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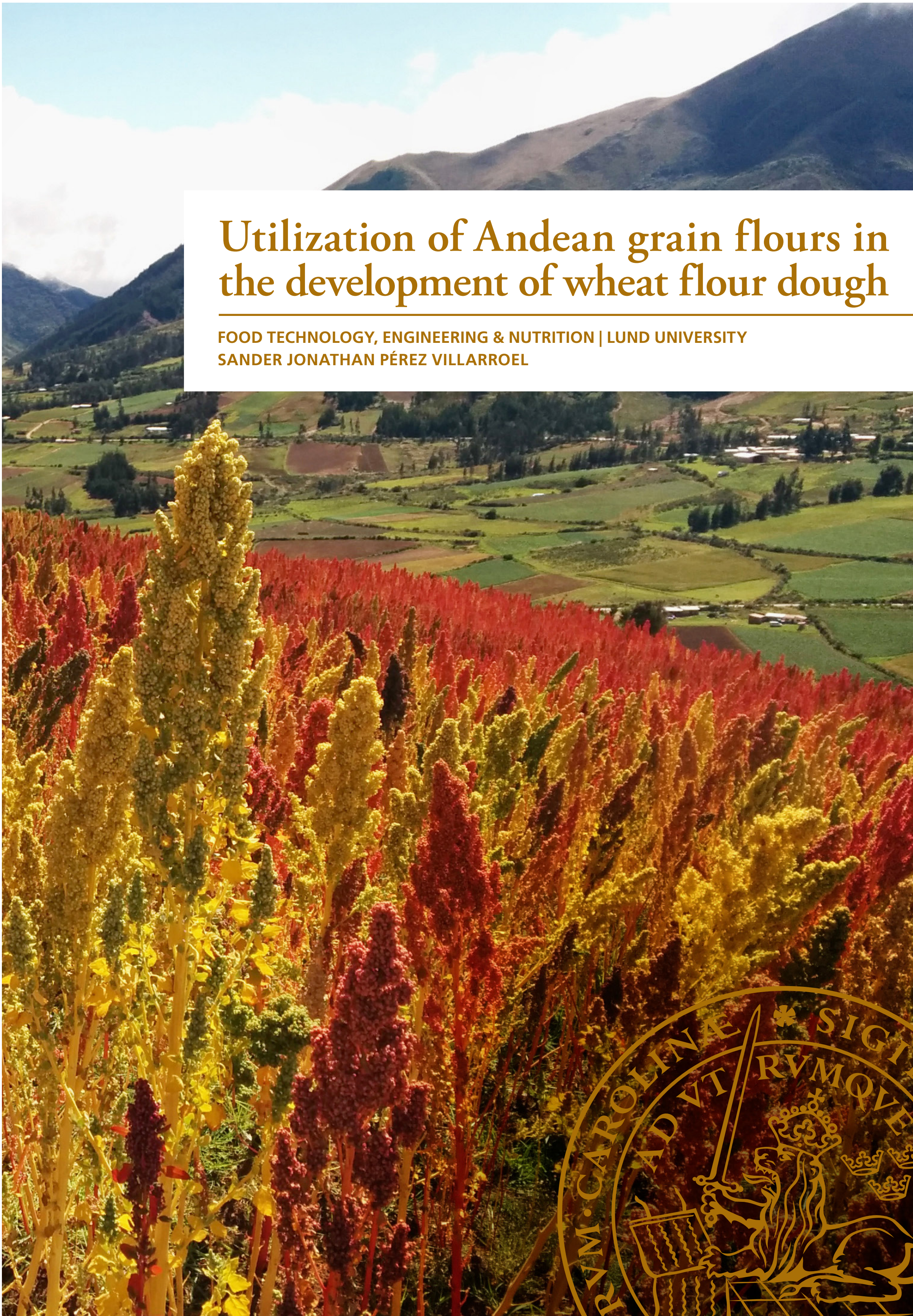

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FOOD TECHNOLOGY, ENGINEERING & NUTRITION | LUND UNIVERSITY

SANDER JONATHAN PÉREZ VILLARROEL

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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The thesis presents the study of the separation of the main components of dough based on thermodynamic incompatibility and ultracentrifugation, and thereby dough structures and properties. Properties were evaluated by means of light microscopy, ultracentrifugation (including relative volume fraction of the separated phases), and differential scanning calorimetry (DSC for water and thermal properties of each sample and its separated phases). The separation of dough showed direct information about the relative amount of each phase in dough, and the volume fraction of all of them, including liquid, gel, gluten, and starch phases. These were found to be characteristic for the different samples of flour and highly affected by flour combinations. The freezable water of each phase had high impact on the freezable water of the dough. The gluten phase dominates the melting behaviour, and therefore the water and freezable water properties. The addition of Andean grain flours modified the phase separation, water and thermal properties. At a level of 25% of substitution, doughs gave four separated phases as for pure wheat dough, but with different volume fractions highly affected by the type of Andean grain flour. At 50% of substitution, the phase separation was substantially affected. Pure Andean grain flour dough was characterized using the same approach, resulting in eight separated phases for amaranth, nine phases for quinoa, as well as four phases for canahua, where the separation was not complete.

The second part of the thesis deals with textural analysis combined with multivariate analysis. The symbiosis of textural and multivariate analysis gave the increased understanding of dough rheology. The selected methods were D/R dough inflation system, Kieffer extensibility rig, Hoseney/Chen stickiness cell, the textural profile analysis (TPA), and the A/DP dough preparation set. All of these methods are based on different kinds of deformation, namely, biaxial extension, uniaxial extension, unlubricated compression, and penetration, which gave in total 26 parameters relevant for breadmaking, such as hardness, strength, extensibility, elasticity, and stickiness. The textural properties described by the methods were different. For example, extensibility was better described by uniaxial extensibility, unlike stickiness and hardness whose results were fully independent. A partial least squares (PLS) model revealed that both uniaxial and biaxial extensional methods are the most robust when dough matrix is modified under the conditions analysed.

Finally, the thesis deals with the optimization of the use of the three Andean grains flours combined together as partial replacement for wheat flour at a level of 25%. The results showed that the combination of these can avoid impairment of crumb texture and specific volume.

Key words
Wheat, Andean grains, Amaranth, *Amaranthus caudatus*, Canahua, *Chenopodium pallidicaule*, Quinoa, *Chenopodium quinoa*, Dough, Substitution, Ultracentrifugation, Phase separation, Dough composition; Macroscopic separation, Freezable water, Textural properties, Compression, Uniaxial tension, Biaxial tension, Principal component analysis, Partial least square regression, Bread

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MADE IN SWEDEN

To my God, my family and my beloved grandparents

Abstract

Bread dough was studied in relation to three Andean grain flours, quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus caudatus*) and canahua (*Chenopodium pallidicaule*), as partial replacements for wheat flour with the perspectives of dough and breadmaking. This thesis deals with the understanding of both dough as a complex system, consisting of various water-filled polymers, and dough as a viscoelastic material, with different responses to deformations, in order to contribute to possibilities for improvements in dough analysis and development of novel products.

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Finally, the thesis deals with the optimization of the use of the three Andean grains flours combined together as partial replacement for wheat flour at a level of 25%. The results showed that the combination of these can avoid impairment of crumb texture and specific volume.

Popular Scientific Summary

Breadmaking has evolved over centuries through deliberate experimentation by mixing water and different crop flours, mainly wheat. The bread made from wheat flour was the staple food for Egyptians, Romans and many old civilizations in the Middle East and Europe.

While wheat was the significant grain for both Europe and the Middle East, quinoa was the main grain for South America. For centuries, quinoa and its relative, canahua, were the staple food for average Andean people, while amaranth, another Andean grain, was said to be reserved only for the Inca (King). Sadly, all these grains were banned during the conquest of America as the Spaniards did not like their taste at all. Moreover, the Spaniards were disgusted that Andean people revered these grains as sacred and regarded them as pagan. Peanuts and potatoes were instead taken to Europe and around the world. Even so, Andean people still preserve these grains as a food even to this day.

The United Nations General Assembly declared 2013 as the "International Year of Quinoa" in recognition of the ancestral practices of the Andean people, who have preserved it as a food for present and future generations, through knowledge and practices of living in harmony with nature. The objective was to draw the world's attention to the role that quinoa could play in providing food security, nutrition and poverty eradication in support of achieving the Millennium Development Goals.

Andean grains are very nutritious. They contain all 20 amino acids, particularly lysine, an essential amino acid that the human body cannot produce and is scarce in wheat flour. They are rich in minerals and vitamins. They also contain lipids, and fatty acids, particularly omega-3. They are a non-allergenic and also diabetic-friendly food, especially canahua.

Andean grains are highly resistant to drought, frost, and saline soils. They are highly efficient in photosynthesis, fast-growing, and do not require much care. Basically, they easily adjust to soil and environmental conditions in which very few other species can survive. NASA identified quinoa as an ideal candidate for the Controlled Ecological Life Support System (CELSS), i.e. for long-term human space missions, because of its resistance, high protein quality and desirable amino acid composition, as well as the ease with which it can be prepared and combined with other crops.

The partial substitution of wheat flour with other kind of flours could bring nutritional improvements. However, the substitution of wheat flour could also reduce some bread properties like volume and softness. Nevertheless, consumers want to enjoy a greater wealth of healthier food products nowadays.

The aim of this PhD work was to investigate different aspects of the partial substitution of wheat flour with flours from the Andean crops mention above. The results present new methodology for characterizing wheat flour properties and their change when Andean grain flours are blended with wheat flours. Textural analysis of wheat dough is crucial for evaluating dough quality and predicting breadmaking, so under this premise, the results also present the uncertainty of textural properties analysis when dough is formed from blended flours. The thesis includes optimization of the use of wheat and Andean grain flour to show that it is possible to optimize the concentration of amaranth, canahua and quinoa to preserve and enhance the volume and softness of wheat pan bread so that the desirable properties of pure wheat flour bread can still be enjoyed. And quinoa, canahua and amaranth may even be involved in the conquest of space during this century.

Resumen científico popular

La panificación ha evolucionado a lo largo de los siglos por medio de la experimentación deliberada al mezclar agua y harina de diferentes cultivos, principalmente trigo. El pan hecho de harina de trigo era el alimento básico para egipcios, romanos y muchas civilizaciones antiguas en el Medio Oriente y Europa.

Mientras que el trigo era el grano significativo tanto para Europa como para el Medio Oriente, la quinua era el grano principal para América del Sur. Durante siglos, la quinua y su pariente, la cañahua, fueron el alimento básico para la gente andina común, mientras que el amaranto, otro grano andino, se decía que estaba reservado solo para el Inca (Rey). Lamentablemente, todos estos granos fueron prohibidos durante la conquista de América ya que a los españoles no les gustó en absoluto. Además, los españoles estaban disgustados porque los pueblos andinos veneraban estos granos como sagrados y por lo tanto los consideraron paganos. En cambio, los maníes y las papas fueron llevados a Europa y al mundo. Aun así, los pueblos andinos aún conservan estos granos como alimento incluso hasta el día de hoy.

La Asamblea General de las Naciones Unidas declaró el 2013 como el "Año Internacional de la Quinua" en reconocimiento de las prácticas tradicionales de los pueblos andinos, que lo han preservado como alimento para generaciones presentes y futuras, a través del conocimiento y las prácticas de vivir en armonía con la naturaleza. El objetivo era llamar la atención del mundo al papel que la quinua podría desempeñar en la seguridad alimentaria, nutrición y erradicación de la pobreza en apoyo para lograr las Objetivos de Desarrollo del Milenio.

Los granos andinos son muy nutritivos. Contienen los 20 aminoácidos, particularmente la lisina, un aminoácido esencial que el cuerpo humano no puede producir y escasea en la harina de trigo. Son ricos en minerales y vitaminas. También contienen lípidos y ácidos grasos, particularmente omega-3. Son un alimento no alergénico y también apto para diabéticos, especialmente la cañahua.

Los granos andinos son altamente resistentes a la sequía, las heladas y los suelos salinos. Son altamente eficientes en la fotosíntesis, de rápido crecimiento y no requieren mucho cuidado. Básicamente, se adaptan fácilmente a las condiciones del suelo y del medio ambiente en las que muy pocas especies pueden sobrevivir. La NASA identificó a la quinua como un candidato ideal para el Sistema de soporte de vida ecológica controlada (CELSS), es decir, para misiones espaciales humanas de larga duración, debido a su resistencia, alta calidad de proteínas y composición deseable de aminoácidos, así como la facilidad con la que puede ser preparada y combinada con otros cultivos.

La sustitución parcial de la harina de trigo con otro tipo de harinas podría traer mejoras nutricionales. Sin embargo, la sustitución de la harina de trigo también podría reducir algunas propiedades del pan, como el volumen y la suavidad. Sin embargo, hoy en día los consumidores desean disfrutar de una mayor riqueza de productos alimenticios más saludables.

El objetivo de este trabajo de doctorado fue investigar diferentes aspectos de la sustitución parcial de la harina de trigo con harinas de los cultivos andinos mencionados anteriormente. Los resultados presentan una nueva metodología para caracterizar las propiedades de la harina de trigo y su cambio cuando las harinas de granos andinos se mezclan con harinas de trigo. El análisis de textura de la masa de trigo es crucial para evaluar la calidad de la masa y predecir la fabricación de pan, por lo que, bajo esta premisa, los resultados también presentan la incertidumbre del análisis de las propiedades de textura cuando la masa se forma a partir de harinas mezcladas. La tesis incluye la optimización del uso de harina de trigo y granos andinos para demostrar que es posible optimizar la concentración de amaranto, canahua y quinua para preservar y mejorar el volumen y la suavidad del pan de trigo para que las propiedades deseables de la harina de trigo puro aún se puedan disfrutar. Y quizás durante este siglo la quinua, la cañahua y el amaranto puedan estar involucrados en la conquista del espacio.

List of publications

The thesis is based on the following papers, which will be referenced in the text through use of their Roman numeral. The papers are appended at the end of the thesis.

Paper I: Pérez, S., Rojas, C., Eliasson, A-C., Sjöö, M. (2019) Water and thermal properties characterized in phases of wheat flour dough during thawing and baking conditions, Advances in Food Science and Engineering, vol. 3, no 3. To be released in September 2019

Paper II: Pérez, S., Rojas, C., Eliasson, A-C., Sjöö, M. (2018) Phase separation, water and thermal properties of Andean grain flours and their effect on wheat flour dough, Journal of Food Processing & Technology, vol. 10, no:2. doi: 10.4172/2157-7110.1000779

Paper III: Pérez, S., Sjöö, M., Rojas, C., Purhagen J. Methods for analysing textural properties of wheat flour dough, Manuscript submitted to Journal of Food Process Engineering

Paper IV: Pérez, S., Sjöö, M., Rojas, C., Purhagen J. Texture analysis of wheat flour dough substituted with Andean grains flour. A chemometric approach, Manuscript submitted to Journal of Food Processing and Preservation

Paper V: Pérez, S., Rojas, C., Antezana, R., Portanta, V., Jimenez, N., Purhagen J., Sjöö, M A breadmaking study for optimizing the use of blends of flours from wheat and Andean grains. Manuscript

Author’s contribution to the publications

Paper I: Sander Perez planned the study together with the co-authors. Sander Perez performed the experimental work. Sander Perez and co-authors analysed the results. Sander Perez wrote the first draft of the paper and corrected it together with the co-authors.

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Paper III: Sander Perez planned the experimental design together with the co-authors. Sander Perez performed all the experimental work. Sander Perez and co-authors analysed the results. Sander Perez wrote the major part of the paper.

Paper IV: Sander Perez planned the experimental design together with the co-authors. Sander Perez performed all the experimental work. Sander Perez and co-authors analysed the results. Sander Perez wrote the major part of the paper.

Paper V: Sander Perez planned the study together with the co-authors. Sander Perez and co-authors analysed the results. Sander Perez wrote the major part of the paper.

Abbreviation and symbols

A	Amaranth flour
A-25	Amaranth 25%
A-50	Amaranth 50%
AD	Anno Domini
AX	Arabinoxylans
BC	Before Christ
C	Canahua flour
C-25	Canahua 25%
C-50	Canahua 50%
CELSS	Controlled Ecological Life Support System
d	Diameter of particle
DIS	Dough Inflation System
DSC	Differential scanning calorimetry
f	Force developed on extensional flow
F1	Swedish wheat sample
F2	Bolivian wheat sample 1
F3	Bolivian wheat sample 2
FAO	Food and Agriculture Organization of the United Nations
f_e	Internal energy component
f_s	Entropic component
FW	Freezable water
g	Relative centrifugal force
HMW-GS	High molecular weight glutenin subunits

k_B	Boltzmann constant
LMW-GS	Low molecular weight glutenin subunits
M	Mean
MASL	Metres above sea level
NASA	National Aeronautics and Space Administration
NSP	Non-starch polysaccharides
PCA	Principal component analysis
PLS	Partial least squares
Q	Quinoa flour
Q-25	Quinoa 25%
Q-50	Quinoa 50%
R	Rotor radius
r_n	Relative length of the macromolecules
S	Sedimentation coefficient
SD	Standard deviation
SMS	Stable Micro Systems
To	Melting of ice
Tp	Peak temperature
TPA	Texture profile analysis
UFW	Unfreezable water
V	Velocity of particle
VF	Volume fraction
w	Rotor speed
W	Wheat
WC	Water content
WEAX	Water-extractable arabinoxylans
WHO	World Health Organization
WUAX	Water-unextractable arabinoxylans
$\Delta H_{a\text{-}l}$	Amilose lipid melting enthalpy

ΔH_g	Gelatinization enthalpy
ΔS_M	Combinatorial entropy of the possible arrangement of the macromolecules
ΔT	Temperature range
μ	Dynamic viscosity of the medium
ρ_l	Density of the medium
ρ_P	Density of particle
ϕ_n	Volume fraction of macromolecule

Table of Contents

1 Introduction

Among Andean peoples, quinoa (*Chenopodium quinoa*) has been treated in ritual ceremonies, highly respected and known as “chisaya mama” or “mother of all grains” (1). Determined to destroy Andean culture, in 1532 Inca Empire conqueror Francisco Pizarro and his 158 men suppressed traditions, banned the production of quinoa and destroyed the intricate agricultural system (1-5). But, quinoa survived hidden away at high altitude at the edges of salt lakes, at the Andean Plateau and mountains, where the Spaniard would not find it, and the native people continued to cultivate quinoa. Nearly 480 years after that, the United Nations General Assembly declared 2013 as the “International Year of Quinoa” in recognition of ancestral practices of the Andean people, who have managed to preserve quinoa in its natural state as food for both present and future generations (6).

Nowadays, the Food and Agriculture Organization of the United Nations (FAO) has been dynamically involved in supporting the cultivation of quinoa in countries outside the Andean region with the aim of strengthening food and nutrition security (7). Like quinoa, other disregarded food plants are being rediscovered in the world. Quinoa and other relatives that followed the same path, such as amaranth or kiwicha (*Amaranthus caudatus*) and canahua or kañiwa (*Chenopodium pallidicaule*), are now paid attention owing to their remarkable resistance to abiotic stresses, their nutrition qualities, and their technological attributes.

With this in mind, this thesis aimed to contribute to the understanding of the use of quinoa, amaranth and canahua as whole grain flours in combination with wheat, and the effect on dough procedures for making bread.

In order to understand the structure and the function of dough, methods were combined to study the structure, phase separation, water, and thermal properties of wheat dough by using ultracentrifugation, microscopy and differential scanning calorimetry (DSC) (Paper I).

These methods were subsequently applied, for the first time, to study the corresponding properties of dough made from Andean grains, and further to understand their effect upon addition to wheat dough properties (Paper II).

To understand rheological methods, more specifically, texture analysis methods, for the evaluation and characterization of dough and how these methods may be related to each other, wheat flour was analysed as a model system (Paper III).

Since these methods were generally developed for wheat dough characterization, a further objective was to better understand which of these texture analysis methods was more robust and suitable to analyse the rheological properties of dough when Andean grain flours have been added (Paper IV).

Finally, the effect on final bread properties was evaluated by studying the use of flours from amaranth, canahua and quinoa to optimize proportions of them when used in combination in order to obtain a partially substituted wheat bread with the volume and crumb texture corresponding to a pure wheat bread (Paper V).

2 Andean grains and wheat

2.1 Origin and adaptability of Andean grains

The progress of old societies was possible due to the development of agriculture, especially wheat and other grains, and the technology of bread production. The large-scale production of leavened bread in the Egyptians and Romans societies is still surprising. Wheat has been adapted to a wide range of environments in all continents except Antarctica. Wheat culture was introduced to the Americas in 1529 by Spanish colonists in Mexico (8). Wheat is currently the most important crop in the world and it is highly appreciated owing to its ability to form dough, which is transformed into noodles, pasta, and bread.

For thousands of years, quinoa was domesticated in the Andean regions, mainly Bolivia and Peru. Like the potato and peanuts, quinoa was one of the main foods of the Andean peoples (9), known as the “mother grain” owing to its importance for their civilization. Quinoa is a granifer species belonging to the *Amaranthaceae* family (the same as spinach and beetroot), but possesses cereal characteristics (*Poaceae* family), resulting in it being designated as a “pseudo-cereal”(10). Quinoa, amaranth and canahua have been underutilized and neglected for many years, and mainly used as traditional crops by farmers on a local scale. Quinoa was prevalently produced in Bolivia and Peru, but there have been small productions in other Andean countries like Ecuador, Chile, Argentina, and Colombia. Bolivia, Peru, and Ecuador increased their production of quinoa by approximately 300% from 1980 to 2011, with the largest increase (from ca. 9 to 38 metric tons) in Bolivia, followed by Peru (11). Until the early 1980s, the production of quinoa was specific to the Andean countries. There are about 60 species of *Amaranthus* spread around the world in tropical, subtropical and temperate regions. Amaranth is native to Peru and other Andean countries (12, 13). Canahua is a relative to quinoa and also has its origins in the Andes of Peru and Bolivia. It is reported to be the plant most resistant to cold climates.

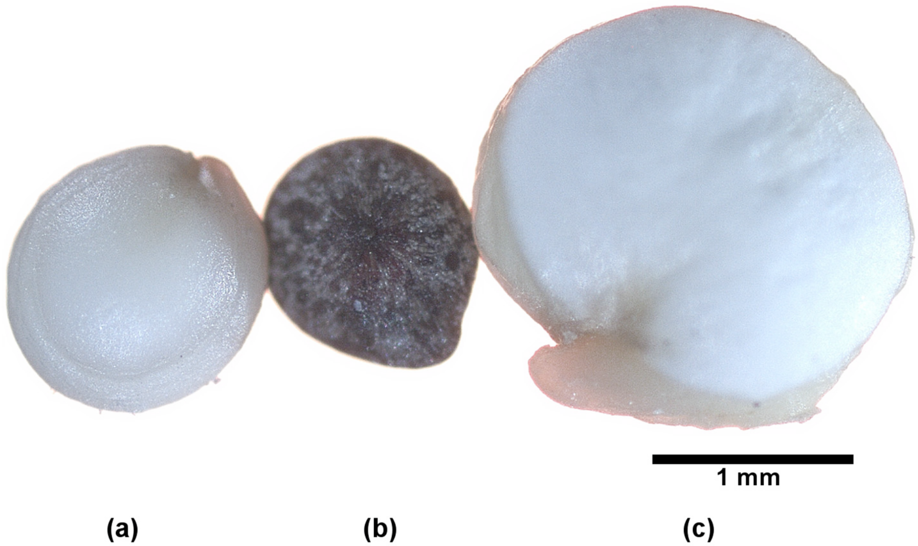

Figure 1: (a) Amaranth; (b) Canahua; (c) Quinoa

Nowadays, the conditions for conventional crop production are rapidly deteriorating as a result of climate change. The FAO has therefore given special recognition to an ancient grain like quinoa, which has been designated as a good candidate to offer food security in the face of the predicted future world scenario of increasing salinization and aridity which are affecting the conditions under which crops can be grown as a result of global climate change (14, 15). It is also predicted that the human population will reach nine billion within the next few decades. To keep pace with this growth, it is urgent for food production to increase, even though there is limited cultivable land and water (16). Another important aspect is the affordable costs of sustainable diets that can provide nutritious foods with low impact on the environment (17).

First of all, Andean grains are a stress-tolerant species and represent new opportunities for farmers in a drier climate (16, 18, 19). Quinoa, like amaranth, has a vast genetic diversity resulting from its fragmented and localized production over the centuries in the Andes, from sea level to the highland (at more than 4,000 MASL). Quinoa can be adapted to various agroecological conditions worldwide (9). USA and Canada have been producing quinoa since the 1980s. Brazil has been using quinoa to improve the soil organic matter (20). In Denmark and the Netherlands, quinoa has been improved through breeding with Chilean and Peruvian lines with potential for drought and salt stress conditions. The breeding of quinoa, especially in Europe and North America, is concentrated on decreasing the time to maturity (around 150 days) and reducing the content of saponins (21-

26). The cultivation of quinoa has reached other countries like Sweden, Italy, UK, France, Tibet, Morocco, India and China (4, 27, 28).

After the "International Year of Quinoa" (2013), the FAO was actively involved in promoting and evaluating the adaptability of selected quinoa genotypes under different environments outside the Andean region, with the aim of strengthening food security and nutrition (4, 18). Today, quinoa is cultivated or tested in around a hundred countries, and this expansion is continuing (4).

Amaranth species has been garnered attention since the 1950s, but canahua is much less known in the world and even in the Andean countries. There are more than 60 species of *Amaranthus*, which are grown in tropical regions of Africa, South, and North America and Southeast Asia (especially in India). The three major species are annual herbaceous plants domesticated in ancient times, *A. cruentus* was cultivated over 4000 years BC in Mexico, and *A. hypochondiracus* was grown about 500 years AD. There is an archaeological record of *A. caudatus* found in the north of Argentina dating back 2,000 years. Nowadays, there are about 21 germplasm collections worldwide, the most important in America, China and India (29, 30). Efforts have been made to improve amaranth production, e.g. Kenya and Tanzania are developing and promoting new varieties (31). Amaranth has also been considered as a substrate for biodiesel production (32). No detailed official sources of volumes produced were found, but there has been a notable increase in the category of "other non-milled cereals" in the world exports in recent years, which is the category amaranth falls under. Peru and Bolivia remain as the main exporting countries, and Germany, France, Lithuania, Poland and China have been the most important consumer markets (33).

Very recently, the National Aeronautics and Space Administration (NASA) has looked at canahua for the diet of astronauts (34, 35). Nowadays, canahua is mainly produced in Peru and Bolivia.

All of this effort to introduce Andean grains to other regions is also lead by their beneficial nutritional properties.

2.2 Nutritional properties

The chemical composition of Andean grains and wheat can be seen in Table 1.The nutritional value of any food is substantially determined by its protein quality, which depends primarily on its amino acid content, digestibility, influence of anti-nutritients, and the tryptophan to a large neutral amino acids ratio.

Carbohydrates have a nutritional function with different physiological health effects, such as provision of energy, effects on satiety, control of blood glucose and insulin metabolism, protein glycosylation, cholesterol and triglyceride metabolism (36).

Table 1: Composition of wheat and Andean grains (g/100g dry weight).

	Wheat (37)	***Amaranth (38)***	***Cañahua/Kañiwa(39)***	***Quinoa (40)***
Protein	14.3	14.5	19	16.1- 17.3
Carbohydrates	78.4	71.5	67.7	75.8-73.6
Lipids	2.3	6.4	10.6	5.3- 5.7
Dietary fibre	2.	5.0	5.5	9.6-11.4
Ash	2.2	2.6	2.7	2.9- 3.5
Fe (mg/100g) (41)		5.00	4.91	2.95
Zn (mg/100g) (41)		1.25	2.15	2.95
Ca (mg/100g) (41)		27.90	29.76	68.55

Lipids provide the human body with essential fatty acids, such as linoleic (omega-6) and linolenic (omega-3), which are precursors to other substances involved in inflammatory responses and cardiovascular health. Andean grain oils have been reported to be rich in unsaturated fatty acid, especially linolenic acid (37, 38, 42). Table 2 describes the content of the main unsaturated fatty acids in wheat and Andean grain oils.

Table 2: Composition of the main unsaturated fatty acid in wheat and Andean grain (% of total fatty acid).

	Wheat (43)	***Amaranth (42)***	***Canahua (38)***	***Quinoa (37)***
Linoleic acid (omega-6)	54.2%	38.2%	42.6%	53.1%
Linolenic acid (omega 3)	3.5%	1.0%	6.0%	6.2%
Oleic acid (omega-9)	22.3%	33.3%	23.5%	23.3%

Nutritional properties are also characterized by micronutrients, including vitamins and minerals, which are important for the correct development of bones and immune system. Important minerals include calcium and zinc. Andean grains are known to contain anti-nutritional factors, such as phytate and oxalate. The total mineral content in Andean grains is higher than in conventional grains (Table 1) (41, 44), but their absorption can be reduced by the anti-nutritional factors.

2.2.1 Protein

Traditionally, the Osborne fractionation procedure has been used to classify cereal proteins according to their solubility as either albumins (soluble in distilled water), globulins (soluble in dilute salt solution), prolamins (soluble in 70% aqueous ethanol), and glutelins (soluble in dilute acid) (45). Each class of proteins is made up of heterogeneous groups of proteins.

In wheat, the first two fractions, albumins and globulins, make up about 20% of the total proteins. Their concentration in the starchy endosperm is relatively low, but is high in the germ, bran and aleurone layer. They are metabolic proteins, i.e., enzymes or enzyme inhibitors, such as α- and β-amylases, proteases, lipases, lipooxygenases and phosphatases. In contrast, prolamins and glutelins are located only in the starchy endosperm with the biological function of supplying the seedling with nitrogen and amino acids during germination. Wheat prolamins and glutelins are called gliadins and glutenin, respectively. Gliadins are monomeric proteins, relatively small, without cross-links between gliadin proteins, i.e. gliadins are single-chains, but intra-molecular disulphide bonds are present. Their molecular weights are in the range of 30,000 to 100,000 Da. Glutenin is a polymeric protein with intermolecular disulphide bonds between the two basic glutenin subunits, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). Their molecular weights are in the range of 80,000 to 120,000 Da, and of 40,000 to 55,000 Da, respectively. Both gliadin and glutenin are the main component of gluten.

But the ingestion of gluten causes injuries of intestinal mucosa to certain genetically-predisposed individuals. This sensitivity to gluten is diagnosed as Celiac disease. So far, the only therapy for this disease is to avoid products that contain gluten (46, 47). Andean grain flours are a good option for patients with celiac disease since Andean grains are free of gluten. The average protein content reported for quinoa is 12–23% (38, 48, 49), while amaranth grain contains about 13-17% (50, 51), and canahua about 18% (52). With an amino acid profile close to the optimum suggested by FAO/WHO (53, 54) and with their high lysine content, they are ideal for combination with cereals (19, 55, 56). The use of Andean grains in blends with cereals was reported to improve protein quality (50). The first limiting amino acid in amaranth is leucine (57, 58). It has been reported that raw seeds of various Andean grains caused poor growth in a rat feeding study (59), but processing like cooking has been shown to improve the protein properties. Albumins and globulins are the major fractions of Andean grain protein (38, 51, 52).

2.2.2 Carbohydrates

Carbohydrates can be classified according to their degree of polymerization into three principal groups: sugars (monosaccharides, disaccharides, polyols), oligosaccharides, and polysaccharides (starch and non-starch) (36).

The major carbohydrate in wheat flour is starch (80%), followed by minor constituents such as arabinoxylans (1.5–8%), β-glucans (0.5– 7%), sugars (about 3%), cellulose (about 2.5%), and glucofructans (about 1%) (60). Starch is a storage carbohydrate and nutritionally important. Wheat, rye, and barley starches usually have two granule populations differing in size. Small spherical B-granules with an average size of 5 µm can be distinguished from large ellipsoid A-granules with mean diameters of around 20 µm. Small granules tend to pack more densely, resulting in efficient packing. Starch is comprised of two water-insoluble homoglucans, amylose and amylopectin. Amylose is almost linear and consists of α-(1,4)-linked D-glucopyranosyl units. Amylopectin is responsible for building the granular nature of starch and is a highly branched polysaccharide consisting of linear α-(1,4)-linked D-glucopyranosyl units and α-(1,6)-linked branches.

Andean grain starches are small (2 µm) with irregular shape (polygonal and angular). Amaranth has been reported to be free of amylose (61), while canahua is approximately 14–18 % amylose (39), and quinoa 12% (61) (Table 3). Starch is the major component of quinoa carbohydrates and is present as between 32% and 69% of the grain composition (62, 63). In vitro digestibility (α-amylase) of raw quinoa starch has been reported to be 22%, while that of autoclaved, cooked, and drum-dried samples was 32%, 45%, and 73%, respectively (49). Saponins did not affect the digestibility of the starch. The total dietary fibre content in quinoa flour is affected by thermal treatment, while the insoluble dietary fibre fraction does not change with thermal treatment (49).

Table 3: Properties of wheat and Andean grain starch.

	Wheat	*Amaranth*	*Canahua*	*Quinoa*
Amylose (% starch)	27.4-29.9 (64, 65)	0-12% (61, 66)	11.7-18.9	3.5-22.7% (39, 63, 67-69)
Amylopectie (% starch)	72-75			77.5 (39, 63, 67-69)
Granule sizs (µm)	2-35(Bimodal) (70)	1-2 (66, 68)	<2(39)	0.6-3.5 (39, 56, 63, 67, 68)
Melting temperature (ºC)	65-67.5	59.4-86.9 (61, 68)	49.8-65.5(61, 68)	44.6-86.4 (39, 63, 68)

The non-starch polysaccharides (NSP) are primarily constituents of the cell walls. Thus, a higher extraction rate is associated with a higher content of NSP. Arabinoxylans (AX) or pentosans are the major fraction (85–90%). Wheat contains only 1.5–2% AX. AX can be subdivided into a water-extractable (WEAX) and a water-unextractable fraction (WUAX). WEAX makes up 25–30% of total AX in wheat and has considerable functionality in breadmaking.

The unique technological properties of AX are attributable to the fact that AX are able to absorb 15–20 times more water than their own weight and, thus, form highly viscous solutions, which may increase gas holding capacity of wheat doughs via stabilization of the gas bubbles during dough mixing. However, they are considered to have a negative impact on wheat breadmaking as they form physical barriers against the gluten network and thus actually destabilize the gas bubbles. The baking performance can be affected by adding endoxylanases, which preferentially hydrolyse WUAX. This produces solubilised WUAX, which have techno-functional effects comparable to WEAX.

2.2.3 Andean grain flours as nutraceutical foods

Quinoa is considered a nutraceutical food because it has shown beneficial hypoglycaemic effects and induced lowering of free fatty acids (71). Studies have shown that amaranth has many health benefits including decreased plasma cholesterol levels, stimulation of the immune system, the exercise of anti-tumour activity, reduced blood glucose levels, and improvement in conditions of hypertension and anaemia (13). Canahua also has excellent potential for a health promoting profile since the total content of polyphenolic compound of canahua was reported to be 2.5 mg GAE/g, which indicates that canahua could have favourable effects on thrombosis and tumorigenesis. In addition, canahua has exceptionally high levels of flavonoid, ranging from 46.2 to 144.3 (52, 72).

2.3 Technological uses of Andean grains in bread

Andean grain flours have already been used in blends with cereals. Amaranth has been used with wheat and maize for making cookies, tortillas, pasta, marzipan and breads (73). The use of low amounts of Amaranth (10-15%) has been reported as having a good flavour and being preferred over wheat bread (19). It has further been reported to provide an anti-staling effect (12). The use of quinoa with wheat in bread has been widely studied. Addition of low amounts of quinoa (10%) has been reported to produce an acceptability score similar to that of a control. The use of 20% quinoa has been reported to be acceptable, but with different crumb structure (74). Quinoa has been reported to increase the digestibility of sourdough bread when used at a substitution level of 20% (75). It has further been reported that low amount of canahua (12.5%) could increase the specific volume of wheat bread (76), and that bread staling would occur at a slower rate when canahua flour was included (77).

3 Bread dough

3.1 Thermodynamics of mixing

Dough is a dispersion of macromolecules having specific properties, and where water is more than a simple diluent. Upon hydration and mixing of dry flour particles, part of the water added becomes distributed among macromolecules (proteins, starch, and pentosans) and, with the help of small amounts of surface-active lipoprotein and glycolipids, becomes molecularly bonded, resulting in a filled-water-macromolecule system.

The degree of freedom of the macromolecules depends on their distances to the bonding-force sites existing during mixing. If the number of macromolecules free to move independently is high, it is more likely that they will interact or separate into co-exiting phases. Thus, the entropy of the mixing is more significant if water is present in more than sufficient amount for the macromolecules to move. The Flory-Huggins equation (Eq. 1) describes the entropy of mixing e.g. for a binary system (78, 79),

$$\Delta S_M = -k_B \left(\frac{\emptyset_1}{r_1} \ln \emptyset_1 + \frac{\emptyset_2}{r_2} \ln \emptyset_2\right) \quad (1)$$

where ΔS_M is the combinatorial entropy of the possible arrangement of the macromolecules, k is the Boltzmann constant, ϕ_1 and ϕ_2 are the volume fractions, and r_1 and r_2 are the relative length of the macromolecules 1 and 2, respectively. More specifically, during mixing of wheat flour and with the help of water, both gliadin and glutenin can freely move or be transported. As internal constraints are removed, the mixing entropy increases to a maximum. It is then possible for charged monomers close to the surface of these macromolecules to eventually come in contact and cause aggregation through disulphide linkages and hydrogen bonds (80), producing the gigantic structure of the poorly charged gluten matrix (81). Gluten can therefore be considered a concentrated dispersion of gliadins and glutenin, embedding granules of starch (82). One might think that both water addition and mixing regime should be optimized in order to generate the best possible water distribution and water-binding of the macromolecules. In this work, the condition of water content and mixing regime was the same for all the prepared doughs (Paper I and II). Mixing beyond the optimum is thought to damage the dough by changing the water-binding capacity of macromolecules and disrupting the gluten structure (83). The mixing of the non-gluten Andean grain flour dough under the same conditions of water content resulted in stable solid-in-water dispersions (Paper II).

3.2 Phase separation

Phase separation is a phenomenon observed between different macromolecules in solution. When a macromolecule moves towards another macromolecule, they may adhere to each other if they have sufficient attraction or affinity, while other macromolecules may be excluded and left out. If this happen, there will be a decrease in the number of ways macromolecules can be arranged in space, resulting in a loss of mixing entropy. The phase separation depends on a number of parameters, such as the formation of insoluble "inter-polymers" complexes, thermodynamic incompatibility of biopolymers, and physical-chemical differences (84).

The formation of gigantic and stiff gluten due to the strong interactions between gliadins and glutenins (85, 86) results in a loss of mixing entropy so great that the gluten phase separates from the other macromolecules even when the repulsion is very weak (87). Moreover, gluten has limited solubility in water (88), thus the formation of gluten excludes water, which increases the amount of water available for the starch-pentosans system. Therefore, the addition of too much water leads to an increase in the amount of water covering the starch granules in dough as reported earlier by Létang *et al*., who studied the microstructure of wheat flour-water doughs by adding different amounts of water during mixing (89). These findings also agree with Larsson *et al* (90).

Another reason for phase separation in dough is the incompatibility between gluten and starch, which can be exemplified by the way dough can be washed under cold water to remove starch and other soluble components in order to isolate gluten. One also can therefore understand that there is a low affinity between gluten and starch granules and other soluble components. The example above is a separation of phases, but is not a phase separation. Thermodynamic incompatibility between two macromolecules arises from the low entropy of their mixing when they just tend to occupy different phases. In general, the thermodynamic incompatibility of different macromolecules determines food structure and properties. Last, but not least, separation of phases takes place through the physical and chemical properties of the co-exiting phases, such as viscosity, molecular weight, and density.

3.2.1 Ultracentrifugation

As the macromolecular motion in a mixed aqueous solution is slow, the phase separation of dough is thermodynamically possible but kinetically too slow to be seen on realistic timescales owing to the high viscosity of the continuous gluten phase. Dispersions are thermodynamically unstable because of the large surface free energy, and they will eventually phase separate.

The phase separation behaviour of dough from different flours was investigated by means of ultracentrifugation, microscopy, and DSC (differential scanning calorimetry) as combined methods for characterising dough (Paper I) and applied to understand the effect of different flours in wheat dough (Paper II).

There are various mechanisms of phase separation, including gravity. Either by rest or centrifugation, the coexisting phases eventually settle in different layers owing to the difference in density (84). Ultracentrifugation relies on the principal property of phases and the fundamental laws of gravitation according to the equation of sedimentation velocity developed by Svedberg and his collaborators (Eq. 2) (91),

$$S = \frac{V}{w^2R} = \frac{d^2(\rho_P - \rho_1)g}{18\mu} \tag{2}$$

where S is the sedimentation coefficient, V is the velocity of particle, w is the rotor speed (rad/s) and R is the rotor radius, d is the diameter of particle, ρ_P is the density of particle, ρ_l is the density of the medium, g is the relative centrifugal force, and μ is the dynamic viscosity of the medium. The only way to accelerate the sedimentation of a dispersion like dough and to make it kinetically viable is through the modification of the gravitational force (g). Ultracentrifugation of dough for one hour at 100,000 x g resulted in the isolation of dough components (Papers I and II). The main benefit of ultracentrifugation is to avoid conditions that could modify the chemical and physical properties of dough components (90, 92).

3.3 Dough components

In Papers I and II, doughs were prepared at a ratio of 50% water content (by dough weight). After ultracentrifugation, four sharply separated phases were observed in all wheat flour doughs (Paper I). The same results were obtained by other researchers, who noted that a sharp separation is dependent on the water content of the dough (90). In Paper I, each sample had a different volume fraction (VF) of the separated phases, Figure 2 and Figure 3. Ultracentrifugation of Andean grain flours is shown in Figure 4. These flours separated into substantially different phases. These results showed the main structural differences among these flours and in relation to wheat. Amaranth dough separated into eight fractions, canahua into four fractions (partially unseparated), and quinoa into nine fractions (Paper II). This was the first work to ultracentrifuge dough from Andean grain flours.

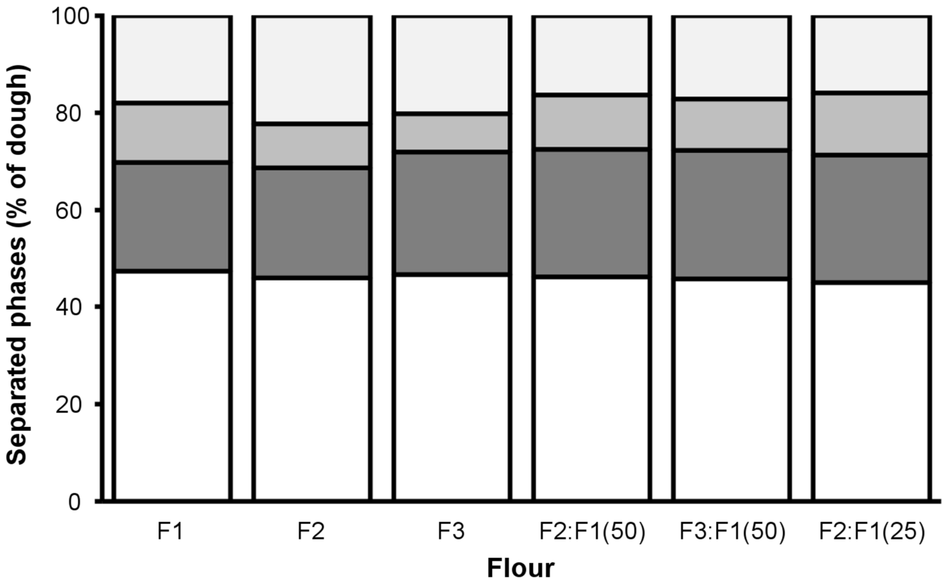

Figure 2:. Phase separation behavior of dough systems made with water and flour. F1, pure Swedish wheat flour; F2, pure Bolivian wheat flour 1; F3, pure Bolivian wheat flour 2; F2:F1(50), 50% of F2 blended with 50% of F1; F3:F1(50) 50% of F3 blended with 50% of F1; and F2:F1(25), 25% of F2 blended with 75% of F1. From top to bottom: liquid, gel, gluten and starch.

In wheat, from top to bottom (Figure 3), the first phase was an aqueous solution "the liquid phase" (90). The liquid phase is a multicomponent phase. MacRitchie *et al*., who studied the liquid phase, reported the presence of soluble carbohydrate (7%), protein (3.4%), sodium chloride (3%) and lipids (0.3%) (93). The protein reported in this phase belongs to the water soluble proteins, i.e. albumin and globulin (as described in Chapter 2), like enzymes and glycoproteins. These proteins do not contribute to the properties of gluten or the dough forming properties.

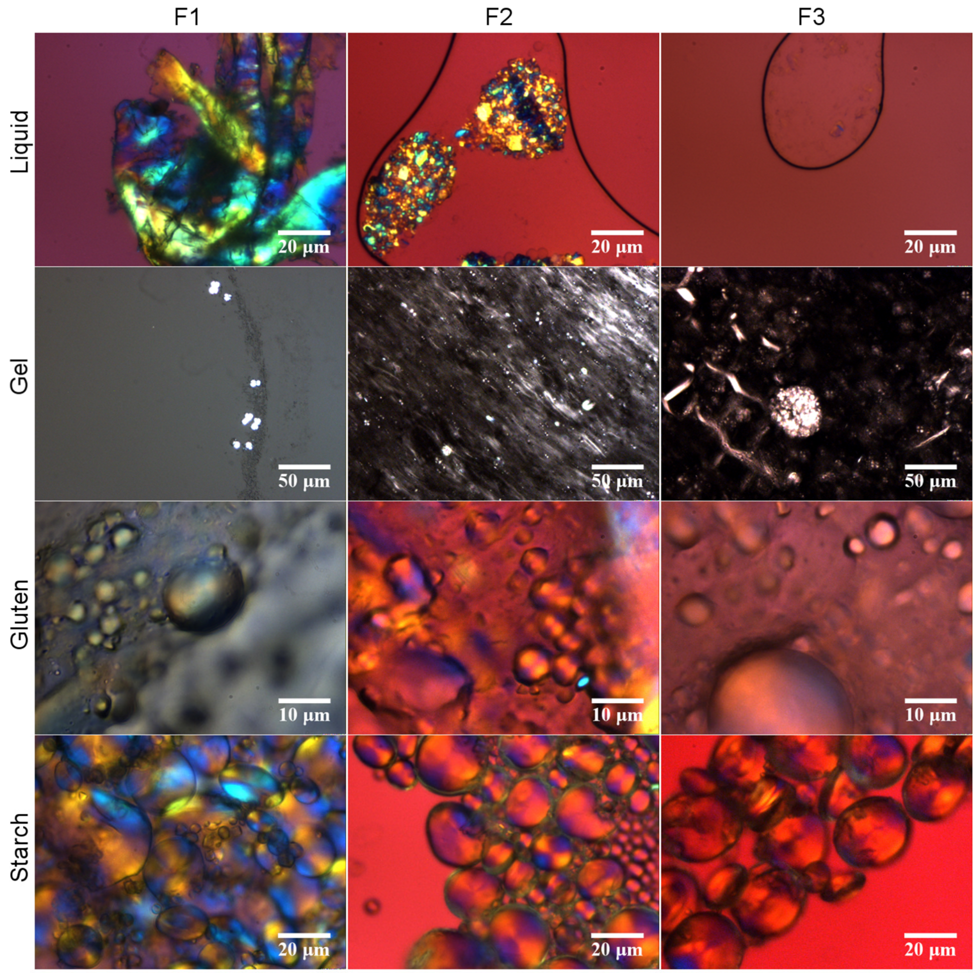

Figure 3: Images of the separated phases. From top to bottom: liquid, gel, gluten, and starch phases. From left to right: F1 (Swedish wheat flour), F2 (Bolivian wheat flour 1) and F3 (Bolivian wheat flour 2).

Macroscopically, the liquid phase of the Swedish and Bolivian wheat flour samples did not show significant differences, only the volume fraction (Figure 2), whereas the microscopy analysis of the liquid phases revealed that the Swedish wheat sample (F1) contained more and larger particles, while one of the Bolivian wheat samples (F3) almost did not have particles and the other Bolivian sample (F2) contained aggregation of small and crystalline particles related to starch in the liquid phase (Figure 3).The following phase was identified as “gel” owing to its macroscopic appearance (90). The macroscopic and microscopic analysis revealed interesting characteristics. The Swedish flour gel was brighter and larger (in volume fraction), while both Bolivian flour gels appeared darker and more compacted. The microscopic difference between them are shown in Figure 3. The main difference was the size and shape of the particles included. In the F1 gel, they were small and round, with a small amount of starch granules, while F2 contained fibrous particles and F3 more cell walls and aggregation of crystalline remains. The third phase was the gluten phase, which appeared as a continuous phase containing embedded starch granules, bran, and large cell remnant particles. The microscopy analysis revealed that more small starch particles were found in the gluten phase together with a few very large starch granules (Figure 3). The fourth phase was comprised of starch granules. The microscopy analysis also showed different size of starch granules, and relative more small starch granules in F1 compared to F2 and F3.

All of the wheat flour doughs were characterized by these four phases, the volume fraction of them and their microstructure. Additional analysis of blends of these wheat flours at a ratio of 50:50, and 75:25 were performed. The blending of flours modified the volume fractions of the liquid, gel and gluten phases, but only the gel phase seemed to directly follow the proportions of the individual flours used.

All of the Andean grain flours produced a film phase at the top of the test tube, which was related to the presence of lipids (Figure 4). Andean grain flours have a relative high lipid content. The liquid phases were different in colour and odour, i.e. light yellow for quinoa, bright pink for amaranth, and dark brown for canahua, the latter with a pleasant sweet taste. In addition, the separation of amaranth flour dough resulted in several liquid phases, with the first mainly containing floating particles. Quinoa flour produced a transparent gel phase with some presence of starch, and rather high water content (WC), freezable water (FW), and unfreezable water content (UFW). The solid phases of the Andean grain flour doughs were even more different, especially for canahua, since it only had one unseparated phase with a thin layer of free starch at the top, and the microscopy analysis showed relative large particles of bran and aggregates of starch. Quinoa and amaranth were slightly more similar, with several fractions distinguished by colour and volume. The canahua seed is very small and hard, whereas quinoa and amaranth seeds are larger and easier to mill, which could be an additional reason for the differences and presence of additional separated phases.

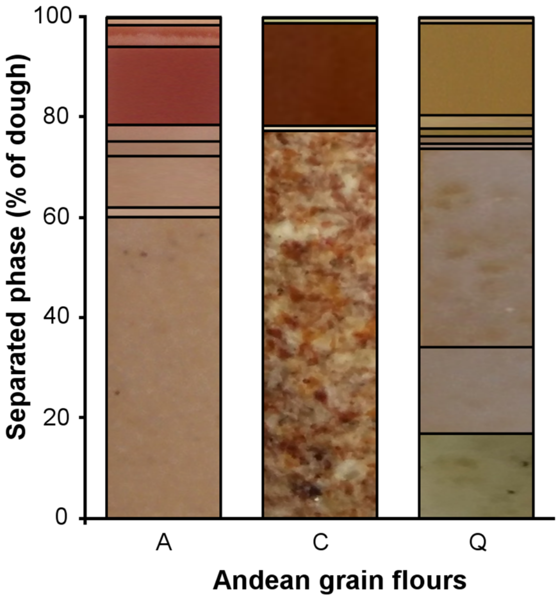

Figure 4: Phase separation behavior of doughs prepared from amaranth flour (A), canahua flour (C) and quinoa flour (Q).

Moreover, Andean grain flours would be expected to affect the formation and phases formed in wheat dough. Paper II therefore also included the substitution of wheat flour with 50% or 25% Andean grain flour to understand the effect on the normally four phases of wheat dough (Figure 5). At a 50% substitution, the doughs became more complex and separated into more than four phases. These results suggest that the components did not mix together and could be separated into further phases. This was valid for all Andean grain flours. At 25% substitution of wheat flour, all doughs were still separated into the main four phases (Figure 5). However, even though the four phases typical for wheat flour were present, the VF differed. The presence of canahua increased the VF of the liquid phase, and the gel phase was substantially reduced. All Andean grains contributed to increasing the VF of the gluten phase through the combined effects of dilution by the inclusion of gel, small starch granules, and bran particles, as also confirmed by microscopy. The boundaries between separated phases, especially with canahua, were not as sharp as for wheat. The addition of different flour than wheat in total reduced both the proportion of gliadins and glutenins and the availability of water during mixing, which increased the entropy of mixing and reduced the motion of gliadins and glutenin. The formation of a gluten matrix was confirmed by ultracentrifugation, but there is a possibility that the formation of gluten could not be completed in the timescale of mixing, and, more so, that prolonging mixing in order to complete the formation of gluten could damage the gluten that had already formed.

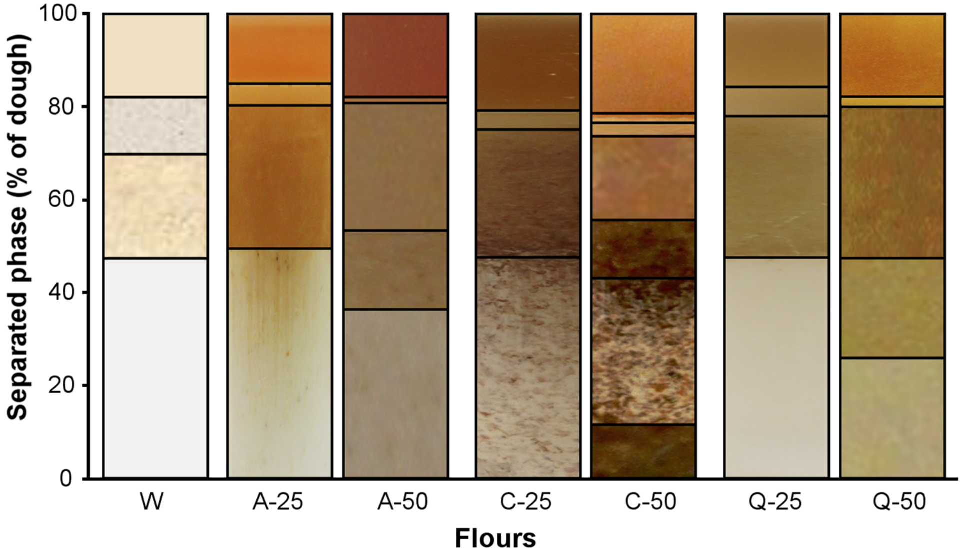

Figure 5: Phase separation behavior of dough and separated phases for wheat (W), 25% (A-25) and 50% (A-50) amaranth, 25% (C-25) and 50% (C-50) canahua, 25% (Q-25) and 50% (Q-50) quinoa.

The ultracentrifugation coupled with microscopy was useful to better understand the components of dough and the differences related to volume fraction and microstructure.

3.3.1 Water relationships

Ice crystallization temperatures, amount of frozen water, and its melting enthalpy as a function of total water content can be investigated using DSC. Calorimetry is an appropriate technique for the study of the physical state and properties of water in biological systems.

Papers I and II also describe water behaviour in all selected doughs and their separated phases. Water was characterized by properties in terms of freezable water (FW), unfreezable water (UFW), water content (WC), melting of ice (To), peak temperature (Tp), and melting range (ΔT)

The water content of the liquid phases ranged from 87.40 to 88.99% (Paper I). The results of Paper I also show an interesting difference in freezable water (FW). More FW occurred in the Swedish wheat sample F1, suggesting a lower presence of soluble components. Another interesting feature was the melting range, which was shorter for F1 than for the Bolivian wheat flours. These results indicate higher water availability in the liquid phase of F1. The main difference between flours observed in the gel phases is that F1 had more WC and FW. The different liquid and gel phases generally had the highest values of UFW, which was expected since pentosans, which are known to have high water affinity, were expected to be present. UFW is sometimes called unavailable water and includes the saturated monolayer associated with ionic, polar and non-polar groups on the surface of proteins and other polymers. The WC values for the gluten phases were similar and higher than 50%. Long periods of mixing can increase the presence of water in the gluten phase and change properties. Again, the gluten phase of F1 tended to be higher in FW and ΔT, which suggest greater availability of water. Moreover, the water inside the gluten structure of F1 could have a different physical accommodation which allowed recrystallization during rewarming. The starch phase of F1 had higher WC and FW values in comparison to those of F2 and F3. It seems reasonable to suggest that water was more available in the F1 starch phase, and also that the F1 starch phase seemed to have a higher affinity for water or a better distribution of water inside the interstitial alloys formed by the starch granules.

The amount of FW was similar for all pure doughs, wheat and Andean grains, ranging from 71.41 to 72.46%. The lowest amount of FW was present in amaranth dough, however, the difference was not significant. The quinoa lipid phase had a notably lower WC (34%) and higher FW (90%) compared to amaranth and canahua (Table 4), and an extremely low UFW content (0.05 mg/mg). This may suggest that this lipid phase was more hydrophobic. The WC and FW of canahua liquid were rather low compared to the liquid phases of the other Andean grains, suggesting a high presence of solids and soluble components with high affinity for water. The Quinoa gel phase had rather high WC, freezable water FW, and UFW. The solid fraction of the separated dough had different water properties, but the three different solid phases of amaranth and quinoa had similar water properties.

Table 4: Volume fractions (VF) and water properties (Water content (WC), Freezable water (FW), Unfreezable water (UFW), Peak temperature of ice melting (Tp)) of separated phases of doughs prepared from amaranth, canahua, quinoa and wheat. Mean (M) and standard deviation (SD).

	Volume fraction VF (%)	***Water content WC (%)***	***Freezable water FW (%)***	***Unfreezable water UFW (mg/mg)***	***Peak temperature T_P (°C)***
	M SD	**M SD**	**M SD**	**M SD**	**M SD**
Amaranth					
Lipid	1.0 ± 0.2	56.87 ± 0.75	75.74 ± 0.83	0.32 ± 0.01	0.70 ± 0.25
Liquid 1	4.4 ± 0.7	86.70 ± 0.17	90.16 ± 1.88	0.64 ± 0.12	2.21 ± 0.55
Liquid 2	15.7 ± 0.2	86.40 ± 0.07	90.37 ± 1.45	0.61 ± 0.09	1.72 ± 0.24
Solid 1	3.3 ± 0.3	67.89 ± 0.45	89.74 ± 0.73	0.22 ± 0.02	0.77 ± 0.13
Solid 2	2.9 ± 1.1	53.48 ± 0.20	80.00 ± 0.18	0.23 ± 0.00	0.31 ± 0.30
Solid 3	10.4 ± 0.2	46.19 ± 0.49	70.58 ± 1.58	0.25 ± 0.02	0.59 ± 0.28
Solid 4	1.9 ± 1.3	42.37 ± 0.61	65.55 ± 2.85	0.25 ± 0.02	1.27 ± 1.04
Solid 5	60.5 ± 1.6	34.93 ± 1.07	56.34 ± 1.19	0.23 ± 0.01	0.91 ± 0.14
Canahua					
Lipid	0.4 ± 0.1	56.87 ± 0.75	78.85 ± 1.57	0.26 ± 0.06	-0.16 ± 0.39
Liquid	20.7 ± 2.4	82.71 ± 0.01	87.64 ± 0.54	0.59 ± 0.03	0.75 ± 0.24
Solid 1	0.5 ± 0.1	60.15 ± 0.66	81.99 ± 1.44	0.27 ± 0.02	0.00 ± 0.22
Solid 2	78.4 ± 2.2	36.09 ± 0.49	57.80 ± 1.19	0.24 ± 0.00	-0.46 ± 0.14
Quinoa					
Lipid	0.4 ± 0.0	34.27 ± 6.33	89.95 ± 1.58	0.05 ± 0.01	-0.16 ± 0.39
Liquid	18.5 ± 0.7	91.77 ± 0.07	93.72 ± 4.09	0.70 ± 0.46	0.75 ± 0.24
Gel	2.7 ± 0.8	89.04 ± 0.42	93.30 ± 1.63	0.54 ± 0.13	0.00 ± 0.22
Solid 1	1.6 ± 0.4	73.65 ± 1.52	95.08 ± 0.36	0.14 ± 0.02	-0.46 ± 0.14
Solid 2	1.5 ± 0.2	51.77 ± 2.85	81.36 ± 2.83	0.20 ± 0.01	-0.16 ± 0.39
Solid 3	1.0 ± 0.2	44.45 ± 1.80	69.63 ± 1.10	0.24 ± 0.01	0.75 ± 0.24
Solid 4	39.9 ± 0.7	38.91 ± 1.84	63.56 ± 3.39	0.23 ± 0.00	0.00 ± 0.22
Solid 5	17.4 ± 2.8	35.42 ± 1.78	55.18 ± 2.85	0.25 ± 0.00	-0.46 ± 0.14
Solid 6	17.1 ± 3.2	27.89 ± 1.85	33.52 ± 6.04	0.26 ± 0.00	-0.16 ± 0.39
Wheat					
Liquid	18.0 ± 1.3	87.40 ± 0.04	96.86 ± 3.56	0.22 ± 0.25	2.10 ± 0.94
Gel	12.3 ± 0.4	84.13 ± 0.20	89.95 ± 0.79	0.53 ± 0.05	2.17 ± 0.21
Gluten	22.4 ± 0.9	52.89 ± 0.71	72.36 ± 0.75	0.31 ± 0.01	4.68 ± 1.28
Starch	47.4 ± 0.6	30.94 ± 2.98	37.23 ± 1.85	0.28 ± 0.04	2.47 ± 1.28

The addition of Andean grain flour to the wheat flour dough resulted in interesting changes in water properties. Amaranth flour at 25% substitution substantially reduced the amount of FW in the dough, i.e. to 62% compared to 72% in wheat. The starch-water interface is then higher due to the presence of the small-sized starch, which may therefore facilitate water molecules absorbing on and penetrating into the starch granules. Meanwhile, canahua was comprised of many large bran particles, which could explain the different behaviour between quinoa and canahua. Amaranth flour has been reported to increase bread moisture by increasing the water retention capacity. High water retention is also associated with improved crumb structure and greater product acceptability. Amaranth caused a reduction of liquid phase, while canahua tended to increase the liquid phase. When canahua was added at 50%, the gluten phase had a lower water content (47.2%). These results suggest that the gluten structure was not well developed. Larsson *et al*, reported that ultracentrifugation of well-developed dough yielded gluten with a water content higher than 50% (90). The presence of quinoa at low and high levels slightly modified the WC of the gluten phase. Another interesting result is the effect of levels of substitution and the type of flour on the FW of dough, which suggest a minimum for each flour.

Water plays a crucial role in any system owing to the association between water and other compounds. Water affects the performance of systems, while the performance of water can be changed in the presence of different compounds, depending on the degree of chemical or physical interactions between the water and the specific compounds.

3.3.2 Thermal properties

The addition of water is crucial for any thermodynamic phenomenon in dough, from the mixing, through the baking process, to the shelf life of the final bread.

The thermograms for the wheat samples (Figure 6) were typical for doughs, with gelatinization of starch at approximately 55-85°C. Nevertheless, F1 gelatinized at the lowest temperatures, and F3 at the highest. Since F1 had the lowest onset and peak temperature and concurrently the highest amount of freezable water, these results suggest that F1 seems to present the best conditions for baking.

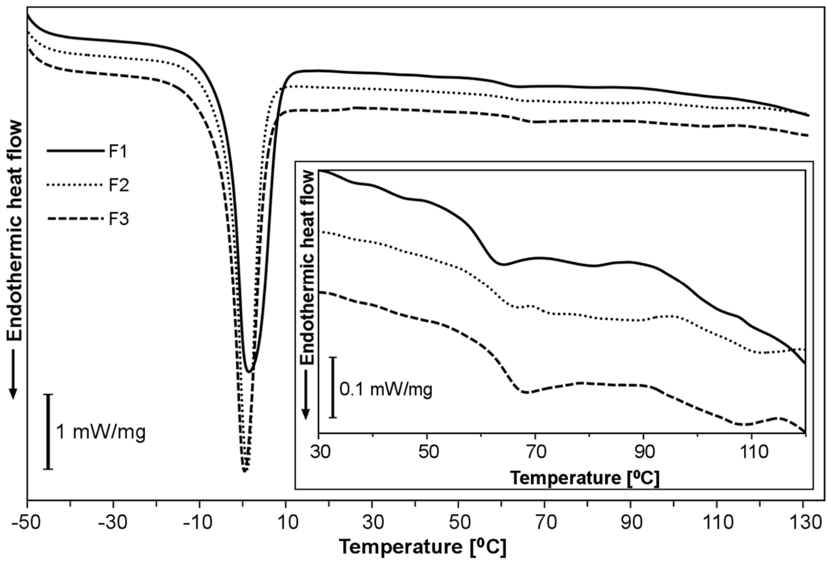

Figure 6: DSC thermograms at ice melting, baking and higher temperatures of dough systems made with water and flour. (a) Swedish wheat flour (F1), Bolivian wheat flour 1 (F2), Bolivian wheat flour 2 (F3).

Thermal properties were further analysed in each separated phase of dough. Two thermal transitions in the liquid phase were observed at 45 and 90^{o}C, which could be caused by the soluble components. The former could be related to globulins and albumins, known to occur around 50^{o}C, and a second baseline shift caused by higher order components. The thermal behaviour of the gel phase, on the other hand, was characterized by small transitions without characteristic temperatures, hardly possible to distinguish from the noise. Generally, the thermal behaviour of gel revealed that this phase is thermally stable during baking. The gluten phases presented a single endothermic peak that appeared at the temperature of 73, 70 and 71^{o}C for samples F1, F2 and F3, respectively. Glutenin denaturation is known to occur at around 64–80^{o}C since these are the more ordered structure proteins, followed by gliadins at 58^{o}C. The broad peaks observed in this study could be attributed to the overlapping of glutenin denaturation and starch gelatinization. The starch phases had very low water content and the starch granules therefore did not gelatinize. Thus, the starch phases were diluted to 10% on dry basis in order to analyse starch gelatinization properties. Generally, the starch granules of F2 gelatinized at a lower temperature. As expected, the thermal transitions in the starch phase generally corresponded to the transitions of the dough.

Amaranth, canahua, and quinoa doughs had slightly different thermal behaviour as seen from the DSC thermograms (Figure 7). The gelatinization enthalpy is an indicator of thermal stability of starch. Since quinoa flour dough had the highest gelatinization enthalpy (Table 5), this indicates that quinoa starch is more thermally stable during baking. Amaranth dough had the highest gelatinization peak temperature, which is another measure of thermal stability. The stable structure of small starch granules, as found in these Andean grains, in combination with polar lipids can increase the stability of gas bubbles during baking.

Table 5: Thermal properties of doughs prepared from amaranth, canahua, quinoa, and wheat.

	Gelatinization Enthalpy ΔH (J/g)	*Onset Temperature T_O (℃)*	*Peak Temperature T_P (℃)*	*Temperature Range ΔT (℃)*
	M SD	**M SD**	**M SD**	**M SD**
Wheat	6.38 ± 0.33	56.90 ± 0.82	64.5 ± 0.75	28.65 ± 0.49
Amaranth	6.20 ± 0.45	64.27 ± 0.59	76.57 ± 2.80	24.87 ± 3.10
Canahua	4.09 ± 0.37	54.01 ± 1.06	67.24 ± 0.57	26.87 ± 3.82
Quinoa	8.13 ± 0.85	56.63 ± 0.28	64.39 ± 0.38	28.08 ± 0.50
25% Substitution				
Amaranth 25%	5.13 ± 0.87	59.35 ± 0.88	65.90 ± 0.78	28.20 ± 3.20
Canahua 25%	5.13 ± 0.22	57.90 ± 0.82	65.79 ± 1.20	25.97 ± 0.08
Quinoa 25%	4.43 ± 0.45	58.24 ± 0.75	64.31 ± 0.07	27.98 ± 0.47
50% Substitution				
Amaranth 50%	6.39 ± 0.54	60.03 ± 0.68	68.18 ± 0.82	27.22 ± 0.85
Canahua 50%	4.19 ± 1.41	58.77 ± 1.68	65.48 ± 0.25	26.71 ± 5.63
Quinoa 50%	5.44 ± 0.34	58.57 ± 0.10	64.99 ± 0.63	25.70 ± 3.99

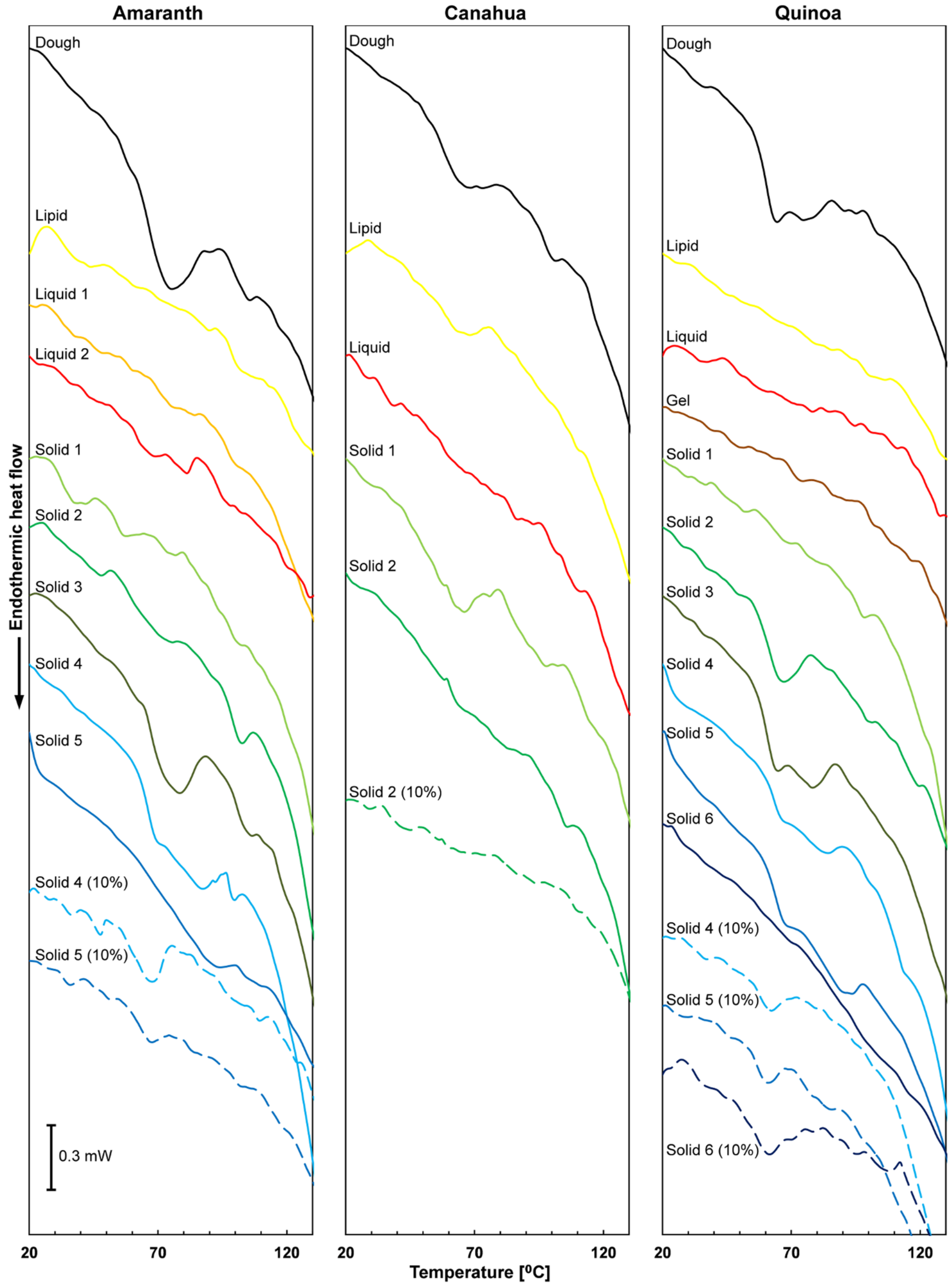

Figure 7: DSC endotherms at temperatures relevant for baking for dough and separated phases for (a) amaranth, (b) canahua, and (c) quinoa. Dotted lines indicate that the phase was diluted to 10% of solid content.

The combination of DSC at higher temperatures and microscopy showed that the first solid (Solid 1) phase of amaranth, as achieved through ultracentrifugation, mainly contained starch and protein. The second solid (Solid 2) phase contained starch and cell remnants, and the WC (53.5%) was similar to the gluten phase of wheat flour dough (WC 52.9%). The melting of amylose-lipid complexes, with a transition at 105^{o}C, was detected in Solid 2 (Figure 5). The DSC curve of the diluted fourth (Solid 4) phase showed one major peak for starch gelatinization at 67.5^{o}C (ΔH_g=18.46 J/g), a small peak for the amylose-lipid complex at 109.3^{o}C ($\Delta H_{a\text{-}l}$=1.34 J/g) and another two peaks related to protein denaturation (37.2^{o}C and 47.5^{o}C). The diluted fifth (Solid 5) phase, on the other hand, had two endothermic peaks, one for starch gelatinization at 67.6^{o}C (ΔH_g=8.24 J/g) and one for protein denaturation (35.9^{o}C). The above results confirmed the sensitivity of the separation, particularly since the fifth solid fractions were comprised of more agglomerated starch granules and brand particles.

DSC curves for canahua dough showed lipid melting (17^{o}C), starch gelatinization (67^{o}C), and melting of the amylose-lipid complexes (100^{o}C, 107^{o}C). On top of the unseparated phase (Solid 2), starch gelatinization was determined by DSC (To=66^{o}C, ΔH_g=3.44 J/g). The unseparated phase was also diluted to a concentration of 10%, and DSC analysis showed a tendency for starch gelatinization to occur at 67^{o}C (ΔH_g=3.47 J/g).

The DSC curves of the lipid phase from quinoa dough displayed an endothermic peak at -27^{o}C, which could be related to the melting of polyunsaturated lipids. The DSC curve for the Solid 1 of quinoa revealed the presence of the amylose-lipid complex. The Solid 3 fraction showed a broad transition with two peaks (65^{o}C and 78^{o}C) related to the gelatinization of starch at low water content. The solid fractions 4, 5 and 6 were low in WC and FW. Upon dilution, the endothermic peak increased towards the bottom (Figure 6). The Solid 6 fraction also showed a clear transition for amylose-lipid complexes at 108.25^{o}C.

The DSC curves for the dough from blends of wheat flour with Andean grain flour (25%) was dominated by two endothermic peaks, the starch gelatinization (59.4^{o}C) and the amylose-lipid complex (107.1^{o}C). The ΔH_g for starch gelatinization for wheat with 25% amaranth substitution was also reduced (5.1 J/g, from 6.4 J/g in the wheat dough), whereas ΔH_g was the same as for wheat flour dough for the higher level of substitution (50%). Dough prepared with 25% canahua showed a slight change of FW, reduced gelatinization enthalpy and a large melting peak for amylose-lipid complexes compared to wheat flour dough. The reduction of FW could not be the main reason for this change in thermal behaviour, but the presence of polar lipid from canahua could hinder starch gelatinization and induce a rapid and strong formation of amylose-lipid complexes. Quinoa in wheat dough reduced the FW of doughs in a similar way as canahua. Starch gelatinization dominated the thermal analysis, although a shallow melting peak for the amylose-lipid complex was detected for 50% quinoa dough. The presence of quinoa reduced the gelatinization enthalpy compared to wheat dough.

4 Texture of dough

4.1 Rheology

Any change in shape that a substance undergoes when energy is applied to it, either mechanical or by heat, is referred as “deformation”. Controlled deformation, in rate and nature, is used to characterize important physical properties of materials (94). The study of deformation and flow of matter was first referred as “rheology” by Professor Bingham (95, 96). In rheology, the matter can be classified as liquid, solid, and a mix of both, which are referred to as viscoelastic materials and present both solid-like and liquid-like properties. Furthermore, the rheology of dispersions can be considered at two levels: the macro-rheological and the micro-rheological.

4.2 Deformation and viscoelastic functions

Wheat dough is a viscoelastic material. Moreover, it is a dispersion comprising a bicontinuous phase (liquid and gluten) with embedded starch granules and macromolecules, such as arabinoxylans.

Gluten is the main factor controlling the viscoelastic behaviour during deformation (97). The balance of gliadins/glutenin is known to affect the viscoelastic properties of doughs during mixing, handling, and baking (98, 99). Gliadins, the monomers, must be cross-linked to form a network and act to constrain the system, and are related to the viscous component of dough. Glutenins, on the other hand, are polymers that control elastic behaviour.

Moreover, the addition of more or less water to the system modifies the consistency, i.e. consistency is increased with less water. Furthermore, free water molecules along with the soluble flour components form the liquid phase within which the thermal and colloidal properties of components determine the relative thickness of the liquid phase. The amount of liquid phase of wheat dough was modified by the presence of Andean grain flours (Paper II), but a change in consistency was also observed (Paper IV).

Rheological knowledge of these materials is essential in order to control the processes and produce high quality final products (54). The samples used in Paper III and IV were therefore prepared using the same condition of consistency in a Farinograph.

Mechanical deformations are commonly achieved in three ways: extension, bulk compression, and shearing as depicted in Figure 8 (100). Dough responds quickly to an applied force owing to the molecular conformation, cross-links, and hydrogen bonds. When dough is subjected to stress, it can reach a state of equilibrium with the external stress if this remains constant for an appropriate timescale. At equilibrium the rheological properties of dough can be described thermodynamically.

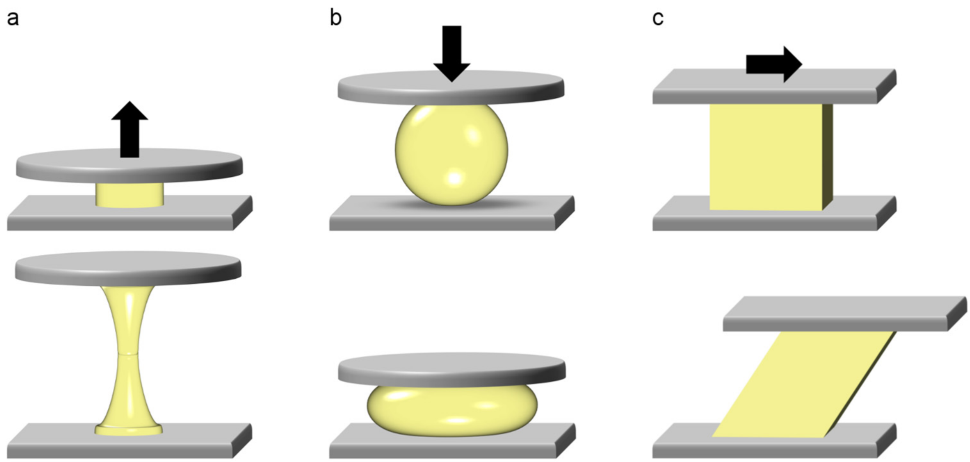

Figure 8: Schematic representation of deformation, (a) extension, (b) compression, and (c) shearing.

4.3 Extensional flow

The force developed during extensional flow has two components (Eq. 3), an entropic component and an internal energy component, f_s and, f_e, respectively.

$$f = f_s + f_e \tag{3}$$

f_e is attributed to strain in the bonds, whereas f_s is related to a decrease in the number of possible conformations in the lowest energy state, i.e., f_s arises from the loss of entropy. If dough is stretched, entropy is reduced from a high entropy state, since the random coil conformation is constrained to adopt a restricted number of conformations (Figure 9).

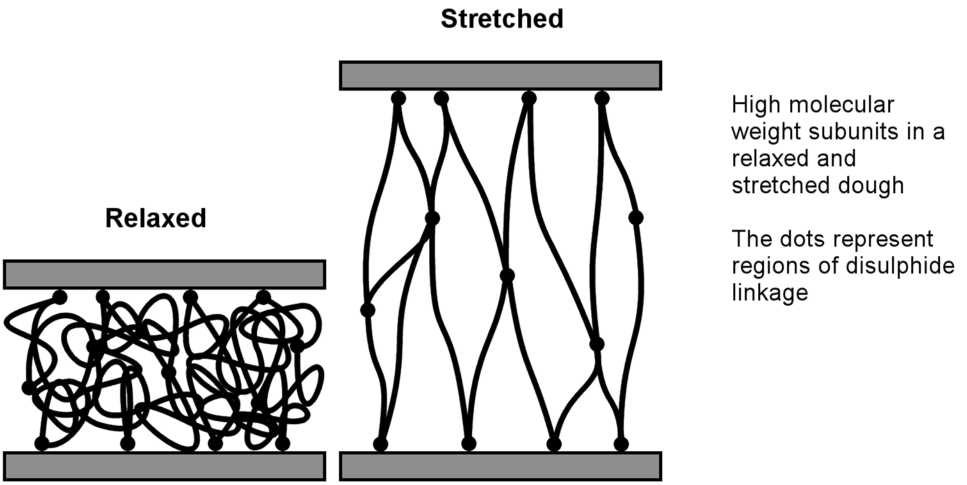

Figure 9: Different macromolecular arrengement upon extension.

The entropy loss provides the restoring force. If a state of equilibrium is not reached, the stretched conformation of dough will store elastic energy and can be used to increase the resistance to extension. The restoring force causes the macromolecules to return to their original state if the applied stress has been removed, returning to a high entropy random coil conformation. There is an increase in the temperature due to stretching, and a cooling when dough is allowed to contract. The release of heat correlates with the loss of motion, and in turn with a decrease in entropy on extension. The internal energy component increases if the strain on bonds increases, which could result in breakage.

4.2.1 Uniaxial extension

The method used for the uniaxial extension measurements in this work was the Stable Micro Systems (SMS) Kieffer Dough and Gluten Extensibility Rig, which provided useful information on the rheological properties, such as resistance to extension and extensibility (101, 102). In these tests, force-distance curves were obtained, and the area under the curve can be related to the protein quality of gluten (103). The maximum resistance to extension has been highly correlated with the concentration of glutenins, especially high-molecular-weight glutenin subunits (104).

4.2.2 Biaxial extension

An alternative to the uniaxial extension measurements is the bubble inflation or Dough Inflation System (DIS) (Figure 10) (105, 106). In this test, a sample in the shape of a thin disc is inflated into a balloon with pressurised air. A state of equi-biaxial tension is accomplished at the top of the bubble (107, 108). This method can be used to obtain the strain hardening properties, where strain hardening is the phenomenon in which the stress increases more than proportionally to the strain. From the curve of Hencky stress vs stress, it is possible to calculate the index and the coefficient of strain hardening, which can be related to the final bread volume (109). This method was developed to mimic the condition of cell expansion during proofing and baking.

Figure 10: Dough inflation system.

4.3 Compression

The compression test is by far the most commonly used method as it is very simple to perform. The most common test is the Textural Profile Analysis (TPA), which represents the relationship between force and time during two subsequent compressions (Figure 11) (110, 111). This method provides several textural parameters that may be correlated with other methods (112).

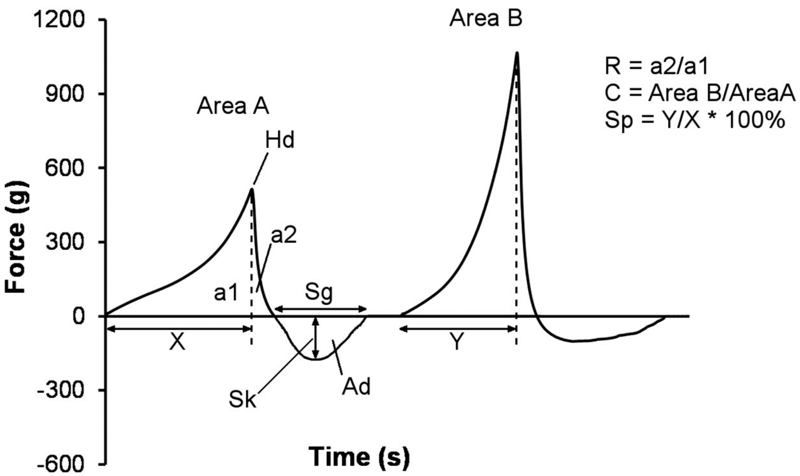

Figure 11: Interpretation of TPA parameters: Hardness (Hd), resilience (R), stickiness (Sk), stringiness (Sg), adhesiveness (Ad), springiness (Sp), and cohesiveness (C).

4.4 Texture measurements of wheat dough with and without Andean grain flour

Paper III tries to resolve the question of if and how methods using different deformations are correlated and their usefulness in measuring wheat dough properties.

The rheological parameters, 26 in total, were measured using 5 different methods. Principal component analysis was performed to understand their underlying relation (Figure 11).

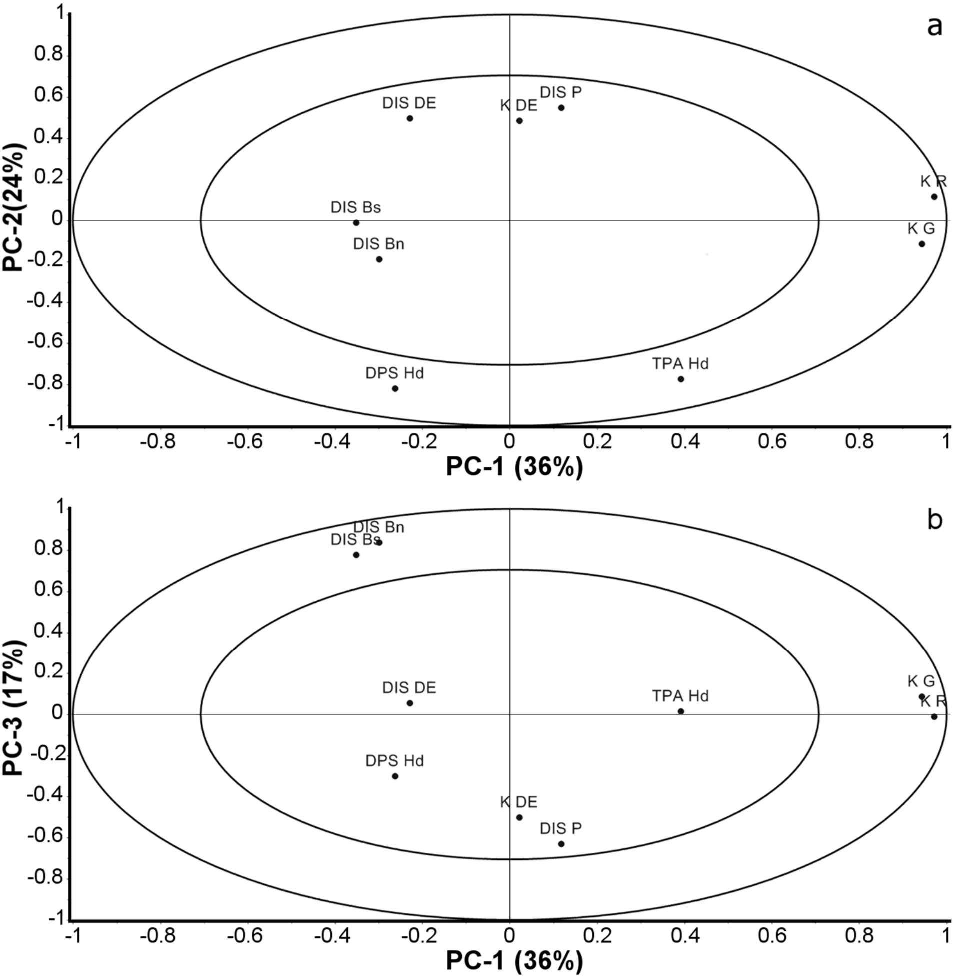

Figure 12: Correlation loading of variables related to hardness shown for the first principal components (PC) a) PC1 vs PC2, and b) PC1 vs PC3, TPA Hd (Hardness in TPA), DPS Hd (Hardness in DPS), DIS Bn and DIS Bs (bubble strain and bubble stress in DIS), K DE, K G and K R (deformation energy, gradient, and resistance in K).

All the methods were found suitable for evaluating the textural properties of dough, but they are all highly influenced by the intrinsic characteristics of the specific method and sample. Using hardness as an example, all of the methods gave independent results according to the deformation applied, i.e. extension, bulk compression, and shearing (penetration). This independence could also be related to the loss of entropy upon extensional measurements and the gain of entropy upon compression (Figure 13).

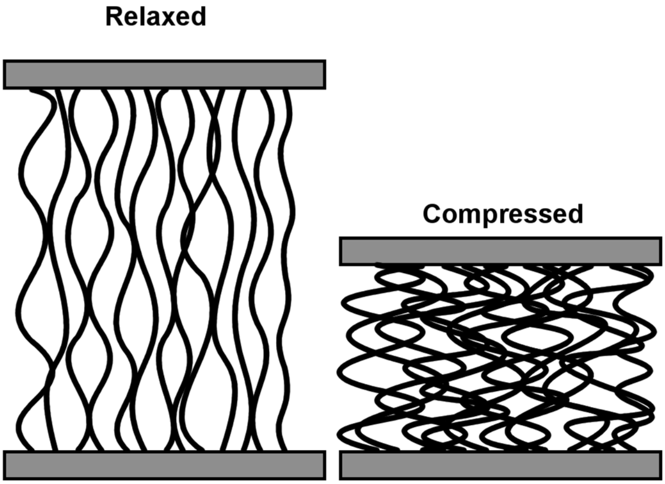

Figure 13: Different macromolecular arrengement upon compression.

Further analysis was performed to determine the robustness of the methods for the evaluation of flour blends with Andean grains in order to understand which methods are more suitable for the study of these kind of systems. In Paper IV, the same methods were used as in Paper III, although dough was prepared with different flour blends. Wheat flour was substituted with amaranth, canahua, and quinoa, respectively, at the levels 10, 20, and 30%. The results were evaluated using partial least squares regression analysis (PLSR). The results revealed that the most robust parameters were related with the biaxial extension, followed by the uniaxial deformation (Figure 14).

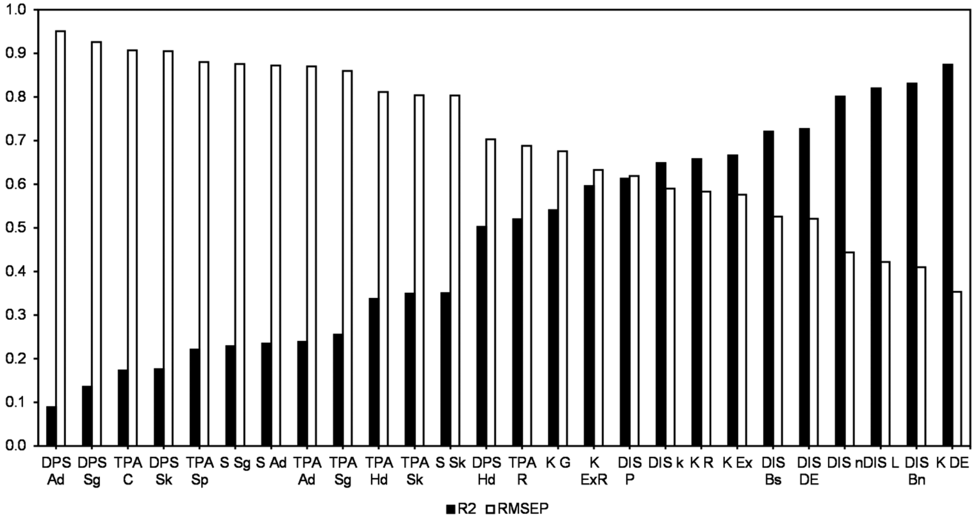

Figure 14: Comparison of robustness of the 26 textural variables based on Calibration R2 (R-Squared) and RMSE. Improved quality of parameters from left to right with R2 increasing (black columns), and RMSE decreasing (white columns).

In extensional measurements, the internal energy component increases (f_e) to a maximum to the break point, whereas f_s is reduced as the extension continues to the break, assuming that the timescale of the tests is sufficient. At this point, the macromolecules have a more stable conformation, whereas, the more stable conformation could not be reached during compression. The main disadvantage of compression tests is the presence of friction between the sample and the loading surfaces, which can lead to a non-homogeneous stress.

5 Andean grain flours in bread

Optimization of the use of Andean grain flours in bread

The transformation of dough into bread is a dramatic change involving physical, chemical and biochemical processes. Under the appropriate conditions, yeast activity causes dough to expand, resulting in a spongy-cellular structure within the dough. During the first stages of baking, the centre of the dough continues to expand, compressing the outer layer. Meanwhile, there is a loss of cellular structure in these regions where crust is formed. Thermodynamically, there is a temperature gradient from the region near the crust to the centre of the dough. The gradient is the driving force for the heat transfer, which is conducted along the dough structure or cell walls to the centre, whose temperature will eventually approach the boiling point. The change of moisture is not significant at the loaf centre.

The addition of Andean grain flours modifies dough structure that is intimately related to liquid phase, gluten quality and quantity, and the presence of particles such as starch and bran.

Paper V deals with bread properties when amaranth, canahua and quinoa are used alone and in binary and ternary combinations. A simplex-centroid design with seven trials (treatment) was applied for modelling the use of mixtures. Each trial was prepared under the same formulation with 25% each of Andean grain flours, or their combinations, blended with commercial wheat flour (by weight of total flour). The level of water was added according to Farinograph WA.

The trace plot for each Andean grain flour (Figure 15a for firmness and 15b for volume) shows the rather surprising results that the addition of quinoa (Q) increased both hardness and volume. Amaranth (A), on the other hand, presented the opposite trend as both volume and hardness decreased as the amount of A was increased. Finally, increasing additions of Canahua (C) led to a decrease in volume and an increase in hardness.

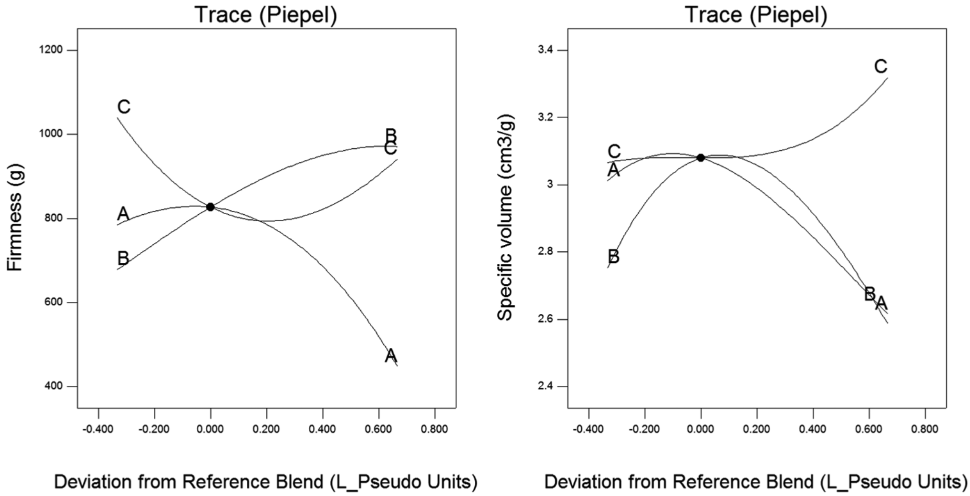

Figure 15: Trace plot showing the effects of Amaranth (A), Canahua (C), Quinoa (Q) on (a) firmness, and (b) specific volume, when replacing 25% of the wheat flour in bread.

The poor volume of canahua and amaranth bread could be related to the disruption of the gas cell owing to the bran particles (Paper V). The softer crumb with amaranth could be due to the reduced presence of amylose related to the initial retrogradation of starch and the consistency of fresh crumb. Quinoa provided the best volume, even higher than with wheat. Martines *et al*. related the small starch granules with better bubble cell stabilization owing to Pickering stabilization (113). Another interesting result was the stickier crumb with A, which could be related to the fact that its presence increased the amount of UFW, which is related to its water holding ability.

The combined results of paper II, IV and V strengthened the hypothesis that the combination of Andean grain flours could provide the same desirable attributes of a common wheat bread as regards volume and texture.

The binary combination of Q with A and C, namely AQ and CQ, but especially the ternary combination of them, ACQ, were similar to the control in relation to crumb structure (Figure 16)

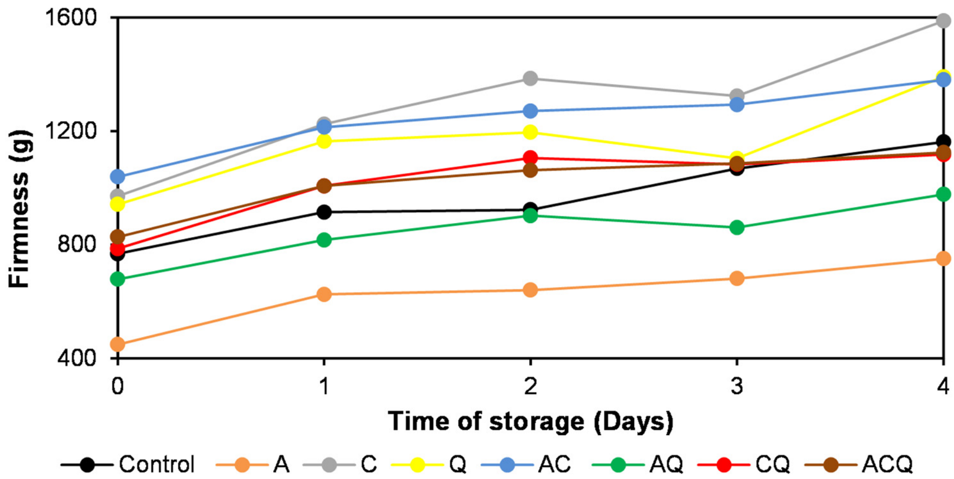

Figure 16: Bread firmness measured on day 0, 1, 2, 3, and 4 after baking for wheat flour bread (Control), and bread with amaranth (A), canahua (C), and quinoa (Q) flour, including pure Andean grain flours (A, C, Q), binary mixtures (AC, AQ, CQ), and ternary mixture (ACQ) replacing 25% of the wheat flour.

Contour curves obtained for hardness and volume (Figure 17 a and b, respectively) show the influence of each Andean grain flour and their combinations (Figure 17 c), and that it was possible to estimate the best combination of them to keep the volume and the hardness of the control.

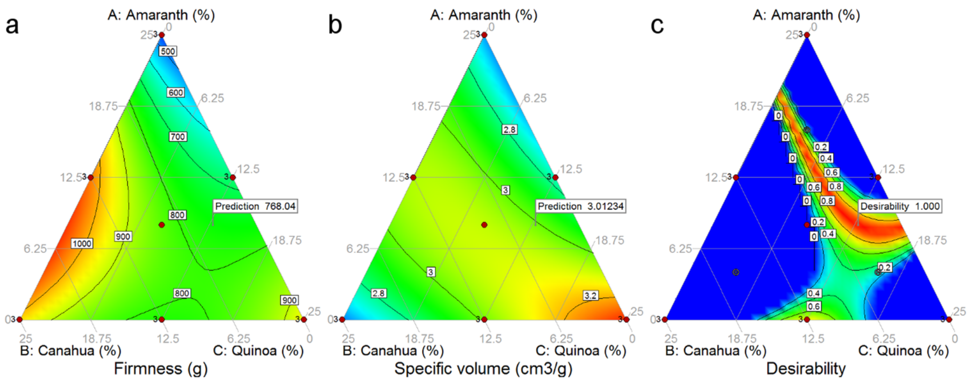

Figure 17:. Contour plots showing the effects of amaranth (A), canahua (C), quinoa (Q) on (a) firmness; (b) specific volume, (c) optimal area for mixtures of these considering firmness and volume of bread when replacing 25% of the wheat flour.

The coefficients for hardness showed that amaranth with quinoa reduced the firmness of crumb (Eq. 4), whereas canahua increased the values of firmness.

These results agreed with their water properties presented in Paper II. Volume was improved by the presence of quinoa alone and by the combination of amaranth and canahua (Eq. 5).

$$\text{Firmness} = 448.20*A + 970.94*C + 942.33*Q + 1317.22*AC - 67.45*AQ - 683.48*CQ \quad (4)$$

$$\text{Volume} = 2.62*A + 2.59*C + 3.32*Q + 1.86*AC - 0.86*AQ + 0.24*CQ \quad (5)$$

These results indicated that the optimal area for obtaining the firmness and volume desired, or equal to those of the control, corresponded to a proportion of Andean grains of A=8.58%, C=4.14% and Q=12.28%.

6 Conclusion

Andean grain flour doughs showed substantial differences in dough formation. Functional characteristics including phase separation, water, and thermal properties differed among the flours and when compared to wheat flour dough. The Andean grains contributed to different phases formed in the dough, and to differences in water behaviour, thereby resulting in different effects on wheat dough. There were more similarities between quinoa and amaranth, whereas the effects of canahua differed more.

The rheological properties of wheat dough and wheat dough substituted with Andean grain flours can be measured using different methods, but the most reliable are those performed under uniaxial and biaxial extension, which proved to be robust. Considering the deformation of these kinds of dough helped to improve understanding of the importance of unifying the methods used in order to obtain better and more reliable results for conclusions regarding dough systems when using flour mixtures.

The knowledge of phase separation and water, thermal, and rheological properties of the Andean grain flour doughs and their combination with wheat flour has increased the understanding of their function in breadmaking. This can lead to an increase in the possibilities of finding better systems to increase the addition of Andean grains and other type of flours into wheat in a better way.

Multivariate analysis was an important tool in exploring and understanding the relationship existing among the rheological responses, the nature of deformation, and the samples analysed. The multivariate analysis coupled with the design of experiments made it possible to gain a better understanding in the use of blends of wheat and Andean grain flours in breadmaking technology.

The use of Andean grain garnered attention owing to their important nutritional qualities and as a method of facing ongoing climate change. Their uses will gain increased importance with time, and this thesis has added important knowledge for the benefit of many.

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Eternal life to those who *by persistence in doing good*
seek glory, honour and immortality

Romans 2:7

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