Prevention of Rancidity in Experimental Rat Diets for Long-Term Feeding

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ABSTRACT

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Experimental rat diets containing casein as the sole protein source were aged at 24 and 37°C for six months. Test variables included antioxidant effect and types of casein, fat, and iron. Samples were analyzed by a trained, experienced sensory panel for rancid odor intensity and by gas chromatography for pentanal and hexanal formation. Antioxidants in the diets effectively prevented rancid odor development and formation of pentanal and hexanal. Diets without the antioxidant combination of butylated hydroxyanisole and tertiary butylhydroquinone developed rancid odors and generated large amounts of the two aldehydes after four days of storage at 37°C and after eight days of storage at 24°C. Neither iron nor fat type had a significant effect on rancid odor development or on the content of aldehydes in the aged diets. The diets containing vitamin-free casein (alcohol-washed) had higher intensities for rancid odor and greater contents of pentanal and hexanal than did diets prepared with regular lactic casein. Logs of pentanal and hexanal contents correlated significantly with odor intensity values for rancidity in diets without antioxidants.

Rats are used extensively in feeding studies, and the oxidative stability of their diets is important if the introduction of undesirable variables is to be avoided.

Although rat diets of excellent stability have been formulated with soy protein (Hayes et al 1977) even without antioxidants (Bookwalter 1977), no information is available on the stability of other types of rat diets. Rancidity development in rat diets such as those with casein has not been investigated, probably due to the short-term duration of most feeding tests and to the use of fresh diet preparations. Long-term animal feeding studies using unstabilized diets could be adversely affected by the animals' preference for either rancid or nonrancid diets or by physiological differences resulting from ingestion of lipid oxidation products. Rapid development of rancidity after one week at ambient temperature in casein control diets formulated for a two-year feeding study prompted an investigation of the problem. The objectives of this research were to identify the factors responsible for the oxidative instability, to modify the formulations to provide stable casein diets, and to develop a method for detecting rancidity that correlated with odor analyses.

MATERIALS AND METHODS

Diet Composition

Experimental rat diets containing casein as the sole protein source were prepared using 34.5% casein (lactic or vitamin-free), 15.0% glucose, 5.0% nonnutritive fiber, 5.0% fat (corn oil or a lard-corn oil blend), 33.3% dextrin, 2.0% vitamin mixture, 0.2% choline chloride, and 5.0% mineral mixture (ferric citrate or ferrous fumarate). All diets were prepared at Purina Test Diets, Richmond, IN, with individual commercial ingredients of the same lot. Sixteen experimental formulations were statistically designed to test combinations of two types each of casein, fat, and iron, with and without antioxidants (Table I). Some of the diet components were chosen because of their possible prooxidant activity factors, eg, ferrous iron (Borenstein 1974), or their antioxidant effect, eg, tertiary butylhydroquinone (TBHQ). An Eastman Chemical (Kingsport, TN) product (Tenox 24) containing 20% each of TBHQ, butylated hydroxyanisole (BHA), corn oil, acetylated monoglyceride, and glycerol monoleate was added to half the diets at a level of 200 ppm based on a dietary fat content of 5%.

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Storage Conditions

Diets were packaged in 4-oz, wide-mouth jars with screw closures and then tightly sealed. A sufficient number of jars was placed in storage at 24 and 37°C to allow seven examinations of the diets, at 0, 10, 22, 30, 56, 90, and 182 days. Controls held at −18°C were used as blanks during odor evaluations.

Sensory Evaluations

Diets were evaluated for rancid odor intensity by a 12-member experienced sensory panel. Panelists used an odor intensity scale that rated rancid odor as 0 = none, 1 = weak, 2 = moderate, and 3 = strong. Standards for the four intensity levels were reviewed by the judges at the testing sessions. Panelists were instructed to open the storage containers and sniff the air in the headspace. Four samples of known identity (two blanks with no rancid odor and two diets with moderate rancid odor) were included with the 16 experimental diets as a check on panel consistency. Odor intensity values (OIV) for rancidity were calculated by the following formula (Rackis et al 1972):

$$\text{OIV} = \frac{1 \times \text{no. weak responses} + 2 \times \text{no. moderate responses} + 3 \times \text{no. strong responses}}{\text{no. panel members}}$$

TABLE I
Experimental Formulations

Sample	Casein Type[a]	Fat Type[b]	Antioxidant[c]	Iron Type[d]
1	X	L + CO	···	FC
2	X	L + CO	···	FF
3	X	L + CO	A	FC
4	X	L + CO	A	FF
5	X	CO	···	FC
6	X	CO	···	FF
7	X	CO	A	FC
8	X	CO	A	FF
9	Y	L + CO	···	FC
10	Y	L + CO	···	FF
11	Y	L + CO	A	FC
12	Y	L + CO	A	FF
13	Y	CO	···	FC
14	Y	CO	···	FF
15	Y	CO	A	FC
16	Y	CO	A	FF

[a] X = lactic casein (1.4%) fat, Y = alcohol-washed casein (0.1% fat).
[b] L = lard, CO = corn oil; blends are 1:1.
[c] A = Butylated hydroxyanisole + tertiary butylhydroquinone (200 ppm).
[d] FC = ferric citrate, FF = ferrous fumarate; 40 mg of Fe per kilogram of diet.

The samples were evaluated before aging at 24 and 37°C and at seven intervals from zero to 182 days of storage.

Volatiles Analyses

Casein diets were analyzed by gas chromatography (GC) with a modified direct technique for eluting volatile components (Fore and Dupuy 1972, Honig et al 1979, Rayner et al 1978). A Packard instrument with flame ionization detector was fitted with a glass column, 3 ft × ⅛ in. i.d., packed with Porapak P. Flow rates were 400 ml/min for air, 40 ml/min for hydrogen, and 40 ml/min for helium carrier gas. The inlet temperature was 120°C and the detector temperature was set at 230°C. The 40 mg of diet, layered with glass wool, was packed into a glass precolumn (7 cm × 4 mm i.d.). The internal standard (2 μl of 0.1% *n*-butanol) and 20 μl of distilled, deionized water were injected onto the precolumn before connection with the Porapak P column. *n*-Butanol was chosen as a standard because its retention time did not overlap those of any of the volatiles detected in the diet. In initial tests, 80 μl of water was added to the precolumn, but this amount partially clogged the precolumn; later tests showed better separation occurred with only 20 μl of water. The packed precolumn was placed into the inlet block and heated for 20 min at 120°C to sweep the volatiles onto the separation column. The precolumn was then replaced with a blank, and the oven was programmed from 50 to 100°C at 10°C/min and from 100 to 220°C at 5°C/min, with a final hold at 220°C for 10 min to clear the column. Pentanal and hexanal were chosen as markers for rancidity because of their prominence in the rancid diets. Peaks due to pentanal and hexanal were identified by comparing retention times with those of reference compounds. Quantitations were achieved by standardizing peak areas with a known amount of *n*-butanol. The response factors for 1 ppm of each aldehyde relative to 1 ppm of *n*-butanol were 1.25 for pentanal and 1.47 for hexanal. Integration counts were adjusted accordingly. Pentanal eluted at 120°C and hexanal at 138°C.

Statistical Analyses

Analyses of variance were computed to estimate effects of time, temperature, casein, fat, antioxidant, and iron type. Variation due to higher order interactions was used to test the signficance of lower order effects and interactions (Cochran and Cox 1964). Logs of pentanal and of hexanal concentrations were used in the analyses. Correlation coefficients were calculated for rancid odor intensity vs logs of the aldehyde contents.

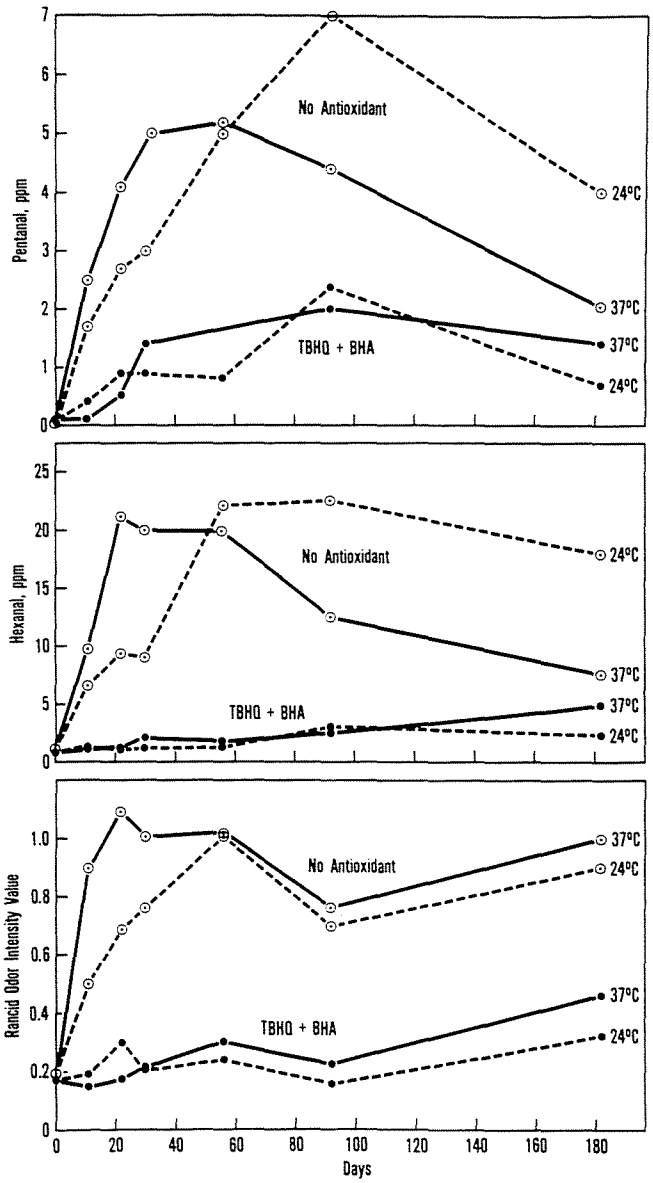

Fig. 1. Effects of antioxidant, storage temperature, and time on rancid odor intensity (LSD = 0.30), hexanal formation (ratios of hexanal >1.75 significant at 0.05 level), and pentanal formation (ratios of pentanal >1.99 significant at 0.05 level). **TBHQ** = tertiary butylhydroquinone, **BHA** = butylated hydroxyanisole.

RESULTS AND DISCUSSION

This study began as a result of a two-year research project initiated in 1979 to investigate the effects of various diets on rats. After the first batch of diets was formulated, a rancid odor was detected in the three control diets, which contained 10, 20, and 30% casein as the sole protein source, when stored at 24°C for 7–10 days and at 4°C for seven additional days. No rancidity developed in other diets containing either raw and toasted soy flours or raw and heated soy protein isolates as sole protein sources or in diets of soy flour blended with up to 20% lactic casein. The other dietary components, which included glucose, nonnutritive fiber, corn oil, lard, dextrin, vitamins, and minerals, were not rancid before being mixed with casein. The fat sources did not contain antioxidants.

The unprecedented development of rancidity in the casein diets after only a week of moderate storage conditions was considered to be a serious problem because the diets were to be the controls in the two-year study. The casein diets with soy appeared to be inherently more stable than those with no soy. Hayes et al (1977) reported that antioxidants are not needed to stabilize diets containing soy. Therefore other factors in the diets, such as casein, iron, fat, as well as the presence of antioxidants, were tested to determine their effects on rancidity development during storage. The antioxidant

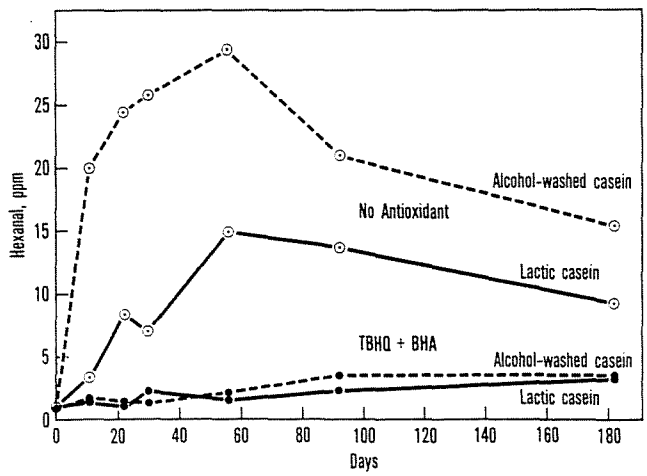

Fig. 2. Effects of antioxidant, casein type, and storage time on hexanal formation. **TBHQ** = tertiary butylhydroquinone, **BHA** = butylated hydroxyanisole.

system of BHA and TBHQ was chosen because in previous work (Bookwalter et al 1971) these additives were effective in stabilizing aged full-fat soy flour processed under extremely adverse conditions. Additional tests under less severe conditions showed that antioxidants were not needed.

Figure 1 shows the effect of antioxidants, storage time, and temperature on pentanal and hexanal formation and rancid odor intensity. Statistical analyses identified significant interactions of storage time, storage temperature, and antioxidant in controlling rancid odor and aldehyde development. Data were pooled to combine values for iron, fat, and casein type. Each data point reflects the values for eight diets. The variable in the diet formulations with the most pronounced effect for limiting rancid odor development and aldehyde formation was the antioxidant system containing BHA and TBHQ. The rancid odor intensity and pentanal and hexanal levels followed similar patterns for all the diets stabilized with BHA and TBHQ. After six months at either 24 or 37° C, the diets with antioxidants showed only a slight increase in rancid odor and volatiles formation. After 10 days of storage, the diets without antioxidants showed significantly more aldehyde formation and rancid odor development than did the stabilized diets. The diets aged at 37° C reached their highest rancid odor intensity and hexanal formation after 22 days, whereas their pentanal content peaked at 56 days. The diets aged at 24° C did not reach maximum aldehyde levels until 90 days of storage. After the aldehyde contents peaked, they decreased significantly on subsequent storage, indicating that some of the volatiles may be lost through interactions with diet components (Karel et al 1975) or be further decomposed. On the other hand, rancid odor intensity reached a peak and decreased slightly. The diets held at −18° C did not increase in rancid odor intensity during storage.

Figure 2 shows the effects of antioxidant, casein type, and time on hexanal formation. The type of casein in the diets was the other variable that had an effect on aldehyde formation and rancid odor development. Hexanal production in the antioxidant-free diets containing lactic casein was signficantly lower than in the corresponding diets containing the alcohol-washed (vitamin-free) casein. The removal of fat in the alcohol-washed casein did not increase oxidative stability. The pattern was similar for pentanal formation and rancid odor intensity. The type of casein had no effect in diets with antioxidants because the BHA and TBHQ prevented significant increases in the aldehydes and rancid odor. Table II shows the effect of casein type, antioxidant, and storage temperature on hexanal development. The data were pooled to combine the effects of fat and iron types and storage time. The amount of hexanal in the vitamin-free casein without TBHQ and BHA is significantly higher than that detected in lactic casein without additives. The pattern is the same at both 24 and 37° C. The alcohol washing of casein apparently altered the ability of the casein to resist oxidation and aldehyde formation. The mechanism for this effect was not investigated.

Fat and iron type were two variables in the diets that had statistically little effect on rancidity development and no significant interactions with the other variables, so data for fat or iron type were pooled according to casein type and antioxidant. The diets with corn oil showed similar rancid odor development and aldehyde formation throughout the storage periods as did the diets containing a blend of corn oil and lard. The diets with ferric acid citrate did not differ significantly in either odor or aldehyde development from the diets with ferrous fumarate in the presence or absence of antioxidants.

Table III shows the correlation coefficients calculated for pentanal and hexanal levels vs rancid odor intensity. Because the diets with antioxidants developed only a slightly rancid odor and had less than 5 ppm of either of the aldehydes, the coefficients were not statistically significant. In the absence of antioxidants, significant correlations between rancidity and aldehyde levels were observed at both storage temperatures and for both types of casein. Because the GC analyses of aldehydes correlated highly with rancid odor development, this instrumental method can be used to monitor rancidity in the diets when the use of sensory panels is inappropriate for large numbers of samples. Other researchers have also used pentanal and/or hexanal as measures of rancidity in low-fat foods and in vegetable oils (Fritsch and Gale 1977, Rayner et al 1978, Warner et al 1978).

The diet components responsible for the oxidative rancidity in the original diets and in the experimental formulations were not specifically determined but were thought to be either the casein or the unprotected fat source or an interaction between the two. The soy protein in the diets had a sufficiently strong antioxidant effect to overcome the factors causing the oxidative rancidity.

Based on this research and previously mentioned studies (Hayes et al 1977), we conclude that antioxidants are not needed to stabilize diets containing normally processed full-fat and defatted soy flours, concentrates, and isolates. However, if casein is the only protein source, antioxidants are needed. The antioxidant system containing TBHQ and BHA was the most significant factor in preventing oxidative deterioration in this study.

The use of pentanal and hexanal as indicators of rancidity correlated significantly with rancid odor intensities detected by the sensory panel. Although pentane has been used an an indicator of rancidity by other researchers (Jarvi et al 1971, Scholz and Ptak 1966, Warner et al 1974), it was not used as a marker in this study because only small amounts of this hydrocarbon were detected in the rancid diets and because a methanol peak eluted near the pentane peak. The use of pentane as an indicator is not appropriate for soy protein analyses when this solvent is used in processing or if the processing history of the products is unknown. The direct GC sampling technique is an acceptable method of monitoring rancidity in casein-based diets but should be used in conjunction with a trained panel.

As a result of this study, the original rat feeding project was continued with casein and soy diets stabilized with antioxidants. The factors of physiological impact of oxidation products in the rancid casein diets and preference of either rancid or stable diets by the rats were thus eliminated by the feeding of oxidatively stable diets.

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TABLE II
Effect of Casein Type and Antioxidant on Hexanal Development (ppm) in Diets After Storage at 24 and 37°C

Casein Type	Antioxidant	Storage at 24°C	Storage at 37°C
Alcohol washed (vitamin-free)	None	17.5[a]	14.9
	TBHQ + BHA[b]	1.6	1.9
Lactic	None	4.8	7.8
	TBHQ + BHA	1.5	1.8

[a] Average of four diets at seven storage periods.
[b] Tertiary butylhydroquinone + butylated hydroxyanisole.

TABLE III
Correlation Coefficients for Pentanal (C_5) and Hexanal (C_6) Values vs Odor Intensity Values for Oxidative Rancidity

Odor Intensity in Diets	Volatiles at 37°C C_5	Volatiles at 37°C C_6	Volatiles at 24°C C_5	Volatiles at 24°C C_6
Lactic casein				
No antioxidant	0.613[a]	0.611[a]	0.607[a]	0.719[a]
BHA + TBHQ[b]	0.120[c]	0.146[c]	0.031[c]	0.168[c]
Alcohol-washed casein				
No antioxidant	0.747[a]	0.831[a]	0.646[a]	0.684[a]
BHA + TBHQ	0.185[c]	0.235[c]	0.152[c]	0.200[c]

[a] Significant at the 99% confidence level.
[b] Butylated hydroxyanisole + tertiary butylhydroquinone.
[c] Not significant.

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