UNIVERSITI PUTRA MALAYSIA

EFFECTS OF EXTRACTION CONDITIONS AND ALTERED SOLVENT ENVIRONMENT ON THE FUNCTIONALITY OF THE FOOD MACROMOLECULES OF PIGEONPEA (Cajanus cajan L.) AND COWPEA (Vigna unguiculata L.)

MWANJALA ALFRED MWASARU

FSMB 1996 7

EFFECTS OF EXTRACTION CONDITIONS AND ALTERED SOLVENT ENVIRONMENT ON THE FUNCTIONALITY OF THE FOOD MACROMOLECULES OF PIGEONPEA (*Cajanus cajan* L.) AND COWPEA (*Vigna unguiculata* L.)

BY

MWANJALA ALFRED MWASARU

Dissertation Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia.

October, 1996

This dissertation is dedicated to my late father Mr. Jacob Mwasaru Kambu who was called to be with the Lord in August 1990.

ACKNOWLEDGEMENTS

The author is indebted to a number of individuals and institutions without whose intervention this endeavour would have been rendered infeasible. I would first like to express my unqualified gratitude to Dr. Sharifah Kharidah Syed Mohammed for her guidance and assistance in the preparation and execution of the project and to Dr. Jamilah Bakar, Assoc. Prof. Dr. Yaakob Che Man and Dr. Nasir Azudin for conscientiously serving as members of my supervisory committee. I would also like to thank my wife Mercy and our children Margaret Saru, Josephine Mkachia and Jacob Mwasaru for their patience and understanding during my absence from home.

The enabling environment in terms of financial support from Japan International Cooperation Agency (JICA) and Jomo Kenyatta University of Agriculture and Technology (JKUAT) is gratefully acknowledged. Special thanks to Mr H. Koaze, JICA Kenya Office for his assistance for arranging the shipment of the legumes to Malaysia and the Country Representative and staff of JICA Malaysia Office for their cooperation.

Finally I would like to register massive appreciation and a mega-style cheer to all my friends and colleagues in the Biochemistry and Microbiology Laboratories, Department of Food Science for their friendship, assistance and encouragement in the course of the project. Special thanks are also due to the administrative staff of the Faculty of Food Science and Biotechnology for handling the procurement of the requisite materials and reagents, Assoc. Prof. Dr Asbi Ali for literature material, Mr Razali Mustaffa for helping out with SAS, Mrs Faridah

Hussin and Mr Amin Ismail for helping with the Bahasa Malaysia translation, Dr. C.C. Seow of the Food Technology Division, Universiti Sains Malaysia for assistance with DSC analysis and the Dean and staff of the Graduate School, Universiti Pertanian Malaysia for their cooperation whenever I needed assistance.

Above all, praise to God The Almighty for watching over me all the time.

TABLE OF CONTENTS

LIST OF TABLES

Figure

LIST OF FIGURES

LIST OF PLATES

Abstract of dissertation submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

EFFECTS OF EXTRACTION CONDITIONS AND ALTERED SOLVENT ENVIRONMENT ON THE FUNCTIONALITY OF THE FOOD MACROMOLECULES OF PIGEONPEA (*Cajanus cajan* L.) AND COWPEA (Vigna *unguiculata* L.)

By

MWANJALA ALFRED MWASARU

OCTOBER 1996

Chairman: Dr Sharifah Kharidah Syed Muhammad.
Faculty: Food Science and Biotechnology.

The extractability of the proteins from pigeonpea and cowpea seeds was influenced by extraction techniques and conditions; the micellization technique extracted 40.2 and 36.7% of the total seed protein, respectively. The isoelectric precipitation technique extracted 35.1 to 58.1% and 36.4 to 53.5% of the seed protein, respectively at extraction pH 8.5–12.5. The purity of the isolates was in the range of 78.1 to 92.9%, and for the isoelectric isolates, it was inversely correlated to extraction pH. The isolates were free of the antinutrients associated with the legume seeds, however, extraction technique had no effect on the subunit composition and electrical mobility.

An inverse relationship was evident between the lightness colour value (L) of the isoelectric isolates and the pH of extraction for pigeonpea (R^2=0.76) and cowpea (R^2=0.77), and the micelle isolates were lighter in colour than the isoelectric isolates. All the isolates presented typical solubility profiles, however, significant quantitative differences were observed; the micelle isolates exhibited superior solubility characteristics to the isoelectric isolates and for the latter an inverse relationship

between solubility and extraction pH was apparent. The reduction in solubility with increasing extraction pH was attributed to the increased degree of denaturation as determined by differential scanning calorimetry. Micelle isolates exhibited better emulsifying, whipping and gelation functionalities than the isoelectrically precipitated isolates. Regression analysis indicated that the emulsion stability and whipping properties of the isolates were best predicted by solubility and exposed hydrophobicity, and the gelation properties by ln(solubility x exposed hydrophobicity). All the isolates, except those extracted at pH 12.5 exhibited emulsifying, whipping, and gelation functionalities that were similar or superior to those of a commercial soy isolate. Altering the solvent environment in terms of pH and NaCl concentration generally resulted in an improvement in the emulsifying and whipping properties of the pigeonpea isolate but the reverse was observed for cowpea isolate. All combinations of pH (2–8) and salt concentration (0.1–0.5M) improved the emulsifying activity of pigeonpea isolate but had no effect on cowpea isolate. They also improved the emulsion stability for both isolates except at pH 2 and low salt, and at pH 8 and high salt concentrations. Improved foam expansion but reduced foam stability for both isolates were observed under all combinations of pH and salt concentation. Increasing the pH and salt concentration up to 0.3M impaired the gelation properties of the isolates.

Pigeonpea and cowpea starches presented mixed size and shape granule population with diameters in the 6–36μm range, cowpea starch was higher in total amylose (31.0%) than pigeonpea starch (29.6%), the latter exhibited higher swelling power and lower solubility than the former, and both exhibited the restricted Brabender pasting pattern C. Cowpea starch, however, exhibited higher paste consistencies at characteristic reference points than pigeonpea starch. Incorporation of surfactants increased the initial pasting temperatures of the starches and the increase paralleled the hydrophile–lipophile balance (HLB) of the surfactants, reduced the paste consistency of pigeonpea starch, reduced the strength, elastic modulus and stickiness of the starch gels. Surfactants of HLB 1.8–11.0 significantly increased the freeze–thaw stability of cowpea starch gels but decreased it for pigeonpea starch. Addition of corn oil and protein increased the initial pasting temperature of pigeonpea starch as did pH 2 and 10–40% sucrose for cowpea starch. Corn oil increased the cold paste consistency of cowpea starch, and pH 2 caused hydrolysis but the starches were stable to acid conditions at pH 4.

It was concluded that extraction technique and conditions had significant influence on protein extractability, the emulsification, whipping, and gelation functionalities of pigeonpea and cowpea protein isolates and that these functional properties can be manipulated by altering the solvent environment. Surfactant incorporation and chemical additives had significant effects on the rheological properties and related phenomena of legume starch gels.

Abstrak dissertasi yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Doktor Falsafah

KESAN KEADAAN PENGEKSTRAKAN DAN PERUBAHAN PERSEKITARAN PELARUT TERHADAP FUNGSI MAKROMOLEKUL PIGEONPEA (*Cajanus cajan* L.) DAN COWPEA (VIgna unguiculata L.)

Oleh

MWANJALA ALFRED MWASARU

OKTOBER 1996

Pengerusi: Dr Sharifah Kharidah Syed Muhammad.
Fakulti: Sains Makanan dan Bioteknologi.

Kebolehan mengekstrak protin daripada biji pigeonpea dan cowpea adalah dipengaruhi oleh teknik dan keadaan pengekstrakan; teknik miselisasi dapat mengekstrak sebanyak 40.2 dan 36.7% daripada jumlah keseluruhan protin daripada biji masing–masing. Teknik pemendakan isoelektrik dapat menghasilkan 35.1 hingga 58.1% dan 36.4 hingga 53.5% masing–masing pada pH pengekstrakan 8.5–12.5. Ketulenan isolat adalah di antara 78.1 hingga 92.9%, manakala isolat isoelektrik, ketulenannya berkadar sonsang dengan pH pengekstrakan. Isolat–isolat ini adalah bebas dari anti–nutrien yang berhubungkait dengan biji kekacang, walaubagaimanapun teknik pengekstrakan tidak mempunyai sebarang kesan ke atas komposisi subunit dan pergerakan elektrik.

Perkaitan sonsang adalah ketara di antara nilai kecerahan (L) bagi isolat isoelektrik dan pH pengekstrakan pigeonpea (R^2=0.76) dan cowpea (R^2=0.77), dan isolat misel mempunyai warna yang lebih cerah daripada isolat isoelektrik. Semua isolat menunjukkan profil kelarutan yang tipikal, walaubagaimanapun, perbezaan kuantitatif yang ketara dapat dilihat di antara isolat; isolat misel menunjukkan ciri kelarutan yang lebih tinggi dibandingkan dengan isolat isoelektrik dan bagi isolat

isoelektrik ianya berkadar sonsang dengan kelarutan dan pH pengekstrakan. Penurunan kelarutan dengan peningkatan pH pengekstrakan adalah disebabkan oleh penambahan kadar nyahasli seperti ditentukan dari `differential scanning' kalorimetri. Isolat misel menunjukkan sifat pengemulsian, pembusaan dan gelatinasi yang lebih baik daripada isolat yang dimendakkan secara isoelektrik. Analisis regrasi menunjukkan bahawa ciri kestabilan pengemulsian dan pembusaan isolat boleh dianggarkan dengan baik menggunakan kelarutan dan pendedahan hidrofobik dan ciri pengelatinasi melalui ln(kelarutan x pendedahan hidrofobik). Kesemua isolat kecuali yang diekstrak pada pH 12.5 menunjukkan kefungsian pengemulsian, pembusaan dan pengelatinan yang sama atau lebih baik berbanding dengan isolat soya komersil. Perubahan persekitaran pelarut seperti pH dan kepekatan NaCl secara amnya memberikan kesan positif terhadap ciri pengemulsian dan pembusaan isolat daripada pigeonpea tetapi sebaliknya untuk ciri yang sama bagi isolat daripada cowpea. Penggabungan semua pH (2–8) dan kepekatan garam (0.1–0.5M) meningkatkan aktiviti emulsi isolat pigeonpea tetapi tidak mempunyai kesan ke atas isolat cowpea. Ianya juga meningkatkan stabiliti emulsi untuk keduanya, kecuali pada pH 2 dan kepekatan garam rendah, dan pada pH 8 dan kepekatan garam tinggi. Peningkatan pengembangan busa tetapi pengurangan kestabilan busa bagi kedua–dua isolat diperolehi dibawah semua gabungan pH dan kepekatan garam. Peningkatan pH dan kepekatan garam sehingga 0.3M memberi kesan negatif terhadap ciri pengelatinan isolat tersebut.

Kanji pigeonpea dan cowpea menunjukkan populasi saiz dan rupabentuk granul yang berbagai dengan diameter dalam julat 6–36μm, kanji cowpea mempunyai kandungan amilos yang lebih tinggi (31.0%) berbanding dengan kanji pigeonpea (29.6%) yang menunjukkan daya pengembangan yang lebih tinggi dan kelarutan yang lebih rendah berbanding dengan kanji cowpea, dan keduanya menunjukkan ciri pengelatinan Brabender bentuk C. Walaubagaimanapun, kanji cowpea menunjukkan konsistensi gel yang lebih tinggi pada titik rujukan berbanding dengan kanji pigeonpea. Penambahan surfaktan meningkan suhu permulaan pengelatinan bagi semua kanji dan peningkatan adalah selaras dengan imbangan hidrofil–lipopil (HLB) bagi surfaktan, mengurangkan konsistensi gel kanji pigeonpea, mengurangkan kekuatan, kekenyalan modulus dan kelekitan gel kanji. Surfaktan HLB 1.8–11.0 meningkatkan dengan ketara kestabilan sejukbeku gel kanji cowpea tetapi mengurangkannya bagi kanji pigeonpea. Penambahan minyak jagung dan protin meningkatkan suhu awal pengelatinan kanji pigeonpea seperti juga pada pH 2 dan 10–40% kandungan sukrosa untuk kanji cowpea. Minyak jagung meningkatkan konsistensi gel pada masa penyejukan bagi kanji cowpea, dan pH 2 menyebabkan hidrolisis tetapi kanji tersebut adalah stabil kepada keadaan acid pada pH 4.

Adalah disimpulkan bahawa, teknik dan keadaan pengekstrakan mempunyai pengaruh yang ketara terhadap kebolehan mengekstrak protin, pengemulsian, pembusaan dan sifat pengelatinan isolat protin dari pigeonpea dan cowpea, dan kesemua ciri kefungsian boleh dimanipulasikan dengan mengubah persekitaran pelarut. Penambahan surfaktan dan aditif memberi kesan yang ketara terhadap sifat reologi dan fenomena yang berkaitan dengan gel kanji kekacang.

CHAPTER I

GENERAL INTRODUCTION

Food legumes and cereals provide the bulk of calories and proteins for many people inhabiting Africa, Asia, Latin America and the Near East. There is little doubt that the importance of cereals and legumes in the diet, particularly of the poorest, will increase rather than decline. Virtually all cereal proteins are deficient in the essential amino acid lysine. The nutritional quality of cereal grains can be improved by the addition of grain legumes which are comparatively rich in lysine. The comparatively low levels of methionine and cystine in legumes is offset by the higher proportions of these amino acids in most cereal grains. A combination of cereal and legume proteins, therefore, comes very close to providing an ideal source of dietary protein for human nutrition.

Food legumes such as pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) are crops that are well adapted to the arid and semi-arid zones of Kenya because of their drought tolerance. Considerable research has been done in breeding varieties most suited to the different ecological zones found in the arid and semi-arid regions in Kenya. There is, therefore, a need to exploit the potential of these legumes at the household and industrial level in order to enhance their role in the country's food system. This objective can be achieved through postharvest processing of the legume grains into convenient forms that can induce sustained demand by consumers particularly those domiciled in the urban areas.