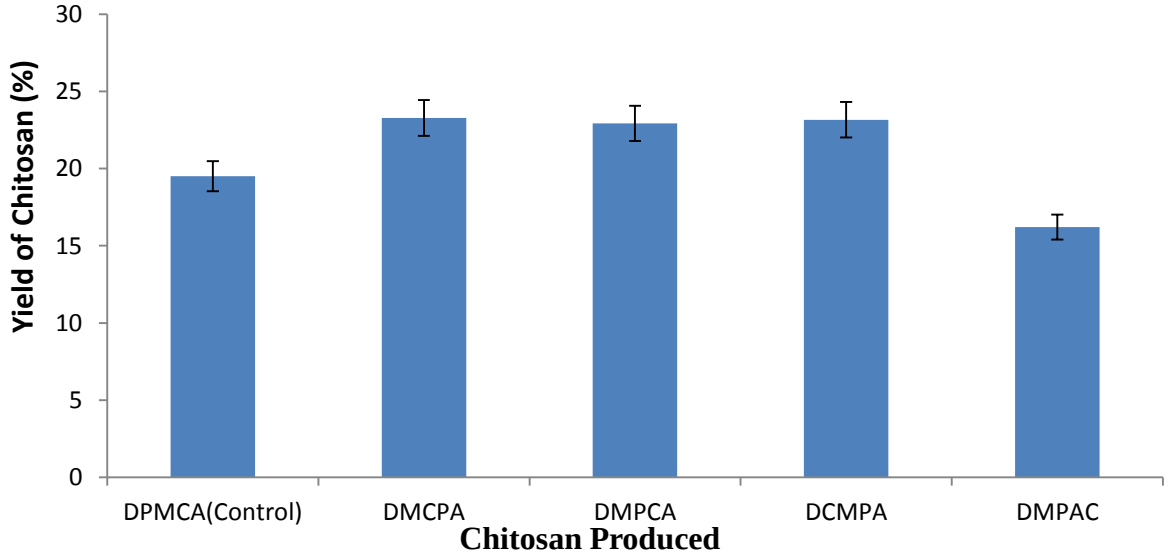

Figure 2b: Yields of Chitosan produced from fish scales using different production process

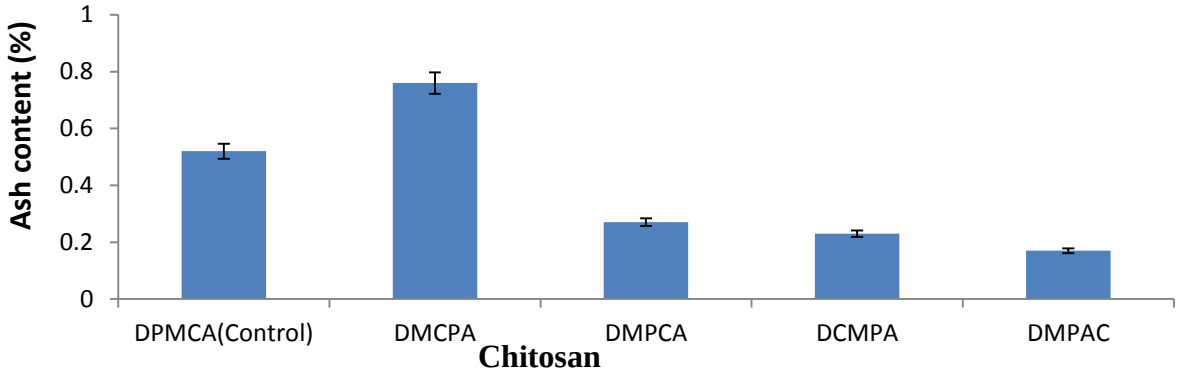

Figure 3: Percentage composition of Ash content in Chitosan produced from fish scales with different production process

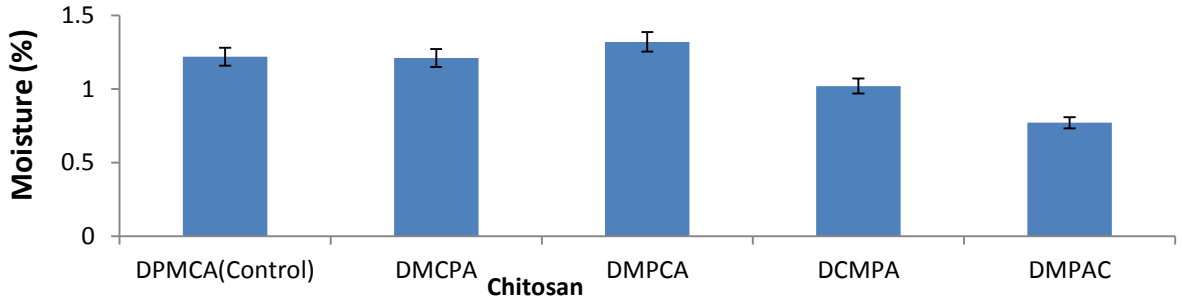

Figure 4: Percentage composition of Moisture content in Chitosan produced from fish scales with different production process

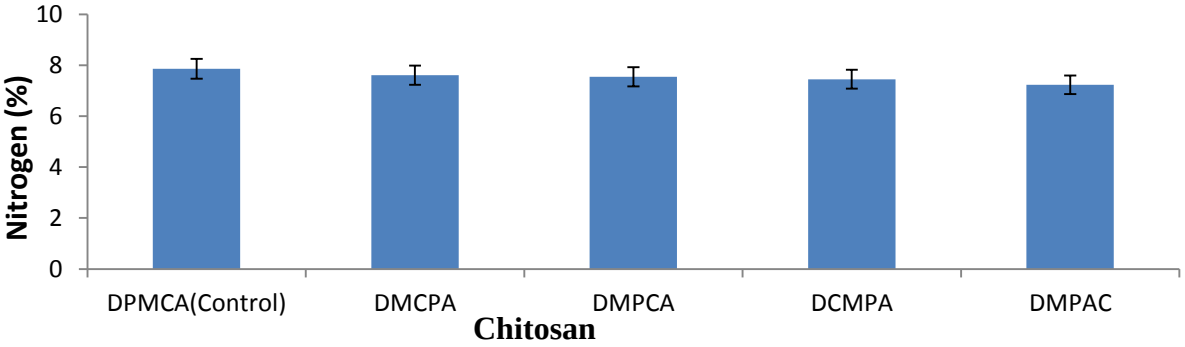

Figure 5: Percentage composition of Nitrogen content in Chitosan produced from fish scales with different production process

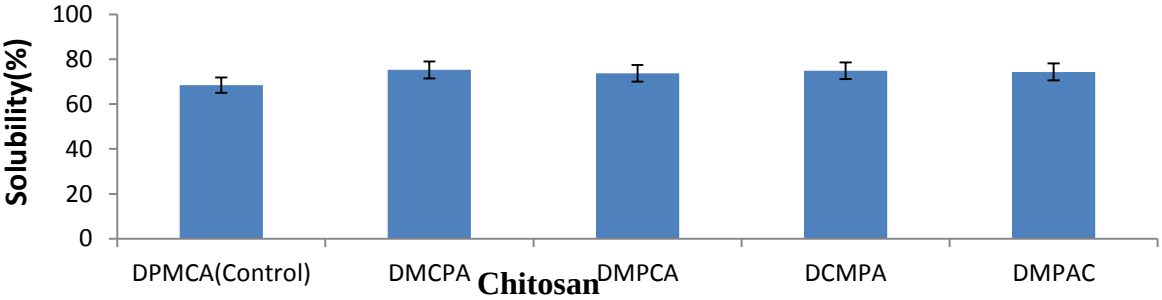

Figure 6: Solubility of Chitosan produced from fish scales with different production processes.

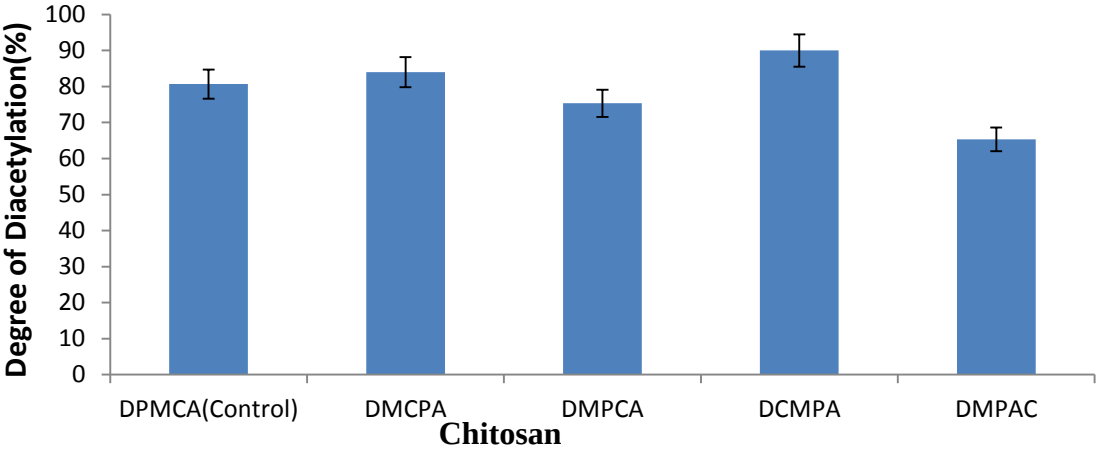

Figure 7: Degree of Deacetylation of Chitosan produced from fish scales with different production process

vi. Emulsion Capacity

An emulsion capacity ranged from 4.61 to 9.66 % was obtained in this work (Table 8). The least emulsion capacity (4.61%) was recorded with DMPAC as production method (4.61%) while highest (9.66%) was obtained through DMPCA production sequence (Figure 8). 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.

vii. Viscosity

Production protocols through DCMPA recorded highest viscosity (96cP) while the least (37.33 cP) was found in DMPAC methods, this showed a decrease in molecular weight (Figure 9). The significant differences were found between the viscosity of DCMPA samples (96 cP) and others sequential process methods in this study (Figure 9). The viscosity found in chitosan of fish scale in this work with DCMPA as production sequence was 2 and 3-folds higher than with the DMCPA (56.67 cP) and DPMCA (35 cP) methods (Figure 9). Compared to other crustaceans (Tharanathan and Kittur, 2003) the viscosity of Chitosan obtained from fish scales was higher. The application of chitosan as a thickening and suspending agent for medical, cosmetic and food applications depends on higher viscosity as it enhances the thickened in aforementioned field.

viii. Bulk Density

In this study, bulk density of chitosan ranged between 0.78 to 1.04 g/ml (Figure 10). The highest bulk density (1.04g/ml) was recorded with DMPAC production sequence (Figure 10). 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.

xi. Water Binding Capacity (WBC)

Water binding capacity observed in this study for fish scales chitosan ranged from 682 to 926% (Figure 11). A similar result has been reported by Cho *et al.* (1998) but No *et al*. (2003) reported lower results of 355 - 611%. The highest WBC was observed for DCMPA (926%), followed by DMPCA, DMCPA, DPMCA and DMPAC (894, 782.67, 746.33 and 682%, respectively) (Figure 4.11). As shown in Figure 11, reversing the sequence of steps had marked effects on WBC. An increase in WBC was observed when demineralization was conducted prior to deproteinization followed by deacetylation, whilst this was not detected when deproteinization was performed prior to demineralization, followed by deacetylation. A similar result has been reported by Rout (2001) in crawfish. He also reported that the process of decoloration causes a decrease in WBC of chitosan than those of unbleached crawfish chitosan.

x. Fat Binding Capacity (WBC)

The fat binding capacity chitosan was determined using olive oil. As shown in Figure 4.12, FBC of fish scales chitosan ranged from 363.33 to 516.9%. The range of FBC in this study (363.33 to 516.9 %.) was slightly similar to that reported by Cho *et al.* (1998) and 217 - 403% observed by Li *et al*. (1992).

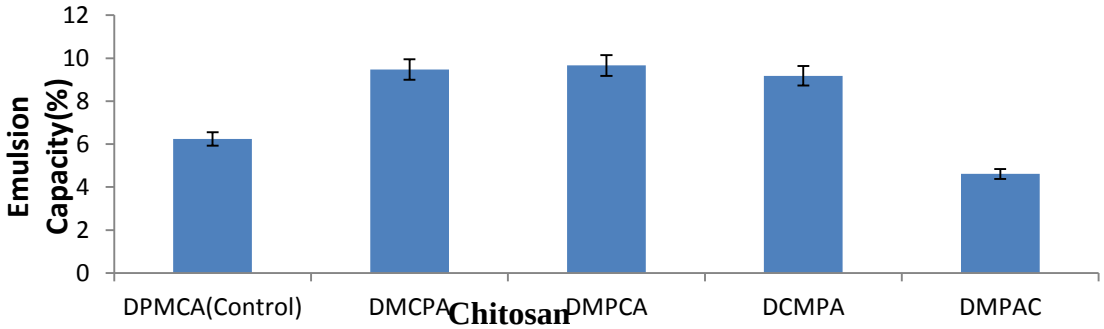

Figure 8: Emulsion Capacity of Chitosan produced from fish scales with different production process

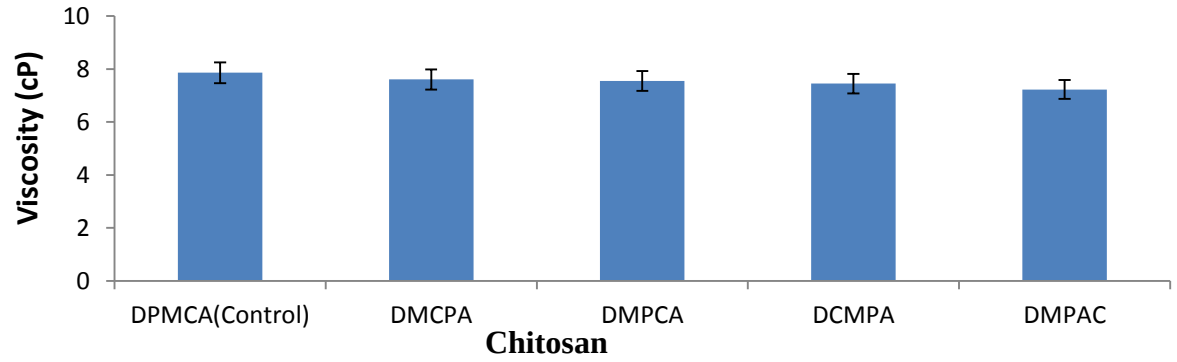

Figure 9: Viscosity of Chitosan produced from fish scales with different sequential modification process

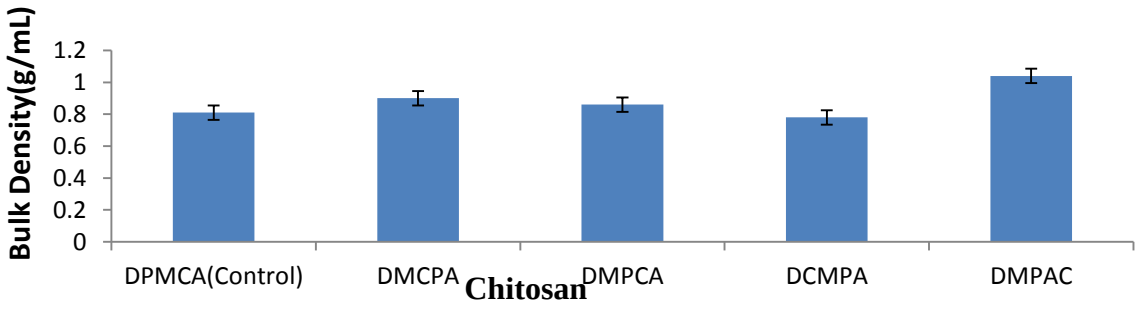

Figure 10: Bulk Density of Chitosan produced from fish scales with different sequential modification process

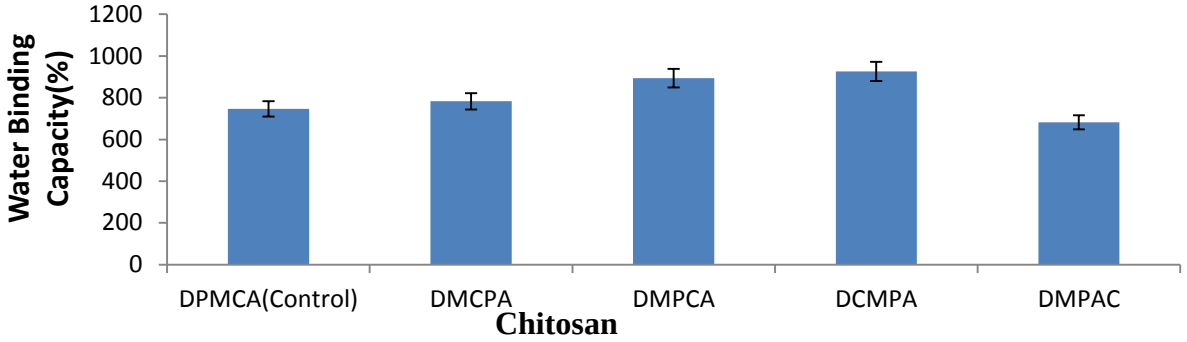

Figure 11: Water Binding Capacity of Chitosan produced from fish scales with different sequential modification process

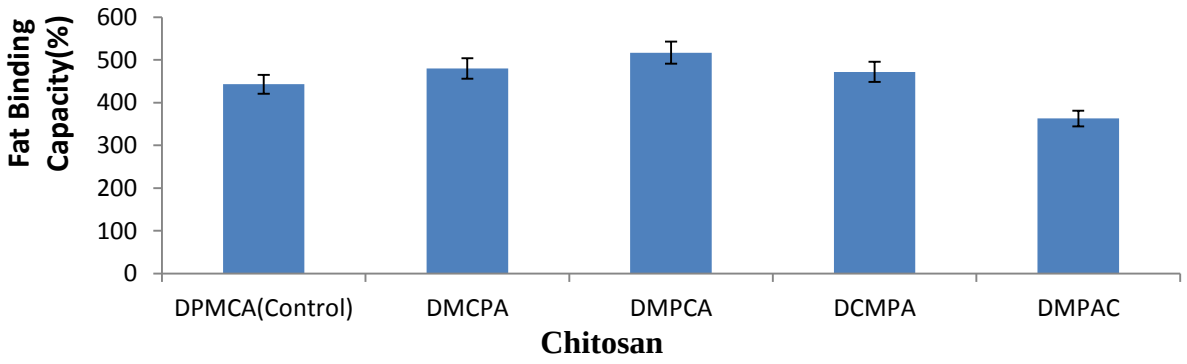

Figure 12: Fat Binding Capacity of Chitosan produced from fish scales with different sequential modification process

IV. CONCLUSION

The main emphasis on chitosan throughout the previous work is on its quality and physicochemical properties through existing traditional production protocols. Despite the arrays of applications of chitosan in industry, the quantity and quality of chitosan produced is yet to meet the industrial needs. The current work was attempted to monitoring and altering the production processing protocols of the chitosan using fish scale shell as starting materials. The chitosan were produced through different production process in a bid to improve on the quality and quantity of chitosan produced. From our results, we observed that physicochemical and functional properties of chitosan affected by process protocol alteration/modification. Overall, the results indicated that different production process in fish scales chitosan production yields improved quality and quantity of chitosan.

REFERENCE

AOAC (1990) *Official methods of analysis of the Association of Official Analytical Chemistry,* 15th ed. *The Association of Official Analytical Chemistry Inc:* Washington, DC.

Bhatnagar, A. and Sillanpää M. (2009). Applications of chitin- andchitosan-derivatives for the detoxification of water and wastewater - a short review, *Advances in Colloid and Interface Science*. 152: 26–38.

Brine, C and Austin P. R (1981) Chitin variability with species and method of preparation, *Comparative Biochemistry Physiology*, 69B: 283-286.

Chandumpai, A. Singhpibulporn, N.; Faroongsarng, D and Sornprasit, P.(2004) Preparation and physicochemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*. *Carbohydrate Polymer*., *58*, 467–474, 2004

Cho, Y.I.; No, H.K.; Meyers, S.P. (1998) Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *Journal Agriculture. Food Chemistry*., *46*, 3839-3843.

Cho. No, H. K and Meyers, S. P. (1999) Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *J. Agric. Food Chem.* 1999, *4 6* ,3839-3843.

Crini, G (2008). Non conventional low cost adsorbents for dye removal; a review. *Bioresource Technology* ; 97: 953-71.

FAO (2015). Food and Agriculture Organization of the United Nations, Rome, Italy

Fernandez-kim, S (2004). Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols. Dissertation of MSc.: *Graduate Faculty of Seoul National University*;. p. 10.

Gaikwad, A. (2015). Assessment of hematological, biochemical effects and genotoxicity among pesticide sprayers in grape garden. *Journal of occupational medicine and toxicology,* 10, 11.

Hsu, J. (2011) A novel method to identify cooperative functional modules: study of module coordination in the Saccharomyces cerevisiae cell cycle. *BMC Bioinformatics* 12:281, 2011

Kim, J., Cho, A., Yin, H. and Schafer, D.A. (2011). Mouneimne, G., Simpson, K.J., Nguyen, K.V., Brugge, J.S., Montell, D.J. Psidin, a conserved protein that regulates protrusion dynamics and cell migration. Genes Dev. 25(7): 730-741.

Kjartansson, G (2008) Extraction and functional properties of ultrasonicated chitin and chitosan from crustacean by-products. Doctor of philosophy thesis, Graduate School of the University of Massachusetts Amherst, p 158.

Knor, D (1982). Functional properties of chitin and chitosan, *Journal of Food Science.47*, 593- 595.

Kuddus, S. M and Ahmad, R. Z. (2013) Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase, *Journal of Genetical Engineering Biotechnology* 11:39-46.

Li, X. (2013) Comprehensive in vivo RNA-binding site analyses reveal a role of Prp8 in spliceosomal assembly. *Nucleic Acids Res* 41(6):3805-18.

Li., L., Dunn, E.T.; Grandmaison, E.W.; Goosen, M.F.A.(1992). Applications and properties of chitosan. *Journal of Bioactivity Compat. Pol*., *7*, 370–397.

Martino, R., Malet-Martino, M., Gilard, V. and Balayssac, S.(2005). Counterfeit drugs: analytical techniques for their identification. *Analytical Bioanalysis Chemistry*, 398, 77–92.

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

No, N Park, N.Y.; Lee, S.H.; Meyers, S.P. (2003) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Food Microbiol*ogy *74*, 65-72.

Ocloo, F (2011). Quayson ET, Adu-Gyamfi A, Quarcoo EA, Asare D, Serfor-Armah Yand Woode BK.. Physicochemical and functional characteristics of radiation-processed shrimp chitosan. *Journal Radiatory Physical Chemistry*; 80:837–84.

Qiu B (2014). C-terminal motif within Sec7 domain regulates guanine nucleotide exchange activity via tuning protein conformation. *Biochemical Biophyscical Research Commun* 446(1):380-6.

Rabea, E., Badawy, M.E.I., Steurbaut, W. and Stevens, C.V. (2009). In vitro assessment of N- (benzyl) chitosan derivatives against some plant pathogenic bacteria and fungi. *European Polymer Journal* 45:237–245.

Ray, S., Samantaray, S., Smith, J.A., Matzelle, D.D., Das, A. and Banik, N.L.(2011). Inhibition of cysteine proteases in acute and chronic spinal cord injury, *Neurotherapeutics*; 8:180–186, 2011.

Rinaudo, M (2006) Chitin and chitosan: Properties and applications, *Progress in Polymer Science* 31: 603- 632. doi: 10.1016/J.progpolymsci, 2006.

Rout, S (2001) Physicochemical, functional, and spectroscopic analysis of crawfish chitin and chitosan as affected by process modification. *Louisiana State University: Baton Rouge*, LA, USA.

Shahidi, F and Synowiecki, J. (1991). Isolation and characterization of nutrients and value-added products from snow crab (*Chionecetes opilio*) and shrimp (*Pandalus borealis)* processing discards. *Journal of Agricultural Food Chemistry* , *39*, 1527-1532.

Shi Y (2016) Rpn1 provides adjacent receptor sites for substrate binding and deubiquitination by the proteasome. *Science*, 351(6275).

Tajik, K and Kohara, T. A (2008) conductometric method for colloid titrations. *Anal. Chim. Acta.* 1976, *83*, 59-65.

Tan, T. (2013). Mcp1 and Mcp2, two novel proteins involved in mitochondrial lipid homeostasis, *Journal Cell Science* 126(Pt 16):3563-74.

Tharanathan, R and Kittur, F.S (2003).Chitin -the undisputed biomolecule of great potential. *Criital. Reviws Food. Science Nutrition 43*, 61-87.

Toshkova, R.N., Manolova, N. E., Gardeva, D., Ignatova, M. Yossifova, L. Rashkov, I and Alexandrov, M.(2010) Antitumor activity of quaternized chitosan-based electrospun implants against Graffi myeloid tumor, *International Journal of Pharmaceutics*, 400, 221–233, 2010.

Upadhyay, A, Kandachar, V., Zitserman, D., Tong, X. and Roegiers, F. (2013) Sanpodo controls sensory organ precursor fate by directing Notch trafficking and binding γ-secretase. Journal of Cell Biology. 201(3): 439--448.

Wang, J. C. and Kinsells, J. E. (1976) "Functional Properties of Novel Proteins: Alfalfa Leaf Protein," Journal of Food Science, Vol. 41, No. 2, pp. 286-292. doi:10.1111/j.1365-2621.1976.tb00602.x

Xing, Y. Su, T.T. and Ruohola-Baker, H. (2015). Tie-mediated signal from apoptotic cells protects stem cells in Drosophila melanogaster. *Natural Community* 6(): 7058.

Yasumatu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T. and Ishii, K. (1972). Whipping and emulsifying properties of soybean product, *Agricultural Biology Chemistry*., 36, 719 – 726.

Yen, Y., Ko, C. H., Yen C. F., Chen C. S. and Chen C. C.(2009) The association between harmful alcohol use and Internet addiction among college students: comparison of personality. *Psychiatry Clinical Neuroscience* 63 218–224, 2009.