

# Influence of Various Enzyme Combinations on Njangsa (*Ricinodendron heudelottii*)



## Seed Oil Extraction, Recovery and Quality



Mary Besong<sup>1</sup>, Immaculate Arrey<sup>2</sup>, Anh Nguyen<sup>2</sup> and Alberta N.A. Aryee<sup>2</sup>

<sup>1</sup> The Henry P. Becton School of Nursing & Allied Health, Fairleigh Dickinson University, Teaneck, NJ 07666, <sup>2</sup>College of Agriculture & Related Sciences, Delaware State University, Dover, DE 19901

**Abstract:** Njangsa (*Ricinodendron heudelottii*) seeds are a staple ingredient used in cuisine throughout Africa. Njangsa seed oil (NSO) has been found to be rich in polyunsaturated fatty acids (PUFA). Solvent extraction, commonly employing hexane is one of the main conventional methods used for oil extraction from oilseeds. However, exposure to solvents pose health risk and has negative effects on the environment. To preclude these repercussions, enzymatic oil extraction emerges as one of the most environmentally friendly alternative method. In this study, four enzymes; hemicellulase, amylase, protease and pectinase and a combination of these were used to extract NSO, and the yield and quality indices of the oil recovered were determined and compared to hexane extraction. Oil yield ranged between 4.91% and 41.78%. The quality indices of the extracted oils such as free fatty acid (FFA) content and peroxide value (PV) were significantly ( $P < 0.05$ ) affected by the type of enzyme used, whilst thiobarbituric acid (TBA) value was not significantly ( $P > 0.05$ ) affected by the type of enzyme used. Free fatty acid content, PV and TBA value, were 1.61 - 13.06%, 7.89 - 121.17 mEq of peroxides/kg of oil and 0.03 - 0.2 mg of malonaldehyde/kg of oil, respectively. The results obtained from this present study indicate that oil extracted using these enzymes were qualitatively and quantitatively comparable to hexane extraction.

## Introduction

*Ricinodendron heudelottii* commonly known as Njangsa, are the seeds of a tropical tree found in Central and West Africa. Njangsa is considered a nutritious food used in many sauces, soups, and condiments due to its characteristic spicy flavor [1]. It is mainly cultivated in West Africa for its content of good quality oil. Oil content of Njangsa seed range from 45 - 67% out of which PUFAs constitute ~75% of the total fatty acids [2]. Seed oils are mainly extracted by conventional pressing or solvent extraction (commonly with hexane). However, there is a growing concern that even insignificant amounts of hexane residues in oil, could pose health problems in the long run. Enzymes are receiving considerable interest in the oil industry due to their specificity and milder operating conditions. Studies have shown that enzymes are environmentally friendly and safe [3,4], and extracted components and by-products require minimal to no additional purification processes. The treatment of mustard seeds with cellulolytic enzymes resulted in 20 - 30% increased in oil yield [5]. The aim of this study was to extract NSO using; hemicellulase, amylase, pectinase and protease or a combination of these enzymes, and to evaluate their effect on the quantity and quality of oil extracted compared to hexane extraction.



## Results & Discussions

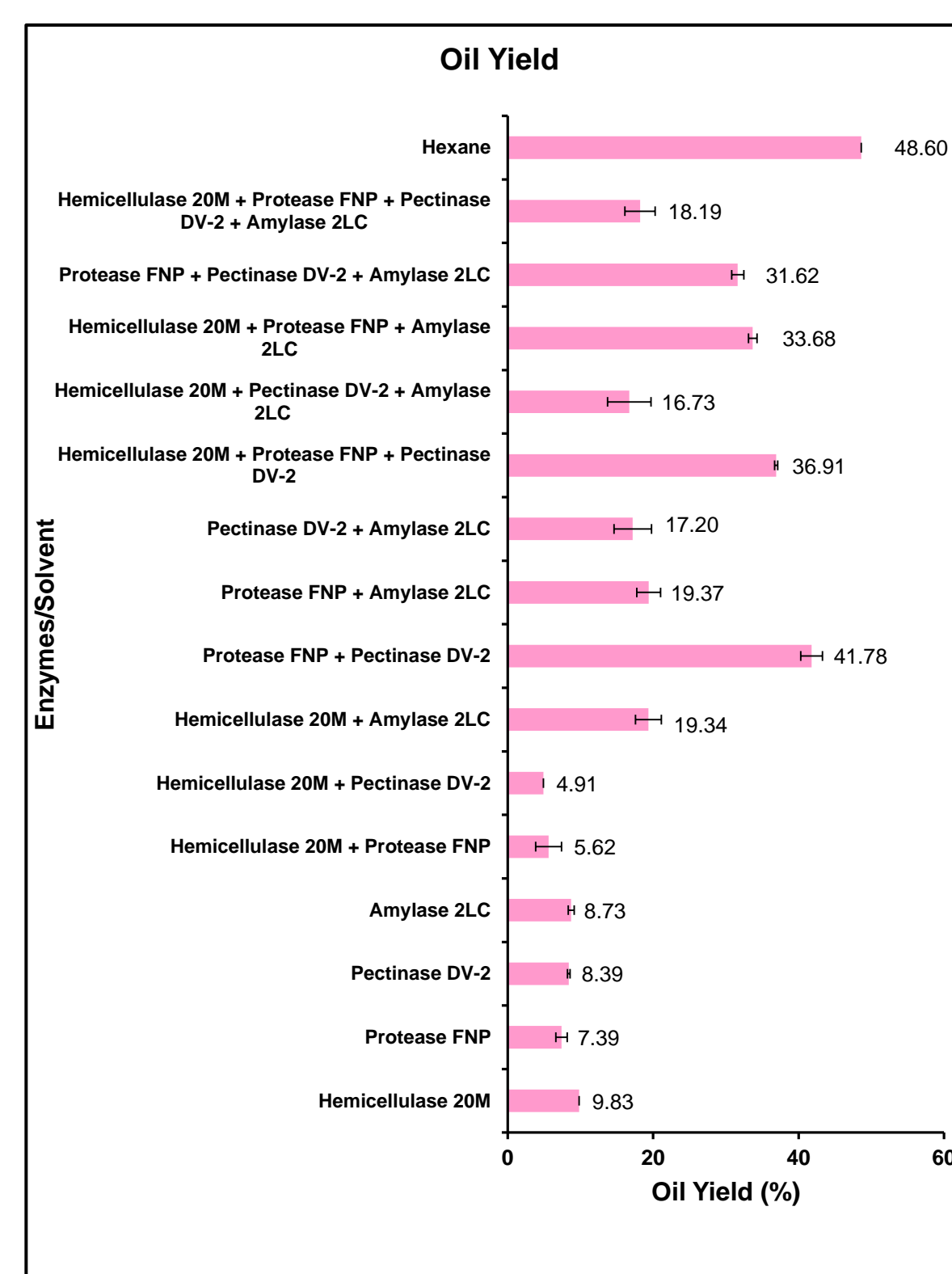


Fig. 1: Effect of enzyme/solvent extraction on the recovery of NSO

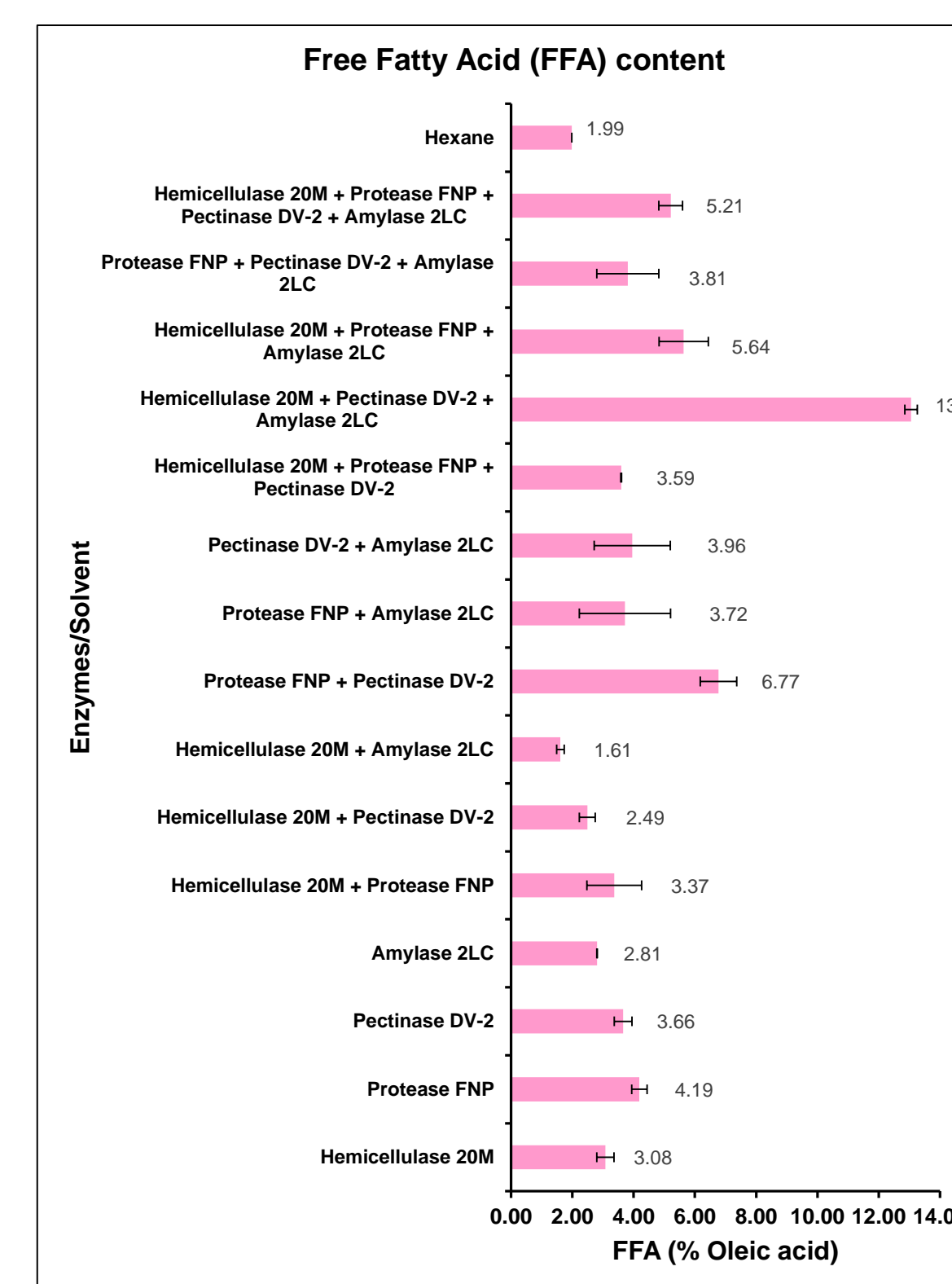


Fig. 3: Effect of enzyme/solvent extraction on FFA content of NSO

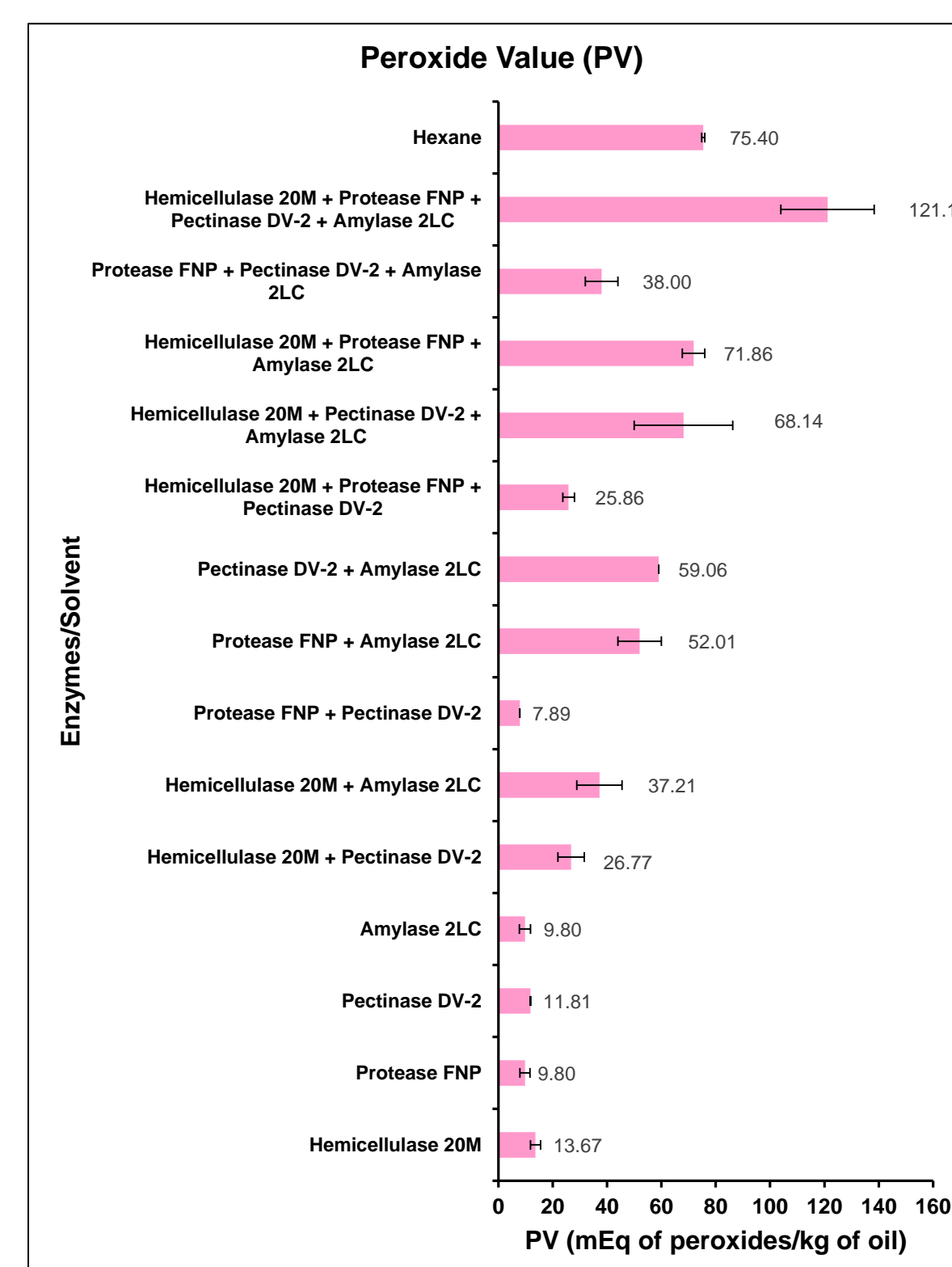


Fig. 3: Effect of enzyme/solvent extraction on PV of NSO

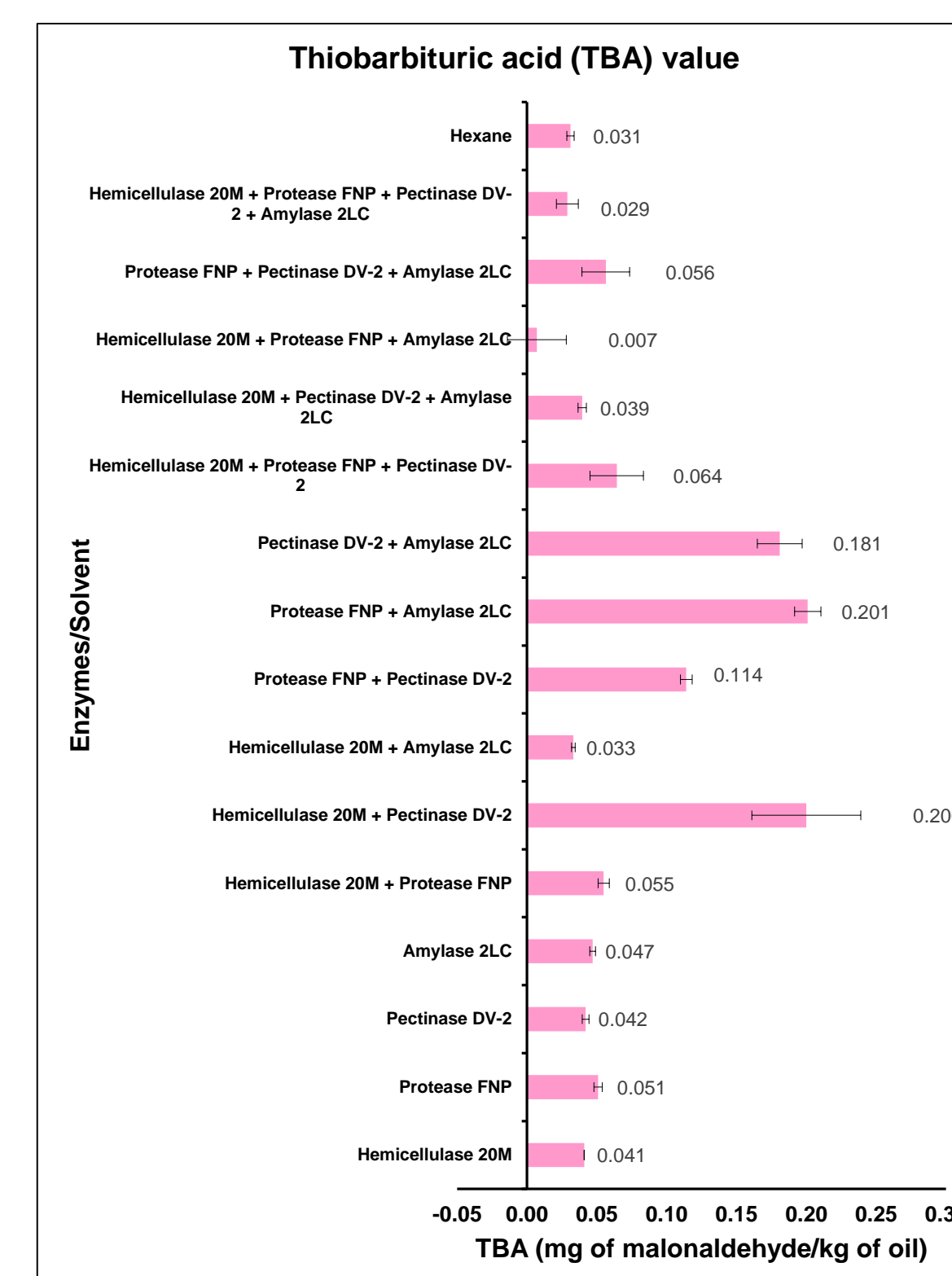


Fig. 4: Effect of enzyme/solvent extraction on TBA value of NSO

## Conclusion

Enzymes, when used individually, resulted in low oil recoveries, but met the acceptable limits of an edible oil.

In spite of the comparably poor quality indices, extracting Njangsa seed oil with a combination of two or three enzymes, increased the overall oil yield, and these yields were comparable to solvent extraction.

Compared to solvent extraction, enzymes are safer to use, non-hazardous and possess little to no environmental, toxicological or health threats.

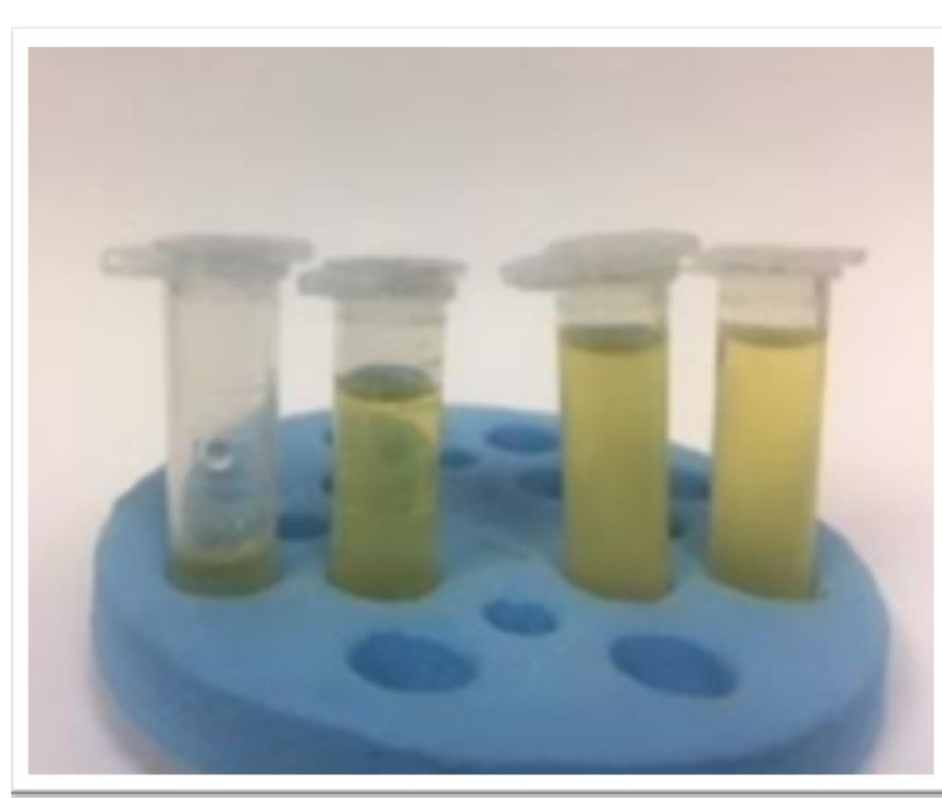
This study has elucidated both individual effect of hemicellulase, amylase, pectinase and protease and a combination of these enzymes, as well as solvent on oil extraction and quality parameters.

## Materials & Methods

Njangsa seeds were obtained from a local African store in Smyrna, DE.

**Oil Extraction:** Njangsa seed was ground using a hand-blender to particle size  $\leq 0.4$  mm. Forty grams of ground Njangsa seed and 1.5 - 4% of the enzyme was either added individually or in various combinations to pH-adjusted distilled water (1:6 w/v). The mixture was incubated for 24 h in a water bath at 55-70°C (depending on the optimum temperature of the enzyme) with agitation (115 rpm). The slurry was centrifuged at 3000 rpm (20°C, 20 min) and transferred to a separatory funnel. The upper oil layer was centrifuged again and oil obtained was stored in amber glass vials until further analysis. During hexane extraction the ground seed was treated with hexane at 1:8 (w/v) for 1 h at 23°C. Hexane was evaporated under vacuum.

**Quality Indices:** Free fatty acid (FFA) content, peroxide value (PV) and thiobarbituric acid (TBA) value of the oil were measured according to Ca 5a-40, Cd 8-53 and Cd 19-90, respectively of the Official Methods of the American Oil Chemists' Society (AOCS) [6], to assess the effect of solvent and enzyme(s) extraction on oil quality.



Njangsa seed oil (NSO)

- The stronger effect of a combination of protease FNP and pectinase DV-2 on NSO recovery can be attributed to the breakdown of protein network and protein-based membrane (oleosins) that surround the lipid bodies, and cell wall of the seed, respectively, liberating the oil (Fig. 1).
- The lower oil yields (Fig. 1) when the enzymes were used individually were plausibly due to limited degradation of the cell wall and the membrane encasing the lipid bodies.
- Enzymes used individually and combinations of two involving hemicellulase during oil extraction resulted in FFA content within the limits for edible oil of 0 - 3.3% (Fig. 2).
- Enzymes used individually produced PVs within the acceptable limits for edible oil of 0 - 15 mEq/kg oil (Fig. 3).
- TBA values of 0.03 - 0.2 mg of malonaldehyde/kg oil indicate minimal secondary oxidation of NSO and the absence of any off-flavors (Fig. 4).

## References

- Ezekwe et al. (2014). Nutritive composition of omega-3 fatty acids-rich *Ricinodendron heudelottii* and its potential for nutrition. *Int. J. Nutr. Metab.* 6(6): 56-62.
- Tchiégang et al. (1997). Amandes de *Ricinodendron heudelottii* (Baill): Matière première potentielle pour les industries Agroalimentaires tropicales. *J. Food Eng.* 32: 1-10.
- Ezekwe et al. (1995). Aqueous processing of sunflower kernels with enzymatic technology. *Food Chem.* 53: 427-434.
- Latif and Anwar (2008). Quality assessment of *Moringa concanensis* seed oil extracted through solvent and aqueous enzymatic techniques. *Grasas Aceites.* 59: 67-73.
- Dobozi et al. (1988). Enhancement of mustard oil yield by cellulolytic pretreatment. *Appl. Microbial Biotechnol.* 29: 39-43
- AOCS (2004). Official Methods and Recommended Practices. Method Additions and Revisions.

## Acknowledgments

This study was made possible by the National Science Foundation EPSCoR Grant No. IIA-1301765, the State of Delaware, and enzymes donation from Enzyme Development Corporation (New York City, NY).