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Phase Separation, Water and Thermal Properties of Andean Grain Flours and their Effect on Wheat Flour Dough

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Abstract

Structural components and their interaction with water are fundamental to dough functionality. By studying phase-separated systems the understanding of new formulas for bakery products can be improved. The phase separation, water and thermal properties of doughs from Andean grain flours and wheat flour substituted by Andean grain flours at two levels (25% and 50%) were investigated. Amaranth, canahua, and quinoa were used. Water and thermal properties at temperatures relevant for baking were analyzed by differential scanning calorimetry. Structures and particles in dough and phases were further observed under a microscope. By ultracentrifugation, amaranth flour dough was separated into nine phases, and quinoa flour into eight. This can be compared to four for wheat flour. Canahua dough remained partially unseparated. The changes in the volume fraction of phases, thermal properties, and water properties were substantially influenced by the specific Andean grain flour and the amount used. The substitution of wheat flour by Andean grain flour at the 25% level affected the properties of the dough phases, whereas wheat flour dominated the overall phase separation into four phases. At higher levels of substitution, the separation behavior was further affected, with more phases and less clear separation. When comparing different levels of substitution, the amount of freezable water in the dough was most affected by the addition of 25% amaranth flour.

Keywords: Pseudocereals; Amaranth; Canahua; Quinoa; Dough; Phase separation

Introduction

Amaranth (*Amaranthus caudatus*), canahua (*Chenopodium pallidicaule*) and quinoa (*Chenopodium quinoa*) are Andean grains which are grown in cool and semi-arid regions where common cereals cannot grow easily. All these Andean grains are highly nutritious and not known to cause allergenic reactions. Although they were a staple food in pre-Hispanic time for many peoples in South America, nowadays their potential benefits have been studied widely [1-6]. Quinoa has an interesting amino acid profile and mineral content [7,8], being selected by the Food and Agriculture Organization (FAO) as one of the crops destined to offer food security in the 21st century [9,10]. Also, the National Aeronautics and Space Administration (NASA) has considered quinoa as a potential 'new' crop for the Controlled Ecological Life Support System (CELSS) [11]. Amaranth (Kiwicha) has protein with high levels of lysine and it is biologically active, furthermore, amaranth was reported to have a great water-binding capacity, similar to trehalose [12,13]. Canahua (cañahua, cañihua, kañiwa) has a protein which composition of essential amino acids is similar to the composition of casein [6]. These Andean grains also contain oil [2]. In addition, the starch granules of quinoa, amaranth, and canahua are quite small (1 to 2-micrometer diameter), polygonal in shape with high water absorption capacity [14-16]. Therefore, flours from Andean grains have interesting attributes which have been promoted through the use of composite flours in different food application [17-19]. Bread in which wheat flour is partially substituted with Andean grain flours principally enhances the content of lysine and other essential amino acids present in a scarce amount in wheat flour bread. Breadmaking with 20% substitution of wheat flour by quinoa flours has been reported to give acceptable bread quality [20]. Amaranth flour at 10% in wheat flour bread has though been reported to give lower loaf volume and lower taste scores [12,21]. On the other hand, the substitution of wheat flour by 25% canahua flour still produces bread with good sensory acceptability but different color [22].

Andean grain flours are quite different from wheat flour regarding their physical properties, especially for breadmaking due to the lack of gluten proteins, since the presence of gluten is critical for the normal development and structure of the dough. The breadmaking process is highly dependent on flour characteristics, water and kneading since water plays important roles from mixing when flour transforms from discrete particles into the cohesive and viscoelastic dough. An effective way to study water properties is by using DSC for analysis during freezing and thawing. Upon freezing the ice formation divides the water into freezable water (FW) and unfreezable water (UFW), where the FW is assumed to be the free water at room temperature [23]. Furthermore, a well-developed wheat flour dough presents certain attributes regarding water, thermal, and phase separation properties which are related to the heterogeneous nature of the dough structure [24,25]. Therefore, the study of phase-separated systems and their water and thermal properties would allow comprehending and hence improving the development of novel baked foods using Andean grains.

Several authors have studied the quality of breadmaking with the substitution of wheat flour by quinoa or amaranth flour in order to improve nutrition, and/or to provide gluten free bread and reduce wheat-allergenic reactions. On the other hand, the addition of Andean grain flours has been showed to reduce the bread quality [13,14,22,26]. Furthermore, the information regarding the use of canahua in breadmaking processes is scarce. To our knowledge, no extensive studies on the principle of dough formation and dough characteristics have been published on the use of amaranth, canahua and quinoa flours.

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This study aims create a further understanding of Andean grain dough properties by characterizing pure Andean grain flour doughs and their blends with wheat flour, based on water, thermal properties, and phase separation by ultracentrifugation, Differential Scanning Calorimetry (DSC), and microscopy.

Materials and Methods

Material

Amaranth whole grain flour (*Amaranthus caudatus*) and canahua whole grain flour (*Chenopodium pallidicaule*) were purchased from Andes Tropico (Cochabamba, Bolivia). Quinoa grains (*Chenopodium quinoa*) were purchased from a local market (Uyuni, Bolivia) and ground to whole grain flour in a laboratory mill (Laboratoy mill 120, Perten Instruments AB, Finland). Before the purchase, the quinoa grains were mechanically pretreated by the producer, involving washing, friction, and drying. Wheat flour (Bagarn's bästa, Lantmännen Food R and D, Malmö, Sweden) was used as a reference and for mixing with Andean grains. The moisture contents of the flours were determined using the AACC method 44-19 (American Association of Cereal Chemist (AACC), 2002).

Dough preparation (Mixing)

In order to assess the Andean grain flours and their influence on wheat flour dough properties, pure amaranth flour (A), canahua flour (C) and quinoa flour (Q) were blended with wheat flour (W) at 50% and 25%. Dough mixing was performed in a kitchen mixer (KitchenAid, St. Joseph, Michigan, USA, Model Artisan KSM 150) at speed 2 for 5 minutes at room temperature. The flour was mixed with water to 50% by dough weight since this has previously been found suitable to obtain a fully developed gluten phase in wheat flour dough and good phase separation [24].

Ultracentrifugation

The study of phase separation properties was accomplished by ultracentrifugation. Doughs at the same water content of 50% were transferred into ultracentrifuge tubes up to a weight of ~10.00 g. The samples were ultracentrifuge (Optima L-90K ultracentrifuge with SW41 Ti rotor, Beckman Coulter, USA) at 24,000 rpm (about 100,000X g) for 1 h. The volume fractions (VF, %) of the separated phases were determined by using a method described by Larsson and Eliasson [24]. The height of the phases and the inside diameter of the tubes were measured with a slide caliper; for the liquid phase, the measurements were read at the bottom of the meniscus. The test tubes had an internal diameter of 8.5 mm and a height of 71 mm.

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) curves of the different doughs and separated phases were recorded and analyzed with a Seiko 6200 DSC (Seiko instruments Inc., Shizuoka, Japan) calibrated with indium (Mp=156.6 °C), and equipped with a cooling device (Haake, EK90/SII, Thermo Fisher Scientific, MA, USA) and EXSTAR6000 Thermal analysis system. At least triplicate samples were analyzed for each sample prepared and each separated phase. The samples (7 mg) were rapidly transferred into aluminum pans (TA instruments, New Castle, Delaware, USA) weighed in a C-30 Microbalance (CAHN Instruments Inc., California, USA) and hermetically sealed. With the use of an empty pan as a reference, the sample was cooled to -50°C at a 10°C/min, and equilibrated until the base lined was stable. The temperature then was linearly increased at a scanning rate of 10°C/min from -50°C to 150°C. The parameters obtained from DSC curves included transition enthalpy (ΔH), onset (T_O), peak (T_p), and temperature range (ΔT) for water properties, i.e. ice melting, and thermal properties relevant for baking and higher temperatures (Figure 1). After the scan, the pans were punctured and dried at 105°C for 24 h to determine the solid and water content (WC) for each sample.

Water properties

DSC results were used to analyze the water properties in the dough and separated phases, distinguishing between freezable (FW) and unfreezable (UFW) [23,27]. The water content (H%) of each dough and each separated phase was determined by puncturing the pan after DSC

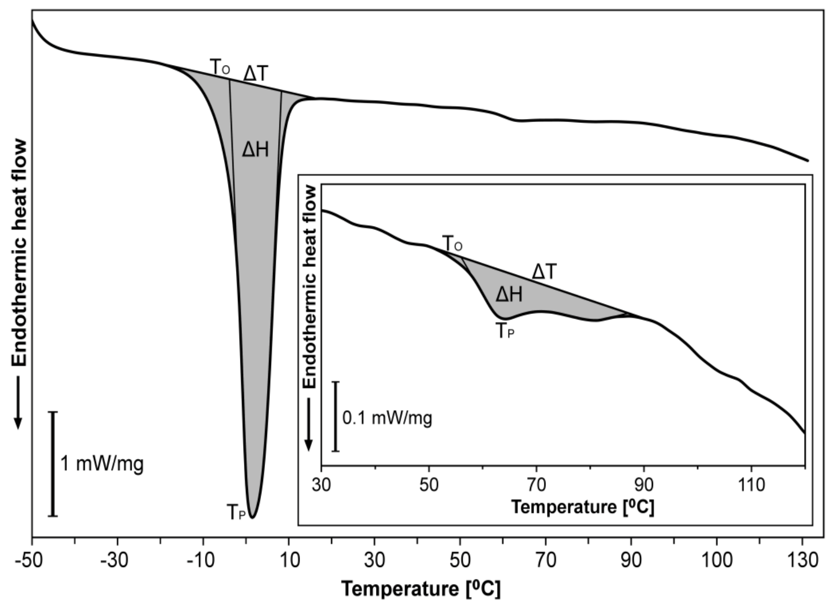

Figure 1: Example of DSC curves for wheat dough showing ice melting (ca 0°C) and starch gelatinization (ca 50-90 °C, enlarged) with transition enthalpy (ΔH), onset (TO), peak (TP), and melting range (ΔT), noted. The endothermic heat flow is indicated as scale factors next to the Y axis.

analysis and drying it at 105°C for 24 h. The endothermic peak around 0°C (Figure 1) corresponded to ice melting, and the enthalpy (ΔH) was used to calculate freezable water content (FW, %) using Eq. 1.

$$FW = \frac{\Delta H}{\Delta H_f} * 100\% \tag{1}$$

where ΔH_f is the latent heat of fusion of ice, taken that of bulk water (333.5 J/g). However, after the measurements of the enthalpy of fusion of deionized water with the same protocol, the latent heat of fusion of ice was measured to 318 J/g and the results adjusted correspondingly. It was assumed that the amount of free water present in the sample at room temperature would correspond to the amount of FW. For a correct measurement of FW, ΔH must be determined during rewarming after a complete cooling process. The unfrozen water content (UFW, mg/mg), measured in mg of unfrozen water (H_2O) per mg of dry solid was determined using Eq. 2.

$$UFW = \frac{\text{mg total water}}{\text{mg total dry solid}} * (1 - \frac{FW}{100}) \tag{2}$$

Thermal properties relevant for baking

The thermal events at temperatures relevant for baking of each dough and each separated phase were determined from the corresponding shift of the baseline of the DSC thermograms (Figure 1). Transitions that could be identified at these temperatures, 30-130°C, are lipid melting, starch gelatinization, protein denaturation and amylose-lipid complex melting [28,29].

Microscopy

The dough and the separated phases were studied with a microscope (Microscopy Olympus BX50, Tokyo, Japan) under bright field and polarized light.

Results

The characterization of Andean grain flour doughs by means of their phase separation, water, and thermal properties was a useful step to understand the characterization of composite flour doughs, and hence the effect of the addition of Andean grain flours to wheat flour with regard to these properties. Different effects were observed from each Andean grain flour, i.e. amaranth, canahua, and quinoa.

Dough preparation

Initial work was carried on by preparing the doughs of wheat flour and Andean grain flours, respectively, with water (50%), under the same regime of mixing. It was clear that each Andean grain dough was substantially different from wheat flour dough. The typical viscoelastic characteristics of wheat flour dough were not obtained using Andean grain flours, and these doughs disrupted easily during handling. Nevertheless, amaranth dough was somewhat viscous and sticky, remaining stuck on the bowl surface after mixing, quinoa dough seemed more elastic and a bit extensible, whereas canahua dough did not form a proper dough, but rather appeared as a darkly concentrated particle agglomerate, which disrupted easily upon handling.

Microscopy analysis showed that the structures of both amaranth (Figures 2a and 2d) and quinoa (Figures 2c and 2f) dough were mainly comprised of cell structures and small starch granules with more discrete bran particles in amaranth dough. The starch granules of amaranth and quinoa are known to be very small (1-3 μm) [16,30]. In this study, the microscopy analysis revealed one continuous phase between particles, i.e., the aqueous phase formed by water and soluble components, but no continuous hydrated matrix similar to wheat gluten was observed. Canahua dough differed very much from amaranth and quinoa doughs since canahua was comprised primarily of large particles of both white endosperm and dark bran. The starch granules were generally seen as individual small granules (less than 10 μm) released from white endosperm cells or aggregates (Figure 2b and 2e). A few larger granules, which resembled those of wheat, were also visible which were mainly seen as contamination, probably from the flour mill. In total, bran and endosperm cell remains differed most between the Andean grains as viewed under the microscope.

Water, thermal properties, and phase separation of Andean grain flour doughs

The water properties in terms of freezable (FW), unfreezable

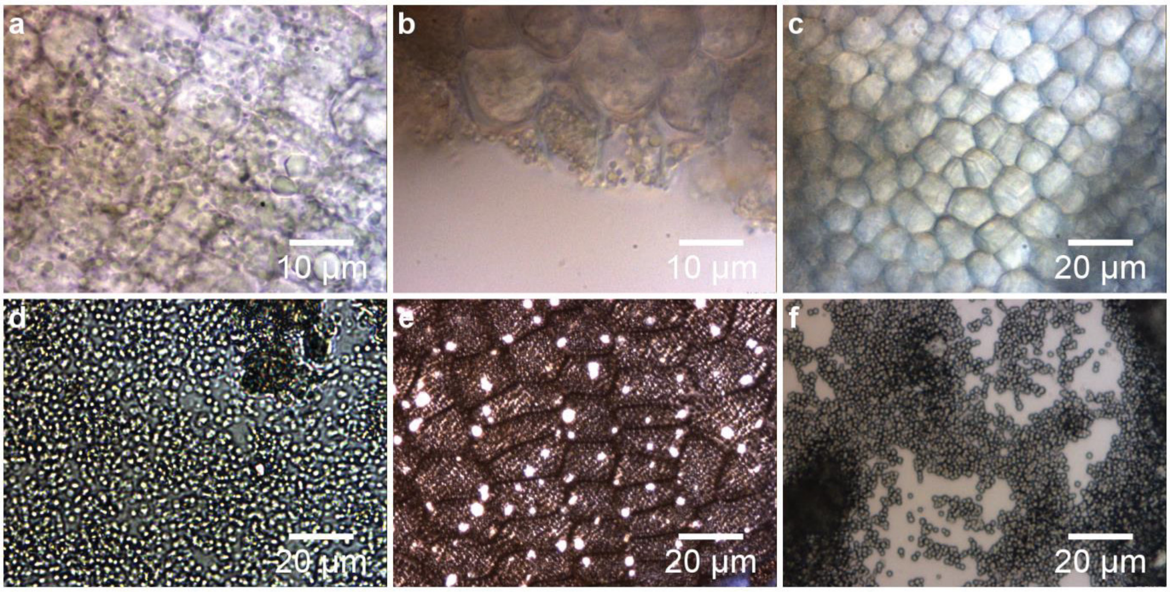

Figure 2: Cellular structures of amaranth (a), canahua (b), and quinoa (b) flour dough, and starch granules of amaranth (d), canahua (e), and quinoa (f) dough.

(UFW), and water content (WC) of the doughs prepared from amaranth, canahua, and quinoa flours were determined, FW and UFW by using DSC (Table 1).

The different doughs made from Andean grains were prepared in the same way as the reference wheat flour dough. Wheat flour dough can be sharply separated into four phases, as described previously [24,25]. The difference in WC among the doughs did not exceed 0.5% with an average of 47.8 ± 0,3%. The WC of doughs was slightly lower than 50% due to water evaporation during mixing and handling.

The interactions between water and flour components are important for the dough quality, and thereby also for the quality of the corresponding bread [31]. Water in the presence of complex materials, such as food components or mixtures of polymers, can be present different states. This, in addition to different ice crystal forms, affects the heat required for the solid-liquid transition [32]. In this study, the water was characterized as FW and UFW. The former represents the portion of water available for the processes following mixing (i.e. fermentation, baking, and shelf life).

The amount of FW was similar for all doughs, around 70% (Table 1). The lowest amount of FW was present in amaranth dough; however, there was no significant difference among the doughs. A reduction of FW would be directly related to an increase of UFW, sometimes also called unavailable water. The UFW includes the saturated monolayer associated with ionic, polar and non-polar groups on the surface of proteins and other polymers [33].

The thermal properties of the Andean grain flour doughs were determined by DSC. The DSC curves, typical for the limited water condition [34,35], are shown in Figure 3 and the corresponding data at temperatures relevant for baking are seen in Table 2. Amaranth, canahua and quinoa doughs had slightly different thermal behavior as seen from the DSC thermograms. Similar gelatinization properties have been reported previously for quinoa flour dough [36]. The gelatinization enthalpy is an indicator of thermal stability of starch. Since quinoa flour dough had the highest gelatinization enthalpy (Table 2), this indicates that quinoa starch is more thermally stable during baking [37]. Concurrently, amaranth dough had the highest gelatinization peak temperature, which is another measure of thermal stability. The stable structure of small starch granules, as found in these Andean grains, along with polar lipids can increase the stability of gas bubbles during baking [21]. In addition, gelatinization of starch granules upon further heating increases the ability of starch interactions through hydrogen bonds, further contributing to the gas bubble stability, and therefore potentially to the improvement of the functional properties of the crumb [38]. Canahua dough, on the other hand, had the lowest values for gelatinization enthalpy and gelatinization temperature. This may be related to starch crystalline properties and entrapment in cellular structures.

Dough prepared form Andean grain flours showed very different phase separation properties compared to the wheat dough.

Amaranth

Table 3 shows the volume fraction, VF, of the separated phases. The ultracentrifugation of amaranth dough yielded eight separated fractions (Figure 4) starting by a lipid phase on the top, followed by two liquid layers, and five solid fractions differentiated by color. The lipid phase (1% VF), which had a rather low WC (56.87%) and FW (75.73%) (Table 3), presented a melting peak at 18.7 °C related to lipid melting (Figure 5). The amount of lipid in amaranth has been reported to range from 6-8% [39,40].

The first liquid (Liquid 1) phase contained floating particles which were seen as clusters of small, mainly crystalline, particles of starch and light bran under the microscope. The second liquid phase was pink (Liquid 2) with similar WC, FW, and UFW as the previous phase, but with more clear transitions as determined by DSC at higher temperatures. The peaks at 58 °C and 80 °C (Figure 5) were related to starch gelatinization and protein denaturation, respectively. The microscopy study confirmed the presence of small starch granules.

The solid phases were not distinctly separated but had gradual color changes. WC and FW decreased with each phase. This was mainly an effect of the separation process but additional differences were noted between the solid phases. The combination of DSC at higher temperatures and microscopy showed that the first solid (Solid 1) phase mainly contained starch and protein. The second solid (Solid 2) phase contained starch and cell remains, and the WC (53.5%) was similar to the gluten phase of wheat flour dough (WC 52.9%). The melting of amylose-lipid complexes [41], with a transition at 105 °C, was detected in solid phase two (Figure 5). The third solid (Solid 3) fraction was whiter than the previous ones but the microscopy analysis still revealed the presence of larger cell structures among the starch. The DSC curves showed starch gelatinization, but the peak was broad and shallow due to the low availability of water [35]. The fourth and fifth solid fractions were whiter, although the bottom fraction was slightly yellow in color. The bottom fraction contained more agglomerated starch granules.

	Water content	Freezable water	Unfreezable water	Onset Temperature	Peak Temperature	Melting range
	WC (%)	FW (%)	UFW (mg/mg)	T_O (°C)	T_P (°C)	ΔT (°C)
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Wheat	46.75 ± 0.75	72.36 ± 1.42	0.24 ± 0.01	-3.27 ± 0.05	2.30 ± 1.51	11.47 ± 0.85
Amaranth	47.48 ± 0.64	71.41 ± 0.96	0.26 ± 0.01	-3.33 ± 0.21	1.98 ± 0.17	10.84 ± 0.54
Canahua	47.83 ± 0.48	72.46 ± 0.48	0.25 ± 0.01	-4.27 ± 0.24	0.52 ± 0.56	9.24 ± 0.81
Quinoa	47.98 ± 0.62	72.46 ± 1.45	0.25 ± 0.01	-2.90 ± 0.13	1.16 ± 0.10	8.88 ± 0.15
25% substitution						
Amaranth 25%	49.91 ± 0.05	61.57 ± 1.09	0.39 ± 0.01	-2.81 ± 0.14	1.39 ± 0.16	9.27 ± 0.42
Canahua 25%	47.54 ± 0.39	70.79 ± 1.19	0.26 ± 0.01	-3.11 ± 0.22	0.82 ± 0.10	8.54 ± 0.42
Quinoa 25%	44.50 ± 0.51	70.26 ± 2.09	0.24 ± 0.02	-2.16 ± 0.18	1.36 ± 0.31	7.53 ± 0.46
50% substitution						
Amaranth 50%	45.90 ± 0.58	69.84 ± 1.84	0.26 ± 0.01	-3.77 ± 0.17	0.44 ± 0.20	8.78 ± 0.10
Canahua 50%	46.58 ± 0.75	72.77 ± 2.09	0.24 ± 0.03	-3.96 ± 0.24	0.46 ± 0.13	8.70 ± 0.47
Quinoa 50%	46.97 ± 0.89	70.58 ± 1.27	0.26 ± 0.02	-3.05 ± 0.05	0.96 ± 0.13	8.98 ± 0.69

Table 1: Water properties of doughs prepared from amaranth, canahua, quinoa, and wheat.

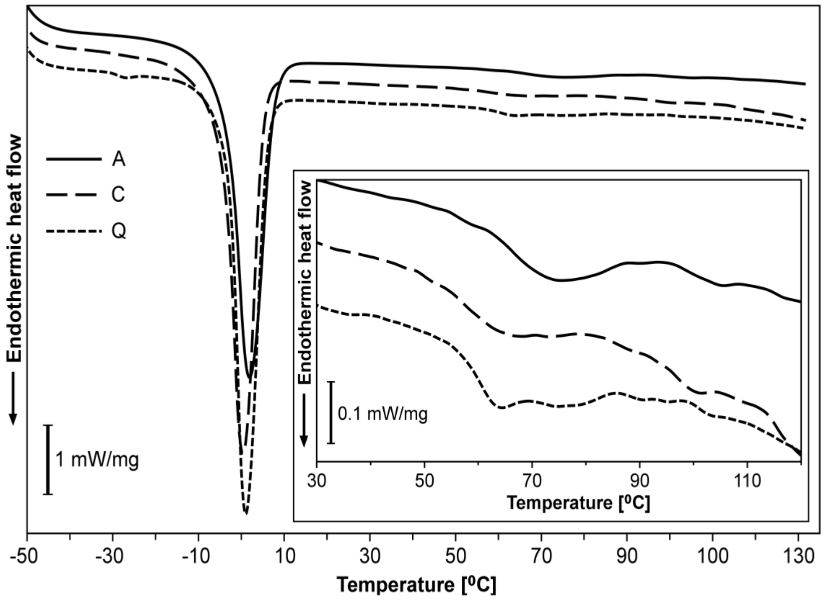

Figure 3: DSC curves at ice melting and baking temperatures of doughs prepared from amaranth flour (A), canahua flour (C), and quinoa flour (Q).

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

Table 2: Thermal properties of doughs prepared from amaranth, canahua, quinoa, and wheat.

Due to the low water content, these fractions were diluted with water to 10% to analyze the starch behavior (Figure 5). The DSC curve of the diluted fourth (Solid 4) fraction showed one major peak for starch gelatinization at 67.5°C (ΔH=18.46 J/g), a small peak for the amylose-lipid complex at 109.3°C (ΔH=1.34 J/g) and other two peaks related to protein denaturation (37.2 °C and 47.5°C). On the other hand, the diluted fifth (Solid 5) fraction presented two endothermic peaks, starch gelatinization at 67.6°C (ΔH=8.24 J/g) protein denaturation (35.9 °C). The above results confirmed the sensitivity of the separation since the fifth solid fractions were comprised of more agglomerated starch granules and brand particles.

Canahua

Canahua dough was hard to separate at these conditions, which mainly resulted in a lipid phase, a liquid phase, and a largely unseparated fraction (Figure 4 and Table 3). The yellow-white lipid fraction was very thin (0.4% VF) and under a microscope, it was seen as fat drops (ca 20 μm) agglomerated with some individual starch granules. Its water properties were similar to the amaranth lipid phase, and DSC curves (Figure 5) showed the presence of lipid melting (17°C), starch gelatinization (67°C), and amylose-lipid complexes (100°C, 107°C).

The liquid phase had a dark brown color and a fruity odor was noted. The WC and FW were rather low compared to liquid phases of the other Andean grains, suggesting a high presence of solid and soluble components with high affinity for water. Microscopy analysis revealed the presence of many and small broken crystals (under polarized light), and agglomeration of particles and oil drops. Minor transitions detected by DSC, (28°C, 38°C, and 45°C) were related to the presence of lipids (confirmed by microscopy) and protein denaturation, and one major transition (86°C) was related to starch (Figure 5). Damaged starch granules were detected under the microscope. On top of the unseparated phase (Solid 2) there was a thin gray layer, Solid phase 1 (Figures 4 and 5) (<0.5%). This phase contained the very small starch granules, but also some larger, as previously noted for the complete dough. Starch gelatinization was determined by DSC (To=66°C, ΔH=3.44 J/g). The unseparated Solid 2 fraction was dark brown with white fragments, mainly comprised of large bran and endosperm particles. The WC (36%) and FW (57%) were low. DSC indicated a small peak at 106⁰C.

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

Table 3: Volume fractions and water properties of separated phases of doughs prepared from amaranth, canahua, quinoa and wheat.

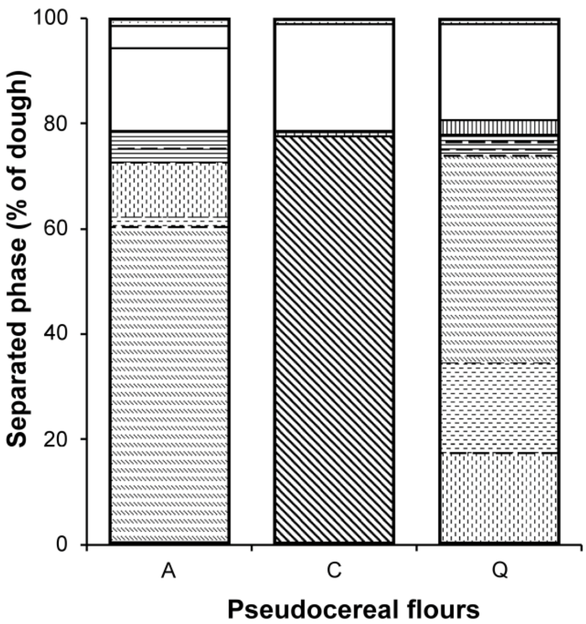

Figure 4: Phase separation behavior of doughs prepared from amaranth flour (A), canahua flour (C) and quinoa flour (Q). Symbols for phases: lipid (dotted), liquid (white), gel (vertical lines), gel-like (zig zag), solid phase more rich in protein (horizontal lines), in starch (dashes), and unseparated solid (diagonal lines).

The unseparated phase was also diluted at a concentration of 10%, and DSC analysis showed small starch gelatinization occurring at 67°C (ΔH=3.47 J/g).

Quinoa

Quinoa dough separated into 9 fractions by ultracentrifugation (Figures 4 and 5) (Table 3). Also, for quinoa, the first fraction was a yellowish lipid phase (0.4% VF), with clustered lipid drops, sometimes with starch granules attached. The quinoa lipid phase had a notably lower WC (34%) and higher FW (90%) compared to amaranth and canahua (Table 3), and an extremely low content of UFW (0.05mg/mg). This may suggest that this lipid phase was more hydrophobic. The DSC curve further displayed an endothermic peak at -27°C, which could be related to melting of polyunsaturated lipids [1,2,7]. The yellow liquid phase, on the other hand, had the highest WC (92%), FW (94%), and UFW among the Andean grains. Some particles were seen under the microscope, but the color and UFW indicated a substantial presence of soluble components. The DSC scan showed transitions that could be related to protein denaturation and starch gelatinization (35°C, 58°C, 90°C, and 68°C, respectively) (Figure 5). Quinoa further presented a transparent gel phase with some presence of starch, and rather high WC, FW, and UFW. The high peak temperature and the wide melting range for ice melting in the liquid and gel phases confirm the presence of soluble components (Table 3). Similar transitions in the gel and the liquid phases were found by DSC.

Quinoa flour dough further revealed six solid fractions upon ultracentrifugation (Figures 4 and 5). The values of WC and FW

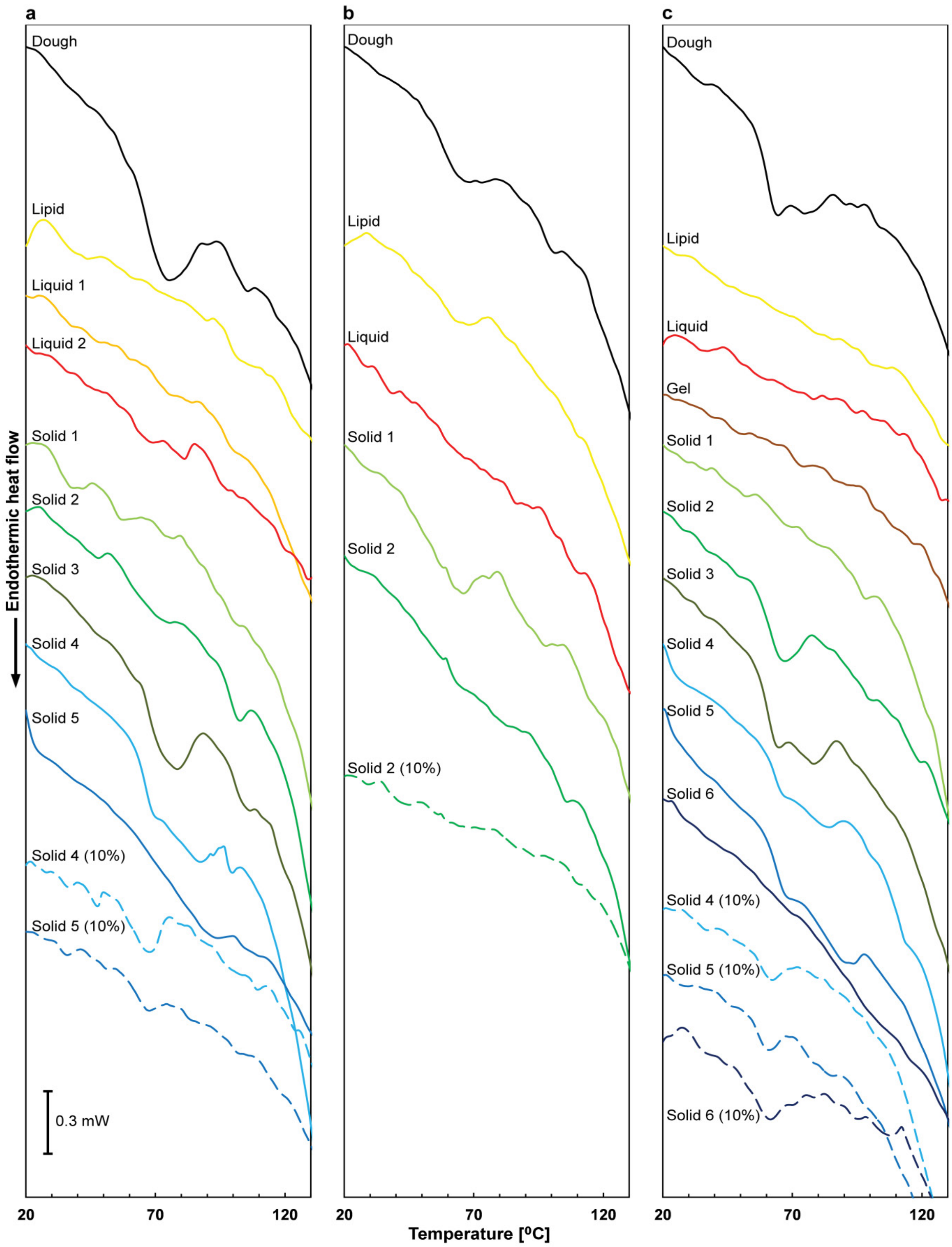

Figure 5: DSC endotherms at temperatures relevant for baking for dough and separated phases for (a) amaranth, (b) canahua, and (c) quinoa. Dotted lines indicate that the phase was diluted to 10% of solid content.

decreased with each phase. Three minor phases (1.6%, 1.5%, 1% VF) were clearly differentiated by color. The Solid 1 fraction was dark yellow, with gel-like consistency and rather low in UFW compared to the gel and other solid phases, and generally no starch. Its DSC curve revealed the presence of the amylose-lipid complex. The Solid 2 fraction was grey in color and contained starch and bran particles (Figure 5). The Solid 3 fraction was slightly yellow and contained also larger starch particles. The DSC curve showed a broad transition with two peaks (65°C and 78°C) related to the gelatinization of starch at low water content. The solid fractions 4, 5 and 6 were low in WC and FW. These phases were differentiated by their color, fractions 4 and 6 being more yellowish. Under the microscope, they looked very similar, with endosperm cells and some large bran particles. The WC was too low for full starch gelatinization, not even detectable in the bottom phase without dilution with water. Upon dilution, the endothermic peak increased towards the bottom (Figure 5). The Solid 6 fraction also showed a clear transition for amylose-lipid complexes at 108.25 °C.

The effect of mixing Andean grain flours into wheat flour dough

The phase separation properties of wheat flour dough were substantially affected by the addition of Andean grain flours. Upon 25% substitution of the wheat flour, all doughs could still be separated into four phases (Figure 6). However, the gel phase was substantially reduced and all Andean grains contributed to increasing the VF of the gluten phase. The boundaries between separated phases, especially with canahua, were not as sharp as for wheat. Upon further addition (50% of substitution) the dough became more complex; i.e. components did not mix together and could, therefore, be separated into further phases for all Andean grain flours.

The effect of amaranth mixed into wheat flour dough

Amaranth flour, at 25% substitution, substantially reduced the amount of FW in the dough, i.e. to 62% compared to 72% in wheat dough and 71% in amaranth flour dough (Table 1). Concurrently, slight increases in WC and UFW were noted. This could be related to the presence and interaction of components with high water affinity, such as fiber and cell structures, and possibly also protein as reported above. Amaranth flour has been reported to increase bread moisture by increasing the water retention capacity [42]. High water retention is related to improved crumb structure and greater product acceptability [43]. Structurally, this can be attributed to the inclusion of significant amounts of insoluble dietary fiber [21]. In this study, whole grain flour was used, and the microscopic analysis of the doughs also revealed a high presence of fiber. Water retention could further be influenced by the small size of starch granules of Andean grains (1-3 μm), much smaller wheat starch granules [44-47]. The starch-water interface is higher due to the presence of the small size Andean grain starch and therefore may facilitate the absorption and penetration of water molecules on and into the starch granules [48]. Additionally, proteins may add further to water holding. Albumins and globulins present in amaranth are known to be more soluble than wheat proteins (mainly insoluble glutenin and gliadins) [46,49,50]. With the increasing rate of substitution (50%), the FW was less affected (69.8%). These values of FW suggest synergistic and concentration-dependent effects rather than an additive effect.

The DSC curve for the blends (25%) was dominated by two endothermic peaks, the starch gelatinization (59.4°C) and the amylose-lipid complex (107.1°C) (Figure 7). The ΔH for starch gelatinization was also reduced (5.1 J/g, from 6.4 J/g in the wheat dough) (Table 2), whereas for the higher level of substitution (50%) ΔH was the same as for wheat flour dough.

The phase separation was notably affected at 25% substitution (Figure 6). The four phases typical for wheat flour were present but with different VF (Table 4). In general, the VF of the liquid and gel phases were reduced but substantially increased for the gluten phase (Table 4). Nevertheless, 50% amaranth doughs showed that the VF of

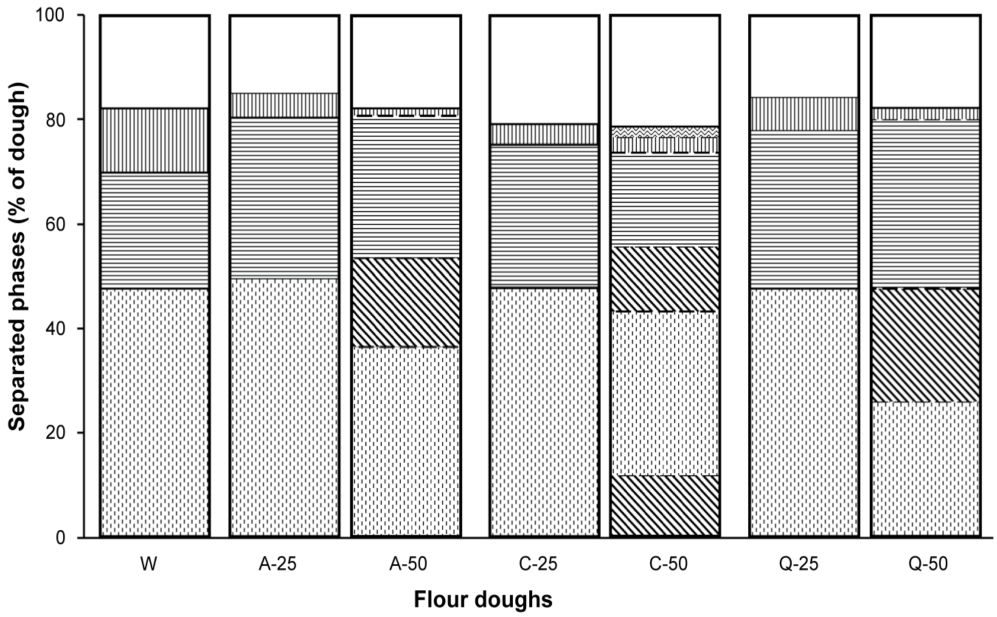

Figure 6: Phase separation behavior of dough and separated phases for wheat (W), 25% (A-25) and 50% (A-50) of amaranth, 25% (C-25) and 50% (C-50) of canahua, 25% (Q-25) and 50% (Q-50) of quinoa. Symbols for phases: liquid (white), gel (vertical lines), gel-like (zig zag), gluten (horizontal lines), starch (vertical dashes), and unseparated solid (diagonal lines).

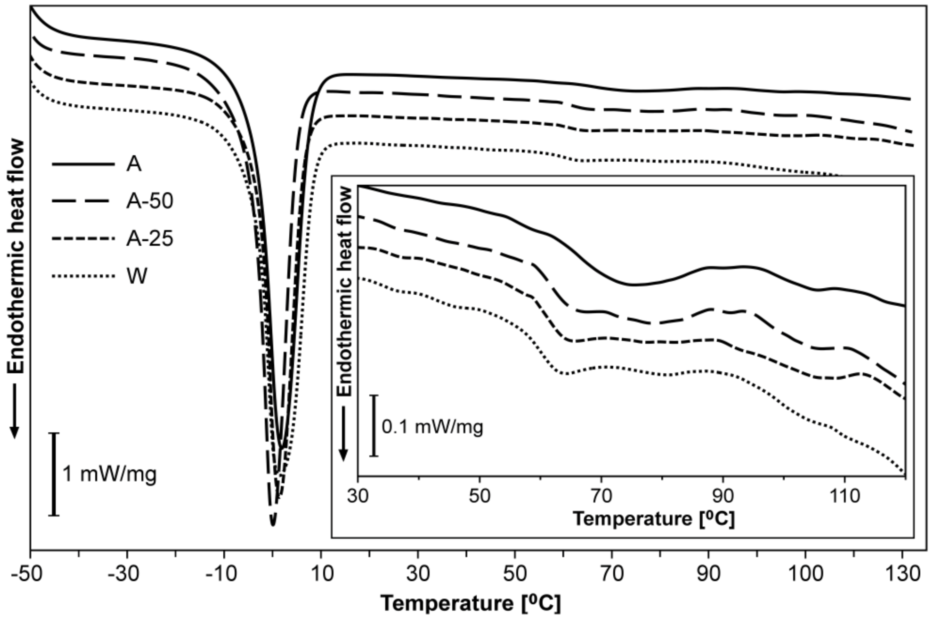

Figure 7: DSC curves at ice melting and temperatures relevant for baking for pure amaranth dough (A), 50% of amaranth (A-50), 25% of amaranth (A-25), and wheat dough (W). The endothermic heat flow, is indicated as scale factors next to the Y axis.

	Volume fraction	Water content	Freezable water	Unfreezable water	Onset temperature	Peak temperature
	VF (%)	WC (%)	FW (%)	UFW(mg/mg)	T_O (˚C)	T_P (˚C)
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Amaranth 25%						
Liquid	15.4 ± 0.4	87.79 ± 0.79	85.34 ± 0.48	1.06 ± 0.11	-3.08 ± 0.03	1.63 ± 0.11
Gel	4.6 ± 1.1	81.85 ± 2.38	81.88 ± 3.18	0.82 ± 0.15	-3.41 ± 0.02	1.22 ± 0.08
Gluten	30.9 ± 0.4	54.52 ± 1.91	70.05 ± 3.00	0.36 ± 0.03	-3.83 ± 0.03	0.57 ± 0.32
Solid	49.5 ± 0.3	31.88 ± 0.30	43.66 ± 2.73	0.26 ± 0.01	-1.91 ± 0.07	0.86 ± 0.05
Amaranth 50%						
Liquid	17.9 ± 0.4	87.25 ± 0.54	87.96 ± 5.99	0.82 ± 0.39	-3.24 ± 0.19	2.30 ± 0.23
Gel	1.3 ± 0.2	77.02 ± 1.89	86.28 ± 0.18	0.46 ± 0.05	-4.06 ± 0.27	0.90 ± 0.45
Gluten	27.4 ± 0.4	52.95 ± 2.23	76.34 ± 2.06	0.27 ± 0.04	-4.31 ± 0.04	0.14 ± 0.29
Solid 1 (Unseparated)	17.0 ± 1.5	44.24 ± 0.99	63.04 ± 1.73	0.29 ± 0.00	-4.58 ± 0.42	0.65 ± 0.49
Solid 2	36.3 ± 1.4	32.60 ± 1.01	48.49 ± 1.89	0.25 ± 0.01	-2.20 ± 0.47	2.61 ± 1.60
Canahua 25%						
Liquid	20.8 ± 0.5	86.95 ± 0.06	91.20 ± 1.73	0.59 ± 0.12	-3.34 ± 0.17	1.87 ± 0.39
Gel	4.1 ± 0.6	78.77 ± 1.28	88.90 ± 0.63	0.41 ± 0.01	-3.66 ± 0.04	0.74 ± 0.23
Gluten	27.5 ± 0.9	52.57 ± 0.18	79.79 ± 0.54	0.22 ± 0.0	-4.04 ± 0.09	0.11 ± 0.26
Solid	47.6 ± 0.9	30.81 ± 1.38	43.35 ± 2.57	0.25 ± 0.01	-3.17 ± 0.40	0.37 ± 0.13
Canahua 50%						
Liquid	21.4 ± 0.2	85.72 ± 0.97	86.39 ± 2.18	0.82 ± 0.16	-4.12 ± 0.26	1.02 ± 0.23
Gel-like	2.1 ± 0.1	69.36 ± 2.71	79.90 ± 1.73	0.46 ± 0.09	-4.48 ± 0.21	0.41 ± 0.31
Gel	2.9 ± 0.1	61.97 ± 6.08	79.58 ± 1.42	0.34 ± 0.11	-4.63 ± 0.20	0.24 ± 0.36
Gluten	18.0 ± 1.3	47.22 ± 1.88	70.89 ± 1.73	0.26 ± 0.02	-5.42 ± 0.22	0.16 ± 0.43
Solid 1 (Unseparated)	12.6 ± 0.8	35.64 ± 3.52	56.44 ± 5.35	0.24 ± 0.05	-5.19 ± 2.08	0.45 ± 0.32
Solid 2	31.5 ± 1.9	32.13 ± 0.41	59.06 ± 1.78	0.19 ± 0.01	-2.57 ± 0.48	0.84 ± 0.08
Solid 3 (Unseparated)	11.6 ± 0.4	21.12 ± 1.09	36.54 ± 4.22	0.17 ± 0.02	-2.64 ± 0.36	0.30 ± 0.05
Quinoa 25%						
Liquid	15.7 ± 0.8	88.63 ± 0.08	91.94 ± 0.96	0.63 ± 0.08	-2.34 ± 0.12	2.77 ± 0.66
Gel	6.3 ± 0.3	81.65 ± 2.07	89.63 ± 0.48	0.47 ± 0.07	-2.63 ± 0.08	2.15 ± 0.42
Gluten	30.5 ± 1.2	53.13 ± 0.93	79.16 ± 0.83	0.24 ± 0.00	-3.45 ± 0.10	0.57 ± 0.04
Solid	47.5 ± 0.1	31.59 ± 1.44	47.96 ± 3.22	0.24 ± 0.01	-2.14 ± 0.22	1.68 ± 1.67
Quinoa 50%						

Liquid	17.8 ± 0.3	90.12 ± 0.32	92.67 ± 0.00	0.67 ± 0.02	-3.29 ± 0.12	1.99 ± 0.32
Gel	2.2 ± 0.6	73.19 ± 1.62	86.28 ± 2.54	0.38 ± 0.08	-3.74 ± 0.12	0.81 ± 0.07
Gluten	32.6 ± 0.3	52.04 ± 1.56	74.24 ± 1.42	0.28 ± 0.01	-4.33 ± 0.16	0.38 ± 0.09
Solid 1	21.5 ± 1.4	32.10 ± 1.60	36.96 ± 2.67	0.30 ± 0.03	-2.96 ± 0.59	0.39 ± 0.03
Solid 2	25.9 ± 1.5	25.40 ± 2.22	28.06 ± 1.12	0.25 ± 0.03	-1.93 ± 0.22	0.47 ± 0.13

Table 4: Water properties of separated phases of doughs prepared from wheat flour substituted by 25% and 50% Andean grain flour, respectively.

the liquid phase was similar to that of wheat dough, 17.9%, and 18.0%, respectively (Figure 6).

The gel phase was reduced as the presence of amaranth increased (Figure 6). The amounts of FW in the liquid and gel phases were significantly reduced, and UFW increased (Tables 3 and 4). These results are consistent with the reduction of FW in the dough. This indicates that the presence of amaranth reduced the availability of water in the liquid and gel phases, which could be attributable to soluble components from amaranth.

The gluten phase of 25% amaranth presented a WC which was higher than that of wheat (Tables 3 and 4). This could indicate that the gluten was developed [24], but its structure allowed a rapid ice melting, revealed by the ice peak temperature, which is related to a gluten structure without capillaries or holes where water could be located [51].

The phase separation of 50% amaranth dough was less efficient for gluten and starch phases since between these phases an unseparated phase occurred (Figure 6). This contained large bran particles, gluten, and starch granules. As for 25%, the gluten phase had a similar WC than that of wheat and a more rapid ice melting.

The effect of canahua mixed into wheat flour dough

Dough prepared with 25% canahua showed a slight change of FW (Table 1), reduced gelatinization enthalpy (Table 2) and a large melting peak for amylose-lipid complexes (Figure 8), compared to wheat flour dough. The reduction of FW could not be the main reason for this change of thermal behavior, but the presence of polar lipid from canahua could hinder starch gelatinization and induce a rapid and strong formation of amylose-lipid complexes [15,52].

The substitution with canahua flour modified the phase separation behavior of wheat dough. The presence of canahua increased the VF of the liquid phase (Figure 6 and Table 4). Besides, at a high level of substitution (50%) an intermediate gel-like phase was located between the liquid and gel phases (Figure 6). The gluten phase VF of 25% canahua dough was increased, which was seen as dilution by the inclusion of gel and bran particles, also confirmed by microscopy. Besides, the gluten phase WC was similar to that of wheat dough (Tables 3 and 4), but with lower ice melting peak. When canahua was added at 50%, the gluten VF was lower and most bran particles were located in a dark unseparated phase between the gluten and starch phases (Figure 6). Apparently, at lower levels of substitution the gluten structure was strong enough to hold the canahua bran but weaker at higher levels of substitution The weakening of gluten structure was confirmed by both its lower water content (47.2%), in relation to a well-developed gluten (>50%) [24,53], and the unseparated phases clearly showing the need for more water during dough formation.

The effect of quinoa mixed into wheat flour dough

Quinoa in wheat dough reduced the FW of doughs in a similar way as canahua (Table 1). Starch gelatinization dominated the thermal analysis (Figure 9), although for 50% quinoa dough a shallow melting peak for the amylose-lipid complex was detected. The presence of quinoa reduced the gelatinization enthalpy compared to a wheat dough (Table 1).

Like the other Andean grains at 25% substitution, quinoa dough was separated into four phases (Figure 6). The boundary between gel and gluten was better defined than for amaranth and canahua. Besides, the accumulation of bran particles at the bottom of the gluten phase was more compact and did not intermix with gluten or starch, however, the VF was too low to be measured separately.

The phase separation behavior of 25% quinoa dough was similar to that of 25% amaranth (Figure 6). The presence of quinoa at low and high levels slightly modified the WC of the gluten phase (Tables 3 and 4). The microscopic analysis revealed that the gluten phase contained substantial amounts of small starch granules and large bran particles. With 50% of quinoa, the phase separation was notably affected. The gel phase had a low WC compared to other quinoa dough or wheat dough. The gluten fraction at 50% substitution had more bran and starch granules, and it disrupted very easily. The starch-rich solid fraction was slightly yellowish at the top and white at the bottom (Figure 7).

Discussion

Andean grain flours could not form dough structures typical for wheat flour. Amaranth and quinoa produced sticky doughs without the viscoelasticity of wheat dough. Canahua did not form a proper dough, instead, an agglomerated particle network was formed. The water properties (WC, FW, UFW) of Andean grain doughs were not statistically different from the wheat dough. Starch gelatinization and melting of amylose-lipid complexes were detected in all dough using DSC. Quinoa and canahua doughs had the highest and lowest starch gelatinization enthalpies, respectively. Each Andean grain behaved differently regarding phase separation. Nevertheless, in contrast to wheat dough, all of them had lipid fractions on the top. Amaranth dough was separated into eight different fractions and quinoa to nine, whereas canahua dough was hard to separate.

The substitution of wheat flour by amaranth, canahua or quinoa flours enabled more wheat-like dough to be formed. At 25% substitution, these doughs were also separated into four fractions, like wheat, although the VF of the respective fractions differed. The gel phase was reduced, whereas the gluten phase increased in VF, mainly due to bran particles and small starch granules interspersed in the gluten network. The UFW increased in the liquid phase due to soluble components and light particles from the Andean grain flours compared to the wheat dough. The phase separation further showed a reduced VF of the liquid phase for amaranth and quinoa, but an increase for canahua. At a higher level of substitution, 50%, the main effect was observed on the phase separation properties, with more phases and less clear separation. When comparing different levels of substitution, FW was most affected by the addition of amaranth at a concentration of 25% (Figure 10).

Conclusion

Simple water-flour dough prepared from Andean grain flours, namely, amaranth, canahua, and quinoa flours, and wheat flour dough

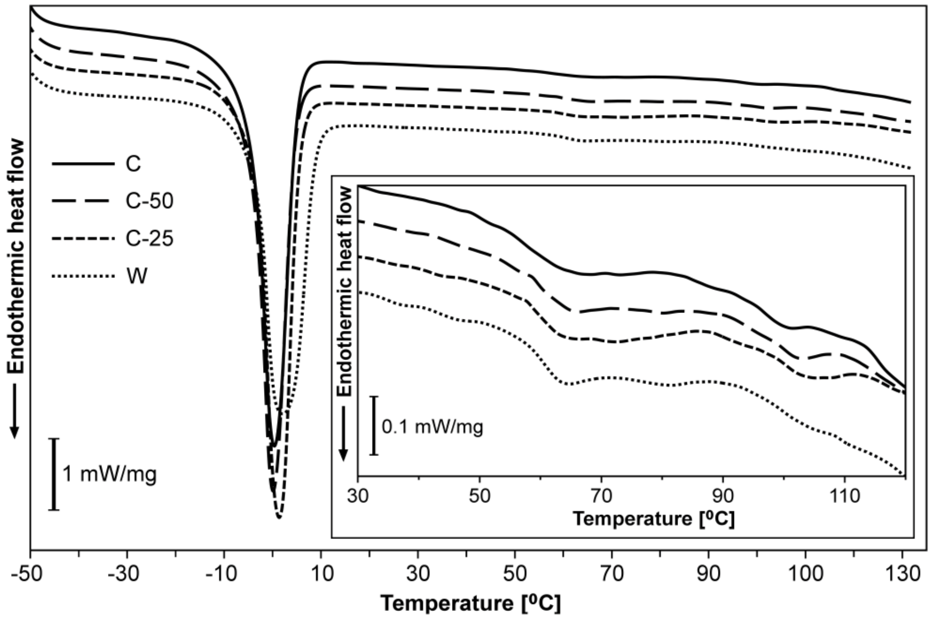

Figure 8: DSC curves at ice melting and temperatures relevant for baking for pure canahua dough (C), 50% of canahua (C-50), 25% of canahua (C-25), and wheat dough (W). The endothermic heat flow, is indicated as scale factors next to the Y axis.

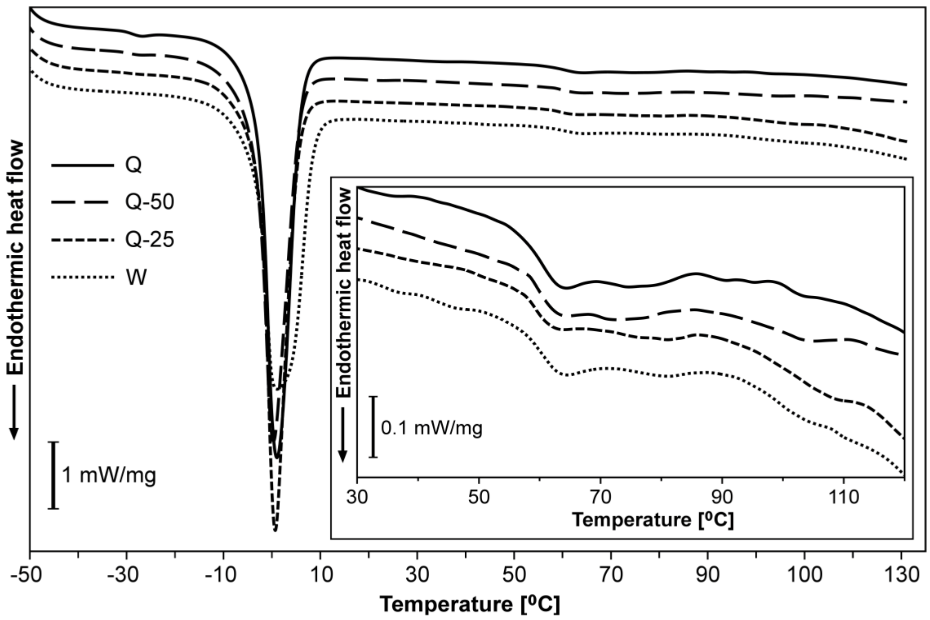

Figure 9: DSC curves at ice melting and temperatures relevant for baking for pure quinoa dough (Q), 50% of quinoa (Q-50), 25% of quinoa (Q-25), and wheat dough (W). The endothermic heat flow, is indicated as scale factors next to the Y axis.

substituted at two levels, 25%, and 50%, by Andean grain flours, was characterized by ultracentrifugation, DSC, and microscopy. Dough structure in combination with water properties is known to contribute substantially to functionality. Ultracentrifugation of these doughs prepared under the same water content and mixing regime yielded different fractions with main structural differences. Each fraction would correspond to the dough as composites of structural elements, ranging from coarser particles and aggregates to macromolecules and monomers dissolved in water. The changes in VF, thermal properties and water properties were determined by the amount and the specific Andean grain flour. At the lower level of substitution, wheat flour dominated the overall phase separation behavior, whereas the respective Andean grain rather influenced the properties of each specific phase. The use of phase-separated systems is helpful for understanding the effect of new formulas for bakery products.

Acknowledgment

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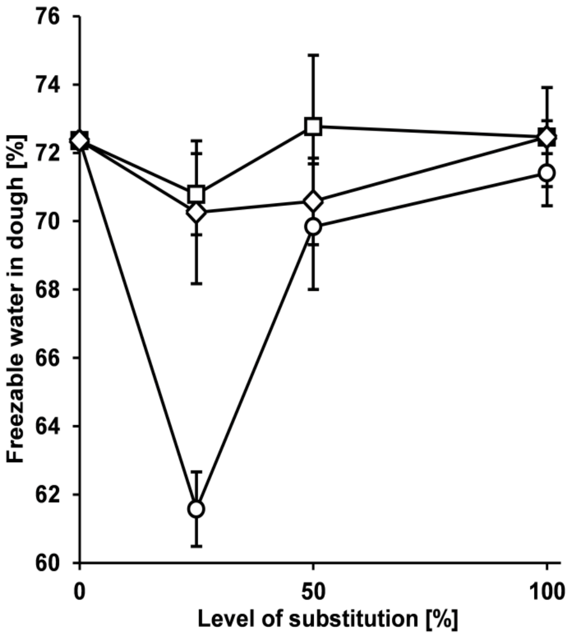

Figure 10: Freezable water content behavior according to the level of substitution for amaranth (white circle), canahua (white square), and quinoa (white diamond).

Conflict of Interest

The authors declare that they have no conflict of interest.

Human and Animal Rights Statement

This article does not contain any studies with human or animal subjects.

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