Effect of transglutaminase and cyclodextrinase on the rheological and shelf-life characteristics of oat bread

Patrick Hermaan Nitcheu Ngemakwe

Thesis presented in partial fulfilment of the requirements for the degree of

Master of Technology (Food Technology)

Department of Food Technology

Faculty of Applied Sciences

Cape Peninsula University of Technology

Supervisor: Prof. V. A. Jideani

Co-Supervisor: Dr. M. Le Roes-Hill

September 2014

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not previously, in its entirely or in part, been submitted at any other university for a degree.

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Patrick Hermaan Nitcheu Ngemakwe   Date

ABSTRACT

The aim of this study was to evaluate the effect of transglutaminase (TG) and cyclodextrinase (CG) on the rheological characteristics of oat dough and shelf-life characteristics of oat bread with a view to developing oat bread with improved texture and shelf-life. Firstly, the effects of yeast, carboxylmethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) and cyclodextrinase (CG) on the mixing, pasting, thermal, quantification of free amino acid groups and protein crosslinking properties of oat dough were investigated through a 2^{5-2} fractional factorial design resolution III with yeast (1.25, 3.25%), CMC (1, 2%), YG (10.75, 33.75%), TG (0.5, 1.5%) and CG (10, 40 µl) as independent variables. Among all the ingredients, only CMC, YG, and TG exhibited significant ($p < 0.05$) effects on the mixing properties of oat dough while yeast and CG slightly affected it. TG addition increased water absorption (34.80 - 38.45%) and peak resistance (696.40 - 840.30 FU) but decreased the dough softening (93.20 - 67.75 FU) as its level varied from 0.5 to 1.5 g. CG did not significantly ($p > 0.05$) affect the mixing properties of oat dough. As its level increased from 10 - 40 µl, the water absorption (38.45 - 34.80%), energy at peak (11.45 - 3.75 Wh/kg), peak resistance (840.30 - 696.40 FU) slightly decreased while the softening of oat dough increased from 67.75 to 93.20 FU. The addition of yeast and YG showed significant ($p < 0.05$) impacts on the pasting properties of oat dough compared to CMC, TG and CG. The storage modulus of oat dough was slightly ($p > 0.05$) increased by adding TG (180.37 - 202.78 kPa) and CG (170.75 - 175.71 kPa). TG decreased the loss modulus (65.95 - 62.87 kPa) of oat dough while CG increased it from 62.01 - 64.61 kPa. The thermal properties of oat dough were slightly affected by all the ingredients. The denaturation temperature was increased by incorporation of TG (6.53 - 8.33°C) and CG (6.42 - 8.33°C) but there was a decrease of enthalpy due to addition of TG (from 0.76 to –4.05 J/g) and CG (1.11 to –4.05 J/g). Only CG decreased the number of free amino acid groups (0.94 - 0.62) confirming that it catalysed the protein crosslinking of the oat glutelin while other ingredients increased it. Secondly, as CMC, YG and TG affected the mixing, pasting and thermal properties of oat dough, oat bread was baked with carboxylmethylcellulose (CMC), yoghurt (YG) and transglutaminase (TG) following a 3^3 Box-Behnken design consisting of CMC (1, 2 g), YG (10.75, 33.75 g) and TG

(0.5, 1.5 g) as independent variables. The physical and textural analysis of oat bread showed that CMC, YG and TG addition did affect oat bread. TG decreased the springiness (6.47 - 4.14 mm), specific volume (1.61 - 1.54 ml/g) and increased hardness (537.85 - 692.41 N) of oat bread. No significant effect was observed on the colour parameters of crust and crumb of oat bread. Despite the optimal oat bread exhibited a high desirability, its high hardness and low springiness remain some challenges associated with oat bread production. Since it was well established that TG increased hardness and decreased springiness of the optimal oat bread, improvement was needed for the production of best oat bread. Thirdly, Psyllium husks (PH) and cyclodextrinase (CG) were added in five (05) best oat bread formulations such as (1) PH + CG, (2) CG, (3) TG + CG, (4) TG + PH and (5) TG + PH + CG. The best oat bread formulation with low hardness containing PH and CG was further used for sensory and shelf-life studies. The combination of ingredients psyllium husks and cyclodextrinase significantly ($p < 0.05$) improved the textural properties of best oat bread. It decreased the hardness (94.88 N) and increased the springiness (10.97 mm) of the best oat bread. Fourthly, the sensory evaluation showed that the consumers highly appreciated the crumb colour and texture of the best oat bread than the ones of wheat bread. In addition, they found that there was a strong correlation in crust and crumb colour between wheat and the best oat bread. However, some differences existed between the wheat and best oat bread. The best oat bread exhibited a less preference in taste than its wheat counterpart. The best oat bread positively received an overall acceptability (4.07) as wheat bread (4.22). Fively, the shelf-life studies of the best oat bread revealed that the pH and TVC of the best oat bread were more affected by the time, temperature and the interaction of both parameters (time and temperature) than Total Titratable Acidity (TTA), yeasts and mould as the storage time passed. The best oat bread could safely be stored up to 21 days at refrigeration temperature (5°C) with a Total Viable Count (TVC) load of 10^5 cfu/g. Finally, using survival analysis for the shelf-life studies of the best oat bread, the mathematical model revealed that the risk of deteriorating increased with the temperature.

CONTENTS

Language and style used in this thesis are in accordance with the requirements of the International Journal of Food Science and Technology. This thesis represents a compilation of manuscript where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

DEDICATION

Dedicated to the Lord God my Heavenly Father who made me for His own glory, and my family, my mother Marceline Mboyo, late father, Michael Ngemakwe, my siblings Bertrand, Laurent and Frank with bottomless gratitude for their love, support and believing in me, last but not least Knowledge Marcelle for being there.

CHAPTER 1
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Bread in its various forms is one of the most common staple foods consumed in the world. It is traditionally based on flour derived from the cereal, wheat. Many other types of cereals, pulses and legumes can be milled to give flour. However, the ability of the wheat proteins (gluten) to transform the gruel of wheat flour and water into a glutinous mass, which upon baking becomes bread, is currently limited to wheat and a few other commonly used cereal seeds (Cauvain & Young, 2007). Gluten or wheat protein is important to retain gas in order to obtain the desired volume and texture in a dough system. It is essential to develop a strong protein network required for the visco-elasticity and hence good dough rheology. The major fractions of gluten are glutenin and prolamin. Prolamin provides viscosity and extensibility in a dough system, whereas glutenin is responsible for elastic and cohesive properties of dough (Gujral & Rosell, 2004; Demirkesen *et al.,* 2010). Beyond improving the bread appearance, gluten is also important for the crumb structure of wheat-based products (Demirkesen *et al.,* 2010).

The non-wheat flours, such as oat flour, are characterized by the lack of gluten. Dough manufactured from gluten-free formulations such as oat dough does not have the cohesive and elastic properties, because of the absence of gluten (Lazaridou & Biliaderis, 2009). As a result, the baked bread has a crumbling texture, poor colour and other post-baking defects (Torbica *et al.,* 2010). However, oat flour is a new substitute for wheat flour in the preparation of products consumed by wheat-intolerant or individuals suffering from celiac's disease. Oats is unique among the common cereals used because oat bread taste is nutty, soft and pleasant (Flander *et al.,* 2007). It could compete successfully as a healthy alternative to consumers who are used to eating wheat bread because of its great health benefits. Whole grain oat contains high amounts of valuable nutrients such as total dietary fibre (5 - 13%), soluble fibres, proteins (15 - 17%), starch and sugars (59 - 70%), fat (4 - 9%), β-glucan (2 - 6%), unsaturated fatty acids, vitamins, minerals and phytochemicals (Flander *et al.,* 2007; Kaukovirta-Norja & Lehtinen, 2008). The dietary fibre complex with its antioxidants may

prevent cardiovascular disease and some types of cancer (Thompson, 1994; Jacobs *et al.,* 1998a, 1998b; Slavin *et al.,* 2000). The highly viscous β-glucan fraction of oat has the ability to lower blood cholesterol and the intestinal absorption of glucose (Wood, 1993; Malkki, 2001). Therefore, oat bread will have rich nutritional value with regards to soluble fibers, proteins, unsaturated fatty acids, vitamins, minerals and phytochemicals (Jacobs *et al.,* 1998a; 1998b). Oat bread also helps in the treatment of diabetes and hypertension (Butt *et al.,* 2008) and it can also prevent certain types of cancer (Thompson, 1994; Jacobs *et al.,* 1998a; 1998b; Slavin *et al.,* 2000).

The lack of gluten in oats is the reason for its use as porridge, flakes, or as a breakfast cereal instead of it being processed into bread (Butt *et al.,* 2008). In order to produce bread similar in texture to wheat bread the functionality of proteins from gluten-free flours such as oat flour could be modified by enzyme action to improve their baking properties (Renzetti *et al.,* 2008). Enzymes are safe alternatives to chemical compounds because they are labeled as Generally Recognized As Safe (GRAS) and do not have negative health effects associated with their excessive consumption (Onyango *et al.,* 2010). Furthermore, the enzymes are denatured during baking and cannot be detected in the final product (Rosell, 2009). Among the enzymes used in the food industry, transglutaminase has been successfully used in several food systems (Kuraishi et *al.,* 2001) because of its unique ability to modify protein functionality and promote protein cross-linking (Babiker, 2000; Babin & Dickinson, 2001; Basman *et al.*, 2002; Renzetti *et al.*, 2008). The use of transglutaminase in gluten-free products modifies the visco-elasticity properties of the batters and improves the rheological behaviours (Gujral & Rosell, 2004; Moore *et al.*, 2006; Renzetti *et al.,* 2008) and shelf-life (Zhu & Tramper, 2008) of the resulting gluten-free breads. The addition of cyclodextrinase produces a reduction in dough consistency and elasticity and improves bread quality, specific volume, shape index, crumb texture and shelf-life of bread (Gujral *et al.,* 2003a, 2003b).

1.2   Statement of the Research Problem

Renzetti *et al.* (2008) and Nitcheu (2010) had demonstrated the possibility of making oat bread. However, the appearance of oat bread is unattractive because of the lack of dough cohesiveness and elasticity (Cauvain, 1998). Furthermore, the shelf-life of gluten-free products such as oat bread is usually short mainly due to the tendency of these products to easily stale as compared to wheat products (Gallagher, 2009). It is thought that transglutaminase and cyclodextrinase will modify protein functionality and promote protein cross-linking thereby improving the viscoelastic property of oat dough and the resulting bread. However, the effect of transglutaminase and cyclodextrinase on the rheology of oat dough and bread is not well-documented. In this study, the effect of transglutaminase and cyclodextrinase on the rheological and shelf-life characteristics of oat dough and bread was investigated.

1.3   Broad Objectives of the Research

The aim of this project was to evaluate the effect of transglutaminase and cyclodextrinase on the rheological characteristics of oat dough and shelf-life characteristics of oat bread with a view to developing oat bread with improved texture and shelf-life.

1.3.1   Specific objectives

The specific objectives that were addressed include to:

1.   Determine the individual effect of transglutaminase and cyclodextrinase on oat dough rheology.

2.   Establish the amount of cyclodextrinase and transglutaminase required, for optimal oat bread production through the use of Response Surface Methodology (RSM).

3.   Establish the shelf-life of oat bread produced with transglutaminase and/or cyclodextrinase.

1.4   Hypotheses

The hypotheses of the research were:

1.   Transglutaminase will improve the rheological characteristics of oat dough.

2.   Cyclodextrinase will improve the rheological characteristics of oat dough.

3.   Transglutaminase will improve the rheological and shelf-life characteristics of oat bread.

4.   Cyclodextrinase will improve the rheological and shelf-life characteristics of oat bread.

1.5   Delineation of the Research

Only one variety of oats (*Avena sativa),* Transglutaminase Activa WM (*Streptomyces mobaraense*) and Cyclodextrinase or Cyclomaldextrin glucanotransferase, Toruzyme (*Bacillus licheniformis*) will be used for this research.

1.6   Significance of the Research

Novel form of oat cereal utilization might encourage its wide production, hence improving its economic status. Oat flour is a new substitute for wheat flour in the preparation of products consumed by wheat-intolerant or individuals suffering from celiac disease as it is gluten-free.

Developing oat bread with good rheological and shelf-life characteristics using enzymes such as transglutaminase and cyclodextrinase will be beneficial to bakery industries because enzymes are safe alternatives to chemical compounds. Enzymes are Generally Recognized As Safe (GRAS) and do not have negative health effects (Onyango *et al.,* 2010) because the enzymes are denatured during baking and cannot be detected in the final product (Rosell, 2009). The industry experiences huge losses as between 8 and 10% of wheat bread production are unsalable due to staling (Stampfli & Nersten, 1995). However, the beneficiaries of the completed research will be bakeries and consumers, particularly patients suffering from cardiovascular disorders, diabetes and hypertension, as well as patients suffering from celiac disease because lifelong adherence to a gluten-free diet remains the cornerstone treatment for celiac disease (Gallagher *et al.,* 2004).

1.7   Expected Outcomes, Results and Contributions of the Research

Novel knowledge on the possibility of producing oat bread from oat flour through using enzymes will be generated. Information regarding the effect of transglutaminase and cyclodextrinase on oat dough rheology and the establishment of the amount of cyclodextrinase and transglutaminase required for optimal oat bread production will be generated. The shelf-life of oat bread produced with transglutaminase and/or cyclodextrinase will be established.

At least one journal article will be published in DOE accredited journal and an attended international conference.

1.8   Thesis Overview

The present thesis included six (06) chapters. Chapter 1 highlighted the motivation and the design of the study. Chapter 2 covered the literature review relevant to this research. Chapter 3 (first research chapter) pointed out the effects of yeast, carboxymethylcellulose, yoghurt, transglutaminase and cyclodextrinase on the mixing, pasting, thermal and protein modification properties of oat dough. Chapter 4 (second research chapter) was based on the optimization of oat bread production process. It investigated the effects of carboxymethycellulose (CMC), yoghurt (YG) and transglutaminase (TG) on the physical, textural and colour characteristics of oat bread with a view to optimize the level of these ingredients and established the amount of CMC, YG and TG required, for optimal oat bread production using Response Surface Methodology (RSM). Chapter 5 (third research chapter) investigated the effects of psyllium husks (PH) and cyclodextrinase (CG) on the textural characteristics of the best oat bread, established the quality, consumer acceptability and shelf-life of the best oat bread. Chapter 6 focuses on general discussions and conclusion of the different findings.

Keywords

Rheology, Shelf-life, Oat bread, Transglutaminase, Cyclodextrinase.

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CHAPTER 2
LITERATURE REVIEW

2.1   Background Information on Oats

Oat is a multi-purpose crop which mainly grows up in cool, moist regions (Hareland & Manthey, 2003).   According to Hareland & Manthey (2003), archeological discoveries trace oat grain back to the Greeks, Romans, and Chinese from the first century.   However, the grain may have originated in areas surrounding the Mediterranean sea in countries of the Middle East.   The world oats crop includes thousands of commercial cultivars which are grown for various uses.   Oat is traditionally used as a cheap source for farm livestock feed, forage, and bedding (Coffman, 1997; Hareland & Manthey, 2003).   Of the worldwide commercially grown oats, approximately 70% is used as livestock feed, 20% for human consumption, and 5% for industrial usage (Hareland & Manthey, 2003).   Hareland & Manthey (2003) pointed out that more than half of the oats crop never leaves the farm where it is produced.   Oats is often used as an alternate crop to break cycles of soil-borne insects and crop diseases.   Compared to other cereal crops, oats relatively remains low market cost and confines to growing on marginal soils associated with poor drainage and low fertility (Welch, 1995; Hareland & Manthey, 2003).   High-quality oats is still in demand for human consumption although oats production has decreased in recent years (Hareland & Manthey, 2003).

2.2   Structure of Oat Grain, Oat Milling and Nutritional Composition of Oat Flour

The grain of oat is composed of four parts: the hull, the bran, the endosperm and the germ (Figure 2.1).   The hull is normally separated from the kernel before use (Butt *et al.,* 2008).   Oat grain proportionally consists of pericarp, testa and aleurone together 12%, endosperm 84% and germ 3.7% (Kent & Evers, 1994; Flander *et al.,* 2012).

Oat is mainly processed to produce oat flour, oat flakes, oat bran or endosperm flour for human consumption.   The conventional processes of oat milling include dehulling, kiln drying, cutting, steaming and flaking/milling to oat flour (Girardet & Webster, 2011).   The milling process of oat is performed from groats already after kiln drying or flaking. Oat bran is separated from flour in one or several

grinding and sieving operations to a coarse fraction (bran) and fine fraction (endosperm flour) (Paton & Lenz, 1993).

Hull   Bran   Endosperm   Germ
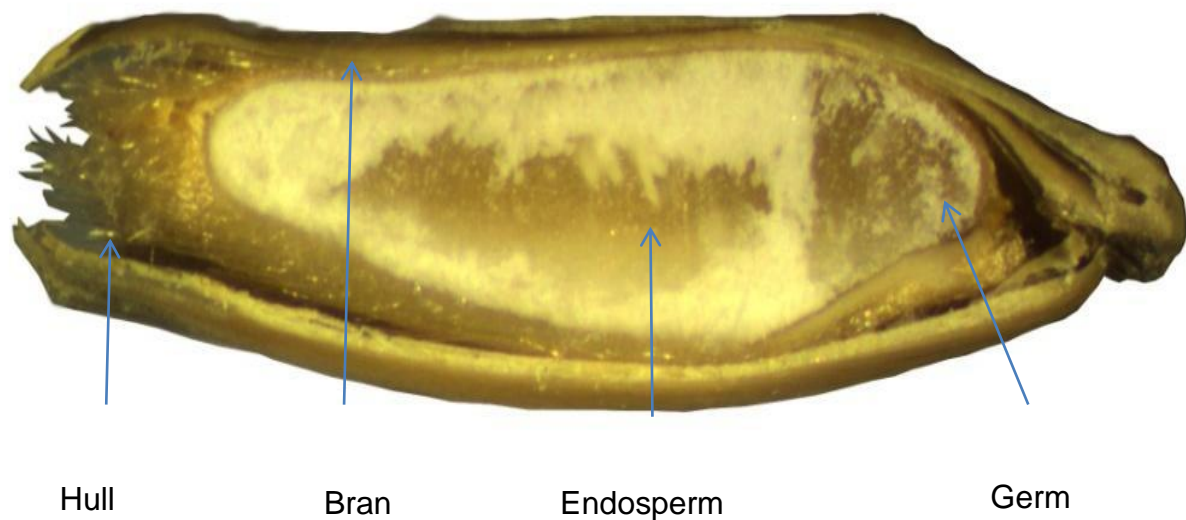
Figure 2.1   Major structural features of an oat grain (Flander *et al.,* 2012).

During kiln drying, the groats are heated with steam to 100 - 102°C, during which the moisture content of the groats increases from 12 - 14 to 17 - 20%. Thereafter, the groats are dried to 8 - 10% moisture content by dry heating and lastly by cooling air (Ganssmann & Vorwerk, 1995). Kiln drying stabilizes the groat by inactivating all enzymes such as lipase and peroxidase and prevents the development of oat rancidity during storage (Girardet & Webster, 2011). A pleasant nutty and toasted flavour is developed by oat after kiln drying. Oat flaking operation can be performed immediately after kiln drying if the process involves a steaming period that is long enough for enzyme inactivation. Usually, an additional steaming stage is performed after kiln drying groats and this plasticises the groats after storage (Girardet & Webster, 2011). Oat is flaked by rolling it between cast iron rolls.

Whole grain of oat flour contains high amounts of valuable compounds such as soluble fibres, proteins, unsaturated fatty acids, vitamins, minerals and phytochemicals. Its nutritional composition is presented in Table 2.1.

Table 2.1   Nutritional composition of oat flour

Component	Range (% of flour weight)
Carbohydrate (without dietary fibre)	55.7 - 62.4
Dietary fibre (with β-glucan)	10.6 - 17.2
β-glucan	1.8 - 8.1
Protein (N x 5.83)	9.6 - 16.9
Fat	4.5 - 9.0
Ash	1.7 - 2.0
Moisture	8.2 - 14.1

Source: Flander *et al.* (2012)

2.2.1 Protein content of oat flour

Oat is characterized by higher protein content compared to other cereals (Zhou, 1999). The protein content varies from 10 to 12% in the whole oat grain (Zarkadas *et al.,* 1995) or 13 to 22% in groats (Youngs, 1972). The approximate protein concentration in individual fractions of cultivated oat variety is: groat, 12 - 25%; embryonic axis, 25 - 40%; scutellum, 24 - 32%, bran, 18 - 32% and starchy endosperm, 9 - 17%. Most of the protein is commonly located in the bran and endosperm. The bran contains approximately twice the protein concentration as the endosperm, but only approximately half of the total groat protein because of the difference in relative size. Oat protein has a well-balanced amino acid content, with changes in protein content depending on the variety (Robbins *et al.,* 1971). Oat groats contain protein of high quality compared to other cereals.

2.2.2 Lipid content of oat flour

The lipid content in the groat is the highest among all the common cereal grains (Acker & Becker, 1971; Youngs, 1974; Youngs *et al.,* 1977; Morrison, 1978; Morrison *et al.,* 1984; Gudmundsson & Eliasson, 1989; Becker, 1992). It varies from 4 to 16% (Frey & Hammond, 1975; Schipper & Frey, 1991) which is 2 - 5 times greater than that of wheat. The lipid content is distributed throughout the grain and may be as high as 10% of total oat mass although values are dependent on the method of analysis. In the case of solvent extraction, it varies considerably depending on the nature of the extracting solvent (Matz, 1991). Oat lipids are considered as

nutritionally important because they are highly unsaturated and contain several essential fatty acids (Youngs, 1986) and a very high level of antioxidants (Peterson, 1992). Estimated contents of free lipids in oat fractions are: hull, 2%; endosperm, 5.2%; aleurone and bran, 6.4%; scutellum, 20.4%; and embryonic axis, 10.6%. The embryonic axis and scutellum together contain the highest concentration of lipids in the oat kernel. However, due to its relative size compared with other oat fractions, lipid quantity is low. The endosperm layer contains low lipid concentration, but contains over 50% of the lipids in the groat. The aleurone layer is very rich in lipids and represents the major source of bran lipids. Oat lipids are nutritionally important because of the high concentration of polyunsaturated fatty acids, especially linoleic acid, oleic acid and essential fatty acids. Essential fatty acids are utilized in the synthesis of prostaglandins which function to regulate smooth muscles such as the heart. The approximate contents of fatty acids in oat lipids are: myristic, 0.4 - 4.9%; palmitic, 15.6 - 25.8%; stearic, 0.8 - 3.9%; oleic, 25.8 - 47.5%; linoleic, 31.3 - 46.2%; and linolenic, 0.9 - 3.7% (Zhou, 1999).

2.2.3 Carbohydrates of oat flour

The occurrence of reducing sugars in oat is quite low. It is usually less than 0.1%, while total sugars are often near 1% (Henry, 1985). Nevertheless, the monomers are of extreme importance as components of polysaccharides. Glucans [$(1{\rightarrow}3)$, $(1{\rightarrow}4)$-β-D-glucans] represents 2 - 6% of the total groat mass and as much as 7% to the starchy endosperm of the oat grain (Fincher & Stone, 1986; Bhatty, 1992). The high content of β-glucans is of advantage in human nutrition. It is considered to be responsible for lowering serum cholesterol levels (Hurt *et al.,* 1988; Wood *et al.,* 1989). β-glucans form viscous gums with water (Autio *et al.,* 1987) and contributes significantly to water retention and textural properties. The higher content of gums, especially β-glucans, in the wet-milled oat bran has a pronounced effect on the viscosity of heat- and α-amylase-treated bran slurries (Jaskari *et al.,* 1995). Nonetheless, starch remains the most abundant component in oat where it represents 60% of the dry matter of the entire oat grain. The iodine affinity of oat starch at about 19.5 g/100 g is analogous to that of wheat, barley and rye (Banks & Greenwood, 1967). It indicates a similar amylose/amylopectin ratio as found in these cereals.

Starch is the major carbohydrate in oat (Hareland & Manthey, 2003). The starch is widely located in the endosperm of the oat granule with only trace amounts in the embryo and other organs. The oat starch granules are not implanted in a continuous protein matrix as in the case of wheat and barley. There is no starch industry using oat as feedstock analogous to those based on maize, as in the United States, or wheat, as in Australia (Zhou, 1999). As such, there are relatively few references in cereal chemistry literature on the functionality of oat starch. However, the knowledge of the morphology and functionality of oat starch is important to achieving maximum use of this cereal.

The starch of oat exists as discrete granules. It is solid, optically clear bodies as presented in other cereal grains. However, oat starch granules are identical to those of other cereals (Zhou, 1999). They are only weakly birefringent, irregular in shape (often polyhedral but sometimes ovoid or hemispherical). They exist in clusters and do not fall into discrete size distributions as in wheat and barley. The surfaces of the granules are smooth with no evidence of fissures (Hoover & Vasanthan, 1992). The average size of individual oat starch granules ranges from 3 to 10 μm (Reichert, 1913; Matz, 1969; Lineback, 1984; Paton, 1986; Gudmundsson & Eliasson, 1989; Hartunian-Sowa & White, 1992; Hoover & Vasanthan, 1992). The range and size are both much smaller than starch granules of wheat, rye, barley and corn (Hoseney *et al.,* 1971).

Known as compound granules, the individual granules develop in compact spherical bundles or clusters of 60 μm in diameter (Reichert, 1913; Matz, 1969; Hoover & Vasanthan, 1992). Most of the granules are round on one side and polygonal on the opposite side, presenting the growth of the granules in clusters.

The amylose and amylopectin starch components are present in a ratio of about 1:3, respectively (Zhou, 1999). The main variation in composition of oat starch is mainly due to the relative proportions of amylose and amylopectin in the starch granules, the chain length distribution (Hoover *et al.,* 1994) and the frequency and spacing of branch points within the amylopectin molecule. The amylopectin molecule has a profound influence on the properties of the starch. The iodine affinity of oat amylose varies from 18.4 to 19.5 g/100 g and that of amylopectin from 0.30 to 0.58 g/100 g (Banks & Greenwood, 1967; Wang & White, 1994) reflecting the capability of the amylose to form a complex with iodine. Reports of the content of

amylose in oat starch vary from a low of 18% (Paton 1979) to a high of 26 - 29% depending on oats variety (MacArthur & D'Appolonia, 1979; Morrison *et al* 1984; Doublier *et al.,* 1987; Gudmundsson & Eliasson. 1989).

2.3 Health Benefits of Oats

Usually consumed as a whole grain cereal, oats are valuable part of our daily diet and may even lower the risk of several chronic diseases. The lack of dietary fibre in diets may be associated with the increase of occurrence of obesity, type 2 diabetes and cardiovascular diseases. Furthermore, it has been recently reported that cereal fibre intake may lower the risk of death from cardiovascular, infectious, and respiratory diseases by 24 - 56% in men and by 34 - 59% in women (Park *et al.,* 2011). Although the components responsible for the beneficial effects of whole grain foods are still under investigation, substantial evidence indicates that consumption of oat can decrease high plasma cholesterol, which is a major risk factor for heart disease. The decreases in serum cholesterol and plasma insulin responses are attributed to the main water-soluble polysaccharide of oat, β-glucan (Wood, 1993; Malkki, 2001; Jenkins *et al.,* 2002; Liatis *et al.,* 2009; Juvonen *et al.,* 2009). Based on numerous clinical studies, the European Commission has recently allowed the following health claim in Article 14(1) (a) for foods which provide at least 1 g β-glucan per quantified portion (3 g/day): "β-glucan of oat has been shown to lower/reduce blood cholesterol. The risk factor in the development of coronary heart disease is attributed to high cholesterol" (European Commission, 2011). In addition, the U.S. Food and Drug Administration allows a health claim for products containing whole oat flour and a minimum of 0.75 g of β-glucan per portion: "Soluble fiber from foods such as whole oat flours as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" (FDA, 1997a). The lowest suggested daily intake of β-glucan for achieving the health effects is 3 g per day, which requires four portions with 0.75 g of β-glucan (FDA, 1997b).

The lowering effects of β-glucan on cholesterol and postprandial glucose levels in blood is mostly related to its capability to increase the viscosity of digesta in the gut (Battilana *et al.,* 2001; Kerckhoffs *et al.,* 2002; Wolever *et al.,* 2010). The molecular weight (MW) of β-glucan also affects the digestibility of starch in food products (Regand *et al.,* 2011). β-glucan must be soluble, and its concentration and

MW must be sufficiently high in order to be physiologically active and form viscous solutions in the gut (Aman *et al.,* 2004; Wood, 2007; Wolever *et al.,* 2010,).

Oats have recently been approved by the European Commission as an ingredient in gluten-free labelled products (European Commission, 2009). The high amount of beneficial fibres ((1→3)(1→4)- β-D-glucan), proteins, unsaturated fatty acids, vitamins, minerals and bioactive compounds renders oat flour a healthy alternative to starch-based ingredients in gluten-free breads.   Since prolamins damage the small intestinal mucosa, celiac disease patients must adhere to a lifelong gluten-free diet by avoiding wheat gluten and prolamins of barley and rye, (Facano & Catassi, 2001; Maki & Collin, 1997).

2.4   Different Uses of Oats

Oat is mostly used as an ingredient for making various food products. Hot breakfast cereals are the number one application for oat flakes. The instant products are generally fortified and are sold in a wide variety of flavours. Oat flour is also used as as an ingredient in cold cereals. Oat is used in many bakery items, to which they add important benefits. First of all, oat has excellent moisture-retention properties that keep breads fresh for longer periods of time (Mc Kechnie, 1983). Dodok *et al.* (1982a) concluded that oat improved product consistency and shelf life. Dodok *et al.* (1982b) reported that in addition to the favourable physical effects, oat flour is capable of stabilising the fat component. Oat flour at levels of up to 30% is used to replace wheat flour, with the primary benefit being moisture retention and freshness. Oat is a main ingredient in many cookie mixes (Smith, 1973; Mc Kechnie, 1983). Incorporation of oat as an ingredient in cookie mixes influences the water absorption of dough, as well as the flavour and the texture of the final product. Smith (1973) showed that the incorporation of oat confers crispness to a cookie and allows for the reduction of the shortening. Oatmeal or oat flour is a main component of many infant foods where it is used as a thickener. Oat usage as an antioxidant is proposed by Peters & Musher (1937). Especially fine ground oat flour is marketed for antioxidant purposes. It is effective to some extent in milk and milk powder, butter, ice cream, fish, bacon, frozen fish or sausage, cereals and other products that are sensitive to fat oxidation during storage. Several researchers proposed the use of oat starch in adhesive applications (Kessler & Hicks, 1949; Secondi & Secondi, 1955; Waggle, 1968).

2.5 Technology of Gluten-free Bread Production

The technology of gluten-free bread production includes the conventional ingredients, gluten replacers, optional ingredients and breadmaking processes.

2.5.1 Conventional ingredients

The most basic gluten-free bread ingredients consist of gluten-free flour, water, yeast and salt. The sugar addition is not essential since flour amylases convert starch to sugars. These sugars are metabolized by yeast for gas production. Nevertheless, even those most skilled in the art of baking agree that at the very least it is difficult to make bread of a high, consistent quality from only these raw materials. The baker always adds small amounts of extra ingredients to enhance dough performance during processing or to improve the quality of the finished product. The principal benefit is related to the properties of the final baked product and the modification of the dough during processing. Besides these ingredients psyllium husks, dairy products (yoghurt), soya, eggs, and pea proteins are commonly used as gluten replacers in gluten-free bread formulations. Each ingredient has a specific role in the baking of gluten-free bread. The functionalities of the ingredients and production processes for gluten-free bread making are the main topic of discussion in this section.

Water

Water influences the dough consistency, dough rheology and dough temperature (Brown, 1993; Wang *et al.,* 2004). It hydrates proteins and carbohydrates for the development of dough and hence acts as a dispersing agent bringing the ingredients into contact with each other and dissolving the soluble ingredients (Mani *et al.,* 1992; Brown, 1993; Wang *et al.,* 2004; Chieh, 2006). Dough is formed when mixing water and flour, resulting in activating enzymes such as amylases (Mani *et al.,* 1992; Wang *et al.,* 2004) for starch degradation and sugar production. Water hydrates the protein fractions which also assist in the development of dough viscoelasticity (Chieh, 2006) and affects starch gelatinization during baking (Mani *et al.,* 1992; Brown, 1993; Wang *et al.,* 2004). The amount of water added during dough mixing mainly depends on water absorption of the gluten-free flour. Thus, water absorption increases with increase in protein content and with increase in flour extraction. Water binds flour and other dough ingredients into a coherent mass and dissolves

certain ingredients for development of yeast and for leavening action at the baking stage. Water addition reduces the viscosity and increases dough extensibility. If the water volume is too low, the dough becomes brittle, not consistent and highlights a marked "crust" effect due to the rapid hydration. Water content and its distribution therefore play an important role in textural properties of bread such as softness of crumb, crispness of the crust and shelf-life.

Salt

Salt is added at about 1.5% of gluten-free flour weight for taste (Zobel & Kulp, 1996; Gray & BeMiller, 2003; Chieh, 2006; Lemmer, 2009) and to improve dough handling. Salt slows down water imbibition and swelling of flour proteins, reduces dough extensibility, and improves gas retention, bread crumb and slicing properties.

Yeast

Saccharomyces cerevisiae added at about a 2% concentration (gluten-free flour basis, weight/weight) is used in the baking industry (Zobel & Kulp, 1996; Williams & Pullen, 1998) for its ability to produce gas through the metabolism of glucose. Yeast ferments glucose to produce carbon dioxide and ethanol under anaerobic conditions (Zobel & Kulp, 1996; Williams & Pullen, 1998; Gray & BeMiller, 2003). Brown (1993) reveals that the carbon dioxide goes into the dough/water phase when it becomes saturated and is released into a gas cell that is formed during dough mixing. Yeast also contributes to the flavour of baked products by the fermentation by-products produced (Lemmer, 2009) through releasing reducing sugars that react with the amino groups of proteins during baking. *S. cerevisaie* is considered to be amongst one of the major yeasts used in dough fermentation and has an important effect on dough rheological properties. Research has shown that the effect of the yeast on rheological properties is similar to the effect of hydrogen peroxide (Mirsaeedghazi *et al.,* 2008). This fact indicates that the effect of yeast on rheological properties is due to the production of hydrogen peroxide by the yeast. The carbon dioxide produced during fermentation dissolves in water, resulting in a decrease in pH. Hence, carbon dioxide affects rheological properties of the fermented dough such as gluten-free dough (Spies, 1997). Furthermore, Salvador *et al.* (2006) demonstrated that dough samples containing yeast shows lower elastic, viscous and viscoelastic moduli than the control sample and greater frequency dependence, particularly at the higher

frequencies, within the period studied. However, Wehrle & Arendt (1998) reported that yeasted dough had a lower recovery capacity. In other words, they were less elastic than unfermented dough. Yeast at 2% and 4% (w/w) concentrations showed very similar behaviour but the 8% yeast sample presented the lowest viscoelastic constant values. This behaviour and the lower moduli values indicated a weaker, less structured gel with a more viscous-like behaviour. In all the yeast dough samples, the viscoelastic moduli showed a similar behaviour in relation to the temperature. The presence of yeast does not appear to induce delays in the gelatinisation onset temperatures.

Gluten-free flour

Gluten-free flour such as fonio, rice, rye, maize, oats, buckwheat, teff and blends consists of various components, namely protein, starch and minerals (Sultan, 1990; Sluimer, 2005). The starch and protein components are essential because they are important for the transformation of a gluten-free dough foam-type system to a bread-like system (Hug-Iten *et al.,* 1999). Starch is relatively inert during dough mixing, but contributes to increased dough viscoelasticity through its filling function. Starch is situated in spherical granules (Pateras, 1998; Karim *et al.,* 2000) and is made up of two polymers, namely, amylose and amylopectin. Amylose is a linear polymer and a determining key factor for initial loaf volume whereas amylopectin is a branched polymer (Blanshard, 1986; Pateras, 1998; Karim *et al.,* 2000). Amylopectin is responsible for crystallinity, while amylose is in a more amorphous state (Blanshard, 1986; Pateras, 1998; Karim *et al.,* 2000). The solubilised amylose forms a continuous network during cooling by which swollen and deformed starch granules are embedded and interlinked. Consequently, bread loses its freshness during cooling and stales (Eliasson & Larsson, 1993). The crust toughens and the crumb becomes more firm and less elastic losing moisture and flavour (Hoseney, 1994). Starch retrogradation involves the re-association of starch component molecules into a partially crystalline, ordered structure (Ronda & Roos, 2011). As a result, the aging of bread diminishes cohesiveness (Gomez *et al.,* 2007) usually due to the loss of intermolecular attractions between ingredients responsible for crumb formation, and is usually associated with the loss of water (Gomez *et al.,* 2007). Water migrates from crumb to crust during staling and leads to a glass to rubber transition

of the two components. Zobel & Kulp (1996) described the mechanism of starch retrogradation leading to staling (Figure 2.2).
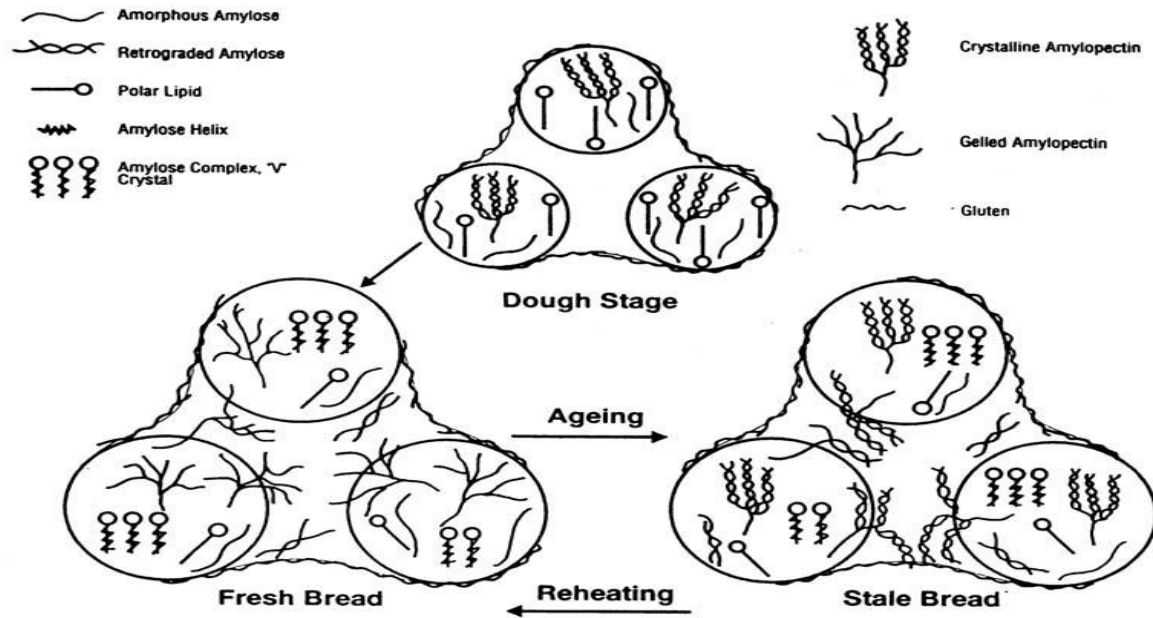
Figure 2.2   Starch retrogradation model (Source: Zobel & Kulp, 1996).

Due to the formation of double helical structures and crystalline regions, the migration of water and amylopectin retrogradation is considered to be primarily the cause of bread staling during aging (Zobel & Kulp, 1996; Gray & BeMiller, 2003). Changes in the firming rate of bread are due to hydrogen bonding between protein and starch granules, where protein is cross-linked by gelatinized starch (Martin *et al.,* 1991). In one study, bread baked from flours with low protein (10.4%) content staled at a faster rate than those baked from flours with a higher (13.1%) protein content. Maleki *et al.* (1980) concluded that the gluten-free flour component primarily responsible for the shelf life of bakery products is, therefore, protein. Acting as a diluent, protein slows the staling rate of starch (Zobel & Kulp, 1996; Pateras, 1998). However, Gray & BeMiller (2003) and Kestin *et al.* (2004) suggested that the starch-protein interaction is responsible for the firming process because swollen starch and protein cross-link during baking (Kamel & Ponte, 1993; Kestin *et al.*, 2004). The kinetic energy of crumb decreases during staling which allows cross-linkages to increase both in number and in strength thus resulting in the firming of the crumb

(Figure 2.3) (Kamel & Ponte, 1993; Kestin *et al.*, 2004). Hence, Martin *et al.* (1991) proposed a model of bread staling that incorporates the role of starch and protein. They argued that bread firming results from interaction between the continuous protein matrix and discontinuous remnant of starch granules. Poor quality flour has more hydrophilic properties than good quality flour (He & Hoseney, 1991; Martin *et al.,* 1991). Consequently, poor quality protein interacts more strongly with starch granules in dough. These interactions are stronger during and after baking, increasing the tendency of bread firming.

It is known that cereal flours other than wheat do not contain gluten. Hence, certain ingredients have been used as gluten replacers. Such ingredients are discussed below.
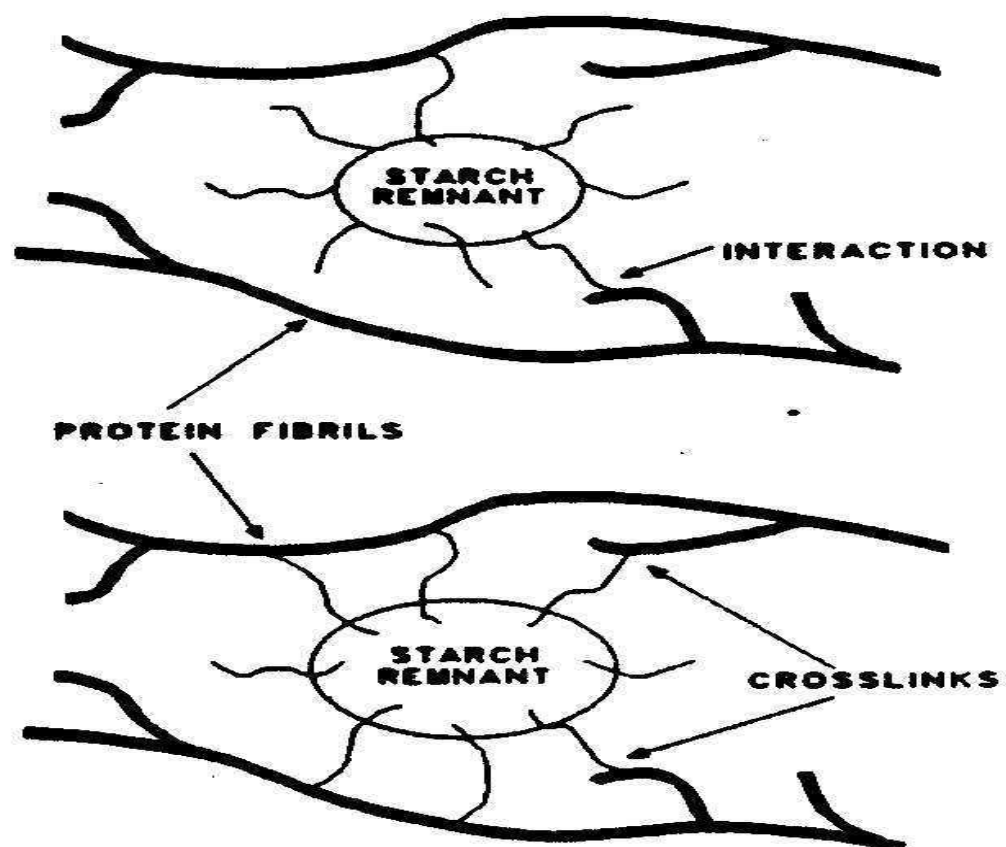
Figure 2.3 Starch-Protein interaction during staling (Source: Kamel & Ponte, 1993).

2.5.2 Common gluten replacers

Psyllium husks

Psyllium husks or Ispaghula is a common name used for several members of the plant genus *Plantago* whose seeds are used commercially for the production of mucilage. It develops "weak gel" networks which traps carbon dioxide generated during proofing and therefore increases gas retention and loaf volume (Zandonadi *et al.,* 2009). It is stable at various pH levels and temperatures and it is similar to gluten in food. Therefore, Zandonadi *et al.* (2009) suggested that psyllium can replace gluten in recipes because of its contribution in developing dough viscoelasticity.

Considered as fibre, psyllium does not modify the Dough Development Time (DDT) or the stability. However, Laurikainen *et al.* (1998) reported an increase in DDT and a decrease in stability with 5% rye bran. Greater effects are observed on the Mixing Tolerance Time (MTT) which is the difference in Brabender Units between the top of the curve at the peak and the top of the curve measured 5 minutes after the peak is reached. Both MTT and elasticity are reduced by the addition of fibres. The extent of the decrease depends on the type of fibre. These results can be explained by the interactions between fibres and protein, as described by Chen *et al.* (1988).

The addition of fibre to gluten-free flour modifies the rheological properties of the dough to a lesser extent compared to bran (Wang *et al.,* 2002), increases the configuration curve ratio (P/L) (P = tenacity or resistance to extension; L = dough extensibility), improves proofing stability and increases dough stability (Annon, 1999). Fibre in gluten-free dough interacts with the proteins resulting in increased dough resistance to deformation or tenacity (P) (Wang *et al.,* 2002). .

Dietary fibre such as psyllium husks is the edible portion of plants (or analogous carbohydrates). It is resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Gelroth & Ranhotra, 2001). From the technological view, fibre incorporation improves the texture, sensory characteristics and shelf-life of foods due to their water binding capacity, gel forming ability, fat mimetic, texturizing and thickening effects (Thebaudin *et al.,* 1997; Gelroth & Ranhotra, 2001). The addition of dietary fibre in gluten-free formulations improves gas retention ability yielding breads with significantly higher loaf volume and crumb softness compared to the control

(Zandonadi *et al.,* 2009). The gluten-free breads with dietary fibre provide the consumer with higher amounts of total dietary fibre and have an appealing dark crust and a uniform and finely grained crumb texture (Sabanis *et al.,* 2009).

Dairy products

The incorporation of dairy ingredients is well-established in the baking industry (Zadow & Hardham, 1981; Stahel, 1983). Dairy proteins are significantly functional ingredients and due to their versatility can be readily incorporated into many food products. They are used in bakery products for both nutritional and functional benefits including flavour and texture enhancement, and storage improvement (Cocup & Sanderson, 1987; Mannie & Asp, 1989; Kenny *et al.,* 2000). Dairy products such as yoghurt used in gluten-free bread formulas increases water absorption and, therefore, enhance the handling properties of the dough (Gallagher *et al.,* 2004a; Nunes *et al.,* 2009). However, Houben *et al.* (2012) showed that the main whey proteins contained in yoghurt, the α-lactalbumin (four disulphide bonds) and the ß-lactoglobulin, which can be a monomer, dimer and an oligomer depending on pH value, ionic strength and temperature, have a globular structure and hydrophobic, compact folded polypeptide chain. Hence, decreases water absorption of dough.

Dough viscosity is one of the key elements which determine the rheological quality of gluten-free bread. Gallagher *et al.* (2003) showed that increasing water addition in the dough resulted in increased loaf volumes. These findings are confirmed by Schober *et al.* (2005) in their study on gluten-free dough made from sorghum flour. Dairy proteins possess functional properties similar to gluten, as they are able to form networks and have good swelling properties (Gallagher, 2009). However, dough with added dairy product displays a much higher ability to resist deformation and led to greater solid-like behaviour under the applied testing conditions (Bertolini *et al.,* 2005). This might be mainly due to the presence of proteins such as milk protein isolates and sodium caseinate in the dairy product, decreasing water absorption of dough (Bertolini *et al.,* 2005). This finding nicely correlates with studies performed by Nunes *et al.* (2009) on rice flour. Bertolini *et al.* (2005) also showed that the storage modulus (G′) increased when sodium caseinate was present in the composite flours. This effect seemed to be clear, mainly for rice starch, at the lower starch concentration, suggesting that the viscoelastic properties

of the sodium caseinate are more important in the systems with low starch concentration. Despite the conflicting results, it seems clear that changes in the viscoelastic properties of the systems could be attributed to the limitation of starch swelling and gelatinization by sodium caseinate (Bertolini *et al.,* 2005). The effect of sodium caseinate on starch swelling was indicated by water restricted in the system and is more evident in starches with high amylose content (Bertolini *et al.,* 2005).

In general, dairy powders with high protein/low lactose content (sodium caseinate, milk protein isolate) give breads with an improved overall shape and volume (Gallagher *et al.,* 2003), and a firmer crumb texture (Arendt *et al.,* 2008). When optimal water is added to the gluten-free formulation these breads exhibits increased volume and a much softer crust and crumb texture than the controls. Supplementing the gluten free formulation with high protein-content dairy powders doubles the protein content of the breads (Gallagher *et al.,* 2004a). Whole and skimmed milk powders improve sensory characteristics. Sodium caseinate and hydrolysed casein display beneficial functional properties in bread making including low proof time, high volume and low firmness (Kenny *et al.,* 2000). Therefore, breads containing dairy ingredients exhibit the best quality and resemble a wheat bread most closely (Gallagher *et al.,* 2003). The high quality of bread that contains dairy ingredients is attributed to the capacity of these ingredients to form a network similar to gluten.

The colour analysis of gluten-free bread shows that the bread supplemented with dairy products, has lower crust L* (value given a darkness to lightness indicator for products) values than the control (Gallagher *et al.,* 2003). This is due to the small amount of lactose contained in dairy powder which is involved in Maillard browning and caramelisation reactions (Gallagher *et al.,* 2003). These reactions are affected by the distribution of water and the reaction of reducing sugars and amino acids resulting in a darker crust colour (Gallagher *et al.,* 2003). However, crumb colour is not significantly affected by dairy product (Gallagher *et al.,* 2003). Gluten-free bread supplemented with dairy products has an appealing dark crust and white crumb appearance (Gallagher *et al.,* 2004b).

The incorporation of dairy products in gluten-free bread renders it "looking more like real bread" (Gallagher *et al.,* 2003). The crumb is "more even and more airy than the control" and the loaves have "better volume and crust colour, like wheat bread" (Gallagher *et al.,* 2003). As a result, most of the panelists show interest in

bread supplemented with dairy products than the control (Gallagher *et al.,* 2003) leading to good acceptability scores in sensory tests (Gallagher *et al.,* 2004a).

Soya

Soybeans belong to the *Fabaceae* family, which contains legumes or pulses. Soybeans have a number of properties that renders them an attractive ingredient for functional foods. Soya has positive impacts on bone tissue and hence reduces osteoporosis, the risk of cardiovascular disease and prevents breast cancer. It is used to increase the protein content and the structural properties of gluten-free products. Soya protein show strong gel-forming properties and is used for the production of emulsions and foams. Its functionality depends on the environmental factors such as pH value, ionic strength and temperature. The soya proteins are classified into two groups, globulins (representing 90% of the total amount) and albumin (representing 10% of the total amount).

The addition of soya in gluten-free formulations improves the pasting profile of dough. Soya proteins promote significant decreases of 14% and 61% of the final viscosity and setback, respectively when compared to the control of rice dough (Marco & Rosell, 2008a). However, it significantly increases by 69% the storage or elastic modulus (G') of rice dough (Marco & Rosell, 2008a). As reported by Marco & Rosell (2008a), the same tendency is noted on viscous modulus (G'') with independent frequency. The increase of dough viscosity in the presence of soya proteins could be attributed to its higher water binding capability.

The presence of soya proteins hardly modifies the emulsifying properties of rice dough. Marco & Rosell (2008a) pointed out that the incorporation of soya proteins increase the emulsifying properties of rice dough. It may be due to the process followed while it is produced, since this process can impact the solubility and the degree of hydrophobicity, modifying the emulsion properties of the proteins (Petruccelli & Anon, 1994).

Supplementing gluten-free formulations with soya proteins leads to the improvement of the quantification of free amino groups of the resulting products. As is expected its incorporation results in an increase in the amount of the free amino groups, as the result of the increase in the protein content (Marco & Rosell, 2008a). This increase can be explained by the increase in solubility of the soya proteins resulting from the deamidation reaction (Babiker, 2000).

Soya proteins addition in gluten-free products improves crumb grain score, bread volume and overall bread score (Sanchez *et al.,* 2002) and increases water absorption of gluten-free dough. Similar findings are shown by Houben *et al.* (2012) on gluten-free flours.

Eggs

Egg proteins such as albumins and globumins are able to form strong, cohesive and viscoelastic films essential for stable foaming (Moore *et al.,* 2007). They form viscous solutions, a film-like continuous protein structure similar to that of wheat gluten. Used as gluten replacer in baking, eggs proteins mainly act as a foaming agent, as a crumb stabilizer and for creating a good shape because of their border areas activity.

The incorporation of egg proteins improves the pasting properties of gluten-free dough. Egg albumin significantly decreases by 34% the breakdown of rice dough compared to the control and significantly increases the viscosity of gluten-free dough (Marco & Rosell, 2008a). Egg albumin proteins exhibit a slight increase in elastic modulus (G') and viscous modulus (G'') with frequency (Marco & Rosell, 2008a). The swelling ability of the egg albumen proteins in gluten-free dough leads to a viscous fluid showing a similar network protein structure function than the one known from gluten (Houben *et al.,* 2012).

The presence of egg albumin increased the emulsifying properties of rice flour (Marco & Rosell, 2008a). These emulsifying properties were induced by the heat coagulation of the protein and the egg yolk containing phospholipids and lipoproteins. As a result, they facilitate the dispersion and stabilization of gas bubbles in the gluten-free dough systems (Houben *et al.,* 2012); because egg white proteins containing ovalbumin (54%) includes four thiol and one disulphide group able to stabilize the gel by polymerization via the thiol disulphide exchange. Egg yolk is often used as emulsifier in the baking industry because of its coagulation power during the thermal treatment creating similar thermo-irreversible gels.

The incorporation of egg albumin proteins modifies the quantification of free amino groups of gluten-free formulations. It results in an increase in the amount of the free amino groups, increasing the protein content of gluten-free product (Marco & Rosell, 2008a). As soya proteins, this increase might be also attributed to the

increase in solubility of the egg albumin proteins resulting from the deamidation reaction (Babiker, 2000).

Eggs improve the rheological properties of gluten-free bread. They increase bread volume and the amount of pores per square centimeter (Moore *et al.,* 2006). Egg albumin increases the gas-binding capacity by connecting the starch granules (Jonagh *et al.,* 1968). According to Houben *et al.* (2012), the best crumb texture of gluten-free bread is reached by the addition of full egg powder compared to the other protein sources. The increase in interface of liquid and air is induced by the denaturation and aggregation of egg white inside the ovalbumin during the pitching. Stable gas foam is formed, which loses the dough and stabilizes the dispensation of further ingredients (Houben *et al.,* 2012). The fibrillar structures of gas bubbles are stabilized by the egg white protein ovomucin. Therefore, the protein network reduces the swelling and gel forming of the starch leading to the coagulation and prevention of the coincidence of the bread during baking.

Pea protein

Proteins from different sources such as pea protein are traditionally added in gluten-free products to increase its nutritional benefits because gluten-free products have a very low protein content and are lysine deficient (Marco & Rosell, 2008a). However, its presence in gluten-free formulations impacts other properties. The addition of pea protein significantly affects the faringraph water absorption (FWA) of gluten-free dough. The higher the amount of pea protein added, the higher the water absorption of rice dough (Marco & Rosell, 2008b; Mariotti *et al.,* 2009). This increase in water absorption might be attributed to pea protein water holding capacity, which is about 2.7 - 2.8 g/g (Marco & Rosell, 2008b).

The incorporation of pea protein significantly impacts the viscoelasticity properties of gluten-free formulations. According to Marco & Rosell (2008b), the increase in the amount of pea protein produces a significant linear decrease in the storage or elastic (G') and viscous modulus (G'') of rice dough derived from a significant quadratic effect. The decrease in G' and G'' could be mainly due to the high water holding capacity of pea protein, since constant water absorption is used (Marco & Rosell, 2008a). Findings presented regarding the effect of pea protein on the water absorption confirmed this assumption. However, Marco & Rosell (2008a) noted a significant increase in G'' and G' of rice dough while adding 5% of pea

protein. In addition, Mariotti *et al.* (2009) demonstrated that the higher amount of pea protein decreases the elastic modulus (G') and its impact is more evident on G' at temperatures lower than the starch gelatinization temperatures. The final viscosity of rice dough decreased by 20% with added pea protein (Marco & Rosell, 2008a).

Rheological assessment is a good tool to evaluate the polymer molecular structure and the end-use performance of gluten-free products. The level of pea protein significantly affects gluten-free dough hardness (Marco & Rosell, 2008b). The increase of the amount of pea protein increases the resulting dough harness (Marco & Rosell, 2008b). However, pea protein content does not significantly affect the dough cohesiveness (Marco & Rosell, 2008b). The supplementation of gluten-free formulations with pea protein leads to dough with higher springiness when protein level increases and results in a positive linear effect on gumminess. Marco & Rosell (2008b) pointed out that stickiness shows a quadratic positive dependence on the incorporation of pea protein.

The presence of 5% of pea protein hardly modifies the emulsifying properties of rice dough. It could be attributed to its hydration capacity since water acts as a plasticizer improving the functional properties of the dough (Marco & Rosell, 2008b). Thus, pea protein relatively possesses good emulsifying properties with low emulsion stability compared to the properties of soybean protein (Marco & Rosell, 2008a).

The microstructure analysis of rice dough containing pea protein shows that pea proteins present aggregates of distorted spherical structures (Marco & Rosell, 2008b). Besides, when the higher amount of pea isolate (6%) was added, as expected, more green area was proportionally seen, highlighting a larger protein matrix (Mariotti *et al.,* 2009).

The protein analysis of rice dough reveals that the addition of pea protein isolate produces an increase in proteins extracted in the albumin-globulin fraction (Marco et al., 2007).

Therefore, the incorporation of pea protein in gluten-free formulations improves the experimental gluten-free dough from both a physical (rheological and ultrastructural) and nutritional point of view, and a delay in the staling phenomenon of these samples might be expected (Mariotti *et al.,* 2009).

2.5.3 Optional ingredients

The main optional ingredients used in the gluten-free baking industry are fat, sugar, emulsifiers, oxidizing agents, reducing agents, enzymes and fermentation accelerators (Cauvain, 1998). Fat makes the crumb finer and silkier in small amounts (up to about 3%, flour weight) and increases loaf volume and freshness retention. Sugar improves both fermentation and browning; improves dough stability, elasticity, and shortness; and makes the baked product somewhat mellow (Cauvain & Young, 2007). Emulsifiers can be dispersing agents (lecithin, hydroxylated lecithin), volume improvers, dough strengtheners (polysorbate 60), and/or crumb softeners (monoglycerides and diglycerides). Oxidizing agents such as potassium iodate, calcium peroxide and calcium iodate oxidize protein. They enhance the gas retention of dough by strengthening the gluten and increasing absorption. Reducing agents ensure the dispersal of proteins to reduce mixing requirements (e.g., L-Cysteine). Enzymes such as amylases provide fermentable carbohydrates which stimulate gas production in dough (diastatically active malt preparations). Fermentation accelerators (ammonium phosphate, ammonium sulphate, etc.), provide nitrogen sources for yeast metabolism.

2.5.4 Gluten-free breadmaking processes

Different gluten-free processes had been reported in the literature. All the processes almost include the same unit operations as described in Table 2.2. The production of gluten-free breads is slightly different to that of standard wheat breads in terms of the regulation of physical parameters and the absence of gluten. As wheat dough, gluten-free dough is traditionally mixed, bulk fermented, divided/molded, proofed, and finally baked. However, several studies show that gluten-free dough have the tendency to contain higher water levels and tend to have a more fluid-like structure (Bernadin & Kasarda, 1973). Therefore, they require shorter mixing, proofing and baking times than their wheat counterparts (Table 2.2). The function of main steps in gluten-free bread making process is described below.

Weighing and mixing

All the ingredients are weighed according to the defined standard or formulation

Table 2.2   Gluten-free bread production processes

Cereal flour	Unit operation	Authors
Fonio (acha)	Dissolving sugar in 80% warm water; Adding yeast and allow to stand for 10 minutes, Pre-mixing the remaning sugar with other dry ingredients; Mixing of dry ingredients, water containing yeast mixture using wooden spoon (5 min); First proofing in the bowl for 30 min); Moulding and panning; Second proofing (45 - 50 min); Baking (215°C, 5 min; 180°C, 40 - 50 min); Cooling (15 min); Packaging in polyethylene bag.	Jideani *et al.* (2007)
Wholegrain oat	Dissolving baker's yeast and sugar in a solution of water (30°C, 10 min); Placing remaining dry ingredients and activated baker's yeast in a mixing bowl; Mixing all the ingredients (level 2 for 30 s and level 4 for 1.5 min); Dough scaling (450 g) and panning; Proofing (30°C, 85 % relative humidity, 30 min) in a proofer; Baking (190°C, 45 min); Depanning; Cooling (2 h, room temperature).	Huttner & Arendt (2010)
Sorghum and pregelatinised cassava	Mixing all the ingredients (low gear, 10 min); Dough weighing (1200 g) and panning; Proofing (33°C, 85 % relative humidity, 55 min); Baking (210°C, 35 min); Depanning; Cooling (2 h, 25°C); Packaging in polythene bag.	Onyango *et al.* (2010)
Rye	Dissolving TG in water; Mixing all the ingredients (30 °C, 60 s, 53 rpm; 120 s, 106 rpm); Proofing (60 min, 30°C, 80% relative humidity); Baking (60 min, 240°C).	Beck *et al.* (2011)
Rice	Dissolving yeast in warm water (35 °C); Adding yeast to dry ingredients and sunflower oil; Mixing all the ingredients (2 min, speed 3); Proofing (25-30 min, 20 min); Baking (215°C, 20 min); Cooling (1 h, room temperature); Packaging in polypropylene bag.	Lazaridou *et al.* (2007)
Buckwheat + rice + corn + oat + sorghum + teff	Dissolving dried yeast and sugar in a solution of water (22 - 26°C); Pre-fermenting the mixture in a proofer (30°C, 85% relative humidity, 10 min); Mixing all the ingredients (2 min, speed 2 of out 6); Dough scaling (400 g) and panning; Proofing (30°C, 85% relative humidity, 30 min); Baking (190°C, 35 min); Depanning; Cooling (90 min, room temperature).	Renzetti *et al.* (2008a)
Rice + buckwheat	Mixing flours and salt (speed 1, 5 min, 30 °C); Dissolving yeast in a portion of water (30°C); Mixing all the ingredients (speed 2, 5 min); Dough scaling (250 g) and panning; Proofing (30°C, 85 % relative humidity, 45 min); Baking (200°C, 50 min).	Peressini *et al.* (2011)

(including minor ingredients). The ingredients are blended and hydrated with water in order to develop the dough, and incorporate air bubbles. Dough connotes a semi-solid mass that resists mixing. Stauffer (1998) revealed that a typical mixogram shows the various stages of dough formation namely, hydration, blending, and breakdown.

Hydration

In flour, most of the protein is considered as flinty material. The mixer firstly hastens the conversion of the flinty protein bodies into soft, hydrated (but not truly dissolved) protein dispersions. The protein is further modified during gluten development due to the absorption of water from the water-soluble flour components (and added water-soluble ingredients such as salt and sugar). When water is brought into contact with the flour particles and the process is observed under a microscope, the particles seem to explode; strands of protein are rapidly expelled into the aqueous phase (Bernadin & Kasarda, 1973). Movement of the cover glass stretches the protein indicating their extensibility (Amend & Belitz, 1990). The input of mechanical energy is important to dough formation. For instance, a thick slurry that has no dough-like properties when stirred increases in consistency, forming soft (undeveloped) dough. Hydration alone is not sufficient for dough making.

Blending

Particles of flour are agglomerates of starch granules embedded in a network of protein. As the protein network is softened by hydration and agitated by mixing, the starch granules become less firmly attached to the protein but remain associated with the protein fibers. Most of the starch is removed by washing and kneading the dough but it cannot be totally removed. During this early stage of mixing, all the ingredients in the dough are blended to give homogeneous dough mass. Lipids are uniformly distributed and brought into contact with the protein fibers, and soluble materials are fully dissolved and distributed in the aqueous matrix.

Breakdown

Peak development is reached when the dough becomes softer and less resistant to mixing action. During this peak development process, the dough loses its ability to retain gases during proofing (Cauvain, 1998). The viscosity of dough proteins

extracted into 1% sodium dodecyl sulfate solutions is lowered in over mixed dough compared to optimally mixed dough, indicating a smaller average molecular weight (Danno & Hoseney, 1982). The breakdown phenomenon is due to the presence of ferulic acid in the water soluble fraction of flour (Schroeder & Hoseney, 1978). Dough breakdown simply appears to be a continuation of the process by which flour protein is converted to medium-length protein polymers that impart the desired rheological properties to dough.

Other food process operations in breadmaking

During the mixing, bulk fermentation occurs, which produces flavour development and allows dough development. Punching expels gas and subdivides the existing gas cells, thereby incorporating air into the dough mass. The dough mass is divided according to the standard process defined, rounded by the shaping of the dough piece into a shape to allow proofing to occur. During the first proof, stresses in the dough relax, resulting in improved handling properties. Prior to a final proofing process, the dough piece is shaped into a cylindrical form and placed into bread pans. For the final proofing, the production of CO_2 by the yeast allows the dough to rise while in the bread pans. The exposure to heat during baking sets the loaf structure and develops the baked flavour and colour of the bread. The final baked product is removed from the tins during the depanning step. Cooling allows for the slicing of the bread and prevents any moisture migration onto wrapping or packaging (cooled at a temperature of 27°C in cold air; with a residence time of approximately 2 hours). Bread loaves are sliced and wrapped for hygienic, aesthetic and convenient presentation to the consumer (reciprocating frame of blades, followed by automatic wrapping).

2.6 Use of Enzymes and Hydrocolloids in Gluten-free Breadmaking Technology

Besides the ingredients used in wheat breadmaking; psyllium husks, soya and eggs are commonly used as gluten replacers in gluten-free bread formulations to solve the above rheological challenges but with limited success. Recently, the effects of transglutaminase (TG) and cyclodextrinase (cyclodextrin glycosyl transferase, CG) on gluten-free formulations have been studied (Gujral *et al.,* 2003; Huang *et al.,* 2010). TG catalyses cross-link formation while CG degrades starch. The effects of

hydrocolloids such as CarboxyMethylCellulose (CMC) and HydroxyPropylMethylCellulose (HPMC) in wheat-free formulations have also been documented and have been found to modify the functional properties of gluten-free bread products through their action as water binders. This section gives an overview on the functional properties of these additives and their role in gluten-free formulations.

2.6.1 Effect of TG on the functional properties of gluten-free dough

Transglutaminase (TG) is a family of enzymes (EC 2.3.2.13), which catalyzes reactions over a temperature range of 0-65°C (with optimal temperature 50-55°C) and over a pH range of 4 - 9 (with optimal pH 6 - 7). It catalyzes an acyl-transfer reaction between the g-carboxamide group of peptide-bound glutamine residues (acyl donor) and a variety of primary residues (acyl-acceptors) (Figure 2.4) (Jaros *et al.,* 2006; Gallagher, 2009).

Acyl-transfer reaction

$$
\text{Gln} - \text{C} - \text{NH}_2 + \text{RNH}_2 \quad \rightarrow \quad \text{Gln} - \text{C} - \text{NHR} + \text{NH}_3
$$

Cross-linking reaction

$$
\text{Gln} - \text{C} - \text{NH}_2 + \text{NH}_2 - \text{Lys} \quad \rightarrow \quad \text{Gln} - \text{C} - \text{NH} - \text{Lys} + \text{NH}_3
$$

Deamidation

$$
\text{Gln} - \text{C} - \text{NH}_2 + \text{HOH} \quad \rightarrow \quad \text{Gln} - \text{C} - \text{OH} + \text{NH}_3
$$
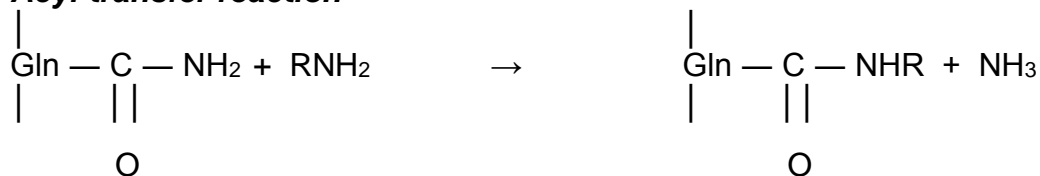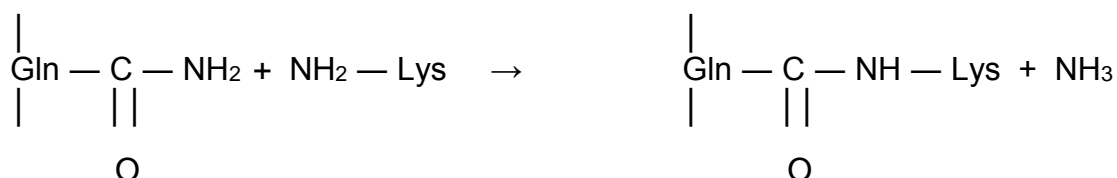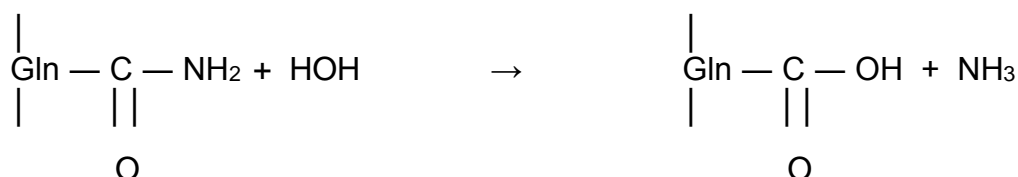
Figure 2.4 Reactions catalyzed by transglutaminase. Acyl group transfer between the γ-carboxamide group of a protein or peptide bound Gln residue and a primary amine, cross-linking between protein bound Gln and Lys residues to form a ε-(γ -glutamic)-lysine isopeptide bond, deamination of the Gln residue by water (Source: Gallagher, 2009).

Formation of an isopeptide bond between a free amine group (e. g. protein- or peptide-bound lysine residues) and the γ-carboxamide group of protein- or peptide-bound glutamine residues causes the formation of high molecular weight polymers. It seems to be the predominant reaction caused by TG in nature (Jong & Koppelman, 2002). In the absence of primary amines, water molecules are used as acylacceptors and the γ-carboxamide side chains are deamidated, forming glutamic acid residues (Motoki & Seguro, 1998). Table 2.3 summarizes the effect of TG on the functional properties of wheat and gluten-free doughs.

Many studies with wheat flour show that TG has a dough-strengthening effect. Thus, TG increases the extensibility and modifies the elasticity of dough by improving the protein network (Basman *et al.,* 2002; Bauer *et al.,* 2003; Caballero *et al.,* 2007) and water-holding capacity, reducing therefore the required work input during mixing (Basman *et al.,* 2002).

The addition of TG at less than 1% significantly increases the elastic (G'), viscous (G'') and viscoelasticity (|G*|) modulus values of buckwheat dough (Kohajdova & Karovicova, 2009). This increase of dough elasticity and viscosity suggests that TG leads to protein cross-linking and network formation of buckwheat dough, thereby modifying its viscoelasticity properties (Han *et al.,* 2011). This modification is said to be due to the formation of non-disulfide covalent cross-links between peptide bound γ-glutamyl residues and ε-amino groups of lysine residues in proteins (Shin *et al.,* 2010). Similar results have been reported by Renzetti et al. (2008a) and Shin *et al.* (2010) in their studies on rice dough. However, upon increasing the TG amount in the buckwheat dough from 1.0 to 1.5%, the values of |G*| and G'' were not significantly changed (Han *et al.,* 2011). It is probably due to the restricted reactivity of the TG owing to sufficiency of lysine but rather the relative content of glutamine in the buckwheat flour (Renzetti *et al.,* 2008a). Huang *et al.* (2010) reported that the mechanical spectrum of all oat dough indicates the elastic modulus (G') is always higher than the viscous modulus (G''), and both increase with increasing levels of TG. Consequently, TG leads to protein cross-linking and the formation of a network structure which causes a modification in the viscoelastic properties of the oat dough and other gluten-free dough. The viscous modulus (G'') shows a higher increase in amplitude than the elastic modulus (G'). The highest viscoelastic dough is obtained when the enzyme is added at a final concentration of

Table 2.3   Effect of TG on the functional properties of wheat and gluten-free dough

Characteristics	Cereal	Effect	Authors
Pasting	Buckwheat	Increased elastic (G') and viscous modulus and viscoelasticity	Kohajdova & Karovicova, 2009
	Rice	Increased elastic (G') and viscous modulus	Renzetti *et al.* (2008b)
	Rice	Only a significant increase of the elastic modulus	Marco & Rosell (2008a)
	Oat	Increased elastic (G') and viscous modulus; elastic modulus is always higher than the viscous ones	Huang *et al.* (2010)
	Rye	Stronger character of elastic modulus than the viscous ones; Increased relative elasticity by 31% and relative viscosity	Beck *et al.* (2011)
	Pregelatinised cassava + sorghum	Stronger character of elastic modulus than the viscous ones; Increased relative elasticity and relative viscosity	Onyango *et al.* (2010)
Thermal	Oat	Slight variation in transition onset and peak temperatures; improvement of dough stability and increased enthalpy	Huang *et al.* (2010)
	Wheat+barley+soy	Decreased enthalpy	Ahn *et al.* (2005)
Mixing	Oat	Decreased water absorption and increased torque peak, development time and stability	Huang *et al.* (2010)
	Wheat	Decreased water absorption	Basman *et al.* (2002)
	Buckwheat	Increased torque peak, development time and stability and decreased water absorption	Han *et al.* (2011)
	Oat	Structural changes of oat globulin, no significant differences in oat starch and cooking stability	Siu *et al.* (2002)
Quantification of free amino acid groups	Oat	Decreased number of free amino acid groups	Huang *et al.* (2010)
	Rice	Decreased number of free amino acid groups	Gujral & Rosell (2004) and Bonet *et al.* (2005)
	Buckwheat	Decreased number of free amino acid groups	Han *et al.* (2011)
SDS-PAGE	Oat	Globulin and avenin: good substrates for TG. Cross-linking of avenalin and glutelin by TG	Huang *et al.* (2010)

1.0% (Huang *et al.,* 2010), implying that TG modifies the anti-deformation ability of the oat dough. Similarly, rye dough also shows a stronger elastic character than a viscous one due to protein aggregation (probably due to isopeptide cross-links) (Beck *et al.,* 2011). The relative viscosity significantly decreases by 40% with an increase in TG concentration (Beck *et al.,* 2011). At the same time, the relative elasticity of rye dough significantly increases by 31%. These properties almost remain constant when TG concentration is above 1000 Ukg^{-1} for different reasons namely (Beck *et al.,* 2011): (a) no additional protein aggregation in the protein network of the dough system occurs, (b) additional protein network formation occurs, but these changes do not contribute to significant changes in dough rheology any more or (c) other enzymatic reactions (deamination of glutamine to glutamic acid) negates additional protein aggregation.

Beck *et al.* (2011) showed that zero shear viscosity was observed for the controls in rye dough production due to the relatively low molecular weight of proteins because of the lack of further cross-links by isopeptide bonds or rather no further protein aggregation. Due to the above reasons, the relative viscosity of gluten-free dough steadily rises with increasing TG until 1000 Ukg^{-1} (Beck *et al.,* 2011). These results are consistent with the work reported by Onyango *et al.* (2010) on pre-gelatinised cassava and sorghum. Marco & Rosell (2008a) reported that there is only a significant increase on the elastic modulus of the rice-protein blends. Other parameters such as viscous modulus and viscoelasticity do not show significant difference. Therefore, there is an increase in G' (elastic modulus) values when cereal proteins are treated with TG (Larre *et al.,* 2000; Demirkesen *et al.,* 2010). Nevertheless, TG has negative effects on corn flour where its application is detrimental for the elastic properties of the dough (Arendt *et al.,* 2008).

The thermal properties of oat dough measured by DSC (Differential Scanning Calorimeter) show that a single endothermic peak is obtained between 60 and 70°C after TG addition (Huang *et al.,* 2010). A slight variation in transition onset temperature (T_o) and transition peak temperature (T_p) is observed between samples treated with TG compared to a control sample that does not contain TG (Huang *et al.,* 2010). This variation indicates that TG improves the thermal stability of the dough (4.53 - 5.03 min). Furthermore, enthalpy (ΔH) of the dough samples significantly increases (0.56 - 0.70

J/g) with TG treatment (Huang *et al.,* 2010) because flour is heterogeneous material, hence the value of enthalpy reflect a combination of the transition of all components in the flour sample. These findings are not consistent with those of Ahn *et al.* (2005) who reported that TG's impacts on protein denaturation of pure protein samples leading to a decrease in enthalpy due to protein unfolding. Therefore, the lower ΔH values of soy flours or wheat–soy blends are due to the high protein content of soy (Huang *et al.,* 2010). Larre *et al.* (2000) similarly demonstrated that TG has significant impacts on the thermal stability of gluten. This is mainly due to the covalent cross-linkage promoted by the network and enzyme, which renders them insensitive to the temperature (Huang *et al.,* 2010).

The mixing analysis of oat dough by Mixolab, highlights that the water absorption decreases (66.1 - 65.2%) as the level of TG increases (Huang *et al.,* 2010). Similar findings are reported by Basman *et al.* (2002) and Han *et al.* (2011) on wheat and buckwheat dough. These results are attributed to acyl-transfer reactions that introduce new functional groups leading to changes in the structure, charge, and hydrophobicity of the buckwheat proteins (Han *et al.,* 2011). The increase in developing time (0.65 - 0.82 min) and stability (4.53 - 5.03 min) indicates that the elasticity of the oat dough is increased by the TG action (Huang *et al.,* 2010). Similar results are reported by Han *et al.* (2011) on buckwheat dough. These findings are due to the presence of storage proteins (2S albumin and 8S and 13S globulin) of buckwheat flour that are cross-linked after a TG treatment. Moreover, the dough extensibility increases due to modification of the cross-link between the oat proteins by TG, thereby increasing the stability of the protein network. The rise in water-holding capacity are attributed to the cross-linking after TG addition that changes in secondary structure or, possibly, due to changes in protein hydrophobicity from the formation of glutamic acid residues from glutamine hydrolysis (Gerrard *et al.,* 1998). Structural changes in TG treated oat globulin were also reported by Siu *et al.* (2002). No significant difference has been noted in the oat starch after TG treatment (Siu *et al.,* 2002). TG does not significantly affect the setback value and cooking stability of the oat flour (Siu *et al.,* 2002). There is only an increase in cooking stability at a TG level of 1.5%. The presence of TG promotes an increased

torque peak and decreases the water absorption of oat dough (Huang *et al.,* 2010). These findings are mainly attributed to the cross-links catalysed by TG.

The reaction between an ε-amino group on protein bound lysine residues and a γ-carboxyamide group on protein bound glutamine residues leading to covalent cross-linking of the proteins is catalysed by TG (Huang *et al.,* 2010). The implication of the amino groups in the cross-linking reaction reduces the number of these groups (Figure 2.5).
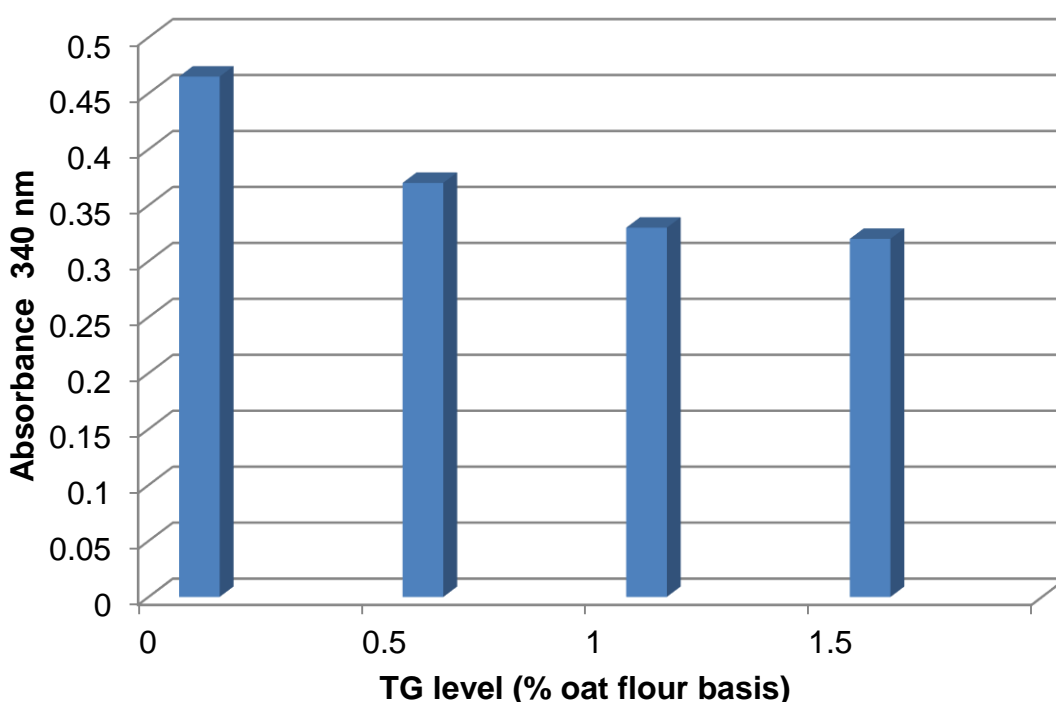
Figure 2.5   Effect of increasing TG concentrations on the number of free amino groups available for cross-linking in oat dough formulations (Adapted from Huang *et al.,* 2010).

This decrease in the number of the free amino groups is noticed when rice (Demirkesen *et al.,* 2010) and buckwheat (Han *et al.,* 2011) proteins are treated with TG. However, no significant change of the number of free amino groups is observed when TG level exceeds 1.0% (Han *et al.,* 2011). This is relatively due to the limited reaction of

glutamine coming from the additional TG in the dough. The free amino groups of the proteins of oat flour are measured to assess the effect of TG. The protein modification made due to TG addition is measured by changes in the number of free amino groups before and after TG treatment (Huang *et al.,* 2010). A reduction in the number of free amino groups is progressively noticed when TG is added up to 1.0%. No significant differences in the number of free amino groups are observed beyond 1.0% (Huang *et al.,* 2010). Gujral & Rosell (2004) reported similar findings and proposed that this phenomenon is due to a low amount of lysine limiting the action of the additional TG. Although oat protein is rich in lysine at a TG level of 1.5%, the number of free amino groups decreased significantly because oat samples have a low protein content, which limits the TG action (Huang *et al.,* 2010).

2.6.2 Effect of TG on the functional properties of gluten-free bread

By promoting the cross-linking effect on different flours (Larre *et al.,* 2000; Gerrard *et al.,* 2001; Bauer *et al.,* 2003; Rosell *et al.,* 2003; Autio *et al.,* 2005), TG widely modifies bread rheological properties (Caballero *et al.,* 2007) as summarized in Table 2.4.

According to Caballero *et al.* (2007), TG significantly decreases loaf specific volume but results in no change in the loaf shape. TG addition in rice flour strongly improves volume (565 - 633 ml) (Shin *et al.,* 2010). The highest specific volume of rice bread is obtained with TG at 1 U/g (Autio *et al.,* 2005).

The incorporation of TG leads to a significant increase in crumb hardness, cohesiveness, gumminess, chewiness and resilience of wheat bread (Caballero *et al.,* 2007). Similar results have been reported by Salmenkallio-Marttila *et al.* (2004) on oat bread. In contrast, the hardness of rice bread decreases (6.836 - 5.731 N) with the addition of TG but the springiness is not affected (Shin *et al.,* 2010). Increasing TG concentration significantly increases chewiness (9.190 - 12.133 N) and crumb firmness (17.696 - 21.808 N) but does not affect springiness, cohesiveness and resilience of pregelatinised cassava and sorghum bread (Onyango *et al.,* 2010). These variations may be due to the molecular weight of the proteins formed during the cross-linking action of this enzyme (Marco *et al.,* 2007; Marco *et al.,* 2008a). The protein and TG result in the formation of a network in the dough and forms the structure of gluten-free

Table 2.4   Effect of TG on the functional properties of wheat and gluten-free bread

Cereal	Effect	Authors
Wheat	Decreased loaf specific volume, but no change in the loaf shape; Increased rate of bread staling during storage, limitation of availability for starch and acceleration of retrogradation, increased crumb hardness, cohesiveness, gumminess, chewiness and resilience.	Caballero *et al.* (2007)
	Extension of bread shelf-life and sensory deterioration	Collar & Bollain (2005)
Rice	Improvement of volume, decreased hardness, but no effect on springiness	Shin *et al.* (2010)
Pregelatinised cassava + Sorghum	Increased crumb firmness, no effects on springiness, cohesiveness and resilience	Onyango *et al.* (2010)
Oat, sorghum and tef	No effect	Arendt *et al.* (2008) and Renzetti *et al.* (2008b)
Oat	Increased crumb hardness, cohesiveness, gumminess, chewiness and resilience	Salmenkallio-Martilla *et al.* (2004)

bread retaining carbon dioxide gas. Although TG is shown among other enzymes to enhance gluten-free bread texture depending on the raw material, Arendt *et al.* (2008) reported that no impact of TG could be observed on breads from oat, sorghum or tef. Similar results have been reported by Renzetti *et al.* (2008a). TG extends bread shelf-life by lowering crumb staling kinetics and sensory deterioration during storage when used in combination with α-amylase (Collar & Bollain, 2005). However, a certain study revealed that TG increases the shelf-life of certain foods and reduces their allergenicity (Zhu & Tramper, 2008). The single presence of TG increases the rate of bread staling during storage (Caballero *et al.,* 2007) and specifically affecting bread hardness, chewiness and gumminess. It is due to the interaction between starch granules and the

protein network actively contributing to crumb firming. The microscopic analysis of bread crumb points out significant differences in the starch-protein matrix during the course of storage (Blaszczak *et al.,* 2004). TG promotes the affinity for water and therefore limiting water availability for starch and accelerating its retrogradation (Caballero *et al.,* 2007). Hence, this starch retrogradation induced by TG decreases bread shelf-life.

2.6.3 Effect of CG on the functional properties of gluten-free dough

Cyclodextrinase or cyclodextrin glycosyl transferase (EC 2.4.1.19) acts at a pH range of 5.0 - 5.5, the temperature should not exceed 80 - 90 °C and the enzyme reaction can be terminated by lowering the pH. It catalyses four different reactions: cyclization, coupling, disproportionation and hydrolysis (Ohnishi et al., 1997; Feng *et al.,* 2011) with the production of cyclodextrins at the end of these reactions (Figure 2.6). Cyclodextrins are made from the hydrolysis and cyclization of starch, releasing closed circular molecules of six, seven, or eight glucose units, referred to as α-, β-, or γ- cyclodextrin, respectively (Gujral *et al.,* 2003). These molecules possess a polar surface responsible for the aqueous solubility and a hydrophobic inner core (Gujral *et al.,* 2003). Thus, their most characteristic property is that they have a hydrophilic exterior. This property allows them to dissolve in water while having a hydrophobic cavity that forms inclusion complexes with a wide variety of hydrophobic guest molecules. The cyclodextrins form complexes with fatty acids and emulsifiers influencing the rheological properties of starch and the functionality of the resultant starch (Rosell, 2001). Consequently, the pasted starch containing cyclodextrins lower elastic and viscous behaviours of doughs (Gujral *et al.,* 2003). Table 2.5 summarizes the effect of CG on the functional properties of rice dough.

The incorporation of CG lowers dough consistency when increasing CG to 40 μl/100 g flour. This indicates that CG brings about some breakdown in the starch during the mixing process (Gujral *et al.,* 2003). The elastic modulus of rice dough is higher than the viscous modulus (Gujral et al., 2003). The CG addition to the dough lowers the elastic modulus and complex viscosity of the dough but does not seem to influence the viscous modulus. According to Gujral *et al.* (2003), the tan δ (G''/G') or viscoelasticity
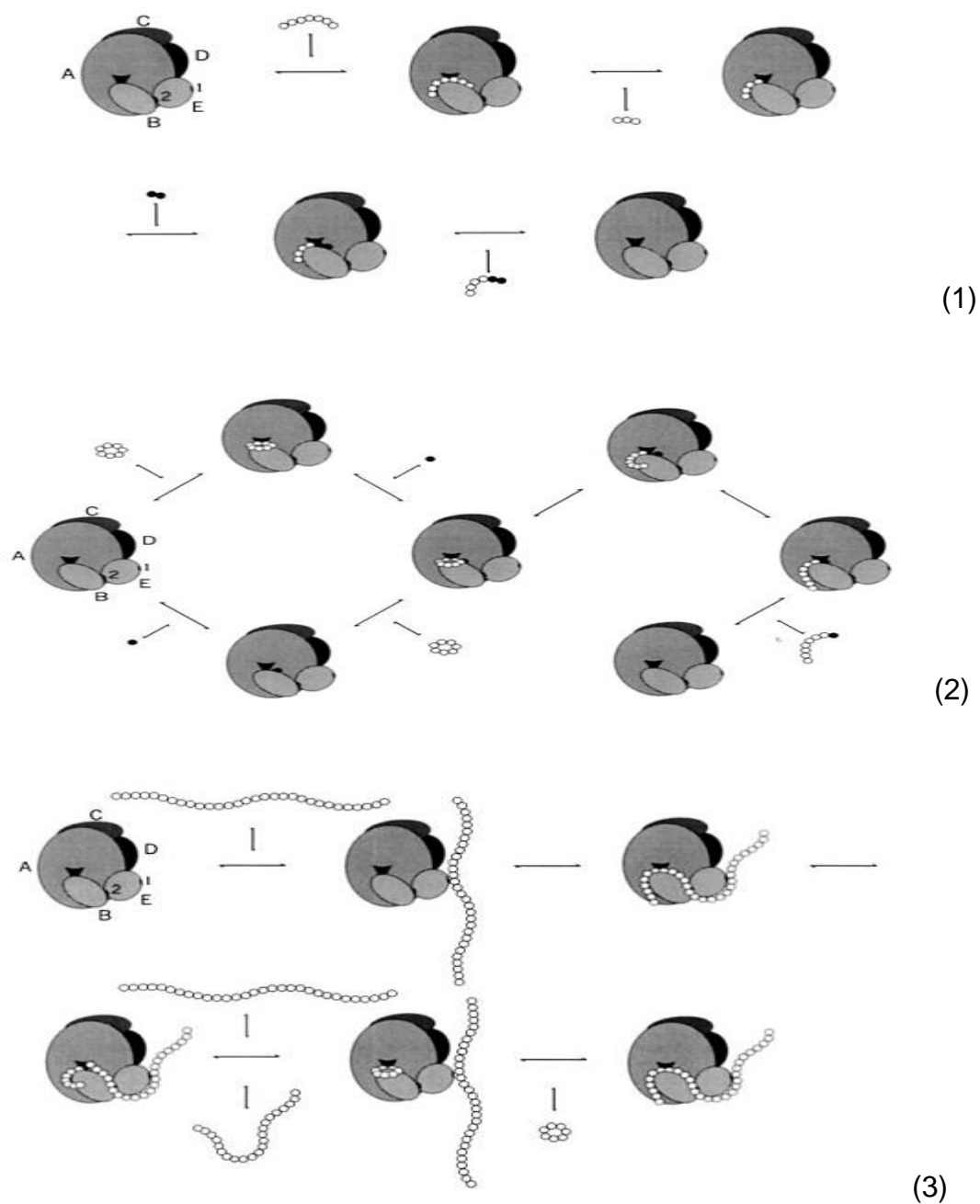
Figure 2.6 Proposed model of the events taking place in the CG-catalyzed reactions. (1) Disporportionation, (2) Coupling, (3) Cyclization. The different CG domains are indicated (A, B, C, D and E). 1 and 2 indicate the maltose binding sites on the E-domain. The triangle indicates the cleavage site in the active site. Circles represent glucose residues; acceptors residues are represented in black. (Source: Feng *et al.,* 2011).

Table 2.5 Effect of CG on the functional properties of rice dough and bread (Gujral *et al.,* 2003)

Product	Effect
Dough	Decreased consistency, elastic modulus, peak viscosity and final viscosity
	Increased viscoelasticity and breakdown
	Elastic modulus higher than the viscous modulus
Bread	Increased specific volume by 73%, shape index and volume
	Negative correlation between crumb firmness and specific volume
	Decreased crumb firmness by 53%, resulting in soft bread
	Extension of shelf-life by retarding amylopectin retrogradation

increases in the presence of the enzyme, suggesting that the relative contribution of the solid character (G') decreases.

It acts on the damaged starch during the mixing process and proofing time (30°C). This action brings about some hydrolysis, which impacts the dough rheology. A decrease in the elastic modulus and an increase in the tan δ in wheat flour dough from sprouted wheat flours are highlighted by Singh *et al.* (2001) and are due to higher amylase and protease activities.

CG incorporation (20 μl/100 g of rice flour) lowers the peak viscosity (2424 cP) and slightly affects the final viscosity (3147 cP), indicating that the enzyme acts on the starch, hence lowering the viscosity (Figure 2.7) (Gujral *et al.,* 2003). According to Gujral *et al.* (2003), CG also increases the breakdown (1250 cP), indicating that the paste is less resistant to heating and shear stress because starch is hydrolyzed. When adding CG at higher levels (40 µl/100 g of rice flour), a further decrease of the peak viscosity (2136 cP) along with an increase of the breakdown (1399 cP) is observed (Figure 2.7). The setback defined as the difference between the peak viscosity and the viscosity at 50°C, is related to the starch retrogradation. It relates to amylose helix interaction, and is one of the most important parameters in predicting rice bread characteristics (Nishita & Bean, 1979). CG decreases the final viscosity, although the setback reduces at 40 μl /100 g of flour (Gujral *et al.,* 2003). It is possible that at a low

enzyme dosage, some hydrolysis products are associated during cooling as the amylose does, and at high enzyme level a high amount of cyclodextrins is present, which physically interferes with the amylose complex as suggested by Gujral *et al.* (2003) and Liang *et al.* (2002). From the effect of the highest concentration of CG on the gelatinization property of rice flour, it is predicted that a softer crumb texture is obtained by adding this enzyme, which agrees with bread-making results (Gujral *et al.,* 2003). The cyclizing activity of the CG promotes an additional impact on the gelatinization behaviour of rice flour. It is mainly due to the fact that the resulting cyclodextrins form complexes with different compounds.
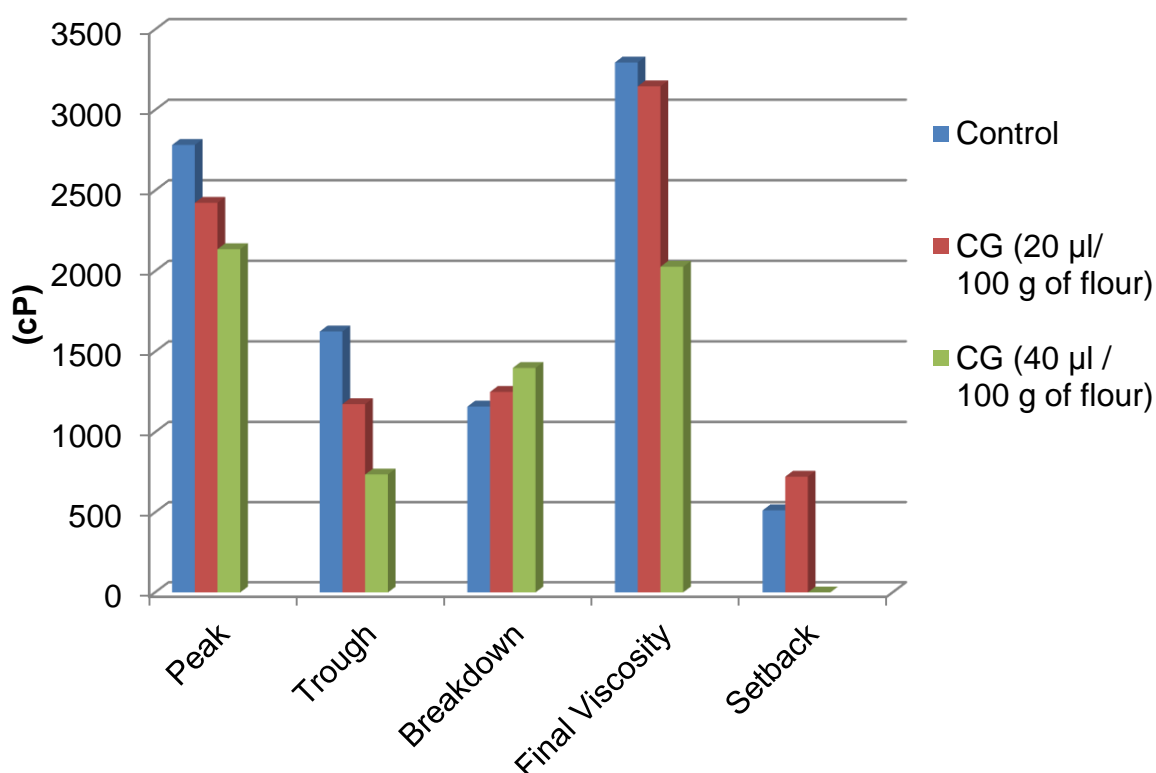
Figure 2.7   Effect of CG on the pasting properties of rice dough determined with Viscoanalyzer (Adapted from Gujral *et al.,* 2003).

According to Rosell (2001), CG degrades starch of gluten-free dough by its hydrolyzing and cycling activities. The hydrolysis reaction releases cyclodextrins which

are able to form complexes with lipids and proteins. The necessary substrates for the complex formation between lipids and proteins with cyclodextrins are provided by the cyclization reaction (Rosell, 2001). Therefore, the hydrophobic environment of gluten-free dough is reduced by CG through starch hydrolyzing and cyclizing activities and also through the hydrolysis products that can form complexes with a variety of solid, liquid and gaseous compounds.

2.6.4 Effect of CG on the functional properties of gluten-free bread

Preliminary experiments indicated that CG has positive impacts on rice bread volume (Gujral *et al.,* 2003). Increasing the CG concentration from 0 to 20 μl/100 g of flour increases the specific volume by 73% (Gujral *et al.,* 2003). This effect is probably due to the release of fermentable sugars utilized by the yeast, as a result of the hydrolysis of starch, which is catalysed by CG. Regarding the crumb texture, the crumb firmness shows a negative correlation with specific volume. The incorporation of increasing CG dosage considerably lowers firmness by 53%, obtaining a very soft bread crumb (Gujral *et al.,* 2003). The identical decrease in the crumb firmness is obtained with the addition of α-amylase, but the crumbs are very sticky (Gujral *et al.,* 2003). Hence, the improvement produced by CG can be attributed to the starch hydrolysis that yields fermentable sugar but also to the cyclization of the hydrolysis products, which form complexes with lipids and also proteins (Gujral *et al.,* 2003). CG possesses multiple catalyzing activities; therefore, the improvement of rice bread results from the combined effect of those activities. Despite CG having a cyclizing activity, it is important to validate that the breadmaking conditions are favourable for that reaction to take place. No detectable amount of cyclodextrins is observed in the crumb from rice bread obtained in the absence of CG. The cyclodextrin dosage increases by increasing the CG concentration. The presence of cyclodextrins validates the cyclizing activity of CG during the breadmaking process. Therefore, the hydrolyzing activity of the CG during breadmaking is identical to the effect of α-amylase. The starch hydrolysis yields fermentable sugars which are metabolized by yeast. Triglyceride molecules and lipids with inclusion complexes reducing interfacial tension are formed by cyclodextrin molecules which act as emulsifiers (Shimada *et al.,* 1992; Liang *et al.,* 2002). The

softening effect of the emulsifiers on the crumb of wheat bread is well-known (Collar *et al.,* 1998). Furthermore, cyclodextrins possess the capability to interact with hydrophobic proteins, leading to increased solubility (Lee & Fennema, 1991). The complexes with the hydrophobic proteins (globulin and glutelin) of rice formed by cyclodextrins increase their solubility (Gujral *et al.,* 2003) improving CO_2 retention, increasing volume and enhancing texture of bread. Table 2.5 summarizes the effect of CG on the functional properties of rice bread.

CG contributes to extending the shelf-life of rice bread. Its shelf-life extension is due to its ability to decrease amylopectin retrogradation during storage through its hydrolyzing and cyclizing activities (Gujral *et al.,* 2003). This anti-staling effect is attributed to the low molecular weight dextrins produced as a result of starch hydrolysis. Those dextrins interfere with the capability of the amylopectin to retrograde (Lin & Lineback, 1990; Defloor & Delcour, 1999; Rojas *et al.,* 2001; Leon *et al.,* 2002), or with other interactions also, namely starch-protein or protein-protein entanglement involved in firming (Lin & Lineback, 1990; Martin & Hoseney, 1991).

2.6.5 Effect of CMC on the functional properties of gluten-free dough

CMC (E466) is a derivative of cellulose with carboxymethyl groups bound to some of the hydroxyl groups present in the glucopyranose monomers that form the cellulose backbone. Its molecular structure is based on the β-(1⟶4)-D-glucopyranose polymer of cellulose. Different formulations could exist with different degrees of substitution, but it is mainly in the range 0.6 - 0.95 derivatives per monomer unit (LSBU, 2013). The functional effects of CMC on dough are highlighted in Table 2.6. The addition of hydrocolloids such as CMC increases the water absorption of rice dough. It is attributed to the hydrophilic character of these polymers (Leon *et al,* 2002). The highest absorption is observed for CMC (63.4%), followed by the control at 60.5%. The DDT increases with CMC incorporation (26.5%) whereas control decreases it (4.0%). Thus, CMC exhibits a stronger negative effect on the farinograph curve (with an increase of DDT, 26.5 min) compared to the control (4.0 min) (Lazaridou *et al.,* 2007). These findings are consistent with the studies performed by Sivaramakrishnan *et al.* (2004) in

Table 2.6   Effect of CMC on the functional properties of gluten-free products

Product	Cereal	Effect	Authors
Dough	**Rice**	Increased water absorption	Leon *et al*. (2002)
		Increased development time, elastic modulus, viscous modulus and viscoelasticity Elastic modulus higher than viscous modulus	Lazaridou *et al.* (2007)
		Increased development time	Sivaramakrishnan *et al.* (2004)
		Decreased water absorption	Bertolini *et al.* (2005)
		Increased consistency	Sciarini *et al.* (2010)
Bread	**Rice**	Increased volume No significant effects on crumb firmness and crust yellowness Decreased hardening rate, retarding staling Increased "a value" or redness colour of crust and crumb	Lazaridou *et al.* (2007)
		Lighter colour of crust	Sciarini *et al.* (2010); Mezaize *et al.* (2009)
	Fonio (acha)	Increased loaf volume, crumb texture, crust colour, crumb colour, general acceptability No significant effect on specific volume	Jideani *et al.* (2007)
	Gluten-free	Decrease of crumb hardness	Arendt *et al.* (2008)
	Gluten-free	Comparable to wheat bread in terms of sensory attributes	Ylimaki *et al. (*1991)

rice flour fortified with the addition of 4.5% HPMC. However, Bertolini *et al.* (2005) showed that a non-starch polysaccharide such as CMC decreases water absorption of rice starch gel. This addition of non-starch polysaccharides such as CMC to starch-water systems limited the hydration of the starch and, as water had a plasticizing effect

in amorphous regions of the starch, the mobility of the plasticizer was also restricted. Therefore, non-starch polysaccharides may have an "anti-plasticizing" effect (Bertolini *et al.,* 2005).

The hydrocolloid incorporation increases the dynamic elastic modulus (Lazaridou *et al.,* 2007). CMC renders rice dough more elastic (70 Brabender Units) than the dough control (60 Brabender Units). However, the increasing effect of hydrocolloid level on G' values is not clear because the added water also increases and CMC becomes stronger (higher G' values) with increasing concentration affecting the rheological properties of dough more than the increasing content of water (Lazaridou *et al.,* 2007). Although gluten-free dough exhibits an elastic modulus higher than the viscous modulus, the dough made with CMC has a higher viscosity than the ones containing no hydrocolloid (Lazaridou *et al.,* 2007). Moreover, the viscoelasticity of gluten-free dough is lower with CMC. The consistency of gluten-free dough supplemented by CMC is greater (419.5) than the control (285.4) (Sivaramakrishnan *et al.,* 2004).

2.6.6 Effect of CMC on the functional properties of gluten-free bread

Table 2.6 summarizes the effect of CMC on the functional properties of gluten-free bread. Thus, hydrocolloids such as CMC improve the volume of gluten-free formulations. The greatest volume is exhibited for rice bread supplemented with CMC (267 cm^3/100 g bread) among hydrocolloids used (Lazaridou *et al.,* 2007). This improvement is attributed to the increase of dough viscosity by hydrocolloids leading to the enhanced dough development and gas retention (Rosell *et al.,* 2001). The modified polysaccharide derivatives such as CMC contain hydrophobic groups imparting additional properties which increase interfacial activity of the dough system during proofing, and producing gel networks on heating during the bread making process. These network structures increase viscosity and strengthen the boundaries of the expanding cells in the dough, thereby increasing gas retention during baking which leads to a better loaf volume (Bell, 1990). Furthermore, the addition of CMC on Fonio or Acha flour increases the loaf volume by 40-59.5% of the resulting bread but its specific loaf volume does not significantly differ (2.60-2.73 ml/g) (Jideani *et al.,* 2007). Small bread volume of control (219 cm^3/100 g bread) might be attributed to highest

strength and elasticity of dough which cause a limited and slow expansion of the gas cells during proofing similar to bread supplemented with xanthan gum (Lazaridou *et al.,* 2007). Consequently, the dough becomes too rigid to incorporate gases (Lazaridou *et al.,* 2007).

The addition of CMC does not significantly affect the crumb firmness of rice bread compared to control formulations (Lazaridou *et al.,* 2007). The strengthening effect of CMC on crumb structure appears to be consistent with the low rigidity highlighted by dough containing it. Thus, a low crumb hardness is observed for breads supplemented by CMC.

The sensory evaluation by an untrained consumer panel revealed that gluten-free bread containing 2% CMC is highly acceptable because of its low crumb hardening compared to the ones containing other hydrocolloids (Arendt *et al.,* 2008). In addition, the loaf of acha bread with 4 % CMC is significantly better in terms of appearance, crust colour, crumb texture, crumb colour and general acceptability compared to other loaves of acha bread (Figure 2.8) (Jideani *et al.,* 2007).

The analysis of stored bread quality showed that a low rate of hardening is observed for bread supplemented with CMC when added at a concentration of 2%. After 3 days of storage, the softest crumb is associated with CMC-supplemented bread (Lazaridou *et al.,* 2007). Crumb texture, crumb colour and general acceptability of acha bread containing 4% CMC is not significantly different from wheat bread (Jideani *et al.,* 2007). In addition, Ylimaki *et al.* (1991) found that gluten-free bread containing CMC is comparable to a reference wheat bread on sensorial attributes from a trained panel.

The colour analysis of the crust of gluten-free bread reveals that bread supplemented with CMC has a lighter crust compared to the control (Sciarini *et al.,* 2010). This could be attributed to the effect of the hydrocolloid on water distribution which affects the Maillard reaction and caramelisation. Similar findings were obtained by Mezaize *et al.* (2009) studying the colour of gluten-free breads. The colour analysis of rice bread demonstrated that the redness value of crust is higher for a CMC formulation as compared to that of the control (Lazaridou *et al.,* 2007). No significant difference in crust yellowness was found. The presence of CMC at 2% concentration

showed a significant difference of the redness parameter for crumb among gluten-free breads (Lazaridou *et al.,* 2007).
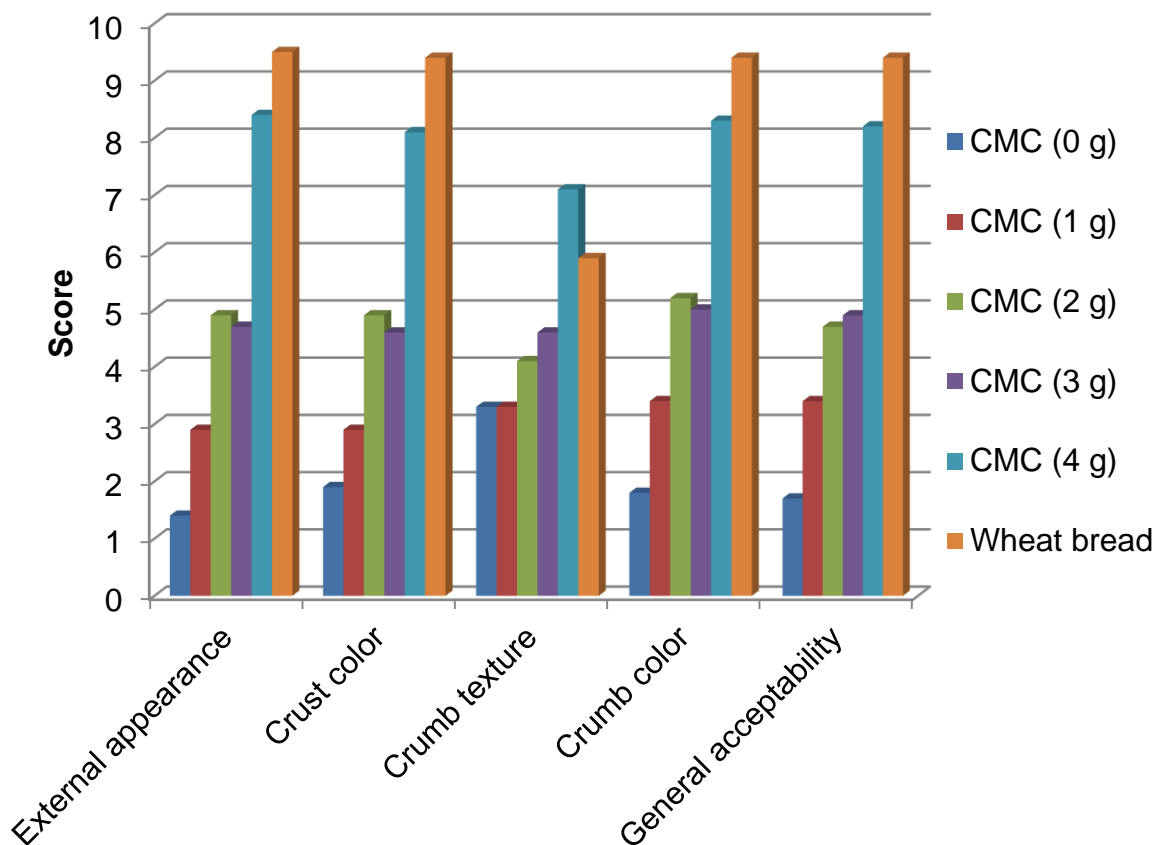
Figure 2.8   Effect of CMC on the sensory properties of Fonio or Acha bread (Adapted from Jideani *et al.,* 2007).

2.6.7 Effect of HPMC on the functional properties of gluten-free dough

HPMC (E464) is produced by the addition of methyl and hydroxypropyl groups to the cellulose chain (Shhuiguang, 2013) leading to a polymer with high surface activity and unique properties concerning its hydration-dehydration characteristics in the solution state and during temperature changes (Kohajdova & Karovicova, 2009). Its effects on the functional properties of wheat and gluten-free doughs are summarized in Table 2.7. The incorporation of HPMC improves gas retention and water absorption of gluten-free

dough, a property which is usually conferred by gluten (Huttner & Arendt, 2010). It also increases the viscosity of aqueous systems interfering with the diffusion phenomena (Barcenas & Rosell, 2005). According to Bell (1990), the substitution of the hydroxyl groups of cellulose by methoxyl and hydroxypropyl increases the water solubility and the affinity to the non-polar phase enhancing the hydrophilic character of HPMC.

Table 2.7   Effect of HPMC on the functional properties of wheat and gluten-free products

Product	Cereal	Effect	Authors
Dough	**Gluten-free**	Improvement of water absorption and gas retention	Huttner & Arendt (2010)
	Wheat	Increased viscosity	Barcenas & Rosell (2005)
Bread	**Gluten-free**	Increased crumb moisture content	Bell (1990); Dziezak (1991)
		Improvements of sensorial properties, crumb texture and softness	Kohajdova & karovicova (2009)
	Wheat	Increased volume	Rosell et al. (2001)
		Decreased crumb hardening rate and staling	Guarda et al. (2004); Collar et al. (2001)
		Improvement of crumb texture (soft crumb)	Barcenas & Rosell (2005)

The incorporation of HPMC improves gas retention and water absorption of gluten-free dough, a property which is usually conferred by gluten (Huttner & Arendt,

2010). It also increases the viscosity of aqueous systems interfering with the diffusion phenomena (Barcenas & Rosell, 2005). According to Bell (1990), the substitution of the hydroxyl groups of cellulose by methoxyl and hydroxypropyl increases the water solubility and the affinity to the non-polar phase enhancing the hydrophilic character of HPMC. This double role allows keeping the dough uniform and the emulsion stable during breadmaking. Therefore, it increases the water absorption of dough. The improvement of gas retention is attributed to the formation of interfacial films at the boundaries of gas cells conferring some stability against gas expansion (Huttner & Arendt, 2010). The optimal level of HPMC in gluten-free bread formulation consisting of rice flour and potato starch was determined to be 2.2% (McCarthy *et al.,* 2005). The addition of HPMC in gluten-free bread formulations increases the moisture content of the crumb (Bell, 1990; Dziezak, 1991), bread volume (Rosell *et al.,* 2001), reduces the crumb hardening rate (Guarda *et al.,* 2004) and improves crumb texture (Barcenas & Rosell, 2005) and sensorial properties (Kohajdova & Karovicova, 2009) as summarized in Table 2.7.

The capability of HPMC to improve bread volume/mass ratio is due to the release of water molecules allowing a stronger interaction between the chains when exposed to higher temperatures. As a result, there is the creation of a temporary network that disintegrates under cooling conditions (Bell, 1990). Dough expands during baking, the gas losses are reduced and volume increased due to gas cells strengthening of dough created by the HPMC network. This barrier for gas diffusion of HPMC decreases water vapor losses (Bell, 1990) and increases the final moisture content of the loaf (Barcenas & Rosell, 2005) and provides better crumb texture and softness without any adverse impact on the palatability of the fresh product (Kohajdova & Karovicova, 2009).

2.6.8 Effect of HPMC on the functional properties of gluten-free bread

The microstructure analysis showed a possible interaction between HPMC and the bread constituents (Barcenas & Rosell, 2005) and its capacity to interact with the effective water present in the system (Schiraldi *et al.,* 1996) thereby performing an anti-staling function. The softening impact of HPMC is attributed to its water retention ability and its inhibition of amylopectin retrogradation since HPMC preferentially binds to

starch (Collar *et al.,* 2001), avoiding starch-gluten interactions and therefore reducing crumb hardening rate and staling (Table 2.7, see page 50).

The improving effect of HPMC on the sensory quality of bread is attributed to its impact on the crumb texture through the production of softer crumbs (Barcenas & Rosell, 2005).

2.7   Conclusion

The cornerstone treatment for patients with celiac disease is a lifelong elimination of gluten in their diet. Hence, the biggest challenge for food technologists remains the production of high quality gluten-free bread with properties similar to that of gluten-containing bread. The majority of gluten-free breads currently on market shelves exhibit a very poor quality due to the lack of gluten. Good quality gluten-free bread can only be made with polymeric substances which mimic the viscoelastic behaviours of gluten. Dairy-based ingredients have shown to be the most promising among protein-based ingredients in the improvement of gluten-free bread properties. Water is one of the most important ingredients in any gluten-free formulation and therefore needs to be optimized to achieve optimal results. Recently, research has focused on the application of TG, CG, CMC and HPMC to improve the texture of gluten-free bread. TG in gluten-free bread products modifies the viscoelasticity properties of the dough and improves the rheological behaviours and shelf-life of the resulting bread. CG produces a reduction in dough consistency and elastic modulus and enhances specific volume, shape index, crumb texture and shelf-life of bread. CMC increases dough elasticity, bread volume and does not alter the crumb firmness. HPMC is an important ingredient for gluten-free bread production because of its ability to mimic the viscoelasticity properties of gluten to a certain extent. It reduces staling, improves water binding and overall structure of the resulting bread but further research is required to optimize the application of this hydrocolloid in gluten-free systems. Although research on gluten-free bread is still in its infancy, researchers should develop the high-quality products by the right selection and combination of the additives in order to obtain breads with a desirable quality.

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CHAPTER 3

EFFECTS OF YEAST, CARBOXYMETHYLCELLULOSE, YOGHURT, TRANSGLUTAMINASE AND CYCLODEXTRINASE ON MIXING, PASTING, THERMAL, QUANTIFICATION OF FREE AMINO ACID GROUPS AND PROTEIN CROSSLINKING PROPERTIES OF OAT DOUGH

Abstract

The effects of yeast, carboxylmethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) and cyclodextrinase (CG) on the mixing, pasting, thermal, free amino acid groups and protein crosslinking properties of oat dough were investigated. A 2^{5-2} fractional factorial design resolution III with yeast (1.25, 3.25%), CMC (1, 2%), YG (10.75, 33.75%), TG (0.5, 1.5%) and CG (10, 40 µl) as independent variables was implemented. The changes in the oat dough mixing, pasting, thermal, free amino acids and protein crosslinking were determined using a DoughLab, rheometer, Differential Scanning Calorimeter, spectrophotometry and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS - PAGE), respectively. The mixing properties of oat dough were significantly ($p < 0.05$) affected by CMC, YG and TG. CMC significantly ($p < 0.05$) increased stability (7.40 - 16.40 min), energy at peak (11.45 - 20.65 Wh/hg), development time (4.95 - 9.45 min), but decreased water absorption (38.45 - 35.75%), peak resistance (840.30 - 736.50 FU), softening (67.75 - 0.00 FU) as CMC level increased from 1 to 2 g. TG significantly increased water absorption (34.80 - 38.45%), peak resistance (696.40 - 840.30 FU) but decreased the softening (93.20 - 67.75 FU) while increasing its level from 0.5 - 1.5 g. YG decreased all the parameters measured as its level varied from 10.75 - 33.75 g, with the exception of softening, which was increased from 67.75 to 93.20 FU. Principal component analysis indicated that 85.5% of the variation in the data could be explained by two components. Component 1 explaining 52.3% of the variation loaded highly on dough strength (stability and departure time). Component 2 contributing 33.2% of the variation loaded on dough resistance (water absorption and peak resistance). CMC significantly increased dough strength while YG reduced it significantly. TG

significantly ($p < 0.05$) increased the resistance of the dough to mixing while CMC and yoghurt reduced it significantly ($p < 0.05$). Among all the ingredients, only yeast and YG significantly ($p < 0.05$) affected the pasting properties of oat dough. Yeast decreased shear stress (1.62 - 1.34 kPa), storage (202.78 - 132.09 kPa) and loss (62.87 - 52.31 kPa) modulus and torque (0.05 - 0.04 Nm) as its level increased from 2.25 to 3.25 g. YG increased storage modulus (139.42 - 202.78 kPa), but significantly decreased the damping factor (0.72 - 0.54). Yeast, CMC, YG, TG and CG had no significant ($p > 0.05$) effects on the thermal properties of the oat dough. The dough also exhibited a significant ($p < 0.05$) decrease in the number of free amino acid groups from 0.94 to 0.62 while CG level increased from 25 to 40 µl, confirming protein crosslinking catalyzed by CG. However, yeast, CMC, YG and TG significantly increased the number of free amino acid groups. The SDS-PAGE showed that the albumin, globulin and avenin of oat flour were good substrates for CG. Therefore, CMC, YG and TG were ingredients of choice for modifying mixing properties of oat dough.

3.1   Introduction

The dough making performance of oat flour (*Avena sativa*) is mainly based on the swelling properties of endogenous pentosans. Pentosans are able to bind water and increase the viscosity of the dough at low pH, thus improving the flow properties and shape of the dough during proofing and baking. In contrast to wheat proteins, oat proteins are not capable of forming three dimensional structures. Two possible reasons are assumed to be responsible for the different behavior of oat and wheat proteins. Firstly, oat and wheat proteins show qualitative as well as quantitative differences. For example, the wheat gluten network consists of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits stabilized by intermolecular disulfide bonds (Wieser & Kieffer, 2001), whereas oat proteins lack LMW glutenin subunits. Although oat also contains HMW subunits, the ability of forming intermolecular disulfide bonds is inferior as compared to the HMW glutenin subunits of wheat (Koehler & Wieser, 2000). The second reason for the limited aggregation behaviour of oat proteins is the low pentosan fraction, which leads to the formation of a gel-like layer on the flour particles thereby hindering protein aggregation (Wang, 2003). This low pentosan fraction of oat flour results in a dough with a higher plasticity than wheat

dough and its surface is more moist and sticky. Consequently, manual dough processing and machinability of oat dough is more difficult compared to that of wheat dough.

The mixing, pasting, thermal, free amino acid groups and protein crosslinking properties of oat dough may be modified by any one of the following components: yeast, carboxymethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) or cyclodextrinase (CG). *Saccharomyces cerevisaie* is considered to be one of the major yeasts used in dough fermentation and has an important effect on dough rheological properties. Research has shown that the effect of the yeast on rheological properties is similar to the effect of hydrogen peroxide (Mirsaeedghazi *et al.,* 2008). This fact indicates that the effect of yeast on rheological properties is due to the production of hydrogen peroxide by the yeast. Salvador *et al*. (2006) demonstrated that dough samples containing yeast shows lower elastic, viscous and viscoelastic moduli than the control sample and greater frequency dependence, particularly at the higher frequencies, within the period studied.

CMC is a derivative of cellulose with carboxymethyl groups bound to some of the hydroxyl groups present in the glucopyranose monomers that form the cellulose backbone. The addition of hydrocolloids such as CMC increases the water absorption and dough development time (DDT) of rice dough, and has been attributed to the hydrophilic character of these polymers (Leon *et al.,* 2002). The incorporation of CMC increases the dynamic elastic modulus (Lazaridou *et al,* 2007). CMC makes rice dough more elastic than the dough control. Although gluten-free dough exhibits an elastic modulus higher than the viscous modulus, the dough made with CMC has a higher viscosity than the ones containing no hydrocolloid (Lazaridou *et al.,* 2007). Moreover, the viscoelasticity of gluten-free dough is lower when CMC is added.

The main whey proteins contained in yoghurt, the α-lactalbumin (four disulphide bonds) and the ß-lactoglobulin, which can be a monomer, dimer and an oligomer (depending on the pH, ionic strength and temperature), have a globular structure and a hydrophobic, compact folded polypeptide chain, which results in a decrease in the water absorption of dough (Houben *et al.,* 2012). Dough samples that contain dairy products such as yoghurt, display a much higher ability to resist deformation and lead to greater solid-like behaviour under the applied testing

conditions (Bertolini *et al.,* 2005). Bertolini *et al.*(2005) also showed that the storage modulus (G′) of samples showed an increasing trend when sodium caseinate was present in the composite flours.

Commercial TG preparations for food applications have been available for several years. In industrial processes, this enzyme is mostly used in the meat and fish processing industry for the production of restructured meat as well as for dairy and tofu products (Herrero *et al.,* 2008). Transglutaminases are a family of enzymes (EC 2.3.2.13) that catalyzes an acyl-transfer reaction between the γ-carboxamide group of peptide bound glutamine residues (acyl donor) and a variety of primary residues (acyl-acceptors) (Jaros *et al.,* 2006). TG improves the water-holding capacity and reduces the required work input during mixing (Basman *et al.,* 2002). A study confirms that TG improves the pasting, thermal properties of oat dough through the protein cross-linking catalysed by TG, decreasing the number of free amino acid groups because of the catalysis of the cross-linking of avenin or gliadin and glutelin of oat flour by TG (Huang *et al.,* 2010). The addition of TG at less than 1% significantly increases the elastic (G'), viscous (G'') and viscoelasticity (|G*|) modulus of buckwheat dough (Kodajdova & Karovicova, 2009), thereby modifying its viscoelasticity properties. This modification is said to be due to the formation of non-disulfide covalent cross-links between peptide bound γ-glutamyl residues and ε-amino groups of lysine residues in proteins (Shin *et al.,* 2010). Consequently, TG leads to protein cross-linking and the formation of a network structure which causes a modification in the viscoelastic properties of the oat dough and other gluten-free doughs.

Cyclodextrinase or cyclodextrin glycosyl transferase (EC 2.4.1.19) catalyses four different reactions: cyclization, coupling, disproportionation and hydrolysis (Ohnishi *et al.,* 1997). Cyclodextrins are the end-products from these reactions. They are formed due to the hydrolysis and cyclization of starch releasing closed circular molecules of six, seven, or eight glucose units that are referred to as α-, β-, or γ- cyclodextrin, respectively (Gujral *et al.,* 2003). The cyclodextrins form complexes with fatty acids and emulsifiers influencing the rheological properties of starch and functionality of the resultant starch (Rosell, 2009). Although it is reported that the optimum activity of cyclodextrinase is 70°C, it must be acting on the damaged starch during the mixing process and also during

the proofing stage (30°C), bringing about some hydrolysis, which affects the dough rheology.

The application of yeast, CMC, yoghurt, TG and CG still remain to be evaluated with oat dough. Monitoring the enzymatic reaction by screening accurate measurement of the dough properties, while using relevant processing conditions, is consequently important in the development of improved oat dough. Therefore, the aim of the present study was to investigate the effects of yeast, CMC, YG, TG and CG on the mixing, pasting, thermal, free amino acids and protein crosslinking properties of oat dough, with a view to establish the combination for improved rheological properties.

3.2   Materials and Methods

3.2.1 Source of materials

Commercial oat flour was purchased from Health Connection Wholefoods (Cape Town, South Africa). Transglutaminase (TG) (Activa WM) and cyclodextrinase (CG) were respectively donated by Maccallum & Associates (Cape Town, South Africa; representing Ajinomoto Company in South Africa) and Novozymes (Johannesburg, South Africa). DATEM (diacetyl tartaric acid ester of mono- and diglycerides or E472e), fat and carboxymethylcellulose (CMC) were kindly donated by Danisco/Dupont (Cape Town, South Africa). Plain yoghurt (YG), instant dry yeast, sugar, and salt were purchased from a local supermarket. All ingredients used in this study were of food grade.

This chapter pointed out the effects of yeast, carboxymethylcellulose, yoghurt, transglutaminase and cyclodextrinase on the mixing, pasting, thermal and protein modification properties of oat dough (Figure 3.1).

3.2.2 Proximate analysis of oat flour

All chemical analyses of the oat flour were performed in triplicates. Moisture, total ash and protein contents were respectively determined according to the standard methods AACC 44 - 15A (AACC, 1994), AACC 08 - 02 (AACC, 1994) and AACC 46 - 30 (AACC, 1994).
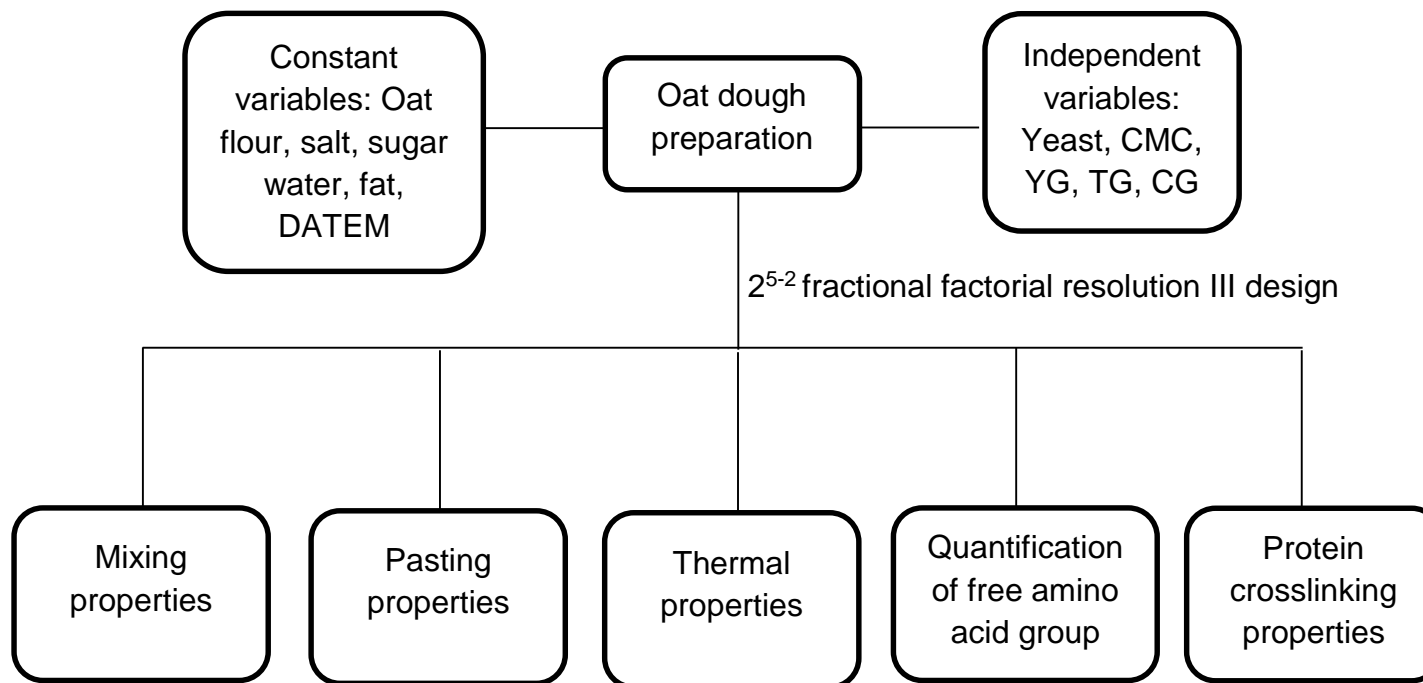
Figure 3.1 Overview of chapter three.

3.2.3 Particle size distribution of oat flour

Particle size distribution of oat flour was determined using a U.S. standard sieve (212 μm mesh). A known weight of oat flour (100 g) was placed on the sieve, and the weight of samples retained on a sieve after 10 min of shaking at 1400 rpm, was recorded (Chen *et al.*, 1988). The particle size was expressed as the percentage of particles retained on each sieve (Toma *et al.,* 1979). The particle size distribution analysis of the flour was performed in triplicates.

3.2.4 Determination of protein concentrations of TG and CG

Protein concentration of samples was measured by the Bradford method (Bradford, 1976) on a PerkinElmer ultraviolet/visible spectrophotometer. This method is mainly based on the binding of Coomassie Brilliant Blue G-250 dye to proteins. The enzyme samples at different stages of purification were diluted (1/10, 1/100 and 1/1000). Bovine Serum Albumin (BSA) reagent (1.0 ml) and dye reagent (0.5 ml) were added to 0.1 ml of the enzyme sample. The protein concentration was determined at 595 nm in a PerkinElmer ultraviolet/visible spectrophotometer. The analysis was performed in triplicates.

3.2.5 Experimental design

A 2^{5-2} fractional factorial resolution III design was used to determine the main effects of independent variables on the mixing, pasting, thermal, free amino acid groups and protein crosslinking properties of oat dough. The independent variables (yeast (X_1), CMC (X_2), plain yoghurt (X_3), transglutaminase (X_4) and cyclodextrinase (X_5)), and their levels are detailed in Table 3.1. The outline of the experimental design (11 runs) with the coded levels (-1 = low, 0 = middle, +1 = high values of independent variables) are summarised in Table 3.2. Each design point was performed in triplicates with the centre in four replicates. Following the combination of the ingredients as per the design, dough samples were produced following the process described in Section 3.2.7. The experiment was carried out in randomized order. The dependent variables of the experimental design were mixing (Section 3.2.6), pasting (Section 3.2.8), thermal (Section 3.2.9), free amino acid groups (Section 3.2.10) and protein crosslinking variables (Section 3.2.11).

Table 3.1 Process variables and their quantities used in the 2^{5-2} fractional factorial design for oat dough preparation[1, 2]

Factor (/100 g flour)		Lower level (-1)	Upper level (+1)
Yeast (g)	X_1	1.25	3.25
CMC (g)	X_2	1	2
YG (g)	X_3	10.75	33.75
TG (g)	X_4	0.5	1.5
CG (µl)	X_5	10	40

[1]Transformation of coded variable (x_i) to uncoded variable (X_i) levels could be obtained from: $X_1 = x_1 + 2.25$; $X_2 = 0.5x_2 + 1.5$; $X_3 = 11.50x_3 + 22.25$; $X_4 = 0.5x_4 + 1$; $X_5 = 15x_5 + 25$.
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodextrinase.

Table 3.2 Independent variables and levels used for the 2^{5-2} fractional factorial design for oat dough formulations[1, 2]

Run	Ingredients				
	Yeast	CMC	YG	TG	CG
1	-1	-1	-1	+1	-1
2	+1	-1	-1	+1	+1
3	-1	+1	-1	-1	+1
4	+1	+1	-1	-1	-1
5	-1	-1	+1	-1	+1
6	+1	-1	+1	-1	-1
7	-1	+1	+1	+1	-1
8	+1	+1	+1	+1	+1
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower, middle and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 µl) per 100 g of oat flour.
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodextrinase.

3.2.6   Determination of mixing properties of oat dough

The mixing properties of the oat dough were determined using DoughLab (Perten Instruments, Warriewood, Australia) following the method reported by McCann & Day (2013) with some modification. Following the design in Table 3.2, all the ingredients were placed in the DoughLab mixing bowl, and were mixed. After tempering the solids, the water required for optimum consistency was added. Particular attention was given to the determination of water absorption to ensure the complete hydration of all the components. The settings used as required by the equipment were 200 g flour (as base amount), 13.1% as sample moisture, 30% expected absorption, 500 FU as target peak resistance. Water absorption and peak resistance were automatically adjusted at the end of analysis. The total amount of solid material added and the amount of liquid material added were dependent on each point of the experimental design. The mixing time of each assay was 20 minutes.

Each design point was performed in triplicates with the centre in four replicates. The experiment was carried out in randomized order. The parameters described in Table 3.3 were obtained from the software version DLW 1.0.7.58 to assess the dough mixing properties.

3.2.7   Oat dough preparation

Oat dough was produced as described in Figure 3.2. The basic recipe consisted of: oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g) and water (78 g). Other ingredients (yeast, CMC, YG, TG and CG) at variable quantities were added following the fractional factorial design outlined in Table 3.2. The oat flour with all the ingredients were mixed for 5 min (speed 1: 2 min and speed 2: 4 min) using a Kenwood dough mixer. The oat dough was wrapped in cling film and allowed to rest for 10 min at room temperature. Following the procedure (Figure 3.1), different combinations of oat dough preparations were obtained. Each combination was analysed for pasting (Section 3.2.8), thermal behaviours (Section 3.2.9), free amino acid groups (Section 3.2.10) and protein profile (Section 3.2.11).

3.2.8   Determination of pasting properties of oat dough

Dynamic rheological measurements of the dough were assessed on the Physica MR 501 Rheometer (Anton Paar Germany GmbH, Osfildern, Germany).

Table 3.3   Mixing parameters assessed by DoughLab

Parameters	Definition
Water absorption	Percentage of water required for the dough to produce a torque of 1.1 ± 0.07 Nm or Water absorption corrected for target peak resistance and actual flour moisture content (typically to 14% moisture basis).
Arrival time	The time required for the top (maximum) curve to reach the peak resistance. This value is related to the rate at which water is taken up by the flour.
Stability	The elapsed time at which the torque produced is 1.1 ± 0.07 or the difference between the arrival and departure times. Stability indicates the flour's tolerance to mixing.
Development time	The time to reach the maximum torque at 30°C or the time taken for the dough to reach the peak resistance, between times T1 and T2. Development time is related to the protein content and quality of the flour sample, and the test conditions used.
Departure time	The required time for the top (maximum) curve to fall below the peak resistance. A longer departure time indicates stronger flour.
Softening	The difference in torque between the peak resistance and the middle (average) curve at the specified time after the development time (typically 12 minutes).
Bandwith at peak	The difference in torque between the top (maximum) and bottom (minimum) curves at the development time.
Peak resistance	The maximum torque attained, as measured from the middle (average) curve, between times T1 and T2.
Energy at peak	Maximum energy required to stretch the test piece to its rupture point or the accumulated mechanical energy applied to the dough up to the development time.

Source: DoughLab (2009)
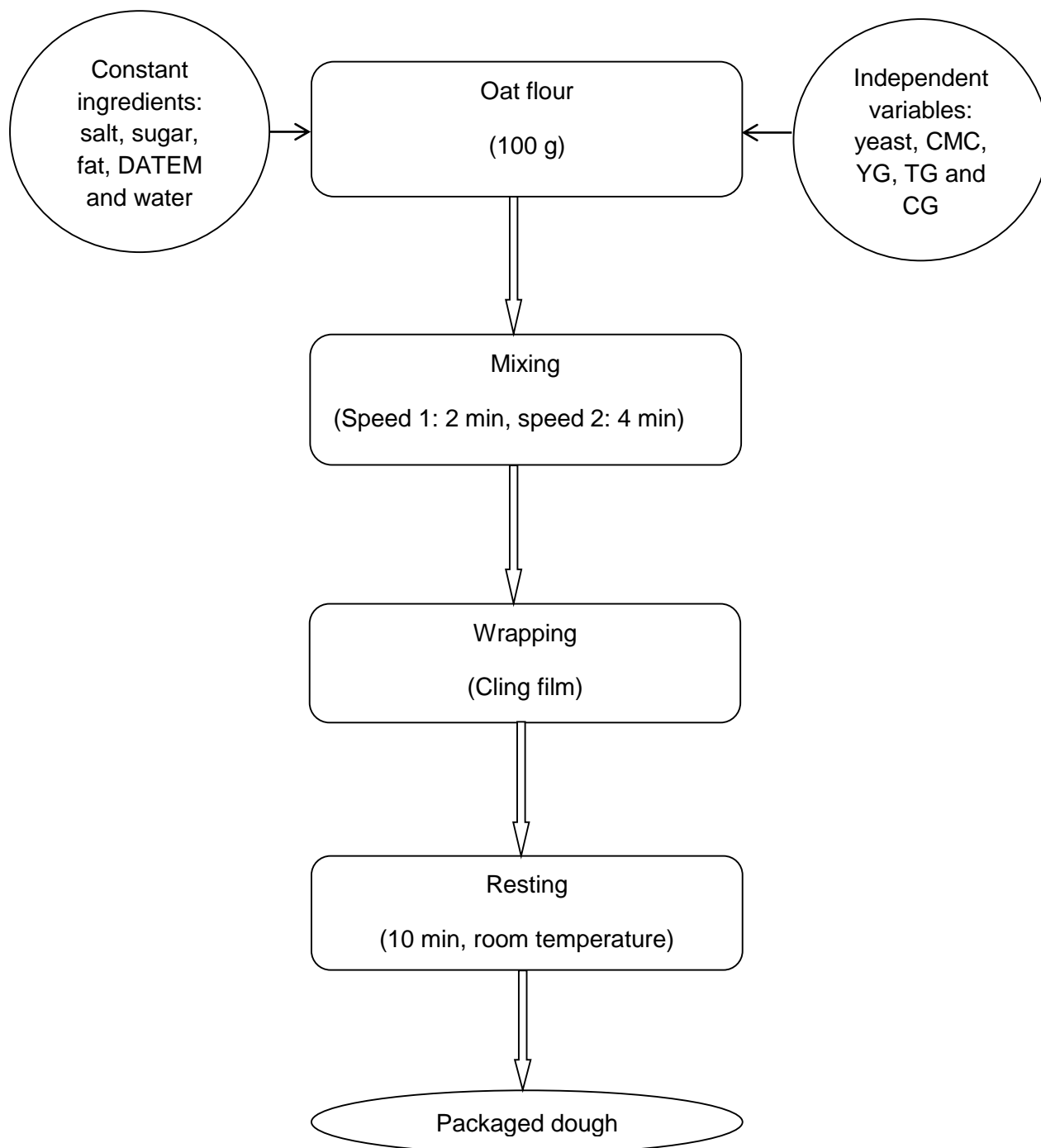
Figure 3.2 Oat dough production process.

The method of Huang *et al.* (2010) was modified and used to determine the pasting properties of the oat dough. The measuring system consisted of parallel geometry (25 mm diameter, 1 mm gap). The dough (20 g) was placed between the plates within 10 minutes after mixing, and the test was started after the dough had rested for 5 minutes. The attachment for temperature control was used to prevent evaporation while the measurements were being taken. Measurements were performed at 30°C. The linear viscoelasticity zone was assessed by stress sweeps at a frequency of 1 Hz. Amplitude (amplitude gamma) sweep tests were performed from 0.01 to 100% to determine the strain (%), shear stress (kPa), storage modulus (kPa), loss modulus (kPa), damping factor, reflection (rad) and torque (Nm) as a function of amplitude.

3.2.9   Determination of thermal properties of oat dough

A Pyris-1 DSC thermal analyzer (PerkinElmer, Waltham, USA) was used to assess the thermal properties of oat dough samples. Different oat dough (3.0 mg) preparations were weighed with a microbalance into aluminium pans, hermetically sealed, and heated from 30 to 110°C at a rate of 5°C/min. Onset (T_o), peak (T_P), end (T_c), and denaturation temperatures with enthalpy (ΔH) were automatically determined by Pyris software. A sealed, empty pan was used as a reference (Huang *et al.,* 2010).

3.2.10   Quantification of free amino acid group in oat dough

The formation of TG-catalysed covalent bonds was confirmed by determining the decrease in the amount of free amino groups. The method was based on the reaction between primary groups and *o*-phthaldialhedyde (OPA) (Dinnella *et al.,* 2002; Gujral & Rosell, 2004; Huang *et al.,* 2010). Oat dough samples (0.2 g) were re-suspended in 2 ml 0.1 M HCl (pH1.0), vortexed and centrifuged for 10 minutes at 10,000 g. OPA reagent (2.5 ml) was added to 0.1 ml of the clear supernatant. The mixture was allowed to react for 2 minutes. The absorbance was determined at 340 nm in a PerkinElmer ultraviolet/visible spectrophotometer.

3.2.11 Determination of protein crosslinking properties of oat dough

The Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE) was used. It was consisted of two main steps: protein extraction from oat dough and polyacrylamide gel electrophoresis.

Protein extraction from oat dough

Globulins and albumins were extracted from 0.5 g oat dough by adding 1.5 ml of 400 mM NaCl, vortexing for 5 minutes and centrifuging for 10 minutes at 10,000 g. The supernatant was removed and stored at -10°C. The precipitate was washed using distilled water and was used to extract the gliadin (avenin). Avenins were extracted from the pellet by adding 1.5 ml of 60% (v/v) ethanol; the sample was vortexed and centrifuged as described above. Glutelins were extracted by adding 1.5 ml SDS buffer (62.5 mM Tris-HCl, pH 6.8, 2.3% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol) to the above residue (Huang *et al.,* 2010).

Polyacrylamide Gel Electrophoresis

The effects of TG and CG on the protein fractions of oat flour were analyzed by SDS-PAGE. The method of Huang *et al.* (2010) was modified and used for the electrophoresis analysis of protein extracted from oat dough. The discontinuous gel was prepared with a 12.5% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). The protein samples (20 µl) were mixed with 10 µl sample buffer (0.01 M Tris-HCl, pH 6.8, including 10% (w/v) SDS, 5% (v/v) DTT, 10% (v/v) glycerol, 0.1% (w/v) bromophenol blue), heated for 5 minutes at 95°C and centrifuged for 10 min at 4,000 g. Sample volumes of 20 µl were loaded into each well. The electrophoresis was performed at 120 mA until the tracking dye was less than 0.5 cm away from the base of the gel. The gels were stained with 0.25% Fermentas pre-made Coomassie brilliant blue (PAGE Blue) dye solution, de-stained in distilled water and analysed.

3.2.12 Statistical analysis

Multivariate Analysis of Variance (MANOVA) was used to determine the differences between treatments for determining significant effects. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS, 2010).

The system behavior was described by a linear factorial model regression, carried out using Design-Expert software (Minneapolis, Minnesota, USA) and given by:

$$Y = \beta_0 + \sum_{k=1}^{5} \beta_i X_i + \sum_{k=1}^{5} \beta_{ij} X_i X_j + \varepsilon \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\text{Equation (1)}$$

Where Y is the response variable, β_0 is a constant; β_i and β_{ij} are the linear and interactive coefficients, respectively; X_i and X_j are the levels of the ingredients and ε is the random error. The quality of the fit of the linear model equation was evaluated by R^2, adjusted R^2, adequate precision (AP) and lack of fit.

The optimisation objective was to minimise energy at peak and development time of oat dough while maximising water absorption and peak resistance of oat dough.

Design Expert-8 was used to estimate desirability, an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimisation found a point that maximizes the desirability function. Principal component analysis was used to extract the components that explained the variability in the data.

3.3   Results and Discussion

3.3.1 Proximate composition and particle size distribution of oat flour

The proximate composition of oat flour used in this study consisted of moisture (13.10 ± 0.00%), ash (1.04 ± 0.02%), protein (9.13 ± 0.06%), and particle size < 212 µm (67.4 ± 0.41%) and 212 µm (31.68 ± 0.64%). All these values were not within the range reported in the literature (Flander *et al.,* 2007). It could be due to the use of different varieties of oats cereals and some processing factors such as degree of milling.

3.3.2 Protein concentrations of TG and CG

The protein concentrations of transglutaminase (TG) and cyclodextrinase (CG) were 35.75 ± 0.40 µg/ml and 51.75 ± 0.42 µg/ml, respectively. The low protein concentration of TG might be explained by the presence of insoluble protein polymers inhibiting the proteolytic activity whereas CG contains soluble proteins. CG proteins were easily degraded during the proteolysis and therefore yielding higher protein concentration.

3.3.3 Effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

Model adequacy

The effects of independent variables on the mixing properties of oat flour are outlined in Tables 3.4 and 3.5. The linear model regression coefficients for mixing properties of oat dough is detailed in Table 3.6. The model p-value ranged from <0.0001 to 0.0105 indicating that the linear models were significant ($p < 0.05$) for each response in explaining the variation between the independent and dependent variables. The adequacy of precision (estimation of the signal to noise ratio) ranged from 7.12 to 22.35. A ratio of 4 is desirable. These values being greater to 4 indicated that the models were adequate. The significant lack of fit ranged from 0.09 to 8.07 indicated that lack of fit was not significant ($p > 0.01$) and hence the model was adequate. The adjusted R^2 ranged from 0.43 to 0.91 indicating variation in the fit for the models. The adjusted coefficients of correlation (R^2_{adj}) of water absorption (0.84), energy at peak (0.89), peak resistance (0.84) and development time (0.91) exhibited better goodness of fit compared to other parameters (Table 3.6). In general the models were adequate in explaining the variation between the independent and dependent variables and could be used to navigate the design space. However, for optimising the effects models with higher R^2 were used.

Main effect of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

The linear model regression coefficients for mixing properties of oat dough for each response variable are shown in Table 3.6. Yeast did not have significant ($p > 0.05$) effect on all the mixing properties of oat dough. However, it slightly decreased the water absorption ($F_{(1, 24)} = 1.98$, $p = 0.1773$) (Figure 3.3) and peak resistance ($F_{(1, 24)} = 1.64$, $p = 0.2160$) (Figure 3.3) of oat dough when its level was increased from 1.25 - 3.25 g (Tables 3.4 and 3.5). The yeast consumed water as a nutrient to achieve the fermentation process of glucose and therefore it

Table 3.4   Effect of Yeast, CMC, YG, TG and CG on the water absorption, arrival time, stability, energy at peak and peak resistance of oat dough[1, 2]

	Ingredients					Response variables				
Run	Yeast	CMC	YG	TG	CG	Water absorption (%)	Arrival time (min)	Stability (min)	Energy at peak (Wh/kg)	Peak resistance (FU)
1	-1	-1	-1	+1	-1	38.45 ± 0.49	2.95 ± 0.21	7.40 ±0.85	11.45 ± 2.33	40.30 ± 19.09
2	+1	-1	-1	+1	+1	37.70 ± 0.57	2.80 ± 0.71	7.50 ± 2.69	10.45 ± 0.46	816.00 ± 20.08
3	-1	+1	-1	-1	+1	35.75 ± 0.07	3.60 ± 0.85	16.40 ± 0.85	20.65 ± 1.91	736.50 ± 4.24
4	+1	+1	-1	-1	-1	35.15 ± 0.07	2.90 ± 0.14	16.90 ± 0.42	15.55 ± 4.03	712.90 ± 2.83
5	-1	-1	+1	-1	+1	34.80 ± 0.99	1.55 ± 0.07	2.05 ± 0.64	3.75 ± 0.78	696.40 ± 40.87
6	+1	-1	+1	-1	-1	33.70 ± 0.14	1.95 ± 0.07	5.75 ± 0.35	4.25 ± 0.21	652.00 ± 5.66
7	-1	+1	+1	+1	-1	33.90 ± 0.57	2.25 ± 0.21	7.21 ± 2.69	5.65 ± 0.78	663.95 ± 21.99
8	+1	+1	+1	+1	+1	34.80 ± 0.71	2.00 ± 0.42	10.06 ± 10.82	4.85 ± 0.78	699.90 ± 25.60
9	0	0	0	0	0	35.77 ± 0.55	2.92 ± 0.31	4.22 ± 0.83	9.17 ± 1.39	737.77 ± 22.44

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 µl).

[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase

Table 3.5 Effect of Yeast, CMC, YG, TG and CG on the development time, departure time, softening and bandwith at peak of oat dough[1, 2]

	Ingredients					Response variables			
						Development time	Departure time	Softening	Bandwith at peak
Run	Yeast	CMC	YG	TG	CG	(min)	(min)	(FU)	(FU)
1	-1	-1	-1	+1	-1	4.95 ± 0.78	10.40 ± 0.71	67.75 ± 11.95	51.25 ± 0.49
2	+1	-1	-1	+1	+1	4.75 ± 0.21	10.25 ± 1.91	73.50 ± 22.20	54.35 ± 10.25
3	-1	+1	-1	-1	+1	9.45 ± 0.78	20.00 ± 0.00	0.00 ± 0.00	45.90 ± 3.25
4	+1	+1	-1	-1	-1	7.80 ± 1.56	19.80 ± 0.28	10.70 ±15.13	47.85 ± 1.34
5	-1	-1	+1	-1	+1	2.10 ± 0.28	3.65 ± 0.78	93.20 ± 0.57	44.15 ± 4.17
6	+1	-1	+1	-1	-1	2.65 ± 0.21	7.50 ± 0.14	39.40 ± 2.26	40.50 ± 0.71
7	-1	+1	+1	+1	-1	3.35 ± 0.35	9.40 ± 2.55	43.10 ± 0.99	41.00 ± 0.28
8	+1	+1	+1	+1	+1	2.80 ± 0.57	12.05 ± 11.24	58.85 ± 26.80	41.70 ± 2.97
9	0	0	0	0	0	4.59 ± 0.55	7.14 ± 0.95	73.04 ± 13.31	45.42± 2.20

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively.
Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 µl).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase

Table 3.6   Regression coefficients of linear model for mixing properties of oat dough[1,2]

Coefficients	Water absorption (%)	Arrival time (min)	Stability (min)	Energy at peak (Wh/kg)	Peak resistance (FU)	Development time (min)	Departure time (min)	Softening (FU)	Bandwith at peak (FU)
Response variable									
Linear									
β_0	41.1127	3.0080	6.7408	15.9406	910.0339	5.0751	8.4989	94.1279	52.0907
β_1	-1.0000	0.3625	0.8937	1.2250	-37.4375	0.7500	0.7687	-30.6375	-0.9375
β_2	-2.9510*	0.6333	4.4833*	4.8208*	-93.4531*	3.6156*	4.5083*	-59.4979*	-1.9167
β_3	-0.1071*	-0.0489*	-0.2516*	-0.4304*	-4.2766*	-0.1755*	-0.3027*	0.8978	-0.3478*
β_4	1.3625*	-	-2.2375	-2.9500*	55.5875*	-1.5625*	-	24.9750*	2.4750
β_5	-0.0133	-0.0258	-0.1592	-0.1217	0.6637	-	-0.1808	1.9008	0.2458
Interaction									
β_{12}	0.5375	-0.3000	-	-1.3500	20.2625	-0.6625*	-	18.6250	0.8000
β_{25}	0.01917	0.0167	0.0992	0.0967	-	5.5000E-003	0.1142	-0.9083	-0.1333
R^2	0.8883	0.6582	0.5697	0.9228	0.8808	0.9328	0.6061	0.6817	0.6587
Model p-value	<0.0001	0.0017	0.0105	<0.0001	<0.0001	<0.0001	0.0020	0.0026	0.0044
Adjusted R^2	0.8423	0.5443	0.4263	0.8910	0.8411	0.9104	0.5025	0.5507	0.5181
AP	15.2500	9.0450	7.124	18.5830	16.1950	22.3500	8.5740	8.4060	7.4390
Lack of fit	1.0600	3.6100	8.0700	0.3600	1.0700	0.2200	5.0000	18.7500	0.0900

[1]significant at $p < 0.05$, β= constant, β_1 = effect of yeast, β_2 = effect of CMC, β_3 = effect of YG, β_4 = effect of TG, β_5 = effect of CG,
R[2]: regression coefficient, AP: Adequate Precision.
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase
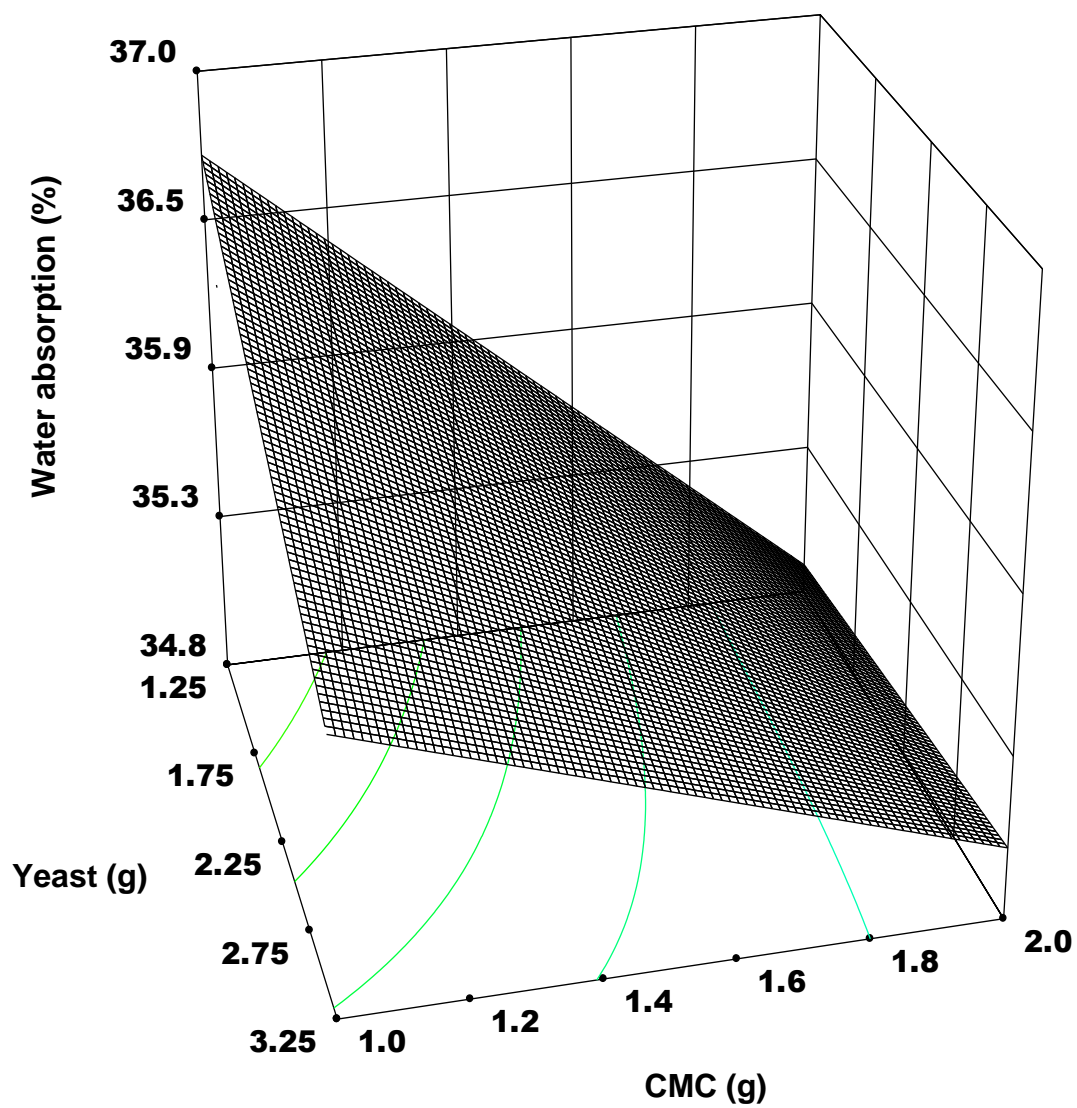
Figure 3.3   Effect of CMC and yeast on the water absorption of oat dough based on 100 g of oat flour.

slightly decreased the water absorption (38.45 - 37.70%) and peak resistance (840.30 - 816.00 FU) of oat dough (Figure 3.4). As the level of yeast decreased from 3.25 - 1.25 g, the energy at peak slightly (F = (1, 24) = 3.96, p = 0.0629) increased (10.45 - 11.45 Wh/kg) (Tables 3.4 and 3.5, Figure 3.5) while the development time of the oat dough slightly (F = (1, 24) = 2.31, p = 0.1472) increased (2.10 - 2.65 min) as yeast level varied from 1.25 to 3.25 g (Tables 3.4 and 3.5, Figure 3.6). These variations could be explained by the yoghurt decreasing dough pH and limiting starch degradation of oat flour into dextrins or simple sugars used as substrates by yeast during the mixing process. Therefore, the yoghurt acidity could be slowing down the significant effect of yeast.

The mixing parameters such as stability (F (1, 24) = 12.49, p = 0.0024), energy at peak (F (1, 24) = 26.27, p = 0.0001), development (F (1, 24) = 49.69, p < 0.0001) and departure (F (1, 24) = 23.66, p = 0.0001) times, water absorption (F (1, 24) = 21.10, p = 0.0003), peak resistance (F (1, 24) = 19.34, P = 0.0004) and softening (F (1, 24) = 16.91, p = 0.0007) were significantly (p < 0.05) affected by CMC (Table 3.6). Stability (7.40 - 16.40 min), energy at peak (11.45 - 20.65 Wh/kg), development (4.95 - 9.45 min) and departure (10.40 - 20.00 min) times, increased with increase in CMC from 1 to 2 g (Tables 3.4 and 3.5). Conversely, water absorption (38.45 - 35.75%), peak resistance (840.30 - 736.50 FU), softening (67.75 - 0.00 FU) and bandwidth at peak (51.25 - 45.90 FU) decreased with increased amounts of CMC from 1 to 2 g (Tables 3.4 and 3.5). The addition of CMC lowered water absorption (38.45 - 35.75%) as its level increased from 1 to 2 g (Tables 3.4 and 3.5, Figure 3.3). These changes were caused by a decrease of the absorption capacity of oat flour indicating that CMC resulted in flour hydrating processes that were slower, causing the dough to have a lower hydrating ability. Lazaridou *et al.* (2007) showed that the addition of CMC increased the water absorption of rice flour. This was attributed to the hydrophilic character of CMC (Leon *et al.,* 2002). However, the results presented here differ. CMC effectively bound water molecules through hydrogen bond formation at 6.5 ≤ pH ≤ 9. Furthermore, the addition of non-starch polysaccharides such as CMC to starch-water systems limited the hydration of the starch and, since water has a plasticizing effect in amorphous regions of the starch, the mobility of the plasticizer will also be restricted. Thus, the non-starch polysaccharides might have an "anti-plasticizing" effect (Bertolini *et al.,* 2005).
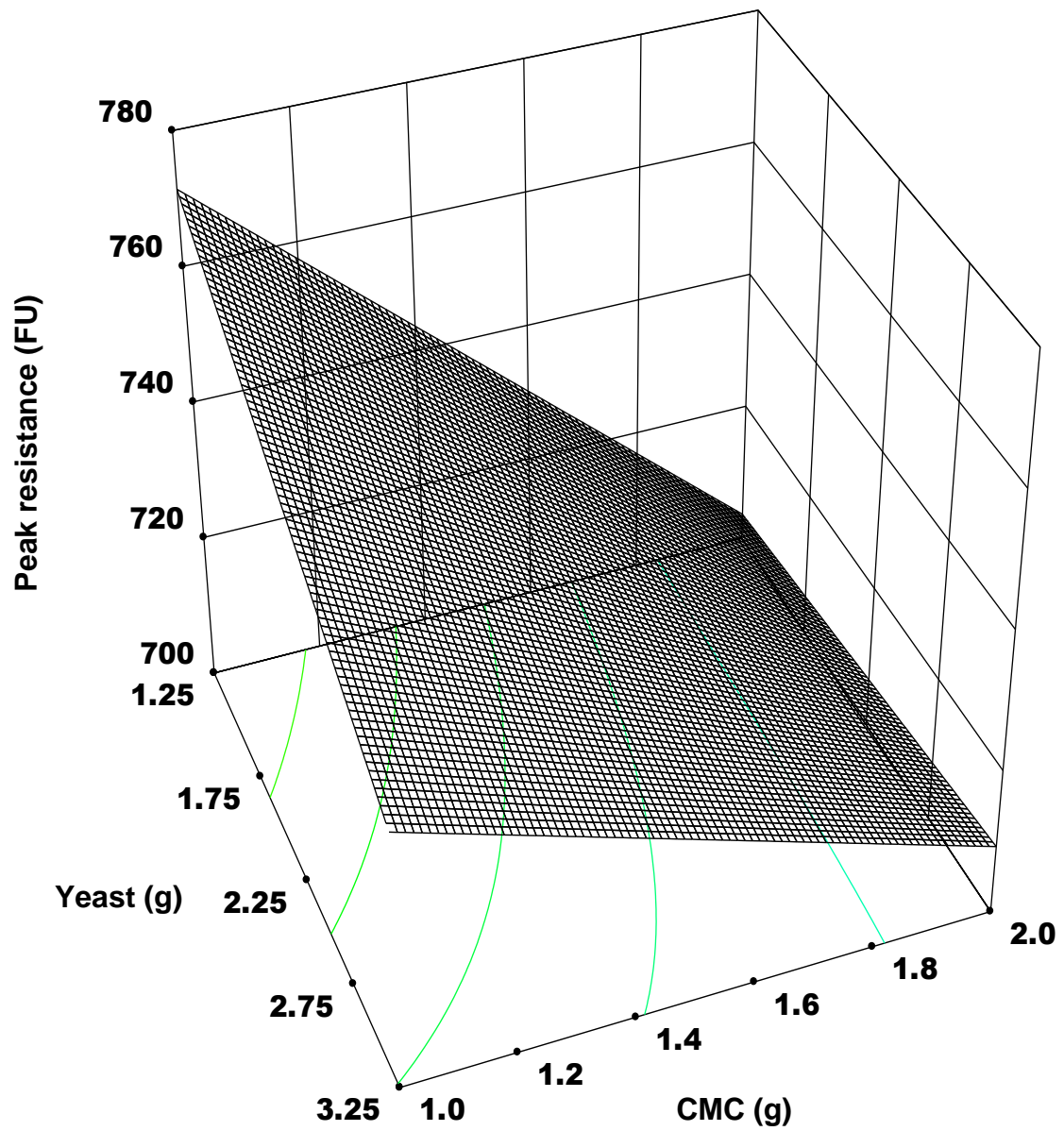
Figure 3.4   Effect of CMC and yeast on the peak resistance of oat dough based on 100 g of oat flour.

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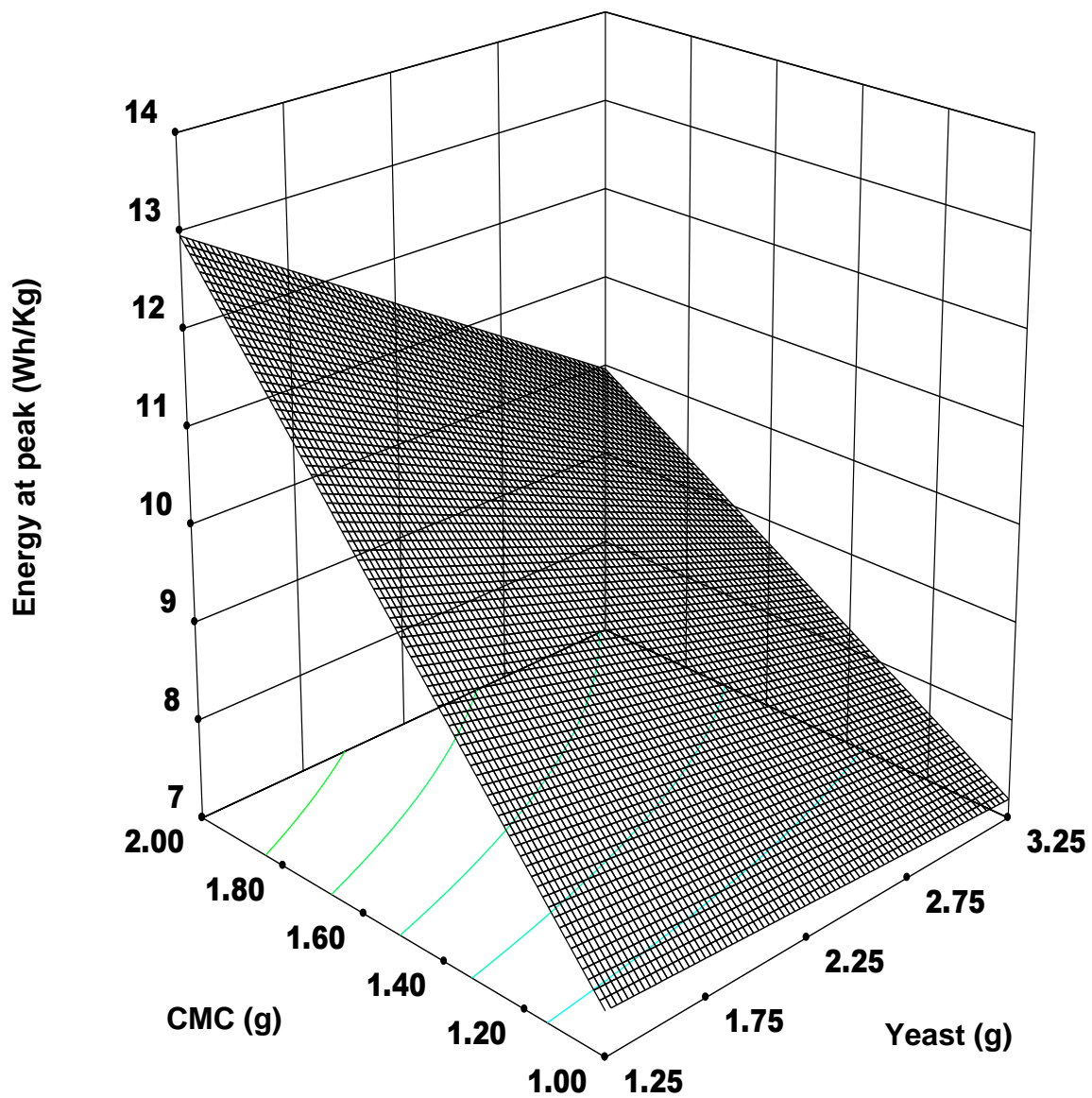
Figure 3.5   Effect of CMC and yeast on the energy at peak of oat dough based
on 100 g of oat flour.

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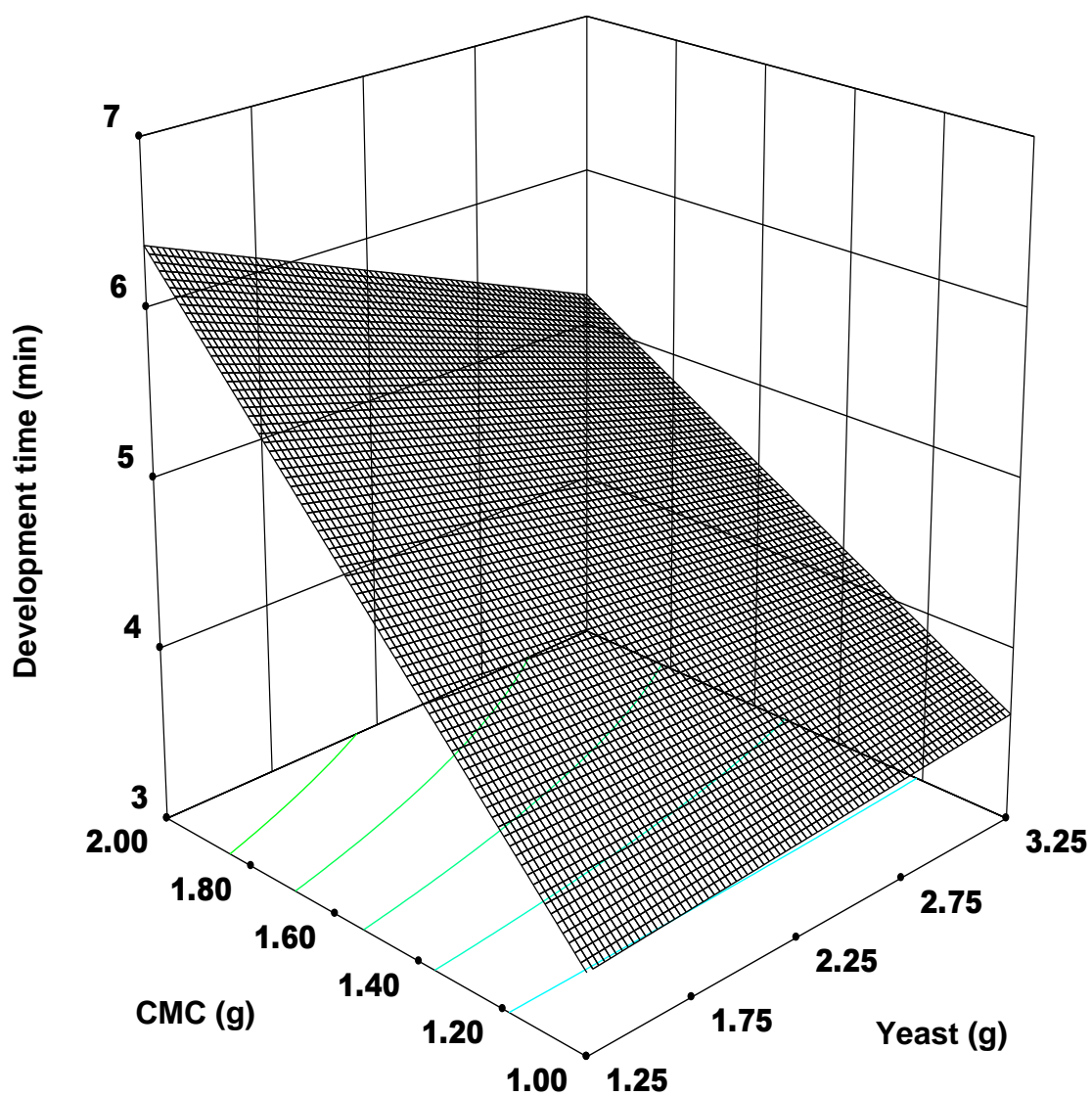
Figure 3.6   Effect of CMC and Yeast on the development time of oat dough based on 100 g of oat flour.

A decrease in pH was noted during the mixing process and was attributed to the lactic acid present in the yoghurt. The lactic acid could inhibit any hydrogen bond formation and therefore resulted in limited water absorption by CMC. This decrease in water absorption lead to increased energy at peak (Figure 3.5), a decreased peak resistance (Figure 3.4) as well as an increased development and departure times (Figure 3.6), due to the fact that the oat flour required less water, less resistance and more energy to reach a dough consistency of 500 FU. These findings were not consistent with the study of Lazaridou *et al.* (2007) which was based on rice flour. However, the presence of yoghurt in this study could result in the observed inconsistency comparatively. According to Lazaridou *et al.* (2007), the addition of CMC increased the water absorption capability of rice dough. This was attributed to the ability of CMC to bind large amounts of water in a gluten-free system. In addition, the rice dough exhibited an increase in dough development time (Lazaridou *et al.*, 2007). This increase of development time is consistent with the studies performed by Sivaramakrishnan *et al.* (2004) on rice flour fortified with 4.5% HydroPropylMethylCellulose (HPMC). The stability of oat dough measured by DoughLab improved flour strength, with higher values being related to stronger doughs (Rosell et al., 2001) such as wheat dough as the level of CMC increased from 1 to 2 g. Therefore, stability of oat dough was clearly positively affected by CMC, indicating that CMC acted as a gluten replacer (Tables 3.4 and 3.6). Therefore, CMC rendered the oat flour dough tolerant to mixing. The dough softening was significantly decreased by the presence of CMC as the dough water absorption decreased.

Yoghurt significantly ($p < 0.05$) decreased all responses (Table 3.6), with the exception that it caused significant ($F_{(1, 24)} = 4.44$, $p = 0.05$) increase in softening as its level increased from 10.75 to 33.75 g. The decreased water absorption (38.45 - 33.90%) and increased dough softening (67.75 - 93.20 FU) were respectively due to the decreased pH caused by the lactic acid in the yoghurt and dairy proteins acting as gluten replacers. In addition, the main whey proteins contained in yoghurt, the α-lactalbumin (four disulphide bonds) and the ß-lactoglobulin, which can be a monomer, dimer and an oligomer depending on pH value, ionic strength and temperature, have a globular structure and hydrophobic, compact folded polypeptide chain, hence decreasing water absorption of oat dough (Houben *et al.,* 2012). Conversely, dairy products such as yoghurt, that have been

used in gluten-free bread formulas, increased water absorption as its level decreases and, therefore, enhanced the softening properties of gluten-free dough (Nunes *et al.,* 2009; Gallagher *et al.,* 2004). According to Gallagher (2009), dairy proteins possess functional properties similar to gluten, as they are able to form networks and have good swelling properties. Some of the useful properties of dairy proteins are the emulsifying and stabilizing ability of caseinates, the gelling properties of whey protein concentrates and isolates, as well as the water-absorption capacity of high-heat, non-fat dry milk (Chandan, 1997).

TG significantly ($p < 0.05$) affected water absorption ($F_{(1, 24)} = 24.49$, $p = 0.001$), peak resistance ($F_{(1, 24)} = 25.60$, $p < 0.001$), softening ($F_{(1, 24)} = 6.50$, $p = 0.0208$), energy at peak ($F_{(1, 24)} = 13.47$, $p = 0.019$) and development time ($F_{(1, 24)} = 24.61$, $p = 0.001$) (Table 3.6). Water absorption (34.80 - 38.45%) and peak resistance (696.40 - 840.30 FU) increased with the increase of TG from 0.5 to 1.5 g. Conversely, the energy at peak (11.45 - 3.75 Wh/kg) and development time (4.95 - 2.10 min) decreased when TG concentration was decreased from 1.5 to 0.5 g. In addition, TG decreased the dough softening (93.20 - 67.75 FU) as its level varied from 0.5 to 1.5 g. The rise in water-holding capacity was attributed to the cross-linking that occurred after TG addition, which caused changes in secondary structure or, possibly, due to changes in protein hydrophobicity from the formation of glutamic acid residues from glutamine hydrolysis (Gerrard *et al.,* 1998). However, this increase in water absorption is not consistent with the findings of Basman *et al.* (2002), Huang *et al.* (2010) and Han *et al.* (2011) whose studies were based on wheat dough, oat dough and buckwheat dough, respectively. This increase of water absorption of oat dough could be due to the added yoghurt. According to Huang *et al.* (2010), TG decreased the water absorption of oat dough. This decrease of water absorption was attributed to acyl-transfer reactions that introduced new functional groups leading to changes in the structure, charge, and hydrophobicity of the proteins (Han *et al.,* 2011). The increased development time and peak resistance of oat dough increased the dough extensibility because of the modification of the cross-link between the oat proteins catalysed by TG. Similar findings were reported by Huang *et al.* (2010) on oat flour. These findings are mainly attributed to the cross-linking catalysed by TG. TG did not significantly affect cooking stability of the oat dough because no significant difference was observed in the oat starch after TG

treatment. Similar results are reported by Siu *et al.* (2002) from research based on oat flour. Hence, TG did improve the mixing properties of oat dough.

CG did not significantly (p > 0.05) affect water absorption ($F_{(1, 24)}$ = 2.82, p = 0.1113), arrival time ($F_{(1, 24)}$ = 0.014, p = 0.9084), stability ($F_{(1, 24)}$ = 0.025, p = 0.8757), energy at peak ($F_{(1, 24)}$ = 0.76, p = 0.3960), peak resistance ($F_{(1, 24)}$ = 3.28, p = 0.0866), softening ($F_{(1, 24)}$ = 2.72, p = 0.1177), bandwith at peak ($F_{(1, 24)}$ = 0.70, p = 0.4150) of oat dough during the mixing process (Table 3.6). As its level increased from 10 - 40 μl, the water absorption (38.45 - 34.80%), arrival time (2.95 - 1.55 min), stability (7.40 - 2.05 min), energy at peak (11.45 - 3.75 Wh/kg), peak resistance (840.30 - 696.40 FU) and bandwith at peak (51.25 - 44.15 FU) slightly decreased (Tables 3.4 and 3.5) while softening of oat dough increased from 67.75 to 93.20 FU. CG did not have any effect on development time of oat dough (Table 3.4). These variations were attributed to the decreased dough pH limiting starch degradation of oat flour into dextrins or simple sugars caused by CG activity. This decreased the availability of simple sugars which were used as substrates by yeast during the mixing process. The optimum pH range of CG is between 7.5 and 8.5. As the yoghurt was added to oat flour, the lactic acid present decreased the pH, thereby inhibiting the significant action of the CG. Therefore, CG did not enhance the mixing properties of oat dough.

Interaction effect of yeast, CMC, YG, TG and CG on the mixing properties of oat dough

There were two types of interactions during the mixing of oat dough. The first one was between yeast and CMC and the second one between CMC and CG. The combined effect of yeast and CMC was significantly (p < 0.05) decreased the development time of oat dough during the mixing process (Table 3.6). However, yeast and CMC slightly (p > 0.05) increased water absorption, peak resistance, softening and bandwith at peak and decreased arrival time, energy at peak of oat dough (Tables 3.4 and 3.5). Their interaction effect did not affect the departure time of oat dough. The efficiency of the interaction between yeast and CMC might be explained by the hydrophilic character of CMC and production of carbon dioxide and ethanol by yeast increasing dough water absorption (Table 3.6). Yeast released gas into cells contributing to the evaporation of water and hence increased the water absorption.

In combination CMC and CG slightly ($p > 0.05$) increased water absorption, arrival time, stability, energy at peak, development time and departure time but decreased softening and bandwith at peak of oat dough (Tables 3.4 and 3.5). This could be due to the hydrophilic character of CMC and starch degradation by CG increasing dough water absorption (Table 3.6).

Optimisation of ingredients

The optimisation goal was to maximise water absorption and peak resistance while minimising energy at peak and development time. The quantity of ingredients for preparing the optimal oat dough formulation per 100 g of oat flour was: yeast (1.25 g), CMC (1 g), yoghurt (20.66 g), TG (1.50 g) and CG (40 µl) with desirability of 0.83. Under this formulation, the model predicts a maximum of water absorption of 37.6%; maximum peak resistance of 816.7 FU; minimum energy at peak of 6.3 Wh/kg and minimum development time of 2.3 minutes. To verify the results, an experiment was performed using optimal conditions. An average values of water absorption (36.15 ± 0.64%), peak resistance (752.9 ± 24.46 FU), energy at peak (10.45 ± 0.35 Wh/Kg) and development time (5.10 ± 0.14 min) were obtained, which is close to the model predicted values. This confirms that the model was adequate to predict the effects of yeast, CMC, YG, TG and CG on the mixing properties of oat dough. Among all the ingredients, only TG induced low pressure which remarkably contributed to the dissociation of protein network and weakness of hydrophobic electrostatic bonds, increasing water absorption and consistency of oat dough.

Components explaining the variation in mixing properties of oat dough as affected by yeast, CMC, YG, TG and CG

Principal component analysis (PCA) indicated that 85.5% of the variation in the data could be explained by two components (Figure 3.7). Component 1 explaining 52.3% of the variation loaded highly on dough strength (stability and departure time). Component 2 contributing 33.2% of the variation loaded on dough resistance (water absorption and peak resistance). CMC significantly increased dough strength while yoghurt reduced it significantly. TG significantly ($p < 0.05$) increased the resistance of the dough to mixing while CMC and yoghurt reduced it significantly ($p < 0.05$). Hence, CMC, TG and yoghurt were ingredients of choice for the modification of oat dough.
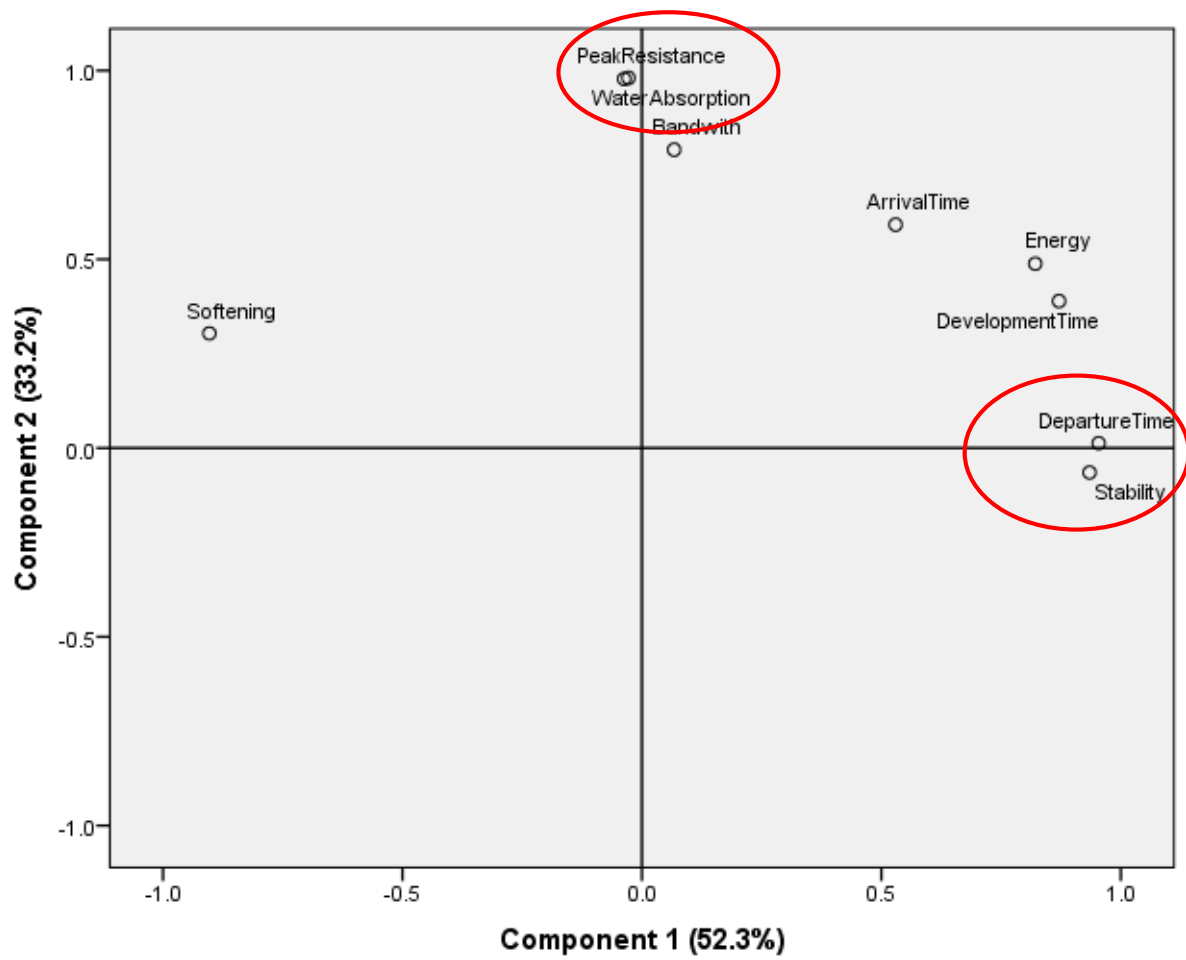
Figure 3.7   Principal component analysis of dough mixing parameters.

3.3.4 Effect of yeast, CMC, YG, TG and CG on the pasting properties of oat dough

The mechanical spectra of all the samples showed that yeast significantly affected the pasting properties of the oat dough, decreasing strain ($F_{(1,18)}$ = 5.66, p = 0.029), shear stress ($F_{(1,18)}$ = 6.51, p = 0.02), storage modulus ($F_{(1,18)}$ = 8.17, p = 0.01), loss modulus ($F_{(1,18)}$ = 7.67, p = 0.013) and torque ($F_{(1,18)}$ = 6.56, p = 0.02) as yeast levels increased from 2.25 to 3.25 g (Table 3.7). Yeast decreased the shear stress (1.62 - 1.34 kPa), storage modulus (202.78 - 132.09 kPa), loss modulus (62.87 - 52.31 kPa) and torque (0.05 - 0.04 Nm) of oat dough as its level increased from 2.25 to 3.25 g. The effect of yeast on pasting properties was attributed to the production of hydrogen peroxide by the yeast acting as an oxidant and making flour-water more elastic. The carbon dioxide produced during the fermentation, dissolved in water, resulted in decreased pH which was good for the formation of cross-links. Hence, carbon dioxide decreased pasting properties of the fermented dough. These findings were consistent with the results reported by Salvador *et al.* (2006) in studies focusing on wheat dough. Furthermore, the storage modulus was always higher than the loss modulus. This indicated that the presence of yeast led to protein cross-linking and the formation of a network structure, which, therefore, decreased the strain, shear stress, storage modulus, loss modulus and torque. Similar results were reported in other studies on gluten-free flours (Renzetti *et al.,* 2008a).

CMC did not significantly (p > 0.05) affect the pasting properties of the oat dough when its level increased from 1.0 - 2.0 g (Table 3.7). However, it slightly increased the storage modulus ($F_{(1,18)}$ = 1.364, p = 0.258) from 156.42 to 190.04 kPa and loss modulus $F_{(1,18)}$ = 3.050, p = 0.098) from 56.37 to 70.25 kPa of oat dough as CMC level increased from 1 to 2 g. The increases were mainly due to the hydrophilic character of CMC. Lazaridou *et al.* (2007) showed that CMC increased the dynamic elastic modulus (storage modulus) and viscous modulus (loss modulus) of rice dough, rendering the rice dough more elastic (70 Brabender Units) than the dough control (60 Brabender Units). However, the increasing effect of hydrocolloid level on G' was not clear because the added water also increased. Thus, CMC became stronger (higher G' values) with increasing concentration affecting the rheological properties of the dough more than the increasing content of water (Lazaridou *et al.,* 2007). Although gluten-free dough exhibited an elastic modulus

Table 3.7   Effect of Yeast, CMC, YG, TG and CG on the pasting properties of oat dough[1, 2]

	Ingredients					Responses			
Run	Yeast	CMC	YG	TG	CG	Strain (%)	Shear stress (Kpa)	Storage modulus (Kpa)	Loss modulus (Kpa)
1	-1	-1	-1	+1	-1	12.55 ± 0.00	1.40 ± 0.28	143.50 ± 4.95	64.15 ± 13.36
2	+1	-1	-1	+1	+1	12.54 ± 0.00	1.05 ± 0.24	87.45 ± 41.79	44.50 ± 15.56
3	-1	+1	-1	-1	+1	12.54 ± 0.00	1.81 ± 0.64	215.50 ± 6.36	91.80 ± 27.15
4	+1	+1	-1	-1	-1	12.54 ± 0.00	1.41 ± 0.54	111.25 ± 70.36	55.15 ± 28.21
5	-1	-1	+1	-1	+1	12.55 ± 0.00	1.82 ± 0.10	232.50 ± 3.53	64.70 ± 0.57
6	+1	-1	+1	-1	-1	12.54 ± 0.00	1.31 ± 0.52	162.25 ± 101.47	52.15 ± 23.69
7	-1	+1	+1	+1	-1	12.54 ± 0.00	2.72 ± 0.37	266.00 ± 0.00	76.60 ± 0.28
8	+1	+1	+1	+1	+1	12.54 ± 0.00	1.58 ± 0.64	167.40 ± 95.60	57.45 ± 22.56
9	0	0	0	0	0	12.54 ± 0.00	1.62 ± 0.50	202.78 ± 63.19	62.87 ± 12.40

	Ingredients					Responses		
Run	Yeast	CMC	YG	TG	CG	Damping factor	Reflection angle (rad)	Torque (Nm)
1	-1	-1	-1	+1	-1	0.78 ± 0.02	0.10 ± 0.00	0.01 ± 0.00
2	+1	-1	-1	+1	+1	0.69 ± 0.00	0.10 ± 0.00	0.00 ± 0.00
3	-1	+1	-1	-1	+1	0.72 ± 0.04	0.01 ± 0.00	0.00 ± 0.00
4	+1	+1	-1	-1	-1	0.70 ± 0.02	0.10 ± 0.00	0.00 ± 0.00
5	-1	-1	+1	-1	+1	0.51 ± 0.01	0.10 ± 0.00	0.00 ± 0.00
6	+1	-1	+1	-1	-1	0.55 ± 0.06	0.10 ± 0.00	0.00 ± 0.00
7	-1	+1	+1	+1	-1	0.49 ± 0.04	0.10 ± 0.00	0.01 ± 0.00
8	+1	+1	+1	+1	+1	0.59 ± 0.03	0.10 ± 0.00	0.00 ± 0.00
9	0	0	0	0	0	0.54 ± 0.03	0.10 ± 0.00	0.00± 0.00

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 μl).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase.

higher than the viscous modulus, the dough made with CMC had a higher viscosity than the ones containing no hydrocolloid (Lazaridou *et al.,* 2007). Moreover, the viscoelasticity of gluten-free dough was lower when CMC was incorporated. The consistency of gluten-free dough supplemented by CMC was greater (419.5 BU) than the control (285.4 BU) (Sciarini *et al.,* 2010). The results presented are similar and thereby confirming that CMC action was not limited by pH decrease due to the presence of lactic acid and carbon dioxide from yoghurt and yeast, respectively. CMC could contribute towards the swelling, the gelatinization and the gelling properties of the dough and the retrogradation of the starch. The water molecules could bind to CMC via hydrogen bonds at low pH (induced by yoghurt) and the reaction could be catalyzed effectively. Therefore, CMC could interact with the water molecules included in the system, limiting the diffusion and system stability.

Yoghurt significantly ($p < 0.05$) increased the strain ($F_{(1, 18)} = 4.82$, $p = 0.041$) from 12.542 to 12.543%, storage modulus ($F_{(1, 18)} = 5.52$, $p = 0.03$) from 139.42 to 202.78 kPa, but significantly ($F_{(1, 18)} = 68.25$, $p = 0.00$) decreased the damping factor 0.72 to 0.54 of oat dough as its level increased from 10.75 to 22.25 g (Table 3.7). The samples displayed a much higher ability to resist deformation leading to greater solid-like behaviour under the applied testing conditions. This might be mainly due to the presence of proteins such as milk proteins (sodium caseinate) in the yoghurt, resulting in decreased water absorption of the oat dough. This result correlated with studies performed by Nunes *et al.* (2009) on rice flour. Furthermore, Bertolini *et al.* (2005) also showed that the storage modulus (G′) of samples showed an increasing trend when sodium caseinate was present in the composite flours. This effect seemed to be clear, mainly for rice and wheat starches, at the lower starch concentrations, suggesting that the viscoelastic properties of the sodium caseinate are more important in dough systems with low starch concentrations. Despite the conflicting results, it seems clear that changes in the viscoelastic properties of the systems could be attributed to the limitation of starch swelling and gelatinization by sodium caseinate addition, as for most non-starch polysaccharides (Bertolini *et al.,* 2005). The effect of sodium caseinate on starch swelling seems to be similar to when water is restricted in the system (see mixing results, Section 3.3.3, Tables 3.4 & 3.6) and is more evident in starches with a high amylose content such as oat starch (Bertolini *et al.,* 2005). As the swelling behaviour of the starches was the main feature that was altered in these systems, it

was expected that this feature would be related to the botanical origin of starch responsible for starch swelling, such as amylose/amylopectin ratio, molecular weight of amylose and amylopectin, their distribution on the granule starch, lipid content, minor components (such as minerals and salts), granule size, and affinity of starch for water should play an appreciable role in changes promoted by the sodium caseinate. Nevertheless, the contribution of sodium caseinate to the pasting properties of starch gels is strongly related to the concentration and the botanical origin of the starch. Therefore yoghurt acted as a gluten replacer because it was able to improve the swelling character of oat dough and form network (Gallagher, 2009).

TG did not significantly ($p > 0.05$) affect the pasting properties of the oat dough when its level was increased from 0.5 - 1.0 g (Table 3.7). However, it slightly increased the storage modulus ($F_{(1,18)} = 0.246$, $p = 0.626$) from 180.37 to 202.78 Kpa but decreased the loss modulus ($F_{(1,18)} = 0.441$, $p = 0.515$) from 65.95 to 62.87 kPa of oat dough as TG level increased from 0.5 to 1.0 g. These variations might be attributed to the slight amount of protein cross-links induced by TG. Kohajdova & Karovicova (2009) found that the addition of TG at less than 1% (w/v) significantly increased the elastic (G'), viscous (G'') and viscoelasticity (|G*|) modulus of buckwheat dough. This increased dough elasticity and viscosity suggested that TG led to protein cross-linking and network formation of buckwheat dough, thereby modifying its viscoelasticity properties (Han *et al.,* 2011). This modification was said to be due to the formation of non-disulfide covalent cross-links between peptide bound γ-glutamyl residues and ε-amino groups of lysine residues in proteins (Shin *et al.,* 2010). Similar results were reported by Renzetti *et al.* (2008b), Huang *et al.* (2010) and Shin *et al.* (2010) in their studies on rice and oat dough. However, upon increasing the TG amount in the buckwheat dough from 1.0% to 1.5%, the |G*| and G'' were not significantly changed (Han *et al.,* 2011). The results presented here was similar to these findings. It was probably not only due to the restricted reactivity of the TG owing to the lack of lysine residues present, but rather the relative content of glutamine in the oat flour. Similar results were reported by Renzetti *et al.* (2008a) on buckwheat flour. This low level of lysine might have lead to: (a) no additional protein aggregation occurred in the protein network of the dough system, and (b) additional protein network formation occurred, but these changes did

not contribute to significant changes in dough rheology (Beck *et al.,* 2011). Therefore, TG did improve the pasting properties of oat dough.

CG did not significantly (p > 0.05) affect the pasting properties of the oat dough when its level was increased from 10.0 - 40.0 µl (Table 3.7). However, it slightly increased the storage modulus (F (1,18) = 0.030, p = 0.865) from 170.75 to 175.71 kPa and the loss modulus (F (1,18) = 0.107, p = 0.747) from 62.01 to 64.61 kPa of oat dough as its CG level varied from 10 to 40 µl. These slight changes were attributed to the hydrophobic environment of oat dough which was not significantly reduced by CG through starch hydrolyzing and cyclizing activities. Conversely, the CG addition to the dough lowered the elastic modulus and complex viscosity of rice dough but did not seem to influence the viscous modulus (Gujral *et al.,* 2003). According to Gujral *et al.* (2003), the tan δ (G''/G') or viscoelasticity increased in the presence of the enzyme, suggesting that the relative contribution of the solid character (G' or elastic modulus) decreased. It acted on the damaged starch during the mixing process. This action brought about some hydrolysis, which impacted the dough pasting. A decrease in the elastic modulus and an increase in the tan δ in wheat flour dough from sprouted wheat flours were highlighted by Singh *et al.* (2001) and were due to higher amylase and protease activities. According to Rosell (2009), CG degraded starch of gluten-free dough through its hydrolyzing and cyclization activities. The hydrolysis reaction released cyclodextrins which were able to form complexes with lipids and proteins. The necessary substrates for the complex formation between lipids and proteins with cyclodextrins were provided by the cyclization reaction (Rosell, 2009). Therefore, the hydrophobic environment of gluten-free dough was reduced by CG through starch hydrolysis and cyclizing activities and also through the hydrolysis products that could form complexes with a variety of solid, liquid and gaseous compounds. However, the results presented here differ. This was attributed to the low pH of the oat dough (as indicated earlier), limiting the CG action and lowering the level of amylase and protease activities hydrolyzing starch and hence impacting the dough pasting behaviours. Therefore, the hydrophobic environment of oat dough was not reduced by CG through starch hydrolyzing and cyclizing activities and also through the hydrolysis products that could form complexes with a variety of solid, liquid and gaseous compounds, known

to improve the pasting properties of oat dough. CG did not enhance the pasting properties of oat dough.

3.3.5 Effect of yeast, CMC, YG, TG and CG on the thermal properties of oat dough

Yeast, CMC, YG, TG and CG had no significant ($p > 0.05$) effects on the thermal properties of the oat dough (Table 3.8). However, there were slight variations in all the parameters upon increasing levels of ingredients such as yeast (1.25 - 2.25 g), CMC (1.0 - 1.5 g), YG (10.25 - 22.25 g), TG (0.5 - 1.0 g) and CG (10 - 25 µl). Yeast incorporation slightly affected onset temperature ($F_{(1,18)} = 0.081$, $p = 0.779$), peak temperature ($F_{(1,18)} = 0.170$, $p = 0.685$), conclusion temperature ($F_{(1,18)} = 0.255$, $p = 0.620$), denaturation temperature ($F_{(1,18)} = 0.570$, $p = 0.460$) and enthalpy (($F_{(1,18)} = 0.040$, $p = 0.844$) of oat dough. As yeast varied from 1.25 to 2.25 g, there were increases of onset temperature (57.11 - 58.66°C), peak temperature (61.72 - 63.11°C), conclusion temperature (64.45 - 66.99°C), denaturation temperature (7.34 - 8.33°C) and decrease of enthalpy (from 0.04 to –4.06 J/g) (Table 3.8).

CMC did not significantly ($p > 0.05$) affect the thermal properties of oat dough. However, CMC slightly increased the onset temperature ($F_{(1,18)} = 0.091$, $p = 0.776$) (57.54 - 58.66°C), peak temperature ($F_{(1,18)} = 0.371$, $p = 0.550$) (61.94 - 63.11°C), conclusion temperature ($F_{(1,18)} = 0.0906$, $p = 0.354$) (65.03 - 66.99°C), denaturation temperature ($F_{(1,18)} = 0.791$, $p = 0.385$) (7.49 - 8.33°C) but slighly decreased the enthalpy ($F_{(1,18)} = 0.017$, $p = 0.898$) from 0.29 to –4.06 J/g of oat dough as CMC level increased from 1.0 to 1.5 g (Table 3.8). In accordance with many reports by various researchers (Lai & Kokini, 1991; Kokini *et al.,* 1992; Fanta & Christianson, 1996 Rojas *et al.,* 1999), the presence of CMC did not significantly influence melting, gelatinization, fragmentation and retrogradation of starch. These effects were shown to affect the pasting properties of oat dough, which was in agreement with the findings of Armero *et al.*(1995) and Rojas *et al.*(1999). According to Kohajdova *et al.* (2009), it is generally accepted that hydrocolloid such as CMC affects the pasting properties of starch in a different way. The most important factor involved in this, is the molecular structure of hydrocolloids and/or ionic charges of both starch and hydrocolloid (Kohajdova *et al.* 2009). These sufficient ionic charges of both starch and CMC in oat dough were not limited by the low pH of the oat dough. In addition, it was reported that the changes in the thermal

Table 3.8   Effect of yeast, CMC, YG, TG and CG on the thermal properties of oat dough[1, 2, 3]

	Ingredients					Responses				
Run	Yeast	CMC	YG	TG	CG	T_o (°C)	T_p (°C)	T_e (°C)	ΔT (°C)	Enthalpy (J/g)
1	-1	-1	-1	+1	-1	57.65 ± 2.59	65.93 ± 8.60	67.03 ± 7.59	9.37 ± 5.00	0.55 ± 0.57
2	+1	-1	-1	+1	+1	56.27 ± 0.14	57.93 ± 3.32	62.95 ± 2.35	6.68 ± 2.33	-0.47 ± 1.60
3	-1	+1	-1	-1	+1	58.78 ± 3.77	61.67 ± 3.37	67.00 ± 8.88	8.21 ± 5.11	0.37 ± 0.07
4	+1	+1	-1	-1	-1	56.87 ± 1.27	60.28 ± 0.41	60.86 ± 0.78	3.99 ± 2.06	-0.19 ± 0.74
5	-1	-1	+1	-1	+1	55.84 ± 1.13	59.1 ± 0.96	62.54 ± 2.50	6.70 ± 3.63	-0.99 ± 1.57
6	+1	-1	+1	-1	-1	60.39 ± 6.05	64.79 ± 5.90	67.61 ± 3.19	7.22 ± 2.86	3.86 ± 4.02
7	-1	+1	+1	+1	-1	56.15 ± 0.63	60.19 ± 0.09	61.22 ± 0.18	5.06 ± 0.81	0.22 ± 0.12
8	+1	+1	+1	+1	+1	56.57 ± 0.09	60.30 ± 0.02	61.06 ± 0.76	4.49 ± 0.85	0.34 ± 0.28
9	0	0	0	0	0	58.66 ± 3.01	63.11 ± 4.35	66.99 ± 5.76	8.33 ± 5.99	-4.06 ± 12.53

[2]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 μl).
[2]T_0: Onset temperature; T_P: Peak temperature; T_e: End temperature; ΔT: Denaturation temperature.
[3]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase.

properties of starch caused by the addition of the non-starch polysaccharide, CMC, to the starch is markedly high at starch/solvent ratios of 1:10 (or above) and that the end temperature increased as the non-starch polysaccharide concentration increased in the system (Bertolini *et al.,* 2005).

YG did not significantly (p > 0.05) affect the thermal properties of oat dough. As YG increased from 10.25 to 22.25 g, samples containing YG showed slight increases in the onset temperature ($F_{(1,18)} = 0.011$, p = 0.918) (57.39 - 58.66°C), peak temperature ($F_{(1,18)} = 0.027$, p = 0.872) (61.45 - 63.11°C), conclusion temperature ($F_{(1,18)} = 0.265$, p = 0.613) (64.46 - 66.99°C), denaturation temperature ($F_{(1,18)} = 0.268$, p = 0.611) (7.06 - 8.33°C) and slight decrease in the enthalpy ($F_{(1,18)} = 0.035$, p = 0.853) from 0.06 to –4.06 J/g of oat dough (Table 3.8). The decrease in the enthalpy of gelatinization, suggesting that milk protein such as sodium caseinate present in the YG had the same decreasing effect on the enthalpy of gelatinization as all the other ingredients. This might be attributed to significant interactions between the starch and sodium caseinate as the gelatinization onset temperature, the peak temperature, and the end temperature increased (Table 3.8). In samples with sodium caseinate addition, a shift in the gelatinization peak was observed, with increases in the onset temperature, the peak gelatinization temperature, and the end temperature, in agreement with the results obtained by Erdogdu *et al.* (1995) for casein-wheat starch samples. Changes in the gelatinization onset and peak temperatures appeared to be higher in cereal starches, even if the changes did not always follow this trend. In contrast to the wheat and rice starch samples, the endothermic peak at 100°C (relative to the complex amylose-lipid in the cereal starch samples) was not shown in the sodium caseinate-starch samples. Nevertheless, it is not clear whether these results were not, at least in part, caused by a dilution effect (Erdogdu *et al.,* 1995). According to Bertolini *et al.* (2005), the presence of non-covalent hydrogen interactions or chemical bonds between starch and caseinate were suggested because these interactions seemed to be more stable at a casein/starch ratio of 1:1. Hence, the presence of some proteins such as sodium caseinate in the systems impacted swelling and starch gelatinization. These changes determined some of the characteristics of the matrix, depending on the botanical origin of the starch, the caseinate/starch ratio, and the minor components.

TG slightly ($p > 0.05$) increased the onset temperature ($F_{(1,18)} = 0.792$, $p = 0.385$) (57.97 - 58.66°C), peak temperature ($F_{(1,18)} = 0.029$, $p = 0.867$) (61.40 - 63.11°C), conclusion temperature ($F_{(1,18)} = 0.0301$, $p = 0.590$) (64.50 - 66.99°C), denaturation temperature ($F_{(1,18)} = 0.003$, $p = 0.955$) (6.53 - 8.32°C) but slightly decreased the enthalpy ($F_{(1,18)} = 0.020$, $p = 0.888$) from 0.76 to –4.06 J/g of oat dough as TG level increased from 0.5 to 1.0 g (Table 3.8). TG did not significantly affect the thermal behaviours of oat dough because the covalent cross-linkage promoted by the network and enzyme did not make them sensitive to the temperature, confirming the covalent cross-link character of TG. However, slight increases in onset temperature (T_o), peak temperature (T_p), end temperature and denaturation temperature were observed between samples as TG levels increased from 0.5 - 1.0 g. Similar results were reported by Huang *et al.* (2010) on studies involving oat dough. This variation indicated that TG improved the thermal stability of the dough. However, enthalpy (ΔH) of the dough samples slightly decreased with TG because TG did not significantly act on starch during the thermal treatment (Huang *et al.,* 2010). These findings were consistent with those of Ahn *et al.* (2005) who pointed out that TG impacts on protein denaturation of pure protein leading to a decrease in enthalpy due to protein unfolding. Therefore, the lower enthalpy of soy flours or wheat - soy blends are due to the high protein content of soy (Huang *et al.,* 2010). Larre *et al.* (2000) similarly demonstrated that TG has significant impacts on the thermal stability of gluten. Therefore, TG did not improve the thermal properties of oat dough.

As CG increased from 10 to 25 µL, there was slight increase in the onset temperature ($F_{(1,18)} = 0.376$, $p = 0.548$) (57.76 - 58.66°C), peak temperature ($F_{(1,18)} = 1.962$, $p = 0.178$) (62.79 - 63.11°C), conclusion temperature ($F_{(1,18)} = 0.091$, $p = 0.766$) (64.18 - 66.99°C), denaturation temperature ($F_{(1,18)} = 0.002$, $p = 0.963$) (6.41 - 8.33°C) and slight decrease the enthalpy ($F_{(1,18)} = 0.094$, $p = 0.763$) from 1.11 to –4.06 J/g of oat dough (Table 3.8). The slight effect of CG on oat dough indicated that the dough was too resistant to heating and shear stress because oat starch was not totally hydrolyzed by CG. This was attributed to the hydrophobic environment of oat dough, thereby limiting CG action. Hence, CG did not enhance the thermal properties of oat dough.

3.3.6 Effect of yeast, CMC, YG, TG and CG on the amount of free amino acid groups present in oat dough formulations

The spectra of all the samples showed that all the parameters such as yeast ($F_{(1, 18)} = 9.37$, $p = 0.007$), YG ($F_{(1, 18)} = 68.58$, $p = 0.00$), TG ($F_{(1, 18)} = 9.86$, $p = 0.006$) and CG ($F_{(1, 18)} = 6.63$, $p = 0.019$) significantly affected the presence of free amino acid groups of oat dough except CMC ($F_{(1, 18)} = 2.020$, $p = 0.172$) (Table 3.9).

Yeast increased the number of free amino acid groups from 0.59 to 0.94 when its level increased from 1.25 - 2.25 g (Table 3.9), indicating that it limited the catalysis of covalent cross-linking of the protein present in the oat dough. This could be because it is rich in some specific amino acid groups such as glutamic acid contributed by albumin, globulin and avenin, thereby contributing to an increase in the amount of free amino acid groups.

Presence of CMC in oat dough led to the slight increase in the number of free amino groups from 0.61 to 0.80 as CMC level increased from 1 to 2 g (Table 3.9). This increase might be explained by the lack of interaction between the oat dough proteins and CMC via van der Waals' interactions or other transient interactions due to charge. A change in pH would lead to such interaction and thereby increasing the number of free amino acid group of oat dough. This finding is documented in literature.

YG increased the number of free amino acid groups from 0.36 - 0.94 as its level increased from 10.75 - 33.75 g, pointing out that it limited the catalysis of covalent cross- linking of the protein present in the oat dough. This might be attributed to the high amount of amino acid groups present in milk proteins contributed by albumin, globulin and avenin, thereby contributing to an increase in the amount of free amino acid groups.

TG increased the number of free amino acid groups from 0.59 - 0.94 when its level increased from 0.5 - 1.0 g (Table 3.9), revealing that it limited the catalysis of covalent cross-linking of the protein present in the oat dough, thereby contributing to an increase in the amount of free amino acid groups. Conversely, Huang *et al.* (2010) found that there was a reduction in the number of free amino groups when TG was added up to 1.0% in oat flour. No significant differences in the number of free amino groups were observed beyond 1.0% (Huang *et al.,* 2010). This was attributed to the reaction between an ε-amino group on protein bound lysine residues

Table 3.9   Effects of yeast, CMC, YG, TG and CG on the amount of free amino acid groups present in oat dough formulations[1, 2]

Run	Ingredients					Results
	Yeast	CMC	YG	TG	CG	Number of free amino acid groups
1	-1	-1	-1	+1	-1	0.41 ± 0.02
2	+1	-1	-1	+1	+1	0.46 ± 0.13
3	-1	+1	-1	-1	+1	0.29 ± 0.08
4	+1	+1	-1	-1	-1	0.27 ± 0.00
5	-1	-1	+1	-1	+1	0.38 ± 0.05
6	+1	-1	+1	-1	-1	1.43 ± 0.02
7	-1	+1	+1	+1	-1	1.31 ± 0.00
8	+1	+1	+1	+1	+1	1.35 ± 0.23
9	0	0	0	0	0	0.94 ± 0.33

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. Yeast (1.25, 2.25, 3.25 g); CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g); CG (10, 25, 40 μl).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase, CG: Cyclodextrinase.

and a γ-carboxyamide group on proteinboundglutamine residues leading to covalent cross-linking of the proteins, which was catalysed by TG. The results presented here differ because TG promoted the cross-links between proteins, thereby decreased the number of free amino acid groups of dough . The contradiction in the results reported here may be due to a very low pH and temperature of oat dough, thermodynamic incompatibility between polar and non-polar surfaces of the milk proteins, limiting the amount of lysine for the cross-linking reaction and therefore increasing the number of free amino acid groups of oat dough. These findings confirm the insignificant effects of TG on the pasting and thermal properties of oat dough. However, Moore *et al.* (2006) showed that the addition of an external source of protein increases the number of lysine residues, which is the limiting factor of the cross-linking reaction. Exogenous protein sources such as soya milk, skim milk powder or egg powder were (12.5% composite flour basis) added to a gluten-free formulation in the presence of increasing levels of TG. Consequently, the confocal laser scanning micrographs confirmed cross-linking of dairy proteins, although varying amounts of TG was needed mostly due to the thermodynamic incompatibility between polar and non-polar surfaces of the milk proteins (Moore *et al.,* 2006).

CG decreased the number of free amino acid groups from 0.94 to 0.61 as its level increased from 25 - 40 µl. The implication of the amino groups in the cross-linking reaction reduced the number of these groups, confirming the covalent cross-link character of CG.

3.3.7 Effect of yeast, CMC, YG, TG and CG on the cross-linking of oat proteins

The SDS-PAGE of the different oat dough formulations as outlined inTables 3.1 and 3.2 were similar after the quantification of free amino acid revealed that CG cross-linked oat protein among albumin and globulin, avenin and glutelin. This was observed in SDS-PAGE analysis through a loss of staining intensity or vanishing of protein bands of the known proteins found in the dough and an increase in high molecular weight protein bands. The typical gel photograph of treatment 3 (yeast, 1.25 g; CMC, 2 g; YG, 10.75 g; TG, 0.5 g and CG, 40 µl) was retained because of the significant effect of CG on the decrease of the number of free amino acid groups due to the catalysis of protein cross-linking (Figure 3.8).
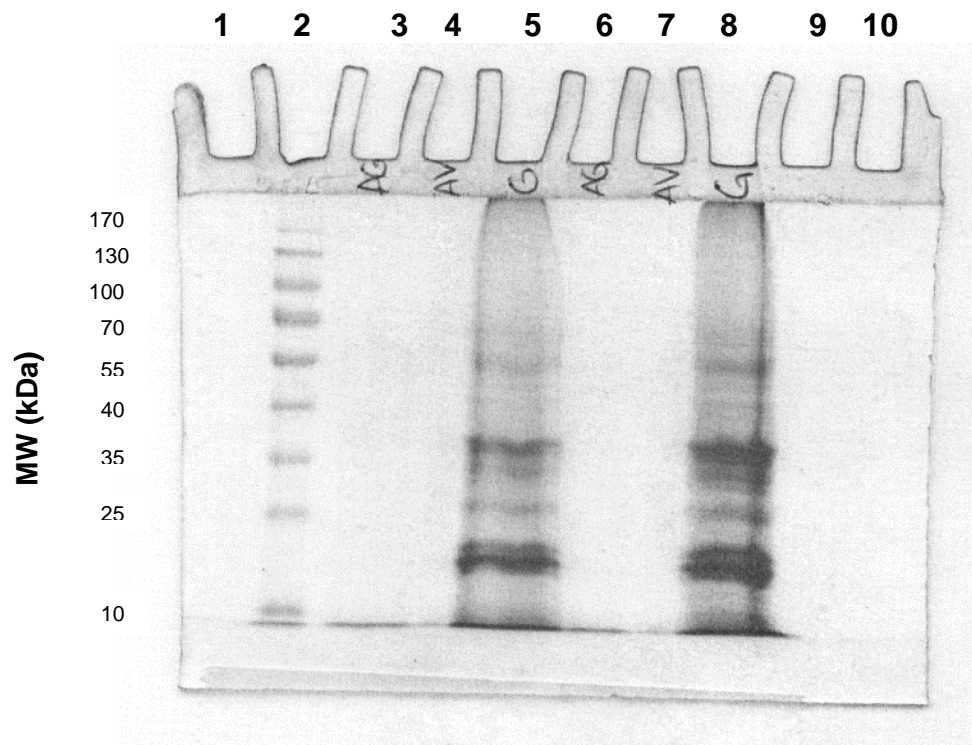
Figure 3.8   SDS-PAGE analysis of protein fractions present in oat dough based on the treatment 3 of the experimental design. Lane 2, marker; lanes 3 and 6, albumin and globulin; lanes 4 and 7, avenin, lanes 5 and 8, glutelin.

Figure 3.8 shows the effect of CG at 40 µl on the different oat protein fragments as indicated the number of free amino acid groups which signicantly decreased. After adding CG, the molecular weight of glutelin (typically XkDa in size) increased (10 - 37 kDa) due to the catalysis of CG, which polymerized low molecular weight protein into high molecular weight protein polymers (Lanes 5 and 8), confirming that glutelin was significantly cross-linked by CG . In addition, CG slighlty increased the molecular weight of glutelin from 55 to 60 kDa. Other proteins such as albumin, globulin and avenin were not cross-linked as shown in the electrophoregram.

3.4 Conclusion

The aim of this chapter was to determine the effects of yeast, CMC, YG, TG and CG on the mixing, pasting, thermal, number of free amino acid groups and protein crosslinks properties of oat dough. Among all the ingredients, only CMC, YG, and TG exhibited significant improvements on the mixing properties of oat dough. Yeast and YG showed greater enhancements in the pasting properties of oat dough compared to CMC, TG and CG. The thermal properties of oat dough were slightly affected by all the ingredients. Only CG decreased the number of free amino acid groups confirming that it catalysed the protein crosslinking of the oat glutelin while other ingredients increased it. Therefore CMC, YG, and TG are ingredients of choice in modifying the mixing properties of oat dough.

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CHAPTER 4

OPTIMISATION OF OAT BREAD PRODUCTION PROCESS

Abstract

Oat bread was baked with carboxylmethylcellulose (CMC), yoghurt (YG) and transglutaminase (TG) following a 3^3 Box-Behnken design consisting of CMC (1, 2 g), YG (10.75, 33.75 g) and TG (0.5, 1.5 g) as independent variables. The dependent variables of interest for the physical and textural properties were hardness, chewiness, cohesion energy, cohesion force, gumminess, springiness and specific volume. The colour parameters included lightness, redness, yellowness of crumb and crust. Increasing CMC (1 - 2 g) significantly ($p < 0.05$) increased all the textural parameters of bread, except its specific volume (1.49 - 1.46 ml/g). YG significantly ($p < 0.05$) decreased the hardness (786.01 - 593.39 N), chewiness (36.44 - 20.22 N) and gumminess (2.28 - 1.21 N), but sightly ($p > 0.05$) increased the cohesion energy (0.003 - 0.02), cohesion force (0.04 - 0.44), springiness (11.28 - 11.41 mm) and specific loaf volume (1.51 - 1.67 ml/g) of the resulting bread. TG did significantly ($p < 0.05$) affect all the textural parameters of oat bread; increased bread hardness (537.85 - 692.41 N) but decreased other parameters such as chewiness (79.60 - 35.68 N), cohesion energy (0.008 - 0.003), cohesion force (0.03 - 0.02), gumminess (4.66 - 2.14 N), springiness (6.47 - 4.14 mm) and specific loaf volume (1.61 - 1.54 ml/g) as TG level increased from 0.5 - 1.5 g. CMC, YG and TG had no significant ($p > 0.05$) effect on any parameters of colour characteristics of crumb and crust of oat bread. The objective of the optimisation was to establish the amount of carboxymethycellulose CMC, yoghurt (YG) and transglutaminase (TG) required, for optimal oat bread production. Hardness, chewiness, gumminess and springiness were used for numerical optimisation to estimate the optimal level of ingredients for the production of oat bread. The predicted optimal ingredients for preparing oat bread were: CMC (1 g), yoghurt (33.75 g) and TG (0.98 g) with a desirability of 0.93.

4.1   Introduction

The nearly ubiquitous consumption of bread places it in a position of great importance in human diet. Wheat (*Triticum aestivum*) is the most important crop for bread making due to its supreme and unique baking performance compared to other cereals (Dewettnick *et al.,* 2008). The protein component in wheat called "gluten" plays an excellent role during baking. It is important to retain gas in a typical bread production process in order to obtain the desired volume and texture. Gluten is essential to form a strong protein network required for the desired viscoelasticity. The major fractions of gluten are glutenin and prolamin. While glutenin is responsible for elastic and cohesive properties of dough, prolamin provides viscosity and extensibility in dough system (Gujral & Rosell, 2004). The gluten is not only important for appearance but also crumb structure of wheat based products. Nevertheless, gluten has to be eliminated from the nutrition of patients suffering from celiac disease because its ingestion causes intestinal damage (Sciarini *et al.,* 2008).

The absence of gluten often leads to batter rather than dough. Resulting in baked bread with a crumbling texture (very dry crumb structure), poor color and other post-baking quality defects such as short shelf-life, unattractive appearance and taste as well as poor mouthfeel (Gallagher, 2009). Bread dough without gluten cannot retain gas. The diversification of gluten-free raw materials which might be used is limited because some of them might need the rheological modification done by certain food ingredients such as enzymes, emulsifiers or hydrocolloids to the traditional production process (Marconi & Careca, 2001). Attempts to improve the rheological properties of gluten-free breads have focused on adding carboxymethylcellulose (CMC), plain yoghurt (YG) and transglutaminase (TG) to the oat dough. Chapter 3 of this thesis reported that CMC, YG and TG have the ability to modify the mixing oat dough.

However, nothing is known as the optimal levels of these ingredients that would produce an optimal oat bread. Hence, our objective was to (1) investigate the effects of carboxymethycellulose CMC, yoghurt (YG) and transglutaminase (TG) on the physical, textural and colour characteristics of oat bread with a view to optimize the level of these ingredients and (2) establish the amount of carboxymethycellulose CMC, yoghurt (YG)

and transglutaminase (TG) required, for optimal oat bread production using Response Surface Methodology (RSM).

4.2   Materials and Methods

4.2.1 Source of materials

The source of materials was diverse as indicated in Chapter 3, Section 3.2.1.

This chapter was based on the optimization of oat bread production process. It investigated the effects of carboxymethycellulose (CMC), yoghurt (YG) and transglutaminase (TG) on the physical, textural and colour characteristics of oat bread with a view to optimize the level of these ingredients and established the amount of CMC, YG and TG required, for optimal oat bread production using Response Surface Methodology (RSM) (Figure 4.1).

4.2.2 Proximate analysis of oat flour

The proximate analysis of oat flour was done as reported in Chapter 3, Section 3.2.2.

4.2.3 Particle size distribution of oat flour

The particle size distribution of oat flour was done according to the method described in Chapter 3, Section 3.2.3.

4.2.4 Determination of protein concentrations of TG and CG

The determination of protein concentrations of TG and CG followed the method described in Chapter 3, section 3.2.4.
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4.2.5 Experimental design

A 3^3 Box-Behnken design for three (3) independent variables (CMC (X_1), YG (X_2) and TG (X_3)) that significantly affected the dough rheology in the previous chapter was used to determine the optimum ingredients for the optimal oat bread production and their levels are shown inTable 4.1. The outline of experimental design (15 runs) with the coded levels (-1 = low, 0 = middle, +1 = high values of independent variables) is given in Table 4.2. Each design point was performed in triplicates with the centre in four
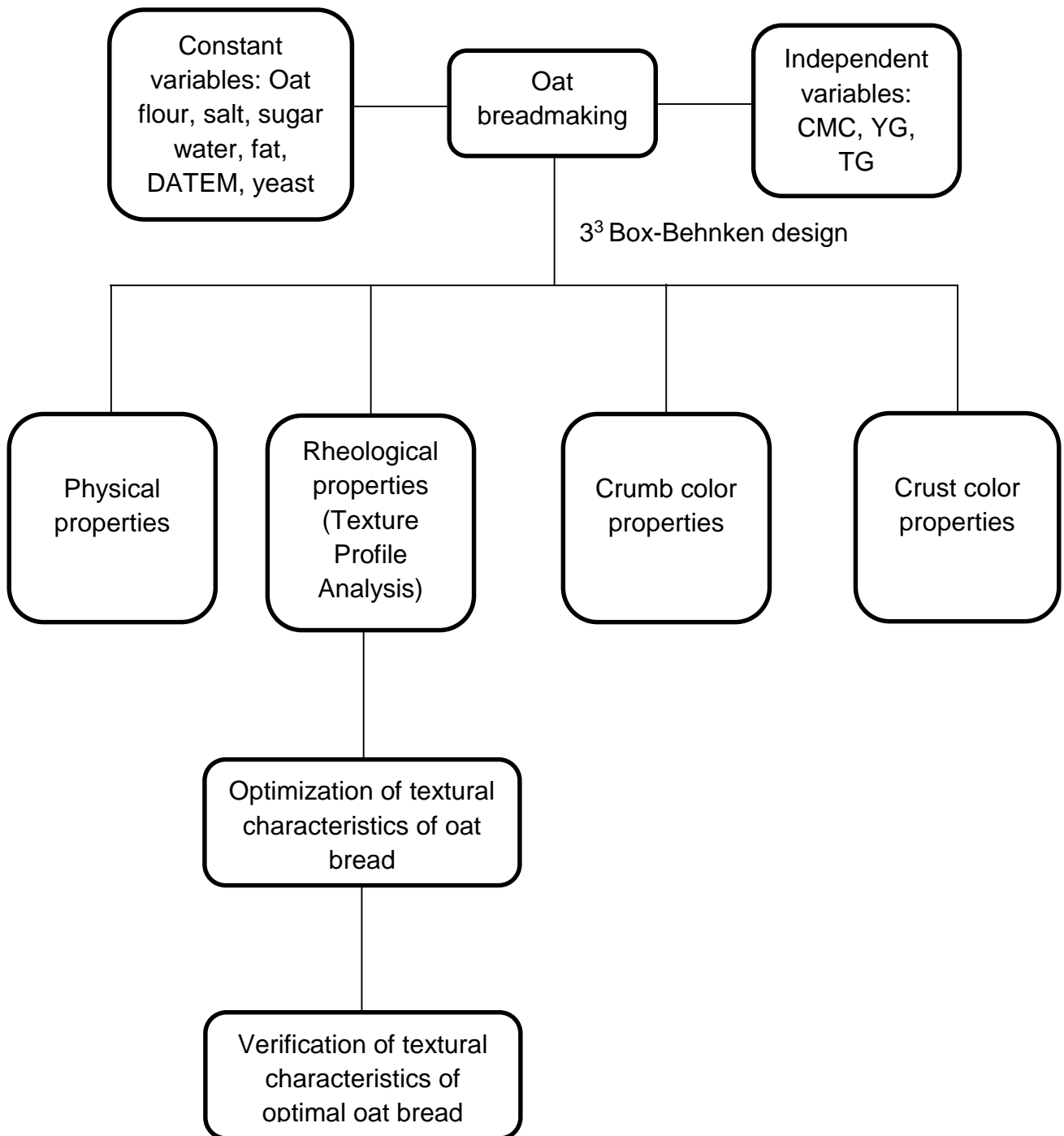
Figure 4.1 Overview of chapter four.

Table 4.1 Process variables used in the 3^3 Box-Behnken design for oat dough preparation[1, 2]

Factor (g/100 g flour)	Coded (x_i)		
	-1	0	+1
CMC (X_1)	1	1.5	2
YG (X_2)	10.75	22.25	33.75
TG (X_3)	0.5	1.0	1.5

[1]Transformation of coded variable (x_i) to uncoded variable (X) levels could be obtained from: $X_1=0.5x_1+1.5$; $X_2=11.50x_2+22.25$; $X_3=0.5x_3+1$
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Table 4.2 Independent factors and levels used for 3^3 Box-Behnken design for optimization of oat bread formulation[1, 2]

Run	Factors		
	CMC	YG	TG
1	-1	-1	0
2	-1	+1	0
3	+1	-1	0
4	+1	+1	0
5	-1	0	-1
6	-1	0	+1
7	+1	0	-1
8	+1	0	+1
9	0	-1	-1
10	0	-1	+1
11	0	+1	-1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to low level, middle level and high level respectively. CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

replicates. Following the combination of the ingredients as per the design, bread samples were produced following the process described in Section 4.2.6.   The experiment was carried out in randomized order.   The dependent variables of the experimental design were specific volume, hardness, chewiness, cohesion energy, cohesion force, gumminess, springiness, lightness, redness and yellowness.

4.2.6 Oat bread production

The method of Nitcheu (2010) was modified and used to bake oat bread. Oat bread was produced as described in Figure 4.2. The basic recipe consisted of: oat flour (100 g), yeast (2.25 g) salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g) and water (78 g).   Other ingredients (CMC, YG and TG) at variable quantities were added following the Box-Behnken design outlined in Table 4.2. All the ingredients were placed in a stainless steel bowl mixer (Kenwood chef, UK) and mixed for 6 min (speed 2: 2 min and speed 3: 4 min). The sides of the bowl were scrapped down half way and the dough was allowed to rest for 10 min at room temperature. A 150 g of dough was weighed, mechanically sheeted, rolled, panned (96 mm × 51 mm × 33 mm), proofed (Macadams, South Africa) (40°C, 50 min, 80% relative humidity), and baked in rotated oven (Macadams, South Africa) at 230°C for 30 min. After baking, the loaf was removed from the pan, cooled on the rack for 2 h at room temperature, packaged in a sealed polyethylene bag, stored at room temperature for 24 h and analysed for physical (weight, volume and specific volume), rheological (texture profile analysis) and colour analysis.

4.2.7 Physical, textural and colour analysis of oat bread
Weight determination of oat bread

After cooling, each loaf was weighed using a laboratory scale (Model: AV3102 precision scale, Capacity: 3,100 g, Readability: 0.01 g, Adventurer OHAUS, China) (Renzetti *et al.,* 2008).

Loaf volume and specific loaf volume determination of oat bread

The rapeseed displacement method of Jideani *et al.* (2007) was modified and used to determine the loaf volume of oat bread. A 1000 ml cylinder was filled with 500 ml quinoa seeds. The 500 ml quinoa seeds from the measuring cylinder were poured
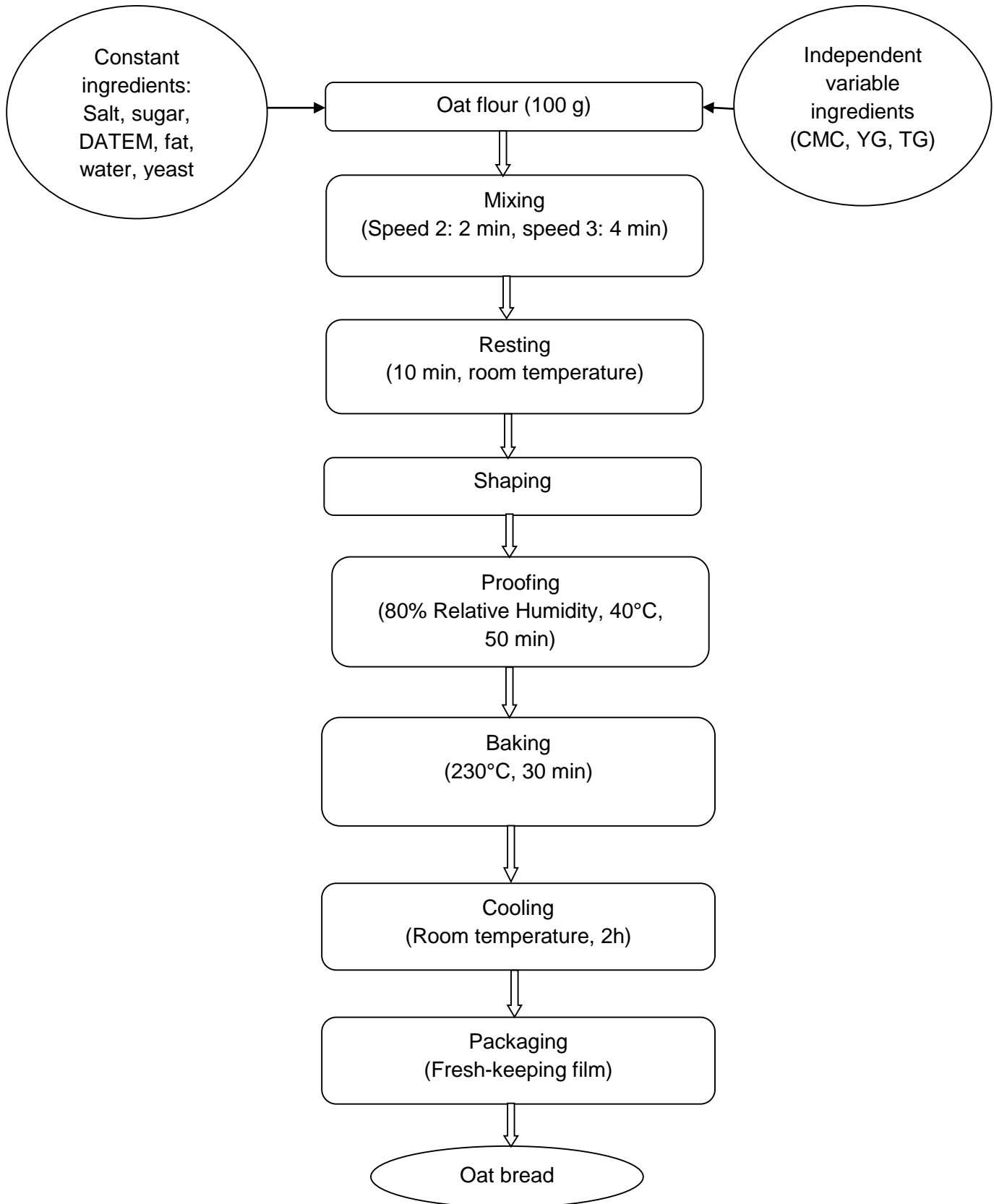
Figure 4.2   Oat bread production process.

over the bread loaf in another cylinder and then the difference in height from the original volume (500 ml) is expressed as the volume of the loaf. The specific loaf volume of the bread was calculated as the loaf volume per weight of the loaf (ml/g) (Jideani *et al.,* 2007).

Texture analysis of oat bread

The texture of each loaf was determined using INSTRON Texture Analyser with the software "Blue Hill Software", and equipped with an aluminium 55 mm diameter cylindrical probe. The methods of Caballero *et al.* (2007) and Lazaridou *et al.* (2007) were modified and used to determine the texture profile of the oat bread. Rectangular loaf of 44 mm width, 43 mm thickness and 9 mm height after cooling for 24 h, was compressed to 80% of the original height in a "Texture Profile Analysis", double compression test (TPA), at 100 mm/min rate, with a 30 sec delay between first and second compression. The characteristics such as hardness, chewiness, cohesion energy, cohesion force, gumminess and springiness were automatically estimated by the Blue Hill Software.

Crumb and crust colour determination of oat bread

Crumb and crust colours were measured using a Hunter Lab, ColorFlex (Hunter Lab, ColorFlex, Reston, Virginia, USA). The colour analysis of crumb and crust of oat bread was performed after 24 h cooling. A 50 g of crumb and crust was individually removed from bread loaf and poured into the sample cup. Colour values, L^*, a^* and b^*, were recorded. The method of Skendi *et al.* (2010) was modified and used to determine the color characteristics of the oat bread. L^* is the lightness variable from 100 for perfect white to 0 for black, whilst a^* and $b*$ values were the chromaticity values, +redness/-greenness and +yellowness/-blueness, respectively.

4.2.8 Statistical analysis

Multivariate Analysis of variance (MANOVA) was used to determine the differences between treatments. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS, 2010).

The system behavior was described by a quadratic polynomial model regression, carried out using Design-Expert software (Minneapolis, Minnesota, USA) and given by:

$$Y = \beta_0 + \sum_{k=1}^{3} \beta_i X_i + \sum_{k=1}^{3} \beta_{ii} X_i X_i + \varepsilon$$ ....Equation (1)

Where Y is the response variable, β_0 is a constant; β_i and β_{ii} are the linear and interactive coefficients, respectively; X_i is the level of the ingredient and ε is the random error. The quality of the fit of the linear model equation was evaluated by R^2, adjusted R^2, adequate precision (AP) and lack of fit.

The optimisation objective was to minimise hardness, chewiness, gumminess and springiness of oat bread. Design Expert-8 was used to estimate desirability, an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimisation found a point that maximizes the desirability function.

4.2.9 Verification of optimal oat bread

The hardness, chewiness, gumminess and springiness of the optimal oat bread were compared to the predicted model for verification purpose. The model predicted a maximum hardness of 572.35 N; minimum chewiness of 20.33 N; minimum gumminess of 1.21 N and minimum springiness of 5.25 mm.

4.3 Results and Discussion

4.3.1 Proximate composition and particle size distribution of oat flour

The proximate composition and particle size distribution of oat flour were reported in Chapter 3, Section 3.3.1.

4.3.2 Protein concentrations of TG and CG

The protein concentrations of TG and CG were reported in Chapter 3, Section 3.3.2.

4.3.3 Effects of CMC, YG and TG on the physical and textural properties of oat bread

Model adequacy

The effects of independent variables on the physical and textural properties of oat bread are outlined in Table 4.3. The regression coefficients of the response surface linear and quadratic model for physical and textural properties of oat bread for each response variable in terms of coded values are shown in Table 4.4. The model p-value ranged from < 0.0001 to 0.0074 indicating that the linear and quadratic models were significant (p < 0.05) in explaining the variation between the independent and

Table 4.3   Effect of CMC, YG and TG on the physical and textural properties of oat bread[1,2]

	Ingredients			Response variable						
Run	CMC	YG	TG	Hardness (N)	Chewiness (N)	Cohesion energy (resilience) (ratio)	Gumminess (N)	Springiness (mm)	Cohesion force (resilience) (ratio)	Specific volume (ml/g)
1	-1	-1	0	786.01 ± 4.96	36.44 ± 3.44	0.00 ± 0.00	2.28 ± 0.21	5.68 ± 0.75	0.02 ± 0.00	1.50 ± 0.14
2	-1	+1	0	593.93 ± 20.45	20.22 ± 16.96	0.00 ± 0.00	1.21 ± 1.02	4.75 ± 0.62	0.01 ± 0.00	1.43 ± 0.42
3	+1	-1	0	728.39 ± 66.15	230.59 ± 29.97	0.02 ± 0.00	13.68 ± 1.98	11.33 ± 0.16	0.04 ± 0.00	1.47 ± 0.17
4	+1	+1	0	686.38 ± 15.33	200.57 ± 24.04	0.02 ± 0.00	11.84 ± 1.30	11.04 ± 0.64	0.04 ± 0.00	1.46 ± 0.16
5	-1	0	-1	537.85 ± 22.14	79.60 ± 6.25	0.01 ± 0.00	4.66 ± 0.31	6.47 ± 0.34	0.03 ± 0.00	1.61± 0.71
6	-1	0	+1	692.41 ± 4.88	35.68 ± 11.39	0.00 ± 0.00	2.14 ± 0.69	4.14 ± 0.66	0.02 ± 0.00	1.54 ± 0.69
7	+1	0	-1	828.92 ± 39.04	373.64 ± 26.86	0.03 ± 0.00	21.94 ± 1.28	12.13 ± 0.89	0.04 ± 0.00	1.70 ± 0.32
8	+1	0	+1	880.32 ± 16.61	338.29 ± 24.29	0.02 ± 0.00	19.90 ± 1.38	11.87 ± 0.29	0.04 ± 0.00	1.60 ± 0.28
9	0	-1	-1	749.00 ± 43.63	264.29 ± 51.24	0.02 ± 0.00	15.42 ± 3.04	11.28 ± 1.16	0.04 ± 0.00	1.51 ± 0.15
10	0	-1	+1	886.97 ± 37.96	129.43 ± 1.30	0.01 ± 0.00	8.27 ± 0.06	7.30 ± 0.29	0.03 ± 0.00	1.33 ± 0.00
11	0	+1	-1	583.58 ± 35.05	229.73 ± 29.07	0.02 ± 0.00	13.46 ± 1.60	11.41 ± 1.24	0.04 ± 0.00	1.67 ± 0.01
12	0	+1	+1	648.25 ± 0.48	180.54 ± 36.95	0.02 ± 0.00	10.64 ± 2.00	9.73 ± 1.15	0.04 ± 0.00	1.57 ± 0.11
13	0	0	0	724.70 ± 121.64	226.66 ± 79.75	0.02 ± 0.00	13.59 ± 4.84	9.90 ± 1.89	0.04 ± 0.00	1.53 ± 0.05

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase.

Table 4.4   Regression coefficients of polynomial model for physical and textural properties of oat bread[1,2]

Coefficients	Hardness (N)	Chewiness (N)	Cohesion energy (resilience) (ratio)	Gumminess (N)	Springiness (mm)	Cohesion force (resilience) (ratio)	Specific volume (ml/g)
Response variable[1]							
Linear							
β_0	578.4954	-26.5221	-0.2404	-6.5814	-11.02538	-0.0885	1.6155
β_1	128.5875*	45.2720*	0.4296*	11.1855*	24.1274*	0.1348*	-0.1102
β_2	-6.9450*	0.6553	5.0717E-003	0.1557	0.0855	1.8801E-003	0.0298
β_3	102.1075	-12.4499*	-0.1094*	-2.8798*	-4.0081*	-6.8337E-003*	-0.6868*
Quadratic							
β_{11}	-	-11.6015	-0.1152	-2.8863	-5.93251	-0.0391	0.0481
B_{22}	-	-0.0152	-1.0824E-004	-3.6431E-003	-1.5944E-003	-3.9180E-005	-5.8953E-004
β_{33}	-	4.9121	0.0396	1.1424	0.97282	-	0.2871
R^2	0.4978	0.8607	0.8797	0.8588	0.8068	0.8923	0.4678
Model p-value	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0074
Adjusted R^2	0.4459	0.8285	0.8520	0.8262	0.7623	0.8723	0.3450
AP	9.692	17.393	18.593	17.186	13.541	19.489	7.014
Lack of fit	*1.39*	*0.69*	*1.21*	*0.57*	*1.07*	*2.24*	*1.81*

[1]significant at p < 0.05,
[2]β = constant, β_1 = effect of carboxymethylcellulose (CMC), β_2 = effect of yoghurt (YG), β_3 = effect of transglutaminase (TG), β_{11} = carboxymethylcellulose^2 (CMC^2), β_{22} = effect of yoghurt^2 (YG^2), β_{33} = effect of transglutaminase^2 (TG^2)

dependent variables. The adequacy of precision (estimation of the signal to noise ratio) ranged from 7.014 to 19.489. A ratio greater than 4 is desirable. These values being greater than 4 indicated that the models were adequate. The significant lack of fit ranged from 0.57 to 1.81 indicated that lack of fit was not significant ($p > 0.01$) and hence the models were adequate. The adjusted R^2 ranged from 0.44 to 0.87 indicating variation in the fit for the models. The adjusted coefficients of correlation (R^2_{adj}) for chewiness (0.83), cohesion energy (0.85), gumminess (0.83), springiness (0.76), and cohesion force (0.87) exhibited better goodness of fit (Table 4.4). In general the models were adequate in explaining the variation between the independent and dependent variables and could be used to navigate the design space. However, for numerical optimisation of the effects dependent variables such as hardness, chewiness, cohesion energy, cohesion force, gumminess, springiness and specific volume with higher R^2 were used.

Main effect of CMC, YG and TG on the physical and textural properties of oat bread

The linear model regression coefficients for physical and textural properties of oat bread for each response variable are shown in Table 4.4. CMC did have significant ($p < 0.05$) effect on physical and textural properties of oat bread. It significantly ($p < 0.05$) affected hardness ($F_{(1, 32)} = 9.06$; $p = 0.0054$) (Figure 4.2), chewiness ($F_{(1, 32)} = 121.02$; $p < 0.0001$), cohesion energy ($F_{(1, 32)} = 129.91$; $p < 0.0001$), cohesion force ($F_{(1, 32)} = 110.98$; $p < 0.0001$), gumminess ($F_{(1, 32)} = 119.05$; $P < 0.0001$) and springiness ($F_{(1, 32)} = 88.50$; $p < 0.0001$) but it slightly impacted the specific loaf volume ($F_{(1, 32)} = 0.62$; $p = 0.4389$) of oat bread (Table 4.4) when its level increased from 1.00 - 2.00 g (Table 4.3). CMC increased hardness (537.85 - 686.38 N) (Figure 4.3), chewiness (36.44 - 230.59 N), cohesion energy (0.003 - 0.02), cohesion force (0.02 - 0.03), gumminess (2.28 - 13.68 N) and springiness (5.68 - 11.33 mm) but decreased the specific loaf volume (1.49 - 1.47 ml/g) of oat bread. The specific loaf volume is defined as the volume occupied by a unit of mass of the loaf. This decrease of specific loaf volume mathematically implied that CMC decreased loaf volume. Small bread volume might be attributed to highest strength and elasticity of dough which caused a limited and slow expansion of the gas cells during proofing similar to bread supplemented with xanthan gum (Lazaridou *et al.,* 2007). Consequently, the dough became too rigid to incorporate gases (Lazaridou
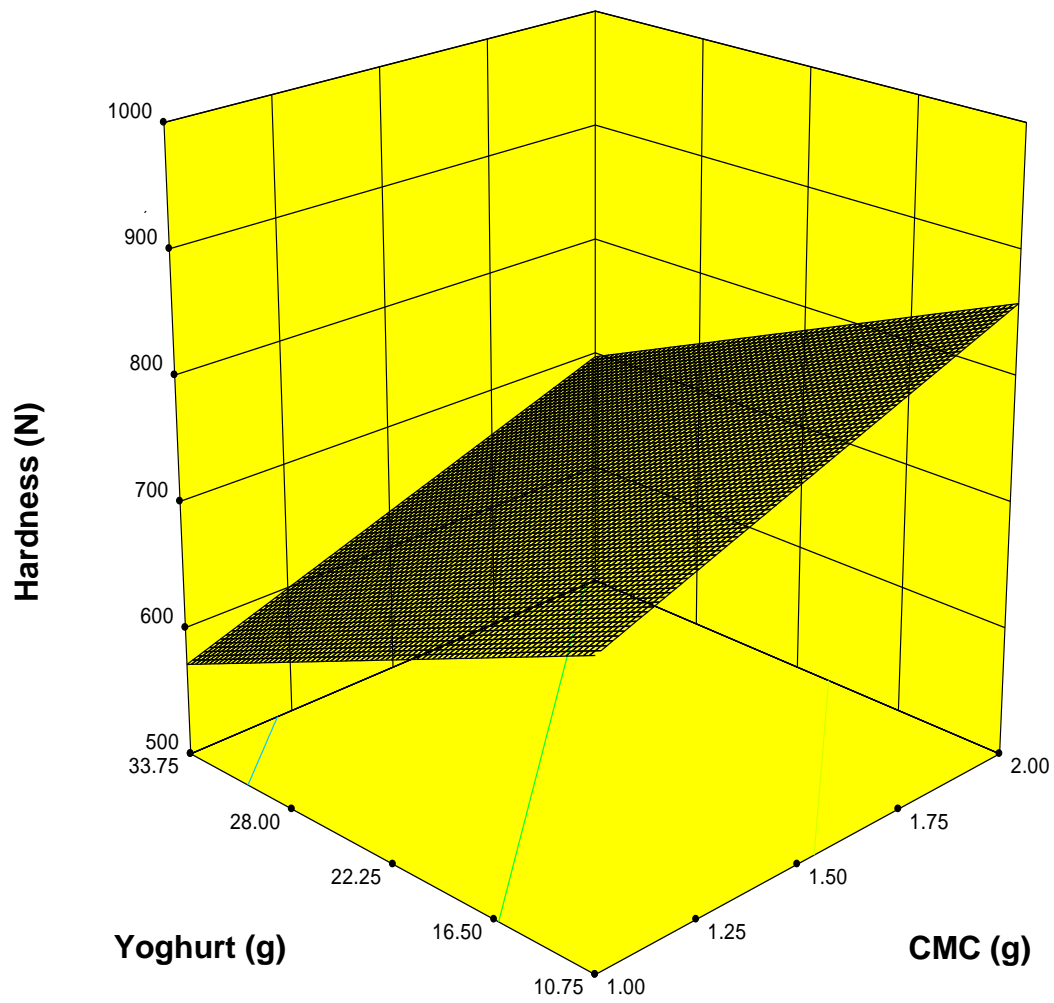
Figure 4.3   Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the hardness of oat bread based on 100 g of oat flour.

et al., 2007). This change might be the consequence of a decrease of the absorption capacity of oat flour indicating that CMC resulted in flour hydrating processes that was slower, causing the dough to have a lower hydrating ability. Consequently, oat bread exhibited low specific loaf volume bread and high hardness, chewiness, gumminess, springiness and resilience (cohesion energy and cohesion force). However, the results presented here differ. CMC effectively bound water molecules through hydrogen bond formation at $6.5 \leq pH \leq 9$. Furthermore, the addition of non-starch polysaccharides such as CMC to starch-water systems limited the hydration of the starch and, since water has a plasticizing effect in amorphous regions of the starch, the mobility of the plasticizer was also restricted. These decrease of specific loaf volume and increased hardness, chewiness, gumminess, springiness and resilience (cohesion energy and cohesion force) of oat bread were significantly associated with the low water absorption of oat dough induced by CMC during the mixing process (Chapter 3, Section 3.3.3, Figure 3.2). By decreasing dough pH, the lactic acid of yoghurt inhibited any hydrogen bond formation and therefore resulted in limited water absorption and specific loaf volume of bread leading to high hardness, chewiness, gumminess and springiness of oat bread. Thus, the physical and textural properties of oat bread were negatively affected by CMC due to the acidification of lactic acid inhibiting CMC action.

YG significantly ($p < 0.05$) impacted the hardness ($F_{(1, 32)} = 13.98$; $p = 0.0008$), but sightly affected the chewiness ($F_{(1, 32)} = 0.23$; $p = 0.6341$), cohesion energy ($F_{(1, 32)} = 0.63$; $p = 0.4337$), cohesion force ($F_{(1, 32)} = 3.54$; $p = 0.0709$), gumminess ($F_{(1, 32)} = 0.41$; $p = 0.5271$), springiness ($F_{(1, 32)} = 0.25$; $p = 0.6225$) and specific loaf volume ($F_{(1, 32)} = 3.59$; $p = 0.0695$) of the resulting oat bread (Table 4.4) as its level increased from 10.75 to 33.75 g (Table 4.3). YG decreased the hardness (786.01 - 593.39 N), chewiness (36.44 - 20.22 N) and gumminess (2.28 - 1.21 N) but increased the cohesion energy (0.003 - 0.02), cohesion force (0.04 - 0.44), springiness (11.28 - 11.41 mm) and specific loaf volume (1.51 - 1.67 ml/g) of oat bread. These results might be attributed to the increased softening (Chapter 3, Section 3.3.3, Table 3.6), strain and storage modulus of oat dough induced by YG (Chapter 3, Section 3.3.4, Table 3.7). Dairy products such as yoghurt, that are used in gluten-free bread formulas, increase water absorption as its level decreases and, therefore, enhance the softening properties of gluten-free dough (Gallagher *et al.,* 2004a; Nunes *et al.,* 2009). According to Gallagher (2009),

dairy proteins possess functional properties similar to gluten, as they are able to form networks and have good swelling properties. In addition, dairy products as yoghurt gave breads with an improved overall shape and volume (Gallagher *et al.,* 2003), and a firmer crumb texture (Arendt *et al.,* 2008) thereby decreasing bread hardness. Sodium caseinate and hydrolysed casein of yoghurt displayed beneficial functional properties in breadmaking including high volume and low firmness (Kenny *et al.,* 2000). Therefore, the hardness and specific loaf volume of oat bread was positively affected by YG, indicating that YG formed a network similar to gluten.

TG did significantly ($p < 0.05$) affect all the textural parameters of oat bread. It increased bread hardness ($F_{(1, 32)} = 5.71$; $p = 0.0236$) but decreased other parameters such as chewiness , ($F_{(1, 32)} = 7.62$; $p = 0.0105$), cohesion energy ($F_{(1, 32)} = 16.77$; $p = 0.0004$), cohesion force ($F_{(1, 32)} = 16.72$; $p = 0.0003$), gumminess ($F_{(1, 32)} = 6.60$; $p = 0.0163$), springiness ($F_{(1, 32)} = 9.40$; $p = 0.0050$) and specific loaf volume ($F_{(1, 32)} = 6.67$; $p = 0.0158$) (Table 4.4) while its level increased from 0.5 - 1.5 g (Table 4.3). TG addition increased the hardness (537.85 - 692.41 N) but decreased chewiness (79.60 - 35.68 N), cohesion energy (0.08 - 0.03), cohesion force (0.03 - 0.02), gumminess (4.66 - 2.14 N), springiness (6.47 - 4.14 mm) and specific loaf volume (1.61 - 1.54 ml/g) of oat bread as TG level varied from 0.5 - 1.5 g. These findings might result from the slight amount of protein cross-links induced by TG during oat dough formation. Consequently, TG decreased the development time of oat dough (Chapter 3, Section 3.3.3). This confirms that TG limited the catalysis of covalent cross-linking of the protein present in the oat dough, thereby contributing to an increase in the amount of free amino acid groups. Acyl transfer reactions might not significantly introduce new functional groups leading to changes in the structure, charge, and hydrophobicity of the proteins. However, by promoting the cross-linking effect on different flours (Larre *et al.,* 2000; Gerrard *et al.,* 2001; Bauer *et al.,* 2003; Rosell *et al.,* 2003; Autio *et al.,* 2005), TG widely modifies bread textural properties (Caballero *et al.,* 2007). The incorporation of TG led to a significant increase in crumb hardness, cohesiveness, gumminess, chewiness and resilience of wheat bread (Caballero *et al.,* 2007). Similar results were reported by Salmenkallio-Marttila *et al.* (2004) on oat bread. Although TG was shown among other enzymes to enhance gluten-free bread texture depending on the raw material, Arendt *et al.* (2008) reported that detrimental impact of TG could be observed on breads from oat, sorghum or tef. Similar results were reported by Renzetti *et al.*

(2008). Therefore, the textural properties of oat bread was negatively affected by TG as oat bread became more hard and less springy.

Quadratic effect of CMC, YG and TG on the physical and textural properties of oat bread

The regression coefficients of quadratic model for physical and textural properties of oat bread for each response variable in terms of coded values are shown in Table 4.4. The quadratic effect of CMC affected all the textural parameters except bread hardness. CMC did not significantly ($p > 0.05$) decreased the chewiness (Figure 4.4), cohesion energy (Figure 4.5), cohesion force (Figure 4.6), gumminess (Figure 4.7), springiness (Figure 4.8) but increased the specific loaf volume (Figure 4.9) of oat bread (Table 4.4) as its level decreased from 2 to 1 g (Table 4.3). It means that the quadratic term was exerting a positive influence on the curve resulting in a concave curve for chewiness (Figure 4.4), cohesion energy (Figure 4.5), cohesion force (Figure 4.6), gumminess (Figure 4.7) and springiness (Figure 4.8). This implies that there was a maximum turning point beyond which CMC decreased chewiness, resilience (cohesion energy or cohesion force), gumminess and chewiness of bread. However, CMC incorporation slightly increased the specific loaf volume of oat bread (Table 4.4) as its level decreased from 2 - 1 g (Table 4.3 & Figure 4.9). Similar finding was confirmed by Lazaridou *et al.* (2007) studying the specific loaf volume of rice bread. This improvement of specific loaf volume was attributed to the increase of dough viscosity by CMC leading to the enhanced dough development and gas retention (Rosell *et al.,* 2001). The modified polysaccharide derivatives such as CMC contain hydrophobic groups imparting additional properties which increase interfacial activity of the dough system during proofing, and producing gel networks on heating during the breadmaking process. These network structures increased viscosity and strengthened the boundaries of the expanding cells in the dough, thereby increasing gas retention during baking which therefore leaded to a better loaf volume (Bell, 1990) and high specific loaf volume. Furthermore, the addition of CMC to Fonio or Acha flour increased the loaf volume by 40 - 59.5% of the resulting bread but its specific loaf volume did not significantly ($p > 0.05$) differ (2.60 - 2.73 ml/g) (Jideani *et al.,* 2007). Furthermore, the addition of CMC did not significantly ($p > 0.05$) affect the crumb firmness of rice bread compared to control formulations (Lazaridou *et al.,* 2007). The strengthening effect
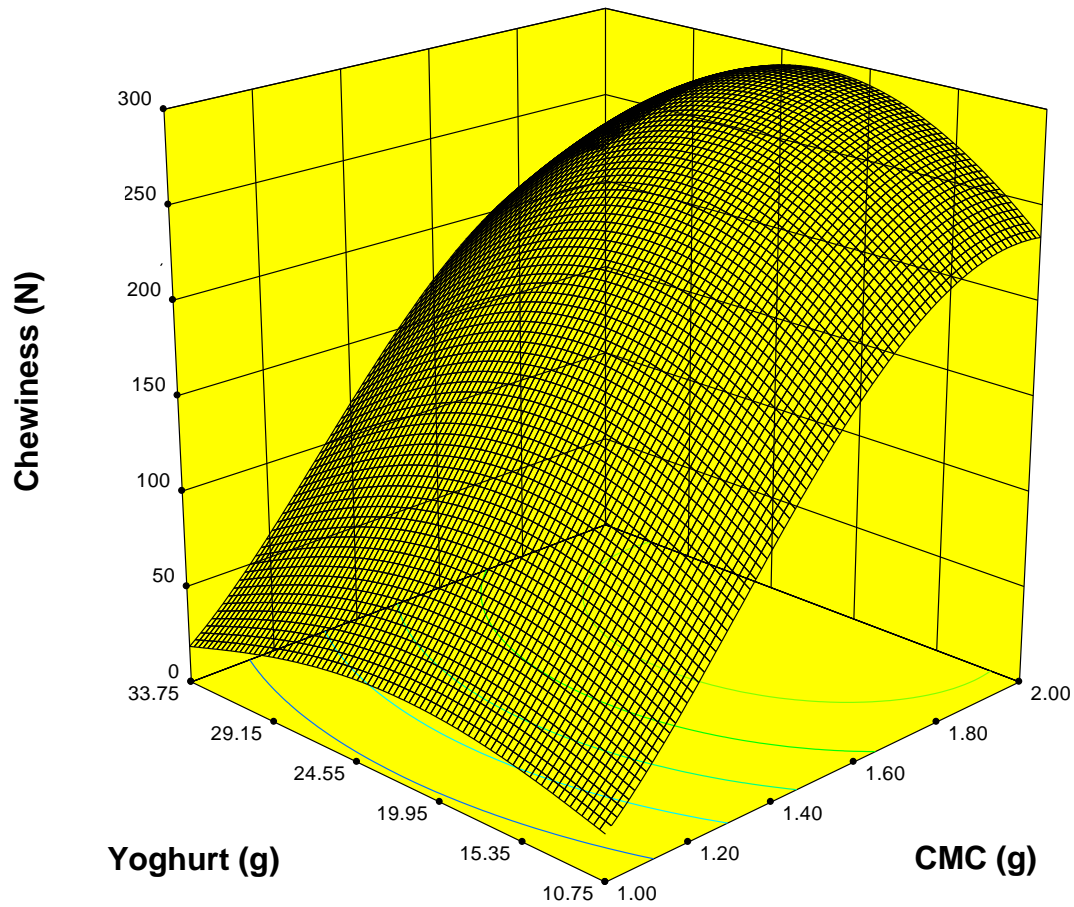
Figure 4.4   Effect of carboxymethycellulose (CMC) and yoghurt (YG) on the chewiness of oat bread based on 100 g of oat flour.
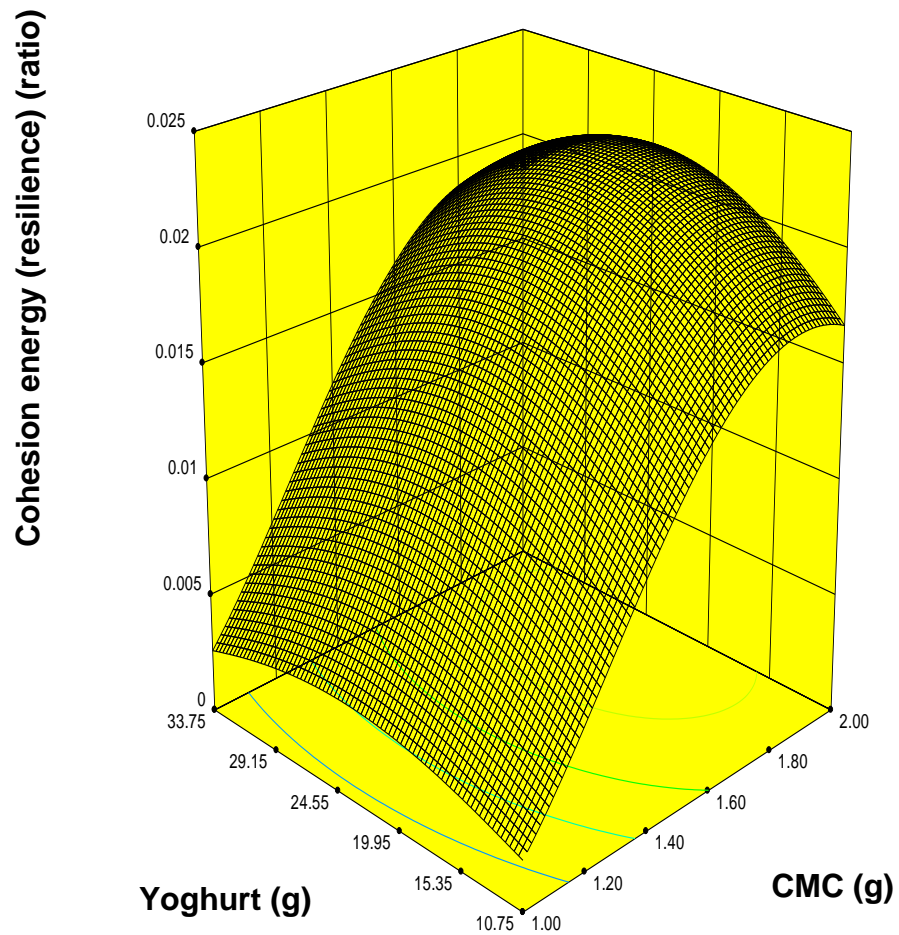
Figure 4.5 Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the cohesion energy (resilience) of oat bread based on 100 g of oat flour.
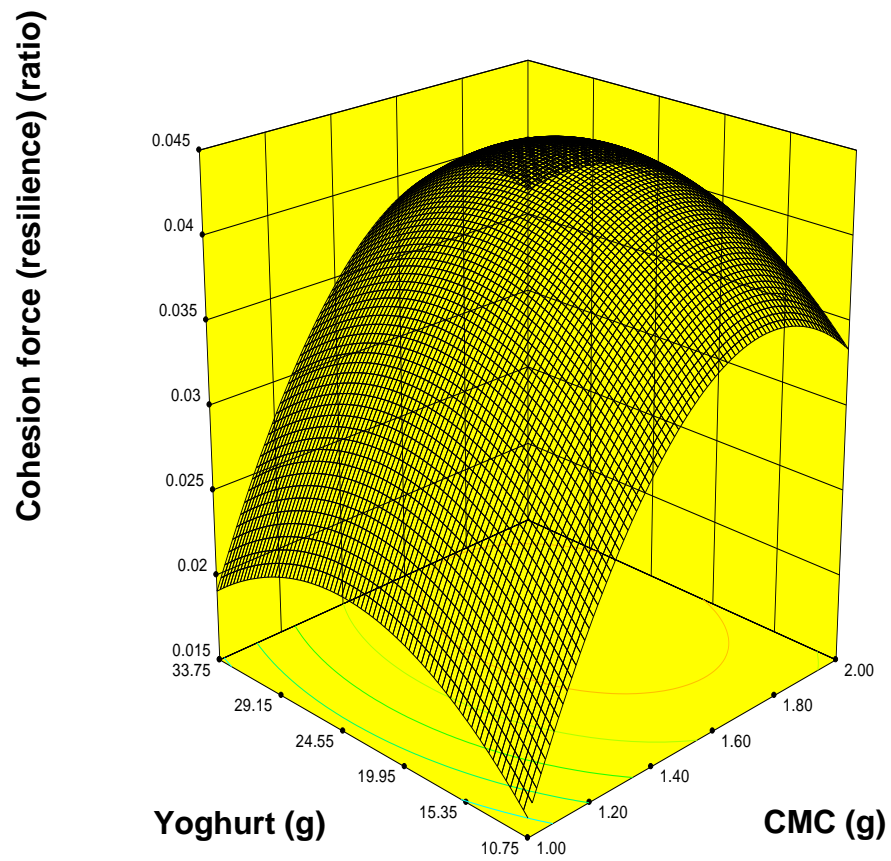
Figure 4.6 Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the cohesion force (resilience) of oat bread based on 100 g of oat flour.
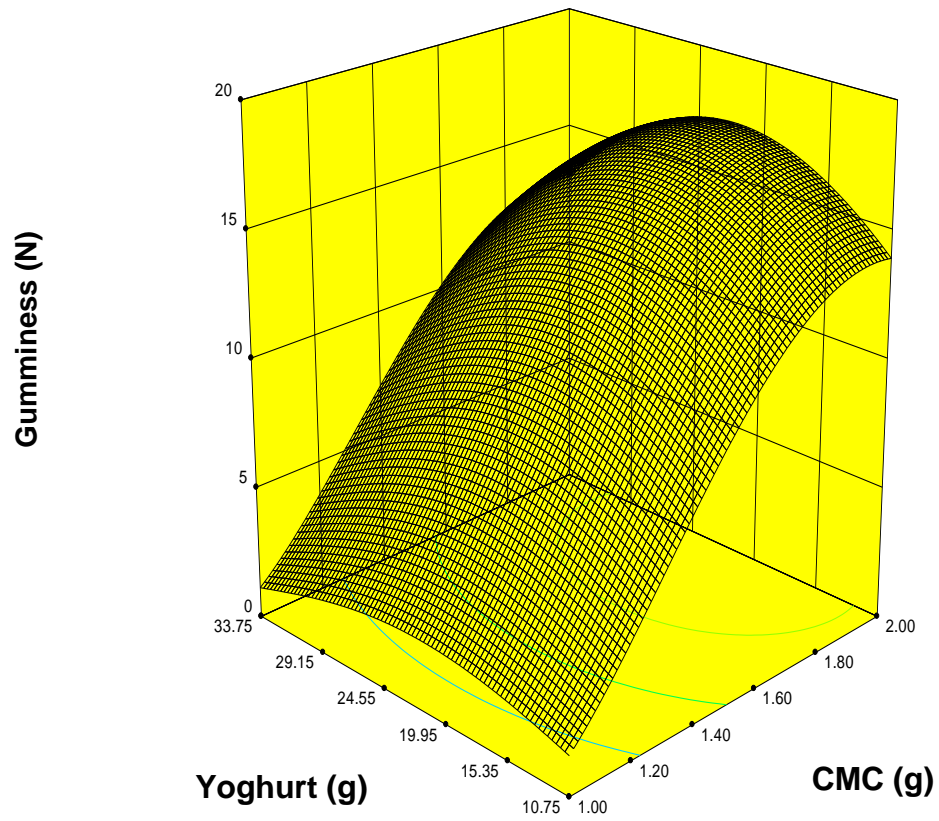
Figure 4.7   Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the gumminess of oat bread based on 100 g of oat flour.
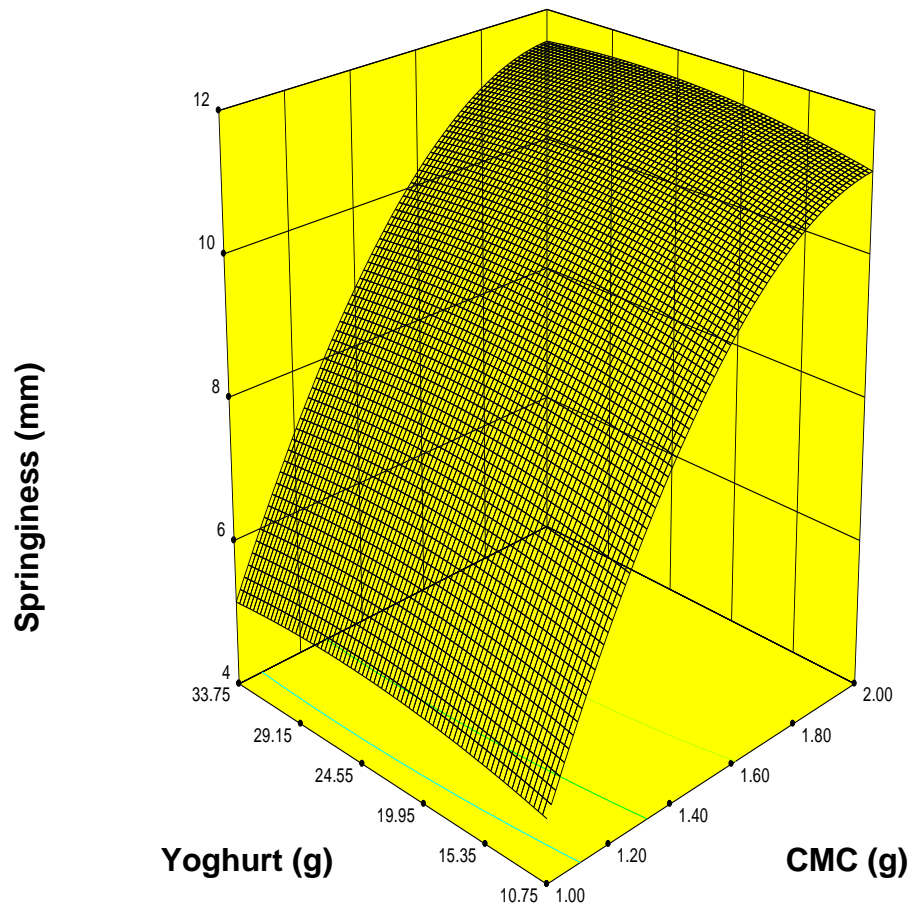
Figure 4.8   Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the springiness of oat bread based on 100 g of oat flour.
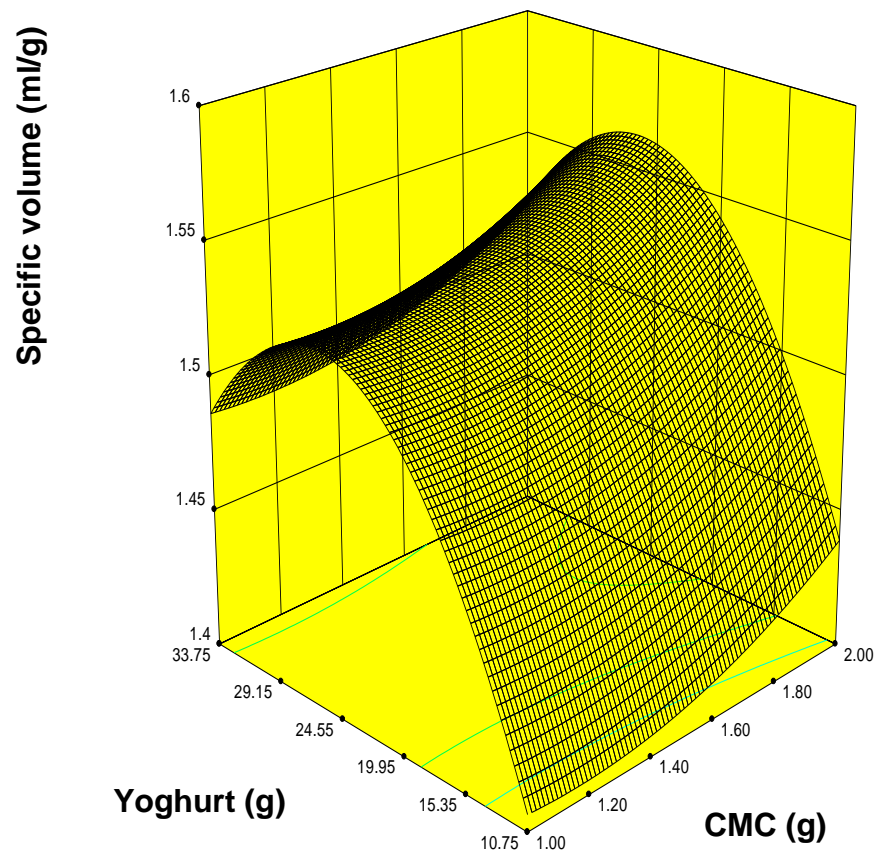
Figure 4.9   Effect of carboxymethylcellulose (CMC) and yoghurt (YG) on the specific volume of oat bread based on 100 g of oat flour.

of CMC on crumb structure appears to be consistent with the low rigidity highlighted by dough containing it. Thus, a low rate of crumb hardness was observed for breads supplemented by CMC. Therefore, the quadratic effect of CMC improved chewiness, resilience (cohesion energy and cohesion force), gumminess and springiness of oat bread, indicating that CMC did efficiently act as a gluten.

The quadratic effect of YG in oat bread formulation revelaled that it did not significantly ($p > 0.05$) decreased all the physical and textural parameters but did not affect bread hardness (Table 4.4). Chewiness, cohesion energy, cohesion force, springiness, gumminess and specific loaf volume decreased while YG level increased from 10.75 to 33.75 g (Table 4.3) explaining that the quadratic term was exerting a positive influence on the curve resulting in a concave curve for chewiness (Figure 4.4), cohesion energy (Figure 4.5), cohesion force (Figure 4.6), gumminess (Figure 4.7) , springiness (Figure 4.8) and specific volume (Figure 4.9). This implies that there was a maximum turning point beyond which YG decreases chewiness (Figure 4.4), cohesion energy (Figure 4.5) , cohesion force (Figure 4.6), gumminess (Figure 4.7), springiness (Figure 4.8) and specific volume (Figure 4.9) of oat bread. The decrease in specific loaf volume might be explained by limited water absorption capacity of YG. This decrease of water absorption of oat dough was due to the decrease in pH caused by the lactic acid in the yoghurt, decreasing the water absorption of dairy proteins acting as gluten replacer. Thus, the main whey proteins contained in yoghurt, the α-lactalbumin (four disulphide bonds) and the ß-lactoglobulin, which can be a monomer, dimer and an oligomer depending on pH value, ionic strength and temperature, have a globular structure and hydrophobic, compact folded polypeptide chain, hence decreasing water absorption of oat dough (Houben *et al.,* 2012). This might be mainly due to the presence of proteins such as milk proteins (sodium caseinate) in the yoghurt, resulting in decreased water absorption of the oat dough. This result correlates with studies performed by Nunes *et al.* (2009) on rice flour. Furthermore, Bertolini *et al.* (2005) also showed that the storage modulus (G′) of samples showed an increasing trend when sodium caseinate was present in the composite flours. This effect seems to be clear, mainly for rice and wheat starches, at the lower starch concentrations, suggesting that the viscoelastic properties of the sodium caseinate were more important in dough systems with low starch concentrations. Despite the conflicting results, it seemed clear that changes in the viscoelastic properties of the systems could be attributed to

the limitation of starch swelling and gelatinization by sodium caseinate addition, as for most non-starch polysaccharides (Bertolini *et al.,* 2005). The effect of sodium caseinate on starch swelling seems to be similar to gluten when water is restricted in the system and is more evident in starches with a high amylose content such as oat starch (Bertolini *et al.,* 2005). Consequently, this limited water absorption capacity of YG led to oat bread with low specific volume, low springiness, low chewiness, low gumminess and low resilience (cohesion energy and cohesion force).

TG had a quadratic effect on all the parameters except bread hardness and cohesion force. It did not significantly (p > 0.05) increased the chewiness, cohesion energy, gumminess, springiness and the specific loaf volume of oat bread (Table 4.4) as its level decreased from 1.5 to 0.5 g (Table 4.3). These quadratic effects might be attributed to the increase of water absorption of oat dough (Chapter 3, Section 3.3.3, Table 3.6). This rise in water-holding capacity of oat gluten-free dough induced by the cross-links that occurred after TG addition, which caused changes in secondary structure or, possibly, due to changes in protein hydrophobicity from the formation of glutamic acid residues from glutamine hydrolysis (Gerrard *et al.,* 1998). As result, this reaction increased the molecular weight of the proteins formed during the cross-linking action of this enzyme (Marco *et al.,* 2007; Marco *et al.,* 2008) and thereby retaining carbon dioxide gas and improving bread the physical (specific volume) and textural properties of oat bread. Similar findings have been reported by Salmenkallio-Marttila *et al.* (2004) on oat bread. In addition, TG addition significantly increased chewiness (9.19 - 12.13 N) and crumb firmness (17.70 - 21.81 N) but did not affect springiness, and resilience of pregelatinised cassava and sorghum bread (Onyango *et al.,* 2010). In contrast, the springiness was not affected by TG addition (Shin *et al.,* 2010).

4.3.4 Effects of CMC, YG and TG on the crumb and crust colour properties of oat bread

The colour analysis of all the samples showed that CMC, YG and TG had no significant (p > 0.05) effect on crumb and crust colour characteristics of oat bread. However, the incorporation of CMC in oat bread formulation slightly decreased crust lightness (L*) ($F_{(1, 20)}$ = 1.05, p = 0.32) (47.00 - 46.34) and crust yellowness (b*) ($F_{(1, 20)}$ = 0.92, p = 0.35) (28.14 - 26.71) as CMC level increased from 1 - 2 g, but slightly increased crust redness (a*) ($F_{(1, 20)}$ = 2.72, p = 0.11) (12.37 - 14.89) while

CMC was added from 1 - 1.5 g (Table 4.5). In addition, CMC slightly decreased crumb lightness ($F_{(1, 20)} = 0.18$, $p = 0.68$) (64.38 - 63.79) and crumb yellowness ($F_{(1, 20)} = 1.87$, $p = 0.19$) (24.55 - 22.58) but slightly increased crumb redness ($F_{(1, 20)} = 0.28$, $p = 0.60$) (5.21 - 6.88) as its level increased from 1 - 1.5 g (Table 4.5). These decreases of crust and crumb lightness could be explained by the the high extent of Maillard reaction and caramelization. The high amount of amino acid and reducing sugars contained in CMC increased amino acids and reducing sugars contents of oat dough leading to the browning reaction with heat as catalyst. Being entirely different process from Maillard browning, the caramelization also increased the extent of browning reaction because of the pyrolysis of certain sugars contained in dough. Conversely, the colour analysis of the crust of gluten-free bread revealed that bread supplemented with CMC has a lighter crust compared to the control (Sciarini *et al.,* 2010). This could be attributed to the effect of the hydrocolloid on water distribution and low baking temperature and time which affect the Maillard reaction and caramelization. Similar findings were obtained by Mezaize *et al.* (2009) studying the colour of gluten-free breads. The colour analysis of rice bread demonstrated that the redness of crust is higher for a CMC formulation as compared to that of the control and no significant difference in crust yellowness was found (Lazaridou *et al.,* 2007). Similar findings were obtained on oat bread. However, the presence of CMC at 2% concentration showed a significant difference of the redness parameter for crumb among gluten-free breads (Lazaridou *et al.,* 2007).

The effect of YG on oat bread formulation showed that it did not significantly increased crust lightness (L*) ($F_{(1, 20)} = 1.40$, $p = 0.25$) (47.00 - 49.60) and crumb yellowness (b*) ($F_{(1, 20)} = 2.95$, $p = 0.10$) (22.93 - 23.14) but slightly decreased crust redness (a*) ($F_{(1, 20)} = 0.01$, $p = 0.92$) (12.37 - 12.32) , crust yellowness (b*) ($F_{(1, 20)} = 0.02$, $p = 0.89$) (28.14 - 27.35) , crumb lightness (L*) ($F_{(1, 20)} = 0.35$, $p = 0.56$) (64.38 - 63.40) and crumb redness (a*) ($F_{(1, 20)} = 0.59$, $p = 0.45$) (6.88 - 6.31) as its YG level increased from 10.75 - 33.75 g (Table 4.5). This increase of crust lightness might be attributed to the effect of yoghurt pH on Maillard reaction and caramelization of oat bread. The low pH of yoghurt might inhibit Maillard reaction and caramelization of oat bread as these reactions were efficient at higher pH. However, gluten-free bread CMC was added from 1 - 1.5 g (Table 4.5).

Table 4.5   Effect of CMC, YG and TG on the crumb and crust colour properties of oat bread[1,2]

	Ingredients			Response variable					
				Crumb colour			Crust colour		
Run	CMC	YG	TG	Lightness (L*)	Redness (a*)	Yellowness (b*)	Lightness (L*)	Redness (a*)	Yellowness (b*)
1	-1	-1	0	64.38 ± 0.93	5.21 ± 1.02	24.55 ± 0.80	47.00 ± 0.39	12.37 ± 1.01	28.14 ± 2.64
2	-1	+1	0	63.4 ± 0.34	5.35 ± 1.03	23.62 ± 0.62	49.60 ± 2.40	12.32 ± 1.35	27.35 ± 2.82
3	+1	-1	0	63.70 ± 0.60	5.92 ± 0.33	22.93 ± 0.39	49.28 ± 2.11	11.19 ± 0.61	26.71 ± 2.42
4	+1	+1	0	64.14 ± 0.37	4.60 ± 0.30	23.14 ± 0.13	48.73 ± 0.96	12.28 ± 2.08	26.23 ± 1.01
5	-1	0	-1	63.68 ± 0.15	4.23 ± 0.08	24.3 ± 0.00	50.05 ± 0.61	10.54 ± 0.03	23.96 ± 3.68
6	-1	0	+1	63.79 ± 0.96	4.71 ± 0.52	22.89 ± 1.60	49.40 ± 1.62	10.31 ± 0.85	25.64 ± 2.46
7	+1	0	-1	63.79 ± 1.56	5.25 ± 0.05	23.32 ± 0.78	46.34 ± 2.62	12.01 ± 1.37	22.28 ± 3.01
8	+1	0	+1	64.35 ± 0.17	4.76 ± 0.15	23.49 ± 0.54	46.07 ± 0.16	14.37 ± 0.77	23.52 ± 0.15
9	0	-1	-1	63.95 ± 0.27	6.88 ± 0.65	22.58 ± 0.40	47.78 ± 0.40	14.89 ± 0.81	26.33 ± 1.70
10	0	-1	+1	64.39 ± 0.80	6.15 ± 0.30	23.74 ± 0.73	44.99 ± 1.46	15.25 ± 0.63	23.66 ± 0.66
11	0	+1	-1	64.5 ± 0.55	6.31 ± 0.56	22.12 ± 1.26	49.09 ± 2.43	15.05 ± 0.46	26.31 ± 1.03
12	0	+1	+1	63.36 ± 1.40	6.40 ± 1.90	21.82 ± 1.20	48.17 ± 1.25	14.30 ± 2.01	24.06 ± 4.08
13	0	0	0	63.55 ± 0.94	5.27 ± 1.22	23.22 ± 0.99	46.80 ± 3.88	14.09 ± 1.50	25.89 ± 4.29

[1]Coded levels of the quantity of ingredients (-1, 0, +1) corresponds to lower level, middle level and upper level respectively. CMC (1, 1.5, 2 g); YG (10.75, 22.25, 33.75 g); TG (0.5, 1, 1.5 g).
[2]CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase.

In addition, CMC slightly decreased crumb lightness ($F_{(1, 20)} = 0.18$, $p = 0.68$) (64.38 - 63.79) and crumb yellowness ($F_{(1, 20)} = 1.87$, $p = 0.19$) (24.55 - 22.58) but slightly increased crumb redness ($F_{(1, 20)} = 0.28$, $p = 0.60$) (5.21 - 6.88) as its level increased from 1 - 1.5 g (Table 4.5). These decreases of crust and crumb lightness could be explained by the the high extent of Maillard reaction and caramelization. The high amount of amino acid and reducing sugars contained in CMC increased amino acids and reducing sugars contents of oat dough leading to the browning reaction with heat as catalyst. Being entirely different process from Maillard browning, the caramelization also increased the extent of browning reaction because of the pyrolysis of certain sugars contained in dough. Conversely, the colour analysis of the crust of gluten-free bread revealed that bread supplemented with CMC has a lighter crust compared to the control (Sciarini *et al.,* 2010). This could be attributed to the effect of the hydrocolloid on water distribution and low baking temperature and time which affect the Maillard reaction and caramelization. Similar findings were obtained by Mezaize *et al.* (2009) studying the colour of gluten-free breads. The colour analysis of rice bread demonstrated that the redness of crust is higher for a CMC formulation as compared to that of the control and no significant difference in crust yellowness was found (Lazaridou *et al.,* 2007). Similar findings were obtained on oat bread. However, the presence of CMC at 2% concentration showed a significant difference of the redness parameter for crumb among gluten-free breads (Lazaridou *et al.,* 2007).

The effect of YG on oat bread formulation showed that it did not significantly increased crust lightness (L*) ($F_{(1, 20)} = 1.40$, $p = 0.25$) (47.00 - 49.60) and crumb yellowness (b*) ($F_{(1, 20)} = 2.95$, $p = 0.10$) (22.93 - 23.14) but slightly decreased crust redness (a*) ($F_{(1, 20)} = 0.01$, $p = 0.92$) (12.37 - 12.32) , crust yellowness (b*) ($F_{(1, 20)} = 0.02$, $p = 0.89$) (28.14 - 27.35) , crumb lightness (L*) ($F_{(1, 20)} = 0.35$, $p = 0.56$) (64.38 - 63.40) and crumb redness (a*) ($F_{(1, 20)} = 0.59$, $p = 0.45$) (6.88 - 6.31) as its YG level increased from 10.75 - 33.75 g (Table 4.5). This increase of crust lightness might be attributed to the effect of yoghurt pH on Maillard reaction and caramelization of oat bread. The low pH of yoghurt might inhibit Maillard reaction and caramelization of oat bread as these reactions were efficient at higher pH. However, gluten-free bread supplemented with dairy products had an appealing dark crust and white crumb

appearance (Gallagher *et al.,* 2004b). According to Gallagher *et al.* (2003), the colour analysis of gluten-free bread showed that the bread supplemented with dairy products, had lower crust lightness (L*) (value given a darkness to lightness indicator for products) than the control (Gallagher *et al.,* 2003). This was due to the small amount of lactose contained in dairy powder which is involved in Maillard browning and caramelization reactions (Gallagher *et al.,* 2003). These reactions were affected by the distribution of water and the reaction of reducing sugars and amino acids resulting in a darker crust colour (Gallagher *et al.,* 2003). Crumb colour was not significantly ($p > 0.05$) affected by YG. Similar results were shown by Gallagher *et al.* (2003) on her studies in gluten-bread formulations.

The color characteristics of oat bread supplemented by TG shown that TG treatment did not significantly ($p > 0.05$) decreased the crust lightness (L*) ($F_{(1, 20)} = 0.71$, $p = 0.41$) (50.05 - 49.40) but increased crust redness (a*) ($F_{(1, 20)} = 0.45$, $p = 0.51$) (14.89 - 15.25) as its level increased from 0.5 - 1.5 g. In addition, crumb lightness (L*) ($F_{(1, 20)} = 0.71$, $p = 0.41$) (63.68 - 63.40) and crumb redness ($F_{(1, 20)} = 0.11$, $p = 0.74$) (6.88 - 5.27) of oat bread did not significantly ($p > 0.05$) decreased due to TG addition. However, crumb yellowness ($F_{(1, 20)} = 0.04$, $p = 0.83$) (22.58 - 23.22) and crust yellowness ($F_{(1, 20)} = 0.09$, $p = 0.77$) (23.96 - 27. 35) did not significantly ($p > 0.05$) increased while increasing TG level from 0.5 - 1.00 g (Table 4.5). The decreases in crust and crumb lightness (L*) might be the result of a low extent of Maillard reaction and caramelization due to an increase in the amount of available lysine because of limited cross-link induced by TG catalysis reaction in oat dough. This blockage of lysine residues via enzymatic cross-linking of oat dough proteins had a limited effect on the Maillard reaction. However, the release of ammonia during the TG-catalyzed cross-linking reaction might also participate in the Maillard reaction and therefore slightly contribute to the changes in colour properties of oat bread.

4.3.5 Optimal oat bread

The optimisation goal was to target hardness at 522.2 N, while minimising chewiness, gumminess and springiness. The predicted optimal ingredients for preparing oat dough were: CMC (1 g), yoghurt (33.75 g) and TG (0.98 g) with a desirability of 0.93. Under

this formulation, the model predicts a maximum hardness of 572.35 N; minimum chewiness of 20.33 N; minimum gumminess of 1.21 N and minimum springiness of 5.25 mm. Average value of hardness (562.87 ± 11.86 N), chewiness (21.67 ± 5.89 N), gumminess (1.96 ± 1.65 N) and springiness (6.51 ± 0.50 mm) were obtained, which is close to the model predicted values. This confirms that the model adequately predicted the texture of oat bread.

4.4   Conclusion

The aim of this study was to determine the effects of CMC, YG and TG on the physical, textural and color characteristics of oat bread and to optimise the oat bread production process through the establishment of the amount of carboxymethycellulose CMC, yoghurt (YG) and transglutaminase (TG) required, for optimal oat bread production. The physical and textural analysis of oat bread showed that CMC, YG and TG addition did affect oat bread. CMC increased all the textural parameters of bread, except its specific volume. YG decreased the hardness, chewiness and gumminess but increased the resilience (cohesion energy and cohesion force), springiness and specific loaf volume of the resulting bread. TG decreased all the physical and textural parameters of oat bread with exception of hardness which increased. No great change was observed on the colour parameters of crust and crumb of oat bread. Optimal oat bread could be produced using CMC (1 g), yoghurt (33.75 g) and TG (0.98 g). Its high hardness and low springiness remain some challenges.

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CHAPTER 5

EFFECTS OF PSYLLIUM HUSKS AND CYCLODEXTRINASE ON TEXTURAL, SENSORY AND SHELF-LIFE CHARACTERISTICS OF BEST OAT BREAD

Abstract

TG was taken out of the formulation of the best oat bread as it was well established that TG significantly ($p < 0.05$) increased the hardness and decreased the springiness of the optimal oat bread. The effects of psyllium husks and cyclodextrinase on textural, sensory and shelf-life characteristics of the optimal oat bread were investigated. The optimal oat bread was baked with psyllium husks (15 g), cyclodextrinase (5 µl) and transglutaminase (0.98 g) as independent variables for screening the best individual or combination ingredients of the best oat bread formulation. The change in the optimal oat bread texture was determined using a Texture Analyzer. Sensory evaluation and Accelerated Shelf-Life Testing were respectively used for the consumer acceptability and shelf-life studies of the best oat bread. The dependent variables of interest for the textural properties of the optimal oat bread were hardness, chewiness, gumminess and springiness. The sensory characteristics of the best oat bread included appearance, crust colour, crumb colour, aroma, taste, texture and overall acceptability. pH, Total Titratable Acidity (TTA), Total Viable Count (TVC), Yeast and mould were used as dependent variables for the shelf-life studies of the best oat bread. The combination of ingredients psyllium husks and cyclodextrinase significantly ($p < 0.05$) decreased bread hardness (94.88 N) and increased bread springiness (10.97 mm). Thus, the formulation of best oat bread included psyllium husks (15 g) and cyclodextrinase (5 µl). The sensory evaluation showed that there was a significant ($p < 0.05$) correlation between crust and crumb colour of the best oat bread and wheat bread. As a result, there was no significant ($p < 0.05$) difference between the best oat bread and wheat bread in terms of overall acceptability. However, some significant differences existed between the best oat and wheat bread. The taste of wheat bread received high score compared to the best oat bread. The shelf-life studies of the best oat bread revealed that the pH

and TVC of the best oat bread were significantly (p < 0.05) affected by the time, temperature and the interaction of both parameters (time and temperature) than TTA, yeasts and mould as the storage time passed. Using survival analysis for the shelf-life studies of the best oat bread, the medians of storage time or the time corresponding to 50% probability of end of shelf-life were 21, 12 and 12 days for breads kept at 5°C, 27°C and 44°C, respectively. The mathematical model of the best oat bread shelf-life was established using Total Count Viable (TVC) and it revealed that the risk of deteriorating increased with the temperature as shown its hazard function.

5.1   Introduction

Belonging to dietary fibre, psyllium husks is the edible portion of plants (or analogous carbohydrates). From the technological view, fibre incorporation as pysllium husks improves the texture, sensory characteristics and shelf-life of foods due to their water binding capacity, gel forming ability, fat mimetic, texturizing and thickening effects (Thebaudin *et al.,* 1997; Gelroth & Ranhotra, 2001). The addition of dietary fibre as psyllium husks in gluten-free formulations improves gas retention ability yielding breads with significantly higher loaf volume and crumb softness compared to the control (Zandonadi *et al.,* 2009). The gluten-free breads supplemented psyllium husks provide the consumer with higher amounts of total dietary fibre and have an appealing dark crust and a uniform and finely grained crumb texture (Sabanis *et al.,* 2009).

Preliminary experiments indicated that CG has positive effects on rice bread volume (Gujral *et al.,* 2003). This impact is probably due to the release of fermentable sugars utilized by the yeast, as a result of the hydrolysis of starch, which is catalysed by CG. Concerning the crumb texture, the crumb firmness shows a negative correlation with specific volume. The incorporation of CG considerably lowers firmness by 53%, obtaining a very soft bread crumb (Gujral *et al.,* 2003). The similar decrease in the crumb firmness is obtained with the addition of α-amylase, but the crumbs are very sticky (Gujral *et al.,* 2003). The cyclizing activity of CG is validated by the presence of cyclodextrins during the breadmaking process. Hence, the hydrolyzing activity of the CG during breadmaking is identical to the effect of α-amylase. Its shelf-life extension is due to its capability to lown downamylopectin retrogradation during storage through its

hydrolyzing and cyclizing activities (Gujral *et al.,* 2003). This anti-staling impact is attributed to the low molecular weight dextrins produced as a result of starch hydrolysis. Those dextrins interfere with the ability of the amylopectin to retrograde (Lin & Lineback, 1990; Defloor & Delcour, 1999; Rojas *et al.,* 2001; Leon *et al.,* 2002), or with other interactions also, particularly starch-protein or protein-protein entanglement involved in firming (Lin & Lineback, 1990; Martin & Hoseney, 1991).

Little published work is available on the sensory and shelf-life profile of gluten-free breads. It is only in recent years that the prevalence of celiac disease and the need for research and development on the quality of the gluten-free breads has been investigated (Gallagher *et al.,* 2003). It has also been showed that gluten present in wheat bread decrease the movement of water by forming an extensible protein network, hence keeping the crumb structure together (Gallagher *et al.,* 2003). Therefore, the absence of gluten should increase the movement of water from the bread crumb to crust, leading in a firmer crumb and softer crust (Gallagher *et al.,* 2003).

The previous chapter shows that the optimal oat bread exhibits a high hardness and low springiness although it has a high desirability (0.93) (Chapter 4, Section 4.3.5). However, attempts to improve the rheological, sensory and shelf-life characteristics of the gluten-free bread have focused on adding Psyllium husks (PH) and Cyclodextrinase (CG) to the gluten-free dough.

As psyllium husks (PH) and cyclodextrinase (CG) affect the rheological, sensory and shelf-life characteristics properties of gluten-free bread, nothing is known as to the effect of psyllium husks (PH) and cyclodextrinase (CG) on the rheological (texture profile), sensory and shelf-life characteristics of the optimal oat bread. Hence, our objective was to (1) investigate the effects of psyllium husks (PH) and cyclodextrinase (CG) in on the textural characteristics of the optimal oat bread, (2) establish the quality and consumer acceptability of the best oat bread and (3) establish the shelf-life and the mathematical model for its determination of the best oat bread.

5.2   Materials and Methods

5.2.1 Source of materials

Psyllium Husks (PH) was purchased from Health Connection Wholefoods (Cape Town, South Africa). The source of materials of other ingredients was diverse as revealed in Chapter 3, Section 3.2.1.

This chapter investigated the effects of psyllium husks (PH) and cyclodextrinase (CG) on the textural characteristics of the optimal oat bread, established the quality, consumer acceptability and shelf-life of the best oat bread (Figure 5.1).

5.2.2 Proximate analysis of oat flour

The proximate analysis of oat flour was according the method described in Chapter 3, Section 3.2.2.

5.2.3 Particle size distribution of oat flour

The particle size distribution of oat done as reported in Chapter 3, Section 3.2.3.

5.2.4 Determination of protein concentration of TG and CG

The determination of protein concentrations of TG and CG followed the procedure described in Chapter 3, section 3.2.4.

5.2.5 Experimental for the effect of psyllium husks and cyclodextrinase on optimal oat bread

Psyllium husks (PH), cyclodextrinase (CG) and transglutaminase (TG) were used to produce five (5) best oat bread formulations such as (1) PH + CG, (2) CG, (3) TG + CG, (4) TG + PH and (5) TG + PH + CG (Tables 5.1 and 5.2). The variables (TG, PH and CG), and their levels are detailed in Table 5.1. Each design point in Table 5.2 was performed in triplicates. Following the combination of the ingredients as per the design, bread samples were produced following the process described in the Section 5.2.6. The experiment was carried out in randomized order. The dependent variables of interest were hardness, chewiness, gumminess and springiness. The best oat bread
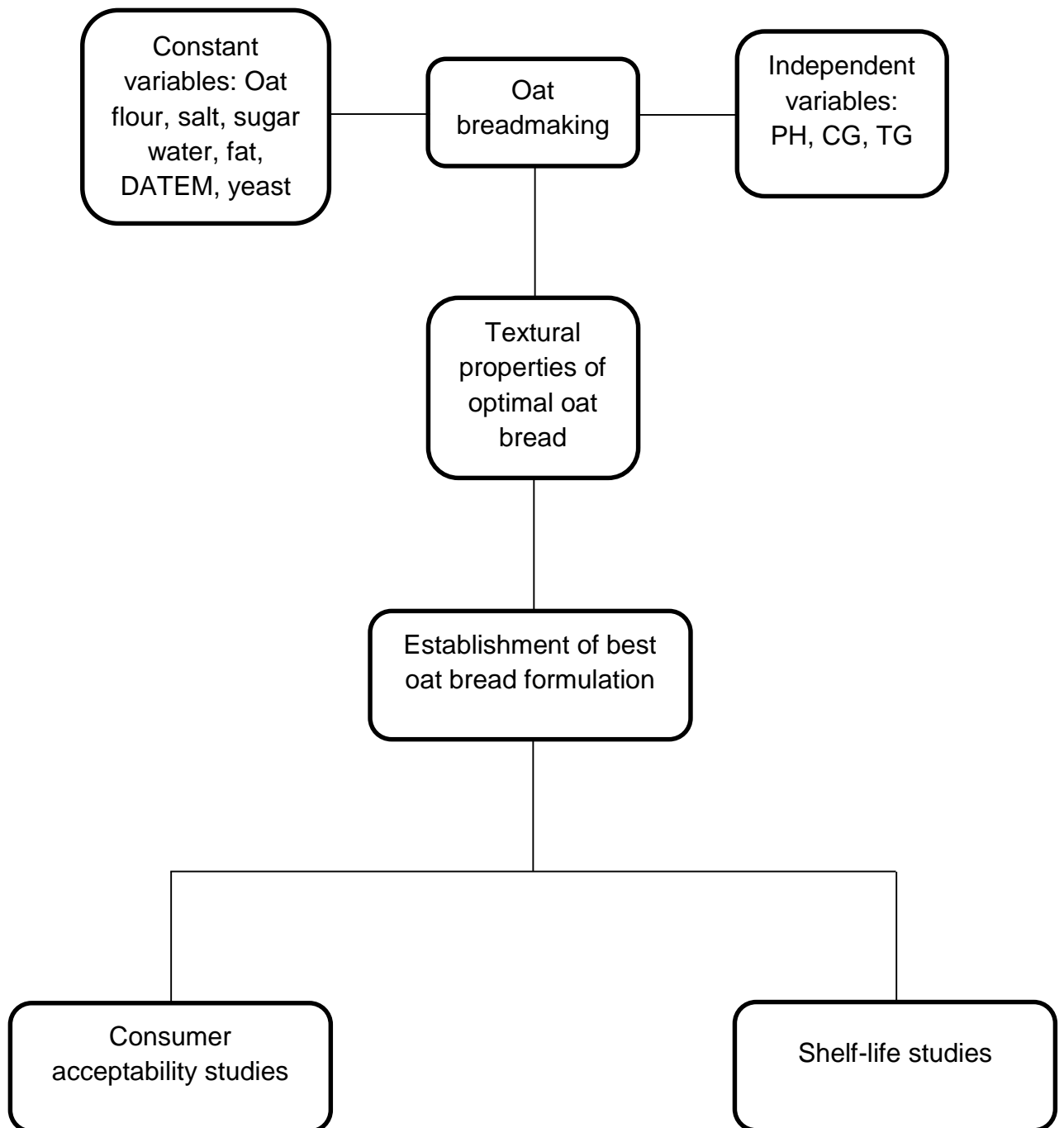
Figure 5.1   Overview of chapter five

Table 5.1　Process variables used for best oat bread preparation

Variable	Level (/100 g flour)
Transglutaminase	0.98 g
Psyllium husks	15 g
Cyclodextrinase	5 µl

Table 5.2　Variable and levels used for the best oat bread formulation

Run	Variable		
	Transglutaminase	Psyllium husks	cyclodextrinase
1	-	15 g	5 µl
2	-	-	5 µl
3	0.98 g	-	5 µl
4	0.98 g	15 g	-
5	0.98 g	15 g	5 µl

formulation that exhibited a low hardness and high springiness was designated as the best oat bread and was used for sensory and shelf-life studies.

5.2.6 Effect of psyllium husks and cyclodextrinase on optimal oat loaf

The method of Nitcheu (2010) was modified and used to bake oat bread. Oat bread was produced as described in Figure 5.2. The basic recipe consisted of: oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g), water (78 g), yeast (2.25 g), YG (33.75 g) and CMC (1 g). Other ingredients (TG, PH and CG) at variable quantities were added following the design outlined in Table 5.2. All the ingredients were placed in a stainless steel bowl mixer (Kenwood chef, UK) and mixed for 6 min (speed 2: 2 min and speed 3: 4 min). The sides of the bowl were scrapped down half way and the dough was allowed to rest for 10 min at room temperature. 150 g of dough was weighed, mechanically sheeted, rolled, panned (96 mm x 51 mm x 33 mm), proofed (Macadams, South Africa) (40°C, 50 min, 80% relative humidity), and baked in rotated oven (Macadams, South Africa) at 230°C for 30 min. After baking, the loaf was removed from the pan, cooled on the rack for 2 h at room temperature, packaged in a sealed polyethylene bag, stored at room temperature for 24 h and analysed for rheological (texture profile analysis) properties.

5.2.7 Textural analysis of optimal oat bread

The textural analysis of oat bread was done according to the method defined in Chapter 4, Section 4.2.7. The best oat bread formulation with low hardness and high springiness was designated as the best oat bread and used for sensory and shelf-life studies.

5.2.8 Sensory evaluation of best oat bread

The method of Torbica *et al.* (2010) was modified and used for the sensory analysis of oat bread. Wheat bread was used as the control of the sensory evaluation of oat bread. The basic recipe of wheat and oat bread consisted of: wheat or oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g), water (78 g), yeast (2.25 g), YG (33.75 g), CMC (1 g), PH (15 g) and CG (5 µl). Wheat and oat breads were baked according to the same baking process as described in this chapter, Section 5.2.6,

```
  ┌─────────────────┐                                      ┌─────────────────┐
  │  Salt, sugar,   │         ┌──────────────────┐         │     Other       │
  │    DATEM,       │────────▶│ Oat flour (100 g)│◀────────│  ingredients:   │
  │  CMC, YG, fat,  │         └──────────────────┘         │   (TG, PH,      │
  │  water, yeast   │                  │                   │      CG)        │
  └─────────────────┘                  ▼                   └─────────────────┘
                          ┌───────────────────────────┐
                          │          Mixing           │
                          │ (Speed 2: 2 min, speed 3: │
                          │          4 min)           │
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │          Resting          │
                          │ (10 min, room temperature)│
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │          Shaping          │
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │         Proofing          │
                          │ (80% Relative Humidity,   │
                          │       40°C, 50 min)       │
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │          Baking           │
                          │     (230°C, 30 min)       │
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │          Cooling          │
                          │  (Room temperature, 2h)   │
                          └───────────────────────────┘
                                       │
                                       ▼
                          ┌───────────────────────────┐
                          │         Packaging         │
                          │   (Fresh-keeping film)    │
                          └───────────────────────────┘
                                       │
                                       ▼
                            ┌─────────────────────┐
                            │     Oat bread       │
                            └─────────────────────┘
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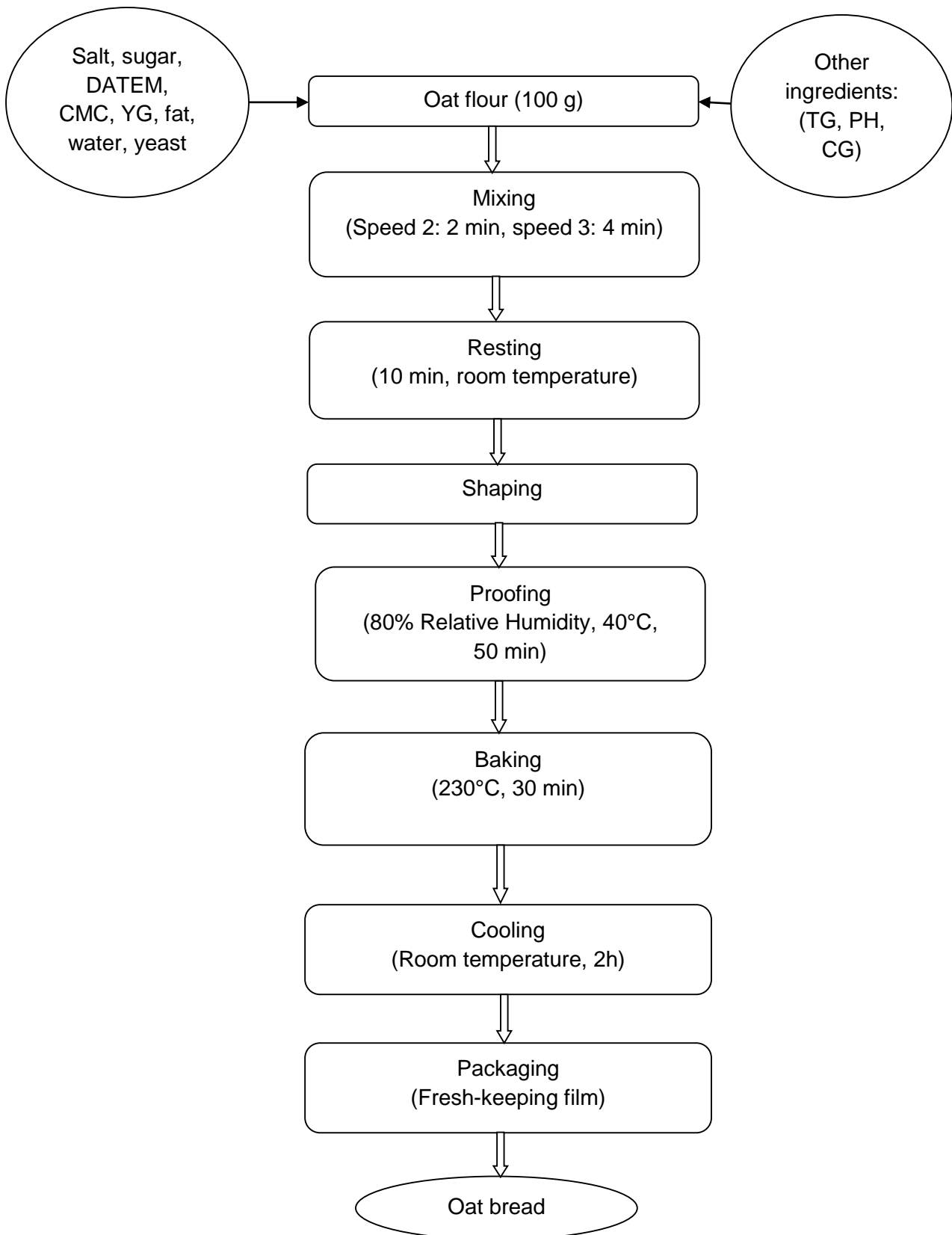
Figure 5.2   Best oat bread production process.

Figure 5.1. The sensory evaluation was carried out 24 h after bread cooling by 40 untrained panellists in lighted room and at room temperature. Slices of approximately 10 g of weight and 3 mm thick of each type of bread (wheat and oat bread) packaged in polyethylene zip lock bag were served on odourless green or black plastic tray and coded with three-digit random numbers. The panellist was instructed to take a sip of water before starting tasting and in between tasting the different samples. The following sensory attributes were evaluated: appearance, crust colour, crumb colour, aroma, taste, texture and overall acceptability. For each parameter, five-point hedonic scale was used ranging from 1 (dislike very much) to 5 (like very much). Products were found acceptable if their mean scores for the acceptability were above 2.50.

5.2.9 Statistical analysis
Multivariate Analysis of variance (MANOVA) was used to determine the differences between treatments. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS, 2010).

5.2.10 Shelf-life evaluation of best oat bread
A 24 loaves of best oat bread were produced and the basic formulation was consisted of: oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g), water (78 g), yeast (2.25 g), YG (33.75 g), CMC (1 g), PH (15 g) and CG (5 µl). Best oat bread was baked according the breadmaking process described in Section 5.2.6 and Figure 5.1. The bread loaves were packaged in polyethylene zip lock bag. Eight (08) bread loaves were stored at each storage temperature (5, 27 and 44°C). Bread loaves were removed at 3-day interval over 21 days and analysed for pH, total titratable acidity (TTA), total viable count (TVC) and yeast and mould in triplicates.

Determination of pH and Total Titratable Acidity (TTA) of best oat bread
The method of Simonson *et al.* (2003) was modified and used to determine the pH and TTA of the stored oat bread. The pH was determined using a GLP 21 pH – meter, CRISON INSTRUMENTS, S.A (Barcelona, Spain) at room temperature (21 ± 2°C). After, the bread sample was taken out from the incubator and it was unpackaged. 10 g of bread was weighed, added to 100 ml of distilled water, mixed

with a Bamix mixer (Bamix, Mettlen, Switzerland) for 3 minutes, filtered and the pH of the fluid was measured.

Five (5) grams of oat bread was added to 100 ml of distilled water and mixed with a Bamix mixer (Bamix, Mettlen, Switzerland) for 3 min and filtered. After filtering, 20 ml of supernatant was used for TTA determination. The TTA value was estimated by recording the volume of 0.1N NaOH required to increase the pH to 6.0.

Determination of Total Viable Count (TVC), yeast and mould of best oat bread

The method of Degirmencioglu *et al.* (2011) was modified and used to determine the TVC, yeast and mould of the oat bread. The microbial analysis was performed in duplicate after 0, 3, 6, 9, 12, 15, 18 and 21 days of storage. The samples of oat bread were weighed aseptically (10 g) and homogenized in a Stomacher (AES CHEMUNEX, AES LABORATOIRE, Comburg, France) for 60 sec at room temperature ($20 \pm 2°C$) with 90 ml sterile maximum recovery diluent (Oxoid CM0733). Decimal dilutions (from 10^{-1} to 10^{-6}) were prepared by using maximum recovery diluent. Plate Count Agar (Oxoid CM0325) was used for Total Viable Counts (TVC) and incubated at 37 °C for 48 h.

Yeast and mould counts were examined with the method given by Rodriguez *et al.* (2003) and Pascall *et al.* (2008). Rose Bengal Chloramphenicol Agar (Oxoid CM0549 supplemented with SR0078) was used for yeasts and moulds and incubated at 25°C for 4 days. TVC, yeasts and moulds counts were expressed as log cfu/g.

Data analysis and modeling of shelf life of best oat bread

Analysis of variance (ANOVA) was used to determine the differences between oat bread stored at the different temperatures. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS, 2010).

Survival analysis was used to determine and model the shelf-life of oat bread. It includes Kaplan Meier analysis and Cox regression. Kaplan Meier analysis is the univariate version of survival analysis used to analyse censored and uncensored data for the survival time. The multivariate analysis was performed using Cox regression (IBM SPSS, 2010). It was used to predict variables in model by estimating coefficients for each covariates and thereby assessing the multiple

covariates in the same model. All the results were reported as mean of three independent trials.

5.3 Results and Discussion

5.3.1 Proximate composition and particle size distribution of oat flour

The Proximate composition and particle size distribution of oat flour are indicated in Chapter 3, Section 3.3.1.

5.3.2 Protein concentration of TG and CG

The protein concentrations of TG and CG were reported in Chapter 3, Section 3.3.2.

5.3.3 Effects of PH, CG and TG on the textural properties of the optimal oat bread

The different ingredients (1) PH + CG, (2) CG, (3) TG + CG, (4) TG + PH and (5) TG + PH + CG had a significant ($p < 0.05$) effect on hardness, chewiness, gumminess and springiness of the best oat bread (Table 5.3). The bread hardness of all the formulations were different (Table 5.3). As the ingredient combinations changed from PH + CG to TG + PH + CG, the hardness of the best oat bread increased from 94.88 to 786.01 N (Table 5.3). Defined as force required to compress a substance between molar teeth (in the case of solids) or between tongue and palate (in the case of semi-solids), hardness is mainly characterized by strong intermolecular bonds. In addition, hardness is mainly attributed to the amylose and amylopectin matrix which contribute to overall bread texture (Schiraldi & Fessas, 2000). Gomez *et al.* (2003) revealed that bread hardness is due to interactions between gluten and fibrous materials. Therefore, bread hardness of different formulations can be explained by the specific interaction between gluten replacer (combination of ingredients), fibrous materials and water avaibility. This strong intermolecular bonds showed that the combination TG + PH +CG decreased dough water absorption, thereby increasing bread hardness. As the combination PH + CG increased dough water absorption, the resulting oat bread became softer or firmer.

Bread samples supplemented with ingredient combinations such as PH + CG and TG + PH + CG showed that they were not significantly ($p > 0.05$) different in terms of chewiness (57.87 - 36.44 N) and gumminess (3.66 - 2.28 N) whereas other formulations were significantly ($p < 0.05$) different in terms of chewiness and.

Table 5.3   Effects of PH, CG and TG on the hardness, chewiness, gumminess and springiness of the best oat bread[1,2]

Formulation	Hardness (N)	Chewiness (N)	Gumminess (N)	Springiness (mm)
PH + CG	94.88 ± 0.89[a]	57.87 ± 1.05[a]	3.66 ± 0.05[a]	10.97 ± 0.00[a]
CG	317.27 ± 15.96[b]	-35.21 ± 14.48[b]	-2.12 ± 0.89[b]	3.39 ± 0.83[b]
TG + CG	488.37 ± 17.37[c]	-79.93 ± 9.7[c]	-4.8 ± 0.52[c]	0.04 ± 0.06[c]
TG + PH	560.05 ± 9.8[d]	156.66 ± 27.78[d]	9.65 ± 1.73[d]	10.62 ± 0.41[a]
TG + PH +CG	786.01± 4.96[e]	36.44 ± 3.44[a]	2.28 ± 0.21[a]	5.68 ± 0.75[d]

[1]Mean ± standard deviation of three replicates; values followed by the different letter in the same column are significantly different ($p < 0.05$)
[2]PH: Psylliums Husks; CG: Cyclodextrinase; TG: Transglutaminase

gumminess (Table 5.3). Considered as length of time (in sec) required to masticate the sample, at a constant rate of force application, to reduce it to a consistency suitable for swallowing, bread chewiness mainly depends on dough elasticity. Although the combinations PH + CG and TG + PH + CG acted as gluten replacers, they still exhibited high chewiness because of the lack of wheat protein confering high dough viscosity and therefore high chewiness of the resulting bread. Known as denseness that persists throughout mastication; energy required to disintegrate a semi-solid food to a state ready for swallowing, gumminess also depended on the tenacity and extensibility of the dough, the flour protein content, and the falling number. Thus high gumminess of bread formulated with the ingredient combinations PH + CG and TG + PH + CG might be explained by the high extensibility of dough. A research work of Wang *et al.* (2002) also showed similar trend for breads with added fibers since they caused an increase in gumminess and chewiness of the resulting bread.

Similarly, springiness (degree to which a bread returns to its original shape after being deformed between the teeth) of the bread samples were significantly (p < 0.05) increased by the addition of PH +CG and TG + PH in the formulations (Table 5.3). Bread samples supplemented with the ingredient combinations PH +CG and TG + PH showed that they were significantly (p < 0.05) similar in terms of springiness (10.67 - 10.62 mm) (Table 5.3). However, the springiness of other bread samples were significantly (p < 0.05) different. According to Hoseney (1994), interaction between gelatinized starch and protein cereal caused dough to be more elastic could form continuous sponge structure of bread after heating. Therefore, the high springiness could be attributed to dilution of the ingredient combinations (PH + CG and TG + PH) in composite breads. Other ingredient combinations (CG, TG + CG and TG + PH + CG) had lower ability to hold gases which caused an elasticity reduction in breads.

The optimal oat bread supplemented with the ingredient combination (PH + CG) showed that psyllium husks (PH) and cyclodextrinase (CG) positively improved the textural properties (less hard and more springy) of the optimal oat bread compared to others (Table 5.3, Figure 5.3). Therefore, this formulation of the best oat bread including oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g), water (78 g), yeast (2.25 g), YG (33.75 g), CMC (1 g), PH (15 g) and CG (5 µl)

Figure 5.3   Effects of different ingredient combinations on the textural properties of the optimal oat bread formulation[1].

[1](1) Psyllium husks (15 g) + Cyclodextrianse (5 µl), (2) Cyclodextrianse (5 µl), (3) Transglutaminase (0.98 g) + Cyclodextrianse (5 µl), (4) Transglutaminase (0.98 g) + Psyllium husks (15 g), (5) Psyllium husks (15 g) + Cyclodextrianse (5 µl) + Transglutaminase (0.98 g).

was designated as the best oat bread and used for sensory evaluation and shelf-life studies.

5.3.4 Sensory evaluation of wheat and best oat bread

The demography of the panellists is indicated in Table 5.4. There were 40 panellists 62.5% of whom were females, 100% were students, 10% were international students and 95% were less than 30 years old and 5% within 30 - 34 years of age and 0% within 40 and above years of age.

The sensory evaluation of wheat and best oat bread showed that wheat bread was rated higher than the best oat bread in terms of appearance, crust colour, aroma, taste and overall acceptability (Figure 5.4). However, the best oat bread received a higher rating in terms of crumb colour and texture compared to wheat bread (Figure 5.4). There was slight correlation between all the sensory parameters assessed except in crust and crumb colour. A significant ($p < 0.05$) correlation in crust and crumb colour was noted between wheat bread and the best oat bread. In addition, wheat and best oat bread exhibited a slight difference in appearance, crust colour, crumb colour, aroma, texture and overall acceptability but a significant ($p < 0.05$) difference in taste (Figure 5.4). The population consisted of persons from different walks of life. The difference in rating amongst the panellist was expected, since people from different age groups (< 30 - 40 years), and occupation (staff, student) participated in the study.

The significant ($p < 0.05$) correlations in the crust colour and crumb colour indicated that the panellists who rated the crust and crumb colour of wheat bread high also rated the oat bread high in these attributes, consistently (Figure 5.4). These correlations might be attributed to the similar content of substrates reacting during Maillard reaction and caramelization in wheat and oat doughs. As Maillard reaction and caramelization is one main event occurring during baking, the free amino group of lysine and/or other amino acids and the carbonyl groups of reducing sugars such as glucose and maltose lead to compounds responsible of brown colour and sensory properties of bread. The findings efficiently showed that the ingredient combination PH + CG impact the best oat bread crumb and crust as gluten replacer. However, some differences existed between wheat and oat bread. Negative values indicated the attributes where wheat bread was rated lower (crumb colour and texture) though not significant ($p > 0.05$). These differences in crumb colour and

Table 5.4   Demography of the panellists[1]

Item	Frequency (Percentage)
Gender	
Male	25 (62.5)
Female	15 (37.5)
Staff/student?	
Staff	0 (0)
Student	40 (100)
International students?	
No	36 (90)
Yes	4 (10)
Age category	
< 30	38 (95)
30-39	2 (5)
40 & above	0 (0)

[1]Numbers are frequency and percentage in bracket.
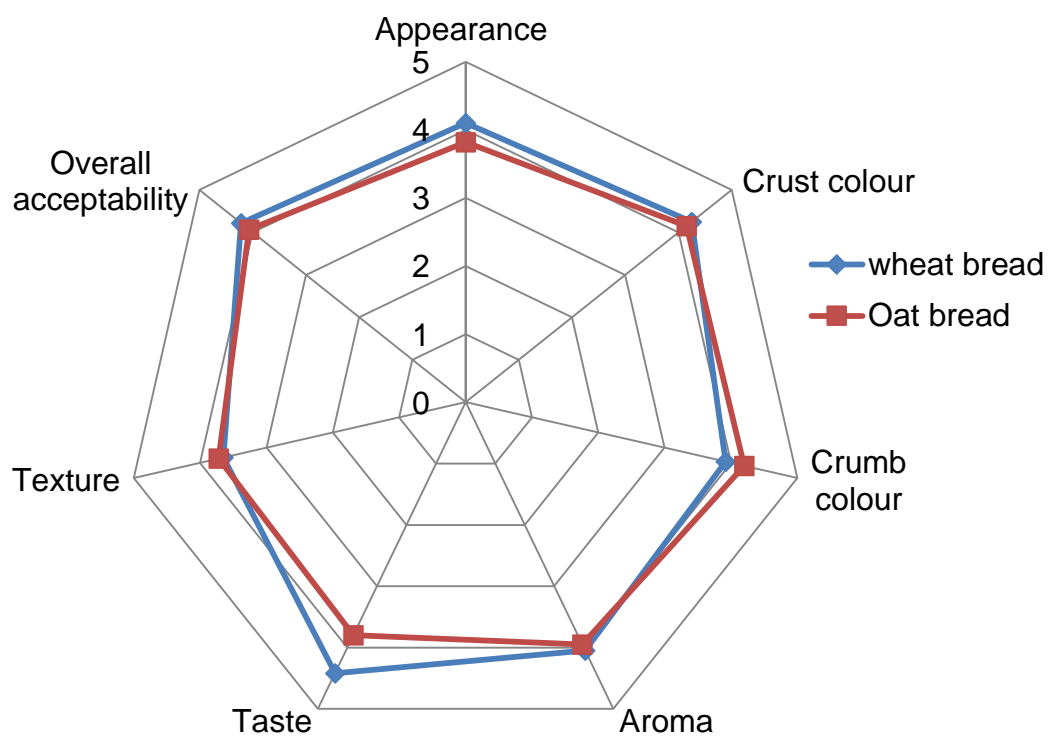
Figure 5.4   Panellist scores for sensory characteristics of wheat and best oat bread.

Wheat and best oat bread significantly ($p < 0.05$) differed in taste with wheat bread rated higher in taste than oat bread. This was mainly attributed to the presence of wheat gluten protein improving the taste of wheat flour. Wheat and best oat bread had overall acceptability of 4.22 and 4.07, respectively (Figure 5.4). Since, bread sample which received score higher than 2.50 (neither like nor dislike) was considered as acceptable, best oat bread was therefore acceptable.

5.3.5 Shelf-life study of best oat bread

Effect of storage time and temperature on pH and TVC of best oat bread

Storage time, temperature and the interaction of both parameters significantly ($p < 0.05$) affected bread pH whereas neither time, temperature nor the interaction of both parameters significantly ($p > 0.05$) affected TTA (Total Titratable Acidity) of oat bread (Table 5.5).

As the storage time varied from day 9 to day 21, the pH of oat bread significantly ($F_{(7, 13)} = 29.06$, $p = 0.00$) increased from 5.59 to 5.67 (Table 5.5). This might be attributed to the presence of psyllium husks in oat bread formulation, dietary fibre mainly rich in soluble fibre. Its addition increased the water absorption capacity of oat dough due to its hydrophilic character. This property led to the development of "weak gel" networks which trapped carbon dioxide generated during proofing and therefore increased gas retention and loaf volume (Zandonadi *et al.,* 2009). The high retention of carbon dioxide by oat bread might increase bread pH during the storage time because it reacted with water and thereby releasing carbonic acid acting mainly as a weak acid. The carbonic acid diluted the hydronium ions released by the strong acids such as lactic and acetic acid (induced by lactic acid bacteria of yoghurt) and therefore increased bread pH during the storage time of oat bread.

In addition, bread pH significantly ($F_{(2, 13)} = 72.99$, $p = 0.00$) increased (5.59 - 5.67) while keeping the storage temperature at 5°C (Table 5.5). This increase in pH implied that the temperature did affect bread pH because the carbonation intensity was significantly perceived at lower temperature (Yau & Mc Daniel, 1991). As yeast and yoghurt cultures are inactive at lower temperatures, the bread pH increased because lower temperature accelerated the carbonation process. Carbon

Table 5.5   Effect of storage time and temperature on pH, TTA, TVC, yeast and mould growth of the best oat bread[1,2]

Storage time (day)	Storage temperature (°C)	Chemical parameters		Microbiological parameters	
		pH	TTA	TVC (log cfu/g)	Yeast & mould (log cfu/g)
0	5	5.45 ± 0.08	3.60 ± 0.00	0.65 ± 0.92	0.5 ± 0.71
	27	5.45 ± 0.08	3.60 ± 0.00	0.65 ± 0.92	0.5 ± 0.71
	44	5.45 ± 0.08	3.60 ± 0.00	0.65 ± 0.92	0.5 ± 0.71
3	5	5.53 ± 0.03	4.20 ± 0.85	3.62 ± 0.21	1.45 ± 0.16
	27	5.51 ± 0.06	4.20 ± 0.85	4.52 ± 0.01	2.22 ± 0.09
	44	6.26 ± 0.04	6.00 ± 0.00	6.35 ± 0.01	1.90 ± 0.04
6	5	5.56 ± 0.00	3.00 ± 0.85	4.14 ± 0.09	3.30 ± 0.03
	27	6.01 ± 0.01	4.80 ± 0.00	6.47 ± 0.01	2.60 ± 0.01
9	5	5.59 ± 0.07	3.00 ± 0.85	4.41 ± 0.04	2.97 ± 0.02
12	5	5.64 ± 0.03	3.00 ± 0.85	4.49 ± 0.00	3.10 ± 0.01
15	5	5.64 ± 0.01	3.00 ± 0.85	4.68 ± 0.00	3.13 ± 0.01
18	5	5.66 ± 0.01	3.00 ± 0.85	4.71 ± 0.01	3.24 ± 0.02
21	5	5.67 ± 0.08	3.00 ± 0.85	4.99 ± 0.05	2.36 ± 0.97

[1]Mean ± standard deviation of three replicates.
[2]TTA: Total Titratable Acidity; TVC: Total Viable Count

dioxide solubility was particularly influenced by the temperature. Dough allowed more carbon dioxide to stay in solution at lower temperature. In general, the lower the temperature and the higher the pressure, the more carbon dioxide gas stays in solution.

Bread pH significantly ($F_{(3, 13)}$ = 57.74, p = 0.00) increased (5.59 - 5.67) while simultaneously varing storage time from day 9 to day 21 and keeping storage temperature at 5°C. This was attributed to the presence of psyllium husks in oat bread formulation. Its incorporation improved the carbonation intensity of oat bread at lower temperature because of the solubility of carbon dioxide and the production of carbonic acid in oat bread

As the storage time varied from day 9 to day 21, the TTA of oat bread did not ($F_{(7, 13)}$ = 1.83, p = 0.16) vary at all as TTA was constant (3.00) (Table 5.5). Similar findings were obtained while keeping the storage temperature at 5°C ($F_{(2, 13)}$ = 3.66, P = 0.06) or varing storage time from day 9 to day 21 and keeping storage temperature at 5°C ($F_{(3, 13)}$ = 3.25, p = 0.06) (Table 5.5). It was due to the fact that neither storage time, storage temperature nor both parameters affected TTA of oat bread during storage.

Effect of storage time and temperature on TVC, yeast and mould of best oat bread

The microbiological analysis of the best oat bread revealed that the time, temperature and the interaction of both parameters significantly ($p < 0.05$) affected bread TVC whereas neither time, temperature nor the interaction of both parameters significantly ($p > 0.05$) affected yeast and mould growth in oat bread (Table 5.5).

As the storage time varied from day 0 to day 3, the TVC of oat bread significantly ($F_{(7, 13)}$ = 63.53, p = 0.00) increased from 0.65 to 6.35 log cfu/g (Table 5.5). Total Viable Count (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mould in a sample. This increase of TVC showed that oat bread was significantly deteriorated by the microorganisms such as bacteria, yeast and mold as pH was greater than 4.5. As the pH increased as a function of time, due to the carbonation process induced by psyllium husks, oat bread simultaneously was slimy at day 9 while stored at 44°C. It might be due to the combined effect of the proteolytic and amylolytic enzymes produced by some

Bacillus strains (Saranraj & Geetha, 2012), accelerating the development of the spores of microrganisms and thereby increasing TVC of oat bread.

In addition, TVC significantly ($F_{(2, 13)}$ = 17.32, p = 0.00) increased (0.65 - 6.35 log cfu/g) while increasing the storage temperature from 5°C to 44°C (Table 5.5) because increasing temperature of oat bread increased pH. As a result, the microorganisms increased while the temperature increased. TVC significantly ($F_{(3, 13)}$ = 10.37, p = 0.00) increased (0.65 - 6.35 log cfu/g) while varing storage time and temperature storage from day 0 to day 3 and 5°C to 44°C, respectively because of the increase of pH induced by the carbonation process.

As the storage time varied from day 9 to 18, yeast and mould of oat bread slightly ($F_{(7, 13)}$ = 0.69, p = 0.68) increased from 2.97 to 3.24 log cfu/g (Table 5.5). In addition, yeast and mould slightly ($F_{(2, 13)}$ = 0.80, p = 0.47) increased (2.97 - 3.24 log cfu/g) while keeping the storage temperature at 5°C (Table 5.5). Bread pH slightly ($F_{(3, 13)}$ = 2.36, p = 0.12) increased (2.97 - 3.24 log cfu/g) while varing storage time day 9 to 18 and keeping the storage temperature at 5°C. It is attributed to the bread pH which was greater than 4.5. Yeast and mould did not significantly (p > 0.05) affect oat bread because certain foods with pH value greater than 4.5 can be spoiled by bacteria and are not more susceptible to yeast and mold spoilage. TVC was used to model the shelf-life as it did significantly (p < 0.05) affect the microbiological properties of oat bread during the storage.

Modelling the shelf-life of best oat bread

Table 5.7 shows the number of events at each storage temperature, namely the number of cases was 1, 12 and 14, with the percentage of censored cases being 93.8%, 25.0% and 12.5% respectively at 5°C, 27°C and 44°C (Table 5.6). Table 5.7 shows the means and medians of survival time. The medians of storage time or the time corresponding to 50% probability of end of shelf-life were 21, 12 and 12 days respectively at 5°C, 27°C and 44°C. The survival curves were compared using the log rank test. It was used to test the null hypothesis that there is no difference in survival time (i.e. the probability of an event occurring at any time point is the same for each population) between the temperatures studied. Table 5.8 showed that the storage times at 27°C and 44°C significantly (p < 0.05) differed compared to the ones at 5°C. However, there was no difference between the storage times at 27°C and 44°C. In addition, more information on the percentage of the survival for

Table 5.6   Case processing summary

Storage temperature (°C)	Total N	Number of events	Censored N	Percent
5	16	1	15	93.8%
27	16	12	4	25.0%
44	16	14	2	12.5%

Table 5.7   Medians for survival time

Storage temperature (°C)	Median[a]		95% confidence interval	
	Estimate	Std. Error	Lower bound	Upper bound
5	21.00	-	-	-
27	12.00	2.60	6.91	17.09
44	12.00	2.78	6.56	17.44

[a]Estimation was limited to the largest survival time if it was censored.

Table 5.8   Pairwise comparisons

Storage temperature (°C)	1		2		3	
	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
5	-	-	11.86	0.01	13.93	0.00
27	11.86	0.00	-	-	0.23	0.63
44	13.93	0.00	0.23	0.63	-	-

different storage times were accessed by referring to the specific storage time and looking for the associate survival rate. Figure 5.5 shows the survival function S (t) defined as the probability of surviving at least to time (t). The probability of survival of oat bread samples stored at 27°C and 44°C were significantly (p < 0.05) lower than those at 5°C (Figure 5.5). Hence, the loaves stored at 27°C and 44°C deteriorated faster with lower shelf-life. It was confirmed by the hazard function (the conditional probability of deteriorating at time t having survived to that time) of the storage time of oat bread (Figure 5.6) as the risk of deteriorating increased with the temperature.

The log rank was used to test whether there was difference between the survival times of different temperatures but it did not allow other explanatory variables to be taken into account. Cox's proportional hazards model is analogous to a multiple regression model and enables the difference between survival times of the loaves of oat bread kept at different temperatures. In this model, the response (dependent) variable is the 'hazard'. The hazard is the risk (probability) of deteriorating (or experiencing the event in question) given that the bread have survived up to a given point in time, or the risk for deterioration or end of shelf life at that moment.

The Ominibus test of model coefficient (Table 5.9) showed that the model was significant with chi square value of 10.89 and p < 0.05. Table 5.10 provided the p-values and the hazard ratio (Exp(B)) of the variables with temperature at 44°C as the reference. The hazard ratio is the predicted change in the shelf-lfie for a unit increase in temperature. All SE values in Table 5.10 were small, and the problem of multicolinearity was therefore under control (Hoon, 2008). However, the regression coefficient for 27°C did not significantly (p = 0.695) differ when with that at 44°C. The negative signs on the coefficients meant that the hazard (risk of deterioration) was lower and shelf life higher for bread stored at 5°C and 27°C compared to that at 44°C. The results revealed that the p-value was 0.011, 0.695 and 0.039 for bread respectively kept at 5°C, 27°C and 44°C (Table 5.10). The hazard ratio (Exp(B) = 0.071) for bread at 5°C was significantly (p = 0.011) 0.07 times as likely to reach the end of shelf life as that at 44°C. It appeared that bread stored at 5°C reduced the shelf life by 93% [(1 - 0.071) *100)]. That at 27°C was 0.86 times as likely to reach the end of shelf life as that at 44°C (p = 0.695), reducing the shelf life by 14.3%. However, the confidence interval contained 1 and the p > 0.05 indicated that there
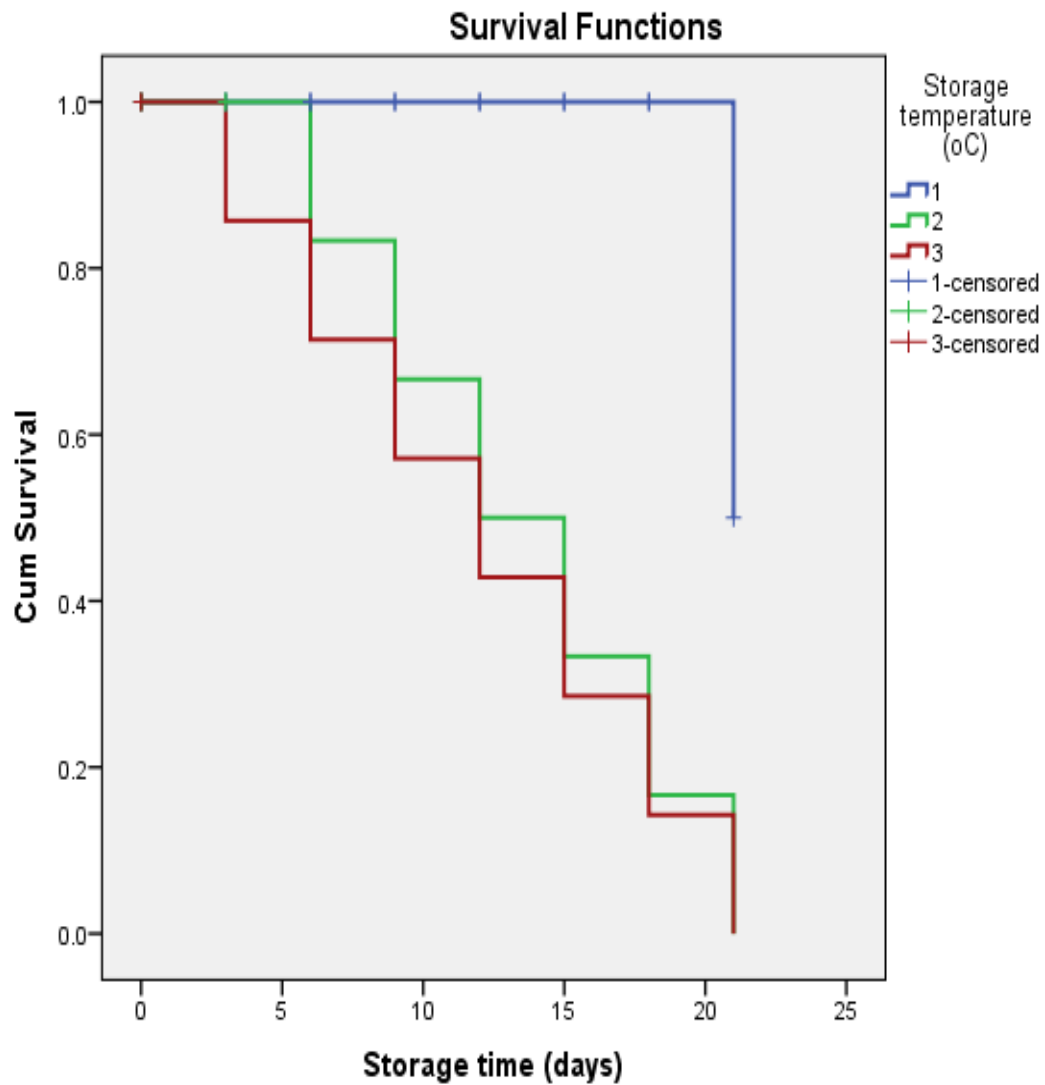
Figure 5.5   Survival function of the storage time of best oat bread[1].

[1]1 = 5°C, 2 = 27°C, 3 = °C
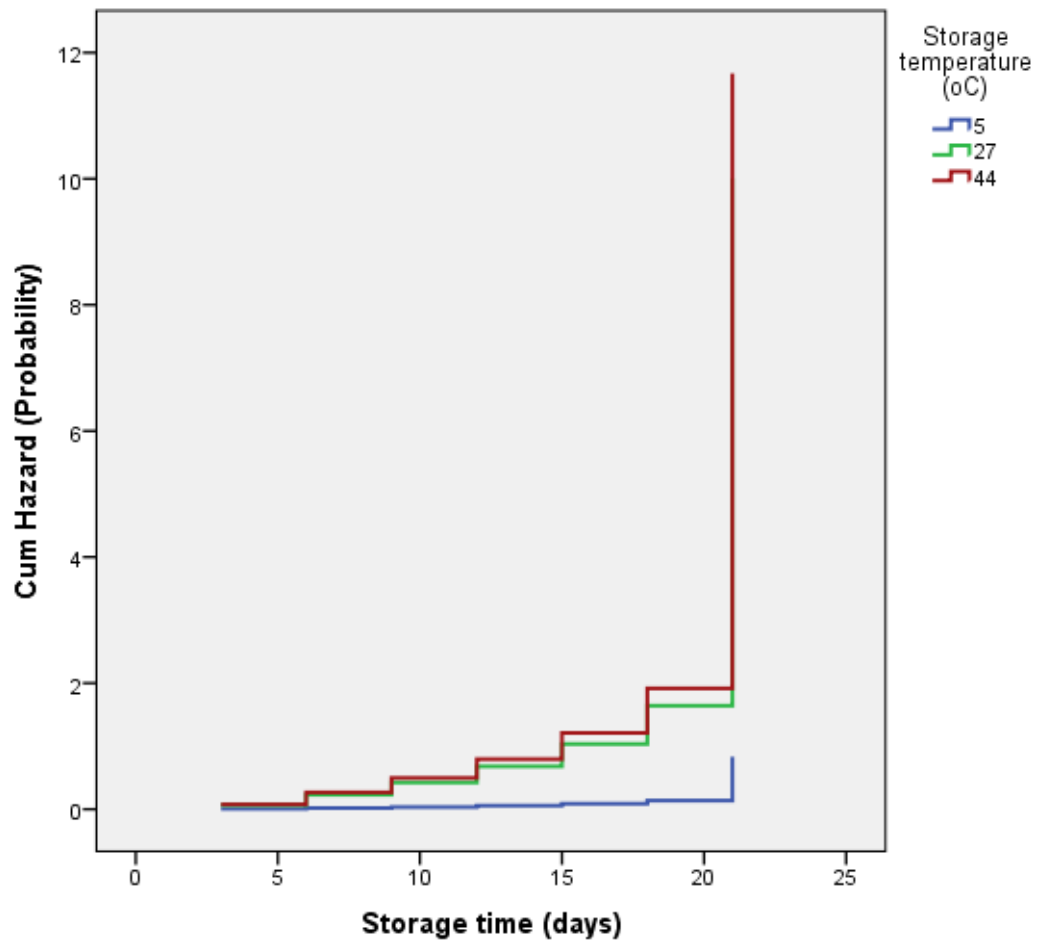
Figure 5.6   Hazard function of the storage time of best oat bread.

Table 5.9   Omnibus tests of model coefficients[1, 2]

-2 Log Likelihood	Overall (score)			Change from previous step			Change from previous block		
	Chi-square	df	Sig.	Chi-square	df	Sig.	Chi-square	df	Sig.
142.29	10.89	2	0.00	14.89	2	0.00	14.88	2	0.00

[1]Beginning Block Number 0, initial Log Likelihood function: -2 Log likelihood: 157.173.
[2]Beginning Block Number 1. Method = Enter

Table 5.10   Variables in the equations

Variable	B	SE	Wald	df	Sig.	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Temperature*			6.50	2	0.039			
Temperature (1)	-2.639	1.04	6.50	1	0.011	0.07	0.01	0.54
Temperature (2)	-0.154	0.39	0.15	1	0.695	0.86	0.40	1.85

*Temperature at 44°C was the reference; 1 = 5°C; 2 = 27°C

was no significant difference in associated risk between the temperatures (27 and 44°C). There are three possibilities for reporting hazard ratio, namely (1) a value of '1' means that there is no differences between two groups in having a shorter time to event; (2) a value of 'more than 1' means that the group of interest is likely to have a shorter time to event as compared to the reference group, and (3) a value of 'less than 1' means that the group of interest is less likely to have a shorter time to event compared to the reference group (Hoon, 2008). The hazard ratios for bread at 5°C, and 27°C were all less than one. Hence, less likely to have shorter shelf-life compared to that at 44°C. Oat bread stored at 5°C resulted to longer shelf life of 21 days, right censored compared to 12 days at 27°C and 44°C. Therefore, the risk of bread deterioration increased with the temperature (Figure 5.6) and consequently reduction in shelf life.

5.4   Conclusion

The aim of this chapter was to determine the effect of psyllium husks and cyclodextrinase on the textural, sensory and shelf-life characteristics of oat bread. The combination of psyllium husks and cyclodextrinase positively improved the textural properties of oat bread. It decreased the hardness and increased the springiness of the optimal oat bread. The sensory evaluation showed that the consumers highly accepted the crumb colour and texture of the best oat bread than the ones of wheat bread. In addition, it was found that there was a strong correlation in crust and crumb colour beween wheat and the best oat bread. However, some differences existed between the wheat and best oat bread. The best oat bread exhibited a less preference in taste than its wheat counterpart. The best oat bread positively received an overall accepatability as wheat bread. The shelf-life studies of the best oat bread revealed that the pH and TVC of the best oat bread were more affected by the storage time, temperature and the interaction of both parameters (time and temperature) than TTA, yeasts and mould. Using survival analysis for the shelf-life studies of the best oat bread, the mathematical model revealed that the risk of deteriorating increased with the temperature. Therefore, oat bread stored at 5°C resulted to longer shelf life of 21 days compared to 12 days at 27°C and 44°C.

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CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

The aim of this study was to evaluate the effect of transglutaminase and cyclodextrinase on the rheological characteristics of oat dough and shelf-life characteristics of oat bread with a view to developing oat bread with improved texture and shelf-life. This objective was achieved by undertaking the following: (1) investigated the effects of yeast, carboxylmethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) and cyclodextrinase (CG) on the mixing, pasting, thermal, quantification of free amino acid groups and protein crosslinking properties of oat dough, with a view to establish the combination for improved mixing properties, (2) investigated the effects of carboxymethycellulose CMC, yoghurt (YG) and transglutaminase (TG) on the physical, textural and colour characteristics of oat bread with a view to optimize the level of these ingredients, (3) established the amount of cyclodextrinase and/or transglutaminase required, for optimal oat bread production using Response Surface Methodology (RSM), (4) investigated the effects of psyllium husks (PH) and cyclodextrinase (CG) on the textural characteristics of the optimal oat bread, (5) established the quality and consumer acceptability of the best oat bread and (6) established the shelf-life of the best oat bread.

The effects of yeast, carboxylmethylcellulose (CMC), plain yoghurt (YG), transglutaminase (TG) and cyclodextrinase (CG) on the mixing, pasting, thermal, quantification of free amino acid groups and protein crosslinking properties of oat dough were investigated through a 2^{5-2} fractional factorial design resolution III with yeast (1.25, 3.25%), CMC (1, 2%), YG (10.75, 33.75%), TG (0.5, 1.5%) and CG (10, 40 µl) as independent variables. Among all the ingredients, only CMC, YG, and TG exhibited significant improvements on the mixing properties of oat dough confirming the enhancement ability of TG on the mixing properties of oat dough (Chapter 1, Section 1.4) while yeast and CG slightly affected it, rejecting the enhancement capacity of CG on the mixing properties of oat dough (Chapter 1, Section 1.4). TG increased water absorption (34.80 to 38.45%) and peak resistance (696.40 - 840.30 FU) of oat dough. However, CG decreased water absorption (38.45 - 34.80%) and peak resistance

(840.30 - 696.40 FU) of oat dough. The optimisation goal was to maximise water absorption and peak resistance while minimising energy at peak and development time. The quantity of ingredients for preparing the optimal oat dough formulation was: yeast (1.25 g), CMC (1 g), yoghurt (20.66 g), TG (1.50 g) and CG (40 µl) with desirability of 0.83. Under this formulation, the model predicted a maximum of water absorption of 37.6%; maximum peak resistance of 816.7 FU; minimum energy at peak of 6.3 (Wh/kg) and minimum development time of 2.3 minutes. Those findings showed that TG and CG improved the mixing properties of oat dough since TG increased the water absorption of oat dough while CG decreased its peak resistance. Yeast and YG showed greater enhancements on the pasting properties of oat dough compared to CMC, TG and CG. The storage modulus of oat dough was slightly increased by adding TG (180.37 - 202.78 kPa) and CG (170.75 - 175.71 kPa). TG decreased the loss modulus (65.95 - 62.87 kPa) of oat dough while CG increased it from 62.01 - 64.61 kPa. As TG and CG did not significantly increased the storage modulus of oat dough, they did not enhance the pasting properties of oat dough and therefore, rejecting their improvement abilities on the pasting properties of oat dough as hypothesized in Chapter 1, Section 1.4 The thermal properties of oat dough were slightly affected by all the ingredients. The denaturation temperature was increased by TG (6.53 - 8.33°C) and CG (6.42 - 8.33°C) but there was a decrease of enthalpy due to addition of TG (from 0.76 to –4.05 J/g) and CG (1.11 to –4.05 J/g), confirming that TG and CG did not improve the thermal properties of oat dough and hence, rejecting the improvement capacity of TG and CG on thermal properties of oat dough as hypothesized in Chapter 1, Section 1.4.. Only CG decreased the number of free amino acid groups (0.94 - 0.62) confirming that it catalysed the protein crosslinking of the oat glutelin while other ingredients increased it. This showed that only CG enhanced the protein modification of oat dough.

As CMC, YG and TG affected the mixing properties of oat dough, oat bread was baked with carboxylmethylcellulose (CMC), yoghurt (YG) and transglutaminase (TG) following a 3^3 Box-Behnken design consisting of CMC (1, 2 g), YG (10.75, 33.75 g) and TG (0.5, 1.5 g) as independent variables. TG decreased the springiness (6.47 - 4.14 mm), specific volume (1.61 - 1.54 ml/g) and increased hardness (537.85 - 692.41 N) of

oat bread. These results confirmed that TG did not improve the physical and textural properties of oat bread. No great change was observed on the colour parameters of crust and crumb of oat bread, confirming that TG slightly enhanced the colour of crust and crumb of oat bread. The optimisation goal was to target hardness at 522.2 N, while minimising chewiness, gumminess and springiness. The predicted optimal ingredients for preparing optimal oat dough were: CMC (1 g), yoghurt (33.75 g) and TG (0.98 g) with a desirability of 0.93. Under this formulation, the model predicts a maximum hardness of 572.35 N; minimum chewiness of 20.33 N; minimum gumminess of 1.21 N and minimum springiness of 5.25 mm. Despite the optimal oat bread exhibited a high desirability, its high hardness and low springiness were due to TG, rejecting the enhancement ability of TG on the rheological (textural profile) of oat bread as hypothesized in Chapter 1, Section 1.4. These high hardness and low springiness were a challenge, the improvement was needed for the manufacture of the best oat bread.

Psyllium husks (PH) and cyclodextrinase (CG) were added in five (05) best oat bread formulations such as (1) PH + CG, (2) CG, (3) TG + CG, (4) TG + PH and (5) TG + PH + CG. Among all the five (5) formulations, only the combination of psyllium husks and cyclodextrinase exhibited a lowest hardness (94.88 N) and the highest springiness (10.97 mm). It was therefore designated as the best oat bread. This best oat bread formulation included oat flour (100 g), salt (2 g), sugar (8.5 g), fat (2.5 g), DATEM (2 g), water (78 g), yeast (2.25 g), YG (33.75 g), CMC (1 g), PH (15 g) and CG (5 µl) and it was further used for sensory and shelf-life studies.

Consumers highly appreciated the crumb colour and texture of the best oat bread than the ones of wheat bread. In addition, it was found that there was a strong correlation in crust and crumb colour beween wheat and the best oat bread. However, some differences existed between the wheat and best oat bread. The best oat bread exhibited a less preference in taste than its wheat counterpart. The best oat bread positively received an overall accepatability as wheat bread.

The shelf-life studies of the best oat bread revealed that the pH and Total Viable Count (TVC) of the best oat bread were more affected by the time, temperature and the interaction of both parameters (time and temperature) than Total Titratable Acidity (TTA), yeasts and mould as the storage time passed. Using survival analysis for the

shelf-life studies of the best oat bread, the mathematical model revealed that the risk of deteriorating increased with the temperature.

The following conclusions can therefore be made from this research project:

1. CMC and YG improved the mixing properties of oat dough by decreasing its peak resistance.

2. TG improved the mixing properties of oat dough since TG increased the water absorption of oat dough.

3. As TG and CG did not significantly increased the storage modulus of oat dough, they did not enhance the pasting properties of oat dough.

4. TG and CG did not improve the thermal properties of oat dough.

5. Only CG enhanced the protein modification of oat dough as it catalysed the protein crosslinks.

6. TG did not improved the physical and textural properties of oat bread.

7. The combination of ingredients psyllium husks and cyclodextrinase positively improved the textural properties of best oat bread.

8. The best oat bread positively received an overall accepatability as wheat bread in terms of consumer acceptability..

9. The best oat bread could safely be stored up to 21 days at refrigeration temperature (5°C) with a TVC load of 10^5 cfu/g.

10. The mathematical model revealed that the risk of deteriorating increased with the temperature.

11. A manuscript written from this study has been accepted for publication in Journal of Food Science and Technology on 19 February 2015:

    ▪ Nitcheu N., P. H., Le Roes-Hill, M. & Jideani, V. A. (2015). Effects of yeast, carboxymethycellulose, yoghurt, transglutaminase and cyclodextrinase on mixing properties of oat dough. *Journal of Food Science and Technology.* DOI: 10.1007/s13197-015-1776-5 (Appendix 16).

12. A manuscript written from this study has been published in Food Science and Technology International:

- Nitcheu N., P. H., Le Roes-Hill, M. & Jideani, V. A. (2014). Advances in gluten-free bread technology. *Food Science and Technology International*, **0 (0)**, 1 - 21. DOI: 10.1177/1082013214531425 (Appendix 17).

13. Conferences attended:

- Nitcheu N., P. H., Le Roes-Hill, M. & Jideani, V. A. (2014). Effects of carboxymethylcellulose, yoghurt and transglutaminase on textural properties of oat bread. In: *Book of abstracts*. IUFOST Conference, 17 - 21 August 2014, Palais de Congres, Montreal, Canada.

- Nitcheu N., P. H., Le Roes-Hill, M. & Jideani, V. A. (2014). Effects of yeast, carboxymethylcellulose, yoghurt, transglutaminase and cyclodextrinase on pasting, thermal and protein modification properties of oat dough. In: *Book of abstracts*. IFT Conference, 22 - 24 June 2014, New Orleans Morial Convention Center, New Orleans, USA.

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As recommendation, the effects of yeast, CMC, yoghurt transglutaminase and cyclodextrinase on pH of oat dough should be investigated.

APPENDICES

Appendix 1 ANOVA for water absorption – Optimisation of oat dough water absorption

Source	Sum of squares	df	Mean square	F-value	p-value
Model	41.00	7	5.86	19.32	< 0.0001
Yeast	0.60	1	0.60	1.98	0.1773
CMC	6.38	1	6.38	21.03	0.0003
YG	24.26	1	24.26	80.00	< 0.0001
TG	7.43	1	7.43	24.49	0.0001
CG	0.86	1	0.86	2.82	0.1113
Yeast * CMC		1	1.16	3.81	0.0676
CMC * CG		1	0.33	1.09	0.3110
Residual	5.15	17	0.30		
Lack of Fit	0.32	1	0.32	1.06	0.3193
Pure Error	4.84	16	0.30		
Cor Total	46.15	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 2 ANOVA for arrival time – Optimisation of oat dough arrival time

Source	Sum of squares	df	Mean square	F-value	p-value
Model	6.36	6	1.06	5.78	0.0017
Yeast	0.12	1	0.12	0.67	0.4245
CMC	0.56	1	0.56	3.07	0.0970
YG	5.06	1	5.06	27.59	< 0.0001
CG	2.500E-003	1	2.500E-003	0.014	0.9084
Yeast * CMC		1	0.36	1.96	0.1783
CMC * CG		1	0.25	1.36	0.2583
Residual	3.30	18	0.18		
Lack of Fit	1.03	2	0.51	3.61	0.0508
Pure Error	2.28	16	0.14		
Cor Total	9.66	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 3 ANOVA for stability – optimisation of Oat dough stability

Source	Sum of squares	df	Mean square	F-value	p-value
Model	369.93	6	61.66	3.97	0.0105
Yeast	12.78	1	12.78	0.82	0.3762
CMC	193.91	1	193.91	12.49	0.0024
YG	133.98	1	133.98	8.63	0.0088
TG	20.03	1	20.03	1.29	0.2709
CG	193.91	1	0.39	0.025	0.8757
CMC * CG		1	8.85	0.57	0.4599
Residual	279.37	18	15.52		
Lack of Fit	140.28	2	70.14	8.07	0.0038
Pure Error	139.09	16	8.69		
Cor Total	649.30	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 4 ANOVA for energy at peak – Optimisation of oat dough energy at peak

Source	Sum of squares	df	Mean square	F-value	p-value
Model	525.31	7	75.04	29.03	< 0.0001
Yeast	10.24	1	10.24	3.96	0.0629
CMC	70.56	1	70.56	27.30	< 0.0001
YG	392.04	1	392.04	151.68	< 0.0001
TG	34.81	1	34.81	13.47	0.0019
CG	1.96	1	1.96	0.76	0.3960
Yeast * CMC		1	7.29	2.82	0.1114
CMC * CG		1	8.41	3.25	0.0890
Residual	43.94	17	2.58		
Lack of Fit	0.96	1	0.96	0.36	0.5583
Pure Error	42.98	16	2.69		
Cor Total	569.25	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 5 ANOVA for peak resistance – Optimisation of oat peak resistance

Source	Sum of squares	df	Mean square	F-value	p-value
Model	64246.02	6	10707.67	22.18	< 0.0001
Yeast	793.83	1	793.83	1.64	0.2160
CMC	9163.28	1	9163.28	18.98	0.0004
YG	38700.73	1	38700.73	80.15	< 0.0001
TG	12359.88	1	12359.88	25.60	< 0.0001
CG	1586.03	1	1586.03	3.28	0.0866
Yeast * CMC	1	1642.28	3.40	0.0817	
Residual	8690.88	18	482.83		
Lack of Fit	1026.89	2	513.45	1.07	0.3657
Pure Error	7663.99	16	479.00		
Cor Total	72936.89	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 6 ANOVA for development time – Optimisation of oat dough development time

Source	Sum of squares	df	Mean square	F-value	p-value
Model	99.10	6	16.52	41.62	< 0.0001
Yeast	0.95	1	0.95	2.40	0.1391
CMC	20.48	1	20.48	51.60	< 0.0001
YG	65.21	1	65.21	164.32	< 0.0001
TG	9.77	1	9.77	24.61	0.0001
Yeast * CMC	1	1.76	4.42	0.0498	
CMC * CG	1	0.95	2.40	0.1391	
Residual	7.14	18	0.40		
Lack of Fit	0.19	2	0.094	0.22	0.8071
Pure Error	6.95	16	0.43		
Correct Total	106.25	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 7 ANOVA for departure time – Optimisation of oat dough departure time

Source	Sum of squares	df	Mean square	F-value	p-value
Model	432.25	5	86.45	5.85	0.0020
Yeast	9.46	1	9.46	0.64	0.4338
CMC	216.83	1	216.83	14.67	0.0011
YG	193.91	1	193.91	13.12	0.0018
CG	0.33	1	0.33	0.022	0.8827
Yeast * CMC		1	11.73	0.79	0.3842
Residual	280.91	19	14.78		
Lack of Fit	135.93	3	45.31	5.00	0.0124
Pure Error	144.98	16	9.06		
Cor Total	713.15	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 8 ANOVA for softening – Optimisation of oat dough softening

Source	Sum of squares	df	Mean square	F-value	p-value
Model	13987.11	7	1998.16	5.20	0.0026
Yeast	116.64	1	116.64	0.30	0.5888
CMC	6496.36	1	6496.36	16.91	0.0007
YG	1705.69	1	1705.69	4.44	0.0503
TG	2495.00	1	2495.00	6.50	0.0208
CG	1043.29	1	1043.29	2.72	0.1177
Yeast * CMC		1	1387.56	3.61	0.0745
CMC * CG		1	742.56	1.93	0.1824
Residual	6530.35	17	384.14		
Lack of Fit	3523.21	1	3523.21	18.75	0.0005
Pure Error	3007.13	16	187.95		
Cor Total	20517.45	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 9 ANOVA for bandwith at peak – Optimisation of oat dough bandwith at peak

Source	Sum of squares	df	Mean square	F-value	p-value
Model	355.34	7	50.76	4.69	0.0044
Yeast	1.10	1	1.10	0.10	0.7536
CMC	47.61	1	47.61	4.40	0.0513
YG	256.00	1	256.00	23.63	0.0001
TG	24.50	1	24.50	2.26	0.1509
CG	7.56	1	7.56	0.70	0.4150
Yeast * CMC	1	2.56	0.24	0.6331	
CMC * CG	1	16.00	1.48	0.2408	
Residual	184.15	17	10.83		
Lack of Fit	0.99	1	0.99	0.087	0.7721
Pure Error	183.16	16	11.45		
Cor Total	539.49	24			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase; CG: Cyclodexrinase

Appendix 10 ANOVA for hardness – Optimisation of oat bread hardness

Source	Sum of squares	df	Mean square	F-value	p-value
Model	2.099E+005	3	69967.94	9.58	0.0001
CMC	66138.98	1	66138.98	9.06	0.0054
YG	1.021E+005	1	1.021E+005	13.98	0.0008
TG	41703.77	1	41703.77	5.71	0.0236
Residual	2.117E+005	29	7301.23		
Lack of Fit	81423.94	9	9047.10	1.39	0.2575
Pure Error	1.303E+005	20	6515.58		
Cor Total	4.216E+005	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 11 ANOVA for chewiness – Optimisation of oat bread chewiness

Source	Sum of squares	df	Mean square	F-value	p-value
Model	581.55	6	96.93	26.76	< 0.0001
CMC	438.28	1	438.28	121.02	< 0.0001
YG	0.84	1	0.84	0.23	0.6341
TG	27.58	1	27.58	7.62	0.0105
CMC^2		1	69.22	19.11	0.0002
YG^2		1	33.13	9.15	0.0055
TG^2		1	12.41	3.43	0.0755
Residual	94.16	26	3.62		
Lack of Fit	16.12	6	2.69	0.69	0.6613
Pure Error	78.04	20	3.90		
Correct Total	675.71	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 12 ANOVA for cohesion energy – Optimisation of oat bread cohesion energy

Source	Sum of squares	df	Mean square	F-value	p-value
Model	0.041	6	6.895E-003	31.70	< 0.0001
CMC	0.028	1	0.028	129.91	< 0.0001
YG	1.375E-004	1	1.375 E-004	0.63	0.4337
TG	3.647E-003	1	3.647E-003	16.77	0.0004
CMC^2		1	6.825E-003	31.38	< 0.0001
YG^2		1	1.686E-003	7.75	0.0099
TG^2		1	8.067E-003	3.71	0.0651
Residual	5.655E-003	26	2.175E-004		
Lack of Fit	1.509E-003	6	2.514E-004	1.21	0.3406
Pure Error	4.147E-003	20	2.073E-004		
Correct Total	0.047	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 12 ANOVA for gumminess – Optimisation of oat bread gumminess

Source	Sum of squares	df	Mean square	F-value	p-value
Model	33.92	6	5.65	26.36	< 0.0001
CMC	25.54	1	25.54	119.05	< 0.0001
YG	0.088	1	0.088	0.41	0.5271
TG	1.42	1	1.42	6.60	0.0163
CMC^2	1	4.28	19.97	0.0001	
YG^2	1	1.91	8.90	0.0061	
TG^2	1	0.67	3.13	0.0887	
Residual	5.58	26	0.21		
Lack of Fit	0.81	6	0.14	0.57	0.7513
Pure Error	4.77	20	0.24		
Cor Total	39.50	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 13 ANOVA for springiness – Optimisation of oat bread springiness

Source	Sum of squares	df	Mean square	F-value	p-value
Model	196.67	6	32.78	18.10	< 0.0001
CMC	160.27	1	160.27	0.25	< 0.0001
YG	0.45	1	0.45	9.40	0.6225
TG	17.01	1	17.01	0.0040	0.0050
CMC^2	1	18.10	9.99	0.6568	
YG^2	1	0.37	0.20	0.6086	
TG^2	1	0.49	0.27		
Residual	47.09	26	1.81		
Lack of Fit	11.48	6	1.91	1.07	0.4103
Pure Error	35.61	20	1.78		
Correct Total	243.76	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 14 ANOVA for springiness – Optimisation of oat bread springiness

Source	Sum of squares	df	Mean square	F-value	p-value
Model	2.499E-003	5	4.998E-004	44.73	< 0.0001
CMC	1.240E-003	1	1.240E-003	110.98	< 0.0001
YG	3.950E-005	1	3.950E-005	3.54	0.0709
TG	1.868E-004	1	1.868E-004	16.72	0.0003
CMC^2		1	7.861E-004	70.36	< 0.0001
YG^2		1	2.211E-004	19.79	0.0001
Residual	3.016E-004	27	1.117E-005		
Lack of Fit	1.325E-004	7	1.893E-005	2.24	0.0746
Pure Error	1.691E-004	20	8.456E-006		
Correct Total	2.801E-003	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 15 ANOVA for specific volume – Optimisation of oat bread specific volume

Source	Sum of squares	df	Mean square	F-value	p-value
Model	0.17	6	0.029	3.81	0.0074
CMC	4.692E-003	1	4.692E-003	0.62	0.4389
YG	0.027	1	0.027	3.59	0.0695
TG	0.051	1	0.051	6.67	0.0158
CMC^2		1	1.192E-003	0.16	0.6952
YG^2		1	0.050	6.59	0.0164
TG^2		1	0.042	5.58	0.0259
Residual	0.20	26	7.594E-003		
Lack of Fit	0.069	6	0.012	1.81	0.1486
Pure Error	0.13	20	6.401E-003		
Cor Total	0.37	32			

CMC: Carboxymethycellulose; YG: Plain yoghurt; TG: Transglutaminase

Appendix 16 Acceptance letter

From Pichan Prabhasankar

To me

19 February 2015

CC: bhask.jfst@gmail.com

Ref.: Ms. No. JFST-D-14-00830R2

Effects of yeast, carboxymethylcellulose, yoghurt, transglutaminase and cyclodextrinase on mixing properties of oat dough

Journal of Food Science and Technology

Dear Mr. Nitcheu Ngemakwe,

I am pleased to tell you that your work has now been accepted for publication in Journal of Food Science and Technology.

You will soon receive an e-mail from Springer production team. Please respond to this e-mail in the timely manner to help us process your manuscript to the next stage. Please note that the article will be processed and the proofs be sent to you only after we receive your responses to this e-mail. If you have any queries on the same please contact the editor.

Thank you for submitting your work to this journal.

With kind regards

Pichan Prabhasankar, M Sc, Ph D

Editor-in-Chief

Journal of Food Science and Technology

Appendix 17

Journal Article Published in

Food Science & Technology International