THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Enhancing Composite Cassava Bread Quality

Effect of cassava pre-treatment and baking improvers

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Front cover: Cassava root, picture taken by L. Tivana.

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Abstract

Due to unfavourable climatic conditions, the production of wheat in Mozambique is not sufficient to satisfy national food industrial needs and substantial quantities must be imported at high cost. Bread is currently produced basically using wheat flour. Therefore, this thesis examined the partial replacement of wheat flour by cassava-maize flours in the Mozambican bread manufacturing context and looked for ways of improving bread quality at high wheat flour substitution.

The effect of cassava pre-treatment (sun drying, roasting and fermentation), cassava level (20, 30 and 40%) and the addition of a baking improver, high methoxyl pectin (HM pectin) at two levels 1 and 3%, were evaluated for the bread quality parameters of loaf volume, crumb firmness and moisture, and crust colour. The loaf volume decreased by 20 to 30% in comparison with the wheat reference bread as a result of added cassava flour that had been pre-treated in different ways. Increasing the cassava level reduced the loaf volume except for bread with roasted cassava, which even increased in volume with the addition of high level HM pectin; the crumb firmness was higher in composite bread with sun-dried and fermented cassava flour compared with wheat bread, although the composite bread with roasted cassava flour with 3% HM pectin had a crumb firmness similar to wheat bread. Bread baked with roasted cassava flour also had a crust colour similar to wheat bread. The roasting pre-treatment of cassava flour along with baking improvers was indicated to have a good potential to improve the baking quality of composite cassava-maize-wheat breads.

Two hydrocolloids, HM pectin and carboxymethylcellulose (CMC), were added alone or in combination with three different emulsifiers (DATEM, LC and SSL) in formulation of composite cassava-maize-wheat (ratio 40:10:50) breads in order to gain knowledge of their effect on bread quality characteristics. Each emulsifier was tested in combination with the hydrocolloids at the levels of 0.1, 0.3 and 0.5%, while hydrocolloids were used at the level of 3%. It was concluded that the hydrocolloids in combination with emulsifiers had a greater effect than hydrocolloids alone in increasing the specific volume (from 7.5 to 22%) and the brownness index (from 81.8 to 86.6%) and reducing the crumb firmness of the breads (from 14 to 36%).

Two composite cassava-maize-wheat breads with either CMC/DATEM or HM pectin/LC, both at levels of 3/0.3%, were assessed for their acceptability and sensorial attributes among

Mozambican consumers. The consumption pattern, willingness to buy and attributes of the composite breads were also collected. It was concluded that the optimized composite bread with 40% roasted cassava and CMC/DATEM was highly acceptable (score of 7.47 out of 9) and comparable to commercial wheat bread (score of 7.82 out of 9). Instrumental analysis of crust and crumb colour and firmness correlated highly with their perceived sensorial properties of its counterpart.

Texture, moisture content and starch retrogradation (recrystallized amylopectin) of optimized composite bread were evaluated during storage up to four days under controlled conditions (23^{o}C and 50% RH). In addition to the improvers used in the sensory analysis, monoglyceride (MG) was evaluated for its role on the bread quality during storage of those composite breads. DATEM and MG showed a softening effect, while the melting enthalpy was significantly lower in the composite bread with the hydrocolloids and emulsifiers compared to composite bread (without improvers).

Keywords: cassava flour, pre-treatment, composite bread, baking improvers, sensory evaluation, bread quality

”… work in quiet fashion and eat your own bread.”

2 Thessalonians 3:12

LIST OF PUBLICATIONS

This thesis is based on the work contained in the following papers, referred to in the text by their Roman numerals.

I. Eduardo, M., Svanberg, U., Oliveira, J. and Ahrné, L. (2013). Effect of cassava flour characteristics on properties of cassava-wheat-maize composite bread types. *International Journal of Food Science* Volume 2013, Article ID 305407, 10 pages. http://dx.doi.org/10.1155/2013/305407

II. Eduardo, M., Svanberg, U. and Ahrné, L. (2014). Effect of hydrocolloids and emulsifiers on baking quality of composite cassava-maize-wheat breads. *International Journal of Food Science* Volume 2014, Article ID 479630, 9 pages. http://dx.doi.org/10.1155/2014/479630

III. Eduardo, M., Svanberg, U. and Ahrné, L. (2014). Consumers' acceptance of composite cassava-maize-wheat breads using baking improvers. *African Journal of Food Science* **8**:390-401.

IV. Eduardo, M., Svanberg, U. and Ahrné, L. Effect of hydrocolloids and emulsifiers on the quality of composite cassava-maize-wheat breads after storage. *Submitted manuscript.*

CONTRIBUTION REPORT

Paper I — The author, Maria Eduardo (ME), designed the experiment together with the co-authors. The author ME planned the work, performed the baking and experimental work, except microscopy examinations, and evaluated the results together with the co-authors. The author ME wrote the first draft of the manuscript, which was finalized with contributions from the co-authors.

Paper II — The author ME designed the experiment together with the co-authors. The author ME was responsible for planning the work, performed the baking and experimental work and evaluated the results together with the co-authors. The author ME wrote the first draft of the manuscript, which was finalized with contributions from the co-authors

Paper III — The author ME designed the experiment together with the co-authors. The author ME was responsible for planning the work. The author ME also conducted the laboratory work and consumer studies together with the technical staff of the Chemical Engineering Department of Eduardo Mondlane University, Mozambique, and evaluated the results together with the co-authors. The author ME wrote the first draft of the manuscript, which was finalized with the contribution from the co-authors.

Paper IV — The author ME designed the experiment together with the co-authors, performed the experimental work and baking. The author ME was responsible for evaluation and interpretation of the data and wrote the first draft of the manuscript, which was finalized with the contribution from the co-authors.

ABBREVIATIONS

Abbreviation	Description
ANOVA	Analysis of variance
BF	Bright field
CB	Composite bread
CBA	Composite bread with CMC/DATEM
CBB	Composite bread with HM pectin/LC
CMC	Carboxymethylcellulose
DATA	Diacetyl tartaric acid ester of glycerides
DATEM	Diacetyl tartaric acid ester of monoglycerides
DE	Degree of esterification
DGMS	Distilled glycerol monostearate
DMG	Distilled monoglyceride
DS	Degree of substitution
DSC	Differential Scanning Calorimetry
GMS	Glycerol monostearate
GRAS	Generally Recognized As Safe
ΔH	Enthalpy of the recrystallized amylopectin measured by DSC
HLB	Hydrophilic-lipophilic balance value
HM pectin	High methoxyl pectin
HPMC	Hydroxypropylmethylcellulose
LC	Lecithin
MG	Monoglyceride
MDG	Monodiglyceride
PM	Polarizing microscopy
PLM	Polarized light microscopy
PS80	Polysorbate 80
SSL	Sodium stearoyl lactylate
T_o	Onset temperature
T_p	Peak temperature
Agro.Ges	Sociedade de Estudos e Projectos
FAO	Food Agriculture Organization
IITA	International Institute of Tropical Agriculture

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1. INTRODUCTION

Since ancient times, bread has been a highly appreciated product of wheat flour and has become one of the most important food items in the human diet. Although a number of ingredients can be used for the production of bread, the most important ones are wheat flour, water, yeast and salt. Contrary to all other cereals, wheat flour contains gluten proteins that have a capacity to entrap carbon dioxide in separate gas cells in the dough. During baking off, this structure is stabilized into a light, soft and porous crumb structure. In Mozambique, bread is at the present time produced basically using wheat flour. However, due to climatic conditions, there is not a great enough production of wheat to satisfy national food industrial needs and substantial quantities must be imported at a high cost.

The use of locally grown crops such as starchy tubers (cassava, yam or sweet potatoes) or cereals (maize, sorghum and millet) and oil seeds (soy, peanuts) to partially substitute wheat in high-quality products such as bread would therefore help to reduce dependence on expensive wheat imports. For this reason, the use of composite flour in breadmaking has recently been promoted by the Mozambican Government in collaboration with local research institutions; this composite flour, which consists of wheat flour in combination with flours from the above mentioned crops, has mainly been based on mixtures with cassava (Dias, 2012). In this context, baking experiments were carried out with an addition of either 10% or 25% cassava flour to wheat flour, and an acceptable bread quality (texture and taste) was reported with 10%, based on a sensory panel evaluation (Donovan *et al*., 2011).

Cassava and maize are suggested to be used for producing composite flours because of their crucial role for the food security of Mozambique. Cassava is widely grown in the region and constitutes the most important crop, along with maize, rice, beans and millet. It is a rich source of carbohydrates but low in the content of protein, fat, some minerals and vitamins (Montagnac *et al*., 2009). Although maize is predominantly starchy, it also provides some fat, iron and fibre (Begum *et al*., 2013) as well as β-carotene (a provitamin A) in the yellow type variety (Bibiana *et al*., 2014). The protein quality is comparable to that of wheat, and nutritionally it is comparable to other cereals (FAO, 1992). On the other hand, these non-wheat flours lack gluten forming proteins and, when used alone or in combination with wheat flour, result in poorer bread quality, that is, they mainly give a more stiff and rigid product with an irregular texture because of the lower ability of the dough to retain gas during proofing (Grace, 1977).

There are relatively few studies in the literature that report the effects of composite flours on bread quality, i.e. wheat flour in combination with starchy tubers, cereals and oil seeds. In the studies that exist, the substitution level for non-wheat flours varied between 5 and 50%, and inferior bread quality characteristics (low loaf volume, lack of flavour, coarse crumb, hard texture, and black specks) were generally reported with substitution levels above 20%. Therefore, baking improvers such as enzymes, hydrocolloids and emulsifiers need to be added to composite dough formulations in which a high amount of wheat flour is substituted in order to improve bread quality characteristics and to slow down the firming of the resulting bakery products.

Hydrocolloids are used in bakery products as baking improvers to increase water retention capacity and loaf volume, to decrease firmness and starch retrogradation, and to enhance the overall quality of the products during storage (Kohajdová and Karovičová, 2009).

Emulsifiers are commonly used in bakery products because of their ability to interact with different components of the flour and other ingredients in the dough, resulting in softer crumbs (Demirkesen *et al*., 2010). They are composed of both hydrophobic and hydrophilic residues, which allow the interaction and formation of complexes with starch, protein, shortening and water. The improving effect of emulsifiers seems to be related to their effect in reducing the repulsing charges between gluten proteins by causing them to aggregate in composite dough flour as the wheat gluten has been diluted. For instance, interaction of an emulsifier with the protein can improve dough strength and allow better retention of carbon dioxide (Demirkesen *et al*., 2010).

However, a combination of hydrocolloids and emulsifiers might have synergistic effects on bread quality, but no studies on composite bread have been found in literature. With the overall objective to produce a high quality composite bread with a wheat flour substitution level of 50%, a study was initiated to evaluate the effect of varying levels of cassava flour obtained from three different processing methods (sun drying, roasting and fermentation) in combination with high methoxyl pectin (HM pectin). Paper I reports the effects on the composite bread quality characteristics, specific volume, crust colour and crumb firmness.

On the basis of the findings reported in Paper I, a substitution level of 40% roasted cassava flour with an addition of baking improvers was used in the study reported in Paper II. Two hydrocolloids, HM pectin and carboxymethylcellulose (CMC), and three different types of emulsifiers (diacetyl tartaric acid ester of monoglycerides (DATEM), sodium stearoyl-2-lactylate (SSL) and lecithin (LC)) were used as improvers in the attempt to improve the baking quality of composite bread. Each emulsifier was tested in combination with HM pectin or CMC.

In the study described in Paper III, two improved composite breads selected from Paper II were assessed for their acceptability by local consumers in Mozambique in comparison with commercial wheat bread.

In the study discussed in the final paper, Paper IV, the aim was to examine the role of HM pectin and CMC and its combination with DATEM, LC and monoglyceride (MG) as baking improvers in the quality of composite bread during storage.

The baking procedure took place in two different processes, laboratory baking (Papers I-II, IV) and semi-industrial baking (Paper III).

The results of these studies may be of great importance for Mozambique in efforts to increase the utilization of locally produced food crops such as cassava and maize in composite flours for the bakery industry and thus contribute to reducing imports of wheat flour.

2. OBJECTIVES

The main objective of this work was to partially substitute wheat flour in breadmaking with locally grown crops such as cassava and maize in order to reduce the dependence on expensive wheat imports. The challenge in substituting wheat flour lies in the fact that the bread quality is mainly governed by the gluten content of wheat, which becomes gradually lower with increasing amounts of alternative flours (> 20%) in the composite dough, leading to a lower breadmaking potential due to the poorer viscoelastic properties of the composite dough. The proteins and the starch components of the alternative flours thus need to provide a structure that compensate for the lower gluten content of the dough. This can be achieved by pre-treatment of the alternative flours in combination with the addition of baking improvers that enhance viscosity, dough properties and the quality of the baked composite product.

To achieve this main purpose, an investigation was carried out with the following aims:

- to evaluate to what extent cassava flour produced from sun drying, roasting and fermentation methods in mixtures with maize and wheat flours affects composite bread quality such as loaf volume, crust colour and crumb firmness (Paper I),

- to investigate the effect on the quality of composite bread (specific loaf volume, crumb moisture and firmness, and crust colour) of the addition of hydrocolloids, carboxymethylcellulose (CMC) and high methoxyl pectin (HM pectin), and different types of emulsifiers, diacetyl tartaric acid ester of monoglycerides (DATEM), sodium stearoyl lactylate (SSL) and lecithin (LC) (Paper II),

- to assess consumers' acceptance of two optimized composite cassava-maize-wheat bread characterized in Paper II, to explore the most important attributes for consumer acceptance and to collect general information on bread consumption pattern, the intention to purchase and attitudes to composite breads of Mozambican consumers (Paper III), and

- to evaluate the role of HM pectin and CMC and their combinations with emulsifiers, DATEM, LC and monoglyceride (MG), on the starch retrogradation and quality characteristics of composite cassava-maize-wheat breads after storage (Paper IV).

3. BACKGROUND

3.1. Bread making process

Bread can generally be produced using four breadmaking methods: (1) Straight dough bulk fermentation, which is the traditional method, where all the ingredients are mixed together to form a dough and are left to ferment for long hours before baking; (2) Sponge and dough method, which includes a two-stage process in which part of the total quantity of flour, water and other ingredients from the formulation are mixed to form a homogeneous soft dough, referred to as the sponge, where this sponge is left to rest in bulk for a prescribed time, which depends on flavour requirements, after which the sponge is mixed with the remainder of the ingredients to form a homogenous dough where the final dough is immediately processed; (3) Rapid processing method, which uses the different combinations of active ingredients and processing methods, and where a common element in this procedure is the inclusion of improvers to assist in dough development; and (4) Mechanical dough development method, e. g. where the Chorleywood Bread Process (CBP) is a baking process with no need of a fermentation period in bulk and where dough development is achieved during high-speed mixing by intense mechanical working of the dough (Cauvain, 2003; Cauvain, 2007b; Sedláček and Horčička, 2011).

However, there is little information on the specific effects of the different methods for bread made from the composite flours. Onuegbu *et al*. (2013) studied the effect of different bread making methods (rapid process, straight dough, sponge and dough, sourdough) on baking and the sensory properties of composite wheat-maize bread using varied levels of an addition of maize flour (0 to 20%). The authors found that rapid processing was the best method in most of the sensory attributes and overall acceptability. The sponge and dough method gave the best crumb texture, while crust colour did not show a significant difference in the rapid processing and sourdough methods. In addition, they found that crumb texture did not show a significant difference in composite breads baked using the rapid processing dough, straight dough and sour dough methods.

The current study applied breadmaking with the straight dough bulk fermentation method. According to Eliasson and Larsson (1993), the breadmaking process when using composite flour is considered to be the same as that described for wheat flour, which mainly includes three major stages – mixing or dough formation, fermentation and baking. Each stage in the processing is critical in influencing the final bread quality. Figure 1 shows the flowchart of bread processing.

Figure 1 Flow sheet diagram of bread processing

3.1.1. Mixing

Mixing is a critical step that blends all ingredients together into homogeneous dough mass, occludes air into the dough, develops the gluten proteins into a continuous phase (Cauvain, 2007b) and yields a dough with optimum consistency, which is referred to as the height of the mixing curve at the peak. Water is added to the dry ingredients and hydrates the flour through diffusion; here, the hydrated flour particles rub against each other and, in the process, the outer, hydrated layer is removed. As this process continues, all the flour particles – mainly the protein and starch – become hydrated, and this means that the dough has been optimally mixed or developed (Eliasson and Larsson, 1993). Belton (1999) proposed a model they called "loops and trains" (Figure 2) to explain the behaviour of dough with respect to the hydration of gluten proteins. With a low level of hydration (Fig. 2a), the proteins are disordered and have close interactions via hydrogen bonds but no regular structure. In the intermediate hydration, plasticization of the system facilitates the formation of hydrogen-bonded structures, which is described as a low loop-to-train ratio (Fig. 2b). In high hydration, there is a formation of hydrogen bonds that results in the formation of regions in which interchain interactions are broken. This is observed as a high loop-to-train ratio (Fig. 2c). That is, the more hydrated the flour is, the more viscoelastic properties the dough has.

a)
b)
c)

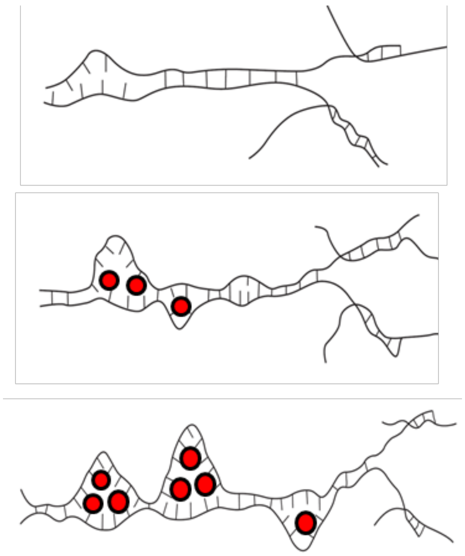


Figure 2 Levels of hydration of the gluten proteins in the wheat dough: (a) Low hydration; (b) Intermediate hydration; (c) High hydration (source: Belton, 1999)

3.1.2. Fermentation

The developed dough that was formed while mixing expands as the gas is retained in its structure during proofing and will be set during baking (Cauvain, 2003; Marsh and Cauvain, 2007). There are two phases in the fermentation stage, bulk fermentation (first proof, floortime) and the main fermentation (known as the final proof).

Dough has a resting period (floortime) in bulk after mixing and before dividing, which varies from 0.5 to 3 h (Ćurić *et al.*, 2014). The objective of this stage is to transform a piece of dough in a more elastic and more resistant form to being stretched without tearing (Brown, 1993) in the moulding stage (Gould, 2007).

After a dough resting period, the bulk dough may be divided into individual pieces by weighting or volumetrically (which is more common) and then shaped.

Two successive steps are responsible for the final shape of the dough: laminated and curled. The moulded dough is placed either in tins or on a baking tray and kept in a proofing cabinet to continue fermentation (final proof). This step is necessary to work the fermented dough, divide the alveolus, and provide a uniform redistribution.

During the final proof, which lasts about 30-60 minutes, starch from the flour is progressively converted into sugars and dextrins by enzyme action (Ćurić *et al.*, 2014). The sugars feed the yeast, and the breakdown products are carbon dioxide and ethanol. As carbon dioxide is produced, the dough expands and retains it, and it is important that the skin remains flexible. There is a relationship between produced and retained gas that depends on the quality of the gluten structure. The more retained gas, the greater the bread volume (Cauvain, 2007b).

3.1.3. Baking process

Baking is the last operation in the bread production, where, by the action of heat, the dough is transformed into bread by firming (stabilization of the structure) and by the formation of the characteristic aromatic substances. In bread, it is achieved at baking temperatures around 220-250^{o}C, although at the centre temperature of the loaf reaches only 92-96^{o}C, which is accepted as being necessary for an adequate rigid structure throughout the loaf, due partly to the loss of water (Cauvain, 2003; Ćurić *et al.*, 2014).

The dough undergoes a series of changes due to the rise in temperature while it is in the oven. Initially, yeast activity ceases when a temperature of 55^{o}C is reached. Subsequently, the stability of the structure is sustained due to the expansion of entrapped gas. As the temperature nears 60^{o}C, the starch starts to gelatinize. The starch granule first absorbs any free water from the dough and later from the protein membranes until it is fully gelatinized (Cauvain, 2003; Wiggins and Cauvain, 2007). A final internal temperature in the range of 92-96^{o}C should be achieved for an adequately baked loaf (Cauvain, 2003; Ćurić *et al.*, 2014).

The different temperatures reached inside and outside the dough cause the formation of the crust and crumb of the bread. The different phases during baking are described as oven spring (enzyme active zone), gelatinization of starch, and browning and aroma formation. Crust formation is of great importance to both the strength of the bread loaf and flavour development (Wiggins and Cauvain, 2007).

3.2. Composite bread

Composite flour technology is viewed as the process of mixing a proportion of two or more flours (grains, tuberous plants or legumes) with or without wheat flour to produce bread with desired quality attributes that is referred to as composite bread. Composite flour has been used in developing countries since the 1960s (Grace, 1977; AllAfrica.com, 2014) where wheat does not grow due to the unfavourable agronomic conditions (Grace, 1977; Edwards, 2007; AllAfrica.com, 2014), and bread costs can be reduced due to lower wheat imports (Abdelghafor *et al*., 2011). Edwards (2007) reported that typical substitution levels of wheat flour are in the range of 15-20% for sorghum flour and millet flour, 20-25% for maize flour; and 10-20% for cassava flour (Eriksson *et al*., 2014).

3.2.1. Different flours in composite bread

Different flours such as cereals (maize, rice, sorghum, millet) and tubers rich in starch (cassava, cocoyam, sweet potato, yam) and protein-rich flours (cowpea, soybean) have been used in breadmaking to partially substitute wheat flour in bread (Siddiq *et al*., 2009; Oladunmoye *et al*., 2010; Mongi *et al*., 2011; Nindjin *et al*., 2011; Rai *et al*., 2012; Begum *et al*., 2013; Bibiana *et al*., 2014; Trejo-Gonzalez *et al*., 2014). In those studies, features such as loaf weight and volume, specific volume, specific weight, texture and colour parameters have been evaluated to characterize the quality of the composite baked products. All of them have indicated that the optimum amount of wheat to be substituted is about 10% without an impairment of the quality characteristics of wheat bread. However, the percentage limit seems to be dependent on the source of the non-wheat flour.

Mongi *et al*. (2011) evaluated bread characteristics of cocoyam/wheat composite breads and found that loaf volume and specific volume decreased, whereas loaf weight increased with an increased amount of cocoyam to 30%. Similar results were reported for composite wheat breads with increasing amount of maize and sweet potato flours (Bibiana *et al*., 2014) and for composite bread with added maize flour (Siddiq *et al*., 2009). Trejo-Gonzalez *et al*. (2014) found that hardness increased with an increased amount of maize and sweet potato flour up to 20%. This was due to a gluten dilution effect, which consequently affected the retention of carbon dioxide. In addition, Siddiq *et al*. (2009) reported a significant decrease in the crust lightness of bread with addition of maize flour (above 10%).

Sensory studies have also been widely reported to describe the sensory attributes and acceptability of the composite breads. Various researchers found decreased sensory attributes in the scores for appearance, crust and crumb colour, taste and flavour with an increased ratio of non-wheat flour (cassava, cocoyam, yam, sweet-potato, soy, maize, rice), leading to a decreased overall acceptability of the composite bread (Almazan, 1990; Khalil *et al*., 2000; Sabanis and Tzia, 2009; Mongi *et al*., 2011; Nindjin *et al*., 2011; Rai *et al*., 2012; Bibiana *et al*., 2014; Trejo-Gonzalez *et al*., 2014). The reported levels of wheat substitution were in the range of 20-50%. On the other hand, a good breadmaking potential could be obtained with partial substitution of wheat flour by cassava flour up to 20 and 30% with an addition of 1 % malt (Khalil *et al*., 2000), 30% yam starch and 20% cassava starch (Nindjin *et al*., 2011).

The replacement of wheat by composite flours has currently been encouraged in Mozambique in order increase the consumption of locally grown crops (Agro.Ges, 2007; Donovan *et al*., 2011).

Nonetheless, the use of composite flours with a high amount of non-wheat flour (> 20%) presents considerable technological difficulties due to the low levels or the absence of gluten (Cato *et al*., 2004), if volume, crumb characteristics and flavour similar to that of wheat bread are required (Cauvain, 2007a). There is thus a need to find solutions to enhance quality of composite breads.

3.3. Characterization of baking ingredients

The minimum formula of bread is flour, yeast, salt and water. The ingredients in the formula are usually expressed as a percentage of the flour by weight.

The following section reports the main ingredients in the manufacture of bread, including those added to influence the structural and physicochemical characteristics of the flour constituents, such as sugar, oxidizing agent and shortening; dough stabilizers and crumb softeners such as emulsifiers, and polymeric substances with viscoelastic properties such as hydrocolloids.

3.3.1. Flour

Flour is the product obtained from cereal grains, roots etc. after milling. McKevith (2004) reported that several mechanical changes during milling of cereal grains are related to the starch reflected in an increased proportion of damaged starch in the flour that is more susceptible to enzymatic attack. In addition, the author reported that these changes are important in bread making because they provide access for α-amylase to work, and the extent of the referred change will depend on the quality of the grain and the parameters of milling. Furthermore, proteins can denature due to excessive heating (50 – 60^{o}C), leading to lower wet gluten yield, and consequently a decrease in the water absorption capacity of the flour. In the case of cassava flour the changes that occur in production are due to different root processing methods. This results in a decrease in the product yield, which is of low quality (e.g. in terms of vitamins, minerals) compared to fresh cassava roots (IITA, 1992; FAO, 1998).

Table 1 summarizes the chemical composition of flours from wheat, maize and cassava. It shows that there is a major difference in the amount of protein between the three flours, where cassava flour shows a lower amount compared to the other two. Cassava is composed mainly of starch, making it a product of inferior quality (Agro.Ges, 2007). However, the wheat proteins are of superior breadmaking quality due to their gluten protein content, which is considered important in terms of its gluten quality (gluten index) and quantity (wet and dry gluten). According to Fig. 2, gluten is the substance that has the ability to form cohesive and viscoelastic properties of the dough when hydrated, and it allows retaining the gas formed during the fermentation stage.

Contrary to wheat flour, maize and cassava have non-gluten forming proteins which do not interfere with gluten development during dough mixing. According to Mohamed *et al.* (2010), a lack of fully developed gluten has a direct effect on dough formation, mixing time and bread quality.

Table 1 Chemical composition of wheat flour compared to maize flour and cassava flour

	Flours		
Nutrient (g per 100 g)	Wheat[1]	Maize[2]	Cassava
Water	11.6	13.0	11.7
Protein	10.3	9.0	1.8
Fat	1.5	3.5	0.5
Total carbohydrates	73.1	68.4	82.2
Fibre	3.0	4.6	1.9
Ash	0.5	1.5	1.9

Source: Korkalo *et al*. (2011)
[1] 75% extraction rate. [2]Whole flour

3.3.1.1. Wheat flour

Wheat (*Triticum aestivum* L.*)* flour is the basic ingredient in the production of the bread. The wheat grain is composed of three components: bran, which represents 14% of the grain, contains the majority of the grain fibre (cellulose and pentosans); germ represents approximately 3% of the grain; and endosperm, the main part of the grain with 83%, contains mostly starchy endosperm with lower protein content and lipid content compared to the germ and the bran (Bushuk and Scanlon, 1993; Rosell, 2011). There are mainly two types of wheat flour, which are distinguished by their extraction rate (degree of milling). White wheat flour has an extraction rate in the range of 76-78% whereas in whole meal the extraction rate is between 85 and 90%. Brown flour can be produced during milling by mixing white wheat flour and whole meal in the proportion of 50:50% (Catterall and Cauvain, 2007).

Wheat proteins can be grouped according to their functionality in breadmaking in two main groups: the non-gluten proteins (albumins and globulins), with either no or just a minor role in affecting breadmaking performance, and the gluten proteins (gliadins and glutenins) with a major role in breadmaking (Goesaert *et al*., 2005; Cauvain and Young, 2006). In contrast, the glutenins (known as glutelins) fraction provides strength (resistance to deformation) and elasticity (springiness) to the dough, while the gliadin (known as prolamins) is responsible for the viscous properties of the dough (Stauffer, 2007), which act as plasticizers in the glutenin network (Goesaert *et al*., 2005).

Starch is the largest fraction of wheat flour, making up about 65%, and exerts an influence on dough elasticity (Stauffer, 2007). It consists of amylose and amylopectin. Amylose is a linear polymer, while amylopectin is a branched polysaccharide (Eliasson and Larsson, 1993). The amylose/amylopectin ratio varies between cereal species, but typical levels are 25-27% of amylose and 72-75% of amylopectin (McKevith, 2004; Goesaert *et al*., 2005; Cornell, 2004). The shape of the starch granules is either spherical or lenticular with an average size range between 5 and 20 µm (Goesaert *et al*., 2005). The contribution of starch in breadmaking is related to its three important properties, which includes water absorption (dough preparation phase), gelatinization (baking phase) and retrogradation (in cooling and storage stages).

Gelatinization is the process that occurs above the gelatinization temperature, and this is characterized by the loss of molecular order and crystallinity of the starch granule, whereas retrogradation is the opposite process, which can occur even when no moisture is lost from the product (Goesaert *et al*., 2005; Cauvain and Young, 2006).

In the stage of dough preparation, starch absorbs up to 46% water, and it mainly acts as an inert filler in the protein matrix of the dough; however, its exact role has not been completely clear. During baking, the starch granules gelatinize and swell, while a small amount of amylose leaches out into the inter granular phase. In this phase, amylose is located in the centre of the large granules, while amylopectin is in the outer granule layers. Some of the solubilized amylose forms inclusion bodies with endogenous or added polar lipids (Goesaert *et al*., 2005).

Immediately upon cooling, the solubilized amylose molecules start to crystallize and interlink, forming a continuous network with embedded starch granules. Thus, amylose, due to its rapid retrogradation, is an important process in breadmaking and may be crucial for the initial firmness of the bread (Goesaert *et al*., 2005). In addition, the amylopectin fraction also contributes to the crumb firming, which affects retrogradation.

3.3.2. Non-wheat flours

The production of wheat in Mozambique for the last five years since 2009, on average, was around 0.5% with respect to its importation of about 595 000 ton/year (CIMMYT, 2014). There is therefore a need of using locally grown crops (non-wheat crops) for breadmaking to replace wheat flour, which is imported.

The non-wheat flour can be used either alone or mixed with wheat flour, and the latter is known as composite flour. In this thesis, composite flour based on cassava and maize is tested for their potential use as ingredients in breadmaking. These non-wheat crops are the two major staple crops produced in Mozambique and more than 80% of each crop is consumed by the Mozambican population (Dias, 2012; Dias, 2013). Their production has been estimated to be 5.7 million tons for cassava and 1.9 million tons for maize.

3.3.2.1. Cassava

Cassava (*Manihot esculenta* Crantz), a root, consists of two main varieties, which can be distinguished on the basis of the taste of the raw roots as sweet (low in cyanogenic glucosides) and bitter (high in cyanogenic glucosides) (Westby, 2002; Wheatley *et al*., 2003; Montagnac *et al*., 2009).

Despite their cyanogenic glucosides, both fresh cassava varieties are rich sources of energy due to their high content of carbohydrate. About 64-72 % of the carbohydrate is starch, mainly in the form of amylose (~17%) and amylopectin (~83%) (Rawel and Kroll, 2003; Charles *et al*., 2005). However, cassava starch differs from that of cereal starch in its granular structure, amylose content and branch chain length distribution, granule size and shape. In fact, spherical or lenticular granules with a truncated end and a well-defined hilum characterize cassava starch. The granule size is between 5 and 45 μm (Tester *et al*., 2004).

Cassava is deficient in protein content, particularly in sulphur-containing amino acids (methionine and cysteine) (Montagnac *et al*., 2009), with no ability to form a network that retains gas during dough development as wheat flour does. However, fresh cassava is generally considered to have a high content of dietary fibre (Westby, 2002) and water (about 70%) (Dias, 2012).

Due not only to the higher amounts of water but also to the presence of cyanogen substances, cassava roots are limited for human consumption. There is therefore a need to use processing techniques to detoxify and reduce the cyanogen substances to safe levels (maximum 10 mg/kg of dry weight), and simultaneously to extend the shelf-life of the cassava roots (Niba *et al*., 2001).

Methods used to process cassava roots into flour

Cassava flour is obtained by milling the dried roots. The production of flour involves washing and/or peeling roots to remove the outer parts consisting of the periderm and the cortex, soaking, grating, fermenting, chipping or slicing and then heating to avoid microbial contamination (FAO, 1998; Wheatly *et al*., 2003; Agro.Ges, 2007). However, processing sequences may have similar starting steps and then differ in different cassava products.

In Mozambique, however, the traditional processing methods and the choice of the final product vary accordingly to geographic region. For example, dry chips are produced in areas of greater cultivation of cassava – this is a characteristic of the Northern and central regions – while dry-roasted cassava (also known as rale), a product similar to West African gari, is mainly produced in the Southern region (Agro.Ges, 2007; Tivana *et al*., 2009; Donovan *et al*., 2011).

Chip production gives an unfermented and a dried cassava product obtained by peeling, chipping or cutting the peeled roots into pieces and sun drying (Agro.Ges, 2007; FAO, 2010). Chipping is done to expose the maximum surface of the starchy flesh and promote rapid drying while sun drying is done to reduce the moisture content of the product to about 13%, which is considered safe for long-term storage (FAO, 1998).

Rale (or gari) is a creamy-white, partly gelatinized, dried granular product with an irregularly shaped granule. The production of rale involves natural fermentation for one to six days, which is done with grated or soaked cassava roots; as a result, the pH decreases (Tivana *et al*., 2007; Montagnac *et al*., 2009), while the protein content slightly increases (Tivana *et al*., 2007). It has also been reported that fermentation increases the swelling index, which was attributed to the organic acids and amylose released by microorganisms that degrade starch granule (Irtwange and Achimba, 2009). Moreover, a breakdown of starch granules invariably loosens up the starch network and allows a higher moisture absorption capacity (Irtwange and Achimba, 2009). However, the difference between rale (=gari) and cassava flour is in the length of fermentation, in which rale is left for longer than flour, thus conferring it a sour flavour (Lancaster and Coursey, 1984). The garification and drying combined with frying are steps also included in the production of gari and take place in the range of 80-85^{o}C for about 30-35 min. The moisture content of the product is reduced to about 12-18% (FAO, 1998).

3.3.2.2. Maize

Maize (*Zea mays* L.), a cereal, consists of about 82-83% endosperm, 10-11% germ, 5-6% bran (pericarp), and 0.8-1% tip cap. The endosperm contains around 85% of the starch, which consists of amylose and amylopectin (Singh *et al*., 2011; Singh *et al*., 2014), 8.5% protein and a low amount of fat (1%) (Singh *et al*., 2014). During milling, the germ and bran, which are rich in fat, protein and dietary fibre, are removed from the endosperm.

The total protein content of maize varies between 8 and 11% on a dry basis of the kernel weight (FAO, 1992). However, the proteins of maize, on hydration, do not form a glutenous substance as do those of wheat and, therefore, breads made from maize flour are of the unleavened or flat bread type (Edwards, 2007) and are granular rather than porous (Kent-Jones and Amos, 1967).

Starch is the major chemical component of the maize kernel. The starch is made up of about 25% amylose with the remainder being amylopectin (Singh *et al*., 2014), which are very similar in composition to those of wheat starch. However, they behave differently in baking (Eliasson and Larsson, 1993). The size of the starch granules of maize ranges from 1 to 20 µm in diameter (Singh *et al*., 2014) and they do not show a distinct bimodal size distribution as does wheat starch (Goesaert *et al*., 2005). Moreover, maize starch granules are angular or spherically shaped (Singh *et al*., 2014).

3.3.3. Water

Water is of great importance in the production of bread, since it hydrates flour particles and helps flour components to interact, producing a homogeneous mass of dough.

During the mixing process, water is necessary for formation of the dough and becomes distributed between the flour components for its fluidity. The rest of the added water remains as "free" water and forms the so-called water phase. In this phase, the flour proteins are hydrated, partially absorbed by the flour starch, particularly the damaged starch fraction of the flour; soluble solids such as sugars, salt, soluble proteins are also dissolved, and the yeast cells are dispersed as well (Brown, 1993). The amount of water absorbed into the dough is mainly controlled by the quality of the flour. Therefore, the type and quality of the flour are key factors in water absorption.

During the fermentation step, water acts as a solvent in the dough, and many of the reactions that take place at this phase cannot occur if there is no solvent. For example, water acts as a solvent for some of the released carbon dioxide gas to form carbonic acid. Carbonic acid contributes to the acid pH of the dough, providing a feasible atmosphere for the action of enzymes and yeast in the dough system.

Water governs the major changes that take place during baking (starch gelatinization, protein denaturation, yeast and enzyme inactivation, and flavour and colour development). At the baking stage, the degree of starch swelling and gelatinization depends of the total amount of water present in the dough. For this, the temperature in the crumb does not exceed $100^{o}C$ while the final temperature to completely gelatinize the starch must be below $100^{o}C$ (Cauvain and Young, 2003).

Based on the dependence between the level of added water and the quality of flours, it can be concluded that if too little water is added to the dough, the dough will be firm and difficult to mould. As a consequence, the bread will have a small volume and poor external appearance while, if there is too much water, the dough will be soft and difficult to mould, hence producing bread of poor quality (Cauvain, 2003). However, water also has plasticizer effects that increase softness and decrease bread firmness (Mohammadi *et al*., 2014).

3.3.4. Bakers' yeast

Bakers' yeast (*Saccharomyces cerevisiae*) is used in the manufacture of bread, due to its ability to metabolize fermentable sugars (glucose, fructose, sucrose and maltose) present in the dough and thus produce carbon dioxide and alcohol towards fermentation; the carbon dioxide produced is an important product since it enables the dough to expand to the required volume through its action on internal pressure of the gluten network while the alcohol formed and other compounds released from secondary fermentation such as organic acids, aldehydes, ketones and other carbonyl compounds act as precursors in the development of taste and flavour (Rose and Vijayalakshmi, 1993; Poitrenaud, 2004).

Other products of fermentation are reducing sugars, which react with the dough proteins on the dough surface under the influence of the oven heat to give the characteristic browning of the bread crust, which is known as the Maillard reaction that contributes greatly to the flavour of bread (Brown, 1993).

3.3.5. Salt

Salt (sodium chloride) generally forms part of the dough ingredients. In breadmaking, the usage levels are around 1.0-2.0 % based on flour weight (Brown, 1993; Eliasson and Larsson, 1993). A few percent of salt stiffens the dough and makes it less sticky.

It has been reported that salt increases the mixing tolerance of wheat dough, extends the dough development time and increases the dough resistance, elasticity and extensibility (He *et al*., 1992; Uthayakumaran *et al*., 2011). Salt has also been related with part of the strengthening effect of the dough, and this effect has been attributed to the decrease in the water absorption of the flour (Preston, 1989).

3.3.6. Sugar

Sugar is a disaccharide composed of two units, one of glucose and another of fructose. In typical bread production, 2-3% sugar is adequate to sustain yeast activity. Later, more sugar is released for gas production by the action of enzymes in the flour.

Due to the affinity with water, it has been reported that sugar exerts a limiting effect on gluten formation during the dough preparation stage. This limitation of water availability is partly responsible for the effect on starch gelatinization (Cauvain and Young, 2006). Sugar is used as a substrate for the yeast during the early stages of fermentation. When added to dough, sugar is hydrolysed almost instantly into glucose and fructose by the yeast enzyme invertase. Sugar acts as antistaling ingredients inhibiting starch recrystallization (Levine and Slade, 1990). In the case of microorganism growth, this is restricted through increasing levels of sugar in the dough formulation (Cauvain and Young, 2006).

Sugar increases product volume and will increase crust colour unless the oven temperature is adjusted (Brown, 1993). Sugar that remains unfermented by yeast appears as residual sugar in the finished products (Nip, 2006). Residual sugar takes part in caramelization and the Maillard reaction (i.e., the reaction between reducing sugar and the proteins of flour to promote rapid colour and taste formation).

3.3.7. Ascorbic acid

Ascorbic acid (vitamin C) is a slow acting oxidant agent that works in the proofing and early oven stage. It has been used to enhance the strength of gluten, handling and baking properties of dough, and gas retention (Goesaert *et al*., 2005) by oxidation of –SH groups of gluten protein to –SS groups (ratio of ~1:20, respectively). The disulphide bonds thus established within and between proteins chains lead to a firmer gluten structure (Narvhus and Sørhaug, 2006; Williams and Pullen, 2007).

Ascorbic acid should be added at 25 mg/kg of flour (normal use) and 100 mg/kg of flour (intense mechanical dough development) (Eliasson and Larsson, 1993). Addition of oxidative improvers at optimum levels results in both optimized dough handling properties and bread quality (large volume, great oven spring, better quality of the grain). The amount of oxidative improver required for optimum results depends firstly on the properties of the flour, the choice of oxidants and the processing conditions (Preston and Yamada, 1991).

3.3.8. Shortening

Shortening, which is lipids in the form of either fat or oil, consists of 25% solid fat at room temperature. It has been used in bread production to impart tenderness, to give moister mouthfeel, confer structure, lubricate during chewing and contribute to flavour. Due to the liquid phase of shortening, the tenderizing effect slows the staling process compared to the same dough formulation without shortening. Typical usage levels are in the range of 3-4% all-purpose shortening or 2-3% vegetable oil, although up to 5% (flour weight basis) can also be used (Stauffer, 1993).

With the addition of shortening in the dough formulation, a larger bread volume has been reported due to improved oven spring, softer crumb, less crisp crust and better keeping quality of the bread (Stauffer, 1993). In fact, studies by Campbell (1972) showed a greater increase in loaf volume and improved crumb grain with additions up to 1.5 g (flour basis) of shortening in the dough formulation. This effect was attributed to the lubrication on the gluten matrix during mixing that improves the uniformity of the dough by lowering its resistance to diffusion while increasing the oven spring (dough expansion) (Eliasson and Larsson, 1993; Stauffer, 1993).

3.3.9. Baking improvers

In this work, baking improvers refers to hydrocolloids and emulsifiers that are used to improve the quality of composite bread.

Comparing research on the last five years regarding the effect of baking improvers on quality characteristics of composite bread based on mixtures of wheat with any non-wheat flours (Table 2), it is observed that there are some improved effects.

Table 2 Effect of baking improvers on quality characteristics of composite bread

Type of improver	Type of composite flour	Effect on bread quality	References
Pectin **Gum Arabic** **(0, 1, 2 and 3%)**	Wheat/Corn (20% corn)	No hydrocolloids: Decreased volume. No difference in weight. Decreased crumb moisture. Sign low scores in all sensory characteristics. Increased staling rate. With hydrocolloids: Sign increased volume and specific volume. Sign improved of the sensory characteristics. Increased crumb moisture. The best levels: 3% pectin and 2% gum Arabic.	(Yaseen, Shouk & Ramadan, 2010)
PS80 **DATEM** **SSL** **(0.5% and 1%)**	Resistant starch high-maize/Wheat (12.5% resistant starch)	Increased loaf volume. Sign increased hardness (except 0.5% PS80). Increased retrogradation enthalpy (7d storage).	(Gómez, Buchner, Tadini, Añón & Puppo, 2013b)
DATEM **DMG** **(2%)**	Millet/Wheat (up to 50%)	Composite bread up to 30% millet flour: Sign increased specific volume. Sign decreased firmness. Increased darkness crust colour.	(Schoenlechner, Szatmari, Bagdi & Tömösközi, 2013)
Xylanase **Transglutaminase** **(1%)** **+ DATEM or DMG**		Sign increased specific volume (particularly transglutaminase) Slight decreased firmness (xylanase) whereas firmness Increased (transglutaminase) No sign effect crust colour.	
Xanthan **(1 and 2%)**	Cassava/Wheat (10% cassava) 1%	Sign increased specific volume. Sign increased softness index. Decreased firming rate. Slight decreased crumb moisture Reduction moisture loss. Sign decreased L/b ratio crumb value. Increased L crust value.	(Shittu, Aminu & Abulude, 2009)
	2%	Sign decreased specific volume. Increased crumb moisture. Increased L/b ratio crumb value. Increased L crust value.	

According to above (Table 2), studies have been carried out to show the use of different baking improvers in composite baking technology, including approaches such as the use of hydrocolloids (pectin, gum arabic, xanthan), emulsifiers (PS80, DATEM, SSL, DMG) and enzymes (xylanase, transglutaminase) or combinations thereof (emulsifier/enzyme) to improve loaf volume, crumb structure, sensory qualities, mouthfeel and tolerance to staling (Shittu *et al.*, 2009; Yaseen *et al.*, 2010; Gómez *et al.*, 2013b; Schoenlechner *et al.*, 2013).

3.3.9.1. Emulsifiers

Emulsifiers can function as dough stabilizers when the emulsifier interacts with gluten protein in the dough and/or as crumb softeners when the emulsifier complexes with the gelatinizing starch during baking (Goesaert *et al.*, 2005). They can be used in bread production to either increase or aid dough stability and gas retention, improve bread volume and allow weaker flours to be used (Brown, 1993). The most widely used emulsifiers include lecithin or phospholipids, diacetyl tartaric acid esters of glycerides/ monoglycerides (DATA/DATEM), sodium stearoyl lactylate (SSL), glycerol monostearate (GMS) and diglycerides (Gomez *et al.*, 2004).

Emulsifiers are normally categorized based on their ionic charge, their solubility in certain solvents and their hydrophilic-lipophilic balance (HLB). HLB is a number that determines the ratio of the molecular weight of the hydrophilic portion of the emulsifier divided by the total molecular weight of the emulsifier. The values for HLB range from 0 (completely lipophilic) to 20 (totally hydrophilic) for each type of emulsifier, where emulsifiers with low HLB values in the 3-6 range are soluble in oil (w/o emulsions), whereas high values in the range of 8-18 are soluble in water (o/w emulsions) (Msagati, 2013).

The mechanisms of action of emulsifiers in bread manufacturing are not fully understood. They can influence the quality of bread in different ways depending on type of emulsifier and the bread formulation (Goméz *et al.*, 2004).

Sodium stearoyl lactylate

Sodium stearoyl lactylate (SSL), an anionic oil-in-water (o/w) emulsifier that has both starch and protein complexing properties can be used to improve dough stability and gas retention, and thereby enhances crumb softness and keeping properties (Brown, 1993). Due to its dual functionality, SSL interacts with gluten proteins during dough mixing, which provides strength (resistance to deformation) and forms complexes with amylose and amylopectin, which slows down the staling process (crumb firming) while improving the crumb softness (De Stefanis *et al.*, 1977).

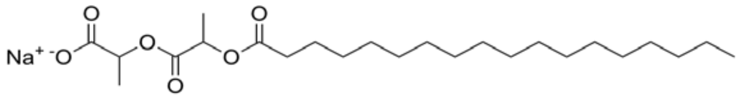


Figure 3 Chemical structure of sodium stearoyl lactylate (Gómez *et al.*, 2013a)

Diacetylated tartaric acid esters of mono- and diglycerides of fatty acids

Diacetylated tartaric acid esters of mono- and diglycerides of fatty acids (DATA esters, DATEM) are an anionic o/w emulsifier. This property allows DATEM to complex with the flour proteins and increases the strength of the dough, giving it better stability and improved gas retention properties. Adding this emulsifier to low protein flour enables higher volume, aids dough stability and tolerance for dough handling properties as well as aids gas retention through final proof and the early stages of baking (Brown, 1993). Up to 0.3% (flour weight) is usually added based on flour type and variety of bread (Kohajdová *et al.*, 2009).

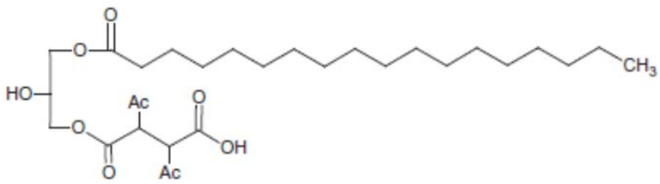


Figure 4 Chemical structure of the diacetyl tartaric acid esters of monoglycerides (Gómez *et al*., 2013a)

Mono- and diglycerides

Mono- and diglycerides are considered GRAS (Generally Recognized As Safe) materials, which are extensively used in bakery products. They are mainly dependent on the nature and characteristics of their fatty acid content.

Glycerol (GMS) and distilled (DGMS) are also mono- and diglycerides constituted by fatty acids of different chain lengths. They are used as a softening agent and, based on their ability to complex with amylose (component of starch), monoglycerides can then be used to slow the rate of the staling process (Brown, 1993; Cauvain and Young, 2006).

(a) (b)

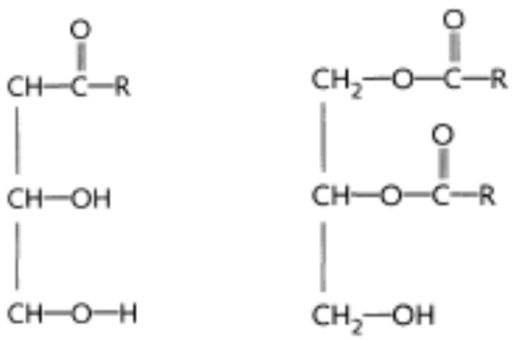


Figure 5 Chemical structure of (a) monoglyceride and (b) diglyceride (where R may either be stearic or oleic acid) (Zielinski, 1997)

Lecithin

Lecithin (LC) is also a GRAS substance approved in some countries (e.g. USA) and meets standards of the Food Chemicals Codex. LC is used for its multifunctional properties, which are emulsifying, viscosity modifying and so on (Szuhaj, 1983). The effects of lecithin in baking include improved dough stability, tolerance and fermentation behaviour of yeast doughs, crumb structure and increased loaf volume (Brown, 1993). Hydrolysed lecithins are used to retard staling upon storage. It is added as an improver in breadmaking in concentrations up to 6 g/kg of flour (Helmerich and Koehler, 2005).

Lecithin is a natural phospholipid emulsifier containing choline. It is an amphoteric emulsifier, and unlike SSL or GMS does not form complex with starch (Stampfli and Nersten, 1995).

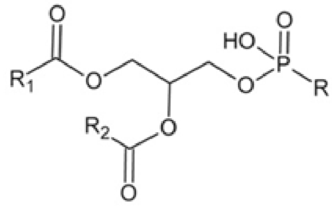


Figure 6 Chemical structure of lecithin phospholipids with R1, R2 representing fatty acid chains and the R group mostly being a choline (Pareyt *et al*., 2011)

3.3.9.2. Hydrocolloids

Hydrocolloids (gums) are starch-based substances from various plants of high molecular weight characterized by their ability to act as polymeric substances in forming viscoelastic dispersions and/or gels when dispersed in water (Bemiller, 2008; Milani and Maleki, 2012), modifying starch gelatinization (De Leyn, 2006) and retarding the hardening of bread crumb (Guarda *et al*., 2004). These substances have been used to regulate water distribution and water holding capacity and thereby improve yield, although the mechanism is not completely understood. Guarda *et al.* (2004) suggested that the hydrocolloids have a weakening effect on the starch structure that causes better water distribution and retention as well as a decrease in the crumb resistance.

The most well-known hydrocolloids include guar gum, xanthan gum, methylcellulose, hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC) and pectins. Other hydrocolloids used are derived from natural sources such as gum arabic, kappa carrageenan (κ-carrageenan) and locust bean gum. This study focused mainly on pectin and CMC as baking improvers of the quality of bread.

Pectin

Pectins are anionic heteropolysaccharides that contain at least 65% D-galacturonic acid units in the sodium salt form, which are joined with α-(1,4) bonds and are partially esterified by methanol. This heteropolysaccharide is divided according to its esterification degree – DE (%

of galacturonic acid methylation in methyl-ester form, $-COOCH_3$) – to high esterified pectins (HM) with a DE above 70% and to low esterified pectin (LM) with a DE below 50% (Bemiller, 2008; Mikuš *et al*., 2011). This hydrocolloid is soluble in water and insoluble in most organic dissolvents. Solubility in water drops with a growing molecular weight and degree of esterification of carboxyl groups. High esterified pectins are soluble in a warm environment. Moreover, pectin molecules have in a neutral environment negative charge; therefore they react with positively charged polymers (proteins) (Mikuš *et al*., 2011).

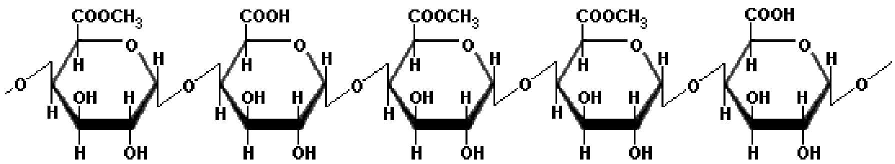


Figure 7 Section of a high ester content pectin molecule (Nussinovitch, 1997)

Carboxymethylcellulose

Carboxymethycellulose (CMC) is a modified and an anionic polysaccharide that belongs to cellulose with carboxymethyl, either in groups in the sodium salt form ($-O-CH_2-COO^-Na^+$) or to some of the hydroxyl groups present in the glucopyranose monomers that form the cellulose backbone (Bemiller, 2008). Structurally, it is a polymer chain composed of repeated anhydro glucosyl units joined with β-(1,4) glycoside bonds. Each anhydro glucosyl unit contains three hydroxyl groups, which can be substituted. This cellulose derivative is used because of its ability to retain moisture, to improve the mouthfeel of the products, to increase volume and the uniformity of baked products, to soften bakery texture, to prolong the freshness of bakery products and to reduce physical damage caused by ice particles during storage at very low temperature (Kohajdová *et al*., 2009; Mikuš *et al*., 2011).

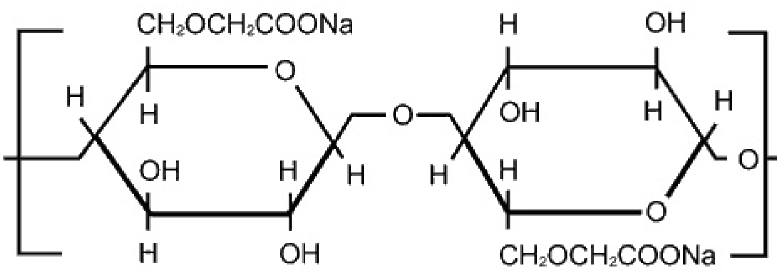


Figure 8 Idealized unit structure of carboxymethylcellulose (Nussinovitch, 1997)

3.4. Bread quality

Bread is traditionally made with wheat flour (Cauvain, 2007a), whereas composite bread is produced from flours of other types of grains, legumes and tubers, both combined with salt, water and yeast (*Saccharomyces cerevisiae*) (Baiano *et al*., 2009). In the cases of different flours and other ingredients, bread can be described as a product with different qualities that vary accordingly to weight, size (loaf volume), shape, crust thickness and crispness, colour, crumb texture and softness, flavour and tolerance to staling (Brown, 1993; Cauvain, 2007a; Rosell, 2011; Hadiyanto and Boxtel, 2011). The quality of bread depends on the properties of the raw materials as well as on the baking process. The most frequently quality parameters assessed are the volume, weight, and specific weight (true density) of bread (Różyło and Laskowski, 2011). There are a large variety of bread products with large differences in volume, crumb structure and texture, and crust thickness or crispness. The optimal bread quality depends therefore on the type of bread product. Różyło and Laskowski (2011) suggested that structure, moisture and mechanical properties (e.g. firmness/hardness) are important aspects of crumb quality, whereas the quality of bread crust is often based on colour and thickness/crispness of the crust.

According to Brown (1993), in order to compare the quality of a particular bread type with another of the same type, it must be known what characteristics are desirable for that type of bread. That is, a characteristic that is desirable in one bread type may be described as a fault in another bread type. For instance, standard sliced and wrapped pan bread has a fine, soft, even crumb cell structure with a thick crisp crust, whereas a French baguette has a very open random crumb cell structure with a thick crust and double the volume of standard sliced pan bread (Brown, 1993). Rosell (2011) highlighted that the quality attributes provide objective values that are very useful for comparison of bread features. The size of the bread provides a quantitative measurement of baking performance, where light and not so dense breads are desired (Rodriguez-Sandoval *et al*., 2012). Texture includes the crispness and softness of the baked product and depends on the product rigidity/elasticity and structure. Colour is the result of the Maillard reaction, which involves the production of melanoidins as colouring components (Hadiyanto and Boxtel, 2011). In fact, studies by Rózyto and Laskowski (2011), who compared loaf volume and crumb texture from different wheat cultivars, found that the bread quality differed mainly due to differences in flour protein content, dough extensibility (loaf volume) and strength of the flour (hardness).

3.4.1. Staling of bread

Starch is the main component of bread, and its reorganization during aging is known to contribute significantly to bread staling (firming of the bread crumb). Staling is known to influence consumer acceptance of bakery products owing to changes in bread due to loss of moisture and the retrogradation of starch (Narvhus and Sørhang, 2006). Although the mechanism of this phenomenon is, to date, not completely understood (Gray and Bemiller, 2003), the major change in the staling process is starch retrogradation (Schiraldi and Fessas, 2001; Narvhus and Sørhang, 2006). Starch consists of amylose and amylopectin compounds, and it is important with respect to retrogradation. Thus, on cooling and aging of bread, rearrangements in the starch fraction lead to a series of changes including gelation and crystallization. This transformation is called retrogradation.

During the retrogradation process, amylose and amylopectin have different behaviour in which the retrogradation of amylose is the first to be observed a few minutes after gelatinization is completed and forms an irreversible (above 100^{o}C) crystallinity (Eliasson, 2010). In contrast, retrogradation of amylopectin occurs over a long time period and forms a reversible crystalline structure. Amylopectin retrogradation is often measured by differential scanning calorimetry, where the retrogradation temperatures (T_o, T_p) as well as the energy required to melt the recrystallized amylopectin ΔH (expressed in J/g) are studied.

3.4.2. Sensory quality

Sensory evaluation is defined as a science that is used not only to measure and evaluate the sensory properties of food but also to analyse and interpret the responses to those characteristics of foods as they are perceived by the human senses (sight, smell, touch, taste) (Stone and Sidel, 2004). The sensory quality of food products plays a key factor in the selection of food. Hedonic testing (e.g. 5, 7 or 9-point scale) is often used to determine consumers' attitude towards the food by measuring a degree of acceptability, overall liking, preference or sensory attributes (appearance, flavour, texture, aroma etc.) of a new product or improving the existent food product (Meilgaard *et al*., 1999). The data obtained from this test can help to identify potential buyers of the product and the way in which the product can be introduced into the food market (Cordonnier and Delwiche, 2008; Kemp *et al*., 2009; Gámbaro, 2012).

Overall appearance includes all visible sensory attributes such as colour, size and shape as well as surface texture (Meilgaard *et al*., 1999). Flavour involves sensory attributes such as taste, specific flavour, aroma and sweetness. Aroma is the odour of a food product, resulting from the process that involves the course of volatiles through the nasal passages when a person inhales them (voluntarily or otherwise) (Meilgaard *et al*., 1999).

Texture is referred to as the tactile feel properties, measured as geometrical particles, and mechanical and moisture properties, by the tactile nerves in the surface of the skin of the hand, lips or tongue (Meilgaard *et al*., 1999). This can be described as aerated, crispy, crumbly, gummy, soft, hard and moist (Setser, 1993). Overall liking can be defined as a complex expression of liking the product as a whole.

The willingness to buy represents the extent to which a consumer has a positive attitude towards purchasing a product. Roininen *et al*. (1999) concluded in a quantitative study of important predictors of food that food choice is mainly predicted by taste, although appearance and smell are also important hedonic aspects for consumers' quality perception.

Recent studies have shown that baking improvers can be used to improve the sensory qualities of various type of breads. Lazaridou *et al*. (2007) reported the highest score, which was in the range of like moderately and like very much, for the overall acceptability of gluten-free bread prepared with CMC (2% level). Sabanis and Tzia (2011) also reported that the sensory properties (crust colour, crust crispiness, appearance, crumb texture, flavour and taste) of gluten-free bread with added hydrocolloids were improved compared to that of control bread; however, the major improvement was obtained with HPMC. This was attributed to the fat mimetic and texturizing effects of HPMC that affected mouthfeel, flavour release and texture perception during consumption. Moreover, the authors reported that gluten-free breads formulated with 1.5% of hydrocolloids received a higher score than those formulated with 1%. Shittu *et al*. (2009) investigated the effect of xanthan gum on composite bread using 10% cassava flour mixed with wheat flour as well as the sensory acceptability and found that the xanthan gum gave a distinct taste perception; furthermore, as the level of xanthan increased from 1% to 2%, the crust appearance, taste and overall acceptability were improved while the surface of the crust became drier and coarser compared to composite bread without xanthan.

3.4.3. Effect of baking improvers on loaf volume

Various types of emulsifiers are widely used in different bread formulas because they can improve or aid dough stability and gas retention, thereby improving bread volume (Brown, 1993). Azizi and Rao (2005) reported that the use of sodium stearoyl-2-lactylate (SSL), DATEM, glycerol monostearate (GMS) and distilled glycerol monostearate (DGMS) surfactant (at 0.5%) improved the loaf volume of fresh wheat bread; however, adding shortening (up to 2%) reduced the volume increasing effect. Helmerich and Koehler (2005) found that crude and defatted soybean and rapeseed lecithin were the most effective in increasing the wheat loaf volume by up to 37%, at concentrations between 0.6 and 1.0%.

Hydrocolloids have been used as baking improvers in different types of breads and, depending on the type of hydrocolloid, various effects on the volume of bread have been obtained. The specific loaf volume of wheat bread was improved with κ-carrageenan and HPMC (Rosell *et al*., 2001; Guarda *et al*., 2004). Correa *et al*. (2012) compared the effect of LM pectin and HM pectin at levels ranging from 0.25% to 2% and found that wheat bread containing HM pectin had a larger specific volume. Higher levels of different kinds of hydrocolloids in gluten-free bread have been shown to give a negative effect on bread quality. Lazaridou *et al*. (2007) studied the effect of added pectin, CMC, agarose or xanthan up to 3%. Pectin or xanthan at 1% resulted in a lower loaf volume in comparison with pectin (2%) and CMC (1%). Sabanis and Tzia (2011) also found that HPMC and guar gum, both at 1.5%, improved the loaf volume of gluten-free bread. Yaseen *et al*. (2010) reported that gum arabic (2%) or pectin (3%) improved the loaf volume of composite bread based on corn-wheat (20:80) flours. Cato *et al*. (2004) studied the effect of the addition of various hydrocolloids (CMC, HPMC, guar gum) on the characteristics (loaf volume, texture and crust and crumb colour) of the resulting breads. The formulations were based on rice flour/potato starch (a gluten-free bread) and on wheat/rice mixture flours. The authors concluded that HPMC most favourably affected bread qualities while CMC had little effect and guar gum had no effect.

A combination of hydrocolloids and emulsifiers might have synergistic effects on bread quality, although only few studies of wheat bread have been reported. Mettler and Seibel (1993) used a response surface methodology to investigate the effects of emulsifiers and hydrocolloids on whole wheat bread quality. An increase in specific volume and crumb firmness, and a reduction in crumb elasticity during storage were obtained by adding 0.3 parts monodiglycerides (MDG), 0.6 parts DATEM, 0.15 parts guar gum (GG), and 0.5 parts CMC. Bollaín and Collar (2004) showed that a combination of DATEM and HM pectin improved wheat dough strengthening, which is related to bread with a high specific volume and a lower rate of staling during storage (Martínez *et al*., 1999).

3.4.4. Effect of baking improvers on texture

Emulsifiers, hydrocolloids or emulsifiers/hydrocolloids have been applied to the dough formulation in order to decrease the crumb firmness of fresh bread or retard the staling of stored bread.

The functionality of different emulsifiers on wheat bread quality was evaluated by Gómez *et al*. (2004), and they found that SSL, sucrose ester, lecithin and enriched lecithin had the greatest crumb-softening effects, explained by the formation of smaller gas cells that resulted in a finer crumb grain structure of the fresh product. Furthermore, Gómez *et al*. (2004) found that emulsifiers such as monoglyceride (MG) and lecithin enriched in lysophospholipids delay the hardening of wheat bread. This effect has been attributed to the interference action of MG on the swelling starch, thereby avoiding the diffusion of water, and also by the complex formation with amylose. Lecithin also forms complexes with starch amylose. The addition of emulsifiers in combination with shortenings produces a softer wheat bread than emulsifiers alone (Azizi and Rao, 2005). Onyango *et al*. (2009) also found that adding different emulsifiers at a concentration of 2.4% had a protective effect on the staling of gluten-free bread prepared from sorghum and gelatinized cassava starch.

Concerning hydrocolloids, it has been reported that the crumb hardness of fresh wheat bread was decreased by κ-carrageenan and HPMC (Rosell *et al*., 2001). Mohammadi *et al*. (2014) evaluated the texture of fresh gluten-free bread and found that the firmness was significantly decreased by xanthan gum and CMC.

Starch retrogradation and crumb firming have been used as indicators for the staling of bread. Guarda *et al*. (2004) found that the moisture losses in wheat bread during storage were smaller and that bread staling was retarded by HPMC and alginate. A texture profile analysis showed that pectin softened the crumb of wheat bread during storage (Correa *et al.*, 2012), and this effect was attributed to the interactions between pectin with gluten protein that led to the ability to establish more hydrophobic bonds due to the large proportion of methoxyl groups. According to Collar *et al*. (2001), the softening effect of HPMC could be attributed firstly to its high water retention capacity, and therefore possible inhibition of the amylopectin retrogradation, and secondly, since HPMC preferentially binds to starch, it may inhibit starch-gluten interactions. Sabanis and Tzia (2011) also reported that hydrocolloids (HPMC, κ-carrageenan and guar gum) with the exception of xanthan reduced the crumb firmness of stored gluten-free bread. This was attributed to the water binding capacity of the hydrocolloids that avoids water loss during storage and with possible hydrogen bonding between hydrocolloids and starch that would delay starch retrogradation (Sabanis and Tzia, 2011). Guar gum has been added to composite flour based on wheat flour/whole meal spelt and resulted in bread with reduced staling (Kohajdová and Karovicova, 2008). According to Yaseen *et al*. (2010), hydrocolloids such as gum arabic (2%) or pectin (3%) retarded staling/crumb firming of stored bread (up to two days) based on corn-wheat (20:80) flours. In contrast, Lazaridou *et al.* (2007) reported that pectin and CMC did not alter the crumb firmness and that xanthan had an unfavourable influence on this parameter during storage of gluten-free bread. The investigations of Purhagen *et al*. (2012) also showed that DATEM reduced crumb firming, and the bread retrogradation rate of amylopectin in gluten-free bread during storage was less with an addition of emulsifier.

However, studies of the interaction of hydrocolloid/emulsifier on the texture characteristics of bread are scarce as compared to when those baking improvers are used alone. Mettler and Seibel (1993) found that the crumb elasticity and crumb firmness during storage were reduced by a combination of baking improvers, emulsifiers DATEM and MDG, and hydrocolloids GG and CMC. Those improving effects were suggested to be due to the high amount of water required for bread preparation when GG was added and due to the thinning of the crumb walls surrounding the air spaces in the high volume bread in the presence of DATEM.

3.4.5. Effect of baking improvers on colour

There are studies that show that various hydrocolloids affect bread crust colour differently. Sciarini *et al*. (2010) and Sabanis and Tzia (2011) showed that xanthan gum, HPMC, and κ-carrageenan make the crust of bread darker while CMC and xanthan make it lighter. Shittu *et al*. (2009) also reported a lighter crust colour with xanthan gum in composite cassava-wheat bread. Lazaridou *et al*. (2007) found that xanthan, pectin and CMC had no effect on crust colour. This may be explained by the reduction in the rate of Maillard's browning reaction at the crust layer between amino acids and reducing carbohydrates (Kent and Evers, 1994).

4. MATERIALS AND METHODS

Several different materials and methods were used in the studies reported in Papers I-IV. These are summarized in Figure 9.

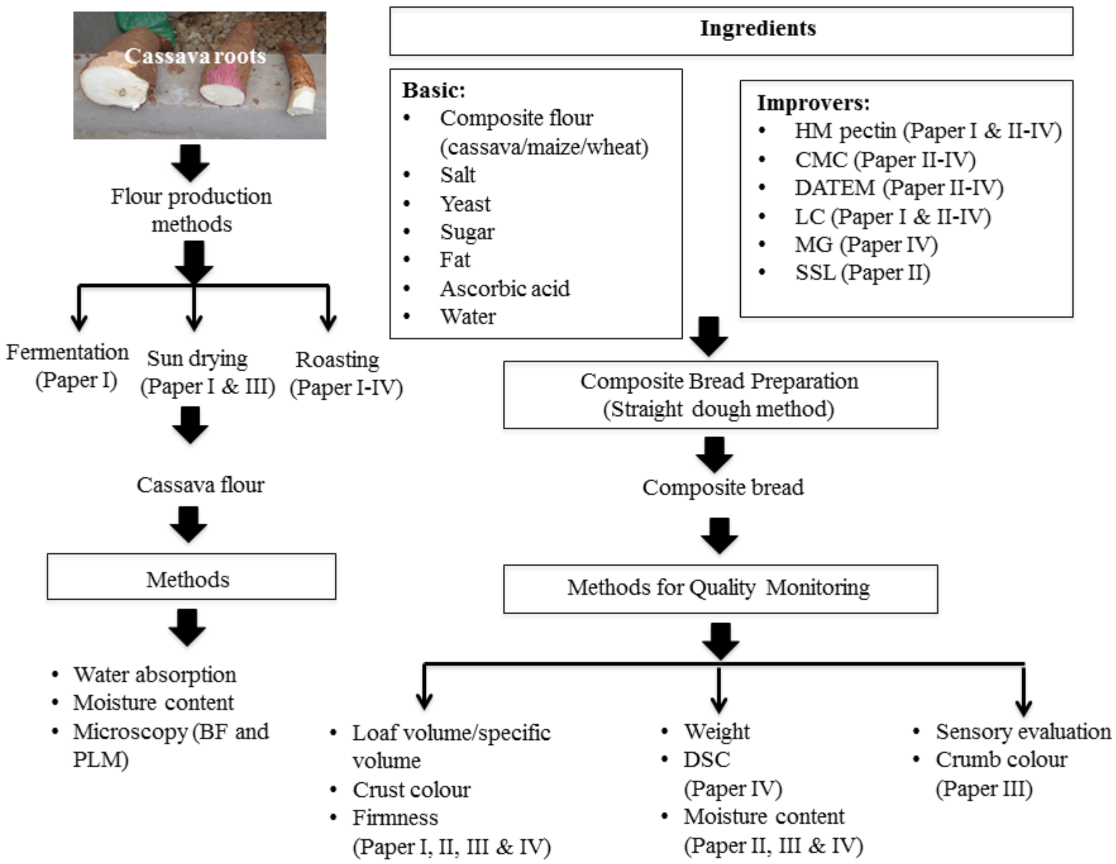


Figure 9 Schematic diagram of the materials and methods used in Papers I-IV

4.1. Materials

This study used materials including cassava flour obtained from different processing methods, maize flour, basic ingredients (wheat flour, water, yeast and salt), other ingredients (sugar, fat and ascorbic acid), hydrocolloids (HM pectin and CMC), and emulsifiers (DATEM, LC, MG and SSL). The materials used in the four studies will be presented here. A short background to why they were chosen will also be given here.

Table 3 Description and specification of different baking improvers

Baking improvers	Abbreviation	Description	Specification	Company	Paper
GENU® pectin type BIG (E440)	HM pectin	High ester pectin from citrus peel and standardized by addition of sucrose	Degree of esterification: 68-75%	CP Kelco, DANISCO CP Kelco, Denmark	I II, III and IV
CEKOL® 50000 W Cellulose gum (E 466)	CMC	Highly purified sodium carboxymethyl cellulose	Degree of substitution: 0.75-0.85	CP Kelco, Denmark CP Kelco, Finland	II and III IV
Lecithin (E 322)	LC	Sunflower lecithin (fluid) Deoiled soya lecithin (powder)	-	Lecico, GmbH Sternchemie, Germany Lecico GmbH, Germany	I II and III IV
Sodium stearoyl lactylate MULTEC SSL 3000 (E 481)	SSL	SSL made from edible vegetable oil	-	MULTEC, Puratos, Belgium	II
Diacetyl tartaric acid esters of monoglycerides (E 472e)	DATEM	DATEM made from edible vegetable oil	-	MULTEC, Puratos, Belgium Panodan A2020, DANISCO, Denmark	II and IV III
Monoglyceride (Dimodan® PH 200) (E 471)	MG	MG is a distilled form made from edible, refined vegetable oil	Total monoglyceride min. 90%	DANISCO, Denmark	IV

4.1.1. Flours

Flour in this thesis is a product that is obtained from milling grain cereals or tubers. Generally, these flours are rich sources of starch but have a variable quantity and quality of protein. However, from a technological point of view, there exist structural differences between those flours due to their physicochemical composition.

4.1.1.1. Wheat flour

Wheat flour was used in the four studies to produce composite flours. The protein content was 10.5%, and is in the range of the 10-12% minimum protein content used for the production of white bread (Bushuk and Scanlon, 1993).

4.1.1.2. Cassava flour

The cassava roots were subjected to three different production methods (sun drying, fermentation and roasting) to obtain flour (Fig. 10). According to bright-field and polarized light microscopy examinations, the three cassava flours were different in their structure, with roasted cassava flour showing partially swollen starch granules and the other two intact starch granules (Paper I). All the three cassava flours were used in the study reported in Paper I, and wheat flour was partially substituted by two levels (20% and 40%). Roasted and sun-dried cassava flour were used in Paper III, and only roasted cassava flour was used in Papers II and IV with a substitution level of 40%. Cassava was chosen because it is widely grown in Mozambique (§ 3.2.2, pp. 9).

Processing methods for cassava flour

Three processing methods were used to transform the fresh cassava roots (~ 70% water) (Dias, 2012) into a stable product such as flour. Flour is the most widely used product and can be processed in a variety of different ways. Effective processing, essentially involving root disintegration and removal of the cyanogenic compounds with water, ensures the safety of products. Sun drying produces flour with intact starch granules; fermentation of cassava roots improves the internal stability of the starch granules, thereby reducing swelling and decreasing the amylose released during heat treatment (Numfor *et al.*, 1995); the starch granules in cassava flour are partly gelatinized when roasting is used. The differences in the characteristics of cassava flour are expected to influence the resulting composite dough, and thereby the quality of the bread. This was evaluated in Paper I.

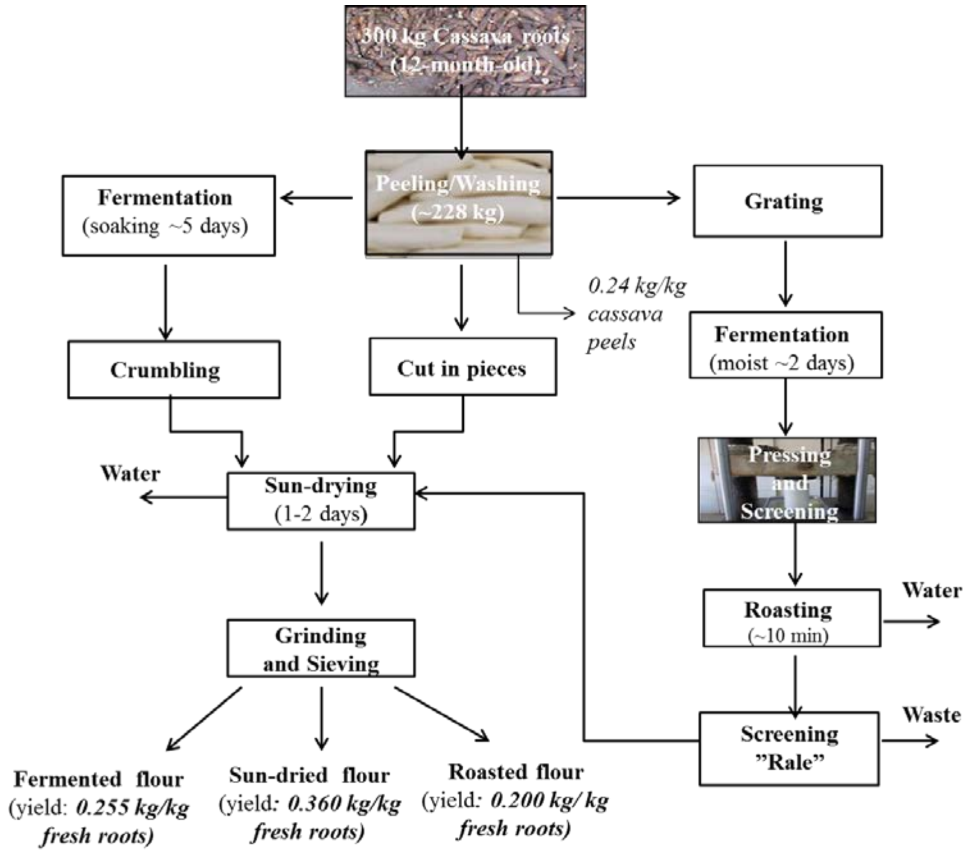


Figure 10 Production of cassava flour by different methods

Approximately 100 kg of cassava roots (~12 months old; *Xinhembwe* variety) per type of pre-treatment (sun drying, roasting and fermenting) were used to produce cassava flour. For sun-dried flour, the cassava roots were washed, peeled and sliced in small pieces which were sun-dried. For roasted flour, the peeled cassava roots were chipped followed by moist fermentation for about 2 days, pressing and screening in a mechanical machine and then toasted over fire in an open frying pan until cooked and crisp (~10 minutes). For fermented flour, the peeled cassava roots were immersed in water for 5 days, and then the fermented roots were crumbled and sun-dried. All dried pieces were ground into a flour with a laboratory mill, and excess fibre was removed by passing the ground material through a sieve DIN 4188 (0.125 mm aperture sieve). The dried products per pre-treatment yield approximately 36 kg, 20 kg and 25.5 kg, respectively.

4.1.1.3. Maize flour

Maize flour, being the second most important staple crop, was used to partially replace wheat flour by 10% and 30% levels (Paper I) and 10% (Papers II, III and IV). Yellow maize flour was used in the studies reported in Papers I, II and IV. White maize was used in Paper III.

4.1.2. Hydrocolloids

Hydrocolloids are high molecular weight compounds formed with long chain polymers derived from fruits and plants, among others from sources that are capable of forming viscous dispersions or gels when dispersed in water (Milani and Maleki, 2012). Their functionality depends on their source, extraction process, original chemical structure and modifications, concentrations, and interactions with other food polymers and ingredients; in the present study they include high methoxyl pectin (HM pectin) and carboxymethylcellulose (CMC).

4.1.2.1. High methoxyl pectin

High methoxyl pectin (HP pectin) is a heteropolysaccharide from plants with a degree of esterification (DE) above 50%. DE is the percentage of esterified carboxyl groups (Correa *et al*., 2012; Milani and Maleki, 2012). The HM pectin used in the studies reported in all the four papers was extracted from citrus peel and standardized by the addition of sucrose (CP Kelco, **Table 3**). The DE was in the range of 68-75%.

4.1.2.2. Carboxymethylcellulose

Carboxymethylcellulose (CMC) is an anionic, water soluble cellulose derivative that is obtained by chemical modification of cellulose (Kohajdová *et al*., 2009). The degree of substitution (DS) with the carboxyl groups is in the range of 0.6-0.95 per monomeric unit (Milani and Maleki, 2012). The CMC used in the studies reported in the Papers II, III and IV is high purified sodium CMC and was obtained from CP Kelco (**Table 3**). The DS was between 0.75 and 0.85.

4.1.3. Emulsifiers

Emulsifiers are molecules that consist of a polar (hydrophilic = head group) part and a non-polar (hydrophobic = tail) part, in which their functionality depends on their chemical structure; that is, it consists of a hydrocarbon chain that is lipophilic and the hydrophilic polar group.

Another property that will affect the functionality of the emulsifiers to water is the hydrophilic-lipophilic balance (HLB).

4.1.3.1. Lecithin

Lecithin (LC) is a natural anionic phospholipid emulsifier (HLB=3-4) (Ahmad *et al*., 2014).

Two different sources of LC were used in the work reported in Papers I-IV (**Table 3**). The fluid sunflower lecithin, obtained from Lecico, was used in Papers I and IV while deoiled soya lecithin powder, obtained from Sternchemie, was used in Papers II and III.

4.1.3.2. Sodium stearoyl-2-lactilate

Sodium stearoyl-2-lactilate (SSL) is an anionic oil-in-water emulsifier (HLB=18-21) that carries a negative charge in the hydrophilic head. The SSL made from refined fatty acids of vegetable origin (Multec 3000, Belgium) (**Table 3**) was used in the study reported in Paper II.

4.1.3.3. Diacethyl tartaric acid ester of mono- and diglyceride

Diacethyl tartaric acid ester of monodiglyceride (DATEM) is also an anionic oil-in-water emulsifier (HLB=9.2) that is formed by the esterification of mono and diacylglycerols with the mono- and diacetyl tartaric acid (Ahmad *et al*., 2014). The DATEM used in this work was made from edible vegetable oil (**Table 3**). DATEM (MULTEC, Belgium) was used in the study reported in Papers II and IV while DATEM (A2020, DANISCO, Denmark) was used in Paper III.

4.1.3.4. Monoglyceride

Monoglyceride (MG) is an ester of glycerol and fatty acid, which is a nonionic water-in-oil (w/o) emulsifier (HLB = 2.8-3.8) (Kamel and Ponte Jr., 1993). MG (Dimodan® PH 200) was used in the study reported in Paper IV. It is a distilled form produced from edible and refined vegetable oil, consisting of at least 90% monoglyceride (**Table 3**).

4.2. Processing and analytical methods

In order to evaluate the quality (loaf volume, colour, crumb moisture, firmness, weight, density, starch retrogradation and sensory attributes) of composite bread, different experimental techniques were employed. The experimental details are described in each paper. The techniques are described below in terms of why they were chosen.

4.2.1. Processing methods

4.2.1.1. Preparation of the composite doughs

A brief summary of the composite flour preparations used in the studies is reported in Papers I-IV.

Paper I

Blended flours of cassava roots (fermentation, roasting or sun drying methods), maize and wheat flour, which are referred as composite flour, were produced. Composite flours were made with different additions of maize-cassava flours to wheat flour. The amount of wheat flour was always 50% while the ratio cassava-to-maize flour was 20:30 and 40:10% (w/w), respectively. The composite flour was also blended with HM pectin at concentrations of 1% and 3% (w/w) based on the total amount of flour. To ensure homogeneity, composite flour samples were mixed in a kitchen mixer for approximately 10 minutes. The composite flours were stored in sealed polythene bags until use. To produce bread, an experimental full factorial design was used in which the composite flour with 30% (w/w) sun-dried, pre-treated cassava flour was the central point, replicated three times. Volume, crust colour and texture were determined, and the results were analysed with factorial ANOVA.

Paper II

Optimization of the quality of composite bread was studied in mixtures with roasted cassava flour. The ratio cassava:maize:wheat flour was 40:10:50% (w/w), respectively. The same procedure as in Paper I was used in the preparation of composite flour. Hydrocolloids such as HM pectin and CMC, and hydrocolloid/emulsifier (DATEM, LC and SSL) combinations, were used. Three different concentrations of emulsifiers were used: 0.1, 0.3 and 0.5% (w/w). The concentration of hydrocolloid was 3% (w/w). A full factorial experimental design without replicates was used to produce bread. Specific volume, texture, crust colour and moisture content were measured, and the results were evaluated with ANOVA and post hoc multiple range tests.

Paper III

A consumer test of composite cassava-maize-wheat (40:10:50%, w/w) breads was made in this paper.

Four composite breads were evaluated sensorially in this paper based on overall acceptance, sensory attributes and attitudes. The two breads that in study II were found to have optimal quality attributes were selected. These were based on 40% roasted cassava with either 3% CMC and 0.3% DATEM or 3% HM pectin and 0.3% LC.

The other two breads were based on either 40% roasted cassava or on a mixture of 20% roasted/20% sun-dried cassava, and were prepared with 3% HM pectin and 0.4% LC.

Paper IV

The effect of hydrocolloids, emulsifiers and their interactions on weight, crumb firming, moisture content and starch retrogradation (recrystallized amylopectin) after storage of composite cassava bread was investigated in this paper. The40% level of roasted cassava was used in composite maize-wheat flours. HM pectin or CMC was added to dough at a level of 3% (w/w) whereas emulsifiers (DATEM, LC and MG) were added at a level of 0.3% (w/w). After the cooling process and before analysis, the composite breads were unpacked and stored in a room with controlled relative humidity (50%) and temperature (23^{o}C). Bread samples were removed from storage after 0 and 4 days.

4.2.1.2. Breadmaking process

The baking experiments were planned according to a full factorial design with 3 levels for type of cassava flour (sun-dried, roasted and fermented) with 2 levels for amount of cassava and HM pectin, plus the center point (for sun-dried pre-treated cassava flour only), replicated three times. This resulted in 15 experiments that were performed in random order. The design is shown in Figure 11.

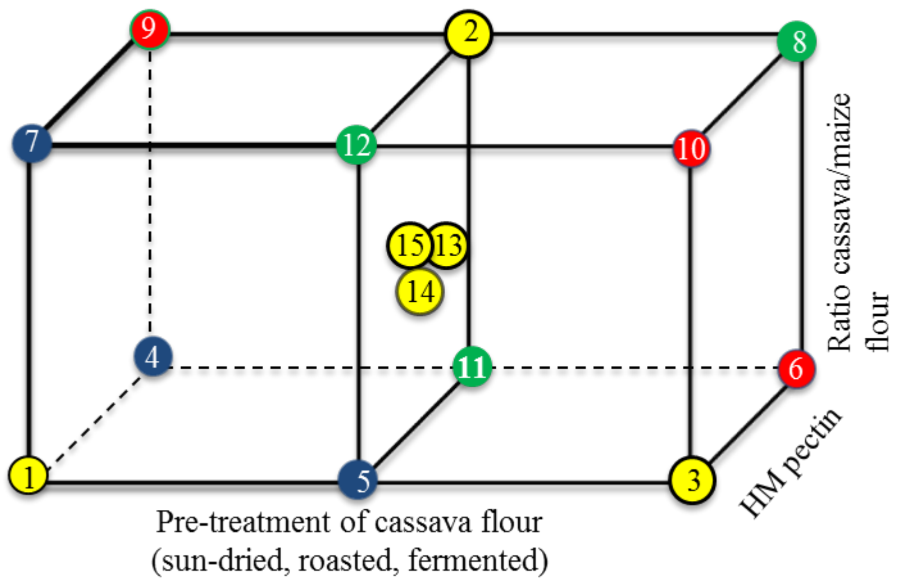


Figure 11 Full factorial experimental plan design (Paper I)

The breadmaking process used was the straight dough method (Figure 12), in which all the ingredients are mixed together to form a dough and left to ferment before baking (Papers I, II, III and IV).

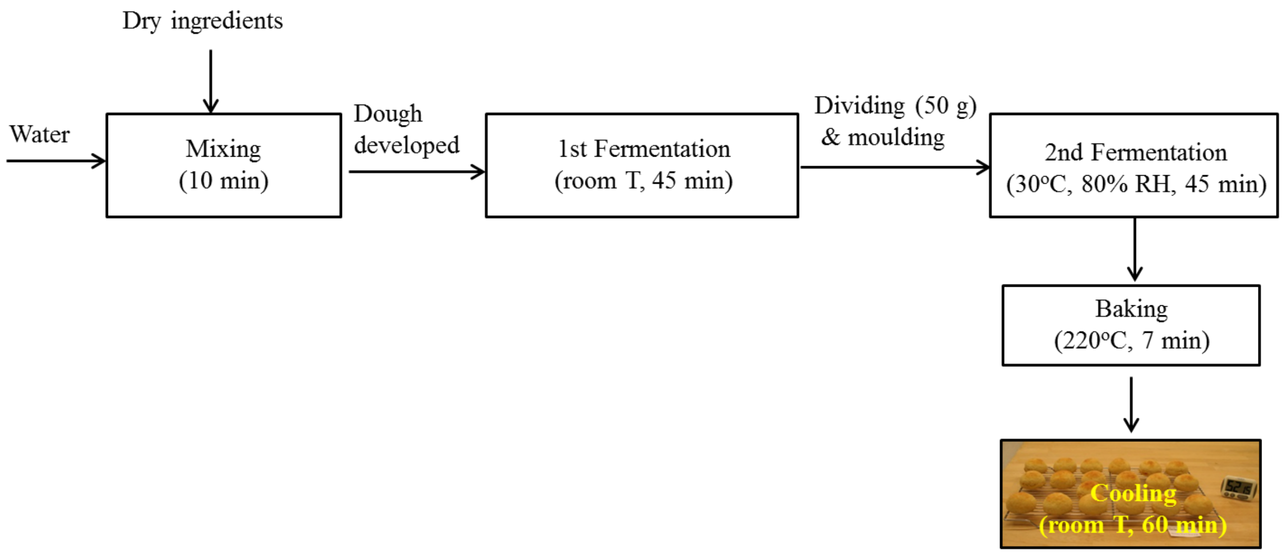


Figure 12 Flowsheet diagram of straight dough method for preparation of composite bread

4.2.2. Analytical methods

4.2.2.1. Bread quality

The concept of bread quality is related to instrumental attributes, which can be assessed objectively. The instrumental attribute has been grouped into external bread characteristics (product dimensions, volume, appearance, colour and crust formation), internal bread characteristics (number and distribution of cells in the crumb grain, the crumb colour) and textural bread characteristics (firmness and resiliency) (Cauvain, 2007a). Rosell (2011) pointed out that these measurements provide objective values, which are very useful for comparison of bread features.

Volume and specific volume

Loaf volume is the most important bread quality that affects overall consumer acceptance and provides a quantitative measurement of baking performance. It is generally measured by rapeseed displacement. This method was used to evaluate the volume of composite breads (Papers I, II and IV). However, a bread volume apparatus (TextVol Instruments, BVM-L370, CE, Sweden) was used in Paper III. BVM-L370 uses a laser sensor to measure a product's volume, and its advantage over the seed displacement method is that there is no compression of the sample (Cauvain and Young, 2006). Measurement of volume is used for the determination of the specific volume (= volume/weight of bread, cm^3/g) as well as for the determination of density of bread (= weight of bread/volume, g/cm^3).

Firmness

Firmness, resistance of the bread crumb to deformation, is the textural property of bread that determines how bread is accepted by the consumer (Abu-Shakra and Sherman, 1984). Bread firmness can be evaluated in a compression test with the Instron machine (UTM), as this is widely used. UTM is the method that determines the force required to compress wheat bread a preset distance. This principle can be extended to other loaf types (AACC, 1995).

Colour

The digital colour imaging system (DigiEye) is a digital colour imaging system that measures the colour appearance of the product using a non-contact and non-destructive method. The imaging system uses controlled illumination conditions to capture high resolution images of the product surface. The International Commission on Illumination (CIE) recommended colour measurement in terms of *L*, *a* and *b* values, which form the basis of any colour measurement system (Vyawahare *et al*., 2013). Thus, the DigiEye 2.53b software (Cromocol Scandinavia AB, Borås, Sweden) allows for storage of specific colour standards with a given L^* (lightness), a^* (redness-greenness) and b^* (yellowness-blueness) - values according to the CIELab system definition. The method was used for evaluation of the crust colour appearance of the composite breads reported in Papers II and IV.

The Minolta Color Reader (Minolta CR-10) also uses the CIE $L^*a^*b^*$ colour space system, but differs from the DigiEye in terms of the storage CIELab parameters (Papers I and III).

The crust colour of the breads was reported as brownness index (BI), calculated according to Maskan (2001):

$$BI = \frac{[100 \cdot (x - 0.31)]}{0.17} \qquad (1)$$

where,

$$x = \frac{a + 1.75L}{5.645L + a - 3.01b} \qquad (2)$$

Thermal properties

Differential scanning calorimetry (DSC) is a technique for direct assessment of heat energy uptake that occurs in a sample and a reference sample in a regulated increase or decrease in temperature. This is used to determine the temperature and heat flow associated with transitions in a sample material as a function of time and temperature (Gill *et al*., 2010). A peak in the DSC is caused by a change in the differential heat flow, which is attributed to the sample absorbing or evolving heat. The area under the peak is proportional to the change in enthalpy, and its direction indicates whether it is endothermic or exothermic. DSC can be used to detect the melting temperature, which is seen as a peak, and the enthalpy associated with this transition can be measured (Gray and Bemiller, 2003). DSC is the method used for quantitative measurements, since this produces a time-based plot in which the peak area is directly proportional to enthalpy (Stevens and Elton, 2006).

DSC was used to study the starch retrogradation (amylopectin recrystallization) of composite bread with hydrocolloids (HM pectin and CMC), emulsifiers (DATEM, LC and MG) and their interactions after storage (Paper IV).

However, according to earlier work, the objective values of these bread attributes do not reflect consumer preferences or freshness perception (Rosell, 2011). Consumer studies were performed for this reason (Paper III).

4.2.2.2. Sensory properties

Sensory evaluation can be useful for assessing the response of a determined product, and for gathering a product idea or feature from potential consumers. It can also provide information about how many consumers say they like the product when they are consuming it by using the scale based on nine labelled categories; it has actually become a standard tool in determining consumers' acceptance of food products (Gámbaro, 2012).

Two consumer studies were performed in the work reported in Paper III. The first is a consumer acceptance test (overall liking) and the other is also a consumer acceptance test based on the evaluation of sensory attributes (appearance, texture, crust and crumb colour, flavour and smell) and attitudes. The sensory evaluation method was based on a nine-hedonic scale (Meilgaard *et al*., 1999).

4.2.2.3. Other quality characteristics measured

Several other methods and tools than those described above were used in the studies described in Papers I-IV.

Microstructure of starch granules

The microstructure of the three cassava flours (Paper I) was examined by bright-field (BF) and polarized light microscopy (PLM). BF and PLM are the two traditional imaging techniques and were developed to achieve higher resolution, enabling soft matter research at length scales ranging from the molecular to the macroscopic level (Lee *et al.*, 2011). In BF, the sample is illuminated by unpolarised white light, and the contrast in the image results from direct interaction of the probing light with the sample, while polarizing microscopy (PM) uses polarized light for imaging of birefringence (Lee *et al.*, 2011).

In the BF and PLM, the cassava flour samples were stained with Lugol's iodine solution, and the slurries were then smeared and dried. After drying, the samples were stained with Lugol's iodine solution. The samples were further examined with a Microphot FXA light microscope (Nikon, Tokyo, Japan) using a 10x and a 40x objective. Images were taken with an Altra 20 Soft Imaging System camera (Olympus, Tokyo, Japan).

Weight, crumb density and crumb grain structure

Weight and crumb density were used to evaluate respectively the percentage of weight loss and the density of the material cells (porosity) after storage (Paper IV). To investigate the crumb grain structure of the composite breads with different improvers, the mean cell area (mm^2) was used, which gives an idea of the size of the cells of the bread crumb (Paper II).

4.3. Statistics

A factorial ANOVA was used only to quantify the relative significance of each of the control factors (pre-treatment, cassava ratio and HM pectin) and all two-way interactions between factors (pre-treatment x cassava ratio, pre-treatment x HM pectin and cassava ratio x HM pectin). The data were subjected to a least squares regression analysis with a multifactorial model (Paper I). One-way analysis of variance (ANOVA) and Tukey´s HSD post hoc multiple range test ($p < 0.05$) were used to evaluate the statistical differences owing to addition of improvers to the composite bread (Paper II), sensory attributes (Paper III) and quality parameters after storage (Paper IV).

5. RESULTS AND DISCUSSION

In the first part of this thesis, the effect on the quality of composite bread was examined by partial substitution of wheat flour with a combination of maize flour and three different pre-treated cassava flours, obtained by fermentation, roasting and sun drying (Paper I). Different concentrations of the three cassava flours in combination with two levels of HM pectin were investigated in a full factorial design. In two subsequent studies, roasted cassava flour was selected and different baking improvers added, two hydrocolloids (CMC and HM pectin) and three emulsifiers (DATEM, LC and SSL), with the aim of optimizing quality characteristics such as specific loaf volume, crust colour and crumb firmness in fresh composite bread and after storage (Papers II and IV). Finally, a consumer acceptance study was carried out with two selected optimized composite breads (Paper III).

This chapter presents and discusses results from the work reported in Papers I-IV.

5.1. Wheat flour substitution with cassava flour on bread properties of composite bread (Paper I)

The results of the factorial ANOVA for the level of cassava, type of pre-treatment of the cassava flour, HM pectin and their interactions are summarized in Figure 13. The factorial ANOVA represents how much of the variability of the data is explained by each factor and interaction, in terms of the percentage of the total sums of squares. This analysis revealed significant effects on bread firmness by type of pre-treatment, cassava level and the interactive effects (Fig. 13a), explaining 71% of the effect. The major effect on firmness was related to cassava pre-treatment (40%). On the other hand, pre-treatment had only a minor effect on bread volume (6%) while the major effect was related to the cassava level (27%) (Fig.13b). However, the interactive effects of pre-treatment with the level of cassava and with the HM pectin content were high (33%). For more detail on the results in terms of the analysis of the nature of these interactive effects, see Paper I in the appendix.

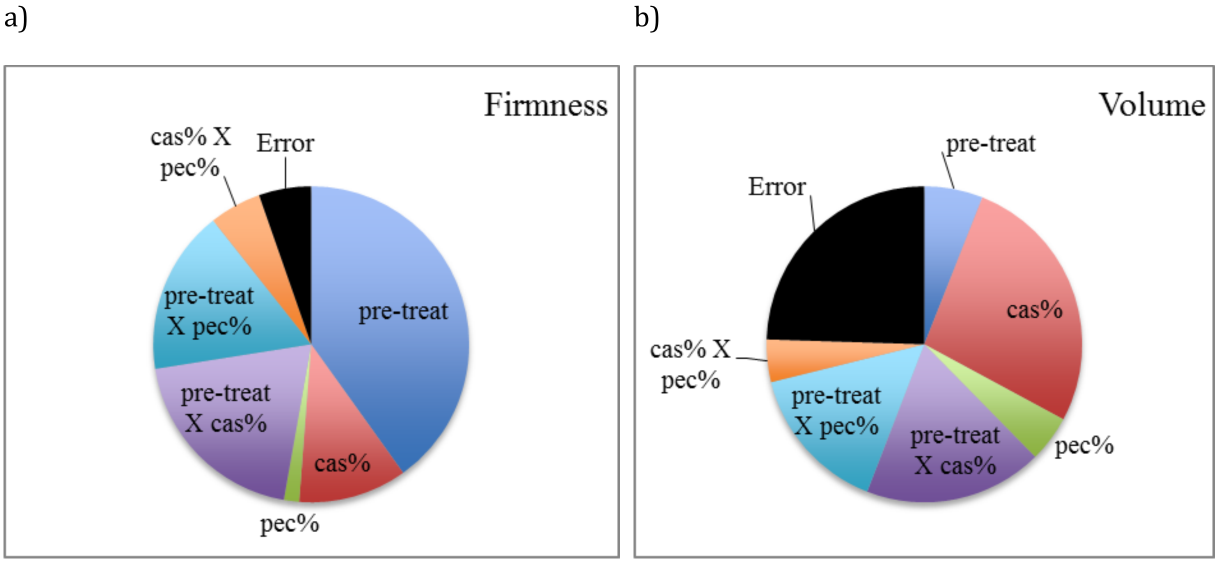


Figure 13 Percentage of the total sum of squares of the data for (a) bread firmness and (b) bread volume that is explained by each factor and interaction. ***"pre-treat" denotes the cassava pre-treatment used, "cas%" the level of cassava (%) in the recipe and "pec%" the pectin level (%). All effects were statistically significant at a 90% confidence level.***

The use of three differently processed cassava flours resulted in composite breads with varying effects on volume and crumb firmness (Table 4). Bread loaves with an addition of different cassava flour types had a lower volume than wheat bread (135.0 ± 4.5 cm^3). At a low cassava level (20%), the volume of the breads was similar. However, with flours from sun-dried and fermented cassava, the volume of bread loaves decreased with a higher cassava level. A higher cassava level of roasted cassava flour did not affect the loaf volume. Negative effects of non-wheat flours on the volume of composite breads can be explained by their dilution effect, which leads to reduced flour strength and a lower ability of the gluten network to enclose the carbon dioxide produced during fermentation. Our findings agree with the work on composite breads reported by Siddiq *et al.* (2009), Abdelghafor *et al.* (2011), Schoenlechner *et al.* (2013) and Trejo-González *et al.* (2014) who found a reduced loaf volume with an increase in non-wheat flour (maize, sorghum, millet and sweet potato) in the dough made from wheat flour.

Table 4 Mean values of firmness and volume for composite bread types

	Effect on	
Type of cassava flour	Volume (cm^3)	Crumb firmness (N)
Sun-dried (%):		
20	107.5±4.1	8.6±0.4
30	90.5±2.9	11.0±0.3
40	82.4±3.4	11.7±0.2
Roasted (%):		
20	102.0±2.2	7.8±0.3
40	103.7±2.4	7.3±0.2
Fermented (%):		
20	108.8±4.3	8.8±0.5
40	90.9±5.4	9.7±0.6

The means for the different cassava levels with the standard error being due to white noise and to the influence of HM pectin content

With regard to bread texture, it was found that the effect of the pre-treatment along with the higher cassava level had a significant effect on crumb firmness, with the flour of sun-dried cassava giving the firmer breads and roasting the softer ones. With the lower level of cassava, roasting gave a slightly softer bread, while the other two pre-treatments resulted in breads with a similar crumb firmness. Bread with roasted cassava appeared to have the same firmness as the wheat control bread (7.3±0.5 N). The positive effects of roasted cassava flour on bread crumb firmness can be explained by their previous gelatinization, which provides a high swelling capacity at the dough phase. Increased resistance against disintegration and formation of soft agglomerates at high temperatures (90^oC) has been reported by Tivana *et al.* (2009).

The colour of the crust, expressed as the brownness index (BI), has been also an important parameter for characterizing composite bread made from different cassava flours. The wheat reference bread had a BI value of 91.8±2.3. With sun-dried or fermented cassava flour in the composite flour, the brownness value decreased with a higher amount of cassava flour (BI ≈ 58) while it increased with roasted cassava flour compared with the reference wheat bread.

Overall, our findings suggest that the addition of any of the above mentioned cassava flours (sun-dried, fermented and roasted) reduced the loaf volume and increased the crumb firmness (except for bread with roasted cassava) compared with wheat bread. However, an increased cassava level, from 20% to 40% had a variable effect on the bread quality. The volume of bread with roasted cassava flour did not change, while it was further reduced with the other two cassava flours (sun-dried and fermented). The crumb firmness remained the same and was similar to wheat bread in bread with roasted cassava and increased in bread with the other two cassava flours.

In summary, these results indicate that, depending on the pre-treatment of the cassava flour, there is a potential for using cassava flour in breadmaking. In particular, roasted cassava flour showed a high potential, even at high level of substitution, to improve the quality characteristics of composite bread. This pre-treatment was further investigated at a high level of addition (40%) for optimization of the baking improvers. The quality characteristics evaluated were specific volume, crust colour, crumb moisture and firmness.

5.2. Improving composite breads by addition of baking improvers (Paper II)

Five different baking improvers were included in this study. Two hydrocolloids and three emulsifiers were used. The hydrocolloids were CMC and HM pectin, which have different functionalities in dough processing. CMC is a cellulose derivative that is mainly used for controlling viscosity without gelling (Kohajdová and Karovicová, 2009) and as a thickening agent (Milani and Maleki, 2012). HM pectin is a high polysaccharide substance and is used as a gelling agent (Milani and Maleki, 2012). The emulsifiers SSL, DATEM and LC differed in their hydrophilic-lipophilic balance (HLB) and varied in the range of 3 (LC) and 21 (SSL).

5.2.1. Specific loaf volume

The specific volume of the composite reference bread (without improvers) was 1.94±0.06 cm^3/g. The addition of hydrocolloids (CMC and HM pectin) and their combination with emulsifiers (DATEM, LC and SSL) at different levels (0.1%, 0.3% and 0.5% on a flour basis) increased the specific volume compared with the reference bread. The addition of CMC caused an increase in volume of 10.8% while 7.5% was obtained with HM pectin. Kohajdová and Karovičova (2009) previously reported that CMC improved the volume yield of certain doughs as a result of the drop in viscosity during baking by imparting improved elasticity to the dough through encouraging gas bubble formation.

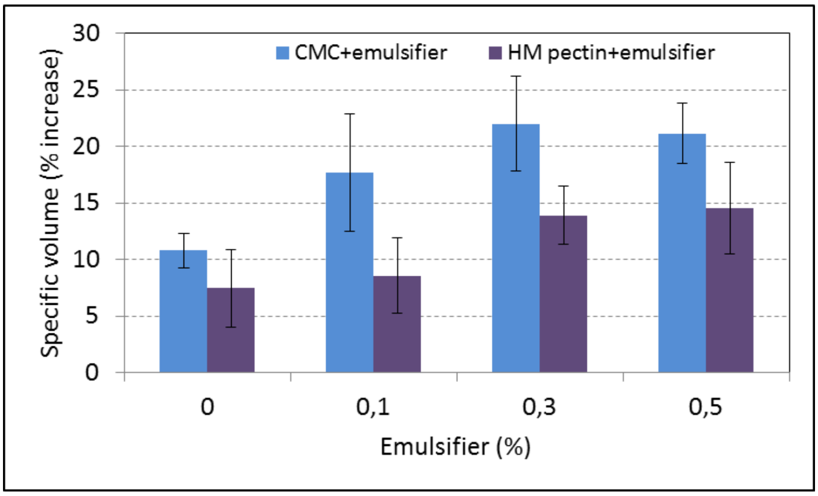


Figure 14 Effect of hydrocolloids (CMC and HM pectin) and their combination with emulsifiers (DATEM, SSL and LC) on the specific loaf volume of composite cassava-maize-wheat (40:10:50%) breads. Error bars indicate the standard deviation.

The specific volume was significantly higher for breads prepared with combinations of CMC/emulsifiers than for breads with HM pectin/emulsifiers (Fig. 14). The specific volume results for bread samples prepared with CMC/emulsifiers and HM pectin/emulsifiers are presented as average values. The addition of emulsifiers to composite bread further increased the specific volume of the breads containing hydrocolloids, from 10.8% to between 17.7% and ~22% for CMC, and from 7.5% to between 8.6% and 14.5% for HM pectin. A possible explanation of these results can be based on the two different chemical components of these emulsifiers, the lipophilic part (L) and the hydrophilic part (H), expressed as the HLB value. SSL is an ionic molecule and is less hydrophobic (HBL = 21.0) than DATEM (HLB= 9.2) (Armero and Collar, 1996) and LC (HLB=3.0-4.0) (Kamel and Ponte Jr., 1993). According to Gómez *et al*. (2013b), the hydrophilic chain of SSL can favourably interact with ionic bonds in the gluten proteins, thereby producing gluten agglomeration and strengthening of the dough structure during baking. On the other hand, DATEM may interact cooperatively with gluten proteins and flour lipids at the air/water interface, and may therefore improve the gas holding ability of the dough.

5.2.2. Crumb firmness

Crumb firmness measured by the Instron Universal Testing Machine (UTM, model 5542) revealed that the addition of baking improvers such as hydrocolloids (CMC and HM pectin), emulsifiers (DATEM, SSL and LC) and their combinations was found to be significantly effective in lowering composite bread firmness (Figure 15).

The firmness of reference composite flour bread without baking improvers was 7.1±0.3 N, which was reduced by 34.0% with the addition of CMC and by 13.9% with HM pectin. Rosell *et al*. (2001) similarly observed a decrease in the crumb firmness of wheat bread with an addition of hydrocolloids (κ-carrageenan and HPMC), and Crockett *et al*. (2011) reported similar responses in gluten-free breads based on rice/cassava starch made with high methoxy (HM) HPMC.

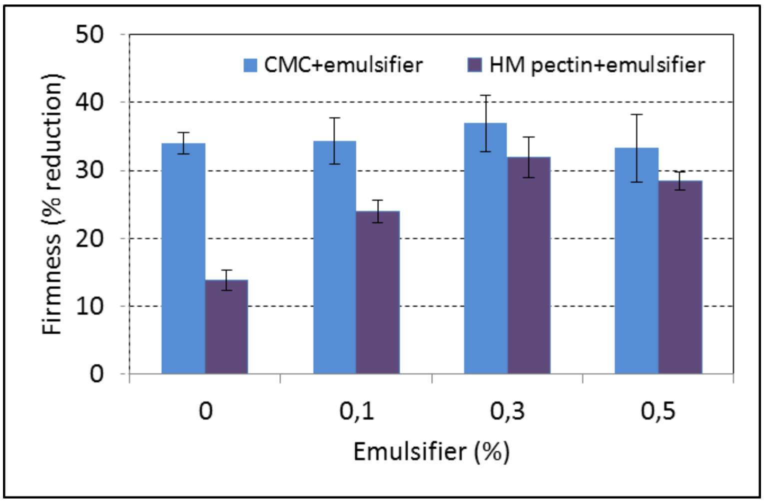


Figure 15 Effects of hydrocolloids (CMC and HM pectin) and their combination with emulsifiers (DATEM, SSL and LC) on the firmness of composite cassava-maize-wheat (40:10:50%) breads. Error bars indicate the standard deviation.

Adding emulsifiers to composite breads with HM pectin, had a further softening effect, the firmness was reduced by about 28%, while no further effect was obtained by adding emulsifiers to CMC bread (Figure 15). The firmness of bread samples prepared with CMC/emulsifiers and HM pectin/emulsifiers is presented as average values.

The positive effect of emulsifiers is due to their ability to aggregate gluten proteins, which create a gluten network that can improve the entrapment of air and result in an increased bread volume and thereby reduced crumb firmness (Stampfli and Nersten, 1995).

5.2.3. Crust colour

The BI value of the reference bread was 57, while the BI value of loaves with either CMC or HM pectin were increased by 40% and 16%, respectively, which is in agreement with previous findings in gluten-free bread with xanthan (Sciarini *et al.*, 2010) and HPMC and carrageenan (Sabanis and Tzia, 2011). With the addition of emulsifiers, the BI values significantly increased in all bread containing HM pectin. However, there were no varying effects on BI values of bread containing CMC by adding emulsifiers. For more detail on the results in terms of the effects of emulsifiers on the BI of composite bread, see Paper II in the appendix.

In summary, this study showed that addition of hydrocolloids or combinations of hydrocolloids and emulsifiers as baking improvers could significantly improve the quality characteristics (loaf specific volume, crumb texture and crust colour) of composite cassava-maize-wheat breads. In the presence of HM pectin, the best specific volume, firmness and browning index values were obtained in breads containing emulsifier at 0.5% and 0.3%. However, the desired bread quality results could be obtained for breads prepared with CMC containing emulsifier at varying levels. Therefore, to obtain the acceptable quality values in composite bread produced from cassava-maize-wheat flours, emulsifier at 0.3% level can be recommended to be used with CMC. An acceptability test was carried out by a Mozambican consumer panel in order to investigate the sensory quality of the composite cassava-maize-wheat bread at the proportion 40:10:50 that was optimized with respect to high specific loaf volume and low crumb firmness according to results in Paper II. Therefore, two composite breads were selected with either CMC or HM pectin and with 0.3% emulsifier (Paper III).

5.3. Consumers' acceptance of composite breads using baking improvers (Paper III)

The sensory test was carried out with untrained Mozambican consumers in two different consumer studies. In the first consumer study, the overall liking of composite bread was evaluated by 79 consumers while 52 were used in the second study to evaluate consumer acceptance and attitudes to optimized composite bread (Paper III). The sensory attributes evaluated were appearance, texture, smell, flavour, crust and crumb colour, and overall quality. The overall quality was calculated as an average of the attributes evaluated.

The sensory evaluation method was based on a nine-point hedonic scale. The classification of the sensory attributes was done according to the scale ranging from one (dislike extremely) to nine (like extremely), with five being neither like nor dislike (Peryam and Pilgrim 1957; Lawless and Heymann 1999, 2010). Bread was considered acceptable if the mean value was above five. The results of the first consumer study showed that composite cassava bread was ranked similarly to wheat bread.

In the second study, the results showed a generally favourable response to the composite bread in comparison to commercial wheat bread. However, appearance and crust colour of the composite breads obtained significantly lower sensory scores compared with commercial wheat bread.

The following paragraphs present the results of the sensory evaluation of quality attributes, the relationship between sensory evaluation and instrumental analysis and general information on the bread consumption pattern and intention of purchase.

5.3.1. Sensory evaluation of quality attributes

There was a significant difference ($p<0.05$) in appearance and crust colour between the commercial wheat bread and the two composite breads made with CMC/DATEM (CBA) and with HM pectin/LC (CBB) (Table 5). No differences were observed between the composite breads and the reference wheat bread in the attributes of smell and flavour, which might be expected as the improvers used are not supposed to influence the sensory attributes of fresh composite bread. These results agree with the findings reported by Guarda *et al*. (2004) in their study of different hydrocolloids as bread improvers. In general, CBA bread was scored higher for the majority of the attributes evaluated as compared with CBB bread, which was scored lower for most of the sensory attributes.

Table 5 Mean scores of hedonic sensory attributes and consumer attitudes to purchasing composite cassava-maize-wheat breads with hydrocolloids and emulsifiers as compared with wheat bread (n=52)

Hedonic scale[1)]	Bread type		
	Commercial wheat	CBA	CBB
Appearance	8.33^{a}	7.67^{b}	6.80^{c}
Texture	7.73^{a}	7.40^{ab}	6.98^{b}
Smell	7.38^{a}	7.35^{a}	7.24^{a}
Flavour	7.35^{a}	7.50^{a}	7.04^{a}
Crust colour	8.08^{a}	7.38^{b}	6.80^{b}
Crumb colour	8.08^{a}	7.50^{ab}	7.09^{b}
Overall quality	7.82^{a}	7.47^{a}	7.01^{b}
Intention of consumption[2)]	4.23^{a}	4.08^{a}	3.47^{b}

[a,b,c]Mean values in the same row followed by a different letter differ significantly ($p < 0.05$). CBA (composite bread with CMC/DATEM); CBB (composite bread with HM pectin/LC). [1)]Hedonic scale (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much and, 1=dislike extremely). [2)]Scale of attitudes to consumption (5 = consume whenever had the chance, 3 = would consume if it was accessible, but not strive for it; 1 = consume only if forced).

The hedonic ratings for the quality attributes of composite cassava bread made with CMC/DATEM and wheat bread ranged from 7.35 (like moderately) to 8.33 (like very much) for all attributes. However, composite bread with HM pectin/LC received ratings for all attributes ranging from 6.8 (like slightly) to 7.24 (like moderately). The results showed that the overall quality score of CBA bread was similar to that of wheat bread but significantly higher than for CBB bread. The lower quality score of CBB bread is probably due to its lower score in the attributes of appearance and crust colour (Fig. 16). In our study, bread with an acceptable quality was defined to have a score > 5 on nine-point hedonic scale and highly acceptable with a score larger than 7 (Paper III). Therefore, although CBB bread was rated lower than CBA bread, it received an overall quality score of 7, which makes this bread also highly acceptable.

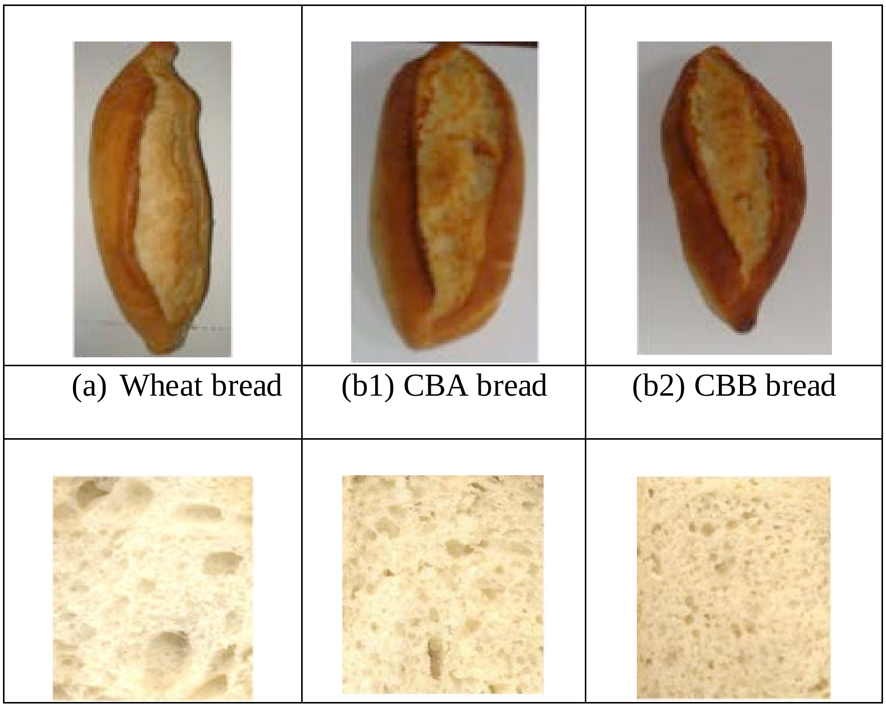


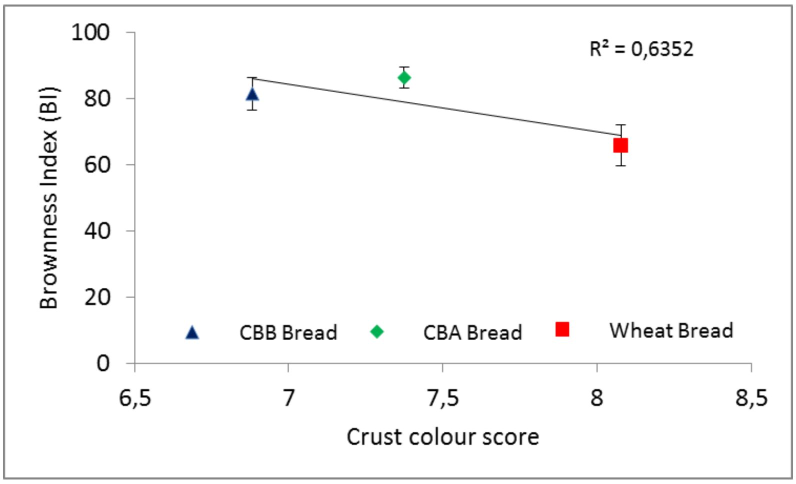
Figure 16 Crust appearance (upper) and breadcrumb (lower) of (a) commercial wheat bread; (b) composite bread: (b1) with 3% CMC and 0.3% DATEM (CBA) and (b2) with 3% HM pectin and 0.3% LC (CBB).

The intention (attitudes) of consumption to composite bread among the studied group of consumers was also verified (Table 5), and CBA bread obtained a similar score for intention of consumption as for wheat bread.

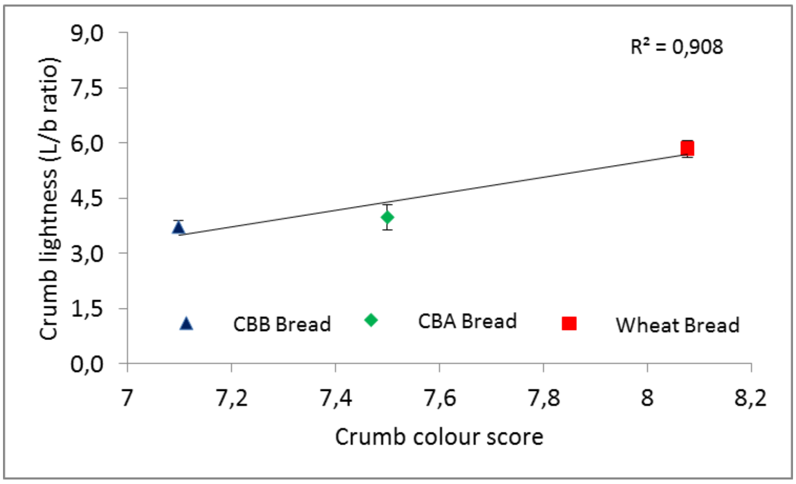
5.3.2. Relationship between the sensory evaluation and instrumental analysis

Figure 17a, 17b and 17c show the relationship between the sensory evaluation and instrumental analysis for the composite bread attributes. Significant correlations were found between the crust colour score and BI (R^2 = 0.64) (Fig. 17a). It can be seen from Figure 16a that the wheat bread had a higher crust colour score and lower BI values as compared with the two composite breads. These results indicate that the lower brownness crust colour is more preferred by the consumers, which is in accordance with a high acceptability of this attribute for wheat bread (Table 5). However, the parameters of crumb colour (R^2 = 0.91) (Fig. 17b) and firmness (R^2 = 0.87) (Fig. 17c) showed stronger correlations with corresponding sensory scores. In conclusion, these results indicate that consumers prefer lighter bread (lower white-to-yellow ratio) and bread with a softer crumb texture (lower crumb firmness).

(a)



(b)



(c)

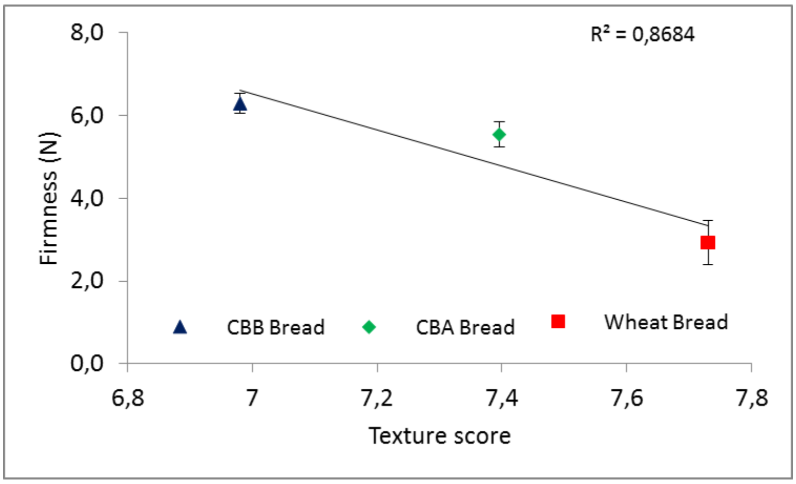


Figure 17 Relationship between instrumental and sensory scores for the quality attributes of composite cassava-maize-wheat (40:10:50%) breads with hydrocolloids and emulsifiers. (a) crust colour; (c) crumb colour; and (c) bread firmness. Error bars indicate the standard deviation.

5.3.3. General information on bread consumption pattern and purchase intention

The distribution of respondents in the purchase and consumer pattern for wheat bread (Table 6) revealed that 34.6% of the respondents pay for a bread loaf of 200 g less than 1.00 US$ and that 82.7% buy bread more than three times per week. Of those who consume bread, about 32.7% ate bread more frequently, while 8% ate less than three times a week. Appearance was the most important criterion that respondents used to decide whether bread was no longer edible, followed by texture, length of storage and odour.

The majority of the respondents (94.2%) said that they would accept bread with an addition of cassava flour, while the remaining 5.8% claimed that they would not accept it. Further, when asked why it would be accepted, while 50% of the respondents considered nutritional quality to be very important in accepting a composite bread, 28.8% regarded variety to be of great importance and 15.4% were concerned with the price of bread. However, a greater proportion (92.3%) expressed their willingness to purchase bread made of a mixture of two or more flours while 84.6% were unwilling to pay for a mixture of flours.

Table 6 Distribution of respondents based on purchase and consumer pattern in wheat bread among the study population (n=52)

Variables	Frequency	Percent
How much do you normally spend to pay (in US$) for the bread (a loaf of bread of 200 g)[*]:		
Less than 0.33	13	25.0
0.33-0.67	18	34.6
0.67-1.34	10	19.2
1.34-1.67	6	11.5
Greater than 1.67	5	9.6
How often do you buy bread:		
Less than once a week	0	0.0
Once a week	3	5.8
Twice a week	3	5.8
Three times a week	3	5.8
More than three times a week	43	82.7
Bread consumption:		
Once a day	12	23.1
More than once a day	11	21.2
1-2 times a week	4	7.7
3-4 times a week	8	15.4
5-7 times a week	17	32.7
How do you decide if your bread is no longer edible:		
Appearance (mould, colour)	34	65.4
Texture (too hard)	8	15.4
Length of storage	7	13.5
Odour	3	5.8

*) 1US$=29.91 MT (source: Banco de Moçambique, 17th of February 2013)

5.4. Effect of storage on the quality of composite breads with baking improvers (Paper IV)

Changes in the quality parameters such as weight, crumb density, moisture content, firmness and starch retrogradation (recrystallized amylopectin) were measured in composite bread after storage for four days (d) at 23°C and 50% relative humidity (RH). The composite cassava-maize-wheat breads were prepared with an addition of 3% hydrocolloids (CMC or HM pectin) and/or 0.3% emulsifiers (DATEM, LC and MG), and the results were compared with composite reference bread (without improvers) (Paper IV). Significant differences (*p<0.05*) (Table 7) were obtained in these parameters when baking improvers were added.

Table 7. Crumb moisture, crumb firmness and melting enthalpy of composite bread samples after four days of storage, as affected by hydrocolloids, emulsifiers and combinations of both improvers

Bread formulations	Crumb moisture (% wet basis)		Crumb firmness (N)		$\Delta H_{retr.}$ (J/g dry crumb)
	Fresh bread	4-d storage	Fresh bread	4-d storage	4-d storage
No emulsifier or hydrocolloid	48.6^{abc}	27.7^{c}	7.0^{e}	33.4^{g}	20.0^{g}
Emulsifiers (0.3%):					
DATEM	48.8^{bc}	29.3^{cd}	5.1^{c}	23.0^{d}	14.0^{def}
LC	47.8^{ab}	28.8^{cd}	6.1^{d}	36.5^{h}	23.2^{h}
MG	48.6^{abc}	24.8^{a}	5.7^{d}	29.8^{f}	15.6^{f}
Hydrocolloids (3%):					
CMC	47.5^{a}	29.5^{cd}	4.2^{b}	24.6^{e}	12.8^{cd}
HM pectin	48.0^{ab}	27.5^{bc}	3.8^{ab}	22.4^{d}	14.8^{ef}
Hydrocolloids+ Emulsifiers (0.3/3%):					
CMC/DATEM	49.7^{c}	31.2^{d}	3.6^{a}	12.4^{a}	10.6^{b}
HM pectin/DATEM	48.2^{ab}	29.3^{cd}	4.1^{ab}	17.0^{b}	10.3^{b}
CMC/LC	47.8^{ab}	27.3^{bc}	3.8^{ab}	16.6^{b}	6.7^{a}
HM pectin/LC	48.1^{ab}	29.0^{cd}	3.7^{a}	20.1^{c}	12.8^{cd}
CMC/MG	47.9^{ab}	25.3^{ab}	4.3^{b}	21.8^{d}	11.1^{bc}
HM pectin/MG	47.7^{ab}	24.7^{a}	5.7^{d}	29.2^{f}	15.8^{f}

[a-h]Values in the same column followed by different letters are significantly different ($p < 0.05$).
$\Delta H_{retr.}$: enthalpy of melting of the amylopectin recrystallization.
CMC: carboxymethyl cellulose; HM pectin: high methoxyl pectin; DATEM: diacetyl tartaric acid esters of monoglycerides; LC: lecithin; MG: monoglycerides.

The changes observed on the quality characteristics of composite bread after four days of storage were decreased moisture content and weight, and increased firmness and enthalpy of recrystallized amylopectin retrogradation.

The extent of starch retrogradation in reference composite bread after four days of storage was equivalent to a melting enthalpy of 20 J/g (dry basis), which was significantly higher (p<0.05) than that measured for breads produced with emulsifiers (except LC) and/or hydrocolloids alone. Taking into account that, in general, the weight/moisture loss was approximately the same for all the breads analysed (with the exception of breads prepared with MG, HM pectin/MG, CMC/MG and CMC/DATEM), the high enthalpy of the reference bread was probably a result of a significant increase in the crumb hardness.

5.4.1. Weight

The weight values for the composite bread decreased upon storage because of weight/moisture loss. The weight values of bread varied in the range of 44.2-45.5 g, 38.7-40.4 g and 30.8-33.9 g at 0 day, 1st day and 4th day of storage, respectively. In most cases, the addition of baking improvers did not affect the weight values (except for bread samples DATEM, MG, HM pectin, HM pectin/LC and CMC/MG at day 0, CMC/MG and HM pectin/MG at day 1 and LC, MG, CMC, CMC/MG and HM pectin/MG at day 4) compared with reference bread (day 0: 45.4 g, day 1: 40.0 g and day 4: 32.2 g).

5.4.2. Crumb moisture

The moisture value of crumbs with improvers varied within the ranges of 47.5 to 49.7% (wet basis) on the day of baking, which was similar to that of reference bread (without improvers) (48.6%, wet basis). In contrast, the moisture content of all breads decreased significantly with storage time (Table 7), although the decrease from day 0 to day 1 was not significant (p>0.05) (data not shown). Barrett *et al*. (2005) and Sabanis and Tzia (2011) also observed a reduction in the moisture content of crumbs due to storage. This observation was attributed to moisture migration from the crumb to the crust and subsequent evaporation. After four days of storage and compared with the reference bread, the moisture content tended to be lower in breads containing MG, and to be similar in breads with LC (Table 7). In breads with DATEM, however, the moisture content tended to be higher.

5.4.3. Firmness

The crumb firmness of the reference composite bread (day 0) was significantly higher than that of bread with baking improvers. Adding emulsifiers alone resulted in a softer bread crumb that was further reduced by the addition of hydrocolloids. Firmness values of bread crumbs prepared with CMC, HM pectin and their combinations with emulsifiers (DATEM, LC and MG) were similar (Fig. 18). The bread crumb hardness (Fig. 18) increased significantly in all composite breads with the length of storage time due to the starch retrogradation process and moisture loss (Table 7). After four days of storage, bread containing CMC/emulsifiers was in general softer (lower crumb firmness) than bread containing HM pectin/emulsifiers. However, bread containing only LC had the highest firmness value after four days of storage. This may be attributed to a lower ability of LC to interact with the amylose and amylopectin fraction of the starch, which is the major component responsible for firming of the crumb (Gray and Schoch, 1962). In the studies of Collar *et al*. (2001) and Correa *et al*. (2012), CMC and HM pectin reduced the crumb hardness with respect to wheat bread. This might be explained by the possible hydrogen bonding between hydrocolloids and starch that would delay starch retrogradation (Sabanis and Tzia, 2011). The crumb hardness in breads with either DATEM or MG alone followed the same trend as the crumb hardness of bread with hydrocolloids. Our results agree with those of Collar *et al*. (2001) and Azizi *et al*. (2003), who found an anti-staling effect of an addition of DATEM or MG in wheat bread, but they conflict with those of Azizi *et al*. (2003), who observed a delay in the staling rate of wheat bread with the addition of lecithin. The positive effect of these emulsifiers was attributed to the adsorption of emulsifier to the starch granule, as well as the formation of a starch-emulsifier complex, which prevent starch from taking up water released from gluten during the aging of bread (Pisesookbunterng and D'Appolonia, 1983).

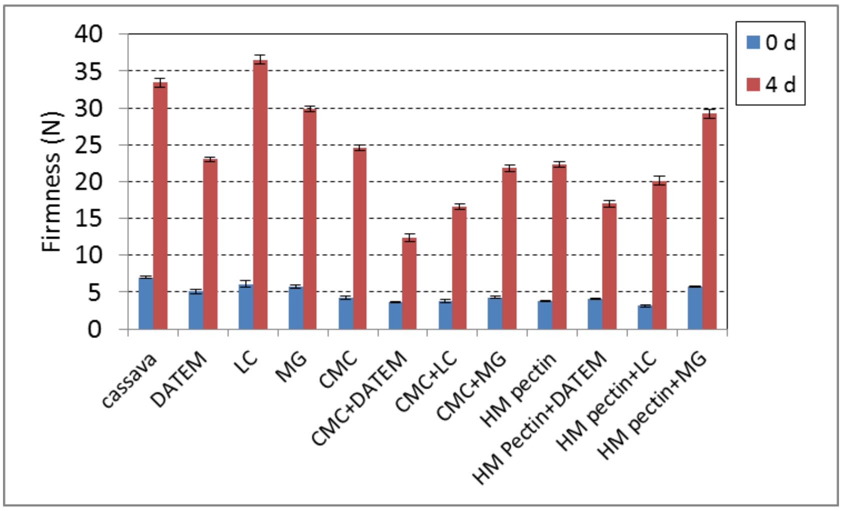


Figure 18 Effects of hydrocolloids (CMC and HM pectin), emulsifiers (DATEM, LC and MG) and their interactive effect on the crumb firmness of composite cassava-maize-wheat (40:10:50%) breads after storage. *Error bars indicate the standard deviation.*

5.4.4. Starch retrogradation

An increase in crumb hardness is associated with an increase in the melting enthalpy of retrograded amylopectin (Miyazaki *et al*., 2005). Concerning the onset temperature of stale composite bread (after four days of storage), this occurred in the range between 50.6 and 53.4^{o}C, and it corresponded to a melting enthalpy of 6.7 to 23.2 J/g dry crumb (Paper IV) (fresh bread with or without improvers showed no transition in the range of 35 and 70^{o}C, indicating that starch was gelatinized and that amylopectin was not yet retrograded). The reference bread with a high firmness value also had a high enthalpy value, and thereby a high amylopectin retrogradation after four days of storage. Composite bread containing LC had similarly high firmness and enthalpy values. In contrast, CMC combined with emulsifiers had a significantly lower firmness and melting enthalpy values, followed by HM pectin/emulsifiers and CMC (Figure 19). Purhagen *et al*. (2012) found that the addition of emulsifier gave less retrograded starch in gluten-free bread, and thereby increased the firmness of those breads.

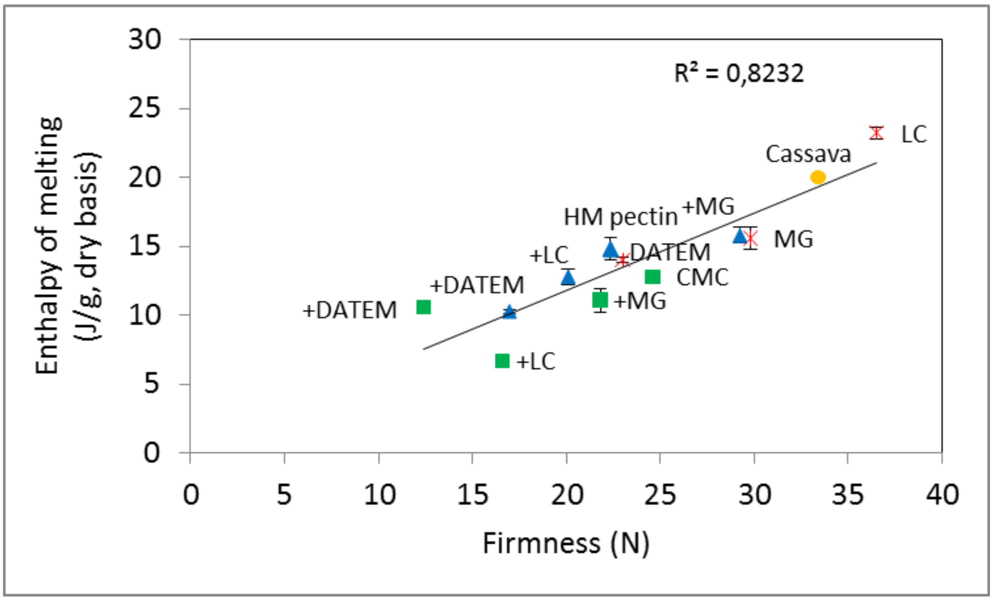


Figure 19 Melting enthalpy of retrograded amylopectin compared with the firmness of stale composite cassava-maize-wheat (40:10:50%) bread prepared with hydrocolloids (CMC and HM pectin), emulsifiers (DATEM, LC and MG) and their combination and stored for four days. Error bars indicate the standard deviation.

The melting enthalpy of retrograded amylopectin in stale composite bread had a linear relationship with the firmness of stale bread (R^2 = 0.82) (Fig. 19). This indicates that the firming of composite bread prepared with different baking improvers is related to recrystallization of amylopectin.

In summary, bread containing CMC/emulsifiers had lower firmness than bread containing HM pectin/emulsifiers or HM pectin alone. Bread containing LC and the reference bread had the highest firmness compared with DATEM, CMC and MG. Thus, the greatest single softening effects were provided by hydrocolloids, but a combination of hydrocolloid/emulsifier gave softer breads.

5.5. Cost of ingredients of improved composite breads

The sensory analysis indicated that the improved composite breads have a high overall quality acceptance (Paper III). Table 8 shows the comparative cost of the ingredients used in bread manufacturing to produce 1 kg of dough for improved cassava bread (CBA and CBB) and commercial wheat bread.

Table 8 Comparative estimated cost of the ingredients between the improved composite cassava-maize-wheat breads and commercial wheat bread

Ingredients	Price (US$/kg)	Added amount (%)			Cost/kg of dough (US$)		
		CBA[g]	CBB[i]	Wheat	CBA	CBB	Wheat
Wheat flour[a,e]	1.14	50	50	100	0.57	0.57	1.14
Cassava flour[b,e]	0.26	40	40	0	0.10	0.10	-
Maize flour[a,e]	0.91	10	10	0	0.09	0.09	-
Baking improver[c,f]	7.77	0	0	0.3	-	-	0.02
CMC[d]	2.74	3.0	0	0	0.08	-	-
DATEM[d]	2.95	0.3	0	0	0.01	-	-
HM pectin[d]	5.50	0	3.0	0	-	0.17	-
LC[d]	0.88	0	0.3	0	-	0.003	-
Total					0.86	0.93	1.17
Cost of the composite dough as a % of wheat dough					73.5	79.5	

Source: [a] SIMA (2014). [b]AJM (2013). [c]Pão Bom bakery (2013). [d]Alibaba.com (Global Trade) (2014). [e]Tax rate: US$/MT=30.65 (Banco de Mocambique, April, 2014). [f]Composition: soy flour, oxidant agent E300 and baking enzymes. [g]CBA composite bread made with CMC/DATEM. [i]CBB composite bread made with HM pectin/LC.

The estimated cost to make CBA and CBB breads is about 74% and 80% of the cost for making wheat bread. It appears that it would be economic to partially substitute wheat flour by cassava-maize flour combinations in breadmaking. However, a full cost analysis must be made in order to study the viability of the present project.

6. CONCLUSIONS

The results in this thesis show that cassava flour derived from different processing techniques (sun drying, fermentation and roasting) had varying effects on the quality characteristics of composite bread (loaf volume, crumb firmness, crust colour). Compared with the wheat reference bread the different cassava breads had about 20 to 30% lower volume. Breads with low cassava level (20%) had similar volumes independent of the type of cassava flour, but increasing the cassava level (from 20% to 40%) resulted in a significant decrease of volume for all breads, except for bread formulated with roasted cassava flour. For sun-dried and fermented breads, increasing the cassava level resulted in firmer bread crumb compared with roasted bread.

In order to compensate for the lower gluten content of the composite flour breads, the effect of HM pectin at 1 and 3% (w/w) on bread quality was evaluated. Increasing both the HM pectin content and the level of roasted cassava flour resulted in a softer crumb and a higher loaf volume compared with bread of the other two cassava flours. Bread with roasted cassava flour had significantly more attractive yellow brown crust colour compared with the other two breads (sun-dried and fermented). In relation to the important objective of achieving bread similar to that made with wheat flour in terms of volume, firmness, and crust colour, roasted cassava flour is the most promising pre-treatment.

Bread produced from composite flours of roasted cassava, maize and wheat (40:10:50%, respectively) could be improved by the addition of hydrocolloids (CMC and HM pectin) in combination with emulsifiers (DATEM, LC and SSL). The specific loaf volume was significantly higher in comparison with composite bread baked with hydrocolloids or emulsifiers alone. The crumb firmness was significantly lower by a combination of hydrocolloids and emulsifiers. However, the combination of hydrocolloids and emulsifiers that will result in the highest quality characteristics (loaf volume and crumb firmness) of cassava composite bread, was CMC with emulsifier at 0.3% level.

A consumer sensory analysis of optimized composite bread formulations (40% roasted cassava/10% maize/50% wheat) with respect to improvers (CMC/DATEM and HM pectin/LC at a ratio of 3:0.3%) showed quality attributes similar to those of wheat bread in terms of smell and flavour. Composite bread with CMC/DATEM showed a higher overall quality compared to HM pectin/LC bread; however, both composite breads were highly acceptable in all sensory attributes as they received scores between seven and eight on a nine-point hedonic scale. Crust brownness index, crumb colour and firmness measured instrumentally correlated well with their hedonic sensory properties counterparts, which suggests that consumers prefer bread with characteristics similar to those of wheat bread. The general information on the bread consumption pattern indicated a high acceptability of and willingness to purchase composite bread based on cassava flour.

Bread staling during storage of composite bread with baking improvers showed that the main effect of hydrocolloids was to reduce crumb firmness and to delay the retrogradation peak temperature. CMC/LC, HM pectin/DATEM and CMC/DATEM (at 3:0.3% w/w, respectively) were especially effective in retarding amylopectin recrystallization (decreased melting enthalpy) in composite bread.

The estimated cost of the ingredients, based on the composite bread samples considered in sensory evaluation, is much lower than the cost of wheat bread. There is therefore an advantage in using composite flours in breadmaking.

This study showed that the composite maize-wheat bread quality with a high level of roasted cassava (40%) could be improved by using emulsifiers in combination with hydrocolloids. Taking into account the bread quality (loaf volume, crumb texture and colour), bread staling and the estimated cost for the composite bread with the highest consumer acceptability, the following composition is suggested: 40% roasted cassava flour, 10% maize flour, 50% wheat flour improved with 3% CMC and 0.3% DATEM. However, further work must be carried out to explain the mechanisms that lead to the augmented effects.

7. FUTURE OUTLOOK

The aim of this thesis was to study the partial substitution of wheat flour by locally grown material (cassava and maize) in a high quality bread product. However, these non-wheat flours are flours that do not form a gluten structure. This induces a gradual loss of gluten in the composite flour mixtures, leading to a lower bread making potential (e.g. reduction in loaf volume). This is due to the reduction of viscoelastic properties of composite dough, which might be explained by the reduced capacity of the gluten network to slow the rate of carbon dioxide diffusion. Therefore, the use of different treatments of cassava roots before their being processed into flour and the application of baking improvers were also important parameters of the present study. This work thus provides insight into how the composite roasted cassava-maize-wheat bread (40:10:50%, respectively) can be improved.

Some further investigations of the following issues are suggested:

- Cassava flour was produced with three different methods and it was found that the roasting treatment was best for the substitution of a higher amount of wheat flour and the quality characteristics of the composite bread. Further study of other heat treatments for cassava roots, e.g. blanching (hot water and steam), which is a short and mild heat treatment prior to the main process, could provide valuable information on the quality characteristics of composite bread.

- It would be rewarding to further study the role of other improvers, e.g. enzymes, in improving the quality of composite bread. It has been demonstrated that transglutaminase has good results for increasing the technological quality of gluten-free bread based on rice (Shin *et al.*, 2010). Amylases retards amylopectin retrogradation via modifications on the structure of starch (Gray and Bemiller, 2003).

- Cassava flour has a low protein content, which affects the overall nutritional aspect of bread when high levels of cassava flour are used. It would therefore be important to investigate how to increase the protein content/nutritional value of the composite bread by using locally grown legumes rich in proteins.

- To be able to implement the production of the composite cassava-maize-wheat bread (40:10:50%) it would be interesting to make a feasibility study to evaluate costs versus the quality of the composite bread.

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