Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours

By

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DEDICATION

This thesis is dedicated to the memory of my beloved mother, late Dorothy Irene Mvula
and
To the Lord my God, my ever present help in times of need;
   I lift my eyes to the hills
where does my help come from
   my help comes from the Lord
maker of heaven and earth.
DECLARATION

I declare that the thesis which I hereby submit for the degree PhD (Food Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another university or institution of higher education.

Agnes Mbachi Mwangwela
2006
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ABSTRACT

Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours

By

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Promoter: Professor Amanda Minnaar
Co-promoter: Professor Ralph D. Waniska (Texas A & M University)
Department: Food Science
Degree: PhD (Food Science)

Cowpea (Vigna unguiculata L. Walp) is an important source of protein in some parts of sub Saharan Africa. In southern Africa, it is mainly boiled into a stew, and long cooking time is a concern. Micronisation of preconditioned seeds has been used to reduce the cooking time of other dry legume seeds such as lentils. Hence micronisation (moisture conditioning and infrared heating) presents an opportunity for processing cowpeas to alleviate long cooking time and provide a convenient product as well as diversify cowpea products. In addition, potential exists for using flour milled from micronised (moisture conditioned and infrared heated) seeds in food systems. However, variations in raw material physicochemical properties (seeds) and micronisation temperature would affect the efficacy of the process to produce products with desired properties. Mild (130 and 153 °C) and severe (170 °C) final surface temperatures were used to determine the extent of micronisation-induced changes in cowpea structure and physicochemical properties and functionality of the resultant flours.

Two cowpea varieties (Bechuana white and Var. 462; 41 % moisture) micronised to 153 °C were used to study the effect of micronisation on physicochemical and structural properties of cowpea seeds. Bechuana white (41 % moisture) micronised to three
temperatures (130, 153 and 170 °C) was used to study the micronisation temperature effect on physicochemical properties of cowpeas and functional properties of resultant flours. Scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) were used to study seed structure, while light microscopy was used for the flour. Gel permeation high performance liquid chromatography (GP-HPLC), differential scanning calorimetry (DSC), and a rapid visco-analyser (RVA) were used to study starch-related properties, while fluorescence spectroscopy and electrophoresis (SDS-PAGE) were used to study physicochemical properties of isolated proteins. These physicochemical and structural properties were determined to aid in explaining the possible micronisation-induced changes in cooking characteristics of seeds and functional properties of the resultant flours.

Micronisation (41 % moisture, 153 °C) reduced cooking time (Bechuana white > Var. 462) and increased splitting (Var. 462 >Bechuana white) of the cowpeas during subsequent cooking. The micronised (41 % moisture, 153 °C) seeds were relatively softer than the unmicronised samples following subsequent cooking. The mild temperatures (130, 153 °C) were more effective in reducing cooking time than the higher temperature (170 °C). Micronisation (41 % moisture, 153 and 170 °C) caused physical fissuring of the seed coat, cotyledon, and parenchyma cell wall and reduced the bulk density of treated seeds. These changes in the physical structure improved the hydration rate of the seeds during cooking. There is a possibility that micronisation (41 % moisture, 130, 153, 170 °C) also caused the degradation of pectic substances of the middle lamella, since shorter cooking time was required for cotyledon parenchyma cells to separate during cooking.

Mild micronisation (41 % moisture, 130 and 153 °C) temperatures caused the disruption of the native starch granular order leading to retrogradation of amylose, while at higher micronisation (41 % moisture, 170 °C) temperatures the retrogradation of amylose was possibly accompanied by endodegradation of starch. Simultaneously, micronisation (41 % moisture, 130 and 170 °C) led to increased surface hydrophobicity and crosslinking of protein, which was more pronounced in M-170 °C samples. SDS-PAGE
indicated that disulphide bonds were formed in micronised (41 % moisture, 130 and 170 °C) samples; while isopeptide bonds, dityrosyl bonds and Maillard derived crosslinks are possibilities especially in the M-170 °C sample. The pronounced crosslinking of protein and possible depolymerisation of starch contributed to hardening of the cotyledon structure, consequently impacting negatively on the effectiveness of the micronisation (41 % moisture, 170°C) treatment in reducing cooking time.

These changes in seed structure and physicochemical properties of starch and protein contributed towards the reduction in cooking time and increased splitting of seeds and modified flour functionality. Cowpea flour foaming capacity was lost following micronisation (41 % moisture, 130 and 170 °C) possibly due to reduced solubility and crosslinking of protein. Micronisation (41 % moisture, 130 and 170 °C) reduced flour gelling and pasting properties while increasing the water absorption capacity, more so in M-170 °C samples than in M-130°C. Hence micronisation to mild temperatures (130 °C) has the potential of producing cowpeas with shorter cooking time, which can also be milled into flour with modified functionality. Thus micronisation of moisture-conditioned cowpeas to mild temperatures would contribute towards increased utilisation of cowpeas as well as improving household nutrition status.
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**Figure 3.3.10** Effect of micronisation temperatures (130 °C, 153 °C and 170 °C) on cowpea flour from micronised (41 % moisture, infrared heating) seeds stained for damaged starch with
Congo red: a = M-130 °C, b = M-153 °C and c = M-170 °C, Bar ~ 50 nm.  

**Figure 3.3.11** Effect of micronisation temperatures on cowpea flour from micronised (41 % moisture, 130 °C and 170°C) seeds during sequential enzyme digestion (stained with acid Fuchsin): 1 = No enzyme treatment, 2 = α –amylase, 3 = α –amylase - protease, and 4 = α –amylase-protease- pectin; a = unmicronised, b = M-130 °C and c = M-170 °C, Bar ~50 nm...  

**Figure 3.4.1** Effect of high (170 °C) and low (130 °C) final micronisation temperature for cowpea seeds (41 % moisture) on recovery of protein rich fraction (PRF) from cowpea seeds and its protein content (purity) (Yield has been expressed as percentage of crude protein content of the cowpeas)..........................................................  

**Figure 3.4.2** SDS-gradient gel electrophoresis profiles of cowpea protein extracts from unmicronised (raw) and micronised (41 % moisture, 130 and 170 °C) seeds under non reducing (a) and reducing conditions (b); lane 1 = molecular markers; lane 2 = unmicronised; lane 3 = micronised to 130 °C and lane 4 = micronised to 170 °C; changes in protein band profile are denoted with (i) to (vi)..........................................................  

**Figure 4.1** Proposed mechanisms of the effect of micronisation (41 % moisture, 130, 153 and 170 °C) on cowpea seed structure and the physicochemical properties as related to cooking characteristics of cowpea seeds ....................................................  

**Figure 4.2** Postulated effect of cowpea seed micronisation (41 % moisture, 130 and 170 °C) on physicochemical properties of starch and protein and functional properties of flour milled from the micronised (41 % moisture, 130 and 170 °C) seeds; NSI = Nitrogen solubility index, WAC = Water absorption capacity..........................................................
Figure 4.3  Diagram showing the suggested changes in cowpea structure at the microscopical level that lead to softening of texture during cooking of unmicronised (raw) cowpeas (Based on the mechanism proposed by Liu et al., 1992; Liu et al., 1993a; and Liu et al., 1993b).

Figure 4.4  Diagram showing the suggested changes in cowpea structure that lead to softening of texture during cooking of micronised (41% moisture, 130, 153 and 170 °C) cowpeas.