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Editorial

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(**Dr. Kaniz Fizza Azhar**)
Executive Editor

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Short Communication

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Deposition and Characterization of ZnS Thin Films Using Chemical Bath Deposition Method in the Presence of Sodium Tartrate as Complexing Agent

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Abstract. ZnS thin films were deposited on indium tin oxide glass substrate using the chemical bath deposition method. The deposited films were characterized by X-ray diffraction and atomic force microscopy. The influence of bath temperature on the structure and morphology of the thin films was investigated at three different bath temperatures of 60, 70 and 80 °C in the presence of sodium tartrate as a complexing agent. The XRD results indicated that the deposited ZnS thin films exhibited a polycrystalline cubic structure. The number of ZnS peaks increased from three to four peaks as the bath temperature was increased from 60 to 80 °C based on the XRD patterns. From the AFM measurements, the film thickness and surface roughness were found to be dependent on the bath temperature. The grain size increased as the bath temperature was increased from 60 to 80 °C.

Keywords: chemical bath deposition, thin films, zinc sulphide, atomic force microscopy

Introduction

Zinc sulphide thin films are wide band gap semiconductors which have been used in phosphors, catalysts, solar cells, electro-luminescent devices and many other optoelectronic devices. The ZnS thin films have been deposited using various methods such as RF reactive sputtering (Shao *et al*., 2003), chemical bath deposition (Goudarzi *et al*., 2008; Noikaew *et al*., 2008; Antony *et al*., 2005), atomic layer epitaxy (Oikkonen *et al*., 1998), pulsed-laser deposition (Yano *et al*., 2003) and electrodeposition (Lokhande *et al*., 1998). Chemical bath deposition method is considered a cheap method for producing large area thin films. Up-to-date, chemical bath deposition method has been successfully used to deposit various thin films including FeS_2 (Anuar *et al*., 2010), PbS (Raniero *et al*., 2010), CdTe (Garadkar *et al*., 2010), CdS (Li *et al*., 2005) and As_2S_3 (Mane *et al*., 2004). Chemical bath deposition method is based on controlled precipitation from solution of a compound on a suitable substrate. The substrate is immersed in either alkaline or acidic solution containing the metal ion, chalcogenide source and a complexing agent. Several complexing agents have been utilized in the deposition of thin films such as ammonium sulphate (Soundeswaran *et al*., 2004), sodium citrate (Esparza-Ponce *et al*., 2009), triethanolamine (Gumus *et al*., 2005), disodium ethylene diamine tetra-acetate (Anuar *et al*., 2009), nitrilotriacetic acid (Khallaf *et al*., 2008) and sodium tartrate (Anuar *et al*., 2004).

The present work reports preparation and physical characterization of ZnS thin films onto indium tin oxide glass substrates using chemical bath deposition method. The chemical bath contains zinc sulphate and sodium thiosulphate which provide Zn^{2+} and S^{2-} ions, respectively. It is the first time that the influence of bath temperature ranging from 60 to 80 °C on the ZnS thin film in the presence of sodium tartrate solution is reported. Thin films were analyzed by X-ray diffraction and atomic force microscopy.

Materials and Methods

All the chemicals used for the deposition were analytical grade reagents and all the solutions were prepared in deionised water (Alpha-Q Millipore). Zinc sulphide thin films were prepared from an acidic bath using aqueous solutions of zinc sulphate ($ZnSO_4$) and sodium thiosulphate ($Na_2S_2O_3$) as a source of Zn^{2+} and S^{2-} ions, respectively. Sodium tartrate ($Na_2C_4H_4O_6$) was used as complexing agent to chelate with Zn^{2+} for obtaining Zn-tartrate complex solution. Indium tin oxide (ITO) glass was used as the substrate for deposition of ZnS thin films. Before deposition, indium tin

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oxide glass was degreased with ethanol for 10 min. Then, ultrasonically cleaned with distilled water for another 10 min and finally dried in desiccator. Deposition of ZnS thin films was carried out using the following procedure: 25 mL of zinc sulphate (0.3 M) was complexed with 25 mL of sodium tartrate (0.5 M) solution. To this, 25 mL of sodium thiosulfate (0.3 M) was added slowly. pH was adjusted to 3 by addition of hydrochloric acid with constant stirring. Hydrochloric acid also prevents the formation of hydroxyl species and insoluble compounds. The cleaned indium tin oxide glass was immersed vertically into a beaker. The deposition process was carried out at different bath temperatures (60, 70 and 80 °C) in order to determine the optimum conditions for deposition of ZnS thin films. During deposition, the beaker was kept undisturbed. After completion of deposition (120 min), the indium tin oxide glass was removed, washed several times with distilled water and dried naturally in desiccator.

The structural characterization of films was carried out using a Philips PM 11730 diffractometer with $CuK_{á}$ radiation (λ=0.15418 nm) in the scanning angle from 25° to 70°. The surface morphology of films was investigated by atomic force microscopy (Quesant Instrument Corporation, Q-Scope 250). It was operated in a contact mode with Si_3N_4 cantilever. The value of root mean square (RMS) roughness was calculated from the height in the atomic force microscopy images using commercial software.

Results and Discussion

Figure 1 shows X-ray diffraction (XRD) patterns of ZnS thin films deposited on indium tin oxide glass substrate at various bath temperatures. The XRD patterns were found to be polycrystalline with cubic structure. Films deposited at 60 and 70 °C show three peaks at 2θ =28.5°, 33.3° and 56.6° corresponding to (111), (200) and (311) planes, respectively. The observed *d*-spacing values and the standard values are in good agreement with the Joint Committee on Powder Diffraction Standard (JCPDS) values (Reference code: 00-065-0309) (Dubrovin *et al*., 1983) which confirms the deposition of ZnS thin films under the proposed deposition conditions. The lattice parameter values are a=b=c=5.4 Å. XRD results confirm that the films deposited at higher bath temperature proved more favourable as the peak intensity corresponding to ZnS increased. Furthermore, the number of peaks assigned to ZnS also increased. As the bath temperature was increased to 80 °C, additional peak corresponding to (220) plane was obtained. Meanwhile, as the bath temperature was increased from 60 to 80 °C, the intensity of the peak corresponding to (111) plane increased. The (111) plane seems dominant at this stage of experiment. Similar (111) plane was found to be prominent for zinc sulphide thin films reported elsewhere (Hoa *et al*., 2009; Laukaitis *et al*., 2000; Tran *et al*., 2000).

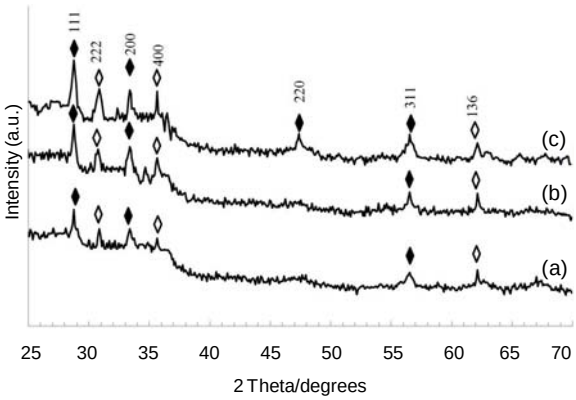

Fig. 1. X-ray diffraction patterns of ZnS thin films deposited at different bath temperatures (a) 60 °C (b) 70 °C (c) 80 °C (◊ $In_{1.875}O_3Sn_{0.125}$; ♦ ZnS)

From the X-ray diffraction results, it can be seen that the presence of indium tin oxide peaks (Reference code: 01-089-4597) in the X-ray diffraction patterns are due to the glass substrate used in the analysis. All three peaks, corresponding to (222), (400) and (136) reflection, were observed. The peaks marked with solid diamonds are associated with reflections of ZnS and those marked with open diamonds can be ascribed to indium tin oxide (Nadaud *et al*., 1998).

The surface morphology of zinc sulphide thin films deposited on indium tin oxide glass substrate was investigated by atomic force microscopy (AFM). Figures 2(a), 3(a) and 4(a) show two-dimensional while Fig. 2(b), 3(b) and 4(b) display three-dimensional AFM images of ZnS thin films deposited at 60, 70 and 80 °C, respectively. The AFM images (Fig. 2a and 2b) indicate that the films deposited at 60 °C have smaller grain sizes (0.5-0.8 μm) and the substrate surfaces are well covered with spherical grains. It is seen that the films deposited at 70 °C (Fig. 3a and 3b) are more homogeneous and more uniform compared to the films

deposited at other bath temperatures. Also, the grain sizes are larger (1.5 and 2 μm) as compared to the films deposited at 60 °C. The ZnS grains are formed on the substrate in an irregular distribution pattern at 80 °C. This observation suggests irregular growth rate of grains. As a result, the films consist of majority of larger grains and a few smaller grain sizes. The size distribution of grains seems to be very broad with the maximum size reaching 3 μm.

Root mean square (RMS) roughness is defined as the standard deviation of the surface height profile from the average height and is the most commonly reported measurement of surface roughness (Jiang *et al.* 2005). The surface roughness values of 63, 124 and 205 nm were observed for the films prepared at 60, 70 and 80 °C, respectively, indicating that the surface roughness increased with the increasing bath temperatures. That means the films deposited at lower bath tempera-

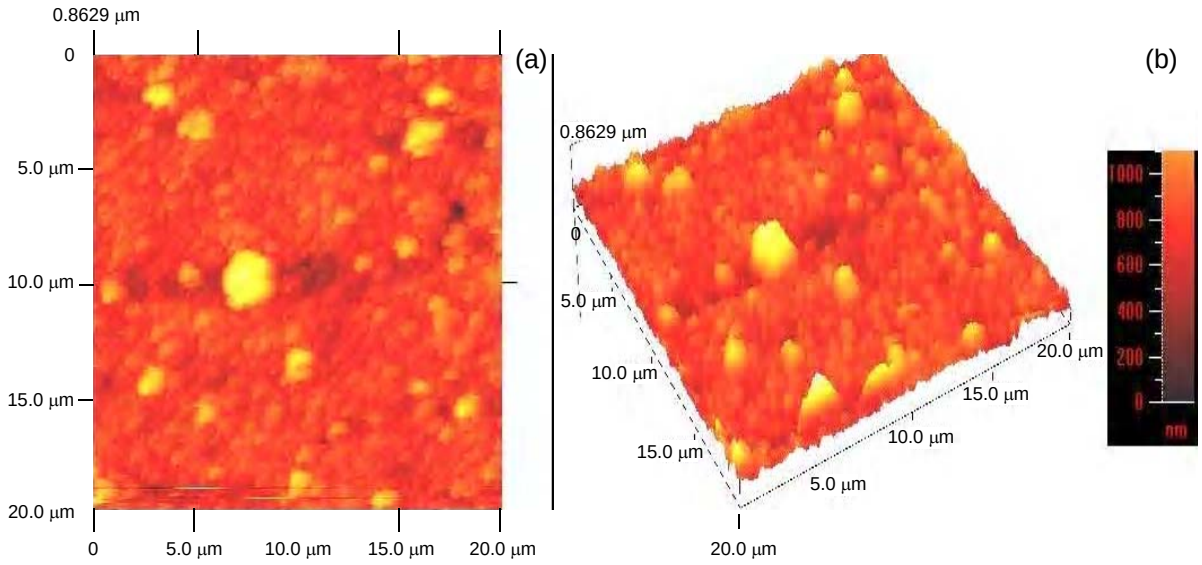

Fig. 2. Two-dimensional (a) and three-dimensional (b) atomic force microscopy images of ZnS thin films deposited at 60 °C.

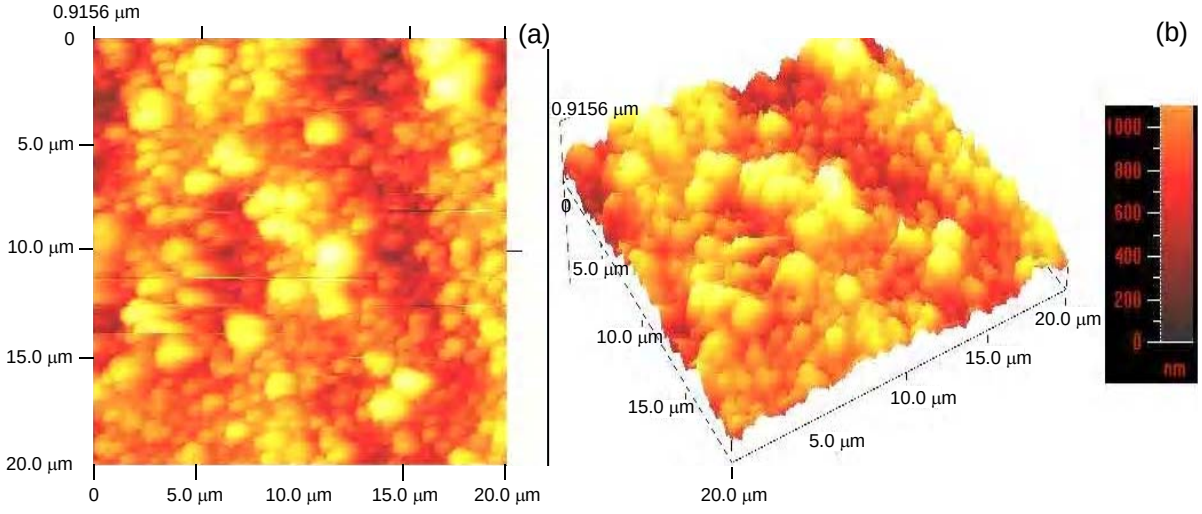

Fig. 3. Two-dimensional (a) and three-dimensional (b) atomic force microscopy images of ZnS thin films deposited at 70 °C.

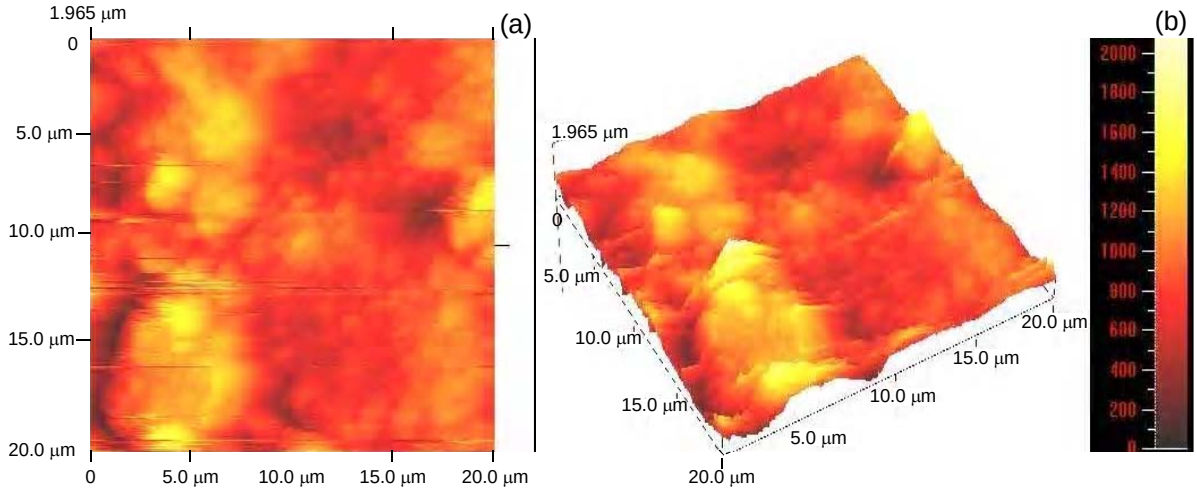

Fig. 4. Two-dimensional (a) and three-dimensional (b) atomic force microscopy images of ZnS thin films deposited at 80 °C.

ture have smoother surface (smaller grain sizes) while the films prepared at higher bath temperature have rougher surface (larger grain sizes). Similarly, the corresponding values of thickness are 863, 916 and 1965 nm, respectively. We can conclude that more materials deposited onto the substrates and thicker films are formed for the films prepared at the higher bath temperature.

Conclusion

The zinc sulphide thin films could be deposited on indium tin oxide glass substrate using zinc sulphate, sodium thiosulphate and sodium tartrate solutions. Based on the X-ray diffraction results, the thin films produced were polycrystalline in nature. The X-ray diffraction patterns showed that the most intense peak corresponded to (111) plane of ZnS. The number of ZnS peaks increased from three to four peaks as the bath temperature was increased from 60 to 80 °C. From the AFM measurements, the grain size, film thickness and surface roughness were found to be dependent on the bath temperature. The films deposited at 60 °C have smaller grain sizes. However, at higher bath temperature (80 °C), larger grain sizes and thicker films are be formed.

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Rapeseed Lipase Catalyzed Synthesis of Butyl Butyrate for Flavour and Nutraceutical Applications in Organic Media

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Abstract. Butyl butyrate, a short chain ester with fine fruity pineapple odour, is a significant flavour compound. Recent investigations show that butyrate esters also have anticancer activity. Factors influencing the synthesis of butyl butyrate by organic phase biocatalysis were investigated. Maximum ester yield of 89% was obtained when 0.25 M butanol and butyric acid were reacted at 25 °C for 48 h in the presence of 250 mg rape seed lipase acetone powder in hexane. Addition of water did not affect synthesis, while a water activity of 0.45 was found optimum. Of 15 different alcohols evaluated, isoamyl and (*Z*)-3-hexen-1-ol were esterified most effectively with molar conversion yields of 92.2 and 80.2%. Short chain primary alcohols such as methanol and medium-long chain alcohols, such as heptanol and octanol were esterified more slowly. The results show that rape seed lipase is versatile catalyst for ester synthesis with temperature stability range 5-50 °C.

Keywords: flavour, butyl butyrate, rape seedling, biocatalysis, esterification, anticancer agent

Introduction

Esters of butyric acid are important as flavour compounds (Leblanc *et al*., 1998). Ethyl butyrate and isoamyl butyrate are found in the aroma of strawberry and banana. The butyrate ester of isoamyl alcohol is a valuable, high demand flavour and fragrance compound widely used in the food, beverage and pharmaceutical industries. The world market for flavours is thought to account for a quarter of the total food additive market. An emerging area of application of butyrate esters is as nutraceutical agents. Naturally occurring butyrate esters such as tributyrin as well as synthetic esters have been shown to possess antiproliferative action against a wide variety of cancer cell lines. Anti-tumour activity was also demonstrated *in-vivo* (Kuefer *et al*., 2004).

Direct synthesis of esters from fatty acids and alcohols by enzymatic methods has been suggested as a good alternative route to industrial catalysis. Butyrate esters and other short chain flavour esters can be synthesized by organic phase biocatalysis (OPB) to satisfy commercial demands (de Baros *et al*., 2009; Pires-Cabral *et al*., 2009; Torres *et al*., 2009; Ben Salah *et al*., 2007; Romero *et al*., 2005). Fungal lipases are preferred for organic phase biocatalysis (OPB) owing to their ready availability and low cost (Abbas and Comeau, 2003; Krishna *et al*., 2000; Langrand *et al*., 1999). Lipases from higher vegetative plants including wheat germ (Xia *et al*., 2009), papaya (Miyazawa *et al*., 2008; Caro *et al*., 2000) and rapeseed lipase (Mukherjee and Jachmanián, 1996; Ncube *et al*., 1993; Hills *et al*., 1990) have also been used for various purposes in OPB. The cost of biocatalyst remains an important consideration in OPB as purified enzymes are expensive. Crude seedling powder is potentially inexpensive alternative form of biocatalyst for OPB. Procedures for preparing acetone powder are simple, making it quite suitable for technical use (El *et al*., 1998). Earlier, we had evaluated various plant seedlings in OPB and results showed that acetone powder obtained from day 4 germinated rape seed was potentially useful biocatalyst for the synthesis of low molecular weight flavour esters (Liaquat and Apenten, 2000). Butyl butyrate was amongst the esters formed in a good yield.

In the present study, the impact of several parameters on synthesis of butyl butyrate catalyzed by crude rape seedlings powder was carefully investigated first time, which included effect of added water, water activity, substrate concentration, temperature and incubation time. The ability of enzyme to catalyze the synthesis of

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organic esters commonly used in the flavour industry by esterification of butyric acid as acylating agent with various alcohols in hexane was also studied.

Materials and Methods

Reagent grade chemicals, acids, alcohols, organic solvents (HPLC grade), salts and esters were obtained from Sigma-Aldrich Co. Ltd., Poole, England. Hexane and heptane were obtained from Fisher Scientific Ltd. (Loughborough, UK). Hexane was dried over molecular sieves (3A, 8-12 mesh; both from Sigma-Aldrich Co. Ltd.) for at least 24 h prior to use. Seeds were supplied by Nickerson Seeds Ltd., Lincoln, UK.

Acetone powder preparation from rape seedlings. Dry whole rapeseeds were surface sterilized by soaking in 0.1% sodium hypochlorite solution for 30 sec., rinsed thoroughly with running tap water and soaked for 24 h at 26 °C (designated as day 1st) in a dark incubator. Germination was achieved by placing rapeseed on moist filter paper towels, on top of moist perlite (Silvaperl graded horticultural) in shallow plastics trays, and then covering with perforated aluminium foil. Samples of seedlings were withdrawn on day 4 after germinating for further processing. In preliminary studies, lipase activity reached to a maximum at 4-6 days after germination. Germinated rapeseed was washed with distilled water three times, equilibrated in a refrigerator at 4 °C for 10 min, cut into small pieces, and then homogenized with 5 volumes of cold acetone (-18 °C or less) for 1 min. The resulting solid was recovered by vacuum filtration using a Buchner funnel, fitted with a Whatman No. I filter paper. Rapeseed lipase acetone powder was washed with 4 volumes of cold acetone and air dried under a hood for 10 h. The light greyish powder was kept in sealed bottles at -20 °C until used.

Direct esterification conditions. Unless otherwise stated, 0.25 g of seedlings powder was added to 5 mL of hexane containing 0.25 M of acid and 0.25 M of alcohol. Synthesis was performed by shaking reaction vessels at about 100 rpm at a constant temperature of 37 °C. The concentrations of ester formed were determined by withdrawing samples (1 mL). These were then centrifuged (1300 × g for 5 min at room temperature) to remove the residual lipase. Aliquots of 0.5 mL reaction mixture were taken from the supernatant and stored at -10 °C until analysed (usually within 24 h). The frozen samples were allowed to warm to room temperature and then analyzed by gas chromatography to determine the concentration of ester, alcohol and acids. Esters synthesis is expressed as percentage molar conversion of acids. All synthesis experiments were performed in duplicate using separate reaction vials. A control experiment was also carried out to check spontaneous esterification. Products of synthesis were analyzed by GC.

Gas chromatographic analysis. The gas chromatography system consisted of Carlo Erba apparatus (Model 5160) equipped with a flame ionization detector. Separation involved a BP-20 fused silica capillary column (SGE, UK, 25 m × 0.32 mm ID; film thickness 1 micron) operated with helium gas as carrier (2 mL/min, split ratio 1:15). The oven temperature was maintained at 50 °C for 2 min and then increased to 210 °C at a rate of 15 °C/min and held for 4 min. The injector temperature was fixed at 250 °C and detector temperature at 240 °C. The GC was connected to an integrator (Hewlett Packard 3395 integrator) which recorded the peak areas and retention times in a chromatogram.

Esters identification and quantification. Esters, alcohol and acids were identified according to their retention times on chromatograms and from comparisons with results obtained with standards. A calibration graph of known acid concentration *vs* corresponding peak area was constructed. Various concentrations of acid (0.0125 M-1 M) were prepared by diluting in *n*- hexane and 0.2 μL of each was injected into GC. Injection was repeated twice for each vial. The percentage conversion of acids and alcohols were calculated by the following formulae:

$$\text{Ester yield (\%)} = \frac{\text{(molar ester produced)} \times 100}{\text{(molar acid added)}}$$

$$\text{Molar conversion (\%)} = \frac{100\,([\text{Acid}]_0 - [\text{Acid}]_F)}{[\text{Acid}]_0}$$

where subscripts 0 and F denote initial and final concentrations, respectively.

Effect of added moisture and water activity (a_w). Varying amounts of distilled water (0-30% v/v) were added to the reaction medium containing rapeseed lipase acetone powder 25 mg (50 g/L), alcohol and acid. Ester synthesis was performed as above. To examine the influence of a_w on ester yield, first reactants, enzyme and organic solvents were equilibrated with standard saturated salt solutions at room temperature

(21 °C) in separate desiccators for 7 days as described by Goderis *et al.* (1987). The salt standards were $MgCl_2$ (a_w = 0.113), Mg $(NO_3)_2$ (a_w = 0.45), NaCl (a_w = 0.74), KCl (a_w = 0.86), $ZnSO_4.7H_2O$ (a_w = 0.90), and molecular sieves (a_w = 0.04). Ester synthesis was initiated by mixing the three reaction components followed by incubation at 40 °C with shaking (100 rpm) for 48 h. Synthesis was also carried out without enzyme (controls).

Reaction temperature. The effect of temperature on ester synthesis was studied by incubating reaction mixtures at various temperatures (0-80 °C). For temperatures below 20 °C, a thermo controller (cooled refrigerator) was used. For temperatures above 40 °C, an oil bath filled with Dow Corning silicon oil was used for incubation.

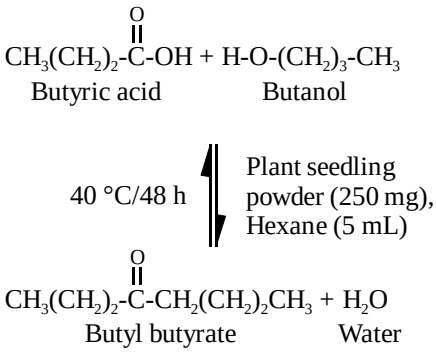
Effect of substrate concentration. The effect of increasing the concentration of one of the substrates was evaluated, while keeping the other constant. The concentration of butyric acid added was 0.0625, 0.125, 0.25, 0.4, 0.5, 1 M while keeping the alcohol concentration and other variables constant. Similarly concentration of alcohol (butanol) was varied while keeping the acid concentration constant (0.25 M). Studies were carried out in hexane at 40 °C (optimum temperature for ester synthesis as determined above).

Time course studies. For the time course experiment, the esterification reaction was monitored at different intervals until the reaction reached equilibrium. Samples of the reaction medium were drawn at given timed intervals and analyzed for butyl butyrate (percent acid molar conversion) concentration. Equilibrium was reached when the product concentration remained constant.

Alcohol specificity for ester synthesis. The specificity of lipase from rape seedlings for different alcohols was checked using butyric acid as acyl donor. The alcohols used were ethanol, propanol, 2-propanol, butanol, ter-butanol, pentanol, isopentanol, hexanol, (*Z*)-3-hexen-1-ol, ter-hexanol, heptanol, 3-heptanol, octanol and geraniol.

Results and Discussion

Optimization of butyl butyrate synthesis in *n*-hexane. Butyl butyrate was formed by direct esterification of butanol with butyric acid in hexane as shown in the given scheme. Esters were identified by comparing their retention times with those of authentic standard esters and by matching their mass spectra with those of standard esters as well as with the NBS library of flavours and fragrances as described previously (Liaquat and Apenten, 2000).



Time course of butyl butyrate synthesis. The reaction catalyzed by the rape seed lipase acetone powder reached equilibrium after 48 h at 40 °C with a final ester yield of 68% (Fig. 1). Both ester yield and substrate molar percentage yield are shown. Compared to reaction for butyl caprylate using cold-adapted lipase from psychrotrophic *Pseudomonas* P38 lipase in *n*-heptane at 20 °C (Tan *et al.*, 1996) which reached equilibrium after 96 h, this is a considerably faster reaction. High temperature organic phase biocatalysis is expected to be associated with a faster rate of reaction and lower organic solvent phase viscosity.

Reaction time and product yield are two important process endpoints in this study. A short reaction time reduces overall process cost, decreases substrate

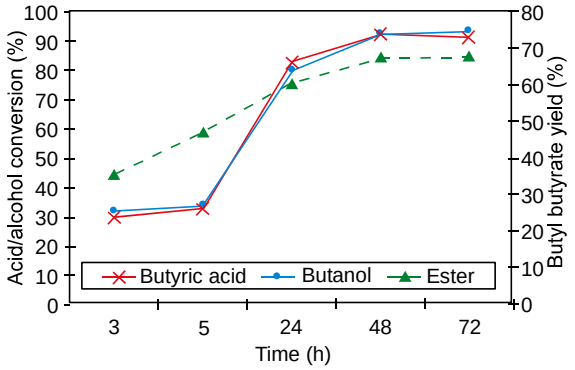


Fig. 1. Time course of the synthesis of butyl butyrate. The reaction mixtures consisted of 0.25 M of butyric acid and 0.25 M butanol in 5 mL of hexane. Reaction medium was incubated at 40 °C in the presence of 250 mg rape seed acetone powder.

inventory and reduces the requirement for energy. The time of reaction is dependent on kinetic factors such as, enzyme specific activity, amount of biocatalyst used, concentrations of co-substrates, reaction temperature, choice of organic solvent, and the degree of stirring, shaking or sonication that affects mass transfer limitations and also the reaction rate (Halling, 1994; Takahashi *et al*., 1985).

Effect of added water on butyl butyrate synthesis. Ester yield of 96% was obtained with up to 30% (v/v) added water (Fig. 2) and the yield was not affected by the amount of water in the reaction mixture. The acetone powder used in the present study contained 8.7 to 9.5% water measured by oven drying the sample to a constant weight at 105 °C overnight. Water contents greater than 20% appeared to produce agglomeration of lipase powder but did not have any effect on the synthesis.

Compared to this, P38 lipase activity was optimum at an organic phase water concentration of 0.25% (v/v) to catalyze the synthesis of butyl caprylate. At a higher or lower water concentration, the yield of ester decreased (Tan *et al*., 1996). The optimum amount of water required for organic phase biocatalysis may depend on factors such as the type of organic phase and the choice of enzyme (Zaks and Russell, 1988). An organic phase water content of 0.1-0.6% (v/v) has commonly been adopted (Shaw and Lo, 1994) and 1% for goat pregastric lipase (Lai and O'Connor, 1999).

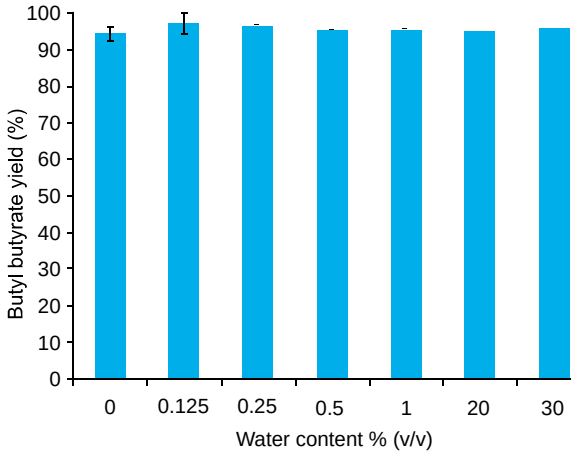


Fig. 2. Effect of added water on butyl butyrate synthesis. The reaction mixture consisted of 0.25 M of butyric acid with 0.25 M butanol in 5 mL of hexane. Reaction medium was incubated at 40 °C for 48 h in the presence of 250 mg rape seed acetone powder.

Higher water content levels have been shown to reduce the product yield of lipase catalyzed reactions in organic phase (Valivety *et al*., 1993).

Effect of water activity (a_w) on the synthesis. The effect of water activity (a_w) on the synthesis of butyl butyrate is shown in Fig. 3. Crude rape seedlings lipase showed maximum ester synthesis activity at a_w = 0.45. Lipases from different sources vary widely in dependence of catalytic activity on a_w (Valivety *et al*., 1992). Wheat germ lipase had a high activity and enantioselectivity in *n*-hexane with a high initial water activity 0.97 (Xia *et al*., 2009). Rape seedling lipase is thought of belonging to a group of lipases that function medium a_w. This feature is useful for synthetic applications in order to suppress hydrolytic side reactions.

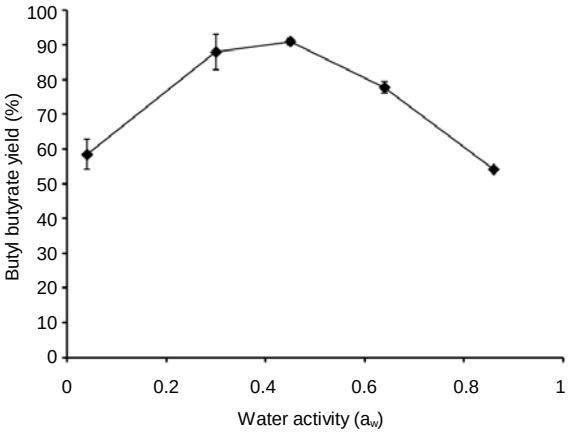


Fig. 3. Effect of water activity ($a_{w)}$ on the butyl butyrate synthesis. The reaction mixture consisted on 0.25 M of butyric and 0.25 M butanol in 5 mL hexane. Reaction medium was incubated at 40 °C for 48 h in the presence of 250 mg rape seed acetone powder.

Effect of reaction temperature on butyl butyrate synthesis. The reaction was optimal at 30-50 °C (Fig. 4). However, rape seedling powder can also be used to synthesize esters in good yield even at low temperatures (5-25 °C) leading to a yield of 61-78% for butyl butyrate. The decrease in ester yield at temperature above 50 °C is probably a result of the catalyst instability. Optimum temperature for a given enzymatic reaction depends on the enzyme source, type of immobilization (if any) and the pH of the reaction mixture (Dordick, 1989). Esterification yield of 90% was reported for the same ester in hexane with

immobilized porcine pancreatic lipase (deCastro *et al*., 1999). However, temperature optimum for butyl caprylate synthesis, using *Pseudomonas* P38 lipase was 20 °C (Tan *et al*., 1996). The decrease in ester synthesis above 20 °C was associated with lipase inactivation at higher temperature. In general, thermostability within an organic solvent is achieved if the enzyme is intrinsically rigid, or if the environment (e.g. low water activity) prohibits enzyme flexibility.

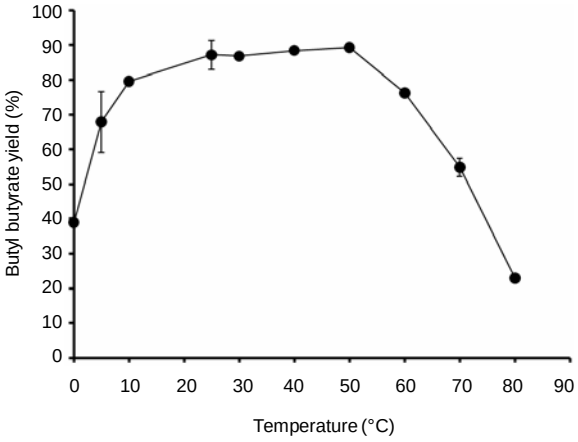


Fig. 4. Effect of reaction temperature on butyl butyrate synthesis. The reaction mixture consisted of 0.25 M of butyric acid and 0.25 M butanol in 5 mL of hexane. Reaction medium was incubated at 40 °C for 48 h in the presence of 250 mg rape seed acetone powder.

Effect of alcohol and acid on ester synthesis. With a system containing a fixed concentration of 0.25 M of alcohol, increasing the acid concentration from 0.0625-0.25 M increased ester yield up to 86.46%. Further increase did not lead to improvement (Fig. 5). The lower conversion obtained below 0.25 M of butyric acid is probably a simple consequence of the lower concentration of acid substrate. Likewise the ester yield increased with increasing the butanol concentration until 0.25 M (Fig. 6). Increasing butanol concentration above 0.25 M adversely affected the yield. Loss of synthetic activity at high alcohol concentrations might be due to its dehydrating effects on rape seed lipase stability. Such results indicate that butanol may act as a competitive inhibitor to the reaction, or a high concentration of the alcohol might be detrimental to rape seed lipase stability. Alcohols are reported to be terminal inhibitors of lipases (Alvarez-Macarie and Baratti, 2000; Chowdary *et al*., 2000; Chulalaksananukul *et al*., 1993) and acids may cause acidification of the microaqueous interface leading to enzyme inactivation. However, the acidification of the microaqueous interphase may be less pronounced in the present case, since butyric is a comparatively weak acid and more hydrophobic than the other low molecular weight acids such as acetic and propionic acids.

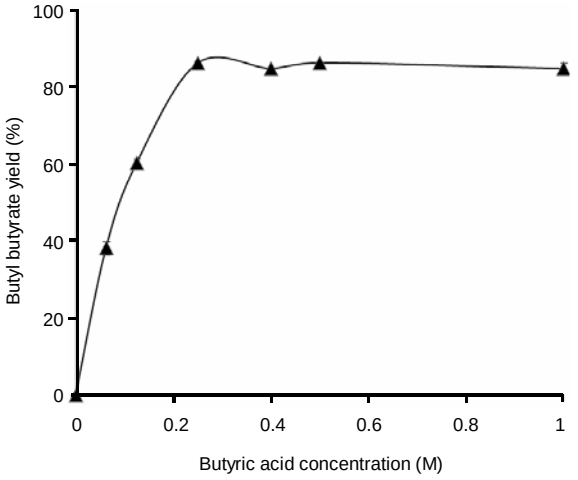


Fig. 5. Effect of butyric acid concentration on ester butyl butyrate synthesis while butanol concentration of the respective system remains constant at 0.25 M. 250 mg of rape seedling acetone powder was suspended in 5 mL of hexane containing the substrates. All reactions were carried out over period of 48 h at 40 °C with no added water.

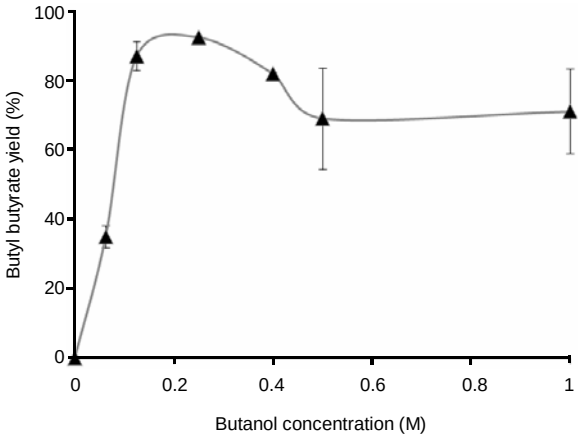


Fig. 6. Effect of butanol concentration on the butyl butyrate synthesis, while butyric acid concentration of the respective systems remain constants at 0.25 M. 250 mg of rape seed acetone powder was suspended in 5 mL of hexane containing the substrates. All reactions were carried out over period of 48 h at 40 °C with no added water.

Effect of alcohol structure on the esters synthesis. Short chain primary saturated alcohols (1- propanol and 1- butanol) were esterified by rape seedling powder in higher yields than *sec*-alcohol (2-propanol). The molar conversion yield of butyric acid with branched chain isopentanol and (*Z*)-3- hexen-1-o1 was 92.2 and 80.5%, respectively, which are the highest yields obtained in the present study (Table 1). These results demonstrate that rapeseed lipase has good specificity for both isoamyl alcohol and (*Z*)-3-hexen-1-ol. Tertiary alcohols were either not esterified by the rapeseed lipase or showed little reactivity. There was a decrease in reactivity as the chain length of the alcohol increased. The greater affinity of crude rape seedling lipase for isoamyl and (*Z*)-3-hexen-1-ol alcohols as compared to their reaction with 1-pentanol and hexyl alcohol, indicates that these are recognized by the rape seedling lipase as different and not like simple alcohols. Broad specificity of seedling powder in the present study might be due to the crude nature of acetone powder. The crude rape seedling lipase behaves similar to the crude papaya lipase (Gandhi and Mukherjee, 2000) and to microbial lipases (Karra-Chaabouni *et al*., 1998; Rotticci *et al*., 1998) which esterify primary alcohols but not tertiary alcohols. Literature reports also demonstrate that branched substrates (either alcohol or acid) are poor substrates for lipase catalysis (Chowdary *et al*., 2000; Rangheard *et al*., 1992).

Table 1. Effect of alcohol structures on the synthesis of butyric acid esters catalyzed by rape seed lipase. Reaction was carried out at 40 °C for 48 h in 5 mL of hexane containing 0.25 M of alcohols and 0.25 M butyric acid, and 50 g/L of rape seed lipase. Results are average of two independent determinations; highest errors on means were less than 10%

Alcohols	Butyric acid molar conversion (%)
Methanol	35.6
1-Propanol	48.8
2-Propanol/(*iso*-propanol)	20.1
1-Butanol	54.5
Tert-butyl alcohol/(2-methyl-2-propanol)	0
1-Pentanol/(amyl alcohol)	53.6
Iso-amyl alcohol/(3-methyl-1-butanol)	92.2
Hexyl alcohol	27.5
Tert-hexyl alcoho/(2-methyl-2-pentanol)	8.1
(*Z*)-3-hexen-1-ol/*cis*-3-hexen-1-ol	80.5
Trans-2-hexen-1-ol	0
1-Heptanol	26.4
3-Heptanol	25.0
1-Octanol	23.4
Geraniol	49.0

Geranyl esters are essential fragrance compounds used in food, cosmetic and pharmaceutical industries. Rape seedlings powder has also catalyzed the reaction with geraniol and a yield of 49% in 48 h was obtained. This yield is 20% lower than that obtained by Kim *et al*. (1998) after 72 h. Further studies are needed to maximize synthesis of gerenyl esters with this enzyme.

Conclusion

Production of low molecular weight esters as flavour compounds by biotechnological processes has a potential interest for the food industry. Crude acetone powder made from germinating rape seedlings was used for butyl butyrate ester synthesis by direct esterification of butyric acid with butanol in *n*-hexane which was rarely investigated. Different reaction parameters for enhancing ester formation were investigated. Conversion yield of 89% was obtained at 25 °C after 48 h at water activity of 0.45 in *n*-hexane without added water. The esterification reduced by increasing alcohol concentration beyond 0.25 M and by raising reaction temperature above 50 °C. The highest molar conversion yields of butyric acid were obtained with isopentanol and (*Z*)-3-hexen-1-o1 reflecting the enzyme specificity for branched chain alcohols. Alcohol chain length higher than (C_6) reduced butyric acid ester formation. This work illustrates the possibility of using rape seed lipase acetone powder for low temperature biocatalysis. This is particularly important because, commercially, use of ambient temperature (25-30 °C) is economical. Low temperature OPB might in future have applications in the preparation of heat-sensitive, high value products. Finally, certain limitations of the current discussion should be highlighted chief amongst which is the realization that rapeseed acetone powder could conceivably contain more than one lipases species.

Acknowledgement

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Effects of Storage and Packaging Materials on Some Physicochemical Properties and Sensory and Microbiological Parameters of Pineapple Juice *(Ananas comosus)*

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Abstract. Physicochemical, microbiological and sensory parameters of concentrated pineapple juice stored in cans and glass bottles were studied over a period of ten weeks. There was slight increase in pH from 4.2 to 4.7 and to 4.8 and decrease in titratable acidity from 8.1 to 5.1 and to 4.6 mg/100 mL, whereas total solids (%) decreased from 76.23 to 65.47% and to 60.38% in canned and bottled pineapple concentrates, respectively. Over 90% loss of Vitamin C was observed, with the bottled samples retaining more Vitamin C than the canned samples. The microbial counts ranged from 2.0×10^3 to 2.4×10^4 cfu/mL whereas fungi and mesophilic bacteria, were not detected to 6×10^3 cfu/mL. Freshly prepared single strength juices of pineapple were better in terms of taste and colour, while the bottled reconstituted juice concentrate competed favourably with the fresh one in colour. The canned samples lost their colours within 10 weeks of storage. The glass bottled samples had a characteristic desirable aroma. Thus concentrated juice in glass bottles stored at room temperature enhanced the keeping quality of the juice and compared more favourably with the fresh juice than the canned concentrated juice.

Keywords: pineapple juice, packaging materials, physicochemical properties, sensory qualities, microbiological quality, storage

Introduction

Fruits are common food materials, which contribute micronutrients (vitamins and minerals) and natural soluble sugars for energy to support human nutrition (Ihekoronye and Ngoddy, 1985).

Juice is obtained from fruits e.g., orange, pineapples, apple, grape etc., and concentrates can be prepared by partial evaporation of moisture from the juices which are likely to have a longer shelf life than the juices due to lower moisture content (Takahashi *et al*., 2000)

Pineapple (*Ananas comosus*) is one of the most common non-citrus tropical and sub-tropical fruits. It has pleasant flavour and acceptable taste and is a very rich source of vitamin C and organic acids. Pineapple contains high level of sugars and other carbohydrates and is, therefore, a major source of dietary fibre and enzymes and can serve as digestion aids (Takahashi, *et al*., 2000). Pineapple is a member of the Bromiliaceae family (Medina and Garcia, 2005) and is the second largest harvest of importance after bananas, contributing over 20% of the world production of tropical fruits (COVECA, 2002). Nearly 70% of pineapple is consumed as fresh fruit in the producing countries. Thailand, Philippines, Brazil and China are the main pineapple producers in the world, supplying 50% of the total output (FAO, 2004). Other important producers include India, Nigeria, Kenya, Indonesia, Mexico and Costa Rica that account for the remaining 50%.

Pineapple, as a plant is put to a number of uses. One of the best known uses of pineapple juice is as a diuretic in the ailments of kidneys, bladder and prostate. Due to the fibre content of the pulp, pineapple prevents constipation and regularizes the intestinal flora (FAO, 2004). Furthermore, there is evidence of pineapple being an appetite reducer, heat protector and an aid in treatment of fever and sore throats, mouth aches and inflammation. Lightly boiled ground pineapple can be used to clean infected wounds because it eliminates dead tissues without affecting live tissues, acts as a disinfectant and accelerates cicatrisation (Mundogar, 2004).

Unfortunately, this fruit is seasonal, highly perishable and prone to high post-harvest losses. Post-harvest losses

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occur in fruits during transportation, storage and while waiting to be processed. Pineapples, after harvesting are still physiologically active. Deterioration of fruit can result from physical factors such as bruises, action of their own enzymes (proteolytic enzyme), microbial action, or combination of these agents. Extracting and concentrating the juice reduces the losses by enhancing the keeping qualities thereby making it available all the year round (Verma and Joshi, 2000).

Losses in pineapple during air transport can be minimized by careful handling. Mechanical damage includes bruising or puncturing caused by poor handling, dropping or abrasion resulting in localized softening and development of secondary microbial infections. Protective measures are required throughout the handling stages to minimize fruit damage. (GeoCoppens and Ferla, 2000).

Canning is a method of food preservation which involves subjecting food materials in cans to elevated temperature for the purposes of eliminating both pathogenic and spoilage organisms and hermetically sealing the cans to avoid external influence from the environment. Bottling is also a method of preservation which employs the use of sterile glass bottles with lids in food packaging.

Chemical composition and microbial profile of a food are the most important parameters in determining the overall quality of that food. Changes in these parameters which occur during preparation and subsequent storage are indices of the suitability or otherwise of the product for consumption. Although, food preservation is necessary, the maintenance of the nutrients in the food is also of utmost importance. Pineapples is known to be the best source of vitamin C, a major portion of which is lost during storage at room temperature (Egidio *et al.,* 2009).

Previous works on pineapple showed that it contains 81.2 to 86.2% moisture and 13-19% total solids of which sucrose, glucose and fructose are the main components. Carbohydrates are up to 85% of the total solids whereas, fibre makes upto 2-3%; citric acid is the most abundant organic acid. Pulp has very low ash content, nitrogen compounds and lipids (0.1%); 25-30% of nitrogenous compounds are true proteins. Out of this proportion, 80% has proteolytic activity due to a protease known as Bromelain (Dull, 1971). Utilization of by-products from pineapple culture, canning and juice extraction has been encouraged for feed production (GeoCoppens and Ferla, 2000).

The objectives of this research work were to extract and concentrate pineapple juice in the form of thick slurry, store it in tin cans and glass bottles and examine the effects of storage containers on the physico-chemical properties, sensory and microbiological qualities of the juice stored over a period of ten weeks.

Materials and Methods

Pineapple fruits, used for this research work, were purchased from a renowned market called “Oja Koko” in Owo Local Government area, Ondo State, Nigeria. The juice was extracted from the fruits following the procedure (Fig. 1) described by Ihekoronye and Ngoddy (1985). The juice was concentrated to 71% brix using a water bath (40 °C) at a temperature slightly above the room temperature. The concentrate was divided into two parts; one part was placed in tin cans(laminated) while the other, in glass bottles of equal capacity. The juice was stored inside a cupboard (dark) at room temperature of 28 °C on an average. The physico-chemical properties, sensory qualities and microbial content of pineapple juice and concentrate were determined every two week for a period of ten weeks.

Determination of the physicochemical properties of the pineapple juice. The physicochemical properties determined were pH, brix, total solids, specific gravity, total titratable acidity and vitamin C content. The pH of fresh pineapple juice as well as the concentrated juice was determined using pH meter. Brix was determined using Abbe refractometer. Total solids, specific gravity, total titratable acidity and vitamin C were determined using standard methods (AOAC, 2005).

Sensory evaluation of pineapple juice concentrate. Sensory evaluation based on taste, colour and aroma was carried out as described by Larmond (1982) using a team of 10 taste panelists for each set of samples. Score of each panelist was reflected on a nine point Hedonic scale ranging from nine (like extremely) to one (dislike extremely). The result obtained was subjected to statistical analysis of variance and means were calculated using Duncan’s Post Hoc. Test.

Microbiological analyses of pineapple juice concentrate. The media used in carrying out the microbial count of the samples were MacConkey agar (MA) and potato dextrose agar (PDA) for viable plate counts of mesophilic bacteria and yeast and mould, respectively. The medium was prepared according to the method described by Franzetti and Galli (1999).

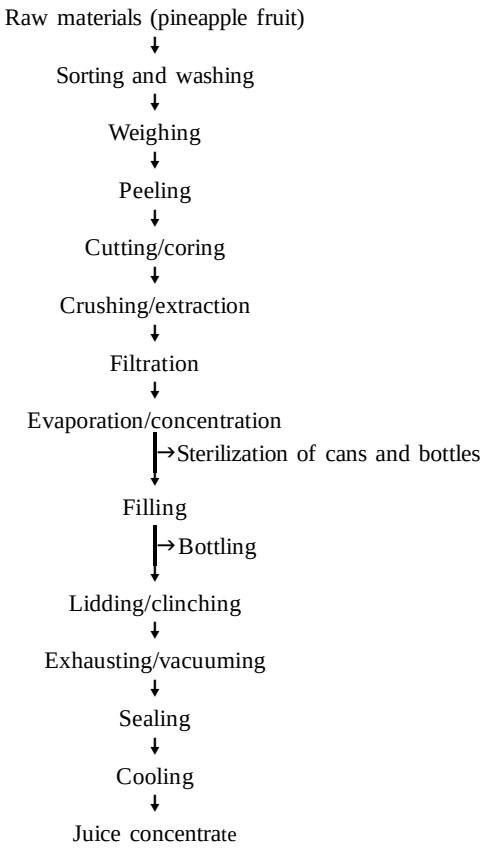

Fig. 1. Flow chart for the production of pineapple juice concentrate.

Results and Discussion

From the result of various analyses carried out during ten weeks of storage, several data trends were observed. The results presented in Table 1 are a combination of trends seen in the experiments (on canned and bottled pineapple concentrates). The means and standard deviations are based on triplicate analyses of each sample from individual container per storage condition.

Table 1 shows some physicochemical properties of pineapple juice and concentrate stored in tin cans and glass bottles for a period of ten weeks**.** The fresh pineapple juice had a pH of 3.70 ± 0.10, ^{0}brix of 12.20±0.427%, total titratable acidity of 1.38 ± 0.03 g/100 mL citric acid, vitamin C content of 153.3±0.4 mg/100 mL, total solids 10.11±0.11% and specific gravity of 1.21±0.033. This is consistent with the findings of Nelson (2005) who obtained 12.8% brix, and 0.724 g/100 mL total acidity. The concentrated juice had a pH of 4.20 ± 0.10, ^{0}Brix of 71.01±0.115%, total titratable acidity of 8.17 ± 0.148 g/100 mL citric acid, vitamin C content of 781.94±0.051 mg/100 mL, total solids of 76.23±0.252% and specific gravity of 1.25±0.104. It was observed that all these parameters (except pH) decreased as the progress of storage period increased in both the canned and bottled concentrates. The pH, brix and vitamin C of bottled concentrate were relatively higher than that of the bottled concentrate while

Table1. Some pysicochemical properties of pineapple juice and concentrate stored in tin cans and glass bottles for a period of ten weeks

Time (weeks)	Pineapple juice sample	*pH	*Brix (%)	*Titratable acidity (g/100 mL citric acid)	*Vitamin C (mg/100 mL)	Total solid (%)	Specific gravity
0	Fresh juice	3.70 ± 0.10	12.20 ± 0.43	1.38 ± 0.03	153.30 ± 0.40	10.11 ± 0.11	1.21 ± 0.03
	Concentrated Juice	4.20 ± 0.10	71.01 ± 0.12	8.17 ±0.15	781.94 ± 0.05	76.23 ± 0.25	1.25 ± 0.10
	Canned concentrate	4.20 ± 0.09	71.30 ± 0.61	8.17 ± 0.02	780.91 ± 0.42	76.23 ± 0.33	1.31 ± 0.01
	Bottled concentrate	4.20 ± 0.07	70.92 ± 0.38	8.17 ± 0.02	781.94 ± 0.05	76.23 ± 0.44	1.31 ± 0.01
2	Canned concentrate	4.40 ± 0.10	63.42 ± 0.29	7.64 ± 0.04	141.03 ± 0.15	74.42 ± 0.42	1.34 ± 0.14
	Bottled concentrate	4.50 ± 0.10	66.30 ± 0.48	7.62 ± 0.02	177.66 ± 0.60	70.37 ± 0.07	1.33 ± 0.03
4	Canned concentrate	4.53 ± 0.15	63.29 ± 0.19	7.04 ± 0.02	72.99 ± 0.43	71.41 ± 0.01	1.32 ± 0.01
	Bottled concentrate	4.60 ± 0.10	67.33 ± 0.15	6.94 ± 0.06	80.29 ± 0.10	66.39 ± 0.09	1.33 ± 0.01
6	Canned concentrate	4.57 ± 0.06	62.92 ± 0.52	6.90 ± 0.02	34.16 ± 0.18	68.39 ± 0.09	1.30 ± 0.05
	Bottled concentrate	4.60 ± 0.20	66.87 ± 0.11	6.58 ± 0.03	41.13 ± 0.54	63.44 ± 0.48	1.30 ± 0.04
8	Canned concentrate	4.63 ± 0.06	63.07 ± 0.07	6.37 ± 0.03	12.06 ± 0.05	66.14 ± 0.09	1.31 ± 0.04
	Bottled concentrate	4.70 ± 0.48	66.25 ± 0.25	6.22 ± 0.026	16.03 ± 0.10	61.82 ± 0.26	1.28 ± 0.02
10	Canned concentrate	4.70 ± 0.10	62.50 ± 0.33	5.11 ± 0.11	7.02 ± 0.05	65.47 ± 0.07	1.31 ± 0.01
	Bottled concentrate	4.80 ± 0.06	66.33 ± 0.14	4.61 ± 0.03	13.02 ± 0.03	60.38 ± 0.07	1.29 ± 0.09

* = mean value ± standard deviation of triplicate determinations.

the reverse trend was noticed for titratable acidity and total solid over ten week storage period. However, the parameters of fresh juice were much lower than that of the concentrated juice (canned and bottled concentrates). This can be attributed to the fact that the concentrated juice contains reduced moisture content (Montero-Calderon *et al.*, 1998).

Over the period of storage, the range values of pH, ^{0}Brix, total titratable acidity, Vitamin C and total solid content of canned concentrate were 4.20-4.70, 62.50-71.30%, 5.11-8.17 g/100 mL citric acid, 7.02 -780.91 mg/100 mL and 65.47-76.23%, respectively, while that of the bottled concentrate were 4.20-4.80, 66.33% -70.92%, 4.61-8.17 mg/100 mL citric acid, 13.02-781.94 mg/100 mL and 60.38-76.23%, respectively.

There was a sharp decrease in the brix content of pineapple juice between the initial time of production and the second week of storage (week 2) while it was relatively steady during the remaining storage period with slight difference. Slight increase in pH with the cor-responding slight decrease in the titratable acidity of pineapple juice in both the containers was observed during the storage which is in agreement with the findings of Montero-Calderon *et al.* (2008). Total solid and Vitamin C were also observed to decrease with time. Vitamin C remarkably decreased to the tune of more than 90% loss, while the total solids slightly decreased over the storage period. Specific gravity of the juice was relatively stable throughout the period of storage with negligible difference.

The colour of the juice concentrate reduced with storage period; the juice concentrates being acidic reacted with the cans and resulted in darkening of the colour. Despite the transparency of glass bottles, the sample in bottle containers competed favourably with the fresh samples in terms of colour. This may be due to the fact that the samples were not exposed to light; exposure of juice to light naturally denatures it. It was also observed that the bottled samples retained more Vitamin C which is the major nutrient in juices than the canned samples.

Table 2 depicts the mean score of sensory qualities. It was observed that there were significant differences in terms of taste, colour and aroma among the samples examined. However, fresh juices were mostly preferred to the reconstituted juices followed by the bottled concentrate, while the canned concentrates were ranked the least in term of colour.

Table 2. Sensory quality of pineapple fresh juice and concentrate samples

Time (weeks)	Attributes	Fresh juice	CPC	BPC
0	Taste	5.8^{b}	5.5^{b}	5.3^{b}
	Colour	7.4^{a}	5.8^{b}	6.9^{ab}
	Aroma	6.3^{b}	5.5^{b}	5.5^{b}
2	Taste	6.2^{a}	4.2^{b}	4.7^{b}
	Colour	6.2^{a}	5.9^{a}	5.7^{a}
	Aroma	7.5^{a}	6.5^{a}	5.8^{a}
4	Taste	7.8^{a}	6.1^{b}	6.1^{b}
	Colour	7.0^{b}	6.8^{b}	8.2^{a}
	Aroma	7.1^{b}	6.4^{b}	6.2^{b}
6	Taste	6.7^{a}	5.1^{b}	4.8^{b}
	Volour	6.6^{a}	7.1^{a}	6.8^{a}
	Aroma	7.9^{a}	6.2^{b}	5.8^{b}
8	Taste	8.0^{a}	5.8^{b}	5.5^{b}
	Colour	7.9^{a}	6.4^{b}	6.7^{b}
	Aroma	7.2^{a}	5.6^{b}	6.2^{b}
10	Taste	5.3^{b}	4.3^{b}	4.1^{b}
	Colour	7.8^{a}	6.4^{b}	6.4^{b}
	Aroma	4.9^{b}	6.0^{ab}	6.2^{ab}

Means with different superscripts in a row for each week are subscript significantly different (P=0.05); CPC = canned pineapple concentrate; BPC = bottled pineapple concentrate.

Table 3. Microbial counts of pineapple juice and concentrate

Time (weeks)	Pineapple sample	Fungi (cfu/mL)	Mesophilic bacteria (cfu/mL)
0	Fresh juice	2.4×10^4	5×10^3
	Concentrated juices	2.0×10^3	1×10^3
	Canned concentrate	2.0×10^3	1×10^3
	Bottled concentrate	2.0×10^3	1×10^3
2	Canned concentrate	6.0×10^3	2.0×10^3
	Bottled concentrate	8.0×10^3	4.0×10^3
4	Canned concentrate	1.2×10^4	3.0×10^3
	Bottled concentrate	2.0×10^4	6.0×10^3
6	Canned concentrate	8.0×10^3	2.0×10^3
	Bottled concentrate	2.0×10^4	6.0×10^3
8	Canned concentrate	6.0×10^3	ND
	Bottled concentrate	1.4×10^4	2.0×10^3
10	Canned concentrate	4.0×10^3	ND
	Bottled concentrate	1.0×10^4	2.0×10^3

ND = not detected.

Table 3 documents the microbial counts of pineapple fresh juice and concentrate. The viable plate counts of mesophilic bacteria, yeast and mould were reduced with concentration process. However, they increased with storage up to the 4th and the 6th week and later started decreasing. The predominant members were mould and yeast; this is due to the fact that the juice concentrate is acidic (Ihekoronye and Ngoddy, 1985). Bacterial contamination may be due to poor handling during processing. In the canned samples, the microbial load was less than that in the bottled samples due to the anaerobic condition in the cans. In any case, the reconstituted juices from the concentrates are microbiologically fit for consumption since the microbial loads were within tolerable limits for human consumption.

Conclusion

Juice concentrates stored in glass bottles had better physicochemical and sensory qualities; due to inert nature of glass it prevented certain reactions. However, concentrated fruit juices in cans contained less microbial load but with unacceptable colour. Conclusively, pineapple juice and concentrate stored in glass bottle retained more nutrients and is preferred to the juice stored in cans in terms of colour, taste and Vitamin C retention.

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Osmotic Dehydration of Pomegranate (*Punica granatum* L.) Using Response Surface Methodology

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Abstract. For studying osmotic dehydration of pomegranate arils, a mathematical model was developed to quantify the responses of water loss, weight reduction and solute gain using response surface methodology. Under the experimental conditions, 15-32% water was lost, whereas 6-13% solids were gained. The high value (> 0.98) for determination coefficient (R^2) and adequate precision (> 38) and a low value for coefficient of variance (< 2.5) was achieved for the developed model. Optimisation of the model with the goal of maximum water loss and minimum solute gain resulted in 24.5% and 9.6% values, respectively, whereas, with the goal of minimum water loss and maximum solute gain resulted in 15.6% water loss and 13.8% solute gain.

Keywords: pomegranate, osmotic dehydration, mathematical modeling

Introduction

Pomegranate (*Punica granatum* L.) is a fruit of tropical and subtropical regions. It is widely cultivated in Iran, Spain, Egypt, Afghanistan and India (Adsul and Patil, 1995). The edible fruit is a berry with a rounded hexagonal shape, and has thick reddish skin and around 400-600 seeds (Al-Said *et al*., 2009). The pulp bearing seeds are called "arils". Dehydrated arils are known as "Anardana" in local language in India and Pakistan and are used in culinary and traditional medicines. The arils are either consumed as fresh or their juice is extracted. The juice may also be used in processed products like jams and jellies.

Drying conditions of pomegranate arils, significantly affect essential functional properties. Pomegranate is usually dried in open environment (sun drying) due to which the resulting product contains dust, insects and other contaminants. Moreover, open environment (sun drying) does not result in consistent product due to varying humidity and temperature conditions (Doymaz and Pala, 2002). Industrial dryers have been proposed (Doymaz, 2004) to avoid these problems. However, industrial dryers are not only expensive but result in low quality product as well due to the use of hot air for drying the product. An alternate drying method is osmotic dehydration.

Osmotic dehydration (OD) is widely used to remove water from fruits and vegetables by dipping them in aqueous solutions of low molecular weight compounds e.g., sucrose at high concentration. During OD, water is lost from the product, whereas solids are transferred from the dipping medium to the product simultaneously (Madamba, 2003). OD thus results in energy saving and improved product quality (Raoult-Wack, 1994).

Rate of OD depends on several variables including temperature, immersion time and solute concentration. Successful application of osmotic dehydration requires mathematical modelling of process variables. Mathematical modelling helps in dealing with multiple factors to optimise the desired outcome by simulating the process variables and allowing the quantification under various conditions (Jalali *et al*., 2008).

Using response surface methodology (RSM), the aim of this work had been to study the effects of temperature, immersion time and concentration on the weight reduction (WR), water loss (WL) and solute gain (SG) during the OD of pomegranate arils. The OD parameters were simulated and optimised using mathematical model.

Meterials and Methods

Sample preparation. Pomegranate fruits of approximately same size, weight and maturity level were purchased from the local market. The arils were manually separated from the fruits and the peel was discarded. The arils were then subjected to osmotic treatment.

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Osmotic dehydration. Osmotic solutions were prepared with commercial sucrose. For every experiment, 150 g sample (arils) was dipped in 1 L osmotic solution for different time intervals, temperatures and concentrations (Table 1). During this treatment the solution was continuously stirred, on shacking water bath. After the treatment, arils were superficially dried with an absorbent paper, manually. The samples were weighed after the process to calculate the percentage of weight reduction. (WR), water loss (WL) and solute gain (SG), according to the following formulas:

$$SG = \frac{W_{st} - W_{so}}{W_o} \times 100 \quad (1)$$

$$WR = \frac{W_o - W_t}{W_o} \times 100 \quad (2)$$

$$WL = SG + WR \quad (3)$$

Where:

W_{so} = weight of solids present in pomegranate arils before treatment,

W_{st} = weight of solids present in pomegranate arils after treatment,

W_o = weight of pomegranate arils before treatment and

W_t = weight of pomegranate arils after treatment.

All the experiments were conducted in triplicate and average values were taken for calculation.

Table 1. Variables and experimental design levels of osmotic dehydration

Variable	Coded symbol	Levels				
Coded value		-2	-1	0	1	2
Time (min)	A	25.5	49.0	72.5	96	119.5
Temperature (°C)	B	28.5	35	41.5	48	54.5
Concentration (°Bx)	C	25	34	43	52	61

Experimental method and statistical analysis. A central composite experimental design was used to study the effects of time, temperature and concentration on the OD parameters. This experiment design allows the modelling of a second-order polynomial that describes the responses. Data were analysed by multiple regressions through the least square method to fit in the following equation.

$$Y = b_o + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2 + b_3C + b_{13}AC + b_{23}BC + b_{33}C^2 \quad (4)$$

The analysis of variance (ANOVA), response surfaces and other statistics were executed using design expert software (1996, V.5.0.3).

Results and Discussion

The results of water loss, solute gain and weight reduction for the 20 trials generated by the central composite design are shown in Table 2.

Relative extent of solute uptake is usually expressed in terms of dehydration efficiency index (DEI) which is the ratio of water loss/solid gain (WL/SG). The value of WL/SG in this study was found to be approx. 2 (2.15-2.57). Thus the water removed was almost double the solute gained under the process conditions studied. Value of DEI may be desired either high or low depending on the end use. In case of Anardana, a higher DEI is required as the end use is in culinary. On the other hand, low DEI value is preferred in case of "candying". Structure of the raw material had a significant effect on SG and WL (Lazarides *et al*., 1997; Lazarides and Mavroudis, 1996). Since there is no reported study on pomegranate, values are not available for comparison. Mujica-Paz *et al*. (2003), working on osmotic dehydration of melons, got the WL/SG values ranging from

Table 2. Effect of osmotic treatment on water loss (WL), solid gain (SG), weight reduction (WR) and WL/SG

Treatment No.	Time (min)	Temperature (°C)	Concentration (°Bx)	WL (%)	SG (%)	WR (%)	WL/SG
1	49.0	35.0	34.0	15.66	6.44	9.22	2.43
2	96.0	35.0	34.0	17.85	7.86	9.98	2.27
3	49.0	48.0	34.0	23.55	9.52	14.03	2.47
4	96.0	48.0	34.0	25.90	10.65	15.25	2.43
5	49.0	35.0	52.0	20.89	8.51	12.38	2.45
6	96.0	35.0	52.0	22.83	9.80	13.03	2.33
7	49.0	48.0	52.0	28.56	11.46	17.11	2.49
8	96.0	48.0	52.0	31.61	13.22	18.39	2.39
9	25.5	41.5	43.0	19.46	8.44	11.02	2.31
10	119.5	41.5	43.0	24.30	11.30	13.00	2.15
11	72.5	28.5	43.0	16.45	6.81	9.64	2.42
12	72.5	54.5	43.0	32.77	13.01	19.75	2.52
13	72.5	41.5	25.0	18.08	7.02	11.06	2.57
14	72.5	41.5	61.0	29.03	11.66	17.37	2.49
15	72.5	41.5	43.0	22.56	8.97	13.59	2.52
16	72.5	41.5	43.0	22.50	8.92	13.58	2.52
17	72.5	41.5	43.0	22.98	9.30	13.68	2.47
18	72.5	41.5	43.0	22.92	9.25	13.67	2.48
19	72.5	41.5	43.0	23.45	9.67	13.78	2.42
20	72.5	41.5	43.0	23.19	9.47	13.72	2.45

1.3 to 2.2 depending on the concentration of the immersion solution.

The results of WR, WL and SG were fitted on a quadratic model and analyzed statistically (Table 3A, 3B and 3C). High F-value for model (>132) and low F-value for lack of fit (<0.4) implies that the model is significant. This is strengthened by low Fisher F-test value ("Pmodel > F" < 0.0001). The determination coefficient (R^2) – a measure of how well the responses are likely to be predicted by the model – was found to be >0.98 which reveals the good fitness of the model (Table 4). Adjusted R^2 (Adj. R^2) is the value of R^2 adjusted for the number of explanatory terms and sample size in a model. The value of the Adj. R^2 was also found to be high (>0.97), showing that the high value of R^2 is not just due to added terms. The difference between the Predicted R^2 and Adj. R^2 was also found to be low (<0.01). Low values of coefficient of variance (<2.5) that was found in this study indicate that the deviation between the experimental and the predicted values is low. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this work, the ratio was found to be >38, which indicates an adequate signal. Petchi and Manivasagan (2009), working on osmotic dehydration of radish, obtained similar statistical parameters.

Table 3A. ANOVA for response surface quadratic model: water loss

Source	Sum of squares	df	Mean square	F value	p-value prob > F
Model	410.408	9	45.601	507.304	< 0.0001
A-Time	23.062	1	23.062	256.566	< 0.0001
B-Temperature	264.235	1	264.235	2939.580	< 0.0001
C-Concentration	114.600	1	114.600	1274.910	< 0.0001
AB*	0.203	1	0.203	2.263	0.1634
AC*	0.026	1	0.026	0.286	0.6045
BC*	0.033	1	0.033	0.363	0.5603
A^2	1.606	1	1.606	17.865	0.0018
B^2	4.628	1	4.628	51.488	< 0.0001
C^2	0.687	1	0.687	7.645	0.0200
Residual	0.899	10	0.090	-	-
Lack of fit*	0.229	5	0.046	0.341	0.8685
Pure error	0.670	5	0.134	-	-
Cor total	411.307	19	-	-	-

* = not significant.

Table 3B. ANOVA for response surface quadratic model: solid gain

Source	Sum of squares	df	Mean square	F value	p-value prob > F
Model	66.930	9	7.437	132.311	< 0.0001
A-Time	8.019	1	8.019	142.664	< 0.0001
B-Temperature	37.940	1	37.940	675.015	< 0.0001
C-Concentration	19.761	1	19.761	351.588	< 0.0001
AB*	0.004	1	0.004	0.074	0.7913
AC*	0.030	1	0.030	0.541	0.4791
BC*	0.031	1	0.031	0.552	0.4747
A^2	0.636	1	0.636	11.321	0.0072
B^2	0.722	1	0.722	12.851	0.0050
C^2*	0.019	1	0.019	0.337	0.5744
Residual	0.562	10	0.056	-	-
Lack of fit*	0.146	5	0.029	0.350	0.8630
Pure error	0.416	5	0.083	-	-
Cor total	67.492	19	-	-	-

* = not significant.

Table 3C. ANOVA for response surface quadratic model: water reduction

Source	Sum of squares	df	Mean square	F value	p-value prob > F
Model	153.3327	9	17.0370	4309.2478	< 0.0001
A-Time	3.8834	1	3.8834	982.2432	< 0.0001
B-Temperature	101.9250	1	101.9250	25780.4213	< 0.0001
C-Concentration	39.1849	1	39.1849	9911.2313	< 0.0001
AB	0.1494	1	0.1494	37.7931	0.0001
AC*	0.0002	1	0.0002	0.0496	0.8283
BC*	0.0000	1	0.0000	0.0051	0.9444
A^2	4.2640	1	4.2640	1078.5051	< 0.0001
B^2	1.6937	1	1.6937	428.3952	< 0.0001
C^2	0.4780	1	0.4780	120.8938	< 0.0001
Residual	0.0395	10	0.0040	-	-
Lack of fit*	0.0095	5	0.0019	0.3141	0.8852
Pure error	0.0301	5	0.0060	-	-
Cor total	153.3722	19	-	-	-

* = not significant.

Table 4. Statistical parameters for quadratic model

Parameters	WL	SG	WR
SD	0.2998	0.2371	0.0629
Mean	23.2271	9.5641	13.6629
C.V. %	1.2908	2.4788	0.4602
Press	2.6741	1.6848	0.1150
R-squared	0.9873	0.9917	0.9997
Adj R-squared	0.9758	0.9842	0.9995
Pred R-squared	0.9622	0.9750	0.9993
Adeq precision	38.4352	40.0759	237.2569

The diagnostic curves (normal probability, predicted *vs* actual and residual *vs* run) help to find out any abnormality in the data points. Normal probability curve should not show any pattern; it should be a straight line with few scattered points (Fig. 1A). Predicted *vs* actual and residual *vs* run (Fig. 1B and 1C) help to detect any value or group of values giving large deviation in the model. In our case, these curves show that all the data points are adequately explaining the model.

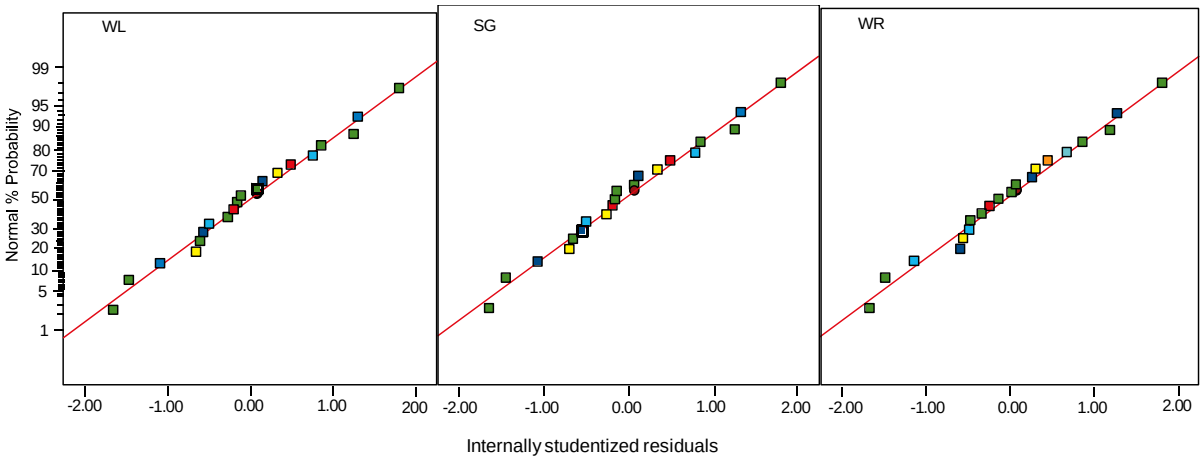

Fig. 1A. Diagnostic curves, normal plot of residuals (WL = water loss, SG = solid gain, WR = weight reduction).

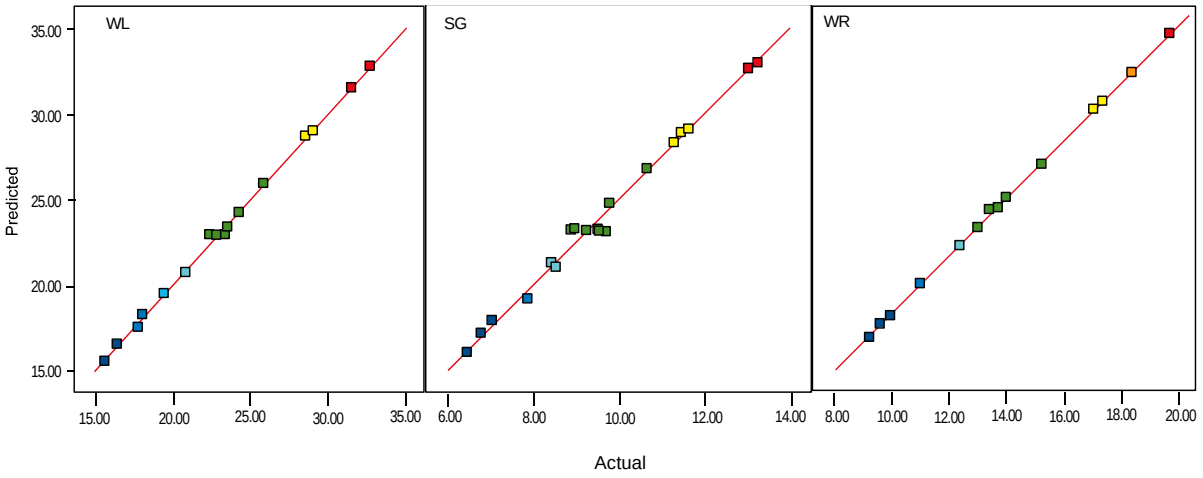

Fig. 1B. Diagnostic curves, predicted *vs* actual curves.

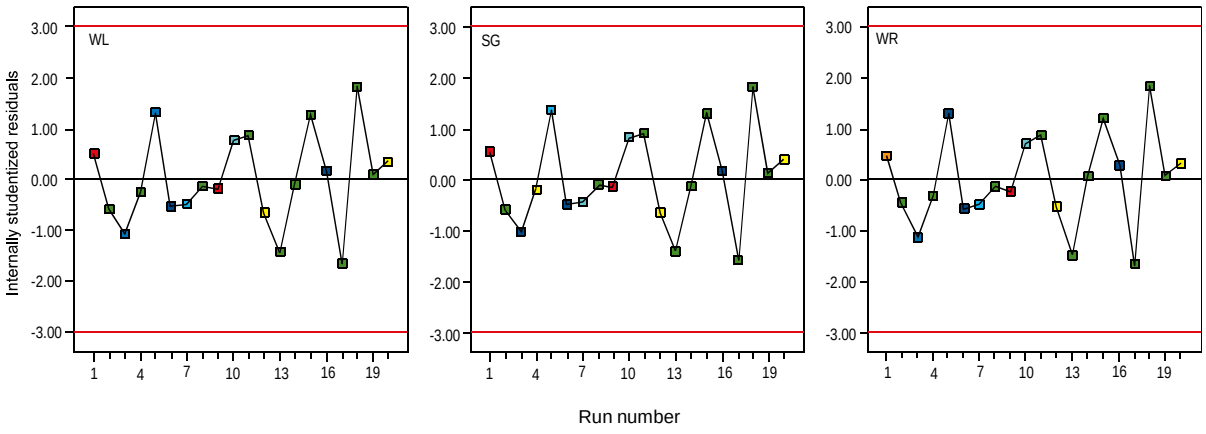

Fig. 1C. Diagnostic curves, residual *vs* run curves.

However, the generated model was not significant with respect to all the factors studied (Table 5). In all cases, interaction effects were not found to be significant except for WR for which time, temperature interaction was significant. This fact can also be observed in Fig. 2(A-F). These surfaces are rectangular planes without any elliptical curvature.

For WL, linear time factor was found to be positive whereas quadratic time factor was negative. This can be observed in Fig. 2A. This response surface curved with a plateau by increase in time. Alam and Singh (2010) working on osmotic dehydration of aonla fruit also found positive sign for linear time term and negative sign for quadratic time term. This indicates that initially water is lost quickly, while this loss gets lower and lower with time. In comparison, for SG, linear time term was found to be negative whereas quadratic term was positive. This type of behaviour is the result of continuous uptake of solute (Raoult-Wack, 1994). The linear temperature term was found to be negative but quadratic term was positive for both WL and SG. This implies that at low temperature, the mass transfer phenomenon is low whereas it increases at higher temperature not only due to the higher kinetic energy of molecules but also due to the change in the structure of the fruit membrane at higher temperature (Torreggiani, 1993). Both the linear and quadratic concentration terms were found to be positive for WL and SG; however, in case of SG the quadratic term was

Table 5. Coefficients of model equations

Factor	WL	SG	WR
Intercept	5.29859	4.26041	1.03818
Time	0.06260	-0.03037	0.09297
Temperature	-0.34025	-0.15265	-0.18761
Concentration	0.05711	0.02904	0.02806
Time* Temperature	0.00104*	0.00015*	0.00089
Time* Concentration	0.00027*	0.00029*	-0.00002*
Temperature* Concentration	0.00109*	0.00106*	0.00003*
Time^2	-0.00046	0.00029	-0.00075
Temperature^2	0.01015	0.00401	0.00614
Concentration^2	0.00204	0.00034*	0.00170

* = not significant.

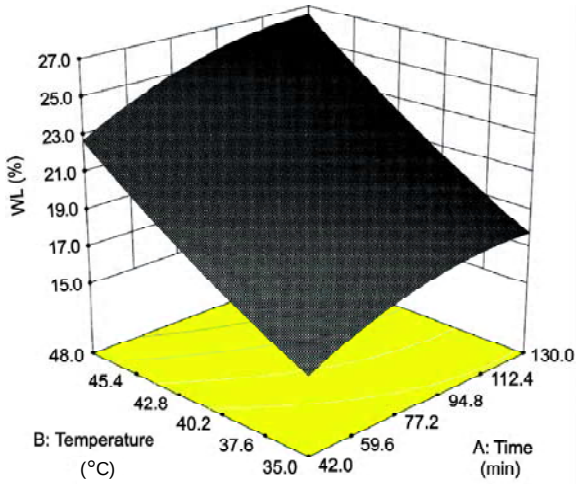

Fig. 2A. Response surface for water loss (WL) at 34° Brx.

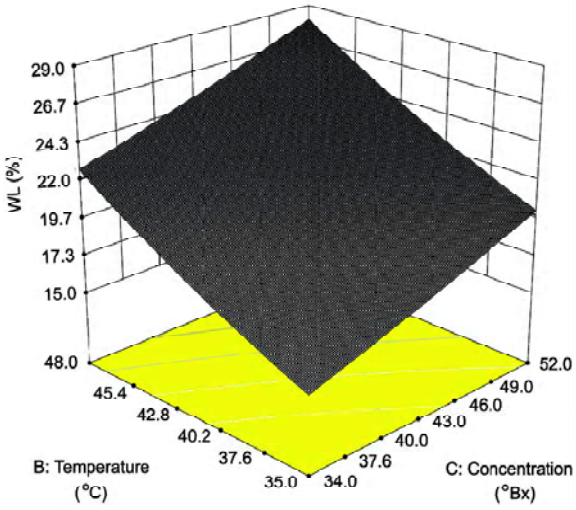

Fig. 2B. Response surface for WL at 42 min.

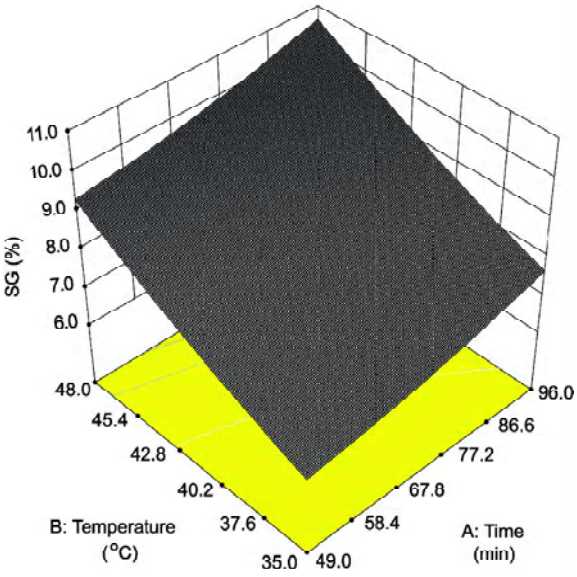

Fig. 2C. Response surface for solid gain (SG) at 34° Brix.

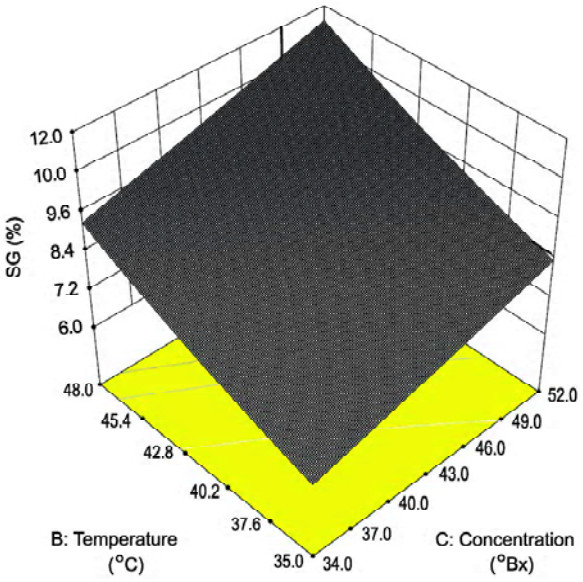

Fig. 2D. Response surface for SG at 42 min.

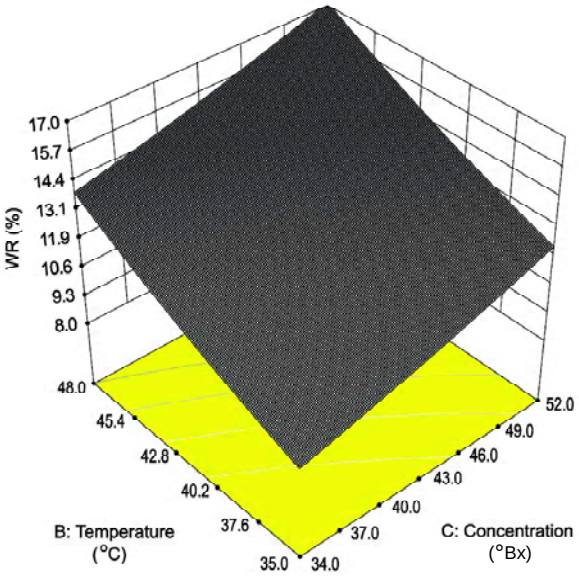

Fig. 2F. Response surface for WR at 42 min.

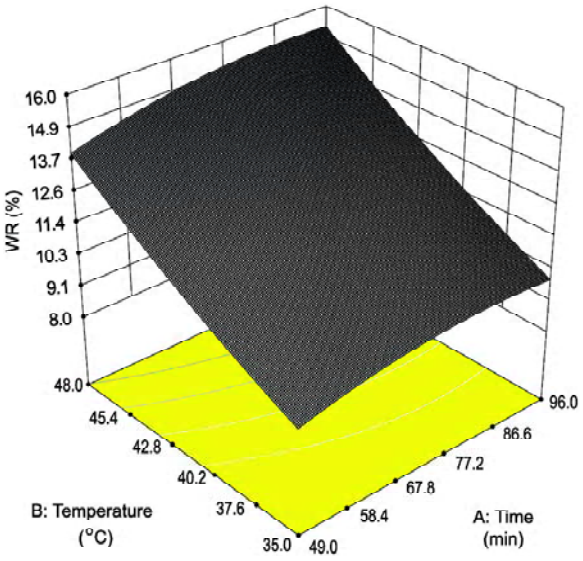

Fig. 2E. Response surface for weight reduction (WR) at 34° Brix.

not significant. Higher concentration results in greater difference of osmotic pressure, therefore, greater removal of water but increase in concentration results in steady increase in solid gain. This might be due to increase in external resistance. Borsato *et al*. (2009) working on osmotic dehydration of pineapple pieces showed that external resistant is also important along with the major resistance caused by the semi-permeable membrane of plant material.

Table 6 shows the optimum conditions under two different scenarios. This optimisation was performed on Design Expert software which uses the desirability function as described by Myers *et al*. (2009).

Table 6. Optimisation of model under different conditions

Factor	Goal	Solution	Goal	Solution
A: Time	is in range	65.96	is in range	188.41
B: Temperature	is in range	48.00	is in range	35.05
C: Concentration	is in range	34.00	is in range	34.04
WL	maximize	24.48	minimize	15.62
SG	minimize	9.66	maximize	13.84

Two different goals were chosen on the basis of two different possible end uses. The first goal was to maximize the WL, keeping the SG lowest. It is to be used when osmotic dehydration will be used as pre-treatment of conventional drying for production of anardana. Whereas the second goal was chosen for possible application of candied pomegranate arils. For this purpose, minimum WL was used as it results in shrinkage, keeping SG the maximum. From Table 6 it is obvious that this goal can be achieved when the temperature and concentration are low and time is high. This result is in agreement with the traditional candied fruit (*Murabba*) making practices.

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Effect of Roasting Temperature on the Fatty Acid Composition and Physicochemical Characteristics of Extracted Oil *Carthamus tinctorius* Thori-78 of Pakistani Origin Seeds

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Abstract. Study of *Carthamus tinctorius* L. (safflower) oil extracted from unroasted and roasted seeds (at temp 120-180 °C) showed, on roasting, significant increase in free fatty acid, acid value, unsaponifiable matter, rancidity, peroxide value, colour development and oxidative deterioration, while refractive index and density were relatively constant. The iodine value of oil of seeds roasted at 160 and 180 °C was reduced. The concentration of oxidation-sensitive linoleic acid reduced from 75.42 to 73.41% but that of palmitic and stearic acids increased, showing no adverse effect on the nutritional value of the roasted seed-oil. But at higher temperature (180 °C) the browning of seeds occurred.

Keywords: *C. tinctorius* Thori-78, roasted-seed oil, fatty acids, vegetable oil

Introduction

Carthamus tinctorius L. (safflower) is an annual herb belonging to the family Compositae. It is widely distributed through out the world such as Asia, USA, Canada, Africa and Australia. India, Africa and USA are the main producers of safflower oil. It has long been extensively grown for obtaining a dye from the flowers as well. This oil crop was introduced into Japan from China, where it became an important source of cooking oil (Oyen and Umali, 2007; Knights *et al*., 2001; Kaffka *et al*., 2000; Sastri, 1950). From Middle East, the crop also spread to Europe and then to America and Africa. *C. tinctorius* flowers, seeds and oil have a wide range of medicinal uses in different countries (Kaffka *et al*., 2000; Sastri, 1950). In northern America, the plant is cultivated for using as bird seed, animal meal and industrial applications (Oyen and Umali, 2007; Mündel *et al*., 2004; Oelke *et al*., 1992).

C. tinctorius seeds are edible and are also eaten after roasting like sunflower seeds (Duke, 1983). The seed is rich in edible oil and oil content is similar to olive, sunflower and peanut oils. The oil content varies from 24 to 36%, depending on the variety of *C. tinctorius*, soil texture, climate and other conditions (Pritchard, 1991; Swern, 1964). The oil is composed of linoleic acid (67.7-83.2%), the essential fatty acid that the human body is unable to biosynthesize (Hamrouni *et al*., 2004, Lee *et al*., 2004). Hence the seeds and seed oil are therapeutically important.

C. tinctorius oil can be used in cosmetics, foods, nutritional supplements, personal care products, soap and shampoos. Developed countries have the most significant market for *C. tinctorius* oil as salad oil, margarine and cooking oil, as it is non-allergenic and is considered to be one of the healthiest oils for human consumption with a high ratio of polyunsaturated/ saturated fatty acids.

Roasting process is the primary step for making condiment oil since the colour, flavour, composition and quality of oil are all influenced by the processing conditions. *C. tinctorius* oil is used as condiment oil along with sesame, red pepper and perillar oils in Korea (Lee *et al*., 2004; Kim *et al*., 2002a; 1998; Yoshida and Takagi, 1997). Recently, use of roasted *C. tinctorius* safflower seed was investigated as medicinal food for bone formation in Korea and the powder of roasted *C. tinctorius* seeds was found to help in recovery of bone repair in rats (Kim *et al*., 2002b; 1998).

In Asia roasted seeds of *C. tinctorius* are commonly consumed. Hence, the objective of the present study was to investigate the changes in physical and chemical indices of unroasted and roasted seed oil of *C. tinctorius* Thori-78 and correlate the results of physicochemical parameters of oil to evaluate the

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quality of seed for edible purpose. At high temperatures, free fatty acids, peroxide value and oxidative deterioration of oil was found to be high; it was further confirmed by fatty acid profile that linoleic acid content decreased while that of palmitic, stearic and oleic acids slightly increased whereas iodine value was reduced upon roasting. This is evident from the results at high temperature (180 °C).

Materials and Methods

Plant material. *Carthamus tinctorius* Thori-78 seeds were purchased from Oilseeds Botanist Agriculture Research Institute Tandojam, Sindh, Pakistan. The fruit is an achene (dry, one seeded with a thin hull). It resembles sunflower seed but is smaller in size and of creamy color. It is irregularly pear-shaped, smooth, shiny and up to 10 mm long.

Reagents. Solvents and chemicals such as *n*-hexane (95%), *n*-heptane (99%), ethanol (95%), carbon tetrachloride (95.5%), chloroform (99.5%), methanol (98.8%), sulphuric acid (95.98%), hydrochloric acid (37%), acetic acid (100%), glacial acetic acid (99.5%); sodium hydroxide (98%), potassium hydroxide (98%), sodium thiosulphate penta hydrate (R.G.), oxalic acid (extra pure), potassium dichromate (extra pure), potassium iodide (extra pure), iodine mono-chloride (R.G.) and anhydrous sodium sulphate were purchased from E. Merck (Darmstadt, Germany) and Labscan (Bankok, Thailand). Standards of fatty acid methyl esters were purchased from Supelco (Bellefonte, PA, USA) and Sigma Aldrich Co. (St. Louis, MO, USA).

Apparatus. The instruments used included gas chromatograph with flame ionization detector, Model Clarus 500, purchased from Perkin Elmer Instruments LLC (Shelton, CT, USA), capillary column Rtx-2330 (60×0.25 mm $\times$ 0.20 μm, film thickness) from Supelco (Bellefonte, PA, USA), UV spectrophotometer Nicollet Evolution 300 (Cambridge, UK), Lovibond model E tintometer (Salisbury, UK), Abbé Refractometer Model 2W (Shijiaahuang, China), Electric oven Model LR-271C with thermostat sets (Florida, USA) and Vacuum oven (Melrose Park, IL, USA).

Roasting of seeds and oil extraction. *C. tinctorius* Thori-78 seeds were roasted at 120, 140, 160 & 180 °C for 20 mins each using electric oven. Unroasted and roasted seeds (200 g of each batch) were separately crushed and finely ground to flour and then subjected to extraction with *n*-hexane in a Soxhlet extractor for 8 h (AOCS, 2004). Fat was recovered using a rotary evaporator and the extracted fat was dried over anhydrous sodium sulphate, transferred to a capped bottle and stored at 4 °C until used for analysis.

Colour determination. Oil samples extracted from roasted and unroasted seeds were placed in a 1 inch cell, and the colour was determined using a Lovibond tintometer model E (Salisbury, England) at room temperature (35 °C) through making the best possible match with the standard colour slides of red and yellow indices.

Determination of colour development. Colour development was measured through absorbance at 420 nm of 5.0% (W/V) solutions of oils in chloroform, using UV Spectrophotometer (Nicolet Evolution 300, Cambridge, UK).

Determination of oxidative deterioration of oil. Deterioration of the roasted and unroasted extracted oil was determined following the analytical method 2.505 described in IUPAC (1987). Oil samples were dissolved in isooctane. A Nicolet Evolution 300 UV Spectrophotometer, with the software "VISION*pro*", was used to determine absorbtivity at $\lambda_{(max)}$ 232 and 270 nm.

Fatty acid composition. Methyl esters of fatty acids were prepared according to standard IUPAC method 2.301 (IUPAC, 1987). Chemical composition of fatty acid methyl esters was accomplished with a Perkin Elmer gas chromatograph model Clarus 500 fitted with a polar capillary column Rtx-2330 (60×0.25 mm $\times$ 0.20 μm, film thickness) and a flame ionization detector. Nitrogen was used as carrier gas at flow rate of 3 mL/min. Other conditions were as follows: initial oven temperature, 70 °C for 5 mins ramped at 10 °C/min to 180 °C and then ramped at 3 °C/min to final temperature 220 °C where it was held for 8 mins; injector temperature, 270 °C; detector temperature, 270 °C. A sample volume 0.3 μL was injected; (split less). Fatty acid methyl esters were identified by comparing their relative and absolute retention times of standards of fatty acid methyl esters. The quantification was done by a built-in data-handling programme, provided by the manufacturer of the gas chromatograph. The analyses were performed in triplicate.

Physical and chemical analysis of the extracted oil. The oils extracted from unroasted and roasted oil samples were analyzed by adopting the methods of

American Oil Chemist Society (AOCS, 2004). For determination of refractive index, density, free fatty acid, acid value, peroxide value, rancidity, iodine value, unsaponifiable matter, colour, colour development and deterioration of oil, UV-spectrophotometer was used. The colour of oil was determined by Lovibond Tintometer (Tintometer Ltd., Salisbury, UK) using 1″ cell.

Results and Discussion

Seeds were roasted at 120, 140, 160 and 180 °C for 20 mins. The hexane-extracted oil contents of unroasted and roasted samples of *C. tinctorius* Thori-78 are shown in Table 1. Physical and chemical characterization of unroasted and roasted seed-oil samples are depicted in Tables 1 and 2. It was noticed that as the roasting temperature increased, slight increase in oil content from 28.33 to 29.64% occurred which may be due to rapid evaporation of moisture at high temperature.

The values of FFA (%) as OA (oleic acid) and acid value also increased with the increase in roasting temperature (Fig. 1). Seed samples showed a significant increase in peroxide value and rancidity at high roasting temperature (160 and 180 °C), as depicted in Fig. 2, as a result of formation of more hydroperoxides at these temperatures. The absorptivity at 232 and 270 nm, due to the formation of conjugated dienes and trienes, was a good index for measuring the oxidative degradation of oil. The extent of oxidative deterioration of the oil samples were measured by UV spectrophotometer. The values of absorptivity at 232 nm and 270 nm increased from the room temperature to 180 °C (Fig. 3). As the roasting temperature increased, the oxidative deterioration of oil also increased resulting in formation of primary and secondary products in the seeds (Khatoon and Krishna, 1998; Vieira *et al*., 1998; Albi *et al*., 1997; Yen and Shyn, 1989).

The colour of unroasted and roasted oil samples was measured on Lovibond Tintometer; the result showed increase in red and yellow units with the increase in temperature (Fig. 4). Intense color of the vegetable oil depends mainly upon the presence of various colouring pigments of plants such as carotenoids, chlorophyll etc. which are effectively removed during refining and bleaching steps of oil processing. The vegetable oils with minimum values of colour index are good for edible purpose.

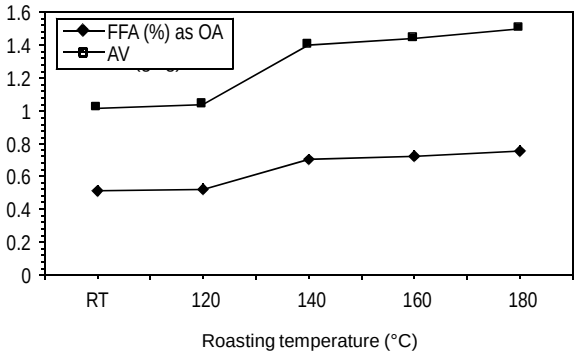

Fig. 1. FFA and AV values at different roasting temperature (RT = 35 °C).

Table 1. Characteristics of unroasted and roasted seed-oil of *C. tinctorius* Thori-78

Parameters	Unroasted seed oil	Roasted-seed oil at different temperatures			
	(at room temperature)	120 °C	140 °C	160 °C	180 °C
Oil content (%)	28.33	28.63	28.74	29.12	29.64
Free fatty acid (% as OA)	0.51	0.52	0.70	0.72	0.75
Acid value (g/kg)	1.12	1.04	1.40	1.44	1.50
Peroxide value (meq/kg)	17.2	19.35	19.40	40.14	42.42
Rancidity	Incipient	Rancid	Rancid	Highly rancid	Highly rancid
Iodine value (g of I_2/100 g oil)	134.82	135.10	134.90	133.57	132.99
Unsaponifiable matter (%)	0.4132	0.5601	0.7025	0.7210	0.8980
Refractive index at 40 °C	1.4734	1.4732	1.4730	1.4730	1.4730
Density at 24 °C	0.9100	0.9101	0.9121	0.9151	0.9154
Density at 40 °C	0.9064	0.9087	0.9088	0.9097	0.9100
Colour 1″ cell (red unit)	1.4	1.5	1.6	2	4.3
(yellow unit)	25	26	26	35	70
(neutral unit)	0.71	0.1	0.8	0	0
Colour determination					
$\lambda_{(max)}$ 420 nm (w/v 5%)	0.018	0.031	0.068	0.073	0.118
Absorbtivity at $\lambda_{(max)}$ 232 nm	4.585	4.611	4.714	4.726	4.779
at $\lambda_{(max)}$ 270 nm	3.399	3.481	3.571	3.611	3.700

Table 2. Fatty acid composition (wt %) of unroasted and roasted high linoleic seeds oil of *C. tinctorius* Thori-78

Fatty acids	Unroasted seed oil (room temperature)	Oil from seeds roasted at different temperatures			
		120 °C	140 °C	160 °C	180 °C
Palmitic acid ($C_{16:0}$)	6.45	7.35	7.17	7.03	7.04
Stearic acid ($C_{18:0}$)	1.99	2.18	2.01	2.03	2.46
Oleic acid ($C_{18:1}$)	15.55	14.57	14.22	14.12	14.96
Linoleic acid ($C_{18:2}$)	75.42	75.75	75.64	75.84	73.41
Others	0.59	0.15	0.96	0.98	2.17
Total saturated fatty acids	8.44	9.53	9.18	9.06	9.5
Total unsaturated fatty acids	90.97	90.32	89.86	89.96	88.37

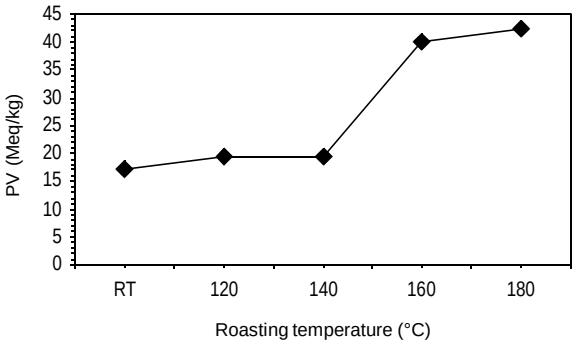

Fig. 2. PV values at different roasting temperatures (RT = 35 °C).

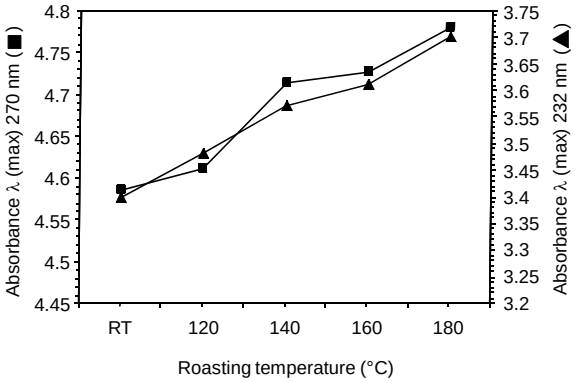

Fig. 3. Absorbance at 232 and 270 nm at different roasting temperatures (RT = 35 °C).

The colour formation during roasting of *C. tinctorius* Thori-78 seeds at 120, 140, 160 and 180 °C (Fig. 5) with the increase in temperature, resulted in significant

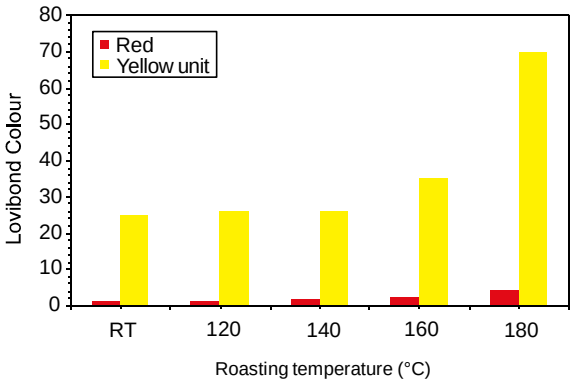

Fig. 4. Red and yellow units of unroasted and roasted seed oil samples (RT = 35 °C).

increase in the absorbance at 420 nm from 0.018 to 0.118 (light yellow to dark brown). As the temperature increased, the increase in colour of oils, seemed to be due to maillard type non-enzymatic reactions between sugars and free amino acids or amides in the seeds (Lee *et al*., 2004) resulting in brown colour substances in that seed samples. This result is in concordance with the earlier literature, reporting significant increase in colour of oils of rice germ, sesame seed and safflower (Lee *et al*., 2004, Kim *et al*., 2002a; Yoshida and Takagi, 1997; Yoshida, 1994; Yen, 1990).

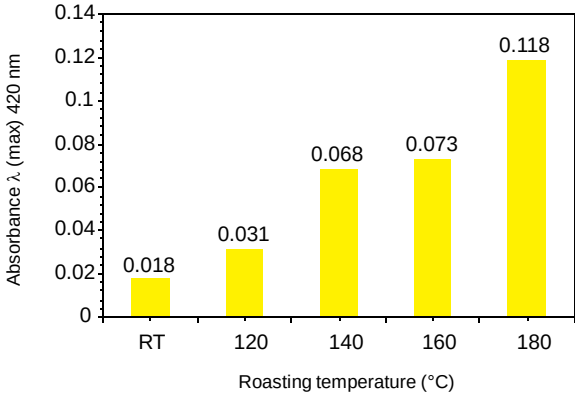

Fig. 5. Absorbance at 420 nm at different roasting temperatures (RT = 35 °C).

There was no observable significant variation in the refractive index determined at 40 °C; this showed that the high temperature did not affect the structure of oils of unroasted and roasted seeds. Thus the oils are fit for edible purposes.

Densities of all samples were determined at 24 and 40 °C. There was no significant variation observed in density with rise in temperature as shown in Fig. 6; the values are in concordance with the reported values for vegetable oils (Rossell, 1991).

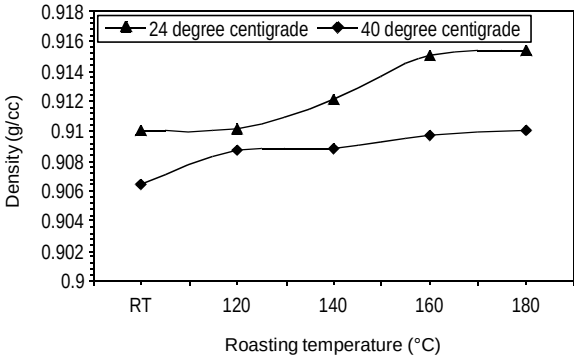

Fig. 6. Oil density at different roasting temperatures (RT = 35 °C).

With the increase in roasting temperature, unsaponifiable matter slightly increased (Table 1) but the values are concordant with the previous reports about unsaponfiable matter of vegetable oils (Rossell, 1991).

High iodine values of *C. tinctorius* is due to the presence of high content of unsaturated fatty acids and are comparable to the values obtained for poppy, soybean and sunflower oils (Rossell, 1991). High iodine values show that seed oil has good qualities of edible oil and drying oil (Eromosele *et al*., 1994); thus it can be put to different industrial uses. Iodine values of roasted and unroasted oil samples were more or less similar except the slight decrease at 180 °C. This is also confirmed by fatty acid composition of sample oil roasted at 180 °C (Table 2) with decrease in linoleic acid concentration from 75.42 to 73.41% and slight increase in stearic acid from 1.99 to 2.46%. Similar observation was reported in the previous studies on roasted pumpkin seeds (Markovic *et al*., 2004). Fatty acid composition of all the extracted oil samples was determined using gas chromatography; one of the fatty acid profile of *C. tinctorius* is shown in Fig. 7 with the components depicted in Table 2. The principle fatty acid components in Thori-78 were palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Linoleic acid is predominantly present. The fatty acid composition was more or less similar to that of sunflower, soybean, corn and cotton seeds and *C. tinctorius* originating from different geographic regions (Cosgse *et al*., 2007, Rossell, 1991). *C. tinctorius* Thori-78 variety is high in linoleic acid content as compared to the other varieties, grown in Pakistan (Sultana *et al*., 2010).

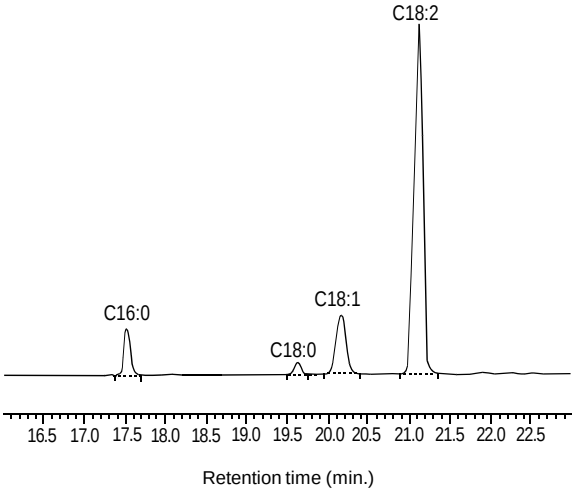

Fig. 7. Gas chromatogram of fatty acids of *C. tinctorius* (Thori-78) seed oil.

Characteristic parameters of roasted seed oil compared well with those of the oil samples of seeds roasted upto 160 °C, with no significant difference in the nutritional quality of oils. At the highest temperature (180 °C), however, maillard and non-enzymatic reaction occurred and the seeds were slightly burnt.

Conclusion

It is concluded that with the increase in roasting temperature the physicochemical characteristics of the roasted seed oil of *C. tinctorius* Thori-78 variety such as free fatty acid, acid value, rancidity, peroxide value and oxidative deterioration significantly increased while iodine value showed decreasing trend at 180 °C. This was also confirmed by fatty acid composition of sample oil roasted at 180 °C in which linoleic acid decreased and slight increase occurred in palmitic and stearic acids. Refractive index and densities of seed oil were relatively constant.

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Effect of Moisture Content and Heat Treatment on Peroxide Value and Oxidative Stability of Crude Palm Kernel Oil

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Abstract. Effect of moisture content, roasting time and temperature on peroxide value (PV) and oxidative stability (OS) of unrefined palm kernel oil was studied using response surface methodology at five levels of moisture content (4, 7, 10, 13 and 16% wet basis), roasting time (5, 10, 15, 20 and 25 min) and roasting temperature (50, 70, 90, 110 and 130 °C). Within the studied range, mean PV of palm kernel oil was recorded to be 13.06±5.13 meq/kg. Least PV of 7.3 meq/kg was obtained at 10% moisture content, 15 min roasting time and 130 °C temperature. Maximum stability time of 27.0 h was achieved at 10% moisture content, 15 min roasting at 130 °C. This treatment produced unrefined palm kernel oil stable for 388 days. All the studied parameters significantly influenced flavour rating and shelf life of unrefined palm kernel oil at $P \leq 0.05$.

Keywords: palm kernel oil, oxidative stability, peroxide value, moisture, heat treatment

Introduction

Vegetable oil, as a valuable part of a well-balanced diet, contains a range of fat soluble vitamins (A, D, E and K) and essential fatty acids, both necessary for the healthy functioning of the body (Fellows and Hampton, 2003). Good quality palm kernel oil serves the aforementioned nutritional significance. Industrial application of palm kernel oil is also fast increasing. Among the 17 commodity covered in the data provided by Oil World (2009), palm kernel oil occupied eleventh production level, after four major vegetable oils (soy, palm, rape, and sunflower), three animal fats (tallow, lard and butter) and three minor oils (groundnut, cotton seed and coconut).

Palm kernel oil is a yellowish white fat containing 82% proportional weight of saturated fatty acid and 18% unsaturated fatty acid (O'Brien, 2008). It is classified by the nutritional experts as saturated oil and has the advantage of having solid texture at room temperature (Rossell *et al.*, 1985). Chemicals and physical properties of palm kernel oil resemble those of the coconut oil. It belongs to members of a group called lauric oils due to high level of lauric acid (46-52%) present in proportional weight (Tat and Eng, 1985).

Preferences for fat and oil products with fresh bland flavours and odours require keeping quality and rancidity evaluations both during development and processing. Peroxide value (PV) is one of the most widely used chemical tests for the determination of fats and oils quality. Peroxide value is a measure of rancidity in its early stage, this test showed good corelation with organoleptic flavour scores (O'Brien, 2008). Although a linear relationship has been observed between peroxide values and flavour scores during the initial stages of lipid oxidation, this method alone is not a very good flavour quality indicator because the peroxide value increases to maximum and then decreases as storage time increases (Hill, 1994). Hence, oils and fats are subjected to oxidative stability test which is a good quality parameter assessment that complements PV analysis (Shahidi and Naczk, 2004; Holser and Isbell, 2000). Most fat and oil products are tested for flavour stability as part of quality control programmes to assure that customer specifications are satisfied. The purpose of this evaluation is to satisfactorily determine the product shelf life. Studies have been carried out on oxidative stability of edible fats and oils such as sesame oil (Lee *et al.*, 2010), safflower oil (Lee *et al.*, 2004), olive husk oil (Lucas *et al.*, 2002), meadow foam oil (Holser and Isbell, 2000), cotton seed (Hill, 1994) and lard (Kikugawa *et al.*, 1983). In summary, these reports showed oxidative stability of fats and oils dependent on the method used for determination of chemical composition and processing parameters.

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For oil expression, raw material preparation involves operations such as decortication, winnowing, cracking, pulping, and conditioning. Conditioning of oil seeds is an important operation in the production line of palm kernel oil. These activities include roasting, flaking and size reduction, cooking, pre-pressing and drying. Dehydrating and roasting of palm kernel before oil expression improves palm kernel oil yield (Akinoso *et al.*, 2006; Ajibola, 1989). There is a need to establish the degree of influence of these parameters on storage life of the expressed crude palm kernel oil. This work investigated effects of moisture content, roasting time and temperature on the initial quality and the oxidative stability of unrefined palm kernel oil using response surface methodology. Calabar variety of palm kernel was used for the research work. The choice of the species was influenced by NIFOR (2008) report which stated that tenera variety of oil palm of calabar will soon be the major oil palm available for kernel crushing in Nigerian market due to its acceptability by organised palm oil industries in the country.

Materials and Methods

Experimental design. The experiment was conducted using central composite rotatable design as described by Montgomery (2005). Moisture content, roasting time and temperature were independent variables, while peroxide value and oxidative stability of the oil were the dependent variables. Fixing of independent parameter levels were based on preliminary work (Akinoso *et al.*, 2006). Fifteen level combinations were used; the last level was repeated 6 times (Table 1) thus a total of 20 runs were conducted.

Material preparation. Palm kernel was obtained from Ogun State Ministry of Agriculture and Cooperative (Apoje oil mill), Nigeria. It was cleaned manually to remove foreign materials such as shell, broken kernels and stone.

Determination of moisture content. Initial moisture content of the seed was determined using ASABE (2008) method. Three samples each weighing 15 g, were placed in an oven set at 130 °C for 6 h. The samples were then cooled in a glass jar containing silica gel as desiccant. The dried samples were weighed and the difference in weight before and after drying was taken as the moisture loss. Ratio of moisture loss to weight of wet material (percentage) was recorded as moisture content on wet basis. The desired moisture content levels were achieved by adding distilled water as calculated from below mentioned equation 1 (Akinoso *et al.*, 2006). Samples were individually sealed in separate polyethylene bags and kept at 5 °C in a refrigerator for 12 h for distribution of moisture uniformly throughout the samples. Five moisture content levels *viz.* 4, 7, 10, 13 and 16% (wet basis) were prepared for each set:

$$Q = A(b - a)/(100-b) \quad \text{..... (1)}$$

where:

A = Initial mass of the sample
a = Initial moisture content of the sample (% wet basis)
b = Final (desired) moisture content of sample (% wb)
Q = Mass of water to be added (kg)

Pre-heating time and roasting temperature. Roasting temperature stability was achieved by Akinoso and Igbeka (2007) method. The product's initial temperature was raised to equilibrium with roasting temperature. This was achieved by wrapping them in polythene bags and placed in convection oven (Model LDO-25OF, Win Science, Seoul, Korea) at the desired roasting temperature level. These samples were later heated by spreading thinly on a heat conductor tray in the oven at the preset temperature. The samples were heated at specified temperature and time. Stopwatch was used to monitor the time. Roasting time was 5, 10, 15, 20 and 25 min, while roasting temperature was 50, 70, 90, 110 and 130 °C.

Oil expression. The expeller used was Tite 002 manufactured by Tiny Tech Plant, India, of a rated capacity of 180 kg/h, powered by a 30 kW electric motor with interchangeable speed. It consisted of a helical thread that revolved within a stationary perforated cylinder. The experiment was conducted through running the screw press for about 3 min before loading the pretreated samples of palm kernel. The oil seed was forced through the barrel by the action of the revolving worms. The volume was axially displaced by the worm, from the feeding end to the discharge end, thus compressing the meal as it passes through the barrel. The expelled oil drained through the perforation of the lining bars of the barrel, while the deoiled cake was discharged through the annular orifice. The expressed oil was collected and clarified by allowing it to stand for 96 h. The clarified oil was then bottled and labelled.

Peroxide value. The peroxide value (PV) of unrefined palm kernel oil was determined by modified American

Oil Chemists' Society standard method Cd 8-53 (AOCS, 1997). About 5 mL of oil was placed on a test paper (Matsushita, 1978). About 10 mL of water was added to the paper on which the oil has been placed. The intensity of the blue colour, developing on the test paper, was proportional to the peroxide value of the oil. Colour of the paper was compared to a predetermined standard as reported by Matsushita (1978).

Oxidative stability. Oxidative stability (OS) was evaluated by AOCS method Cd 12b-92 (AOCS, 1997) using Rancimat 679 apparatus (Metrohm AG, Herison, Switzerland). The evaluation procedure was performed by placing 2 g of the oil in the sample tube, which was preheated to 120 °C, connected on one side to the air source and on the other side to 50 mL cell of deionized water. Conductance of the water over time was measured automatically with data logger. Stability was expressed as the oxidation induction period in hours.

Data analysis. The experimental procedures were repeated thrice for each treatment and mean values of the data were recorded. Interaction between the independent and dependent variables was analysed by response surface methodology (RSM) using Design-Expert® 8 software. Analysis of variance (ANOVA) and regression analysis were carried out and model equations were also developed. Level of significance was preset at 0.05 (95 % confidence level) for the entire analytical values.

Results and Discussion

Peroxide value. Within the studied range, recorded mean PV of palm kernel oil was 13.06 ± 5.13 meq/kg (Table 1). Least PV of 7.3 meq/kg was obtained at 10% wb moisture content, 15 min roasting time and 130 °C roasting temperature while 16% wb moisture content, 15 min roasting time and 90 °C roasting temperature gave the highest PV of 21.9 meq/kg. Difference in the PV may be associated with variation in the roasting temperature and the moisture content of the samples. It is known that factors such as temperature, light, moisture, metals and oxygen affect the rate of oxidation (Salunkhe *et al.,* 1992). Peroxide value of 7.3 meq/kg is within recommended/permissible PV of edible oil by FAO/

Table 1. Central composite rotable design of the experiment showing variables and response

MC (%wb) Coded	RD (min.) Coded	RT (°C) Coded	MC (%wb) Real	RD (min.) Real	RT (°C) Real	PV (meg/kg)	OS (h)
-1	-1	-1	7	10	70	10.6 ± 1.2	19.4 ± 5.7
-1	-1	1	7	10	110	9.0 ± 1.1	23.1 ± 3.6
-1	1	-1	7	20	70	8.6 ± 0.9	22.0 ± 5.8
-1	1	1	7	20	110	8.8 ± 2.1	25.9 ± 3.9
1	-1	-1	13	10	70	16.2 ± 3.3	15.0 ± 3.7
1	-1	1	13	10	110	19.1 ± 1.7	14.7 ± 2.1
1	1	-1	13	20	70	18.3 ± 2.9	16.3 ± 3.2
1	1	1	13	20	110	14.8 ± 3.7	17.6 ± 4.5
1.682	0	0	16	15	90	21.9 ± 4.1	2.3 ± 1.1
-1.682	0	0	4	15	90	7.5 ± 1.5	24.6 ± 2.6
0	1.682	0	10	25	90	17.2 ± 2.9	21.1 ± 4.2
0	-1.682	0	10	5	90	19.9 ± 3.8	16.3 ± 3.8
0	0	1.682	10	15	130	7.3 ± 1.9	27.0 ± 5.1
0	0	-1.682	10	15	50	14.2 ± 1.1	18.3 ± 3.8
0	0	0	10	15	90	11.2 ± 1.2	21.3 ± 3.2
0	0	0	10	15	90	11.8 ± 2.1	21.9 ± 3.8
0	0	0	10	15	90	10.9 ± 1.3	20.9 ± 4.1
0	0	0	10	15	90	11.7 ± 1.9	21.6 ± 3.2
0	0	0	10	15	90	11.3 ± 1.8	20.6 ± 3.0
0	0	0	10	15	90	11.4 ± 1.9	21.5 ± 3.6

MC = moisture content; RD = roasting time; RT, roasting temperature; PV = peroxide value; OS = oxidative stability.

WHO codex alimentarious standard. The maximum permissible level is 10 meq/kg (CAC, 2001).

Statistical analysis of the collected data showed the model to be significantly fit for the response surface quadratic model. Mathematical expression of relationship between the actual values of the variables and the response is shown as below given equation 2. Coefficient of determination R^2 of the model was 0.8. High coefficient of determination implies that, as a result of application of this mathematical equation errors will be minimal. Significant model terms of the equation at P = 0.05 were moisture content and the 2nd order of roasting time. This agreed with the findings of Gunstone (2002) that isolated moisture content is the major stimulant of rancidity in some edible oils.

$$PV = +35.14 - 1.64\,m - 2.25\,r - 0.08\,t - 2.15\,x\,10^{-16}mr + 1.67x10^{-3}mt - 5.75x10^{-3}rt + 0.14\,m^2 + 0.09r^2 + 5.65x10^{-4}t^2 \quad (2)$$

Three dimensional view of the response to the variables are shown in Fig. 1 to 3. These plots help to visualize the shape of the response surface and give useful information about the fit model. The outlook of the response surface plots (Fig. 1-3) indicated that the centre was saddle and was neither maximum nor minimum. This explained why response surface quadratic model was fit to predict the PV of expressed palm kernel oil as affected by treatments.

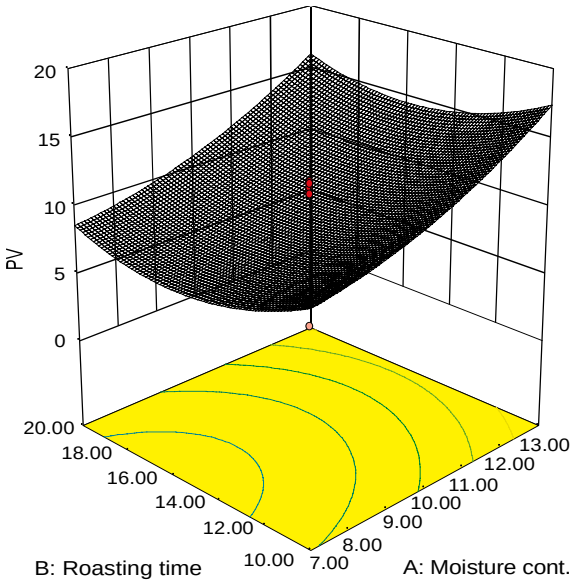

Fig. 1. Peroxide value of crude palm kernel oil as influenced by moisture content and roasting time.

It could be seen in Fig. 1 and 2 that the PV of oil increased with the moisture content. According to Goh (2002), disadvantage of using palm kernel oil in the presence of moisture and lipase enzyme is the hydrolytic reaction that liberates short-chain fatty acids

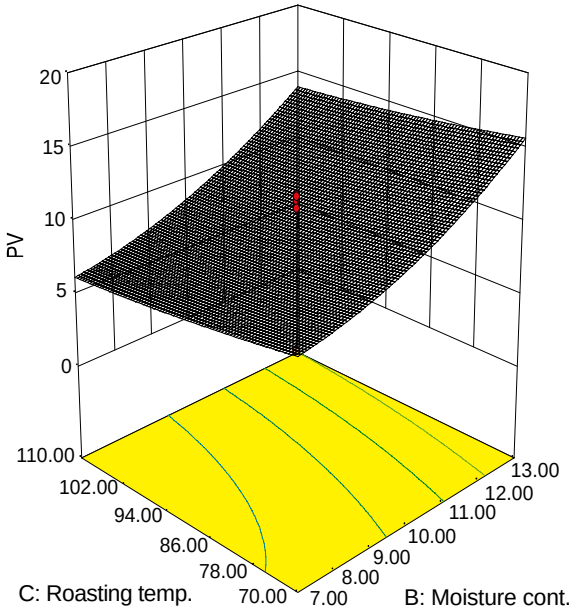

Fig. 2. Peroxide value of crude palm kernel oil as influenced by moisture content and roasting temperature.

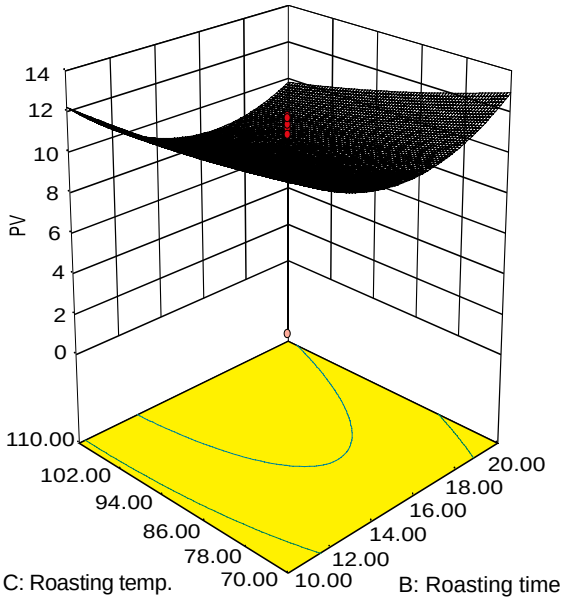

Fig. 3. Peroxide value of crude palm kernel oil as influenced by roasting time and temperature.

(C 6:0 to C 12:0) and gives rise to unpleasant soapy off-flavour. This phenomenon explained the observed trend of the plots (Fig. 1 and 2).

Response to the heat treatment as shown in Fig. 3 was non-proportional variation. That is, neither continuous increase nor decrease in PV of the oil samples was experienced with the increase in roasting time and temperature. This suggested unsteady thermal influence on the reaction. As expected, a biological material like palm kernel displayed non homogeneous behaviour; as behaviour similar to the one shown in Fig. 3 was reported by Guillen and Cabo (2002) on PV of safflower, sunflower, rape seed and olive oils. Formation of PV is a chemical reaction, and like most reactions, it is influenced by heat, light and impurities.

Oxidative stability. With treatment at 16% moisture content for 15 min roasting time at 90 °C roasting temperature, minimum oxidative time of 2.3 h was recorded while maximum oxidative time of 27.0 h was achieved at 10 % moisture content, 15 min roasting time and 130 °C roasting temperature. One hour of active oxygen is equivalent to 15 days (O'Brien, 2008); using this conversion factor, the oil in its form can retain its flavour for 388 days. This quality may be traced to the presence of tocopherol in palm kernel oil and also to its saturation. The degree of saturation is known to significantly influence lipid oxidation. Tocopherol is a natural antioxidant and has impact on oxidative stabi-lity of oils (Otal, 2001; Kamal-Eldin and Appelqvist, 1996).

A response surface quadratic model was used to illustrate the relationship between the oxidative stability (response), moisture content, roasting time and temperature (variables) (equation 3). Lack of fit and F-value of the model are significant. Also the coefficient of determination R^2 (0.99) of the model was high.

$$OS = -4.69 + 4.17m + 0.87r + 0.1t - 1.0\,x10^{-2}\,mr - 1.37x10^{-2}mt + 2.25x10^{-3}rt - 0.21m^2 - 0.02r^2 + 9.5\,x\,10^{-4}\,t^2 \quad \text{..... (3)}$$

The coefficient of determination R^2 (0.99) of the model indicated suitability of the application of the model in predicting effect of moisture content, roasting time and temperature on OS of palm kernel oil. ANOVA of the data revealed that the three independent variables and the 2nd order of moisture content were significant model terms at P = 0.05. This implies that variation in any of the variables, will bring noticeable difference in oxidative stability of palm kernel oil.

Visual outputs of the interaction are shown in Figs. 4-6. Reduction in OS time was observed with the increase in moisture content of palm kernel oil (Fig. 4 and 5). Moisture naturally accelerates hydrolysis reaction (Hui, 1996). This accounts for the reduced oxidative stability time with the increase in moisture content.

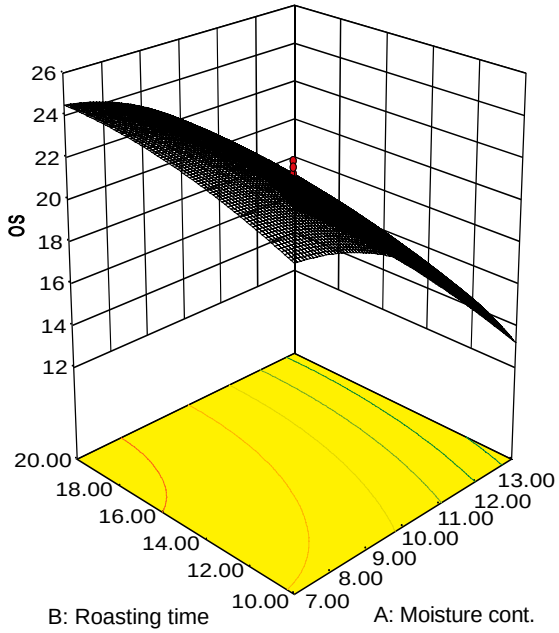

Fig. 4. Oxidative stability (OS)of crude palm kernel oil as influenced by moisture content and roasting time.

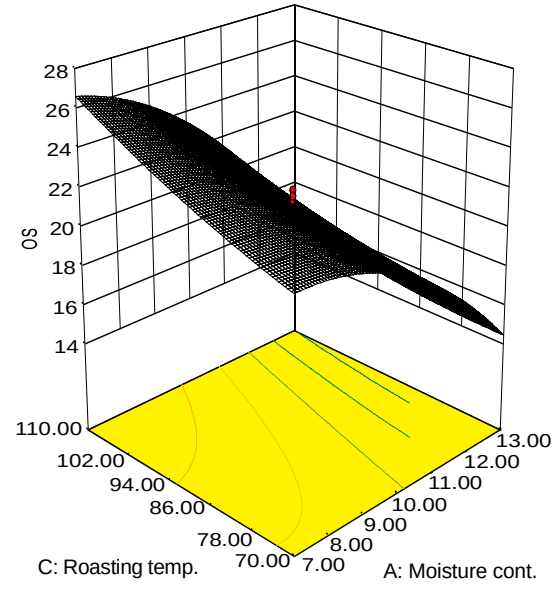

Fig. 5. Oxidative stability (OS) of crude palm kernel oil as influenced by moisture content and roasting temperature.

Heat treatment (Fig. 6) showed a trend converse to as shown in Fig. 4 and 5. Potencies of tocopherol, a natural antioxidant in palm kernel, are temperature dependent (Hove and Hove, 1944). This may account for the observed behaviour.

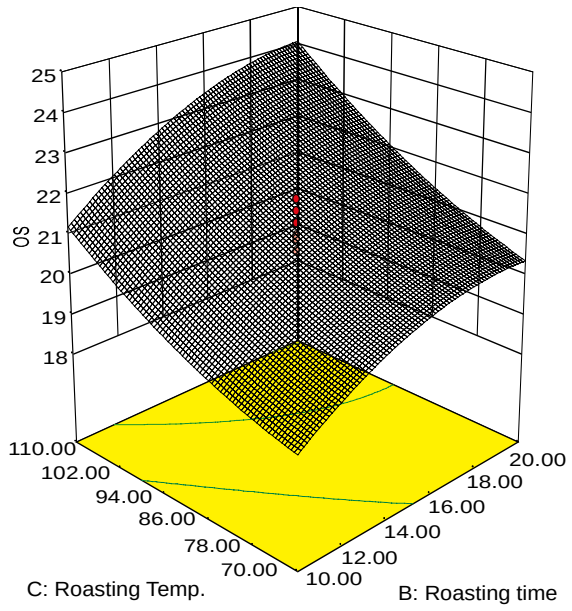

Fig. 6. Oxidative stability (OS) of crude palm kernel oil as influenced by roasting time and temperature.

Conclusion

Initial moisture content of palm kernel and conditioning of palm kernel by heating significantly affected the flavour rating *viz* peroxide value and stability of unrefined palm kernel oil at $P \leq 0.05$. The degree of influence of these treatments on peroxide value and oxidative stability of palm kernel oil can be predicted using response surface quadratic model. Maximum oxidative time of 27.0 h was achieved at 10% moisture content, 15 min roasting time and 130 °C roasting temperature. This treatment produced unrefined palm kernel oil that will be stable for 388 days before usage.

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Effect of Citric Acid and Storage Containers on the Keeping Quality of Refined Soybean Oil

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Abstract. Free fatty acids (FFA), acid value (AV), peroxide value (PV) and iodine value (IV) of the soybean oil in tinned cans, transparent white glass and plastic bottles were monthly monitored for one year at room temperature. The results revealed that the oil stored in tin containers had the highest FFA and AV of 0.334±0.054% oleic acid and 0.653±0.104 mg KOH/g oil, respectively, while that in plastic containers had the lowest value of 0.252±0.033% oleic acid and 0.495±0.064 mg KOH/g oil for the FFA and AV. Addition of food grade citric acid (FGCA) at 0.2% level increased the keeping quality of refined soybean oil stored in glass and plastic bottles both with respect to hydrolytic stability of the oil. However, it reduced the peroxide value and slightly increased the iodine value of oil in all the containers. The additive (FGCA) led to a higher reduction in the oxidative rancidity of the oil stored in plastic bottles as compared to that stored in glass and tinned cans. There was significant difference at $P<0.05$ in FFA and AV of oil stored in tin and glass containers as well as in PV and IV of the oil stored in all the containers. The additive enhanced the shelf life with respect to oxidative stability of the oil the most in plastic bottles and the least in tinned cans.

Keywords: soybean oil, containers, citric acid, free fatty acids, acid value, peroxide value, iodine value

Introduction

Soybean oil remains in high demand due to low cholesterol level making it safer for human consumption (Arawande, 2008). Crude soybean oil is produced from soybean seeds which are cracked, adjusted for moisture content, rolled into flakes and solvent-extracted with commercial hexane. The crude oil is further subjected to refining (Wikipedia, 2007) through degumming, neutralization, bleaching and deodorisation (Arawande and Abitogun, 2009a).

Refined soybean oil is the predominant vegetable oil used domestically in edible oil products (Erickson *et al*., 1980). Application of soybean oil falls into two main categories: edible fat products, meant for human consumption, and industrial fat products, used for technical purposes (ASA, 1996). The oil is unique among vegetable oils due to its high content of unsaturated fatty acids and remains in liquid form much below the room temperature. It contains potential natural antioxidants which are not removed during processing thereby preventing the oxidative rancidity which may occur in the lipids present in the oil (Haumman, 1994). It contains 7-8% linolenic acid which can be reduced during processing. The high content of linolenic acid is responsible for the development of off-flavour and off-odour during degradation of the oil.

Nowadays, there is a drastic shift from the consumption of common red palm oil and other edible oils to refined soybean oil (Arawande, 2008).Vegetable oil merchants sometimes purchase the oil when it is cheap and store it in different containers, such as plastic bottles, glass bottles and tinned cans, and later sell it during off season when it becomes expensive, without taking into consideration the deterioration of oil quality.

One of the major problems confronting the producers, sellers and consumers of oil is associated with the deterioration of oil during storage where in the oil turns rancid owing to high content of unsaturated fatty acids. However, the use of antioxidants reduces deterioration during the storage (Arawande and Abitogun, 2009b).

Several studies have been conducted earlier and reported on soybean oil. Carlson and Scott (1991) and Erickson *et al*. (1980) reported processing and utilisation of soy oil, whereas Abitogun *et al*. (2009) studied effects of phosphoric acid on physicochemical parameters of soybean oil. Arawande (2008) investigated effects of storage containers on the shelf life of refined soybean oil. The use of soybean oil as insect repellent has been

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established (Barnard and Xue, 2004; Fradin and Day, 2002). Arawande and Amoo (2009) reported effects of light and dark storage conditions on some quality parameters of refined soybean oil. The use of citric acid as a potential antioxidant on crude palm kernel oil was reported by Arawande and Abitogun (2009a). However, little or no work has been done on the combined effects of both citric acid and storage containers on the shelf life of refined soybean oil. The aim of the present work was to investigate the effects of storage containers (tinned can, glass and plastic bottles) and 0.2% food grade citric acid on the hydrolytic and oxidative stability of refined soybean oil, stored over a period of twelve months.

Materials and Methods

Refined soybean oil was obtained from the deodouriser of the Vegetable Oil Division Plant (Refinery) of Jof Ideal Family Farms Limited, Owo, Ondo State, Nigeria.The oil was obtained before it was fortified with vitamin A. Two sets of three different containers (tinned can, transparent white glass bottle and plastic bottle) of equal capacity (750 mL) were obtained, washed and dried. Each of these containers was filled with 700 mL of refined soybean oil leaving 50 mL air space. Food grade citric acid (0.2%) was added to the oil in a set of three different containers which were thoroughly shaken while the other set contained only the oil sample and citric acid was not added. Free fatty acid (FFA), acid value (AV), peroxide value (PV) and iodine value (IV) of the oil samples were monthly determined using standard methods (AOCS, 1989) over a period of twelve months.

Statistical analysis. The results obtained were subjected to one-way analysis of variance (one-way ANOVA) and the Duncan multiple range test (DMRT) was used to separate the means.

Results and Discussion

Table 1 shows mean values of free fatty acid (FFA), acid value (AV), peroxide value (PV) and iodine value (IV) of refined soybean oil stored with and without 0.2% food grade citric acid (FGCA) in different containers (tinned can, transparent white glass bottle and plastic bottle) over a period of twelve months. The results revealed that the FFA and AV of the oil in all the storage conditions were linearly correlated. Both the FFA and AV of the oil stored in plastic bottles were the lowest while that in the tin containers were the highest. FGCA (0.2%) lowered the FFA and AV of refined soybean oil stored in all the containers except that in tin. There was significant difference at $P< 0.05$ in both FFA and AV of refined soybean oil stored in tin and glass containers. FFA and AV both are measures of hydrolytic rancidity of oils; the higher their values in any lipid, the higher the degree of hydrolytic rancidity (Ihekoronye and Nggody, 1985). The overall assessment showed that 0.2% FGCA reduced hydrolytic rancidity of refined soybean oil stored in plastic and glass containers but increased it in tin container. Incorporation of the additive at the specified concentration level enhanced the keeping quality with respect to hydrolytic stability of the oil stored in plastic and glass bottles.

The PV of refined soybean oil stored for twelve months was the highest in tin and the lowest in plastic bottles. It was significantly different at $P< 0.05$ in all the storage

Table 1. Mean values of quality properties of refined soybean oil stored in different storage conditions for twelve months

Storage condition	*Free fatty acid (FFA) (% oleic acid)	*Acid value (AV) (mg KOH/g oil)	*Peroxide value (PV) (Meq O_2/kg oil)	*Iodine value (IV) (g/100 g oil)
Tin	0.334±0.054^{d}	0.653±0.104^{d}	125.420±27.725^{f}	137.675±1.195^{a}
Tin + 0.2%CA	0.352±0.059^{e}	0.676±0.117^{e}	108.380±23.729^{e}	138.355±0.953^{ab}
Glass	0.308±0.046^{c}	0.614±0.094^{c}	87.241±19.110^{d}	139.220±0.767^{b}
Glass + 0.2%CA	0.286±0.077^{b}	0.560±0.154^{b}	46.614±9.072^{b}	140.725±0.376^{bc}
Plastic	0.252±0.033^{a}	0.495±0.064^{a}	53.586±10.120^{c}	140.550±0.412^{c}
Plastic+ 0.2%CA	0.244±0.033^{a}	0.479±0.064^{a}	37.132±6.942^{a}	141.125±0.288^{d}

Within each column, mean values followed by the same superscript are significantly different at $P<0.05$ level according to Duncan Multiple Range Test (DMRT); * = mean value of quality properties ± standard error; CA= citric acid.

conditions. 0.2% FGCA lowered the PV of oil in all the containers. PV of the oil samples containing 0.2% FGCA was the least in plastic containers followed by glass containers and the highest in tin containers. The IV of refined soybean oil stored for twelve months was the highest in plastic bottles and the lowest in tin containers. IV of the oil was significantly different at $P< 0.05$ in all the containers. Oil samples containing 0.2% FGCA had slightly higher IV in all the containers. The values obtained for both PV and IV under the same storage conditions indicated that the two parameters (PV and IV) were indirectly correlated. The storage condition that had the highest IV gave the least PV and *vice versa.* Increase in iodine value (IV) is always accompanied with decrease in peroxide value (PV) owing to more C=C unsaturated double bonds that are present in the lipid which are left to be oxidised, therefore, leaving more C=C unsaturated double bond in the lipids for iodination reaction during iodine value determination (Arawande and Ademulegun, 2009). Goli *et al*. (2005) and Rossel (1994) reported that PV and IV were measures of oxidative stability of lipids and that the lower the value of PV of any lipid, the more the lipid is oxidatively stable. 0.2% FGCA inhibited oxidative rancidity of refined soybean oil in all the containers and thereby enhanced the keeping quality of oil, which was the most oxidatively stable in plastic bottles, followed by glass bottles and the least in tin containers.

Figures 1 and 2 show free fatty acid (FFA) and acid value (AV), respectively, of refined soybean oil stored with and without 0.2% FGCA in tin, glass and plastic containers for a period of twelve months. The plots of these parameters show a consistent trend. Considering

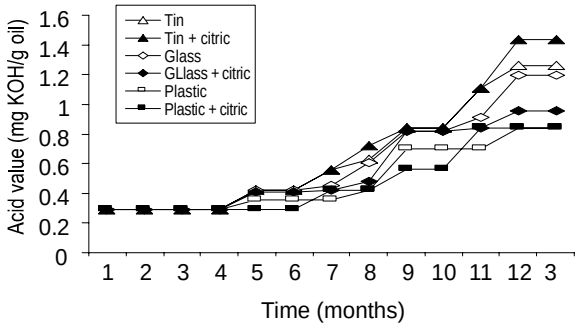

Fig. 2. Acid value of refined soybean oil stored in different conditions for twelve months.

the first six months of storage, it was observed that the oil stored in plastic bottles had the longest induction period while during the last three months of storage, refined soybean oil stored in tin containers had the highest value of FFA and AV, followed by the oil stored in glass bottles and the least in plastic bottles. In all the containers, the FFA and AV of refined soybean oil stored with 0.2% FGCA had lower values than those not containing additive. From the plots, it is obvious that the hydrolytic stabilitiy of refined soybean oil increased in the order; plastic + citric > plastic > glass + citric > glass > tin + citric > tin.

Figures 3 and 4 depict peroxide value (PV) and iodine value (IV), respectively, of refined soybean oil stored with and without 0.2% FGCA in tin, glass and plastic containers for a period of twelve months. It was observed that as the PV of the oil increased, there was a slight decrease in the IV of the oil in all the storage conditions. This was due to reduction in the C=C unsaturated double bonds in the hydrocarbon chain of the oil structure with

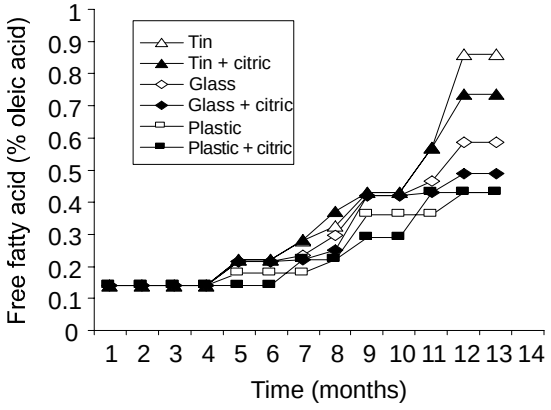

Fig. 1. Free fatty acid or refined soybean oil stored in different conditions for twelve months.

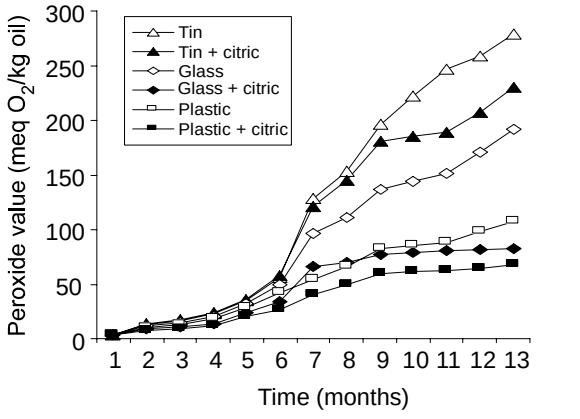

Fig. 3. Peroxide value of refined soybean oil stored in different conditions for twelve months.

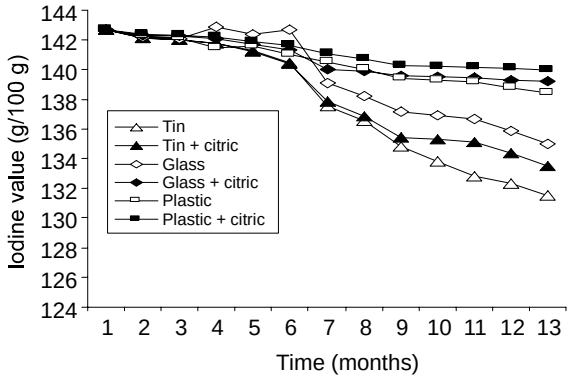

Fig. 4. Iodine value of refined soybean oil stored in different conditions for twelve months.

the increase in the oxidation processes thereby reducing the C=C unsaturated double bond sites for iodine reaction (iodination) (Arawande and Amoo, 2009). In the first four months of storage, there was no sharp noticeable increase in the values of PV and IV of the oil in all the containers. Beyond this point, there were sudden incremental changes in the PV of the oil as the storage period increased while there was slight sudden decrease in the IV of the oil as the storage period increased. During the last five months of storage, addition of 0.2% FGCA to the oil remarkably reduced the PV of the oil in all the storage conditions, consequently resulting in a sharp increase in IV of the oil. PV of oil is the measure of oxidative stability of the oil which means that the lower is the value, the less is the oxidative rancidity of the oil. Therefore, the oxidative rancidity of refined soybean oil decreased in the order of storage conditions: plastic + citric < glass + citric < plastic < glass < tin + citric < tin during the last five months of storage.

Conclusion

Storage conditions (such as containers and citric acid additive) of refined soybean oil affected the keeping quality of oil in varying degrees. Food grade citric acid 0.2% was an effective antioxidant against hydrolytic and oxidative rancidity of the refined soybean oil in all the containers under study. Refined soybean oil stored in plastic bottles was the least prone while those stored in tin containers were the most prone to both the hydrolytic and the oxidative rancidity. However, further research work is required on the soybean oil as well as on some other edible oils using varying concentrations of citric acid with different packaging materials for a longer period of time.

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Soil Micronutrient Status in Hazro Area of District Attock, Pakistan

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Abstract. Study of micronutrients in the soil of Hazro area of District Attock (Potohar), Pakistan, revealed micronutrient deficiency in the order of Fe> Mn> Zn> Cu. All the soils were low to medium in Fe and Mn followed by Zn content, whereas only 8% samples had low Cu content. 92% and 18% soils in Hazro area had satisfactory to adequate Cu and Zinc contents, respectively, Thus soils were deficient in Fe, Mn and Zn, whereas Cu was in medium to adequate range.

Keywords: micronutrients, Hazro, soil micronutrients, Attock

Introduction

Micronutrients are as important in plant nutrition as the macro nutrients and plants grown on soils deficient in micronutrients can exhibit similar reduction in growth and yield (Havlin *et al.*, 2004). To get optimum yield, a balance dose of macro as well as micronutrients are required. Deficiency of various micronutrients is related to soil type and crop. The introduction of new high yielding hybrids or cultivars demanding a higher level of soil fertility has further accentuated the incidence of micronutrient deficiencies. Zn deficiency is the most widespread disorder in the country. Soil analyses revealed that > 50% of the cultivated soils of the country are unable to provide sufficient Zn to meet the needs of many crops (Khattak, 1995). The information obtained from 329 soil samples collected from various depths throughout the country during the period of seven months revealed widespread deficiency of Zn and B followed by Fe (Zia *et al.*, 2004b).

District Attock of Potohar comprises of six tehsils, i.e., Attock, Hazro, Fatehjang, Pindi Gheb, Jand and Hasan Abdal. The district lies between latitude 32.35° N and longitude 72.55° E. The climate is sub-humid to semi-arid with 400-700 mm annual rainfall. Hazro tehsil is among the most productive tehsils of Attock with sizeable contribution to agriculture. The soils of Hazro are medium (loam) to light (sandy loam) textured with most of the soils poor in fertility status (Mehmood *et al.*, 2008).

Keeping in view the low fertility, nutritional disorder and importance of micronutrients for successful cropping in Hazro area, a study was conducted to assess the extent of micronutrients *viz.*, zinc, iron, copper and manganese deficiencies in soils of Hazro area of District Attock.

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Materials and Methods

Soil samples were collected from different field locations of Hazro area of District Attock, air dried, sieved and stored in plastic bottles. Samples were analyzed by diethylene triamine pentacetic acid (DTPA) extraction method. Twenty (20) grammes of soil were shaken with 40 mL of 0.005 M DTPA solution for 2 h, and double filtered with Whatman filter paper # 42. A series of standard DTPA extraction solutions for micronutrients were also prepared. Zn, Fe, Cu and Mn were measured directly in the filtrate and the standard solutions by atomic absorption spectrophotometer using appropriate lamp for each element (Ryan *et al.*, 2001).The data was subjected to statistical analysis in MS Excel-2007.

Soil micronutrients have been characterized (Table 1) according to the generalized guidelines (Martens and Lindsay, 1990) used for interpretation of soil micronutrient analysis data in Pakistan (Zia *et al.*, 2004a).

Table 1. Guidelines for interpretation of soil micronutrient status

Parameters	DTPA extractable micronutrients (mg/kg)		
	Low	Medium	Adequate
Zinc	< 0.5	0.5-1.0	> 1.0
Iron	< 4.5	-	> 4.5
Copper	< 0.2	-	> 0.2
Manganese	< 1.0	1.0 – 2.0	> 2.0

Results and Discussion

Generally, soils of Hazro are light to medium textured, calcareous in nature, having normal pH but low in fertility. Mehmood *et al.* (2008) reported that 74.0% of soils in Hazro tehsil were loam textured while, 99.0% soils had normal pH (7.5-8.5) and EC (< 4.0 dSm^{-1}) status. They also reported that 89.0% and 99.0% soils in Hazro tehsil were poor in organic matter and available phosphorus, respectively.

The results of the micronutrient analyses are given in Table 2 and 3 and Fig. 1 and 2, discussed as under:

Zinc. The data revealed that 41 (62%) soils samples of Hazro area had low to medium while, only 9 (18%) had adequate zinc contents (Table 3). Zn contents were found in the range of 0.32 to 1.40 with a mean value of 0.94 mg/kg at upper soil depth.

Iron. Soils of Hazro area had very low Fe status (Table 2). All the 50 samples had iron content less than the critical value of 4.5 mg/kg. Fe was observed in the range of 1.02 to 2.70 mg/kg with mean value of 1.70 mg/kg at upper 0-15 cm soil depth.

Copper. The data in Table 2 indicated adequate concentration of copper in the surveyed samples. About 92% samples showed adequate, whereas, only 8% had low Cu contents. The range was 0.04-2.16 mg/kg with mean value of 1.32 mg/kg at 0-15 cm soil depth (Fig. 2).

Table 2. Micronutrients in Hazro soil samples

Parameter	DTPA extractable micronutrients (mg/kg)		
	Low	Medium	Adequate
Zinc	10	31	09
Iron	50	-	0
Copper	4	-	46
Manganese	05	45	0

Table 3. The maximum and minimum quantities of micronutrients in Hazro soils

Parameter	Depth (cm)	Minimum (mg/kg)	Maximum (mg/kg)
Zinc	0-15	0.32	1.40
	15-30	0.22	0.32
Iron	0-15	1.02	2.70
	15-30	0.98	2.68
Copper	0-15	0.04	2.16
	15-30	0.02	2.12
Manganese	0-15	0.36	1.90
	15-30	0.32	1.82

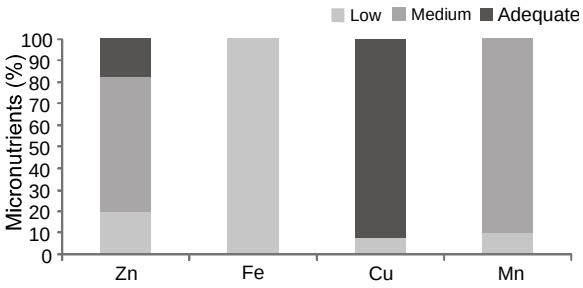

Fig. 1. Micronutrients (%) in soil samples of Hazro area.

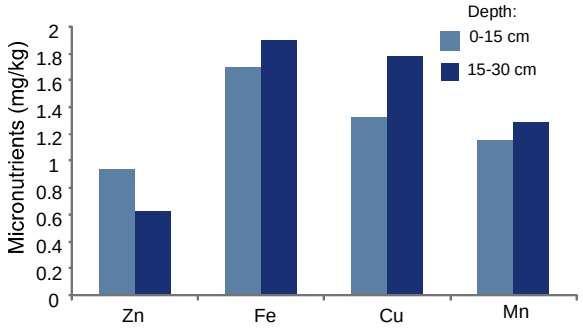

Fig. 2. Mean quantity of micronutrients (mg/kg) in Hazro soils.

Manganese. All the soil samples had low to medium Mn content (< 2.0 mg/kg); 45 (90%) samples had Mn in the medium range (1.0-2.0 mg/kg) with mean value of 1.28 mg/kg.

The results of the present study revealed deficiency of micronutrients in the soil in the order of Fe> Mn> Zn> Cu. The higher Fe, Cu and Mn contents at the depth of 15-30 cm while higher Zn content in the upper 0-15 cm soil layer was observed (Fig. 2).

These are in accordance with Rashid and Qayyum (1990) who encountered Zn and Fe deficiencies in most of the soils of Potohar. Similar results were also reported by Tariq *et al.* (2004) who found that more than 60% of soils in Punjab are deficient in Zn while, Fe deficiency was also observed in most of the soils. However, Rashid *et al.* (1994) recorded none of the fields in Attock deficient in Mn. Micronutrient deficiency is more often observed in high pH, calcareous soils with low organic matter content (Havlin *et al.*, 2004). Presence of low quantities of micronutrients (Fe,

Zn, and Mn) in the study area might be due to alkaline (high pH) calcareous nature of soils and low organic matter in this area. Greater incidence of Fe deficiency and low solubility of soil Fe (Havlin *et al.*, 2004) is encountered in the soils containing $CaCO_3$ in the pH range of 7.3-8.5. The deficiency of available Fe and Mn may also be attributed to the antagonistic effect of Zn fertilizer application (Imtiaz *et al.* 2003).

Conclusion

Deficiency of Fe, Mn & Zn was observed in the Hazro area soils in the Attock District, whereas Cu was in the satisfactory range.

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Contribution of Different Global Varieties of Cotton towards Water Hardness in Textile Wet Processing

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Abstract. Specimens of nineteen different global varieties of cotton were studied to determine their contribution to water hardness through calcium and magnesium impurities, resulting in various problems during textile pretreatment, colouration and finishing. Pakistani cotton was found to be the second most contaminated cotton in terms of calcium and magnesium impurities, whereas Elisa variety from Uzbekistan was the cleanest.

Keywords. cotton, water hardness, magnesium, calcium

Introduction

Cotton is the backbone of the world's textile trade. It has many countless end uses, which make it one of the most abundantly used textile fibres in the world (Wilson, 2006; Yafa, 2006; Becerra, 2000; Cook, 1984). It is seed hair of plant of genus *Gossypium* (Lewin, 2006) and the purest form of cellulose found in nature. Although cotton may be as much as 96% cellulose, there are always some other components present in it as impurities. The level of impurities in cotton is affected by geology of the cultivation area, soil constitution, weather conditions during the maturing period, cultivation techniques, chemicals, pesticide, fertilisers and harvesting techniques. Among the impurities present in cotton, the elements that pose the greatest threat in textile wet processing are alkaline earth and heavy metal contaminants such as iron, manganese, calcium and magnesium.

Textile wet processes comprise of desizing, scouring, bleaching, dyeing, printing and chemical finishing. Water hardness caused by calcium and magnesium impurities in cotton, may cause several problems in each of the textile wet processes. In desizing process, effectiveness of the wetting agents used may be reduced in the presence of calcium and magnesium ions. In scouring processes, calcium and magnesium ions may precipitate the soaps used, forming a sticky insoluble substance which deposits on the cotton fabric (Losonczi *et al.*, 2005). These deposits impair the fabric handle, cause resist-spots in dyeing, attract soil to the material and cause inconsistent absorbency in subsequent processes. Although most synthetic detergents today used in scouring do not precipitate in the presence of calcium and magnesium ions, the fatty acid hydrolysis products formed by the saponification of natural waxes, fats, and oils in the fibres will precipitate. Formation of complexes with alkaline and alkaline earth salts drastically reduces the solubility and the rate of dissolution of surfactants, thus impairing the wash removal ability of the surfactants (Bille, 1987).

Although magnesium produces beneficial effects when present in hydrogen peroxide bleaching solutions, the presence of calcium may result in decreased stability of peroxide bath due to blockage of stabilisers, harsh handle of the fabric due to deposition of insoluble salts and decrease in fabric whiteness due to formation of insoluble products with optical brighteners. In bleaching cotton with hydrogen peroxide, sodium silicate is used as stabiliser, which may be converted to calcium silicate in the presence of calcium impurities. This calcium silicate has poor water solubility and is not washed-off the fabric easily, resulting in harsh fabric handle (Topalovic, 2007).

In dyeing, the presence of calcium and magnesium ions may result in lowering of solubility of dyes and staining due to formation of insoluble products with dye, change in dyeing shades, and difficulties in the removal of hydrolysed reactive dye ensuing in low washing fastness. Most water-soluble anionic dyes are sodium salts of sulphonic acid which, in the pre-sence of calcium and magnesium impurities, may be converted into their respective salts with lower water solubility, and thus staining. In the washing-off stage, the removal of unfixed hydrolysed reac-

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tive dye is also reduced in the presence of calcium impurities (Akcakoca *et al.*, 2007).

Due to so many problems associated with the calcium and the magnesium ions, their absence is imperative during wet processing of cotton material. It is usually suspected that the major source of these impurities is the water used in textile processing. However, this may not always be the case, as these impurities may also come with the cotton itself (Hashem, 2007). The impact of these impurities has been explained above. In order to prevent the deleterious effect of calcium and magnesium impurities in cotton, an additional demineralisation process may become necessary leading to an extra burden on the processing cost. Alternatively, suitable sequestering agents may be used to alleviate the problems associated with calcium and magnesium impurities in cotton. However, this again adds to the processing cost. The present study was undertaken to compare the hardness characteristics of various global varieties of cotton to determine how much these different varieties contribute to the hardness of water used in the textile processing.

Materials and Methods

Samples of nineteen varieties of cotton fibres originating from different countries were obtained. Four of them were from the USA (SJV Pima, Elpaso, Memphis, Mote), three each from Egypt (Giza 70, Giza 86, Giza 88) and Brazil (Lot 992, Lot 1017, Lot 1832), two each from India (MCU 5, Shankar 6) and Commonwealth of Independent States (Elisa, Sultop), and one each from Mali, Greece, Ivory Coast and Pakistan. As all the collected samples were in raw form, they contained varied content of trash such as dust, seed-coat fragments, leaves, and stems etc. In order to eliminate the influence of these impurities on the test results, the non-lint trash content of each sample of cotton was removed by passing the samples through a laboratory machine "Shirley Analyzer" as per standard method (ASTM D2812-07, 2009). The machine operates on mechanical-pneumatic principles and segregates trash and cotton fibres in separate chambers.

After the preliminary removal of trash, samples of each variety of cotton were conditioned as per standard method (ASTM D1776-08, 2009) and the representative specimens of 10 g of each cotton variety were boiled separately in 500 mL of distilled water. Initial hardness of the distilled water used was zero. After 10 min of boiling in distilled water, cotton fibres were removed from water with tweezers and the excess liquid was allowed to drip back to the extract. The extract was allowed to cool down at room temperature. Then the hardness of each extract was determined according to the method described elsewhere (Vogel and Bassett, 1999).

Results and Discussion

The hardness characteristics of different varieties of cotton tested in this study are presented in tabular and graphic forms (Table 1 and Fig. 1).

As can be seen, CIS Elisa cotton was found to be the best in terms of hardness characteristics, resulting in absolutely zero water hardness. The worst among the tested varieties, in terms of total hardness, was Brazil Lot 992, while Pakistani cotton was only the second worst. However, Pakistani cotton was found to contribute the highest towards the magnesium hardness of water rather than the calcium hardness. Since presence of magnesium is beneficial in hydrogen peroxide bleaching, it means that Pakistani cotton behaves the best as far as bleaching process is concerned. However, along with calcium, magnesium may contribute to other

Table 1. Hardness of different varieties of cotton

Cotton varieties	Country of origin	Calcium hardness (ppm)	Magnesium hardness (ppm)	Total hardness (ppm)
Elisa	Uzbekistan	0	0	0
Shankar 6	India	18	17	35
Mote	USA	23	14	37
MCU-5	India	21	17	38
Mali	Mali	33	7	40
Barkat	Sudan	26	15	41
Giza-88	Egypt	12	30	42
SJV Pima	USA	20	22	42
Brazil Lot 1832	Brazil	32	10	42
Ivory Coast	Ivory Coast	35	8	43
Giza-86	Egypt	18	28	46
Sultop	Uzbekistan	18	29	47
Giza-70	Egypt	31	21	52
Elpaso	USA	29	26	55
Memphis	USA	34	22	56
Brazil Lot 1017	Brazil	45	13	58
Greece	Greece	52	14	66
CIM-443	Pak	27	47	74
Brazil Lot 992	Brazil	77	2	79

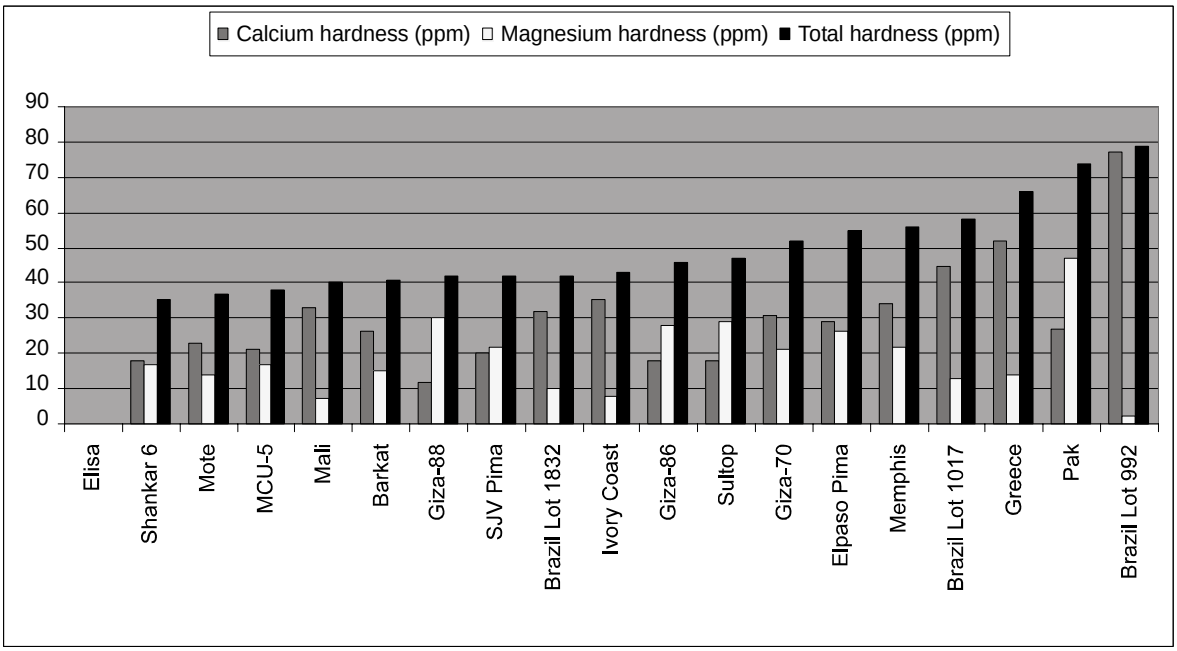

Fig. 1. Hardness characteristics of different varieties of cotton.

problems in wet processing such as decreased efficiency of desizing and scouring as well as irregularities in dyeing.

The results indicate that most of the cotton varieties have significant amount of salts of calcium and magnesium, which can contribute to hardness of the water used in wet processing. The ratio of the water and cotton used in this study was 50:1. In most of the textile wet processes, much lower liquor ratio is employed. Hence, it is expected that at lower level of liquor ratio, the contribution of cotton to water hardness may be much higher. This means that water softening plants alone are not sufficient to guarantee that no problem associated with water hardness will occur during textile wet processing since the salts causing water hardness may also come with the cotton even when initially the water is absolutely free from the calcium and magnesium ions. Therefore, to be on the safe side, it is always advisable to subject cotton either to a demineralization process in order to remove the naturally occurring hardness-causing salts of calcium and magnesium or to make use of sequestering agents during textile wet processing (Strohlen, 2007).

Conclusion

The level of impurities such as calcium and magnesium is different in different global varieties of cotton. Due to the presence of calcium and magnesium salts, different varieties of cotton contribute to water hardness in different degrees. Calcium and magnesium impurities present in cotton may cause several problems in textile wet processing, including reduction in the efficiency of desizing, scouring and bleaching operations, shade change and staining in dyeing and poor washing fastness of dyed and printed fabrics. In order to avoid these problems in textile wet processing, appropriate measures must be taken such as demineralization of cotton or use of sequestering agents during wet processing even when soft water is used for processing.

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Study of Tannery Wastewater Treatability by Precipitation Process

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Abstract. A study was conducted for the removal and recovery of chromium from tannery wastewater, using NaOH, MgO, $Ca(OH)_2$ and $Al_2(SO_4)_3.18H_2O$ as precipitating agents and comparing their effect on pH, total dissolved solids (TDS), total suspended solids (TSS), sludge volume and chromium removal. MgO and $Ca(OH)_2$ produced least amount of sludge and dewatering of sludge was also easy as compared to $Al_2(SO_4)_3.18H_2O$ and NaOH. The chromium removal of MgO and $Ca(OH)_2$ was 95% and 96%, respectively.

Keywords: precipitating agents, chromium removal, tannery wastewater, sludge volume

Introduction

Pakistan leather industry is one of the major foreign exchange earners of the country. About 90% of leather products are exported in finished form. However, the operation of tanneries is causing severe environmental degradation due to the disposal of untreated effluent on land and in water bodies. High chromium concentration is harmful for environment and human health (Zayed and Terry, 2003).

In tanning process, chromium compounds are commonly used for processing of hides, 60-70% of which react with the skin and the remaining amount is discharged as effluent (Mant *et al*., 2005; Sreeram and Ramasami, 2003).

The remaining chromium (about 30-40%) in the solid and liquid waste contributes to the environmental pollution. Considering the high cost of chromium metal, it would be preferable to recover it from the wastewater (Kocaoba and Akcin, 2002; Ludvik, 2000; Fabiani *et al*., 1997).

Various methods have been used for removing toxic metal ions from aqueous solutions including chemical precipitation, ion exchange, reverse osmosis, evaporation, solvent extraction, electroprecipitation, coagulation and adsorption (Dhungana and Yadav, 2009; Rashed, 2008; Kongjao *et al*., 2007; Esmaeili *et al*., 2005; Kocaoba and Akcin, 2002). Among these, chemical precipitation is the most commonly used method. Many factors affect the process of chemical precipitation including the type of precipitation agent, pH, nature, velocity of precipitation, sludge volume, time of mixing and complexing agents (Patterson, 1985). Precipitation can be followed by coagulation and flocculation, in order to enhance sedimentation. The process is very effective for the removal of precipitated solids and is used to treat the industrial effluent before discharging them into receiving water (Noyes, 1994). The precipitation process is not always perfect and chemical characteristics of the treated wastewater may not meet the standards. Consequently, a further treatment is often necessary.

Kasur town is located 55 Km southeast of Lahore in the province of Punjab, Pakistan. The city is well known for its tanning industry with more than 250 tanneries discharging large volumes of untreated tannery waste in the form of wastewater, sludge and solid waste. There is an urgent need for the treatment of tannery effluents prior to their disposal. The main purpose of this research was to compare pH, chromium concentration, sludge volume, colour, TDS and TSS using $Al_2(SO_4)_3.18H_2O$, $Ca(OH)_2$, NaOH and MgO in the precipitation process, so as to find the best precipitating agent for chromium removal and recovery.

Materials and Methods

The study was carried out at Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore, in June 2009. Samples were brought from inlet of Kasur treatment plant and

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transported to the laboratory for determination of the most important parameters which included biological oxygen demand (BOD_5), chemical oxygen demand (COD), pH, total suspended solids, total dissolved solids, colour, conductivity and chromium concentration. Each test was performed in triplicate and the average value was reported. Analysis was carried for 12 metals namely Mn, Cd, Pb, Se, Zn, Cr, Cu, Ba, Fe, B, Ag and Hg.

All chemicals used were of analytical reagent grade; Jar test was conducted as described by Esmaeili *et al*. (2005). In the first step, 600 mg/L of each precipitating agent was added to the samples. In the second step, samples were mixed for 30 min at 670 rpm. In the last step, after 6 h settling time, samples were taken from the supernatant. To evaluate the efficiency of precipitating agent of tannery wastewater, the parameters measured were sludge volume, pH, conductivity, total dissolved solids (TDS), total suspended solids (TSS), BOD_5, colour and chromium volume.

In order to determine the sludge volume, samples were poured in calibrated cylinder and after 6 h the height of supernatant and sludge volume was read. pH was measured by digital pH meter (JENO 6173). Standard solution with pH 4, 7 and 10 were used for calibration. Conductivity, TDS, TSS, BOD_5 and COD were measured and calculated according to the standard methods for water and wastewater testing (APHA, 2005).

The colour of samples before and after treatment was measured by tintometer (LOVIBOND PFX 995). The path length of cell used was 100 mm. Before the measurement, samples were filtered to prevent turbidity.

For metal analysis, samples were digested (Iqbal *et al*., 2009) and different standard of chromium, prepared for calibration curve, were used. Atomic absorption spectrophotometer (Perkin Elmer Analyst 300) was used to measure chromium concentration in wastewater before and after the treatment. The analysis of effluent metal was carried out by inductive couple plasma (Perkin Elmer Optimum 5300).

Results and Discussion

Tannery effluents are highly polluted fluids and contain protein, colloids, fats, fragments of flesh, hair, lime, colouring matter, sulphide and chromium left after chemical tanning. It was found that no special treatment process was used for the high polluted wastewater, generated in the tannery area. The chemical characteristics of effluent (Table 1) showed high values of pH, conductivity, TDS, TSS, BOD_5, COD, chromium and colour concentration as compared to national environmental quality standard (GOP, 2000). If this wastewater is discharged without treatment, it may cause severe environmental problems. Most of the metals in the effluent were below NEQS level (Table 2). However, the concentration of chromium and iron were 25.01 ppm and 3.740 ppm, respectively, being higher than the NEQS limits.

Table 1. Characterisation of effluent of tannery treatment plant, District Kasur number of samples=6

Parameters	Range (effluent) (mg/L)	NEQS limits (mg/L)
pH (unsettled effluent)	7.5-10	6-10
Conductivity, (ms/cm)	16.0-18.0	—
Total dissolved solid*, (mg/L)	9214-12000	3500
Total suspended solid*, (mg/L)	400-600	150
Chloride*, (mg/L)	2000-3000	1000
BOD_5*, (mg/L)	800-4000	80
COD*, (mg/L)	1300-6500	150
Chromium*, (mg/L)	25-50	1.0
Colour, Pt-Co/Hazan	500-510	—
Unsettled effluent		—

* = (30 min settling).

Table 2. Results of metal analysis of tannery effluent treatment plant, District Kasur

Metals	Measured value (mg/L)	NEQS limits (mg/L)	Metals	Measured value (mg/L)	NEQS limits (mg/L)
Mn	0.0172	1.5	Cu	0.078	1.0
Cd	N.D	0.1	Ba	0.231	1.5
Pb	0.041	0.5	Fe	3.740	2.0
Se	0.012	0.5	B	0.698	6.0
Zn	0.030	5.0	Ag	0.001	1.0
Cr	25.01	1.0	Hg	ND	0.01

ND = not detected.

Effect of precipitating agents on pH. pH of solution is an important factor in precipitation process. pH was greatly affected when different precipitating agents were added (Fig. 1). pH increased to 12.76 after 50 min of adding NaOH. However, the values of pH in case of MgO, $Ca(OH)_2$ and $Al_2(SO_4)_3.18H_2O$ were 9.99, 12.18 and 7.45, respectively. The literature shows that the optimum pH for chromium removal in case of MgO is 7-8 (Pansward *et al*., 1995).

Effect of precipitating agents on chromium concentration. Same amount of each precipitating agents was

added for comparison of maximum chromium removal and minimum sludge production. The percentage removal of chromium in case of NaOH was much less (Fig. 2). Since NaOH increases the pH causing peptizing and chromium is re-dissolved, hence chromium concentration in supernatant increases. However, with MgO, $Ca(OH)_2$ and $Al_2(SO_4)_3.18H_2O$ percentage removal of chromium was 95%, 96% and 94%, respectively (Table 3).

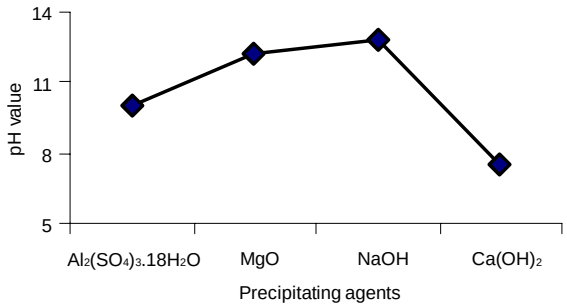

Fig. 1. Variation of pH for the four precipitating agents.

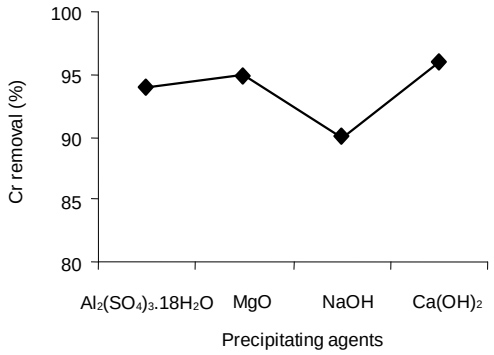

Fig. 2. Percentage removal of chromium (—◆—) for the four precipitating agents.

Effect of precipitating agents on TDS and TSS. Percentage removal of TDS and TSS are shown in Fig. 3. Better results were obtained in case of MgO and $Ca(OH)_2$ as compared to NaOH and $Al_2(SO_4)_3.18H_2O$. The results of BOD_5 for all precipitating agents were very good and below NEQS level.

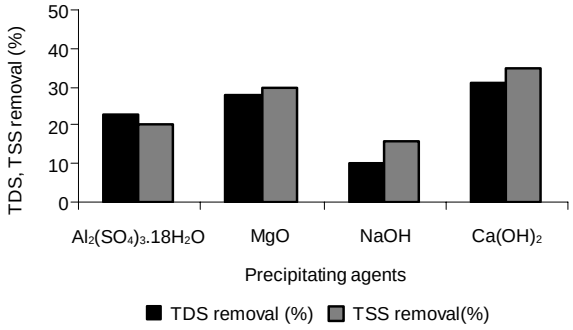

Fig. 3. Effect of precipitating agents on TDS and TSS removal.

Effect of precipitating agents on colour removal. The effect of precipitation on colour removal was good and showed that this process was effective for the colour reduction. Substances producing colour consist either of colloidal metallic hydroxides (e.g., iron hydroxides) or of organic compounds (e.g., dyestuff), which have a much smaller particle size. These substances can be removed by coagulation, which serves to agglomerate very small particles into sizes that can be settled or can be removed by filters or absorption. (Aboulhassan *et al.*, 2008); Fig. 4 shows the percentage removal of colour, 78% colour was removed when $Ca(OH)_2$ was used as precipitating agent and 40% in case of NaOH. Colour removal of MgO and $Al_2(SO_4)_3.I8H_2O$ were 60% and 63%, respectively.

Table 3. Residual values and percentage removal of various parameters after treatment of tannery wastewater with different precipitating agents

Parameters	$Al_2(SO_4)_3.18H_2O$		MgO		NaOH		$Ca(OH)_2$	
	Residual value	Percentage removal	Residual value	Percentage removal	Residual value	Percentage removal	Residual value	Percentage removal
Colour	85	63	209	60	305	40	109	78
TDS (mg/L)	9200	23	8600	28	9900	10	8181.8	31
TSS (mg/L)	490	20	400	30	598	16	400	35
BOD_5 (mg/L)	35	98	28	99	44	90	4	99
Cr (mg/L)	0.986	94	1.015	95	1.376	90	0.935	96

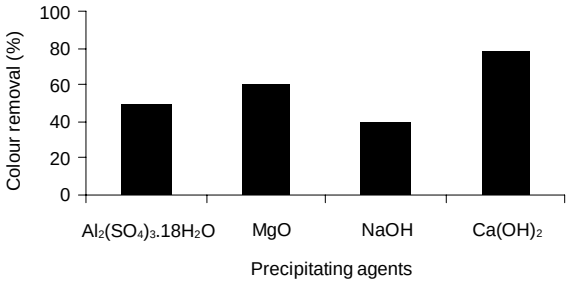

Fig. 4. Effect of precipitating agents on colour removal.

Effect of precipitating agent on sludge volume. $Al_2(SO_4)_3.I8H_2O$ and NaOH produced very large amount of sludge, as compared to MgO and $Ca(OH)_2$ (Fig. 5). The sludge from MgO and $Ca(OH)_2$ was grainy, dense, easily settleable and de-watering could be easily carried out for recovery of chromium. However, sludge obtained from NaOH and $Al_2(SO_4)_3.18H_2O$ was very gelatinous and dewatering of sludge was difficult which created problem in the recovery of chromium and large area was required for sludge storage (Esmaeili *et al*., 2005; Pansward *et al*., 1995). Thus MgO and $Ca(OH)_2$ produced the least amount of sludge and the removal of colour, TDS and TSS was better as compared to NaOH and $Al_2(SO_4)_3.18H_2O$.

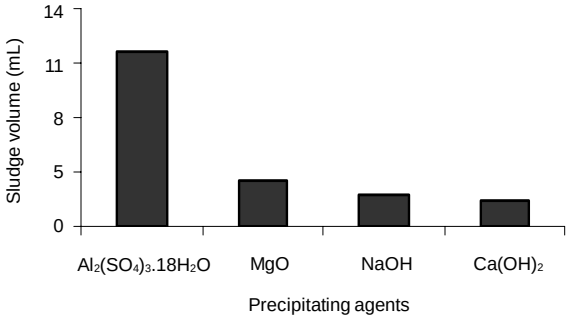

Fig. 5. Effect of precipitating agents on sludge volume.

Conclusion

The results showed that MgO and $Ca(OH)_2$ as precipitating agents produce less amount of sludge and chromium removal from the supernatant yielded good result. For the recovery of chromium dewatering of sludge was also easily done. However, NaOH and $Al_2(SO_4)_3.18H_2O$ produced large volume of sludge and dewatering of the sludge was difficult. The percentage removal of colour was 78%, 40% when $Ca(OH)_2$ and NaOH were used. Colour removal of MgO and $Al_2(SO_4)_3.I8H_2O$ were 60% and 63%, respectively. It is concluded that for the removal and recovery of chromium, MgO and $Ca(OH)_2$ are much more desirable precipitating agents than NaOH and $Al_2(SO_4)_3.18H_2O$.

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Short Communication

Quantitative Status of Heavy Metals in Soils of Quetta Irrigated by Sewage Water

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Abstract. In soils of different areas of Quetta city, irrigated by sewage water, the highest concentration of heavy metals was found to be as follows: lead (1.38 ppm), copper (0.86 ppm), chromium (0.036 ppm), cadmium (0.29 ppm), iron (10.50 ppm), nickel (0.74 ppm), zinc (19.45 ppm) and arsenic (0.001 ppm) on average basis. The sewage water contained lead (53.26 ppb), copper (22.5 ppb), chromium (1.33 ppb), cadmium (0.53 ppb), iron (127.7 ppb), nickel (51.14 ppb), manganese (17.08 ppb), zinc (31.38 ppb) and arsenic (0.011 ppb). At each site the concentration of heavy metals and sewage water showed positive relationship.

Keywords: heavy metals, sewage water, bio-accumulation, bio-magnification

Heavy metals in environment affect human life and bio-diversity and ultimately deteriorate sustainable development. Human activities have drastically changed the bio-chemical cycles through discharge of heavy metals into the environment. Heavy metals, being non-degradable, tend to accumulate in soil, sea water, fresh water and sediments and pose risks to human consumers of the sea foods, vegetables and also to many other organisms at the same level.

Nowadays large amount of untreated sewage/industrial water is being discharged into surface bodies for disposal (Saleemi, 1993) which may contain non-essential heavy metals in large amounts and which could be transferred to animal and human beings through food chain (Malla *et al.*, 2007; Ghafoor *et al.*, 1994). Sediments are ready sink or reservoir of pollutants including trace metals (Becker *et al.*, 2001; Muohi *et al.*, 2003).

The main anthropogenic sources of heavy metals are various industrial sources including former and present mining activities, foundries and smelters, and defuse sources such as piping, combustion bio-products, traffic, detergents, welding, batteries and leather tanneries.

For determining the status of heavy metals in soil of Quetta, in summer, soil samples were collected from Habib Nala, Hudda, Samungli and Barori and sewage water samples were collected from Habib Nala, Hudda, Samungli, Mariaabad and Angle Road. Drinking water samples were also collected from Shahbaz town, Jinnah town, Pashtoonabad ,Mariaabad and Satellite Town. After removing the stones, drying and grinding, the soil was sieved through 2 mm wire mesh. (MAFF, 1986). The sample were then digested and analyzed using atomic absorption spectrometer.

All the analyzed sewage water samples (40 in number) contained on an average (ppb) lead 53.26, copper 22.5 chromium 1.33, cadmium 0.53, iron 127.7, manganese 17.08, nickel 51.14, zinc 31.38 and arsenic 0.011 ppb (Table 1).

It was observed that the results relating to heavy metals in the sewage water were quite below the National Environmental Quality Standards of EPA for sewage

Table 1. Heavy metal concentration in sewage water of Quetta and NEQS

Elements	Average conc. (ppb)	NEQS (ppb)
Lead	53.26	500
Copper	22.50	2000
Chromium	1.33	1000
Cadmium	0.53	100
Iron	127.7	2000
Manganese	17.08	1500
Nickel	51.14	1000
Zinc	31.38	5000
Arsenic	0.011	1000

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and industrial effluents. The sludge soil which was irrigated with the sewage water contained (ppm) lead 1.38, copper 0.86, chromium 0.036, cadmium 0.29, iron 10.50, manganese 3.11, nickel 0.74, zinc 19.45 and arsenic 0.001 ppm on an average. However, the values did not exceed the National Environmental Quality Standards of EPA, which are lead 60, copper 100, chromium 120, cadmium 1.6, nickel 32, zinc 220 and arsenic 14 ppm (Table 2).

Table 2. Heavy metal concentration in sludge soil of Quetta and NEQS

Elements	Average conc. (ppm)	NEQS (ppm)
Lead	1.38	60
Copper	0.86	100
Chromium	0.03	120
Cadmium	0.29	1.6
Iron	10.50	-
Manganese	3.11	-
Nickel	0.74	32
Zinc	19.45	220
Arsenic	0.001	14

Though small quantities of such heavy metals are found in the natural environment but the anthropogenic activities augment their concentration.

There could be a number of sources responsible for the presence of these heavy metals in the sewage water and sludge and subsequently in soil. All of these heavy metals, individually or in combination, are used in many products and activities. Most of the manufacturing plants and service providers discharge the effluents resulting from their activities as such without any treatment into the environment.

Some of such responsible sources/activities , for example, may include metal and chrome plating, dyes, paints, leather processing, fertilizers, pesticides, wood preservatives, PVC, automotive parts, electrical and electronic equipment, petrol and lubricating oils, mineral ores and alloys etc. and the list goes on. Wear and tear of many products and consumer items also release heavy metals into the environment. The former and the present mining activities carried on in Quetta may also add to the problem.

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