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Use of amaranth, quinoa, kañiwa and lupine for the development of gluten-free extruded snacks

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ACADEMIC DISSERTATION

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ABSTRACT

Amaranth (*Amaranthus caudatus*), quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*) have been cultivated in the Andean region of South America since Pre-Hispanic times. They are regarded as formidable nutritious alternatives due their high content of protein (rich in lysine), dietary fibre and bioactive compounds such as tocopherols, phenolic compounds and folate. Despite this, the academic research conducted on their utilisation for human consumption is relatively low. Conversely, lupine (*Lupinus angustifolius*) is a well-known legume used for animal feed in most of the Nordic countries. The aim of this research was to incorporate amaranth, quinoa, kañiwa and lupine to corn-based snacks, and study their physical, chemical and sensory properties. A co-rotating twin screw extruder was used to obtain corn-based extrudates containing amaranth, quinoa, kañiwa and lupine. In preliminary studies (incorporation up to 20% of solids), Box-Behnken experimental design with three predictors was used: water content of mixture (WCM, 15-19%), screw speed (SS, 200-500 rpm) and temperature of the die (TEM, 150-170 °C). Subsequent studies were conducted using partial least squares regression (PLSR) and L-partial least squares regression (L-PLSR) with nine predictors: Grain type, grain content (20-50% of solids), temperature of die (140-160 °C), screw speed (200-500 rpm), water content of mixture (WCM, 14-18%) as well as contents of protein, ash, fibre and sum content of main fatty acids of blend.

In general, WCM and screw speed had the greatest importance for response variables such as torque and pressure at the die during extrusion, sectional expansion index (SEI), stiffness and water content of extrudate; the content of protein and dietary fibre in the blend was particularly relevant during the extrusion of extrudates containing kañiwa and lupine. Regarding the most expanded extrudates, those containing 20, 35 and 50% amaranth, quinoa or kañiwa presented comparable SEIs and stiffness while those containing above 20% lupine suffered from structural collapse. Extrusion reduced the content of fatty acids and tocopherols in the solids but it had a slight effect on the content of total phenolic compounds and folate. In sensory studies, extrudates with higher contents of amaranth, quinoa and kañiwa were rated less crispy, less crunchy and less adhesive with less hard particles. Temporal analysis showed that with increasing contents of amaranth, quinoa and kañiwa, crispiness and crunchiness became the most dominant attributes during mastication while the dominance of roughness reduced considerably. Porosity and wall thickness, measured by X-ray microtomography, were linked to the perception of crispiness and crunchiness, respectively. In storage, whole extrudates containing 20% amaranth, quinoa or kañiwa and exposed to RH of 76% presented the lowest formation of hexanal compared to milled extrudates exposed to RH of 11%.

This study showed that expanded corn-based extrudates containing up to 50% amaranth, quinoa and kañiwa and at most 20% lupine of solids can maintain key mechanical and textural properties as well as added nutritional value. This study applied successfully PLSR and L-PLSR modelling techniques to study the incorporation of amaranth, quinoa, kañiwa and lupine to corn-based snacks. This research has expanded the knowledge linked to the development of gluten-free extrudates with added nutritional value.

PREFACE

This thesis work was mainly carried out in the General Food Technology group at the Department of Food and Environmental Sciences during 2012-2015. The work was supported by the University of Helsinki Research Foundation, Hämäläisten Ylioppilassäätiö and ETL (Elintarviketeollisuusliitto).

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Ramos Diaz, J.M., Kirjoranta, S., Tenitz, S., Penttilä, P.A., Serimaa, R., Lampi, A.M., Jouppila, K. 2013. Use of amaranth, quinoa and kañiwa in extruded corn-based snacks. Journal of Cereal Science, 58, 59-67.

II. Ramos Diaz, J.M., Sundarrajan, L., Kariluoto, S., Lampi, A.-M., Tenitz, S., Jouppila, K. 2015. Effect of extrusion-cooking on physical and chemical properties of corn-based snacks containing amaranth and quinoa: Application of Partial Least Squares Regression. Revised version.

III. Ramos Diaz, J.M., Sundarrajan, L., Kariluoto, S., Lampi, A.-M., Tenitz, S., Jouppila, K. 2015. Partial Least Squares Regression modelling of physical and chemical properties of corn-based snacks containing kañiwa and lupine. Submitted version.

IV. Ramos Diaz, J.M., Suuronen, J.-P., Deegan, K.C., Serimaa, R., Tuorila, H., Jouppila, K. 2015. Physical and sensory characteristics of corn-based extrudates containing amaranth, quinoa and kañiwa flour. LWT-Food Science and Technology, 64, 1047-1056.

Contribution of the author to Studies I-IV

I, IV Jose Martin Ramos Diaz planned the study together with the other authors. He conducted extrusion experiments and sample pre-conditioning. He was responsible for the chemical and sensory analyses, and co-responsible for physical analyses involving micro- and nanostructures, as well as interpretation of results. He was the main author of the papers.

II, III Jose Martin Ramos Diaz planned the study together with the other authors. He conducted extrusion experiments and sample pre-conditioning. The chemical analyses were conducted partly as a Master's thesis (Lakshminarasimhan Sundarrajan). He was responsible for the physical analyses, and co-responsible for the exploratory data analyses and interpretation of results. He was the main author of the papers.

ABBREVIATIONS

CD	Coeliac disease
CoF	Content of amaranth or quinoa flour of solids
ETC	Easy to change
FA	Main fatty acids of blend
GS	Gluten sensitivity
HTC	Hard to change
L-PLSR	L-Partial least squares regression
PCA	Principal component analysis
PLSR	Partial least squares regression
SEI	Sectional expansion index
Total SME	Total specific mechanical energy
VIP	Value of importance in the projection
WCE	Water content of extrudate
WCM	Water content of mixture
WPC	Whey protein concentrate

1 INTRODUCTION

Amaranth (*Amaranthus caudatus*), quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*) are grains cultivated by pre-Hispanic civilisations for thousands of years. These grains are endemic to Andes of South America. Cultivation areas extend from Southern Colombia to Northern Chile and Argentina. Currently, Peru and Bolivia are the two largest producers and exporters of quinoa worldwide (Suca Apaza and Suca Apaza, 2008). Despite belonging to the same genus as quinoa, kañiwa is much lesser known than quinoa, and mostly consumed by local population from indigenous background (Woods Páez and Eyzaguirre, 2004). As kañiwa's genetic pool is still rather heterogeneous compared to quinoa (kañiwa is still not fully domesticated), the phenotypical variation has become a burden for the commercialisation of a grain that possesses, otherwise, superior nutritional characteristics to quinoa. Amaranth belongs to the same family tree as quinoa and kañiwa (*Amaranthaceae*) and, due to its wide range of edible species and cultivation areas; it is a better known commodity than quinoa and kañiwa. *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* are probably the most popular species of edible amaranth grains in the Americas (Tucker, 1986). Unlike amaranth, quinoa and kañiwa, lupine (*Lupinus angustifolius*) is a legume comparable to soybean, navy beans, faba beans and green peas. Lupine is a legume cultivated worldwide (from Finland to Australia) mostly for animal feed (Gade 1970; Bhat and Karin, 2009).

According to the Thorogood et al. (1994), Jew et al. (2009), Garnett (2009), American Dietetic Association (2003) and Micha et al. (2010), the overreliance on conventional cereals like corn or wheat, and meat products may not only have negative impact on human health but increase carbon emissions associated to the food industry. Therefore, the adoption of alternative grains, legumes and tubers will become a necessity in the coming years as the population grows into nine billion by 2050, water become increasingly scarce and new food-borne diseases appear (Eckstein, 2009; Suk and Semenza, 2011). Alternative foods such as amaranth, quinoa and, particularly, kañiwa are naturally resistant to extreme weather conditions, high salinity soils and require little water for its cultivation (Jacobsen, 2003). In fact, quinoa's potential to contribute to world food security has been widely acknowledged (National Research Council, 1989; FAO, 2011), and amaranth is considered as 'food for a future' given its high quality protein and resistance to drought and heat (European commission, 2011).

Amaranth, quinoa, kañiwa and lupine are gluten-free grains and, therefore, attractive food alternatives for those suffering from coeliac disease (CD) and/or gluten sensitivity (GS). CD is a systematic immune-mediated disorder of the small intestine due to the ingestion of gluten (i.e., prolamines from crops of the tribe triticeae such as wheat, barley and rye), which occurs in genetically susceptible individuals. Most patients with CD present a combination of gluten-dependent clinical manifestations such as discomfort in the digestive tract, chronic constipation, diarrhoea, failure to thrive etc. and the presence of specific antibodies (e.g., anti-tissue transglutaminase). According to Troncone and Jabri

(2011), it is difficult to draw a line between CD and GS as there are various degrees of clinical symptomatology. GS has been defined by the presence of morphological, functional and immunological disorders (e.g., epithelial distress, mucosal inflammation) as a consequence of gluten ingestion but without the key features of CD such as the absence of anti-tissue transglutaminase and histological enteropathy (Troncone and Jabri, 2011).

Despite being gluten-free and having formidable nutritional properties, amaranth, quinoa, kañiwa and lupine are currently underexploited by the food industry (Izquierdo and Roca, 1998). Amaranth and quinoa possess high quality protein (10-15%, albumin and globulin), considerably amount of fibre (8-10%) and bioactive compounds such as phenolic compounds, tocopherols and folate (Ranhotra et al., 1993; Guzman-Maldonado and Paredes-Lopez, 1998; Repo-Carrasco-Valencia, 2011b). Repo-Carrasco-Valencia et al. (1993) reported that quinoa var. Amarilla de Marangani had no limiting amino acids. Also, Ranhotra observed that protein quality in quinoa (protein digestibility, protein efficiency ratio and nitrogen balance) was equivalent to that of milk protein casein. Kañiwa has generally higher content of protein (15-20%), fibre (10-15%) and bioactive compounds (e.g., phenolic compounds, tocopherols) than amaranth and quinoa (Repo-Carrasco-Valencia et al., 2003; Siger et al., 2012) while lupine possess a very high content of protein (25-35%), fibre (40-50%) and comparable contents of bioactive compounds to amaranth, quinoa and kañiwa.

Little is known about the nutritional status of these grains after processing. Thus, there is scarce information on the physical and sensory characteristics of processed food products containing amaranth, quinoa, kañiwa and lupine. Some studies (Brennan et al., 2008; Willis et al., 2009) have confirmed that there is a notable reduction in carbohydrate digestibility and increase in satiety upon ingestion of cereal breakfast high in fibre. In that sense, amaranth, quinoa, kañiwa and lupine could be attractive ingredients for the development of snacks or cereal breakfast with added nutritional value. Yet the high content of protein and fibre represents a technological challenge for the development of food with appealing sensory characteristics (Lue et al., 1991; Liu et al., 2000).

Extrusion is a versatile low-energy technology that alters the physicochemical characteristics of cereal-like products, containing starch and protein, through a process that involves high pressure and temperature; such changes result from the rotational movement of a screw against the internal walls of the barrel. The degree of change of granulated solid materials (e.g., flours) into a melt (liquefied matter), generally resulting from starch gelatinisation, depends strongly on various process conditions such as the screw configuration (length of the metering section, compression ratio, number of flights, metering depth, feed depth, multiple stage screws etc.), screw speed, water content of the mixture, temperature profile and chemical composition of raw materials (content of starch, size of starch granules, content of fibre etc.). The changes in these extrusion conditions may have strong effects on the physical characteristics of extrudates such as sectional expansion index (SEI), density and hardness; these are good indicators of, for example, the degree of starch gelatinisation, consumer appeal and some particular sensory attributes. Alvarez-Martinez et al. (1988) suggested that the excess of water may greatly reduce elastic characteristics of amylopectin network thereby decreasing sectional expansion. Bhattacharya and Hanna (1987) observed that starch gelatinisation reduced at greater content of water of mixture during the extrusion of corn starch. This may potentially reduce the degree of expansion thereby hardening the extrudates. Besides, Suknark et al. (1999) studied the effect of temperature (90, 95 and 100 °C) and screw speed (100, 250 and 400 rpm) on the shear strength (expressed in N/g) of tapioca-fish and tapioca-peanuts snacks. The authors observed that increasing temperature and screw speed decreased shear strength and bulk density (mass of various pieces of extrudates divided by the volume they occupy).

Some research has been conducted regarding the incorporation of amaranth and quinoa to extruded snacks (Coulter and Lorenz, 1991; Ilo and Liu, 1999; Dokic et al., 2009). For instance, Coulter and Lorenz (1991) found that the incorporation of quinoa flour (10-30% quinoa) to corn-based extrudates reduced SEI and increased product density (mass of an individual extrudate divided by the volume that it occupies). Dokic et al. (2009) incorporated up to 50% amaranth to corn-based extrudates and observed a four-fold reduction in SEI and a three-fold increase in product density. It seems that extrudates with higher contents of protein and fibre tend to have lower SEI, higher product density and greater hardness (Lue et al., 1991; Yanniotis et al., 2007; Brennan et al., 2008).

Various studies (Broz et al., 1997; Grela et al., 1999; Suknark et al., 2001; Repo-Carrasco-Valencia et al., 2009) have shown that changes in the extrusion parameters had negative or positive effects on the retention of different bioactive compounds. Grela et al. (1999) found that extrusion of grass pea reduced the content of α- and β-tocopherol by 50 and 67%, respectively. Also, Anton et al. (2009) and Brennan et al. (2008) observed that the detectable content of total phenolic compounds increased (relative to the raw material) after the extrusion of common bean (*Phaseolus vulgaris L.*) and white wheat flour, respectively. Kariluoto et al. (2006) found that there was a loss of folate between 26 and 28% during the extrusion of rye and Håkansson et al. (1987) reported a loss of 20% of folate after the extrusion of white wheat flour. Also, amylose and fatty acids may form complexes during extrusion, thereby reducing the detectability of free fatty acids (Bhatnagar and Hanna, 1994a).

Multiple linear regression (MLR) has been commonly used in various extrusion studies, and for the development of snack products. When predictors (controllable and easy-to-measure variables) are few and have a relatively well-understood relationship to response variables (variables whose behaviour is attempted to be explained), MLR can be a useful tool to turn data into information (Randall, 1995). Nonetheless, if any of these conditions are unfulfilled, MLR may be an inappropriate tool for predicting modelling. In that sense, partial least squares regression (PLSR) is method for constructing predictive models, particularly, when response variables are many, noisy, missing and present high collinearity.

According to Wold et al. (2001), the solution of PLSR to a regression problem is statistically more robust than the solution given by MLR, leading to more reliable predictions.

The objective of this study was to evaluate the effects of amaranth, quinoa, kañiwa and lupine on the physical and sensory properties, as well as chemical composition of corn-based extruded snacks. The application of relatively new modelling techniques such as PLSR and L-partial least squares regression (L-PLSR) was a key part of the study.

2 LITERATURE REVIEW

2.1 Andean crops

Amaranth (*Amaranthus caudatus*), quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*) do not belong to the same family (*Poaceae* or *Graminea*) as well-known cereals grains like wheat, oat or barley (**Figure 1**). Yet they can be ground and used as conventional cereals; there comes the name 'pseudo cereal'. In this regard, there is some controversy among Peruvian scholars about the term 'pseudo cereal' as it, probably, overemphasises the crops' condition of 'non-cereal' thereby belittling their potential as food (Agraria, 2013). The preferred term remains 'Andean grains'.

Amaranth, quinoa and kañiwa were staples for many pre-Hispanic cultures that inhabited in the Andean regions. The capacity of these plants to resist very low temperatures, high salinity soils and poor terrains made them essential to secure nutrition in, otherwise, inhospitable places. *Amaranthus caudatus* was domesticated and cultivated in South America (Pickersgill, 2007) and, according to Tapia (1979), Andean farmers used to intercrop *Amaranthus caudatus* and *Amaranthus mantegazzianus* with corn and quinoa. Unlike quinoa, these species of amaranth could not be cultivated in areas of greater elevation (Tapia, 1979).

Despite the nutritional and cultural importance of quinoa, there is little historical evidence regarding its domestication (Nuñez 1970). Some traces of quinoa seed found in Northern Chile may suggest that its use as food may date back to 3000 B.C. Some other evidence found in the region of Ayacucho (Central Peru) suggests that quinoa's domestication may date back to 5000 B.C. (Uhle, 1919). There is even less archaeological evidence regarding the origin of kañiwa. It seems that most historians and chroniclers of the 17th century mistook kañiwa for quinoa, and it was not until 1929 that the Swiss botanist Paul Aellen proposed the scientific name *Chenopodium pallidicaule* for kañiwa.

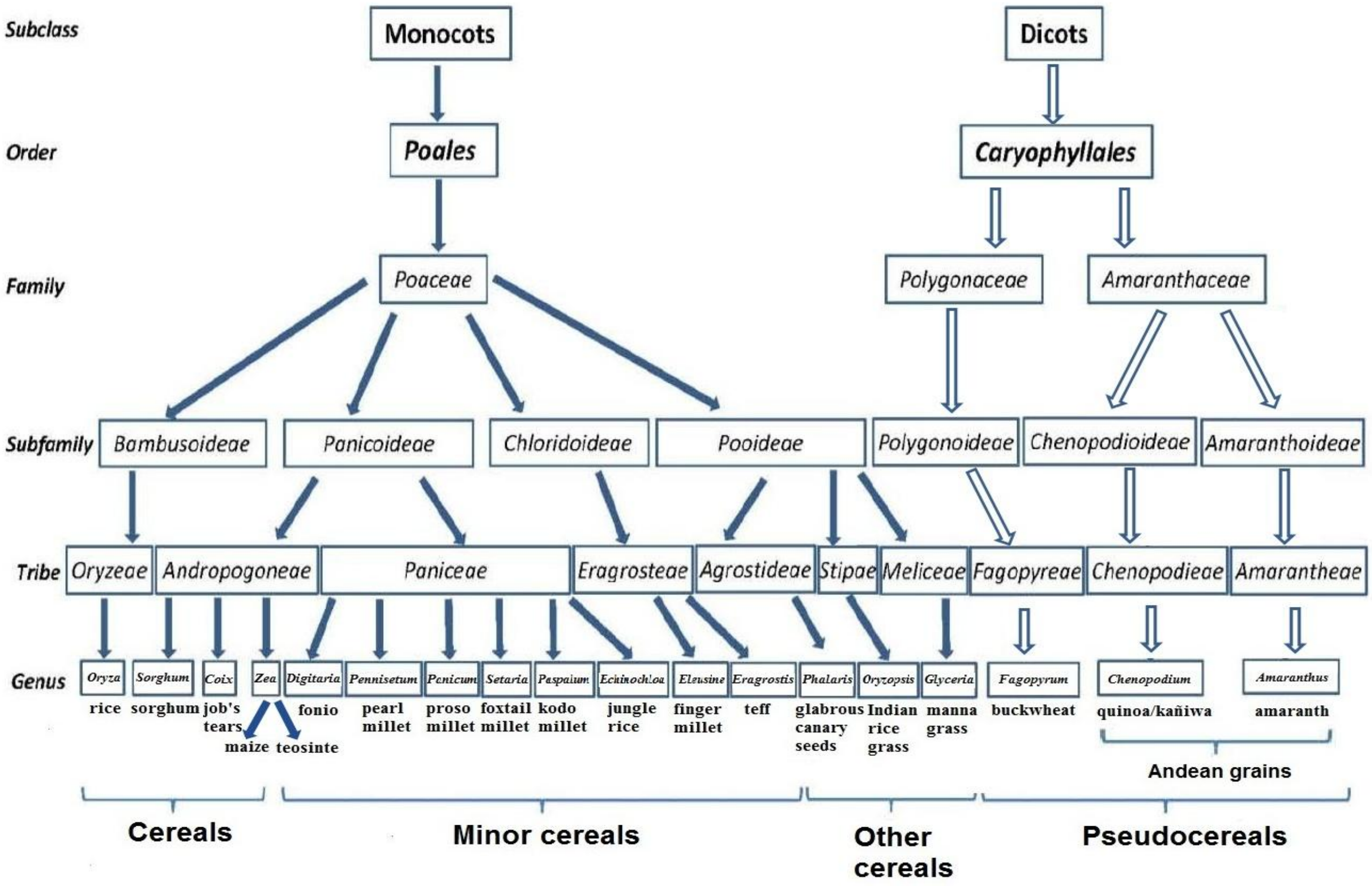


Figure 1. Taxonomic relation of known non-toxic cereals, minor cereals, pseudocereals and other cereals [modified from Moreno et al. (2014)].

2.1.1 Amaranth

Amaranth is a cosmopolitan genus of annual perennial plants that are cultivated as leaf vegetables, edible grains and ornamental plants. Since there are around 60 species of amaranth, the present dissertation will focus only on *Amaranthus caudatus*, a species endemic to the Andean region of South America. The plant can grow to up to 2.5 m and has veined lance-shaped leaves with purple on the under face. One of the plant's iconic features is red cymes of densely packed flowers (**Figure 2A**); the red colour is mainly due to the high content of betacyanins. The seeds of amaranth have round shape with a diameter between 1-1.5 mm (**Figure 2B**). As there are many varieties within the same species, some grains can have ivory colour while others may be red or dark brown.

2.1.2 Quinoa

Quinoa is a dicotyledonous annual plant that usually grows to up to 2 m. This plant has pubescent and lobed leaves whose colour can vary among green, red or purple, depending on the variety. Flower panicles grow from the top of the plant or from the leaf axils. Panicles have a central axis with secondary or tertiary axis from which flowers emerge (**Figure 2C**). The grain is disk-shaped with a flat equatorial band around its periphery. The size of the grain can vary considerably depending on the variety. A large seed has a diameter between 2.2-2.6 mm, a medium seed between 1.8-2.1 mm and a small seed lower than 1.8 mm (**Figure 2D**).

2.1.3 Kañiwa

Kañiwa is a terophyte plant that is branched from the base. This plant can grow between 0.2 and 0.7 m. The upper part of the stem, leaves and flowers are covered by white or pink vesicles. Leaves have a rhomboid form with a length between 1 and 3 cm. The upper part of the leave presents three lobes with veined under face (**Figure 2E**). The grain has a lenticular form with a diameter between 1-1.2 mm. The colour of the grain is light brown or black with a fine episperm (**Figure 2F**).

2.2 Finnish grain legumes

Peas (*Pisum sativum L.*), faba bean (*Vicia faba L.*) and blue lupine (*Lupinus angustifolius*) are the most cultivated grain legumes in Finland. According to Huurre (2003), one of the first cultivation sites for peas was located in Niuskala (near Turku) and may date back to around 500 BC, while faba bean was probably grown in Laitila and Hattula around AD 600-800. As a measure to counteract erosion, Nootka lupine was introduced to Iceland during the mid-20th century (Pylvänäinen, 2010). From there, it may have spread to the

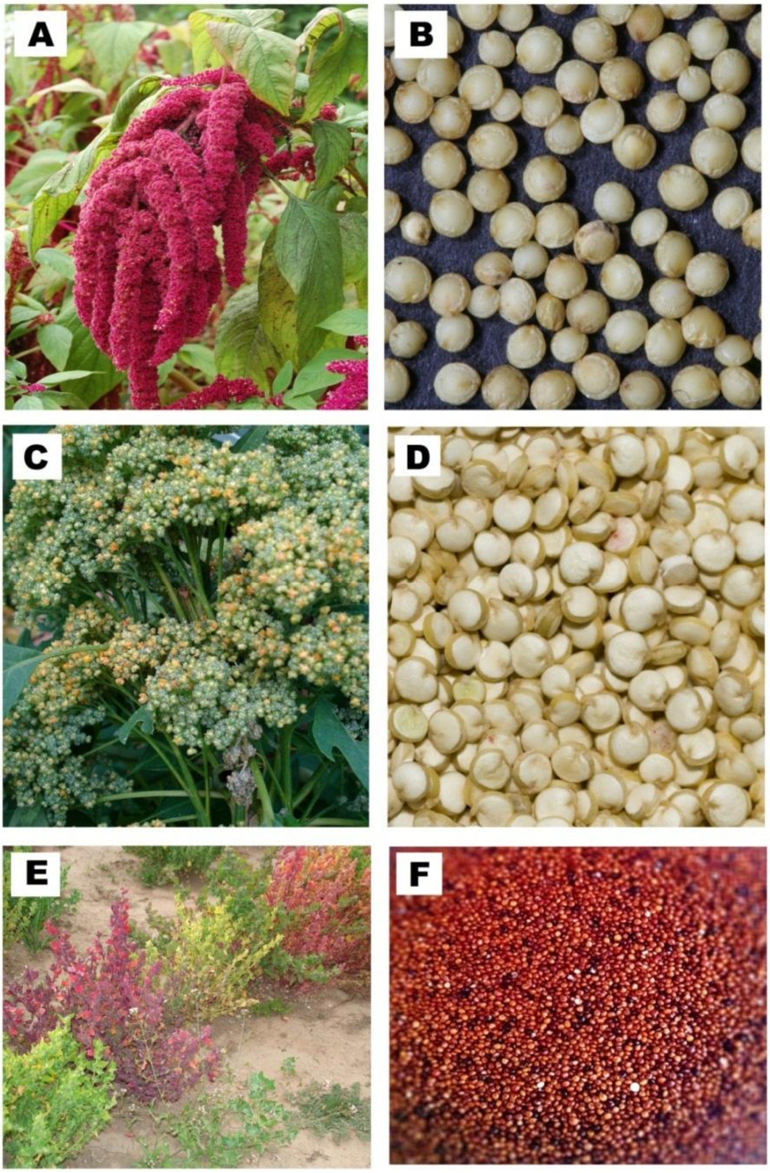


Figure 2. Plant and grain corresponding to *Amaranthus caudatus* (A, B), *Chenopodium quinoa* (C, D) and *Chenopodium pallidicaule* (E, F) [Photos reprinted under the permission of Vivian Polar (E), Misti Sayani (F); the rest of the photos (A, B, C and D) are licensed for non-commercial reuse].

neighbouring countries like Norway, Sweden and Finland. Despite its long history as food for human consumption, less than 1% of Finnish arable land is devoted to the cultivation of grain legumes nowadays (Stoddard, 2009). This responds to a sharp decline in the cultivation of grain legumes in Finland between the late 1930s to the late 1960s in order to give place to animal farms as living standards improved. At present, Finland is strongly dependent on soybean imports from the USA for feed or food uses (Stoddard, 2009; Ekroos, 2010). Replacing soybean imports for home-grown grain legumes could not only reduce costs but allow farmers to benefit from biologically fixed nitrogen, improved soil structure (prevention of erosion) and disrupt cereal disease cycles (Stoddard, 2009).

Blue lupine is a narrowed-leafed plant that, like other legumes, fixes nitrogen in a symbiotic interaction with *Bradyrhizobium lupini* and *Kribbella lupine* (Trujillo et al., 2006). Lupine is an erect and branching herb that grows around one meter. The inflorescence bears flowers in shades of blue, violet, pink or white (**Figure 3**). Legume pods are containing grain seeds of colours ranging from brown to white or speckled (FAO, 2012). Even though blue lupine contains naturally a large amount of alkaloids, varieties with low content of alkaloids (sweet lupine) have been bred.

Figure 3. Plant (left) and seed (right) corresponding to *Lupinus angustifolius* (Photos licensed for non-commercial reuse).

2.3 Nutritional properties of amaranth, quinoa, kañiwa and lupine

From a general perspective, the nutritional importance of amaranth, quinoa, kañiwa and lupine relies on their high quality protein, and high content of fat and fibre compared to cereal grains such as wheat, rice and rye (**Table 1**). For instance, lupine seeds had the highest content of protein, only comparable to soybeans and beans, but had relatively low content of fat. Conversely, amaranth seeds had higher contents of fat than quinoa, kañiwa, lupine and most cereal grains, but almost half as much as in soybeans. Regarding fibre, lupine (e.g., var. Borre) had comparatively higher contents of crude fibre than any other grain included in **Table 1**. Amaranth, kañiwa and some varieties of quinoa (e.g., ‘sweet’,

Table 1. Chemical composition of amaranth, quinoa, kañiwa, lupine and cereals (g/100 g dry matter)

Grain/cereal	Variety/ type	Content (% d.m.)					References
		Protein	Fat	Crude Fibre	Ash	CHO*	
Amaranth							
	Centenario	14.6	10.1	7.43[a]	2.4	65.6	Repo-Carrasco-Valencia et al., 2009a
	Oscar Blanco	14.7	10.2	7.27[a]	2.6	65.3	
Quinoa							
	40057[4]	14.1	9.7	n.m.	n.m.	72.5	Gonzales, 1989
	Bitter	15.7	5.7	10.3[a]	3.1	66.5	Wright et al., 2002
	Huancayo	14.4	6.0	4.0	2.9	72.6	Repo-Carrasco-Valencia, 1992
	Red	15.4	7.5	2.5	3.1	68.4	De Bruin, 1963
	Sajama	11.2	4.0	n.m.	3.0	77.2	Gonzales, 1989
	Sweet	14.8	5.3	8.8[a]	2.6	69.1	Wright et al., 2002
	Unkown	16.5	6.3	3.8	3.8	69.0	Valencia-Chamorro, 2003
	White	14.1	7.2	2.1	2.4	74.3	De Bruin, 1963
	Yellow	16.0	6.2	3.1	3.7	68.5	
Kañiwa							
	Blanca	18.8	7.6	6.1	4.1	63.4	Repo-Carrasco-Valencia, 1992
	Cupi	14.4	5.7	11.24[a]	5.0	63.6	Repo-Carrasco-Valencia et al., 2009b
	Ramis	14.9	7.0	8.18[a]	4.3	65.7	
	Unknown	16.9	8.8	3.9	2.8	67.6	De Bruin, 1963
L. Angustifolius							
	Boregine	29.4	n.m.	n.m.	3.7	0.7[b]	Lizarazo et al., 2010
	Borre	36.4	4.8	14.6	3.5	36.4	Oomah and Bushuk, 1983
	Haags[3] Blaue	31.0	n.m.	n.m.	3.7	0.6[b]	Lizarazo et al., 2010
	Unknown	39.1	7.0	14.6	4.0	35.3	Repo-Carrasco-Valencia et al., 2008
Wheat[2]							
	Manitoba	16.0	2.9	2.6	1.8	74.1	Kent, 1983
Rye		13.4	1.8	2.6	2.1	80.1	
Rice		9.1	2.2	10.2	7.2	71.2	
Corn		11.1	4.9	2.1	1.7	82.0	
Oat[2]		11.6	5.2	10.4	2.9	69.8	
Soybean		36.1	18.9	5.6	5.3	34.1	Valencia-Chamorro, 2003
Bean		28.0	1.1	5.0	4.7	61.2	

[a] Value expressed in dietary fibre

[b] Starch content

*CHO, carbohydrates; n.m. = not measured ‘bitter’, ‘red’, ‘yellow’) presented substantially lower content of total carbohydrates than rye and corn. Regardless of their variety, lupine had the highest contents of non-starch polysaccharides (Rubio et al., 2005).

2.3.1 Protein and amino acid content

The grains of amaranth, quinoa and kañiwa have distinct morphological characteristics compared to cereals like wheat, barley and sorghum. Unlike cereals, the embryo surrounds the perisperm where starch and storage proteins, such as glutelins and prolamins, are located. The proteins located in the embryo are albumin and globulin; these are metabolically active as they participate in the generation of new cellular structure. Several studies (Tellerias, 1976; Scarpati De Briceño and Briceño, 1980; Romero, 1981; Ballon et al., 1982) showed that quinoa presented a much larger fraction of albumins and globulins, and lower fraction of glutelins and prolamins compared to maize, rice and wheat (**Table 2**). Watanabe et al. (2003) studied the protein fraction of quinoa and observe that the content of globulins were comparatively higher than albumins (**Table 2**). In contrast, amaranth seems to have a larger fraction of albumins (albumin-1 and albumin-2) and glutelins than globulins and prolamins (Segura-Nieto et al., 2004). Bressani and García-Vela (1990) studied the protein fraction of *Amaranthus caudatus*, *Amaranthus hypocondriacus* and *Amaranthus cruentus*. The authors found that *A. caudatus* had the largest fractions of albumins and globulins, and they all had comparable fractions of glutelins and prolamins (**Table 2**). Scarpati de Briceño (1979) observed that the contents of albumins + globulins and glutelins in kañiwa are comparable to those of quinoa. It seems that the content of prolamins in kañiwa is among the highest, similar to those of wheat or maize (**Table 2**).

Legumes like lupine are known for accumulating large amounts of protein during their development and having very small content of starch (<2% d.m.). Globulins are the major protein components while albumins are only minor components (Cerletti et al., 1978). According to Osborne’s classification, the rate of albumin and globulin fraction is around 1 to 9 (Blagrove and Gillespie, 1975; Duranti et al., 1981) (**Table 2**). Storage proteins such as globulins are bound to organelles in cotyledonary parenchyma cells. During hydrolysis (e.g., germination), these proteins provide ammonia and carbon skeleton to the developing seedlings but, unlike albumins, are devoid of catalytic activity. In legumes, globulins were empirically classified according to their molecular size and sedimentation coefficient as 11S (legumin) and 7S (vicilin) (Duranti and Gius, 1997). The 11S proteins are oligomers (hexamers), resistant to dissociation at high ionic strength (low pH) while 7S are also oligomer (trimers), but sensitive to changes in ionic strength. Larger protein aggregates like 15-18S were found in soybean proteins but not in lupine seeds (Koshiyama, 1983). Nowadays, it is possible to classify unequivocally globulin fractions as α-, β-, γ- and δ-conglutins on the basis of their electrophoretic mobility, amino acids and nucleotide sequences.

Amino acids are the ‘building blocks’ during the process of biosynthesis, and are classified as indispensable, conditionally indispensable and dispensable depending on their biological activity and availability. Indispensable or essential amino acids cannot be synthesized by the organism, and their absence hinders the synthesis of protein-based tissues and enzymes. The chronic deficiency of essential amino acids (protein deficiency) has been shown to affect seriously body organs and systems which includes immunodeficiency, brain function of infants and young children, gut mucosal function and absorption of nutrients, kidney function etc. (Gomez et al., 1958 ; Zeisel et al., 1991; Massey et al., 1998; Otten et al. 2006). The importance of conditionally indispensable amino acids is strictly related to an individual pathophysiological condition such as prematurity or catabolic distress (Zeisel et al., 1991; Massey et al, 1998; Otten et al. 2006). Dispensable amino acids are synthesised from non-amino acid sources of nitrogen and they are commonly found in staple food such as rice and wheat. Some indispensable amino acids found in food protein fall short of meeting the amino acid requirement for humans, bearing the name of ‘limiting amino acids’. Generally, animal protein contains all the essential amino acids in appropriate proportion while plant proteins tend to have an insufficient amount of essential amino acids. In fact, Thomas B. Osborne was the first scientist proposing lysine as the first-limiting amino acid in maize and wheat flour (Osborne and Leavenworth, 1913; Osborne and Mendel, 1915, 1916). In addition, Nollau (1915) showed that rice, oat and barley had very low contents lysine. In contrast, White et al. (1955) and Mahoney et al. (1975) observed that lysine is not a limiting amino acid in quinoa and kañiwa. The authors also suggested that the protein quality of quinoa and kañiwa is comparable to casein.

Repo-Carrasco-Valencia (1992) observed that amaranth had a slightly higher content of lysine than quinoa and kañiwa, and way above than rice and wheat. Moreover, Pisarikova et al. (2005) found one variety of amaranth (Elbus) that had substantially higher content of lysine and arginine compared to other varieties of quinoa and lupine, and kañiwa (**Table 3**). Prakash and Pal (1998) studied the amino acid composition of three varieties of quinoa, originally from Guatemala, Bolivia and India. The authors found that the varieties from Guatemala and Bolivia had higher content of lysine and arginine than the variety from India, which, in contrast, had the highest content of valine and leucine (**Table 3**). According to Oomah and Bushuk (1983) and Sujark et al. (2006), the content of lysine in lupine is easily comparable to those in amaranth, quinoa and kañiwa (**Table 3**) while the content of arginine in lupine is higher than in amaranth (except for var. Elbus), quinoa and kañiwa. Lupine and quinoa presented the lowest contents of methionine compared to amaranth, kañiwa, rice and wheat (**Table 3**). From all these grains, amaranth var. Elbus had the lysine content, closest to milk.

Table 2. Protein fractions (as percentage of total protein) of amaranth, quinoa, kañiwa, lupine and common cereals.

		Content of protein fraction (% of total protein)					
Grain/ cereal	**species/ variety**	**Albumins + globulins**	**Albumins**	**Globulins**	**Glutenins/ glutelins**	**Gliadins/ prolamins**	**References**
Amaranth	*A. caudatus*	43.4	22.9	20.5	44.3	1.7	Bessani and Garcia-Vela, 1990
	A.hypochondriacus	37.3	19.2	18.1	42.3	2	
	A. cruentus	39.1	20	19.1	46.3	2.8	
Quinoa		45	n.m.	n.m.	32[a]	23	Scarpati de Briceño, 1979
Quinoa		55	n.m.	n.m.	39.8[a]	5.2	Tellerias, 1976
Quinoa		43.6	n.m.	n.m.	29.2	27.2[a]	Scarpati de Briceño and Briceño, 1980
Quinoa		76.6	n.m.	n.m.	12.7	7.2	Romero, 1981
Quinoa		10.9	n.m.	n.m.	59.4	0.5	Ballon et al., 1982
Quinoa		68	31	37	n.m.	0.8	Fairbanks et al., 1990
Quinoa		62.6	28.5	34.1	n.m.	n.m.	Watanabe et al., 2003
Kañiwa		41	n.m.	n.m.	31[a]	28	Scarpati de Briceño, 1979
Lupine	*L. angustifolius* v. Borre	72	3	69	21	n.m.	Oomah and Bushuk, 1983
Lupine	*L. angustifolius* v. Unicrop	76	4	72	17	n.m.	
Lupine	*L. albus* v. Kali	86	8	78	8	n.m.	
Lupine	*L. albus* v. Neuland	84	2	82	6	n.m.	
Lupine	*L. albus*	99	12	87	n.m.	n.m.	Duranti et al., 1981
Maize		38.3	n.m.	n.m.	37.2	24.5	Lasztity, 1984
Rice		19.2	n.m.	n.m.	71.9	8.9	
Wheat		17.1	n.m.	n.m.	54.4	28.5	

[a] Fraction combined with insoluble protein residues

n.m. = not measured

2.3.2 Fatty acid and tocopherol content

The biggest difference between amaranth, quinoa, kañiwa and other grains detailed in **Table 4** is the content of α-, β-, γ-tocopherol and, perhaps, a slightly higher degree of unsaturation. Wood et al. (1993) and Ryan et al. (2007) observed that the percentage of unsaturated fatty acids of quinoa was around 90%; this was higher than in amaranth (Bruni et al., 2001), kañiwa (Repo-Carrasco-Valencia et al., 2003) and cereal grains such as rye and barley (**Table 4**). Regarding the fatty acids, barley had higher fraction of palmitic acid than amaranth and kañiwa, and twice as much as quinoa and lupine. Lupine (*L. albus*) had the highest fraction of oleic acid (almost 50%) while amaranth, quinoa and kañiwa had around half of it (**Table 4**). Except for *L. albus* and safflower, all the grain shown in **Table 4** had fractions of linoleic acid above 40%. Compared to oleic and linoleic acid, linolenic acid had the smallest fraction in almost all grain shown in **Table 4**. However, two varieties quinoa and one of kañiwa presented high fractions of linolenic acid (around 25%), shifting the ratio of linoleic/linolenic acid (omega 6/omega 3) from 10/1 (most cereal grains and legumes in **Table 4**) to almost 2/1. According to Simopoulos (2002), humans evolved on a diet where the ratio of linolenic/linoleic acid was around 1 while, in the current western diets, the ratio is about 15/1 to 16.7/1. Such misbalance has been associated with many chronic conditions, cardiovascular disease, diabetes, cancer, obesity, rheumatoid arthritis, asthma and depression (Patterson et al., 2012).

Simopoulos (2002) explained that the increasing amounts of linoleic acid lead to greater accumulation of eicosanoid metabolic products such as prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acids and lipoxins. Such compounds happened to be biologically active in very small quantities but, in large amounts, they contribute to the formation of thrombus, atheromas, inflammatory disorders, cell proliferations and decreasing of bleeding time, which is common among patients with hypercholesterolemia and hyperlipoproteinemia (Joist et al., 1979; Brox et al., 1983).

Traditional cereal grains and legumes, including lupine, have generally very low content of α-, β- and γ-tocopherol (**Table 4**). In contrast, quinoa and kañiwa have substantially higher contents of α- and γ-tocopherol than cereals and legumes (Repo-Carrasco-Valencia et al., 2003) (**Table 4**). Regardless of the variety, amaranth showed the highest content of β-tocopherol (**Table 4**). Amaranth, quinoa and kañiwa could increase dramatically the intake of tocopherols only if their frequency of consumption increases. Simopoulos (1999) claimed that the presence of tocopherols (vitamin E) in an average human diet (e.g., western diet) has reduced considerably since the early 19th century, which parallels the start of the industrial age. The adequate consumption of tocopherols along with vitamin C serves to scavenge and, eventually, destroy harmful reactive oxygen species (ROS). The human body is constantly exposed to such oxidants (e.g., breathing), and it has evolved an antioxidant system that, though effective, fails to protect the body against the

Table 3. Content of essential amino acids (g amino acid / 16 g of nitrogen) in quinoa, kañiwa, amaranth, lupine and cereals

	Content of essential amino acids (g amino acid / 16 g of nitrogen)												
	Amaranth (var. Elbus)[4]	**Amaranth** (var. Oscar Blanco)[2]	**Quinoa** (var. Huancayo, Peru)[2]	**Quinoa** (Guatemala)[3]	**Quinoa** (Bolivia)[3]	**Quinoa** (var. album, India)[3]	**Quinoa**[5]	**Kañiwa** (var. Blanca)[2]	**Lupine** (*L. albus* v. Kali)[1]	**Lupine** (*L. angustifolius* v. Borre)[1]	**Lupine** (*L. Angustifolius* v. Baron)[5]	**Rice**[1] **-2**	**Wheat**[1] **-2**	**Milk**[6]
Arg[b]	13.5	8.2	8.1	6	4.8	3.5	n.m.	8.3	9.6	10.7	11.7	6.3	4.8	n.m.
Cys[b]	3.1	2.3	1.7	1.1	1.4	0.8	n.m.	1.6	n.m.	n.m.	1.5	2.5	2.2	n.m.
His[a]	1.7	2.4	2.7	2.7	3.3	1.8	3.2	2.7	2.6	2.7	3.2	2.2	2	2.7
Ile[a]	3.4	3.2	3.4	4.1	3.8	3.9	4.9	3.4	5.2	4.3	4	3.5	4.3	10
Leu[a]	5.9	5.4	6.1	6.2	6.1	6.9	6.6	6.1	8.6	6.7	6.8	7.5	6.7	6.5
***Lys**[a]	7.6	6	5.6	5.8	5.6	5.1	6	5.3	6	5.1	5	3.2	2.8	7.9
Met[a]	2	3.8	3.1	0.4	0.7	0.6	5.3	3	0.7	0.7	0.7	3.6	1.3	2.5
Phe[a]	n.m.	3.7	3.7	3.2	3	3.3	6.9	3.7	4.4	4	3.8	4.8	4.9	1.4
Val[a]	4.7	3.8	4.2	5	5.5	6	4.5	4.2	5	4.2	3.9	5.1	4.6	7
Thr[a]	4.7	3.3	3.4	3.5	3.7	3.5	3.7	3.3	4.3	3.5	3	3.2	2.9	4.7
Trp[a]	n.m.	1.1	1.1	n.m.	n.m.	n.m.	0.9	0.9	n.m.	n.m.	0.7	1.1	1.2	1.4
Tyr[b]	n.m.	2.7	2.5	1	1.4	1	n.m.	2.3	3.7	2.8	1.6	2.6	3.7	n.m.

[a] Indispensable amino acids

[b] Conditionally indispensable amino acids

*Limiting amino acid in cereals

n.m. = not measured

[1]Oomah and Bushuk, 1983; [2]Repo-Carrasco-Valencia, 1992; [3]Prakash and Pal, 1998; [4]Pisarikova et al., 2005; [5]Sujark et al., 2006; [6]Jancurova et al., 2009

Table 4. Degree of unsaturation, fatty acid and tocopherol content in quinoa, kañiwa, amaranth, lupine, cereals and seeds

		Content							
	Unsaturated fatty acids, %	**C 16:0, %**	**C 18:1, %**	**C 18:2, %**	**C 18:3, %**	**α-tocopherol, µg/g**	**β-tocopherol, µg/g**	**γ-tocopherol, µg/g**	**Reference**
Amaranth[a]	75.7	16.5	26.2	46.9	n.m.	47.84	61.6	2.53	Bruni et al., 2001
Amaranth[b]	79.7	12.3	32.9	46.3	n.m.	32.1	41.5	5	
Amaranth	n.m.	15	27	43	1	110	295	105	Repo-Carrasco-Valencia et al., 2003
Quinoa	82.7	n.m.	n.m.	50.2	26	721.4	n.m.	797.2	
Quinoa	88.8	9.2	29.5	48.1	8	n.m.	n.m.	n.m.	Ryan et al., 2007
Quinoa	n.m.	0.6-1.1	22.8-29.5	48.1-52.3	4.6-8	n.m.	n.m.	n.m.	Abugoch James, 2009
Quinoa	89	8.5	23	52.3	24.2	n.m.	n.m.	n.m.	Wood et al., 1993[2]
Quinoa	n.m.	9.6	21.1	56	6.7	n.m.	n.m.	n.m.	Przybylski et al., 1994[3]
Kañiwa	72.9	n.m.	n.m.	42.6	23.5	726	n.m.	788.4	Repo-Carrasco-Valencia et al., 2003[5]
L. angustifolius	84.9	7.6	31.2	48.3	5.4	0.3	n.m.	12.7	Hansen and Czochanska, 1974; Fernandez-Orozco et al., 2006
L. albus	n.m.	7.6	47.6	20.3	9.2	n.m.	n.m.	n.m.	Uzun et al., 2007
Sesame	n.m.	9.4	36.8	45.3	0.4	7.4	n.m.	280.9	Uzun et al., 2007; Cooney et al., 2009
Barley	77.5	20.4	14.9	58	4.4	15	1[c]	n.m.	Ryan et al., 2007
Rye	83.6	15	17.4	58.7	6.8	Tr	1	n.m.	
Peas	85.3	10.4	28.2	47.6	9.3	104	57[c]	n.m.	
Soy	n.m.	10.7	22	56	7	42.6	n.m.	26.5	Abugoch James, 2009; Ujiie et al., 2005
Corn	n.m.	10.7	26.1	57.7	2.2	n.m.	n.m.	n.m.	Abugoch James, 2009
Safflower	n.m.	4.8	75.3	14.2	n.m.	n.m.	n.m.	n.m.	Wood et al., 1993

[a] *A. caudatus* var. Macas (Ecuador)

[b] *A.caudatus* (Italy)

[c] β- and γ-tocopherol; Tr, traces; n.m. = not measured

accumulation of ROS. Long-term exposure to ROS may lead to various pathological changes such as Alzheimer's and Parkinson's disease, cardiovascular disease, cancer, arthritis, cirrhosis and cataract (Benzie, 2003).

2.3.3 Carbohydrates and dietary fibre

Amaranth and quinoa seeds contain between 60 and 75% carbohydrates (of solids) (**Table 1**), from which between 48 and 69% is starch (Qian and Kuhn, 1999). The chemical composition of starch granules from amaranth, quinoa, cereal grains and legumes is detailed in **Table 5**. The starch of amaranth and quinoa had the greatest content of crude fat and protein, respectively (Stone and Lorenz, 1984; Lorenz, 1990). The starch of cereals such as barley, corn, wheat and wild rice presented amylose contents between 20 and 35% while quinoa and amaranth presented between 9-19% and 0.2-13% amylose, respectively (**Table 5**). Information on starch granules from kañiwa and lupine could not be found in the literature; however, it is plausible that starch granules from kañiwa have similar chemical characteristics to quinoa as they belong to the same family (*Chenopodium*)

The size and morphology of starch granules from amaranth and quinoa are particularly distinct compared to granules from cereal grains such as barley, rye and corn (**Figure 4**). In general, starch granules from cereal grains, legumes and tubers (e.g., potato) are larger than those from amaranth and quinoa. Potato presented the widest range of granule sizes, with the largest granules measuring up to 75 µm. Legumes such peas and beans also presented a wide range of granule sizes, with a maximum of 45 µm. Barley (var. large granule) presented the largest granule size (45 µm) compared to other cereal grains such as rye, wheat, oat, rice and corn (descending order; **Table 5**). Amaranth and quinoa had the smallest starch granules of all, ranging from 0.5 and 2 µm (**Table 5**).

The morphology of starch granules may vary considerably depending on the type of grain, variety or chemical composition. For instance, Lindeboom (2005) noticed that as the content of amylose increased, starch granules of quinoa lost their sharp edges and acquired a smoother surface (**Figure 4D, E**). In contrast, starch granules of amaranth (*A. cruentus*) seemed sharper at increasing contents of amylose (**Figure 4A-C**). In addition, Jane et al. (1994) compared the starch morphology of various cereal grains, legumes, tuber and amaranth grains. The authors found that amaranth (*A. caudatus*) had the smallest starch granules (**Figure 4G**) while barley and rye showed considerable variation in granule sizes (**Figure 4H, I**); even the smallest rye or barley granule seemed larger than any granule of

Table 5. Chemical composition of starch granules[a]

		Content (g/100 g d.m.)					
Starch	**Variety/type**	**Crude Fat, %**	**Nitrogen, %**	**Protein[b], %**	**Amylose, %**	**Average granule size, µm**	**Reference**
Amaranth	*A.caudatus*	n.m.	n.m.	n.m.	n.m.	0.5-2	Jane et al., 1994
Amaranth	*A.cruentus*	1.1	0.08	0.49	0.2	n.m.	Stone and Lorenz, 1984
Amaranth	*A. cruentus var.* R159 (US)	n.m.	n.m.	n.m.	8.7	1.25	Kong et al., 2009
Amaranth	*A. cruentus var.* V69 (China)	n.m.	n.m.	n.m.	12.5	1.28	
Amaranth	*A. cruentus var.* Japan19 (Japan)	n.m.	n.m.	n.m.	8.6	1.12	
Amaranth	*A. cruentus var.* K112 (Mexico)	n.m.	n.m.	n.m.	5.7	1.21	
Amaranth	*A. cruentus* (Bolivia)	n.m.	n.m.	n.m.	7.8	1-2	
Quinoa		0.11	0.16	0.91	9.28	n.m.	Lorenz, 1990
Quinoa	AAFC-1	n.m.	n.m.	0.14	4.6	n.m.	Lindeboom, 2005
Quinoa	Ames 22155	n.m.	n.m.	0.27	11.5	n.m.	
Quinoa	WMF	n.m.	n.m.	0.57	14.4	1.5	
Quinoa	QC	n.m.	n.m.	1.23	19.6	1.5	
Quinoa		n.m.	n.m.	n.m.	12.2	1-2	Qian and Kuhn, 1999
Normal corn		n.m.	n.m.	0.69	25.4	14	Lindeboom, 2005
Waxy corn		n.m.	n.m.	0.82	1	14	
Barley		0.5	0.08	0.45	25.7	n.m.	Stone and Lorenz, 1984
Barley		n.m.	n.m.	n.m.	n.m.	15-32	Wang et al., 2002
Barley	Small granule	n.m.	n.m.	n.m.	27.8	0.9-5.1	Tang et al., 2001
Barley	Medium granule	n.m.	n.m.	n.m.	31.4	5.1-26.1	
Barley	Large granule	n.m.	n.m.	n.m.	31.9	7.7-44.9	
Rye		n.m.	n.m.	n.m.	n.m.	22-36	
Wheat		0.27	0.06	0.34	21.7	n.m.	Stone and Lorenz, 1984
Wheat		n.m.	n.m.	n.m.	n.m.	22-36	Jane et al., 1994
Oat		n.m.	n.m.	n.m.	n.m.	2-15	
Rice		n.m.	n.m.	n.m.	n.m.	3-8	
Wild Rice		0.2	0.01	0.06	2.04	n.m.	Stone and Lorenz, 1984
Wild rice		n.m.	n.m.	n.m.	19-20.5	2-8	Wang et al., 2002
Potato		n.m.	n.m.	n.m.	n.m.	15-75	Jane et al., 1994
Green pea		n.m.	n.m.	n.m.	n.m.	10-45	
Lentin bean		n.m.	n.m.	n.m.	n.m.	10-20	
Lima bean		n.m.	n.m.	n.m.	n.m.	10-45	

[a] Dry weight basis; [b] Protein = %N x 5.7; n.m. = not measured

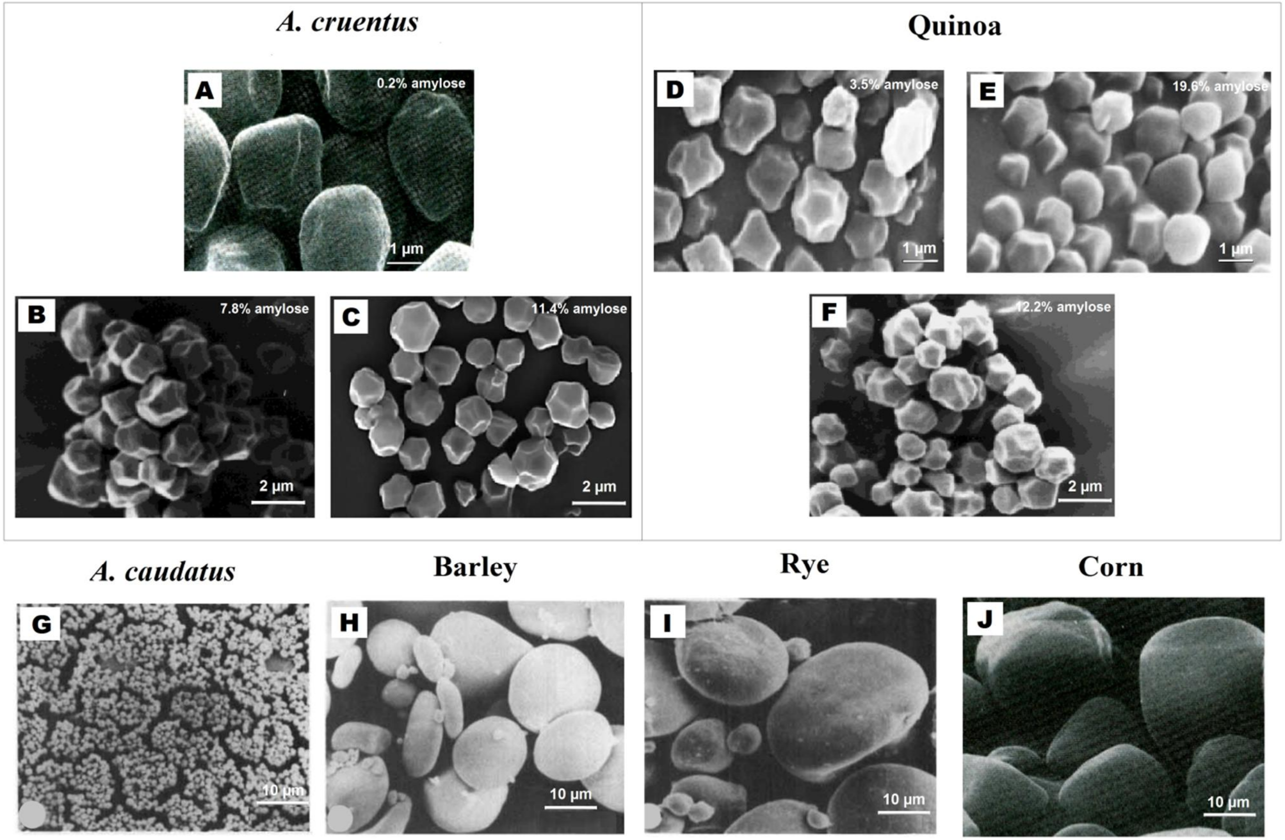


Figure 4. SEM images of starch granules belonging to amaranth, quinoa, barley, rye and corn. Photographs from: A, J. Stone and Lorenz (1984); B, F. Qian and Kuhn (1999); C. Kong et al. (2009); D, E. Lindeboom (2005); G-I. Jane et al. (1994).

amaranth (**Figure 4G-I**). Starch granules of corn were, on the other hand, as large as those from rye and barley, and showed more consistent sizes (**Figure 4J**). As photographs were compiled of various studies (except for Lindeboom, 2005), the morphological changes shown in **Figure 4** may arise from various external factors like settings and type of instrument. Hence, caution should be exercised during visual comparison and interpretation.

Amaranth, quinoa and, in particular, kañiwa and lupine are characterized for their high content of fibre and non-starch polysaccharides (NSP). For instance, kañiwa had a substantially higher content of lignin than amaranth and conventional cereal grains (**Table 6**). However, in terms of β-glucan, barley and oat had by far the highest contents. β-glucans are polysaccharides that contain glucose as their structural components, which are linked by β-glycosidic bonds. According to the scientific opinion given by EFSA (EFSA, 2010), oat β-glucan has been shown to reduce or lower blood cholesterol which is strongly linked to a lesser risk of coronary heart diseases. Thus, food should be provided with at least 3 g oat β-glucan per day (EFSA, 2010). While evidence demonstrated that the primary factors for a low glycemic response to oat food is the viscosity of β-glucan (Jenkins et al., 1978; Panahi et al., 2007), there is no compelling evidence to claim that the consumption of β-glucan from other grain sources has direct connection to health benefits (Björklund, 2005; Wood, 2007). Wood (2007) suggested that if viscosity is important for bioactivity, then changes in source, processing, cooking or storage must be considered.

Repo-Carrasco-Valencia et al. (2009a, 2009b) found that kañiwa had a high content of total and insoluble dietary fibre compared to quinoa and amaranth. Regarding soluble dietary fibre, kañiwa had a slightly higher proportion in comparison to amaranth and quinoa (**Table 6**). Some investigations have focused on the role of insoluble dietary fibre in modulating satiety using food samples such as unrefined whole grains (Levine, 1989; Burley and Blundell, 1990). Still, the complex nature of the food matrix and their various components (e.g., soluble fibre, NSP) made difficult to pinpoint the sole effect of insoluble dietary fibre. Various authors such as, Duncan et al. (1983), Solum et al. (1987), Rigaud et al. (1990), Anderson (1990) and Slavin and Green (2007) have observed the notorious effects of soluble and insoluble dietary fibre on the suppression of satiety.

In general, lupine had a greater content of low-molecular-weight sugars than conventional cereals (**Table 7)**. Still, lupine had comparable content of arabinose and lower content of xylose than conventional cereals. The content of galactose and uronic acid in lupine is many times superior to cereal grain and chickpeas (**Table 7**). The content of starch in lupine is distinctively low compared to conventional cereals such as maize and wheat, and compared to legumes like chickpeas.

Table 6. Content of lignin, beta-glucans and resistant starch for varieties of amaranth and kañiwa (g/100g d.m.)

		Content (g/100 g d.m.)						
	Variety	**Lignin, %**	**Beta-glucans, %**	**Resistant starch, %**	**IDF[a]**	**SDF[b]**	**TDF[c]**	**References**
Amaranth								
	Centenario	3.95	0.97	0.12	13.9	2.4	16.4	Repo-Carrasco-Valencia et al., 2009a; Repo-Carrasco-Valencia and Astuhuaman Serna, 2011a
	Oscar Blanco	3.97	0.63	0.1	12.2	1.7	13.8	
Quinoa								
	Blanca de Juli	n.m.	n.m.	n.m.	12.2	1.5	13.7	Repo-Carrasco-Valencia and Astuhuaman Serna, 2011a
	Kcancolla	n.m.	n.m.	n.m.	12.7	1.4	14.1	
	La Molina 89	n.m.	n.m.	n.m.	14.4	1.6	16	
	Sajama	n.m.	n.m.	n.m.	12	1.6	13.6	
Kañiwa								
	Cupi	6.88	0.07	0.24	22.3	3	25.2	Repo-Carrasco-Valencia et al., 2009b; Repo-Carrasco-Valencia and Astuhuaman Serna, 2011a
	Ramis	7.98	0.04	0.26	23.2	2.8	26	
Wheat[4]		0.8	0.8	n.m.	n.m.	n.m.	11.4	Frolich et al., 2013[4]
Rye[4]		1.1	1.5	n.m.	n.m.	n.m.	14.4	
Oat[4]		1.4	5	n.m.	n.m.	n.m.	10.3	
Barley[4]		0.7	4.6	n.m.	n.m.	n.m.	10.3	

[a] IDF, insoluble dietary fibre;

[b] SDF, soluble dietary fibre;

[c] TDF, total dietary fibre; n.m. = not measured

Table 7. Carbohydrates and starch content in grain cereals and legumes (g/kg d.m.)

	Content (g/kg d.m.)									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**Total sugars**	**Starch**
Lupine[2a]	21.3	7.9	39.1	28.1	25.4	112.9	100.2	36.1	399.2	16
Barley[1]	12	5	1	22	50	2	8	4	207	587
Chickpeas[2]	18.3	5	22.8	28.3	5	9.7	23.4	11.7	164.7	405
Maize[1]	13	2	1	19	28	4	9	6	117	690
Oat[1]	11	3	2	15	78	5	5	7	249	468
Rye[1]	19	4	3	24	41	4	20	3	184	613
Wheat[1]	11	4	2	22	38	2	7	4	138	651

I = sucrose, II = raffinose, III = stachyose, IV = arabinose, V = xylose, VI = galactose, VII = glucose, VIII = uronic acid

[a] *L. angustifolius*

[1] Bach Knudsen, 1997; [2] Rubio et al., 2005

2.3.4 Mineral composition and vitamins

The mineral composition and vitamins for amaranth, quinoa, kañiwa, lupine and cereals was summarised in **Table 8**. Based on this information, there is no one grain that presents distinctively superior mineral or vitamin content. For instance, lupine (*L. angustifolius* and *L. albus*) and amaranth presented the highest contents of calcium while oat and quinoa (Ruales and Nair, 1993) presented the highest contents of phosphorous. According to Miranda et al. (2010) and Ruales and Nair (1993), quinoa presented the highest content of potassium and, according Gamel et al. (2006), amaranth had the highest content of magnesium. Regarding minerals with minor content, iron, manganese and copper were more abundantly found in kañiwa, wheat and quinoa (Koziol, 1992), respectively, while zinc and sodium were mostly found in wheat and quinoa, respectively (Miranda et al., 2010).

In terms of vitamins, kañiwa and quinoa were more predominant than the rest of the grains, except for wheat. Kañiwa (White et al., 1955) presented the highest contents of thiamine (B_1) and riboflavin (B_2) while quinoa (Ruales and Nair, 1993) had substantially greater content of ascorbic acid, pyridoxine (B_6) and folate than any other grain. Interestingly, wheat, brown rice and barley had the highest contents of niacin.

Table 8. Content of minerals and vitamins for amaranth, quinoa, kañiwa, lupine and cereals **(µg/g)**

	Content (µg/g)															
	Minerals									**Vitamins**						
	Ca	**P**	**K**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**	**Na**	**B_1**	**B_2**	**B_3**	**Ascorbic Acid**	**B6**	**Folate**	**Reference**
Amaranth	1760	5820	4870	2880	140	13	9	39	250	n.m.	2.4	12.5	29.8	n.m.	n.m.	Gamel et al., 2006
Amaranth	2360	4530	n.m.	2440	75	n.m.	12.1	37	310	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	Becker et al., 1981; Collazos et al., 1993
Quinoa	565	4689	11930	1760	140	23	2	28	266	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	Miranda et al., 2010
Quinoa	1487	3837	9267	2496	132	n.m.	51	44	n.m.	3.8	3.9	10.6	40	n.m.	n.m.	Koziol, 1992
Quinoa	874	5300	12000	260	81	n.m.	10	36	n.m.	4	2	n.m.	164	n.m.	0.8	Ruales and Nair, 1993
Quinoa	700	4620	8550	1610	63	35	7	32	2.7	2.9	3	12.4	n.m.	4.9	1.8	Ranhotra et al., 1993
Kañiwa	1100	3750	n.m.	n.m.	150	n.m.	n.m.	n.m.	n.m.	7.8	5.5	13.4	n.m.	n.m.	n.m.	White et al., 1955; Collazos et al., 1993
Lupine[a]	2500	4600	n.m.	1500	55	40	6.6	38	n.m.	7.1	2.4	n.m.	n.m.	n.m.	n.m.	Hove, 1974; Torres et al., 2005
Lupine[b]	2700	4800	n.m.	2100	73	115	Tr	54	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	Hove, 1974
Barley	290	2210	2800	790	25	13	4	21	9	1.9	1.1	46	n.m.	2.6	0.2	USDA, 2005
Brown Rice	320	2210	2140	n.m.	16	n.m.	n.m.	n.m.	90	3.4	0.5	47	n.m.	n.m.	n.m.	Houston and Köhler, 1970
Oat	580	7340	5660	2350	54	56	4	31.1	4	7.6	1.34	9.6	n.m.	1.2	0.6	USDA, 2005
Wheat	982	7.73	10.43	4.01	104	156	11.3	76.1	n.m.	3.93	1.07	54.5	n.m.	n.m.	n.m.	Calhoun et al., 1960

[a] *L. angustifolius*

[b] *L.albus*

n.m. = not measured

2.3.5 Phenolic compounds

Based on gallic acid equivalence method (GAE equivalents), kañiwa and lupine have the greatest anti-oxidant capacity *in vitro* compared to quinoa and amaranth (**Table 9**). In contrast, cereals such as oat, rye, wheat and barley showed a very low antioxidant capacity (**Table 9**). The reagent used to conduct this assay (Folin-Ciocalteu reagent, FCR) not only measured phenols but any reducing compound such as nitrogen containing hydroxylamine and guanidine, thiols, vitamins, dihydroxyacetone and some inorganic ions (Everette et al., 2010). Accordingly, GAE equivalent is insufficient to ascertain the content of phenolic compounds in a given sample.

Table 9. Total phenolic compounds, as GAE equivalents, in amaranth, quinoa, kañiwa lupine and cereals

	Variety	GAE equivalent µg/g d.m.	Reference
Amaranth			Repo-Carrasco-Valencia et al., 2009a
	Centenario	1130	
	Oscar Blanco	990	
Quinoa			Repo-Carrasco-Valencia and Astuhuaman Serna, 2011
	Blanca de Juli	1420	
	Kcancolla	1570	
	La Molina 89	1970	
	Sajama	1630	
Kañiwa			Repo-Carrasco-Valencia et al., 2009b
	Cupi	2540	
	Sajama	2430	
Lupine[a]		954	Siger et al., 2012
	Bojar	2697	
	Zeus	2584	
Oat		300	Kähkönen et al., 1999
Rye flour		500	
Wheat		200	
Barley		400	

[a] *L. angustifolius*

On the other hand, Repo-Carrasco et al. (2009) quantified soluble and total phenolic acid contents (as aglycones) in different varieties of amaranth, quinoa and kañiwa (**Table 10**). The authors observed that kañiwa var. Ayara and Wila had the highest contents of

Table 10. Total phenolic acid contents (µg/g d.m.) in amaranth, quinoa, kañiwa, lupine and grain derivates (Mattila et al., 2005[1]; Repo-Carrasco-Valencia et al., 2009[2]; Siger et al., 2012[3])

	Variety	I	II	III	IV	V	VI	VII	VIII	Total
Amaranth[2]	Unknown 1	8.5	83.2	8.1	31.6	66.7	3.2	128	n.m.	329.3
	Unknown 2	8.7	64.6	9.9	19.7	42.8	0.9	62.8	n.m.	209.4
	Unknown 3	7	62.1	8	31.9	63.8	0.9	n.d.	n.m.	173.7
Quinoa[2]										
	INIA-415 Pasankalla	6.1	200	275	24.4	91.9	n.d.	n.d.	n.m.	597.4
	Salcedo INIA	2.5	123	80.2	31.7	146	n.d.	n.d.	n.m.	383.4
	Commercial 1	5.7	186	28.4	33.8	119	n.d.	n.d.	n.m.	372.9
	03-21-1181	5.9	137	95	19.2	107	n.d.	n.d.	n.m.	364.1
Kañiwa[2]										
	Kello	11	261	13.4	17.7	43.4	n.d.	n.d.	n.m.	346.5
	Wila	21.6	298	10	17.7	36.1	n.d.	n.d.	n.m.	383.4
	Guinda	23.7	260	17.4	15.5	30.4	n.d.	n.d.	n.m.	347
	Ayara	70.4	234	7	19.7	69.5	n.d.	n.d.	n.m.	400.6
	Commercial sample	11	120	3.7	15.4	32.3	n.d.	n.d.	n.m.	182.4
Lupine[3a]										
	Butan	0.6	n.m.	0.1	22.8	n.m.	n.m.	13	3.5	36.5
	Boros	0.09	n.m.	0.2	27.8	n.m.	n.m.	15	3.4	43.09
Lupine[3b]										
	Lord	1	n.m.	0.03	1.1	n.m.	n.m.	36	3.5	38.13
	Parys	1.2	n.m.	0.7	2.2	n.m.	n.m.	73.6	4.2	77.7
Lupine[3c]										
	Bojar	0.8	n.m.	0.4	43.7	n.m.	n.m.	12.5	0.6	57.4
	Zeus	0.6	n.m.	0.3	42.7	n.m.	n.m.	13.8	0.6	57.4
Rye[1]		11.1	954.6	45.51	7.5	24.4	133.2	10.43	n.d.	1186.8
Wheat[1]		n.d.	136.1	4.3	2.4	4.5	9.1	n.d.	n.d.	156.3
Pasta[1]		n.d.	132.9	4	2.7	n.d.	18.8	n.d.	n.d.	158.4
Corn[1]		29.2	427.4	34.9	6.4	5.2	64.1	n.d.	n.d.	567.3
Barley[1]		2.3	339.5	54.3	4.2	9.6	14.9	2.2	n.d.	427.1

I = caffeic acid, II = ferulic acid, III = p-coumaric acid, IV = p-Hydroxybenzoic acid, V = vanillic acid, VI = sinapic acid, VII = protocatachuic acid, VIII = gallic acid; n.d. = not detected; n.m. = not measured

[a] L. albus[3]

[b] L.luteus[3]

[c] L.angustifolius[3]

caffeic and ferulic acid, respectively while quinoa var. INIA-415 Pasankalla and Salcedo presented the highest content of p-coumaric acid and vanillic acid (**Table 10**). Besides, amaranth had the highest contents of p-Hydroxybenzoic, sinapic and protocatachuic acid. Interestingly, the contents of phenolic acids in three species of lupine were among the lowest, even compared to cereal derivates such as pasta or wheat flour (Mattila et al., 2005; Siger et al., 2012). Barley and corn flour presented similar amount of total phenolic acids to those of amaranth, quinoa and kañiwa, but rye flour had twice as much phenolic acids as quinoa var. INIA-415 Pasankalla (the highest phenolic-acid containing grain type compared to amaranth and kañiwa). Rye flour was generally low in all phenolic acids analysed, except for ferulic acid (**Table 10**). It is evident that there are many other compounds in lupine, other than phenolic acids, that contribute to their anti-oxidant capacity (**Table 9** and **10**).

2.3.6 Saponins, alkaloids and phytates

Several studies refer to saponins and alkaloids in grain foods as antinutrients given that, at high concentrations, they have shown some signs of toxicity on birds (Improta and Kellems, 2001), small mammals (Gee et al., 1993), aquatic crustaceans (e.g., brine shrimps; Ma et al., 1989), fungi and bacteria (Wink, 2006; Woldemichael and Wink, 2001). Saponins and alkaloids are widely distributed in the plant kingdom (Raffauf, 1996; Vinckens et al. 2007). For instance, triterpene saponins are found in different parts of the quinoa plant such as leaves, flowers and seeds, mainly, in seed coat (Kuljanabhagavad et al., 2008). According to Koziol (1992), the concentration of saponins in quinoa may range from 0.01% to 4.65% d.m. Triterpene saponins are glycosides (sugar molecules bound to a different functional group via a glycosidic bond) that can be classified according to their aglycone molecule (non-sugar molecule) as: oleanolic acid, hederagenin, phytolaccagenic acid, serjanic acid, 3b-hydroxy-23-oxo-olean-12-en-28-oic acid, 3b-hydroxy-27-oxo-olean-12-en-28-oic acid and 3b, 23a, 30b-trihydroxy-olean-12-en-28-oic acid (Kuljanabhagavad and Wink, 2009). Triterpene saponins can also be classified according to the number of sugar molecules bound to the aglycone as: mono-, bi- and tridesmosidic triterpene saponins.

An *in vivo* study showed that monodesmosidic saponins had a substantial anti-inflammatory activity in the exudative and proliferative phases of inflammation in doses between 25 and 100 mg/kg (Ghosh et al. 1983). Gee et al. (1993) investigated the effects of quinoa grain at high and low levels of saponins *in vitro* and *in vivo*. The authors found that, in vivo, high levels of saponins were membranolytic against cells of the small intestine while, in vitro, saponins were found to increase mucosal permeability. This effect, according to Kuljanabhagavad and Wink (2009), has a pharmaceutical potential as quinoa saponins may aid the absorption of particular drugs. It appears that triterpene quinoa saponins are extremely toxic to insects, birds and cold-blooded animals but their toxicity to mammals (e.g., laboratory rats) is considerably low.

According to Oleszek et al. (1999), the content of saponins (mostly monodemosidic saponins) in seeds of *Amaranthus cruentus* was between 0.09 and 1% d.m. Toxicity tests with highly purified extracts showed no physiological effects on hamsters, unless the dose increased to 1100 mg/kg of body weight (lethal dose). The authors concluded that amaranth-derived products pose no hazard for consumers.

Wild varieties of blue lupine (*Lupinus Angustifolius*) are known for their high levels of alkaloids (chemical compounds containing basic nitrogen atoms) in seeds (1-2%) which were shown to cause severe mammalian toxicity (Gladstones, 1970). Low-alkaloid varieties of blue lupine (e.g., sweet) were eventually developed in order to increase their human consumption. According to Harris and Jago (1984), the mean alkaloid content in a commercial variety of blue lupine, 'sweet', cultivated in Western Australia was around 0.015% (between 1982 and 1985); yet the alkaloid profile of commercial varieties of blue lupin may vary considerably. For instance, Petterson and Mackintosh (1994a) reported that the alkaloid fraction in 'sweet' blue lupin was 42-59% lupanine, 24-45% 13-hydroxylupanine, 7-15% angustifoline and 1-1.5% α-isolupanine while Erdemoglu et al. (2007) reported the following alkaloid fraction for blue lupine var. Fabaceae: 50.8% 13-hydroxylupanine, 23.6% lupanine, 2.9% angustifoline and 1.7% α-isolupanine. Butler et al. (1996) tested the toxicity of lupine alkaloids by feeding rats with a spiked lupine-based diet (55.4 g lupine flour/100 g diet; 250, 1050 or 5050 mg lupine alkaloids/kg diet) for a period of 90 days. The authors observed that male rats given the highest doses of lupine alkaloids presented lower haemoglobin levels and mean cell volume compared to the control (50 mg alkaloids/kg). Conversely, liver weights of female rates showed a dose-related increase. Butler et al. (1996) concluded that the hepatic lesions identified in their study correspond mostly to top-dose female rats (25% total sample population) while top-doses of alkaloids showed little evidence of consistent effect on the lowering haemoglobin levels in male rats.

Phytic acid (saturated cyclic acid) is mostly present in the bran fraction of grains and is the principal storage form of phosphorus in plant tissues (Schachtman et al., 1998). This compound is known for its capacity to form chelates with minerals such as iron, zinc and calcium thereby reducing their bioavailability. Repo-Carrasco-Valencia et al. (2010) studied the *in vitro* mineral bioavailability (dialyzability) in raw, roasted (190 °C for 3 min) and boiled (boiling water for 20 min) amaranth, quinoa and kañiwa seeds. The authors found that boiling increased the iron, zinc and calcium dialyzability in kañiwa, and zinc dialyzability in amaranth and quinoa. Regardless of the treatment, all samples showed high calcium dyalizability (21-29 % DCa) but low iron dyalizability (0.5-6 % DCa). Petterson et al. (1994b) compared the zinc absorption capacity (in humans) of various lupine-based meals, such as lupine milk, against soy-bean-based milk. The authors observed that lupine milk showed a substantially higher absorption of zinc (26.3%) than soybean milk (17.6%). Other lupine-based meals showed comparable zinc absorption capacity to soybean meals. It appears that commercial varieties of lupine grains have almost undetectable content of lectins, and relatively low content of trypsin inhibitors (0.14 mg/g) and phytate (4.4 mg/g) (Horton et al., 1990).

2.4 Extrusion

Extrusion is a food processing method that alters the physicochemical characteristics of macromolecules such as starch and protein (e.g., starch gelatinisation, protein denaturation) through high temperature and shear in order to form a solid structure, termed extrudate. During processing, food ingredients are subjected to various mixing conditions, heating and shear, designed to form and puff-dry the melt at die point. Extrusion is a highly versatile unit operation that can be applied to various food processes such as cooking, forming, mixing and texturizing of food products in conditions that favour high productivity, energy efficiency and low cost (Riaz, 2000). According to Darrington (1987) and Riaz (2000), extrusion can lead to substantial savings of raw material (19%), labour (14%) and capital investment (44%), and extrusion processing may require less space (per unit operation) than more traditional cooking methods. Since extrusion is a high-temperature/short-time (HT/ST) heating process, the chances of degrading food nutrients are minimised while the digestibility of protein and starch improves. Hameed (1993) observed that extrusion (single screw extruder) at 105 °C (die point) might lower the content of aflatoxins in infected ground corn (10% moisture content) from 50 to 80%. In that sense, extruders perform many functions that could be applied to a wide range of food, animal feed and other industrial applications.

2.4.1 Single-screw extruders

The main characteristics of single-screw extruders are detailed in **Figure 5**. There, it was observed that the product volume decreases from feed to the end of the barrel as the screw root thickens. The dry ingredients are generally preconditioned (e.g., moistened) prior to their processing, mixed in the first feed section, compressed in the transition section and cooked in the metering section. The product is finally discharged through a die and, if required, cut into desired lengths by a rotating knife. The barrel(s), which act as a retainer of the screw, can be jacketed to allow heating or cooling during extrusion (**Figure 5**). Single-screw extruders can be classified based on the extent of the shear as: *Low-shear cooking extruders,* moderate-shear machines with high compression screws allowing enhanced mixing or cooking (if heat is applied). This is ideal for the manufacture of soft-moist food and meat-like snacks. *Collet or pellet extruders,* high-shear equipment with grooved barrels, multiple shallow flights and short length: diameter (L/D) screw ratio (3-10:1). This extruder relies on heavy-induced heat to produce collets (broad meaning but generally used to describe coarse pieces obtained from extruding oilseeds to enhance solvent extraction characteristics) or pellets (also referred as collet); this is ideally used as pre-treatment before solvent extraction. *High shear cooking extruders*, high-shear equipment with various channels depths and screw pitch (**Figure 6**) that has the capacity to achieve high compression ratios and temperatures along the barrel (L/D = 15-25:1). Commonly, a large

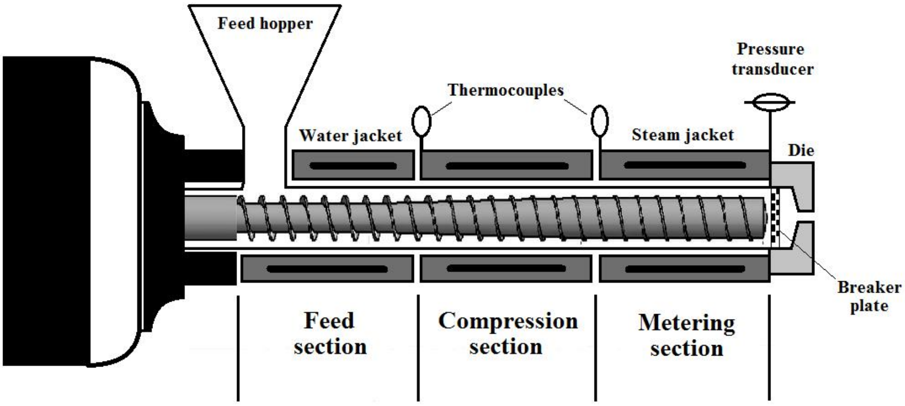


Figure 5. Classic view of a single-screw extruder [modified from Riaz (2000)]

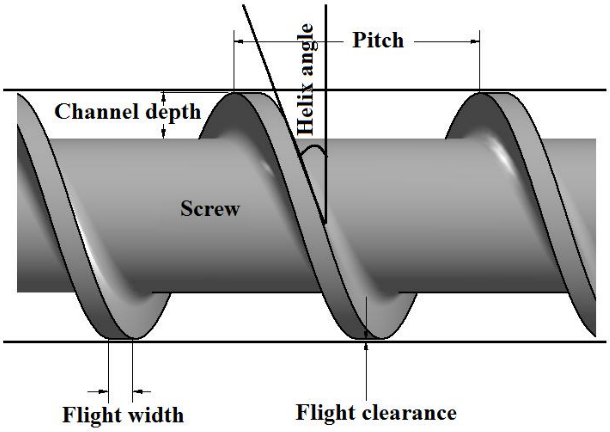


Figure 6. Basic geometry of a screw [modified from Eslami (2015)]

proportion of its heat is supplied by conversion of mechanical energy. High shear extrusion allows gelatinisation in very short time at very high cooking temperatures (>150 °C); this condition is conducive to puffing (expansion of the extruded product as it emerges from the extruder) and highly dextrinised microstructure. In this regard, the extruded products possess textural characteristics that make them edible right after processing (e.g., cereal breakfast, extruded snacks). Further details can be found in Riaz (2000) and Guy (1994).

2.4.2 Twin-screw extruders

The design of twin-screw extruders resulted from the necessity for higher quality products (Riaz, 2000). Some of the advantages of this equipment over single-screw extruders are its pumping efficiency, good control over residence time, self-cleaning mechanism, process uniformity and their capacity to handle very viscous materials with up to 25% fat; some general features are detailed in **Figure 7**. Screws can either rotate in opposite directions (counterrotating) or in the same direction (corotating) as shown in **Figure 7**. These extruders can also be classified based on their screw position and proximity as intermeshing (the flight of one screw engages or penetrates the channels of the other screw as shown in **Figure 7**) and non-intermeshing (the screws are not engaged, and so one screw turns without interfering the other, as if two single-screw extruders sat side by side).

2.4.3 Extrusion of Andean grains and legumes in US and Latin America

Before 1970, most of the information found on quinoa and kañiwa was in Spanish language and, even though some authors emphasize their high quality protein (Cardozo Gonzales, 1959; Oros Villegas, 1965), these grains were studied mostly as animal feed or traditional food with minimum processing (Cardozo Gonzales, 1959; Arévalo Gumucio, 1961; Benavides Varela, 1961; Granier-Doyeux, 1962). During the late 1970s and early 1980s, Colorado State University and the office of International cooperation and development (at USDA) joint efforts to evaluate the use of small low-cost (single-screw) extrusion cookers (LECs) for the manufacture of legume- and cereal-based nutritious food in developing countries. Pilot plants using LEC technology were designed, constructed and operated successfully in Sri Lanka, Tanzania, Costa Rica, and Guyana (Harper and Jansen, 1981). Scientists involved in the LEC project such as Crowley (1979) experimented mostly with a blend of soy and corn; widely available crops in the countries involved. In 1982, a “new crop” programme was initiated by Colorado State University in cooperation with growers, bankers, industrialists and researchers (Johnson, 1990). The project had identified the necessity to introduce crops for oil, gourmet foods and bulk ingredients to the United States. Quinoa was then considered to have potential as a gourmet commodity in specialized markets due to its high nutritional value; this based on a handful of research articles available at that time (White et al., 1955; De Bruin, 1963; Mahoney et al., 1975). Simmonds (1965) was among the first describing the traditional uses of quinoa as an ingredient for bread, porridge, etc.; or for the production of “chicha”, a fermented drink traditional from the Peruvian Andes. Probably, Romero et al. (1985) was among the first to study the effect of extrusion-cooking on the functional characteristics and protein quality of quinoa,

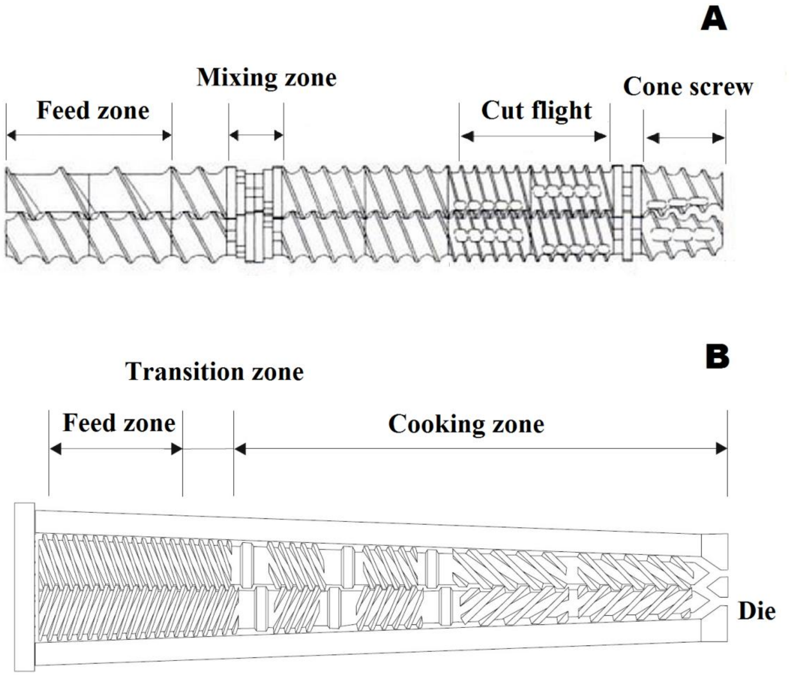


Figure 7. Screw configuration of corotating (A) and counterrotating (B) twin-screw extruder [modified from Riaz (2000) and Ilo et al. (2000)]

compared to other processes such as popping and boiling. The authors observed that boiling and extrusion led to higher protein efficiency ratios (PERs, 2.6 and 2.43, respectively) relative to popping (2.16), and closer to casein (3). The authors also observed that the extrudates obtained were acceptable from a sensory point of view, and could be consumed without any major modification. Nowadays, various companies in Peru manufacture low-tech single-screw extruders, which are purchased by small food companies, located in the highlands, and/or by state-run food aid programmes (e.g., Qali Warma).

2.4.4 Physicochemical changes of starch during extrusion

Cereal flours such as wheat, rice and corn are common ingredients in extrusion as their starch granules possess distinct chemical composition and physicochemical characteristics that allow a greater sectional expansion of extrudates. Corn has become the most popular extrusion ingredient worldwide and this could be linked to its high content of starch (around 70% of solids), amylose (around 25% of solids) and large granule sizes (around 14 µm), as well as its availability, compared to other cereals and grains (**Table 5**; **Figure 5**). Indeed, the main component of those ingredients for extrusion is starch, specifically, amylose

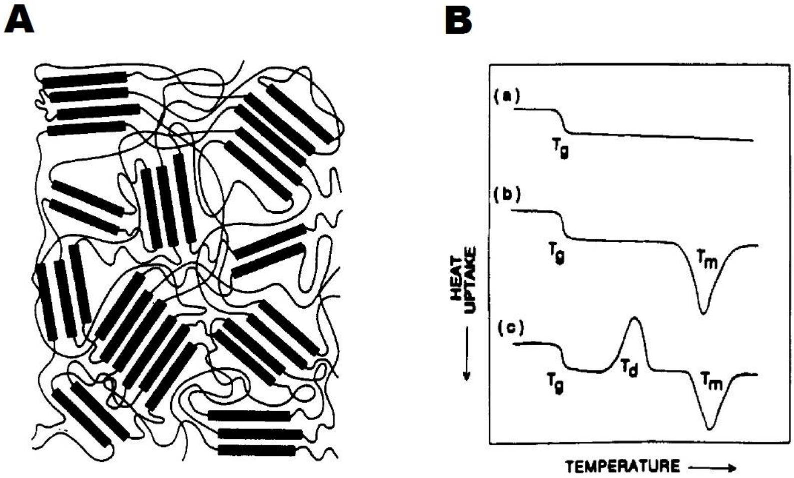


Figure 8. A. Model for crystalline-amorphous structure of a native starch polymer. Thicker lines represent amylose while the thinner and crossed lines represent amylopectin (Slade and Levine, 1987); B. Differential scanning calorimetry (DSC) heat flow curves showing T_g (temperature of heat uptake to change from glass into rubber) for an amorphous material (a), T_g and T_m (temperature of melting/decrystillisation) for a partially crystalline material, similar to starch (b) and T_g, T_d (temperature of crystallisation) and T_m for an amorphous but crystallisable material during rewarming followed by melting (c) (Lai and Kokini, 1991).

and amylopectin. Amylose is a linear polymeric compound that is made of glucose molecules linked by α D 1-4 glucosidic bond while amylopectine is a branched polymeric compound made of glucose molecules linked by α D 1-4 glucosidic bonds in the linear section and α D 1-6 glucosidic bonds at the branching point. While the content of amylose or amylopectin may vary widely depending on the type and source of the starch (e.g., waxy starch, 0% amylose), it is possible to identify key structures in the starch network such as amorphous (generally as glass state) and crystalline components. As proposed by Slade and Levine (1987), these crystalline components may be covalently cross-linked to the flexible chain segments of amorphous regions as shown in **Figure 8A**.

During extrusion, high temperatures, pressure, shear force and water content of mixture alters considerably the physicochemical characteristics of amylose and amylopectin leading to loss of crystallinity, gelatinisation, melting, molecular fragmentation and retrogradation (right after extrusion). These changes can be monitored, for instance, by differential scanning calorimetry (DSC) trough heat flow curves (**Figure 8B**) as the state of matter changes. In this regard, a vital parameter is the *glass transition temperature* (T_g) which indicates the temperature of heat uptake to change from glass into rubber state. Indeed, starch undergoes irreversible gelatinisation during extrusion with network entanglement and fragmentation or dextrinisation. Gomez and Aguilera (1984) proposed a model of starch degradation during extrusion, which is shown in **Figure 9**.

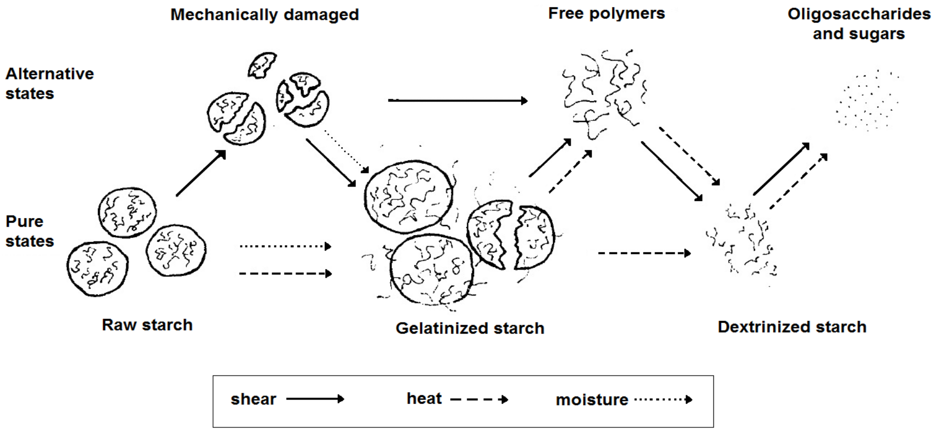


Figure 9. Proposed model of starch degradation during extrusion [modified from Gomez and Aguilera (1984)]

According to Gomez and Aguilera (1984), raw starch should be mechanically damaged as consequence of shear force, which, along with heat and moisture, may lead to gelatinisation (loss of birefringence). Then, shear force and heat may produce free polymers, dextrinised starch and oligosacharides. Even though this model seems reasonable, it does not contemplate the formation of glass structures post-extrusion. Babin et al. (2007) measured the elastic modulus along rising temperatures for extruded starches containing from 0 to 70% amylose. The authors observed that the elasticity of all extrudates was similar in the glassy domain, but then it dropped radically in the rubber domain (T_g = 137 °C) as the content of amylose increased. Babin et al. (2007) and Della Valle et al. (1996) suggested that the linear configuration of amylose might have the ability to form entanglements that should restrict mobility while the highly branched amylopectin molecules are more likely to adopt a more compact conformation. This might shed some light on why corn starch with higher content of amylose leads to greater sectional expansion and lower shrinking at die point. Chinnaswamy and Hanna (1988) studied the effect of various contents of amylose in blends (10, 25, 50 and 65% amylose resulting from blending native starches with various contents of amylose), pure native starches (0, 25, 50 and 70% amylose) and amylose/amylopectin mixes on the sectional expansion ratio of extrudates (barrel temperature, 140 °C; screw speed, 160 rpm; feed rate, 60 g/min) (**Figure 10**). In general, the authors found that blends containing 50% amylose had greater sectional expansion than at lower or higher amylose contents. Chinnaswamy and Hanna (1988) also found that those blends with higher proportion of native starches, high in amylose (I<II<III<IV; **Figure 10**), had in average higher sectional expansion. Also, pure native starches showed the highest sectional expansion at 50% amylose, and among the lowest at 75% amylose. The amylose/amylopectin mixes confirmed the previous results as it showed a sectional expansion peak at 50% amylose, and the lowest expansion of all at 0% and 100% amylose (**Figure 10**).

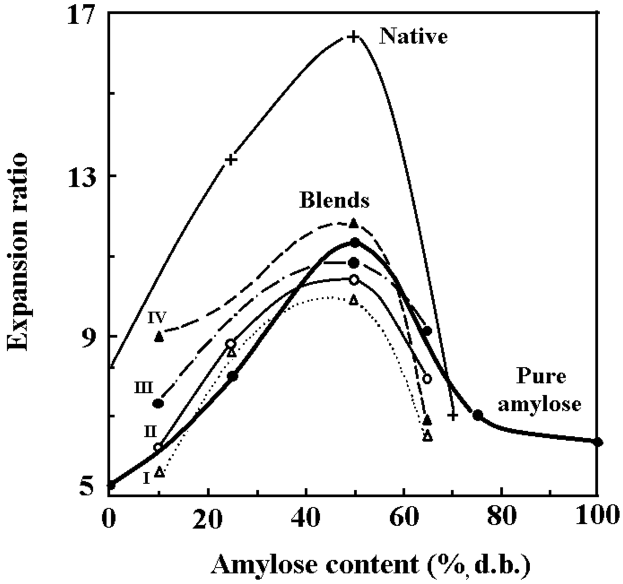


Figure 10. Relationship between amylose content and expansion ratio of native starches, starch blends and amylose/amylopectin mixes. (Chinnaswamy and Hanna, 1988)

Despite having the same amylose content, the samples examined by Chinnaswamy and Hanna (1988) has distinct expansion rations. This might be explained by changes in the molecular weight and structure of amylopectin, which is likely to change depending on the raw material (wheat, barley, quinoa, amaranth, kañiwa etc.) and as the fraction of amylose varies. Chinnaswamy and Bhattacharya (1983, 1984, 1986) found that rice varieties with high mean molecular weight amylopectin showed the greatest sectional expansion after extrusion.

According to some authors (Chiang and Johnson, 1977; Gomez and Aguilera, 1984; Lai and Kokini, 1991) the most important variables affecting starch during extrusion are temperature and water content of melt. For instance, Gomez et al. (1988) compared the starch granules of sorghum-based extrudates processed at low and high water content of mixture (17-45%). The authors observed that starch granules processed at high water content retained some of their birefringence. This might be explained by an increase in the fluidity of the melt as water content increases, leading to a reduction in shear force during extrusion (Gomez et al., 1988). While various authors have observed that low water content of melt during extrusion leads to greater sectional expansion, Ilo et al. (2000) suggested that at very low content of water (<5-10%) starches may overheat at metal surfaces, creating problems with flow behaviour and leading to an eventual blockade. According to Guy (1994), starch dispersal at very low contents of water is achieved merely by mechanical shearing of starch granules. In this regard, starch is less degraded and confers greater viscosity to the melt fluid. Temperature in combination with water content has a tremendous effect on the physicochemical changes of starch during extrusion. It appears that in excess of water and shear-less conditions starch crystallites are pulled apart during swelling with few or none of them melting at higher temperatures. Donovan (1979) observed that, under limited water and high-shear conditions, the effect of swelling forces on the degradation of starch crystallites is not as decisive as the effect of temperature and shear force (**Figure 9**).

2.4.5 Effect of extrusion on fibre, protein and lipids

A common way to enrich extruded snacks has been through the addition of fibre. Fibre, in cereals or Andean grains, is generally made of soluble (e.g., soluble β-glucan, inulin, raffinose, xylose) and insoluble (e.g., cellulose, hemicellulose, lignin) fibre. These may go through severe structural changes resulting from high-shear and high-temperature extrusion. Sharma and Gurjal (2013) studied the effect of extrusion (temperature of die, 150 and 180 °C; water content of the blend, 15 and 20%) on the solubility of β-glucan (soluble/insoluble ratio), and observed that at the highest temperature and lowest water content of mixture the solubility of β-glucan had increased substantially compared to non-extruded samples. According to Wood et al. (1989), Gaosong and Vasanthan (2000) and Brennan and Cleary (2005), the increase in soluble β-glucan is probably connected with the high shearing action and the set of temperatures applied during extrusion. Regarding cellulose and hemicellulose, Artz et al. (1990) extruded a corn-starch blend containing 0, 25, 50, 75 and 100% corn fibre (16.6% cellulose and 55.7% hemicellulose), and tested five temperature profiles (same temperature in the last three sections), 90, 105, 120, 135 and150 °C, and five screw speeds, 200, 275, 350, 425 and 500 rpm. The authors observed that extrusion at 150 °C led to greater disruption and porosity of the bran compared to extrusion at 90 °C. Besides, water-holding capacity decreased considerably at greater content of fibre and at higher screw speed. Artz et al. (1990) concluded that an increase in residence time, resulting from lower screw speed, may provide enough time to complete starch gelatinisation and/or encouraged hydrolysis of hemicellulose or cellulose thereby increasing water-holding capacity.

During extrusion, various changes in protein structure occur. Although such changes (like denaturation, aggregation and insolubilisation) have been widely reported, to date there is no consistent explanation on the three-dimensional network formed after extrusion. Several studies (Harper 1986; Ledward and Mitchell, 1988; Dahl and Villota, 1991; Koh et al., 1996) have observed and concluded that upon extrusion proteins denature thereby weakening tertiary and quaternary structures through a combination of high shear and heat. During extrusion, proteins are also known to form cross-links like disulphide bonds, which, according to Koh et al. (1996), are responsible for the textural characteristics of extruded products. In contrast, Alonso et al. (2000) extruded pea and kidney bean at 148 and 156 °C (temperature of die; screw speed, 100 rpm), respectively, and observed that, after extrusion, protein solubility in 2% mercaptoethanol (chemical agent known to cleave disulphide bonds) decreased by more than 50%, meaning that there was an apparent decrease (or accessibility to) disulphide bonds. Moreover, the protein solubility in buffer containing 1% SDS (chemical agent known to cleave hydrogen bonds and disrupt hydrophobic interactions) reduced considerably meaning that extrusion could have promoted very strong hydrophobic interactions and/or the formation of intramolecular disulphide bonds (covalent bonds). Regarding albumin and globulin, Alonso et al. (2000) found extrusion increased the levels of low-molecular-weight protein components (based on SDS-PAGE results). Besides, Anderson and Ng (2000) studied the effect of extrusion (temperature of die, 120, 140 and 160 °C; screw speed, 240, 320 and 400 rpm) on flour proteins present in wheat flour samples [9, 20 and 30% protein (gluten)]. The authors observed that after reduction with 2% mercaptoethanol bands in the high-molecular-weight protein region (in the SDS-PAGE patterns) showed no visible difference between non-extruded and extruded samples; yet patterns in the middle and lower region were notoriously lighter and darker for extruded samples, respectively. It is likely that proteins in the middle region depolymerized into lower molecular weight units during extrusion. Interestingly, extruded samples processed at higher screw speed (400 rpm) and temperature of die (160 °C) showed lighter patterns in the lower region indicating that a combination of shearing forces and high temperatures may have encouraged the formation of disulphide bonds through cross-linking reactions (aggregation) (Anderson and Kg, 2000; Koh et al., 1996). It appears that depolymerisation of protein was followed by protein aggregation as screw speed and temperature increased.

Several studies (Mercier et al., 1980; Galloway et al., 1989; Bhatnagar and Hanna, 1994a; Bhatnagar and Hanna, 1994b; Kaur and Singh, 2000; De Pilli et al., 2008) have found that starches and lipid are likely to form complexes during extrusion. The formation of these complexes involves amylose and fatty acids such as mystiric, palmitic and stearic acid or monoglycerides. The formation of amylose-lipid complexes and/or its type of crystal structure, V_h and E_h, will depend strongly on the moisture content before extrusion and the molecular weight and length of the available fatty acid (Mercier et al., 1980). For instance, Mercier et al. (1980) added between 2 and 4% of various lipids (acetic, lauric, mystiric, palmitic, stearic, oleic and linoleic acid, and monoglycerides) to native manioc starch for extrusion (barrel temperatures between 70 and 225 °C). The authors observed that the extruded native manioc starch showed absence of amylose-lipid complexes while the blend containing linear fatty acids showed consistent formation of V_h and E_h crystal forms. The blend containing monoglycerides presented mostly E_h crystal forms. Interestingly, moisture reconditioning from 20 to 30% transformed unstable E_h crystal forms into stable V_h forms (Mercier et al., 1980). De Pilli et al. (2008) extruded a blend containing almond and wheat flour blend (barrel temperature, 62-120 °C; moisture content, 21-27%) and concluded that the greatest formation of amylose-lipid complexes happened at low moisture content. Van Soest et al. (1996) found comparable results to De Pilli et al. (2008).

2.5. Microstructure of extruded snacks

The porous structure of extruded snacks results from the fast escape of water vapour (conventional extrusion) or carbon dioxide (supercritical fluid extrusion), imbedded in a starch-based molten fluid (or melt), at die point. The dynamic of nuclei formation, bubble growth and contraction has been modelled by Schwartzberg et al. (1995), Alavi et al. (2003a, 2003b) and Nowjee (2004) (**Figure 11**). Schwartzberg et al. (1995) explains that starch granules contain, naturally, microscopic pores thereby providing nuclei at which expansion of vapour bubbles start; this is the case of popcorn. However, starch granules and nuclei are inevitably destroyed during extrusion, and so few nuclei are present at die point resulting in large bubbles prone to coalesce (**Figure 11B**). In order to form bubbles, the pressure exerted by vapour in the pore (P) has to be sufficient to exceed the pressure at the outer surface of the domain (P_s) (**Figure 11B**). A detailed mathematical description of the necessary pressure to overcome the flow yield stress (ΔP_v), elastic stresses (P_c) and surface tension ($2\sigma/R$) is shown below :

$$\Delta P = P - P_s - \Delta P_v - P_c - (2\sigma/R). \tag{1}$$

Even though the negative effect of added water content on the overall sectional expansion of extrudates has been acknowledged, Alavi et al. (2003b) conducted an experiment where water vapour -with different coefficients of diffusion in starch- allowed the drive of bubble expansion. Interestingly, the authors observed that the faster the diffusion of water across the starch-based structure, the smaller the bubble size. Probably, water molecules that are chemically linked to the starch have lower diffusion coefficient (compared to added water) thereby promoting more noticeable structural changes in starch (e.g., slightly larger cells). Another interesting aspect is the time it takes before quasi-exponential bubble growth. According to Schwartzberg et al. (1995), it takes around 17 ms of very slow growth before rapid bubble expansion during the vapour-induced puffing of grains (**Figure 12**). There is, in fact, a critical ΔP at which expansion starts to occur.

The degree of contraction and cell coalescence is linked to sectional expansion of extrudates, which is probably the most important measurable physical property to assess the quality of extruded snacks, and can be defined as the ratio between the diameter of the cross-sectional extruded snack and the diameter of the die. Sectional expansion generally occurs at high barrel temperatures and low water content of feed, resulting into a structural transformation of its native ingredients (like starch gelatinisation, protein denaturation). The combination of extrusion parameters such as temperature, screw speed and water content of mixture as well as chemical composition of the blend may have a considerable effect on the microstructures and, inescapably, sectional expansion of extrudates.

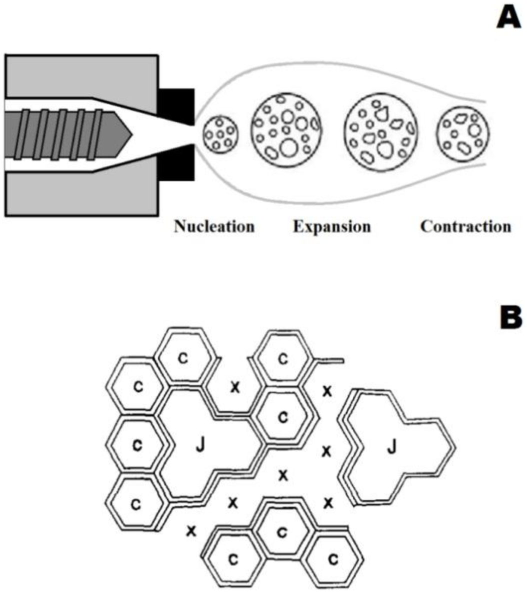


Figure 11. A.Stages of bubble growth at die point [modified from Nowjee (2004)]; B. Formation of open cells and cell coalescence near the end of the expansion process: c, closed cells; x, open cells; J, joined closed cells; ⬡, domain (Schwartzberg et al., 1995).

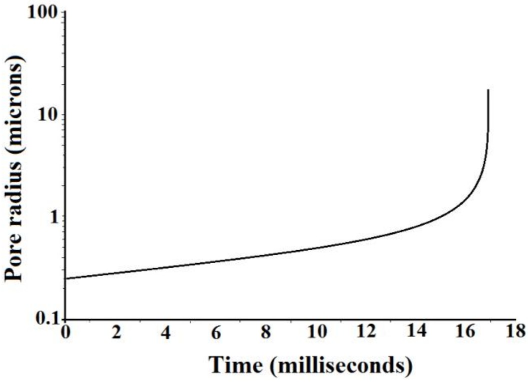


Figure 12. Semi-log plot of calculated pore radius versus time at standard diffusivity of water in cell wall (Schwartzberg et al., 1995).

2.6. Study of microstructures with X-ray micrographs

Digital video imaging, light microscopy and scanning electron microscopy (SEM) have the potential for superficial visual inspection. They have, nevertheless, failed to provide quantitative data in relation to the microstructure of food foams. The main disadvantages of these techniques are their 2-dimentional output and destructive characteristics as sample preparation involves cutting to expose the cross-sectional area for inspection, thus altering structural features (Trater et al., 2005). In contrast, X-ray microtomography (XTM) is more suitable for image analysis because of their greater contrast between solid and void areas in the cross-section area, and its ability to generate multiple 2-D cross-sectional images at incremental depths.

Initially, X-ray micrographs are obtained by placing samples on a stage and rotating them (e.g., 180 °C). The image is then reconstructed by using reconstruction software (e.g., Volumetric Reconstruction, MicroCT instruments) with a filtered back-projection algorithm. According to Trater et al. (2005), the reconstruction time may take up to 4.7 s per cross-section. A set of slices of infinitesimal thickness are taken perpendicularly to the axis of extrudate. These slices should cover the entire cylindrical volume of the sample after reconstruction. **Figure 13** shows what images look like at the different stages of processing: (1) raw images, (2) representative slice after reconstruction and (3) slices corresponding to particular volumes of interest (VOI). As explained by Trater et al. (2005), measurements may be very time-consuming, thus a certain number of VOI containing the entire sample cross-section must be chosen.

Trater et al. (2005) investigated the microstructure of starch-based extrudates containing whey protein concentrate (WPC, 5 and 15% of solids) and processed at two water content of melt (26 and 34%). The authors observed that increasing water content of mixture reduced mean cell diameter and increased cell density (number of cells per unit of volume of unexpanded extrudates). The effect of WPC on the microstructure of the extrudates seemed dependent on the water content of mixture. For instance, an increase of WPC in extrudates at 26% water content of melt caused an increase of mean cell diameter and reduction in cell density while an equivalent increase of WPM in extrudates at 34% water content of melt led substantial decrease in mean cell diameter, wall thickness and increase in cell density (**Figure 14**). This shows the potential of this technique in order to conduct microstructure analysis in extruded snacks (Babin et al., 2007; Parada et al., 2011).

2.7 Effect of extrusion parameters on the physical and textural properties of extrudates

There are various studies dealing with the effect of water content of blend, temperature of die, screw speed and feed rate on the physical properties of extruded snacks (**Table 11**). For instance, Chavez-Jauregui et al. (2000), Gearhart and Rosentrater (2014), Ding et al. (2005), Coulter and Lorenz (1991) and Repo-Carrasco-Valencia et al. (2009b) have all consistently observed that greater water content of blend leads to lower sectional expansion of extrudates and higher product density. Besides, sectional expansion of extrudates was found to increase

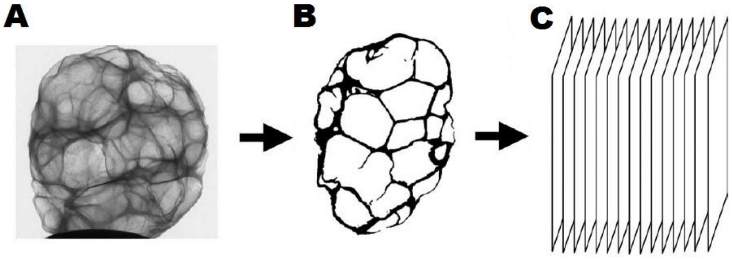


Figure 13. Construction scheme of the volumes of interest (VOI); A. Raw image, B. Representative slice after reconstruction, C. Volume of interest (15 consecutive slices from the middle of the sample) [modified from Trater et al. (2005)]

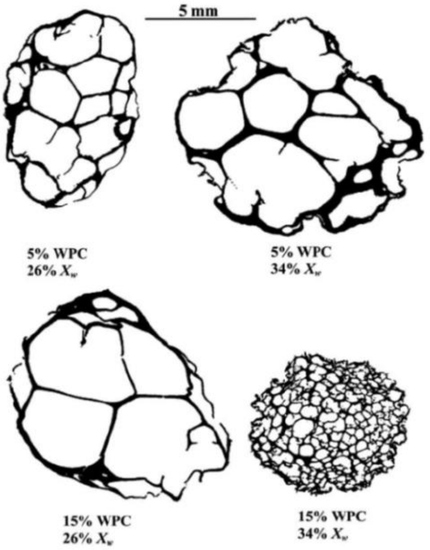


Figure 14. X-way microtomography cross-sectional images of a chosen slice from each treatment (Trater et al., 2005)

in proportion to screw speed (Coulter and Lorenz, 1991; Chavez-Jauregui et al., 2000; **Table 11**).Coulter and Lorenz (1991) extruded a corn-based blend containing up to 30% quinoa by using screw speeds between 100 and 200 rpm. The authors reported that low water content of blend (15%) and high screw speed resulted in greater sectional expansion of extrudates (regardless of the level of quinoa incorporation). Conversely, high water content of blend (20%) and screw speed resulted in slightly lower sectional expansion for extrudates containing 30% quinoa (**Table 11**). Coulter and Lorenz (1991) explained that greater water content of blend may reduce the viscosity and the temperature of the melt at the die thereby increasing bubble contraction after it leaves the die.

Greater water content of mixture has also been found to increase the breaking force (N/mm^2) in extrudates (Ilo and Liu, 1999; Chavez-Jauregui et al., 2000; Ding et al., 2005). For instance, Ilo and Liu (1999) incorporated up to 50% amaranth to rice-based extrudates at water content of mixture ranging from 12 to 15%. The authors found that, regardless of the degree of incorporation, extrudates conteining amaranth increased their breaking force as the water content increased (**Table 11**). Similarly, Ding et al. (2005) observed that increasing water content of mixture from 14 to 20% led to a substantial increase in product density and breaking force of rice-based extrudates (**Table 11**). Some authors have also indicated a relationship between feed rate and density, sectional expansion and breaking force. For instance, Coulter and Lorenz (1991) observed positive correlation between increasing feed rate and product density and sectional expansion of extrudates whereas Ilo and Liu (1999) found opposite results involving product density and breaking force (**Table 11**).

Although Ding et al. (2005) found that temperature of die had no effect on the sectional expansion of extrudates containing 100% rice, the authors reported that greater temperature of die (100-140 °C) had a considerably negative effect on product density and breaking force. The authors also reported that high temperature of die and lower feed moisture increased the instrumental crispiness (area under a force-distance curve). Chen et al. (1991) studied the effect of screw speed (100-300 rpm), temperature at the fourth and fifth section (100-200 °C) and water content of the mixture (20-30%) on the sensory properties of corn-based extrudates. The authors found that the increasing temperature at the fourth and fifth section led to crispier, less chewy and less hard extruded snacks. Chen et al. (1991) explained that, at a given temperature, increasing the water content mixture led to decreased crispiness in extrudates (interaction effect). The authors claimed that the melt containing greater content of water became less viscous (due to the plasticising effect of water) leading to a reduction in the pressure differential between the die and the atmosphere (see section 2.5).

2.8 Effect of fibre, protein and lipids on the physical and textural properties of extrudates

The incorporation of amaranth, quinoa, kañiwa and lupine to the production of corn-based extruded snacks increases inevitably the content of the protein, fibre and fat in the blend. This usually leads to severe changes in the sectional expansion, hardness and pore-size distribution of extruded snacks. For instance, Coulter and Lorenz (1991) found that the incorporation of up to 30% of quinoa to corn grits caused a reduction of sectional expansion and increase in product density (mass of an individual extrudates divided by the volume that it occupies) (**Table 11**). Dokic et al. (2009) incorporated up to 50% amaranth to a corn-based blend and observed that there was four-fold decrease in sectional expansion and two-fold increase in hardness (expressed in N) relative to the control sample (100% corn grits)

(**Table 11**). Gearhart and Rosentrater (2014) extruded pure quinoa and amaranth on a single screw extruder and observed that the expansion ratio (calculated as the division between diameter of extruder and diameter of die) was roughly around 1 for either amaranth or quinoa (**Table 11**). The low sectional expansion found by Coulter and Lorenz (1991), Dokic et al. (2009) and Gearhart and Rosentrater (2014) might be attributed to the low starch gelatinisation resulting, probably, from excessive water content of mixture (15-40%), low screw speed (< 200 rpm) and/or relatively low temperatures (25-150 °C) (**Table 11**). Even if the conditions were ideal for greater starch gelatinisation, the intervention of macromolecules such as protein or fibre may disrupt sectional expansion and alter hardness. Ilo and Liu (1999) extruded rice-based blends containing 30, 40 and 50% amaranth using counter-rotating twin screw extruder, and observed that density and breaking force (expressed in N/mm^2) increased at higher contents of amaranth. Even though the authors did not specify the sectional expansion, they claimed that sectional expansion index reached a maximum of 22.8 (**Table 11**). Apparently, the high temperature at the last section and low water content of mixture had a proportional effect on sectional expansion and breaking force, but the content of amaranth had the greatest overall effect (Ilo and Liu, 1999).

Fibre is abundant in quinoa, amaranth and particularly in kañiwa and lupine. In fact, various authors (Lue et al., 1991; Yanniotis et al., 2007; Brennan et al., 2008) have observed that extrudates with higher content of dietary fibre have lower expansion, higher product density and greater hardness. Brennan et al. (2008) incorporated dietary fibre rich ingredients -wheat bran, inulin and guar gum- to a wheat-based mixture. The authors observed that wheat bran led to lower sectional expansion, greater water loss, low density and hardness (expressed in g/s), while inulin and guar gum presented milder effects. From this, one can inferred that soluble fibre had milder effect on the physical properties of extrudates compared to insoluble fibre. According to Moraru and Kokini (2003), lower fibre contents may contribute to the stability of the matrix thereby reducing the longitudinal expansion of extrudates. By contrast, larger fibre fractions might have an inverse effect on the radial and longitudinal expansion of extrudates. Moraru and Kokini (2003) also suggested that fibre’s capacity to bind water might reduce their availability for starch gelatinisation and greater expansion.

Apparently, the particle sizes of flours have considerable effects on the physical characteristics of extrudates. For instance, Desrumaux et al. (1998) tested the effect of corn flour with various particles sizes (200-500 µm) on the sectional expansion and hardness (expressed in N) of extrudates. The author observed that an increase in particle size gave harder and less expanded extrudates. Similarly, Alam et al. (2013) observed that larger particle size (28-440 µm) of rye bran (26-30% total dietary fibre; 13-14% protein) gave less expanded and harder (expressed in N) extrudates. Conversely, Carvalho et al. (2010) reported that the extrusion of corn flours with larger particle size (180-710 µm) led to extrudates with greater expansion and less hardness (expressed in N). In general, there are more studies showing the technological advantages of using flours with smaller particle size for extrusion (Garber et al. 1997; Onwulata and Konstance 2006)

Faubion and Hoseney (1982), Onwulata et al. (1998, 2001), and Moraru and Kokini (2003) have found strong links between the type and content of protein with physical properties such as sectional expansion and hardness. Faubion and Hoseney (1982) increased the sectional expansion of wheat-based extrudates by adding up to 8% soy protein. Similarly, Onwulata et al. (1998) increased the sectional expansion of rice-based extrudates by adding up to 25% whey protein concentrate and reducing simultaneously the water content of flour blends. The authors observed, though, that sectional expansion reduced dramatically when the incorporation of whey protein concentrate went above 25%. As suggested by Moraru and Kokini (2003), proteins have the ability to affect water distribution in the matrix leading up to changes in the extensional properties of the dough (see section 2.4.5).

Most studies (Galloway et al., 1989; Ruy et al., 1993; Bhatnagar and Hanna, 1994a and 1994b; Ruy et al., 1994; Singh et al., 1998; Dextrumaux et al., 1999) agreed on the negative effect of lipids, such as monoglyceride, on the sectional expansion of extrudates. A major content of lipids in the flour blends may reduce shear force and starch gelatinisation due to their lubrication effects. Additionally, Bhatnagar and Hanna (1994a, 1994b) have found that the formation of amylose-lipid complexes during extrusion could be linked to low sectional expansion and greater bulk density. According to Mercier et al. (1980), the formation of amylose-lipid complexes might take place during the gelatinisation and cooling (retrogradation) of starch-based materials. In fact, several types of lipids including monoglycerides, fatty acids and their esters may complex with the amylose fraction of the starch (Ilo et al., 2000). The generic name of this complex is V amylose, being Vh and Eh amylose the most studied and best described (Goubet et al., 1998; Naknean and Meenune, 2010). According to Ilo et al. (2000), the formation of amylose-lipid complexes during extrusion may alter the melt viscosity and flow behaviour of the melt. For instance, Willett et al. (1994) reported a slight increase in viscosity of the melt as the content of monostearate (in a starch-based blend) increased up to 5 % (of solids). The authors attributed this behaviour to unmelted helical amylose-lipid crystals. In this regard, the formation of amylose-lipid complex may generate a less elastic and rigid matrix (Colonna et al, 1989).

Various authors (Berlung et al., 1994; Dar et al., 2014; Saeleaw et al., 2012; Alam et al., 2015) have studied the effect of incorporating grains and vegetables, rich in fibre, on the textural characteristics of extruded snacks. For instance, Berlung et al. (1994) extruded various rice- or wheat-based blends containing four barley cultivars (Wanubet, Apollo, Bowman and Tupper) using a twin-screw extruder with constant screw speed (410 rpm) and barrel temperatures between 123 and 125 °C. The authors found that, in general, the perceived crispiness of extrudates containing up to 65% barley was more consumer-appealing than of extrudates containing 100% rice while tenderness was less consumer-appealing in extrudates containing barley. Saeleaw et al. (2012) tested the effect of barrel temperature (150-190 °C) and water content of blend (12-16%) on the physical and textural properties of rye-based extruded snacks. The results showed that sound intensity of crunchiness correlated with perceived hardness, product density, maximum force (expressed in kg) and area under force-distance curve; these textural characteristics were strongly associated with high water content of the blend and low temperature. Interestingly, authors observed that crunchiness was inversely correlated to the sound intensity of crunchiness. Dar et al. (2014) extruded a rice-based blend containing pomace and pulse (ratio 83.5:16.5; water content of blend, 19.2%) at constant screw speed (310 rpm) and various barrel temperatures (100-140 °C), and observed that higher barrel temperature led to substantially greater hardness (expressed in N) and low crispiness (calculated as the total number of peaks from force-distance curve). It seems that the absence of standardized methods for the calculation of instrumental or sensory characteristics contributes to contrasting results among authors. Alam et al. (2015) stated that differences in the raw material and particular extrusion conditions can have a tremendous impact on the outcome of the texture evaluation.

Table 11. Effect of the chemical composition (g/100g dry weight) and extrusion parameters on the physicochemical characteristics of extrudates containing amaranth, quinoa and kañiwa flour as a single ingredient or in blend.

	Content (%)			Extrusion Parameters				Physical characteristics				
Raw material	**Fat**	**Protein**	**CHO**	**Water content of feed, %**	**Feed Rate, g/min**	**Temperature of die, °C**	**Screw speed, rpm**	**Density, g/cm3**	**Breaking force, N/mm2**	**Sectional Expansion index (SEI)***	**Longitudinal expansion index (LEI)**	**References**
Defatted Amaranth flour	0.18	15.82	80.77	13	70	135	200	0.239	13.1*	2.45	n.m.	Chavez-Jauregui et al., 2000
	0.18	13.82	80.77	15	70	150	200	0.227	13.8*	2.84	n.m.	
	0.18	15.82	80.77	17	70	135	200	0.254	13.6*	2.47	n.m.	
50% amaranth-50% corn	n.m.	n.m.	n.m.	16	n.m.	160	120	0.346	24.5*	1.83	n.m.	Dokic et al., 2009
20% amaranth-80% corn	n.m.	n.m.	n.m.	16	n.m.	160	120	0.132	20.8*	2.83	n.m.	
100% corn grits	n.m.	n.m.	n.m.	16	n.m.	160	120	0.095	13.2*	4.03	n.m.	
100% amaranth	6.0-8.0	13-18	63	20	n.m.	30	50	1.11	n.m.	1.07	n.m.	Gearhart and Rosentrater, 2014
	6.0-8.0	13-18	63	20	n.m.	25	100	0.90	n.m.	1.18	n.m.	
	6.0-8.0	13-18	63	40	n.m.	43	50	1.43	n.m.	1.04	n.m.	
	6.0-8.0	13-18	63	40	n.m.	38	100	1.25	n.m.	1.07	n.m.	
Amaranth 30%-rice flour 70%	2.65	9.94	81.78	12.9	26	180	74	0.12	0.2	6.2-22.8	0.38-0.91	Ilo and Liu, 1999
	2.65	9.94	81.78	13.5	25	170	70	0.125	0.21	6.2-22.8	0.38-0.92	
	2.65	9.94	81.78	14	22	159	66	0.13	0.23	6.2-22.8	0.38-0.93	

Amaranth 40%-rice flour 60%	3.28	10.54	79.76	12.4	28	150	78	0.12	0.23	6.2-22.8	0.38-0.94	Ilo and Liu, 1999
	3.28	10.54	79.76	13.7	25	168	70	0.13	0.24	6.2-22.8	0.38-0.95	
	3.28	10.54	79.76	14.6	20	188	60	0.14	0.27	6.2-22.8	0.38-0.96	
Amaranth 50%-rice flour 50%	3.91	11.15	77.74	12	27	190	74	0.13	0.28	6.2-22.8	0.38-0.97	Ilo and Liu, 1999
	3.91	11.15	77.74	13.5	26	170	68	0.14	0.3	6.2-22.8	0.38-0.98	
	3.91	11.15	77.74	14.5	18	150	60	0.15	0.33	6.2-22.8	0.38-0.99	
100% Rice flour	1.2	7.6	77.4	14	20-32	120	150	0.1	10.58*	3.87	n.m.	Ding et al., 2005
	1.2	7.6	77.4	16	20-32	120	150	0.19	27.26*	3.41	n.m.	
	1.2	7.6	77.4	20	20-32	120	150	0.35	43.94*	2.48	n.m.	
100% quinoa	5.0-10.0	12.0-19.0	61-74	20	n.m.	54	50	1.16	n.m.	1.12	n.m.	Gearhart and Rosentrater, 2014
	5.0-10.0	12.0-19.0	61-74	20	n.m.	45	100	1.14	n.m.	1.09	n.m.	
	5.0-10.0	12.0-19.0	61-74	40	n.m.	54	50	1.1	n.m.	1.06	n.m.	
	5.0-10.0	12.0-19.0	61-74	40	n.m.	55	100	1.51	n.m.	0.92	n.m.	
Quinoa 10%-corn grits 90%	0.49	8.95	n.m.	15	119	150	100	0.06	n.m.	3.15	n.m.	Coulter and Lorenz, 1991
	0.49	8.95	n.m.	15	149	150	150	0.09	n.m.	3.36	n.m.	
	0.49	8.95	n.m.	15	174	150	200	0.08	n.m.	3.67	n.m.	
	0.49	8.95	n.m.	25	126.8	150	100	0.27	n.m.	1.78	n.m.	
	0.49	8.95	n.m.	25	173.8	150	150	0.22	n.m.	1.78	n.m.	
	0.49	8.95	n.m.	25	216.2	150	200	0.26	n.m.	2.1	n.m.	

Quinoa 20%-corn grits 80%	0.8	9.94	n.m.	15	112.8	150	100	0.09	n.m.	2.73	n.m.	Coulter and Lorenz, 1991
	0.8	9.94	n.m.	15	172.8	150	150	0.08	n.m.	3.15	n.m.	
	0.8	9.94	n.m.	15	206.8	150	200	0.15	n.m.	3.36	n.m.	
	0.8	9.94	n.m.	25	130	150	100	0.3	n.m.	1.68	n.m.	
	0.8	9.94	n.m.	25	208.8	150	150	0.31	n.m.	1.89	n.m.	
	0.8	9.94	n.m.	25	276	150	200	0.33	n.m.	1.89	n.m.	
Quinoa 30%-corn grits 70%	1.1	10.38	80.56	15	140.4	150	100	0.14	n.m.	2.62	n.m.	Coulter and Lorenz, 1991
	1.1	10.38	80.56	15	181.2	150	150	0.12	n.m.	2.52	n.m.	
	1.1	10.38	80.56	15	210	150	200	0.08	n.m.	2.83	n.m.	
	1.1	10.38	80.56	25	166	150	100	0.26	n.m.	2.1	n.m.	
	1.1	10.38	80.56	25	232.8	150	150	0.17	n.m.	1.78	n.m.	
	1.1	10.38	80.56	25	307.2	150	200	0.35	n.m.	1.89	n.m.	
100% Corn grits	0.18	7.96	n.m.	15	110.2	150	100	0.08	n.m.	3.35	n.m.	Coulter and Lorenz, 1991
	0.18	7.96	n.m.	15	197.6	150	150	0.1	n.m.	3.04	n.m.	
	0.18	7.96	n.m.	15	175.2	150	200	0.08	n.m.	3.99	n.m.	
	0.18	7.96	n.m.	25	100	150	100	0.23	n.m.	2.62	n.m.	
	0.18	7.96	n.m.	25	120.4	150	150	0.16	n.m.	2.1	n.m.	
	0.18	7.96	n.m.	25	115.6	150	200	0.23	n.m.	2.62	n.m.	

100% kañiwa	5.68	14.41	63.64	12	10-12 **	180	254.5	0.1	n.m.	1.98	n.m.	Repo-Carrasco-Valencia et al., 2009b
	5.68	14.41	63.64	14	10-12 **	180	254.5	0.2	n.m.	1.77	n.m.	
	5.68	14.41	63.64	16	10-12 **	180	254.5	0.3	n.m.	1.61	n.m.	

* Force in Newtons (N)
** Residence time in seconds (s)
n.m. = not measured

2.9 Flavour formation during extrusion

Gomez and Aguilera (1983) suggested that during extrusion dextinisation was a predominant mechanism of starch degradation, thereby increasing the amount of soluble solids. This could have an effect on the flavour of extrudates. For instance, Sacchetti et al. (2004) incorporated 20, 30 and 40% chestnut flour to rice-based extrudates, and observed that extrudates with higher content of chestnut flour were perceived as having stronger bitter taste. The authors explained that the bitter taste could be related to off-flavour development resulting from Maillard reactions. Miller (2009) used online monitoring setup of a Raman spectrometer and non-destructive ultrasound device during the extrusion (temperature profile: 90/90/90/90/100/110/120/90,100,110 °C) of flour blends containing chickpea, corn, oat, corn starch, tomato powder and ground raw hazelnuts. The author found that increasing temperature during extrusion produced various structural changes in the melt (**Figure 15**). When the temperature in section C rose above 90 °C, C-O-H bending of starch and C-O vibration of hydrocarbons chains band at 1125 cm^{-1} disappeared from the spectra as shown in **Figure 15**. Ester groups, CH_2 of lipids and primary, secondary and tertiary amide appeared to have gone through severe breakdown as temperature increased. Even though, the author did not conduct a sensory test on the samples, it is plausible that such changes in the molecular structure of the mass increased the perception of taste and overall flavour.

In this regard, Chen et al. (1991) studied the effect of temperature at the fourth and fifth section (100-200 °C) and water content of the mixture (20-30%) on the taste of corn-based extruded snacks. The authors found that higher temperature (at the fourth and fifth section) led to a stronger taste and aroma of toasted corn. It seems, however, that an increase in water content of mixture reduced considerably the perception of toasted corn. Regarding the pleasantness of the flavour, Mäkilä et al. (2014) extruded an oat and barley-based blend containing either non- or enzymatic-treated press residue from blackcurrants (27-28% of solids) with constant temperature along the barrels (95 °C) and screw speed (400-420 rpm).The authors found that extrudates containing non-enzymatic-treated press residue were best liked. These samples presented the highest content of glucose, fructose, total organic acids, succinic acids and citric acid. As expected, Mäkilä et al (2014) observed that extrudates containing greater content of fibre and stiffness (expressed in N/mm) were the least liked.

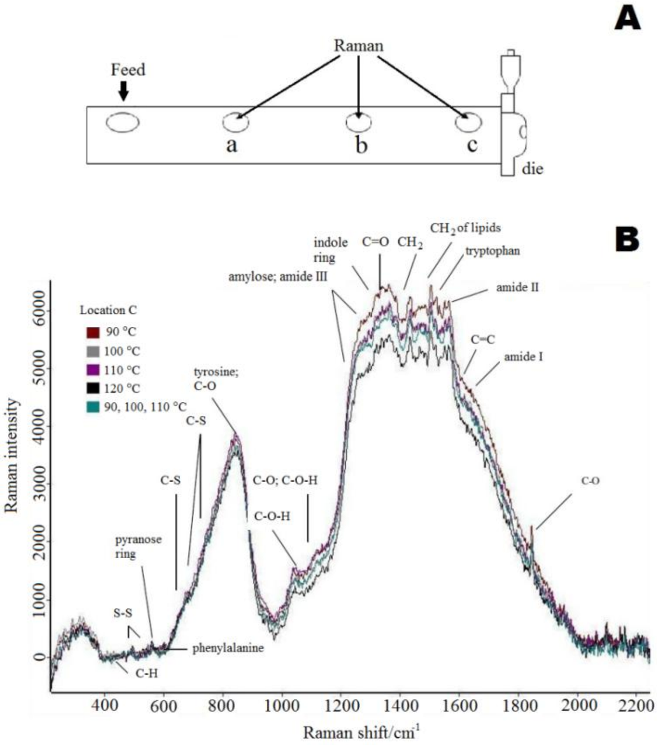


Figure 15. Online monitoring setup of a Raman spectrometer and non-destructive ultrasound device during extrusion (A). Raman spectra of temperature changes at location 'c' in the extruder (B) [modified from Miller (2009)].

2.10 Lipid stability of extrudates during storage

The interaction between amylose and fatty acids has been a topic of intensive research in order to understand its effect on aroma release and lipid oxidation (Kim and Maga, 1994; Gray et al., 2008; Naknean and Meenune, 2010). Yet scientific reports on lipid oxidation within extruded starch-based matrices are still limited. Gray et al. (2008) incorporated linoleic acid to waxy corn starch during extrusion, and stored samples at 50 °C. The authors observed that initial lipid oxidation occurred near the sample surface, and also found a substantial difference in the onset of oxidation between bulk oil (low) and extruded starch-based matrices containing linoleic acid (high). According to Gray et al. (2008), the surface area of extruded samples was 10 times as large as surface area of the bulk oil, which may explain differences in their oxidative sensitivity. The authors also observed that rubbery material allowed oxygen diffusion within the matrix (probably due to its low viscosity) during storage, leading to prompt oxidation. In comparison, samples in glassy state were found to protect linoleic acid from oxidation.

The low sensitivity towards lipid oxidation in starch-based structures can result from small free volume within a glass matrix, thus reducing the ability of oxygen to diffuse towards lipids, and hexanal to diffuse out of the matrix (Voilley and Le Meste, 1985; Kollengode and Hanna, 1997; Parker et al., 2002). At higher viscosity, the chances of reactants to collide and promote oxidation reduce considerably (Orlein et al., 2000). El-Magoli et al. (1979), Su (2003) and Gray et al. (2008) found that temperature and humidity conditions have an influence on lipid oxidation with hexanal as marker. El-Magoli et al. (1979) reported a peak in the production of hexanal followed by a steady decrease when storage temperatures were around 50 °C. The authors claimed that hexanal changed into hexanoic acid due to high temperature during storage. Su (2003) tested the sensitivity of quinoa flour to lipid oxidation during storage at 25, 35, 45 and 55 °C, and found similar results as El-Magoli et al. (1979).

Lampi et al. (2015) studied the effect of four extrusion conditions (the first three compartments were set at 80 °C while the seven remaining compartments plus die were set at: a. 70 °C; b. 130 °C; c. 110 °C; d. 110 °C) on the lipid oxidation of oat grains subjected to 15-week storage. The authors observed that oat flour extruded at 70 °C had greater lipid stability, reflected in the low contents of secondary oxidation compounds such as octane, hexanal and 2-pentylfuran. In contrast, oat flour extruded at 110 °C presented a rapid increase in hexanal between 6- and 15-week storage, while those extruded at 130 °C presented an exponential increase in hexanoic acid between 6- and 12-week storage. Lampi et al. (2015) suggested that 70 °C is enough to stabilize lipids, and that high extrusion temperature such as 130 °C should be avoided as it promotes extensive lipid oxidation and degradation of triacyl glycerol and free fatty acids. Moreover, Moisio et al. (2015) investigated the effect of water content of mixture (13-30%) and temperature (80-140 °C) on the lipid stability of extruded ray bran. The authors found that increasing water content of mixture and temperature led to greater production of hexanal and 2-pentylfuran over ten-week storage. According to Moisio et al. (2015), low water content of mixture may encourage the formation of Maillard reaction products, which could act as antioxidants during storage. Another possible reason for greater lipid stability at lower content of mixture might come from the low hydration of transition metal cations and propagation reactions of lipid hydroperoxides (Labuza et al., 1972). Moisio et al. (2015) also observed that rye bran particle size had an effect on the lipid stability of extrudates. The authors found that finer particles were much less sensitive to lipid oxidation compared to coarser ones. Moisio et al. (2015) suggested that grinding may lead to the exposure of binding sites from degraded protein and polysaccharides (e.g., Maillard reaction products), which could bind oxidation compounds such as hexanal and 2-pentylfural.

2.11 Effect of extrusion cooking on the nutritional profile of extruded snacks

Various studies (Killeit, 1994; Håkansson et al., 1987; Suknark et al., 2001; Repo-Carrasco-Valencia et al., 2009; Brennan et al., 2011) have indicated that exposure to high temperature and pressure may lead to a substantial loss of nutritional compounds in extruded snacks. For instance, Suknark et al. (2001) used flour blends containing starch-fish meal and starch-partially defatted peanut flour in order to produce extruded snacks (temperature profile: 60/90/120/100/100 °C). Even though the authors observed a substantial loss of tocopherols resulting from extrusion, a lower retention was found in extrudates containing fish meal. Suknark et al. (2001) hypothesised that the greater loss of tocopherols in extrudates containing fish meal may be due to the concentration of oxidation-prone polyunsaturated fatty acids. Furthermore, Håkansson et al. (1987) studied the effect of extrusion on the content of tocopherols in white wheat flour under mild (148 °C and 24.6% moisture content) and severe conditions (197 °C and 14.6% moisture content). The authors observed that the losses of α-tocopherol accounted for around 86% under mild conditions and 94% under severe conditions. Accrodingly, the losses of β-tocopherol were about 65 % under mild conditions and 78% under severe conditions.

Repo-Carrasco-Valencia et al. (2009) studied the effect of extrusion (single screw extruder) on the total phenolic compounds (GAE equivalents) present in two varieties of amaranth (*Amaranthus caudatus*), Centenario and Oscar Blanco. The extrusion parameters were: screw speed, 254 rpm; residence time, 10-13 s; work temperature, 180 °C. The authors observed that extrusion reduced considerably the content of total phenolic compounds in amaranth var. Centenario (80.3%) and Oscar Blanco (64.4%). Repo-Carrasco-Valencia et al. (2009) explained that such loss phenolic compounds during extrusion could be attributed decarboxylation. Nonetheless, Anton et al. (2009) reported a high retention (>85%) of total phenolic compounds after the extrusion (twin-screw extruder; screw speed, 150 rpm; feed rate, 1.8 kg/h; temperature profile, 30/80/120/160/160 °C) of corn-starch containing 15, 30 and 45% navy bean flours; in contrast, those extrudates containing small red bean flour presented low retention (<35%). The authors explained that the effect of extrusion on phenolic compounds could be strongly dependent on the cultivar; they also suggest extrusion could promote the polymerisation of phenolic acids and tannins thereby affecting the extractability of such compounds.

Apparently, folate is very sensitive to high temperatures during food processing. For instance, Charlton and Ewing (2007) claimed that temperatures above 95 °C could reduce the content of folate by almost 100%. Håkansson et al. (1987) and Kariluoto et al. (2006) have reported moderate losses of folate (20-30%) resulting from the extrusion of white wheat flour and rye flour, respectively; the study conducted by Kariluoto et al.

(2006) involved the extrusion of rye flour at 120 and 140 °C thereby contradicting the claims of Charlton and Ewing (2007). It seems that the level of retention could depend on the complexation capacity of folate and/or its interaction with particular food matrices during extrusion.

2.12 Modelling techniques

Multiple linear regression (MLR) is a very common modelling technique used to study, for example, the effect of more than one independent extrusion variable (X, predictors) on measurable characteristics of extrudates or process parameters (Y, response variables). MLR includes parameter estimates (i.e., slope, β) for each predictor variable in the model, and a variable representing the error ($\boldsymbol{\varepsilon}$). The MLR model is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k + \varepsilon \tag{2}$$

MLR has been a useful tool when predictors are generally controllable, easy-to-measure, few and have a relatively well-understood relationship to response variables (Randall, 1995). However, if any of these conditions are unfulfilled, MLR may be unsuitable for predicting modelling (Tang et al., 1999; Tang et al., 2000; Adhikari et al., 2009). Even though the MLR model is likely to fit the sampled data, it will be incapable to predict new data (over-fitting). Conveniently, partial least square regression (PLSR) is a valid method to construct predicting models, particularly, when data contains fewer samples than variables (e.g., sensory studies, spectroscopy) or when there is a great deal of noisy and/or high collinearity among response variables. A two-block PLSR is a relatively new distribution-free generalisation of the MLR method. PLSR focused on the detection of few underlying or latent factors that explain most of the variation in the response variables thereby postulating models with high predicting abilities. As shown in the **Figure 16**, the overall goal of PLSR is to predict response variables, indirectly, by extracting latent variables T (*X-scores*) and U (*Y-scores*) from predictors and response variables, respectively. T is then used to predict U, and this predicted U is used to construct predictions for response variables.

A three-block PLSR or L-PLSR is used to combine two matrices of predictors (X and Z) with one matrix Y of response variables (Martens et al., 2005). Even though X- and Z-variables share no physical matrix size-dimension and, therefore, cannot be correlated directly to each other, they are connected via Y. The schematic construction of L-PLSR is shown in **Figure 17**. The L-PLSR may reveal patterns in Y that correspond to patterns in both X and Z thereby acting as a filter against noise in Y. Should $X/Y/Z$ patterns be found, they could be used to predict Y from information easy to obtain (i.e., X and Z). For instance, Martens et al. (2005) applied successfully L-PLSR to the hedonic study of apples (X-variables) combined with product descriptors (Y-variables), and by

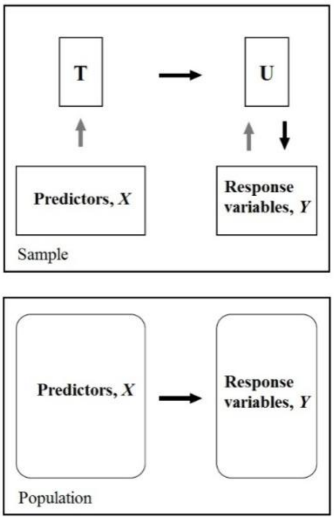


Figure 16. PLSR method, indirect modelling (Randall, 1995)

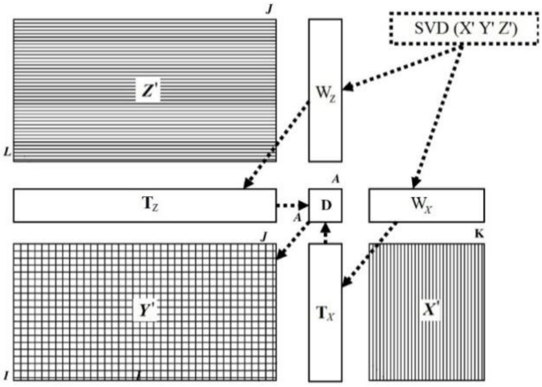


Figure 17. L-PLSR method. Mean-centred data: *Y'*, *X'* and *Z'*. Singular value decompositions (SVD) of *Y'*, *X'* and *Z'* yield W_X (X weights) and W_Z (Z weights). Scores (T_X and T_Z) are then defined as and $T_X = X' W_X$ and $T_Z = Z' W_Z$ The matrix D describe the *Y'*-relevant interaction structures between *X'* and *Z'* ($Y' = T_X D T_Z + R$) [modified from Martens et al. (2005)]

the considering background preferences of the panellists (*Z*-variables). The authors managed to model *Y* by a bi-linear interactions of latent variables from both *X* and *Z* thereby providing an interpretable overview of rather complicated and noisy empirical data.

3 JUSTIFICATION OF THE STUDY

A gluten-free diet is one that excludes wheat, rye and barley due to their contents of toxin prolamines such as gliadin (wheat), secalin (rye) and hordein (barley). As mentioned before, these prolamins may lead to various degrees of CD in those individuals with genetic predisposition to an autoimmune response and/or those exposed to gluten-containing diet since early age (Ascher and Kristiansson, 1997; Greco, 1997; Ivarsson et al., 2002). Various studies have demonstrated that coeliac patients suffer from serious nutritional deficiencies involving the intake of calories, dietary fibre, minerals and vitamins (Kinsey et al., 2008; Niewinski, 2008; Hallert et al., 2002; Bardella et al., 2000; Mariani et al., 1998, Saturni et al., 2010).

Saturni et al. (2010) proposed that, at diagnosis, subject suffering CD present deficiency in fibre, iron, calcium, magnesium, vitamin D, zinc and vitamins such as folate, niacin, vitamin B12 and riboflavin while those with a long-term gluten-free diet still present deficiencies in fibre and vitamins such as folate, niacin and vitamin B12. For instance, Tikkakoski et al. (2007) compared the nutritional status of individuals with previous diagnosis of CD with those of screen-detected patients (cohort of 1900 adults, aged 18-64 years). The authors found that the proportion of women suffering from CD was higher than that of the total study population (1:46 and 1:53, respectively). Tikkakoski et al. (2007) also reported that screen-detected patients that tested positive for CD were deficient in iron and folate compared to those individuals previously diagnosed with CD. It seems that the vast majority of undiagnosed individuals suffering from GS and/or some degree of CD are at substantially greater risk of nutritional deficiency. In contrast, Mariani et al. (1998) found that adolescents (aged 10-20 years) that follow a strict gluten-free diet tend to increase considerably their intake of protein and lipids relative to another group that follows a gluten-containing diet. The authors observed that those adolescents following the gluten-free diet were more likely to be overweight or obese (72%) in comparison to those following a gluten-containing diet (51%). In addition, McFarlane et al. (1995) measured the bone mineral density (BMD) at lumbar spine and femoral neck over 12 months in 45 women and 10 men (all ≥ 18 years old) with CD. The authors found that 45% women and 50% men with CD had a BMD below or equal to 2 SD (times less than a young adult BMD mean), which is defined as osteoporosis.

As shown in previous chapters (2.3), amaranth, quinoa, kañiwa and lupine are gluten-free grains that provide good quality protein, dietary fibre and lipids rich in unsaturated fatty acids. Furthermore, they contain bioactive compounds such as saponins, phytosterols, squalene, fagopyritols and polyphenols, and an adequate balance of minerals and vitamins (Valcarcel-Yamami and da Silva Lannes, 2012; Repo-Carrasco-Valencia, 2011b; Petterson, 1998). Some *in vivo* studies have suggested specific health effects linked to the consumption of amaranth, quinoa and lupine (Sirtori et al., 2003; Pasko et al., 2010; Cazarin et al., 2012). For instance, Cazarin et al. (2012) tested the impact of extruded amaranth supplement on the intestinal bile and fatty acids of normolipidemic (having a normal amount of lipid in the blood) rats. The authors observed that extruded amaranth promoted the reduction of total and LDL serum cholesterol, and increased the production of butyric acid (help to regulate the process of cell differentiation and stimulate the immunogenicity of cancerous cells) in cecum and excretion of deoxycholic acid (undesirable secondary bile acid) in faeces. In another study, Pasko et al. (2010) investigated the effect of diet supplemented with quinoa on the oxidative stress in plasma, heart, kidney, liver, lung, testis and pancreas of fructose administered rats (310 g/kg for 5 weeks). The authors observed that the diet containing fructose led to an increase in lipid peroxides and a decrease in antioxidant activity while the diet containing quinoa was found to improve the effectiveness of the oxidative system of plasma, heart, kidney, lung and pancreas. The authors concluded that quinoa is able to reduce the oxidative stress thereby alleviating the generation of free radicals during pathological states. Sirtori et al. (2003) investigated the effect of lupin protein extract on plasma VLDL and LDL cholesterol in rats fed with a casein-based diet. The authors observed that lupin protein, mostly conglutin γ, reduced VLDL and LDL cholesterol by 21 and 30%, respectively. Based on these results, the authors concluded that lupin has hypocholesterolemic activity similar to other leguminous proteins.

Conducting a study involving Andean grains and lupine that covers key aspects of snack development such as engineering, storage, physical, chemical and sensory studies was deemed necessary in order to provide safe and nutritious alternatives to those suffering from CD or GS.

4 AIMS OF THE STUDY

The overall aim was to evaluate the effect of amaranth, quinoa, kañiwa and lupine on physical and sensory properties, as well as chemical composition of corn-based extruded snacks. The specific aims were to:

- Evaluate lipid stability in extruded snacks containing 0 and 20% amaranth, quinoa and kañiwa (I)
- Expand the knowledge linked to the development of extrudates through the application of advanced modelling techniques such as Partial Least Squares Regression (II, III)
- Compare the effects of processing conditions on the physical properties of extrudates containing 20, 35 and 50% amaranth, quinoa, kañiwa and lupine (II, III)
- Quantify the content of total phenolic compounds, fatty acids, tocopherols and folate before and after extrusion (II, III)
- Identify texture and taste attributes specific to extrudates containing up to 50% amaranth, quinoa and kañiwa (IV)
- Evaluate the relationship between microstructures and texture attributes of extrudates containing up to 50% amaranth, quinoa and kañiwa (IV)

5 MATERIALS AND METHODS

This section summarizes the materials and methods, which are described in more detailed in the original publications (I-IV):

5.1 Materials

5.1.1 Grains and bulk ingredient

Amaranth (*Amaranthus caudatus* var. Oscar Blanco), quinoa (*Chenopodium quinoa* var. Rosada de Huancayo) and kañiwa (Chenopodium pallidicaule var. Cupi) were cultivated in Peru and supplied by Andean Cereal programme at the National Agrarian University 'La Molina' (UNALM, Peru). The grains were milled (500 µm mesh size) prior to vacuum packing and delivery to Finland. Corn flour was supplied by Limagrain (France) and had a particle size of about 150 µm (I).

Commercial varieties of amaranth, quinoa and kañiwa were delivered from South America as grains (Aduki Ltd, Finland), while lupine (var. boruta) was cultivated in Finland. They were all milled as whole grains with a pin disc grinding device (100 UPZ-lb, Hosokawa Alpine, Augsburg, Germany) at VTT Technical Research Centre of Finland. The median particle size of amaranth, quinoa, kañiwa and lupine was around 285, 575, 240 and 800 µm, respectively. Pregelatinised corn flour (median particle size of around 750 µm, Risenta AB, Sweden) was purchased in from a local store in Helsinki, Finland (II, III and IV). The chemical composition of amaranth, quinoa, kañiwa and lupine was determined as part of this research, and is detailed in **Table 12**. Varietal changes may give rise to slight differences in the chemical composition of grains, as shown in the present study (I compared to II-IV, **Table 12**). The calculated content of protein or fibre in the flour blends (flour blend is defined in this study as a mechanical blending of amaranth, quinoa, kaniwa or lupine and corn flour) is detailed in **Table 13**.

Other ingredients of extrudates were distilled water (distilled water) and sodium chloride (NaCl; Meira Ltd., Helsinki, Finland). In the present study, mixture is defined as the material to be extruded including flour blend, water and sodium chloride.

5.1.2 Preparation of extruded samples

The extrusion was conducted in a twin-screw laboratory extruder (Thermo Prism PTW24 Thermo Haake, Polylab System, Germany) that consisted of seven sections with individual temperature control in six of them (96 mm in length each). The diameter and length of the screw were 24 and 672 mm, respectively, and consisted of six conveying areas, five mixing areas, one transition element and one extrusion element (Kirjoranta et

Table 12. Chemical composition of amaranth, quinoa, kañiwa, lupine and corn flour

			Content (g/100 g d.m.)						
	Moisture content (%)		**Protein[1]**		**Ash[2]**		**Dietary fibre[3]**		**Fat[4]**
	I	**II, III, IV**	**I**	**II, III, IV**	**I**	**II, III, IV**	**I**	**II, III, IV**	**I**
Amaranth	10.6±0.1	11.3±0.5	12.2±0.2	16.1±1.3	2.1±0.002	2.4±0.04	8.6±0.1	8.3±1.9	5.9±0.2
Quinoa	11.6±0.1	11.8±0.4	16.4±0.6	13.1±0.4	3.2±0.06	2.2±0.3	11.5±0.2	9.1±2.6	5.6±0.3
Kañiwa	9.1±0.2	11.4±0.4	15.6±0.004	16.7±0.03	4.0±0.03	2.3±0.2	20.5±0.9	16.1±2.8	7.9±0.4
Lupine	n.m.	11.9±0.3	n.m.	28.7±0.4	n.m.	3.6±0.03	n.m.	50.1±2.6	n.m.
Corn	9.4±0.03	14.1±1.0	n.m.	8.2±1.1	n.m.	0.4±0.1	n.m.	5.8±0.3	n.m.

Measured according to:

[1] AOAC (1995)

[2] Schneider (1967), Mattila et al. (2001)

[3] Cho et al. (1997), Mattila et al. (2001), AOAC (2002)

[4] Lampi et al. (2015)

n.m. = not measured

Table 13. Calculated content of protein and fibre of solids in amaranth, quinoa, kañiwa, lupine, corn and flour blends

	Content (% of solids)	
	Protein	**Fibre**
Amaranth (A)	16.1	8.3
20% A : 80% C	9.8	6.3
50% A : 50% C	12.2	7.1
Quinoa (Q)	13.1	9.1
20% Q : 80% C	9.2	6.5
50% Q : 50% C	10.7	7.5
Kañiwa (K)	16.7	16.1
20% K : 80% C	9.9	7.9
50% K : 50% C	12.5	11
Lupine (L)	28.7	50.1
20% L : 80% C	12.3	14.7
50% L : 50% C	18.45	28.0
Corn (C)	8.2	5.8

al., 2012). The feed rate was maintained at 84 g/min (I) and 86 g/min (II, III, IV) throughout extrusion. For the study I, the temperature profile was fixed at 40 °C (section 1), 70 °C (section 2 and 3) and110 °C (sections 4, 5 and 6) (I). Three predictors were used: temperature of die (150, 160 and 170 °C), screw speed (200, 350 and 500 rpm) and water content of mixture (15, 17 and 19%). For studies II and III, the temperature profile was set at 90 °C (section 1), 95 °C (section 2), 95 °C (section 3), 100 °C (section 4), 110 °C (section 5) and 140°C (section 6). The predictors were the content of amaranth, quinoa, kañiwa and lupine (20, 35 and 50% of solids), temperature of die (140, 150 and 160 °C), screw speed (200, 350 and 500 rpm), and water content of mixture (14, 16 and 18%). Salt was dissolved in distilled water to ensure an even distribution in the extrudates, and for adjustment at 0.5% of solids (I, II, III).

For sensory evaluation, nine extrudates samples varying in grain type (amaranth, quinoa and kañiwa) and content (20, 35 or 50% of solids) were prepared for sensory evaluation and physical measurements (IV). Extrusion parameters such as temperature of die, screw speed and water content of mixture were set at 140 °C (temperature profile was the same as for studies II and III), 500 rpm and 14%, respectively. Total salt added during extrusion was 1% of solids.

5.2 Physical and physicochemical analyses

5.2.1 Determination of physical properties

The diameter of twenty (I) and ten (II, III, IV) samples was measured using a Vernier calliper. Sectional expansion index (SEI) was calculated by dividing the cross-sectional area of the extrudates by the area of the die (5 mm diameter). Five (I) and ten (II, III, IV) randomly collected samples were dried in vacuum at 54 °C for 72 h to determine water content of extrudate (WCE) and as pre-treatment for stiffness measurement. Samples for stiffness measurements were dried in order to avoid the plasticising effect of water on the structure. Stiffness was defined as the slope of force-distance curve when compression was perpendicularly enforced under three-point bending. The universal testing machine (Instron 4465, Instron Ltd., High Wycombe, UK) was equipped with a loading cell (100 N) and a flat rectangular-shaped aluminium probe (**Figure 18**). Samples were positioned perpendicularly over a sample holder. The speed of the aluminium probe was 5 mm/min.

5.2.2 Determination of torque, pressure and total SME

Average values over time for pressure and torque were calculated using data obtained during sample collection, and total SME was calculated as the total mechanical energy per unit of feed needed to drive screws at the chosen speed of rotation:

$$Total\,SME\ (Wh/kg) = \frac{angular\ velocity\ of\ screws\ (rad/s) \times torque\ (Nm)}{feed\ rate\ (kg/h)} \tag{3}$$

5.2.3 Analysis of large-scale structures

The most expanded extrudates containing pure corn, amaranth, quinoa, kañiwa and lupine (at each content of solids) were subjected to visual examination. Images were taken for that purpose with an AxioCam digital camera adjusted to an Olympus Zuiko objective (9-18 mm f/4.0-5.6 Lens; Tokyo, Japan). Images were processed using Axiovision 3.1 software (Carl Zeiss, Thornwood, NY) (I, II, III).

5.2.4 Analysis of microstructures

Wall thickness and porosity were analysed using X-ray microtomography (µCT) (IV). Extrudates were scanned with a voxel size of 6.25 µm, using a custom-made nanofocus microtomography device (Nanotom 180NF; phoenix|xray Systems + Services GmbH, Wunstorf, Germany). Three-dimensional (3D) reconstructions were

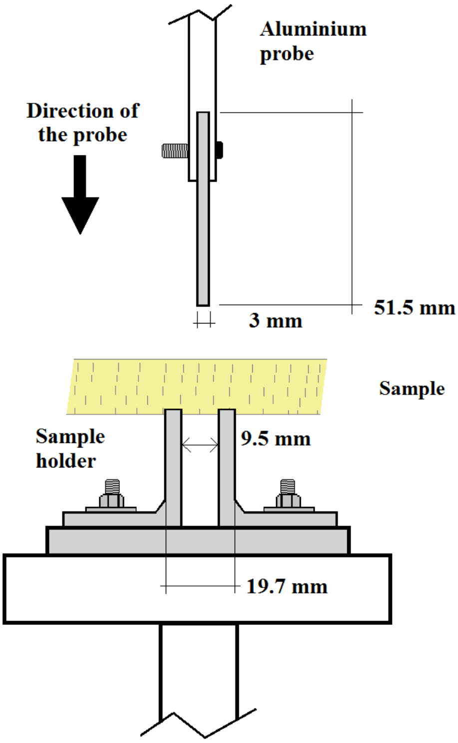


Figure 18. Experimental set-up for stiffness measurement.

made using datos|x rec-software with the X-ray microtomography (µCT) device. Porosity values and wall thickness distributions were then calculated from these reconstructions, using Avizo Fire Edition and ImageJ softwares. The voxel data were first segmented using simple gray-value thresholding, and median filtered twice with a 3 × 3 × 3 voxel kernel to remove noise-originated small islands and holes in the segmented data set. The porosity values are obtained according to following equation:

$$Porosity = \frac{Number\ of\ voxels\ in\ pores}{Total\ number\ of\ vocels\ in\ the\ sample} \times 100 \qquad (4)$$

The distribution of volume-weighted wall thickness (d_W) was determined from the segmented reconstructions by using the watershed and Euclidean distance transform algorithms in Avizo and the local thickness plug-in for the program ImageJ (Dougherty and Kunzelmann, 2007). The analysis was based on fitting spheres with maximal radius inside the segmented material, and d_W was obtained as the diameter of these spheres. This method was very similar to the one used by Kirjoranta et al (2012).

5.2.5 Analysis of nanostructures

Ground extrudates obtained at specific extrusion conditions (WCM 15%, SS 500 rpm, TEM 160 °C) (I) were pressed into metal rings of a thickness of 1mm and covered with a Mylar foil. Wide- and small-angle x-ray scattering (WAXS and SAXS) were measured under perpendicular transmission geometry using Cu Ká radiation ($\lambda = 1.542$ Å) and an image plate detector (MAR345, Marresearch) for WAXS and a wire detector (HI-STAR, Bruker AXS) for SAXS. In SAXS, the magnitude of the scattering vector $q = (4\ \pi\ \sin\theta)/\ \lambda$ ranged from 0.2 to 0.34 Å^{-1}. The data treatment procedures, including background subtraction and other corrections, were similar as Penttilä et al. (2011).

5.2.6 Water absorption index (WAI) / Water solubility index (WSI)

The water absorption index (WAI) and water solubility index (WSI) were determined based on the procedure described by Dansby and Bovell-Benjamin (2003). Specimens were randomly collected and milled (< 60 mesh) using an ultra-centrifugal mill (Retsch ZM 200, Haan, Germany) at 10000 rpm. Ground extrudate (2.5 g) was mixed with 30 ml of distilled water in a 50-ml tared centrifuge tube. The centrifuge tube was vortexed for 1 min, shaken intermittently for 30 min and then, centrifuged at 3000 rpm for 10 min (IV).

While the sediment that remained in the centrifuge tube was weighed for WAI, the supernatant was poured into a tared evaporating dish, dried overnight and weighed for WSI. WAI and WSI were calculated as:

$$WAI\ (\%d.b.) = \frac{Weight\ of\ sediments}{Weight\ of\ dry\ solids} \times 100 \qquad (5)$$

$$WSI\ (\%d.b.) = \frac{Weight\ of\ dissolved\ material}{Weight\ of\ dry\ solids} \times 100 \qquad (6)$$

5.3 Chemical analyses

5.3.1 Headspace analysis

One set of samples was milled using an ultra-centrifugal mill (Retsch ZM 200, Haan, Germany) at 12000 rpm while a second set of samples was cut into pieces of 15 mm in length (I). Milled and whole extrudates were divided and placed into 20-ml headspace vials as follows: 2 g of milled extrudates per vial and 0.5 g of whole extrudates per vial. Sample-containing vials were stored open in vacuum desiccators at relative humidity (RH) of 11 and 76% obtained using saturated salt solutions containing LiCl and NaCl, respectively (LiCl and NaCl, p.a., Merck, Germany) at 20 °C for one week before being sealed and stored for 0, 2, 5 and 9 weeks at room temperature (20 °C) in the dark.

Hexanal content was analysed using static headspace gas chromatography (HS-GC). The Autosystem XL gas chromatograph was equipped with an HS40XL headspace sampler (Perkin-Elmer, Shelton, CT; column NB 54, Nordion). The injection and detection temperature was 250 °C. Flame gases were synthetic air and hydrogen, and carrier gas was helium. The column had a length and inner diameter of 25 m and 320 µm, respectively. Vials were thermostated at 80 °C for 18 min prior to injection. The run temperature was 60 °C and the run time 10 min per sample. An external standard curve was plotted using a solution of hexanal (> 98% hexanal, Merck) in isopropanol (HPLC-grade Rathburn Chemicals Ltd, Scotland, UK) (10^7 ng hexanal/l). This solution was added to open vials containing pure corn extrudates (4, 6, 8, 10, 12, 32, 42, 52, 72 µl hexanal solution/vial). The vials were stored in a shaking incubator overnight (12 h) before GC analysis (I).

5.3.2 Analysis of fatty acids and bioactive compounds

Determination of fatty acids: The lipid fraction of the sample (flour or milled extrudate) was extracted by accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA; pressure 1000 psi) with acetone at 100 °C (Lampi et al. 2015). Further solvent evaporation took place at 37 °C. The extracted lipids were treated in order to provoke partial hydrolysis of triacylglycerols (cleavage of ester link) and methylation in order to make fatty acids volatile compounds (methyl esters), and so detectable by gas chromatography (GC) (Lampi et al. 2015). The fatty acid samples were analysed using GC-system (Hewlett Packard 5890, Palo Alto, USA) having a flame ionisation detector (FID) and autosampler. The samples were separated using the silica-fused capillary column (DB-FFAP, 30 m × 0.32 mm, 0.25 µm, Agilent technologies Inc., Palo Alto, CA). Fatty acids were quantified by an internal standard method (Reference standard solution GLC-63, Nu Check Prep, Inc., Elysian MN, USA).

Tocopherols: The content of α-, β- and γ-tocopherol were analysed by HPLC (HPLC system, Waters Corporation, Milford, MA, USA) according to the procedure of Schwartz et al. (2008). Tocopherol standards were purchased from Merck (für biochemische Zwecke, Art no. 15496).

Total phenolic compounds: The determination of total phenolic content in the flours and extrudates were carried out according to Gorinstein et al. (2007). The methanol-water treatment allowed the determination of free phenolics present in the flours and extrudates while the acid hydrolysis treatment released phenolic compounds bound to the cell matrix by breaking the glycosidic bond with the addition of 1.2 M hydrochloric acid.

Folate: Folate was determined by a microbiological assay on microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the growth indicator organism and 5-formyltetrahydrofolate (Eprova AG, Schaffhausen, Switzerland) as the calibrant (Kariluoto et al. 2004). The sample preparation procedure included heat extraction followed by trienzyme treatment with amylase and hog kidney conjugase (EC3.2.1.1, A-6211 Sigma, St. Louis, MO) at pH 4.9, and protease (EC 3.4.24.31, P-5147, Sigma) at pH 7.0; this was done to liberate folate from the matrix (Kariluoto et al. 2004; Piironen et al. 2008). Except for folate (n = 2), chemical analyses were conducted in triplicate to all flours and extrudates.

5.4 Sensory analyses

5.4.1 Assessors

Ten students (three men and seven women between 20 and 30 years old) from the department of Food and Environmental Sciences at University of Helsinki participated in the sensory evaluation of extruded snacks containing 20, 35 and 50% amaranth, quinoa or kañiwa. Panellists were trained in sensory profiling and temporal dominance techniques for a maximum of 12 h. Training and sensory evaluation sessions took place at the food sensory laboratory of the University of Helsinki in Viikki (IV).

5.4.2 Sensory profiling

Each panellist evaluated nine extruded samples at each profiling session. Sensory profiling was repeated once (duplicate) with at least a 30-min break in between. Each sample was made of 4 pieces (5-cm in length) that were presented in a 100-ml porcelain container and covered with plastic foil. Samples were randomized and coded using three-digit numbers. Evaluations were conducted in individual booths at room temperature (25 °C). The attributes for taste and aftertaste were: overall taste, sweet taste, bitter taste, overall aftertaste, sweet aftertaste and bitter aftertaste; while the attributes for texture were: crispiness, crunchiness, hardness, hard particles and adhesiveness. Each of these well-defined attributes was rated in a 10-cm line scale with indented anchors: “not at all” and “very”.

5.4.3 Temporal dominance of sensation (TDS) test

Panellists were also presented with nine extruded samples at each TDS session which was then repeated once (duplicate) with at least 30-min break in between. Each sample was made of two pieces (5-cm in length) and were presented in a 100-ml porcelain container, covered with plastic foil. The following texture descriptors were presented for evaluation: hardness, crackliness, crunchiness, crispiness, roughness, stickiness and gooeyness. The countdown timer started running (time limit: 90s) when the panellists selected the first dominant attribute and stopped at swallowing or spitting the sample. The experiment was conducted with FIZZ Sensory Evaluation Software, Version 2.45 (Biosystemes, Courternon, France).

5.5 Statistical analyses

A Box-Behnken experimental design with three predictors (water content of mixture, screw speed and temperature of die) was used, following the increasing temperature of die (I). For studies II and III, a face-centred split-plot central composite experimental design for two hard-to-change (HTC) and two easy-to-change (ETC) predictors (Vining et al. 2005) was applied in the extrusion processing. Content of amaranth, quinoa, kañiwa or lupine and temperature of die were the two HTC predictors, and screw speed and water content of mixture were the two ETC predictors in the design (II, III).

The calculated contents of four chemical constituents in the dry flour blend [protein content of blend; ash content of blend; dietary fibre content of blend; sum content of main fatty acids of blend, FA (palmitic, oleic, linoleic and linolenic acid)] were also included as predictors in matrix X (II, III). After that, a two-block PLSR model (see **Figure 16**) was computed from the data now consisting of nine predictors and six response variables (SEI, stiffness, WCE, torque, pressure and total SME). The significances of the regression coefficients B in t-test were computed using the jack-knife technique (Martens and Martens 2000). The cumulative variable importances in the projection (VIPs) for each predictor were computed (Eriksson et al. 1999). The number of significant PLS components in the model was found using the criterion $PRESS_i/SS_{i-1} < 1.0$, in which $PRESS_i$ is the predicted sum of squares of the i:th component and SS_{i-1} is the residual sum of squares of the previous component added in the model (Eriksson et al. 1999) (II, III).

A three-block PLSR (i.e., L-PLSR) model was computed using a different combination of predictors and response variables compared with those in the two-block model (see **Figure 17**). The predictors in the matrix X were content of amaranth or quinoa of solids, temperature of die, protein content of blend, ash content of blend, fibre content of blend and FA. The response variables (Y) were, correspondingly, the contents of palmitic acid, oleic acid, linoleic acid, linolenic acid, α-tocopherol, β-tocopherol, γ-tocopherol, total phenolic compounds and folate in extrudates. The additional data matrix Z consisted of the values of the response variables in the dry matter of flour blends containing 20 or 50% amaranth or quinoa of solids. The Unscrambler X 10.1 (CAMO Software AS, Norway), Matlab P2013a (MathWorks, Inc., USA) and Modde 10.1 (Umetrics AB, Sweden) softwares were applied in the computation and graphing. The physical characteristics of the most expanded extrudates were statistically analysed by analysis of variance (ANOVA) (I), Least Significant Difference (I) and post-hoc Tukey's test with a significant level *p* of 5% (II, III).

The effect of the type of grain (amaranth, quinoa and kañiwa), flour content (20, 35 and 50% of solids), replicate (2) and their interaction on the sensory attributes of extrudates was studied at a significance level *p* of 5% (IV). The data was statistically analysed by using a three-way repeated-measures ANOVA in SPSS (SPSS 18.0, PASW Statistics, Chicago, IL, USA). These data were combined with instrumental and physicochemical measurements in a PCA plot in order to identify the most important directions of variability of different samples in a multivariate data matrix (The Unscrambler v9.7; CAMO Software AS, Oslo, Norway) (IV). Data for TDS analysis was processed into plots where the *standardized time* (x axis) is the percentage of the normalized test duration and *dominance rate* (Y axis) represented the percentage of panellists deciding on the same attribute at a given time (IV).

6 RESULTS

6.1 Modelling and process measurements

6.1.1 PLSR models

Four PLS components were included in the PLSR model computed (II, III). For sampled data obtained from the extrusion of corn-based flour containing quinoa and amaranth, the cumulative explained calibration (R^2) and validation variances (Q^2) for the response variables were satisfactory: 79.4% and 72.7%, respectively (II). Similarly, for sampled data obtained from the extrusion of corn-based flour containing kañiwa and lupine, the cumulative explained calibration (R^2) and validation variances (Q^2) were high: 82.0% and 78.5%, respectively (III). The R^2 for the response variables in the L-PLSR model was 65.4% (II).

Regarding the experiments involving amaranth and quinoa (II), the predictors WCM and screw speed had the greatest effect on response variables [value of importance on projection (VIP): 1.58 and 1.55, respectively]. Although protein content and grain type did not have remarkable importance in the projection altogether, they were important predictors for torque and specific mechanical energy. For those experiments involving kañiwa and lupine (III), there were various predictors having an important effect on response variables: WCM (1.52), screw speed (1.39), protein content of blend (1.20) and fibre content of blend (1.15). The corresponding PLSR regression coefficients for temperature of die were also found to be non-significant ($p > 0.1$) for all the response variables (II, III) of the model in the t-test.

6.1.2 Torque, pressure at the die and SME

Torque and pressure at the die during the extrusion of the corn-based mixture containing amaranth, quinoa, kañiwa and lupine increased with decreasing WCM and screw speed (I, II, III). Decreasing WCM and increasing screw speed increased total SME (II, III) (**Table 14**). While CoF had a noticeable and minor inverse effect on torque and pressure at the die, respectively, during the extrusion of the mixture containing amaranth and quinoa (II), CoF showed a minor effect on torque, pressure at the die and total SME during the extrusion of the mixture containing kañiwa and lupine (III) (**Table 14**). Protein content, fibre content and ash content of blend showed a remarkable inverse effect on torque and pressure at the die during the extrusion of the mixture containing kañiwa and lupine (III).

Table 14. Regression coefficients and explained calibration (R^2) and prediction (Q^2) variances (%) in the PLSR model for the response variables SEI-SME. The predictors were the grain type (GT), the content of amaranth-quinoa and kañiwa-lupine of solids (CoF), temperature of die (TEM), screw speed (SS), water content of blend (WCM), protein content of blend (PROT), ash content of blend (ASH), fibre content of blend (FIB) and sum content of main fatty acids of blend (FA). Response variables were sectional expansion index (SEI), stiffness (STF), water content of extrudate (WCE) and total specific mechanical energy total SME). The values of the coefficients were computed using autoscaled data.

	Amaranth-Quinoa				**Kañiwa-Lupine**			
	SEI	**STF**	**WCE**	**Total SME**	**SEI**	**STF**	**WCE**	**Total SME**
GT	–0.0353^{ns}	–0.0350^{ns}	0.0801^{ns}	0.3190*	–0.1761***	0.1865***	0.0400^{ns}	–0.1204^{ns}
CoF	–0.0757*	0.0098^{ns}	0.0283^{ns}	–0.0226^{ns}	–0.1583***	0.1738***	0.0164^{ns}	–0.0869^{ns}
TEM	–0.0245^{ns}	0.0336^{ns}	0.0661^{ns}	0.0774^{ns}	–0.0047^{ns}	–0.0011^{ns}	0.0321^{ns}	0.0452^{ns}
SS	0.6581*	–0.5750*	–0.3477*	0.6873*	0.3425***	–0.2841**	–0.2826**	0.7919***
WCM	–0.5599*	0.5932*	0.5721*	–0.1619*	–0.3471***	0.1596^{ns}	0.8546***	–0.3017***
PROT	–0.0390^{ns}	0.0292^{ns}	–0.0304^{ns}	–0.2218*	–0.2238***	0.2489***	0.0099^{ns}	–0.1285***
ASH	–0.0694*	0.0156^{ns}	0.0136^{ns}	–0.0785*	–0.1298***	0.1305***	0.0629^{ns}	–0.0464^{ns}
FIB	–0.0806*	–0.0038^{ns}	0.0480^{ns}	0.1004*	–0.2140***	0.2385***	0.0075^{ns}	–0.1238***
FA	–0.0905***	0.0509^{ns}	0.0568^{ns}	–0.1464*	–0.0262^{ns}	0.0287^{ns}	0.0038^{ns}	–0.0113^{ns}
R^2	84.90%	69.70%	70.80%	87.60%	78.20%	75.28%	82.17%	89.63%
Q^2	81.30%	63.10%	61.90%	83.40%	71.51%	69.81%	70.23%	80.08%

*, **, *** Significant at $p<0.05$, $p<0.01$ or $p<0.001$, respectively

^{ns} Not significant

6.2 Physical and physicochemical properties of extrudates containing amaranth, quinoa, kañiwa and lupine

6.2.1 Physical properties

The incorporation of up to 50% amaranth and quinoa flour of solids had generally a minor importance (based on VIP value) on the physical properties of corn-based snacks such as SEI and stiffness (II). While the incorporation of up to 50% kañiwa had a moderate proportional and inverse effect on SEI and stiffness, respectively, the incorporation of lupine above 20% caused structural collapsed, leading inevitably to very low expansion and high stiffness (**Table 14**) (III). The incorporation of up to 20% amaranth, quinoa or kañiwa led to substantially higher SEI when the bulk ingredient was pregelatinised corn flour (II-III) compared to normal corn flour (I).

Process parameters such as SS and WCM had the greatest effect on SEI and stiffness. SEI increased at increasing SS and decreasing WCM, while stiffness increased at decreasing SS and increasing WCM (**Table 14**). Chemical constituents of the blend such as protein, ash, fibre and sum content of fatty acids seemed to have an overall inverse effect on SEI and proportional effect on stiffness (**Table 14; Figure 19**). Temperature of die was the process parameter with the lowest effect on SEI and stiffness (**Table 14**; **Figure 19**) (II, III). Total SME presented a strong positive and negative correlation with SEI and stiffness, respectively (II, III).

6.2.2 Large scale structures and physical properties of the most expanded extrudates

Extrudates containing up to 20 % amaranth and quinoa showed very similar pore size distribution. They were observed to have well-defined pores of different sizes with semiflat sides (I). By contrast, extrudates containing up to 20% kañiwa presented small and poorly defined pores. Extrudates containing pure corn had a distinct pore size distribution with very large pores and thick walls (I). Despite its observable similarity, the most expanded extrudates containing 20% amaranth or quinoa presented statistically larger SEI than those containing 35 or 50% ($p<0.05$) (II). Substantial differences in stiffness were not found among extrudates containing quinoa, whereas the stiffness of extrudates containing 35% amaranth differed significantly from that of those containing 20 and 50% amaranth (II) (**Figure 20**). When it comes to the most expanded extrudates containing kañiwa, stiffness was remarkably stable regardless of the content of kañiwa but SEI reduced considerably (**Figure 20**). For the most expanded extrudates containing

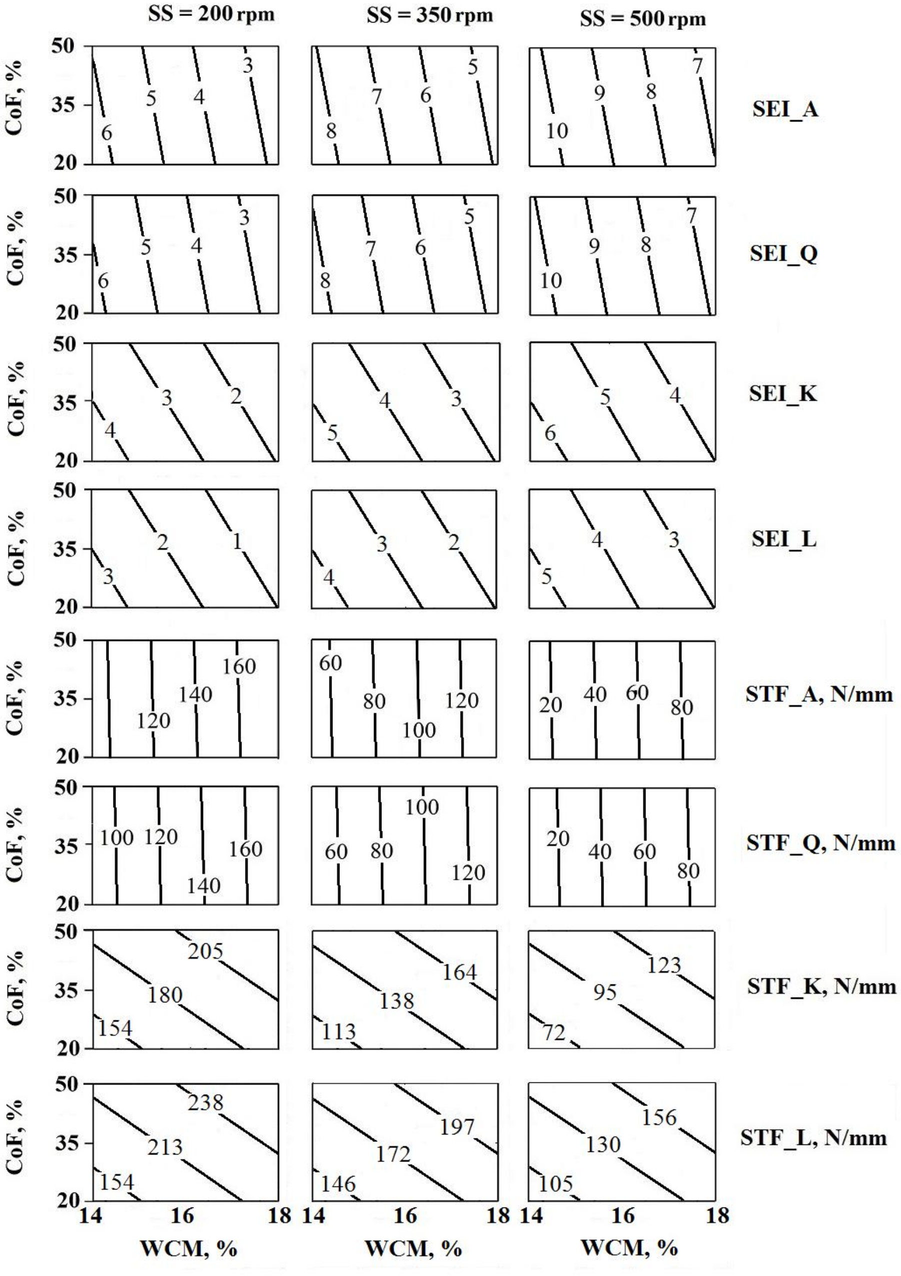


Figure 19. Four-dimensional contour plots for sectional expansion index (SEI) and stiffness (STF) for extrudates containing amaranth (A), quinoa (Q), kañiwa (K) and lupine (L) as a function of screw speed (SS), water content of mixture (WCM) and contents of amaranth or quinoa of solids (CoF).

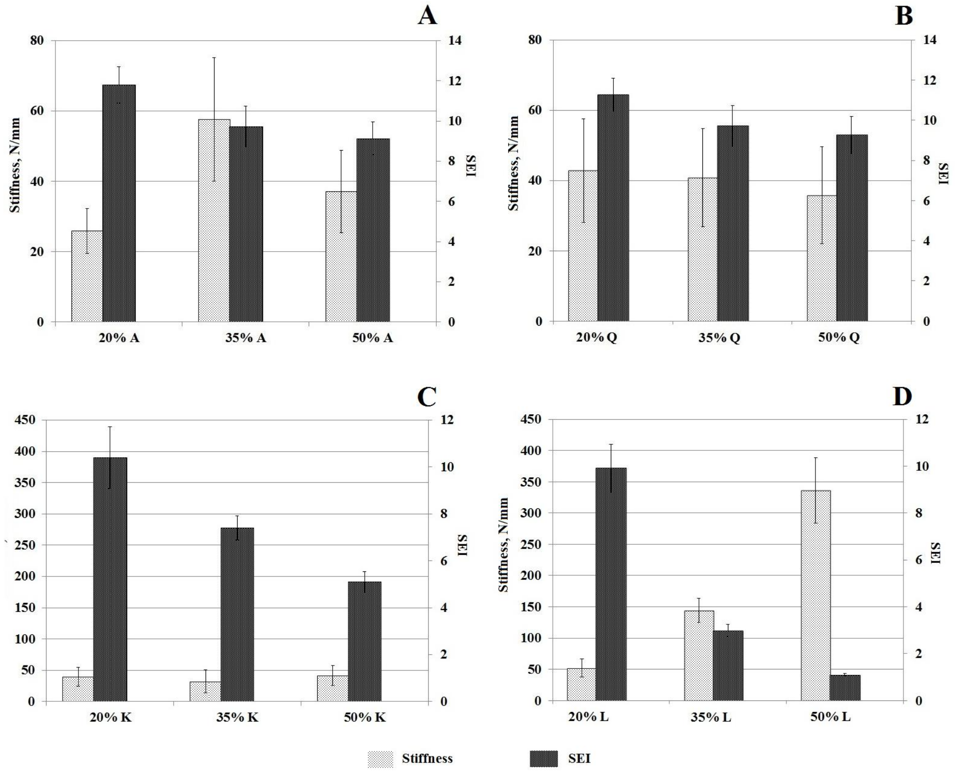


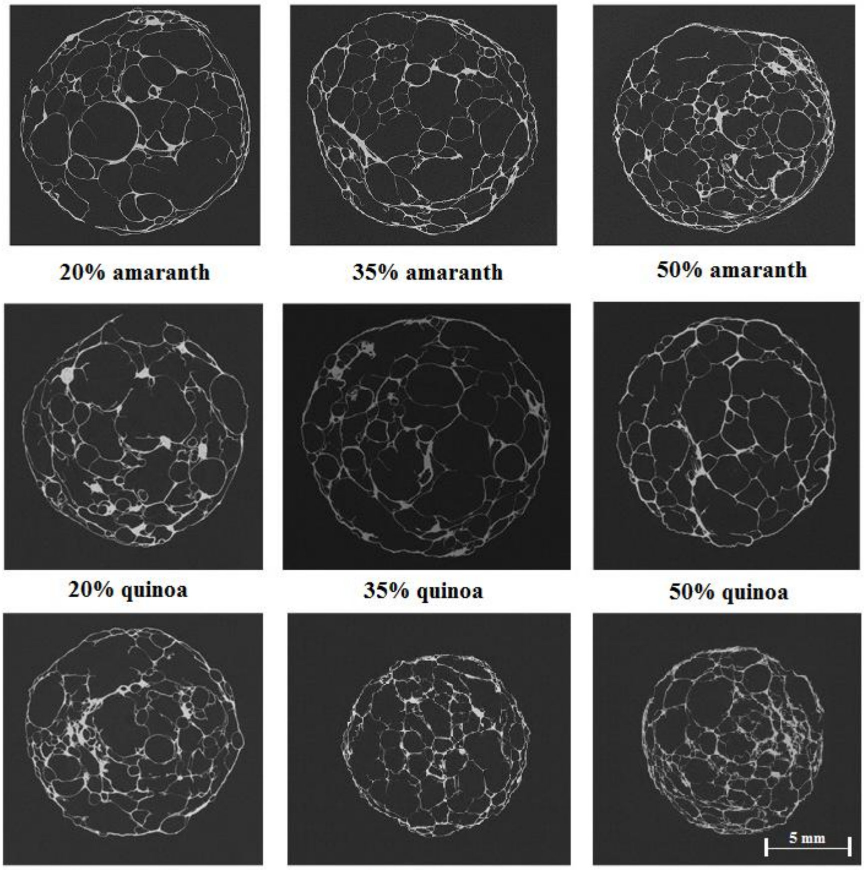
Figure 20. Bar chart for SEI and stiffness of extrudate containing amaranth, quinoa, kañiwa and lupine.

lupine, there was a quasi-exponential increase of stiffness, and decrease of SEI at greater incorporation of lupine (**Figure 20**).

6.2.3 Microstructures of the most expanded extrudates (IV)

Most extrudates presented very stable porosity regardless of the content of amaranth, quinoa or kañiwa (**Figure 21**). Extrudates containing 50% kañiwa were the only ones showing a substantial reduction in porosity; this accounted for 73% compared to 82 and 85% porosity exhibited by extrudates containing amaranth and quinoa, respectively. Despite the apparent stability, increasing content of amaranth, quinoa or kañiwa reduced pore size, progressively (**Figure 22**). Extrudates containing 20% amaranth had about 64% of their pore volume with diameters between 0 and 2000 µm while those containing 50% amaranth had about 94%. In fact, the percentage of pore volumes with diameters between 2000 and 5000 µm reduced from 35% to almost 5% when the content of amaranth increased from 20 to 50% of solids, respectively.

Extrudates containing 20% quinoa had about 77% of their pore volume with diameters between 0 and 2000 µm, and those containing 50% quinoa had around 80%.

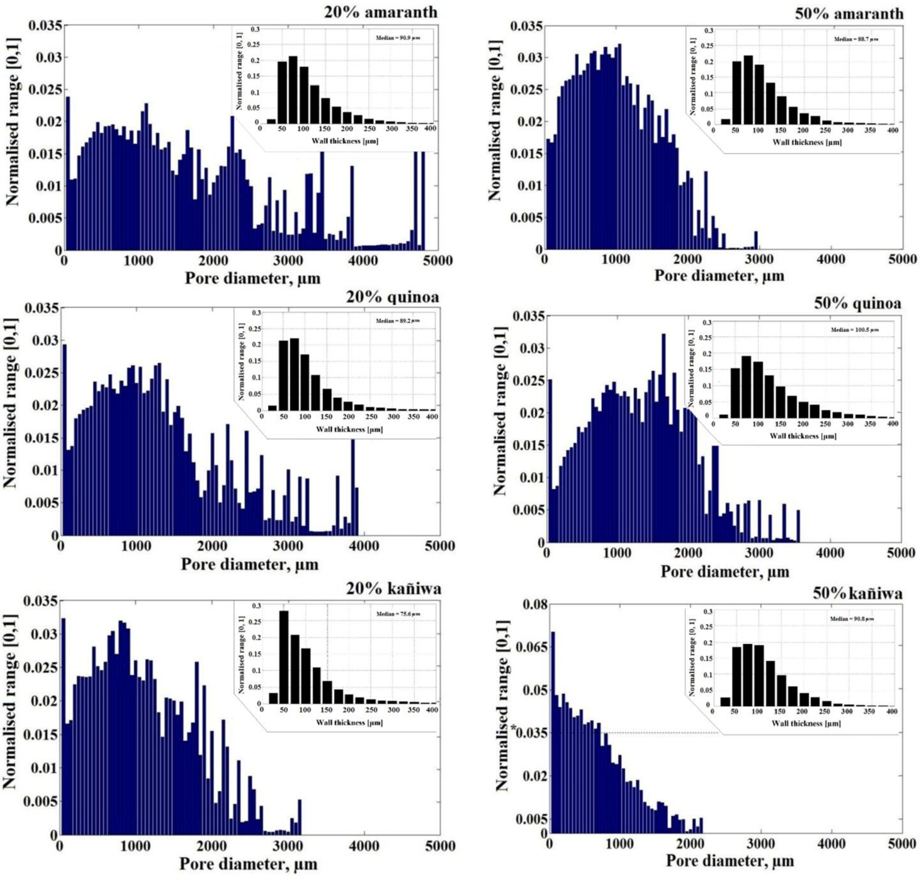


20% amaranth
35% amaranth
50% amaranth
20% quinoa
35% quinoa
50% quinoa
20% kañiwa
35% kañiwa
50% kañiwa

Figure 21. X-ray microtomographs of extrudates containing 20 and 50% amaranth, quinoa and kañiwa.

Extrudates containing quinoa had a remarkable stability concerning the diameters of pores while those containing kañiwa presented smaller pore size diameters as the content of kañiwa increased. For instance, pores with diameters up to 1000 µm increased from 49 to 76% when the content of kañiwa increased from 20 to 50% of solids, respectively. Extrudates containing increasing content of kañiwa presented much thicker walls than those containing amaranth or quinoa.

In fact, extrudates containing amaranth showed almost no difference in wall-thickness as the content of amaranth increased. Extrudates containing kañiwa showed a downfall from 28 to about 18% in the proportion of 50-µm walls. The proportion of walls above 50 µm increased in line with the content of kañiwa. Differences in wall thickness of extrudates containing up to 35% quinoa were hardly observed, but it became noticeable as the content of quinoa reached 50%.



20% amaranth

50% amaranth

20% quinoa

50% quinoa

20% kañiwa

50% kañiwa

Figure 22. Histogram porosity and wall thicknesses in extrudates containing 20 and 50% amaranth, quinoa and kañiwa.

6.2.4 Nanostructures of the most expanded extrudates (I)

The most expanded extrudates containing 20% amaranth, quinoa and kañiwa were milled in order to conduct studies with WAXS and SAXS. WAXS intensities displayed peaks corresponding to Vh and Eh crystal structures. It was observed that Vh structure (peaks at 2θ = 7.4°, 12.9°, and 19.6°) was dominant across the samples while Eh structure (peaks at 2θ = 6.9°, 11.9°, and 18.2°) was the highest in milled pure corn extrudates and the lowest in milled extrudates containing 20% kañiwa (**Figure 23**). SAXS intensities obeyed the power law with an exponent around -3.5 (±0.2). No peak from lamellar repeating structures in the studied length scale (2-30 mm) was observed (**Figure 23**). The combined results indicated that the milled extrudates containing 20% amaranth, quinoa, kañiwa and pure corn presented sheets of crystallised amylose-lipid complexes without the formation of ordered stacks (**Figure 24**).

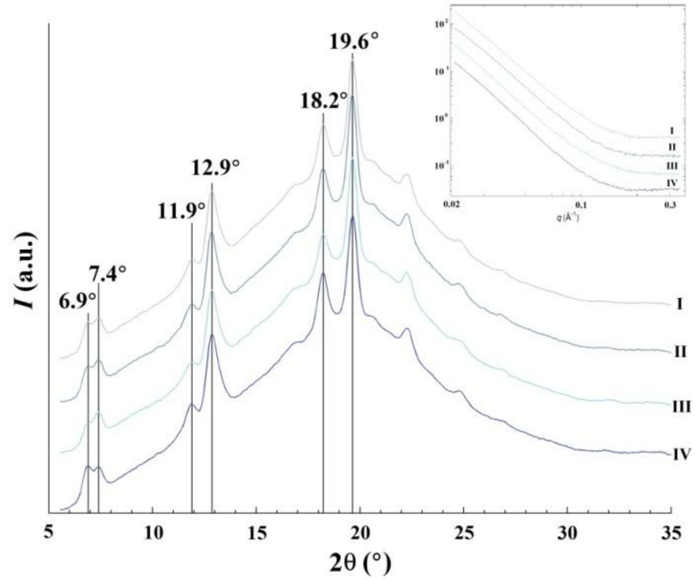


Figure 23. X-ray scattering intensities of milled extrudates containing 20% amaranth (I), 20% quinoa (II), 20% kañiwa (III) and 100% corn (IV)

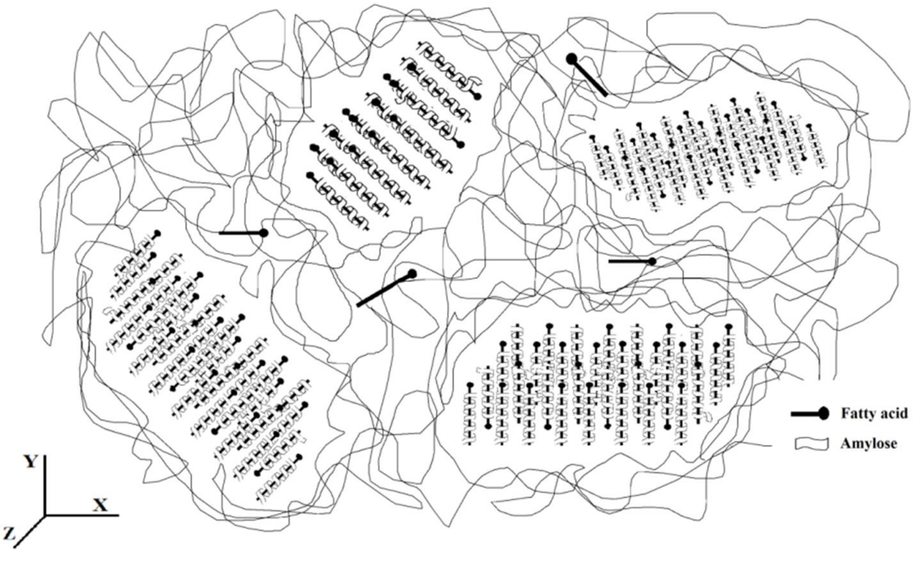


Figure 24. Suggested model for sheets of crystallised amylose-lipid complexes without ordered stacks and fatty acids trapped in the amorphous region

6.2.5 Hexanal formation during storage (I)

In general, the formation of hexanal was substantially higher in milled than in whole extrudates (except for whole extrudates containing quinoa and exposed to RH of 11%). In fact, lipid oxidation was prevalent in extrudates containing quinoa and kañiwa and exposed to RH of 11%. The formation of hexanal in extrudates containing amaranth and pure corn seemed unaffected by changes in the relative humidity (11% and 76% RH). Milled extrudates containing kañiwa and stored at RH of 11% showed a 25-fold increase in hexanal while those stored at RH of 76% showed only a 3-fold increase.

6.2.6 Capacity of water absorption and water solubility

Extrudates with higher content of amaranth presented lower WAI and WSI, while those containing higher content of quinoa had stable WAI and lower WSI (IV). Regarding the incorporation of kañiwa, WAI decreased substantially while WSI was remarkably stable from 20 to 35%, and then reduced slightly at 50% (IV).

6.3 Content of fatty acids and bioactive compounds in extrudates containing amaranth, quinoa, kañiwa and lupine

6.3.1 Fatty acids

After extrusion, there was a substantial reduction of palmitic, oleic, linoleic and linolenic acid compared to flour blends (II, III), and temperature of die was found to have hardly any effect on the content of fatty acids (**Table 15**). It seemed that the retention of oleic acid increased at higher temperature of die, particularly in extrudates with greater content of amaranth (e.g., from 3 to 36 percentage points for extrudates with 50% amaranth, relative to flour blend). Also, the content of palmitic acid in extrudates containing 20% kañiwa or lupine reduced by around 80 percentage points (relative to the flour blend) after extrusion, while those containing 50% kañiwa and lupine presented a retention of 50 and 75 percentage points (relative to the flour blend), respectively. In general, greater incorporation of amaranth, quinoa, kañiwa and lupine led to a greater retention of fatty acids. Traces of linolenic acid were detected in most flours and extrudates (II, III).

6.3.2 Tocopherols

Extrusion reduced considerably the detectable content of α-, β- and γ-tocopherol compared to flour blends, and temperature of die had almost no effect on the content of α-, β- and γ-tocopherol (II, III) (**Table 15**). In general, extrudates containing amaranth presented a higher retention of γ-tocopherol compared to those containing quinoa (II). Extrudates containing 20% kañiwa or lupine showed a great loss of γ-tocopherol (by 75 percentage points relative to the flour blend) while those containing 50% kañiwa and lupine presented a retention of around 50 and 60 percentage points (relative to the flour blend), respectively (III).

6.3.3 Total phenolic compounds

Phenolic compounds showed high retention despite extrusion (II, III) (**Table 15**). Based on the results from methanol-water treatment, extrudates containing 20% quinoa had lower content of phenolic compounds compared to those containing 20% amaranth. Also, extrudates containing 20% kañiwa or lupine presented losses of total phenolic compounds of around 45 percentage points (relative to the flour blend), but the losses reduced to around 20 percentage points at higher content of kañiwa or lupine. Despite this, the increasing contents of amaranth, quinoa, kañiwa and lupine had minor effects on the content of total phenolic compounds. There was a considerable increase in the detectability of phenolic compounds after acid-hydrolysis treatment.

Apparently, the increasing temperature of die had minor or slight effects on the total content of phenolic compounds in extrudates containing amaranth, quinoa, kañiwa or lupine.

6.3.4 Folate

Extrusion had a slight effect on the content of folate in extrudates containing amaranth, quinoa or lupine, and a moderate effect on those containing kañiwa (II, III) (**Table 15**). The increasing contents of amaranth, quinoa, kañiwa and lupine had the expected proportional effect on the content of folate (II, III). In addition, the retention of folate seemed to increase at increasing contents of amaranth, quinoa, kañiwa and lupine. For instance, extrudates containing 20% amaranth presented losses of folate of around 10 percentage points (relative to the flour blend) while those containing 50% amaranth showed an increase in the content of folate of around 5 percentage points (relative to the flour blend). Accordingly, extrudates containing 20% quinoa presented losses between 26 and 31 percentage points (relative to the flour blend) while those containing 50% quinoa showed losses between 10 and 18 percentage points (relative to the flour blend).

Generally, extrudates containing either kañiwa or lupine had comparable or higher detectable content of folate than flour blends (III). Extrusion increased the content of folate in extrudates containing 50% kañiwa while it decreased it in extrudates containing 50% lupine. Extrusion had little or no effect on the content of folate in extrudates containing 20% kañiwa or lupine (III).

Table 15. Content of fatty acids, tocopherols, total phenolic compounds and folate in extrudates containing 20 or 50% amaranth, quinoa, kañiwa and lupine. Temperature of die was set at 140 or 160 °C.

	Fatty acid content (mg/100 g d.m.)				**Tocopherol content (µg/g d.m.)**			**Total phenolic compounds (GAE equivalent µg/g d.m.)**		**Content of folate (ng/g d.m.)***
	C 16:0	**C 18:1**	**C 18:2**	**C 18:3**	**α-TOH**	**β-TOH**	**γ-TOH**	**Methanol-water treatment**	**Acid hydrolysis**	
20% amaranth										
Flour blend**	267.1	404.0	892.7	22.9	2.8	4.1	3.4	307.6	595.5	168
140 °C	52.6±1.0	87.5±0.1	192.4±5.5	6.2±9.0	0.8±0.04	0.8±0.03	1.2±0.1	198.6±24.5	686.1±33.8	152
160 °C	51.3±1.1	88.3±1.1	200.3±0.9	10.1±5.4	0.8±0.1	0.8±0.1	1.1±0.1	251±22.6	680.7±20.0	153
50% amaranth										
Flour blend**	517.2	724.6	1475.9	34.0	4.7	10.2	2.6	286	606	272
140 °C	158.7±0.5	22.8±8.5	460.8±1.0	11.0±0.03	1.7±0.1	3.6±0.2	1.2±0.1	234.6±29.2	643.0±28.2	355
160 °C	180.1±6.6	260.9±0.8	631.3±7.3	16.2±1.0	1.7±0.02	3.6±0.1	1.3±0.02	212.8±5.3	677.0±24.9	371
20% quinoa										
Flour blend**	187.9	498.5	915.5	65.5	3.8	0.2	9.8	332.4	606	346
140 °C	40.7±0.3	109.6±2.6	192.8±0.5	23.9±1.1	1.0±0.1	n.d.	2.2±0.2	149.3±16.8	549.6±9.3	202
160 °C	46.1±2.6	128.6±4.2	213.9±7.0	25.3±0.3	1.1±0.04	n.d.	2.4±0.04	150.1±3.8	562.5±11.4	188
50% quinoa										
Flour blend**	319.2	960.8	1532.8	140.4	7.4	0.5	18.6	348.2	686.8	606
140 °C	94.9±5.3	266.7±13.8	455.9±24.9	43.9±3.0	2.9±0.1	n.d.	6.3±0.3	220.5±6.8	668.5±31.6	495
160 °C	101.6±3.0	286.9±7.1	508.5±18.1	49.3±3.1	3.0±0.1	n.d.	6.4±0.3	212.1±18.2	680.5±58.7	546

20% kañiwa										
Flour blend**	296.8	576	1178	96.96	4.0	0.1	16.9	392.8	774.0	435
140 °C	66.6±4.4	133.3±5.2	252.1±6.1	22.3±2.8	1.0±0.05	n.d.	3.9±0.2	201.2±6.3	684.2±16.4	372
160 °C	63.3±0.7	122.5±1.2	247.2±1.9	20.6±0.1	1.0±0.04	n.d.	3.7±0.2	251.1±22.6	803.3±33.0	441
50% kañiwa										
Flour blend**	590.5	1155	2180	218.1	7.7	0.2	36.4	498.4	1107	1012
140 °C	269.3±3.8	505.6±13.3	968.2±8.1	98.2±2.7	4.2±0.2	n.d.	17.5±0.9	425.4±40.8	1230.3±43.0	1185
160 °C	293.0±8.2	548.4±15.9	1067.1±31.4	109.5±4.3	4.4±0.1	n.d.	18.2±0.4	397.2±55.8	1035.1±36.0	1181
20% lupine										
Flour blend**	194.8	412.0	956.0	91.0	3.2	n.d.	24.6	357.9	704.7	357
140 °C	38.3±1.5	32.2±3.2	154.7±6.8	18.2±0.6	0.7±0.07	n.d.	4.7±0.3	215.7±14.4	689.8±51.1	361
160 °C	38.6±11.3	82.8±24.3	186.9±54.8	18.5±5.4	0.8±0.2	n.d.	4.7±0.5	223.1±32.8	834.8±35.7	383
50% lupine										
Flour blend**	335.5	745.0	1625.0	203.1	5.8	n.d.	55.7	411.1	933.5	818
140 °C	346.6±4.0	747.3±9.8	1656.9±41.9	210.6±8.2	2.8±0.1	n.d.	29.6±0.8	334.1±24.1	1015.4±24.0	654
160 °C	246.2±2.2	556.5±21.8	1160.0±17.6	156.6±21.9	3.4±0.1	n.d.	34.6±1.1	341.1±37.0	1022.0±32.9	694

*Analysis of folate was conducted in duplicate (n = 2)
**Calculated contents in flour blend
n.d. = not detected

Temperature of die had little or no effect on the content of folate in extrudates containing 20, 35 or 50% amaranth, quinoa, kañiwa or lupine (of solids).

6.4 Sensory characteristics of extrudates containing amaranth, quinoa, kañiwa and lupine

6.4.1 Texture and taste profile

Simplified profiles were constructed for texture, taste and aftertaste attributes (**Figure 25**). Extrudates with increasing contents of kañiwa lost more crispiness than those with amaranth and quinoa (**Figure 25**) (IV). Also, the increasing contents of amaranth, quinoa or kañiwa made extrudates less crunchy (**Figure 25**). Extrudates became harder as the content of kañiwa increased while those containing amaranth and quinoa were perceived less hard. The perception of hard particles reduced as amaranth and quinoa increased while the perception remained low at all contents of kañiwa (**Figure 25**). Extrudates containing amaranth and quinoa were perceived as more adhesive (to teeth) than those containing kañiwa.

Kañiwa increased the overall taste intensity, particularly, at the highest content (**Figure 25**). There was also a substantial increase in the perception of sweetness at higher contents of amaranth. Besides, bitterness was perceived stronger as the content of tested flours (i.e., amaranth, quinoa or kañiwa in sensory studies) increased (**Figure 25**).

Generally, overall aftertaste increased at higher content of tested flours, and this became more evident in extrudates containing amaranth. Bitter aftertaste in extrudates containing amaranth and kañiwa were perceived stronger than in those containing quinoa (**Figure 25**).

6.4.2 Textural characteristics during mastication

Six dominance attributes were identified during mastication through TDS testing: crispiness, hardness, crunchiness, stickiness and gooeyness (order of appearance) (IV). An overview of the normalised temporal dominance of sensation curves is shown in **Figure 26**. PCA plots were constructed in order to identify the degree of correlation of dominant attributes based on their dominance of sensation areas (**Figure 27A**). Crispiness was more dominant in extrudates containing amaranth and quinoa than in those containing kañiwa. Similarly, crunchiness was increasingly dominant at greater contents of amaranth or quinoa but it remained stable at increasing contents of kañiwa (**Figure 27A**). Roughness was only dominant at low contents of amaranth and quinoa while it was not dominant at all in extrudates containing kañiwa (**Figure 27A**).

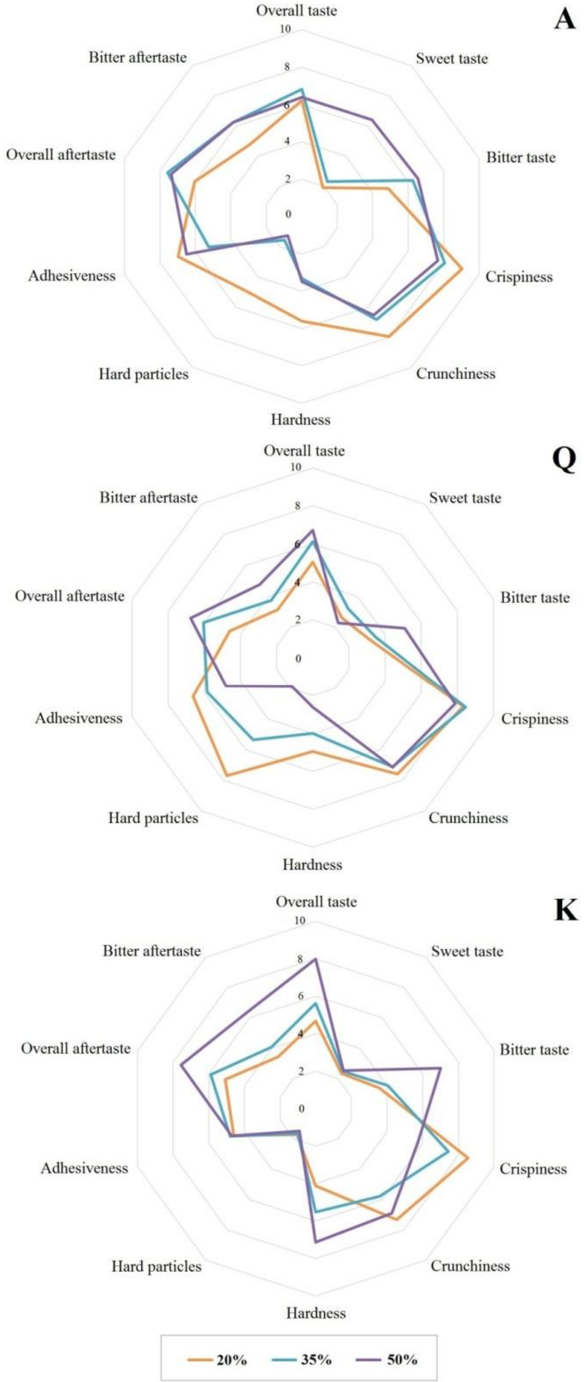


Figure 25. Mean ratings of texture and taste characteristics of extrudates containing 20, 35 and 50% amaranth (A), quinoa (Q) and kañiwa (K)

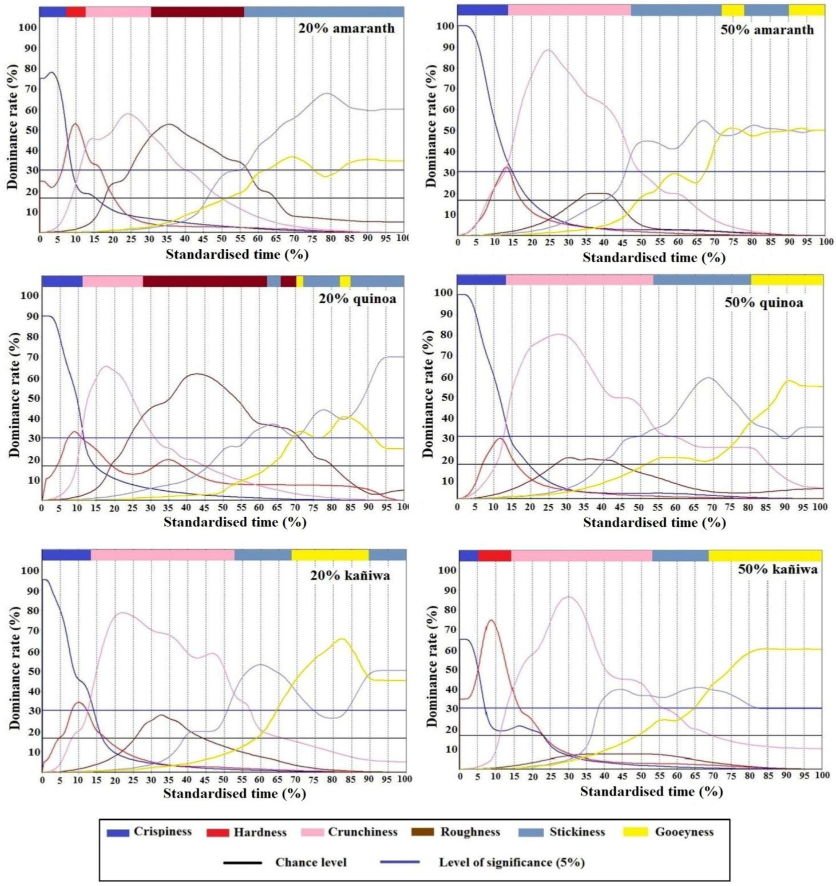


Figure 26. Normalised temporal dominance of sensation curves from texture analysis of corn-based extrudates containing amaranth, quinoa and kañiwa. Values are combined averages from two trials.

Stickiness was also dominant mostly at low contents of tested flours, while gooeyness became slightly more dominant at greater content of tested flours. Hardness was perceived between crispiness and crunchiness, mostly in extrudates containing higher content of kañiwa.

Concerning the length of dominance in the mouth, crunchiness had the longest dominance regardless of the grain type and flour content (**Figure 27B**). Additionally, stickiness was perceived as dominant the longest in extrudates containing amaranth and quinoa while hardness was perceived as dominant the shortest in extrudates containing higher content of kañiwa (**Figure 27B**). For roughness, the length of dominance in the mouth was short and comparable to crispiness.

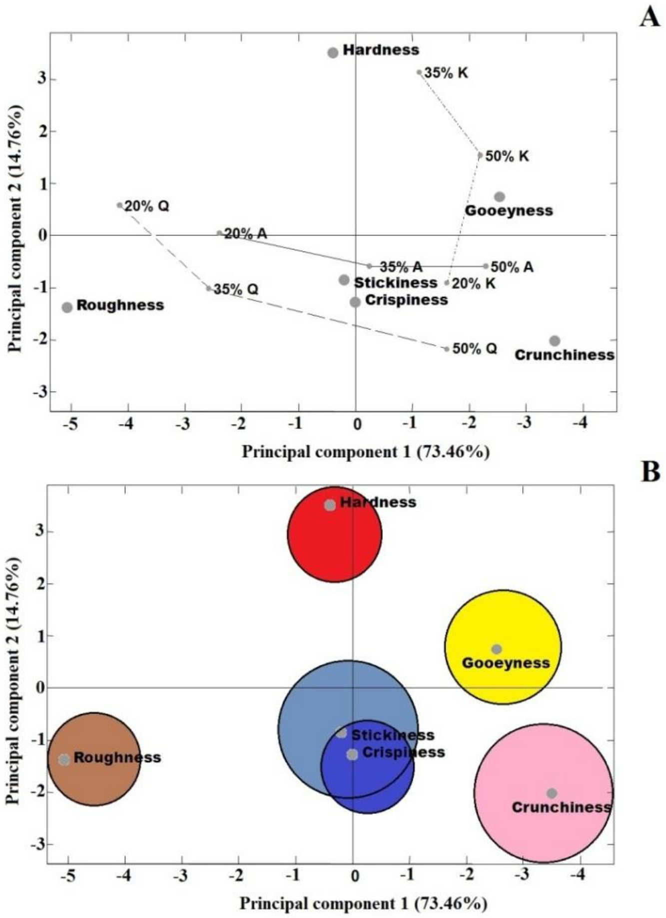


Figure 27. PCA plots based on the area under the dominance of sensation curves. A. Biplot showing changes in the dominant attributes as the content of amaranth, quinoa or kañiwa increases. B. Loading plot depicting comparable dominance of sensation areas: crunchiness>stickiness>gooeyness>roughness>crispiness>hardness (estimation is based on the average area corresponding to individual sensations).

7 DISCUSSION

7.1 Effect of extrusion on process response variables and physical properties of corn-based extrudates

7.1.1 Torque, pressure at die and total SME

In the present study, torque and pressure at the die were mostly inversely proportional to screw speed and WCM (I, II, III) while total SME was proportional to screw speed and inversely proportional to WCM (II, III). This agrees with the results obtained by Ilo and Liu (1999) in which rice-based flour blends containing 20, 40 and 60% amaranth went through extrusion in a twin-screw extruder. The authors observed that torque, pressure and total SME decreased as water content of mixture increased. According to the authors such changes may well result from alterations in the viscosity of the mixture during extrusion. Furthermore, Lin et al. (2000) and Onwulata et al. (2001) also observed that increasing water content of mixture decreased torque, pressure and total SME, considerably. It is plausible that water content may eventually cause surplus plasticisation in the melt, resulting in a lower degree of starch gelatinisation and viscosity. In this regard, Bhattacharya and Hanna (1987) observed that higher water content decreased the percentage of starch gelatinisation during extrusion.

In the present study, increasing contents of amaranth, quinoa, kañiwa and lupine carried an inevitable increase in protein and fibre (**Table 13**), which appeared to have an inverse effect on torque and pressure (II, III). Mohamed (1990) studied the effect of protein content and screw speed on torque and pressure during the extrusion of starch. The author observed that increasing contents of protein and screw speed led to lower values of torque and pressure in the extruder. Additionally, Onwulata et al. (2001) added up to 25% whey protein concentrate to corn-based extrudates, and found that pressure, torque and total SME dropped by 59, 54 and 58%, respectively, at higher contents of whey protein concentrate.

In general, temperature of die showed little or no effect on torque and pressure at the die in the present study. Such results go against the findings of, for instance, Lin et al. (2000) who observed that increasing water content and temperature led to decreasing torque and pressure during the extrusion of soy protein. As expected, Bhattacharya and Hanna (1987) found that gelatinisation increased in direct proportion to the barrel temperature. Except for the temperature of die (140, 150 and 160 °C), the present study maintained the same temperature profile (90, 95, 95, 100, 110, 140 °C) for most experiments (II, III and IV). This may explain the little effect of temperature on the values of torque and pressure.

Despite the temperature at section 6 (preceding the die) being pre-set at 140 °C, the temperature of melt at section 6 was found to vary slightly (around 6 °C) in proportion with torque and total SME (II, III). This shows that pre-set temperature of steel concealed changes that may occur between the temperature of melt and torque or total SME. In most extrusion studies (Coulter and Lorenz, 1991; Ilo and Liu, 1999; Chavez-Jauregui et al., 2000), the relationship between temperature of melt and torque or total SME is not considered.

7.1.2 Sectional expansion

In general, the sectional expansion of extrudates containing amaranth, quinoa, kañiwa and lupine increased in direct and inverse proportion to screw speed and WCM, respectively (I, II, III). Authors such as Coulter and Lorenz (1991) and Chavez-Jauregui et al. (2000) found that increasing screw speed led to greater sectional expansion of extrudates containing quinoa and defatted amaranth flour, respectively. The present study also showed that the sectional expansion of extrudates with higher content of fibre (e.g., extrudates containing kañiwa) became increasingly correlated with screw speed and less correlated with WCM. This shows that mixtures with greater content of fibre were increasingly sensitive to changes in screw speed compared to those in WCM (I). It is plausible that increasing screw speed during extrusion may result in lower melt viscosity and greater elasticity (Fletcher et al., 1985; Ding et al., 2006) leading up to, possibly, more expanded extrudates. Besides, Bhattacharya and Hanna (1987) observed that increasing water content during the extrusion of starch caused considerably less starch gelatinisation thereby hindering sectional expansion of extrudates.

At increasing contents of amaranth or quinoa, substantial reduction in sectional expansion was not observed in the present study (II). While it is true that the actual increase of protein or fibre after the incorporation of amaranth or quinoa varied slightly, authors such as Coulter and Lorenz (1991) observed that the incorporation of just 30% quinoa to a corn-flour blend reduced moderately the sectional expansion of extrudates (2.1-3.99 for extrudates containing 100% corn; 1.78-2.83 for extrudates containing 30% quinoa). Also, Dokic et al. (2009) incorporated up to 50% amaranth to a corn-flour blend, and found that the sectional expansion index reduced from 4.03 (100% pure corn) to 1.83 (50% amaranth). Coulter and Lorenz (1991) and Dokic et al. (2009) used very similar compression rates (3:1 and 4:1, respectively), screw speeds (100-200 and 120 rpm, respectively), temperature of die (150 and 160 °C, respectively), water content (15-25 and 16%of solids, respectively) and single screw extruders. This may explain their similar results, and differences relative to the present study where the screw speed range was wider (200-500 rpm) and a twin screw extruder was used. Possibly, the conditions used in the present study increased our chances to obtained greater sectional expansion (1.9-11.8 for extrudates containing amaranth; 1.9-11.3 for extrudates containing quinoa)

The increasing contents of kañiwa and lupine as well as WCM had an inverse effect on the sectional expansion of extrudates. Interestingly, screw speed, the driving force for the sectional expansion of extrudates containing amaranth and quinoa, had just a slight proportional effect on the sectional expansion of extrudates containing kañiwa and lupine. It seems that the incorporation of flours rich in protein and fibre such as kañiwa and lupine was the main variable hampering sectional expansion (III). Brennan et al. (2008) studied the effects of various contents of wheat bran (5, 10 and 15% of solids) on the sectional expansion of wheat-based extrudates, and showed that increasing bran content reduced, as expected, sectional expansion. In fact, Moraru and Kokini (2003) suggested that the presence of low contents of fibre in the melt may stabilize the sectional expansion thereby increasing the resistance for longitudinal expansion. Although other extrusion studies dealing with the incorporation of kañiwa and lupine to corn-based snacks have yet been conducted, Repo-Carrasco-Valencia (2009) extruded pure kañiwa flour using a single-screw extruder at three different water contents (12, 14 and 16%). Predictably, the lowest water content led to more expanded extrudates (1.98 compared to 1.61), the difference, though, was minor and apparently the aim of the study was to test the effect of extrusion conditions on the status of phenolic compounds rather than to develop an edible extruded snack.

As expected, changes in the bulk ingredient, from normal corn flour to pregelatinised corn four, had a positive effect on the section expansion of extrudates containing up to 20% amaranth, quinoa or kañiwa. Nabeshima and Grossmann (2001) reported that the pregelatinisation of cassava starch through extrusion (profile temperatures, 60-100 °C; screw speed, 100 rpm) led to higher WAI and cold viscosity, particularly, at higher profile temperatures. In the present study, extrudates containing pregelatinized corn flour may have reached greater expansion as a result of faster viscosity development during extrusion, as suggested by Coulter and Lorenz (1991).

7.1.3 Stiffness

Stiffness of extrudates containing amaranth, quinoa, kañiwa and lupine was shown to increase in direct proportion to WCM (I, II, III). Various studies (Fletcher et al., 1985; Ilo and Liu, 1999; Liu et al., 2000; Ding et al., 2006) have reported similar results. For instance, Fletcher et al. (1985) and Ding et al. (2006) suggested that increasing water content of mixture may cause loss of elasticity in the melt thereby contributing to greater hardness (expressed in N) and product density. Greater hardness is generally linked to a lower degree of starch gelatinisation during extrusion and, interestingly, Bhattacharya and Hanna (1987) observed that the degree of starch gelatinisation reduced considerably at increasing contents of water during extrusion. Even though the incorporation of amaranth or quinoa showed a minor effect on the stiffness of extrudates, the incorporation of kañiwa and, particularly, lupine had a distinct effect on stiffness (II, III). As expected, flour blends containing kañiwa and lupine had substantially higher content of protein and fibre compared to amaranth and quinoa. Dokic et al. (2009) incorporated 0, 20 and 50% amaranth to corn-based extrudates, and observed that the hardness (expressed in N) increased in direct proportion to the incorporation of amaranth. This goes clearly against the results obtained in the present study where extrudates containing amaranth had virtually no effect on stiffness (directly proportional to hardness) (II). Unfortunately, there are no studies dealing with the extrusion of quinoa, kañiwa or lupine where hardness or stiffness was measured. Brennan et al. (2008) conducted a comparable study where 5, 10 and 15% bran content was incorporated to wheat-based extrudates. The authors found that hardness (expressed in g/s) increased a maximum of 280% as the content of bran reached 15%. Besides, Onwulata et al. (1998) incorporated WPC or sweet whey solids (SWS) to corn-based extrudates and found that the hardness (expressed in N) reduced slightly at 25% WPC or SWS, and then increased distinctively at 50% WPC or SWS. In the present study, the content of protein was between 10 and 20%, and consisted mostly of glutelins and prolamins, and moderate contents of albumins and globulins for extrudates containing amaranth, quinoa and kañiwa (based on **Table 2**), while those containing lupine had, at least theoretically, greater contents of glutelins, prolamins and globulins and minor contents of albumins (III). In optimal conditions, glutelins/glutenins and prolamins may confer viscosity and elasticity to the melt thereby altering (favourably) the physical characteristics of edible extrudates (Jerez et al., 2005; Hernandez-Izquierdo and Krochta, 2008; Casparus and van der Berg, 2010). The indirect incorporation of albumins and, particularly, globulins into the system (through the addition of amaranth, quinoa kañiwa and lupine) could have, in some way, hindered the development of viscosity and elasticity (II, III).

The most plausible reason for hardening may also come from the proportional reduction of starch available for gelatinisation. For instance, lupine had very low content of starch and mostly fibre, protein, fat and non-starch polysaccharides. This could explain the rapid hardening of extrudates containing 35 and 50% lupine regardless of screw speed or WCM.

7.2 Effect of on the chemical characteristics of expanded corn-based extrudates

7.2.1 Loss of fatty acids

The considerable reduction in the content of fatty acids during the extrusion of flour blends containing amaranth, quinoa and kañiwa could be attributed to the formation of amylose-lipid complexes (Bhatnagar and Hanna, 1994a) (I, II, III). The formation of such complexes was shown in the present study (I) and this could be one of many factors altering the physicochemical characteristics of extrudates at increasing contents of amaranth, quinoa and kañiwa. As lupine flour had a composition devoid of starch, the formation of amylose-lipid complexes may not have taken place. The present study showed clearly that the content of fatty acids in extrudates containing lupine remained quantitatively unaltered after extrusion (III).

Another possible reason for the loss of fatty acids during extrusion is lipid oxidation. Despite the high temperature, pressure, and frictional conditions the mixture goes through during extrusion, the possibility of substantial quantities of oxygen molecules causing instant lipid oxidation is doubtful. Various studies (Rao and Artz, 1989; Lampi et al., 2015; Moisio et al., 2015) have shown that the peroxide values, conjugated oxidation products and hexanal content are close to cero at time cero during storage studies. Therefore, it is reasonable to think that the loss of fatty acids during extrusion was unlikely to result from oxidation. This does not dismiss the possibility of greater sensitivity at particularly extrusion condition such as greater temperature profiles or water contents. For instance, Lampi et al. (2015) observed that oat flour extruded at greater profile temperatures (70, 110 and 130 °C) became remarkably unstable during 15-week storage. In fact, the authors suggested that temperatures such as 130 °C should be avoided as it promotes extensive lipid stability and degradation of triacyl glycerol and free fatty acids. However, the stability of amylose-lipid complexes could also depend on the degree unsaturation. Holms et al. (1983) and Eliasson and Krog (1985) observed that amylose-lipid complexes involving saturated fatty acids were considerably more stable to (enzymatic hydrolysis) than those involving unsaturated fatty acids. In that sense, complexes involving oleic, linoleic and linolenic acid such as the ones in the present study are expected to be more prone to eventual lipid oxidation than those involving, for instance, palmitic acid.

The use of low contents of water during extrusion could, on the other hand, prevent eventual lipid oxidation as suggested by Moisio et al. (2015). The authors claimed that low water content (13%) might encourage the formation of Maillard reaction products thereby acting as antioxidants during storage.

7.2.2 Loss of tocopherols

Previous studies (Grela et al., 1999; Suknark et al., 2001) have shown that extrusion has an adverse effect on the content of tocopherols. This agrees with the results obtained in the present study where the increasing contents of amaranth, quinoa, kañiwa and lupine seemed to have a proportional effect on the retention of tocopherols (II, III). For instance, Grela et al. (1999) tested the effect of increasing temperature profiles on the retention of tocopherols, and observed that even though extrusion reduced the contents of tocopherols, there was no correlation with temperature profiles. In the present study, temperature profiles were maintained constant except for temperature of die (140, 150 and 160 °C). Such differences showed no effect on the content of tocopherol (II, III). It is worth noting that the temperature conditions tested in the present study are in no way comparable with the substantial increase in temperature tested by Grela et al. (1999). Suknark et al. (2001) also found that extrusion reduced considerably the content of tocopherol but still, retention seemed to increase in extrudates containing more partially defatted ingredients.

7.2.3 Increase of total phenolic compounds

The content of total phenolic compounds in extrudates containing amaranth or quinoa was apparently unaffected by extrusion while the content of total phenolic compounds in extrudates containing kañiwa and lupine increased slightly (II, III). There are particular process parameters such as temperature and composition of the raw material that can affect the extractability of phenolic compounds from extruded snacks. For instance, Anton et al. (2009) reported an increase of total phenolic compounds after the extrusion of corn starch containing 15, 30 and 45% navy and read bean flours. In addition, Korus et al. (2007) compared the retention of total phenolic compounds of extrudates containing various cultivars of dry beans. The authors observed that the content of total phenolic compounds increased or decreased (relative to the non-extruded blend) from one cultivar to another. In the present study, the increase of the temperature of die or composition of the blend had practically no effect on the content of total phenolic compounds of extrudates containing amaranth and quinoa (II). The increase of total phenolic compounds was, however, noticeable for extrudates containing kañiwa and lupine (III). This rise in the detection of total phenolic compounds might result from the disruption of the food matrix during extrusion (and acid hydrolysis treatment) thereby increasing their chemical extractability (Zielinski, 2001; Yağci and Göğüş, 2010). Despite having similar initial contents of total phenolic compounds, extrudates containing lupine had moderately lower content of total phenolic compounds relative to those containing kañiwa (III). One plausible explanation could be the formation of complexes with proteins (e.g., globulins) present in the mixtures containing lupine. As suggested by Brennan et al. (2011), lower extractability of phenolic compounds may result from the formation of protein complexes during extrusion.

7.2.4 Increase of folate

The increase of folate in extrudates containing amaranth and quinoa responded to the increase of amaranth and quinoa with minor negative effects of extrusion (II). Conversely, the extrusion of flour blends containing kañiwa and lupine (particularly those containing 50% kañiwa or lupine) showed that extrusion could have a substantial effect on the extractability of folate (III). Despite having comparable contents of folate, extrudates containing 50% kañiwa presented remarkably higher content of folate relative to those containing 50% lupine. The results obtained in the present study contradict previous studies (Håkansson et al., 1987; Broz et al., 1997; Kariluoto et al., 2006; Charlton and Ewing, 2007) where extrusion had generally a negative effect on the contents of folate. For instance, Charlton and Ewing (2007) claimed that temperatures above 95 °C might deplete folate; however, the authors mentioned neither the residence time nor the temperature profile. Kariluoto et al. (2006) used temperatures between 120 and 140 °C and observed a loss of folate between 26 and 28% during the extrusion of rye. Moreover, Håkansson et al. (1987) reported a loss of folate of 20% during the extrusion of white flour. Compared to these studies, the present research has shown that high-temperature-short-time method such as extrusion may lead to minor o no losses of folate. Although the mechanisms allowing increasing contents of folate (during extrusion) are unclear, it is plausible that folate was encased in some large food constituent (e.g., lipid) leading up to very low or no loss during extrusion (III). Another possibility could be the rapid disruption of food constituents thereby allowing a greater release and detectability of folate. For instance, Marchetti et al. (1999) studied the stability of a crystalline and fat-coated vitamin mixture (containing around 6 mg of folate per kg of mixture) during the extrusion of fish meal-based blend. The authors observed that fat-coated vitamins were much less sensitive to extrusion than those in crystalline form. In the present study, food constituents like lipids were likely to prevent further degradation of folate.

7.3 Effect of amaranth, quinoa, kañiwa and lupine on the sensory characteristics of expanded corn-based extrudates (IV)

7.3.1 Texture attributes

Extrudates containing greater contents amaranth, quinoa and kañiwa exhibited perceivable textural changes linked to crispiness, hardness (sensory) and hard particles, and just minor perceivable changes regarding crunchiness and adhesiveness. Crispiness was strongly linked to sectional expansion, porosity and relatively thicker walls. Apparently, extrudates with thicker walls and larger pores were involved in the emission of more noise at rupture with the front teeth. This was generally the case for extrudates with lower contents of amaranth quinoa and kañiwa, and inevitably greater content of starch and lower content of fibre and protein. This goes in line with Liu et al. (2000) who studied the effects of the incorporation of wholegrain oat flour on the physical and sensory properties of corn-based extruded snacks. The results showed a positive correlation between the content of oat and compactness, roughness and hardness, and negative correlation with crispiness and corn flavour. Despite crispiness being a synonym of product quality, it is possible to find less desirable attributes correlating with it. In the present study, the perception of crispiness correlated strongly with the one of hard particles which is, by definition, ‘particles hard to chew’. X-ray micrographs revealed the presence of knot-like formation in extrudates containing lower contents of quinoa, the same samples panellist rated as having the hardest particles. Interestingly, extrudates containing kañiwa and, therefore, greater content of fibre were rated the lowest in hard particles. It is believed that the knots shown in X-ray micrographs may result from starch retrogradation which could have been eventually hindered by the increasing content of dietary fibre, protein and fat. Huang and Rooney (2002) claimed that extensive retrogradation of amylose may produce strong retrogrades that could be even resistant to enzymes.

The perception of hardness was the highest for extrudates containing 50% kañiwa and this attribute was proportional to low porosity and SEI, and inversely proportional to stiffness. This mismatch between these supposedly correlating characteristics may result from the measurement of different physical attributes. For instance, sensory hardness seemed heavily influence by the size of the pores, which inevitably leads to denser extrudates, and greater perception of hardness. Still, stiffness seemed more influenced by wall thickness. Liu et al. (2000) observed a very high correlation between sensory and instrumental hardness of corn-based extrudates containing oat flour. However, it is worth noting that Liu et al. (2000) used a cylindrical probe to compress the extruded sample against a flat surface while in the present study a flat probe was used to compress the extruded samples (vertically oriented) under three-point bending.

Adhesiveness was higher in extrudates containing lower contents of amaranth and quinoa than in those containing kañiwa, and this could be explained by their corresponding values of WSI and WAI. Extrudates containing kañiwa had greater gel-forming capacity (high WAI) and less soluble solids (low WSI) than those containing amaranth or quinoa. In the present study, adhesiveness was strongly correlated with WSI and so it is reasonable to attribute greater adhesiveness to the presence of soluble solids (e.g., sugars) resulting from the dextrinisation of starch. As the contents of amaranth, quinoa and kañiwa increased, there was a mild reduction in adhesiveness, which could be linked to a reduction in the extent of carbohydrates hydrolysis or dextrinisation (less soluble solids). It is plausible that increasing contents in fibre, protein and fat prevented the disruption of gelatinised starch during extrusion.

7.3.2 Taste and aftertaste attributes

Apparently, extrudates containing greater contents of amaranth, quinoa and kañiwa had a stronger overall taste and aftertaste. Such perception may be proportional to the content of sugars, non-starch carbohydrates and, possibly, remnants of saponins and tannins in the flours (Jacobsen et al., 2000; Repo-Carrasco-Valencia et al., 2003). The results obtained in the present study go in line with those of Sacchetti et al. (2004). The authors incorporated up to 40% chestnut flour to rice-based extrudates, and found that there was a stronger perception of bitter taste at greater contents of chestnut flour. However, there is also a good chance that extrusion itself altered the taste and aftertaste of extrudates containing amaranth, quinoa and, particularly, those containing kañiwa. Gomez and Aguilera (1983) suggested that starch dextrinisation was a predominant mechanism of degradation during extrusion cooking, leading to a greater amount of soluble solids. These soluble solids could be easily contributing to the overall taste of extrudates. The technique (Raman spectrometer) used by Miller (2009) showed clearly that various structural changes take place in the melt such as the loss of C-O-H or C-O bonds from starch (probably due to gelatinisation) and breakdown of primary, secondary and tertiary amide. Hofmann (2005) stated that molecules resulting from Maillard reaction such as quinozolate and homoquinizolate are strongly linked to bitterness. Unlike extrudates containing quinoa and kañiwa, those containing amaranth were perceived remarkably sweet at 50% incorporation. Repo-Carrasco-Valencia et al. (2003) affirmed that glucosides (naturally present in amaranth, quinoa and kañiwa) might liberate sugar molecules upon hydrolysis thereby increasing the perception of sweetness. Although this explanation sounds attractive, it is unclear why extrudates containing quinoa and kañiwa were perceived much less sweet than those containing amaranth. It is undeniable that the fact that quinoa and kañiwa belong to the same genus made them more likely to share some underlying sensory characteristics.

7.3.3 Dominant attributes during mastication

The attribute that showed the greatest variations along the incorporation of amaranth and quinoa was roughness. There was a clear reduction in the dominance of roughness as the content of amaranth and quinoa increased. It is possible that the increase of protein and fibre disrupted wall structures thereby reducing abrasiveness during mastication. The disrupted structure of extrudates may have easily contributed to a greater crumbling feeling; this being translated into a lesser perception of roughness. This goes in line with the observations of Liu et al. (2000) where roughness decreased with increasing content of oat. The authors, however, measured roughness as an appearance attribute thereby hindering further comparison with the present study. Even though roughness is generally linked to the perception hardness, this was not the case in the present study. Hardness had a very low dominance rate compared to other attributes such as roughness. This contradicts the findings of Meullenet and Gross (1999) who noticed that roughness during mastication was strongly correlated with sensory hardness. In the present study, hardness was inheritably linked to the degree of expansion and porosity, which could explain why hardness was mostly dominant in less expanded extrudates such as those containing kañiwa. Besides, it is believed that dominance of roughness may be specifically attributed to the glassy state of extrudates at lower contents of amaranth, quinoa and kañiwa. The indirect incorporation of fibre and protein, most possibly, hindered the formation of starch-based glassy structures thereby reducing the dominance of roughness. Gropper et al. (2002) observed that glass transition temperature reduced substantially in proportion to dextran molecular weight in protein-based extrudates. This result suggests that shorter molecules are less likely to form glassy structures, which could be the case for extrudates containing higher content of amaranth, quinoa and kañiwa.

The dominance of stickiness was the last one taking place (before swallowing) at lower content of amaranth, quinoa or kañiwa. Yet this perception changed into gooeyness as the content of these flours increased. It is plausible that the absorption of salivary liquid increased the perception of stickiness. The high degree of starch gelatinisation and protein denaturation could be associated with a greater absorption of salivary liquid. This could be in direct proportion to the high WAIs measured from extrudates containing 20% amaranth, quinoa and kañiwa.

7.4 Methodological considerations

7.4.1 Extrusion

Extrusion was conducted following a pre-established experimental plan (I-IV) under the assumption that the online monitoring setup maintained accurate measurement of process variables. In this regard, temperature at section 6 was fixed at 140 °C for most experiments (II, III, IV), while temperature of melt at section 6 was a response variable, prone to variation resulting from pre-established changes in predictors such as content of flours, screw speed, water content of mixture. Even though temperature of melt changed, generally, in proportion to temperature at section 6, temperature of melt was frequently 6 °C above temperature at section 6.

As explained in section 7.1.1, temperature at section 6 concealed changes that occurred in temperature of melt, probably, associated to torque and total SME (II, III). Unfortunately, most extrusion studies omit information on temperature of melt and its degree of correlation with respond variables such as pressure, torque or total SME (Coulter and Lorenz, 1991; Ilo and Liu, 1999; Chavez-Jauregui et al., 2000; Repo-Carrasco-Valencia et al., 2009a, Repo-Carrasco-Valencia, 2009b).

7.4.2 Experimental design

The chosen experimental design allowed the testing of four variables with three levels per each flour type. Inevitably, the number of experimental runs was large thereby complicating sample collection and randomisation. The ordering of whole plots was altered from the original split-plot design proposed by Vining et al. (2005) in that it followed the increasing content of all tested flours (i.e., amaranth, quinoa, kañiwa or lupine), and the temperature of die. The preparatory stages leading to the experiment made it extremely complicated to fully randomise the experimental design (e.g., blends could not be changed during extrusion). Experiments had to be arranged in a way that content of flours and temperature of die increased stepwise (HTC variables). Rough changes in these experimental conditions would unquestionably incur in the waste of valuable raw material and/or destabilisation of the system. Unfortunately, alterations in experimental setups are commonly underreported thereby covering up potential methodological errors.

The changes made from the original model proposed by Vining et al. (2005) may inadvertently increase the effect of the split-plot structure on the response variables. Næs et al. (2007) described an approach that consisted in obtaining the ratio between the wholeplot and the subplot error variance in order to assess the potential effect of the structure on response variables (II, III). According to Næs et al. (2007), it is reasonable to ignore the effect of split-plot structure if the ratio is lower than 0.4. In the present study, the highest ratio was 0.2 and belonged to WCE in samples containing lupine (III).

7.4.3 Sample collection

The aim of the first study (I) was to test whether or not incorporation of tested flours (up to 20% of solids) was feasible under the available operating conditions, while subsequent studies (II, III) focused on testing the limit of incorporation (20, 35 and 50% of solids). Also, trials to obtain extrudates containing 100% pregelatinised corn flour (bulk ingredient in study II and III) were conducted, but unfortunately, a blockade in the extruder hampered sample collection (temperature of die, 140 °C; screw speed, 500 rpm; water content of mixture, 14-18%).

By definition, the hydration capacity of pregelatinised corn flour is superior to normal corn flour; therefore, the water content of mixture tested in the present study (14-18%; II, III) may have resulted insufficient to obtain pure corn extrudates. Various extrusion studies working with pregelatinised starches set water content of mixture greater than 20% and relatively low temperature profile or screw speed compared to the present study (Garber et al., 1997; Kollengode and Hanna, 1997; Nabeshima and Grossmann, 2001; Mackey et al., 2007). As pure corn extrudates could not be obtained under the same experimental conditions (II, III) as those containing amaranth, quinoa, kañiwa or lupine, they had to be dismissed from further comparison in studies II and III.

7.4.4 PLSR and L-PLSR modelling

In general, the PLSR models obtained had satisfactory prediction abilities (II, III) but this was not the case when response variables involved chemical compounds, particularly, β- and γ-tocopherol and total phenolic compounds (analysed using acid hydrolysis). Probably, sorting the data into smaller groups could have increased the chances to obtain better models, yet decreasing their wholesomeness.

PLSR has been used in various disciplines such as such as organizational and consumer behaviour, marketing and management since the late 1980's (Henseler et al., 2009). Martens and Russwurm (1983) made one of the first accounts on the importance of PLSR as a statistical tool for computer-aided analysis of multivariate data involving chemical composition and microbiological quality of food. Despite its multiple advantages over MLR (see chapter 1), the need for data pre-processing, specialised software and complex graphical results associated to PLSR and, particularly, L-PLSR may discourage its widespread application to food research.

7.4.5 Methodology on physical measurements

Various authors (Ilo and Liu, 1999; Chavez-Jauregui et al., 2000; Dokic et al., 2009) have used simple compression in order to determine values linked to 'hardness' such as breaking strength or maximum force, which, in the case of breaking strength, comes from dividing the maximum peak force by the extrudate cross-sectional area. To our understanding, such results may fail to mimic biting and, instead, provide unrealistic results. In the present study, a three-point bending test provided results that are more in line with front teeth engaged in biting (Vincent, 1998). Stiffness was then defined as the slope of the line 'compression distance vs force' (Vincent, 1998) (II, III, IV).

Although variations in the average particle size of individual flours (e.g., pregelatinised corn flour, 750 µm; amaranth, 285 µm; quinoa, 575 µm; kañiwa, 240 µm; lupine, 800 µm) may have well affected expansion and stiffness of extrudates containing amaranth, quinoa, kañiwa or lupine, blending probably balanced out the overall particle size of the mixture. The corn-based blend containing lupine (III) had inevitably the largest particle size (between 750 and 800 µm) and, upon extrusion, happened to give extrudates with the lowest average expansion and greatest stiffness (> 20% lupine) compared to those containing amaranth, quinoa or kañiwa. Garber et al. (1997) tested the effect of particle size (50-1622 µm) on the expansion ratio of corn-based extrudates, and observed that larger particle sizes were associated with less expanded extrudates and greater breaking strength (expressed in N), particularly at low screw speed (200 rpm). Similarly, Onwulata and Konstance (2006) observed that sectional expansion and breaking strength (expressed in N) of corn-based extrudates reduced and increased, respectively, at larger particle sizes (< 1180 µm). The authors also observed that corn flours with smaller particle size managed to attain greater expansion after the incorporation of 25% WPC. Besides, Alam et al. (2013) found that rye bran with smaller particle size (28-440 µm) gave more expanded, less hard (expressed in N) and crispier extrudates.

Based on the available literature, one can speculate that smaller particle size of corn and lupine flour may have brought greater expansion, and lesser stiffness to the present results.

7.4.6 Methodology on sensory measurements

Sensory profiling was conducted with a panel trained at the food sensory laboratory of the University of Helsinki, and consisting of 70% women under 30 years old (IV). Thus far, the studies dealing with the potential differences in sensory perceptions between gender-groups have shown some consistency regardless of ethnic or cultural differences (Smith and Davies, 1973; Yasaki et al., 1976; Parlee, 1983; Doty et al., 1985). For instance, Smith and Davies (1973) and Yasaki et al. (1976) agreed that males appear to have higher thresholds than females during the testing of quinine and sucrose, respectively. Besides, Henkins (1974) observed that hormonal changes in women could lower the detection threshold for pythiouracil and quinine. Similar results were not consistently obtained by other authors (Glanville and Kaplan, 1965; Wright and Crow, 1973; Aaron 1975). It is unlikely but, still, plausible that gender differences could have some effect on the perception of bitterness and sweetness, in the present study.

Sensory profiling test was conducted in duplicate in order to test potential interactions among replication, and content and type of tested flours. Unfortunately, replicate had significant interaction with ratings of bitter aftertaste and crunchiness. This means that ratings of such attributes were not as reliable as those of overall taste, sweetness, bitterness, crispiness, hardness, hard particles, adhesiveness and overall aftertaste.

In the present study, the TDS test was planned in a way that panellists would choose a dominant attribute from a list of attributes (suggested by themselves) without the simultaneous evaluation of intensity. Still, various authors (Le Révérend et al., 2008; Meillon et al., 2010, Albert et al., 2012) have conducted temporal testing along with the evaluation of intensity. According to Deegan (2014), the mixing of tasks may confuse panellists without creating deeper insights, thereby justifying the decision to focus on temporal dominant attributes, only.

8 CONCLUSIONS

This study has proved that amaranth, quinoa, kañiwa and, to some extent, lupine can be successfully added to corn-based extrudates thereby increasing their nutritional profile and maintaining some key physical and sensory properties. In general, the incorporation of up to 50% amaranth or quinoa had little effect on sectional expansion while the incorporation of up to 50% kañiwa had a moderate effect. The vast increase of fibre and protein had the expected negative effect on the sectional expansion of extrudates containing up to 50% lupine. Despite this, the incorporation of up to 20% lupine was feasible and extrudates presented section expansion comparable to those containing amaranth, quinoa or kañiwa. Sectional expansion and stiffness were negatively correlated regardless of the grain type and, apparently, water content of mixture and, to a less extent, screw speed had a greatest effect on them. The greatest expansion and lowest stiffness was obtained at 14% water content of mixture and at a screw speed of 500 rpm. Despite changes in the temperature of die, this parameter seemed to have no effect on the physical properties of extrudates.

Regarding the chemical composition, extrusion reduced substantially the detectability of fatty acids and tocopherols, and had a minor effect on the contents of total phenolic compounds and folate. Even though the temperature of die showed, in general, a slight effect on the content of fatty acids and tocopherols, total phenolic compounds and folate, it seemed that the temperature of die had some importance on the status of total phenolic compounds and folate in extrudates containing kañiwa and lupine.

Pore size, wall thickness and WSI were the physical/physicochemical characteristics with the strongest influence on texture attributes such as crispiness, crunchiness, hardness and adhesiveness. The increasing content of fibre seemed to disrupt the internal structures of extrudates, leading to smaller pores. Greater pore density could be the main reason for a stronger perception of hardness and reduced perception of crispiness and crunchiness, though slightly. Stiffness was linked to thicker walls rather than to lower porosity, and adhesiveness appeared to increase as the content of soluble material increased. Regarding TDS, the dominance of crunchiness increased and roughness decreased at greater incorporation of amaranth, quinoa and kañiwa. It is believed that the disruption of internal structures contributed to a crumbling feeling during mastication, this being possibly translated into a perception of low roughness. On the other hand, greater incorporation of amaranth, quinoa and kañiwa led to a stronger perception of taste and after taste. It is worth pointing out that extrudates containing 50% amaranth were rated the sweetest. This may result from the hydrolysis of glucosides thereby liberating sugar molecules into the system.

This research proves that Andean grains (amaranth, quinoa and kañiwa) could not only elevate the nutritional status of conventional corn-based snacks but, in most cases, improve or maintain desirable technological and sensory characteristics such as sectional expansion and crispiness, or prevent undesirable characteristics such excessive stiffness and roughness. Special emphasis was given to the textural properties rather than to taste or flavour, as the use of coating agents to boost the taste of snacks is widespread in the food industry. Kañiwa, a strongly neglected Andean grain, worked surprisingly well in combination with corn, and this should be considered by food scientists, food technologists and industrialists for future projects dealing with the design of products with added value.

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