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USE OF AMARANTH, QUINOA AND KAÑIWA IN EXTRUDED CORN SNACKS

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Tiivistelmä — Referat — Abstract

Malnutrition is a common problem in Peruvian highlands and in Bolivia. Amaranth, quinoa and kañiwa are pseudocereals cultivated in these areas and regarded as good sources of protein and non-saturated fatty acids. The literature review deals with the nutritional and technological properties of amaranth, quinoa and kañiwa. The aim of this investigation was to: (1) prepare gluten free corn-based extrudates containing amaranth, quinoa and kañiwa (20% of solids), (2) study the effects of independent extrusion variables on the physical properties of the extrudates and (3) evaluate lipid stability during storage by measuring hexanal production.

Extrudates were made in 4 separate trials using a small scale co-rotating twin screw extruder. Experiments were performed using Box-Behnken’s experimental design in which independent extrusion variables were water content of mass (15, 17 and 19%), screw speed (200, 350 and 500 rpm) and temperature of the die (150, 160 and 170 °C). Samples were collected and their physical properties were analyzed (sectional expansion index, hardness and water content). Ground and whole extrudate samples were stored in open headspace vials at 11 and 76% RH for a week (exposure time) before being sealed and stored for 0, 2, 5 and 9 weeks at room temperature in the absence of light. Hexanal content was analyzed using headspace gas chromatography.

The highest sectional expansion index (SEI) and the lowest hardness were achieved when the water content of mass was 15%, screw speed 500 rpm and temperature of the die 160 °C. Extrudates containing amaranth had the highest SEI (7.6) while extrudates containing quinoa and kañiwa had SEIs of 6.1 and 5.1, respectively. Pure corn extrudates (reference sample) had the lowest SEI (4.5). Extrudates containing kañiwa and pure corn extrudates presented the lowest (28 N/mm) and highest hardness (89 N/mm), respectively. In storage studies, ground extrudates (except samples containing quinoa) showed comparatively higher hexanal production than whole extrudates exposed to 11 and 76% RH. Whole extrudates exposed to 76% RH showed very low hexanal production during storage.

This study proved that it was possible to add amaranth, quinoa and kañiwa to extruded corn snacks and achieve higher expansion than that of pure corn extrudates. Indeed, the results obtained from the evaluation of lipid oxidation during storage suggest a remarkable stability of whole extrudates after being exposed to high relative humidity. Further studies on lipid stability for longer storage would be highly desirable.

Avainsanat — Nyckelord — Keywords
Amaranth, quinoa, kañiwa, extrusion, expansion, lipid oxidation

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PREFACE

This Master's thesis research was conducted in cooperation with Food Chemistry and Food Technology divisions at the Department of Food and Environmental Sciences at University of Helsinki, Finland. Originally, this work was an idea of the Master's thesis writer and took approximately one year for its completion (August 2010-2011). The supervising group was comprised by Ph. D. University Lecturer Kirsi Jouppila, Ph. D. University Lecturer Anna-Maija Lampi and Ph. D. student Satu Kirjoranta.

Contacting external institutions and private companies was necessary in order to obtain the required raw materials. I want to thank Dr. Luz Gomez (head of the Andean cereal project at National Agrarian University in Lima, Peru) for providing the raw material used in the present study. I am also thankful towards Mr. Enrique Torres (General Manager of Jarcon del Peru S.A.) for arranging the cleaning, milling and packing of the raw material.

Finally, big thanks to Ph. D. University Lecturer Ritva Repo-Carrasco-Valencia for sending me a copy of her doctoral thesis.

Helsinki, September 2011

Jose Martin Ramos Diaz

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1. INTRODUCTION

In Peru and Bolivia, the levels of agricultural development do not guarantee a sustainable system of food supply arising problems of food security, specially, in rural areas. Quinoa, amaranth and kañiwa have been consumed in the Peruvian highlands for over 5000 years (Tapia et al., 1979). During Inca's times, these crops provided people with high quality protein during seasons in which conventional crops, such as corn, failed to grow. The extreme resistance of quinoa, amaranth and kañiwa towards harsh environmental conditions attracted some scientific attention in the 1970's and 1980's but it was during the 1990's that their cultivation started to expand in Peru (FAO, 2005). Kañiwa still remains unknown outside the Peru-Bolivian tableland regions and Andes, and it is regarded as a low-class food eaten during droughts and famine only (Mujica, 1994).

The term 'Andean crops' is used by a wide range of authors to refer to cereals, pseudo-cereals, legumes and vegetables cultivated in the Andes as staple food (Tapia et al., 1979; Mujica, 1994). The seeds of quinoa, amaranth and kañiwa can be ground into flour and used in the same way as a cereal grain for either direct consumption or food development. Despite their similarities with cereals, quinoa, amaranth and kañiwa do not belong to the family *Gramineae*. For this reason, they are botanically defined as pseudo-cereals instead of cereals.

The domestication of amaranth and quinoa (certain varieties) has been vital for their classification not only in the Andean and tableland regions of South America but also in the Nordic countries (Jacobsen, 1997). For instance, Blanca of Junín is a variety of quinoa whose principal characteristic is the low content of saponins (alkaloid that gives bitter taste to the seed) (Wright et al., 2002). Blanca of Junín is widely consumed in the Peruvian highlands and neighboring countries such as Ecuador and Bolivia. Similarly, *Amaranthus caudatus* is a species of amaranth extensively cultivated in the Peruvian Andes and North America. Other species of Amaranth such as *A. tricolor* and *A. spinosus* are cultivated in Asia.

Kañiwa, on the other hand, has not been fully domesticated and its industrialization and consumption are limited to the tableland regions of Peru and Bolivia with some minor cultivation areas in the highlands (Tapia et al., 1979). Some scientific evidence suggests that kañiwa may adapt well to external environments (outside the tableland region and the Andes). For example, studies conducted on the ecological conditions to grow kañiwa under prolonged periods of drought and frost led to promising results (National Research Council, 1989). The genetic variability among cultivars may account for the adaptability of quinoa, amaranth and kañiwa to various cultivation areas. This may secure sustainable protein production and diversification of end-products.

With the upcoming of overpopulation and global warming, FAO focused on food security for future generations (FAO, 2002). Projects on the development of alternative crops with higher nutritional properties aimed at reducing overconsumption of corn, rice and wheat. This initiative may offer new nutritional alternatives to the world population regardless of social and economic status.

The nutritional properties of quinoa, amaranth and kañiwa may vary widely. For example, Amarilla de Marangani is a variety of quinoa with high nutritional properties since it has no limiting amino acids (Repo-Carrasco et al., 2003). This characteristic is comparable to the principal milk protein, casein (Ranhotra et al., 1993). Scientific evidence suggests that kañiwa may have a higher percentage of protein than quinoa with similar content of essential amino acids depending on the variety (Repo-Carrasco et al., 1992). The varieties studied by Repo-Carrasco et al. (2009), Cupi and Ramis, appeared to have distinct functional and physico-chemical characteristics compared to commercial grains (rice, corn etc.) under extrusion processing. Other studies on the extrusion of kañiwa are very limited.

Genetic maps of Peruvian ecotypes are yet to be unveiled due to technical and socioeconomic reasons (Mijuca, 1994), and this fact delays the development of industrial processing in order to suit the demand for highly nutritional products. The adoption of low cost technological processes, such as extrusion, was the first step towards the industrialization of Andean grain crops in Latin America. Extrusion is an interesting process for its low-cost and capacity to increase the digestibility of protein and starch through heating. This has been a short-term solution for malnourishment in Peruvian highlands. However, little is done to make more marketable products through technological improvement. The aim of the present research was to study the physical and chemical characteristics of extrudates containing quinoa, amaranth and kañiwa.

2. LITERATURE REVIEW

2.1. General characteristics of quinoa, amaranth and kañiwa

2.1.1. Origin of Andean grain crops

Quinoa, amaranth and kañiwa were important components in the diet of pre-Hispanic cultures in the Peruvian highlands. The capacity of these plants to resist very low temperatures and hail made them irreplaceable in the Andes. Despite this, their consumption reduced after 1940s when countries in South America started the massive importation of wheat.

There is no historical evidence on when and how quinoa was domesticated (Nuñez, 1970). However, some traces of quinoa seeds found in Northern Chile suggest that the use of this seed could date back to 3000 B.C.; Uhle (1919) also found historical evidence in Ayacucho (Peru) which led him to question the antiquity of quinoa. Uhle (1919) claimed that the domestication of quinoa dates back to 5000 BC.

Amaranthus caudatus is originally from South America and it was consumed by pre-Hispanic cultures along with quinoa. Besides, Andean farmers used to intercrop *Amaranthus caudatus* and *Amaranthus mantegazzianus* with corn and quinoa. These species of amaranth, though, could not be cultivated in areas of great elevation, unlike quinoa.

The historical background of kañiwa is not clear and the absence of strong archaeological evidence remains until now. 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 term *Chenopodium pallidicaule* to refer to kañiwa.

2.1.2. Morphological description of quinoa, amaranth and kañiwa seed

The seed of quinoa (*Chenopodium quinoa*) is disk-shaped with a flattened equatorial band around its periphery (Figure 1A). Seeds may vary widely in diameter. A large seed is between 2.2 and 2.6 mm, a medium seed between 1.8 and 2.1 mm and a small one under 1.8 mm in diameter (Figure 1A).

The seed of amaranth (*Amaranthus caudatus*) has a ball-shaped form with a diameter between 1 and 1.5 mm. The colors of the seed may vary depending on the variety but it is generally pale colored (ivory), reddish and dark brown (Figure 1B). On the other hand, the seed of kañiwa (*Chenopodium pallidicaule*) has got a lenticular form with a diameter between 1 and 1.2 mm. The color is light brown or black with a very fine episperm (Figure 1C).

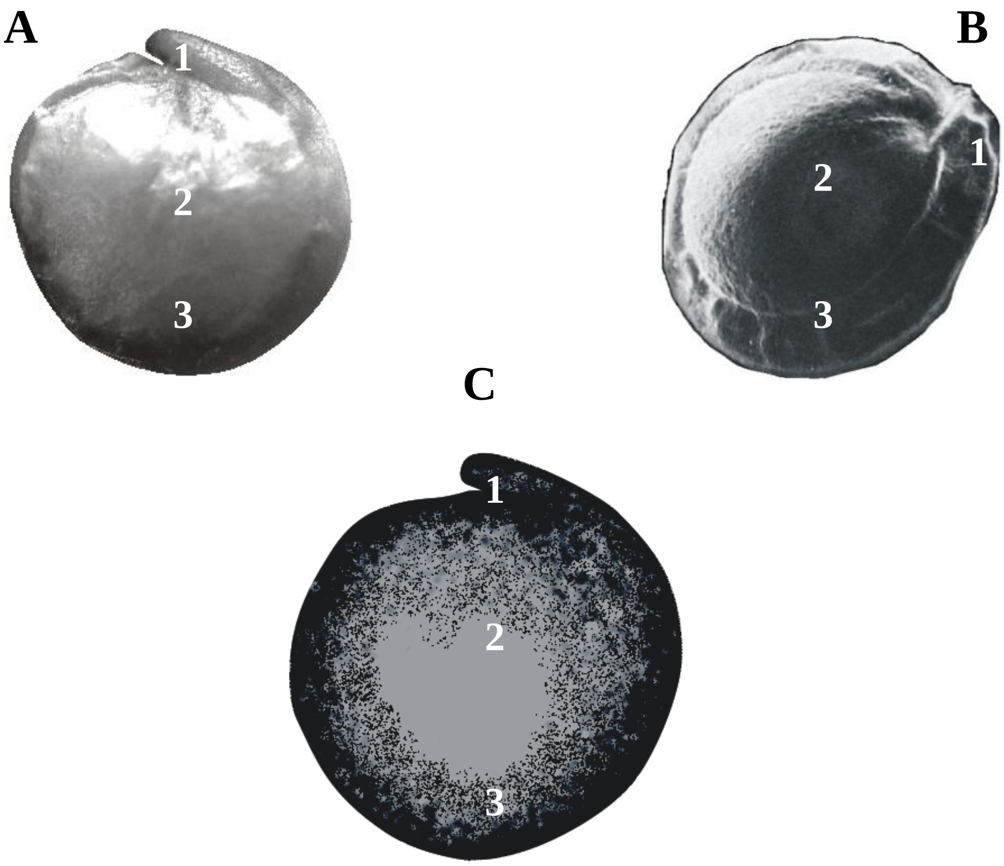


Figure 1. Parts of quinoa (A), amaranth (B) and kañiwa (C) seeds: (1) Radicle, (2) Episperm and (3) Embryo (Image of kañiwa: Tapia et al., 1979)

2.2. Nutritional properties of quinoa, amaranth and kañiwa

Balanced nutrition is a key factor to promote health and well-being. The consumption of food should provide organic compounds in order to encourage favorable physiological and biochemical effects on the human organism. However, nourishment is heavily restricted by socio-economic factors and varies widely.

Quinoa, amaranth and kañiwa present a chemical composition with considerable differences in relation to staples like wheat, corn and rice (Table 1). The nutritional importance of Andean grains relies on their high content of protein, fat and fiber. For this reason, they are an interesting alternative for food development.

Table 1. Composition of cereals and Andean grains (g/100 g dry matter) (Kent, 1983[1]; Gonzales, 1989[2]; Repo-Carrasco et al., 1992[3], 2008[5], 2009[6])

	Variety	Protein	Fat	Raw Fiber	Ash	Carbohydrates
Wheat[1]						
	Manitoba	16	2.9	2.6	1.8	74.1
Rye[1]		13.4	1.8	2.6	2.1	80.1
Rice[1]		9.1	2.2	10.2	7.2	71.2
Corn[1]		11.1	4.9	2.1	1.7	802
Oat[1]		11.6	5.2	10.4	2.9	69.8
Quinoa						
	Sajama[2]	11.22	4.03	n.d.	3.01	77.15
	Huancayo[3]	14.4	6	4	2.9	72.6
	40057[2]*	14.1	9.7	n.d.		72.5
Kañiwa						
	Blanca[3]	18.8	7.6	6.1	4.1	63.4
	Cupi[6]	14.41	5.68	11.24**	5.03	63.64
	Ramis[6]	14.88	6.96	8.18**	4.33	65.65
Amaranth (*A. caudatus*)						
	Oscar Blanco[5]	14,7	10.15	7.27**	2.61	65.27
	Centenario[5]	14.55	10.08	7.43**	2.39	65.55

* Experimental variety of LATINRECO (Quito, Ecuador)
** Value expressed in crude fibre
n.d. = not determined

2.2.1. Protein and amino acid content

The seeds of quinoa, amaranth and kañiwa have distinct morphological characteristics compared to cereals such as wheat, barley and sorghum. The embryo, for instance, is surrounding the perisperm which is the starchy section of quinoa seed and where storage proteins are also present. These proteins are glutelins (glutenins in wheat) and prolamins (gliadins in wheat). Indeed, proteins in the embryo and aleurone cells are albumin and globulin. These are metabolically active since they participate in the generation of new cellular structure.

Despite their morphological similarities, quinoa and amaranth seed present slight differences in their protein fractions. For instance, several studies (Tellerias, 1976; Romero, 1981; Scarpati De Briceño and Briceño, 1980; Ballon, 1982) affirmed that the principal characteristic of protein in quinoa is the high content of albumin and globulin compared to wheat, rice and corn (Table 2). Indeed, the fractions of storage proteins (glutelins and prolamins) in quinoa were considerably lower than in corn, rice and wheat kernels according the same studies (Table 2). On the other hand, Segura-Nieto et al. (1994) observed that amaranth has albumin and glutelin as the major protein fractions with globulin in a third place. Data on the exact protein fraction of amaranth (*A. caudatus*) was not found.

Table 2. Protein fractions (as percentage of total protein). (Romero, 1981[1]; Tellerias, 1976[2]; Scarpati De Briceño and Briceño, 1980[3]; Ballon et al., 1982[4]; Lasztity, 1984[5])

	Albumins + globulins	Glutenins/glutelins	Gliadins/prolamins
Quinoa[1]	76.6	12.7	7.2
Quinoa[2]	55	39.8[a]	5.2
Quinoa[3]	43.6	29.2	27.2[a]
Quinoa[4]	10.9	59.4	0.5
Maize[5]	38.3	37.2	24.5
Rice[5]	19.2	71.9	8.9
Wheat[5]	17.1	54.4	28.5

[a] Fraction combined with insoluble protein residue

Proteins are valuable organic compounds for human nutrition since they intervene in the synthesis of cellular tissues. Amino acids, which are the 'building blocks' during the process of synthesis, are contemporary classified as: indispensable, conditionally indispensable and dispensable, considering their biological activity and availability. The lack of indispensible amino acids halts or limits the synthesis of protein-based tissues. For this reason, indispensible amino acids are also regarded as biologically 'essential'. The nutritional importance of Andean grains is attributed to the high content of indispensable amino acids such as lysine (Table 3).

With regard to conditionally indispensable amino acids, their importance is strictly related to the physiological state of an individual. Arginine and glycine are conditionally indispensable amino acids found in quinoa, amaranth and kañiwa according to Repo-Carrasco (1992). Dispensable amino acids, on the other hand, are synthesized from a non-amino acid source of nitrogen. It was found that rice and wheat have relatively higher content of dispensable amino acids than quinoa, amaranth and kañiwa (Repo-Carrasco, 1992). These Andean grains also presented limitation in one essential amino acid, phenylalanine. This amino acid is strongly linked to allergic symptoms (phenylketonuria) in some individuals.

Several environmental and genetic factors may produce variation in the protein content of quinoa, kañiwa and amaranth within varieties (Table 1). Kañiwa var. Blanca was the Andean grain with the highest protein content in comparison to quinoa and amaranth while quinoa var. Sajama was the grain with the lowest protein content (Gonzales, 1989; Repo-Carrasco et al., 2003, 2008, 2009).

Table 3. Content of essential amino acids in quinoa, kañiwa, amaranth, rice and wheat grains (g amino acid / 16 g of nitrogen) (Repo-Carrasco, 1992)

	Quinoa var. Huancayo	**Kañiwa var. Blanca**	**Amaranth var. Oscar Blanco**	**Rice**	**Wheat**
Arginine[b]	8.1	8.3	8.2	6.3	4.8
Cystine[b]	1.7	1.6	2.3	2.5	2.2
Histidine[a]	2.7	2.7	2.4	2.2	2
Isoleucine[a]	3.4	3.4	3.2	3.5	4.3
Leucine[a]	6.1	6.1	5.4	7.5	6.7
***Lysine**[a]	5.6	5.3	6	3.2	2.8
Methionine[a]	3.1	3	3.8	3.6	1.3
Phenylalanine[a]	3.7	3.7	3.7	4.8	4.9
Valine[a]	4.2	4.2	3.8	5.1	4.6
Threonine[a]	3.4	3.3	3.3	3.2	2.9
Tryptophane[a]	1.1	0.9	1.1	1.1	1.2
Tyrosine[b]	2.5	2.3	2.7	2.6	3.7

[a] Indispensible amino acids
[b] Conditionally indispensible amino acids
*Limiting amino acid in cereals

2.2.2. Lipid and fatty acid content

Amaranth, quinoa and kañiwa are characterized by their high fat content compared to wheat, rice, corn and rye. For instance, Kent et al. (1983) and Repo-Carrasco (1992) presented a chemical composition of cereals and Andean grains in which amaranth had the highest percentage of fat (Table 1).

Repo-Carrasco et al. (2003) observed that kañiwa var. Blanca had higher oil yield compared to quinoa var. Huancayo. However, the type of unsaturated fatty acids should also be considered. Amaranth showed a relatively higher content of oleic acid than quinoa and kañiwa while quinoa showed the highest content of linoleic acid (Table 4). These differences, though, were not significant ($p < 0.05$).

Table 4. Oil yield, degree of unsaturation, fatty acid and tocopherol content in quinoa, kañiwa and amaranth (Bruni et al., 2001[2]; Repo-Carrasco et al., 2003[1])

	Oil Yield, g/100 g seed	**Degree of unsaturation, iodine value (%)**	**Linoleic acid, %**[*]	**Oleic acid, %**[*]	**α-tocopherol, ppm**	**γ-tocopherol, ppm**
Quinoa[1]	4.6	82.7	50.2	26	721.4	797.2
Kañiwa[1]	6.4	72.9	42.6	23.5	726	788.4
Amaranth[2]	n.d.	77.6	46.9	26.2	47.84	2.53

[2] Amaranth var. Macas
[*] Percentage based on fatty acid content
n.d. = not determined

The results obtained by Repo-Carrasco et al. (2003) also suggested that kañiwa had a lower content of unsaturated fatty acids (Table 4) compared to the other Andean grains. Saturated fatty acids like stearic and eicosanoic were found in very small quantities in both kañiwa and quinoa. Even though, data on saturated fatty acids in amaranth was not found, it is believed to be similar to the other Andean grains

The concentration of α- and γ- tocopherol in quinoa and kañiwa is much higher than in amaranth (Bruni, 2001; Repo-Carrasco et al., 2003) (Table 4). This could be associated with a higher sensitivity towards lipid oxidation in amaranth.

2.2.3. Carbohydrates and dietary fiber

The fiber content of kañiwa is considerably higher than mass consumption grains (Table 1). Pedersen et al. (1987) determined dietary fiber content in corn, wheat, sorghum and amaranth, and observed that wheat had the highest dietary fiber content (Table 5). However, Repo-Carrasco et al. (2009) found that kañiwa var. Cupi and Rami had contents of dietary fiber above 20%. Ranhotra et al. (1993), on the other hand, concluded that quinoa was not a significant source of soluble fiber compared to legumes and peas. These results seem to suggest that quinoa and amaranth are not good sources of fiber compared to cereals and legumes, while kañiwa has a much higher content of dietary fiber than wheat, sorghum and corn (Table 5).

Table 5. Dietary fibre composition for Andean grains and cereals (g/100g dry weight) (Pedersen et al., 1987[1]; Ranhotra et al., 1993[2]; Repo-Carrasco et al., 2009[3])

	Variety	Dietary fibre, %	Soluble dietary fibre, %	Insoluble dietary fibre, %
Quinoa				
	D407[2*]	8.9	7.7	1.2
Kañiwa				
	Cupi[3]	25.24	2.98	22.27
	Ramis[3]	25.95	2.79	23.16
Amaranth				
	CAC-064[1**]	8.8	n.d.	n.d.
Wheat[1]		12.1	n.d.	n.d.
Sorghum[1]		8.5	n.d.	n.d.
Corn[1]		9.3	n.d.	n.d.

[*] Variety of quinoa cultivated in San Luis Valley of Colorado, USA

[**] Wild variety of *A. caudatus* collected from the Andes, Peru

n.d.= not determined

On the other hand, Repo-Carrasco et al. (2008, 2009) determined the content of beta-glucans and lignin for kañiwa var. Cupi and Ramis and amaranth var. Oscar Blanco. The results showed a higher content of beta-glucan for amaranth. Nevertheless, amaranth had a lower content of lignin compared to kañiwa. Repo-Carrasco et al. (2008, 2009) also found that the content of resistant starch in kañiwa was twice higher than in amaranth (Table 6).

Table 6. Content of lignin, beta-glucans and resistant starch for varieties of amaranth and kañiwa (g/100g dry weight) (Repo-Carrasco et al., 2008[1], 2009[2]).

		Lignin, %	Beta-glucans, %	Resistant starch, %
Amaranth				
	Centenario[1]	3.95	0.97	0.12
	Oscar Blanco[1]	3.97	0.63	0.1
Kañiwa				
	Cupi[2]	6.88	0.07	0.24
	Ramis[2]	7.98	0.04	0.26

Kañiwa and amaranth are very poor sources of beta-glucan which is well-known for its clinical applications on cancer, arthritis, allergic rhinitis, etc. (Thompson et al., 1987; Kogan et al., 2005).

2.2.4. Micronutrients and antinutrients

Andean grains such as quinoa and amaranth exhibited a higher content of minerals than wheat and brown rice according to the studies carried out by Calhoun et al. (1960), Ranhotra et al. (1993) and Gamel et al. (2006) (Table 7). All these analysis followed the AOAC method for ash determination and chromatographic method for vitamin determination. Information on kañiwa was not found. Gamel et al. (2006) observed that amaranth had very high content of ascorbic acid compared to quinoa, wheat and brown rice (zero content).

Despite its high content of vitamins and minerals, Andean grains also contain antinutrients such as saponins and phytic acid. Quinoa contains three different sapogenins: oleanolic acid, hederagenin and phytolaccagenic acid (Ridout et al., 1991; Cuadrado et al., 1995). Glucose, arabinose and occasionally glactose are bound to these sapogenins in order to form glycosylated secondary metabolites (saponins). The pericarp-seed coat has the highest concentration of saponins which must be removed before the use of quinoa (Repo-Carrasco, 2011). The adverse physiological effects comprise membranolysis of cells in the small intestine and hemolytic activity. Besides this, saponins also have positive effects; it was observed that they reduced serum cholesterol levels, possessed anti-inflammatory and antioxidant activity and exhibited antibiotic and fungicidal properties (Woldemicheael and Wink, 2001). The content of saponins varies according to the quinoa types. In fact, quinoa can be classified as bitter (4.7 to 11.3g/kg) and sweet (0.2 – 0.4g/kg) (Mastebroek et al., 2000).

On the other hand, phytate forms complexes with metal ions like iron, calcium, magnesium and zinc thereby reducing their bioavailability. According to some studies (Ruales and Nair, 1993), the content of phytic acid in quinoa seeds is approximately 1% (of dry matter) which can be reduced by washing. Neither proteases nor tannins were detected in quinoa grains.

Table 7. Content of minerals and vitamins for Andean grains and cereals (Calhoun et al., 1960[1], Houston and Köhler, 1970[2], Ranhotra et al., 1993[3], Gamel et al., 2006[4])

	Quinoa var. D407[3]	**Amaranth**[4]	**Wheat**[1]	**Brown Rice**[2]
Mineral, µg/g				
Calcium	700	1760	982	320
Phosphorus	4620	5820	7.73	2210
Potassium	8550	4870	10.43	2140
Magnesium	1610	2880	4.01	n.d.
Iron	63	140	104	16
Manganese	35	13	156	n.d.
Copper	7	9	11.3	n.d.
Zinc	32	39	76.1	n.d.
Sodium	2,7	250	n.d.	90
Vitamins, µg/g				
Thiamine	0,29	n.d.	3.93	3.4
Riboflavin	3	2.4	1.07	0.5
Niacin	12,4	12.5	54.5	47
Ascorbic Acid	0	29.8	0	0

[1] Variety of Amaranthus caudatus cultivated in The Netherlands

n.d. = not determined

2.3. Physico-chemical characteristics of the major compounds in Andean grain crops

2.3.1. Gelatinization and retrogradation of starch

Starch granules contain amylose and amylopectin, and minor quantities of crude fat and protein. Lorenz (1990) determined the chemical composition of a quinoa starch granule in comparison to starch granules from wheat, barley, wild rice and amaranth (*A. hypochondriacus*) (Table 8). Results indicated that quinoa had the highest content of protein and nitrogen, and the lowest content of crude fiber. Amaranth, on the other hand, showed the highest content of crude fat (10 times more than quinoa) and the lowest content of amylose.

Table 8. Proximate composition of starches granules[1] (g/100 g dry weight) (Lorenz, 1990)

Starch	**Crude Fat, %**	**Nitrogen, %**	**Protein[2], %**	**Amylose, %**
Amaranth	1.1	0.08	0.49	0.2
Barley	0.5	0.08	0.45	25.7
Quinoa	0.11	0.16	0.91	9.28
Wheat	0.27	0.06	0.34	21.7
Wild Rice	0.2	0.01	0.06	2.04

[1] Dry weight basis
[2] Protein = %N x 5.7

Amaranth and quinoa also presented some morphological differences in their starch granules. Lorenz (1990) found that the starch granules of quinoa in the perisperm cells had polygonal shape with a size that ranged from 0.4 to 2 µm (Figure 2 and 3).

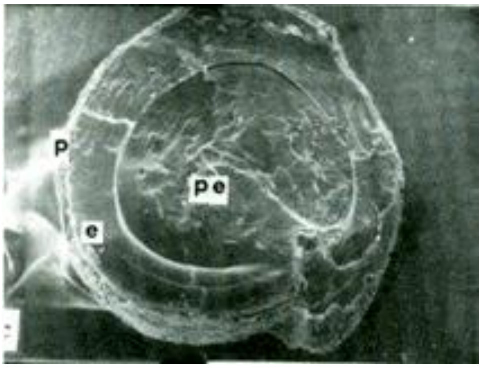


Figure 2. Cross-sectional photo of a quinoa grain: pericarp (p), embryo (e) and perisperm (pe) (Lorenz, 1990)

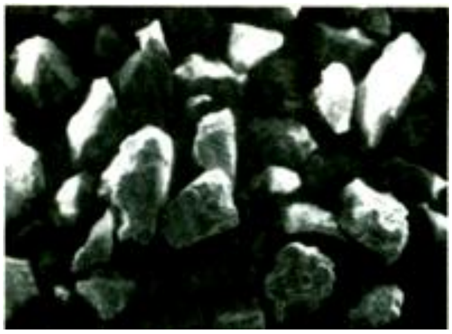


Figure 3. Granules of quinoa starch in the perisperm (Lorenz, 1990)

Lorenz (1984) observed that starch granules of amaranth (*A. cruentus*) are comparatively smaller than starch granules of corn (Figure 5). Under Scanning Electron Microscopy (SEM) starch granules of *A. cruentus* and *A. hypochondriacus* appeared to have similar size but clear differences in their shape. The starch granules of *A. hypochondriacus* were angular and polygonal while the starch granules of *A. cruentus* appeared to be rounded (Figure 5). According to Lorenz (1984, 1990), *A. hypochondriacus* and quinoa presented both polygonal and irregular granules. Nevertheless, granules of quinoa appeared to be more amorphous than *A. hypochondriacus* (Figure 3 and 5B).

Lorenz (1990) and Ahamed et al. (1996) determined the physico-chemical properties of quinoa starch compared to starches of barley, wheat, wild rice, amaranth and corn. Lorenz (1990) could observe that starches with the highest content of amylose presented lower viscosity, swelling power and gelatinization temperature (Table 8 and 9).

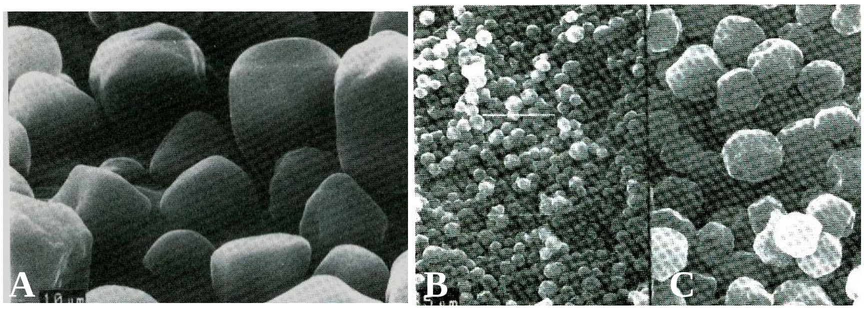


Figure 4. Comparing starch granules of A. *cruentus* and corn: A) Starch granules of corn at 2.500 magnification, B) Starch granules of amaranth at 2.500 magnification and C) Starch granules of quinoa at 10.000 magnification (Lorenz, 1984).

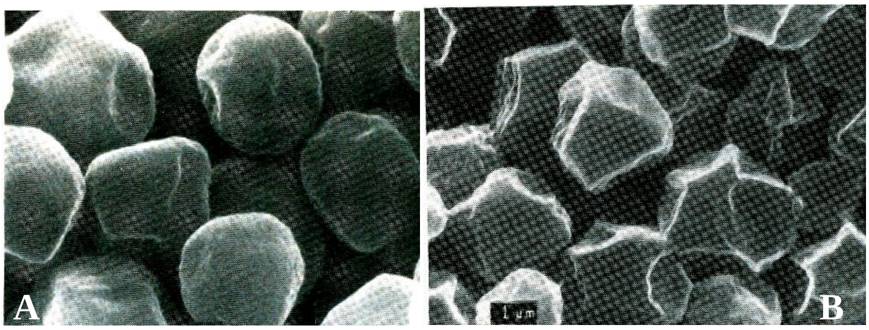


Figure 5. Comparing starch granules of *A. cruentus* and *A. hypochondriacus* starch: A) Starch granules of A. *cruentus* and B) Starch granules of A. *hypochondriacus* (Lorenz, 1984).

Experimental trials on viscosity showed that the quinoa, amaranth and wild rice starch had very similar viscosities at 92 °C (Lorenz, 1990). It seemed that the structural activity of amylose in starch reduced swelling and viscosity of wheat and barley. According to Lorenz (1981, 1984), amylose reinforced the internal network of a granule and, consequently, restricted swelling. This was not observed in quinoa, amaranth and wild rice since their amylose content was very low compared to wheat and barley (Table 8). In fact, the solubility of amaranth and wild rice starch was considerably low compared to other starches at temperatures between 60 and 90 °C (Table 9). This was expected for low-amylose starch granules since amylose is the component that leaches out first at increasing temperatures. In amaranth and rice, the solubilized amylose was possibly trapped in the swollen starch at high temperatures. For this reason, amylose was not decanted and, therefore, measured as solubilized starch.

Table 9. Physico-chemical characteristics of starches in 5% solution (Lorenz, 1990)

Starch Property		Wheat	Barley	Wild Rice	Amaranth	Quinoa
Amylograph Viscosities (BU)						
	at 92 °C	270	180	970	950	980
After 30 min	at 92 °C	340	335	800	820	895
On cooling	to 35 °C	210	355	1110	810	1690
After 60 min	at 35 °C	810	480	1190	840	1900
Swelling power, g H2O/g sample	at 60 °C	2.03	4.39	4.15	5.76	10.61
	at 70 °C	5.39	5.48	6.85	9.71	12.23
	at 80 °C	6.89	n.d	8.18	n.d.	15.75
	at 90 °C	8.36	7.94	10	10.57	19.73
Solubility, %	at 60 °C	0.91	0.95	0.7	1.61	1.14
	at 70 °C	2.02	2.55	0.86	1.25	1.2
	at 80 °C	2.59	n.d.	0.65	n.d.	1.32
	at 90 °C	3.52	3.74	0.12	0.48	4.33
Water binding, %	RT[1]	89.1	92.4	113.9	145.6	118.5
Gelatinization temperature range, °C						
	initial	54	55.5	63	71	61
	midpoint	58	60.5	67	77.5	64.5
	final	61	65	73	82.5	68

[1] Room temperature measurement
[2] Potato starch concentration was 5%, and 9% for other starches.
n.d. = not determined.

Greenwood (1976) reported that starches with low amylose content tended to have higher gelatinization temperatures. Lorenz (1990) confirmed this by obtaining the highest gelatinization temperature for amaranth and the lowest for wheat and barley. According to Atwell et al. (1983), the gelatinization temperatures for quinoa starch were between 57 and 64 °C; this seems to support the results obtained by Lorenz (1990) in which quinoa presented higher gelatinization temperature than wheat and barley (Lorenz, 1990) (Table 9). It is possible that the use of different quinoa varieties caused slight variation in results between Atwell et al. (1983) and Lorenz (1990). The relationship between gelatinization temperature and amylose content seems to depend strongly on the species of quinoa (Lindeboom et al., 2005).

Water binding capacity was the highest in amaranth, quinoa and wild rice. This was attributed to the low content of amylose in the starch granule since fixation of water molecules in a branched structure is more stable. These experiments were conducted at room temperature and do not reflect starch behavior under heating (Lorenz, 1990). Mangels (1933) and Kulp (1973) observed that heat disrupted intergranular bonds during the process of gelatinization thereby increasing water binding capacity.

Ahamed et al. (1996) complemented the studies of Lorenz (1990) by comparing quinoa starch with corn starch. The results obtained seemed to support the findings of Lorenz (1990). However, the values of swelling power and solubility for quinoa were significantly lower than those showed by Lorenz (1990). Ahamed et al. (1996) used a variety developed in India while Lorenz (1990) used the variety D 407 developed in Colorado, USA. Both authors, however, seemed to agree on the stability of quinoa during retrogradation.

During cooling, amylose and amylopectin chains realign themselves into a more crystalline structure, turning a liquid into gel (retrogradation). This phenomenon tends to occur on storage of gelatinized starch pastes at relatively low temperatures (e.g., 4 °C). Lindeboom et al. (2005) conducted experiments on the physico-chemical properties of eight quinoa varieties and indicated retrogradation percentages that ranged from 19.6 to 40.8%. Apparently, there was a positive correlation between retrogradation tendency and amylose content (Table 10). Starch retrogradation seemed to be heavily influenced by the amylose content (Gudmunsson and Eliasson, 1990; Baik et al., 1997; Kaur et al., 2002).

Table 10. Effect of amylose and protein content on retrogradation of quinoa and corn starches after 4-day storage at 4 °C (Lindeboom et al., 2005).

		Starch granule		
Sample	**Variety**	**Amylose [a], %**	**Protein [b], %**	**Retrogradation [c], %**
Quinoa				
	Ames 21926	3.5	0.56	19.6
	AAFC-1	4.6	0.14	25.8
	NQC	6.4	0.41	33.1
	Ames 22155	11.5	0.27	25.9
	Ames 13745	12.7	0.36	28.7
	WMF	14.4	0.57	40.8
	AAFC-2	15.1	0.6	32.4
	QC	19.6	1.23	36.1
Normal corn		25.4	0.69	75
Waxy corn		1	0.82	39.5

[a] Determined by High-Performance size-exclusion chromatography.

[b] N × 6.25

[c] Enthalpy of gelatinization after storage divided by initial enthalpy of gelatinization.

Retrogradation is also affected by other factors such as morphology of the granule (size and shape) and lipid-amylose interaction (Kaur et al., 2002). Amaranth (*A. hypochondriacus*) did not present any sign of retrogradation during the first 4 days of storage at 4 °C compared to corn, wheat and rice (Table 11) (Baker and Rayas-Duarte, 1998). According to Lorenz (1990), the content of amylose in amaranth (*A. hypochondriacus*) was 0.2% which is considerably lower than quinoa (Table 8).

Table 11. Retrogradation of amaranth, corn, wheat and rice in storage at 4 °C (Baker and Rayas-Duarte, 1998)

	Retrogradation [a], %			
Storage time, days	**Amaranth**	**Corn**	**Wheat**	**Rice**
1	0.0	17.4	13.8	16.0
2	0.0	28.2	26.3	27.0
4	0.0	35.5	26.7	35.1
7	14.6	45.3	43.3	43.1
14	34.7	48.5	45.8	43.2
21	45.3	77.0	60.9	44.8

[a] Enthalpy of gelatinization after storage divided by initial enthalpy of gelatinization.

2.3.2. Physico-chemical characteristics of proteins - denaturation

Protein denaturation is a biochemical process in which proteins lose their tertiary and secondary structure due to external stress such as heating or exposure to a strong base or acid. These structural changes in proteins alter their physico-chemical characteristics such as loss of solubility in aqueous medium.

While the content of albumin and globulin in grains of quinoa and amaranth are considerable higher than the content of glutelin and prolamin (Table 2) (Romero, 1981; Segura-Nieto, 1994), staple cereals such as rice and wheat have higher content of glutelin (glutenin for wheat) and prolamin (gliadine for wheat). These differences provoke changes in the behavior of mass (paste made of flour and water) under mechanical stress and heating.

Gorinstein et al. (1996) found that the temperatures of denaturation were not considerably different between salt-soluble amaranth proteins (59 °C) and quinoa proteins (58 °C). And most possibly, albumins were denaturated first since they had less structural stability compared to globulins (Gorinstein et al., 1996). Denaturation temperature for glutenin and glutelin (rice) were 55 °C and 82.2 °C, respectively (Schofield et al., 1983; Ju et al., 2001). Glutenin was thought to be completely unfolded at 75 °C.

According to Köhler et al. (1993), most of the cystine available in wheat was in the glutenins forming part in inter- and intra-molecular disulphide structures. Evans (2001) affirmed that low concentrations of sulphur-containing amino acids like cystine and methionine may provoke low extensibility and poor volume in mass.

Cystine availability had a clear effect on the rheological properties of quinoa and amaranth mass (Lamacchia et al., 2010). In fact, the capacity to form quaternary protein structures through disulphide bonds was observed to confer elasticity in wheat mass (Thatam et al., 2001; Belton, 1999). Lamacchia et al. (2010) conducted trials to test the extensibility of quinoa and amaranth in relation to oat and semolina. The results showed that semolina had a higher degree of extensibility than quinoa, amaranth and oat (Figure 6). The reason why quinoa and amaranth mass is weak and inextensible has not been thoroughly explained. However, differences in the molecular weight distribution of proteins and degree of polymerization seemed to be strongly related to inextensibility.

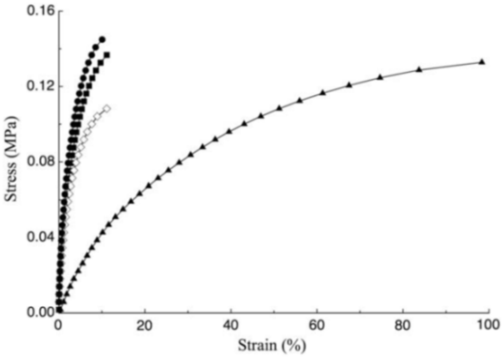


Figure 6. Curve of stress strain for the amaranth (◇), oat (■), quinoa (●) and semolina mass (▲) (Lamacchia et al., 2010)

In order to identify which protein fractions may have some effect on the rheological characteristics in mass, it is worth considering two types of polymeric proteins according to their solubility in sodium dodecyl sulfate-phosphate buffer: soluble fraction and unextractable polymeric proteins (UPP). MacRitchie and Lafiandra (1997) and Weegles (1996) found that there was a good correlation between UPP and mass strength. It was suggested that the polymeric fraction with the highest unextractable molecular weight contributes favorably to the extensibility and tenacity of the mass. Lamacchia et al. (2010) found that the percentage of UPP is much lower in quinoa and oat than in semolina. Amaranth, though, showed values close to those of semolina (Figure 7). The inextensibility of quinoa and amaranth mass was attributed to the polymerization of low-cystine proteins. This polymer tended to be the soluble fraction in the sodium dodecyl sulfate-phosphate buffer while high-cystine proteins were the UPP.

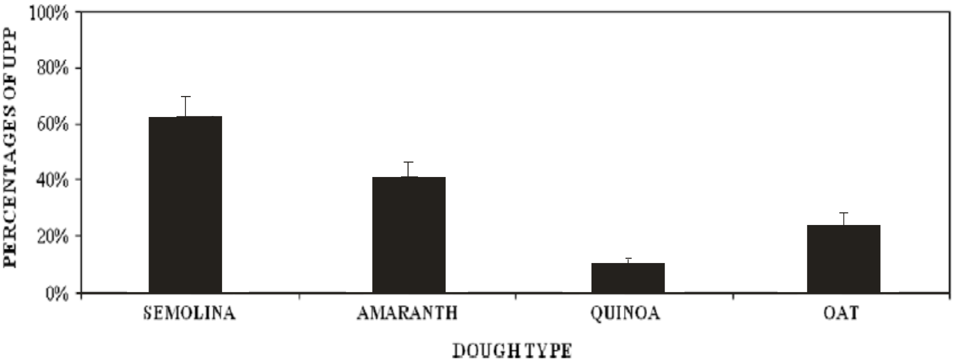


Figure 7. Percentage of total unextractable polymeric protein (UPP) in semolina, amaranth, quinoa and oat mass (Lamacchia et al., 2010).

2.4. Traditional processing of Andean grains

The principal processed product of kañiwa is called *kañiwaco* and its preparation dates back to Incas' time according to Tapia et al. (1979). Kañiwaco is the resultant product of toasted and powdered kañiwa which may be consumed alone or in a mixture with sugar, milk or coffee. This product, however, fails to reach a market position outside the Peruvian mountainous regions due to its status as food for "poor people" (Tapia et al., 1979; Mijuca, 2004). On the other hand, the industrialization of quinoa and amaranth has, to some extent, assured their place in international markets. The main products obtained from quinoa and amaranth are energy bars, bread, biscuits, powder, toasted and popped snacks (Ayala, 2003).

2.5. Principles of extrusion on food development

Extrusion is a process that consists of forcing raw materials through a die under high pressure and temperature in order to form a shaped pellet (extrudate). During the process of extrusion, raw materials are transformed into a fluid (mass) and subjected to mixing. This leads to new physico-chemical and functional properties of the native ingredients. From a nutritional standpoint, the aim of food extrusion is to increase the digestibility of starch and protein through gelatinization and denaturation which results from intense mechanical and heating stress.

The designs of extruders may vary widely. However, the function of certain parts remains the same. Barrel is a stationary component of extruders that protects the screw and maintains the pressure during the processing. Screw is a rotative component of extruders characterized by a helical ridge; the diameter and form of the screw varies along the barrel (this depends on the manufacturer). Die is a stationary component of extruders that shapes the mass at the end of the screw (Figure 8 and 9).

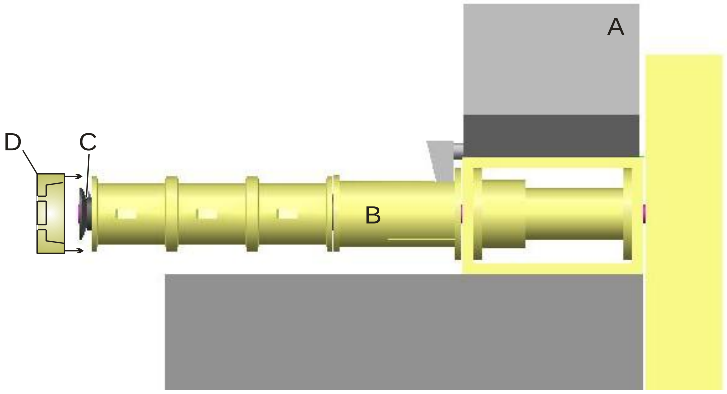


Figure 8. Parts of a single-screw extruder I: A) Feed hopper, B) Barrel, C) Screw and D) Die (Ramos and Quiliano, 2005).

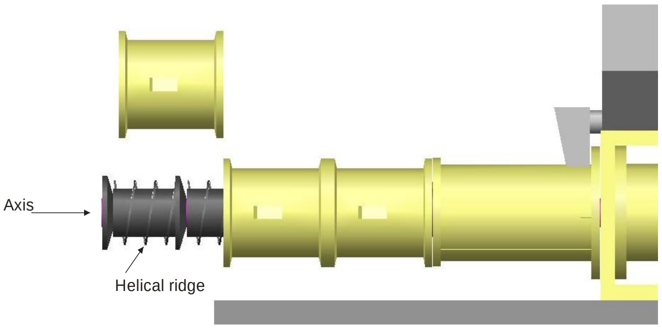


Figure 9. Parts of an extruder II: Screw (Ramos and Quiliano 2005)

Expansion consists of increasing the dimensions of a mass which is subjected to high internal pressure as a consequence of rising temperature. The expansion of an extrudate occurs normally at high temperatures and low water content of feed as a result of a combined structural transformation of its native ingredients. The physical changes comprised during expansion are: nucleation (formation of small bubbles in the mass), expansion (formation of big bubbles under atmospheric pressure) and contraction (shrinkage provoked by bubble breakage or collapse) (Figure 10). There are, however, material and operational parameters that must be taken into consideration during extrusion processing in order to attain expansion such as barrel and die temperature, screw speed and geometry, mass composition and molecular structure. The combined effect of these parameters will have considerable effects on gelatinization (starch section) and denaturation (protein section) of the mass leading to bubble growth and further collapse.

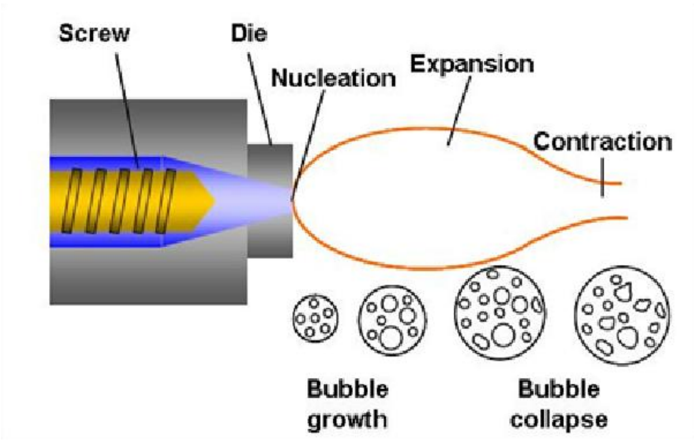


Figure 10. Diagram of extrudate expansion (Nowjee, 2004)

2.5.1. Andean grains and extrusion technology

The introduction of extrusion technology for the inexpensive processing of Andean grains into highly digestible products offer a wide range of possibilities for the production of snack foods, baby food, breakfast cereals etc. (Anderson et al., 1969; Meuser and van Lengerich, 1992; Ding et al., 2005). In fact, the large number of extrinsic (processing parameters) and intrinsic (interaction of raw material components) factors during extrusion processing make this technology difficult to control. For example, screw configuration, barrel temperature, water content of feed and feed rate are extrusion parameters with high degree of influence on the properties of the final product. Physical and functional properties such as density, Water Absorption Index (WAI), Water Solubility Index (WSI), Sectional Expansion Index (SEI) and sensory characteristics (Ding et al., 2005) are usually dependent on the extrusion parameters mentioned above. The determination of the effect of these parameters on the physical and functional characteristic of the final product is one of the aims of research on extrusion technology.

2.5.2. Characteristics of Andean grains extrudates compared to cereal-based extrudates

Andean grains have distinct characteristics compared to cereals such as rice, wheat and corn. Even though, extrusion research is normally conducted on a blend of corn or rice with amaranth or quinoa, single-ingredient extrusion of Andean grains is also found. Corn and rice are well-known bulk ingredients in extrusion and information on their independent and response extrusion variables is widely found.

Cereal flours are commonly used as ingredients in extrusion processing since the physico-chemical characteristic of their starch leads to high expansion. Indeed, starch granules of

Andean grains are morphologically and chemically different from starch granules of cereals (Table 8 and Figure 4). This difference is related to the ratio of amylose/amylopectin.

According to Della Valle et al. (1996) and Kokini et al. (1992), viscosity of the mass increased with the amylose content. Bubble expansion increased at higher internal vapor temperature and pressure just after the product being expelled from the die. Amylose-lipid associations tended to increase the viscosity of the mass leading to short expansion time and limited growth (Della Valle et al., 1997). On the other hand, mass with low amylose content presented low viscosity which led to high sectional and longitudinal expansion and further shrinkage as a consequence of negative pressure of cooling vapor (internal pressure lower than atmospheric pressure) (Chanvrier, 2007)**.** It seems that mass with low amylose content is more sensitive to morphological changes. As mentioned before (Table 9), the content of amylose in quinoa and amaranth is low compared to cereals such as rice, wheat and corn.

Material parameters such as water content of feed, fat and protein content have clear effect on the physical characteristics of extrudates. Kadan et al. (2003) and Ding et al. (2005) obtained data on density, expansion and breaking force for rice extrudates. Considering water content of feed ranging from 14 to 22% and barrel temperature from 100 to 140 °C as independent variables, Ding et al. (2005) observed that density of rice flour extrudates increased with increasing water content of feed and decreased with increasing barrel temperature. Similar results were reported by Cumming et al. (1972) and Harmann and Harper (1973) with extruded corn grits. They also found that higher density correlated well with lower expansion.

Sectional expansion index (SEI) is a response variable that is calculated as the ratio between the cross-sectional area of the final extrudate and the area of the die. Viscosity of the mass may have significant effect on the SEI (Harper, 1981). In this case, rice flour had low content of fat and this characteristic maintained viscosity levels high. Taking into consideration the findings of Coulter and Lorenz (1991), Chavez-Jauregui et al. (2000), Ding et al. (2005), and Repo-Carrasco et al. (2009), it could be observed that the expansion ratio for rice or corn extrudates is comparatively higher than quinoa and kañiwa at higher water content of feed (Table 12).

This difference could be attributed to the high level of fat in Andean grains which weakens the mass, reduces the strength and increases the plasticity of the mass as it escapes through the die (Harper, 1981). This may explain why defatted amaranth flour showed higher expansion ratios during extrusion than quinoa flour (30% in mixture) and milled grain of kañiwa (Table 12) (Chavez-Jauregui et al., 2000). An increase in water content of feed during extrusion may soften the molecular structure of amylopectin by reducing the viscosity and elasticity of the mass which provokes a decrease in expansion and increase in the density of the extrudate (Ilo and Liu, 1999; Ding et al., 2005). It seems that increasing moisture content of feed is strongly associated with decreasing expansion ratio in rice and corn extrudates. Moisture content of feed had a positive effect on density.

Faubion and Hoseney (1982) observed that there is almost a linear relationship between low water content of feed and high expansion ratio for starch-based raw materials such as rice and corn flour. However, Chavez-Jauregui et al. (2000) found that the expansion ratios obtained by extruding defatted amaranth flour (protein-based material) reached a maximum value for a given water content of feed and temperature value (15% and 150 °C) (figure 11). Chavez-Jauregui et al. (2000) also suggested that despite the relatively low protein content (16% dry basis), amaranth showed the behavior of a proteinaceous material in which protein has active participation in the supramolecular network formation during extrusion.

This suggests that protein may limit the expansion of extrudates; this effect was independent from the nature of the protein. Kim and Maga (1987) added whey protein concentrate to a mixture of rice and corn flour which caused a decrease in the expansion of the final product. Similarly, Faubion and Hoseney (1982) noticed that the addition of up to 11% gluten to wheat starch has negative effects on the expansion of extrudates.

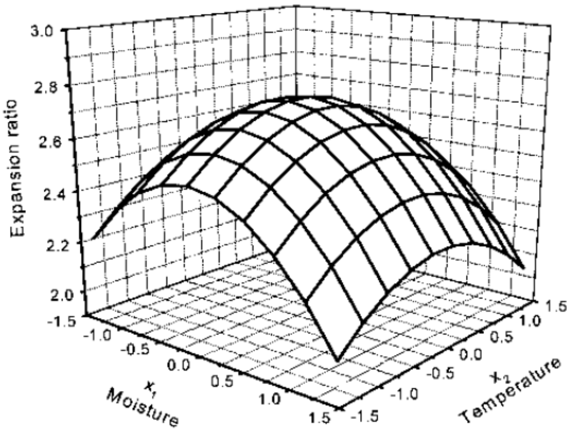


Figure 11. Effect of moisture and temperature on the expansion ratio of amaranth extrudate (Chavez-Jauregui et al., 2000).

Kim and Maga (1993) conducted experiments on the incorporation of isolated soybean protein (ISP) to low (20%) and high (55%) amylose corn starch for extrusion. They suggested that amylose may become reactive with protein causing reduction in starch availability and SEI, specially, when the content of amylose was high. Accordingly, Chang et al. (2001)

conducted trials on a mixture of cassava starch (20% amylose) and ISP. It was observed that there was a positive linear effect between ISP and SEI, contradicting the findings of other authors (Faubio and Hoseney, 1982; Kim and Maga, 1987) (Figure 12)

Chang et al. (2001) increased the content of protein, thereby reducing the content of amylose in the blend, proportionally. It was possible that protein-amylose interactions took place when the content of protein was equivalent to the content of amylose in the mixture (Kim and Maga, 1993). Either amylose or protein seemed to increase the viscosity of the mass but the combined effect (high protein-amylose interaction) provoked low viscosity and SEI. This responds to an antagonist effect between components.

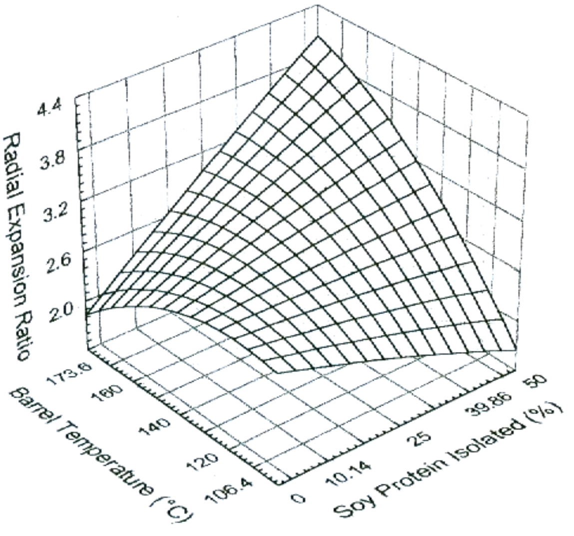


Figure 12. Influence of barrel temperature and isolated soybean protein concentration on the radial expansion of an extrudate at 23% of water content of feed (Chang et al. 2001).

The low amount of amylose and high content of protein in the defatted amaranth flour may account for the peak expansion described by Chavez-Jauregui et al. (2000) (Figure 11). The drop, after peak expansion, might have been caused by structural damage or superheated steam. If the protein content had been higher (>13%), higher viscosity would have prevented structural damage at higher temperatures.

Breaking force, which is the measurement of the force (N) necessary to break an extrudate under the application of a perpendicular force vector, was also considered by Ding et al. (2005) while studying starch-based extrudates. Results showed that a significant decrease in the breaking force was achieved by reducing water content of feed and also feed rate. In fact, low water content of feed at high feed rates (max. 32 kg/h) may provoke a significant increase in the hardness of the extrudate (higher breaking force).

Table 12. Effect of the chemical composition (g/100g dry weight) and extrusion parameters on the physico-chemical characteristics of extrudates made from amaranth, quinoa and kañiwa flour as a single ingredient or in blend.

Raw material	Chemical composition			Extrusion Parameters				Physico-chemical characteristics				References
	Fat, %	Protein, %	CHO, %	Water content of feed, %	Feed Rate, g/min	Temperature, °C	Screw speed, rpm	Density, g/cm3	Breaking force, N/mm2	Sectional Expansion index (SEI)	Longitudinal expansion index (LEI)	
Defatted Amaranth flour	0.18	15.82	80.77	13	70	135	200	0.239	13.1*	2.45	n.d	Chavez-Jauregui et al., 2000
	0.18	13.82	80.77	15	70	150	200	0.227	13.8*	2.84	n.d	
	0.18	15.82	80.77	17	70	135	200	0.254	13.6*	2.47	n.d	
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 & 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 & 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 & 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	
Rice flour	1.2	7.6	77.4	14	20-32	120	150	0.1	10.58*	3.87	n.d	Ding et al., 2005
	1.2	7.6	77.4	16	20-32	120	150	0.19	27.26*	3.41	n.d	
	1.2	7.6	77.4	20	20-32	120	150	0.35	43.94*	2.48	n.d	
Corn grits	0.18	7.96	n.d.	15	110.2	150	100	0.08	n.d.	3.35	n.d.	Coulter & Lorenz, 1991
	0.18	7.96	n.d.	15	197.6	150	150	0.10	n.d.	3.04	n.d.	
	0.18	7.96	n.d.	15	175.2	150	200	0.08	n.d.	3.99	n.d.	
	0.18	7.96	n.d.	25	100.0	150	100	0.23	n.d.	2.62	n.d.	
	0.18	7.96	n.d.	25	120.4	150	150	0.16	n.d.	2.10	n.d.	
	0.18	7.96	n.d.	25	115.6	150	200	0.23	n.d.	2.62	n.d.	

Quinoa 10% - corn grits 90%	0.49	8.95	n.d.	15	119	150	100	0.06	n.d	3.15	n.d	Coulter & Lorenz, 1991
	0.49	8.95	n.d.	15	149	150	150	0.09	n.d	3.36	n.d	
	0.49	8.95	n.d.	15	174	150	200	0.08	n.d	3.67	n.d	
	0.49	8.95	n.d.	25	126.8	150	100	0.27	n.d	1.78	n.d	
	0.49	8.95	n.d.	25	173.8	150	150	0.22	n.d	1.78	n.d	
	0.49	8.95	n.d.	25	216.2	150	200	0.26	n.d	2.1	n.d	
Quinoa 20% - corn grits 80%	0.8	9.94	n.d.	15	112.8	150	100	0.09	n.d	2.73	n.d	Coulter & Lorenz, 1991
	0.8	9.94	n.d.	15	172.8	150	150	0.08	n.d	3.15	n.d	
	0.8	9.94	n.d.	15	206.8	150	200	0.15	n.d	3.36	n.d	
	0.8	9.94	n.d.	25	130	150	100	0.3	n.d	1.68	n.d	
	0.8	9.94	n.d.	25	208.8	150	150	0.31	n.d	1.89	n.d	
	0.8	9.94	n.d.	25	276	150	200	0.33	n.d	1.89	n.d	
Quinoa 30% - corn grits 70%	1.1	10.38	80.56	15	140.4	150	100	0.14	n.d	2.62	n.d	Coulter & Lorenz, 1991
	1.1	10.38	80.56	15	181.2	150	150	0.12	n.d	2.52	n.d	
	1.1	10.38	80.56	15	210	150	200	0.08	n.d	2.83	n.d	
	1.1	10.38	80.56	25	166	150	100	0.26	n.d	2.1	n.d	
	1.1	10.38	80.56	25	232.8	150	150	0.17	n.d	1.78	n.d	
	1.1	10.38	80.56	25	307.2	150	200	0.35	n.d	1.89	n.d	
Kañiwa flour	5.68	14.41	63.64	12	10-12 **	180	254.5	0.1	n.d	1.98	n.d	Repo-Carrasco et al., 2009
	5.68	14.41	63.64	14	10-12 **	180	254.5	0.2	n.d	1.77	n.d	
	5.68	14.41	63.64	16	10-12 **	180	254.5	0.3	n.d	1.61	n.d	

* Force in Newtons (N)
** Residence time in seconds (s)
n.d. = not determine

On the other hand, defatted amaranth flour, which behaved like a proteinaceous material (Chavez-Jauregui et al., 2000), presented a maximum breaking force for a given water content of feed and temperature value (15% and 150 °C) (Figure 13). By comparing expansion ratio and breaking force in defatted amaranth extrusion (Figure 13 and 11), it is possible to notice that the more expanded and greater the volume is (lower density), the higher the breaking force (Chavez-Jauregui et al., 2000). Similarly, Cumming et al. (1972) reported that increasing extrusion temperature produced a protein-based extrudate of higher breaking force but lower density. This is, again, a typical behaviour of protein-based material since they appeared to expand at high temperatures and produced an extrudate which resulted comparatively harder than a starch-based extrudate.

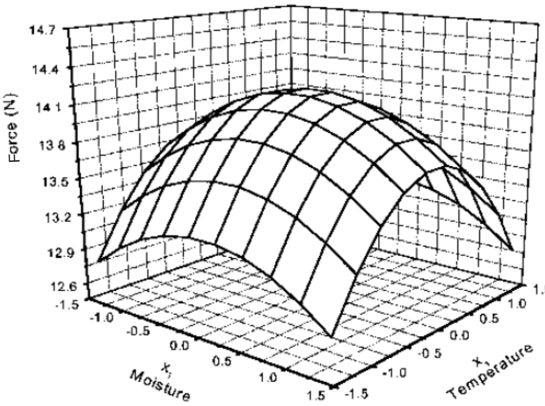


Figure 13. Effect of water content of feed and barrel temperature on the breaking force for amaranth extrudate. (Chavez-Jauregui et al., 2000)

The relationship between breaking force of an extrudate and fat content (raw material) was primarily reported by Faubion and Hoseney (1982). This supported the findings of Coulter and Lorenz (1991) who found that protein and lipid content in a blend of quinoa and corn grits contributed to the decrease in the breaking force. However, Ilo and Liu (1999) encountered opposite results while extruding a blend of amaranth and rice flour. It was observed that at lower levels of amaranth addition (lower fat content) (21 g amaranth/100 g rice), breaking force reached minimum values. This is also in accordance to Pan et al. (1992) who reported a positive effect of fat (oil) on the breaking force of rice extrudate. Probably, proteins and lipids in amaranth had a positive effect on the breaking force in the studies of Ilo and Liu (1999). Indeed, the blend of quinoa and corn had higher content of protein and fat but it seems that they had negative effect on the increase of breaking force (Coulter and Lorenz, 1991). A clear explanation was not found in the literature but the answer could be in the protein fraction of the extrudate.

Hsieh et al. (1989) and Onwaluta et al. (2000) evaluated expansion, density and hardness of extrudates containing varying contents of fiber. Hsieh et al. (1989) extruded yellow corn meals with wheat and oat fiber at screw speed between 200 and 300 rpm. The results indicated a substantial increase in bulk density and hardness thereby decreasing radial expansion. Similarly, Onwulata et al. (2000) extruded a blend of corn meal and wheat fiber (50; 125 g/kg) and found that there was not significant change in density; however, sectional expansion reduced and hardness increased. Another interesting finding was the interaction between fiber and protein. Onwulata et al. (2001) prepared a mixture of corn flour, fiber (50; 125 g/kg) and three different types of milk proteins (250 g/kg casein/whey protein concentrate/whey protein isolate). The results with fiber at 50 g/kg showed a considerable effect of the type of protein on reducing sectional expansion and hardness in equal measure (Figure 14A). In contrast, higher content of fiber (125 g/kg) did not cause considerable variation in sectional expansion among extrudates with different types of protein (Figure 14B).

Lue et al. (1991) reported that the addition of up to 30% of dietary fiber to corn meal did not interfere in gelatinization of the starch. This, however, provoked less sectional expansion than the control sample. Lue et al. (1991) suggested that finer fiber may encourage the formation of new nucleation sites for water vaporization thereby increasing the number and size of air cells. De Souza et al. (2010), in opposition to previous studies, found that the content of fiber has a negative influence on starch gelatinization, concluding that low dietary fiber, high temperature and low humidity may lead to comparatively higher expanded extrudates.

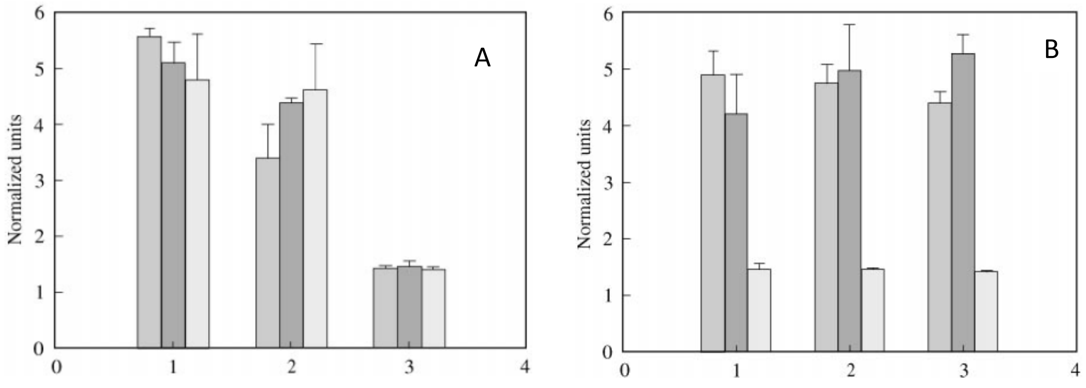


Figure 14. Extruded corn flour with milk proteins and wheat fiber. (1) Corn flour, casein (250 g/kg), fiber A (50 g/kg)/ fiber B (250 g/kg); (2) Corn flour, whey protein concentrate (250 g/kg), fiber A (50 g/kg)/ fiber B (250 g/kg); (3) Corn flour, whey isolate (250 g/kg), fiber A (50 g/kg)/ fiber B (250 g/kg). ▒ Exp: Expansion (mm); ▓ BKS: Breaking strength (N); ░ S-DEN: Substance density (kg/m3). (Onwulata et al. 2001)

2.6. Principles of lipid oxidation in storage

2.6.1. Lipid oxidation and hexanal formation

Oxidation of vegetable oils containing a large amount of unsaturated fatty acids has been a major problem for the food industry since it reduces the nutritional and sensory quality of the final product, leading to economic losses (Min et al., 1989; Ollivon, 2006).

Oxidative stability in storage depends on many factors including the presence of triplet oxygen ($^3O_2$), double bonding (C=C), temperature, light, traces of catalysts, etc. The effect of triplet oxygen has been intensively studied over the last decades in order to understand its role in the initiation step of lipid oxidation and to improve the oxidative stability of food (Min et al., 1989; Choe and Min, 2006). On the other hand, the contribution of non-radical singlet oxygen to lipid oxidation has not been well understood, thus far. In the presence of light, photosynthezisers such as chlorophylls and pheophytins form highly reactive singlet oxygen from triplet oxygen. Singlet oxygen is believed to react with double bonds of fatty acids (Rawls and Van Santen, 1970) leading to the formation of a mixture of conjugated and nonconjugated hydroperoxides. The decomposition of hydroperoxides may produce secondary products such as volatile compounds (hexanal) (Frankel et al., 1985).

During autoxidation, the formation of hydroperoxides and secondary products depends on the reactivity of fatty acids with triplet oxygen. According to Min et al. (1989), the degree of reactivity is: arachidonic acid > linolenic acid > linoleic acid > oleic acid > cholesterol. Specifically, an initiator disrupts a double bond as it abstracts an allylic proton, then the double bond moves to the adjacent position and the lipid radical reacts with triplet oxygen to form trans allylic peroxides. Methyl linoleate hydroperoxides produce a large amount of hexanal, 2-heptanal, pentane, methyl 9-oxononanate and methyl 10-oxo-8-decenoate. During photosensitized oxidation, the formation of isomers from C-10 and C-12 hydroperoxides may lead to a higher production of 2-heptanal, methyl 10-oxo-8-decenoate and 1-octene-3-ol than that by autoxidation (Frankel et al., 1981). In this sense, the absence of light may encourage higher production of hexanal (Koelsch et al., 1991). The autoxidation pathway for linoleic acid is shown in Figure 15.

The mechanisms of lipid oxidation in bulk have been investigated for many years. However, lipid oxidation is still a major problem for the deterioration of food emulsions (Coupland and MacClements, 1996). The physicochemical mechanisms of lipid oxidation in emulsions are more complex than in bulk lipids due to the presence of droplet membranes, interactions between ingredients, partitioning of ingredients and formation of oil-water interfacial regions.

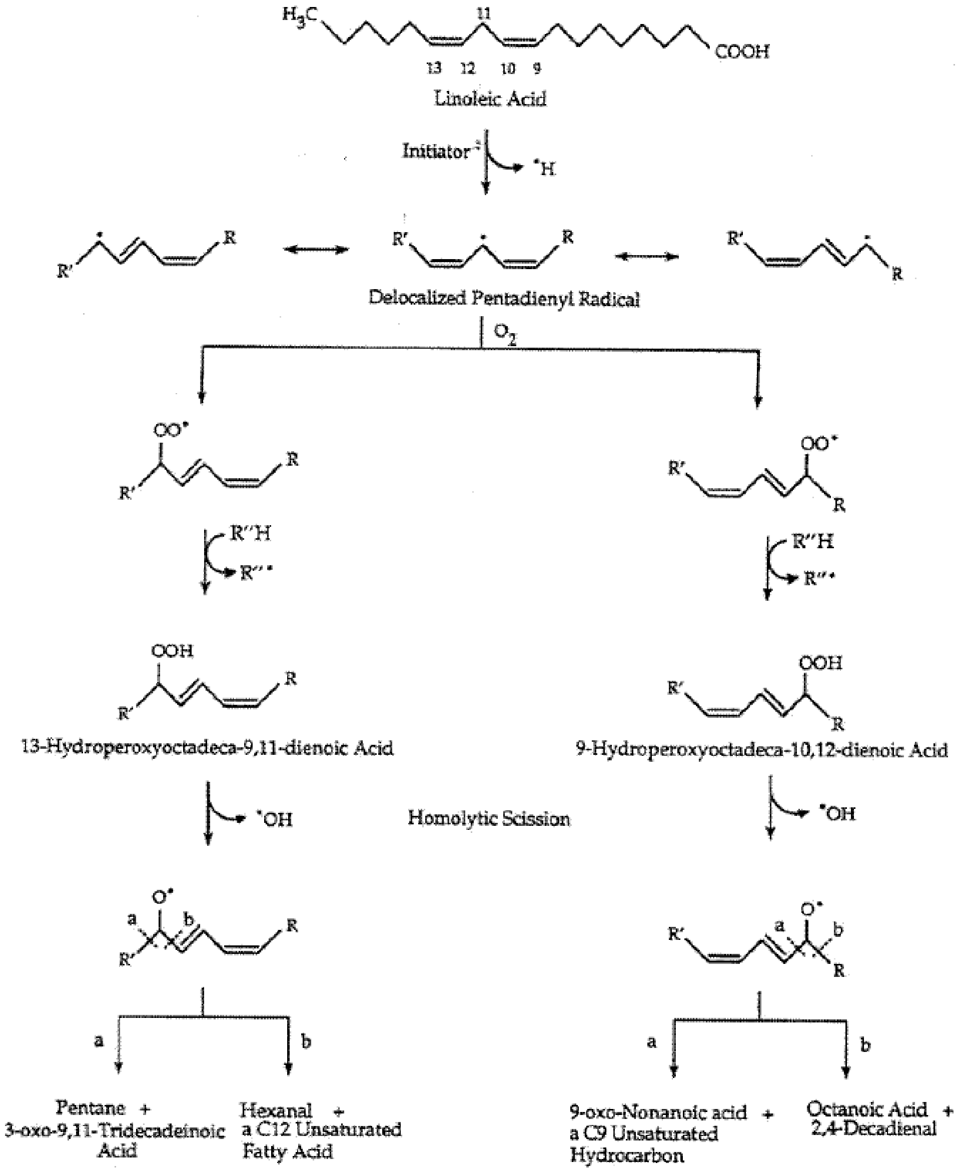


Figure 15. Pathway of autoxidation of linoleic acid to hexanal (Shahidi & Pegg, 1994)

2.6.2. Effect of physical state on lipid oxidation

The interaction between amylose and fatty acids has been intensively studied in order to find out their effects on aroma release and lipid oxidation (Kim and Maga, 1994; Gray et al., 2008; Naknean and Meenune, 2010). Godshall (1997) found that higher aroma volatility was obtained when higher concentration of monosaccharides such as glucose or fructose was added. In contrast, polysaccharides tended to reduce aroma release due to increased viscosity or higher molecular interaction with volatile compounds (Naknean and Meenune, 2010).

Indeed, the amylose fraction of starch is able to form complexes with ligands such as fatty acids, alcohols, ketones, aldehydes and benzene. The generic name of this complex is V amylose, being Vh amylose the most studied and best described (Goubet et al., 1998; Naknean and Meenune 2010). It consists of a sixfold left-handed helix repeating at 0.8 ηm which is surrounding a complexing agent; in this case, the aliphatic part of a fatty acid molecule (Figure 16).

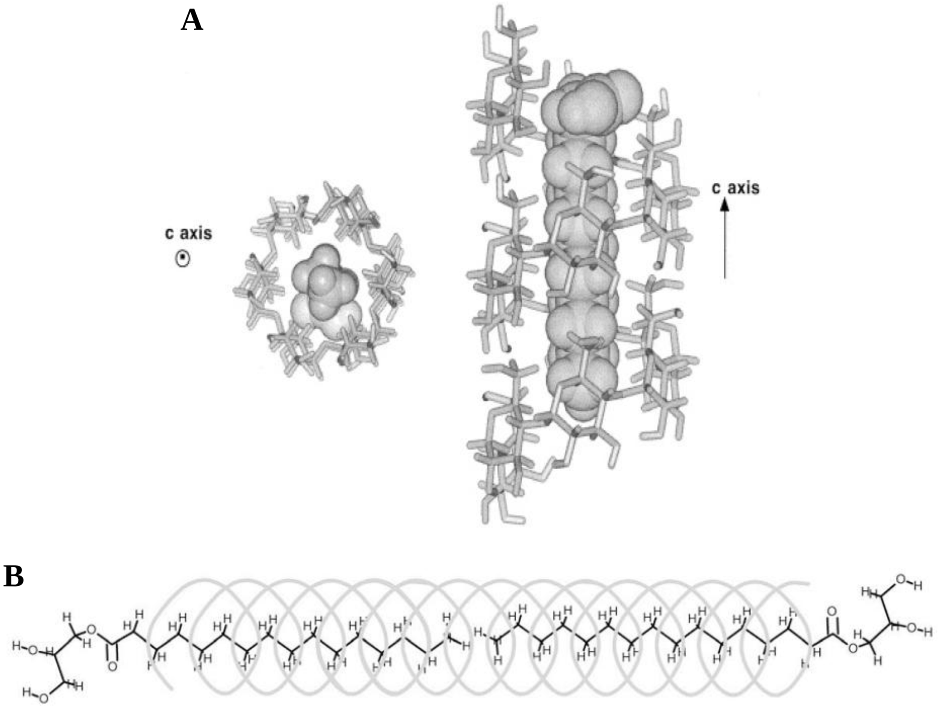


Figure 16. Molecular representation of amylose-fatty acid complexes showing: (A) the inclusion of the aliphatic part (C12) of a fatty acid inside the hydrophobic cavity of the amylose single helix; (B) complex of amylose with monopalmitin molecules. (Buleon et al., 1998; Copeland et al., 2009)

Bhatnagar and Hanna (1994a), Desrumaux et al. (1999) and Naknean and Meenune (2010) claimed that these complexes are reversible and formed during the gelatinization of starch or during cooling. It is also believed that hydrogen bonds are weakened during gelatinization leading to the formation of a helical inclusion complex with amylose around the hydrophobic chain of volatile compounds while amylopectin binds water molecules.

According to Nelson and Labuza (1992), the physical state of a food matrix can play an important role in lipid oxidation kinetics. Physical states of amorphous materials such as "glass" and "rubber" are normally observed when there is a stepwise change in specific heat. A starch-based material such as corn flour can reach glass state if there is supercooling and pressure drop just after starch gelatinization (e.g. extrusion process). A distinguishable feature of a glassy material is its reduced intermolecular volume compared to rubbery material. This volume reduction may lead to a restrictive rate of diffusion which can also provoke limited reactions (Gray et al., 2008).

Reports on lipid oxidation within glassy starch-based matrices formed by extrusion processing are limited. Gray et al. (2008) incorporated linoleic acid to waxy maize starch via extrusion processing, stored the samples at 50 °C and observed that initial oxidation occurred near the surface and a considerable difference in the onset of oxidation between 'bulk oil' (low) and linoleic-acid-containing starch matrices (high). Gray et al. (2008) also mentioned that the surface area of the extruded samples was 10 times greater than that of the surface area of 'bulk oil'. This may explain the considerable differences reported. Another interesting result is the comparable rate of hexanal production between microcracked glassy and rubbery starch structures. Gray et al. (2008) suggested that rubbery material has low enough viscosity to allow oxygen diffusion into the matrix during storage and therefore, provoke oxidation. On the other hand, microcracked glassy structures appeared to expose new areas where lipid can react with oxygen. In contrast, glassy samples without microcracks were observed to protect the linoleic acid from oxidation. At this point, it should be noted that the formation of lipid-amylose complexes is not likely in this experiment since the matrix consisted entirely of amylopectin.

In general, low rate of hexanal production (low sensitivity towards lipid oxidation) in starch-based structures can be the result of small free volume within a glass matrix thereby reducing the ability of oxygen to diffuse towards lipid, and hexanal to diffuse out of the matrix (Voilley and Le Meste, 1985; Kollengode and Hanna, 1997; Parker et al., 2002). In fact, the chances of reactants to collide can also reduce at higher viscosity and limiting molecular mobility (Orlein et al., 2000).

El-Magoli et al. (1979), Su (2003) and Gray et al. (2008) observed the influence of temperature and humidity conditions on lipid oxidation with hexanal as marker. They reported a peak in the production of hexanal followed by a steady decrease when storage temperatures were around 50 °C. El-Magoli et al. (1979) claims that hexanal changed into hexanoic acid due to high temperature during storage. Su (2003) reported similar results while testing the sensitivity of quinoa flour towards lipid oxidation in storage at 25, 35, 45 and 55 °C.

Water activity (a_w), which is the vapor pressure of water in food material divided by that of pure water at the same temperature, appear to have a strong effect on lipid oxidation. Labuza et al. (1972) studied the stability of lipid oxidation in intermediate moisture food reporting high rates of oxidation at a_w lower than 0.2. Labuza et al. (1972) also observed the lowest rates of oxidation in food matrixes at between 0.2 and 0.4 a_w and an increasing trend between 0.4 and 0.7 a_w. After 0.7 a_w, the rate of lipid oxidation started falling again (Figure 17). These differences were associated with the hydration of transition metal cations and propagation reactions of lipid hydroperoxides, which are low for a_w between 0.2 and 0.4 and high for a_w between 0.4-0.7. Gray et al. (2008) tested lipid oxidation in extrudates at a_w levels of 0.95 and observed an increasing trend. Lipid oxidation reports on samples with a_w above 0.9 had not been reported before since there is a high risk of mold growth that may provoke unwanted lipid catalysis.

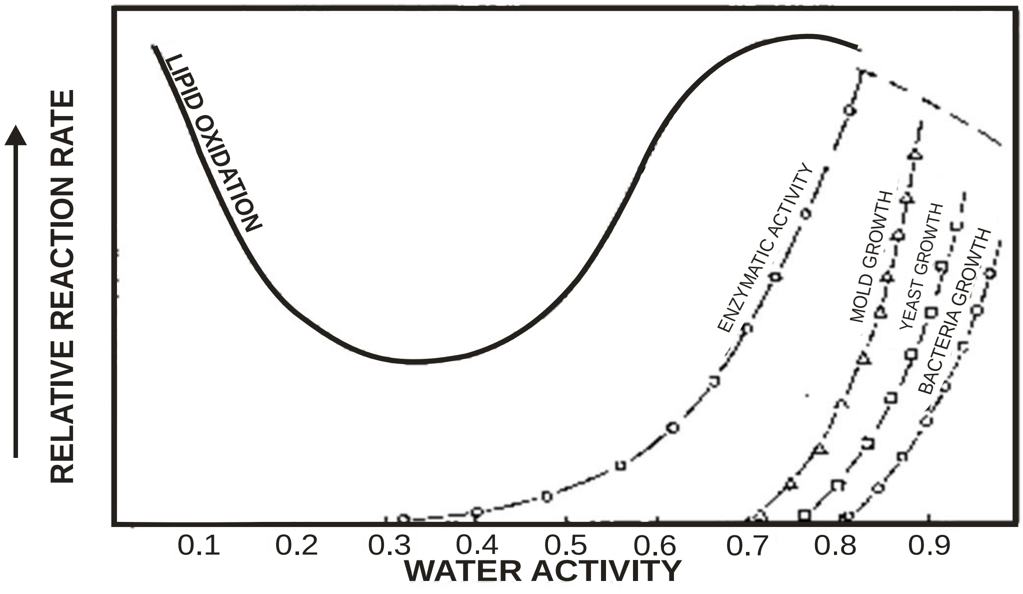


Figure 17. Stability map of food as a function of water content (modified from Labuza et al., 1972)

Matsuno and Adachi (1993), and Gharsallaoui et al. (2007) affirmed that microencapsulation may prevent lipid oxidation by limiting oxygen accessibility through a solid wall (made of protein or carbohydrate). In fact, the distribution of oil droplets in the system might have a tremendous effect on oxidative stability (Marquez-Ruiz et al. 2000). A minor lipid fraction (non-encapsulated oil), which is located on the surface areas or pores of a matrix, is easily extracted with organic solvents without disrupting internal structures. On the other hand, the encapsulated lipid fraction is embedded in the matrix and only extractable if the microstructures are broken up. In fact, very few studies have evaluated the effect of moisture on the oxidation of microencapsulated oils considering their different lipid fractions. For instance, Drusch et al. (2006) studied the oxidative stability of fish oil encapsulated in a starch matrix under different storage conditions. Drusch et al. (2006) suggested that oxidation of total oil might be associated with changes in the physical structure of the matrix as a consequence of different relative humidity.

Ponginebbi et al. (2000) studied the effect of different storage conditions (0%, 32%, 43% and 75% RH) on the oxidation of linoleic acid encapsulated in a starch matrix. It was observed that samples at 0 and 32% RH had a higher rate of oxidation compared to samples at 43 and 75% RH. It seems that free lipid fraction oxidized faster than the encapsulated lipid fraction at 0 and 32% RH. In contrast, data of conjugated dienes indicated higher degree of oxidation in the encapsulated lipid fraction at 43 and 75% RH.

2.7. Justification of the study

The main reason for choosing Andean grain crops was their high nutritional value, high content of bioactive compounds and abundance in impoverished regions of South America. In fact, extrusion is a suitable processing method since it is an inexpensive way of increasing the digestibility of raw material components. In this sense, this investigation may contribute to a more accurate understanding of the effect of extrusion independent variables, raw material components and storage conditions on the physical and chemical characteristics of corn extrudates containing amaranth, quinoa and kañiwa.

3. EXPERIMENTAL RESEARCH

3.1. Aims

The aims were to prepare gluten free corn-based extrudates containing amaranth / quinoa / kañiwa (20% of solids), and study the effects of independent extrusion variables on the physical properties of the extrudates. Chemical analyses were performed in order to characterize the raw materials and evaluate the effects of storage and humidity conditions on lipid oxidation of extrudates.

3.2. Overview of the study

Chemical experiments were conducted in parallel with physical experiments in order to obtain supportive information on the properties of extrudates containing amaranth, quinoa, kañiwa and the control. Extrusion processing was the main experiment of the present study (Figure 18).

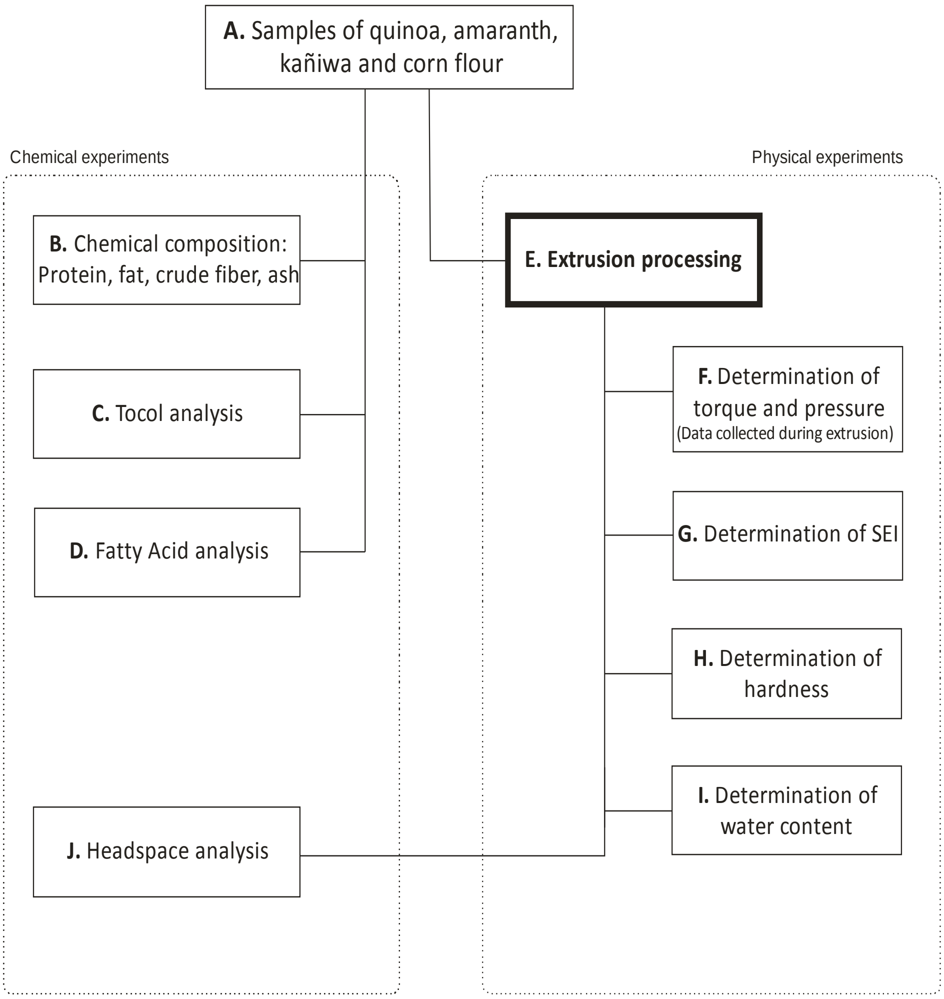


Figure 18. Flow chart of the experiment sequence.

3.3. Materials and methods

3.3.1. Materials

Amaranth (*Amaranthus caudatus*) var. Oscar Blanco was obtained from the province of Carhuaz in Ancash (Northern Peru), quinoa var. Rosada de Huancayo was obtained from the province of Jauja in Junin (Center of Peru) and kañiwa var. Cupi was obtained from the region of Puno (Southern Peru). Samples were supplied by the Andean cereal program at the National Agrarian University (UNALM, Lima) and milled by Jarcon del Peru S.A.C.; grains were cleaned by sieving, and milled (~500 µm) in a stainless steel hammer mill (type MMT-25EX, Jarcon del Peru S.A.C.). Flour was vacuum-packed in high-density polyethylene bags prior to delivery to Finland.

Corn flour was supplied by Limagrain (France). This corn, which was a blend of conventional selected varieties, was degerminated and milled in order to reach a particle size of approximately 150 µm (25-75%). According to the supplier, the corn flour used in the present study was gluten-free since it contained less than 20 ppm of gluten (gluten-free in EU). Corn flour was mixed with amaranth, quinoa and kañiwa flour at a ratio of 80:20 (of solids).

3.3.2. Methods

3.3.2.1. Chemical composition

Determination of protein content was performed according to the AOAC method 976.05 (1995); Kjeltec Analyzer unit (Foss 2300, Höganäs, Sweden) was used for distillation. Determination of fat content (Moreau et al. 2003) was carried out by accelerated solvent extraction (ASE) with heptane at 100 °C. Further solvent evaporation took place at 37 °C.

Table 13. Chemical composition of quinoa var. Rosada de Huancayo, amaranth var. Centenario, kaniwa var. Cupi and corn. Results on dry basis (g/100 g dry weight) except for moisture content (g/100 g fresh weight)

	Moisture	Protein	Fat	Dietary fiber	Ash	Digestible CHO*
Quinoa	11.6 ± 0.14	16.4 ± 0.55	6.3 ± 0.29	11.5 ± 0.19	3.2 ± 0.06	63
Amaranth	10.6 ± 0.13	12.2 ± 0.20	6.6 ± 0.19	8.6 ± 0.12	2.1 ± 0.002	71
Kaniwa	9.1 ± 0.15	15.6 ± 0.004	8.7 ± 0.39	20.5 ± 0.91	4.0 ± 0.03	51
Corn	9.4 ± 0.026	6 - 9[a]	2.05 ± 0.06	< 1[ab]	< 1[a]	>75[ac]

[a] Values provided by Limagrain® on dry basis
[b] cellulose and [c] starch
* Digestible carbohydrates (CHO) = 100 – (Protein + fat + dietary fiber + Ash)

Dietary fiber content was determined by enzymatic-gravimetric method (Cho et al., 1997; Mattila et al., 2001; AOAC, 2002). Ash was determined according to the method described by Schneider (1967) and Mattila et al. (2001). Quinoa, amaranth and kañiwa presented noticeable differences in their content of protein, fat and crude fiber (Table 13). Kañiwa had the highest content of fat, crude fiber and ash compared to quinoa and amaranth. Protein content, however, was the highest in quinoa.

The content of alpha, beta, gamma and delta tocopherol was analyzed using HPLC after volume normalization of extracted lipids with heptane (10 ml). Lipid extraction of the sample (2 g) was carried out in a Dionex ASE 200 accelerated solvent extractor (Sunnyvale, CA) using 11-ml extraction vessels. A cellulose filter was adjusted at the inlet of the stainless steel vessel prior sample addition. The dead volume was filled with sea sand (General purpose grade, Fisher Scientific®). The HPLC-system comprised a Waters 515 HPLC pump (Waters Corporation, Milford, MA, USA), Waters 717 plus Autosampler, Guard-Pak Inserts Resolve Silica precolumn, a Varian Inertsil 5 SI column (250 × 4.6 mm, 5µm particle size, Varian Inc., Palo Alto, CA, USA) and a Waters scanning fluorescence detector (model 474). Millenium 32® software (Version 4.00, 2001 Water Corporation) controlled the system. Detailed information on the method of lipid extraction and HPLC analysis is given by Moreau et al. (2003) and Schwartz et al. (2007), respectively.

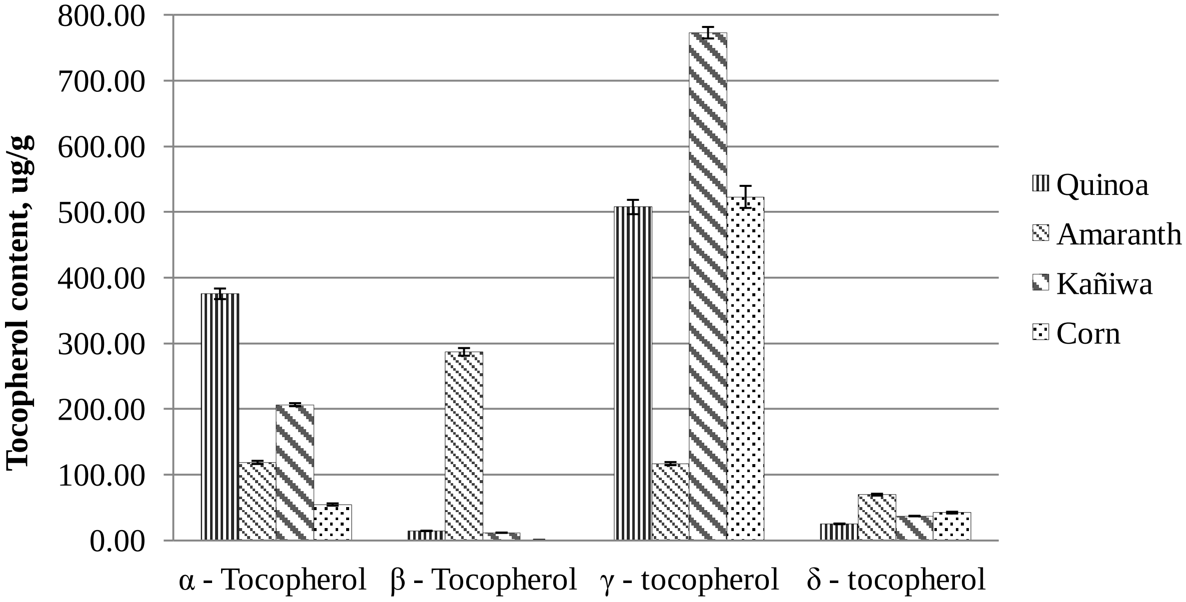


Figure 19. Content of α-, β-, γ- and δ-tocopherol in quinoa var. Rosada de Huancayo, amaranth var. Centenario and kaniwa var. Cupi. Error bar shows +/- standard deviation.

In general, quinoa, amaranth and kañiwa had higher content of tocopherols than corn flour. Quinoa and kañiwa presented high contents of α- and γ-tocopherol compared to amaranth and corn, whereas amaranth presented the highest content of β- tocopherol (Figure 19). The content of γ-tocopherol in corn was not significantly different from quinoa ($p<0.01$). Content of δ-tocopherol was negligible in all the analyzed samples.

For fatty acid analysis, extracted lipid was 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, therefore, detectable by gas chromatography (GC). The method of lipid extraction was the same as for tocopherol analysis (Moreau et al., 2003). However, volume normalization with heptane was 2 ml. The subsequent process of hydrolysis and esterification is detailed by Gunstone et al. (1994) and Mattila et al. (2001). The analysis of volatile compounds was performed with a Hewlett Packard 5890 GC (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The preset conditions were: a fused-silica capillary column (NB-351: 0.2 µm, 25m × 0.32 mm; Hnu-Nordion Ltd., Helsinki, Finland); carried gas: helium at 70 kPa; oven temperature program: 160 °C (0.5 min), 5 °C/min to 240 °C (10 min); detector temperature: 260 °C; injector temperature: 240 °C. There was a split ratio of 1:28 (split injection technique). Rapeseed oil was used as control sample.

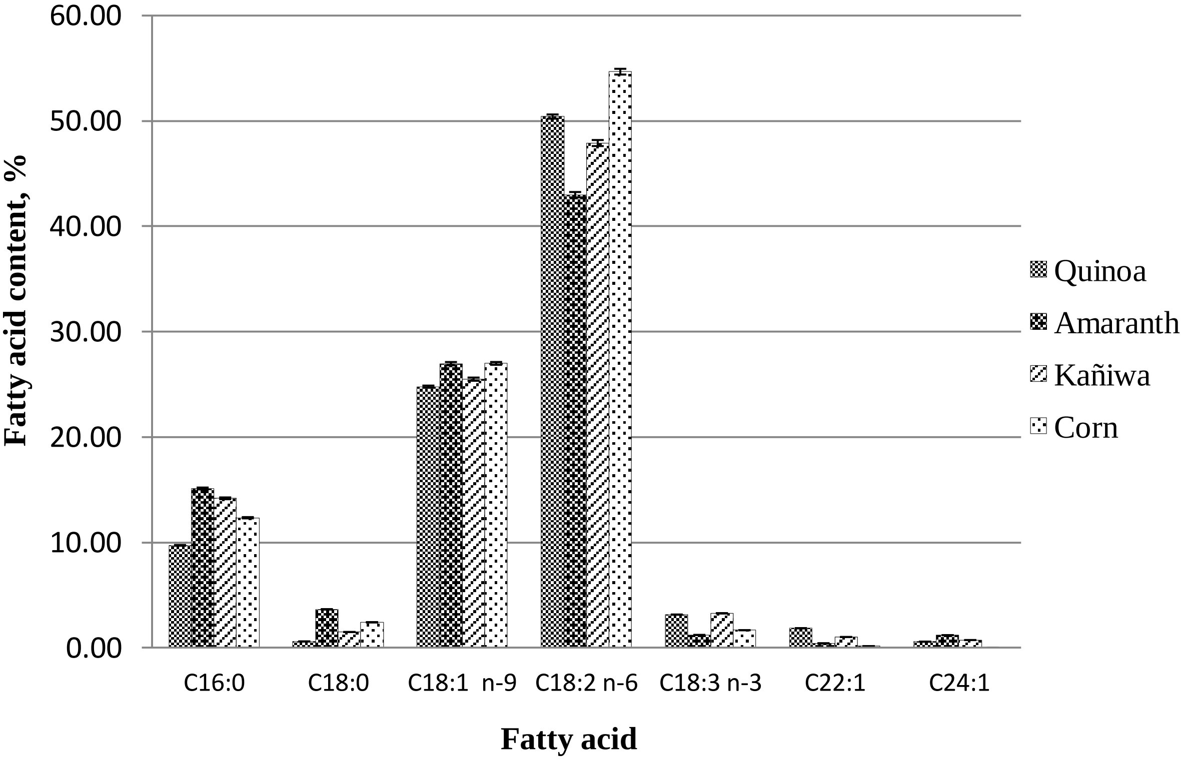


Figure 20. Fatty acid content in quinoa var. Rosada de Huancayo, Amaranth var. Centenario and Kaniwa var. Cupi. Error bar shows +/- standard deviation

All Andean grains and corn presented similar fatty acid composition. There were, however, some differences that were statistically significant. Palmitic, oleic and linoleic acid (ascending order) accounted for more than 80% of total fatty acids.

The content of linoleic acid showed the widest variability among the analyzed samples; linoleic acid was the lowest for amaranth (43%) and the highest for corn (55%). In contrast, quinoa and kañiwa did not show any significant difference ($p<0.01$) in their content of linoleic acid (Figure 20). The second most abundant fatty acid was oleic acid and the analyzed samples showed clear similarities in their content (~25%). The content of palmitic acid was the highest for amaranth and kañiwa (~15%), whereas quinoa and corn presented the lowest (~10%).

Contents of stearic and linolenic acids were around 3% and, despite the statistical differences among the analyzed samples, their actual contents were negligible. There were, also, detectable contents of monounsaturated fatty acids such as C22-1 and C24-1. Their contents were the lowest (< 2%) compared to other fatty acids.

3.3.2.2. Extrusion

The extrusion was carried out in a small scale twin-screw extruder (Thermo Prism PTW24, Thermo Haake, Polylab system, Germany). The following extrusion conditions were fixed based on preliminary experiments: feed rate and barrel temperature. The die diameter was 5 mm. The barrel consisted of 6 independent sections electrically heated and cooled by water. Temperature was monitored along the barrel by thermocouples which supplied signals to a control system. The first section was set at 40 °C, the second and third section at 70 °C, the fourth section at 110 °C and the fifth and sixth section at 110 °C, the temperature of the die varied among 150, 160 and 170 °C. Water content of feed was primarily determined in order to adjust the pump settings (Watson Marlow 505S, Watson-Marlow ltd. Falmouth, Cornwall, UK) and reached the desired water content of mass. Water was pumped through a set of rubber hoses connected to the first section of the barrel during the extrusion processing. In addition, feed rates were also adjusted during extrusion in order to maintain the preset water content of mass (15, 17 and 19%).

Response surface methodology was used to investigate the effects of independent extrusion variables (water content of feed, screw speed and temperature of the die) on the properties of corn-based extrudates containing amaranth / quinoa / kañiwa and the control.

Box-Behnken's experimental design for three independent variables involved 15 experiments that are specified in Table 14. The order of the experiments followed the increasing temperature of the die.

Table 14. Box-Behnken's experimental design with 3 independent variables

Experiments	X_1	X_2	X_3	Water content (%)	Screw speed (rpm)	Temperature of the die (°C)
11	1	0	-1	19	350	150
3	-1	0	-1	15	350	150
5	0	-1	-1	17	200	150
7	0	1	-1	17	500	150
15	0	0	0	17	350	160
1	-1	-1	0	15	200	160
2	-1	1	0	15	500	160
13	0	0	0	17	350	160
9	1	-1	0	19	200	160
10	1	1	0	19	500	160
14	0	0	0	17	350	160
6	0	-1	1	17	200	170
8	0	1	1	17	500	170
12	1	0	1	19	350	170
4	-1	0	1	15	350	170

3.3.2.3. Determination of the response variables

Sectional Expansion Index (SEI)

A total number of 10 specimens per sample were measured using a vernier caliper. Each specimen had a length of 10 cm, approximately. SEI was calculated according to Alvarez-Martinez et al. (1988) and the equation used was the following:

$$SEI = \frac{S_e}{S_d} \tag{1}$$

S_e = Cross Sectional Area of the extrudate, m^2

S_d = Cross Sectional Area of the die, m^2

Hardness

Hardness was the level of resistance per millimeter (N/mm) when penetration was perpendicularly enforced (under three point bending conditions) at 5 mm/min on an extrudate structure. Hardness was measured at 23 °C (50% RH) in a universal testing machine (Instron 4465, Instron Ltd., High Wycombe, UK). Samples (5 specimens per sample) were vacuum-dried at 54 °C for 72 hours prior to hardness measurement.

The collected data (Force vs. distance) were plotted using a scatter graph and a trend line was drawn through areas with seemingly higher correlation in order to determine the slope that was further expressed as N/mm.

Water content of extrudates (WCE)

Water content was calculated from the weight loss in drying. Three specimens per sample were collected just after extrusion processing, weighed and vacuum-dried at 54 °C for 72 hours. Results were calculated using the following equation:

$$M_e = \frac{W_{DS} - W_e}{W_S} \times 100 \tag{2}$$

W_{DS} = Weight of aluminum container and dry sample
W_e = Weight of empty aluminum container
W_S = Weight of sample

Torque and Pressure at the die

The values of torque and pressure were recorded along extrusion every 1.7 centiseconds and mean values were calculated based on torque and pressure registered during sample collecting.

3.3.2.4. Stereomicroscopy inspection

Samples for inspection were obtained using the same extrusion conditions (experiment 2; water content of mass 15%, screw speed 500 rpm, temp. of die 160 °C). A small scale electric saw was used for length (15 mm) and longitudinal (1/2 the initial width) adjustment of the samples. Pictures were taken using a Zeiss Stemi 2000 Binocular Stereo Microscope (Wetzlar, Germany) equipped with AxioCam digital camera. For images at further distance, an Olympus Zuiko objective (9-18mm f/4.0-5.6 Lens for Olympus; Tokyo, Japan) was used. The images were processed using Axiovision 3.1 software (Carl Zeiss Inc.).

3.3.2.5. Headspace analysis

Samples were collected and divided into two groups. One of them was ground using an ultra centrifugal mill (Retsch ZM 200, Haan, Germany) at 12000 rpm in order to reach a particle size of 500 µm. The other group of samples underwent length adjustment of 15 mm. The resulting products were weighed and placed into 20-ml headspace vials as follows: 2g of ground extrudate and 0.5g of whole extrudate. Considering the high specific volume of extrudates and low volume available in vials, standardizing the mass of ground and whole extrudates was unattainable.

Open vials containing samples were stored in vacuum desiccators at 11 and 76% RH at 20 °C for one week before being sealed and stored at room temperature for 0, 2, 5 and 9 weeks. Storage took place in the absence of light.

Samples were analyzed for hexanal content using static headspace gas chromatography (Autosystem XL gas chromatograph equipped with an HS40XL head space sampler, Perkin-Elmer, Shelton, CT; column NB-54, Nordion). The column had a length and diameter of 25 m and 32 µm, respectively. Detection and injection temperature was set at 250 °C. Flame gases were O_2 and H_2, and carrier gas was He. Vials were thermostated at 80 °C for 18 min prior to 60 °C for 10min. The overall run time was 28 min per vial.

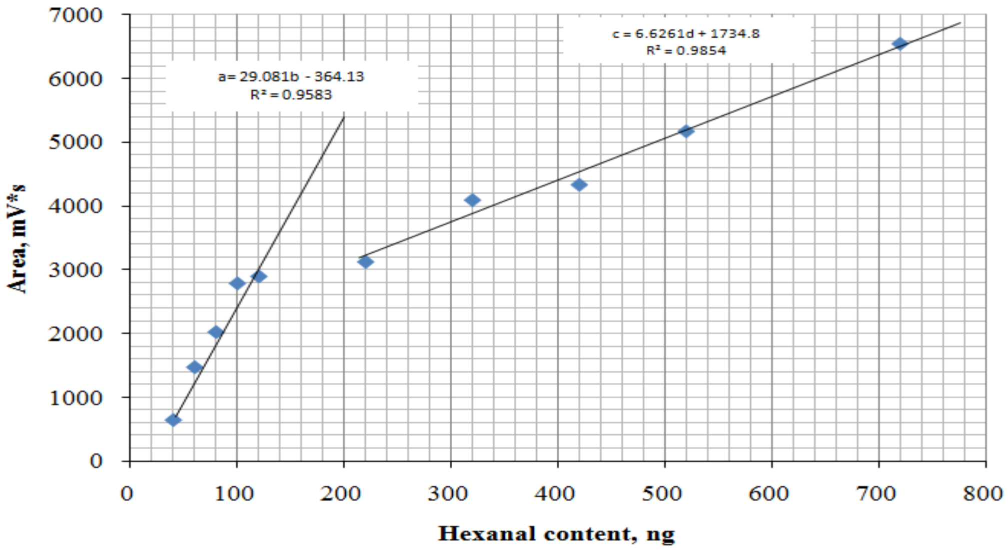


Figure 21. Standard curve for ground extrudate. Sample mass: 2g.

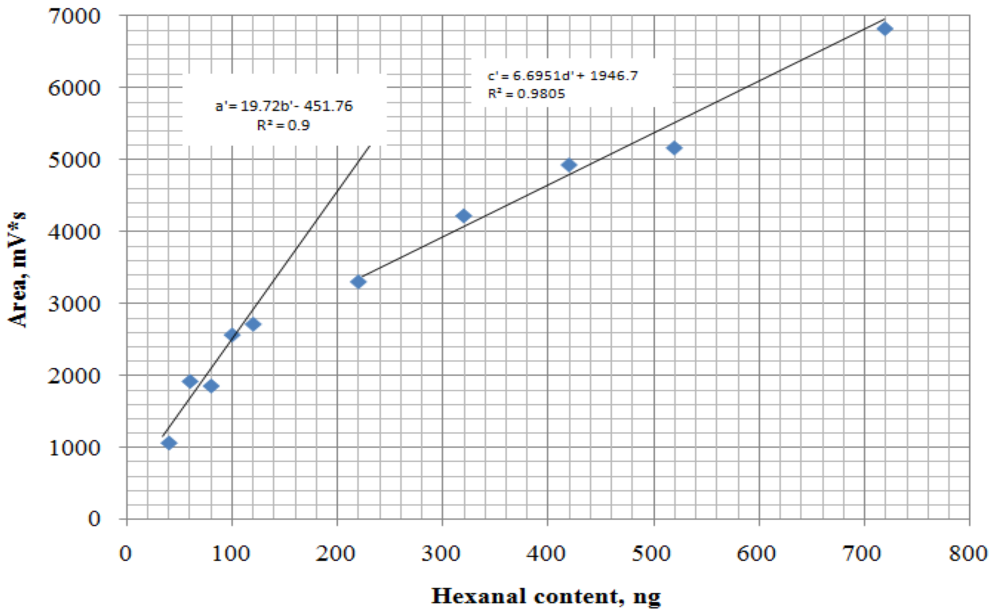


Figure 22. Standard curve for whole extrudate. Sample mass: 0.5g

An external standard curve was plotted using ground (2 g) and whole corn extrudates (0.5 g) with varying contents of hexanal. A solution of hexanal in isopropanol was prepared (10^7 ng hex/L) and added to vials containing the corn extrudates (4, 6, 8, 10, 12, 32, 42, 52, 72 µl hexanal solution/vial).

Prior to GC analysis, samples were stored in a shaking incubator overnight (12 h). Simulation of real sample conditions was enforced in order to reduce the inaccuracy of the results. Two standard curves were plotted: for ground (Figure 21) and whole extrudates (Figure 22).

3.3.2.6. Statistical analysis

A polynomial was fitted to the data in order to obtain the regression equation of independent variables on response variables. Regression analysis and the plotting of response surfaces were performed using Matlab (The Mathworks, Inc., Natick, Massachusetts).

Chemical and physical analyses were done in triplicate and results were expressed as means and standard deviation (SD). The data were statistically processed by analysis of variance and LSD ($p<0.01$; $p<0.05$) using SPSS (version 17.0 SPSS Inc., Chicago, Illinois).

3.4. Results

3.4.1. Physical properties of extrudates

Extrudates containing amaranth exhibited the highest SEIs (compared to other trials) with 7.6 and 6.2 corresponding to the experiments 2 and 3, respectively. It was also observed that the most expanded extrudates containing quinoa presented SEI of 6.1, while extrudates containing kañiwa had a maximum SEI of 5.1 (exp. 2 in both cases). The control, on the other hand, presented SEIs of 4.4 and 5.1 (exp. 2 and 3).

The lowest hardness value (28 N/mm) was for extrudates containing kañiwa (exp. 2), while extrudates containing quinoa and amaranth (also exp. 2) presented hardness values of 39 and 53 N/mm, respectively. In contrast, the control presented a hardness value of 89 N/mm (exp. 2) which is the highest compared to other trials. Water content of extrudates containing amaranth and quinoa was the highest for the experiment 11 (11.1% and 8.9%) and the lowest for the experiments 8 and 1 (4.2% and 3.9%), respectively. Indeed, water content of extrudates containing quinoa and the control was the highest for the experiments 9 and 10 (10.3% and 14.9%). The results obtained from every experiment are shown in Appendix 1.

3.4.2. Effect of independent extrusion variables on response variables

Regression analysis was used to evaluate the effect of water content of mass, screw speed and temperature of the die (independent extrusion variables) on SEI, hardness, water content of extrudate, torque and pressure at the die (response variables) (Table 15). Models that presented non-significant regression were not considered for further evaluation. These regression models were calculated in order to find a pattern of interaction that was graphically represented through response surface plots in which there were two extrusion variables (e.g. screw speed and water content of mass) and one response variable (e.g. SEI or hardness).

Water content of mass and screw speed had the most significant effect on SEI and water content of extrudate. However, the level of significance and direction of the effect changed depending on the raw material used for extrusion. In general, the effect of extrusion variables on torque, pressure and SEI had the highest significance according to the proposed regression models.

Table 15. Parameter estimates from the regression models.

	SEI	**Hardness ^{a}, N/mm**	**Water content of extrudates ^{a}, %**	**Torque ^{a}, N.m**	**Pressure at the die ^{a}, bar**
Control					
X_1: Water content of mass, %	– 0.456***	15.625*	1.175	– 1.375***	– 3.438***
X_2: Screw speed, rpm	0.002	– 0.010	0.013	– 0.018***	– 0.053***
X_3: Temp. of die, °C	– 0.029	– 0.875	0.055	– 0.050	– 0.488***
Intercept	14.92**	– 13.266	– 24.18	71.26***	190.7***
R^2	69.11	46.76	64.80	82.15	97.71
Corn-Amaranth					
X_1: Water content of mass, %	– 0.744***	17.687***	0.888***	– 1.438$^{\text{L.F.}}$	– 6.979
X_2: Screw speed, rpm	0.008**	– 0.081	– 0.007*	– 0.007$^{\text{L.F.}}$	– 0.469**
X_3: Temp. of die, °C	– 0.016	– 0.600	– 0.121**	– 0.188$^{\text{L.F.}}$	– 0.888
$X_1 \times X_2$	-	-	-	-	0.011*
$X_1 \times X_3$	-	-	-	-	– 1.9×10^{-15}
$X_2 \times X_3$	-	-	-	-	0.002
Intercept	16.84*	-82.86	14.22*	93.50***	301.66
R^2	74.83	76.48	83.38	60.53	89.40
Corn-Quinoa					
X_1: Water content of mass, %	– 0.510$^{\text{L.F.}}$	– 39.458	0.794****	– 1.125**	– 2.688***
X_2: Screw speed, rpm	0.005$^{\text{L.F.}}$	– 4.311*	– 0.0004	– 0.024***	– 0.038***
X_3: Temp. of die, °C	– 0.005$^{\text{L.F.}}$	– 10.004	– 0.045	– 0.088	– 0.212*
$X_1 \times X_2$	-	0.062	-	-	-
$X_1 \times X_3$	-	0.200	-	-	-
$X_2 \times X_3$	-	0.020*	-	-	-
Intercept	9.47*	18.6×10^{2}	0.24	79.85***	123.17***
R^2	80.21	70.77	83.58	83.87	89.38
Corn-Kaniwa					
X_1: Water content of mass, %	– 0.312***	11	0.700***	– 0.938**	– 25.604$^{\text{L.F.}}$
X_2: Screw speed, rpm	0.008***	– 0.326**	– 0.005***	– 0.010*	0.975$^{\text{L.F.}}$
X_3: Temp. of die, °C	– 0.006	– 1.063	0.035*	– 0.175*	– 5.967$^{\text{L.F.}}$
$X_1 \times X_2$	-	-	-	-	0.006$^{\text{L.F.}}$
$X_1 \times X_3$	-	-	-	-	0.125$^{\text{L.F.}}$
$X_2 \times X_3$	-	-	-	-	0.012$^{\text{L.F.}}$
Intercept	6.44**	193.84	-7.82**	78.93***	10.8×10^{2}
R^2	93.14	63.20	95.02	72.02	49.69

Significant effect at p< 0.05*, 0.01**, 0.001***, 0.0001****

^{a} Significant lack of fit (L.F.) at p<0.05

3.4.2.1. SEI

SEI increased in direct relation to screw speed. In contrast, SEI decreased as water content of mass increased. The regression models presented significant regression ($p<0.003$), and non-significant lack of fit ($p>0.1$) in all cases except for extrudates containing quinoa. Water content of mass had the most significant effect on SEI (Table 15). The following response surface plots show the direction of the effects on SEI:

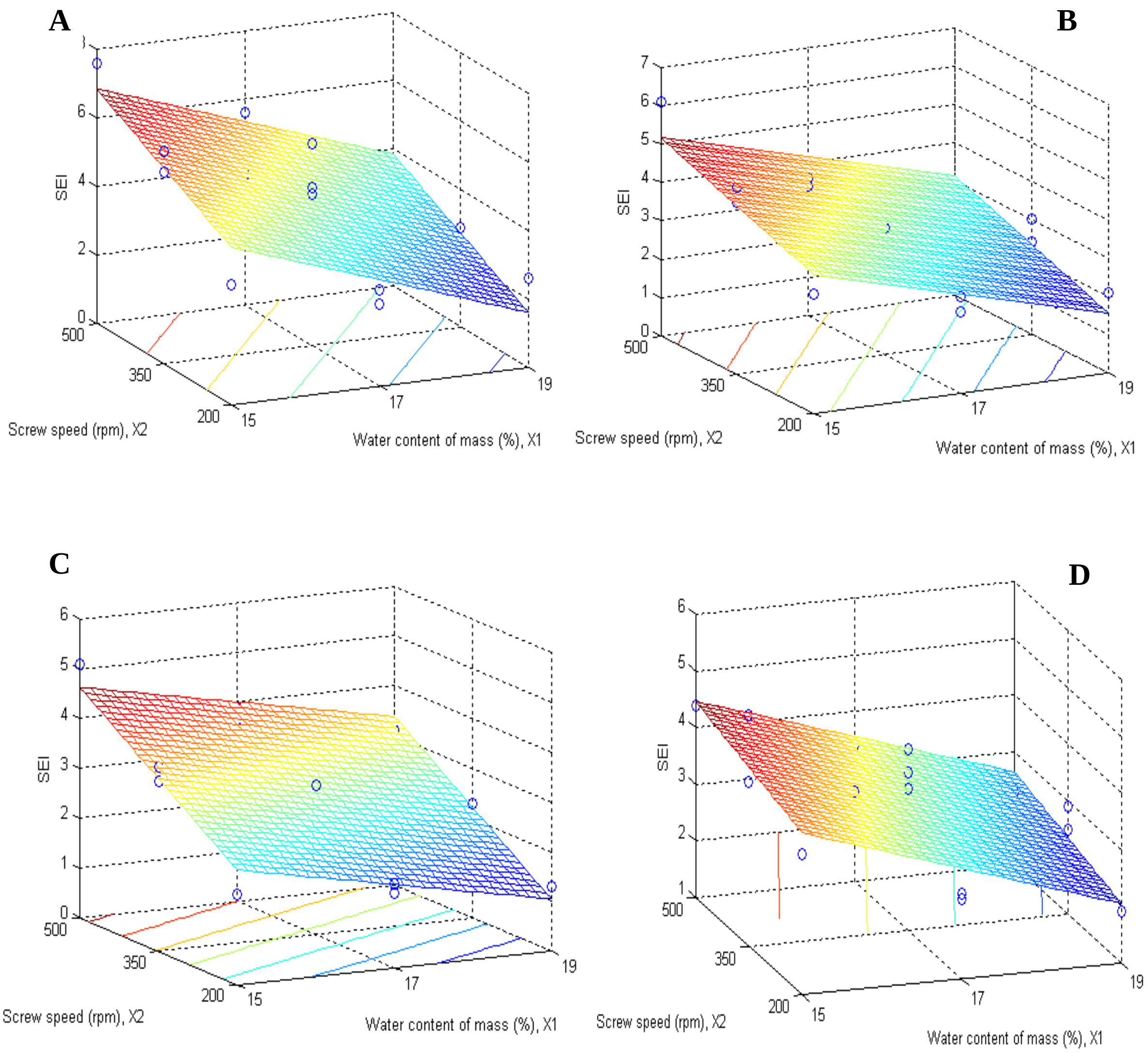


Figure 23. Response surface plots of the effects of screw speed and water content of mass on SEI in extrudates containing amaranth (A), quinoa (B), kañiwa (C) and the control (D).

3.4.2.2. Hardness

Hardness increased as screw speed decreased. Indeed, low hardness was associated with low water content of mass. Regression models presented non-significant lack of fit ($p>0.08$) for all samples and significant regression ($p<0.003$) for extrudates containing amaranth and kañiwa. The effect of water content of mass appeared to be the most significant for extrudates containing amaranth (Table 15). The following response surface plots show the direction of the effects on hardness:

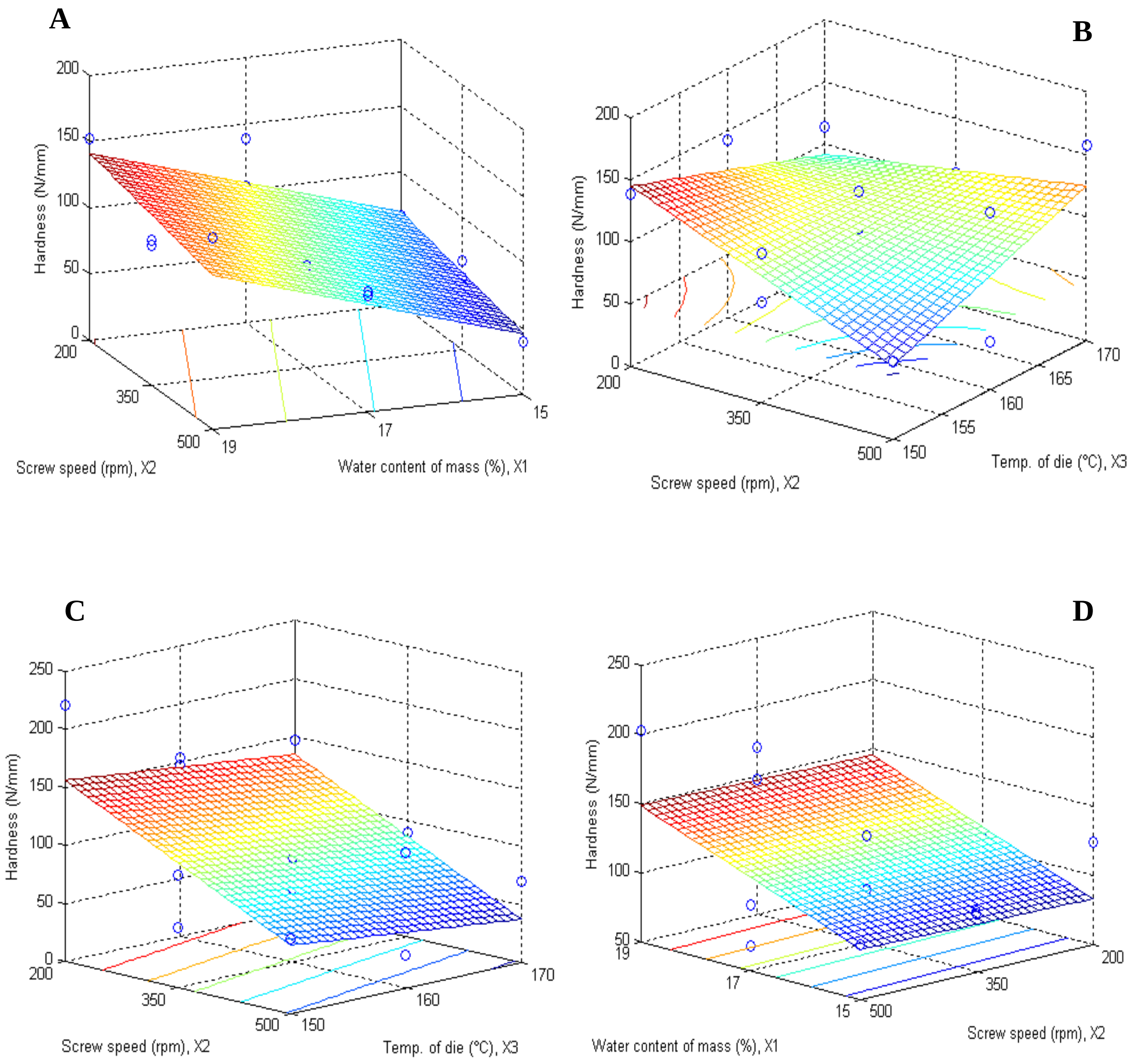


Figure 24. Response surface plots of the effects of screw speed and water content of mass on hardness for extrudates containing amaranth (A), quinoa (B), kañiwa (C) and the control (D).

3.4.2.3. Water content of the extrudates

Water content of the extrudates increased in direct relation to water content of mass. In contrast, water content of the extrudates decreased as screw speed and temperature of the die increased. Regression models presented non-significant lack of fit ($p>0.08$), and significant regression ($p<0.001$) in all cases except for the control. Water content of mass had the most significant effect on water content of the extrudates (Table 15). The following response surface plots show the direction of the effects on water content of extrudate:

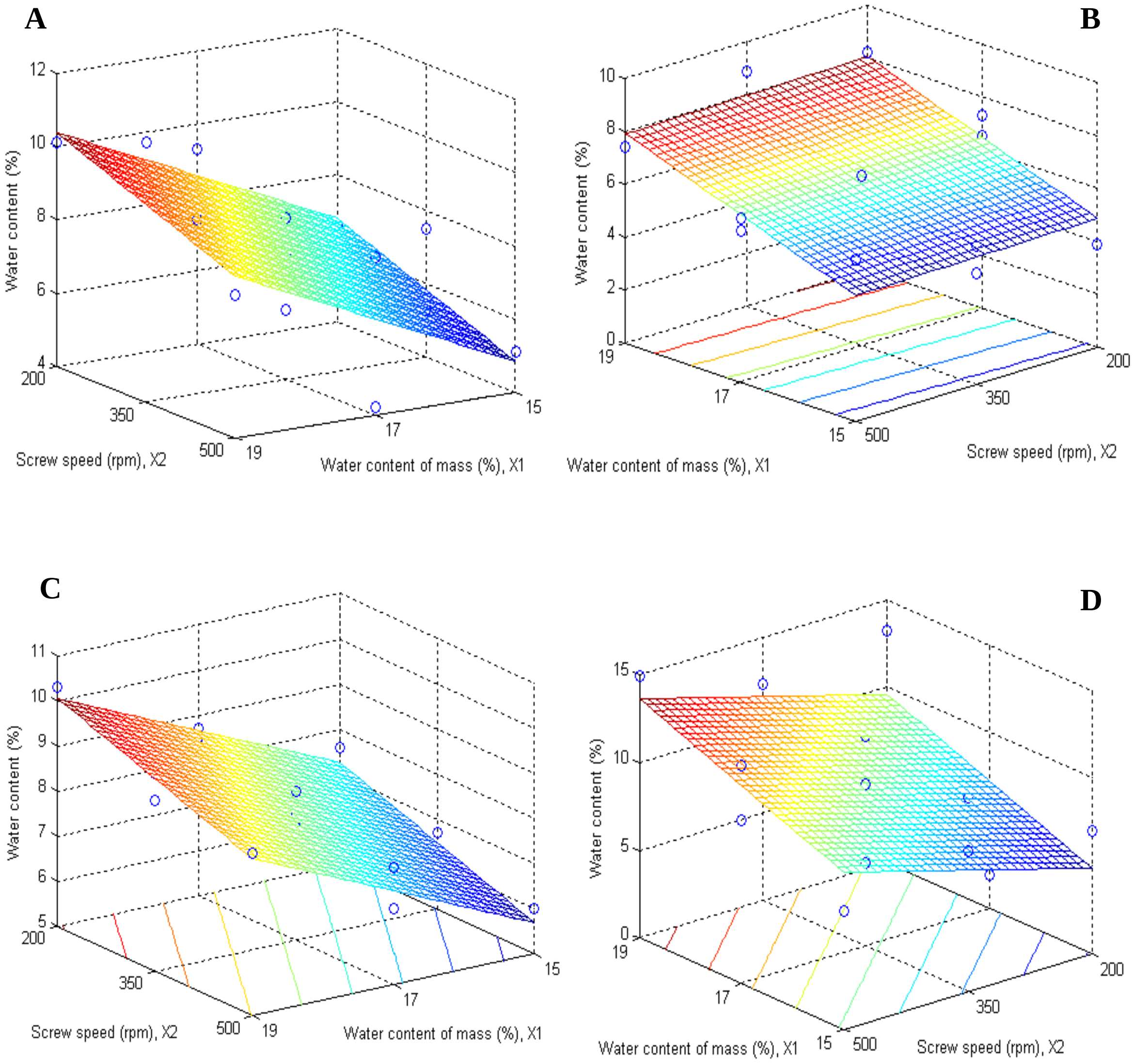


Figure 25. Response surface plots of the effects of screw speed and water content of mass on water content of extrudates containing amaranth (A), quinoa (B), kañiwa (C) and the control (D).

3.4.2.4. Pressure at the die

Pressure at the die increased as extrusion variables decreased. Regression models presented significant regression ($p<0.001$) and non-significant lack of fit ($p>0.05$) in all cases except for extrudates containing kañiwa. In this sense, the effect of water content of mass and screw speed on pressure at the die was the most significant for extrudates containing quinoa and the control (Table 15). The following response surface plots show the direction of the effects on pressure at the die:

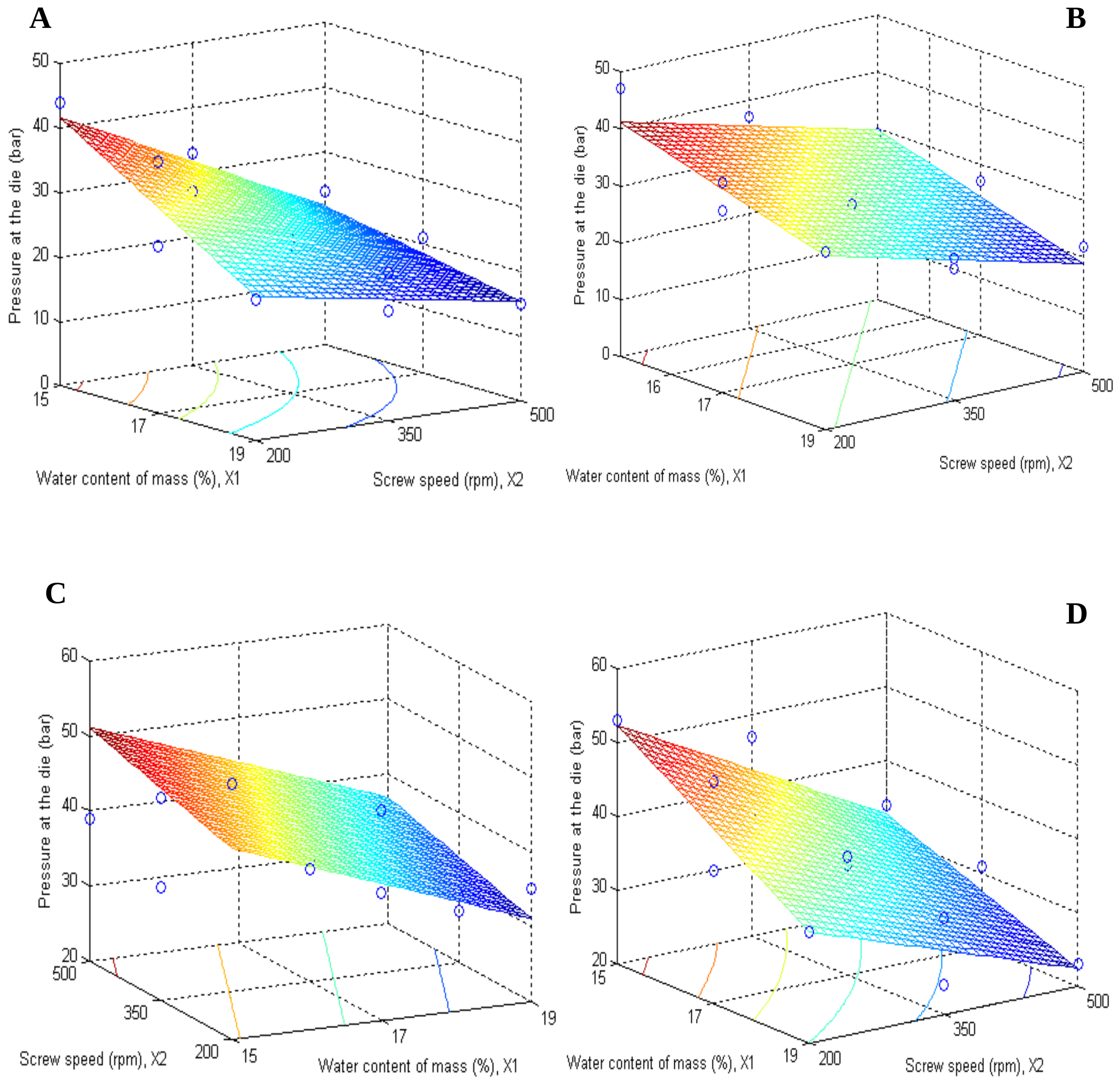


Figure 26. Response surface plots of the effects of screw speed and water content of mass on pressure during extrusion processing of (20% of solids) amaranth (A), quinoa (B), kañiwa (C) and the control (D).

3.4.2.5. Torque

Torque increased as extrusion variables decreased. Regression models presented significant regression ($p<0.001$), and non-significant lack of fit ($p>0.08$) in all cases except for extrudates containing amaranth. The effect of screw speed and water content of mass on torque was significant for all extrudates (Table 15). The following response surface plots show the direction of the effects on torque:

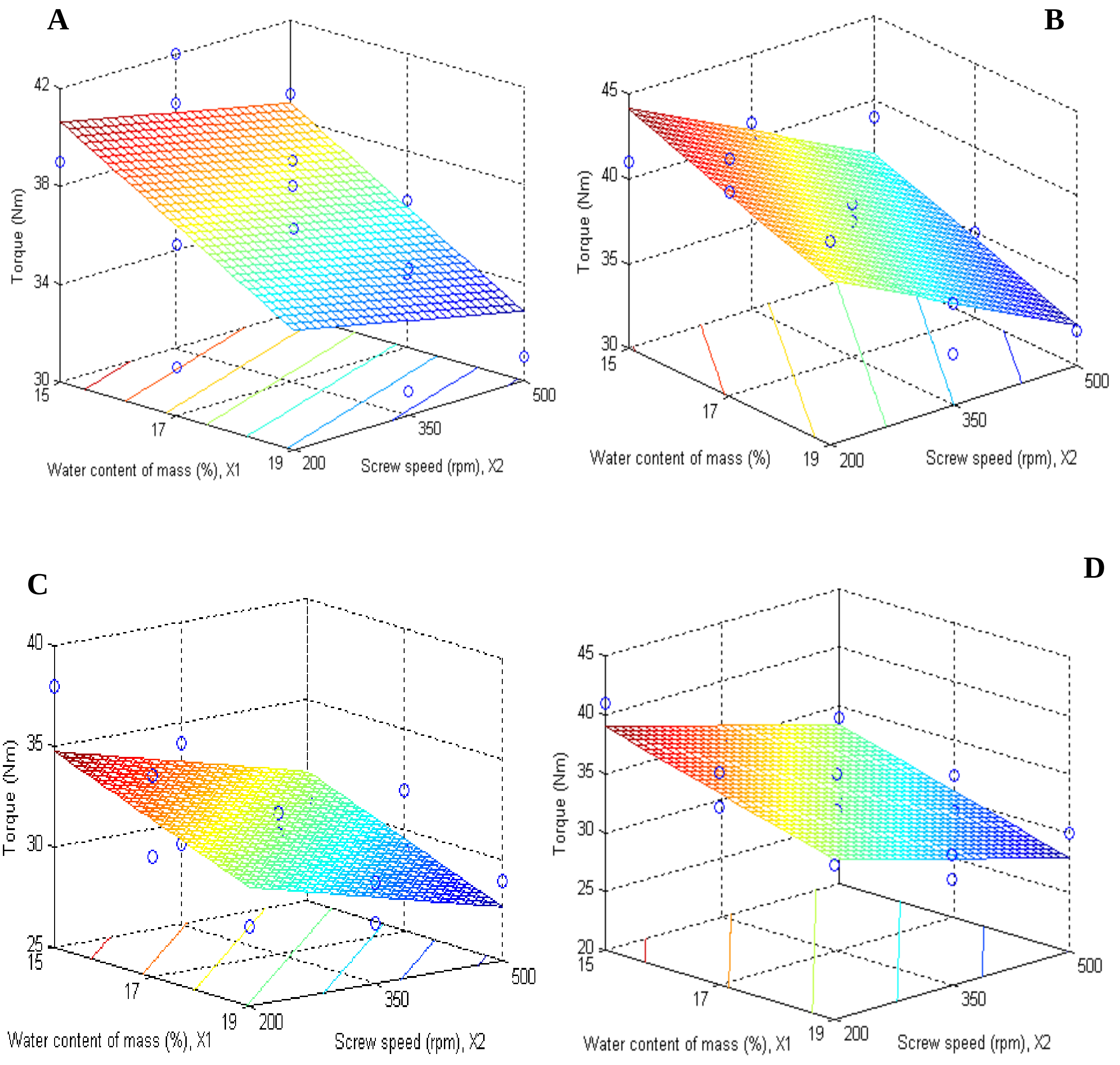


Figure 27. Response surface plots of the effects of screw speed and water content of mass on torque during extrusion processing of (20% of solids) amaranth (A), quinoa (B), kañiwa (C) and the control (D).

3.4.3. Correlation between response variables

Response variables (corresponding to 15 experiments per trial) were statistically analyzed in order to find their degree of correlation. SEI and hardness showed the highest significant correlation (except for the control), followed by SEI and water content of the extrudates (Table 16).

SEI increased as hardness and water content of the extrudates decreased. Besides, hardness increased in direct proportion to water content of the extrudates in all trials except for extrudates containing quinoa. In general, pressure and torque did not present significant correlation with SEI, hardness and water content of extrudates ($p<0.05$). Scatter plots between response variables are presented in Appendix 2 and 3.

Table 16. Correlation coefficients between response variables.

	SEI	Hardness	WCE	Pressure	Torque
SEI					
SEI - A	1	– 0.736**	– 0.575*	– 0.003^{ns}	0.543*
SEI - Q	1	– 0.761**	– 0.547*	0.032^{ns}	– 0.031^{ns}
SEI - K	1	– 0.852**	– 0.796**	– 0.319^{ns}	– 0.280^{ns}
SEI - C	1	– 0.424^{ns}	– 0.514*	– 0.024^{ns}	0.319^{ns}
Hardness					
Hardness - A		1	0.654**	– 0.248^{ns}	– 0.500^{ns}
Hardness - Q		1	0.435^{ns}	– 0.111^{ns}	– 0.098^{ns}
Hardness - K		1	0.613*	– 0.288^{ns}	– 0.267^{ns}
Hardness - C		1	0.603*	0.012^{ns}	0.423^{ns}
WCE					
WCE - A			1	– 0.036^{ns}	– 0.139^{ns}
WCE - Q			1	– 0.543*	– 0.244^{ns}
WCE - K			1	– 0.251^{ns}	– 0.200^{ns}
WCE - C			1	– 0.243^{ns}	– 0.376^{ns}
Pressure					
Pressure - A				1	0.552*
Pressure - Q				1	0.782**
Pressure - K				1	0.882**
Pressure - C				1	0.184^{ns}
Torque					
Torque - A					1
Torque - Q					1
Torque - K					1
Torque - C					1

SEI: sectional expansion Index; WCE: water content of extrudate; A: amaranth 20% (of solids); Q: quinoa 20% (of solids); K: kañiwa 20% (of solids); C: Corn 100% (of solids)

^{ns} Not significant , *Significant at $p < 0.05$, **Significant at $p < 0.01$.

3.4.4. Stereomicroscopy analysis of expanded extrudates

Extrudates used for visual examination were obtained from extrusion processing under the same operational conditions (exp. 2). These extrudates presented the highest SEI (except for the control) and lowest hardness compared to other experiments. Unlike the control, decreasing SEI was associated with decreasing hardness (R= 0.99) (Figure 28). All the examined extrudates presented significant differences in SEI ($p<0.01$) and hardness ($p<0.05$).

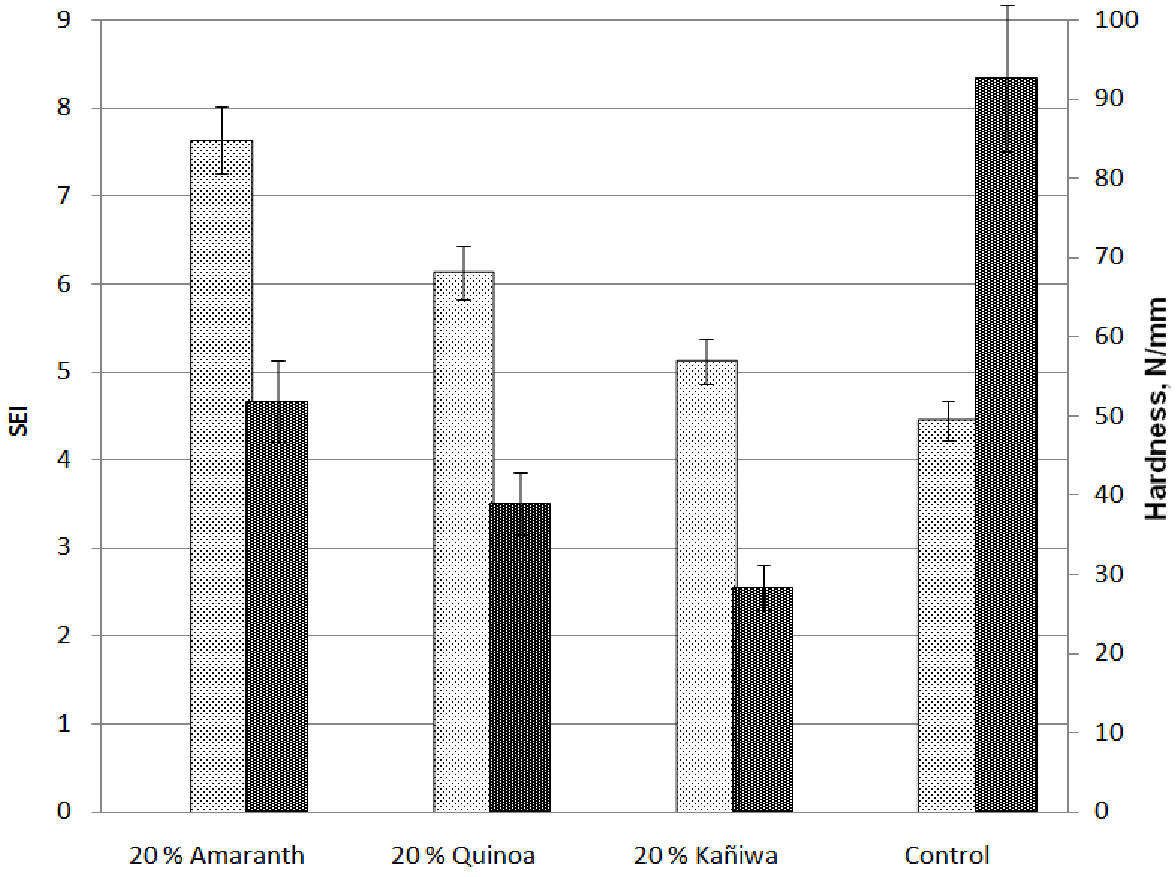


Figure 28. Corn-based extrudate containing amaranth/ quinoa/ kañiwa and the control. ░, SEI; ▓, Hardness (N/mm). Samples from exp. 2. Error bar shows +/- standard deviation.

Apparently, the round structure of the examined extrudates depended considerably on the size, form and distribution of pores (Figure 29). Extrudates containing quinoa and amaranth exhibited well-defined pores of different sizes with semi-flat sides. In contrast, extrudates containing kañiwa presented small amorphous pores that were poorly defined. The control had a rigid structure with comparatively bigger pores and thicker walls than other samples (brittleness).

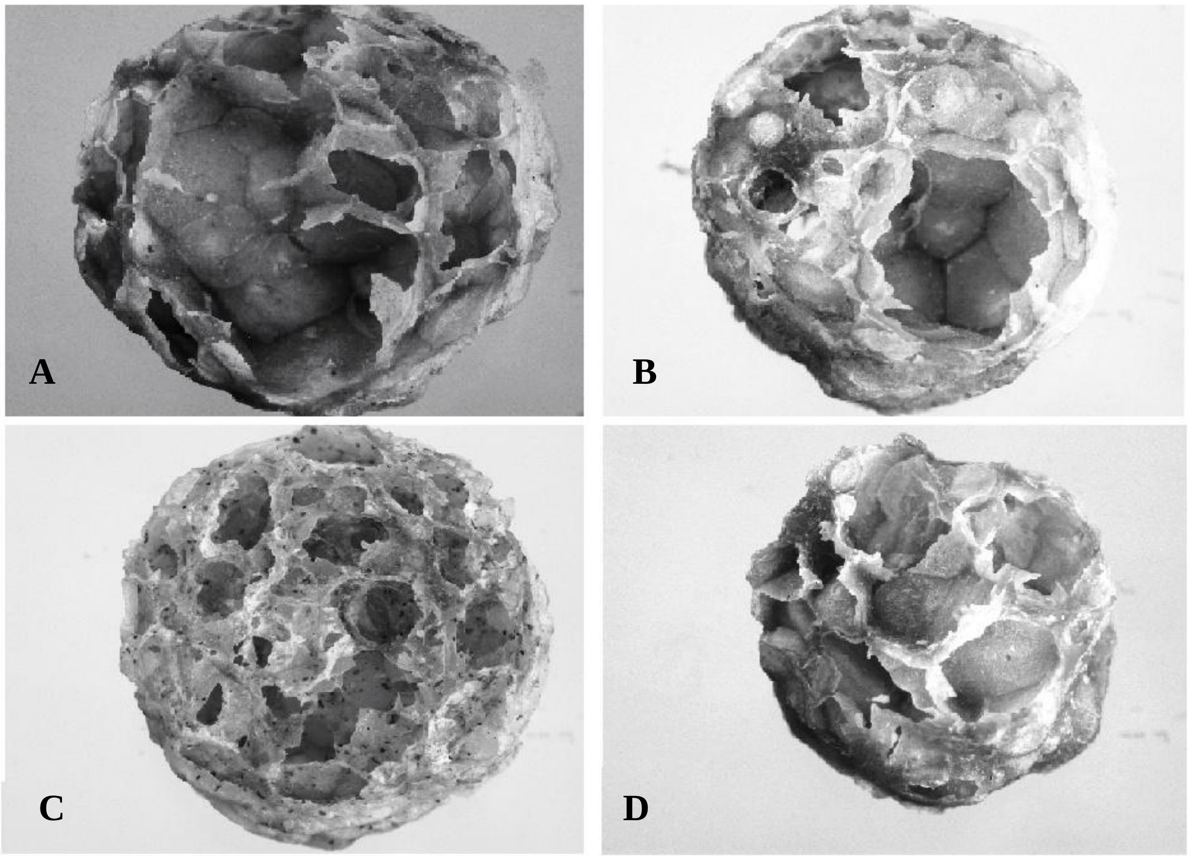


Figure 29. Cross-sectional area of corn-based extrudates containing 20% of amaranth (A), quinoa (B), kañiwa (C) and the control (D) at 100x magnification. Samples from exp. 2.

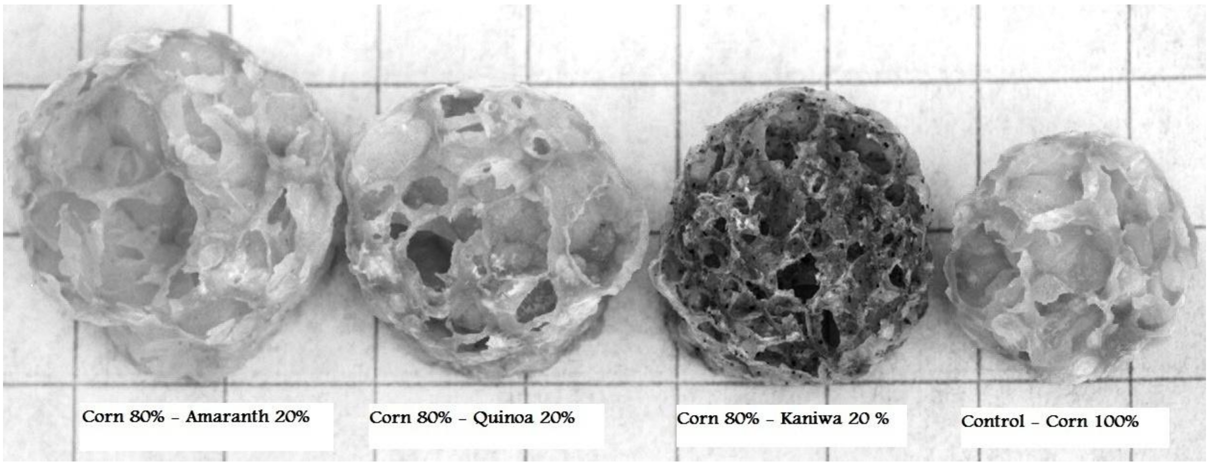


Figure 30. Comparison of cross-sectional areas. Samples from exp. 2.

Differences in color and diameter were attributed to the particular composition of each mixture (Figure 30). Extrudates containing amaranth and quinoa maintained the light color initially presented by raw materials. Kañiwa, though, seemed to have a darkening effect on the final product. The presence of crude fiber (small brown spots) was noticeable in extrudates containing kañiwa (Figure 29C, 30, 31C). The control had a translucent structure with a particular yellowish color which is common among corn-based extrudates.

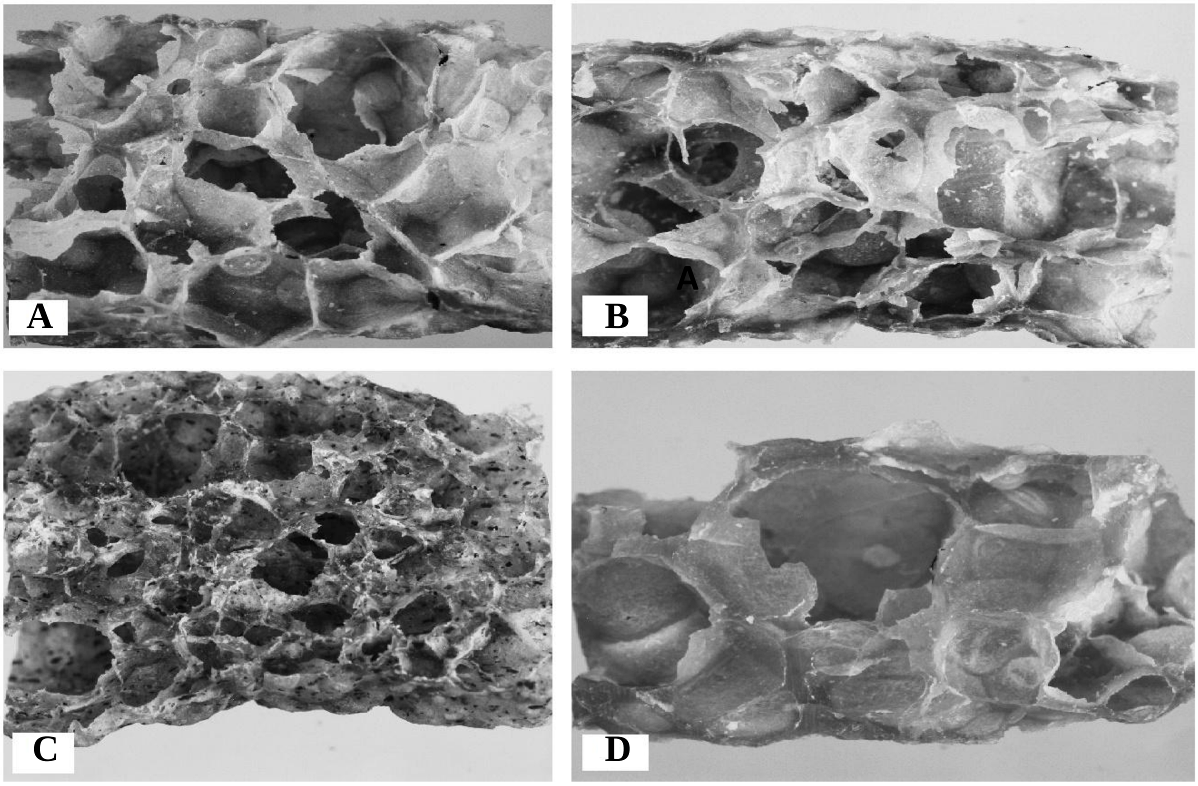


Figure 31. Longitudinal section of corn extrudates containing 20% of amaranth (A), quinoa (B), kañiwa (C) and the control (D) at 100x magnification. Samples from exp. 2.

Considering the Figure 31, there was no visual difference between extrudates containing amaranth and quinoa when it comes to distribution and size of pores. In fact, it was easy to notice that both extrudates had comparable breaking points since they showed very similar damage towards enforced physical modification (longitudinal cutting). Extrudates containing kañiwa exhibited a clear cut surface. In contrast, the control suffered severe structural damage during cutting (brittleness break).

3.4.5. Hexanal production during storage

Ground and whole extrudates were exposed to 11 and 76% RH for one week, followed by storage in sealed head-space vials. There were important changes in the rate of hexanal production during storage. In fact, final hexanal content for ground extrudates was comparatively higher than for whole extrudates (except for whole extrudates containing quinoa and exposed to 11% RH). Extrudates containing quinoa and kañiwa, and exposed to 11% RH showed tendency towards lipid oxidation. However, the stability of extrudates containing amaranth and the control appeared to be independent to humidity exposure and physical modification.

Curves of hexanal production for ground extrudates containing kañiwa (11 and 76% RH) exhibited contrasting slopes (Figure 32). Extrudates containing kañiwa and exposed to 11% RH presented a 25-fold increase in hexanal content along storage, while the ones exposed to 76% RH presented only a 3-fold increase. It was also observed that ground extrudates containing kañiwa and exposed to 11% RH, had lower content of hexanal at zero point (6-fold less) compared to the ones exposed to 76% RH. Differences in trends of hexanal production were particularly marked for ground extrudates containing kañiwa. In contrast, ground extrudates containing amaranth, quinoa and the control did not show significant difference ($p<0.01$) in their curves of hexanal production.

The rates of hexanal production between whole extrudates containing quinoa and kañiwa (11 and 76% RH) showed considerable differences (Figure 33). Hexanal production increased exponentially for extrudates containing quinoa and kañiwa, and exposed to 11% RH (Figure 31A). There was a 200-fold increase in the content of hexanal for extrudates containing quinoa. These results were unexpected, considering the low hexanal production of analogous extrudates. Extrudates containing kañiwa exhibited a 15-fold increase in the content of hexanal after 9-week storage. On the other hand, whole extrudates exposed to 76% RH showed no significant difference ($p<0.01$) in their hexanal content along storage. It was also observed that extrudates containing amaranth and the control (11 and 76% RH) had high degree of stability along storage independent to humidity exposure.

The presence of other volatile compounds such as pentanal and propanal was also measured. Their production, however, was much lower and heavily dependent on hexanal production. Data on pentanal and propanal was not considered for the present research.

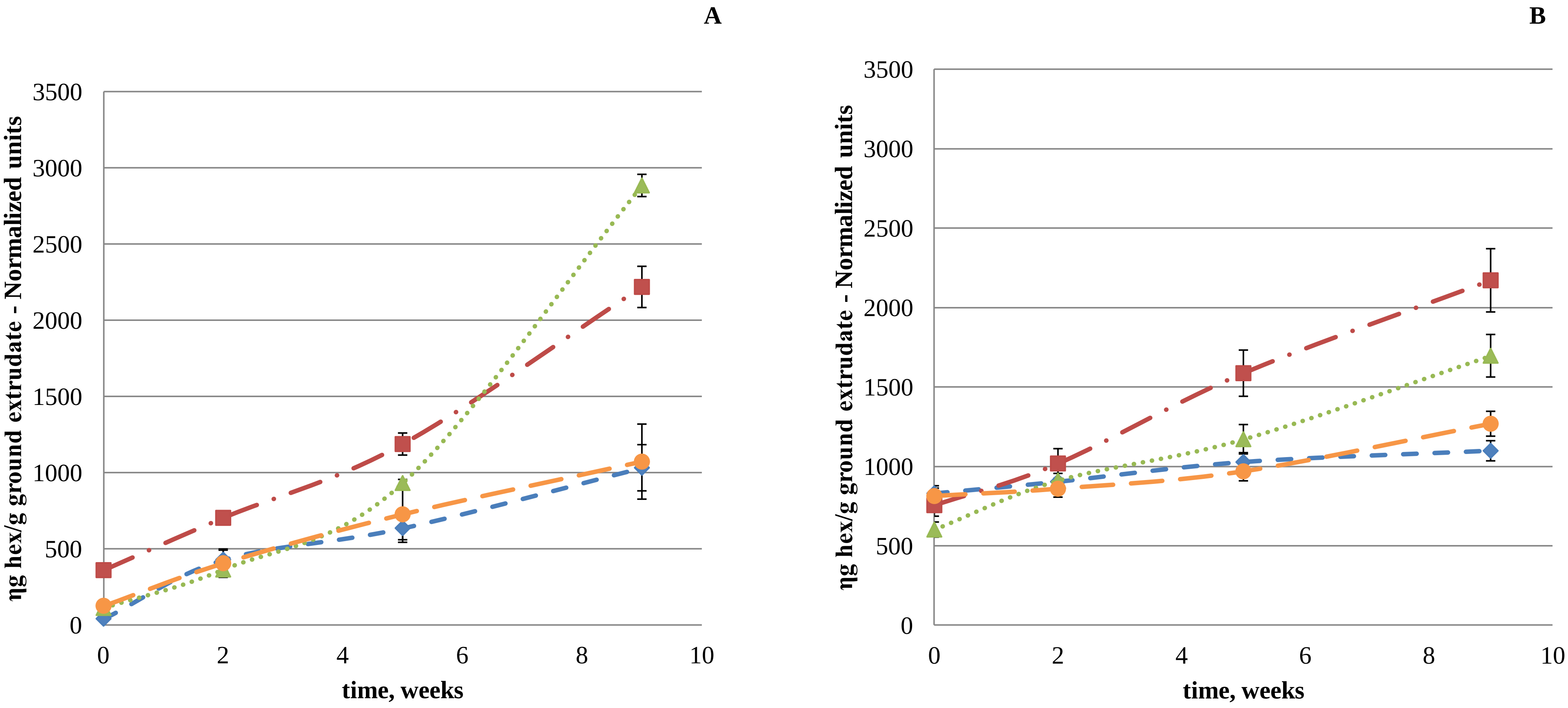


Figure 32. Effect of exposure to 11% (A) and 76% RH (B) on the production of hexanal in **ground extrudates** containing 20% amaranth (– – –), 20% quinoa (—·—), 20% kañiwa (• • • •) and control (— —). Control sample is 100% corn. Original sample mass: 2 g. Error bar shows +/- standard deviation

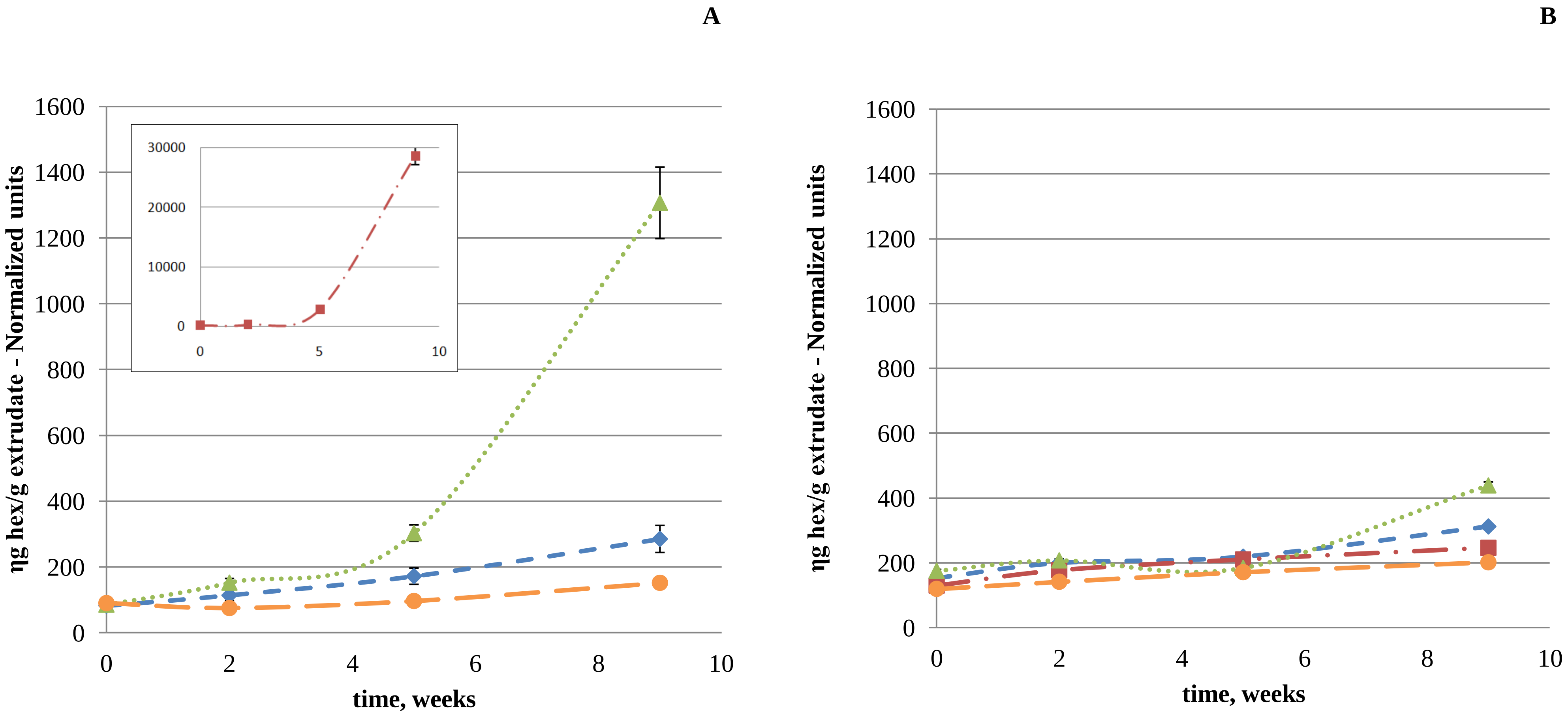


Figure 33. Effect of exposure to 11% (A) and 76% RH (B) on the production of hexanal in **whole extrudates** containing 20% amaranth (– – –), 20% quinoa (–·–), 20% kañiwa (• • • •) and control (— —). Control sample is 100% corn. Original sample mass: 0.5 g. Error bar shows +/- standard deviation

3.5. Discussion

3.5.1. Effect of material and processing conditions on extrudate properties

Expansion of extrudates

It was possible to attain significantly higher SEI ($p<0.01$) with corn-based extrudates containing Andean grains than that with pure corn extrudates. According to similar studies (Coulter and Lorenz, 1991), non-pregelatinized degerminated corn grits gave the highest SEI at comparable extrusion conditions (barrel temperature 100-150 °C, screw speed 100-200 rpm). However, we used corn flour instead of corn grits (Coulter and Lorenz, 1991) and the extruder was considerably smaller with different screw configuration (twin screw diameter 4.5-5 mm; single screw diameter 19.5 mm). Twin screw seemed to increase the degree of gelatinization and development of viscosity, leading to higher SEI (Kirby et al., 1988).

The higher SEI of extrudates containing Andean grains could also be attributed to the plasticizing effect of polymeric and non-polymeric compounds of the mass (Graaf et al., 2003). It is possible that the control reached brittleness at the preset extrusion conditions (exp. 2; 500 rpm, 160 °C, 15% water) due to the very low content of plasticizers such as fat or water during processing. According to Wurzburg (1986) and Graaf et al. (2003), the presence of a homogenously-blended plasticizer such as water, sugars, amides and lipids may prevent the formation of brittle polymers in starch-based extrudates. Unlike extrudates containing Andean grains, the control sample (exp. 2) exhibited brittle-like appearance with significantly lower SEI ($p<0.01$). In fact, the control reached higher SEI in experiment 3 where screw speed and temperature of the die were lower leaving thereby enough room for Brownian movement (higher monomer migration and interaction) as stated by Eyrings ‘free volume theory’ (Bader and Göritz, 1994). This not only agrees with the logic that plasticizers might prevent brittleness but with the effect of extrusion conditions on SEI.

Differences among extrudates containing Andean grains might be attributed to their distinct chemical composition. The effect of fat and dietary fiber seemed to provoke marked differences in sectional expansion, especially between extrudates containing amaranth and kañiwa. It is believed that fat was the main plasticizing compound in the mass that prevented massive crystallization (Wurzburg, 1986; Slade and Levine, 1993; Graaf et al., 2003) at the preset extrusion conditions (500 rpm, 160 °C, 15% water). The formation of amylose-lipid complexes was the main reason for low SEI according to Ilo and Liu (1999), Della Valle et al. (1997) and Coulter and Lorenz (1991). Indeed, the physico-chemical effect of this complex may depend considerably on the polarity of the lipids, type of fatty acid, length of the fatty acid chain and specific heating conditions (Bhatnagar and Hanna, 1994a; Bhatnagar and Hanna, 1994b; Desrumaux et al., 1999). Studies on the formation of amylose-lipid complex with linoleic acid and its effects on extrudate expansion have not been found. However, it is believed that amylose-lipid complexes stabilized the sectional expansion of extrudates containing Andean grains by preventing excessive shrinkage (Della Valle et al., 1997).

Fiber played an important role in the sectional expansion of extrudates containing Andean grains. In fact, Hsieh et al. (1989) and Onwulata et al. (2000) claimed that increasing fiber content in corn flours decreased SEI and increased bulk density. This is in accordance with our findings in which extrudates with the highest content of dietary fiber (kañiwa) presented the lowest SEI compared to analogous extrudates (quinoa and amaranth). Lue et al. (1991) and De Souza et al. (2010) found that the content of dietary fiber, high temperature and water content had a mild effect on starch gelatinization and strong effect on fiber solubilization. This suggests that gelatinization might not have been strongly affected by the content of dietary fiber. Considering the stereomicroscopy analysis, it is believed that crude fiber in kañiwa (lignin and cellulose) disrupted bubble formation and this was reflected in low radial expansion. On the other hand, amaranth and quinoa had much lower content of dietary fiber and crude fiber that possibly prevented bubble disruption. Lue et al. (1991) found similar results.

On the other hand, Kim and Maga (1993) suggested some interaction between amylose (sample A 20%; sample B 55%) and protein (sample A and B 30%) that may reduce starch availability and therefore expansion, especially when the content of amylose was high (Faubio and Hoseney, 1982). In our case, blends had around 9% protein with a content of amylose of approximately 21%. Due to the low ratio protein/amylose, it is possible that protein had a minor effect on expansion.

Water content of the mass had the expected effect on the SEI of extrudates. According to Coulter and Lorenz (1991), Ilo and Liu (1999) and Ding et al. (2005), higher water content of mass produced softening of amylopectin structure (lower viscosity and elasticity) which possibly led to lower sectional expansion. Response surface plots showed that SEI increased as water content of the mass decreased which is in accordance with previous studies. On the other hand, higher screw speed appeared to increase SEI of corn-based extrudates containing Andean grains. This is supported by Coulter and Lorenz (1991) and Chavez-Jauregui et al. (2000) who also found direct relation between screw speed and SEI for corn-based extrudates containing quinoa and defatted amaranth, respectively.

Apparently, temperature of the die did not have effect on SEI neither for corn-based extrudates containing Andean grains nor for the control. In contrast, Park (1976), Bhattacharya et al. (1986) and Coulter and Lorenz (1991) found that higher temperatures resulted in more expanded products. It is believed that slight differences in extrusion conditions may produce contrasting results.

Hardness of extrudates

Results indicated a reasonable degree of correlation between hardness and SEI for every trial (15 experiments); this is in accordance with previous studies (Ilo and Liu, 1999; Chavez-Jauregui et al., 2000; Ding et al., 2005). However, comparative analysis of the most expanded extrudates (exp. 2) showed that increasing hardness was associated with increasing SEI (except for the control). Each blend had particular chemical characteristics and it is not logical to compare results as such but to understand what processing conditions and/or intrinsic factors might have produced an extrudate comparatively less hard than another with higher expansion.

Hardness was significantly higher ($p<0.01$) in the control than in extrudates containing Andean grains. This was mainly attributed to the high degree of crystallization which is characterized by a substantial increase of stiffness (Graaf et al., 2003). In fact, plasticizers such as fat and water may have contributed to the significant changes of hardness among extrudates containing Andean grains. Extrudates containing kañiwa presented the lowest hardness and the highest content of fat and protein. In contrast, extrudates containing amaranth presented the highest hardness (except for the control) and the lowest content of fat and protein compared to other blends. According to Faubion and Hoseney (1982), hardness increased with the removal of lipids and protein, and Coulter and Lorenz (1991) found that addition of quinoa to corn flour caused lower SEI and lower hardness. This supports the results obtained and seemed to confirm the importance of lipids and protein for reduction of hardness.

Hsieh et al. (1989) found that high fiber content in corn flour led to increasing hardness. This was also supported by the findings of Onwaluta et al. (2000, 2001) in which high fiber content led to hard extrudates (corn flour and wheat fiber). However, Onwulata (2001) achieved some hardness reduction by increasing fiber in a system with 25% casein protein. In this sense, our results resemble the ones obtained by Onwaluta et al. (2001) since we also achieved hardness reduction by processing samples with increasing content of fiber (amaranth < quinoa < kañiwa), and high protein content. It is believed that the interaction between fiber and protein might have had some effect on reduction of hardness in our extrudates. The reason for this phenomenon is not clear but it might be associated with the type of protein (Onwulata et al. 2001). On the other hand, the stereomicroscopy analysis showed smaller and disrupted air bubbles within the extrudate containing kañiwa, leading to speculate a possible anti-crystallizing effect of fiber, as well. This might also explain why extrudates containing lower content of fiber presented higher hardness under the preset extrusion conditions.

Water content of the mass had a significant effect ($p<0.001$) on hardness of extrudates containing amaranth and non-significant effect for extrudates containing quinoa and kañiwa. Coulter and Lorenz (1991) reported opposite results in a similar experiment in which various percentages of quinoa were mixed with corn grits. In fact, Mercier and Feillet (1975) and Bhattacharya et al. (1986) observed that higher initial water content had a direct effect on hardness. On the other hand, hardness decreased as screw speed increased for extrudates containing quinoa and kañiwa. Coulter and Lorenz (1991) found comparable results.

In general, the model used to evaluate the effect of independent variables on hardness did not suit all cases. For instance, the effect of water content of mass, screw speed and temperature of the die on the hardness of the control samples was not considered since the model presented non-significant regression. Besides, R^2 for the effect of extrusion variables on hardness was between 0.6 and 0.8 for all the extrudates, except for the control. It is believed that the statistical model was not totally convenient to study extrusion variables on hardness. Apparently, temperature of the die did not have any significant effect on hardness. This was also observed in similar studies (Fabion and Howeney, 1982b; Coulter and Lorenz, 1991).

3.5.2. Oxidative stability of extrudates during storage

Differences in relative humidity and physical state were possibly the main factors affecting lipid stability in extrudates containing Andean grains and the control. Exposure to different relative humidity (11% and 76% RH) may have provoked sharp differences in lipid oxidation due to hydration of catalyzers or hydrogen binding of radicals (Labuza et al., 1972). In fact, brittleness, density levels and wall thickness might have also some effect on the mobility of fatty acids and the way these interact with amylose, oxygen and water molecules (Kim and Maga, 1994; Taylor, 2002; Gray et al., 2008; Naknean and Meenune, 2010) (Appendix 4).

A noticeable characteristic of hexanal production in all the samples was the growing trend along time. Ei-Magoli et al. (1980) observed that hexanal converted into hexanoic acid at temperatures above 50 °C, provoking a substantial decrease in the content of hexanal. In the present study, samples were stored at 20 °C and this may explain why drops in hexanal content were not observed. On the other hand, the content of tocopherol in the samples did not seem to correlate with the rate of hexanal production.

There were not contrasting differences between ground samples exposed to 11 and 76% RH. Ground extrudates containing amaranth and the control presented very similar trends of hexanal production which suggested a slight effect of humidity exposure on these ground extrudates. Two interesting characteristics of these samples were their brittle-like characteristics and low content of crude fiber. However, the importance of these characteristics on lipid stability of ground samples was not clear.

According to Bhatnagar and Hanna (1994), Desrumaux et al. (1999) and Naknean and Meenune (2010) formation of amylose-lipid complexes (V-amylose) took place during the gelatinization and cooling of starch-based materials which resulted from cooking at high temperature and pressure. This suggests that our processing conditions were probably suitable for the formation of this complex. Naknean and Meenune (2010) found that amylose-lipid complexes were reversible which may mean that, depending on the physico-chemical characteristics of the sample such as viscosity, molecular mobility, etc., there could be some release of fatty acids and other compounds that might be target of oxidation (Orlien et al., 2000). Ground extrudates containing amaranth and the control had possibly more limited molecular mobility due to their brittle-like characteristics. In this way, we may try to understand why these ground extrudates had a low, steady and indistinct production of hexanal, despite changes in humidity exposure.

Ground extrudates containing quinoa, and exposed to 11 and 76% RH, did not show noticeable differences in their curve of hexanal production. Hexanal production, though, was comparatively higher than in extrudates containing amaranth and the control. This hexanal production was encompassed by higher content of dietary fiber and ash.

Considerable differences in the curves of hexanal production between ground extrudates containing kañiwa at different relative humidity (11 and 76% RH) suggests a more active role of initiators (cations) and higher interaction between oxygen and fatty acids. Ground extrudates containing kañiwa had the highest content of ash and this may have some effect on the rate of hexanal production during storage at 11% RH compared to other samples. Labuza (1980) proposed that at a_w levels of between 0.4 and 0.7, there is an increased mobility of metal cations and ideal viscosity (compared to 0.2-0.4 a_w) in order to promote lipid oxidation. Above 0.7 a_w, dilution of transition metals cations hinders lipid oxidation. In this sense, it is believed that higher content of ash plus loss of free water (11% RH) in ground extrudates containing kañiwa provided ideal conditions for quasi-exponential hexanal production.

In general, ground extrudates exhibited higher tendency towards lipid oxidation than whole extrudates throughout storage. This seemed to follow the logic: higher surface area, lower lipid stability. However, it remains unclear what triggered the exceptional production of hexanal in whole extrudates containing quinoa.

Unlike ground extrudates, whole extrudates showed marked differences in their hexanal production, particularly in extrudates containing quinoa and kañiwa. As mentioned before, extrudates containing amaranth and the control presented higher hardness and, possibly, thicker walls than extrudates containing quinoa and kañiwa. Extrudates containing amaranth and the control and exposed to 11% RH showed almost no difference in hexanal production between them and against analogous samples exposed to 76% RH. This steady-state trend and low hexanal production may be due to low molecular mobility compared to ground extrudates. On the other hand, whole extrudates containing quinoa and kañiwa (11% RH) presented very similar exponential trends with remarkable differences in their final hexanal contents. Whole extrudates containing quinoa and kañiwa and exposed to 76% RH showed very low levels of hexanal production with almost no differences in their final hexanal contents. These results seemed to suggest a strong effect of humidity despite maintaining their structural characteristics. Nevertheless, it was not possible to understand the mechanism of action under which high humidity prevented lipid oxidation in this system.

According to Gray et al. (2008) lipids within a glassy matrix are protected from oxidation. In fact, the small amount of free volume within a glassy system may considerably reduce the ability of oxygen to diffuse towards lipids and of hexanal to diffuse out of the matrix (Volley et al., 1985; Kollengado et al., 1997; Parker et al., 2002). This seems to support part of the observations made since the control had a brittle-like appearance and the lowest production of hexanal registered in the present study.

The increasing production of hexanal in whole extrudates at 11% RH might be also associated with density and porosity of structures. Possibly, changes in density between ground and whole extrudates had some effect on water losses and oxygen access. In general, whole extrudates had hollowed and cracked structures with surface fatty acids that may react more readily with oxygen. Several studies confirmed the importance of the physical state of a solid system on lipid stability (Matsuno and Adachi, 1993; Gharsallaoui et al., 2007; Gray et al. 2008) (Appendix 4).

3.5.3. Chemical analysis of Andean grains

Studies on Andean grains are very limited and few sources may provide reliable information on the actual chemical composition of the studied varieties. Data obtained in the present research was compared to the results obtained by Repo-Carrasco et al. (1992, 2008, 2009) and substantial differences were found. Considering our results, Repo-Carrasco et al. (1992, 2008, 2009) reported higher content of fat and protein for amaranth, and lower content of fat and protein for kañiwa and quinoa, respectively. Indeed, noticeable differences in total dietary fiber were also observed.

We determined lower fat content for amaranth compared to Repo-Carrasco et al. (2008). This deviation was considered important because it was higher than three units. The method used in the present study combines high temperature, pressure and non-polarity of solvents in order to promote fat extraction (ASE). In contrast, Repo-Carrasco et al. (2008) used soxhlet method with diethyl ether as solvent (AOAC, 1995). This soxhlet solvent (slightly polar) presents several disadvantages for quantitative lipid extraction (Hara and Radin, 1978; Maxwell, 1987; Nelson, 1993; Schafer, 1997) since it may recover small amounts of phospholipids and affect the accuracy of the results which are solely regarded as ‘total fat’. This might explain why fat content of our amaranth sample is comparatively lower than the results primarily obtained by Repo-Carrasco et al. (2008).

This explanation does not seem to adjust to the differences in fat content for kañiwa, in which Repo-Carrasco et al. (2009) obtained lower values. It is not understood which extrinsic factors may provoke marked differences in the content of fat for samples of the same variety. Nevertheless, climate changes, different areas of cultivation and/or domestication, especially of kañiwa, might have caused some deviation.

Compared to our results, Repo-Carrasco et al. (2008) determined higher protein content for amaranth (two units) and lower for quinoa (two unities). These differences may be attributed to the way similar methods were performed. Repo-Carrasco et al. (2008) followed an AOAC method (1995) in which titration was carried out manually, whereas we followed an automated Kjeldahl method (AOAC, 1995). In this sense, we consider that error is more likely if the reliability of the results depends entirely on the skillfulness of the analyst.

It is difficult to find reasons for a four-point deviation of dietary fiber for kañiwa (Repo-Carrasco et al., 2009) since determinations were performed according to the same method (enzymatic-gravimetric method), using the same varieties. Gade (1970) and Bruno (2006) considered that, despite its current status as domesticate, kañiwa still remains 'rustic' since it maintains wild characteristics such as differential maturation. This might have a considerable effect on the chemical characteristics of crops harvested in different seasons and/or geographical conditions.

There were not substantial differences in the content of oleic, linoleic acid and γ-tocopherol, between this study and the one conducted by Repo-Carrasco et al. (2003). In contrast, α-tocopherol did show remarkable differences; the content of α-tocopherol is approximately half (quinoa), and one fourth (kañiwa) of what was reported by Repo-Carrasco (2003). Apparently, Repo-Carrasco et al. (2003) used fresh samples which might have caused such severe variations.

Efforts were made in order to reduce error-prone results. One of the steps taken was the adoption of automated analytical procedures. However, it is still unclear which extrinsic factors led to deviation in the results.

4. CONCLUSIONS

The present study provided information on: (1) the chemical composition of three Andean grains obtained from South America, (2) extrusion conditions to prepare corn-based snacks containing Andean grains (20% of solids) and (3) the effects of storage conditions on lipid oxidation of extrudates.

This study proved that it was possible to attain considerable sectional expansion (SEI) from degerminated corn flour containing amaranth, quinoa and kañiwa flour (20% solid). However, the extent of the expansion was limited probably due to the distinct chemical characteristics of the raw materials. The content of dietary fiber was possibly the main intrinsic factor affecting SEI and, when it comes to independent extrusion variables, water content of mass had apparently the highest significant effect. The pure corn extrudates presented the lowest SEI compared to the analogous blends. For this reason, it is believed that the extrusion conditions might have favored the sectional expansion of raw materials with higher content of fat, protein and fiber. A study of extrudates containing various contents of Andean grains would be highly desirable and may answer questions that arose during the conduction of this investigation.

Hexanal production was considerably higher for all ground samples exposed to either 11 or 76% RH, except for whole extrudates containing quinoa (11% RH). Results seemed to suggest a tremendous effect of low humidity on the rates of hexanal production for whole extrudates (containing quinoa). Indeed, high hexanal production for whole extrudates was not expected but this idea was contradicted by the results obtained. A mechanism of action based on density variations and oxygen access might help to understand this phenomenon to some extent. Indeed, further studies on this topic might elucidate findings on the actual effects of humidity conditions on the oxidative stability of encapsulated fat systems.

Semi automated analytical methods were performed for the determination of fat, protein, tocopherol and fatty acid content. Considerable differences were observed compared to previous studies in which traditional methods were performed. In fact, the results obtained may be used as a new reference for further studies on Andean grains. However, confirmatory studies may help to support the findings of the present investigation.

Neither corn flour nor tested flours (pseudocereals) used in the present study contain nearly considerable amounts of gluten. Hence, this is a step forward in the understanding and developing of edible gluten free products.

REFERENCES

Ahamed, N.T., Singhal, R.S., Kulkarni, P.R. & Pal, M. 1996. Physicochemical and functional properties of *Chenopodium quinoa* starch. Carbohydrate Polymers 31: 99–103.

Alvarez-Martinez, L., Kondury, K.P. & Harper, J.M. 1988. A general model for expansion of extruded products. Journal of Food Science 53: 609-15.

Anderson, R. A., Conway, H. F., Pfeifer, V. F. & Griffin, E. L. 1969. Gelatinization of corn grits by roll and extrusion cooking. Cereal Science Today 14: 4–12.

AOAC. 1995. Official Methods of analysis. 15th ed. Association of Official Analytical Chemists. Washington DC.

AOAC. 2002. Official Methods of analysis. 17th ed. Assciation of Official Analytic Chemists, Washington DC.

Atwell, W.A., Patrick, B.M., Jhonson, L. A., & Glass, R.W. 1983. Characterization of quinoa starch. Cereal Chemistry 60: 9–11.

Ayala, G. 2003. Consumption of quinoa in Peru. Food Reviews International 19: 221–227 pp.

Bader, H. G. & Göritz, D. 1994. Investigations on high amylose corn starch films, part 3: stress strain behavior. *Starch/Stärke*, 435-439.

Baik, M. Y., Kim, K.J., Cheon, K.C., Ha, Y.C., & Kim, W.S. 1997. Recrystallization kinetics and glass transition of rice starch gel systems. Journal of Agricultural and Food Chemistry 45: 4242-4248.

Baker, L.A. & Rayas-Duarte, P. 1998. Retrogradation of amaranth starch at different storage temperatures and the effects of salt and sugars. Cereal Chemistry 75: 308-314.

Ballon, E., De Gomez, V.M. & Cuesta, A. 1982. Estudio de proteínas en variedades de quinua. (Tercer Congreso Internacional de Cultivos Andinos, 8–12 Febrero). La Paz, Bolivia. Ministerio de Asuntos Campesinos y agropecuarios. 29–34 pp.

Belton, P.S. 1999. On the elasticity of wheat gluten. Journal of Cereal Science 29: 103–107.

Bhatnagar, S. & Hanna, M.A. 1994a. Amylose-Lipid complex formation during single-screw extrusion of various corn starches. Cereal Chemistry 71: 582-587.

Bhatnagar, S. & Hanna, M.A. 1994b. Extrusion processing conditions for amylose-lipid complexing. Cereal Chemistry 71: 587-593.

Bhattacharya, M., Hanna, M. A. & Kaufman, R.E. 1986. Textural properties of extruded plant protein blends. Journal of Food Science 51: 988-993.

Bruni R., Medici A., Guerrini A., Scalia S., Poli F., Muzzoli M. & Sacchetti G. 2001.Wild Amaranthus caudatus seed oil, a nutraceutical resource from Ecuadorian flora. Journal of Agricultural and Food Chemistry, 49: 5455–5460.

Bruno, M.C. 2006. A Morphological Approach to Documenting the Domestication of Chenopodium in the Andes. In: Zeder et al. (edits.). Documenting Domestication, *new genetic and archeological paradigms*. Berkeley, California. University of California Press: 32 pp.

Buleon, A., Colonna, P., Planchot, V. & Ball, S. 1998. Starch granules: structure and biosynthesis. International Journal of Biological Macromolecules 23: 85-112.

Calhoun, W.K., Hepburn, F.N. & Bradley, W.B. 1960. The distribution of the vitamins of wheat in commercial mill products. Cereal Chemistry 37: 755–761.

Chang Y.K., Hashimoto J.M., Moura-Alcioli, R. & Martinez-Bustos, F. 2001. Twin-screw extrusion of cassava starch and isolated soybean protein blends. Die Nahrung 45: 234-240.

Chanvrier, H., Appelqvist, I.A.M., Bird, A.R., Gilbert, E., Htoon, A., Li, Z., Lillford, P.J., Lopez-Rubio, A. Morell, M.K. & Topping, D.L. 2007. Processing of Novel Elevated Amylose Wheats: Functional Properties and Starch Digestibility of Extruded Products. Journal of Agriculture and Food Chemistry 55: 10248-10257.

Chavez-Jauregui, R.N., Silva, M.E.M.P. & Areas, J.A.G. 2000. Extrusion cooking process for amaranth (*Amaranthus caudatus* L.). Journal of Food Sciencce 65: 1009–1015.

Cho, S., DeVries, J. & Prosky, L. 1997. Dietary Fiber Analysis and Applications. AOAC Int., Gaithersburg, USA.

Choe, E. & Min, D.B. 2006. Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety 5: 169-186.

Coulter, L.A. & Lorenz, K. 1991. Extruded corn grits – quinoa blends: II. Physical characteristics of extruded products. Journal of Food Processing and Preservation 15: 231–259.

Coupland, J.N. & MacClements, D.J. 1996. Lipid oxidation in food emulsions. Trends in Food Science and Technology 71: 83-91.

Copeland, L., Blazek, J., Salman, H. & Tang, M. C. 2009. Form and functionality of starch. Food Hydrocolloids 23: 1527-1534.

Cuadrado, C, Ayer, G., Burbano, C., Muzquiz, M., Camacho, L., Cavieres, E., Lovon, M., Osagie, A. and Price, K. 1995. Occurrence of saponins and sapogenols in Andean crops. Journal of the Science of Food and Agriculture 67: 169-172.

Cumming, D.B., Stanley, D. W. & Deman, J.M. 1972. Texture Structure relationships in textured soy protein II. Textural Properties and ultrastructure of an extruded soybean product. Journal de l'Institut Canadien de Science et Technologie Alimentaire 5: 124–128.

De Graaf R.A., Karman A.P., and Janssen L.P.B.M. 2003. Material properties and glass transition temperatures of differential thermoplastic starches after extrusion processing. Starch 55: 80-86.

De Souza, L.B. 2010. Effect of the fiber content and operational extrusion parameters on the pasting characteristics of cassava starch and orange pulp mixture. Ciencia e Tecnologia de Alimentos 30: 686-692.

Della Valle, G., Colonna, P., Patria & A., Vergnes, B. 1996. Influence of amylose content on viscous behaviour of low hydrated molten starches. Journal of Rheology 40: 347 – 362.

Della Valle, G., Vergnes, B., Colonna & P., Patria, A. 1997. Relations between rheological properties of molten starches and their expansion behaviour in extrusion. Journal of Food Engineering 31: 277 – 296.

Desrumaux, A., Bouvier, J.M. & Burri, J. 1999. Effect of free fatty acids addition on corn grits extrusion cooking. Cereal Chemistry 76: 699-704.

Ding, Q.-B., Ainsworth, P., Tucker, G. & Marson, H. 2005. The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. Journal of Food Engineering 66: 283–289.

Drusch, S., Serfert, Y. & Schwarz, K. 2006. Microencapsulation of fish oil with n-octenylsuccinate-derivatised starch: Flow properties and oxidative stability, European Journal of Lipid Science and Technology 108: 501-512.

El-Magoli, S.B., Karel, M. & Yong, S. 1979. Acceleration of lipid oxidation by volatile products of hydroperoxide decomposition. Journal of Food Biochemistry 8: 111-123

Evans, E.J. 2001. Cereal production method. In: Owens, G. (edit.). Cereals Processing Technology, University of Newcastle. Woodhead Publishing: 14 pp.

FAO. 2002. International Plant Genetic Resources Institute. <http://www.fao.org-/docs/eims/upload/216774/Neglected_no_more.pdf>. Rome, Italy. Food and Agriculture Organization of the United Nations, visited 27 September 2009.

FAO. 2005. FAO Corporate Document Repository. <http://www.fao.org/DO-CREP/005/AC784E/ac784e-03.htm>. Rome, Italy. Food and Agriculture Organization of the United Nations, visited 27 September 2009.

Faubion, J. M. & Hoseney, R. C. 1982. High-temperature short-time extrusion cooking of wheat starch and flour. I. Effect of moisture and flour type on extrudate properties. Cereal Chemistry 59: 529–533.

Frankel, E.N., Neff, W.E. & Selke, E. 1981. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. Lipids 16: 279-285.

Frankel, E.N., Warner, K. & Moulton, K.J. 1985. Effects of hydrogenation and additives on cooking oil performance of soybean oil. Journal of the American Oil Chemists' Society 62: 1354-58.

Gade, D.W. 1970. Ethnobotany of Cañihua (Chenopodium pallidicaule), rustic seed crop of the Altiplano. Economic Botany 24: 55-61.

Gamel, T.H., Linssen, J.P., Mesallan, A.S., Damir, A.A. & Shekib, L.A. 2006. Effect of seed treatments on the chemical composition of two amaranth species: oil, sugars, fibres, minerals and vitamins. Journal of the Science of Food and Agriculture 86: 82–89.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. and Saurel, R. 1997. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International 40: 1107–1121.

Godshall, M. A. 1997. How carbohydrates influence food flavour. Food Technology 51: 63-67.

Gonzales, J.A., Roldan, A., Gallardo, M., Escudero, T. & Prado, F.E. 1989. Quantitative determinations of chemical compounds with nutritional value from Inca crops: *Chenopodium quinoa* ('quinoa'). Plant Foods for Human Nutrition 39: 331–337.

Gorinstein, S., Zemser M. & Paredes-Lopez O. 1996. Structural Stability of Globulins. J. Agric. Food Chem., 44, pp. 100-105.

Goubet, I., Le Quere, L.J. & Voilley, J. A. 1998. Retention of aroma compounds by carbohydrates: Influence of their physiochemical characteristics and of their physical state. A review. Journal of Agricultural and Food Chemistry 46: 1981-1990.

Gray, D.A., Bowen, S.E., Farhat, I. & Hill, S.E. 2008. Lipid oxidation in glassy and rubbery-state starch extrudates. Food Chemistry 106: 227-234.

Greenwood, C.T. 1976. Starch. In: Pomeranz, Y. (edit.). Advances in Cereal Science and Technology. Saint Paul, Minnesota. American Association of Cereal Chemists.

Gudmunsson, M. & Eliasson, A.C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants, emulsiifiers. Carbohydrates polymers 13: 295 – 315.

Hara, A. & Radin, N.S. 1978. Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry 90: 420.

Harmann, D.V. & Harper, J.M. 1973. Effect of extruded geometry on torque and flow. Transactions of the American Society of Agricultural Engineers 6: 1175–1178.

Harper, J. M. 1981. Extrusion of Foods. CRC Press, Boca Raton.

Houston, D.F. & Köhler, G.O. 1970. Nutritional Properties of Rice. Albany, California. National Academy of Science: 9 pp.

Hsieh, F., Mulvaney, S.J., Huff, H.E., Lue, S. & Brent Jr., J. 1989. Effect of dietary fiber and screw speed on some extrusion processing and product variables. Food Science and Technology 22: 204-207

Ilo, S. E. & Liu, Y. 1999. Extrusion Cooking of Rice Flour and Amaranth Blends. Lebensmittel-Wissenschaft und-Technologie 32: 79–88.

Jacobsen, S.-E. 1997. Adaptation of quinoa (*Chenopodium quinoa*) to Northern Europe agriculture: studies on developmental pattern. Euphytica 96: 41–48.

Ju, Z.Y., Hettiarachchy, N.S. & Rath, N. 2001. Extraction, denaturation and hydrophobic Properties of Rice Flour Proteins. Journal of Food Science 66: 229 – 232.

Kadan, R. S., Bryant, R. J., & Pepperman, A. B. 2003. Functional properties of extruded rice flours. Journal of Food Science 68: 1669–1672.

Kaur, L., Singh, N. & Singh-Sodhi, N. 2002. Some properties of potatoes and their starches. II. Morphological, thermal and rheological properties of starches. Food Chemistry 79: 183-192.

Kent, N. 1983. Technology of cereals. New York. USA. Pergamon Press.

Kim, C. H. & Maga, J. A. 1987. Properties of Extruded whey protein concentrate and cereal flour blends. Lebensmittel-Wissenschaft und-Technologie 20: 311–317.

Kim, C.H. & Maga, J.A. 1993. Influence of starch type, starch protein-composition and extrusion parameters on resulting extrudate expansion. Food Flavours, Ingredients and Composition 32: 957-964.

Kim, C. H. & Maga, J. A. 1994. Chain length and functional group impact on retention during extrusion. In: Parliament, T.H., Morello, M.J. and McGorrin, R.J. (Eds), Thermally Generated Flavours: Maillard, Microwave and Extrusion Processes. American Chemical Society. Washington, DC. ACS Symposium Series 543: 105-153 pp.

Kirby, et al., Ollett, A.-L., Parker, R. & Smith, A.C. 1988. An experimental study of screw configuration effects in the twin-screw extrusion-cooking of maize grits. Journal of Food Engineering 8: 247-272.

Koelsch, C.M., Downes, T.W. & Labuza, T.P. 1991. Hexanal formation via lipid oxidation as a function of oxygen concentration - measurement and kinetics. Journal of Food Science 56: 816.

Kogan, G., Stasko A., Bauerova K., Polovka M., Soltes L., Brezova V. & Navarova J. 2005. Antioxidant properties of yeast (1→3)-β-D-glucan studied by electron paramagnetic resonance spectroscopy and its activity in the adjuvant arthritis. Carbohydrate Polymers 61: 18–28.

Köhler, P., Belitz, H.-D. & Wieser, H. 1993. Disulphide bonds in wheat gluten: further cystine peptides from high molecular weight (HMW) subunits of glutenin and from γ-gliadins. Zeitschrift für Lebensmitteluntersuchung undforschung A 196: 239-247.

Kokini, J.L., Ho, C. & Karwe, M. V. 1992. Food Extrusion Science and Technology. New York, USA. Marcel Dekker Inc. pp. 619–630.

Kollengode, A. N. R. & Hanna, M. A. 1997. Flavor retention in pregelatinized and internally flavored starch extrudates. Cereal Chemistry 74: 396–399.

Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J., & Hurtado, F. 1972. Stability of intermediate moisture foods. 1. Lipid oxidation. Journal of Food Science 37: 154–159.

Labuza, T. P. 1980. The effect of water activity on reaction kinetics of food deterioration. Food Technology 34: 36–41.

Lamacchia, C., Chillo, S., Lamparelli, S., Suriano, N., La Notte, E. & Del Nobile, M.A. 2010. Amaranth, quinoa and oat doughs: Mechanical and Rheological behaviour, polymeric protein size distribution and extractability. Journal of Food Engineering 96: 97–106.

Lasztity, R. 1984. The chemistry of cereal proteins. CRC Press, Boca Raton.

Lindeboom, N., Chang, P.R., Falk, K. C. & Tyler, R. T. 2005. Characteristics of starch from eight quinoa lines. Cereal Chemistry 82: 216-222.

Lorenz, K.1981. Wild rice: the Indian's staple and the white man's delicacy. Critical Reviews in Food Science and Nutrition 15: 281–319.

Lorenz, K. 1984. The starch of amaranthus – Physico-chemical properties and functional characteristics. Starch/Stärke 36: 232 – 237.

Lorenz, K. 1990. Quinoa (*Chenopodium quinoa*) starch – Physico-chemical properties and functional characteristics. Starch/Stärke 42: 81–86.

Lue, S., Hsieh, F. & Huff, H.E. 1991. Extrusion cooking of corn meal and sugar beet fiber: effects on expansion properties, starch gelatinization and dietary fiber content. Cereal Chemistry 68: 227–234.

MacRitchie, F. & Lafiandra, D. 1997. Structure–function relationship of wheat proteins. In: Dekker, S.M. (edit.). Food Proteins and Their Application. New York, USA: 293–323 pp.

Marquez-Ruiz, G., Velasco, J. & Dobarganes, C. 2000. Evaluation of oxidation in dried microencapsulated fish oils by a combination of adsorption and size exclusion chromatography. European Food Research and Technology 211: 13–18.

Mastebroek, H., Limburg, H., Gilles, T. & Marvin, H. 2000. Occurrence of sapogenins in leaves and seeds of quinoa (*Chenopodium quinoa* Willd*)*. Journal of the Science of Food and Agriculture 80: 152-156.

Mattila, P., Piironen, V. & Ollilainen, V. 2001. Elintarvikekemia ja –analytiikka. Yliopistopaino. Helsinki pg. 124

Matsuno, R. and Adachi, S. 1993. Lipid encapsulation technology – Techniques and applications to food. Trends in Food Science and Technology 41: 256-261.

Maxwell, R.J. 1987. Determination of total lipid and lipid subclasses in meat and meat products. Journal of the Association of Official Analytical Chemists 70: 74-77.

Mercier, C. & Feillet, P. 1975. Modification of carbohydrate components by extrusion-cooking of cereal products. Cereal Chemistry 52: 283-297.

Meuser, F., & van Lengerich, B. 1992. System analytical model for the extrusion of starches. In J. L. Kokini, C. Ho, & M. V. Karwe (Eds.), Food Extrusion Science and Technology (pp. 619–630). New York: Marcel Dekker Inc.

Min, D.B., Lee, S.H. & Lee, E.C. 1989. Singlet oxygen oxidation of vegetable oils. In: D.B. Min and T.H. Smouse (Eds.), Flavor Chemistry of Lipid Foods. American Oil Chemists' Society. Champaign, Illinois.

Moreau, R.A., Powell, M.J. & Singh, V. 2003. Pressurized liquid extraction of polar and nonpolar lipids in corn and oats with hexane, methylene chloride, isopropanol, and ethanol. Journal of the American Oil Chemists' Society 80: 1063-1067.

Mujica, A. 1994. Andean Grains and Legumes. In: Hernando J.E. and Leon J. (eds.) Neglected Crops (Plant production and Protection 26). Rome, Italy. FAO. 131–148 pp.

Naknean, P. & Meenune, M. 2010. Factors affecting retention and release of flavor compounds in food carbohydrates. International Food Research Journal 17: 23-34.

National Research Council. 1989. Lost crops of the Incas: Little-known plants of the Andes with promise for worldwide cultivation. Washington, DC. National Academy Press.

Nelson, K. A. & Labuza, T. P. 1992. Water activity and food polymer science: Implications of state on Arrhenius and WLF models in predicting shelf life. In: A. Mullet & B.M. McKenna (eds.) Water in Foods: Fundamental Aspects and Their Significance in Relation to Processing *of* Foods. Peñiscola-Valencia, Spain. Elsevier. 240 pp.

Nelson, C.G.J. 1993. In: E. Perking (ed.) Analysis of Fats, Oils, and Derivatives. Champaign, IL. American Oil Chemists Society. 20 pp.

Nowjee, N.C. 2004. Melt processing and foaming of starch. < http://www.cheng-.cam.ac.uk/research/groups/polymer/RMP/nitin/ProjectPage.html> Visited 26 October 2010.

Nuñez, L. 1970. La agricultura prehistorica en los Andes meridionales. Antofagasta, Chile. Universidad del Norte, Editorial Orbe.

Ollivon, M. 2006. Lipid-flavour interactions. In: A. Voilley and P. Etievant (Eds.), Flavour in food. Boca Raton CRC Press. Cambridge, UK.

Onwulata, C.I., Konstance, R.P., Smith, P.W. & Holsinger, V.H. 2000. High-fiber snacks extruded from triticale and wheat formulations. Cereal Food World 45: 470-473.

Onwulata, C.I., Konstance, R.P., Smith, P.W. & Holsinger, V.H. 2001. Co-extrusion of dietary fiber and milk protein in expanded corn products. Food Science and Technology 34: 424-429.

Orlien, V., Andersen, A. B., Sinkko, T., & Skibsted, L. H. 2000. Hydroperoxide formation in rapeseed oil encapsulated in a glassy food model as influenced by hydrophilic and lipophilic radicals. Food Chemistry 68: 191–199.

Park, K.H. 1976. Elucidation of the Extrusion Puffing Process. Doctoral thesis, University of Illinois, Urbana, IL, USA.

Parker, R., Gunning, Y. M., Lalloue, B., Noel, T. R., & Ring, S. G. 2002. Glassy state dynamics, its significance for biostabilisation and the role of carbohydrates. In H. Levine (Ed.). Amorphous Food and Pharmaceutical Systems. Cambridge, UK: 73–87 pp.

Pedersen, B., Hallgren, L., Hansen, I. & Eggum, B. O. 1987. The nutritive value of amaranth grain (*Amaranthus caudatus*). Plant Foods for Human Nutrition 36: 325–334.

Ponginebbi, L., Nawar, W.W. & Chinachoti, P. 2000. Effect of relative humidity on lipid oxidation in freeze-dried emulsions, Grasas y Aceites 51: 348–354.

Ramos, J. M. & Quiliano, A. 2005. Extruder 500x. Jarcon del Peru S.A.C.

Ranhotra, G.S., Gelroth, J.A., Glaser, B.K., Lorenz, K.J. & Johnson, D.L. 1993. Composition and protein nutritional quality of quinoa. Cereal Chemistry 70: 303–305.

Rawls, H.R. & Van Santen, P.J. 1970. A possible role for singlet oxidation in the initiation of fatty acid autoxidation. Jounal of the American Oil Chemists' Society 47:121–125.

Repo-Carrasco, R. 1992. Andean Crops and Infant Nourishment., Report B 25 Finland. University of Helsinki. Institute of Development studies.

Repo-Carrasco, R. 2011. Andean Indigenous Food Crops: Nutritional Value and Bioactive Compounds. Doctoral dissertation. University of Turku: 14-15

Repo-Carrasco, R., Acevedo, A., Icochea, J.C. & Kallio, H. 2009. Chemical and functional characterization of kañiwa (*chenopodium pallidicaule*) grain, extrudate and bran. Plant Foods for Human Nutrition 64: 94–101.

Repo-Carrasco, R., Esponiza, C. & Jacobsen, S.-E. 2003. Nutritional value and use of the Andean crops: quinoa (*Chenopodium quinoa*) and kañiwa (*chenopodium pallidicaule*). Food Reviews International 19: 179–189.

Repo-Carrasco, R., Peña, J., Kallio & H., Salminen, S. 2008. Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (*Amaranthus caudatus*). Journal of Cereal Science 49: 219–224.

Ridout, C., Price, K., DuPont, M., Parker, M. & Fenwick, G. 1991. Quinoa saponins-analysis and preliminary investigations into the effects of reduction by processing. Journal of the Science of Food and Agriculture 54: 165-176.

Romero, J.A. 1981. Evaluacion de las caracteristicas fisicas, quimicas y biologicas de ocho variedades de quinoa (*Chenopodium quinoa Willd.*). Tesis de Maestro, Instituto de Nutricion de Centro America y Panama. Universidad de San Carlos de Guatemala, Guatemala.

Ruales, J. & Nair, B. 1993. Saponins, phytic acid, tannins and protease inhibitors in quinoa (*Chenopodium quinoa*,Willd) seeds. Food Chemistry 48: 137–143.

Scarpati De Briceño, Z. & Briceño P.O. 1980. Evaluacion de la composición química y nutricional de algunas entradas de quinua *(Chenopodium quinoa* Willd.) del banco de germoplasma de la Universidad Tecnica del Altiplano. Annales Cientifico 18: 125–143.

Schneider, E. 1967. Mineralstoffe. Aufbereitung der Proben, Bestimmung der Gesamtasche, Nachweis und Bestimmung der einzelnen Mineralstoffe. In: Schormuller, J. (ed.). Handbuch der Lebensmittelchemie II/2. Analytic der Lebensmittel. Springer Verlag: 46-81 pp.

Schofield, J.D., Bottomley, R.C., Timms, M.F. & Booth, M.R. 1983. The effect of heat n wheat gluten and involvement of sulphydryl-disulphide interchange reactions. Journal of Cereal Science 1: 241-253

Schwartz, H., Ollilainen, V., Piironen, V. & Lampi, A.M. 2007. Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. Journal of Food Composition and Analysis 21: 152-161.

Segura-Nieto, M., Barba de la Rosa, A.P. & Paredes-Lopez, O. 1994. Biochemistry of amaranth proteins. In: Pared-Lopez, O. (Ed.), Amaranth: Biology, Chemistry and Technology. CRC Press, Boca Raton. 75–106 pp.

Shahidi, F. & Pegg, R.B. 1994. Hexanal as an indicator of the flavour deterioration of meat and meat products. In C.-T. Ho & T.G. Hartman (Eds.). Lipids in food flavors. Washington, DC. American Chemical society. 258-264 pp.

Slade, L. & Levine, H. 1993. Thermoplastic Starches. Carbohydrate Polymers 12: 105-109.

Su C. N. 2003. Effects of accelerated aging on lipid oxidation in quinoa. Master thesis. University of Wisconsin-Stout.

Tapia, M., Gandarinas, H., Alandia, S., Cardozo, A. & Mujica, A. 1979. Quinua y la kañiwa: cultivos andinos. Bogota, Colombia. Instituto interamericano de Ciencias Agricolas. 11–80 / 205–214 pp.

Taylor, A.J. 2002. Food Flavour Technology. 1st edn. CRC Press, UK.

Tellerias, M.L. 1976. Estudio comparativo da Composicao e dos Efeitos de Tratamento Termico em Quatro Variadades de quinua (*Chenopodium quinoa Willd).* Tesis. Facultade de Engenharia de Alimentos e Agricola, Universidade Estadual de Campinas, Brazil.

Thatam, A.S., Hayes, L., Shewry, P.R. & Urry, D.W. 2001. Wheat seed proteins exhibit a complex mechanism of protein elasticity. Biochimica et Biophysica Acta 1548, 187–193.

Thompson, I.M., Spence C.R., Lamm D.L., & DiLuzio N.R.1987. Immunochemotherapy of bladder carcinoma with glucan and cyclophosphamide. The American journal of the medical sciences 294: 294–300.

Uhle, M. 1919. La arqueologia de Arica y Tocta. Quito, Ecuador. Sociedad Ecuatoriana de Estudios Historicos 3: 1–48.

Voilley, A., & Le Meste, M. 1985. Aroma diffusion: The influence of water activity and of molecular weight of the other solutes. In D. Simatos & J. L. Multon (Eds.), Properties of water in foods. Martinus Nijhoff Publishers: 357–373 pp.

Woldemicheael, G. & Wink, M. 2001. Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa.* Journal of Agriculture and Food Chemistry 49: 2327-2332.

Wright, K.H., Pike, O.A., Fairbanks, D.J. & Huber, C.S. 2002. Composition of *Atriplex hortensis*, sweer and bitter *Chenopodium quinoa* seeds. Journal of Food Science 67: 1383–1385.

Wurzburg O. B. 1986. Modified Starches: Properties and Uses. CRC Press, Boca Raton, Fl.

APPENDICES

Appendix 1. Extrusion parameters in relation to physical properties of extrudates

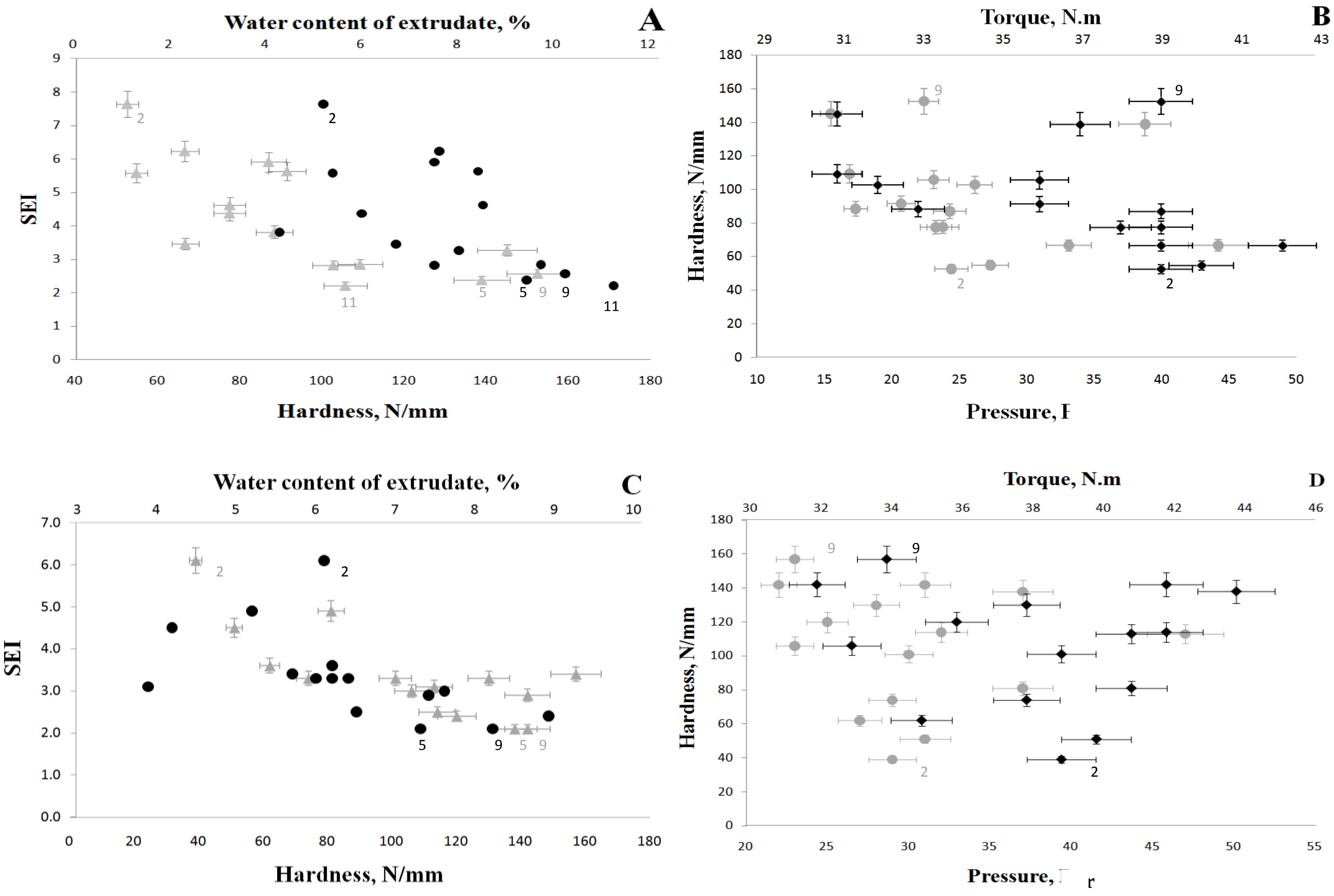
		Extrusion parameters				**Physical properties of extrudates**		
	E	**Pressure**	**Torque**	**Temperature, °C**	**screw speed**	**SEI**	**Hardness, N/mm**	**Water content of extrudate, %***
Corn 80% - Amaranth 20%	1	44.2 ± 8.6	39.2 ± 1.6	160	200	3.5 ± 0.3	67 ± 27.8	6.6
	2	24.4 ± 6.1	38.6 ± 1.2	160	500	7.6 ± 0.6	53 ± 8.4	5.1
	3	33.1 ± 8.1	42.2 ± 1.5	150	350	6.2 ± 0.5	67 ± 15.6	7.5
	4	27.3 ± 6.8	40.0 ± 2.0	170	350	5.6 ± 0.5	55 ± 19.6	5.3
	5	38.8 ± 8.2	37.1 ± 1.8	150	200	2.4 ± 0.3	139 ± 10.3	9.3
	6	26.2 ± 6.0	31.7 ± 1.2	170	200	2.8 ± 0.5	103 ± 39.0	7.4
	7	20.7 ± 4.7	35.9 ± 1.0	150	500	5.6 ± 0.5	91 ± 29.6	8.3
	8	17.3 ± 4.7	32.8 ± 1.0	170	500	3.8 ± 0.3	88 ± 41.0	4.2
	9	22.4 ± 5.5	38.8 ± 1.5	160	200	2.6 ± 0.3	152 ± 32.0	10.1
	10	15.5 ± 4.1	30.9 ± 1.0	160	500	3.3 ± 0.3	145 ± 34.1	7.9
	11	23.1 ± 5.6	36.2 ± 1.2	150	350	2.2 ± 0.2	106 ± 22.0	11.1
	12	16.9 ± 4.8	30.7 ± 1.1	170	350	2.9 ± 0.2	109 ± 26.5	9.6
	13	24.3 ± 5.4	38.8 ± 1.6	160	350	5.9 ± 0.6	87 ± 30.2	7.4
	14	23.3 ± 5.6	38. ± 1.2	160	350	4.4 ± 0.4	77 ± 19.3	5.9
	15	23.8 ± 7.3	39 ± 1.4	160	350	4.6 ± 0.2	77 ± 17.2	8.4
Corn 80% - Quinoa 20%	1	46.8 ± 7.7	41.4 ± 1.2	160	200	3.1 ± 0.5	113 ± 7.7	3.9
	2	29.4 ± 5.5	38.6 ± 1.6	160	500	6.1± 0.5	39 ± 3.7	6.1
	3	37.1 ± 7.5	40.9 ± 1.8	150	350	4.9 ± 0.4	81 ± 8.7	5.2
	4	30.7 ± 6.6	40.1 ± 1.5	170	350	4.5 ± 0.2	51 ± 8.6	4.2
	5	37.6 ± 6.8	44.4 ± 1.5	150	200	2.1 ± 0.2	138 ± 20.9	7.3
	6	32.2 ± 6.8	42.4 ± 1.7	170	200	2.5 ± 0.2	114 ± 8.3	6.5
	7	26.7 ± 5.5	35.4 ± 1.0	150	500	3.6 ± 0.3	62 ± 10.6	6.2

	8	23.2 ± 5.1	34.0 ± 1.1	170	500	3.4 ± 0.2	157 ± 30.3	5.7
	9	31.3 ± 6.2	41.7 ± 1.6	160	200	2.1 ± 0.2	142 ± 14.2	8.2
	10	22.2 ± 4..5	31.8 ± 1.0	160	500	2.9 ± 0.2	142 ± 16.8	7.4
	11	24.8 ± 4.5	35.9 ± 1.0	150	350	2.4 ± 0.2	120 ± 14.4	8.9
	12	22.9 ± 5.8	33.5 ± 1.0	170	350	3.0 ± 0.3	106 ± 5.4	7.6
	13	29.3 ± 7.0	38.3 ± 1.4	160	350	3.3 ± 0.4	74 ± 11.5	6.2
	14	29.9 ± 6.6	39.2 ± 1.2	160	350	3.3 ± 0.2	101 ± 10.2	6.0
	15	27.7 ± 5.1	38.5 ± 1.6	160	350	3.3 ± 0.2	130 ± 24.2	6.4
Corn 80% - Kañiwa 20%	1	54.0 ± 4.8	38.0 ± 0.9	160	200	1.8 ± 0.1	154 ± 12.1	7.6
	2	38.5 ± 8.1	30.5 ± 1.9	160	500	5.1 ± 0.3	28 ± 6.1	6.0
	3	47.1 ± 8.2	33.8 ± 1.3	150	350	3.7 ± 0.3	52 ± 4.3	5.6
	4	35.3 ± 6.8	29.4 ± 0.9	170	350	3.4 ± 0.2	42 ± 12.3	6.7
	5	47.9 ± 4.4	35.3 ± 1.9	150	200	1.5 ± 0.1	221 ± 33.9	8.5
	6	36.6 ± 5.0	31.4 ± 0.9	170	200	1.7 ± 0.2	147 ± 23.6	8.7
	7	34.1 ± 6.0	31.7 ± 1.1	150	500	3.9 ± 0.4	64 ± 4.6	6.7
	8	97.4 ± 5.1	28.6 ± 0.9	170	500	3.5 ± 0.4	70 ± 7.6	7.6
	9	35.4 ± 4.3	29.49 ± 0.9	160	200	1.3 ± 0.1	148 ± 36.2	10.3
	10	26.5 ± 5.0	28.8 ± 0.8	160	500	3.1 ± 0.3	117 ± 8.6	8.6
	11	29.2 ± 5.6	30.1 ± 0.9	150	350	2.3 ± 0.2	97 ± 11.8	8.8
	12	26.7 ± 5.4	28.3 ± 0.8	170	350	2.3 ± 0.2	90 ± 17.2	9.4
	13	35.6 ± 5.7	31.5 ± 0.8	160	350	3.0 ± 0.2	69 ± 11.3	8.3
	14	37.2 ± 6.2	31.2 ± 0.9	160	350	3.0 ± 0.3	63 ± 6.7	7.8
	15	34.9 ±6.3	32.5 ± 0.6	160	350	2.9 ± 0.3	90 ± 10.6	7.5
Corn 100%	1	52.7 ± 7.4	41.1 ± 1.1	160	200	3.5 ± 0.4	124 ± 5.4	7.0
	2	33.8 ± 6.0	34.5 ± 1.1	160	500	4.4 ± 0.4	89 ± 11.5	6.7
	3	47.1 ± 8.6	35.4 ± 1.2	150	350	5.1 ± 0.4	92 ± 19.1	8.0
	4	36.4 ± 7.3	34.7 ± 1.8	170	350	3.9 ± 0.5	94 ± 18.2	11.0
	5	50.5 ± 8.3	37.7 ± 0.9	150	200	2.4 ± 0.2	107 ± 12.3	11.9
	6	38.2 ± 6.6	34.7 ± 1.1	170	200	2.5 ± 0.3	87 ± 16.6	5.2

7	30.6 ± 5.5	32.6 ± 1.0	150	500	3.4 ± 0.4	97 ± 17.9	12.4
8	24.1 ± 5.4	29.2 ± 0.8	170	500	2.6 ± 0.3	68 ± 16.2	8.3
9	35.3 ± 5.9	33.0 ± 0.9	160	200	1.9 ± 0.1	127 ± 6.7	13.2
10	22.9 ± 5.4	30.1 ± 0.9	160	500	2.3 ± 0.2	203 ± 49.7	14.9
11	33.1 ± 6.2	29.4 ± 0.9	150	350	2.9 ± 0.4	171 ± 28.1	11.1
12	23.5 ± 5.1	31.1 ± 1.0	170	350	2.5 ± 0.4	148 ± 27.0	12.3
13	35.0 ± 6.4	35.2 ± 1.3	160	350	3.5 ± 0.3	128 ± 20.9	9.2
14	35.1 ± 7.0	33.2 ± 1.4	160	350	3.8 ± 0.4	114 ± 9.9	11.9
15	36.5 ± 7.0	32.5 ± 1.2	160	350	4.2 ± 0.4	89 ± 15.4	4.7

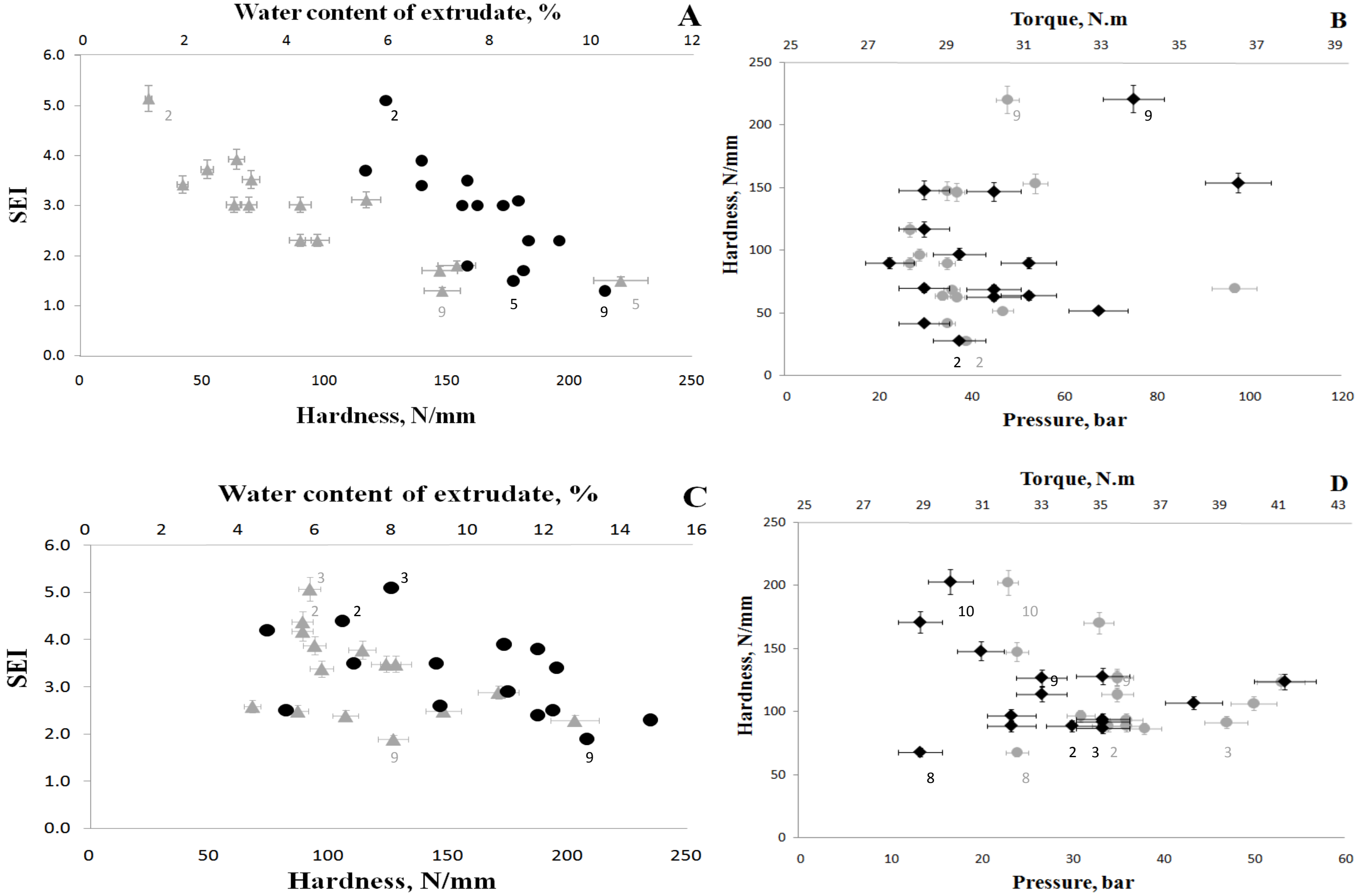
*Analyses were done in duplicate

Appendix 2. Relationship between response variables of extrudates containing amaranth (A, B) and quinoa (C, D). Some experimental points are numbered. Error bars +/- standard deviation.



(▲) hardness vs. SEI; (●) water content of extrudate vs. SEI; (●) pressure vs. hardness; (◆) torque vs. hardness.

Appendix 3. Relationship between response variables of extrudates containing kañiwa (A, B) and the control (C, D). Some experimental points are numbered. Error bars +/- standard deviation.



(▲) hardness vs. SEI; (●) water content of extrudate vs. SEI; (●) pressure vs. hardness; (◆) torque vs. hardness.

Appendix 4. Oxygen access and water evaporation during exposure to low relative humidity