IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 1, Issue 3 (Mar. – Apr. 2015), PP 18-27
www.iosrjournals.org

Comparative Effect of Crude and Commercial Enzyme in Shea Fat Extraction

*Otu, S.A.[1], Dzogbefia, V.P.[1], Kpikpi, E.N.[2], Essuman, E.K.[1]
[1] *Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*
[2] *Department of Applied Biology, University for Development Studies, Navrongo*

***Abstract**: Pectinases are one of the most used enzymes in food industry. Enzymes are widely used to improve upon process parameters such as product yield and rate of product formation. Such biotechnological applications are not currently exploited by industries in Ghana. The objective of the study was to determine the efficacy of locally produced pectinases from S. cerevisiae and other commercial enzymes in the extraction processes of shea butter. The crude pectinase was produced using corn cobs as a substrate and the microorganism Saccharomyces cerevisiae ATCC 52712 in a solid state fermentation process. The crude pectinase had optimal protein concentration of 7.00 mg/ml with enzyme specific activity of 0.86 u/mg. The commercial enzymes were Viscozyme L (beta endo- 1, 3 (4)-glucanase activity with declared activity of 100 FBG/g as well as side activities of xylanase, cellulase and hemicellulases) and Pectinex 5XL (polygalacturonase activity with declared activity of 4500 PECTU/ml as well as arabinase side activity). The application of the crude pectinase gave an optimum oil recovery of 44.00 % with an enzyme dosage of 1.20 % while commercial Pectinex and commercial Viscozyme gave their optimum oil recovery of 58.60 % and 72.00 % respectively at enzyme dosages at 0.80 %. These results indicate the possible use of crude pectinase to improve shea fat extraction processes in Ghana if the enzymes are made available.*

***Keywords:** Aqueous extraction, crude pectinase, corn cobs, Saccharomyces cerevisiae, solid state fermentation*

I. Introduction

Shea butter is a fatty extract from the seed of the shea nut [1] and has approximately 50.00 % (w/w) of poly unsaturated fatty acids (PUFA) consisting of oleic (40.80 %), linoleic acid (6.90 %) and linolenic acid (1.60 %), and a degree of unsaturation of 0.59 % [2,3]. The shea tree, formerly Butryospermum paradoxum, now called Vitellaria paradoxa, is a wild plant and populates the dry Savannah belt of Senegal in the Western part of Africa through Sudan to the foothills of the Ethiopian highlands [4]. In Ghana, shea trees are commonly found in the Upper East, Upper West and the Northern Regions. Shea butter is obtained from the dried kernel of the shea nut and is usually seen as the major occupation of majority of the women in these regions. It is estimated that more than 600,000 women depend on the income from the sales of shea butter and its related products [5].

The vegetable fat of shea nut is second in importance only to palm oil in Africa [6]. The fat is the source of edible oil for majority of the people in Northern Ghana and therefore the most important source of fats and glycerol in their diet [7].

The extraction of shea fat is done by one of two extraction methods broadly described as traditional or modern. The traditional method of extraction is the preferred choice of most shea butter industries in Ghana. This method of shea butter production encompasses many manual unit operations. Each of these units apparently has some problems which renders the whole process tedious and laborious. To address this situation, researchers have identified the application of enzymes in the production of oil from oilseeds as an alternative and environmentally friendly technique for overcoming these challenges [8, 9]. Enzymes are the largest class of proteins and are catalysts that accelerate the rates of biological reactions [10].

Enzyme reactions may often be carried out under mild conditions; they are highly specific and normally have no side effect. The use of enzymes has emerged as a novel and an effective means to improve the oil yield in cold pressing and aqueous extraction techniques [11] and it has been reported by many researchers; for instance Fullbrook [12] in his earlier work reported on soybean and rapeseed; Buenrostro and Lopez-Munguia [13] on avocado; McGleone et al. [14] on coconut and Cheah et al. [15] on palm oil. These studies revealed that treatment of the oil bearing seeds with some enzymes after mechanical milling degrades the cellular structure of the material. Commercial enzymes which have been used for edible oil extraction are cellulase, protease, glucanase, amylase, hemicellulases and pectinases [11, 16, 17].

The use of such commercial enzymes in the shea oil industry in Ghana will be difficult due to the cost involved in acquiring and maintaining the activity of the enzyme as a result of frequent power fluctuation in the country. The alternative approach therefore will be to produce the enzyme locally and make it available to the

shea industry on demand. Pectinase enzyme activities from S. cerevisiae (ATCC52712) have been utilized effectively in fruit juice extractions [18] and cassava starch extraction [19]. It is therefore attractive to investigate into the use of locally produced pectin enzymes in the shea butter industry to improve the technology of shea butter production in Ghana.

II. Materials And Methods

2.1 Sources of Materials

Corn cobs were obtained from Ahomaso a suburb of Kumasi-Ghana. Dried shea kernels were obtained from New Vision Shea Village in Nakolo, a suburb of Kasena-Nankana in the Upper East Region of Ghana. S. cerevisiae (ATCC 52712) was obtained from American Type Culture Collection, Maryland, USA, and had been maintained on agar slants in a freezer.

2.2 Preparation of Samples

The corn cobs were washed, dried, milled using a hammer mill into an average particle size of 0.3 mm and then autoclaved at 121 ^{0}C for 15 min. The dried shea kernels were first roasted at an oven temperature of 120 ^{0}C for 1h and were cracked using a cracking machine. The cracked kernels were further milled into a fine paste using the ordinary corn mill. Samples of this were used in the oil extraction studies

2.3 Culturing of S. Cerevisiae for Pectin Enzyme Production

S. cerevisiae (ATCC 52712) were pre-cultured in malt extract broth for 3 days at room temperature in a UV sterilized room to a cell density of $5.25x10^7$ cells per milliliter prior to inoculation. A 250 ml Erlenmeyer flask served as the containment for the solid state fermentation. Ten ml solution of NaCl (0.3 g), NH_4SO_4 (1.4 g), Na_3PO_4 (2.0 g) and urea (0.3 g) were added to 20 g of milled corn cob in the Erlenmeyer flask and inoculated with 10 ml of S. cerevisiae cell suspension ($5.25x10^7$) and the moisture content adjusted from 40 % to 80 %. The resulting media were incubated at 30 °C for 9 days and cell growth was monitored. The enzymes were extracted from the fermented medium by adding 100 ml of sterilized acetate buffer (pH 5.0), filtered and then centrifuged at 4000 rpm for 15 min. The supernatant obtained was then used as the crude enzyme solution. The total protein content and pectin enzyme activity of the extract were determined, respectively, by the methods of Gornall et al. [20] and Jecfa [21]. This assay method is based on the hydrolysis of pectin, the resulting galacturonic acid being determined spectrophotometrically at 235 nm. One unit of pectinase (PTA) causes an increase of 0.010 of absorbance per minute under conditions of the assay (Pectin 0.5 % and pH 5.8 and 30 ^{0}C).

2.4 Effects of treatment conditions

The effects of pH, enzyme dosage, temperature, hydrolysis time, and water to seed ratio were determined. Single factor experiments were done using the following factor levels: temperature 20 ^{0}C, 30 ^{0}C, 40 ^{0}C, 50 ^{0}C, 60 ^{0}C and 70 ^{0}C with a magnetic stirring hot plate and the extraction was carried out at different time intervals of 30, 60, 90,120 and 150 min. For each run, 50 g of the ground kernel were mixed to get water to seed ratios of 2:1, 4:1, 6:1 and 8:1. The effect of pH was studied at five different pH levels: 3, 4, 5, 6, and 7. for each trial. Five different enzyme dosages 0, 20, 40, 60, and 80 mg (total protein) were used to evaluate the effect of enzyme concentration on the oil yield during EAAE.

2.5 Proximate Composition of Oilseed Residues

After oil extraction, the residue was analyzed for protein, fiber, and ash contents. Physico-chemical Parameters of Oils such as Iodine value, free fatty acid (FAA), density, unsaponifiable matter, peroxides and saponifiable values of the control and enzyme-extracted oils were determined by various standard methods [22].

III. Results And Discussion

Protein concentration and pectinase activity profile during fermentation are shown in Fig. 1. An increase in both protein and enzyme activity was observed from the 2nd day of fermentation, reaching a peak by the 4th day and thereafter began to decline. Enzyme activity was totally lost by the 8th day of fermentation while the protein concentration reached the minimal level by 12 days of fermentation.

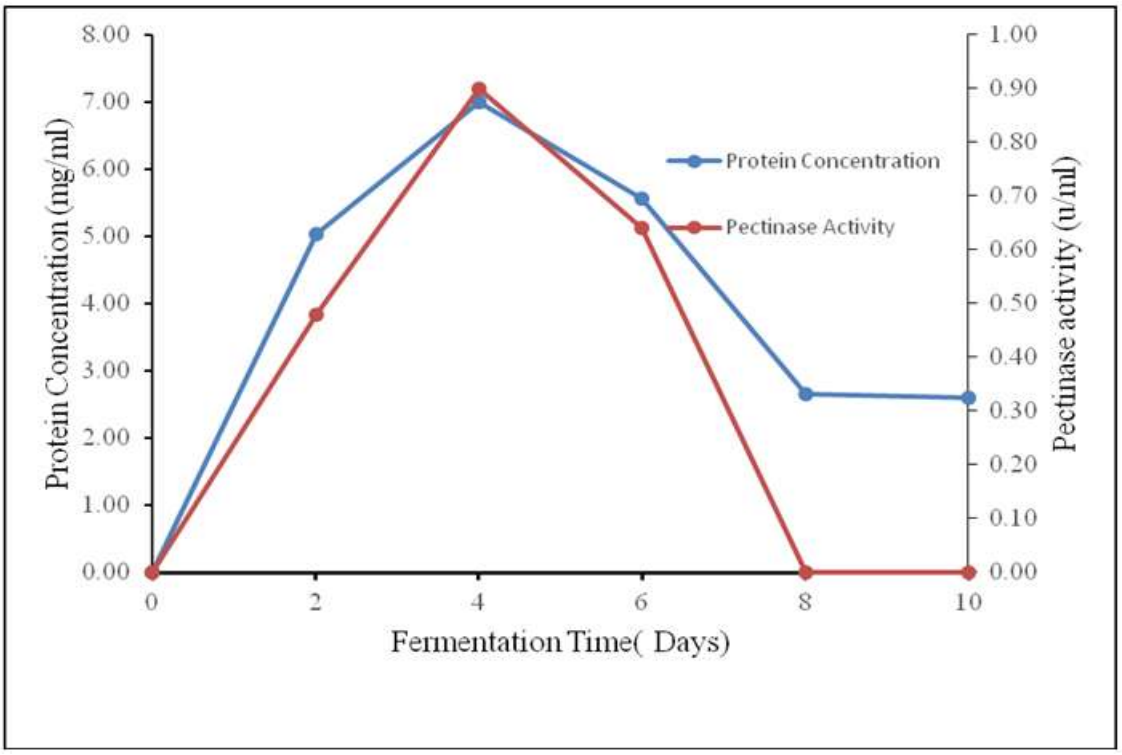

Figure.1: Changes in concentration of protein and pectinase activity produced by Saccharomyces cerevisiae with fermentation time.

Specific activity gives a measurement of enzyme purity in the mixture. It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of total proteins. Specific activity is a measure of enzyme purity. The value becomes larger as an enzyme preparation becomes more pure, since the amount of protein (mg) is typically less, but the rate of reaction stands the same or may increase due to reduced interference or removal of inhibitors [23].

The specific activity for the various pectinase activities was calculated. The specific activity curve as shown in Fig. 2 followed the shape of the pectinase activity curve. It was also at its peak on the fourth day.

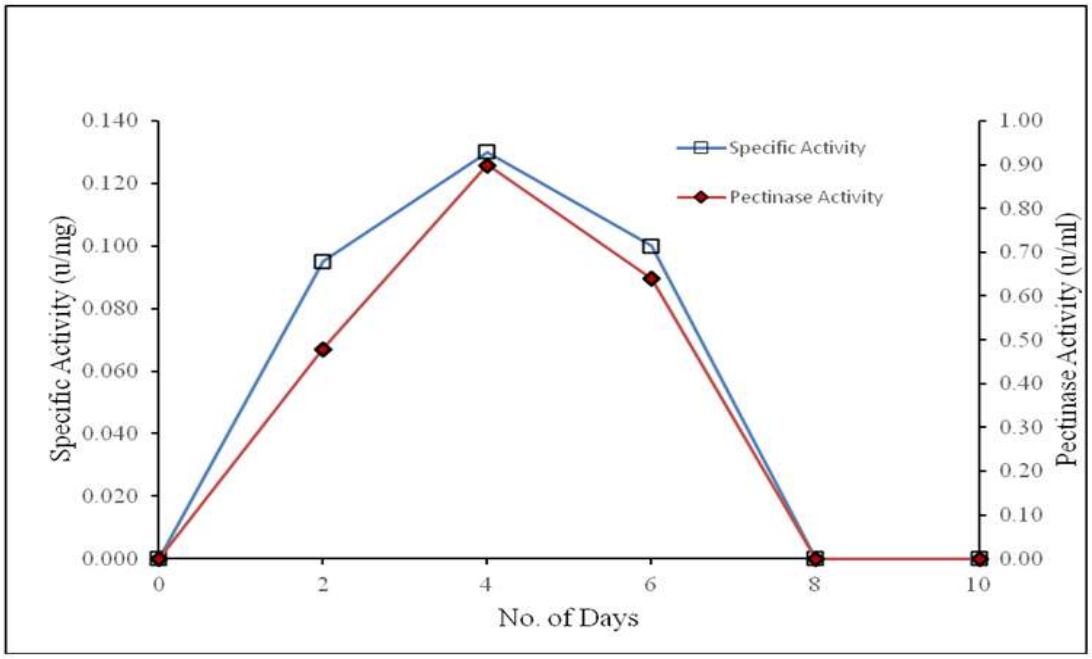

Figure 2: Changes in pectinase activity and Specific activity produced by Saccharomyces cerevisiae with fermentation time

Most crude enzymes usually may have low specific activities due to the fact that they have not been purified. In our work, the peak specific activity (S.A) was as low as 0.130 U/ml. The fourth day gave the highest pectinase activity and therefore was obvious that the same day may give the highest specific activity. In a similar work reported by Dzogbefia et al. [19], had a low specific activity of 0.62 U/ml extract. These therefore support our findings that crude pectinases usually give low specific activities.

3.1 Stability of protein and enzyme activity on storage

Based on the results shown in Fig. 2, subsequent enzyme productions were carried out for 4 days of fermentation. The stability of the protein and enzyme was monitored by storing the crude preparation in refrigerator at 8 ^{o}C. The protein concentration and enzyme activity of the crude pectinase was measured on weekly bases for four weeks. The protein concentration remained unchanged (7.0 mg/ml) for the first two weeks, after which it declined to 5.0 mg/ml on the twenty first day, and further reduced to 3.0 mg/ml by the twenty eighth day (Fig. 3).

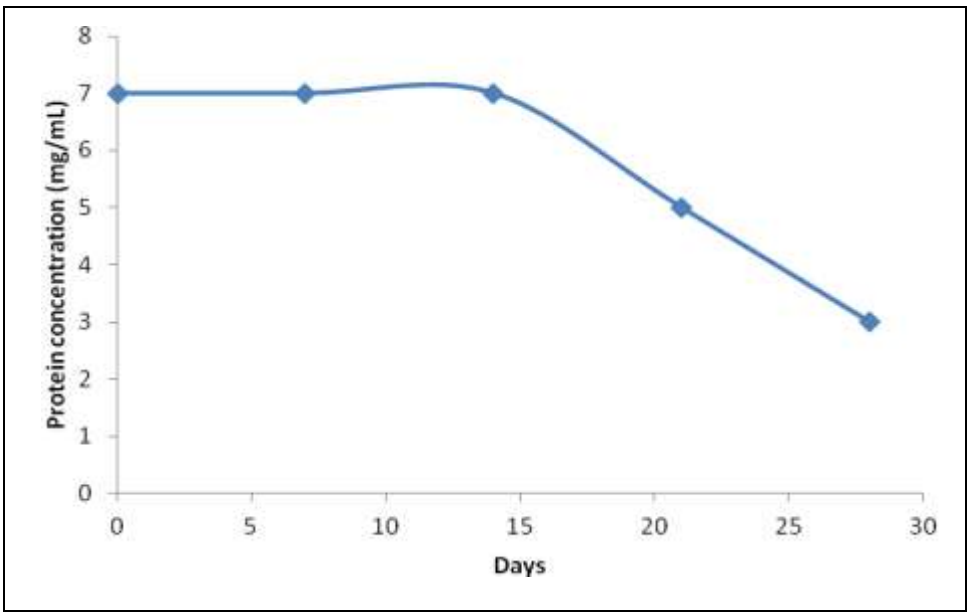

Figure 3: Stability of protein concentration of enzyme extract on storage

Similarly the activity of the crude pectinase was the same from the first day of production to the second week; the enzyme activity began to decline after the second week. No activity was observed after the fourth week of storage (Fig. 4).

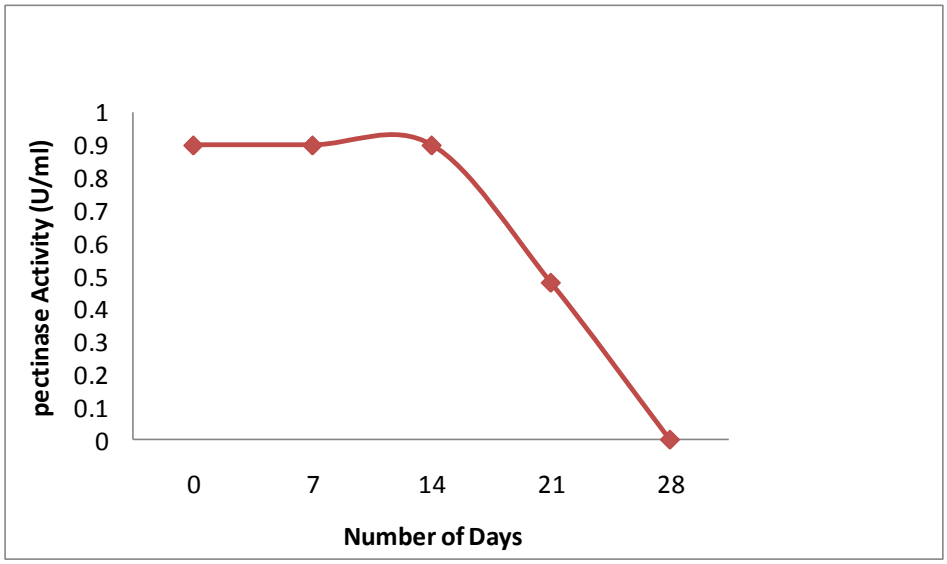

Figure 4: Stability of enzyme extract during storage

From Fig. 4 it was observed that the crude pectinase activity was not effective after 14 days of production, thus the enzyme should not be stored over a long period of time. One of the common practical problems facing an enzymologist is the loss of enzymatic activity in a sample due to enzyme instability [24]. Enzymes, like most proteins are prone to denaturation under many laboratory conditions and specific steps must be taken to stabilize these macromolecules as much as possible [25]. An enzyme can be maintained in stable conditions for several days at 4 °C, however, it should be used in smaller quantities to prevent denaturing [24].

3.2 Oil Extraction of shea seeds during Enzyme-Assisted Aqueous Extraction (EAAE)

3.2.1 Effect of water/seed (w/s) ratio on oil recovery

The effect of water/seed ratio (w/s) on oil recovery was also investigated. The optimum water to seed ratio was found to be 4:1 for crude pectinase, Pectinex and Viscozyme and that of the control was found to be 2:1 (Fig. 9). At a higher w/s ratio (8:1), the viscosity of the mixture decreased. This made it difficult to maintain mixture homogeneity and this decreased the oil recovery. In the present study, a w/s ratio of 4:1 was found to be adequate for this process when enzymes were applied. The amount of water added during the treatment affects the oil recovery. The low water/seed ratio of 2:1 of the control experiment may be due to the fact that, kneading is best done with little water and more water would not give enough fat suspension. The application of enzymes may need enough water for the enzymes to work effectively; this might be attributed to the reason why more water was needed in the case of the enzyme extraction processes. Buenrostro and Lopez-Munguia [13] reported a maximum yield of avocado oil with a 5:1 w/s ratio, while for coconut the best yields was at 4:1 w/s ratio [35]. Tano-Debrah and Ohta [31] also reported that the maximum yield of coconut oil and shea kernel was 4:1 w/s which is consistent with the results of this study.

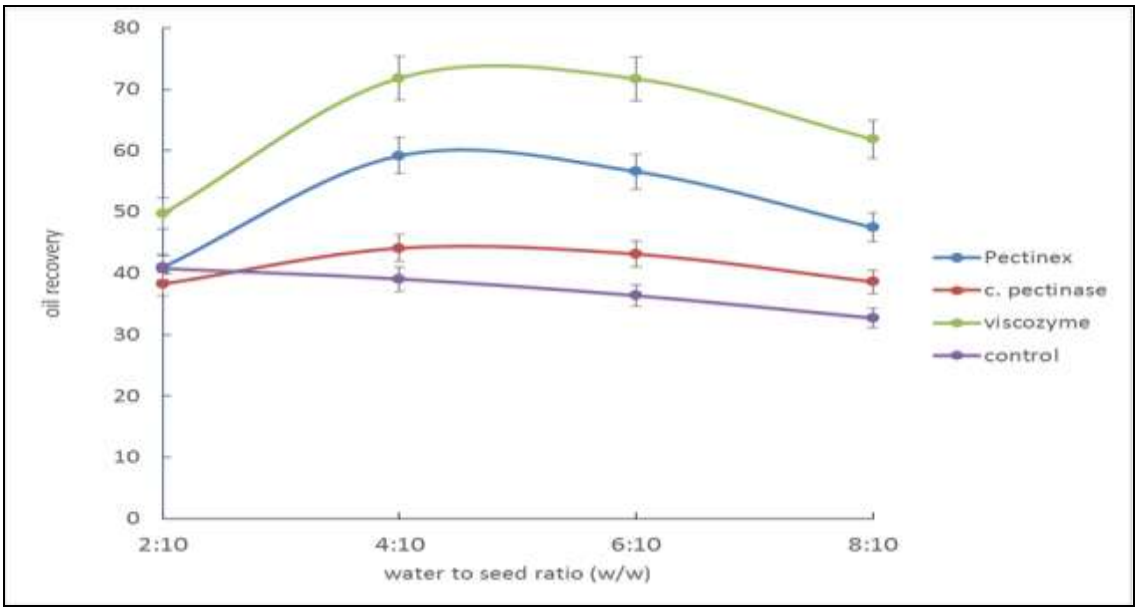

Figure 5: Effect of water seed ratio on oil recovery from shea seeds.

3.2.2 Effect of pH on oil recovery

Fig. 5 shows the effect of pH of the extraction medium on oil yield during EAAE. The pH range of 4-6 was found to be generally suitable in the present study. Crude Pectinase and commercial Pectinex exhibited maximum activity at pH range of 4 to 6 whereas Viscozyme and the control showed maximum activity at pH of 6.0. In earlier extraction trials at unmodified pH 5.4 of the dissolved meal, yield was persistently high and closer to that at pH 6.0 which is the optimum pH of the control. In subsequent experiments, therefore, pH adjustments were not made. This also suggests that in such aqueous extraction processes without the use of commercial enzymes, pH adjustments may not be necessary. Very high and low pH's can result in higher viscosity due to cell degradation [26]. This makes it more difficult to separate solid-liquid phase by centrifugation which results in decrease in the oil yield. It may also result in deterioration of oil quality through saponification [27] and cause several changes to the amino acids, such as the formation of lysinoalanine and lanthionine [28].

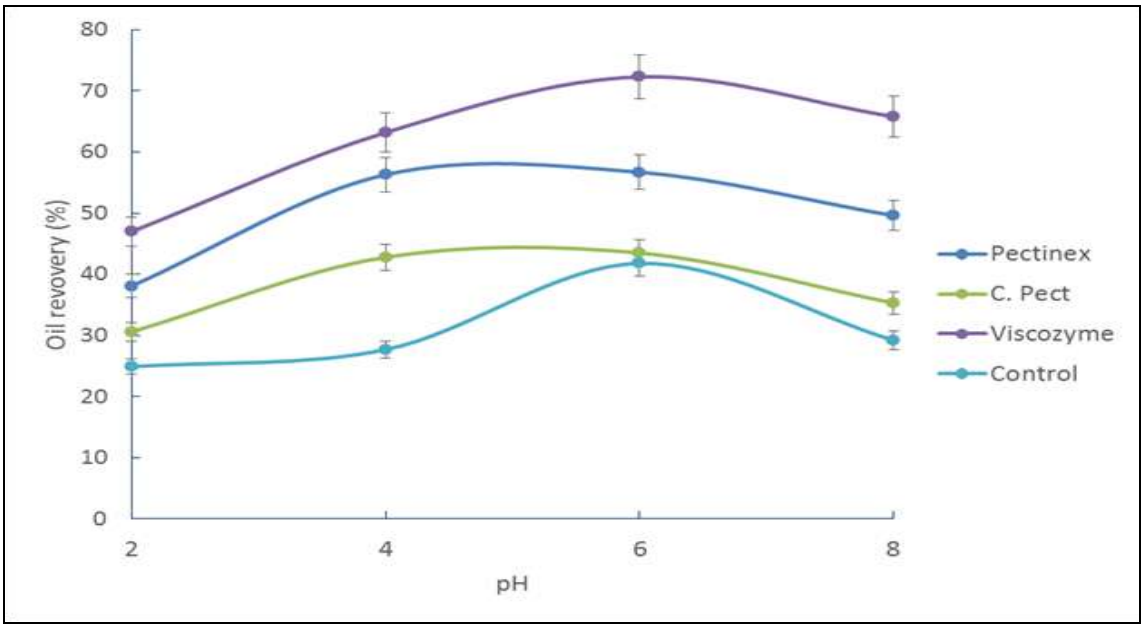

Figure 6: Effect of pH on oil recovery from shea seeds during EAAE.

3.2.3 Effect of enzyme dosage on oil recovery

Fig. 6 illustrates the effect of enzyme dosage on oil yield from shea seeds. The optimum enzyme dosage for crude Pectinase was 1.20 % while commercial Pectinex and commercial Viscozyme were found to be 0.80 %. It has been observed that the oil yield increases with little increment in enzyme dosage [29]. The high enzyme cost associated with high enzyme dosage is a major obstacle for the economy of the process. Depending on the enzyme and the experimental conditions, different oil yields were obtained. Previous studies showed that only 0.02 % of the local pectinase was the dosage required for optimal pineapple juice, pawpaw juice and cassava starch extraction [18,19,30]. Thus the high dosage of 1.20 % observed for shea fat extraction could be attributed to the cell wall structure of the shea kernel.

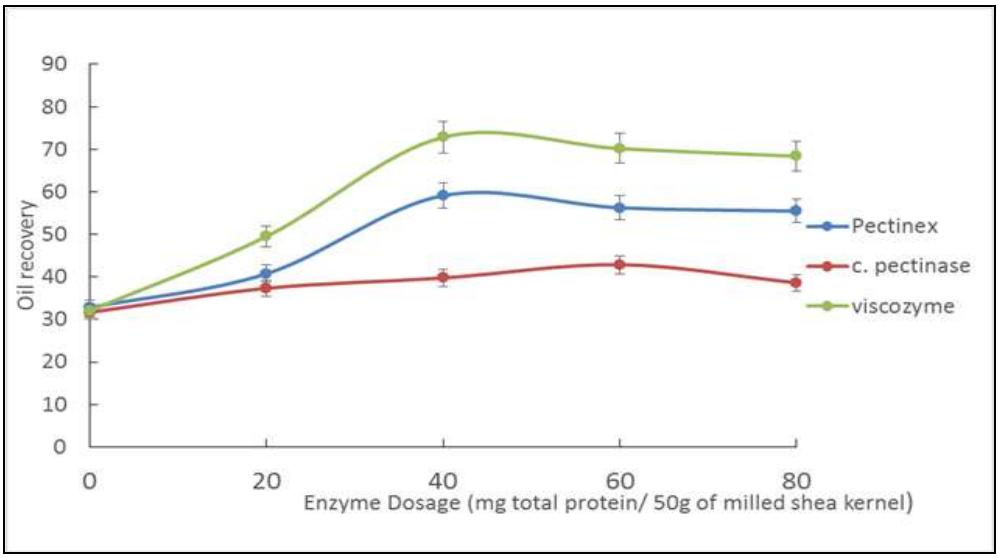

Figure 7: Effect of Enzyme Dosage on Oil Recovery from shea seeds during EAAE.

Enzyme mixtures were found to be more effective than the single enzyme crude pectinase used as shown in Fig. 6. Tano-Debrah and Ohta [31] also examined combined enzymatic effects of acid protease, cellulase, hemicellulase and glucanase, and Sumizymes LP (protease), cellulase and hemicellulase and obtained recoveries of 74.1 and 72.7 % for the extraction of shea and cocoa fat, respectively. A 70.0 % recovery during aqueous-enzymatic extraction of sunflower kernel oil was obtained by using a combination of cellulase and pectinase (Novozymes) [32].

3.2.4 Effect of shea meal hydrolysis time on oil recovery

The effect of hydrolysis time on EAAE is shown in Fig. 7. It can be seen that hydrolysis time beyond 30 min had appreciable effect on oil recovery, but the oil yield decreased after 2.5 h. As evident in Fig. 7, an increase in the incubation time from 1.5 h to 2.5 h did not improve oil yield. It can be seen that oil yields increased slightly when extraction time increased. The maximum oil yield for crude Pectinase and commercial Pectinex were observed at hydrolysis time of 90 min and that of Viscozyme and the control gave their maximum yield at 60 min of hydrolysis. Kim [33] reported 30 min period as an optimum time for palm oil extraction, and 40 min period for extracting both soybean oil and protein [34].

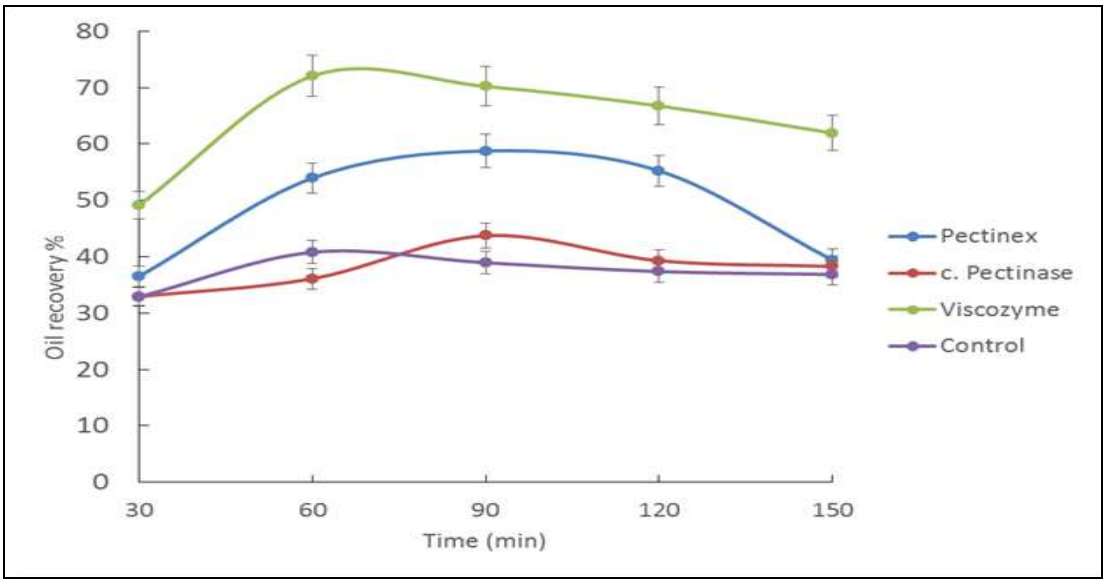

Figure 8: Effect of shea meal hydrolysis time on oil recovery from shea seeds.

3.2.5 Effect of temperature on oil recovery from shea seeds

The effect of temperature on oil yields during EAAE is shown in Fig. 8. When the extraction temperature was raised from 30 to 50 ^{o}C, the oil yield increased significantly. All the enzymes gave maximum oil recovery at 50 ^{o}C. No enhancement in the oil yield was observed above 50 ^{0}C. At high temperatures it was difficult to maintain homogeneity during extraction and this resulted in protein denaturation. Therefore, a temperature range of 40-50 ^{o}C was deemed to be satisfactory for the extraction. Temperature exhibits considerable effect on oil yields from different oilseeds during hot water floatation method [44]. Lusas et al. [34] reported that the temperature is critical for oil extraction from soybeans during aqueous extraction process. They observed maximum oil recovery between 40 ^{o}C

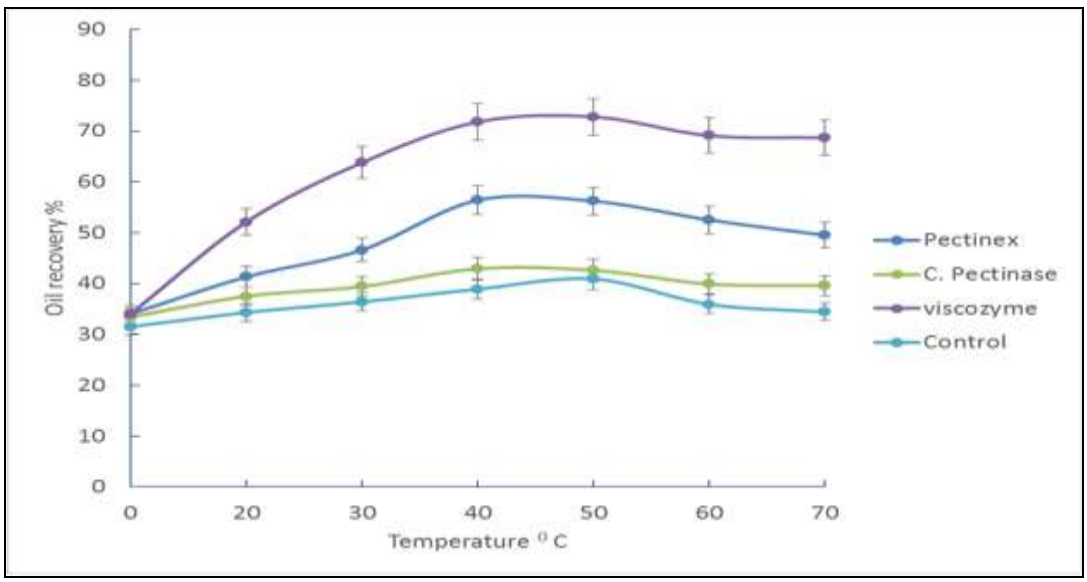

Figure 9: Effect of temperature on oil recovery from shea seeds.

3.2.6 Oil recovered after optimization of all parameters

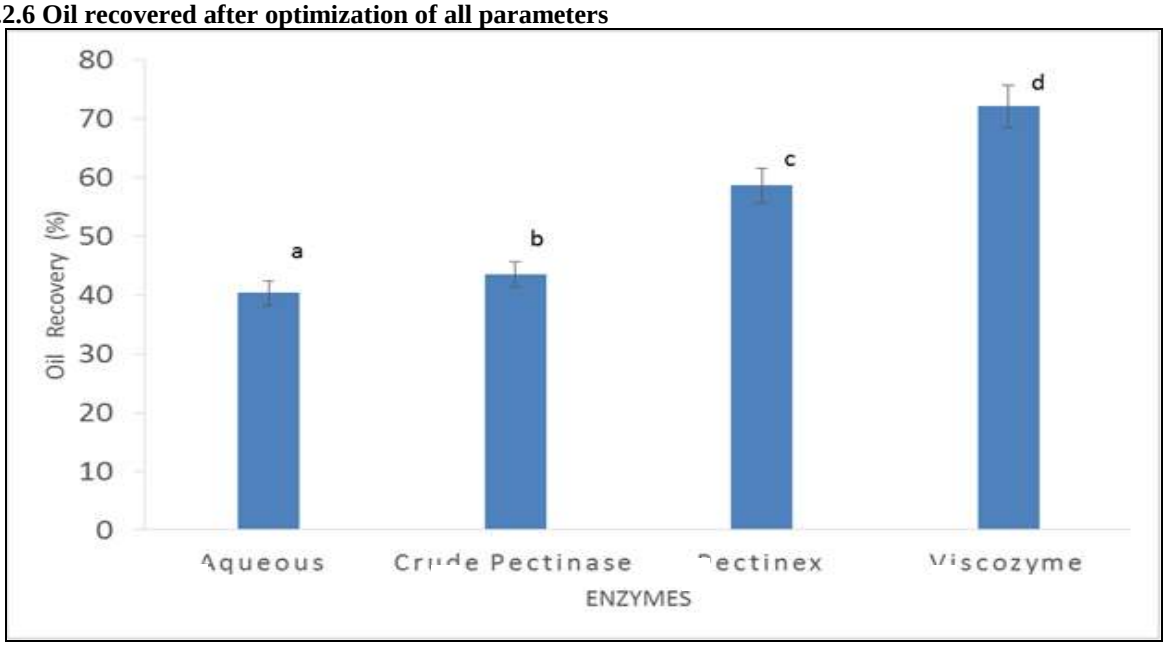

Figure 10: Performance of crude pectinase verses commercial enzymes in shea fat extraction under optimum conditions

The enzyme pre-treatment appreciably increased the oil yields at optimized set of experimental conditions as shown in Fig. 10. Viscozyme gave the highest amount of oil recovery of 72.00 % followed by Pectinex which gave 58.60 %. The oil recovered by the application of the crude pectinase was significantly ($P \leq 0.05$) different from that of the control. The crude pectinase gave 44.00 % oil recovery while that of the control was 40.00 %. Enzyme mixtures offered higher oil recovery due to their combined effect on colloidal and lipoprotein seed structures [36]. This can be attributed to the presence of several potent components in these enzyme mixtures, e.g. arabanase, cellulase, β-glucanase, hemicellulase, xylanase and pectinase (in Viscozyme), and polygalacturonase activity as well as arabinase side activity in Pectinex. Similar results in this study have also been reported by Hernandez et al. [37] for rice bran.

In agreement to the results in this study, enzyme mixtures were found to be more effective than the single enzyme. Tano-Debrah and Ohta [31] also examined combined enzymatic effects of acid protease, cellulase, hemicellulase and glucanase, and Sumizymes LP (protease), cellulase and hemicellulase and obtained recoveries of 74.1 and 72.7 % for the extraction of shea and cocoa fat, respectively. A 70.0 % recovery during aqueous-enzymatic extraction of sunflower-kernel oil was obtained by using a combination of cellulase and pectinase (Novozymes) [32]. The palm mesocarp was treated with cellulase preparation and 57.0 % palm oil was recovered during the aqueous process [38]. Lanzani [39] reported peanut oil yields of 74.0-78.0 % by aqueous extraction using protease, cellulase, and α-1,4-galacturonide glycano hydrolase as compared to the control (72.0 %). Olsen [40] utilized pectinase, cellulase, and hemicellulase combinations to degrade rapeseed during aqueous extraction process. By using cellulolytic enzymes on steamed rapeseed flour, 35.0 % of the oil was extracted [41]. Dominguez et al. [32] observed an enhancement up to 30.0 % in the sunflower oil recovery during aqueous extraction by using mixtures of cellulase and pectinase. A carbohydrase complex was found to be the most effective enzyme when a group of commercial enzymes were utilized for aqueous extraction of corn germ oil [42]. Similarly, 80.0 % oil recovery has been reported by using a commercial carbohydrase complex in the aqueous process of rapeseed [43]. The low effect of the crude pectinase can also be due to the fact that this has not been purified in any way and thus the Specific Activity was as low as 0.130 U/ml.

3.3 Comparison of the Quality of Extracted Shea Butter and Residues

3.3.1 Proximate composition of shea butter

Table 1 shows comparison of proximate composition of Enzyme-assisted Aqueous Extracted Shea butter. The commercial enzyme-assisted aqueous (EAA)-extracted oil yield was found to be significantly ($P < 0.05$) higher than the control (without enzyme) and the use of crude Pectinase. The oil yield obtained by the application of the crude Pectinase was also found to be significantly ($P < 0.05$) higher than the control. Pectinex and Viscozyme were found to be the most efficient enzymes for shea oil recovery from shea kernel seeds, offering 36.40 %, and 45.00 % respectively. Crude protein content from the residue of the application of the Pectinex and Viscozyme are very close (12.98 % and 12.06 %), but higher than that of the control and the crude

enzyme. Protein was significantly ($P \leq 0.05$) different from one residue to the other as shown in Table 1.The highest protein in the aqueous phases of shea seeds was extracted with Pectinex and Viscozyme and this could be due to the fact that they are commercial enzymes with high enzyme activities.

No significant ($P > 0.05$) variations were observed for the fiber and ash contents among enzyme-treated and control oilseed residues. The statistical analysis of the crude fibre content indicated that it was not different ($P \geq 0.05$) across the various residues. The crude fibre content ranged from 19.82 from residues extracted using Pectinex to 22.13 from the residue of the control.

The total ash content presented in Table 1 did not vary significantly among samples across the residues. The total ash content ranged from a minimum of 1.61 % with the control and a maximum value of 2.20 % with residue from Pectinex. The total ash content was statistically ($p \geq 0.05$) not different between the residues from the application of different enzymes although it was higher in the residue of the Pectinex.

Table 1: Comparison of Proximate Composition of Enzyme-assisted Aqueous Extracted Shea butter

	Control (%)	**Local Pectinase (%)**	**Commercial Pectinex (%)**	**Commercial Viscozyme (%)**
Oil content	25.33± 1.40 [a]	26.43 ± 0.38 [b]	36.35± 1.02 [c]	44.52± 0.63 [d]
Protein content	10.54± 0.49 [b]	10.46 ± 0.49 [b]	12.98± 0.47 [a]	12.06 ± 0.31 [a]
Fiber content	22.13 ± 0.32 [a]	20.78 ± 0.19 [bc]	19.817 ± 0.64 [c]	21.38 ± 0.77 [ab]
Ash content	1.61 ± 0.13 [b]	1.88 ± 0.13 [b]	2.20 ± 0.26 [a]	1.75 ± 0.05 [b]

*Values are mean ± SD, calculated as percentage on dry seed weight basis for three shea kernel seed samples for each enzyme, analyzed individually in triplicate. Mean values in the same row followed by the same superscript letters are not significantly different ($P > 0.05$)

Enzymes	Moisture	FFA	PV	Density/g	SMP/c	UM
Aqueous	0.18±0.01a	6.50±0.15a	4.90±0.14a	993.51±4.79a	23.50±0.71a	4.91±0.43a
Crude Pectinex	0.17±0.01a	7.72±0.17b	5.33±0.18b	1016.89±2.71b	22.50±0.71a	5.35±0.30a
Pectinex	0.17±0.02a	7.77±0.09b	5.63±0.18bc	1018.30±3.59b	23.50±0.71a	5.70±0.28a
Viscozyme	0.17±0.02a	8.69±0.34c	5.85±0.07c	1000.07±11.16a	23.50±0.71a	6.67±0.30b

3.3.2 Physicochemical characteristics of oils

Various physical and chemical parameters were investigated on the oil obtained from the extraction using the various enzymes and the control as shown in Table 2.

Table 2: Comparison of Physico-chemical Properties of Enzyme-assisted Aqueous Extracted Shea butter Values are means of duplicate ± standard deviation. The superscript showed that at $P < 0.05$, a significant difference exists. Means within each column that do not share a letter are significantly different. FFA, free fatty acid, PV, peroxide value, SMP, sample melting point, UM, unsaponifiable matter content.

There were no significant ($P > 0.05$) differences for the moisture, density, and sample temperature values between the crude enzyme-assisted-extracted oils and the commercial enzyme assisted extracted oil. The level of free fatty acids was found to be significantly ($P < 0.05$) higher in all the enzyme-extracted seed oils as compared to the control. This increase in the free fatty acids may be attributed to the longer hydrolysis time and enzymatic effect during the Enzyme-Assisted Extraction.

IV. Conclusions

The study showed that crude pectinase, produced from the fermentation of corn cob using Saccharomyces cerevisiae can be used to extract shea fat, but not as efficiently as combination of enzyme mixtures. Whereas commercial enzyme mixtures required 0.8 % dosage for optimal yield, the use of crude pectinase alone was at a dosage of 1.2 % for optimal results.

With the exception of free fatty acids and peroxide value, enzyme treatment has no significant effect on the physic-chemical properties of the butter.

The application of enzymes released protein in the aqueous extraction processes, it is therefore very important that crude enzymes are produced and applied in the shea fat extraction processes.

References

[1]. D. K. Salunkhe, and B. B. Desai, Postharvest biotechnology of oilseeds (CRC, Boca Raton, 1986) Pp 264.
[2]. D. Adomako, Prospects for the development of the shea nut industry in Ghana. Cocoa Research Institute., Ghana, Technical Bulletin, 11, 1985, 8 - 10.
[3]. D. Adomako, Fatty acid composition and characteristics of Pentadesma butyracea fat extracted from Ghana seeds. Journal of the Science of Food and Agriculture, 28, 1977, 384 – 386.
[4]. J.M. Boffa, Agroforestry parklands in Sub-Saharan Africa. Forest Conservation Guide 34. Roma: FAO., 1999.
[5]. www.undp-gha.org/project accessed on 17/09/2013

[6]. J.B., Hall, D.P., Aebischer, H.F., Tomlinson, E., Osei-Amaning and J.R. Hindle, Vitellaria paradoxa: A monograph; School of Agricultural and Forest Sciences Publication No. 8, University of Wales: Bangor, U.K, 1996, p 105.
[7]. K.C. Agyente-Badu, The effect of Cochlospermum planchonii root dye on the shelf- life of shea butter during storage, MPhil thesis submitted to the Department of Biochemistry and Biotechnology. Kwame Nkrumah University of Science & Technology, Kumasi, Ghana, 2010.
[8]. V. A. Barrios, D. A. Olmos, R. A. Noyola and C. A. Lopez-Munguia, Optimization of an enzymatic process for coconut oil extraction, Oleagineux., 45, 1990, 35–42.
[9]. E.W. Lusas, J.T. Lawhon and K.C. Rhee, Producing edible oil and protein from oilseeds by aqueous processing. Oil Mill Gazet, 4, 1982, 28–34.
[10]. H.R. Garrett and M.C. Grisham, Protein. In: Biochemistry, (Saunders College Publishers.USA. 1995), Pp 94.
[11]. K. Tano-Debrah and Y. Ohta, Enzyme-assisted aqueous extraction of fat from kernels of the shea tree (Butyrospermum parkii), Journal of American Oil Chemist's Society, 71, 1994, 979-983.
[12]. P. D. Fullbrook, The use of enzymes in the processing of oilseeds, Journal of American Oil Chemists' Society. 60, 1983, 428-430.
[13]. M. Buenrostro and C.A. Lopez-Munguia, Enzymatic extraction of avocado oil, Biotechnology Letters, 8, 1986, 505-506.
[14]. O.C. McGleone, C.A. Lopez-Munguia and J.V. Carter, Coconut oil extraction by a new enzymatic process. Journal of Food Science, 51, 1986, 695-697.
[15]. S.C. Cheah, M.A. Augustin, and L.C.L. Ooi, Enzymic extraction of palm oil. Palm Oil Research Bulletin, 20, 1990, 30-36.
[16]. N.S.K., Kamar, M. Nakajima and H. Nabetani, Processing of Oilseeds to Recover oil and Protein Using Combined Aqueous Enzymatic and Membrane separation Techniques. Food Sci. Technol. Res, 6(1), 2000, 1-8.
[17]. A. Rosenthal, D.L. Pyle and K Niranjan, Aqueous and enzymatic processes for edible oil extraction, Enzyme Microbial Technology, 19, 1996, 402–420.
[18]. V.P. Dzogbefia, E. Ameko, J.H. Oldham and W. O. Ellis, Production and use of yeast pectolytic enzymes to aid pineapple juice extraction. Food Biotech., 15(1), 2001, 25-34.
[19]. V.P. Dzogbefia, G.A. Ofosum, and J.H. Oldham, Evaluation of locally produced Saccharomyces cerevisiae pectinase enzymes for industrial extraction of starch from cassava in Ghana, Scientific Research and Essay, 3(8), 2008, 365-369.
[20]. A.G. Gornall, C.J. Bardawill and M.M. David, Determination of serum proteinsby means of the biureto reaction. J. Biol. Chem., 177, 1949, 751-766.
[21]. Pectinase from Aspergillus Niger, Var. Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 31st JECFA (1987) and published in FNP 38 (1988) and in FNP 52 (1992). An ADI "Not specified" was established at the 35th JECFA (1989).
[22]. AOAC. Methods of Analysis of the Association of Official Analytical Chemists, (14th Ed., AOAC, Washington, DC, 1994).
[23]. D. Nelson and M. Cox, Lehninger principles of biochemistry, (3rd Edition. Worth Publishers, New York, NY, USA, 2000).
[24]. G. Gomori, In CRC Practical Handbook of Biochemistry and Molecular Biology, (G. D. Fasman, Ed., CRC Press, Boca Raton, FL, 1992), pp. 553—560.
[25]. R.A. Copeland, Methods for Protein Analysis: A Practical Guide to Laboratory Protocols, (Chapman & Hall, New York, 1994).
[26]. S.M. Abdulkarim, O.M., Lai, S.K.S. Muhammad, K. Long and H.M. Ghazali, Use of enzymes to enhance oil recovery during aqueous extraction of Moringa oleifera seed oil, Journal of Food Lipids, 13, 2006, 113–130.
[27]. K.C. Rhee, C.M. Cater and K.F. Mattil, Simultaneous recovery of protein and oil from raw peanuts in an aqueous system, Journal of Food Science, 37, 1972, 90-93.
[28]. A.P. De Groot and P. Slump, Effects of severe alkali treatment of proteins on amino acid composition and nutritive value, Journal of Nutrition 98, 1969, 45-48.
[29]. S.M. Abdulkarim, K. Long, O.M. Lai, S.K.S. Muhammad and H.M. Ghazali, Some physico-chemical properties of Moringa oleifera seed oil extracted using solvent and aqueous enzymatic methods, Food Chemistry, 93, 2005), 253-263.
[30]. V.P. Dzogbefia and D.K. Djokoto, Combined effects of enzyme dosage and reaction time on papaya juice extraction with the aid of pectic enzymes – A preliminary report. J. Food Biochem. 30 (1), 2006, 117–122.
[31]. K. Tano-Debrah and Y. Ohta, Enzyme-assisted aqueous extraction of shea fat: A rural approach, Journal of American Oil Chemists' Society, 72, 1995, 251-256.
[32]. H. Dominguez, M.J. Nunez and J.M. Lema, Aqueous processing of sunflower kernels with enzymatic technology, Food Chemistry, 53, 1995, 427-434.
[33]. H.K. Kim, Aqueous extraction of oil from palm kernel, Journal of Food Science, 54, 1989, 491-492.
[34]. E.W. Lusas, J.T. Lawhon and K.C. Rhee, Producing edible oil and protein from oilseeds by aqueous processing. Oil Mill Gazet, 4, 1982, 28–34.
[35]. M.O. Cintra, A. Lopez-Munguia and J. Vernon, Coconut oil extraction by a new enzymatic process, Journal of Food Science 51, 1986, 695-697.
[36]. H. Dominguez, M.J. Nunez and J.M. Lema, Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review, Food Chemistry, 49, 1994, 271– 286.
[37]. N. Hernandez, M.E. Rodriguez-Alegria, F. Gonzalez and C.A. Lopez-Munguia, Enzymatic treatment of rice bran to improve processing, Journal of American Oil Chemists' Society, 77, 2000, 177-180.
[38]. S.C. Cheah, M.A. Augustin and L.C.L. Ooi, Enzymic extraction of palm oil. Palm Oil Research Bulletin Malaysia Bulletin, 20, 1990, 30-36.
[39]. A. Lanzani, M.C. Petrini, O. Cozzoli, P. Gallavresi, C. Carola and G. Jacini, On the use of enzymes for vegetable-oil extraction. A preliminary report, La Rivista Italiana Della Sostanze Grasse, 52, 1975, 226-229.
[40]. H. S. Olsen, Aqueous enzymatic extraction of oil from seeds, In: Asian Food Conference Proceedings, Bankgok, Thailand, Reprinted by: Novo Industry A/S, pp A-0604la, 1988.
[41]. E. Marek, E. Schalinatus, E. Weigelt, G. Mieth, G. Kerns, and J. Kude, Interbitech '89-Mathematical Modelling Biotechnol. Vol. 6 (Blazej, A. and Ottova, A., Eds.) Elsevier, Amsterdam, 1989, pp 471-474.
[42]. M. Bocevska, D. Karlovic, J. Turkulov and D. Pericin, Quality of corn germ oil obtained by aqueous enzymatic extraction, Journal of American Oil Chemist's Society, 70, 1993, 1273-1277.
[43]. Y. Deng, D. L. Pyle, and K. Niranjan, Studies of aqueous enzymatic extraction of oil from rapeseed. Agriculture Engineering and Rural Development. Conference Proceeding. Vol. 1 Zhang, W., P. W. Giro, S. W. Zhang, Eds. International Academic Publishers. Beijing, 1992, pp 190-195.
[44]. K.H. Southwell, and R.V. Harris, Extraction of oil from oilseeds using the hot water flotation method. Tropical Science, 32, 1992, 251-262.