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Characterization of lipid oxidation products in quinoa (*Chenopodium quinoa*)

Su-Chuen Ng, Alfred Anderson *, Janice Coker, Martin Ondrus

Department of Food and Nutrition, University of Wisconsin-Stout, Menomonie, WI 54751, USA

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Abstract

The oxidative stability of lipids in processed quinoa was investigated in this study. Ground quinoa was subjected to accelerated aging for 30 days at 25, 35, 45, and 55 °C. Three samples were removed from each temperature treatment every 3 days. Free fatty acids, conjugated diene hydroperoxides, and hexanal were used as indicators of lipid oxidation. Storage time and temperature had significant effects ($p \leqslant 0.05$) on all three parameters, while the interaction between storage time and temperature was not significant for conjugated diene hydroperoxides produced. The results from these tests suggest that quinoa lipids are stable for the period of time studied. With vitamin E as a naturally antioxidant occurring abundantly in quinoa, the potential for quinoa to be a new oilseed could be enhanced. This study provided some preliminary information on the oxidative stability of quinoa.
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Keywords: Quinoa (*Chenopodium quinoa*); Lipid oxidation; Free fatty acids; Hexanal; Conjugated diene hydroperoxides

1. Introduction

Quinoa (*Chenopodium quinoa*), a staple food in some ancient civilizations, is a hardy plant that thrives well in high altitudes and poor soil with limited rainfall. Extensive research has been conducted on this seed. Researchers are interested in quinoa due to its complete amino acid profile (Gross et al., 1989; Ruales & Nair, 1992), the microstructure of its starch (Atwell, Patrick, Johnson, & Glass, 1983; Fleming & Galwey, 1995), the functions of its saponins (Ahamed, Singhal, Kulkarni, & Pal, 1998; Ruales & Nair, 1993), and its potential as a future oilseed (Koziol, 1992).

Although a lesser-known plant, interest in quinoa has been increasing, due to its perceived superior nutritional quality compared to other grains, its potential for use as an alternative oilseed crop, and consumers desire to try natural, different, and ethnic foods, as well as interest in functional foods. Quinoa also has excellent reserves of protein and, unlike other grains, is not missing the amino acid lysine, so the protein is more complete.

Ground quinoa is subject to increased lipid oxidation due to the activation of lipolytic enzymes and increased surface area, thus compromising its keeping quality. Quinoa contains relatively high amounts of fat compared to other cereals. It is also rich in vitamin E, which is said to protect its lipids from oxidation. However, no research has actually been conducted to demonstrate this assumption in quinoa.

The fatty acid composition of quinoa is chiefly linoleic acid, followed by oleic acid and palmitic acids, a profile similar to that of corn and soybean oils. Quinoa also has similar amounts of linolenic acid to soybean oil. Although high in unsaturated fatty acids, quinoa oil is stable, due to its high amounts of vitamin E, which acts as a natural antioxidant to prevent rapid lipid oxidation. Because the fat content of quinoa is higher than that of many other cereals, quinoa has potential as an oilseed (Koziol, 1992).

Numerous studies have been conducted to investigate the feasibility of incorporating quinoa into foods. Advantages

* Corresponding author. Present address: Department of Family Sciences, College for Women, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait. Tel.: +1 965 498 3080; fax: +1 965 482 7802.
E-mail address: aanderson@cfw.kuniv.edu (A. Anderson).

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of using quinoa as an ingredient include raising the protein content and improving the taste of the product. Lorenz and Coulter (1991) and Lorenz, Coulter, and Johnson (1995) evaluated the performance of blended quinoa and wheat flours in breads, cakes and cookies. Breads and cakes made with up to 10% quinoa flour were acceptable. When used up to 10% in cakes and 20% in cookies, quinoa contributed a favourable nutty taste to the products. Chauhan, Zillman, and Eskin (1992) investigated the baking performance and overall acceptability of quinoa/wheat breads using quinoa flour or quinoa meal. In general, breads with 10% of water-soaked quinoa meal were more acceptable than were other quinoa variations.

Nutritional properties, sensory evaluation and physical characteristics were examined in extrusion studies blending quinoa and com grits (Coulter & Lorenz, 1991a, 1991b; Lorenz et al., 1995). Quinoa flour was extruded with com grits to produce expanded snack products. Addition of quinoa increased product density and decreased product expansion and shear strength, and produced a darker, less yellow extruded product. The products were rated as moderately acceptable.

Quinoa has been incorporated into wheat noodles (Lorenz, Gifford, & Johnson, 1993). No statistically significant difference was found between noodles made with 10% and 30% quinoa. Noodles with 50% quinoa content were ranked least acceptable.

Penaloza, Davey, Hedger, and Kell (1992) studied selected fermentation parameters to optimize the production of quinoa tempe. No sensory data was available; however, the investigators implied that appearance, flavour, and texture, which were subjectively assessed, were acceptable.

Information on shelf life and lipid oxidation in quinoa is limited in the literature. The objective of this study was therefore to investigate the influence of time and temperature on lipid oxidation in quinoa.

2. Materials and methods

2.1. Materials

Whole, dehulled quinoa was donated by the Northern Quinoa Corporation, Kamsack, Saskachewan, Canada. Hexanal and 2-heptanone standards were purchased from Aldrich Chemical Company (Milwaukee, WI). HPLC grade hexane and reagent grade sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Methods

The whole quinoa was stored at −10 °C until the beginning of the study. Quinoa was ground, using a coffee grinder (Krups, Model 208, Closter, NJ) to pass through a 20-mesh sieve. Approximately 30 g of ground quinoa were placed into half pint sized mason jars (Ball, Muncie, Indiana). The jars were closed and placed in ovens set at 35, 45, and 55 °C. One set of jars was kept at ambient temperature (25 °C) in closed cardboard boxes. Triplicate samples were obtained by removing three jars from each temperature set every 3 days for a period of 30 days. Every time jars were removed from the ovens, the remaining jars were randomly rearranged to ensure even exposure to heat. The jars were flushed with nitrogen and stored at −10 °C until they were analyzed for free fatty acids (FFA), conjugated diene hydroperoxides (CDHP), and hexanal content. The jars were allowed to stabilize to room temperature before quinoa samples were taken for analysis. FFA, CDHP, and hexanal were analyzed within 1 month of storage.

2.3. Free fatty acids and conjugated diene hydroperoxides

Free fatty acids (FFA) and conjugated diene hydroperoxides (CDHP) were analyzed using the method described by Ory, Delucca, St. Angelo, and Dupuy (1980). One-gramme samples were extracted in a 25 ml Erlenmeyer flask using 15 ml of HPLC grade hexane. The mixture was gently swirled by hand for 1 min, loosely stoppered and allowed to stand in the dark at room temperature for 1 h. The mixture was then transferred into centrifuge tubes and centrifuged at 10,000*g* rpm for 20 min. The upper layer, that contained peroxides and free fatty acids (Ory et al., 1980; St. Angelo & Ory, 1975) was carefully decanted and immediately used for CDHP and free fatty acid analyses.

CDHP was determined by measuring the absorbance of the quinoa/hexane extract in a Hewlett–Packard 8452A Diode Array UV–vis spectrophotometer at 234 nm. Pure hexane was used as a blank. The concentration of CDHP was calculated in micromoles per gramme of quinoa by using a molar absorptivity of 24,500 l/mole cm as described by Johnston, Zilch, Selke, and Dutton (1961).

To determine FFA content, 4 ml of the quinoa/hexane extract in 10 ml of 95% ethanol was titrated with standardized 0.00992 N NaOH, using phenolphthalein as an indicator. The endpoint was determined when a light pink solution remained for 1 min. For the blank, 4 ml of hexane in 10 ml of 95% ethanol was used. The blank was subtracted from the total values to obtain the net amount of NaOH needed to neutralize the FFA. FFA was calculated as oleic acid and expressed as percent of quinoa.

2.4. Hexanal standard curve

Hexanal was analyzed by static headspace gas chromatography according to the method of Pershen (1989) with the following modifications: a hexanal standard curve was prepared using a stock solution containing 100 μl of hexanal in 100 ml of ethanol. Hexanal standard solutions were made by adding 1.0, 2.5, 5.0, 10.0, 15.0, and 20.0 ml of the stock solution to 19.0, 17.5, 15.0, 10.0, 5.0, and 0.0 ml of ethanol, respectively, into 1 l volumetric flasks. An internal standard of 25 μl of 2-heptanone was added to each of the standard solutions, and the flasks were filled to volume with Milli-Q water.

The hexanal standards were prepared by placing 2 ml of standard solution and 40 ml of saturated NaCl in a 50 ml serum vial and sealing with a septum cap. The vial was then placed in a 90 °C water bath for 10 min. A 2.5 ml headspace sample was manually obtained using a gas-tight syringe (Hamilton, Reno, Nevada) and injected into the gas chromatograph. A standard curve (Fig. 1) was obtained by plotting the peak area ratio of hexanal and 2-heptanone versus ppm hexanal. Hexanal in the headspace was calculated in ppm using the formula:

$$\text{ppm hexanal} = (Y - b)/a,$$

where Y is the peak area ratio of hexanal to 2-heptanone; a the slope; b the y-intercept.

2.5. Hexanal in Quinoa

To determine the hexanal content of quinoa samples, three-gramme samples were weighed into 50 ml serum vials. A 2-heptanone internal standard solution was prepared by adding 25 μl of 2-heptanone to a 1 l volumetric flask and filling to volume with Milli-Q water. To each vial, 2 ml of 2-heptanone standard solution and 40 ml of saturated NaCl were added and sealed with a septum cap. The vial was then placed into a 90 °C water bath for 10 min. A 2.5 ml headspace sample was manually obtained using a gas tight syringe and injected into the gas chromatograph. The hexanal in quinoa was measured using a Hewlett–Packard 6890 gas chromatograph with flame ionization detector and a 30 m fused-silica capillary column DB 255-30N with 0.25 μm internal diameter (J&W Scientific, Folsom, CA). Column temperature was programmed at 70 °C for 1 min followed by a rise of 6 °C/min to 100 °C with a 4 min hold. The helium flow rate was 1 ml/min with a split ratio of 1:12. The injection port and detector temperatures were held at 200 and 275 °C, respectively. Chromatograms were integrated to obtain hexanal/2-heptanone peak area ratios using the Standard Chemstation G 170 lAA Version A.03.00 programme in a computer data station connected to the gas chromatograph.

2.6. Statistical analysis

Data were analyzed using the statistical package in Microsoft® Excel 2000 (Microsoft Corporation, Redmond, Washington). The 2-factor analysis of variance (ANOVA) with replications was used to determine the effect of temperature and days, as well as interactions between temperatures and days. Where ANOVA showed any significance among means ($p \leqslant 0.05$), the Fisher's least significance difference (LSD) test was used to perform treatments means separation.

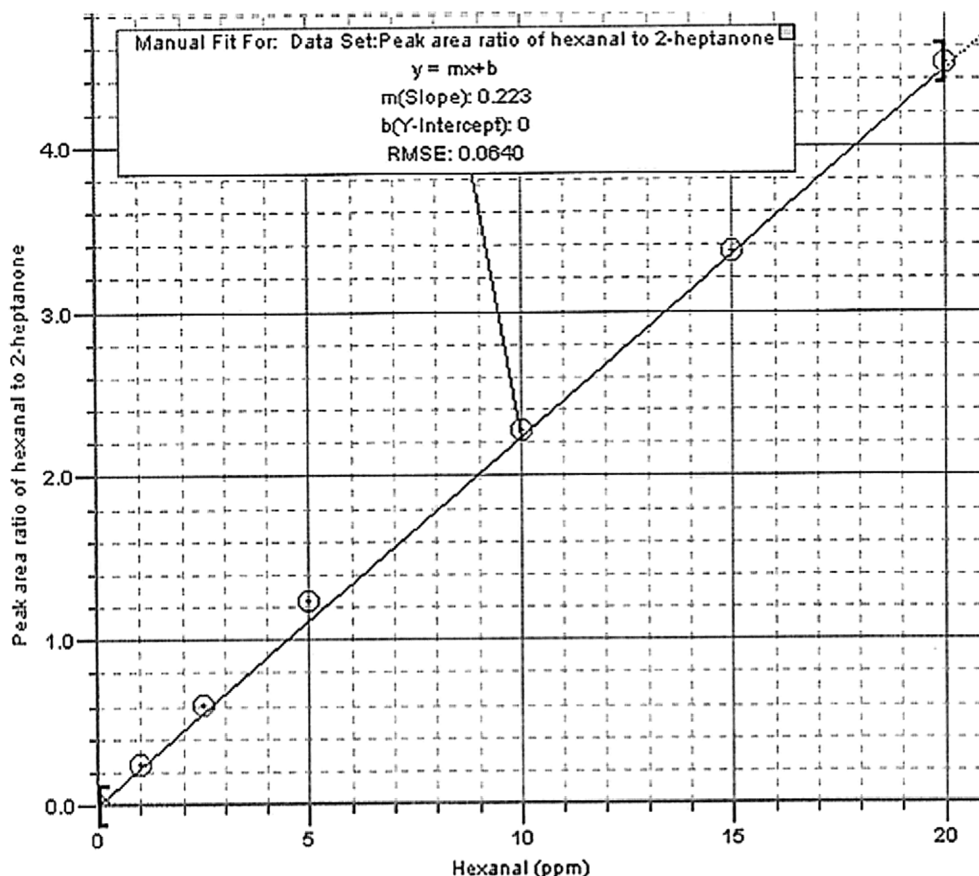

Fig. 1. Standard curve for hexanal.

3. Results and discussion

Fig. 1 shows the linearity of the hexanal standard curve, and Fig. 2 shows a typical chromatogram for a hexanal standard. The peaks showed little or no tailing and in general were sharp and reasonably symmetrical. Baseline resolution between hexanal and 2-heptanone was consistently observed. Similar good peak shape and baseline resolutions were observed in quinoa samples stored at 25, 35, 45, and 55 °C, as shown in Fig. 3.

Since free fatty acids (FFA), conjugated diene hydroperoxides (CDHP) and hexanal were used as indices of lipid oxidation of quinoa in this study, it is important to clarify their relationship. The purpose of selecting these three parameters was to examine the oxidative stability of lipids in quinoa at different stages. Hydroperoxides are the first products of lipid oxidation; thus FFAs are the source of CDHPs. Once formed, hydroperoxides decompose into other products, such as aldehydes, ketones, and alcohols. In this study, the aldehyde of interest was hexanal.

3.1. Free fatty acids

Free fatty acids (FFA) generally showed an increasing trend for the samples kept at 45 and 55 °C over the period of the study (Fig. 4). Quinoa stored at 45 °C showed small increases in FFA, while the samples stored at 55 °C exhibited larger increases. For all samples, percent FFA increased to a maximum around day 6 and then dropped precipitously thereafter. However, after day 9, FFA production in samples stored at 25 and 35 °C showed minimal or no increases, while samples stored at 45 and 55 °C showed dramatic increases in FFA.

Both storage time and storage temperature had significant effects ($p \leqslant 0.05$) on the production of FFA (Table 1). Interaction effects were also significant. This suggests that lipolysis in quinoa is dependent on both temperature and time. Besides lipase hydrolysis, autoxidation may have contributed to lipolysis as well, but probably only up to a certain stage, due to limited oxygen availability in the jars.

Sharp and Timme (1986) observed an increase in free fatty acids when brown rice was stored at 22 and 38 °C using different packaging methods for 9 months. The samples stored at 38 °C exhibited higher amounts of FFA and showed more rapid increases in FFA production than did samples stored at 22 °C. Trawatha, TeKrony, and Hildebrand (1995) examined free linoleic acid content in two soybean cultivars subjected to accelerated aging at 30 and 40 °C for varying periods. Within 30 days, a steady increase in free linoleic acid was observed, with higher values seen in the seeds stored at 40 °C. Free linoleic acid values increased about twice as much in the 30 °C samples, while the values tripled in the 40 °C samples. These observations compare with the increase in FFA in quinoa in this study. Enzymatic reactions in quinoa appear to be increased at higher storage temperatures.

3.2. Conjugated diene hydroperoxides

A steady increase in conjugated diene hydroperoxides (CDHP) was observed at all four temperature treatments up to a maximum on day 9 (Fig. 5). CDHP decreased between day 9 and day 12 for the samples stored at 25 and 45 °C, while samples kept at 35 and 55 °C decreased in CDHP from day 9 to day 15. In general, CDHP readings for samples at all temperatures increased slightly or leveled off from day 18 up to the end of the sampling period.

The interaction between temperature and time of storage did not have a significant effect ($p \leqslant 0.05$) on CDHP produced (Table 1). Since storage time and temperature

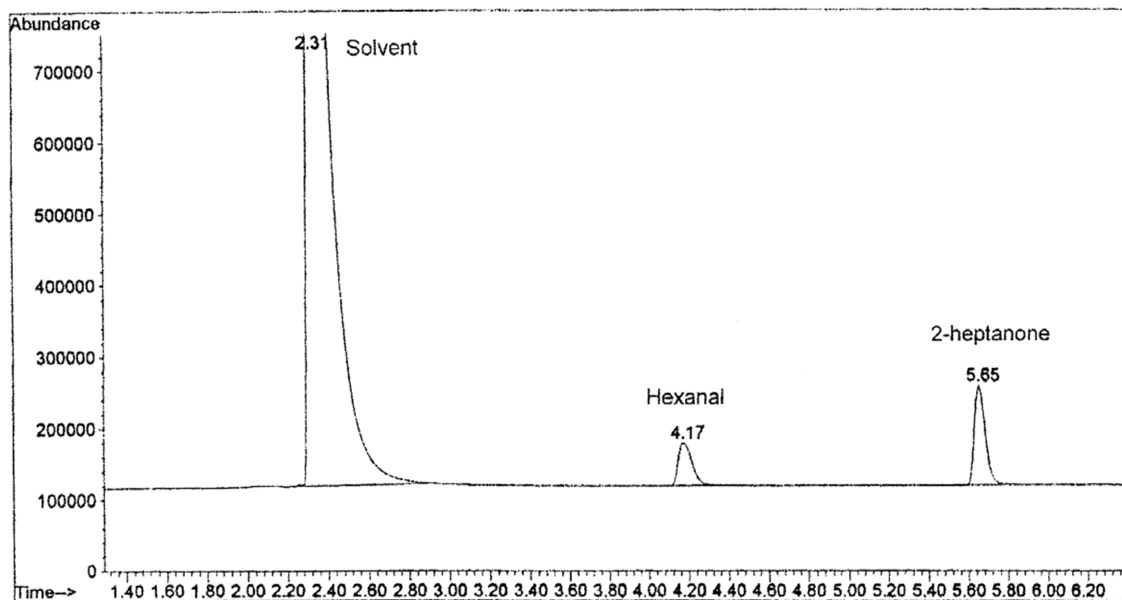

Fig. 2. Chromatogram of peak retention time and area for solvent, hexanal, and 2-heptanone for 20 μl of hexanal standard solution.

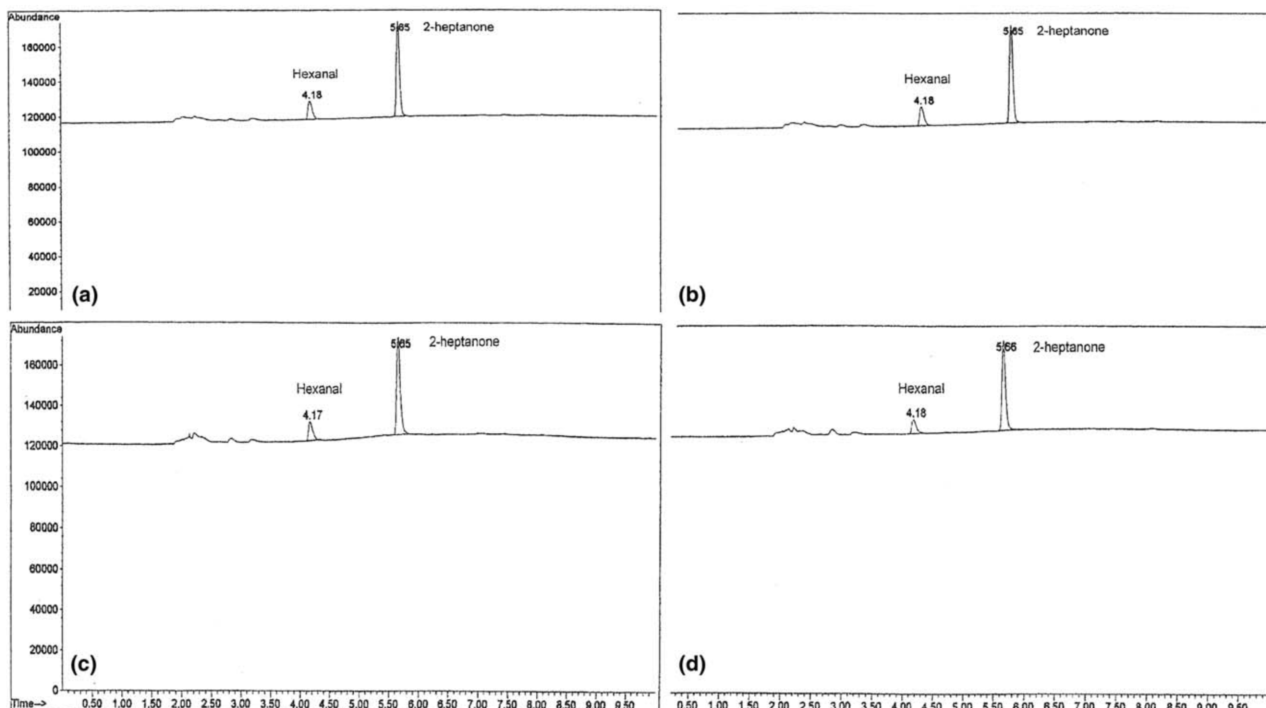

Fig. 3. Chromatogram showing peak retention time and area for hexanal and 2-heptanone for quinoa stored at different temperatures: (A) 25 °C, (B) 35 °C, (C) 45 °C, and (D) 55 °C.

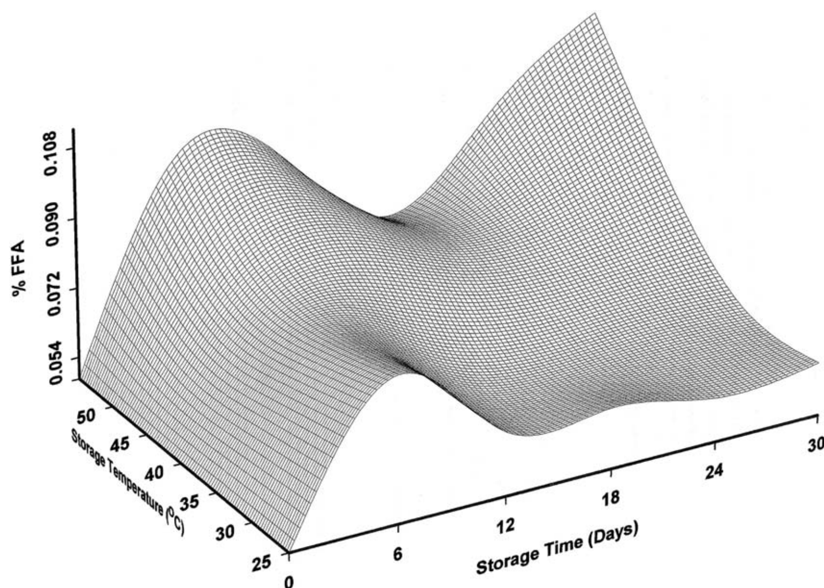

Fig. 4. Three-dimensional plot of the effects of storage time and storage temperature on production of free fatty acids in quinoa.

Table 1
ANOVA probability values (*p-values*)[a] for main and interaction effects of storage time and storage temperature on production of free fatty acids, conjugated diene hydroperoxides, and hexanal in quinoa

	Time	Temperature	Time × temperature
FFA	1.7×10^{-29}	1.99×10^{-19}	8.63×10^{-5}
CDHP	8.79×10^{-20}	1.0×10^{-3}	2.5×10^{-1}
Hexanal	1.0×10^{-3}	3.78×10^{-25}	3.99×10^{-6}

[a] $p \leqslant 0.05$.

showed significant effects ($p \leqslant 0.05$) on CDHP production, means separation by Fisher's least significant difference (LSD) test was performed on main effects to determine the specific points of storage that exhibited significant effects. LSD analysis (data not shown) showed that CDHP values were significantly different ($p \leqslant 0.05$) on days 6, 15, and 24 among the four temperatures.

St. Angelo and Ory (1975) compared peroxide value and CDHP production in peanut butter mixed with selected

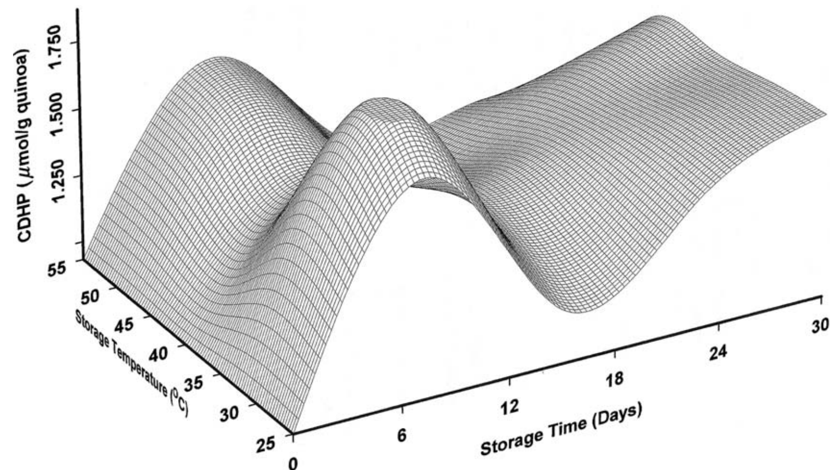

Fig. 5. Three-dimensional plot of the effects of storage time and storage temperature on production of conjugated diene hydroperoxides in quinoa.

additives. The samples were stored in the dark at room temperature, and analyzed at days 7, 14, and 28. In general, CDHP increased throughout storage time. This agrees with the current quinoa CDHP data, although the extent of rise in CDHP values was much less after day 15.

The slower rate of formation of CDHP, compared to the rate of FFA formation, may be explained by a study conducted by El-Magoli, Karel, and Yong (1980). In this study, the formation of conjugated double bonds in methyl linoleate increased with an increase in autoxidation. This suggests that oxygen is the limiting factor in the formation of CDHP, and may be a possible reason for the decrease in CDHP produced in quinoa after day 9, as oxygen in the jars was slowly being depleted in the process of autoxidation.

3.3. Hexanal

All hexanal values dropped slightly at day 3 (Fig. 6). For the 45 and 55 °C samples, only very minor fluctuations were observed over the 30-day sampling period. The largest change in hexanal was observed in samples kept at 25 °C, followed by samples kept at 35 °C. Hexanal increased from day 6 up to day 18 for the 25 and 35 °C samples, and then exhibited a gradual decrease. Storage time and storage temperature had significant effects on hexanal production (Table 1). This agrees with other accelerated shelf life studies in which hexanal was used as an indicator of oxidative rancidity. Cracker-coated and roasted peanuts stored at 40 °C for 110 days (Grosso & Resurreccion, 2002), whey-protein-coated peanuts stored at 40, 50, and 60 °C for 45 days (Lee, Trezza, Guinard, & Krochta, 2002) and 31 weeks (Lee & Krochta, 2002) all exhibited increases in hexanal over the period of time studied. Interaction effects were also significant for quinoa.

A contrasting observation was however, reported by Trawatha et al. (1995) in the study of soybean seed quality. Although the soybeans stored at 30 and 40 °C showed an increase in hexanal production within a 30-day period, more hexanal was produced at 30 °C compared to 40 °C, similar to the hexanal produced by quinoa in this study. For the soybean samples stored at 30 °C, hexanal increased quite sharply up to day 40, and decreased markedly from then onwards. Hexanal values beyond 30 days for samples stored at 40 °C also decreased.

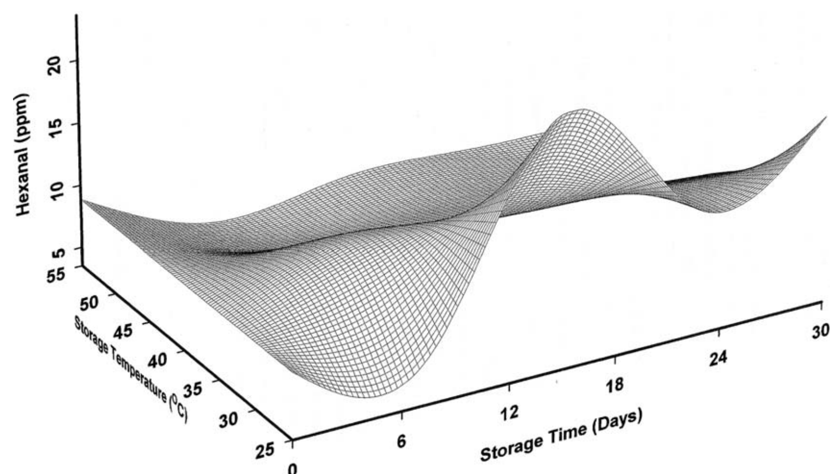

Fig. 6. Three-dimensional plot of the effects of storage time and storage temperature on production of hexanal in quinoa.

The low and almost unchanging amounts of hexanal produced in the 45 and 55 °C treatments observed in quinoa were unexpected. However, studies of peanuts (Grosso & Resurreccion, 2002; Lee & Krochta, 2002; Lee et al., 2002) showed that hexanal levels exhibited only very small increases in the first 30 days. In addition, the fat content of peanuts is about 50%. This is much higher than compared to quinoa, and is likely the reason why more hexanal was produced in peanuts over time.

Limited hydroperoxides were formed at 45 and 55 °C in this study. Since hydroperoxides are the precursors of hexanal, the low amounts of conjugated diene hydroperoxides formed should result in lower hexanal production at these temperatures. El-Magoli et al. (1980) reported that conversion of hexanal to hexanoic acid occurs at 50 °C and above. This could be another possible reason for the low hexanal values seen in quinoa at the higher temperatures.

Because no conclusive studies have been done to show the lipid stability in quinoa, reasons for the observations in the current study can only be postulated. First, it is possible that storage time in this study was not long enough for much oxidation to occur. This conclusion stems from the observation that free fatty acids continued to increase through the sampling period. In the lipid oxidation mechanism, free fatty acids come from triacylglycerols. The free fatty acids undergo oxidation to produce hydroperoxides. Hexanal is then produced from the decomposition of the linoleic acid hydroperoxides. This suggests that the 30 days of this study may have only covered the initial stages of oxidation; therefore, the increasing trend of values as seen in other studies was not observed. Thus, it is hypothesized that a more distinct increase in lipid oxidation byproducts would be observed if the quinoa were stored for a longer period of time.

On the other hand, the fact that minimal increases in oxidation products were observed in this study could also be interpreted as indication of the storage stability of quinoa. Quinoa contains high amounts of vitamin E, which is said to have a protective effect on the polyunsaturated fats in quinoa. Vitamin E acts as a free radical scavenger, terminating the free radical reaction in autoxidation. This could be a reason why little change was seen overall in the lipid oxidation byproducts examined in this study.

4. Conclusion

This study provided some preliminary information on the oxidative stability of quinoa. The amounts of lipid oxidation byproducts in quinoa were dependent on storage temperature and time. Significant differences were observed for storage time and temperature on the production of free fatty acids, conjugated diene hydroperoxides, and hexanal. However, on the whole, the differences appear to be minor, and production of the three byproducts had irregular patterns.

From this study, quinoa flour stored at ambient or a slightly higher temperature for a short period of time resulted in some oxidative deterioration, as measured by the three lipid oxidation tests. Although quinoa is higher in fat than are most cereals, the polyunsaturated fats in quinoa do not appear to oxidize rapidly for the period of time studied, even in the ground state where surface area for oxidative and enzymatic reactions is increased, and at higher temperatures which accelerate enzyme activity. This stability may be linked to the high vitamin E content in quinoa. With vitamin E as a naturally occurring antioxidant in quinoa, and its presence in abundant quantities, the potential for quinoa to be a new oilseed is enhanced, and it should appeal to food product developers interested in food applications focusing on antioxidant qualities naturally present in the raw product.

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