



**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**

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(*Vigna unguiculata* L.)

BY

MWANJALA ALFRED MWASARU

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



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## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS .....	iii
LIST OF TABLES .....	xii
LIST OF FIGURES .....	xv
LIST OF PLATES .....	xvii
ABSTRACT .....	xviii
ABSTRAK .....	xxi
 <b>CHAPTER</b>	
I        GENERAL INTRODUCTION .....	1
II        LITERATURE REVIEW .....	5
Food Legumes .....	5
Pigeonpea .....	7
Origin, Classification and Importance .....	7
Chemical Composition .....	9
Food Macromolecules.....	11
Cowpea .....	12
Origin, Classification and Importance .....	12
Chemical Composition .....	13
Food Macromolecules .....	15
Legume Food Macromolecules Concentration Methods .....	16
Protein Concentration .....	16
Starch Recuperation .....	19

	Physicochemical and Functional Properties of Legume Food Macromolecules.....	21
	Protein .....	21
	Starch .....	25
	Behaviour of Food Macromolecules in Altered Solvent Environment .....	29
	Starch .....	29
	Protein .....	34
III	EXTRACTION, PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERISATION OF PROTEIN FROM PIGEONPEA AND COWPEA .....	37
	Introduction .....	37
	Materials and Methods .....	39
	Materials .....	39
	Proximate Analyses .....	39
	Starch .....	40
	Trypsin Inhibitor Activity .....	41
	Oligosaccharides .....	42
	Phytate .....	43
	Extraction of Legume Proteins .....	44
	Isoelectric Precipitation Technique .....	44
	Micellization Technique .....	45
	Physicochemical and Functional Characterisation of Legume Proteins .....	45
	Chemical Composition .....	45
	Amino Acid Profiles .....	46
	Protein Hydrophobicity .....	46



Sodium dodecyl sulfate-polyacrylamide gel electrophoresis .....	47
Isoelectric Focusing .....	48
Differential Scanning Calorimetry .....	48
Colour .....	48
Nitrogen Solubility Index .....	49
Water and Oil Absorption Indices .....	49
Emulsifying Properties .....	50
Whipping Properties .....	50
Gelation Properties .....	51
Statistical Analysis .....	51
Results and Discussion .....	51
Physicochemical Properties of Pigeonpea and Cowpea Seeds .....	51
Chemical Composition .....	51
Antinutritional Factors Content .....	54
Amino Acid Composition .....	56
Extractability of Protein from Legume Seeds .....	58
Physicochemical Properties of Pigeonpea and Cowpea Isolates .....	63
Chemical Composition .....	63
Amino Acid Composition .....	67
Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Isoelectric Focusing Characteristics . .....	70
Functional Properties of Pigeonpea and Cowpea Protein Isolates .....	73
Colour .....	73





	Nitrogen Solubility and Thermal Properties .....	76
	Water Absorption Capacity .....	83
	Oil Absorption Capacity .....	87
	Emulsifying Properties .....	89
	Whipping Properties .....	97
	Gelation Properties .....	103
<b>IV</b>	<b>EXTRACTION, PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERISATION OF STARCH FROM PIGEONPEA AND COWPEA .....</b>	<b>109</b>
	Introduction .....	109
	Materials and Methods .....	110
	Materials .....	110
	Starch Extraction .....	110
	Physicochemical and Functional Characterisation ...	111
	Chemical Composition .....	111
	Bulk Density .....	111
	Morphological Characterisation .....	111
	Colour .....	112
	Total Amylose .....	112
	Swelling Power and Solubility .....	113
	Differential Scanning Calorimetry .....	114
	Pasting Profiles .....	114
	Water and Oil Absorption Capacities .....	115
	Statistical Analysis .....	115
	Results and Discussion .....	115



	Starch Extractability .....	115
	Physicochemical and Functional Properties .....	118
	Chemical Composition .....	118
	Morphological Characteristics .....	121
	Bulk Density and Colour .....	123
	Swelling Power, Solubility, Water and Oil Absorption Capacities .....	125
	Pasting and Gelatinization Properties .....	129
<b>V</b>	<b>EFFECTS OF ALTERED SOLVENT ENVIRONMENT ON THE VISCOELASTIC PROPERTIES OF PIGEONPEA AND COWPEA STARCH GELS .....</b>	<b>136</b>
	Introduction .....	136
	Materials and Methods .....	137
	Materials .....	137
	Effects of Surfactant Hydrophile–Lipophile Balance (HLB) on the Pasting Characteristics .....	137
	Effects of Surfactant Hydrophile–Lipophile Balance on the Load–Penetration Responses .....	138
	Effects of Surfactant Hydrophile–Lipophile Balance on the Freeze–Thaw Stability .....	139
	Effects of pH, and Additives on the Pasting Properties .....	139
	Statistical Analysis .....	140
	Results and Discussion .....	140
	Effects of Surfactant Hydrophile–Lipophile Balance on the Pasting Characteristics .....	140
	Effects of Surfactant Hydrophile–Lipophile Balance on the Load–Penetration Responses .....	151



	Effects of Surfactant Hydrophile–Lipophile Balance on the Freeze–Thaw Stability .....	159
	Effects of pH, and Additives on the Pasting Properties .....	165
VI	<b>EFFECTS OF ALTERED SOLVENT ENVIRONMENT ON THE FUNCTIONALITY OF PIGEONPEA AND COWPEA PROTEIN ISOLATES</b>	173
	Introduction .....	173
	Materials and Methods .....	174
	Materials .....	174
	Effect of Sodium Chloride Concentration on the pH–solubility .....	174
	Effects of pH and Sodium Chloride Concentration on the Emulsifying and Whipping Properties .....	174
	Effect of Heat Denaturation on the Emulsifying and Whipping Properties .....	175
	Effects of pH and Sodium Chloride Concentration on the Gelation Properties .....	175
	Fractionation of Legume Protein Isolates .....	175
	Statistical Analysis .....	176
	Results and Discussion .....	176
	Effect of Sodium Chloride Concentration on the pH–solubility .....	176
	Effect Sodium Chloride Concentration on the Emulsifying Properties .....	184
	Effect of pH on the Emulsifying Properties .....	187
	Effects of Sodium Chloride Concentration and pH on the Emulsifying Properties .....	191
	Effect of Sodium Chloride Concentration on the Whipping Properties .....	198



	Effect of pH on the Whipping Properties .....	201
	Effects of Sodium Chloride Concentration and pH on the Whipping Properties .....	206
	Effect of Heat Denaturation on the Emulsifying and Whipping Properties .....	213
	Effects of pH and Sodium Chloride Concentration on the Gelation Properties .....	215
VII	GENERAL DISCUSSION .....	220
VIII	SUMMARY AND CONCLUSIONS .....	232
	REFERENCES CITED .....	241
	APPENDICES .....	261
	VITA .....	270



## LIST OF TABLES

Table		
1	Chemical composition of pigeonpea and cowpea seeds .....	52
2	Oligosaccharide, trypsin inhibitor activity and phytate content of pigeonpea and cowpea seeds .....	55
3	Amino acid composition of pigeonpea and cowpea seeds according to the nature of side chains .....	57
4	Extractability of protein from pigeonpea and cowpea seeds by micellization and isoelectric precipitation techniques .....	59
5	Chemical composition of pigeonpea and cowpea protein isolates .....	64
6	Amino acid composition of pigeonpea and cowpea protein isolates according to the nature of side chains.....	69
7	Isoelectric points of pigeonpea and cowpea protein isolates .....	72
8	Hunter colour of soybean, pigeonpea and cowpea protein isolates .....	74
9	Differential scanning calorimetry thermogram values of pigeonpea and cowpea protein isolates ....	81
10	Water absorption capacities of pigeonpea and cowpea protein isolates .....	84
11	Oil absorption capacities of pigeonpea and cowpea protein isolates .....	88
12	Backward stepwise multiple regression models for predicting emulsion stability (ES), foam expansion (FE) and foam stability (FS) of pigeonpea and cowpea protein isolates using exposed (Se) hydrophobicity and solubility (So) parameters .....	96
13	Least gelation concentration of pigeonpea and cowpea protein isolates .....	104



14	Backward stepwise multiple regression models for predicting least gelation concentration (LGC) of pigeonpea and cowpea protein isolates using exposed hydrophobicity (Se) and solubility (So) parameters .....	107
15	Extractability, purity and chemical composition of pigeonpea and cowpea starch fractions .....	116
16	Bulk density and Hunter (L, a, b) colour measurements of pigeonpea and cowpea starches ...	124
17	Swelling power, solubility, water and oil absorption capacities of pigeonpea and cowpea starches .....	126
18	Brabender pasting and DSC gelatinization properties of pigeonpea and cowpea starches .....	130
19	Effect of surfactant hydrophile–lipophile balance value on the load–penetration responses of cowpea and pigeonpea starch gels .....	152
20	Effects of pH, and additives on the Brabender pasting properties of pigeonpea starch pastes at characteristic reference points .....	166
21	Effects of pH, and additives on the Brabender pasting properties of cowpea starch pastes at characteristic reference points .....	167
22	Fraction composition of pigeonpea and cowpea protein isolates .....	183
23	Effect of sodium chloride concentration on the emulsifying properties of pigeonpea and cowpea protein isolates .....	185
24	Effects of sodium chloride concentration and pH on the emulsifying activities of pigeonpea and cowpea protein isolates .....	192
25	Effect of sodium chloride concentration on the whipping properties of pigeonpea and cowpea protein isolates .....	199
26	Effect of heat denaturation on the emulsifying and whipping properties of pigeonpea and cowpea protein isolates .....	214

27	Effects of pH, and sodium chloride concentration on the least gelation concentration of pigeonpea and cowpea protein isolates .....	216
28	Effects of pH and 0.5M NaCl on the least gelation concentration of pigeonpea and cowpea protein isolates .....	218



**Figure**

**LIST OF FIGURES**

1	Effect of alkaline extraction pH on the extractability of protein from pigeonpea and cowpea seeds using 0.1N NaOH .....	61
2	Nitrogen solubility profiles of pigeonpea micelle (MP) and isoelectric (IP) protein isolates .....	77
3	Nitrogen solubility profiles of cowpea micelle (MP) and isoelectric (IP) protein isolates .....	78
4	Emulsifying properties of soy protein (SP), pigeonpea micelle protein (MP), and isoelectric (IP) protein isolates .....	90
5	Emulsifying properties of soy protein (SP), cowpea micelle protein (MP), and isoelectric (IP) protein isolates .....	90
6	Whipping properties of soy protein (SP), pigeonpea micelle protein (MP) and isoelectric protein (IP) isolates .....	98
7	Whipping properties of soy protein (SP), cowpea micelle protein (MP) and isoelectric protein (IP) isolates .....	99
8	Effect of surfactant HLB value on the initial pasting temperature of pigeonpea and cowpea starch pastes	141
9	Effect of surfactant HLB value on the paste consistency at characteristic reference points of pigeonpea starch .....	145
10	Effect of surfactant HLB value on the paste consistency at characteristic reference points of cowpea starch .....	146
11	Effect of surfactant HLB on the freeze–thaw stability of cowpea starch gels .....	160
12	Effect of surfactant HLB on the freeze–thaw stability of pigeonpea starch gels .....	161
13	Effects of sodium chloride concentration on the pH–solubility of pigeonpea protein isolate .....	177





14	Effects of sodium chloride concentration on the pH-solubility of cowpea protein isolate .....	178
15	Effect of pH on the emulsifying properties of pigeonpea protein isolate .....	188
16	Effect of pH on the emulsifying properties of cowpea protein isolate .....	189
17	Effects of sodium chloride concentration and pH on the stability of pigeonpea protein-stabilized emulsion .....	194
18	Effects of sodium chloride concentration and pH on the stability of cowpea protein-stabilized emulsion .....	195
19	Effect of pH on the whipping properties of pigeonpea protein isolate .....	202
20	Effect of pH on the whipping properties of cowpea protein isolate .....	203
21	Effects of sodium chloride and pH on the expansion of pigeonpea protein-stabilized foam .....	207
22	Effects of sodium chloride and pH on the expansion cowpea protein-stabilized foam .....	208
23	Effects of pH and sodium chloride concentration on the stability of pigeonpea protein-stabilized foam .....	210
24	Effects of pH and sodium chloride concentration on the stability of cowpea protein-stabilized foam ..	211



## LIST OF PLATES

Plate		
1	Photograph of pigeonpea seeds .....	10
2	Photograph of cowpea seeds .....	14
3	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis profiles of pigeonpea and cowpea protein isolates .....	71
4	Scanning electron micrographs of pigeonpea (A) and cowpea starches (B) .....	122

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**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  $\ln(\text{solubility} \times \text{exposed hydrophobicity})$ . 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 concentration. 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 6–36 $\mu\text{m}$  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 disertasi 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, ketulennanya 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 songsang 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 regresi menunjukkan bahawa ciri kestabilan pengemulsian dan pembusaan isolat boleh dianggarkan dengan baik menggunakan kelarutan dan pendedahan hidrofobik dan ciri pengelatinasi melalui  $\ln(\text{kelarutan} \times \text{ppededahan hidrofobik})$ . Kesemua isolat kecuali yang diekstrak pada pH 12.5 menunjukkan kefungsiian 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 6–36 $\mu\text{m}$ , 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 meningkatkan suhu permulaan pengelatinan bagi semua kanji dan peningkatan adalah selaras denganimbangan hidrofil-lipofil (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 kefungsiannya boleh dimanipulasikan dengan mengubah persekitaran pelarut. Penambahan surfaktan dan aditif memberi kesan yang ketara terhadap sifat reologi dan fenomena yang berkaitan dengan gel kanji kacang.



## 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.

