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Highlights

- Greater expansion and lower hardness by adding tested flours (e.g. quinoa)
- Water content and screw speed had the greatest effect on expansion and hardness.
- Remarkable stability of lipids in whole extrudates exposed to HR humidity.

USE OF AMARANTH, QUINOA AND KAÑIWA IN EXTRUDED CORN-BASED SNACKS

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Keywords:

Amaranth; quinoa; kañiwa; extrusion

Abbreviations:

SEI (sectional expansion index), WCE (water content of extrudates), WCM (water content of mass), RH (relative humidity), SS (screw speed), TEM (temperature of die)

ABSTRACT

Amaranth (*Amaranthus caudatus*), quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*) are pseudocereals regarded as good gluten-free sources of protein and fiber. A co-rotating twin screw extruder was used to obtain corn-based extrudates containing amaranth/quinoa/kañiwa (20% of solids). Box-Behnken experimental design with three independent variables was used: water content of mass (WCM, 15-19%), screw speed (SS, 200-500 rpm) and temperature of the die (TEM, 150-170 °C). Milled and whole samples were stored in open headspace vials at 11 and 76% relative humidity (RH) for a week before being sealed and stored for 9 weeks in the dark. Hexanal content was determined by using headspace gas chromatography. Extrudates containing amaranth presented the highest sectional expansion index (SEI) ($p < 0.01$) while pure corn extrudates (control) presented the lowest SEI and greatest hardness ($p < 0.01$). SEI increased with increasing SS and decreasing WCM. In storage, whole extrudates exposed to 76% RH presented the lowest formation of hexanal. This study proved that it was possible to increase SEI by adding amaranth, quinoa and kañiwa to pure corn flour. The evaluation of lipid oxidation suggested a remarkable stability of whole extrudates after exposure to high RH.

1. INTRODUCTION

Amaranth, quinoa and kañiwa are pseudocereals that have been cultivated in South America for more than 5000 years. These climate resilient crops were important sources of protein, fiber and fat during pre-Hispanic times. At present, their potential as gluten-free cereal substitute is starting to attract industrial and scientific attention (Gallagher et al., 2004).

Interest in gluten-free products has increased in the developed world; this encompasses the increasing necessity for alternatives with added nutritional value thereby providing consumers with high quality protein, lipid, fiber and bioactive compounds. Repo-Carrasco et al. (2003) reported that quinoa var. Amarilla de Marangani had no limiting amino acids. In fact, Ranhotra et al. (1993) observed that the quality of protein in quinoa (protein digestibility, protein efficiency ratio and nitrogen balance) is equivalent to that of milk protein casein. Scientific studies also suggested that kañiwa and amaranth had protein content and amino acid composition comparable to quinoa as reviewed in Repo-Carrasco (2011). Amaranth, quinoa and kañiwa are also particularly rich in polyunsaturated fatty acids. Sinopoulos (2001) suggested that a high linoleic:linolenic acid ratio (14:1-20:1), which is the case in western countries, could promote the pathogenesis of degenerative diseases (e.g., cancer and osteoporosis). In that sense, linoleic:linolenic acid ratio in quinoa falls within the recommended values (5:1-10:1). When it comes to other bioactive compounds, quinoa and kañiwa are important sources of flavonoids (mainly glycosides of the flavonols kaempferol and quercetin). Kañiwa is especially rich in resorcinols which are not common compounds in plants. In fact, cereal alkylresorcinols were suggested to have an active role in the prevention of cancer and microbial infections (Ross et al., 2003). On the other hand, there is remarkable variation in the content of dietary fiber in pseudocereals mainly due to environmental

75 conditions. In general, soluble dietary fiber is lower than insoluble dietary fiber in amaranth,
76 quinoa and kañiwa (approximately 1:5) (Repo-Carrasco, 2011). Despite their nutritional
77 advantages, there is a lack of interest for their consumption due to socio-economic factors in
78 Latin America.

79

80 The first step towards the industrialization of pseudocereals has been through the adoption of
81 low cost technological processes such as extrusion cooking which is characterised by its
82 capacity of increasing the digestibility of starch and protein. However, the effect of extrusion
83 cooking on micronutrients and bioactive compounds is still a matter of scientific investigation.
84 The potential of these pseudocereals as extrusion ingredients for the production of snack
85 foods, baby foods and breakfast cereals has been recognised by Ding et al. (2005), Mauser
86 and van Lengerich (1992), and Repo-Carrasco (2011).

87

88 Essentially, the extrusion of starchy food provokes gelatinization of starch, denaturation of
89 protein and formation of starch-lipid and protein-lipid complexes (Ho and Izzo, 1992;
90 Mercier et al., 1980). The lipid interaction with carbohydrates and proteins (or both) might
91 occur on a physical (entrapment) or molecular level. In fact, Ho and Izzo (1992) hypothesised
92 that if lipids were simply entrapped within a corn-flour matrix, there should be minimal
93 variation in the enzymatic extractability of different lipids. On the other hand, molecular-
94 level interactions would present different mechanisms, and lead to different enzymatic
95 extractability, depending on the type of lipid (e.g., chain length of fatty acid and polarity) and
96 bonding. During extrusion, the presence of fatty acids and amylose can lead to the formation
97 of complexes that may result in decreasing expansion ratios (Bhatnagar and Hanna, 1994).
98 Lue et al. (1991) observed that the addition of dietary fiber (30% of solids) to corn flour
99 caused lower expansion and greater hardness than those of the control material.

100

101 Even though the mechanisms of lipid oxidation in bulk oil have been investigated for many
102 years, information on lipid oxidation in solid matrices remains limited. Depending on the
103 type of interaction between amylose and fatty acids in solid matrices (such as extrudates),
104 lipid oxidation may lead to the formation of volatile compounds during storage (Gray et al.,
105 2008; Naknean and Meenune, 2010). Nelson and Labuza (1994) affirmed that the physical
106 state of a food matrix can play an important role in lipid oxidation kinetics. For instance, low
107 rate of hexanal production in starch-based solid matrices might be due to small free volume
108 within a glassy matrix, thereby reducing the ability of oxygen to diffuse towards lipids, and
109 hexanal to diffuse out of the matrix (Parker et al., 2002).

110

111 The aims of the present research were to prepare gluten-free corn-based extrudates containing
112 quinoa, amaranth and kañiwa, study the effects of extrusion process variables on the physical
113 properties of the extrudates and evaluate lipid stability after exposure to different humidity
114 conditions. This study proposes novel alternatives for the production and diversification of
115 gluten-free products with added nutritional value by using a low energy technology.

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2. MATERIALS AND METHODS

2.1. Materials

Amaranth (*Amaranthus caudatus* var. Oscar blanco) was obtained from the province of Carhuaz in Northern Peru, quinoa (*Chenopodium quinoa* var. Rosada de Huancayo) from the province of Jauja in the Centre of Peru and kañiwa (*Chenopodium pallidicaule* var. Cupi) from the region of Puno in Southern Peru. Samples were supplied by the Andean cereal program at the National Agrarian University 'La Molina' (UNALM, Peru). The grains were cleaned and milled (500 µm mesh size) prior to vacuum packing and delivery to Finland. Corn flour, supplied by Limagrain (France), had a mean particle size of about 150 µm and it was gluten-free since it contained less than 20 ppm of gluten (EU legislation). The corn flour was mixed with quinoa/amaranth/kañiwa (tested flours) at a ratio of 80:20 (of solids). The chemical analysis of raw materials was conducted in triplicate and detailed results are presented in **Appendix A**. Considerable differences in dietary fiber were observed among amaranth, quinoa and kañiwa (8.6%, 11.5% and 20.5% of solids, respectively).

2.2. Extrusion

The extrusion was carried out in a twin-screw laboratory extruder (Thermo Prism PTW24 Thermo Haake, Polylab System, Germany). The extruder consisted of seven sections (96 mm each) with individual temperature control in six of them; these sections were electrically heated and cooled by water. The total length of the screw was 672 mm and consisted of six conveying screws (144, 49, 47, 95, 72 and 53 mm), six mixing areas (26, 30, 30, 30, 35 and 25 mm) and an extrusion screw element (36 mm); the first mixing area was skipped on feeding. The feed rate (84 g/min), screw speed (SS), water content of mass¹ (WCM) and temperature profile were fixed based on preliminary experiments; water was added into the mass using a peristaltic pump. The section 1 was set at 40 °C, the sections 2 and 3 at 70 °C, the sections 4, 5 and 6 at 110 °C and the temperature of the die (TEM) was either 150, 160 or 170 °C. Box-Behnken experimental design with three independent variables was used: WCM (15, 17 and 19%), SS (200, 350 and 500 rpm) and TEM (150, 160 and 170 °C) (See **Appendix B**). Values of torque and pressure at die were recorded during the extrusion process as a function of time, and their average values were calculated using the data collected during the sample collection.

2.3. Determination of physical properties

The diameter of twenty specimens was measured using a vernier caliper. Sectional expansion index (SEI) was calculated as the ratio between the cross sectional area of the extrudate and the area of the die.

Hardness of the extrudates was measured as the slope of force-distance curve (N mm^{-1}) when compression was perpendicularly enforced under three point bending. A universal testing machine (Instron 4465, Instron Ltd., High Wycombe, UK) was fitted with a flat rectangular-shaped aluminium probe, $3 \text{ mm} \times 29 \text{ mm}$. Samples were placed perpendicularly over a sample holder (distance between two holding points was 12 mm). The speed of the aluminium cross head was 5 mm/min. Five randomly collected samples from each experiment were vacuum dried at 54°C for 72 hours before hardness measurement. This procedure was followed in order to prevent the plasticising effect of water on hardness and, therefore, obtain comparable results. Sizes of all samples were 100-mm in length with diameters between 5 and 14 mm. Water content of extrudates (WCE) was calculated from the weight loss in drying (vacuum drying at 54°C for 72 hours).

2.4. Analysis of large scale structures

Samples for stereomicroscopy inspection were obtained from experiment 2 (WCM 15%, SS 500 rpm, TEM 160 °C). Samples were cut in pieces of 15 mm by using a small scale electric saw. A Zeiss Steni 2000 Binocular Stereo Microscope (Wetzlar, Germany) equipped with an AxioCam digital camera was used in the inspection. For images at longer distance, an olympus Zuiko objective (9-18 mm f/4.0-5.6 Lens for Olympus; Tokyo, Japan) was used. Images were processed using Axiovision 3.1 software (Carl Zeiss, Thornwood, NY).

2.5. Analysis of nanostructures

Milled extrudates from experiment 2 were pressed into metal rings of a thickness of 1 mm and covered with Mylar foil. Wide- and small-angle x-ray scattering (WAXS and SAXS) were measured under perpendicular transmission geometry using Cu K α radiation ($\lambda=1.542$ Å) and an image plate detector (MAR345, Marresearch) for WAXS and a wire detector (HISTAR, Bruker AXS) for SAXS. In SAXS, the magnitude of the scattering vector $q=(4\pi \sin\theta)/\lambda$ ranged from 0.02 to 0.34 Å⁻¹. The data treatment procedures, including background subtraction and other corrections, have been presented by Penttilä et al. (2011).

2.6. Hexanal formation

One set of samples was milled using an ultra-centrifugal mill (Retsch ZM 200, Haan, Germany) at 12000 rpm in order to reach a mean particle size of about 500 μm , while a second set of samples was cut into pieces of 15 mm in length (whole extrudates). Milled and whole extrudates were divided and placed into separate 20-ml headspace vials so there was: 2 g of milled extrudate / 0.5 g of whole extrudate. Due to the low density of whole extrudates, standardizing the mass of milled and whole extrudates was not possible. Open vials containing the samples were stored in vacuum desiccators at 11 and 76% relative humidity (RH) at 20 °C for one week (exposure time) before being sealed and stored for 0, 2, 5 and 9 weeks at room temperature in the dark.

Hexanal content was analysed using static headspace gas chromatography (HS-GC). The Autosystem XL gas chromatograph was equipped with an HS40XL head space sampler (Perkin-Elmer, Shelton, CT; column NB 54, Nordion). The injection and detection temperature was 250 °C. Flame gases were synthetic air and hydrogen, and carrier gas was helium. The column had a length and inner diameter of 25 m and 320 μm , respectively. Vials were thermostated at 80 °C for 18 min prior to injection. The run temperature was 60 °C and the run time 10 min per sample. An external standard curve was plotted using a solution of hexanal in isopropanol (10^7 ng hexanal/l). This solution was added to open vials containing pure corn extrudates (4, 6, 8, 10, 12, 32, 42, 52, 72 μl hexanal solution/vial). The vials were stored in a shaking incubator overnight (12 hours) before GC analysis. The standard curves were plotted for milled and whole extrudates (See **Appendix C1 and C2**).

2.7. Statistical analysis

The Box-Behnken experimental design with three independent variables comprised 15 experiments that were done following the increasing temperature of die for practical reasons (See **Appendix B**). Partial least squares (PLS) regression analysis was used to study the effects of independent variables on response variables (Simca P7.01, Umetrics AB, Sweden). PLS weight plots were used to identify the most important directions of variability of different samples in a multivariate data matrix. Chemical analyses were conducted in triplicate and the results expressed as means and standard deviation (SD). The processing conditions in experiment 2 led to high extrudate expansion. Therefore, the data obtained from that experiment were statistically analysed by analysis of variance (ANOVA), and by computing Least Significant Difference ($p < 0.01$; $p < 0.05$) (v17.0 SPSS Inc., Chicago, Illinois).

3. RESULTS AND DISCUSSION

3.1. Effect of independent extrusion variables on response variables

3.1.1. Torque and pressure at die

Pressure (15.5-97.4 bar) and torque increased (28.3-44.4 Nm) as WCM and SS decreased. Results also suggested that the values of torque and pressure were strongly correlated as they were located on the upper right hand quarter of the PLS weight plot (**Figure 1**). Indeed, WCM and SS were the most influential independent variables, followed distantly by TEM (VIP value, **Table 1**). In **Figure 1**, torque and pressure were located on the opposite side of WCM and SS, indicating strong negative correlation. This partly agrees with the results obtained by Ilo and Liu (1999) in which blends of rice and amaranth flour (20, 40 and 60% of amaranth flour) underwent extrusion in a twin-screw extruder. They claimed that torque and

pressure decreased as water content of feed and SS increased. Also, Martinelli et al. (1983) observed that an increase in SS decreases the length of filled flights, thereby reducing torque and pressure. It seems that the addition of 20% amaranth/quinoa/kañiwa in corn flour (resulting in higher fat and fiber content) had some effect on the viscosity of the mass resulting in considerable changes of torque and pressure.

The predicting and descriptive abilities of the PLS model for torque and pressure were in general good, which appears in high values of prediction coefficient (Q^2) and coefficient of determination (R^2) (**Table 1**). The model of torque for extrudates containing amaranth presented the lowest Q^2 (0.17).

3.1.2. Expansion

In the PLS weight plot (Figure 1), values of SEI were located on the lower right hand quarter and showed negative correlation with WCM and positive correlation with SS. Chavez-Jauregui et al. (2000) and Coulter and Lorenz (1991) found that SEI increases at higher SS for corn-based extrudates containing quinoa and defatted amaranth flour, respectively. It was noticed that as fiber content increased, SEI became increasingly correlated with SS and less correlated with WCM (**Figure 1**). In terms of expansion, flour mixtures containing more fiber seemed less sensitive to changes in water content and increasingly sensitive to changes in SS. This trend was also noticeable by comparing contour plots (**Figure 2**); while SS had minor effect on SEI for extrudates containing pure corn, amaranth and quinoa, small changes in SS caused considerable changes in SEI for extrudates containing kañiwa.

The predicting and descriptive abilities of the PLS model for SEI were in general acceptable, taking into consideration that the Q^2 was between 0.2 and 0.8, and R^2 was generally above 0.6. The PLS model for extrudates containing pure corn possessed the lowest Q^2 (0.2) and R^2 (0.65).

3.1.3. Hardness

Low hardness was associated with low WCM and high SS. In fact, the PLS weight plot showed a high positive correlation with WCM and negative correlation with SS. It was noticed that as fiber content increased, hardness became increasingly correlated with SS and less correlated with WCM (upper left hand, **Figure 1**), showing striking similarity to the trend of SEI. Therefore, the negative correlation between hardness and SEI could be equal if samples with the same composition are compared (e.g., kSEI vs kHAR). Chavez-Jauregui et al. (2000), Ding et al. (2005) and Ilo and Liu (1999) also found strong negative correlation between SEI and hardness in their studies conducted on amaranth and rice.

A comparison of the contour plots (**Figure 2**) indicated that changes in hardness were more associated with SS rather than with WCM for extrudates containing kañiwa. Instead, hardness in extrudates containing amaranth, quinoa and pure corn appeared more associated with changes in WCM.

3.1.4. Water content of the extrudates (WCE)

WCE increased as WCM increased and SS decreased; the effect of temperature on WCE was negligible as appears in the PLS weight plot (**Figure 1**). WCE and hardness were heavily

located on the upper left quarter of the PLS weight plot which indicates a strong positive correlation between them.

In this case, the predicting abilities of the PLS model were considerable, which appear in the low root mean square error of prediction (RMSEP) and moderately high Q^2 (except for pure corn extrudates, **Table 1**). The descriptive abilities of this model, represented by R^2 , were between 0.4 and 0.9.

3.2. Characterization of the most expanded extrudates

3.2.1. Comparison of SEI and hardness

Extrudates obtained from experiment 2 (WCM 15%, SS 500 rpm, TEM 160 °C) presented the highest SEIs (except for pure corn extrudates) and lowest hardness when compared to extrudates from other experiments. Therefore, this and the follow-up sections refer to samples obtained under these specific conditions (experiment 2) unless otherwise specified.

Extrudates containing amaranth, quinoa and kañiwa presented SEIs of 7.6, 6.1 and 5.1, respectively while pure corn extrudates presented a SEI of 4.4; differences in SEIs were significant among samples ($p < 0.01$) and, especially, compared to pure corn extrudates ($p < 0.001$). The higher SEIs of extrudates containing amaranth/quinoa/kañiwa may be attributed to the higher plasticising effect of monosaccharides, oligosaccharides, amines and water (See **Appendix A**) present in the mass (De Graaf et al., 2003). Plasticisers tend to space out molecules and reduce interaction leading to less brittle polymeric structures and greater swelling capacities (De Graaf et al., 2003; Krog, 1973). It is possible that pure corn

extrudates reached the lowest SEI due to the low content of plasticisers. Interestingly, pure corn extrudates reached higher SEI (5.1) in experiment 3 where SS (350 rpm) and TEM (150 °C) were lower. This not only agrees with the logic that plasticisers might cause greater expansion but also suggests that the effect of independent variables on SEI may vary widely depending on the compositional characteristics of the raw material (**Figure 1**).

Except for pure corn, extrudates with higher contents of dietary fiber (kañiwa>quinoa>amaranth) presented lower SEIs. From these results, it is believable that dietary fiber caused disruption of internal structures leading to progressive reduction of SEIs. This logic agrees with the observations of Onwulata et al. (2001) who claimed that increasing fiber content in corn flour decreased SEI and increased bulk density.

Extrudates containing kañiwa presented the lowest hardness (28 N mm⁻¹), while extrudates containing quinoa and amaranth presented hardness values of 39 and 53 N mm⁻¹, respectively. Pure corn extrudates showed the highest hardness value (89 N mm⁻¹). Hardness was significantly higher ($p<0.01$) in pure corn extrudates than in extrudates containing amaranth/quinoa/kañiwa.

While the plasticising effect of monosaccharides, oligosaccharides, amines and water may reduce attractive forces between polymers (e.g., polysaccharides) leading to hardness reduction (De Graaf et al., 2003), lipids might also associate with amylose leading to the formation of complexes and, therefore, increasing the stiffness of polymeric structures (Bhatnagas et al., 1994; Mercier et al., 1980). Mercier et al. (1980) found evidence that amylose-lipid complexation increased hardness as a result of extrusion cooking (manioc starch). Contrasting, though, both explanations are logical in the present context since

physical characteristics, such as hardness, are the result of the interaction of biocomponents (e.g., starch and protein) at a physical and/or molecular level. It is probable that, by adding amaranth, quinoa and kañiwa (20% of solids), plasticizers somehow disrupted polymeric structures, thereby reducing hardness compared to pure corn extrudates. Although the mechanisms leading to hardness reduction are not clear in the present study, it is also suggested that the nucleation capacity of fiber limited the compactness of the structure with possible implications on hardness. From this, it can be deduced that there was limited chain-to-chain interactions between polysaccharides (and proteins) during the extrusion of blends (amaranth/quinoa/kañiwa 20% of solids) leading to progressive hardness reduction (kañiwa<quinoa<amaranth).

3.2.2. Large scale structures

Extrudates containing amaranth and quinoa presented well-defined pores of different sizes with semi-flat sides. In contrast, extrudates containing kañiwa presented small, irregular and poorly defined pores. Pure corn extrudates had a rigid structure with bigger pores and thicker walls than other samples (**Figure 3**). It is plausible that fiber present in kañiwa caused severe bubble disruption leading to lower radial expansion and higher number of pores. Amaranth and quinoa, on the other hand, had lower content of dietary fiber which could limit bubble disruption.

Despite their low radial expansion compared to fibre-free corn extrudates, Lue et al. (1991) observed that extrudates containing higher concentrations of fiber (10, 20 and 30% of solids), at different particle sizes (10, 40, 120 and 200 mesh), had more air cells. Finer ground fiber (10 mesh) had greater water holding capacity leading to more nucleation sites for water

vapour to develop as the mass was expelled through the die (Lue et al., 1991). This seems to agree with the results obtained in the present study.

Visual difference could not be observed between extrudates containing amaranth and quinoa in terms of pore size distribution. Both extrudates suffered similar damage under enforced physical modification (See **Appendix D**). Extrudates containing kañiwa exhibited a clear cut surface whereas pure corn extrudates suffered severe structural damages during cutting. This type of damage could correspond to brittleness break (breakage with minimum deformation) and, therefore, be linked to the lack of plasticisers in the raw material. As stated by De Graaf et al. (2003), the presence of a homogenously blended plasticiser such as water, sugars and amines may prevent the formation of brittle (and hard) starch-based extrudates.

Colour differences were strongly associated with the particular composition of each blend (**Figure 3E**). Extrudates containing amaranth and quinoa maintained a light colour while kañiwa conferred a dark colour to extrudates. Crude fiber (small brown spots) was noticeable particularly in extrudates containing kañiwa (**Figure 3C, E**). Pure corn extrudates, though, had a yellowish translucent structure.

3.2.3. Nanostructures

The nanoscale structure of the milled extrudates from experiment 2 was studied with WAXS and SAXS. The obtained WAXS intensities (**Figure 4**) displayed peaks characteristic of the processing-induced V_h and E_h crystal structures, which are forms of amylose-lipid helical complexes typically obtained in extrusion (Mercier et al., 1980; Penttilä et al., 2011). Based on visual comparison of the heights of the diffraction peaks, the V_h structure (peaks at $2\theta=7.4^\circ$, 12.9° , and 19.6°) dominated in all samples, whereas the proportion of the E_h

structure (peaks at $2\theta=6.9^\circ$, 11.9° , and 18.2°) was highest in the pure corn sample and lowest in the sample containing 20% kañiwa. The SAXS intensities (inset in **Figure 4**) obeyed power laws with an exponent around $-3.5(\pm 0.2)$ and no peak or shoulder from lamellar repeating structures in the studied length scale (2-30 nm) was observed. Combined with the WAXS results, this result indicates that the extrudates from experiment 2 contained sheets of crystallized amylose-lipid complexes without the formation of ordered stacks.

3.2.4. *Formation of hexanal during storage*

The hexanal formation in milled extrudates was comparatively higher than in the whole extrudates (except for whole extrudates containing quinoa and exposed to 11% RH). There was a tendency towards lipid oxidation in extrudates containing quinoa and kañiwa, exposed to 11% RH. The lipid stability of extrudates containing either amaranth or only pure corn, though, appeared to be unaffected by humidity exposure and physical modification (**Figure 5**). Exposure to different RH (11% and 76%) may have caused sharp differences in lipid oxidation due to hydration of catalysers or hydrogen binding of radicals (Labuza et al., 1972). As a matter of fact, density and wall thickness of extrudates might have some effect on the mobility of fatty acids and their interaction with amylose, oxygen and water molecules. In general, the denser the extrudate and the thicker its walls, the less lipids tend to oxidise (Gray et al., 2008; Kim and Maga, 1994).

For milled extrudates containing kañiwa and exposed to 11% RH, the hexanal content increased 25 times during storage whereas, for the ones exposed to 76% RH, the hexanal content increased only 3 times (**Figure 5**). Therefore, differences in trends of hexanal production were considerable in milled extrudates containing kañiwa. In contrast, the trends

of hexanal production corresponding to milled extrudates containing amaranth and quinoa, and pure corn showed no major differences (11% and 76% RH).

Mercier et al. (1980) reported that the formation of amylose-lipid complexes could take place during the gelatinization and cooling of starch-based materials. Less common but still likely, amylopectin and lipids may form complexes depending on the source of amylopectin; Huang and White (1993) found that modified waxy corn starches are likely to associate with lipids and form complexes. In view of this, the chosen processing conditions and raw material used in this study were probably suitable for the formation of amylose-lipid complexes. In fact, x-ray diffraction analysis suggested the presence of E_h and V_h crystal forms (WAXS) in milled samples containing amaranth, quinoa, kañiwa and pure corn, without evidence of stacking (SAXS). Obiro et al. (2012) suggested a model in which amylose-lipid complexes are organised in lamellae interspersed in amorphous regions; this model may resemble ours given the SAXS results obtained.

Naknean and Meenune (2010) suggested that the formation of amylose-lipid complexes could be reversible. This would mean that, depending on the physicochemical characteristics of the sample such as viscosity and molecular mobility, there could be some release of fatty acids and other compounds that might become a target of oxidation. In this sense, it was difficult to understand why milled extrudates containing amaranth, quinoa and especially pure corn had a low, steady and indistinct production of hexanal, despite changes in humidity exposure. On the other hand, the curves of hexanal production for milled extrudates containing kañiwa at different RH (11 and 76%) suggested a more active role of initiators (cations) and higher interaction between oxygen and fatty acids. Extrudates containing kañiwa had the highest content of ash and this might have affected the rate of hexanal production during storage

compared to other samples. Labuza et al. (1980) found that dilution of transition metal cations hinders lipid oxidation above 0.7 a_w .

The trends of hexanal production between whole extrudates containing quinoa and kañiwa (11 and 76% RH) were considerably different from one another (**Figure 5A, B**). There was a 200-fold increase in hexanal content for extrudates containing quinoa and exposed to 11% RH (inset in **Figure 5A**). These results were unexpected, considering the exceptionally lower hexanal production of the rest of the samples. Whole extrudates containing kañiwa exhibited a 15 fold increase in hexanal content after 9 week storage. In contrast, whole extrudates exposed to 76% RH showed no significant difference ($p < 0.01$) in their hexanal content during storage. It was also observed that extrudates containing amaranth and pure corn (11 and 76% RH) had a high degree of stability in storage, independent of humidity exposure and physical modification. This suggests that lipids might have interacted with starch and/or protein at a molecular (e.g., complexation) and physical level (e.g., entrapment).

Even though lipids were likely to be involved in complex formation, the observations made during lipid oxidation tests suggested that lipids, present in extrudates with higher fiber content (and lower hardness), were more prone to oxidation. Unlike extrudates containing amaranth, extrudates containing quinoa and kañiwa showed a marked increase in hexanal production during 9 weeks (storage after exposure to 11% RH). This means that plasticisers (and possibly fiber) might have limited chain to chain interaction, thereby reducing the capacity of macromolecules (e.g., amylose and amylopectin) to form a protective ‘coat’ around lipids (entrapment).

4. CONCLUSIONS

This study showed that it was possible to prepare expanded gluten-free corn-based extrudates containing amaranth, quinoa and kañiwa flour (20% of solids). The SEI was the highest in extrudates containing amaranth and the lowest in pure corn extrudates. Decreasing WCM had probably the greatest effect on extrudates by increasing SEI and decreasing hardness. The increasing contents of dietary fiber (amaranth<quinoa<kañiwa) were proposed to cause the decrease of SEI and hardness.

The hexanal formation was considerably higher in milled samples than in the whole extrudates (exposed to either 11 or 76% RH prior to storage), except for extrudates containing quinoa. The evaluation of lipid oxidation during storage suggested a remarkable stability of fat in whole extrudates after exposure to high RH. Further investigation on the effect of water plasticization on the oxidative stability of starch-complexed lipids would be highly desirable.

A study of extrudates containing various contents of amaranth, quinoa or kañiwa may answer questions on the interaction of biocomponents during extrusion and their effect on SEI and hardness. This investigation was a step forward in the understanding and developing of novel gluten-free snack products.

5. ACKNOWLEDGEMENTS

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Table 1. Parameters estimates, descriptive and predictive abilities from PLS regression models for response variables as a function of water content of mass (WCM), screw speed (SS) and temperature (TEM). Regression models were calculated using coded values of independent variables

	Response variables	x_0 Intercept	x_1 WCM	x_2 SS	x_3 TEM	RMSEP ^f	R ²	Q ^{2g}
100% corn	cSEI ^a	11.422	-0.471	0.001	-0.004	0.525	0.645	0.204
	cHAR ^b	-178.300	14.927	-0.015	0.286	26.856	0.401	0.093
	cWCE ^c	-16.010	1.110	0.003	0.040	2.020	0.407	0.125
	cTOR ^d	79.765	-1.356	-0.016	-0.110	1.334	0.810	0.590
	cPRE ^e	173.630	-3.526	-0.057	-0.363	1.617	0.965	0.859
20% amaranth	aSEI	12.419	-0.762	0.007	0.015	0.829	0.725	0.503
80% corn	aHAR	-156.470	16.518	-0.083	-0.006	15.923	0.724	0.315
	aWCE	0.179	0.828	-0.009	-0.022	1.085	0.671	0.520
	aTOR	79.604	-1.534	-0.010	-0.084	2.305	0.551	0.169
	aPRE	144.320	-3.224	-0.046	-0.300	2.521	0.891	0.715
20% quinoa	qSEI	8.479	-0.517	0.005	0.012	0.470	0.800	0.607
80% corn	qHAR	-103.430	14.357	-0.081	-0.048	26.364	0.427	-0.128
	qWCE	-7.644	0.760	-0.002	0.010	0.685	0.730	0.305
	qTOR	88.091	-1.095	-0.024	-0.142	1.353	0.851	0.703
	qPRE	128.280	-2.655	-0.038	-0.248	2.077	0.892	0.772
20% kañiwa	kSEI	1.805	-0.332	0.007	0.026	0.361	0.869	0.818
80% corn	kHAR	226.610	11.139	-0.321	-1.293	30.501	0.631	0.499
	kWCE	-2.409	0.729	-0.004	-0.004	0.401	0.894	0.765
	kTOR	67.395	-0.983	-0.014	-0.091	1.382	0.714	0.415
	kPRE	158.210	-3.631	-0.041	-0.286	2.460	0.896	0.700
VIP ^h			1.33	1.04	0.38			

^aSEI, sectional expansion index; ^bHAR, hardness (N mm⁻¹); ^cWCE, water content of extrudates (%); ^dTOR, torque (Nm); ^ePRE, pressure (bar);
^fRMSEP, root mean square error of prediction; ^gQ², Coefficient of prediction; VIP^h, variable importance on projection.

FIGURE CAPTIONS

1. PLS weight plot

Figure 1. PLS weight plot for independent extrusion parameters (**WCM**, water content of mass; **SS**, screw speed; **TEM**, temperature) and response variables (SEI, sectional expansion index; HAR, hardness; WCE, water content of extrudate; TOR, torque; PRE, pressure) in which samples codes are a= 20% amaranth/ 80% corn, q= 20% quinoa/ 80% corn, k= 20% kañiwa/ 80% corn, and c= 100% corn.

2. Contour plots

Figure 2. Contour plots for sectional expansion index (SEI) and hardness (HAR, N mm⁻¹) as a function of screw speed (SS) and water content of mass (WCM) at 160 °C; the effect of temperature of the die on the response variables was not relevant (VIP=0.38). a= 20% amaranth/ 80% corn; q= 20% quinoa/ 80% corn; k= 20% kañiwa/ 80% corn; and c= 100% corn.

3. Cross-sectional area of extrudates

Figure 3. Cross-sectional area of corn-based extrudates containing 20% amaranth (A), 20% quinoa (B), 20% kañiwa (C) and 100% corn (D); comparison of cross-sectional areas (E). Samples were obtained from the experiment 2 (WCM 15%, SS 550 rpm, TEM 160 °C).

4. X-ray scattering plot

Figure 4. X-ray scattering intensities of milled extrudates (experiment 2) containing 20% amaranth (a), 20% quinoa (b), 20% kañiwa (c) and 100% corn (d), shifted vertically for clarity.

5. Curves of hexanal production

Figure 5. Production of hexanal in ground and whole extrudates containing 20% amaranth ($\diamond$), 20% quinoa ($\square$), 20% kañiwa ($\triangle$) and 100% corn ($\circ$). Error bar shows $\pm$ standard deviation.

FIGURES

1. PLS weight plot

2. Contour plots

3. Cross-sectional area of extrudates

752 4. X-ray scattering plot

5. Curves of hexanal production

Ground extrudates Whole extrudates

APPENDICES

Appendix A. Chemical composition of amaranth var. Centenario, quinoa var. Rosada de Huancayo, kañiwa var. Cupi and corn. Results on dry basis except for moisture content.

	Moisture content, g/100 g original sample	Content of solids, % of solids						Tocopherol content, µg/g of solid		Fatty acid content, % total fatty acid	
		Protein	Fat	Dietary fiber	Ash	Digestible CHO*	Sugar**	α- Tocopherol	β - Tocopherol	C18:1 n-9	C18:2 n-6
Amaranth	10.6 ± 0.13	12.2 ± 0.20	6.6 ± 0.19	8.6 ± 0.12	2.1 ± 0.002	71	2.5-3.8 ^e	7.84 ± 0.10	19.02 ± 0.26	26.95 ± 1.44	42.99 ± 1.47
Quinoa	11.6 ± 0.14	16.4 ± 0.55	6.3 ± 0.29	11.5 ± 0.19	3.2 ± 0.06	63	2.7 ^f	23.69 ± 0.09	0.76 ± 0.01	24.78 ± 1.10	50.40 ± 1.30
Kañiwa	9.1 ± 0.15	15.6 ± 0.004	8.7 ± 0.39	20.5 ± 0.91	4.0 ± 0.03	51	NF	17.90 ± 0.29	0.95 ± 0.03	25.49 ± 0.35	47.89 ± 0.72
Corn	9.4 ± 0.026	6 - 9 ^a	2.05 ± 0.06	< 1 ^{a, b}	< 1 ^a	>75 ^{a, c}	1.4-2 ^g	5.84 ^d	0.56 ^d	26.98 ± 0.003	54.66 ± 0.02

^a Values provided by Limagrain[®] on dry basis/ ^b Cellulose content/ ^c Starch content/ ^d Analysis in duplicate/ NF=not found

^e Pedersen, B., Kalinowski, L.S., Eggum, B.O., 1987. The nutritive value of amaranth grain (*Amaranthus caudatus*). 1. Protein and minerals of raw and processed grain. Plant Foods for Human Nutrition 36, 309-234.

^f 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.

^g Rooney, L.W., McDonough, C.M., Waniska, R.D. The corn kernel. In: Smith, C.W., Bentrán, J., Runge, E.C.A. (Eds.), Corn origin, history, technology, and production. John Wiley & Sons Inc., New Jersey, pp. 288-289.

*Digestible carbohydrates (CHO) = 100 – (protein + fat + dietary fiber + ash)

**Monosaccharides, disaccharides or oligosaccharides that might act as plasticizers in the mass.

Appendix B. Coded and actual values for 3 independent variables.

Experiments*	Coded values			Actual values		
	X_1^a	X_2^b	X_3^c	A^a	B^b	C^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

^a X_1 and A , water content of mass (%)

^b X_2 and B , screw speed (rpm)

^c X_3 and C , temperature of the die (°C)

*The order of the experiments is following the increasing temperature of the die

Appendix C1. External standard curves for ground extrudate. Sample mass: 2 g.

Appendix C2. External standard curves for whole extrudate. Sample mass: 0.5 g.

Appendix D. Longitudinal section of corn-based extrudates containing 20% amaranth (A), 20% quinoa (B), 20% kañiwa (C) and 100% corn (D). Samples from experiment 2 (WCM 15%, SS 500 rpm, TEM 160 °C)

FOOTNOTES

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819 ¹ Water present in the material fed to the extruder.

APPENDICES

Appendix A. Chemical composition of amaranth var. Centenario, quinoa var. Rosada de Huancayo, kañiwa var. Cupi and corn. Results on dry basis except for moisture content.

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Kañiwa	9.1 ± 0.15	15.6 ± 0.004	8.7 ± 0.39	20.5 ± 0.91	4.0 ± 0.03	51	NF	17.90 ± 0.29	0.95 ± 0.03	25.49 ± 0.35	47.89 ± 0.72
Corn	9.4 ± 0.026	6 - 9 ^a	2.05 ± 0.06	< 1 ^{a, b}	< 1 ^a	>75 ^{a, c}	1.4-2 ^g	5.84 ^d	0.56 ^d	26.98 ± 0.003	54.66 ± 0.02

^a Values provided by Limagrain[®] on dry basis/ ^b Cellulose content/ ^c Starch content/ ^d Analysis in duplicate/ NF=not found

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^g Rooney, L.W., McDonough, C.M., Waniska, R.D. The corn kernel. In: Smith, C.W., Bentrán, J., Runge, E.C.A. (Eds.), Corn origin, history, technology, and production. John Wiley & Sons Inc., New Jersey, pp. 288-289.

*Digestible carbohydrates (CHO) = 100 – (protein + fat + dietary fiber + ash)

**Monosaccharides, disaccharides or oligosaccharides that might act as plasticizers in the mass.

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14 **Appendix B.** Coded and actual values for 3 independent variables.

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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

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16 ^a X_1 and A , water content of mass (%)17 ^b X_2 and B , screw speed (rpm)18 ^c X_3 and C , temperature of the die (°C)

19 *The order of the experiments is following the increasing temperature of the die

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27 **Appendix C1.** External standard curves for ground extrudate. Sample mass: 2 g.

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30 **Appendix C2.** External standard curves for whole extrudate. Sample mass: 0.5 g.

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36 **Appendix D.** Longitudinal section of corn-based extrudates containing 20% amaranth (A), 20%
37 quinoa (B), 20% kañiwa (C) and 100% corn (D). Samples from experiment 2 (WCM 15%, SS 500
38 rpm, TEM 160 °C)

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